AUTHOR INDEX
(**Bold** type denotes presenting author)

Abolencia, Erma	P1, P2, P3, P4, P6, P7
Abraham, Tsadik T.	P33, **P34**
Agrawal, Alpana	P1, P2, P3, P4, P6, P7
Ahlner, Johan	S21
Akrill, Peter	P11, **P29**
Alexander, Russell	P50
Anderson, Daniel T.	S4
Andersson, Malin	S21
Anding, Keith H.	S36
Angier, Mike K.	P43
Annand, Mark	P66
Anne, Lakshmi	**P20**
Avella, Joseph	P54
Augsburger, Marc	S24
Backer, Ronald C.	S5, P61
Baldwin, D.	P11
Ballesteros, Salomé	S8
Baylor, Michael R.	S38, P9, P10, P38, P39
Bell, Suzanne C.	P70
Bell, Wendy C.	**P48**
Benjamin, David M.	**S18, P68**
Benoit, Marc R.	S33, P74
Benson, Paul	S11
Bih, Caroline	P20
Blum, Kristen	P26
Boakye-Agyeman, Felix	P76
Bodepudi, Vani	P20
Boeck, Gert De	P67
Bohulslavek, Jan	P77
Border, Paul T.	P73
Borges, Chad R.	P22, P23
Brainard, Katharine	S32
Brasher, Mary Jo	P79
Brewer, William E.	**S10**
Bruder, Michael F.	P71
Brunsvik, Anders	P24
Buck, M.	P81
Bull, Teresa G.	**P79**
Burrows, David	**P60**
Bush, Donna M.	P9, P10, P38, P39
Cadwallader, Amy B.	**P21**
Callery, Patrick S.	P70
Cangianelli, Leo A.	P38
Cassin, Bader C.	S5
Castella, Vincent	S24
Cathcart, David	P46
Cirimele, Vincent	S27
Clay, David J.	P42, P70
Clelland, Brandi L.	S10
Cody, John T.	P32
Cole, Jason	**P30**
Colman, Skyler	S29
Conoley, Meredith	P30, P31
Cooper, Gail	**S37,** P29
Costantino, Anthony G.	P80
Coulter, Cynthia	S32, P7, P8
Cox, Dawn	P57
Crifasi, Joseph A.	**P71**
Crouch, Dennis J.	P22, P23
Curtis, Travis E.	**S5**
Dahn, Tim	**P27,** P77
Datuin, Manny	P20
Davis, Paul J.	**P36**
Deakin, Anna L.	**S28,** P58
Dettmering, Troy B.	**P79**
Dilek, Işıl	**P40,** P41
Downer, Marc	S11
Dresen, Sebastian	S22
Drewieck, Kristin	P35
Dunkley, Chris	P17
Eagerton, David H.	P48, P49
Eap, Chin B.	S24
Edwards, Jim	P30, P31
Edwards, Randall	S11, P60
Elian, Albert a.	P25, P75
Esposito, Francis M.	P10, **P39**
Favrat, Bernard	S24
Fernadez, Maria del Mar Ramirez	P67
Feldman, Michael	S6
Ferguson, James	P38
Flaxmayer, Chester	**S14**
Flegel, Ronald R.	P38
Fochtman, Fred W.	**P61**
Fowler, David	P44, P57
Frazee, III, C. Clinton	**P45, P46**
Frederick, Donald L.	**S16**
Fritch, Dean F.	P26, P36
Fu, Eucen L.	**S4**
Furr-Holden, Debra	S32
Gantverg, Alex	**S6**
Garg, Uttam	P45, P46

Gassó, S. **P11**
Gebhardt, Myron A. P42
Gines, Leric P20
Giroud, Christian **S24**
Gock, Sue P50
Goodwin, Robert S. S1
Gorelick, David A. S1
Gracia, MªPaz Giménez P14, P53
Grambow, Tim **P49**
Greenberg, Tasha P44

Hackett, Jeffery **P75**
Hägg, Staffan S21
Hahn, Timothy **P54**
Hand, Chris P29
Hansen, L. P81
Hansen, Megan Y. P22
Hansen, Steen Holger P65
Hart, E. Dale P10
Harty, Linda S9
Hayes, Robert J. S1
Hearn, William L. P51
Heffron, Brendan P74
Hepler, Bradford R. S7
Herrin, George P78
Hilden, Malene P65
Hime, George W. P51
Ho, Hsiu-O P69
Holler, Justin P17
Holsinger, Thomas S11
Holt, Lisa A. **P78,** P79
Homan, Joseph W. P80
Honey, Donna **S20**
Huang, Mei-Han S19
Huestis, Marilyn A. S1, S23, P19, P33, P34, P82
Hughes, John **P23**
Hwang, Rong-Jen S20, P52

Isenschmid, Daniel S. **S7**

Jacobs, Aaron P17, P57
Jamerson, Matthew H. P18, P73
Janssen, Kimberly P71
Jehanli, A. P11
Jentzen, Jeffery P50
Johansen, Sys Stybe P64, **P65**
Johnson, Robert D. **P43**
Jufer, Rebecca P44, **P57**
Juhascik, Matthew **S3**

Kacinko, Sherri L. **P19**
Kalscheur, Diane P35
Kaplan, James A. P42
Karas, Roman P. P58
Karschner, Erin L. **S23**
Kazarian, Charles M. **P73**
Kelley-Baker, Tara S32
Kemp, Jesse **S29**
Kemp, Phil **S9**
Kerrigan, Sarah **S25**
Kiely, Elizabeth **S26, P62**
Kim, Insook **P82**
King, Andrea P28
Kintz, Pascal **S27**
Kiscoan, Mike P46
Klette, Kevin L. P18, P73
Kolbrich, Erin A. **S1**
Kraner, James C. **P42,** P70
Kronstrand, Robert **S21**
Kuhlman, Jr., James P60
Kuntz, David S6
Kupiec, Thomas S29

Labay, Laura **P66**
Lacey, John S32
Laloup, Marleen P67
Lambing, Matthew K. P30, **P31**
Langman, Loralie J. **S12, P76**
Larson, Scott **P17**
LeBeau, Marc A. S28, **S30,** P55, P58
Lee, Hei-Hwa S19
Lehrer, Michael P54
Levine, Barry P44, P57
Lewis, Russell J. P43
Lewis, S. Brandon P42
Liddicoat, Laura P35
Lieberman, Edwin P59
Lim, Carol S. P21
Lin, Dong-Liang P69
Liu, Hsiu-Chuan **P69**
Liu, Ray H. **S19,** P69
Lockrey, Lori A. P80
Long, Christopher W. P71
Lowe, Ross H. S23, **P33**
Luckie, Connie S33
Lykissa, Ernest D. S36

Maes, Vivienne P67
Magluilo, Joe P17
Mangin, Patrice S24
Marinetti, Laureen S26, P62
Martínez, María A. **S8**
Mathis, John A. **P80**

McClure III, F. Leland P9, P10
McGrath, Lynn P66
McKinley, Everett P40, **P41**
McKinley, Scott G. P73
McManaway, Daniel **P35**
Meaders, Meredith P9
Meenan, Gerard **P28**
Mireault, Pascal **P72**
Mitchell, John M. S38, P9, P10, P38, P39
Mitra, Swati P20
Monforte, Joseph R. S5, P61
Montgomery, Madeline **P55,** P58
Moore, Christine **S32,** P1, P2, P3, P4, P5, **P6, P7, P8**
Morgan, Stephen L. S10
Morris-Kukoski, Cynthia L. P58
Morrison, A. Michael P78, P79
Mozayani, Ashraf **S17**
Mundy, Lisa A. P37, P59

Nam, Daniel P12
Negrusz, Adam S33, P74
Newland, Gregory P26
Nguyen, Michelle P1, P2, P3, P4, P6, P7
Ngwa, George **P26**
Nielsen, Hanne P65
Nyström, Ingrid S21

Orebaugh, Leslie P15, P16

Patel, Mohan P28
Peace, Michelle R. **P15,** P16
Pearson, Julia M. **S11,** P56
Percy, Susan P35
Petersen, Henning Willads **P64**
Peterson, Bonita P46
Peterson, Joshua **S33,** P74
Pfeifer, Eric A. S12
Philips, Heidi P1, P2, P3, P4, P5
Pirnay, Stephane O. P33, P34
Poklis, Alphonse S31, P15, P16, P63
Pujol, Marie-Laure S27

Quimby, Bruce S34

Raicu, Mihai P28
Rajotte, James W. **P47**
Rana, Sumandeep S32, P7, P8
Razatos, Gerasimos **P52**
Reed, Amy P29
Reed, Claire P29
Repetto, Rosario García **P14,** P53
Rettinger, Joe P41
Rettinger, Mitzi P41
Rizvi, Taquer S36
Robarge, Trisa P30, P31
Rodrigues, Warren P1, P2, P3, P4, P5, P6
Rollins, Douglas E. S2, P21
Romberg, Robert W. **P18**
Ropero-Miller, Jeri D. S38, **P9**
Russell, Todd P40

Saady, Joseph J. S11, **P56**
Saki, Saffia **P44**
Salquebre, Guillaume S27
Samyn, Nele P67
Sánchez, MªLuisa Soria P14, **P53**
Sasaki, Tania A. **P25,** P27
Schaff, Jason E. **P58**
Schmidt, Carl J. S7
Schneider, Randal **P50**
Schreiber, André **S22**
Schwilke, Eugene W. S23
Schwope, David S33, **P74**
Scott, David P45, P46
Sell, Joann P36
Seto, Nora P20
Shacker, Laurie P49
Shakleya, Diaa M. **P70**
Shanks, Kevin P27, **P77**
Shelby, Melinda K. **P22**
Shenoy, Vima P20
Slawson, Matthew H. P22, P23
Smeal, Stacy J. **S2**
Smith, Dustin P49
Snyder, J. Jacob P73
Soares, James P1, P2, P3, P4, P5, P6, P7, P8
Somers, Dale P6
Spirk, Michelle A. **S15**
Sreenivasan, Uma P40, P41
Stadsgaard, Knud P65
Steentoft, A. P81
Stein, Elliot A. S1
Stephenson II, Robert L. P38
Stiess, Kristen D. P80
Stout, Peter R. **S38, P10**
Sukta, Andre J. S33, P74

Taddei, Lisa M. S33, P74
Tarnai, Lisa D. P15, **P16,**
Taylor, C. Mark P13
Teem, Denice M. S7

Terrell, Andrea R.	P27, P77
Tsai, Jin-Lian	S19
Valouch, Tara	P60
Valtier, Sandra	**P32**
Venuti, Susan	P60
Verebey, Karl	P28
Villain, Marion	S27
Vincent, Michael	S32, **P1,** P2, P3, P4, P5, P6, P7, P8, P62
Von Brand, Andrew	P38
Waddell, Kristine	P40
Wall, William H.	P78
Waller, Melissa	**P13**
Walsh, J. Michael	**P38**
Wang, Guohong	P1, **P2, P3, P4, P5,** P6
Wang, Liyuan	S6
Wasserman, Gary	P45
Weinhofer, Lisa	P36
Weinmann, Wolfgang	S22
Whitney, Carolyn	S33, P74
Whitney, Richard	P13
Wiese Simonsen, K.	**P81**
Wilkins, Diana G.	S2
Willette, Robert E.	**S35**
Wilson, Lisa	P29
Wingert, William E.	**P37, P59**
Wolf, Carl E.	**S31,** P15, P16, **P63**
Wong, Raphael C.	**P12**
Wong, Steven	P50
Wood, Michelle	P67
Wu, Chih-Hung	S19
Wu, Meng-Yen	S19
Zahlsen, Kolbjorn	P24
Zaney, M. Elizabeth	**P51**
Zettl, J. Robert	**S13**
Zisman, Arthur	P40, P41
Zoltek, Richard	P12
Zumwalt, Michael	**S34,** P23, **P24**

Key Words

-Phenylpropene	P70
1,1-Difluoroethane (DFE)	P54
1,3-Propanediol	P46
1,4-Butanediol	P55
6-AM conversion	P8
Abnormal Ph	P39
Accident	S16
Acidic and neutral drugs	P81
Adulteration	P58
Alcohol	P52
Alcohol testing	P12
Amphetamines	P3
Anabolic steroids	P21, P22
Analysis program	P65
Androgen receptor	P21
Antemortem casework	P62
Anticoagulants	S10
Anti-estrogens	P22
Antifreeze	P46
Arterial vs. Venous Levels	P68
Artifact	P45
enzodiazepines	S29, P26, P37, P79
Benzoylecgonine	P4, P18
Blood	S12, S32, P14, P35, P36
Blood Analysis	S31, P63
Body Packer	P51
Brown Mixture	P69
Buprenorphine	S21, P5, P19, P50
Cannabinoids	S2, P15, P16
Cannabis	S15, S24
Carbofuran	S9
CEDIA	P14
Chemistry analyzer	P11
Chloroethane	S28
Chlorophene	S5
ChromaLynx	P77
Chromatography	P76
Chronic therapy	S12
Clonazepam	P80
NS depressants	S18
ocaine	S7, S14, S38, P4, P59, P60
Codeine	P48, P69
Comparative Pharmacokinetics	P68
Concentrations	P53, P56
Conjugates	P33
Controlled dosing	S1
Criminal Justice	S35
Cryofocusing	S23
Cutoff	S30
Cyanamide	P53
Database analyses	P38
Deaths	S7
Decontamination	S38
Deconvolution	P13
DFSA	S25, S26, S28, S29, S30, P65, P66
Diesel fuel No. 2	S8
Diphenhydramine	P45
Disposable pipette extraction	S10
Distribution	P43, P49
Driving	S32
Dronabinol	S24
Drug impaired driving	S15
Drug-facilitated crime	P67
Drugs	S25, P64, P65
Drugs and driving	S31, P63
Drugs of abuse	S3, S21, S34, P9, P10, P11, P12, P13, P14, P24
Drug Testing	S36
DUI / DUID	S13, S14, S17
Effects	P56
EI-GC/MS	S33
ELISA	S3, S19, S29, P62
Enzyme assay	P6
Enzyme immunoassay	P15, P16, P20
Equine	P74
Ethanol	S18, P6
Ethyl glucuronide (EtG)	S6
Ethyl sulfate (EtS)	S6
Etorphine	S33
Expert	S17
Extraction efficiency	P7
Fast GC	P18, P73
Fatal	P42, P47, P60
Fatality	S8, P46
Fatal poisonings	P64

Fentanyl S7
Fluoxetine P43
Forensic evidence P59

Gas chromatography S9, P52, P72
GC/MS / GC/MS analysis S19, P13, P18, P28, P29, P30, P34, P36, P69, P74
GC/MS SIM S5
Genetic Polymorphisms S24
GHB P55, P56

Hair / Hair analysis S2, S27, S36, S37, P7, P8, P9
Hair Contamination S38
Hair Testing S38
Hashish P51
Headspace GC P70
Headspace GC/MS S28
Heroin S7
Homogeneous immunoassay P1, P2, P3, P4
Horse Racing S33
Huffing P54
Human Urine P34
Hydrocodone P61
Hydrolysis P33

Immunoassay S20, P5, P7, P19, P35
Impaired driving S15, P63
Impairment S13
Impairment while driving S18
Ingestion S8
Ion trap P24
ISO17025 Accreditation S37

Ketamine S11, S19, P20

LC-APCI-MS assay P82
LC/MS S6, S34, S36, P23, P24, P25, P27, P74, P77
LC/MS/MS P22, P26, P78, P79, P81
Lethal Injection P49
Library S22
LIMS P31
List of Concentrations P64
Lorazepam S26

Marijuana P63
Mass spectrometry P72
MDMA S1, P32, P33
MDMA metabolites P34
Measurement P76
Meclizine P44
Medical Review Officer P38
Melanocytes S2
Metabolism P74
Metabolites P32
Methadone S11, P60, P62
Methamphetamine S14, S16
Methanol P52
Microplate S20
Modafinil P45
Morphine S12, P47, P69

Naloxone P50
Naltrexone P28
Neurotoxicity P32
Nicotine P82
Nicotine metabolites P82
Nitrogen-phosphorus detector P72
Norbuprenorphine P5
Norketamine P20

OH-THC P25
Olanzapine P75
Opiates P1, P29
Opioids P27, P50
Oral fluid P1, P2, P3, P4, P6, P10, P11, P12, S32, S38, P26, P29, P30
Overdose P42, P44, P55
Oxazepam P40
Oxycodone S26, P73

Pain management S11
Papain P17
PCP P2
Pediatric P48
Performance testing P9, P10
Pharmacodynamics S1
Phencyclidine P57
Plasma P82
POCT S35
Poisoning P58
Postmortem S4, S9, P43, P44, P57, P61, P78
Postmortem toxicology P37,

Preferences P68
Prevalence P61
Promethazine P48
Pseudoephedrine S4
Psychosis S24
PT Program S35

QQQ S34, P23
QTrap P27
Quantitation P78, P79
Quetiapine P42

Rat P32
Reference standard P40

Scopolamine S27
Screen / Screening S22, P36, P67, P77
SOFT S30
Software P31
Sexual assault S25
Smuggling P51
Snorting P47
Solid phase extraction / SPE S5, S10, P34, P37, P69, P75
Solution P41
Sonication P8
Specimen validity testing P39
Sports doping P21
Stability P40, P41
Standards P41
Steroids P23
Stir Bar Sorptive Extraction P71
Suicide case P53

Tampering P58
Tandem Mass Spectrometry P80
THC S23, P25, P30
THC-COOH S13, P17, P25
Thermal Desorption P71
Thiopental P49
Time of intake S21
Tissue distribution S4
ToF-MS P67
Topiramate S16
Toxicology P31, P75
Two-dimensional chromatography S23
Twister P71

Ultra performance LC (UPLC™) P80
UPLC-MS/MS S27
Urinary S30
Urine P19, P28
Urine adulterant P17
Urine analysis S21
Urine drug testing P15, P16

Validation S20, S22, P35, P70, P81
Verapamil P74
Veterinary Toxicology S33
Vitreous humor S3, P57
Volatile abuse P54
Vomit P66
Voriconazole P76

Whole blood P81
Workplace drug testing P9, P10, P38, P39

Zolpidem P66

S1 Physiological and Subjective Effects Following Controlled Administration of Oral MDMA to Young Adult MDMA Users

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We conducted a randomized, within-subject, double blind, placebo-controlled study of the physiological and subjective effects of oral 3,4-methylenedioxymethamphetamine (MDMA) in humans. Physiological and behavioral measures were evaluated following controlled administration of placebo, low (1.0 mg/kg) and high (1.6 mg/kg) dose MDMA to six participants with histories of MDMA use. Actual administered doses were 56 to 150 mg MDMA, which are within the range of typical street doses. Four African American (3 male, 1 female) and two Caucasian (2 male) volunteers, ages 18-25, provided written informed consent to participate in this IRB-approved study. Subjects self-reported MDMA use that was confirmed by a positive urine amphetamines test or positive hair test for MDMA. Participants resided on a secure research unit with 24-h nursing coverage for at least 7 days after each MDMA dose; each session was separated by a minimum of one week. Physiological parameters included systolic and diastolic blood pressure (SBP and DBP), heart rate (HR) and body temperature. Visual analog scale (VAS) ratings for high, mind racing, heightened senses, and energy level were collected from -0.25 to 4 h following MDMA administration. Participants completed all dosing sessions without clinically significant adverse events. A safety concern was increased body temperature following MDMA; however, body temperature did not increase more than 1.3°C in any subject, even after the high MDMA dose. Mean ± SE maximum HR increases over baseline were 27.3 ± 7.1 and 35.3 ± 7.8 bpm after the low and high doses, respectively; however, there was high between-subject variability. One participant's HR more than doubled after both active MDMA doses. A significant increase in HR as compared to placebo was observed after the high, but not the low, MDMA dose ($p = 0.017$). There was no significant difference in the mean time of maximum HR increase between the low, 1.2 ± 0.2 h, and high, 2.0 ± 0.5 h, doses. There was a significant increase ($p = 0.011$) in mean SBP after the high MDMA dose (30.2 ± 6.2 mm Hg), but not the low (23.2 ± 5.2 mm Hg) dose, as compared to placebo. The greatest individual increases in SBP were 47 (low) and 43 (high) mm Hg. After the low dose, SBP peaked at a mean time of 2.0 ± 0.5 h and after the high dose, at 1.4 ± 0.4 h. DBP also was increased after MDMA; mean increases of 14.3 ± 5.0 (low) and 21.0 ± 3.3 (high) mm Hg were observed. Only DBP increases after the high dose were significantly different than placebo ($p = 0.049$). Peak DBP increases ranged from 0 to 30 mm Hg after MDMA administration. DBP peaked at a mean time of 1.5 ± 0.3 h after both active doses. Subjects reported significantly higher scores for the "high" VAS after the 1.6 mg/kg dose as compared to placebo ($p = 0.037$). Other VAS ratings did not achieve significant increases as compared to the placebo dose. These controlled MDMA administration data document significant increases in peak HR, SBP and DBP within two hours after oral MDMA at typical recreational doses. Increases in body temperature after MDMA were less than 1.3°C, indicating the importance of environmental influences, such as intense physical activity, water deprivation, and room temperature, in hyperthermia associated with MDMA use.

Supported by the Intramural Research Program, NIH, National Institute on Drug Abuse

Key words: **MDMA, Controlled dosing, Pharmacodynamics**

S2 Mechanism of Cannabinoid Incorporation into Hair.

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Hair pigmentation has been shown to play a role in the concentration of some drugs incorporated into hair. The binding of basic drugs such as cocaine, codeine and amphetamine to melanin is believed to produce a hair color effect through both ionic and non-ionic interactions. However, our present knowledge regarding the characteristics and mechanisms for the incorporation of non-basic drugs or their metabolites remains incomplete. In an abstract presented last year (1) we demonstrated that in an *in vivo* rat model hair pigmentation does not appear to be a critical factor in determining the concentration of Δ^9-tetrahydrocannabinol (THC), 11-nor-9-carboxy- Δ^9-tetrahydrocannabinol (THCA), or cannabinol (CBN) incorporated into hair, thus we studied cellular selectivity and used immunofluorescence to further characterize cannabinoid incorporation into hair. The basic drug cocaine (COC) and the non-basic drug, THC, were analyzed for influx and efflux into and out of pigmented melanocytes (PM) and nonpigmented melanocytes (NPM) to determine their incorporation in hair cells. NPM were of the same melan-a cell line as PM, but made amelanotic by culturing the cells in the presence of phenylthiocarbamide, a tyrosinase inhibitor. Initial influx for COC and THC was established early in the time course (i.e., a few minutes or less). Influx equilibrium for THC was completed and established approximately 100 min, whereas influx equilibrium for COC in pigmented and nonpigmented melanocytes was 60 and 90 min, respectively. Results show PM accumulate much greater amounts of COC at equilibrium than do the NPM. However, there was no difference between THC influx into pigmented and nonpigmented melanocytes. In contrast to the COC, THC uptake into melanoctyes is independent of cellular pigmentation. In addition, our early immunofluorescence studies have demonstrated COC association with melanosomes in pigmented hair follicle cross-sections from COC treated animals. These results are consistent with our previous data for *in vivo* incorporation of THC in pigmented and nonpigmented animal hair. In conclusion, these observations demonstrate that THC and THCA incorporate into hair by mechanism that is independent of pigmentation, or melanin binding.

Supported by NRSA fellowship # 5F31DA017432-02 and NIDA grant # DA07820.

Key Words: **Cannabinoids, Hair, Melanocytes**

(1) Smeal SJ, Wilkins DG, and Rollins, DE. The Incorporation of Delta-9-tetrahydrocannabinol and Its Metabolite, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, into Hair. *Soc Forensic Toxicol Abstracts*, 2005.

S3 Investigation into the Feasibility of Screening for Drugs of Abuse in Vitreous Humor

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Postmortem toxicological analyses can be limited by the integrity and quantity of blood present at the time of autopsy. While urine is an indicator of previous drug use, a biological specimen more closely representing perimortem drug concentrations would be a desirable alternative. The purpose of this study was to determine if an ELISA screen applied to vitreous humor is capable of detecting six drugs/drug classes. Sixty-five vitreous humor specimens that had corresponding postmortem blood specimens were selected for this study. All specimens (blood and vitreous humor) were screened for benzodiazepines, cocaine, opiates, fentanyl, oxycodone, and methadone using the Venture Labs, Inc. (Redwood City, CA) ELISA system. True positive, true negative, false positive, and false negative results were calculated for the vitreous humor as compared to the postmortem blood results. Vitreous humor exhibited the following percent agreement: benzodiazepines, 91%; cocaine, 92%; opiates, 92%; oxycodone, 100%; fentanyl, 71%; and methadone, 88%. Poorly correlated results between the two specimen types most notably occurred at or near the cut-off concentration. This may suggest differing pharmacokinetic distribution rates and patterns between blood and vitreous humor. However, the vitreous humor positive results were observed in cases with toxicologically significant concentrations of the six drugs/drug classes.

Keywords: **Vitreous humor, ELISA, Drugs of abuse**

S4 Distribution of Pseudoephedrine in Over 100 Postmortem Cases

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Pseudoephedrine (PE) is commonly found in over-the-counter cold and allergy medications along with antihistamines and non-narcotic analgesics. PE is the d-isomer of Ephedrine; both occur naturally in *Ephedra* species such as Ma Huang. PE is primarily used as a bronchodilator and nasal decongestant and can be classified as a sympathomimetic amine because of its alpha-adrenergic properties. A typical PE dose ranges between 15 to 240 mg per day. Given the widespread use of PE, it is surprising that there is very little published information concerning the distribution in postmortem specimens, specifically the potential toxic and lethal blood concentrations. The objective of this study was to complete a comprehensive PE analysis on commonly submitted specimens by medical examiners for toxicological analysis and to evaluate all the data for future interpretation.

Cases with PE were initially detected in postmortem specimens during methamphetamine confirmation of cases that were screened positive by ELISA. Extraction of PE was by solid-phase; confirmation and quantitation of PE was performed on a GC/MS following an acylation derivatization.

Over a two-year period, 102 cases were quantitated for PE in at least three of the four commonly submitted autopsy specimens: central blood, peripheral blood, vitreous, and liver. The following are the average PE concentrations detected for each specimen: Central blood 1.1 ug/ml (0.01-16 ug/ml, n=102), femoral blood 0.97 (0.01-14, 100), vitreous 1.0 (0.03-16, 79) and liver 2.6 ug/g (0.02-28 ug/g, 89).

This study provides the necessary information that can ultimately be used by toxicologists around the world for interpretation of their own casework.

Keywords: **Postmortem, Pseudoephedrine, Tissue Distribution**

S5 The Detection of Chlorophene in Postmortem Biological Specimens

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This presentation summarizes the investigation and significance of the presence of chlorophene in three postmortem cases.

Chlorophene, o-benzyl-p-chlorophenol, is a germicide and disinfectant that has wide use in commercial and household settings. Chlorophene is the main ingredient of Ty-D-Bowl™ brand toilet bowl cleaner. This chemical was detected and identified by full scan GC/MS in three postmortem cases processed by Ameritox, Ltd. The identifications were based upon library searches and comparison of the GC and MS properties with those of the commercially purchased material. In one case the finding was supported by the case history, but in the two other cases the result was unexpected. All three cases were submitted by the same death investigation agency.

The quantitative analysis of Chlorophene was accomplished by solid phase extraction and GC/MS SIM. One mL whole blood was placed into a 15 mL glass tube and then 5 mL Biochemical Diagnostics 0.25 M pH 9.1 phosphate buffer and internal standard were added. The calibrator and controls were prepared in an identical manner. All samples were extracted using Biochemical Diagnostics GV-65 solid phase extraction columns. After drying under vacuum for 10 minutes, the samples were eluted into a 5 mL glass tube with a n-butyl chloride/acetonitrile solution (45:55). The eluent was collected and concentrated under a gentle stream of nitrogen, reconstituted with 100 µl ethyl acetate, and 1.5µl of the extract was injected onto an HP 6890 GC equipped with an HP 6973 MSD. The initial temperature was held at 150°C for 2 minutes and then increased to 280°C at a rate of 20°C per minute. The 218 ion was used for quantitation and 183 and 140 ions were used as qualifying ions. Employing a 1000 ng/mL calibrator, the assay was determined to have a linear range of 250 ng/mL to 3000 ng/mL.

In these three cases evaluated for the presence of chlorophene, the concentration of chlorophene in blood ranged from 914 ng/mL to 2203 ng/mL.

Keywords: **Chlorophene, Solid phase extraction, GC/MS SIM**

S6 Ethyl glucuronide and Ethyl sulfate as markers of ethanol consumption.

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Ethyl glucuronide (EtG) urine testing as a marker of alcohol abuse has advantages of prolonged detection of consumption, hours after ethanol itself is been eliminated from blood and urine. It has become a very valuable tool in combination with medical history in evaluating abstinence in patients enrolled in alcohol abstinence programs. However, over two years of running this test we have encountered the problem with about 1% of EtG positive samples. These samples re-aliquoted the following day have shown EtG confirmation levels 2 to 10 fold lower than their initial screening values. Urinary tract infection or possible bacterial contaminations during sample handling are suggested to be the source of β-glucuronidase occurrence in the samples responsible for EtG degradation.

Ethyl sulfate (EtS) is another non-oxidative alcohol metabolite that is known to be unaffected by the presence of bacterial β-glucuronidase. The objective of this study was to add EtS detection capability to our EtG LC-MS/MS method and to establish correlation between EtG and EtS levels in urine in order to consider EtS testing for sample confirmation screened positive for EtG.

Analyses of EtG and EtS urine levels in donor samples were performed in the negative electro-spray mode on an API 3000 triple quadrupole mass-spectrometer (Sciex) coupled with Shimadzu LC-10ADvp pumps and SIL-HTC autosampler. EtG-D5 was used as internal standard for EtG quantitation and Methyl Sulfate – for EtS quantitation. The established LOQ for both markers in urine was 20 ng/mL.

Over 400 donor urine samples were analyzed for EtG and ETS concentration in urine. The highest ever urine EtG and EtS values recorded in our lab were 3,460,000 and 601,000 ng/mL respectively, detected in the urine of a donor admitted to an alcohol addiction clinic. In contrast, donors abstaining from alcohol usually have EtG levels below 100 ng/mL. EtG degradation may be solved by introducing EtS as a confirmatory analyte for ETG. For example, a sample with the initial EtG screening value of 6790 ng/mL was re-aliquoted for confirmation the following day and gave results of 3550 ng/mL of EtG and 1028 ng/mL of EtS. Reconfirmation with the new aliquot the next day gave EtG and EtS values of 421 and 1210 ng/mL respectively. In this specimen EtG degradation was dramatic while EtS value remained consistent within analytical tolerance. Both analytes are stable in urine in the room temperature for at least six months (in the absence of bacterial contamination).

The 356 samples that were within 20 to 1,000,000 ng/mL EtG/EtS concentration range were used for regression analysis. The established linear correlation coefficient between EtG and EtS values was 0.9404. Although the individual variations in the ratio between EtG and EtS were rather wide (0.3 to 17) the average ratio value was 4.0 which had closely matched the slope (3.64) of the regression line between EtG and EtS values.

The established correlation between EtG and EtS permits to substitute EtG with EtS for confirmation testing. Our method sensitivity allows the confirmation of alcohol consumption at the cut-off of a 100 ng/mL of EtS in urine. The combination of ETG and ETS during reconfirmations of initial ETG analysis is attractive. Using different markers for screening and confirmation of ethanol consumption would improve this test reliability and eliminate false negative reports due to EtG degradation.

Key Words: **Ethyl sulfate (EtS), Ethyl glucuronide (EtG), LCMS confirmation**

S7 Postmortem Toxicology Findings Associated With a Rapid Increase in Fentanyl-Related Deaths in the Detroit Metropolitan Area

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Beginning in late August 2005 the toxicology laboratory of the Wayne County Medical Examiner's Office (WCMEO) began detecting the presence of fentanyl in deaths related to cocaine and/or heroin use. As of June 30, 2006, there have been a total of 118 cases of fatalities associated with fentanyl in combination with these drugs of abuse. An additional 36 deaths due to fentanyl or fentanyl and prescription drugs were observed during the same period. (26 additional cases in which fentanyl was detected were excluded from the data as hospital-related administration).

By May 2006 a widespread outbreak of fentanyl-related overdoses were reported in the national media. Through analysis of crime laboratory exhibits and seizures from clandestine laboratories, it was determined that illicit fentanyl was being manufactured and sold by itself or mixed with heroin or cocaine. At what point the drugs were getting mixed is not clear but users are aware of the availability of a "potent heroin" with names such as "suicide", "drop dead", "reaper", "penicillin", "lethal injection" or "crazy" stamped on the packets.

In recent years the WCMEO has seen a gradual increase in the incidence of cases in which fentanyl was identified peaking at 29 cases in 2004. These gradual increases were largely due to wider use of fentanyl patches and abuse associated with them. By the end of 2005 the fentanyl incidence had jumped to 63, with only 13 cases occurring prior to August 2005.

Table 1 shows a statistical break down of the fentanyl concentrations determined by GC/MS-SIM (LOD 1 ng/mL) in cases attributed to the fentanyl-laced deaths. In most cases heart blood was available. When iliac blood was available it was analyzed and the heart blood to peripheral blood ratio was calculated. Some of these ratios were quite high. Although post-mortem redistribution of fentanyl is possible, some of these deaths involved finding the decedent with a syringe still in the arm or groin, suggesting that early, partial drug distribution may also play a role in some high blood to peripheral blood ratios.

Table 1: Fentanyl concentrations (ng/mL) and Heart / Peripheral concentration ratios

	Heart Blood	Iliac Blood	Ratio Heart/ Iliac
Mean	34	17	2.9
Median	22	14	2.1
Range	3 – 190	3 – 69	0.55 – 10.7
N	137	69	64

The demographics of the population was interesting and suggested widespread use of the combination of drugs throughout the metropolitan area. The majority of decedents were male (66%), white (62%), were non-Detroit residents (53%), and between 40-59 years of age (67%). Most were found dead at the scene or in their home (77%).

Key Words: **Fentanyl, Heroin, Cocaine, Deaths**

S8 Investigation of a Fatality Due to Diesel Fuel No. 2 Ingestion

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This paper presents a simple, rapid, reliable, and validated analytical method suited for forensic examination of diesel fuel No. 2 in biological specimens. The proposed methodology has been applied to the investigation of a forensic case due to diesel fuel No. 2 ingestion. Case history and pathological and toxicological findings are described here to illustrate the toxicity of this complex hydrocarbon mixture. The toxicological significance and the possible mechanisms leading to death are also discussed. The toxicological initial screening and quantitation were performed by means of gas chromatography with flame ionization detector and confirmation was performed using gas chromatography-mass spectrometry total ion chromatogram mode. n-Tetradecane peak was selected to estimate diesel fuel No. 2 in all biological samples. Diesel fuel No. 2 analytical methodology was validated at five concentration levels from 5 to 400 mg/L. The method provided extraction recoveries between 89.0% and 97.9%. The limit of detection was 1 mg/L and the limit of quantitation was 5 mg/L. The linearity of the blood calibration curves was excellent with r^2 values of > 0.999. Intraday and interday precisions had a coefficient of variation ≤ 10.9% in all cases. The case reports the suicide of a 64 year-old female due to diesel fuel No. 2 ingestion. Heart blood concentration was 9.1 mg/L and concentration in the abdominal contents was 3500 mg/L (total amount 8.2 mL). Pathological findings were unusual and consisted on gastric and intestinal lesions reminding of effects caused by ingestion of a corrosive agent, and the presence of a dark brownish liquid with strong odor to hydrocarbons. The cause of death was attributed to intoxication with diesel fuel No. 2. The manner of death, according to case history provided by the medical examiner, was considered as suicide. As there are no other similar references regarding analytical and toxicological data, this work provides with evidence about toxic concentrations and is a useful adjunct to the postmortem toxicological interpretation of fatalities if the decedent has been involved in diesel fuel No. 2 use.

Key words: **Diesel fuel No. 2, Ingestion, Fatality**

S9 Postmortem Distribution of Carbofuran in Two Cases From Oklahoma

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This presentation describes two cases of suicidal poisoning with carbofuran. Carbofuran is a broad spectrum insecticide used for controlling a variety of insects and nematodes. It is sold in granular or fluid form for use on such crops as alfalfa, corn, peanuts, and potatoes. Carbofuran is an N-methylcarbamate with reversible cholinesterase inhibitor action.

Case #1 is that of a 37 year old white male with a history of drug abuse. He would use anything he could get his hands on. After becoming ill, the deceased was loaded into a private vehicle to be transported to the nearest emergency room. He arrested in the car en route. The sister stated he might have taken 15 Lortab and 15 Clonopin. Case #2 involves a 92 year old white male with an extensive medical history, the most recent complaint being pneumonia. He stated that he was tired of dealing with doctors and talked about killing himself. His wife found him in a chair with a glass jar containing fluid next to him. The wife recognized the jar as one he used to mix hamburger meat and poison to kill coyotes.

Carbofuran was identified on both base and acid/neutral screens using gas chromatography with nitrogen phosphorous detection, flame ionization detection, and mass spectrometry following liquid-liquid extraction. Quantitation was performed on a variety of fluids and tissue homogenates using a methylene chloride extraction of samples buffered to pH 5.5, followed by gas chromatography/flame ionization detection. Concentrations of carbofuran were determined by comparison to blood calibration curves. The method was linear over a broad range (1.0 – 16.0 mcg/mL). The results of the case analyses are listed in Table 1. The cause of death in each case was carbofuran toxicity and the manner of death was ruled suicide.

Table 1. Postmortem distribution of carbofuran (mcg/mL, mcg/G)

	Heart Blood	Femoral Blood	Subclavian Blood	Vitreous	Liver	Brain	Gastric (mg)
Case #1	5.3	3.2	5.6	7.0	7.9	7.5	251
Case #2	8.2	5.1	n/a	1.4	14.6	5.4	88

Keywords: **Carbofuran, Postmortem, Gas chromatography**

S10 Rapid Extraction of Anticoagulant Rodenticides from Liver Specimens

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The analysis of anticoagulant rodenticides from liver specimens can be very time consuming, mostly due to the time required for sample preparation. Most methods in the literature focus on column chromatography methods to remove unwanted sample matrix components, and these methods require large volumes of solvent. In this method presented, a solid-phase extraction (SPE) method is used that drastically minimizes the amount of solvent required for the analysis as well as the amount of time required for the extraction. The SPE method utilized in this procedure is rapidly performed inside a pipette tip using a technique referred to as disposable pipette extraction (DPX). The DPX tips utilized are composed of styrene divinyl benzene sorbent, which are used to extract hydrophobic analytes.

The method involves first blending 2 g of liver with 6 mL of 0.1% acetic acid in acetonitrile. After blending the sample, 1.5 mL (0.5 g tissue equivalent) is added to 3 mL of water, and the solution is drawn into a 5 mL DPX tip using an attached syringe device (or pipette) and mixed with the sorbent (by drawing in air). After just 30 seconds of equilibration, the sample solution is dispensed back into the test tube, and it is subsequently drawn back into the DPX tip repeating the extraction method to ensure high recoveries and thorough mixing. The sample solution is dispensed to waste, and then 1 mL of water is drawn into the tip and mixed with the sorbent (by drawing in air), and this wash solution is dispensed to waste after 10 seconds. The elution of the analytes of interest is accomplished by drawing in 0.5 mL of 0.1%/0.2% acetic acid/diethylamine in acetonitrile and mixing with the sorbent, waiting 10 seconds, and dispensing into a small test tube, and repeating this elution step. To ensure high recoveries of the more nonpolar anticoagulant rodenticides from the sorbent, the final elution step uses 0.5 mL of methylene chloride as previous. The eluents are then evaporated to dryness using nitrogen gas and heat (70 °C).

A rapid cleanup step was found to be necessary with the use of an ion trap MS detector due to interference from sample matrix components, presumably fatty acids. This was accomplished by using a 1 mL DPX cleanup tip composed of 150 mg basic alumina. This extra cleanup step is accomplished in just a few short minutes due to the limited solvent volumes required.

Nine common anticoagulant rodenticides are extracted using this single method presented. Brodifacoum, bromadiolone, warfarin, dicumarol, coumachlor, diphacinone, chlorphacinone, difenacoum and difethialone are extracted with greater than 70% recoveries with coefficients of variation of approximately 10% at 50 ppb levels. Limits of detection were found to be less than 20 ppb for most of the anticoagulants using HPLC-MS/MS with an ion trap MS detector and approximately 5 ppb or less using a triple quadrupole MS detector.

Key words: **Solid-phase extraction, Disposable pipette extraction, Anticoagulants**

S11 Pain Mismanagement? A Case Report involving Ketamine and Methadone

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This case involves a 40 year old female with a long history of psychiatric and medical disorders including Munchausen's syndrome, scoliosis, Crohn's disease, severe autoimmune skin disorder (pyoderma gangrenosum), anxiety, chronic pain and eight previous suicide attempts. In 2003, her dermatologist began prescribing intravenous ketamine ampules, of which the patient was instructed to dilute and take orally for the treatment of chronic pain associated with her skin disorder. Subsequently, her psychiatrist continued to prescribe intravenous ketamine for oral administration and also began prescribing methadone for the continued intractable pain.

She was found unresponsive on her living room floor. Death investigators located a cardboard box full of medications, pill bottles and injection bottles. Her prescriptions included Cymbalta (duloxetine), alprazolam, clonazepam, Lunesta (eszopiclone), gabapentin, hydroxyzine, Celebrex (celecoxib), trazodone, numerous anti-inflammatory and antibiotic medications, ketamine and methadone. Hundreds of injection bottles of ketamine were recovered in the house. Instructions on the iv ketamine bottles indicated the patient was to dilute 10 mL ketamine with 40 mL water and to take 1 teaspoon of this mixture orally 4 times daily for pain. In the 55 days preceding her death, she had received a total of 370 ten mL iv ampules containing 10mg/mL ketamine (total 37 gm ketamine). In addition, 9 days prior to her death, her psychiatrist began prescribing methadone (10 mg tid or 30mg daily) for pain.

Autopsy results indicated no anatomic findings other than moderate scoliosis of the spine and patchy involvement of pyoderma gangrenosum. Toxicology results were negative for ethanol, methanol, acetone, isopropanol, cocaine/benzoylecgonine, opiates and benzodiazepines. GC/MS analysis of an alkaline extract confirmed the presence of methadone, ketamine and norketamine. No other alkaline extractable drugs were detected. Tissue distribution of methadone was as follows: heart blood 0.4 mg/L, liver 8 mg/kg and gastric 38 mg/L. Tissue distribution of ketamine (norketamine) was as follows: heart blood 0.09 (0.2) mg/L, liver 0.2 (0.8) mg/kg, brain 0.1 (0.2) mg/kg, gastric 3 (2.5) mg/L. By history, the decedent was a relatively naïve user of methadone (<10 days) and her death was determined to be accidental methadone intoxication.

This is an extremely unusual case in that it is the first time we have seen a physician prescribe intravenous ketamine for oral administration in the treatment of chronic pain. A review of recent medical literature indicates a renewed interest in evaluating ketamine for pain management. The treatment involves two very different radical approaches. The first involves using anesthetic doses of ketamine to induce a comatose state for several days and the patient emerges with disrupted or "reset" sensitization to pain. The second involves subanesthetic oral doses of ketamine, an effect postulated to occur by ketamine's antagonism of the N-methyl-d-aspartate (NMDA) receptor. This case also reiterates the danger of dispensing large quantities of methadone for the treatment of chronic pain.

Key words: **Ketamine, Pain management, Methadone**

S12 Post-Mortem Blood Levels of Morphine in Chronic Therapy

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The Federal Medical Center in Rochester (FMC) is an administrative facility providing specialized medical and mental health services to male offenders housed within the Federal Bureau of Prisons. Patients in this facility are often critically ill and may require long term care. The objective of this study is to try to evaluate post-mortem morphine concentrations in patients who were on prolonged morphine treatment and who may have developed tolerance.

Fifty-four cases were initially collected from May 2003 until June 2006. Individuals on morphine for pain management who died from natural disease were identified, resulting in a total of 16. The causes of death in those patients included: myocardial infarction (2), cancer (2), pneumonia (3), and complications of advanced liver disease (9). Free morphine (unconjugated) was measured by Gas Chromatography-Mass Spectrometry using Selected Ion Monitoring Detection.

The whole blood concentrations of free morphine ranged from 0.012 – 2.34 mg/L, and urine total morphine concentrations from 0.59 – 294.60 mg/L. There was no significant correlation between the whole blood and the urine concentrations. For therapeutic drug monitoring purposes, the Mayo Clinic uses therapeutic serum/plasma ranges of free morphine of 0.070 - 0.45 mg/L for analgesia, and suggests that toxic effects could be noted in patients with levels exceeding 0.70 mg/L. Levels of 0.20 mg/L in post-mortem blood samples that have been associated with fatality. But the reported minimum lethal level varies with the route of administration. Of note is the ranges seldom take into account tolerance that an individual may have. In this analysis the cases all had long term morphine therapy, and presumably some degree of tolerance. Seven of the sixteen cases had whole blood morphine levels above the reported serum therapeutic range, and three would be considered to be in excess of the toxic/lethal range for postmortem blood.

Additional patients and information about the dosing history and time of drug administration will be collected. This will allow us to evaluate the relationship between dose, duration of therapy and concentrations.

Key Words: **Morphine, Chronic therapy, Blood**

S13 Can a Positive THC Metabolite in Urine (THC-COOH) Be Used to Prove Impairment in a DUID (Driving Under the Influence) of Cannabis Case?

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Subject was stopped for speeding at 12 am on Interstate 70 at Floyd Hill, which has a 6% grade. Officer observed subjects "speech was slow and thick tongued and he had a brown-green coating on his tongue". Subject stated he had taken a Vicodin earlier due to dental surgery. His right hand was broken and in a heavy cast and he had had recent oral surgery. Subject agreed to perform SFST's, which "he failed to perform, as a sober person would have".

During a search of the subject's vehicle the officer found a partially opened 24 pack of beer with an opened can in the console, and a glass pipe. No DRE.

BrAC was 0.034% at 01:26 am. URINE screened positive for Cannabinoids (Detection Limit 25 ng/ml) and confirmed for Delta-9-THC-COOH (Detection Limit 5 ng/ml) with a Cannabinoids Semi-Quant of 84 ng/ml. Sample also screened positive for Opiates (Detection Limit) 300 ng/ml) - confirmed positive for Hydrocodone.

The subject and persons he spent the day with confirmed he had consumed a MINIMAL amount of beer prior to the stop but had not taken any other drugs than the Vicodin within the prior 6 hours. The Vicodin was provided by his dentist for pain.

? - Could you testify that the drug and drug metabolite found in the subject's urine substantiate that he was impaired by drugs to such a degree that he could not safely operate a motor vehicle.

From a Pro-Perspective - the combination of the alcohol, failure to pass the SFST's (conducted on a 6% incline with cars whizzing by very close and with a full arm cast and other pain), speeding and drugs in his system from consumption some time prior to the event would be cause to conclude that his driving ability was affected to such an extent that he could not safely operate a motor vehicle. ** Urine drug concentrations have no impairing affects on ones ability to safely operate a motor vehicle but can infer.

From a Con-Perspective - Positive Urine Drug Concentrations only infer that a drug was ingested at some prior time (See DOT HS publication 809 642) and as such should never be used to attempt to show subject impairment at time of driving. From a purely toxicology standpoint is it generally accepted that drugs found in urine have no impairing affects on a person's Central Nervous System nor driving ability. Drug(s) in a person's blood at or above a certain concentration can be impairing.

Keywords: **THC-COOH, Impairment, DUID**

S14 “But Officer, I Just Fell Asleep”. Relatively low levels of Methamphetamine and Cocaine in a Fatal Traffic Accident

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The case history and toxicological findings of a DUI related traffic fatality are presented. An individual was seen driving his car on a public city street. According to witnesses, he crossed the raised median and crashed, virtually head-on, into a motorcycle. The rider of the motorcycle was killed. The driver of the car indicated that he “fell asleep.” Blood is drawn several hours after the crash. Subsequent analysis of the blood showed it to contain 14 ng/ml methamphetamine with no amphetamine being found in the sample. The laboratory report indicates that the GCMS report for the methamphetamine represented the total of the undifferentiated d and l enantiomers. The blood also contain 45 ng/ml cocaine and 1000 ng/ml benzoylecgonine. Evidence introduced at trial showed that the defendant had been awake for some 20 hours prior to the accident and the police report indicated that the drugs were consumed some 12 hours prior to the crash. No D.R.E or SFSTs were performed.

The prosecution and defense arguments presented at the trial of the driver for Second Degree Murder will be discussed.

Keywords: **Methamphetamine, Cocaine, Driving under the Influence**

S15 Cannabis Impaired Driving: DRE Blood THC Case, Prosecution and Defense Perspectives

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Presented is a well-documented DUID Cannabis case including significant driving behavior, DRE evaluation, psychoactive parent drug quantitated in blood obtained close to time of driving, that corroborate DRE opinion of cannabis related impairment. Application of Huestis Time-of-Use Models provide additional support of recent cannabis use, consistent with related driving impairment. Case analysis includes both prosecution and defense perspectives.

I. Initial Case Facts: ✓Incident 2-20-05 0044 hrs ✓21 y/o female ✓Busy metro intersection ✓Heavy rain, flooded streets
II. Driving: ✓Completed left turn in wrong lane ✓Observed by unmarked police car ✓Continuously wove inside/outside lane, straddled two lanes before drifting back ✓After emergency lights, drove to next intersection, turned, then stopped ✓No seat belt
III. Observations/Interviews: ✓Attitude cooperative ✓Coordination normal ✓Clothing orderly ✓Face color normal ✓Speech rapid ✓Mouth, faint odor liquor ✓Tired, four hrs sleep ✓No trouble locating license, registration, proof of insurance or exiting vehicle ✓At questioning, side-to-side 1" sway ✓Aware of turning into wrong lane, due to "difficulty noticing lane lines in rain" ✓SFSTs in rain on wet street in moderate traffic at 55-60 ^{o} in athletic shoes ✓Rated self a 1 (sober), on scale of 1-10
IV. SFSTs & DRE Evaluation: ✓HGN 0047, 2/6 clues ✓DRE at PD 0155 to 0237 ✓WAT, OLS, Rhomberg, FTN showed clues of impairment ✓Eyes bloodshot/watery w/red conjunctiva ✓Pupils dilated ✓LOC and Rebound Dilation present ✓Eyelids droopy ✓BP elevated (158/100) ✓Raised taste buds and white coating on tongue ✓Rxn to Light, pupil tracking, muscle tone, body temp, pulse and lack of hippus were normal
V. Statements (chronological)/Possessions: ✓"Shot of raspberry vodka at midnight" ✓"Two hits of marijuana day before" ✓"Two hits with bong at noon" ✓"Usually smoke weed, I get the good shit" ✓"Use two, three times a week for last five years" ✓Has used "Shrooms, acid, ecstacy, coke, crack, GHB, Vicodin, Percoset and Oxycontin" ✓Admitted her pipe w/residue in car
VI. DRE Opinion/DUID Charge: ✓Under influence of Cannabis, unable to operate motor vehicle safely ✓DUID charge: improper turn, failure to drive in single lane, moderate odor alcohol, bloodshot/watery eyes, eyelid tremors, admission to smoking marijuana, poor FSTs
VII. Toxicology: ✓BAC 0.041/0.040 at 56/59 min post driving ✓THC 2 ng/ml, COOH-THC 14 ng/ml 1.85 hrs post driving ✓Time-of-Use Calculations: Model I (w/95% CI) 0.84-4.08 hrs, Model II (w/95% CI) 0.78-5.39 hrs

Prosecution Perspective: case contains much documentation helpful in determining cannabis impaired driving, selected purposefully for teaching. Reality is that few DUID cases are so well-documented and these rarely go to court. Defendant presented with 2 ng/ml THC, 14 ng/ml COOH-THC in blood obtained 1.85 hrs post driving and 0.04 BAC within 1 hr of driving. Significant driving was noted and admission of recent use of alcohol (midnight), marijuana (noon) and possession of pipe w/residue. Exhibited signs and symptoms consistent with cannabis/alcohol use: odor alcoholic beverage; poor balance, poor performance of SFSTs; bloodshot/watery eyes, red conjunctiva, droopy lids, dilated pupils, lack of smooth pursuit, lack of convergence and rebound dilation; BP elevated and raised taste buds/white coating on tongue. Recent use supported by simple presence of 2 ng/ml THC and Huestis Time-of-Use models (w/95% CI) estimating use within 0.78 to 5.39 hrs.

Defense Perspective: case appears ideal, but contains flaws. Driving and many psychophysical tests performed in heavy rain, on flooded streets, in moderate traffic. Defendant provided reasonable explanation for improper turn, presented cooperatively/normally in many respects and admitted to fatigue. No documentation of marijuana odor exists, an unusual/significant omission. Address other assignable causes for signs and symptoms. If 2 ng/ml THC relates to impairment and corresponding LOQ is 2 ng/ml, address method QC and validation. Time-of-use calculations not widely used by forensic toxicologists in USA; address consensus regarding calculations.

Conclusion: this style of forensic case analysis, evaluating strengths and weaknesses of both sides of a DUID case via documentation review, is effective tool for teaching and trial prep.

Key Words: **Cannabis; Marijuana; Impaired Driving; Drug Impaired Driving**

S16 DUI Case of Methamphetamine and Topiramate

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The presence of methamphetamine and topiramate in the blood of a driver suspected of driving under the influence of drugs represents some unusual implications. The driver was involved in a single car accident and could not perform any field sobriety tests because of the injuries received from the accident. Methamphetamine is a central nervous system stimulant causing exhilaration, euphoria, extreme wakefulness, talkativeness, and feelings of increased mental and physical capacity. Blood methamphetamine was measured at 0.50 mg/L while the topiramate was not quantitated. The blood concentration of methamphetamine is sufficiently high enough to indicate the subject was a chronic methamphetamine abuser and probably was not in the withdrawal phase of methamphetamine use although that is still a possibility. The reported behavior of the driver as "unresponsive and non-communicative" is not the typical response of a recent dose of methamphetamine in an experienced user. After unknown injuries experienced in an accident, the extent of drug or injury related symptoms and behaviors may be difficult to separate.

Topiramate is a newer anticonvulsant that the FDA has approved additionally for the treatment of migraines. No concentration was reported in this driver leaving the question unanswered as to over or under dosing. The reason for the prescription is also unknown. Although methamphetamine and topiramate may have been involved with the cause of the accident, with so little information about the driver's condition or accident details a toxicologist can only provide theoretical speculations.

Keywords: **Methamphetamine, Topiramate, Accident**

S17 Marijuana and DUI – Which Expert to Believe?

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In Kansas, an individual ran a stop sign and hit another car, resulting in the death of one of the occupants of the second car. The driver of the first car was found to have a high level of tetrahydrocannabinolic acid (THCA) in his urine. The prosecution expert testified that the urine THCA level was sufficient to prove impairment. The defense expert disagreed. Which expert is correct? Are there techniques that can be used, either by the opposing expert or the opposing attorney, to minimize the impact of the testimony? Does the location of the trial make a difference? This presentation will allow attendees to discuss techniques for establishing limits to expert testimony, determining when those limits have been exceeded and how to counteract an overzealous expert.

Key Words: **DUI, Expert, Marijuana**

S18 The Importance of Developing a Chronology in Determining the Proximate Cause of Impairment in Mixed DUI/DUID Cases

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Operating Under the Influence of ethanol, (OUI), and OUI medications or drugs, and negligent operation of a motor vehicle are three separate charges which may be brought against a citizen stopped by law enforcement for apparent unsafe operation of a motor vehicle in Massachusetts (COM). In COM v. Carol Hendricks, Ms. Hendricks was stopped for unsafe operation. The police report indicated that Ms. Hendricks, who had an established history of a bi-polar psychiatric disorder, admitted to having 2 "Pete's Wicked Ales" earlier in the evening. She also stated that she took prescription medications: nortriptyline, lithium, atenolol, and thyroxine. Ms. Hendricks, , refused to answer the police officer's questions or participate in any field sobriety testing saying. She was charged with OUI ethanol and negligent operation of a motor vehicle. No charge was made regarding the medications.

A chronology of Ms. Hendricks' medication and ethanol-ingestion follows:

11:00 am - lithium, 300 mg, nortriptyline (NT) 50mg - NT therapy initiated 3 days earlier.

7:30 pm - one "Pete's Wicked Ale"

8:30 pm - one "Pete's Wicked Ale"

8:45 pm - lithium, 600 mg and nortriptyline 50 mg

10:05 pm - stopped

Using the Widmark formula, this author calculated Ms. Hendricks' BAC at 10:05 pm, when she was stopped, to have been 0.038% and the Com directs that all calculations be truncated to the two decimal mode, making 0.038% reportable as 0.03%. A burn-off rate of 17/mg/dl/hr and a Widmark distribution coefficient (r) of 0.55 were used. According to the manufacturer, Pete's Wicked Ale contained 4.2% ethanol (v/v), and each container of ale contained 12 oz. to yield a total volume of pure ethanol of 0.50 oz. per container (~15 grams).

The COM was required to prove that the ethanol was the proximate cause of the impaired operation that was alleged. Testimony confirmed that a BAC of 0.03% was not sufficient to produce impairment. Moreover, Ms. Hendricks had just begun taking nortriptyline three days earlier and had experienced "dry mouth, ringing in the ears, and dilated pupils" that afternoon, all side effects of nortriptyline or lithium. She was found Not Guilty of OUI ethanol and because OUI drugs was not charged, she was convicted only of negligent operation.

Key words: **CNS depressants; Ethanol; Impairment while driving**

S19 Performance Characteristics of Enzyme-Linked Immunosorbent Assays for Testing Ketamine Exposure

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Frequently reported misuse of ketamine necessitates the development of an analytical approach that can be effectively applied to high-volume testing environment. Recent availability of commercial enzyme-linked immunosorbent assay (ELISA) for ketamine has made this development possible.

Performance characteristics of the two ketamine ELISA reagents currently available from commercial sources were examined to better understand their analytical parameters, including calibration, cross-reacting characteristics, assay precisions and others. Information thereby derived were applied to the analysis of urine specimens. Resulting ELISA *apparent* analyte concentrations were correlated against ketamine concentrations as determined by GC-MS. Correlation data were examined to determine ELISA apparent analyte concentrations that correspond to specific GC-MS ketamine concentrations.

Neogen ELISA reagent was found to respond very specifically to ketamine, while the product from International Diagnostic Systems (IDS) also responded very significantly to the metabolites of ketamine (norketamine and dehydronorketamine), The IDS reagent allows for the detection of ketamine metabolites in the test specimen at a much lower level; thus, has a much longer detection window. On the other hand, test data derived from the Neogen reagent allow for better correlation with the ketamine concentration as determined by GC-MS.

Neogen ELISA for ketamine can be effectively used as the preliminary test adapting the 2-stage high-volume testing approach now routinely applied to testing the five drug categories regulated by the U.S. Department of Health and Human Services. Using 100 ng/mL of ketamine as the GC-MS cutoff, the corresponding ELISA cutoff value is approximately 120–150 ng/mL. On the other hand, the IDS reagent exhibits significantly higher sensitivity, allowing longer detection time, through the responses derived from ketamine metabolites, which can be advantageous under certain forensic testing environment.

Key Words: **Ketamine, ELISA, GC-MS**

S20 Postmortem and Antimortem Immunoassay Screening Validation Using a Dynex DSX To Detect Seven Classes Of Drugs of Abuse

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A new immunoassay microplate analyzer was recently validated in our laboratory for the purposes of screening postmortem and antimortem samples. We decided to validate the Dynex DSX instrument using Orasure Technologies reagents and kits. The kits used were Benzodiazepine, Cannabinoids, Cocaine, Methadone, Methamphetamine, Opiate and PCP. Target drugs for each kit consisted of Nordiazepam, l-11Nor9-Carboxy-Δ9-Tetrahydrocannabinol (THCA), Benzoylecgonine, Methadone, d-Methamphetamine, PCP and Morphine. Analytical performance of each assay was evaluated using the following criteria: limit of detection, dose response curve, EC50, precision, matrix effect, carryover and binding characteristics at the cutoff concentration. The use of receiver operating characteristic curves was used to determine the appropriate cutoff concentrations. The reliability of each assay was determined using case samples consisting of antimortem and postmortem blood and urine, liver, and brain. All of these cases were run with our cutoff concentrations and compared against GCMS confirmatory data analysis. Sensitivity, specificity, positive and negative predictive values were calculated for each cutoff concentration.

Key words: **Immunoassay, Microplate, Validation**

S21 Estimating time of intake by evaluating the ratio of norbuprenorphine and buprenorphine concentrations in urine.

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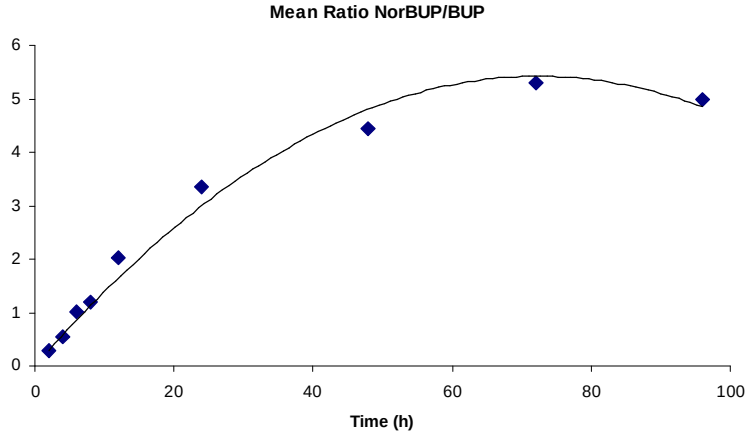
The objective of this study was to describe the relationship between the time of intake of buprenorphine and the measured concentrations of buprenorphine and its metabolite norbuprenorphine in urine.

The study involved 18 subjects (8 males and 10 females) who received 0.4 mg buprenorphine sublingually as a single dose. The data from 14 subjects were used to create models and those from 4 were used to test the models. Urine samples were collected prior to dosing and at 2, 4, 6, 8 12, 24, 48, 72, and 96 hours post dose. Samples were collected in plastic flasks, aliquoted into 10-ml tubes and frozen until analyzed. The samples were screened using CEDIA reagent with a cut-off of 5 ng/ml and quantitation was performed with LC-MS-MS with a cut-off of 0.5 ng/ml. Data analysis was peformed in Microsoft Excel and in MatLab 7.0.

The mean detection time (continuous positive results) with CEDIA was 7 hours whereas for the LC-MS-MS method it was 18 hours for buprenorphine and 65 hours for norbuprenorphine. Still, most subjects had positive results after a negative sample owing to differences in creatinine concentration. The mean time when the ratio norbuprenorphine/buprenorphine exceeded 1 was estimated to 7 hours. The individual ratios showed a considerable variation that became greater the more distant the collection time. During the first 24 hours the relationship between the ratio and the time could be described by a simple linear function whereas the relationship showed a distinct curvature at later times as shown in figure 1. Thus the prediction becomes uncertain at times beyond 72 hours. The use of the ratio instead of the actual concentrations makes the relationship independent of urine dilution and should also correct for different dosages, as the ratio should show the same change over time regardless of the dose.

When a model where $y=0.0019x^2 + 0.1489x$ was tested the predicted times of intake had a mean error of 30 % and a majority of the times were underestimated. More elaborate functions did not improve the predictions. We conclude that the ratio norbuprenorphine/Buprenorphine can be used to estimate the time of intake at least up to 48 hours but thereafter there is a considerable overlap in ratios making predictions uncertain.



Key Words: **Buprenorphine, Time of intake, Urine analysis, Drugs of abuse**

S22 A new MS/MS library of 1000 drugs for drug identification by library searching using LC/MS/MS

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A new LC/MS/MS library of drugs and organic toxins has been developed to utilize Collision Energy Spread with our previously published Multi Target Screening method (MTS) using a Q TRAP LC/MS/MS system (Applied Biosystems/MDS SCIEX).
The new library, based on a former library with 300 drugs, has been enlarged to 1000 compounds such as drugs of abuse, hypnotics, amphetamines, benzodiazepines, neuroleptics, antidepressants, analgesics, cardiac drugs, and also some pesticides and organic toxins. Data have been acquired using Enhanced Product Ion (EPI) scan with three different Collision Energies (CE) of 20, 35 and 50eV, and an EPI scan with Collision Energy Spread (CES 35±15eV).
The acquisition and library search parameters have been optimized and then validated by library searching after injection of different dilutions and re-injection during a three months time period.
Furthermore inter instrument repeatability has been tested using two Q TRAP LC/MS/MS systems (Q TRAP and 3200 Q TRAP).
Results showed high reproducibility of library search fit values in most experiments. Differences in sensitivity of both systems used were not relevant for the quality of the EPI spectra. However, the 3200 Q TRAP is able to detect at lower concentration and dilution might be necessary when very high concentrations of compounds had been injected.

Keywords: **Screening, Library, Validation**

S23 Concurrent Quantification of Δ^9-Tetrahydrocannabinol (THC), 11-Hydroxy-Δ^9-Tetrahydrocannabinol (11-OH-THC), and 11-nor-9-Carboxy-Δ^9-Tetrahydrocannabinol (THC-COOH) in Human Plasma Using Two-Dimensional Gas Chromatography, Cryofocusing, and EI-Mass Spectrometry

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The ability to detect and quantify low concentrations of cannabinoid metabolites in plasma is important in driving under the influence of drugs (DUID) cases, postmortem cases, and pharmacokinetic studies. We developed a method with low limits of quantification and enhanced resolution of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in plasma utilizing two-dimensional gas chromatography/mass spectrometry (GC/MS) with cryofocusing.

Trimethylsilyl (TMS) derivatives of THC, 11-OH-THC, and THC-COOH were prepared and injected onto the primary column of an Agilent 6890 GC equipped with a microfluidic Deans switch. After determination of analyte elution times from the primary column (DB-1ms), a "cut" of each analyte was diverted to the secondary GC column (DB-35ms). Chromatographic signal enhancement was accomplished by cryogenically trapping (Joint Analytical Systems, Marlton, NJ) effluent cuts eluting from the first GC column. THC eluting from the initial GC column was trapped at 100 ^{o}C and released at 275 ^{o}C in an initial temperature cycle. 11-OH-THC and THC-COOH were simultaneously trapped in a second, separate temperature cycle. A second GC oven temperature ramp was applied to further enhance resolution of analyte peaks from plasma matrix in the secondary column. Final elution times were 7.27, 10.98, and 11.66 min. for THC, OH-THC, and THC-COOH, respectively. Signal enhancement by cryofocusing was 119-194% when compared to unfocused chromatograms. On column detection capability of 5 pg of analyte was achieved with a signal to noise ratio > 10:1.

Blank human plasma was spiked with THC, 11-OH-THC, and THC-COOH at concentrations ranging from 0.125 to 100 ng/mL and subjected to solid phase extraction (SPE) using ZSTHC020 columns (United Chemical Technologies, Inc., Bristol, PA). Extracted analytes were derivatized with *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and quantified using electron ionization mass spectrometry (EI-MS). Limits of quantification (LOQ) for THC, 11-OH-THC, and THC-COOH were 0.125, 0.5, and 0.25 ng/mL, respectively. Calibration with split curves allowed quantification up to 100 ng/mL for all analytes. We report a method with enhanced detection and simultaneous quantification of THC, 11-OH-THC, and THC-COOH in human plasma. This method utilizes standard SPE, TMS derivatives, and EI-MS, making it an attractive alternative to current THC quantification methods.

Key Words: **THC, Two-Dimensional Chromatography, Cryofocusing**

S24 Are *CYP, UGT, CNBR1, DRD2, COMT and P-gp* Gene Polymorphisms Contributing to Cannabis and Dronabinol Psychosis Vulnerability?

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During a study designed to evaluate the effects of oral administration of cannabinoids on the ability to drive, 2 out of 8 healthy male subjects, all of them occasional cannabis smokers, over-reacted to medium doses of Δ9-tetrahydrocannabinol (THC) or dronabinol by developing transient psychotic symptoms. Since some candidate genes associated with vulnerability to drug exposure have been suggested for such mental disorders, although the data are controversial, genetic investigations were carried out. The objective was to unfold any association between cannabis psychosis and genetic polymorphisms of enzymes, transporters and brain receptors involved in neurotransmission and THC metabolism. Cytochrome P450 (CYP) allelic variants involved in the oxidative metabolism of THC (2C9/2C19,3A5 and 2D6) as well as UDP-glucuronosyltransferase variants (UGT2B7) catalysing the putative conjugation of THCCOOH were determined. Two genes involved in dopamine metabolism and neurotransmission were also examined: the functional catechol-O-methyltransferase (COMT) polymorphism (Val158Met) with RFLP technology, the C957T and Taq I A polymorphisms of the dopamine D2 receptor (DRD2) gene. A 3-SNP haplotype located in cannabinoid receptor type 1 (CNBR1) intron 2 gene and associated with substance dependence was analysed by real-time PCR with TaqMan. Finally, the polymorphism of the P-glycoprotein (P-gp) drug efflux protein (exon 26 3435C>T and exon 21 2677G>T SNP) which interacts with cannabinoids was determined with TaqMan technology. The whole blood THC, 11-OH-THC and THCCOOH concentrations were measured with GC-MS operating in the NCI mode after solid-phase extraction. All LOQ values were below 1 ng/mL. Large variations between participants were observed in cannabinoid blood levels and concentration ratios time-profiles. However, the kinetics of the 2 subjects who experienced deep anxiety symptoms did not differ significantly from those of the 6 other volunteers. Interestingly, one of the 2 participants with side-effects was predicted as a rare poor metabolizer for CYP2C9 activity (homozygous *2/*2). These results suggest that CYP2C9 is not the limiting factor for 11-oxydation of THC and/or that other pathways (e.g. CYP2C19) may be involved. Furthermore, the same volunteer was the only one to display a Taq IA A1/A2 DRD2 polymorphism. The A1 minor allele has been associated with reduced brain dopaminergic function, substance abuse and mood disorders. These findings suggest that the contribution of genetic markers to unveil cannabis vulnerability warrants further investigations.

Keywords: **Cannabis/Dronabinol, Psychosis, Genetic Polymorphisms**

S25 Challenges in Drug-Facilitated Sexual Assault: The Toxicologists' Perspective

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Drug-facilitated sexual assault (DFSA) investigations can be extremely challenging from a toxicological perspective. A series of case studies are used to demonstrate some of these challenges and discuss possible solutions.

Sexual assault, including those that are drug-facilitated, are under reported and are sometimes subject to lengthy delays in reporting. These delays may result in specimen collection several hours, and sometimes days or weeks after the alleged event, posing a significant challenge from both a detection and interpretation standpoint. It is also recognized that DFSA can involve a myriad of substances, despite the fact a small number of drugs have been popularized as "date rape drugs". Laboratories must be prepared to perform comprehensive toxicological testing for illegal, therapeutic and over-the-counter drugs at appropriate detection limits.

The toxicologist may also face other limitations including selection of the proper specimen, quantity of specimen submitted for testing, preservation and chain of custody issues. Negative toxicological findings are not uncommon and these may require careful interpretation within the context of the case. To complicate these issues victims may experience deficits in physical and/or mental function, including impaired perception, judgment and memory, which may influence their recollection of events. Furthermore, the definition of DFSA and scenarios in which it takes place are frequently misunderstood by investigators and the public. A series of five cases are used to illustrate the many challenges in DFSA casework.

Keywords: **DFSA, Drugs, Sexual Assault**

S26 Case Report: Drug-Facilitated Sexual Assault Cases Involving Lorazepam and Oxycodone

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Last year, drug-facilitated sexual assault requests comprised approximately 5% of the caseload of the Miami Valley Regional Crime Laboratory's toxicology section. Cases are initially screened for alcohol and drugs of abuse (including fentanyl and oxycodone), by headspace gas chromatography and ELISA immunoassay, respectively. Provided a sufficient amount of specimen has been submitted, cases are also screened for basic, acidic, and neutral drugs by gas chromatography/mass spectrometry (GC/MS). Any positive screening results are followed with an appropriate confirmation analysis. Due to poor immunoassay cross-reactivity with some members of the benzodiazepine drug class, analyses are performed by gas chromatography with an electron capture detector. Analysis for GHB and 4-methylGHB is also performed by GC/MS.

Case 1 involves two females that were sexually assaulted by a male acquaintance. A friend of the victims lived with the suspect and both men invited the women to their house after work. The men insisted on picking the women up so they would not have to drink and drive. The suspect gave one of the victims, Victim 1, a beer in the car; however, she found that it tasted strange so she did not drink it. The suspect provided the victims drinks at the house, one of which the suspect claimed was only cranberry juice but Victim 1 found the juice also tasted strange. The group ate dinner and Victim 1 does not remember anything after dinner until waking up naked. Samples of the blood and urine of Victim 1 were taken and sent to our laboratory for testing. Samples of blood and urine taken from Victim 2 were tested at a local hospital. Lorazepam was found in the blood specimen of Victim 1 at a concentration of 0.01µg/mL as well as being present in the urine specimen. The results corroborated a statement provided to the police that the liquid form of Ativan was mixed into the cranberry juice given to the victims.

Case 2 involves two males who were sexually assaulted by a male acquaintance. The victims were invited to go drinking with the suspect. They were both provided with multiple drinks by the suspect at a bar and his residence. Both victims eventually fell asleep, one in an extra bedroom and one on a couch, and each later woke up with the suspect performing oral sex on them. Blood and urine specimens were taken from both victims and sent to the laboratory for testing. Routine testing for Victim 1 resulted in a blood alcohol level of 0.08% and a urine alcohol level of 0.15gm%. Also, oxycodone was detected at a blood level of 0.07µg/mL and was found to be present in the urine specimen. Blood and urine alcohol levels for Victim 2 were 0.08gm% and 0.13gm%, respectively. The urine specimen from Victim 2 was also positive for oxycodone; however, the findings in the blood sample could not be confirmed.

Keywords: **DFSA, Lorazepam, Oxycodone**

S27 Determination of scopolamine-facilitated sedation in three children by hair analysis

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Scopolamine is an alkaloid present in Solanaceae, particularly abundant in Datura species. It has a very close chemical structure to atropine and is a muscarinic blocker, responsible for the anticholinergic effects. Clinical signs involve tachycardia, mucous membranes dryness, urinary retention, quievering and myoclomia. At low dosage, this drug induces sedation and amnesia. At higher doses, major anticholinergic effect produces visual and auditive hallucinations. In therapeutic, scopolamine is used as an anti-spasmodic in some cases of digestive and period pain.

The aim of our study was to develop a procedure to test scopolamine by UPLC-MS/MS in the hair of three children after alleged exposure to FEMINAX®. This pharmaceutical, commercialized in England, contains active ingredients such as paracetamol, codeine, caffeine and scopolamine and is proposed in the treatment of headache, dental pain, related pain and menstrual cramps.

A strand of hair, collected from each child, was decontaminated using dichloromethane, and then segmented (3 segments of 2 cm for the first two subjects and 2 segments of 2.5 cm for the third). Segments were pulverized and overnight incubated in 1ml of pH 8.4 phosphate buffer in the presence of 2.5ng atropine-d_3, used as an internal standard. After liquid-liquid extraction with 5 ml dicloromethane/isopropanol/*n*-heptane (50/17/33, v/v/v) the organic phase was evaporated to dryness. The residue was reconstituted in 100µl of methanol, from which 10 µl were injected into an ACQUITY C18 column (2.1 x 50mm) and eluted with a gradient of acetonitrile and formate buffer delivered at a flow rate of 0.3 ml/min. A Waters® Micromass® Quattro Micro™ API triple-quadrupole mass spectrometer was used for analysis. Ionization was achieved using electrospray in ES+ mode. Each compound was identified using 2 transitions and quantitated with the major daughter ion (*m/z* 304.6 to 138.06 for scopolamine and 293.12 to 127.06 for the internal standard).

The analysis in each hair segment showed the following concentrations of scopolamine:
Subject 1 - 0 to 2 cm : 0.7 pg/mg; 2 to 4 cm : 0.4 pg/mg and 4 to 6 cm : <0.2 pg/mg
Subject 2 - 0 to 2 cm : 0.6 pg/mg; 2 to 4 cm : 0.3 pg/mg and 4 to 6 cm : <0.2 pg/mg
Subject 3 - 0 to 2.5 cm : 0.3 pg/mg and 2.5 to 5 cm : 1.1 pg/mg
Furthermore, codeine (9 pg/mg to 544 pg/mg) was identified in each segment, which confirms the children's repetitive exposure to FEMINAX®.
The combination of scopolamine and codeine was used to sedate the children by the parents, who did not challenge the toxicological results.

Keywords: **Scopolamine, Hair, UPLC-MS/MS**

S28 Case Study: DFSA, Torture, and Homicide Involving Chloroethane

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Chloroethane (ethyl chloride) is a volatile gas that is commonly used as a refrigerant or electronic component cleaner. It has also been used as a recreational drug of abuse, not only due to its depressant effects, but also because it is easy to obtain. The most commonly reported pharmacological effects are that of drunkenness, dizziness, and lack of muscle coordination. Recently, chloroethane was also linked to at least one homicide.

Two male subjects were indicted for several counts of drug-facilitated sexual assault and homicide. The investigation suggested that these two men frequently used GHB to subdue their male victims at bars and then transported the victim to one of the suspects' homes. Once at the home, the men sexually assaulted and tortured the victims. In two cases, the victims were ultimately killed. Numerous photos of victims were discovered at the residence, revealing them to be bound, and at times, with gas masks over their faces. One of the photos revealed an aerosol can of cleaning solvent that contained chloroethane.

This laboratory received a brain sample from one deceased victim. The victim had been discovered severely decomposed in the back of his vehicle. In addition, an aerosol can labeled "Head Cleaning Solvent (Chloroethane)" was also submitted.

An Agilent Headspace GC/MS system was used to identify chloroethane in both samples. Because chloroethane is extremely volatile (bp = 12.3 °C at 760 mmHg) and could not be detected using our usual volatile screening method, a modified method was employed. Approximately 5 grams of brain matter were sampled in a cold room and sealed in a 20 mL headspace vial. All injections were manual headspace injections using a gas-tight syringe into a DB-624 capillary column (30m x 250um x 1.4um). The temperature program was 50°C isothermal with no solvent delay. Identification was by retention time within 2% of the reference standard of chloroethane (2.28 minutes) and a complementary mass spectrum.

Using this method, chloroethane was identified in both the brain sample and the aerosol can. Negative brain samples were also analyzed, but did not contain chloroethane.

Keywords: **DFSA, Headspace GC/MS, Chloroethane**

S29 Evaluation of Fourteen Benzodiazepines and Metabolites in Urine on Three Different Commercial Benzodiazepine Immunoassays

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Objective: The Drug-Facilitated Sexual Assault (DFSA) Committee of the Society of Forensic Toxicologists (SOFT) recently released recommended guidelines for the Maximum Detection Limits (MDL) of the common DFSA drugs and metabolites in urine. The objective of the present study was to evaluate the ability of 3 commercially available enzyme-linked immunosorbent assay (ELISA) benzodiazepine kits to detect the presence of the fourteen listed benzodiazepines and metabolites.

Methods: The fourteen benzodiazepines, metabolites and Citalopram listed in the DFSA guidelines were spiked to twice the suggested MDL, to the MDL and to one-half the MDL in drug free urine. All of the kits were run according to the manufacturer's suggested protocol. Samples were run in neat urine, in triplicate on each kit. Cut-off limits for the assays were set at 80% B/B_O which is ≥4 standard deviations from the mean of the negative control urine to fully differentiate between signal and noise.

Results: Results are provided in Table 1. None of the manufacturer's benzodiazepine kits cross-reacted with Citalopram at the levels spiked into the urine.

Table 1. Evaluation of 3 Commercial Benzodiazepine Immunoassays with 14 Benzodiazepines at 3 Different Concentrations at 80%B/BO Cutoff (≥4SD from Mean of Negative Urine)

Drug or Metabolite	DFSA MDL* (X) ng/mL	Immunalysis			Neogen			Orasure		
		2X	X	0.5X	2X	X	0.5X	2X	X	0.5X
Alprazolam	10	+	+	+	+	+	+	+	+	+
a-hydroxyalprazolam	10	+	+	+	+	+	+	+	+	+
Chlordiazepoxide	10	+	+	+	+	+	+	+	+	+
Citalopram	10	-	-	-	-	-	-	-	-	-
Clonazepam	5	-	-	-	+	+	+	+	+	+
7-aminoclonazepam	5	-	-	-	+	+	+	-	+	-
Diazepam	10	+	+	+	+	+	+	+	+	+
Nordiazepam	10	+	+	+	+	+	+	+	+	+
Flunitrazepam	5	+	+	+	+	+	+	+	+	+
7-aminoflunitrazepam	5	-	-	-	+	+	+	+	+	+
Lorazepam	10	+	-	-	+	+	+	+	-	-
Oxazepam	10	+	+	+	+	+	+	+	+	+
Temazepam	10	+	+	+	+	+	+	+	+	+
Triazolam	5	+	+	-	+	+	+	+	+	+
a-hydroxytriazolam	5	-	-	-	+	+	+	+	+	+

* Denotes "Recommended Maximum Detection Limits (MDL) for Common DFSA Drugs and Metabolites in Urine Samples" as outlined in Tox Talk, 4th Quarter 2005
(+) denotes drug detected at cutoff; (-) denotes drug not detected at cutoff

Conclusions: Each of the manufacturer's ELISA kits coincided well with their respective cross-reactivity data provided. The Immunalysis kit detected all of the benzodiazepines at the DFSA committee MDL with the exception of Lorazepam, Clonazepam, α-hydroxytriazolam, 7-aminoclonazepam, and 7-aminoflunitrazepam. Neogen detected all of the benzodiazepines at the DFSA committee MDL. Orasure detected all of the benzodiazepines at the DFSA committee MDL with the exception of Lorazepam.

Key Words: **Benzodiazepines, ELISA, DFSA**

S30 Overcoming the Challenges of Drug-Facilitated Sexual Assault Through the Use of the SOFT DFSA Committee's Recommended Maximum Cutoff Concentrations for Urine Specimens

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One of the more influential publications for toxicologists performing analyses on specimens related to drug-facilitated sexual assault (DFSA) investigations recommended that the limitations of analytical methods be known and that efforts be made to improve the detection limits of assays. In response, the SOFT DFSA Committee developed a list of drugs commonly encountered in DFSA investigations and recommended maximum cutoff concentrations to use for the analysis of urine specimens for these drugs. This list suggests that over 60 drugs and metabolites should be considered when performing toxicological investigations of alleged DFSA. The drug classes represented by this list include ethanol, GHB and its analogs, benzodiazepines, marijuana, over-the-counter medications, anti-depressants, narcotic and non-narcotic analgesics, miscellaneous central nervous system depressants, and, because of their popularity as recreational drugs of abuse, select stimulants.

This presentation will introduce the committee's list and describe the methods used to develop the list. In addition to the SOFT DFSA Committee recommendations, other suggestions will be provided to help overcome the challenges of DFSA investigations.

Keywords: **DFSA, SOFT, Urinary, Cutoff**

S31 Prevalence of Marijuana in Second Sample Blood Specimens from DUID Arrests in the Commonwealth of Virginia, 2001 – 2002.

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Since marijuana is the most common illicit drug detected in Driving Under the Influence of Drugs arrests, a study was performed to determine what other drugs may be present in drivers with marijuana in their blood. During an 18-month period in 2001 – 2002, 2948 blood samples were received for DUI/D analysis. The blood samples were initially tested using a 3 level testing scheme, all samples were initially analyzed for alcohol. Marijuana was only tested in samples with a blood alcohol concentration < 0.09 gm %. This accounted for ~ 60 % of the samples.

Blood samples were screened for marijuana using either FPIA reagents or EMIT immunoassay reagents. All samples were screened for over 60 common drugs of abuse classes and prescription and over-the-counter medications that may impair driving using either FPIA, EMIT, GC, or GC/MS. All presumptive positives were quantified, and confirmed by GC/MS.

The demographics and drug findings of 593 samples that contained marijuana are presented. The most commonly encountered drugs and range of blood concentrations over the 18-month period are listed below.

Drug	# of Cases	Range	Units
THC		<1 – 26	ng/ml
THC-Acid		2 - > 250	ng/ml
Alprazolam	42	0.02 – 0.22	ng/ml
Nordiazepam	31	0.06 – 0.9	mg/L
Diazepam	24	0.05 - 1.35	mg/L
Cocaine	14	< 0.01 - 0.07	mg/L
Benzoylecgonine	32	0.04 - 1.34	mg/L
Carisoprodol	12	2.5 - 12.1	mg/L
Meprobamate	12	5.6 - 40.7	mg/L
Morphine	12	0.02 - 0.06	mg/L
Phencyclidine	11	0.01 - 0.08	mg/L
Oxycodone	11	0.02 - 0.42	mg/L

	Total #	BAC +THC
BAC (0.00 gm%)	282	199 (70%)
BAC (<0.09 gm%)	155	124 (80%)
BAC (0.09-0.35 gm %)	156	118 (76%)

In two thirds of the samples that contained marijuana, marijuana was not the only drug detected. Alcohol was the predominate additional drug detected. Marijuana was detected in drivers with blood alcohol concentrations as high as 0.35 gm %. However, drugs other than alcohol were only detected in drivers with blood alcohol concentrations as high as 0.26 gm %. Benzodiazepines were the most common additional drug detected. Of the 73 samples that contained both marijuana and benzodiazepine(s), alcohol was also detected in approximately half (37) of these samples. In approximately 50 % (299) of the samples marijuana was detected with only one other drug.

Key Words: **Drugs and Driving, Marijuana, Blood Analysis**

S32 Paired blood and oral fluid specimens from randomly selected nighttime drivers

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The purpose of the study was to determine the feasibility of collecting and analyzing paired blood and oral fluid specimens for drugs and alcohol from a nighttime driving population. Drivers were randomly stopped at six locations in the USA. They were asked to consent to a survey, an oral fluid collection, a blood sample collection and a breath alcohol test. The samples were shipped overnight, the laboratory was blinded to the pairing system, and the data were provided back to the study group as individual specimen results. Six hundred and thirty-nine oral fluid specimens were collected using the Quantisal™ device, and 394 blood samples were taken. All subjects providing a blood sample also provided an oral fluid and the specimens were tested for multiple drugs using ELISA followed by GC/MS or LC/MS/MS.

Overall, 96 samples (15%) were positive for drugs, excluding alcohol. Blood alone accounted for 29 of the total positives, five of those were positive for benzodiazepines, and nine for low-level THC-COOH, with no parent THC present, suggesting marijuana use was not recent.

Drug Class	***# of positive samples***	
	Oral fluid	**Blood**
Amitriptyline/nortriptyline	1	1
Amphetamine/methamphetamine/MDMA/MDA/MDEA	4	3
Barbiturates	2	0
Benzodiazepines	**1**	**6**
Carisoprodol/meprobamate	1	1
Cocaine/benzoylecgonine	**14**	**1**
Fluoxetine	4	8
Methadone	1	0
Opiates (hydrocodone, oxycodone, codeine, morphine, 6-AM)	4	4
Pseudoephedrine/phentermine/Phenylpropanolamine	2	8
Sertraline	5	9
THC (THC-COOH, 11-OH-THC)	**37**	**20**
Tramadol	2	2
Total	***78***	***66***

Oral fluid is an extremely viable biological matrix for the detection of drugs in drivers if sufficient sample volume is collected, and a transportation buffer able to release drugs from the collection pad is used. The individual collection compliance was considerably better than blood, with oral fluid accounting for more total positives. THC and cocaine were present at higher rates and in higher concentration in oral fluid than blood. The low S:P ratio of benzodiazepines caused some difficulty with their detection in oral fluid.

Key Words: **Oral fluid, Blood, Driving**

S33 A Review of Etorphine: A Toxicological Case Study

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Etorphine is a narcotic analgesic that is particularly used in veterinary medicine. Its potency is 1000 times greater than that of morphine. In veterinary medicine it is an ingredient of the mixture called Immobilon. Even very low doses of the drug can produce adverse or fatal effects. This presentation is a case study involving two thoroughbred racehorse track urine samples, containing the compound Etorphine. In addition, several special exhibits confiscated by the Illinois State Police were analyzed as a result of an ongoing investigation.

In October and November of 2005, the UIC Animal Forensic Toxicology Laboratory received two thoroughbred blood and urine track samples. Samples were ran on 65 immunoassay ELISA plates and hit on Etorpine and Flunixin plates. According to the laboratory procedures, both urine samples were then screened and confirmed using solvent extraction after overnight Beta-glucuronidase hydrolysis (pH 5.0 at 37^0C) followed by EI-GC-MS analysis (full scan mode) after derivatization with BSTFA + 1% TMCS and acetonitrile at 65^0C for 30 minutes. The following ions were chosen for identification: *m/z* 272, 396, and 483. In addition, positive control specimens prepared by spiking naïve urine samples with Etorphine (250 ng/ml) were analyzed simultaneously with the samples of interest. Both urine samples and several special exhibits were confirmed for the presence of Etorphine.

Although the outcomes of the processes were positive, there were some obstacles and challenges to overcome, such as having and or acquiring adequate drug standards, low quantity of the drug in the sample, and the type of drug (class 1 or class 2) apparent in the track sample.

Key Words: **Veterinary Toxicology, Horse Racing, Etorphine, EI-GC-MS**

S34 Rapid Analysis of Drugs of Abuse in Oral Fluids by GC/GC/MS and LC/MS/MS

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For drugs of abuse (DOA) testing in the workplace and for those suspected of driving while under the influence of drugs (DUID), oral fluid is an alternative matrix to the industry standard urine and is considered less invasive. Confirmation is required down to specific levels of drug concentration in oral fluid (ng/mL) as set forth by the United States Substance Abuse and Mental Health Services Adminstration (SAMHSA). The analytical challenge is to accurately measure the DOAs in the oral fluid matrix. This work will present two approaches to the problem. For laboratories that use GC/MS, a 2-D GC/MS method is used where the DOAs are heartcut from a non-polar DB-1MS column to a polar DB-17MS column. An air cooled focusing trap is used to improve resolution and sensitivity. Tetrahydrocannabinol (THC), phencyclidine (PCP), and the opiates can all be measured down to the SAMHSA limits. A second approach to the analysis is LC/MS/MS. The use of LC/MS/MS allows for removal of the derivatization step, which is usually required for GC/MS. Furthermore, a rapid-resolution, high throughput, C18 column with a particle size of 1.8 um is used, resulting in elution times under 4.5 minutes for the THC, and under 1.5 minutes for the remaining drug compounds. Finally, the sensitivity of the triple quadrupole (QQQ) mass spectrometer not only results in quantitative determination of the compounds, but as confirmation of their presence through satisfying method-dependent ion ratios of the ion used for quantitation and an ion selected as a qualifier. The sensitivity requirements of SAMHSA, which include 2 ng/mL THC, 8 ng/mL Coc, 5 ng/mL Amp, 5 ng/mL Meth, and 5 ng/mL MDMA, in oral fluids, are easily met.

Keywords: **LCMS, QQQ, drugs of abuse**

S35 Performance Test Monitoring of Drug Abuse Testing with POCT Devices and Onsite Instruments in Criminal Justice Settings

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Beginning in the 1960's, the use of instrumented and non-instrumented onsite or point-of-collection testing (POCT) methods to test for drugs of abuse in arrestees, probationers and parolees has seen an ever increasing use in a wide variety of criminal justice settings, due to the significant advances and availability of these techniques. However, there is or should be concern about the accuracy of such testing unless appropriate quality control and monitoring measures are in place. This paper presents results from an ongoing performance testing (PT) program for 35 criminal justice programs, 19 using non-instrumented POCT devices and 16 using commercial analyzers and immunoassay reagents.

The ongoing PT program involves the submission of identical sets of 12 single-blind quality control samples to both types of sites at 3 to 4 month intervals. The samples are shipped frozen, having remained frozen from the time of their preparation. The sites are instructed to test the samples along with and under the same procedures as those from donors, spreading the testing out at intervals whenever possible. Sites using analyzers send the printed results, instrument calibrations and test data and those using non-instrumented devices send information on the specific device used, drugs tested for and their cutoffs to Duo Research for evaluation.

The samples were prepared with human urine, which is checked for possible interfering substances and microbiologically filtered. They had the following single or multi-drug compositions: benzoylecgonine 375, THC-9-acid 25; benzoylecgonine 225, THC-9-acid 35; benzoylecgonine 150, THC-9-acid 65; benzoylecgonine 330, morphine 150; morphine 375, THC-9-acid 55; morphine 225, d-methamphetamine 750; d-methamphetamine 1250; d-methamphetamine 500; d-amphetamine 1250; oxazepam 500; PCP 35; and one negative. The samples were randomized in each set with the contents blinded to the test site.

Results were received from 19 different POCT sites using 17 different non-instrumented devices, representing 87 separate testing events, an event representing the receipt of results for 6 or 12 samples. The results were scored based on the analytes tested for and the manufacturers' stated cutoffs. If an analyte was not included in the panel, it was counted as a negative. The overall accuracy was 67.1%, sensitivity 86.5%, specificity 55.1%, PPV 0.545, and NPV 0.868. Accuracy for individual sites ranged from 50.0% to 80.0%, and for different vendors' devices 56.2% to 88.9%. Results were also scored based on the GC/MS cutoffs used by the sites' contract laboratories. Accuracy based on GC/MS cutoffs was 94.8%, sensitivity 92.6%, specificity 99.0%, PPV 0.994 and NPV 0.875; and, for individual sites, ranged from 83.3% to 100%, and for different vendors' devices, 88.9 to 97.7%. Of the 1397 individual test results, only 6 true false positive results, where no drug was present, were reported.

Similarly, results from 16 sites, which used various analyzers with commercial immunoassay reagents, based on the manufacturer's cutoffs, had an overall accuracy of 93.8%, sensitivity 89.8%, specificity 97.2%, PPV 0.963, and NPV 0.920. Accuracy for individual sites ranged from 88.9% to 97.2%. Results based on GC/MS cutoffs were: accuracy 95.48%, sensitivity 90.0%, specificity 100%, PPV 1.000, and NPV 0.920. Accuracy for individual sites ranged from 91.7% to 97.2%. There were no false positive results reported.

Although the overall average onsite results using instruments had greater accuracy and PPVs than the devices when compared by the manufacturers' stated cutoffs, accuracy and PPVs differed little based on the GC/MS confirmation cutoffs.

The paper will discuss advantages and disadvantages of both methods and appropriate quality control procedures.

Key Words: **POCT, PT Program, Criminal Justice**

S36 Cocaine, Phencyclidine, Opiates, Amphetamines in Hair by LC/MS

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We have developed a confirmatory method as a follow up to the presumptive Immunalysis positive screens performed in submitted hair samples in our laboratory. We use an Agilent/Brooker MS Trap XCT Electron Spray Ionization(ESI) coupled to Agilent 1100 HPLC system. Zorbax C18 4.6x50mm, 5μm column, mobile phase flow 0.2ml, 80% of 75% Methanol, 25% H2O, 0.001M NH4 HCOOH and 20% of H2O, 0.001 HCOOH. The ESI is run with 30 psi , at 8L/min N2, and 350° C drying temp. It should be noted that for enhanced sensitivity the drying N2 flow maybe increased up to 40 psi and 12L/min flow. Triple stage Fragmentation is set in the trap (3n, AutoMSN). The drugs are detected M+1. i.e cocaine m/z 304 frag2 to182, frag3 to 150,122,108 and 82. PCP M+1 244, frag2 to 159 and 86. Methamphetamine M+1 150, frag2 to 119 and 91. MDMA M+1 194, frag2 to 163, frag 3to 135 and 105. Oxycodone M+1 316 frag2 to 298 frag3 to 280,256,241,213,187. Hydrocodone M+1 300, frag2 to 265, frag3 to 247, 177, 163. Morphine M+1 286 frag2 to 201, frag3 to 207,183,155. Meperidine M+1 248, frag2to 220, frag3 to 174. Codeine M+1 300, frag2 to 265, frag3 to 247, 205.

Sensitivities for these drugs are routinely determined at 0.01 ng/mg of hair, and we have established >95% correlation with the Enzyme Immunoassay Screening Method.

Keywords: **LC/MS, Hair, Drug Testing**

S 37 ISO17025 Accreditation for Drug Testing in Hair and Oral Fluid

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Questions relating to the accuracy or reliability of test results are frequently asked during cross-examination in court or by customers assessing the suitability of a laboratory service. The international quality standard ISO17025, documents the criteria for testing laboratories wishing to demonstrate their technical competence in generating valid test results. As a consequence, laboratories worldwide are recognizing the importance of implementing Quality systems to ensure effective control of the testing services they provide.

The United Kingdom Accreditation Service (UKAS) is the recognised national accreditation body responsible for evaluating analytical testing laboratories for compliance to the ISO17025 standard. This involves assessment of laboratory documentation, participation in proficiency testing schemes, competence of laboratory and support personnel, measurement traceability and uncertainty calculations.

Accrediting methods for the analysis of drugs in hair and oral fluid represents a significant challenge to the analyst with respect to the lack of certified reference material, limited guidelines on best practice and reservations on the efficacy of alternative matrix testing. The experience of a laboratory accredited to ISO17025 in the UK will be presented for both screening and confirmation methods in hair and oral fluid.

Keywords: **ISO17025 Accreditation, Hair, Oral Fluid**

S38 External contamination of hair with Cocaine: evaluation of external cocaine contamination and development of performance testing materials

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Introduction: The National Laboratory Certification Program undertook an evaluation of the dynamics of external contamination of hair with cocaine (COC) while developing performance testing materials for Federal Drug-Free Workplace Programs. This characterization of hair was necessary to develop performance testing materials that could evaluate the efficacy of decontamination procedures used in commercial hair testing laboratories.

Objective: To examine the efficacy of laboratory decontamination procedures through the dry application of cocaine to five different hair types.

Methods: Hair locks (blonde to dark brown/black) from 5 different individuals were contaminated with cocaine HCl, treated with a synthetic sweat solution and then subjected to hygienic treatments to model real-life conditions. The hygienic treatment, which consisted of daily shampooing (Monday through Friday), was continued for 10 weeks. Samples of the hair locks were removed at specified times throughout the 10 week period and analyzed for COC, benzoylecgonine (BE), cocaethylene (CE), and norcocaine (NCOC). Three commercial analytical laboratories analyzed the samples under three separate protocols: no decontamination procedure, individual laboratory decontamination procedures or decontamination by an extended buffer procedure conducted at RTI International.

Results: Results indicated substantial and persistent association of COC, BE and CE with all hair types. While these analyte were present in most hair samples, NCOC presence was variable. Hair that was not decontaminated had significantly greater quantities of COC and BE than did hair that was decontaminated by laboratory procedures. The only hair samples below detection limits for all four compounds were those decontaminated 1 hour after contamination and before being treated with artificial sweat. Additionally, BE/COC ratios increased significantly over the 10 week study (regardless of decontamination treatment). From 21 days post contamination until the end of the study, the mean BE/COC ratio for all hair types exceeded 0.05, the proposed Federal Mandatory Guideline requirement to report a positive cocaine result. The largest variability in results was observed for samples decontaminated by participant laboratories.

Conclusions: These results suggest that current laboratory decontamination strategies will increase variability of performance testing sample results for all hair types. None of the decontamination strategies used in the study were effective at removing all contamination. Some of the contaminated hair in this study would have been reported positive for cocaine use based on the proposed Federal Mandatory Guidelines.

Key Words: **Hair Testing, Hair Contamination, Decontamination, Cocaine**

P1 Development and validation of a homogenous immunoassay for the detection of opiates in oral fluid obtained using the Quantisal™ collection device

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***Background**:* Oral fluid is a useful biological specimen to detect recent usage of drugs, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has many advantages over urinalysis, specifically observed collection and difficulty of adulteration, oral fluid also contains lower concentrations of drugs and sample volume is often limited.

***Sample collection**:* Using the Quantisal™ oral fluid collection device, 1 mL of neat oral fluid is collected (N= 50, x = 0.993 mL, SD =0.029). The collection pad is then immersed in a tube containing extraction buffer (3 mL), capped and sent to a testing facility. To date, oral fluid analysis using a collection device has been limited to an ELISA platform due the lack of a homogenous immunoassay (HEIA) and the need for a pretreatment step to reduce the viscosity problems associated with neat oral fluid.

***Validation**:* Taking into account the dilution factor associated with the collector, a sensitive HEIA for opiates directed at the proposed SAMSHA screening cutoff of 40 ng/mL was developed. The assay uses 20 uL of the specimen lending itself to most commercial chemistry analyzers. The intra assay precision at concentrations of 20, 40, and 60 ng/mL of morphine was determined to be 0.2%, 0.38% and 0.64%; inter assay precision was 1.09%, 0.6% and 0.79% respectively. The extraction efficiency of codeine, morphine and 6-AM from the collection pad was greater than 90%. The buffer also provided stabilization of the extracted drug resulting in negligible losses during transportation. An oral fluid sample fortified with 60 ng/mL of morphine lost only 1 % at room temp for 30 days; a specimen containing 6 ng/mL of 6-AM lost 18 % at room temperature after 30 days. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS.

EIA	**GC/MS**	
	+	-
+	29	0
-	2	123

Sensitivity for: 29/(29+2) = 93.54%; ***Specificity***: 123/(123+0) = 100%. One of the two samples that screened positive contained codeine (32 ng/mL); the other contained morphine (20 ng/mL) and 6-AM (16 ng/mL).

***Cross-reactivity**:* The cross reactivity of codeine was 125% while that of 6-AM was 75% with respect to morphine. Potential interfering substances including toothpaste, cranberry juice, baking soda, orange juice, Cola, mouthwash and cough syrup were fortified at 50% and 150% of the cutoff. No interference from the compounds was detected.

***Summary**:* The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at SAMHSA proposed a cut-off concentration of 40 ng/mL for opiates. The format is compatible with most commercial chemistry analyzers.

Key Words: **Homogeneous immunoassay; Opiates; Oral fluid**

P2 Development and Validation of a Homogenous Immunoassay for the Detection of Phencyclidine in Oral Fluid Obtained Using a Quantisal™ Collection Device

Warren Rodrigues, **Guohong Wang***, Alpana Agrawal, Heidi Philips, Michelle Nguyen, Erma Abolencia, Michael Vincent, James Soares and Christine Moore
Immunalysis Corporation, Pomona, CA, U.S.A.

***Background**:* Oral fluid is a useful biological specimen to detect recent usage of drugs, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has many advantages over urinalysis, specifically observed collection and difficulty of adulteration, oral fluid also contains lower concentrations of drugs and sample volume is often limited.

***Sample collection**:* Using the Quantisal™ oral fluid collection device, which incorporates a volume adequacy indicator, 1 mL of neat oral fluid is collected (N= 50, x = 0.993 mL, SD =0.029). The pad is then immersed in a transportation tube containing 3 mL of extraction buffer, capped and sent to a laboratory facility.

To date, oral fluid analysis using a collection device has been limited in its application to an ELISA platform due the lack of a sensitive homogenous immunoassay and the need for a pretreatment step to reduce the viscosity problems associated with neat oral fluid.

***Validation**:* Taking into account the dilution factor associated with the collector, we have developed a highly sensitive homogenous enzyme immunoassay for phencyclidine at the proposed SAMSHA screening cutoff of 10 ng/mL. This assay uses 20 uL of the diluted oral fluid specimen lending itself to most commercial chemistry analyzers. The intra assay precision of the assay at concentrations of 5, 10, and 15 ng/mL of PCP was determined to be 1.43%, 0.94% and 0.88%; inter assay precision was 0.92%, 0.76% and 0.56% respectively. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS at the proposed SAMSHA confirmation guidelines.

EIA	**GC/MS**	
	+	-
+	9	2
-	0	85

Sensitivity for: 9/(9) = 100%; ***Specificity***: 85/(2+85) = 97.7%. One sample which screened positive was confirmed by GC-MS at 9 ng/mL PCP

***Cross-reactivity**:* In addition the antibody employed in this assay format was challenged with structurally similar and non similar compounds. Dextromethorphan had a cross reactivity of 1% with respect to a 10 ng/mL PCP cutoff. A wide variety of potential adulterants/ ingested substances including sugar dissolved in water, toothpaste, cranberry juice, baking soda, orange juice, Cola, mouth wash and cough syrup were fortified with drug at 50% and 150% of the cutoff. Interference was noted for the OTC cough syrup which contained dextromethorphan at 1 mg/mL.

***Summary**:* The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at SAMHSA proposed a cut-off concentration of 10 ng/mL of PCP. The format is compatible with most commercial chemistry analyzers.

Key Words: **Homogeneous immunoassay; PCP; Oral fluid**

P3 Development and validation of a homogenous immunoassay for the detection of amphetamine and methamphetamine in oral fluid obtained using a Quantisal™ collection device

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Immunalysis Corporation, Pomona, CA, U.S.A.

***Background**:* Oral fluid is a useful biological specimen to detect recent usage of drugs, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has advantages over urinalysis, specifically observed collection, oral fluid also contains lower drug concentrations and sample volume is limited.

***Sample collection**:* Using the Quantisal™ oral fluid collection device, 1 mL of neat oral fluid is collected (N= 50, x = 0.993 mL, SD =0.029). The pad is immersed in a tube containing extraction buffer (3 mL) and capped. To date, oral fluid analysis using a collection device has been limited to an ELISA platform due the lack of a sensitive homogenous immunoassay (HEIA) and the need for a pretreatment step to reduce the viscosity problems associated with neat oral fluid.

***Validation**:* Taking into account the dilution factor associated with the collector, we have developed a HEIA for d-amphetamine and d-methamphetamine at the proposed SAMSHA screening cutoff of 50 ng/mL. The assay uses 20uL of the specimen, lending itself to most commercial chemistry analyzers. The intra assay precision at levels of 25, 50, and 75 ng/mL d-meth was determined to be 0.7%, 1.1% and 1.03%; inter assay precision was 0.4%, 0.46% and 0.38%. Intra assay precision for 25, 50, and 75 ng/mL of d-amp was determined to be 0.42%, 0.64% and 0.55%; inter assay precision was 0.1%, 0.14% and 0.11% respectively. The extraction efficiency of both d-amp and d-meth from the collection pad was greater than 90%. The buffer also provided stabilization of the extracted drug resulting in negligible losses during transportation. A specimen fortified with d-meth (80 ng/mL) lost only 15 % at room temp for 30 days; a specimen containing d-amp (80 ng/mL) lost 5 % at room temp for 30 days. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS.

Methamphetamine	GC/MS			*Amphetamine*	GC/MS	
EIA	+	-		EIA	+	-
+	26	0		+	13	2
-	3	86		-	1	99

d-Methamphetamine: **Sensitivity**: 26/(26+3) = 89.7%; **Specificity**: 86/(86 +0) = 100%
d-Amphetamine: **Sensitivity**: 13/(13+1) = 92.3%; **Specificity**: 99/(99 +2) = 98%

***Cross-reactivity**:* The d-meth antibody employed cross reacts with MDMA at 65%, l-meth at 5% and < 1% with pseudoephedrine and ephedrine. The d-amphetamine antibody cross reacts with MDA at 50% with l-amphetamine at < 2%. Potential adulterants including toothpaste, cranberry juice, baking soda, Cola, mouthwash and cough syrup were fortified with drug at 50% and 150% of the cutoff. No interferences were noted.

***Summary**:* The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at 50 ng/mL for amphetamines. The format is compatible with most commercial chemistry analyzers.

Key Words: **Homogeneous immunoassay; Amphetamines; Oral fluid**

P4 Development and validation of a homogenous immunoassay for the detection of cocaine and benzoylecgonine in oral fluid obtained using a Quantisal™ collection device

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Immunalysis Corporation, Pomona, CA, U.S.A.

***Background**:* Oral fluid is a useful biological specimen to detect recent usage of drugs, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has many advantages over urinalysis, specifically observed collection and difficulty of adulteration, oral fluid also contains lower concentrations of drugs and sample volume is often limited.

***Sample collection**:* Using the Quantisal™ oral fluid collection device, which incorporates a volume adequacy indicator, 1 mL of neat oral fluid is collected (N= 50, x = 0.993 mL, SD =0.029). The pad is then immersed in a transportation tube containing 3 mL of extraction buffer, capped and sent to a laboratory facility. To date, oral fluid analysis using a collection device has been limited in its application to an ELISA platform due the lack of a sensitive homogenous immunoassay and the need for a pretreatment step to reduce the viscosity problems associated with neat oral fluid.

***Validation**:* Taking into account the dilution factor associated with the collector, we have developed a highly sensitive homogenous enzyme immunoassay (HEIA) for cocaine and benzoyleconine (BZE) at the proposed SAMSHA screening cutoff of 20 ng/mL. This assay uses 20 uL of the diluted oral fluid specimen lending itself to most commercial chemistry analyzers. The intra assay precision at concentrations of 10, 20, and 40 ng/mL of BZE was determined to be 1.4%, 1.2% and 0.6%; inter assay precision was < 3% at all three levels. The extraction efficiency from the collection pad was determined to be > 90% for cocaine and > 85% for BZE. The buffer also provided stabilization of the extracted drug resulting in negligible losses during transportation to the laboratory. An oral fluid specimen fortified with 30 ng/mL of BZE lost 5 % when stored at room temperature for 30 days; a control specimen containing 30 ng/mL of cocaine lost 43% when stored at room temperature for 30 days. The assay was further challenged with oral fluid specimens previously confirmed by GC-MS.

EIA	**GC/MS**	
	+	-
+	15	3
-	0	67

Sensitivity: 15/(15+0) = 100%; ***Specificity***: 67/(67+3) = 95.71%
2 of the 3 samples that did not confirm contained cocaine (5 and 7 ng/mL respectively).

***Cross-reactivity**:* The antibody cross reacts with cocaine at 200%. Potential adulterants including toothpaste, cranberry juice, baking soda, Cola, mouthwash and cough syrup were fortified with drug at 50% and 150% of the cutoff. No interferences were noted.

***Summary**:* The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at SAMHSA proposed a cut-off concentration of 20 ng/mL of BZE. The format is compatible with most commercial chemistry analyzers.

Key Words: **Homogeneous immunoassay; Cocaine; Benzoylecgonine; Oral fluid**

P5 Development and validation of an improved homogeneous immunoassay for the detection of buprenorphine and its major metabolite, norbuprenorphine in urine

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***Background**:* Buprenorphine is a semi-synthetic opioid derivative of thebaine. It is approximately 20-40 times more potent than morphine, and is now increasingly being prescribed in the treatment of heroin addiction as an alternative to methadone. Due to its potency, the therapeutic concentration of both drugs is low, so any analytical procedure must be extremely sensitive.

There is an increasing need for high throughput screening method for the detection of buprenorphine and its metabolites in urine. It has been reported that the median ratio of buprenorphine to its metabolite, norbuprenorphine in urine, is approximately 0.25 and that norbuprenorphine may have a slower elimination rate. Therefore, it is highly desirable for an immunoassay screening method to have high cross reactivity toward norbuprenorphine as well as the parent drug.

Methodology: A highly sensitive homogeneous immunoassay (HEIA) method with almost 100% cross-reactivity for both buprenorphine and norbuprenorphine has been developed. The precision of the assay was determined to be less than 1% within run (n = 10), and less than 3% between runs (n = 100). The sensitivity and specificity of the assay are 92% and 96%, respectively. The detection limit for buprenorphine is 2.5ng/mL. The assay was further validated with 39 clinical urine samples, which had also been analyzed using gas chromatography-mass spectrometry. The GC-MS results indicated that the concentrations of free norbuprenorphine in positive urine samples are 5 to 66 times higher than buprenorphine with an average ratio of approximately 35.

	GC/MS				
EIA	+	-		Sensitivity	91%
+	10	1*		Specificity	96%
-	1	27		Accuracy	95%

* Specimen contained 6 ng/mL by GC/MS

***Cross-reactivity**:* Commonly abused other drugs and other unrelated drugs at a concentration of 10,000 ng/mL showed no cross-reactivity or interference with the assay. More importantly, this assay shows no cross-reactivity for dihydrocodeine which has been reported to cause false positive response in other assays for buprenorphine.

Key Words: **Buprenorphine; Norbuprenorphine; Immunoassay**

P6 Correlation of ethanol concentrations in oral fluid by enzymatic assay and headspace gas chromatography

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The performance of an enzymatic assay for the measurement of ethanol in oral fluid was investigated following specimen collection with a Quantisal™ device. The recovery of ethanol from the collection pad, transportation stability, precision of the assay, limit of detection, linearity and correlation with headspace gas chromatography were evaluated.

Extraction efficiency*:* Oral fluid specimens were obtained using a Quantisal™ collection device, which allows 1 mL of neat oral fluid to be absorbed onto a pad. The pad is then placed in 3 mL of transportation buffer, capped and sent to the laboratory for analysis. The extraction efficiency of ethanol from the pad into the buffer was determined at concentrations of 40, 80 and 120 mg/dL. The recovery was 97.24%, 99.78% and 101% at the tested concentrations.

Transportation*:* Drug free oral fluid was spiked with ethanol at 40, 80 and 120 mg/dL. Three aliquots of each concentration were poured into test tubes and a Quantisal™ collection pad was placed in each until the volume adequacy indicator turned blue. The pad was then introduced into the transport tube containing the extraction buffer and capped. One tube of each concentration was stored at 4°C (Reference) while the other two tubes of each concentration were shipped via commercial courier (no ice). A temperature recorder was included with the shipment. Upon return receipt of the shipment, one tube was cooled for 30 min in a refrigerator (4°C) (Extract 1) while the second tube remained at room temperature (Extract 2). After 30 min the tubes were uncapped and analyzed along with the reference sample. No loss of ethanol was observed on either set of extracts compared to the reference samples.

Assay: An aliquot (10 uL) of the oral fluid was placed into a microplate well. Tris buffer with 0.1% sodium azide (100 uL) and a solution containing alcohol dehydrogenase and nicotinamide adenine dinucleotide (NAD) in Tris buffer (100 uL) were added to the sample. The plates were covered and incubated at room temperature for 15 min, after which the absorbance was read at 340 nm.

Validation*:* The assay was linear over the range 0 to 200 mg/dL of ethanol, and the limit of detection was 7 mg/dL. The precision of the assay was < 4% within run, and < 8% between days across all concentrations. The cross reactivity with other alcohols as well as commonly occurring substances was determined. No compounds showed significant interference with the assay.

Correlation: Seventy-three oral fluid specimens were analyzed using both the enzyme assay described and headspace gas chromatography. The overall correlation was $r^2 = 0.951$. The assay showed good linearity around the legally relevant range, as well as excellent precision, specificity and correlation with a standard chromatographic method. The method is suitable for the accurate measurement of ethanol in oral fluid following collection using the Quantisal™ device.

Key Words: **Ethanol, Oral fluid, Enzyme assay**

P7 Evaluation of an aqueous extraction buffer for the recovery of drugs from hair

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Background*:* The efficiency of extraction of drugs from hair is often overlooked as a source of analytical error in laboratory analysis. Extraction efficiency for both screening and confirmation was evaluated using hair specimens from drug users, by re-analyzing residual hair remaining after initial extraction.

Methodology*:* Three positive hair specimens for each drug class, cocaine, amphetamines and phencyclidine were selected. To 10 mg aliquots, 0.025M-phosphate buffer (pH 2.7; 0.5 mL) was added; the tubes were capped and incubated at 75^{o}C for one, two and three hours. After each hour, the supernatant was removed and neutralized. Each extract was diluted 1:5 in PBS (pH 7.0) and an aliquot was screened using ELISA.

Each extract was also analyzed using GC/MS according to routine procedures for hair analysis (overnight acidic incubation, solid phase extraction and derivatization), along with an aliquot of the original hair. The extraction efficiency was determined by comparison of the confirmatory result for original hair, with the GC/MS result for each of the extracts.

Results*:*

Drug Class		***Mean recovery after aqueous incubation (%) (n = 3)***		
		1 hour	*2 hours*	*3 hours*
Cocaine	Cocaine	47.3	74.8	82.7
	Benzoylecgonine	61.0	88.4	93.8
Amphetamines	Amphetamine	64.5	86.6	89.0
	Methamphetamine	69.9	83.4	86.3
PCP	PCP	50.4	76.9	85.5

The optimal incubation time in order to extract adequate drug for screening purposes for the three drug classes was determined to be 2 hours.

Summary*:* After a two-hour aqueous incubation of authentic specimens, the procedure removed 74.8 % of cocaine; 88.4% of benzoylecgonine; 76.9 % of phencyclidine; 83.4 % of methamphetamine and 86.6% of amphetamine from hair.

Key Words: **Extraction efficiency; Hair analysis; Immunoassay**

P8 Conversion of 6-acetylmorphine to morphine during overnight incubation of hair specimens

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The efficiency of extraction of drugs from hair is often overlooked as a source of analytical error in laboratory analysis. During research work on the extraction efficiency of opiates from hair specimens, the conversion of 6-acetylmorphine to morphine was considered. The conversion rate was evaluated using hair specimens from drug users.

Three hair specimens from heroin users were selected. To 10 mg aliquots, 0.025M-phosphate buffer (pH 2.7; 0.5 mL) was added, the tubes were capped and sonicated at 70°C for three hours.

A separate aliquot was incubated with methanol (2 hrs/70°C), then the methanol was decanted, evaporated to dryness and stored. To the remaining hair, 0.1N hydrochloric acid was added and the hair was incubated overnight at 55°C. The following day the acid extract was combined with the corresponding methanol residue. All the solutions were subjected to solid phase extraction, derivatization with BSTFA + TMCS and analysis using GC/MS.

The conversion was determined by comparison of the confirmatory result for overnight extraction, with the GC/MS result for the sonicated specimen.

		Opiate detected (pg/mg)		
Sample		*Codeine*	*Morphine*	*6-acetylmorphine*
A	**Overnight**	0	229	150
	Sonication	0	139	298
	Conversion rate			*49.7%*
B	**Overnight**	1172	4061	2311
	Sonication	912	2713	2893
	Conversion rate			*20.2%*
C	**Overnight**	2883	9330	6424
	Sonication	2795	6810	10775
	Conversion rate			*40.4 %*
			Mean conversion (%)	**36.7%**

The overnight incubation seemed to have little effect on codeine, but losses of 6-acetylmorphine and increasing concentrations of morphine were apparent. The specimens were chosen for the differences in low, medium and high opiate concentrations. The average conversion rate to morphine was 36.7% across the concentration range represented. While the conversion is not important from a screening point of view, the conversion of 6-AM during a routine extraction procedure will have a great impact on proficiency testing results and precise laboratory quantitation.

Key Words: **6-AM conversion; Hair analysis; Sonication**

P9 Preliminary Observations of the NLCP Hair Pilot Performance Testing Program: Confirmatory Analysis of Opioids, Phencyclidine, and Marijuana

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Introduction: In 2000, SAMHSA's National Laboratory Certification Program (NLCP) began a Pilot Performance Testing (PPT) Program to develop appropriate hair samples and assess the participant laboratory confirmatory testing capabilities for this matrix. Since its inception, the NLCP PPT program provided 5 to13 laboratories 21 PT samples for analysis of opioids (codeine, morphine, 6-acetylmorphine), 9 for phencyclidine (PCP) and 22 for the marijuana metabolite, delta-9-tetrahydrocannibinol-9-carboxylic acid (THCA).

Objective: To retrospectively evaluate the effectiveness of the NLCP PPT program: 1) to develop hair performance testing samples, and 2) to assess the ability of laboratories to accurately quantify drugs of abuse in hair.

Methods: Hair included for analysis was either collected from known drug users or fortified with drugs of abuse by NLCP protocols. Samples were sent to the laboratories in a blinded fashion in 8 cycles during a five year period. Approximately 100 mg of each hair sample was stored and shipped overnight at ambient temperature prior to analysis. As directed by the NLCP, some hair samples were subjected to each laboratory's decontamination (wash) procedures prior to extraction and all were analyzed by their standard operating procedures for confirmatory testing. Some specimens were sent to the laboratories in multiple cycles. Generally, fortified samples were targeted for analyte concentrations between 0.5 to 100 times the SAMHSA proposed confirmatory cutoffs for hair specimens as published in the Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs (69 Fed. Reg. 19673, April 13, 2004).

Results: The following table summarizes: 1) the regression analysis (first 5 rows) comparing the achieved results to the targeted values [95% confidence interval (CI) and unweighted y-intercept option] using all data points; 2) the statistical analysis calculations (95% CI) for the proposed cutoff concentrations (last 5 rows).

Value	Codeine	Morphine	6-Acetylmorphine	PCP	THCA
LINEAR REGRESSION ANALYSIS FOR TARGET CONCENTRATIONS (pg/mg)					
Range	100-400	100-2000	100-460	150-1500	0.05-5.0
Slope	0.86	0.83	1.61	0.70	0.70
y-intercept	55	236	-103	89	0.2988
r^2	0.26	0.22	0.23	0.62	0.78
n	83	100	84	104	151
STATISTICAL ANALYSIS AT CUTOFF CONCENTRATION (pg/mg)					
Range	28-410	33-1565	22-543	42-583	0.026-0.60
Mean (cutoff)	229 (200)	325 (200)	209 (200)	274 (300)	0.19 (0.05)
Median	215	240	186	286	0.06
%CV	48	89	68	41	105
n (results reported)	29	27	26	52	15

Conclusions: A summary of observations include: 1) Overall precision and accuracy of hair analysis among laboratories is low given CVs > 20% and large deviations of mean/median values to cutoffs for some analytes (morphine, THCA); 2) Regression analyses demonstrated r^2 <0.8, highly variable y-intercepts (-103 to 236 pg/mg) and highly variable slopes (0.70 to 1.61); 3) Mean inter-laboratory %CVs for 6-acetylmorphine and PCP hair confirmatory testing were lower than those in the urine PPT program (1986-1987). These preliminary data demonstrate that the overall performance of the NLCP hair PPT program to incorporate opioids, PCP, and marijuana into hair specimens and the analytical laboratory performance does not currently achieve standards established in the current maintenance urine PT program for NLCP certified laboratories. The results indicate that the program will require continued effort to improve both laboratory analytical performance and the NLCP's ability to prepare appropriate hair PT samples.

Key Words: **Hair, Performance Testing, Workplace Drug Testing, Drugs of Abuse**

P10 Preliminary Observations of the NLCP Oral Fluid Pilot Performance Testing Program: Confirmatory Analysis of THC, Morphine, Codeine, 6-AM and PCP

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Introduction: In 2000, SAMHSA's National Laboratory Certification Program (NLCP) began a Pilot Performance Testing (PPT) Program to develop appropriate oral fluid samples and assess the participant laboratory confirmatory testing capabilities for this matrix. Since its inception, the NLCP PPT program provided 30 PT samples for analysis of THC, 31 for morphine, 7 for codeine, 25 for 6-AM, and 6 samples for PCP.

Objective: To retrospectively evaluate the effectiveness of the NLCP PPT program: 1) to develop oral fluid performance testing samples, and 2) to assess the ability of laboratories to accurately quantify drugs of abuse in oral fluid.

Methods: Normal human oral fluid was collected from a drug free donor and spiked at concentrations that ranged from 0.20 to 25 times (varied by compound, indicated in the table) the SAMHSA proposed confirmatory cutoff, as published in the Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs (69 Fed. Reg. 19673, April 13, 2004). Once prepared, the samples were stored frozen in silanized 4 mL capped vials and shipped frozen by overnight delivery. Some samples were sent to the laboratories on multiple occasions. All testing was analyte directed by confirmatory test methods.

Results: The following table summarizes: 1) the regression analysis (first 5 rows) comparing the achieved results to the targeted values [95% confidence interval (CI) and unweighted y-intercept option] using all data points; 2) the statistical analysis calculations (95% CI) for the proposed cutoff concentrations (last 5 rows).

Value	THC	Morphine	Codeine	6-AM	PCP
LINEAR REGRESSION ANALYSIS FOR TARGET CONCENTRATIONS (ng/mL)					
Range	1-50	20-400	20-80	2-8	2-20
Slope	0.19	0.71	0.83	0.59	0.83
y-intercept	2.27	3.43	5.5	0.83	0.57
r^2	0.23	0.76	0.67	0.54	0.85
n	188	303	148	218	144
STATISTICAL ANALYSIS AT CUTOFF CONCENTRATION (ng/mL)					
Range	0.18-7.5	11.2-53.7	19.4-136	0.6-8	7.8-16.0
Mean (cutoff)	1.8(2.0)	32 (40)	40.3 (40)	3.1 (4)	9.7(10)
Median	1.34	30.7	37.6	2.9	9.1
%CV	90	31.2	38	42	23.9
n (w/ reported result)	36	91	69	67	36

Conclusions: 1) Regression analyses demonstrated poor analytical performance for all analytes (e.g., performance being r^2 <0.8, y-intercepts greater than ± 10 ng/mL from the origin, slopes not between 0.8 and 1); 2). The poor stability of THC and morphine in human oral fluid contributed to the variability of results found with these analytes; 3) Performance at the cutoff was characterized by high variability (%CV >20%); 4) Manufacture of PT samples containing THC in human oral fluid poses significant issues due to loss of THC from solution. Additionally, high variability in reported results indicates the need for further development both in the manufacture of materials and in the development of improved analytical methodology.

Key words: **Oral Fluid, Performance Testing, Workplace Drug Testing, Drugs of Abuse**

P11 Validation of a New Homogenous EIA for Cocaine and Benzoylecgonine in Oral Fluid.

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Aims:

There is an increasing requirement for the laboratory to provide rapid testing for drugs of abuse in oral fluid. We have recently introduced an improved device for collecting oral fluid which has low retention of abused drugs such as THC and cocaine to the collection material. Homogenous EIA has been the method of choice for high throughput screening for urine drug testing for many years but the detection of low drug levels has been difficult. We describe the development and validation of a homogenous EIA for cocaine and benzoylecgonine which is compatible with the new collection device. The new assay is capable of detecting cocaine and benzoylecgonine in oral fluid with validation against liquid chromatography with tandem mass spectrometry (LC-MS-MS).

Methods:

Standard urine EIA reagents for cocaine metabolite (benzoylecgonine) were modified and adapted to the SPIN180 clinical chemistry analyser. This involved optimisation with respect to sample and reagents volumes, reading kinetics and dose-response profiles. Inter- and Intra-assay coefficients of variation were determined using samples spiked with benzoylecgonine. Cocaine cross-reactivity was determined using samples freshly spiked with methanolic cocaine at a final concentration of 100 and 1000 ng/mL. Oral fluid samples (n = 247, collected from drug dependency and workplace centres) that had been routinely analysed by ELISA microplate, were selected to provide challenging immunoreactive concentrations for analysis on this new platform. Confirmation by LC-MS-MS was also performed.

Results:

The optimal SPIN180 protocol using the modified reagents employed a 30 microlitre sample volume and a total reagent volume 195 microlitres. The kinetic read time employed was 236 seconds with calibrators at 0, 15, 30 (cut off), 45, 150 and 600 ng/mL. Intra-assay precision for each of three controls (n=20) yielded mean concentrations of 22.2, 54 and 124.5 ng/mL with coefficient of variation (CV) of 4.6, 2.2 and 2.0% respectively. Ongoing inter-assay precision from a single calibration curve shows CV's of 10 and 7% at 24 and 54 ng/mL respectively. The fortified cocaine samples yielded consistent cross reactivity of 10.8%. Comparable results between the homogenous EIA and microplate ELISA were observed and histogram analysis showed that 136 samples fell between the 15 and 45 ng/mL calibrators, 62 samples were less than 15 ng/mL and 49 samples were greater than 45ng/mL. The agreement with LC-MS-MS was also good.

Conclusions:

The homogenous EIA described here provides a rapid and reproducible method for analysing drugs in oral fluid. It has good agreement with ELISA microplate and LC-MS-MS and is applicable to most modern clinical analysers. Although the assay showed low cross-reactivity with cocaine, the contribution of the cocaine fraction from in vivo samples would be expected to be higher due to the alkalinity of the collection buffer (pH 8) and time delay in transporting the sample to the laboratory leading to conversion of cocaine to its metabolite benzoylecgonine. We are currently developing this homogenous assay system for other commonly abused drugs.

Keywords**: Drugs of abuse, Oral fluid, Chemistry analyzer**

P12 Evaluations on the Oral Fluid Alcohol and Drug Combination Rapid Test Device – OratectPlus™

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Aims: To evaluate the performance of an onsite oral fluid alcohol and drug combination test device called the OratectPlus™.

Methods and Results: OratectPlus™ combines alcohol and drug testing in a one-step device that integrates oral fluid collection and testing. It consists of a translucent cap and a plastic housing containing a collection pad that is connected to an alcohol test pad (sensitive to 0.02% Blood Alcohol Concentration) and two lateral flow immunoassay test strips that test for up to 6 abused drugs (with cut-off levels of 25 ng/ml for methamphetamine, amphetamine and MDMA, 20 ng/ml for cocaine, 10 ng/ml for opiates, 4 ng/ml for PCP, 5 ng/ml for benzodiazepines and 40 ng/ml for THC). To run the test, the user uncaps the device and rubs the collection pad inside the mouth several times till a blue line in the lateral flow test strip begins to move. The device is then recapped and both drug and alcohol test results are read after 5 min. Presence of a bluish gray color on the alcohol pad indicates alcohol level at or exceeding 0.02% blood alcohol concentration. Drug test results are indicated by the lateral flow strips with the absence of red lines indicating positive results. A laboratory study showed that 98% of 50 subjects rinsing their mouths with a solution containing 0.02% to 0.08% ethanol gave positive alcohol results. In a separate experiment, fifty individuals who did not drink were all tested negative while fifty individuals after consuming 0.8 to 1.2 liters of beer were all found to test positive by OratectPlus™, Alco-screen 2-Minute Saliva Test and a Breathanalyzer (0.020 to 0.066 %BAC). Non-alcoholic drinks were found not to interfere with the test result. Drug test evaluations showed the test results are equivalent to the on-site oral fluid drug screen --Oratect®II.

Conclusion: OratectPlus™ is a viable device to test for alcohol and drug in the oral fluid.

Keywords: **Alcohol testing, Oral fluid, Drugs of abuse**

P13 Low Level GCMS Analysis of Drugs of Abuse from Difficult Matrices

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Low level detection of drugs of abuse in complex matrices, such as hair and urine, has always been a challenge. Numerous hardware configurations have been developed to facilitate the separation of the analyte compounds from the sample matrix once sample clean up has been done. These configurations can increase hardware costs dramatically, and can be difficult and time consuming to use.

Shimadzu GCMS systems have the capability of detecting low levels of drugs of abuse in less time without the addition of special hardware. This poster will demonstrate an innovative novel approach to detection of drugs of abuse in difficult matrices, including hair, at trace levels without the use of additional special hardware. Combining the Shimadzu GCMS system with a newly developed deconvolution software package allows the target compounds of interest to be detected and quantitated in complex matrices with intense co-eluting interferences. The combination of deconvolution software and stable, sensitive GCMS hardware provides a powerful analysis tool for the forensics laboratory.

This poster will demonstrate the detection of THC from hair samples at levels ranging from 100 pg/ mg to 0.5 pg/mg of hair. This analysis is done without the addition of special hardware, using deconvolution software to subtract the peaks of interest from the background interferences, and still have the ability for accurate quantitation. The chromatograms obtained using the deconvolution software will be compared to the same chromatograms that have not been analyzed using the deconvolution software.

Key Words: **Drugs of abuse, GCMS, Deconvolution**

P14 Screening of Drugs of Abuse in Whole Blood by Means of CEDIA Urine Immunoassay. Applications to Drivers Killed in Spanish Road Traffic Accidents ".

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Screening for drugs is generally performed in urine. However, a large number of whole blood specimens are continually being submitted to the forensic laboratory, and sometimes blood is the only specimen available. Therefore, there is a real need of a reliable screening method for drugs in blood samples in case no urine is submitted. Immunoassay techniques are widely applied to the screening of drugs of abuse due to their fast performance and sensitivity. This work deals with the development of a method for detecting the presence of drugs of abuse in blood by the application of cloned enzyme donor immunoassay (CEDIA®), originally developed for urine analysis.

Drugs of abuse studied were opiates, cocaine, methadone and benzodiazepines. The proposed method consisted in mixing 2 mL of acetone with 1 mL of blood followed by centrifugation at 3000 r.p.m. for 5 minutes. Supernatant layers were evaporated to dryness under a N_2 stream and dry residues were reconstituted with saline solution (NaCl, 0.9%). The samples were then analysed according to CEDIA® manufacturer's instructions for urine samples. The intra-day precision study showed a variation in low and high CEDIA® controls display that was always lower than a 3.0% for 21 determinations. The CEDIA® displays values obtained varied in 6 months time in less than an 8 %, with the only exception of %R.S.D. value obtained for the benzodiazepines low control which was 12 %.

Developed method was then applied to blood samples from 737 road traffic accidents. CEDIA® results were then compared with those obtained by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) used as confirmative and quantitative techniques. Obtained results showed that the reproducibility was higher tan 98% for each group of drugs analysed. Negative and positive predictive values were also calculated, being higher than 95% in all groups of drugs analysed. Obtained results yielded 139 cases (18'86%), in which the presence of one of the drugs studied was confirmed: 10 cases were positive for opiates derivatives, 12 for methadone, 46 for cocaine and metabolites and 71 for benzodiazepines.

As a conclusion, we can affirm that obtained results demonstrate the applicability of extending the CEDIA® immunoassays, initially designed for urine, to the analysis of whole blood, which is the specimen typically available in forensic cases.

Key words*:* **CEDIA, Drugs of abuse, Blood**

P15 Evaluation of the Lin-Zhi International Cannabinoid Enzyme Immunoassay for the Detection of Marijuana Cannabinoid Metabolites in Urine.

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We present an evaluation of a new Cannabinoid Enzyme Immunoassay [CEI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of marijuana cannabinoid metabolites in urine. The Lin-Zhi assay is based on competitive antibody binding between cannabinoid metabolites in urine and glucose-6-phospahate dehydrogenase labeled tetrahydrocannabinolic acid (THCA). When cannabinoid metabolites are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with THCA.

The Lin-Zhi CBI was evaluated by testing 666 urine specimens collected from criminal justice clients and pain management patients. All 666 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0, 50 (cut-off calibrator) and 250 ng/mL of THCA. Controls containing 0 ng/mL of THCA and -25% (negative control) and +25% (positive control) of the 50 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All urines were then analyzed by a GC/MS for THCA at a cut-off concentration of 15 ng/mL.

Approximately, 42% (277) of the 666 specimens yielded positive results by the Lin-Zhi assay. Of these 277 specimens, GC/MS confirmed the presence of THCA at >15 ng/mL in all specimens, indicating no false positive results. However, 5 specimens yielding negative Lin-Zhi results were found to contain THCA above the GC/MS cut-off of 15 ng/mL THCA. Therefore the overall agreement of Lin-Zhi and GC/MS results was 99.2%. THCA concentrations in the specimens as determined by GC/MS ranged from 11->4,000 ng/mL. From the presented study, the sensitivity of the Lin-Zhi CBI was 0.982 and the selectivity 1.000. Testing at 1,000 mg/mL of other drugs of abuse or their metabolites such amphetamine, benzodiazepines, benzoylecgonine, morphine and phencyclidine, the Lin-Zhi assay demonstrated no cross reactivity. The within-run precision of the Lin-Zhi assay was determined by the absorbance rates of the negative and positive controls was CV=1% (n=16); while the between-run precision of the controls was CV=<6% (n=16). The assay was found linear from –50% to 150% of cut-off concentration. The Lin-Zhi CBI provides a precise, reliable method for the detection of cannabinoids in urine specimens.

Keywords: **Enzyme Immunoassay, Marijuana, Cannabinoids, Urine Drug Testing**

P16 Comparison of Three Enzyme Immunoassays for the Detection of Marijuana Cannabiniod Metabolites in Urine.

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We present an evaluation of three enzyme immunoassays for the detection of marijuana cannabinoid metabolites in urine: The DRI Marijuana Metabolite Assay [DRI] from Microgenics (Fremont, CA); the Emit Marijuana Metabolite Assay [Emit] from Syva (Palo Alto, CA) and the Cannabinoid Enzyme Immunoassay from Lin-Zhi International, Inc. [LZ] (Sunnyvale, CA). These assay are based on competitive antibody binding between marijuana cannabinoid metabolites in urine and glucose-6-phospahate dehydrogenase labeled tetrahydrocannabinolic acid (THCA). When cannabinoid metabolites are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with THCA.

The assays were evaluated by testing 666 urine specimens collected from criminal justice clients and pain management patients. All 666 specimens were simultaneously tested with all three assays at a cut-off calibration of 50 ng/mL THCA. DRI and LZ analyses were preformed in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY), while the Emit testing was done in a Syva 30-R auto-analyzer (Syva Company) Controls containing 0 ng/mL THCA and –25% (negative control) and +25% (positive control) of the 50 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All urines were then analyzed by a GC/MS for THCA at a cut-off concentration of 15 ng/mL.

In the testing of the 666 urine all three assays demonstrate good overall agreement with GC/MS results: DRI, 99.1%; Emit, 98.8% and LZ, 99.2%. None of the three assays yielded false positive results. However, all three assays yielded false negative results; DRI 6, Emit 8 and LZ 5. From the presented study, the sensitivity of DRI was 0.979; Emit was 0.972 and LZ was 0.982. The selectivity of all three assays was 1.000. The precision of the three assays was determined by the absorbance rates of the negative and positive controls. The within-run precision expressed as %CV (n=16) for all three assays was 1% to 1.3%; while the between-run precision of the controls was 2% to 6%. All three assays provide a precise, reliable method for the detection of marijuana cannabinoid metabolites in urine specimens.

Keywords: **Enzyme Immunoassay, Marijuana, Cannabinoids, Urine Drug Testing**

P17 Screening and Confirmation of Urine Samples Adulterated with Papain

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The adulteration of urine samples is a continuing problem in the detection of drugs or drug metabolites, even in the military where the practice of observed collections is used. These adulterants are used to obtain a false-negative result when specimens are screened for drugs of abuse. Recently, (Burrows, et al., Journal of Analytical Toxicology, Vol. 29, July/August, 2005) it was reported that papain, a cysteine protease, could be successfully used as a urine adulterant for the detection of 11-nor-9-carboxy-tetrahydrocannibinol (THCCOOH) in spiked samples. The current study analyzes the effects of papain (Sigma, 10 mg/mL) and Lawry's Meat Tenderizer (papain is an active ingredient, 10 mg/mL) on different screening assays (FPIA, EMIT, KIMS) as well as a quantitative method (GC/MS) on biological samples. Samples were analyzed at two-to-four hour, one day, three day, seven day, and ten day time intervals by all methodologies. A decrease in response was observed with FPIA (Abbott, 19-23%) and EMIT (Syva Dade Behring, 16-43%, Microgenics, 7-16%) screening assays by the addition of papain to the specimens. In addition, the GC/MS results (18-39% decrease) demonstrate that the papain affects both the screening and confirmation testing. The addition of meat tenderizer caused a decrease in the FPIA (Abbott, 8-15%) screening assay and the GC/MS results (11-40%) similar to the papain (Sigma) while having varied results on the other screening assays. Data will be presented by illustrating the differences in detection based on multiple THCCOOH concentration over time. This study confirms papain could be a potential problem for the Department of Defense's military drug testing program. Further work is needed to combat the detection issue of papain adultered urine samples.

Keywords: **Papain, 11-nor-9-carboxy-tetrahydrocannibinol, Urine adulterant**

P18 Rapid Analysis of Benzoylecgonine in Urine by Fast Gas Chromatography-Mass Spectrometry

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A novel fast gas chromatography-mass spectrometry (FGC-MS) analytical method for benzoylecgonine (BZE) has been developed to improve the efficiency of specimen analysis without diminishing the reliability of metabolite identification and quantification. Urine specimens were spiked with deuterated internal standard (d_8-BZE), subjected to solid-phase extraction, and derivatized with pentafluoropropionic anhydride (PFPA) and pentafluoropropanol (PFPOH). The pentafluoropropyl ester derivative of BZE was identified and quantified using both a standard GC-MS method and the newly-developed FGC-MS method. Shorter GC analyte retention times were made possible in the FGC-MS method by employing a 220 Volt GC oven controller, which allowed an increased temperature ramp rate.

The FGC-MS method was linear between 25 and 10000 ng/mL of BZE yielding a correlation coefficient of 0.9994. The intra-assay precision of a 100 ng/mL BZE standard (n = 15) yielded an average concentration of 99.7 ng/mL and a coefficient of variation of 1.2%. The inter-assay precision of 21 sets of 50, 100 and 125 ng/mL BZE controls was found to be acceptable, with coefficients of variation less than 3.4%. No interference was observed when the FGC-MS method was challenged with cocaine, ecgonine, ecgonine methyl ester as well as nine other drugs of abuse. Analysis of presumptively positive specimens (n = 146) by both analytical methods yielded comparable results with a correlation coefficient of 0.996. The FGC-MS method, when compared with a standard GC-MS method, reduces total assay time by approximately 50% while demonstrating comparable reliability.

Key Words: **Fast GC, GC-MS analysis, Benzoylecgonine**

P19 Preliminary Evaluation of Three Commercially Available Immunoassays for the Semi-Quantitative Analysis of Buprenorphine in Urine

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After 2002 approval by the Food and Drug Administration, buprenorphine (BUP) became the first narcotic drug available for treatment of opiate dependence that can be prescribed in an office setting. A rapid and sensitive method is essential to screen biological specimens for treatment, criminal justice, military and workplace monitoring purposes. Three commercially available immunoassays were evaluated, Microgenics CEDIA® Buprenorphine Assay, and Buprenorphine ELISA kits by Neogen and Immunalysis. The analytical range, precision, and accuracy of each assay were examined along with possible interferences and cross-reactivities. Microgenics included calibrators at concentrations of 0, 5, 20, 50 and 75 ng/mL, and controls at 3 and 7 ng/mL. In-house controls were also prepared at ± 25% of each calibrator. Within-batch precision was calculated using duplicate analyses of 32 pairs of different controls and specimens. Maximum percent difference in concentration between duplicates was 15.4%. Between-batch precision was reported as the percent coefficient of variation (% CV) of control concentrations. The % CV was 11.7, 7.7, and 3.9% at 7.0, 25.0, and 56.3 ng/mL, respectively. Inaccuracy, the percent difference between mean calculated control concentrations and target values, were 0, 1.6 and 8.1% at the same concentrations. Buprenorphine glucuronide cross-reactivity was 160, 85 and 86% at 5, 10 and 25 ng/mL, respectively. Data for the Neogen and Immunalysis ELISA assays were evaluated using in-house calibrators and controls and are included in the table below.

	Neogen ELISA			Immunalysis ELISA		
Analytical Range, ng/mL	0.5 – 7.5			2.5 – 50		
Within-batch precision	(n=10)			(n=10)		
Control Concentration, ng/mL	**0.4**	**3.8**	**6.3**	**3.1**	**12.5**	**37.5**
Concentrations % CV	10.8	11.6	12.0	5.7	9.7	7.3
Absorbance % CV	7.9	7.7	9.0	3.6	6.4	4.7
Between-batch precision	(n=14)			(n=12)		
Concentrations, % CV	16.8	14.2	12.5	22.8	18.9	12.3
Absorbencies, % CV	9.1	26.5	29.1	28.6	21.6	26.0
% Bound, % CV	17.1	31.8	30.7	20.2	18.9	23.3
Inaccuracy	(n=14)			(n-12)		
Concentrations, % difference	8.3	20.8	17.6	11.6	6.9	6.8
Cross-reactivity						
Buprenorphine glucuronide	100.4% @ 3 ng/mL			<0.1% @ 1000 ng/mL		
Norbuprenorphine	0.4% @ 1000 ng/mL			53-84% @ 5-20 ng/mL		
Norbuprenorphine glucuronide	0.3% @ 1000 ng/mL			1.0% @ 1000 ng/mL		

Other opioids, and common drugs of abuse did not interfere in quantification of the low quality control in any assay. We provide validation data for three immunoassays for identification and semi-quantification of BUP in urine. These immunoassays will be used to analyze urine specimens collected in a treatment program for opiate-dependent pregnant women.

We sincerely thank Aaron Jacobs and Justin Holler of the Armed Forces Institute of Pathology, Division of Forensic Toxicology, for supplying the reagents and providing essential technical assistance for the Microgenics CEDIA analyses.

Keywords: **Buprenorphine, Urine, Immunoassay**

P20 Development of a Homogeneous Enzyme Immunoassay for the Detection of Ketamine and Norketamine in Urine

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Ketamine is a dissociative anesthetic drug that is structurally and pharmacologically related to PCP. Ketamine is primarily used in veterinary medicine and in pediatric patients as an anesthetic. Ketamine was developed in 1960's at Parke Davis Laboratories to replace PCP. In recent years, teenagers are abusing Ketamine as a recreational drug and club drug because of its hallucinogenic and stimulant effects. It is also used as "rape drug". Ketamine is known as "K", "Special K", 'Kit-Kat", and "Cat Valium". The plasma half-life of Ketamine is 2-3 hours. Ketamine is metabolized to Norketamine (NK), Dehydronorketamine (DHNK) and several other hydroxylated and conjugated metabolites. The rapid rise in ketamine abuse has created a need for an efficient, convenient and rapid method for the detection of Ketamine and its metabolites. Currently, Ketamine and its metabolites are primarily measured by GC/MS and LC/MS. There is no commercially available homogeneous enzyme immunoassay for the detection of Ketamine and Norketamine. The objective of this study is to develop a homogeneous immunoassay for the detection of Ketamine and its metabolites in urine for the automated clinical chemistry analyzers.

Microgenics DRI® Ketamine Assay is a dual cutoff assay using 100 ng/mL and 300 ng/mL Ketamine as cutoff calibrators. The assay uses a highly specific monoclonal antibody that can detect both Ketamine and its major metabolite Norketamine. The assay is based on competition between drug labeled with glucose-6-phosphate dehydrogenase (G6PDH) and free drug from urine sample for a fixed amount of antibody binding sites. In the absence of free drug from the sample, the specific antibody binds the enzyme labeled drug causing a decrease in enzyme activity. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is determined spectrophotometrically at 340 nm by measuring its ability to convert NAD to NADH.

The reagents and calibrators are liquid ready-to-use. The dynamic range of the assay is 0 to 1000 ng/mL. The performance of the assay was evaluated on the Hitachi 917 analyzer. The within-run and total precision (CV) for the cutoff calibrators and ± 25% controls is <2.0% (qualitative) and <5.0% (semi-quantitative), respectively. The limit of detection is 5.9 ng/mL. The recovery studies yielded recovery ±10% of expected values. The assay has 100% cross-reactivity to Norketamine with no significant cross-reactivity to other commonly abused drugs. No interference was observed with endogenous substances found in urine. Accuracy studies were carried out by testing urine samples that were spiked with Ketamine and Norketamine. The correlation data showed >95% accuracy in the detection of Ketamine and Norketamine.

DRI Ketamine Assay offers a rapid, convenient and sensitive method for the detection of Ketamine and Norketamine. The assay is adaptable to several high throughput automated clinical chemistry analyzers.

Key words: **Ketamine, Norketamine, Enzyme Immunoassay**

P21 Detection of Anabolic-Androgenic Steroids Using a Cell-Based Human Androgen Receptor Assay

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The abuse of anabolic-androgenic steroids (AAS) is a growing problem not only among elite athletes but also in amateur and recreational athletes, and especially among adolescents. Designer steroids are particularly problematic because they are manufactured specifically to avoid detection by current analytical protocols. AAS activate the human androgen receptor (hAR), a transcription factor that is a member of the nuclear receptor superfamily. Upon steroid binding, the hAR shuttles from the cytoplasm to the nucleus and causes subsequent gene transcription. *We hypothesize that AAS of various chemical structures and biological activity will bind the hAR and cause the receptor to be transported into the nucleus as a function of AAS concentration and potency.* To test this hypothesis COS-7 cells were transfected with plasmids containing the hAR labeled with green fluorescent protein (GFP). These cells were then subjected to various concentrations of dihydrotestosterone (DHT) or testosterone (T) and the rate of nuclear transport was determined using live-cell fluorescence microscopy. DHT and T were chosen for these studies because of their differential affinities for the hAR; DHT has a higher affinity (0.1 nM) and T has a lower affinity (0.3 nM). At concentrations of 10 nM (near physiological concentrations of endogenous steroids) and 100 nM (supraphysiologic concentrations seen in the instance of sports doping), DHT and T cause the hAR to be transported into the nucleus at rates and with maximum nuclear intensities that are predictably different based on their affinities for hAR:

		DHT		Testosterone	
	n=number of cells	10 nM (n=16)	100 nM (n=13)	10 nM (n=11)	100 nM (n=16)
a.	mean % nuclear intensity at maximum	33.6	65.6	48.9	64.7
b.	time to maximum intensity (min)	20.7	26.2	51.7	32.9
c.	rate (% nuclear intensity/min)	0.2	1.7	0.6	1.2

Such differential effects may also be apparent in the gene expression caused by these AAS. We predict these findings will hold for more diverse AAS with a wider spectrum of biological activity and affinities for the hAR. By comparing the parameters specified above (a, b, and c) in control (normal) and abuser urines, it may be possible to determine the presence of previously non-detectable and designer AAS. If such a test can be modified to accept biological samples such as urine, it may become useful for detecting the abuse of AAS.

Research supported by NIH grants DA07820 and DK070060.

Key Words: **Anabolic steroids, Androgen receptor, Sports doping**

P22 Detection of Anti-Estrogens and Other World Anti-Doping Agency Banned Substances in Urine by LC/MS/MS.

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The analysis of banned anabolic steroids has traditionally been by GC/MS. However, detection of some banned substances at the World Anti-Doping Agency minimum required performance limits (MRPL) by GC/MS analysis is extremely difficult. Recently, LC/MS has been increasingly used to identify banned substances, including some anabolic agents in urine. A qualitative method for routine screening analysis by LC/MS/MS on a triple quadrupole mass spectrometer has been developed to identify 22 “difficult-to-detect” banned anabolic and anti-estrogenic agents. This method utilizes enzyme hydrolysis, liquid/liquid extraction followed by derivatization in Girard’s Reagent P (GRP). The GRP reacts with ketone groups to produce a quaternary ammonium ion which aids in the ionization of neutral steroids. HPLC coupled to atmospheric pressure chemical ionization triple quadrupole tandem mass spectrometry with selected reaction monitoring is used to identify the individual analytes. MRPLs range from 1 ng/mL for the nandrolone metabolite 19-norandrosterone to 10 ng/mL for others. 19-norandrosterone at 1 ng/mL and clenbuterol at 2 ng/mL were readily detected using this method. Nonsteroidal anti-estrogens, such as tamoxifen, anastrozole, and clomiphene, as well as steroidal anti-estrogens such as exemestane are readily detected at 10 ng/mL. This method provides greater sensitivity over more traditional GC/MS methods and achieved acceptable results when compared to a far more sophisticated, expensive, and time-consuming method utilizing electrospray ionization quadrupole time of flight MS/MS.

Key Words: **Anabolic steroids, Anti-estrogens, LC/MS/MS**

P23 Analysis of Anabolic Steroids in Urine by LC/MS/MS

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This work represents the use of LC/QQQ mass spectrometry for confirmation of performance-enhancing drugs in urine, targeted for sports doping control analysis. LC/MS/MS with high-performance 3.5u rapid resolution columns and ionization by APCI on the QQQ instrument, using MRM analysis, is expected to provide a lower-cost alternative to the current de-facto standard in international doping control, which is the EI-GC/MS high-resolution magnetic sector instrument. Additionally, increased throughput as a result of bypassing the necessary sample derivatization step, without sacrificing the sensitivity required to meet the WADA MRPLs, is also considered an advantage. Confirmation is carried out using designated quantitation ions in MRM mode. Samples are obtained from the Center for Human Toxicology (University of Utah) to generate calibration curves for quantitation. The samples are extracts of control urine spiked at specified levels.

Keywords: **LCMS, QQQ, Steroids**

P24 The use of Ramped Collision Energy for the Confirmation of Drugs of Abuse Compounds in Urine by LC/MS/MS.

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High throughput screening of drugs of abuse is performed at St. Olav's Hospital by LC/MS. Over a million analyses per year are being made. Traditionally, screening is performed by immunoassay, which can be expensive, more time consuming, and less selective than mass spectrometry. The samples that are positive in the screening process are then traditionally confirmed using GC/MS, which requires derivatization as part of the sample preparation process. In this work, we show the use of a unique LC/MS/MS library, consisting of MS2 and MS3 spectra, for automatically confirming the presence of drugs of abuse in urine samples previously screened by an LC/MS single quadrupole mass spectrometer instrument. The library is built by, and the confirmation analysis is carried out on an LC / ion trap mass spectrometer, using ramped collision energy to produce fragment ions over a wide mass range for the purpose of identification. The use of ramped collision energy is also shown to produce consistent product ion spectra among ion trap instruments in different labs.

Keywords: **LCMS, Ion trap, Drugs of abuse**

P25 Analysis of THC, OH-THC, and COOH-THC in Blood Utilizing LC/MS/MS

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Objective

The objective was to develop a simple, rugged method for detection and quantitation of THC and two metabolites, OH-THC and COOH-THC, in blood utilizing LC/MS/MS.

Method

THC, OH-THC, and COOH-THC were analyzed with their respective deuterated internal standards. The IS was spiked at 10 ng/mL. Sample preparation consisted of a simple protein precipitation, evaporation, and reconstitution. LC/MS/MS analysis was performed on a 3200 Q TRAP® instrument interfaced to a Shimadzu LC stack. OH-THC and COOH-THC ionize in both positive and negative modes, though signal intensity is better in negative mode. THC, however, ionizes in positive mode only. Results were compared running in positive mode only, and using pos/neg switching so the two metabolites were analyzed in negative mode. Separation was achieved on a 2 mm x 50 mm Aquasil C18 column and total run time was 3.5 minutes. Mobile phases were water and methanol, with 0.1% formic acid added to both.

Results

Acetone, methanol, ACN, and some various mixtures of ACN with IPA or acetone were tested as extraction solvents for protein precipitation of the blood sample. There did not appear to be significant differences from the various extraction solvents, so cold ACN was used and the extraction efficiency was on the order of 70% or better, depending upon the analyte. Results showed LOQs of 1 ng/mL or better for all analytes when run in positive mode only. When pos/neg switching was implemented, LOQs were around 0.1 ng/mL for all analytes. Concentrations calculated for samples corresponded well with those obtained using a validated GC/MS method. The assay was linear over 3 orders of magnitude and no degradation of data quality was observed throughout hundreds of injections.

Conclusion

An LC/MS/MS method was developed for analysis of THC and two of its metabolites. Sample preparation was greatly simplified vs. GC/MS. LC/MS/MS run times were also much shorter than GC/MS runtimes. Sensitivity was sufficient to detect relevant levels of both the parent drug and the two metabolites.

Key words: **LC/MS, THC, COOH-THC, OH-THC**

P26 Analysis of Benzodiazepines in Oral Fluid by EIA and LC-MS/MS

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A simple procedure for the screening and confirmation of benzodiazepines in oral fluid is presented. The OraSure Technologies, Inc., Benzodiazepine Intercept® MICRO-PLATE Enzyme Immunoassay (EIA) is used for the screening of subjects from a drug treatment center. Oral fluid was obtained using the Intercept® Oral Specimen Collection Device (OraSure Technologies, Inc.), which collects approximately 0.4 ml of oral fluid and dilutes it with 0.8 ml of preservative solution. When specimen or standard is added to an EIA microplate well containing an oral fluid specimen positive for benzodiazepines, there is a competition between the drug in the sample and the enzyme-labeled hapten to bind the antibody fixed onto the EIA well. The EIA wells are then washed, substrate is added, and color is produced. The EIA absorbance (A) at 450nm for each subject was compared with the cut-off. If the A is ≤ to the cut-off, the subject is positive; if the A is > then the cut-off, the subject is negative.

Using part of the oral fluid collected for the same subjects with the Intercept® device, a confirmation of the EIA results for these subjects is done using a novel LC-MS/MS method suitable for the confirmation of 14 Benzodiazepines in oral fluid. The target compounds include the following: diazepam, oxazepam, temazepam, nordiazepam, lorazepam, flunitrazepam, 7-Aminoflunitrazepam, chlordiazepoxide, alprazolam, hydroxyl-alprazolam, clonazepam, 7-aminoclonazepam, desalkylflurazepam, hydroxyethylflurazepam.

The LC-MS/MS procedure is preceded by a solid phase extraction of the drugs on a Varian Bond Elut cartridge. The extracted benzodiazepines are reconstituted in a methanol/water (50/50) mixture. Nordiazepam-D_5 was used as an internal standard for quantitation. The extraction efficiency exceeded 83% for all compounds except for 7-aminoclonazepam, flurazepam and chlordiazepoxide, which had recoveries of 55%, 45% and 48%, respectively.

Upon analysis of 19 Benzodiazepine EIA screened positive subject oral fluids, we found that all were also positive for at least one of the LC-MS/MS analyzed benzodiazepines. We also found that there was a correlation between the absorbance value of the drugs by EIA and the amount of drug (from LC-MS/MS analysis) for the patients screened positive. We therefore conclude that this method is suitable for confirmation of the Intercept® Benzodiazepine Assay.

Keywords: **Benzodiazepines, LC-MS/MS, Oral fluid**

P27 Quantitation of Seven Opioids in Urine Utilizing HPLC-MS/MS

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Objective

The objective was to develop a novel analytical method for the simple preparation and analysis of seven opioids in urine specimens. The drugs to be monitored include hydromorphone, morphine, oxymorphone, 6-MAM, oxycodone, codeine, and hydrocodone.

Method

Sample preparation was based on a previously utilized GC/MS method involving solid phase extraction of the sample. A 250µL aliquot of urine was combined with an internal standard solution followed by hydrolysis using β-glucuronidase. Deuterated analogs of each opioid were used as internal standards at concentrations of 150ng/mL each. Following hydrolysis the specimens were diluted, then a portion was transferred to an autosampler vial for analysis. Ten microliters were injected for HPLC separation, which was performed on an Applied Biosystems 3200 QTrap tandem mass spectrometer interfaced to an Agilent 1100 HPLC system. Separation was achieved on a 2 mm x 50 mm Aquasil C18 column with a total run time of 6.4 minutes. Mobile phases A and B were water and ACN respectively, each containing 0.1% formic acid. Two transitions per analyte and one transition per internal standard were monitored. The mass spectrometry method was divided into two periods, with 3 analytes and associated internal standards in the first period and 4 analytes and internal standards in the second period.

Results

Seven opioids and their respective internal standards were successfully analyzed in a single method. Sample preparation was simplified, as no derivatization is required, in contrast to GC/MS. Run time was reduced from 10 minutes for GC/MS to 6.4 minutes for HPLC. Several sets of samples were run in parallel on GC/MS and LC/MS, with good correlation. The lower limit of quantitation was 5ng/mL, and the upper limit of quantitation was at least 10,000ng/ml all analytes. Detection limits 1ng/ml or lower for each compound. Excellent precision was observed, with a coefficient of variation of less than 10% for all analytes.

Conclusion

A simple and robust LC/MS/MS method for the analysis of opioids in urine was developed. Sample preparation was simplified and run times were significantly reduced versus our GC/MS method. The method has an expanded linear range, good correlation with the GC/MS method, and excellent precision. This method has proven to be superior compared to the GC/MS method.

Key words: **LC/MS, Opioids, QTrap**

P28 Quantification of Naltrexone and 6-β-Naltrexol in Human Urine and Oral Fluid by Gas Chromatography-Mass Spectrometry

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Measurement of naltrexone, an opiate antagonist, can be useful to monitor patient compliance in treatment programs. A gas chromatography-mass spectrometry (GC-MS) method to detect naltrexone (NT) and 6-β-naltrexol (β–OL) in human urine and oral fluid is described.

Paired human urine and oral fluid samples were collected from 10 subjects who had received oral doses of naltrexone over several days prior to collection. Urine samples were hydrolyzed with glucuronidase . Oral fluid samples were collected with Salivette devices from Sarstedt. All specimens were adjusted to a pH between 9.4 and 10.0. Specimens were applied to BondElut Certify columns. The SPE columns were preconditioned with methanol followed by water. After application of the samples the columns were washed in the following order with water; 0.1 M acetate buffer, pH 4.0; and methanol. The columns were dried under vacuum for one minute. After drying, the elution solvent, methylene chloride:isopropanol:ammonium hydroxide, (80:20:2) was applied to the columns.The eluates were collected in glass screw capped tubes. The solvent was evaporated and the trimethylsilyl derivatizing agent: 2% N–trimethylsilyl-imidazole (TSIM) in N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added. The capped tubes were heated at 100 °C for 30 minutes. GC-MS analysis was performed with an Agilent Technologies 5973 GC-MSD System in the selected ion monitoring (SIM) mode. A Varian Factor Four VF-5ms 30 m x 0.25 mm column was used with a temperature programmed run from 100 °C to 310 °C at 40 °C/minute. Standard solutions of naltrexone, d3-naltrexone, and 6-β-naltrexol were obtained from Cerilliant. The selected ions monitored were naltrexone: 542, 557, 484; d3-naltrexone: 545, 560, 487; and 6-β-naltrexol: 544, 559, 372. Subject urine samples were collected over approximately three hours after dosing. All 10 samples demonstrated significant levels for naltrexone, range from 438 to 531 ng/mL and 6-β-naltrexol, range from 596 to 935 ng/mL. The 10 oral fluid samples were collected approximately one and a half hours after dosing. Nine of the 10 oral fluid samples demonstrated 6-β-naltrexol results, range from13 to 115 ng/mL. Only three of the 10 oral fluid samples yielded naltrexone results, range from 34 to 58 ng/ml.

GC-MS analysis detected both naltrexone and 6-β-naltrexol in all urine samples. GC-MS analysis of oral fluid found 6-β-naltrexol in nine of 10 donor samples. The method outlined here can provide a means of monitoring compliance among patients treated with naltrexone.

Key Words: **Naltrexone, GC-MS, Urine**

P29 The validation and use of an accredited routine method for the simultaneous analysis of opiates in oral fluid by GC-MS

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Aims: The analysis of drugs of abuse in oral fluid poses a challenge due to limited sample volume available for analysis and the requirement to detect drugs at lower concentrations. The aim of the work described here was to develop a sensitive and robust method suitable for the routine analysis of codeine, morphine, 6-acetylmorphine and dihydrocodeine in oral fluid in an accredited laboratory.

Methods: The analytes of interest were extracted by solid phase extraction (SPE) using a mixed mode SPE cartridge. Analysis was carried out using an Agilent GC-MS. Each analyte was determined by selected ion monitoring (SIM) of a quantification ion and two qualifying ions (codeine m/z 374, 237, 346; morphine m/z 432, 327, 390; 6-acetylmorphine m/z 402, 343, 290 and dihydrocodeine m/z 379, 288, 318). Deuterated internal standards were used for the quantitation of each analyte. Calibration standards at 0, 15, 30, 60, 90 120 and 180 ng/ml were used, and each sample, standard and control was spiked with deuterated internal standard at 120 ng/ml. The validation of the method involved determination of linearity; detection and quantitation limits; robustness; assay drift and the precision of the method. The method was used for the analysis of 437 samples from criminal justice sources.

Results: The method is linear for each analyte over the range of LOD – 180 ng/ml. The detection limit for each analyte is as low as 1 ng/ml, with the quantitation limit as low as 5 ng/ml. The retention time of the peaks of interest varied by only as much as 0.1. The assay drift was negligible. The intra-assay precision was approximately 5% and the inter-assay precision 9%. Following analysis of 437 samples using this method, 63% were negative; 5% were positive for dihydrocodeine; 10% were positive for codeine; 30% were positive for morphine and 23% were positive for 6-acetylmorphine.

Conclusion: This method is suitably sensitive and robust for the routine analysis of opiates in oral fluid in an accredited laboratory.

Key Words: **Oral fluid, Opiates, GC-MS**

P30 Evaluation of Several Approaches to the GC/MS Confirmation of delta9-THC in Oral Fluid

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The confirmation of delta-9-THC in saliva and oral fluid as based on proposed SAMHSA guidelines is analytically challenging due to the required low limits of detection and quantitation and high matrix concentrations relative to the cutoff. Because of this, successful analysis typically requires alternative approaches when compared to those used for routine urine GC/MS confirmations. Labs can pursue increased sensitivity for the mass spectrometer and a reduction of the affects of the oral fluid matrix. Approaches have therefore included the use of tandem MS, two dimensional GC and chemical ionization.

The work presented details a comparison of different approaches to this method using a GC/MS instrument currently on the market. Because of the variety of approaches available for both sample introduction and mass spectral analyses, techniques compared include constant temperature versus programmed temperature injection, electron impact versus chemical ionization and single stage versus tandem MS. The use of a backflush device, in which heavier components are flushed out of the system through the split vent, was also tested for its suitability to this application. Finally, multiple columns and derivatization reagents were compared for optimal sensitivity and selectivity.

Because this methodology was intended for use in a high through-put laboratory environment, method development and optimization considered a broad spectrum of method performance, including ease of use, method robustness and cost per sample. The selected method was subjected to a full validation, in matrix, and this validation included a linearity study, intra- and inter-day precision study, and an interference study including other THC metabolites and common interferents. The linearity of the method ranged from 0.4 ng/mL to 40 ng/mL, with intra- and inter-day precision less than 10% CV, and no coelution was seen with any of the interferents tested.

Key Words: **THC, Oral fluids, GC/MS**

P31 The New Generation of Mass Spectrometry Software

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A gas chromatography/mass spectrometry software package that bridges the gap between productivity and quality by working with the natural flow of sample data through the toxicology laboratory was evaluated. The toxicologist is offered a dynamic interface that streamlines the path from unknown sample to known result in a manner which is secure, defensible, and aligned with the toxicology laboratory and accrediting agency's specific needs.

The platform can be utilized as a stand alone data reduction program, or as a print engine delivering reports "on-the-fly". The toxicologist is offered a host of over twenty (20) pre-designed reports to effectively give quantitative analytical results in a diverse and versatile format. This application also allows the user to export data in a number of formats, including *.pdf* and *.xml*, making the software integral with LIMS systems.

Analysts experience more productive activities due to bounds-checking features of quality control/quality assurance samples and intuitive batch wizard design. Data integrity is built into the software package, securing the data, local methods, and reports into self-contained folders that are easily executable and archived.

What sets this software ahead of other data packages is the extensive data review section. The user can make changes and see those effects immediately without switching screens, reprocessing the data, or opening other programs.

The user-friendly, layered application software provides an intuitive, complete approach to data acquisition, analysis, reporting, and data review in the clinical and forensic toxicology laboratory.

Key Words: **Toxicology, LIMS, Software**

P32 Analysis of MDMA and its metabolites in urine and plasma following a neurotoxic dose of MDMA

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MDMA (3,4-methylenedioxymethamphetamine) is a commonly encountered drug of abuse. Initially, in addition to its recreational use, MDMA was used as an adjunct to psychotherapy. As its (ab)use increased, it was first scheduled in 1985 as a Schedule I drug. Classified as an entactogen because of its unique properties that lead to feelings of emotional closeness to one's self and others allowing for a breakdown of barriers to communication, thus one of its properties thought by some to be advantageous in psychotherapy. The drug has been shown to cause neurotoxic effects in a variety of studies. Evaluation of urine and plasma concentrations of MDMA and three of its metabolites following administration of a neurotoxic dose (20 mg/kg) to male Dark Agouti rats was accomplished. Currently there are no data available describing urine and plasma concentrations of MDMA and these metabolites over a period of 7 days.

Rats received a single 20 mg/kg i.p. dose of MDMA. Blood and urine samples were collected at 2, 4, 8, 12, 16, 20, 24, 48, 96 and 168 hours following drug administration. Plasma was then separated from the sample and stored at $\leq$20ºC prior to analysis. Urine samples were collected in urine containers and stored at $\leq$20ºC prior to analysis. Urine samples were collected starting 2 hours before each time point.

Plasma and urine samples were extracted using solid phase extraction of a 1 mL aliquot with United Chemical Technologies XTRACKT, XRDAH203 high-flow 200 mg columns using a Zymark RapidTrace. Following extraction, samples were derivatized with MBTFA then analyzed for MDMA and metabolites using GC-MS.

Urine samples showed peak concentrations of MDMA at 4 hours, MDA at 8 hours. Plasma samples showed peak concentrations of MDMA at 2 hours, MDA at 2 hours.

Key words: **MDMA, Rat, Neurotoxicity, Metabolites, MDMA**

P33 Selection and Optimization of Hydrolysis Conditions for the Quantification of Urinary Metabolites of MDMA

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MDMA metabolites must be cleaved from glucuronide/sulfate conjugates prior to extraction and chromatographic analysis to maximize recovery. Two methods are commonly employed to cleave these conjugates: enzymatic hydrolysis with β-glucuronidase and hydrolysis with hydrochloric acid. The objective of this study was to evaluate and optimize hydrolysis conditions for the recovery of MDMA metabolites MDA, HMA, and HMMA from human urine.

Experiments were conducted on human urine specimens collected 2 to 48 hours after oral MDMA administration. Urine specimens were analyzed in duplicate. Enzymatic hydrolysis conditions were 10,000 U/mL of β-glucuronidase incubated for 16 hours at 37°C in a shaking water bath. Acid hydrolysis was performed with 100 or 200 microliters of concentrated hydrochloric acid per mL of urine for varying time and temperature conditions.

Hydrolysis of urine conjugates under acidic conditions and by enzymatic hydrolysis with β-glucuronidase from *Escherichia coli* and *Helix pomatia* was evaluated. Acid hydrolysis yielded 40.0% and 39.3% higher HMA recovery compared to *E. coli* and *H. pomatia* hydrolysis, respectively (n=6; SE=9.8 and 11.4%). *E.coli* β-glucuronidase hydrolysis recovery of MDA was 17.1% and 26.5% greater than acid hydrolysis and *H. pomatia* β-glucuronidase recovery (n=6; SE=3.3 and 6.1%), respectively. Acid hydrolysis recovery of HMMA was 336.1% and 159.8% greater than *E. coli* and *H. pomatia* β-glucuronidase recovery (n=6; SE=72.8 and 31.6%), respectively.

Based on recovery of HMMA and HMA, acid hydrolysis was selected as the preferred hydrolysis method. Studies of the effects of temperature, time, and amount of acid on MDMA metabolite recovery were conducted. HMA and HMMA acid hydrolysis recoveries were improved at temperatures above 100°C (n=3). Effective hydrolysis could be accomplished in a dry block heater, GC oven, or autoclave at temperatures ranging from 110-140°C. Assays were perfomed to evaluate potential interconversions or degradation of analytes at the selected hydrolysis conditions. Blank urine specimens fortified with 2.5 or 10 micrograms of MDMA, MDA, HMA, or HMMA were quantified after acid hydrolysis. Analyte concentrations were within ±20% of target indicating no significant change in analyte concentrations.

Optimal hydrolysis conditions for the measurement of MDMA metabolite conjugates in 1 mL urine specimens were achieved with 0.1 mL of concentrated hydrochloric acid for 40 min at 120°C. Acid hydrolysis was also preferred due to time efficiency and cost effectiveness.

Keywords: **MDMA, Hydrolysis, Conjugates**

P34 Validated Gas Chromatography/Mass Spectrometry Method for the Simultaneous Quantification of 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxymethamphetamine (MDMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyamphetamine (HMA) in Human Urine

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A sensitive GC/MS method was developed and validated for the simultaneous measurement of MDEA, MDMA, and its metabolites, HMA, MDA, and HMMA, in human urine.

MDMA (ecstasy) is excreted primarily as unchanged drug in urine, with additional N-demethylation to MDA, O-dealkylation to 3, 4-dihydroxymethamphetamine (HHMA) and 3, 4-dihydroxyamphetamine (HHA), and O-methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) and 4-hydroxy-3-methoxyamphetamine (HMA).

One mL urine, fortified with MDMA-d_5, MDA-d_5, and MDEA-d_6, was hydrolyzed with 100 µL of concentrated hydrochloric acid at 120°C for 40 min. One hundred µL 10 N sodium hydroxide and 3 mL 0.1 M phosphate buffer (pH 6.0) were added to hydrolyzed urine specimens prior to solid-phase extraction (SPE). SPE columns were preconditioned with methanol, water and 0.1 M phosphate buffer (pH 6.0). Each sample was loaded onto SPE column and washed with water, 1 M acetic acid, and methanol. Analytes were eluted with methylene chloride:2-propanol:ammonium hydroxide (78:20:2 v/v/v) and 50 µL of 1% hydrochloric acid in methanol (v/v) was added prior to evaporation. Extracts were evaporated to dryness under a stream of nitrogen at 37ºC using a Zymark Turbovap LV® evaporator. Extracted residues were reconstituted in ethyl acetate, derivatized with HFAA, and analyzed by GC/MS/EI in selected ion monitoring (SIM) mode. GC/MS analysis was performed using an Agilent 6890 gas chromatograph equipped with DB-35 MS capillary column (15m x 0.32 mm i.d.; 0.25µm-film thickness) and interfaced to a 5973 mass-selective detector. Helium carrier gas a constant pressure of 2.0 psi was used. One µL of derivatized urinary extract was injected with a split ratio of 10:1. The injection port and transfer line temperatures were 250ºC and 280ºC, respectively. The initial column temperature of 70ºC was held for two min, followed by temperature ramps of 20ºC/min to 160ºC, held for 2 min, and 15^{o}C/min to 200^{o}C. Total separation run time was 12.2 min. There was a one minute post run hold at 300^{o}C. The ion source was maintained at 230ºC and the quadrupole at 150ºC.

Limits of quantification were 25µg/L for MDEA, MDMA, and its metabolites. Calibration curves were linear to 5000 µg/L for MDEA, MDMA, HMA, MDA, and HMMA, with a minimum r^2>0.99. At three concentrations spanning the linear dynamic range of the assay, mean overall extraction efficiencies from urine were >85.5% for all compounds of interest. Intra- and inter-assay precision, expressed as CV, were <15% for all drugs at 30, 300, and 3000 µg/L.

This GC/MS assay provides adequate sensitivity and performance characteristics for the simultaneous quantification of MDEA, MDMA and its metabolites HMMA, MDA, and HMA in human urine. The method meets and exceeds the requirements of the proposed Substance Abuse and Mental Health Administration's guidelines for federal workplace drug testing of MDEA and MDMA in urine.

Keywords: **MDMA and Metabolites; GC/MS, Solid-Phase Extraction; Human Urine**

P35 A Method Validation for Drugs of Abuse in Blood on the Dade-Behring Viva-E

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A Dade-Behring Viva-E Drug Testing System™ (Viva-E) was acquired by the Wisconsin State Laboratory of Hygiene Toxicology Section to replace a Syva® ETS® Plus analyzer (ETS) that had been used as a screening instrument for drugs of abuse in blood for many years. Both instruments utilize Syva's Enzyme Multiplied Immunoassay Technique. Prior to installation, a validation process was undertaken to ensure that reliable screening results could be achieved for the following drug classes: barbiturates (BB), benzodiazepines (BZ), cocaine (COC), opiates (OP), phencyclidine (PCP) and cannabinoids (THC).

Whole blood calibrators, controls and samples were extracted with acetone and the supernatant analyzed on the instrument. Cut-off limits were established by calibrating the instrument with an in-house prepared whole blood calibrator. Calibrator drug concentrations with corresponding analyte are as follows: BB (secobarbital) at 100ng/mL, BZ (lorazepam) at 40ng/mL, COC (benzoylecgonine) at 50ng/mL, OP (morphine) at 40ng/mL, PCP at 10ng/mL and THC (carboxy-THC) at 10ng/mL.

Accuracy was determined by analyzing 52 (50 for BZ assay) ante-mortem and post-mortem blood specimens for the above assays by both the Viva-E and the ETS. Further confirmation testing was conducted using GC/MS for specimens originally screened positive by the ETS. The percent agreement from the comparative analysis ranged from 83% to 100% for the drug classes monitored. Agreement improved to 96-100% when discrepant screen results were compared to GC/MS confirmation data.

Intra- and inter-day precision was determined utilizing in-house prepared negative and positive whole blood controls. The negative control is whole blood that has been screened and found to contain no interfering drug substances. The positive control is whole blood fortified with drug at concentrations twice the calibrator cut-off levels. The within day coefficient of variation (CV) was less than 2% at both negative and positive control levels for all assays examined. Total precision (intra- and inter-day) ranged from 0.8% to 1.8% for negative controls and 1.8% – 5.9% for positive controls.

Other validation parameters studied in this process included an examination of limits of detection, specificity, carryover, and interferences. Empirically determined limits of detection were well below the laboratory defined cut-off limits for each assay. Several drugs of concern, including oxycodone, phenytoin and MDMA were analyzed to test instrument specificity. These analytes did not cross-react strongly with the targeted drug class at assay cutoff limits. Carryover was not detected for any of the assays. No positive interferences were observed when analyzing blood fortified with commonly occurring drugs at high concentrations, except when phenytoin was present. Phenytoin at 50µg/mL produced a positive (above cutoff) BZ response. No negative interferences were seen when analyzing fortified blood mixtures with the target analyte present.

We found that the Dade-Behring Viva-E Drug Testing System™ provides rapid and highly reliable screening results for our target drugs in blood and met the specifications and needs of our laboratory. It is a flexible platform that allows assays to be added or removed as the needs of the laboratory change. The limiting factor for the number of assays that may be performed from a single sample extraction is the final sample reconstitution volume.

Key words: **Validation, Immunoassay, Blood**

P36 Solid Phase Extraction Procedure for Screening of Therapeutic and Abused Drugs in Whole Blood by GC/MS

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A procedure for the semi-quantitative identification of therapeutic and abused basic drugs and metabolites in whole blood by GC/MS is presented. Samples are treated with acetonitrile and adjusted to a neutral pH, followed by solid phase extraction using the Caliper Life Sciences RapidTrace® Solid Phase Extraction Workstation with Phenomenex Strata-X-C SPE cartridges. The RapidTrace® conditions the SPE cartridges with elution solvent, methanol, and neutral buffer, adds the sample, washes the cartridges, dries the cartridges with nitrogen, and then elutes the drugs and metabolites with 80% methylene chloride/20% isopropanol which is made alkaline with ammonium hydroxide. The final extracts are evaporated, derivatized with butyric anhydride, and analyzed by GC/MS using the Varian Saturn 2000 Ion Trap. The procedure is useful for the identification and semi-quantitation of over 100 drugs and metabolites. The typical limit of detection is 20 ng/mL with most substances being detectable between 10 and 50 ng/mL. Identification is based on relative retention time to the internal standard (Proadifen) in addition to reverse and forward match of the full scan mass spectrum to the reference spectrum from an in-house generated library. Semi-quantitative results are determined using a historical 5-point calibration curve ranging from 10 to 1000 ng/mL. Correlation coefficients for the calibration curves were generally greater than 0.990.

Key Words: **Blood, Screen, GC/MS**

P37 A Comparison of Two Solid Phase Columns for the Extraction of Ten Benzodiazepines From Whole Blood

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In this presentation, a novel application of the solid phase extraction of various popular benzodiazepines from whole blood is described. Solid phase extraction of drugs from biological matrices (especially postmortem blood) using mixed mode columns is a commonly employed methodology in many laboratories undertaking toxicological analyses; this new procedure utilizes the properties of an endcapped phenyl sorbent for the analysis of this group of compounds in similar materials.

The benzodiazepines are a popular class of pharmaceuticals that contain a common ring structure. The individual drugs each have various substituent groups that give rise to diverse chemical properties, thus complicating simultaneous analytical determination. This poster presents details and NCI-GC/MS data that compare the extraction/analysis of the benzodiazepines diazepam, nordiazepam, oxazepam, temazepam, lorazepam, midazolam, clonazepam / 7-aminoclonazepam, alprazolam, and triazolam from whole blood samples using both UCT CSDAU (mixed mode) and UCT CSBNZ (endcapped phenyl) columns. The data shows how the analytical performance in terms of percentage recovery can be enhanced by the use of the endcapped phenyl columns in the analysis of the benzodiazepines in whole blood.

Keywords: **Benzodiazepines, Solid phase extraction, Postmortem toxicology**

P38 Evaluating Workplace Drug Testing Results from a Medical Review Officer Data Source

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Introduction: Understanding the relationship between laboratory drug test results and Medical Review Officer (MRO) verified results has long been warranted. In 2003 a large drug testing laboratory indices representing roughly 7 million specimens reported an annual positive rate for the Federally Mandated Workforce (Regulated) of 2.3% and a Non-regulated Workforce annual positive rate of 4.9%. These annual indices may not accurately represent illegal drug use rates since they include blind quality control samples and results later reversed through MRO interview with the donor to determine if the donor has a valid medical explanation for the test results.

Objective: To evaluate the relationship between laboratory reported drug test results and MRO verified results reported to employers.

Methods: Records for nearly one million federally Regulated and Non-regulated specimens collected during calendar year 2003 from more than 9,000 companies were obtained from a large MRO data source. The database included donor demographics, employer information, collection site information, laboratory results, and MRO determinations, but did not include agency or employer blind quality control samples. Only the urine data were analyzed for this presentation. Laboratory drug test results were received from 41 labs. Records were transferred from the MRO source in compliance with all HHS Human Subject protection criteria.

Results: Analysis of 2003 data indicated that following MRO review, 5.4% and 18.6% of federally Regulated and Non-regulated drug test results, respectively, were reversed. Table 1 illustrates the breakdown of MRO reversals by Regulated and Non-regulated specimens and by drug class. Additional analyses will address such issues as cancelled or rejected specimens, adulteration, and "invalid" results.

# of Specimens Tested	Regulated			Non-Regulated		
	161,816			664,780		
	Lab +	Verified	Reversed	Lab +	Verified	Reversed
Overall	1.76%	1.67%	5.43%	4.24%	3.45%	18.55%
Amphetamines	0.16%	0.13%	18.68%	0.29%	0.16%	43.49%
Cocaine	0.50%	0.50%	0.12%	0.62%	0.62%	0.10%
Marijuana	1.06%	1.06%	0.23%	2.63%	2.62%	0.22%
Opiates	0.09%	0.03%	72.19%	0.32%	0.06%	80.54%
Phencyclidine	0.01%	0.01%	0.00%	0.02%	0.02%	0.00%
Barbiturates				0.34%	0.06%	83.66%
Benzodiazepines				0.48%	0.12%	74.57%

Conclusions: A significant number of positive test results were reversed in the MRO process. In Regulated testing, this was due primarily to a legitimate medical explanation for the presence of opiates and amphetamines. In the Non-regulated dataset, a greater number of MRO reversals were observed due largely to higher amphetamine reversals [43.5% vs. 18.7%] and the inclusion of the barbiturates [83.7%] and benzodiazepines [74.6%].

Key Words: **Workplace Drug Testing; Database Analyses; Medical Review Officer**

P39 Influence of Basic pH on Federal Regulated Drugs in Urine at Room Temperature

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Objective: Under the Mandatory Guidelines for Federal Workplace Drug Testing, urine specimens are tested for specimen validity and reported as invalid due to abnormal high pH of ≥9.0 and <11. A study was designed to determine the stability of drug analytes in urine slightly below and within this invalid range.

Methods: Drug free human urine was adjusted with hydrochloric acid or sodium hydroxide to pH ranges of <6 (control urine), 8 - 9, 9 - 9.5, and 9.5 - 10. Drug analytes required by the Mandatory Guidelines were added to the specimens at 1.5 to 2 times the initial test cutoff. Twelve certified laboratories under the National Laboratory Certification Program were compensated to perform the analytical work which consisted of two pH meter determinations and a confirmatory quantitative analysis using gas chromatography/mass spectrometry (GC/MS) on each specimen. Percent loss of drug analyte in urine at basic pH was determined by comparing concentrations in basic urine to concentrations in acidic urine (control). To mimic normal delays between specimen collection and analysis, specimens were kept at room temperature and analyzed on two study days (Days 1 and 3).

Results: The pH of specimens remained fairly stable over the three day study period. Amphetamine, methamphetamine, codeine, morphine and THCA did not show loss in drug concentration in basic urine as compared to the acidic urine control at room temperature over the three day study. As shown in the table below, benzoylecgonine, phencyclidine and 6-acetylmorphine decreased significantly in concentration as the urine pH increased in basicity and further loss of these analytes occurred as samples remained at room temperature.

Percent Loss of Drug Analyte and Corresponding pH Values for each Test Day

Analyte & Targeted pH Range	Day 1		Day 3	
	%Loss	Group Mean pH	%Loss	Group Mean pH
Benzoylecgonine				
8.0 – 9.0	4%	8.63	5%	8.38
9.0 – 9.5	13%	9.35	28%	9.33
9.5 – 10.0	37%	9.73	61%	9.70
Phencyclidine				
8.0 – 9.0	18%	8.33	15%	8.06
9.0 – 9.5	43%	9.30	50%	9.21
9.5 – 10.0	53%	9.65	61%	9.65
6-Acetylmorphine				
8.0 – 9.0	22%	8.61	29%	8.41
9.0 – 9.5	58%	9.37	81%	9.27
9.5 – 10.0	82%	9.72	100%	9.73

Note: Values are the group mean (12 laboratories) of two pH meter tests (n=24) and a single GC/MS quantitation (n=12) per specimen.

Conclusions: Loss of benzoylecgonine at high pH values was probably due to hydrolysis to ecgonine at its ester linkage. Loss of phencyclidine was probably due to decreased solubility around its pK_a of 8.5. Loss of 6-acetylmorphine as pH increased was probably due to hydrolysis to morphine. This study supports the current pH range (≥9.0 and <11) used to report invalid specimens in the Mandatory Guidelines due to the loss of drug analytes at a pH between 9.0 and 9.5.

Key Words: **Specimen validity testing, Workplace drug testing, Abnormal pH**

P40 Acetonitrile as an Alternative to Methanol to Address Long Term Degradation of Oxazepam in Solutions: An HPLC Based Stability Study

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Quantitative information on drugs and drug metabolites with pharmacological or toxicological activities is of great interest to the forensic drug testing and the pharmaceutical industries. Reference standard solutions are key components in the successful quantitative analysis of impurities associated with pharmaceutical drugs, identification of specific drugs of abuse in clinical samples as well as determination of the presence of environmental contaminants.

Oxazepam is a member of the drug class known as benzodiazepines. It is a sedative commonly prescribed for anxiety disorders with associated tension, irritability, and depression. Long-term usage generally leads to some form of tolerance and/or dependence. While the abuse liability of oxazepam is found to be less than that of the other benzodiazepine family of sedatives, means for the effective quantitation of this drug are often necessary.

Cerilliant offers oxazepam as a certified reference standard solution. The preferred choice of solvent for all commercially available oxazepam standards has historically been methanol. Recently, Cerilliant has shown that acetonitrile is a more effective solvent for the long term storage of oxazepam standards. This work outlines the outcome of accelerated stability studies using HPLC and LC-MS as the techniques to characterize both oxazepam and its possible degradants when stored in methanol for an extended period of time.

A significant impurity was found during release testing of a 1.0 mg/mL oxazepam standard solution stored in methanol. A potential degradation pathway was proposed based on LC-MS studies. The investigation concluded that oxazepam is unstable in methanol and degrades at approximately 7-8% per year in the presence of a protic solvent. Alternative solvents were evaluated for stability and acetonitrile was selected as the best candidate. An accelerated stability study was performed on methanol and acetonitrile solutions at 40ºC for one month. Samples were pulled at 2 week intervals and tested against fresh standards. At the first testing interval the oxazepam stored in methanol had an analyzed purity of 39.2% by area percent (56% degradation) whereas the purity of the oxazepam in acetonitrile was 98.0% (no degradation). After 4 weeks, oxazepam stored in methanol was 9.5% pure (91% degradation), and the oxazepam in acetonitrile showed only 2% degradation. Comparative oxazepam standard solutions prepared in methanol from different vendors were also investigated, and results are presented.

Keywords: **Reference standard, Stability, Oxazepam**

P41 Multi-Year Stability of Pre-made Solution Reference Standards

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When appropriate parameters are chosen in the design, preparation, packaging, and storage of the solution standards, long term stability is possible. This poster provides examples of pre-made solution reference standards that exhibit multi-year stability.

Five examples were selected for the purpose of this study to exhibit the stability of pre-made solution standards. The compounds selected included a cross section of products offered including morphine, diazepam, fentanyl, meperidine and fluoxetine hydrochloride. Solution stability was determined by comparison of a stability lot to a freshly prepared solution, the existing lot and a calibration curve. Chromatographic analytical techniques were employed to gather this data. Results of these comparisons are included in this poster.

Analytical reference standards prepared in a solvent that promotes stability and packaged under argon in a flame sealed ampules can be stable for many years when stored correctly.

Keywords: **Stability, Solution, Standards**

P42 Quetiapine Distribution in an Acute Fatal Overdose

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The objective of this paper is to present quetiapine concentrations in several biological specimens obtained in a case of fatal acute intoxication. Quetiapine, a dibenzothiazepine derivative, is used for the treatment of schizophrenia and acute manic episodes associated with bipolar disorder. Its activity is derived from selective antagonism with high affinity for serotonin type 2 ($5HT_2$), and dopamine type 2 (D2) receptors. Quetiapine is also an antagonist at serotonin 5-HT_{1A}, dopamine D1 and D2, histamine H1, and adrenergic alpha 1 and alpha 2 receptors. Following oral administration, quetiapine is rapidly absorbed with peak blood reached in 90 min. Plasma protein binding is 83% and quetiapine has a volume of distribution of 10 L/kg. The pKa values for quetiapine are 3.3 and 6.8. Metabolism of quetiapine is catalyzed primarily by cytochrome P450 3A4 to an inactive sulfoxide.

The decedent is a 39-year-old male with a history of bipolar depression with past suicide attempts. One evening at approximately 7 pm, he stated to his wife that he had ingested 50 to 70 quetiapine tablets (300 mg). He was immediately taken to the hospital emergency department and activated charcoal was administered to lessen absorption of the drug. However, at 3 hours post-admission, the patient arrested and at 11:46 p.m., he was pronounced dead.

Blood samples obtained upon admission to the hospital and at autopsy were delivered to the toxicology laboratory where testing was conducted for the presence of alcohol, drugs of abuse and therapeutic drugs. Blood ethanol was analyzed by direct injection GC-FID using *t*-butanol as internal standard. An aqueous-reconstituted acetone blood precipitate was screened for drugs of abuse by EMIT using reagent kits purchased from Dade Behring (Deerfield, IL). Alkaline and acidic/neutral extracts were also screened for drugs by GC-MS with comparison to an in house EI mass spectral library, as well as NIST 2005 and AAFS libraries. Quetiapine was identified in the alkaline extract, with subsequent quantitation/confirmation by LC-MS using methaqualone as internal standard.

No alcohol or drugs of abuse were detected, with quetiapine the only drug identified by GC-MS. Immunoassay of a hospital blood precipitate gave a presumptive positive response for the tricyclic anti-depressant drug class. Structural similarity of quetiapine to tricyclic antidepressants is presumed to be the explanation for this finding. Quetiapine concentrations for several case samples are indicated in Table 1.

Table 1.

Hospital Blood	Subclavian Blood	Liver	Vitreous Fluid	Gastric Contents
3.91 mg/L	6.58 mg/L	46.6 mg/kg	1.99 mg/L	561 mg/L (448 mg)

Conclusion: The blood concentration of quetiapine in the present case is consistent with that in several reported cases of fatal overdose. Information obtained indicated the decedent was not regularly taking the drug prior to the fatal ingestion.

Key Words: **Quetiapine, Overdose, Fatal**

P43 The Distribution of Fluoxetine in Postmortem Fluids and Tissues

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During aviation accident investigations, postmortem specimens from the operator of the aircraft are submitted to the Federal Aviation Administration's (FAA's) Civil Aerospace Medical Institute (CAMI) for toxicological analysis. Due to the violent collisions often associated with aircraft accidents, victims' bodies may be fragmented and/or incinerated. In many cases, the search for remains results in only small fragments of various tissues available for toxicological analysis. In fact, the FAA's Forensic Toxicology and Accident Research Laboratory receives blood specimens in only approximately 70% of fatal aviation accidents. However, therapeutic and toxic concentrations for most drugs are reported in the scientific literature for blood and plasma only. Therefore, it would be helpful for an accident investigator and forensic toxicologist to be able to estimate drug concentrations in a fatal aviation accident victim's blood from the available concentrations in various tissues.

Fluoxetine (Prozac) is a selective serotonin reuptake inhibitor (SSRI) that was introduced in 1986. According to the manufacturer, fluoxetine is the most widely prescribed medication in history for the treatment of depression and obsessive compulsive disorder (OCD). Certain side effects of this medication — drowsiness, dizziness, abnormal vision, diarrhea, and headache — could affect pilot performance and become a factor in an aviation accident. Our laboratory has determined distribution coefficients for fluoxetine and its desmethyl metabolite norfluoxetine in various postmortem tissues and fluids from 10 cases previously found positive for this drug. If available, 11 specimen types were analyzed for each case, including: blood, urine, vitreous humor, bile, liver, kidney, skeletal muscle, lung, spleen, heart, and brain. Specimens were extracted using solid-phase extraction and analyzed by GC/MS. Deuterated fluoxetine and norfluoxetine were used as internal standards to eliminate any possible matrix affects during the extraction. Blood fluoxetine concentrations in these 10 cases ranged from 57 to 739 ng/mL. This concentration range falls within what would normally be expected for an individual taking a prescribed amount of fluoxetine. Distribution data for this compound and its metabolite will be presented.

Keywords: **Fluoxetine, Postmortem, Distribution,**

P44 Case Report: A Meclizine (Antivert®) Related Death

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Meclizine (Antivert®) is a piperazine-derivative H_1-receptor antagonist. It is structurally related to chlorcyclizine and hydroxyzine and has been used as an antiemetic/antivertigo agent since 1955. It is indicated for the management of nausea, vomiting and dizziness associated with motion sickness and vertigo resulting from diseases affecting the vestibular system. Daily oral doses of 25-50 mg are recommended for treatment of motion sickness and doses up to 100 mg daily are recommended for the treatment of vertigo due to disease. In addition to its H_1-antogonist effects, meclizine also produces weak anticholinergic effects, central nervous system depressant effects and local anesthetic effects. Adverse effects that have been reported with meclizine use include drowsiness, dizziness, confusion, dry mouth, fatigue and less frequently, blurred vision.

A peak plasma meclizine concentration of approximately 0.080 mg/L has been reported four hours following administration of a single 25 mg dose; this concentration declined to 0.005 mg/L by 24 hours. The onset of action occurs about one hour after oral administration and its effects can persist for 8-24 hours. There is very limited information on the metabolism of meclizine in humans. The plasma elimination half-life of meclizine has been reported to be about 6 hours. Additionally, it appears that meclizine is extensively metabolized, as one study has identified eleven compounds present in human urine and feces following oral administration.

Case History: A 61-year old female was found unresponsive in her residence. She was lying in a supine position on her bed with photos of her family and a stuffed toy positioned on her chest. The decedent was last seen alive by her neighbor about two days earlier at which time she wanted to sell some of her tools to him. A partial autopsy was performed and the findings were unremarkable. Specimens were submitted for toxicological analysis and analyzed for volatiles, acidic/neutral drugs, basic drugs and free morphine. Meclizine was identified in heart blood at a concentration of 0.33 mg/L. Other significant toxicology findings in the heart blood included oxycodone (0.40 mg/L) and acetaminophen (120 mg/L). Additional specimens were analyzed for meclizine and the results follow:

	Meclizine (mg/L or mg/kg)
Heart Blood	0.33
Liver	0.70
Vitreous Humor	Not detected
Urine	Not detected
Bile	Positive, less than 0.2 mg/L

The heart blood concentration was about four times the peak plasma concentration reported after a single 25 mg dose. Although this is not the ideal comparison, it is the only published reference value; there have been no postmortem case reports published. Analysis of the additional specimens revealed a liver meclizine concentration approximately twice that of blood, while meclizine was not detected in vitreous humor. It was interesting to find that the urine and bile specimens contained undetectable or only trace amounts of meclizine. This suggests that bile and urine may not be the most appropriate postmortem specimens to screen when the cause of death may be related to acute meclizine use. The medical examiner ruled that the cause of death was combined drug intoxication (oxycodone, meclizine) complicating atherosclerotic cardiovascular disease; the manner of death was undetermined.

Key Words: **Meclizine, Postmortem, Overdose**

P45 Modafinil (Provogil) Artifact Vs. Diphenhydramine (Benadryl) Artifact

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Urine was submitted on a 14 year-old patient for a comprehensive urine drug screen. The comprehensive drug screen was performed using EIA, TLC, GC and GC/MS and the urine tested positive for cannabinoids, benzodiazepines, caffeine, diphenhydramine and what the AAFS electronic drug mass spectra library identified as modafinil breakdown product and artifacts. No parent drug spectrum was available or identified. Modafinil is a wakefulness-promoting agent similar in its actions to the sympathomimetic amine class of drugs. Though it has been used experimentally for the treatment of Attention Deficit Disorder, it has been rarely encountered in our hospital's pediatric population. Review of the patient history did not support the presence of Modafinil.

A pharmaceutical grade Provogil tablet was obtained, ground into a powder and analyzed by GC/MS to obtain relative retention time data for modafinil. The results mirrored the observations in the patient urine: two artifact peaks and one breakdown product (no parent drug spectrum). The relative retention time (RRT) and spectrum of the early eluting artifact peak matched the peak identified in the patient urine (RRT +/- 0.05). Further, the modafinil breakdown product and late eluting artifact spectra and RRT data matched those obtained in the patient urine (RRT +/- 0.05). The patient urine was submitted to a reference laboratory for modafinil confirmation testing. The reference laboratory used HPLC for the confirmation and failed to confirm the presence of modafinil. As diphenhydramine was present in a very high concentration in the patient's urine, a possible diphenhydramine artifact was suspected. Interestingly, when the modafinil and diphenhydramine chemical structures were compared, a diphenyl methyl moiety was found to be common to both structures. This diphenyl methyl moiety is also common to benzhydrol and further research indicated benzhydrol as being used for the preparation of both diphenhydramine and modafinil. Benzhydrol has a molecular weight of 184. The modafinil breakdown product observed has 184 as its base m/z ion ratio. Benzhydrol differs from the diphenyl methyl moiety by the addition of one hydroxyl group. When the hydroxyl group is cleaved off, the observed molecular weight becomes 167. This molecular weight corresponds with the modafinil artifact's observed 167 base m/z ion ratio. Modafinil and diphenhydramine have very different metabolic pathways. The breakdown and artifact peaks observed are likely produced exogenously. Regardless of their mode of origin, the diphenyl methyl moiety is not unique to diphenhydramine and modafinil. Interpretive caution should be employed if the electronic drug mass spectra library match indicates modafinil artifact, especially if diphenhydramine is present as well.

Key words: **Modafinil, Artifact, Diphenhydramine**

P46 Fatality Involving 1,3-Propanediol

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The deceased was a 45-year old female with an alleged history of suicidal ideations. She was found decomposing with a suicide note on her presence. Two containers of antifreeze, one labeled "Super Tech" and the other labeled "Prestone", were found in close proximity to the body. The samples submitted to our toxicology laboratory were labeled as "fluid of decomposition". GC/MS screening for a number of basic, acidic and neutral drugs did not show the presence of any drug. Volatile screening by GC-FID showed the presence of ethanol at a concentration of 58 mg/dL. Due to the presence of antifreeze containers near the body, ethylene glycol toxicity was suspected. However, no ethylene glycol was detected using GC-FID. Further scrutiny of the quantitative data indicated an unusually large peak for the internal standard. The internal standard employed was 1,3-propanediol and it was assumed that there was an unknown compound co-eluting with this internal standard.

Propylene glycol was considered as a possibility as it is becoming a common antifreeze ingredient and structurally appeared capable of co-eluting with the1,3-propanediol. The specimen was sent to a reference laboratory for further testing. However, propylene glycol quantitated at a concentration of 1.0 mg/dL. This level was inconsistent with the large internal standard peak area observed on the ethylene glycol quantitation. An internet search was conducted for various antifreeze ingredients and potential active ingredients were purchased from Sigma-Aldrich for further testing. GC/MS was used to screen the sample and cross-reference the results to various standards including ethylene glycol, propylene glycol, 1,2-propanediol and 1,3-propanediol. The decomposition fluid was determined to contain 1,3-propanediol. The 1,3-propanediol was quantitated using ethylene glycol as an internal standard and determined to be present at a concentration of 445 mg/dL. To our knowledge, this is the first report of a death involving 1,3-propanediol. It is important to note that 1,3-propanediol is a commonly used internal standard in the ethylene glycol assay. Therefore, its presence, particularly in small concentrations, is easy to miss and can potentially result in an underestimation of other analytes such as ethylene glycol.

Key Words: **1,3-Propanediol, Fatality, Antifreeze**

P47 Case Report: Death Due to Snorting of Crushed Sustained-Release Morphine Tablets

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MS-Contin® is a sustained-release morphine formulation that is administered orally to treat moderate to severe pain. MS-Contin® is available in tablets containing 15, 30, 60, 100 and 200 mg of morphine sulfate. Within four hours of the administration of 30 or 60 mg tablets of MS-Contin®, the reported peak plasma morphine concentrations are 10 and 30 ng/mL, respectively. Therapeutic plasma morphine concentrations persist for about 12 hours thereafter.

This report documents a morphine-related death in a male prisoner known to be an intravenous drug user who reportedly snorted three crushed 100 mg tablets of MS-Contin® in his jail cell. The prisoner died 8 hours later. Prior to death this individual exhibited symptoms of profound sedation and laboured breathing that progressed to apnea. At autopsy the pathologist observed pulmonary edema as the only significant finding. In addition, two condoms were found in his rectum, one containing three 100 mg tablets of MS-Contin®, the other containing plant material suspected of being marijuana. Absorption of morphine from the condom was ruled out based on a visual assessment of condom integrity and the condition of the tablets. Toxicological examinations of post-mortem blood and urine samples were conducted to determine whether death was related to the presence of illicit substances or pharmaceutical preparations often encountered in death investigations. Analysis for 6-monoacetylmorphine as a marker of heroin use was not performed as there was no investigative information to suggest heroin use. A concentration of 103 ng/mL of free morphine was detected in the femoral blood, and cannabinoid metabolites were indicated by an immunoassay in heart blood. No alcohol, or other substances of toxicological significance were detected.

The reported symptoms, autopsy findings, and the results of the toxicology examination point to a fatal morphine overdose. In the experience of this laboratory, this is the first known death associated with the snorting of a crushed sustained-release morphine tablet.

Key Words: **Morphine, Snorting, Fatal**

P48 Case Study: Pediatric Case of Fatal Codeine/Promethazine Overdose

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Promethazine with Codeine is often prescribed to relieve coughs and other symptoms of allergies and the common cold. Promethazine, an antihistamine, helps reduce itching and swelling and dries up secretions from the nose, eyes, and throat. It also has sedative effects and helps control nausea and vomiting. Codeine, a narcotic analgesic, helps relieve pain and relieves coughing. The combination medication is indicated for use in adults and children 6-12. This report documents the case of a 26 month old (35.5 inches, 37 pound) male who was found unresponsive by relatives. The mother initially reported that she thought the child had been exposed to cleaning products in the home. She later confessed to the police that the child had presented with cold symptoms and she poured some of her narcotic cough medicine into the child's cup to quiet him and help with symptoms. Toxicological analysis revealed the presence of codeine and promethazine in the cup found near the child; 1.2 mg/L codeine and 0.59 mg/L promethazine in subclavian blood; codeine, morphine, promethazine and promethazine metabolites in the urine; codeine and promethazine in gastric contents; 1.5 mg/kg codeine and 0.20 mg/kg promethazine in brain; and 1.4 mg/kg codeine and 0.40 mg/kg promethazine found in liver. No other drugs were detected. All drugs and metabolites involved in this case were quantitated using gas chromatography/mass spectrometry (GC/MS). Based on autopsy findings, case history and toxicological results, the forensic pathologists ruled that the cause of death was acute mixed drug overdose due to additive effects of promethazine and codeine culminating in fatal respiratory depression and the manner of death was homicide. Findings presented are of particular importance due to the limited information available on pediatric overdoses and postmortem distribution in these cases.

Key Words: **Codeine, Promethazine, Pediatric**

P49 Thiopental Levels and Distribution from South Carolina Lethal Injection Cases

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Reports of inconsistent levels of thiopental in blood have led to a controversy over the effectiveness of anesthetics during lethal injections. This study looked at thiopental levels in blood and tissues retrieved at autopsy following lethal injections in South Carolina. Levels were determined using gas chromatography/mass spectrometry. In six cases, the average left ventricular blood level was 22 mg/L while inferior vena cava blood was 9.0 mg/L. The average thiopental levels in the brain and liver were 35 mg/kg and 54 mg/kg, respectively. The variability of post-mortem drug levels dependant on the sampling source within an individual case clearly shows that interpretations of levels must be made carefully with respect to levels of consciousness.

Key Words: **Thiopental, Distribution, Lethal Injection**

P50 A Milwaukee County Medical Examiner's Case of Suspected Manipulation of Suboxone™ at Autopsy

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Suboxone™ sublingual tablets, used for the treatment of opioid dependence, contain buprenorphine HCL and naloxone HCL dihydrate in a ratio of 4:1. Pharmacologically, buprenorphine is a mu (μ) – opioid partial agonist and kappa (κ) – opioid antagonist. Naloxone is an antagonist at the μ- receptor. Naloxone is poorly absorbed via sublingual or oral routes and abuse of suboxone by intravenous administration is deterred by the increased pharmacological activity of naloxone. Side effects of buprenorphine include headache, constipation, vomiting and dizziness and are similar to other opioid agonists. At higher doses, opioid toxicity and death by buprenorphine intoxication is rare due to the partial agonism and poor bioavailability if consumed in large quantities, orally. Buprenorphine, however, has been reported in combination overdoses with other CNS depressants. We report an adult case involving suboxone that was autopsied at the Milwaukee County Medical Examiner's Office (MCMEO).

The decedent, a 21 year-old white male was found unresponsive on his girlfriend's bed. At the scene, no medications or drugs were found. Two small, pint size, bottles of alcohol were noted in the room and were empty. The girlfriend indicated the decedent was drunk earlier in the day. He had recently been diagnosed with schizophrenia and bipolar disorder and was prescribed paroxetine, lithium, geodon, and amitriptyline. No medications were found at the scene but his girlfriend later admitted to giving him a pill of suboxone. The district attorney expressed interest in prosecuting the girlfriend for supplying the decedent with the suboxone if it contributed to his death.

Toxicology results, obtained at the MCMEO, indicated the decedent had a blood alcohol concentration of 0.11g/dL and vitreous alcohol concentration of 0.14g/dL. The comprehensive drug screen for basic drugs was positive for amitriptyline, nortriptyline, and diphenhydramine. Quantification of drugs levels revealed the following:amitriptyline (0.60mg/L), nortriptyline (0.95mg/L, diphenhydramine (0.12 mg/L), lithium (0.51mEq/L), buprenorphine (3.1μg/L), naloxone (3.5 μg/L).

In addition, the observed naloxone levels indicate the decedent manipulated the suboxone tablets for a presumed alternate route of administration although the girlfriend reported that the decedent had not taken any other drugs that day. The forensic pathologist determined the cause of death to be amitriptyline intoxication. A significant contributing condition was an anomalous origin of a right coronary artery. The manner of death was accident.

Suboxone is one formulation that contains the opioid buprenorphine and post-mortem toxicology findings can differentiate the route of administration and or if the medication was manipulated prior to administration. The unique manufacturing of suboxone (buprenorphine/ naloxone) illustrates the need for forensic toxicologist to take into consideration both buprenorphine and naloxone levels. Interpretations can then be made based on the absence or presence of naloxone.

Keywords: **Opioid, Naloxone, Buprenorphine**

P51 Drug Smuggling or Death Sentence? Two Body Packer Deaths in Miami-Dade County, FL

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The Miami-Dade County Medical Examiner Department received two Body Packer cases in just over a month. The following are case reports along with the autopsy and toxicological findings in these two very unique cases.

Case 1 involved a 26 year old white male, who was a state prisoner housed in a single person cell in Miami, FL. During a cell check the decedent was found unresponsive. Medical efforts were unsuccessful and he was pronounced.

Autopsy Findings: Four packages were discovered in the rectal vault of the decedent. Three of these were knotted, white latex glove fingers, and the fourth was a small black zip lock baggie containing marijuana. The first latex package contained cocaine. The second was divided into three sections and contained ecstasy, marijuana and cocaine. The third latex package contained two black baggies, one of which contained marijuana and the other cocaine.

Toxicological Findings: The decedent was positive for the following:
Urine: Amphetamine, Methamphetamine, MDA, MDMA, THC-COOH, BE, Amitriptyline, Metoprolol, Acetaminophen.
Iliac Blood: Cocaine 6.2 mg/L, Methamphetamine 0.15 mg/L, MDMA 0.63 mg/L, MDA 0.46 mg/L, Amitriptyline 0.43 mg/L, Nortriptyline 0.17 mg/L.
Heart Blood: Cannabinoids positive ELISA, Quantitation of THC is pending.
Serum: Benzoylecgonine 10.3 mg/L by TDX.
Gastric: Cocaine 89.6 mg total, BE positive EMIT.
Small Bowel: Cocaine 5.5 mg total, BE positive EMIT, Amitriptyline.

Case 2 involved a 56 year old black male who was a citizen of Jamaica. He had been vacationing in South Florida for one week, and was in Miami International Airport preparing to return to Jamaica. He collapsed in the concourse. Fire Rescue responded and transported him to the Emergency Room where he expired.

Autopsy Findings: Eighty-eight individual packages were discovered in the decedent's colon. The packages were roughly ovoid, approx. 5 cm long and 2 cm in cross sectional diameter, and consisted of an apparent dark, waxy, resinous substance wrapped in clear plastic wrapping. Three of the packages were partially unwrapped with varying lengths of clear polymer strip trailing from the packages. In addition, two long strips of wrapping were found in the colon, along with a single, dark green-brown, firm bolus with a shape consistent with that of the contents of the packages.

Toxicological Findings: One of the packages was submitted to the Toxicology Laboratory for testing. It was positive for Cannabinoids by EMIT and for THC by GC/MS, and was concluded to be Hashish. The decedent's urine was positive for the following: THC-COOH, ASA, Guaifenisen, Dextromethorphan, Dextrorphan. The decedent's blood was positive for Cannabinoids by ELISA and for Chlorpheniramine by GC/MS. Quantitation of THC is pending.

Key Words: **Body Packer, Hashish, Smuggling**

P52 Three Deaths with Acute Methanol Ingestion

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Our laboratory performs drug and alcohol testing on approximately 2800 medical examiner cases each year. Our routine alcohol testing involves analyzing blood, tissue, or vitreous humor for ethanol, methanol, acetone, and isopropanol. Of these analytes, methanol is the most rare compound that is detected. The detection of methanol in Office of the Medical Investigator cases in New Mexico accounted for 29 cases out of the 7344 cases since January 2003.

Methanol has widespread commercial usage as a solvent, especially in paints and varnishes and as a constituent of some antifreeze solutions. Overexposure to methanol can lead to headache, drowsiness, dizziness, vertigo, light-headedness, nausea, vomiting, anorexia, fatigue, pain, visual disturbances/dimness, optic nerve damage, and bilateral blindness. Endogenous concentrations in blood are ≤ 0.0001g/100 mL. Methanol eventually breaks down to form formic acid, which is 6 times more toxic than methanol that may account for the toxicity of methanol ingestion. Initial narcotic effects and toxic syndrome may not appear until 6-30 hours after ingestion. A blood methanol concentration of 0.040 g/100mL is believed to be a minimum lethal level in individuals receiving no medical treatment.

Methanol is detected using headspace gas chromatography. The instruments are a Tekmar 7000 headspace autosampler and an Agilent 6890N gas chromatograph using the 30m Restek BAC 1 and BAC 2 capillary columns. *n*-propanol is used as an internal standard.

Of the 29 cases that were positive for methanol, 3 had exceptionally high results. The manner of death for all three individuals was different, but there were high levels of methanol detected in all three cases. The ranges of methanol in the samples that were provided from the Office of the Medical Investigator ranged from 0.122g/100mL to 0.400 g/100mL. Methanol ingestion contributed to the deaths of these individuals.

Key words: **Blood Alcohol, Methanol, Gas Chromatograph**

P53 Cyanamide concentrations in biological fluids and tissues in an fatal overdose case.

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Calcium cyanamide is an aldheyde dehydrogenase inhibitor used as and adjuvant in the aversive therapy of chronic alcoholism. A case of suicidal attempt is presented here: a 42-years-old male, with a previous history of suicidal attempt, was found unconscious at home, remaining in coma until he died at hospital. Besides him, twelve empty ampoules of Colme® (15 mL, 60 mg/mL calcium cyanamide) were found. Different specimens collected while he was in hospital and after in autopsy were submitted to our laboratory for toxicological investigation.

Cyanamide was extracted from samples analysed with ethyl acetate. Extracts were derivatized by dansyilation prior to HPLC with fluorescence detection analysis. Neither ethanol nor other drugs were detected in samples analysed. Concentrations found in different specimens are shown in the following table. Blood concentrations are low in comparison to estimated dosage. Toxicokinetics parameters and time elapsed since ingestion may explain obtained results. To the authors' knowledge this paper is one of the few cases of suicide after ingestion of cyanamide reported. Toxic or lethal concentrations are unknown.

	Cyanamide concentration
Hospital Blood (mg/L) Urine (mg/L) Stomach contents (mg/L)	 1.58 6.96 3.36
Autopsy Blood (mg/L) Liver (mg/Kg) Kidney	 0.43 0.31 None detected

Keywords: **Cyanamide, Concentrations, Suicide case**

P54 Postmortem Distribution of 1, 1 difluoroethane in Four Cases

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Between April 2002 and June 2006 the Suffolk County Medical Examiner received four cases involving the inhalation of 1, 1 difluoroethane (DFE, Freon 152a). This halogenated hydrocarbon is a propellant compound used as an aerosol dust remover found in canisters such as “Dust Off” or “CRC Duster”. Abuse of DFE is common amongst teens, due to it’s ease of availability and low cost, who “huff” the inhalant for a quick intoxicating high. However, the four cases we investigated involved individuals ranging in age from 20-44 years old which were not characteristic of the established prevalence amongst adolescents. In three of the four cases the death was directly attributed to the arrhythmogenic effects of DFE causing sudden cardiac arrest. The other case involved a motor vehicle accident in which the driver had inhaled DFE immediately prior to impact. Utilizing headspace gas chromatography a complete body distribution pattern of the inhalant was determined for each individual. A comparison of these calculated levels has provided an insight into the pharmacokinetic behavior of DFE in the body. One such postulate involves the correlation of chronic abuse of DFE and elevated levels found in adipose tissue. Additionally, patterns of DFE levels in an intact body following a sudden death may provide a “snapshot” of events at the time of death, leading to a possible mechanism of determining the final time of use of the inhalant. Bases upon our case experiences, it is reasonable to assume that DFE abuse is not strictly found in young individuals and that its abuse carries over into later years. The cases we have encountered show that DFE inhalant abusers may in fact develop a long term dependence on the inhalant through an unknown mechanism.

Keywords: **1,1-Difluoroethane (DFE), Volatile abuse, Huffing**

P55 Fatality due to 1,4-Butanediol Overdose

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The objective of this paper is to present a case of 1,4-butanediol (1,4-BD) overdose, including the postmortem levels of the drug and its major metabolite, gamma-hydroxybutyrate (GHB). 1,4-BD is abused because of its sedative and euphoric properties. It has also been implicated as a drug used to facilitate sexual assaults.

1,4-BD is a pro-drug to GHB. The metabolism of 1,4-BD to GHB is so rapid, that 1,4-BD may not be detected in blood and urine samples after consumption, unless specimens are quickly collected after ingestion. Although several cases of 1,4-BD ingestion have been reported in the literature, there are limited cases that document quantitation of 1,4-BD in multiple biological specimens. Following are 1,4-BD and GHB amounts reported in two fatalities:

	Case A (reported by Duer, et al. in JAT 2001)		**Case B (reported by Lora-Tamayo, et al. in FSI 2003)**	
Specimen	Amount 1,4-BD (µg/mL)	Amount GHB (µg/mL)	Amount 1,4-BD (µg/mL)	Amount GHB (µg/mL)
Peripheral Blood	70	n/a	82[1]	103[1]
Heart Blood	78	303		
Urine	870	1513	401	403
Brain	n/a	260[2]	n/a	n/a
Gastric Contents	n/a	n/a	7.4	n/a

[1] source of blood not specified
[2] units are mg/kg

In the case reported here, several teenagers were drinking what they thought was GHB. The victim, a 16-year old female, reportedly took 10 drinks of the liquid, vomited soon thereafter, and then passed out. Paramedics were called approximately six hours later, and pronounced her dead at the scene. The remaining beverages were tested and found to contain 1,4-BD.

Several biological samples from the decedent were analyzed for the presence of GHB and 1,4-BD. Analysis for GHB was by headspace GC with flame ionization detection and headspace GC/MS, after conversion of GHB to gamma-butyrolactone and solvent extraction. Analysis for 1,4-BD was by GC/MS using both electron impact and chemical ionization modes following solvent extraction and silyl derivitization.

Following are the 1,4-BD and GHB amounts measured in specimens from the victim:

Specimen	Amount 1,4-BD (µg/mL)	Amount GHB (µg/mL)
Blood	500	700
Urine	4400	3700
Vitreous	800	480
Gastric contents	84 mg total	16 mg total

These results clearly implicate 1,4-BD as the substance consumed. Although the amounts of GHB in the various samples may suggest overdose on their own, in cases of 1,4-BD overdose, quantitation of both 1,4-BD and GHB may prove important.

KeyWords: **1,4-Butanediol, GHB, Overdose**

P56 An Examination Of Gamma-Hydroxybutyrate Cases

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Gamma-hydroxybutyrate (GHB) (gamma-hydroxybutyric acid, 4-hydroxybutyrate, sodium oxybate) is a chemical compound which occurs naturally in mammalian tissue, is a minor metabolite in the gamma amino butyric acid (GABA) pathway in the brain, and can also be found in non-neuronal tissues. GHB can also be produced postmortem by a mechanism not fully understood, possibly related to GABA and fatty acid breakdown.

In 1977 GHB gained popularity with bodybuilders as an alternative to anabolic steroid because there was an "unconfirmed" reported steroid enhancing effect and increased human growth hormone but the theory was never proved. Because of the purported bodybuilding, euphoric and sleep aid effects, GHB became more widely available in catalogues, internet and health food stores and thus became more widely abused. GHB is rapidly absorbed after oral administration and significant effects can occur within 15 – 30 minutes depending on the dose. Widespread use of GHB in the US over-the-counter market followed by reports of adverse effects led the Food and Drug Administration (FDA) to issue a warning in 1990 against use of GHB outside of clinical studies.

We examine 27 GHB cases to document effects and drug concentrations. Quantitative results for GHB in blood, urine and/or vitreous fluid for these cases are presented. In cases involving GHB alone there were seventeen separate occasions where most were driving under the influence of drugs (DUID) and one death investigation (DI) case. In the DUID cases, blood concentrations of GHB ranged from 50 to 361 mg/L and urine concentrations ranged from 520 to 2381 mg/L. There was overlap in concentrations noted in the GHB overdose case where blood and urine were 269 and 3700 mg/L, respectively. For the DUID cases there was no consistency or trend on the time of day of the traffic stop.

GHB cases with other toxicology findings present, include DI, DUID, and a hospital emergency room (ER) case. Other substances, aside from the GHB, which were found positive include ethanol, tetrahydrocannabinol and metabolite, methadone, chlorpheniramine, oxycodone, promethazine, zolpidem, cocaine, butalbital, and diazepam and metabolite. The highest DUID GHB blood concentration of 300 mg/L was found in a severely impaired driver. Another ME case showed GHB in blood and vitreous of 410 and 212 mg/L, respectively. One DI case involved the decedent prescribed nine grams per day of Xyrem®.

Five GHB cases involving attempted drug facilitated sexual assault (DFSA). Based on the rapid elimination of the drug, unless a specimen is collected quickly, GHB can not be found in the victim. GHB urine concentrations were found to range from 77 to 209 mg/L in victims when urine was collected.

Most cases involve white male subjects in the age range of 21 to 44 years old. These data show that the amount of drug causing euphoria is similar to the amount which can cause seizures and coma (i.e. there is an overlap of therapeutic and toxic effects). Effects are dose dependent and can range from drowsiness and dizziness to unconsciousness, convulsions, respiratory depression and coma. Tolerance develops in repeat offenders. Most individuals who are treated for the very serious effects can spontaneously regain consciousness within two to four hours. This is a relatively large compilation of GHB related cases involving DUID, DI and DFSA.

Key Words: **GHB, Concentrations, Effects**

P57 Distribution of Phencyclidine (PCP) into Vitreous Humor

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Vitreous humor is a fluid contained in the eye that is largely composed of water. The advantages of vitreous humor as a specimen for postmortem drug analysis include its relatively low susceptibility to contamination and the ability to analyze vitreous humor with little or no pretreatment. The postmortem analysis of ethanol in vitreous humor has been well established. However, studies of drug disposition into vitreous humor are limited. Heart blood, peripheral blood and vitreous humor specimens from 26 phencyclidine positive postmortem cases were analyzed to evaluate the distribution of phencyclidine into vitreous humor. Phencyclidine intoxication was not the cause of death in any of the cases analyzed. Specimens were analyzed by solid phase extraction followed by gas chromatography-mass spectrometry. All positive blood specimens were associated with a positive vitreous humor specimen. On average, the blood phencyclidine concentrations were greater than the vitreous humor phencyclidine concentrations, with average blood/vitreous ratios of 2.85 for heart blood and 2.51 for peripheral blood. However there was considerable variability between cases, which indicates that while vitreous humor is an appropriate specimen for the detection of phencyclidine in postmortem cases, it interpretative value is limited.

Key Words: **Phencyclidine, Postmortem, Vitreous humor**

P58 The Investigation of Cases of Commercial Product Tampering

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Product tampering investigations present a unique set of challenges for the forensic toxicologist. Reported or suspected product tampering is often nothing of the sort, which leaves the analyst in the unenviable position of attempting to prove a negative. The sample matrices involved are usually far removed from those with which most toxicologists are familiar, and many normal analytical procedures may need to be modified in order to account for the resulting complications. Tampering can involve the addition of undesirable materials to a product, the removal of a desirable material from a product, or a combination of the two, and different approaches are required to deal with each of these situations. In many instances, particularly with foodstuffs, there will be very little of the allegedly tampered product left available for analysis, and careful prioritization of analyses becomes critical to the investigation.

We present an overview of these many challenges, and describe the approaches used in dealing with them in our laboratory. Particular attention will be paid to the initial investigative strategies that are often key to directing subsequent chemical analyses and interpreting their results. The importance of analytical techniques not normally a part of the toxicologist's repertoire, including scanning electron microscopy, infrared spectroscopy and microscopy, x-ray diffraction, and x-ray fluorescence, will be discussed. Possible outside sources of information for assistance in developing analytical methodologies and interpreting analytical results will be presented. These points and issues will be illustrated with examples drawn from prior case work at our laboratory.

Keywords: **Tampering, Adulteration, Poisoning**

P59 Unusual Case Evidence with Drugs Concealed on the Head of Matches Seen at a Major City MEO

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***Background**:* A 42 year-old male was found dead lying on his bed by his house mate. Both men appeared to be squatting on the property where they were living. There was no obvious trauma to the body and no suspicion of foul play. At autopsy, triage urine drug screen was positive for cocaine so cause of death was attributed to adverse effect of drugs.

Evidence: Two small plastic packets were retrieved at the scene. Each packet was sealed and contained two match heads.

Toxicology Test Results on Urine and Blood: Urine drug screen for both acid/neural and basic drugs was done by solid phase extraction and analyzed by GC-MS-EI in the scan mode. Positive results were obtained for cocaine and its ecgonine methyl ester metabolite.

Quantitation of cocaine and metabolites in fluoridated blood was done after solid phase extraction and TMS derivatization with GC-MS-EI in SIM mode. Benzoylecgonine concentration = 670 ng/mL.

ELISA screening was negative for opiates, benzodiazepines and fentanyl in blood and opiates and fentanyl in urine.

Manual screening tests for alcohol, cyanide, carboxyhemaglobin and salicylates were also negative.

Toxicology Test Results on Evidence: At the request of the medical examiner, the evidence was tested for drugs. The two match heads were washed with approximately 400 uL of methanol and tested by GC-MS-EI in scan mode. Positive results were obtained for cocaine.

Conclusions: This evidence appears to use a unique method to conceal cocaine, or possibly other drugs of abuse, that forensic investigators need to be aware of. The way in which cocaine was applied to the match heads or the manner in which the drug was ingested are unknown.

Keywords: **Postmortem toxicology, Forensic evidence, Cocaine**

P60 Accidental Deaths Involving Cocaine and Methadone in Virginia

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We examined incidences (N = 20) of accidental deaths involving cocaine and methadone in the four regions of Virginia during 2005-2006. The Western region of Virginia represents 60% of the subjects in our study. Abuse of oxycodone, methadone, hydrocodone, and fentanyl in rural Appalachia has been well publicized and correlates to the increased number of pain management and methadone maintenance clinics in the Western region of Virginia. Concomitant administration of opioids and cocaine ("speedball") elicits an intense sensation of euphoria while simultaneously compensating one another's negative side effects, i.e. hyper-locomotion and respiratory depression. However, the relatively short half-life of cocaine (0.7-1.5 hours) can lead to frequent repeated dosing to obtain a desired state of euphoria. Methadone exhibits a relatively long half-life (15-55 hours) in comparison to other opioids, therefore frequent dosing of methadone and cocaine together may rapidly produce toxic to lethal methadone concentrations in the blood. The average age of the victims was 36 (S.D.= 11); the average methadone and cocaine concentrations in the blood (from all anatomical locations) were 0.42 mg/L (SD = 0.24 mg/L) and 0.10 mg/L (S.D. = 0.14), respectively. The average cocaine concentration and corresponding standard deviation is representative of cocaine's pharmacokinetics and instability in biological matrices. The overwhelming majority of cases represented in the Western region of Virginia in comparison to the other regions may be due to the ubiquitous nature of methadone in rural Appalachia and frequently repeated co-administration of methadone and cocaine, two drugs with dramatically different half-lives.

Key Words: **Methadone, Cocaine, Fatal**

P61 Incidence and Prevalence of Hydrocodone, and Other Drugs in Cases from Multiple Medical Examiner Jurisdictions

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Hydrocodone and other opiate concentrations are reported for death cases from two laboratories that analyze postmortem specimens. Ameritox Ltd, provides toxicology testing for medical examiner offices in six states and reports data on 380 cases. The Forensic Laboratory Division of the Allegheny County Medical Examiner's Office reports data on 25 cases. The various immunoassay methods used for screening specimens will be reported, and the chromatographic, mass spectrometric methods used for quantitation and confirmation will be reported. The range of blood concentrations of opiates are: hydrocodone 20 – 8000 ng/mL, oxycodone 33 – 11,468, and morphine 22 – 22,310. The distribution of opiates in 380 cases is hydrocodone 45%, morphine 41.8%, oxycodone 26.3%, codeine 3.4%, and hydromorphone 1.0%. Data reported will include concentrations of other drugs found in 25 cases. In the 25 cases, alprazolam, diazepam, nordiazepam, and acetaminophen were present in 28%, 16%, 20%, and 76% of the cases, respectively.

Key Words: **Hydrocodone, Prevalence, Postmortem**

P62 Determination of the Prevalence of Methadone in the Miami Valley Region of Ohio in Antemortem Casework

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Methadone is a widely abused analgesic and yet is undetectable in the routine screening of cases received from the Miami Valley Regional Crime Laboratory (MVRCL). Therefore, it was impossible to determine the frequency of occurrences of methadone in MVRCL casework, which includes various types of offenses: DUI, sexual assault, probation violation, automobile accidents, and possession of narcotics. In conjunction with Immunalysis Corporation, a study was conducted to determine the percentage of cases where methadone was present. In order to ensure every case was analyzed for methadone, the Methadone Direct ELISA kit from Immunalysis was added to the standard set of screens for drugs of abuse.

Five hundred twenty-one specimens were screened for methadone over a 10 month period. As the laboratory is not able to dictate the type of specimen submitted by the police agencies, specimens varied between blood and urine. Of the 521 specimens, 11.5% screened positive for methadone, which equates to 60 specimens, at a cutoff level of 25ng/mL for both blood and urine. The 60 positive screens were comprised of 14 blood and 46 urine specimens. All positive screens were then confirmed for methadone by gas chromatography/mass spectrometry and quantitated by gas chromatography with a nitrogen-phosphorus detector. The amount of one blood specimen was insufficient for a confirmation analysis and was, therefore, removed from the data set. Thirty-two of 520 specimens confirmed positive for methadone, resulting in an overall positivity rate of 6.2%.

Of the 13 blood specimens available to be analyzed, 11 were confirmed positive for methadone resulting in an 84.6% confirmation rate. No determination was able to be made regarding the 2 false positive specimens.

The 46 urine specimens provided a more equal division between those that were able to be confirmed and those that were not, with 54.3%, or 25 specimens, resulting in false positives. When the false positive specimens were re-screened with a cutoff level of 50ng/mL, which is equivalent to the limit of quantitation (LOQ) for the confirmation analysis, 20 of the 25 specimens now screened negative. The percentage of urine false positive specimens now falls to 20%, which represents 5 of the total 25 specimens. In using the higher cutoff level, the screen results for one of the specimens that confirmed positive for methadone would now be considered negative. This case had a concentration of methadone lower than the LOQ; therefore, excluding this sample is not unreasonable. With the exclusion of this case, the overall positivity rate for both blood and urine specimens declines to 6.0%.

In attempting to determine the cause for the false positive urine specimens when screening at a cutoff level of 50ng/mL, the issue of cross-reactivity with other drugs present was addressed. Negative urine spiked with diphenhydramine at a concentration of 8000ng/mL gives an equivalent immunoassay response to 25ng/mL of methadone. Some of the urine specimens had very high concentrations of diphenhydramine, accounting for 2 of the 5 false positives. For the 3 remaining false positive specimens, other drugs were evaluated for possible cross-reactivity; however, no conclusions were able to be drawn.

Keywords: **Methadone, ELISA, Antemortem casework**

P63 Prevalence of Drugs in Expanded Second Sample Blood Specimens from DUID Arrests in the Commonwealth of Virginia, 2001 – 2002

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The testing of blood specimens from drivers charged with Driving Under the Influence of Drugs (DUID) in the Commonwealth of Virginia is preformed using three levels of analysis. Initial testing is for ethanol and if 0.09%w/v or greater is detected, testing is stopped and results reported. If ethanol results are negative or <0.09%w/v, then level 2 testing is performed for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates. If none of these drugs is detected, additional level 3 testing is conducted for over 60 prescription and over-the-counter medications that may impair driving. While such a testing scheme is sufficient to provide evidence in DUID prosecutions, it does not document the true incidence of drugs in DUID arrest.

We present the results of a testing scheme for all drugs in 2948 blood samples received for DUID second sample analysis during an 18-month period in 2001 – 2002. Ethanol was determined by gas chromatography (GC). Initial screenings for common drugs of abuse was by either FPIA or EMIT immunoassays. Other initial screening was by GC or gas chromatography/mass spectrometry (GC/MS). All presumptive positive results were quantified, and confirmed by GC/MS.

Of the 2948 blood specimens received for testing, 1777 (60%) exceeded the stop testing value for ethanol of 0.09%w/v or greater. The incidence of the most commonly encountered drugs and range of their blood concentrations found as a result of a complete analysis of the 1777 blood samples is presented below.

Drug	# of Case	Mode	Range	Median
No Drug Detected	1301			
THC-Acid	156	0.011	0.002 - 0.125	0.020
Nordiazepam	118	0.07	< 0.05 - 2.0	0.32
Benzoylecgonine	100	0.07	< 0.05 - 1.07	0.15
Diazepam	64	0.05	< 0.05 - 1.70	0.18
Cocaine	48	0.006	< 0.005 - 0.018	0.007
Alprazolam	47	0.028	< 0.01 - 0.50	0.04
Cocaethylene	47	0.007	< 0.005 - 0.066	0.012
Oxazepam	47	0.12	0.06 - 1.46	0.16
Morphine	43	0.03	< 0.02 - 0.06	0.03
Butalbital	33	0.8	< 0.5 - 11.9	0.8
Diphenhydramine	30	0.05	0.02 - 0.31	0.07

In addition to blood concentration 0.09%w/v or greater of ethanol, at least one other drug was found in 476 (27%) of the specimens subjected to complete drug testing. Marijuana metabolite, benzodiazepines and cocaine or cocaine metabolites were the most commonly encountered additional drugs. The complete testing for all drugs yielded a true incidence of drug and alcohol use in DUID specimens submitted for second sample testing in Virginia.

Key Words: **Impaired Driving, Drugs and Driving, Marijuana, Blood Analysis**

P64 Blood and Tissue Concentrations of Drugs Found in Fatal Poisoning in Eastern Denmark During a Five-year Period (1998-2002)

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The pattern of fatal poisonings in eastern Denmark has been shown in four compilations for the years 1979 to 2002 (1-4). In the last five-year period (1998-2002) specimen from 2996 autopsies were submitted for forensic chemical investigations.

The toxicological analyses were done by state of art methods such as immunoassays, GC, GC-MS and HPLC-DAD screening, followed by verification and quantification by another method such as LC-MS/MS or GC-MS.

Among the fatal poisonings determined in this last five-year period common drugs like morphine/heroin and methadone were the main causes of death among the determined 497 fatal poisonings of drug addicts, accounting for 90% of all fatal drug addict cases. The determined 443 fatal cases among the non addicts were due mainly to medicine, accounting for 73% of the cases. Poisoning in these cases were due to a wide range of strong and weak analgesics, antidepressants and antipsychotic drugs of older and newer origin.

The concentration range of these 50 drugs will be presented together with the frequency and amount of each.

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2. Worm, K. et al. Ugeskr Læger 1994; 156: 3039-43
3. Worm, K. et al. Ugeskr Læger 1999; 161: 622-5
4. Johansen, S.S. et al. Ugeskr Læger 2006 in press.

Keywords: **Fatal poisonings, List of Concentrations, Drugs**

P65 Drug-facilitated sexual assaults (DFSA) in Denmark

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A recent increase in the number of reports of DFSA has been observed in DK as in other countries. Date rape drugs are used for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. A working group with participants from the Police, Centre of Victims of Sexual Assault, and Institute of Forensic Medicine in Copenhagen made instructions for personnel on how to handle DFSA cases as to sampling (time, materials), prevalent substances etc. An analysis step program as illustrated in Table 1 was recommended for DFSA cases in order to keep expenses low. The drug analyses were selected on basis of international guidelines from SOFT, TIAFT etc. The content of each step was prioritised on the basis of drug prevalence in DK and likelihood of association with DFSA according to the literature.

The DFSA drug analysis step program has been used in Denmark since September 2004. The study is based on the routine casework elaborated at the Department of Forensic Chemistry, Institute of Forensic Medicine in Copenhagen, which receives samples and analytical data in cases of DFSA in DK. The aim of this study is to report data on DFSA cases, inclusive the involved drugs in DK, and describe the stepwise analysis program and its applicability.

Table 1. DFSA-analysis step program:

	Analyte	**Materials**	**Screen method****
1. step	**Alcohol**	Blood, urine	GC/FID
(Prime target)	**Benzodiazepines**	Blood, urine	Enzyme-immunoassay
	GHB & GBL, 1,4-BD	Blood, urine	LC/MS/MS
	Basic drugs*	Urine	GC/MS & HPLC/DAD
2. step	**Amphetamines**	Blood, urine	Enzyme-immunoassay
(Narcotics)	**Cannabis**	Blood, urine	Enzyme-immunoassay
	Cocaine	Blood, urine	Enzyme-immunoassay
	Opioids	Blood, urine	Enzyme-immunoassay
3. step	**Acidic & neutral drugs** (incl.	Blood	HPLC/DAD
(versatile -	Barbiturates)		
cases	**Buprenorphine**	Blood, urine	Immunoassay
depended)	**Others (**ex. volatile, potent drugs)	Blood, urine	Ex. HS-GC/MS/LC/MS/MS

*Basic drugs include several hundred drugs such as antidepressants, antihistamines, antipsychotics, analgesics, cough medicine etc.).
**Positive screen results were confirmed and quantified by LC/MS/MS or GC/MS.
The program does not cover carisoprodol, meprobamate, scopolamine, clonidine and other potent drugs. A separate analysis has to be requested for each of these drugs.

Keywords: **DFSA, Analysis program, Drugs**

P66 Analysis of Dried Vomit in a Drug Facilitated Sexual Assault Case and the Finding of Zolpidem

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An 18-year old female claimed to have been sexually assaulted by a relative following the consumption of an alcoholic drink. After consuming the drink, she vomited and some of this vomit was absorbed onto her pants. She asserted that she felt extremely dizzy and numb, and her extremities felt very heavy. In addition, despite being in and out of consciousness, she was aware of the assault. Based upon the symptoms displayed, it was speculated that this drink contained some type of obtunding agent. Two shot glasses, one of them containing a powdery residue, and the pants with vomit were seized from the residence.

The pants were submitted by the investigating agency for toxicological analysis with the purpose of testing the vomit for agents that could cause the symptoms described above. Also provided was a list of medications that had been observed at the residence, which included zolpidem, lorazepam, diltiazem and hydrochlorothiazide.

Prior to the start of toxicological analysis, the pants were photographed. Two near equivalent sections, one containing vomit and the other appearing vomit free, were cut from these pants using a sterilized pair of scissors. The section with vomit was then cut in half and the weights of all three pieces determined and recorded. To make sure that the extraction scheme was appropriate for this type of matrix and to serve the purpose of a positive control, a drug standard containing a mixture of 17 different drug and drug metabolites was added to one of the vomit covered pieces and allowed to dry. Each section was then cut into smaller pieces and these were placed into several appropriately labeled test tubes. All tubes were filled with methanol until the pieces were well covered and repeatedly vortexed over the course of the next 5 hours. The methanol extract was pipetted from each tube and transferred to appropriately labeled test tubes and evaporated to dryness under nitrogen with no heat. Forensic Specimen Diluent (OraSure Technologies, Inc. Cat # 62300) was added to one positive control tube, one negative control tube and one sample tube, and the buffer solution was allowed to interact with the dried residue remaining. Three separate extracts, one for the positive control, one for the negative control and one for the sample were created for testing purposes.

Taking into account the drugs listed above, the samples underwent a comprehensive full scan GC/MS screening procedure that, in addition to many other drugs, would detect the presence of zolpidem, diltiazem and hydrochlorothiazide. A benzodiazepine directed analysis was also performed by SIM-GC/MS. While all the samples tested negative for benzodiazepines, diltiazem and hydrochlorothiazide, the two samples that were created from the vomit-covered material, the positive control and the sample, tested positive for the presence of zolpidem. Zolpidem was not one of the drugs included as part of the positive control drug mixture. The control material tested negative for zolpidem.

To verify these findings, fresh material was prepared in a similar manner as described above and confirmation analysis for zolpidem performed by GC-NPD and GC/MS. Again the findings demonstrated that the vomit on the pants was positive for zolpidem.

This case illustrates a simple yet effective sample preparation technique for specimens (i.e., dried vomit on clothing) that are somewhat atypical in nature. In a practical sense, the analyses provided investigative information important to the case. Based in part upon these laboratory findings, legal proceedings are currently underway.

Key Words: **Vomit, Zolpidem, Drug Facilitated Sexual Assault**

P67 Rapid, Sensitive Screening for Analytes Implicated in Drug-Facilitated Crimes (DFC) using Exact Mass LC-oa-ToF.

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***Introduction**:*

Over the last few years, DFC *e.g.* sexual assault and robbery, have been increasing. The drugs implicated in DFC are wide-ranging and include illegal drugs, prescribed medications and 'over-the-counter' preparations. Owing to the diversity of the analytes, a variety of analytical techniques are usually required, including immunoassay, GC-MS, GC-FID and LC-UV. Our aim was to develop a simple, generic method to screen for these analytes using a single analytical technique based on LC-oa-ToF.

***Methods**:*

Where available, drug standards were used to create a reference library of retention time and spectral data. Analytes were separated using an ACQUITY HSS C18 column (2.1 x 100 mm, 1.88µ) maintained at 30^{o}C. Data was collected using two different voltages within the source region. A low voltage enabled mass measurement of the protonated molecular species. A dual reference sprayer (LockSpray™) was utilised to deliver a constant reference peak, against which, analyte mass spectra were subsequently measured; this enabled mass assignment, accurate to 4 decimal places, over extended periods of time. The higher voltage was used to generate fragments within the source (collision-induced dissociation; CID), for additional confirmatory purposes. Where reference material was unavailable, calculated theoretical monoisotopic masses were utilised.

***Results**:*

Control urine and blood samples were spiked with mixed drug standards and subjected to both qualitative and quantitative analysis using LC-oa-ToF. For the qualitative screen, data was processed using ChromaLynx™, a software program which automates chromatographic deconvolution followed by comparison of the spectral information with the reference library. Additional confidence was achieved using retention time and by measurement of the proximity of the actual acquired mass to the theoretical exact mass; a mass accuracy within 5 ppm was considered acceptable.

Quantitative analysis was performed by spiking biological matrices with increasing amounts of mixed standards. Following analysis, exact mass chromatograms were integrated.

Authentic specimens collected from alleged DFC were analysed and subjected to qualitatative and quantitative analysis.

***Conclusions**:*

We have developed a simple screening method for > 60 of the analytes which have been implicated in DFC. The method is based on chromatographic separation (10 min) in combination with oa-ToF mass spectrometry. Identification was achieved by comparison of spectral and retention time information to the prepared library. Furthermore, the exact mass measurement allowed the prediction of probable elemental composition. The advantage of theoretical monoisotopic masses was demonstrated by the identification of several analytes in the authentic samples where no reference material had previously been available.

Key Words: **Drug-facilitated Crime, Screening, ToF-MS**

P68 A Comparison of the Pharmacokinetics and Pharmacodynamics of Cocaine by Insufflation, Smoking and the Intravenous Routes

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The onset of activity, the intensity of the psychopharmacologic effects and the addiction liability of cocaine administered by various routes has been a topic of interest for both the forensic community and the federal courts. In fact, in 1986 Congress passed new federal sentencing guidelines that required a first-time offender in possession of 5 g of "crack" cocaine with intent to distribute to go to prison for a minimum of five years (21 U.S.C. §841(a)(1)). More severe penalties for possession with intent to distribute "crack" cocaine in contrast to cocaine HCl were passed because legislators believed that smoked "crack" cocaine had a higher addiction liability than did the use of intravenous (IV) cocaine HCl. Do the pharmacokinetic (PK) and pharmacodynamic (PD) data support the legislators or not?

To answer this question, the PK profiles and subjective response of experienced cocaine users to IV cocaine HCl (32 mg), intranasal (IN) cocaine HCl (75-96 mg) and smoked (S) cocaine base (50 mg) have been compiled from the published literature and compared in tabular form.

Comparison of Pharmacokinetic, Pharmacodynamic, and Subjective Properties Among: Cocaine Hydrochloride, Free Base Cocaine, and "Crack" Cocaine

	Cocaine Hydrochloride		Free Base Cocaine	"Crack" Cocaine
Route of Administration	Intravenous	Intranasal	Smoked	Smoked
Amount Used in mg	32	96	50	50
Time of Arterial Cmax	15 seconds		15 seconds	15 seconds
Time of Venous Cmax	3-6 mins	30-40 mins	3-6 mins	3-6 mins
Peak Arterial Conc.(ng/ml)	1500-2,000		1500-2,000	1500-2,000
Peak Venous Conc.(ng/ml)	~170	200	180	180
Time of Max 8HR	mins	15-40	mins	mins
Time of Max. Euphoria	mins	10-20	mins	mins

In the laboratory, subjects preferred S to IV, but when subjects purchased cocaine, they reported "not liking to see it go up in smoke." Based on dose or blood levels, data from scientific studies do not support a rationale for more severe legal penalties for distribution or use of a form of cocaine that can be smoked as opposed to one that can be injected IV.

Key Words: **Comparative Pharmacokinetics, Arterial vs. Venous Levels, Preferences**

P69 Quantitative Determination of Codeine and Morphine in Brown Mixture (Opium Preparations) in Taiwan Using SPE and GC/MS

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Brown Mixture (BM) contains opium powder (10.0-10.5% morphine), opium tincture (0.9-1.1% morphine), or camphorated opium tincture (0.045-0.055% morphine) and is a popular remedy, while heroin use is considered a serious criminal act, the claim of BM use has to be adequately addressed. A sensitive method for simultaneous quantitation of codeine and morphine in BM by gas chromatography-mass spectrometry (GC-MS) is described. Solid-phase extraction (SPE) cartridges were therefore evaluated for their effectiveness in sample preparation. Deuterated analogs (d_3- and d_6-) codeine and morphine were evaluated and conclusions were reached that codeine and morphine can be effectively analyzed, qualitatively and quantitatively, as trimethylsilyl derivative using selective ion monitoring of the following ions: *m/z* 371, 372 and 343 for codeine; 377, 378 and 349 for codeine-d_6; 429, 414 and 401 for morphine; and 435, 420 and 404 for morphine-d_6. The overall protocol achieves the following results when applied to the analysis of 1 mL opiates-free BM specimens fortified with 50–1500 ng/mL codeine and morphine: recovery, 90.00–100.87%; interday and intraday precision ranges, 1.10–6.46% and 1.04–3.91%, respectively; linearity, $r^2 > 0.997$; limits of detections, 30 ng/mL for codeine and 20 ng/mL for morphine; limits of quantitation, 30 ng/mL for codeine and 40 ng/mL for morphine. In this study, BM from 8 different manufacturers (5 tablets and 3 solutions) were analyzed for their morphine and codeine contents. The contents of morphine and codeine in the tablets are very consistent, the morphine to codeine concentration ratios in these BM tablets range from 8.66 to 9.09 with an average of 8.85 ± 0.16 (standard deviation); but vary considerably in the 3 BM solutions, the morphine to codeine concentration ratios were 2.19, 2.34 and 3.28, respectively (Table 1).

Table 1. Quantitation of Codeine and Morphine (in μg/tab or μg/mL) in BM from Different Manufacturers

			Mean ± S.D.	[M]/[C]	
Manufacturer	Dosage form	n	Morphine	Codeine	Ratio
A	Tablet	5	265.06 ± 2.61	29.83 ± 0.27	8.88
B	Tablet	5	244.70 ± 2.95	28.25 ± 0.50	8.66
C	Tablet	5	260.41 ± 15.85	29.63 ± 1.59	8.79
D	Tablet	5	263.09 ± 4.71	29.86 ± 0.50	8.81
E	Tablet	5	294.76 ± 8.16	32.44 ± 1.18	9.09
F	Solution	5	128.78 ± 1.07	58.86 ± 0.48	2.19
G	Solution	5	53.88 ± 1.63	23.03 ± 0.36	2.34
H	Solution	5	44.43 ± 0.84	13.54 ± 0.19	3.28

Key words**: Brown Mixture, Codeine, Morphine, SPE, GC-MS**

P70 Validation of a Headspace GC Method for the Analysis of a Pyrolytic Product of Methamphetamine in Urine

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The aim of the study was to develop and validate a simple and rapid GC/FID method for the identification of 1-phenylpropene in urine. This compound is a pyrolytic product of methamphetamine that has been previously proposed as a marker for smoked methamphetamine. The instrumentation used is the same as employed for blood alcohol determination. The extraction-free procedure is rapid, simple and quantitative using 2-phenylpropene as the internal standard. The method was validated for linearity over a range of 0.1-20 μg/mL with a limit of detection of 0.05 μg/mL, limit of quantification of 0.1 μg/mL, inter-day accuracy within 3.2 to -5.3 %, intra-day accuracy better than 7.5 %, inter-day precision of 7.5 to 10.7 %, intra-day precision of 2 to 8.6 %, and recovery above 80 %. For the robustness determination in urine, the accuracy of four different sources of urine at the mid control level (1 μg/mL) ranged from 1.6 to 19 % error. The % RSD of the different urine sources ranged from 3.1 to 11 %. Urine samples from nine methamphetamine-positive cases investigated by the Office of the Chief Medical Examiner of West Virginia were included in the study. 1-Phenylpropene was found in two methamphetamine positive cases (0.25, 0.44 μg/mL). The advantages of this method are speed, simplicity, and the ability to use existing equipment routinely employed for blood alcohol measurements.

Keywords: **Headspace GC, 1-phenylpropene, Validation**

P71 Performance Evaluation of Thermal Desorption System (TDS) for Detection of Basic Drugs in Forensic Samples by GCMS

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Stir bar sorptive extraction (SBSE) is an innovative sample extraction technique that can be used to process blood, urine, and tissue samples for routine drug screening in the forensic toxicology laboratory. The Gerstel TDU System is a multifunctional desorption unit capable of determining the presence of analytes from liquid samples after extraction using the Twister™ stir bar. The TDU desorption system was evaluated for use in combination with GCMS for determining the presence of basic drugs in forensic samples. Human blood fortified with known quantities of drugs was used to evaluate sample diluents, extraction time, injection parameters and recovery. Case specimens containing drugs typically encountered in forensic samples were evaluated using the desorption method and compared with a liquid-liquid extraction method followed by GCMS analysis. This evaluation demonstrated that the TDU desorptive method worked equally as well as the routine extraction method for the detection of basic drugs in screening forensic samples.

In addition, the described technique avoids the use of extraction solvents and the subsequent centrifugation, transfer, and concentration steps required of liquid-liquid and solid phase extraction methods.

Key Words: **Thermal Desorption, Twister, Stir Bar Sorptive Extraction**

P72 Gas Chromatography Coupled to Nitrogen-Phosphorus and Mass Spectrometry Detectors (GC/NPD/MS): A Simple and Powerful Tool for Forensic Analysis

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Introduction

The aim of this presentation was to evaluate the usefulness of gas chromatography coupled to nitrogen-phosphorus and mass spectrometry detectors (GC/NPD/MS) in routine forensic toxicological casework. Presently, gas chromatography coupled with mass spectrometry (GC/MS) is the most common method used for confirmation and quantification of drugs and their metabolites. Mass spectrometry detection of extracted biological samples generates intense background produced by compounds present in matrices (ex: cholesterol, nicotinamide, fatty acids, putrefaction products, etc), xenobiotics at high concentration (acetaminophen, naproxen, etc) or column bleeding that can mask pharmacologically active compounds at low concentrations. The majority of drugs of abused or prescribed contains at least one nitrogen and can be monitored selectively by NPD detector. The addition of NPD to MS detector will increase the amount of information useful to identification and quantification of xenobiotics

Experimental

The experiments were performed using a Hewlett-Packard 6890 Serie GC equipped with a capillary split/splitless inlet, 7683 autosampler, NPD detector and 5973 MSD controlled by Chemstation. Chromatographic column was obtained from Agilent (DB-5MS, 15 m). The mass detector has been set up in full scan mode (m/z: 41-600). Extracts were injected into a single column and than split post column to MS and NPD detectors.

Results

GC/NPD/MS became a very simple and powerful method for screening analyses of substances with forensic interest. The combination of these two detectors on the same instrument created synergy advantages, whereas disadvantages of both detectors can be minimised. Retention time differences between chromatograms was in the order of 1-3 seconds. This small delay between two chromatograms facilitates the location of an unknown on MS chromatogram and dual detectors permits a double quantification of analytes in a single injection. The used of a Y connector does not affect the symmetry of chromatographic peaks. This technique is particularly useful in routine work and the cost of instrument modification is minor. In our laboratory, GC/NPD/MS has been utilised since 1999, with good results for routine screening of drugs in biological matrices and no problems have been observed. This technique improves the detection and quantification of compounds.

Key words: **Gas chromatography, Mass spectrometry, Nitrogen-phosphorus detector**

P73 Rapid Quantification of Urinary Oxycodone and Oxymorphone Using Fast Gas Chromatography-Mass Spectrometry

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Human urine specimens that were determined to be presumptively positive for oxycodone and its metabolite oxymorphone by immunoassay screening, were assayed using fast gas chromatography-mass spectrometry (FGC/MS) to positively identify and quantify the amounts of oxycodone and oxymorphone. Urine specimens were spiked with deuterated internal standards oxycodone-d3 and oxymorphone-d3, subjected to acid hydrolysis, extracted using a positive-pressure manifold and mixed-bed solid-phase cartridge extraction methodology. Extracts were derivatized using methoxylamine and acetic anhydride. The acetylated-oxime derivatives of oxycodone and oxymorphone were identified and quantified using a selective ion monitoring (SIM) FGC/MS method using SIM ions 386(Q), 343 and 295 for oxycodone (oxycodone-d3: 289, 346) and 372(Q), 414 and 329 for oxymorphone (oxymorphone-d3: 375, 417). Both analytes were linear to 1600 ng/mL with limits of detection for oxycodone and oxymorphone of 40 ng/mL and 20 ng/mL, respectively. Inter-laboratory data comparisons (n=40) showed a correlation coefficient of 0.9999 and 0.9997 for oxycodone and oxymorphone, respectively. Analysis of 28 replicates spiked with 125 ng/mL of oxycodone and oxymorphone in certified negative urine gave an average concentration of 126.82 ng/mL (range = 117.06 – 143.29) for oxycodone with a coefficient of variation (CV) of 5.8% and an average concentration of 119.01 ng/mL (range = 110.38 – 130.96) for oxymorphone with a CV of 4.8%. Similarly, analysis of specimens spiked with 40 ng/mL of oxycodone and oxymorphone in certified negative urine gave an average concentration of 43.62 ng/mL (range = 41.42 – 48.36) for oxycodone with a CV of 4.4% and an average concentration of 40.89 ng/mL (range = 39.31 – 44.29) for oxymorphone with a CV of 3.2%.

Structurally similar compounds were assayed along with derivatized oxycodone and oxymorphone showing no interferences. Finally, exact mass and tandem mass spectrometry techniques were employed to elucidate the structures of the selected SIM ions.

Key Words: **Fast GC, GC-MS analysis, Oxycodone**

P74 The Determination of Verapamil and its Metabolites in Equine-Related Samples

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Verapamil is a commonly prescribed cardiovascular drug used to treat hypertension and cardiac arrhythmia in humans. This calcium-channel blocking agent is also used by some equine trainers and veterinarians in horses exhibiting similar cardiac diseases. Due to its abilities to increase cardiovascular function and exercise capacity, verapamil is prohibited from use by horses participating in any officially sanctioned racing within the State of Illinois. Due to the extensive first-pass metabolism observed after administration of verapamil (<95%), detection of the parent compound in equine urine samples is exceedingly difficult. Therefore, determination of use is only possible by detection of one of several glucuronidated verapamil metabolites. These metabolites were recently discovered by our laboratory in several equine urine samples, and the parent drug was also discovered in an unidentifiable powder mixture recovered from one trainer.

Following a presumptive positive by immunochemical screening, confirmatory determination by GC-MS and was completed. To prepare urine samples for analysis, enzymatic hydrolysis was employed followed by a basic liquid-liquid extraction (dichloromethane:isopropyl alcohol (9:1)) with an acidic backwash. The resulting residue was derivatized with acetic anhydride to facilitate GC-MS analysis. For the unidentified powder, a simple basic extraction into dichloromethane: isopropyl alcohol (9:1) was used to isolate the parent compound, which could not be derivatized. Due to chromatographic difficulties and the resulting lack of sensitivity encountered during our GC-MS confirmation of the parent compound, determination by LC-MS^n is currently being investigated as a preferential technique. Analysis by LC-MS^n would also allow for determination of the metabolites without requiring the derivatization needed for GC-MS analysis.

Key Words: **Equine, Verapamil, Metabolism, GC-MS, LC-MS**

P75 A Novel SPE Procedure for the Extraction and Analysis of Olanzapine from Whole Blood

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Olanzapine (Zyprexa) is a popular pharmaceutical used for in field of anti-psychotic medicine. Concerns have been reported[1,2] about the stability of this drug in biological samples prior to toxicological analysis. This project was developed in order to study this drug at low levels as therapeutic levels of Olanzapine have been reported as low as 9.3 ng per mL (plasma)[3].

In this method, Olanzapine and the internal standard (Prazepam (100 ng)) were spiked into whole blood samples (1mL) over a concentration range 0 through to 100 ng per mL. The samples were buffered with de-ionized (DI) water (9 mL) and the drugs extracted onto endcapped C_4 spe columns (50 mg). The columns were washed with 1% acetic acid in DI water (1x 3 mL) and eluted with 1% acetic acid in methanol (2x 3mL). The eluents were collected and evaporated for further chromatographic analysis. Using GC-MS (SIM), the samples were derivatized prior to analysis using PFPA, for analysis with LC-PDA the samples were reconstituted in 0.1% aqueous TFA.

GC-MS (SIM) analysis was carried out using a Agilent Technologies 6890 GC coupled to a 5975 MSD: Ions = 388, 359,376 (Olanzapine-PFPA) and 269, 295, 324 (Prazepam).

LC-PDA analysis was carried at 260nm using an acetonitrile/ 0.1% TFA gradient on a Phenomenex Luna C_{18} column (150x4.6mm).

From this method LOQ's of 5 ng per mL of sample is easily achievable by either chromatographic system. By using GC-MS (SIM) in EI mode, 1 ng per mL of sample can be detected.

Examples of chromatograms and calibration curves are presented to show the simplicity and efficiency of this methodology.

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Keywords: **Olanzapine SPE, Toxicology**

P76 Measurement of Voriconazole in Serum, Plasma, and Whole Blood

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Voriconazole (VOR) is an azole antifungal that is structurally related to fluconazole, but has enhanced potency and spectrum of activity. *In vitro* activity against most yeasts is 60-fold higher than for fluconazole. VOR has been studied in variety of fungal infections, including Aspergillosis, *Candida* infections, and *Cryptococcus neoformans*. VOR exerts its effect by altering the fungal cell membrane via the inhibition of ergosterol synthesis resulting in increased cellular permeability causing leakage of cellular contents. Infrequent cases of serious hepatic reactions (jaundice, hepatitis, cholestasis, fulminant hepatic failure including fatalities) have occurred. Here we describe an assay for measurement of VOR in serum/plasma and whole blood.

Method: Samples, calibrators and controls as extracted as follows. Briefly following the addition of the internal standard (IS, Phenacetin), the drugs are extracted using 0.2 M borate buffer (pH 8.8), 20% ethyl acetate, 80% chloroform (v/v). The organic layer is removed and evaporated to dryness. The sample is reconstituted in mobile phase. Using a Shimadzu CLASS-VP LC, 30 uL of reconstituted sample is injected onto a Supelcosil LC-18-DB column (25 cm x 4.6 mm, 5 um) held at 50C. Chromatographic separation is achieved using isocratic solvent delivery, using a mobile phase of 60% Acetonitrile, 40% of 0.05M Ammonium Phosphate pH 6.0 (v/v) with a flow rate of1.5 ml/min. Detection was achieved by monitoring the absorbance at 254 nm. VOR eluted at 3.0 min, and IS at 2.4 min. The concentration was calculated by comparison of peak-height ratio of the drug with that of IS against a standard curve.

Results: Linearity is observed from 0.1 – 50 ug/mL; throughout the linear range the intra-run and inter-run CV's were <10%. The recovery of VOR and phenacetin were 89% and 80% respectively, and were consistent through the analytical range.
Serum, plasma, and whole blood were tested in this assay, and using matrix specific controls and calibrators, similar analytical characteristics were seen.

Conclusion: This describes a robust method for the determination of VOR in serum, plasma or whole blood.

Key Words: **Voriconazole, Measurement, Chromatography**

P77 Evaluation of an LC/MS Method to Screen for Drugs in Postmortem Whole Blood Specimens

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Objective

The objective was to evaluate an LC/MS method to screen for drugs in postmortem whole blood samples, and compare this method with our existing GC/MS method.

Method

The extraction method was based on a method developed for GC/MS analysis. A 2.5-mL aliquot of blood was spiked with Proadifen internal standard. An acidic buffer was added and the specimen was vortexed, sonicated, and centrifuged. Samples were loaded onto Cerex solid phase extraction cartridges that had been previously conditioned with Ethyl Acetate and Methanol. The cartridges were washed with a Carbonate Buffer, pH 9.0 then with DI Water and dried for ten minutes. The specimens were eluted with Ethyl Acetate:Ammonium Hydroxide [98:2], dried under nitrogen, and reconstituted with Acetonitrile. Thirty microliters of the sample was injected on a Waters 2695 Liquid Chromatograph, in combination with a Waters Micromass ZQ Mass Spectrometer. The HPLC column used was a Waters XTerra MS C18, 3.5 µm, 2.1 x 150 mm with the appropriate guard column. Mobile phase A was 5 mM Ammonium Formate Buffer, pH 3.0 and mobile phase B was 0.05% Formic Acid in Acetonitrile. The mass spectrometer method, as defined in Waters ChromaLynx software, was comprised of seven scans, each at a different cone voltage. The first six scans were in Electrospray Ionization Positive mode, with the seventh scan in Electrospray Ionization Negative mode. Total run time for the analysis of one specimen was twenty-six minutes.

Results

Several blood specimens were extracted and run in parallel using the LC/MS method with ChromaLynx software and the GC/MS method in full scan mode. The qualitative results of the comparison using actual postmortem blood samples agreed very well. Results of negative whole blood specimens spiked with a wide variety of compounds and analyzed using LC/MS and ChromaLynx compared favorably to the GC/MS method. The ChromaLynx software detected polar analytes with much greater success than the GC/MS method.

Conclusion

The ChromaLynx software displayed some advantages over the GC/MS software. Using the ChromaLynx software more of the polar analytes, such as Amiodarone and its metabolite, Citalopram and its metabolite, and Lamotrigine were able to be qualitatively identified. The ability to qualitatively identify a larger library of analytes was the single most important advantage of using LC/MS and ChromaLynx.

Keywords: **ChromaLynx, LC/MS, Screening**

P78 Simultaneous Quantitation of Multiple Basic Drugs in Post-Mortem Blood Specimens using LC/MS/MS (QTrap) Method

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Using a previously developed method for the rapid detection of drugs in whole blood, the ability of the QTrap to perform quantitation of those same drugs was investigated. The extraction method was the same used for EIA analysis and subsequent liquid chromatography/tandem mass spectrometry of drugs in whole blood, with slight modifications. The extraction technique employed was a simple protein precipitation of whole blood samples with acetone followed by reconstitution with LC mobile phase. The samples were injected onto a MetaSil Basic reversed phase C8 column and eluted using a 200 mcL/min total flow rate with a mobile phase mixture composed of methanol:water containing 0.025% ammonium formate and 0.1% formic acid. The instrument method used in the previously reported assay incorporating electrospray ionization was also evaluated for viability, with some alterations made in the final method of analysis. Various sample volumes were evaluated for acceptability and comparisons to results from ~80 samples previously analyzed by gas chromatography/mass spectrometry were also studied. A wide variety of post-mortem drugs of interest were evaluated, including methadone, EDDP, oxycodone, amitriptyline and fentanyl. The resulting method provides a simple extraction procedure combined with rapid quantitative ability, which can be employed on a wide variety of drugs seen in post-mortem samples with a representative result variability of 21%.

Key words: **Post-mortem, LC/MS/MS, Quantitation**

P79 Evaluating the Quantitation of Certain Benzodiazepines in Whole Blood Specimens using LC/MS/MS (QTrap) Method

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With previous success using a rapid method of analysis for various basic drugs in whole blood, the ability of the QTrap to perform quantitation of certain benzodiazepines using the same method was investigated. The extraction technique employed was a simple protein precipitation of whole blood samples with acetone followed by reconstitution with LC mobile phase. The samples were injected onto a MetaSil Basic reversed phase C8 column and eluted using a 200 mcL/min total flow rate with a mobile phase mixture composed of methanol:water containing 0.025% ammonium formate and 0.1% formic acid. The instrument method used in the previously reported assay incorporating electrospray ionization was also evaluated for viability of benzodiazepine quantitation with diazepam-d5 internal standard. Various samples were evaluated for acceptability using this method while simultaneously analyzed by a liquid-liquid extraction technique followed by gas chromatography/mass spectrometry quantitation. A wide variety of benzodiazepines of interest were evaluated, including diazepam, alprazolam, lorazepam and clonazepam.

Key words: **Benzodiazepines, LC/MS/MS, Quantitation**

P80 Evaluation of Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry for the Analysis of Clonazepam and Related Compounds

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Clonazepam is included in the benzodiazepine class of hypnotic and anti-seizure medications. It has been used for treatment of neuronal discharge in motor seizures. The analysis of clonazepam as well as other benzodiazepine drugs in biological samples has been performed with different instrumental techniques such as gas and liquid chromatography using selective modes of detection. Gas chromatography methods are considered time consuming due to extensive sample preparation. Although traditional liquid phase techniques often improves sample throughput by minimizing the sample preparation method, the separation of multiple analytes with acceptable resolution also requires several minutes per sample. The application of ultra performance liquid chromatography electrospray ionization tandem mass spectrometry (Acquity UPLC™- Quattro Micro API MS/MS, Waters Corp., Milford, MA) allows for the reduction in analysis time and provides both the selectivity and sensitivity required for trace level analysis of this class of drugs in biological samples.

A UPLC™-MS/MS method was developed and compared to a more traditional high performance liquid chromatography (Agilent 1100, Agilent Technologies, Inc., Palo Alto, CA) - MS/MS (HPLC-MS/MS) technique. The aim of the newly developed method was to improve sample analysis time, while assessing accuracy and precision. Liquid phase extraction of the drug substance, metabolites and stable isotope internal standards was performed for the preparation of the calibration, control and test samples. The HPLC, UPLC™ and MS/MS parameters were optimized for the separation and detection of clonazepam and the major metabolite, 7-aminoclonazepam. In contrast to the flow rate of 0.5 mL/minute in the HPLC method the flow was reduced in the UPLC™ to 0.2 mL/minute. A methanol gradient was used with different mobile phase additives in the two separation systems. The UPLC™ method was employed using water and methanol with 0.1% heptafluorobutyric acid (HFBA) added to both solvents. The mobile phase used with the HPLC system contained water and methanol each with 0.1% trifluoroacetic acid (TFA).

The results show that the new UPLC™ method was more rapid despite a lower flow rate. The retention time of the parent drug substance was reduced from 10 to 3 minutes and the metabolite from 5 to less than 2 minutes. The cycle time of the injections was reduced from 12 to 3 minutes. While an analogous sample preparation procedure was used with both separation systems, an apparent recovery enhancement of 10% was discovered. The increased recovery was attributed to enhanced chromatographic performance of the UPLC™ system. These results were consistent with the superior ion-pairing capability of HFBA. Because there was a 3 fold decrease in retention time and a 4 fold decrease in sample cycle time as compared to the traditional HPLC system, the results of the method comparison illustrate that the UPLC™ method enhances sample throughput by reducing the time of sample analysis.

Keywords: **Clonazepam, Ultra performance liquid chromatography (UPLC™), Tandem Mass Spectrometry**

P81 Quantitative Determination of Carisoprodol, Meprobamate, Salicylic acid, Acetaminophen and Phenobarbital in Whole Blood by LC-MS/MS

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Aims

To present a validated LCMS/MS method for quantification of carisoprodol, meprobamate, salicylic acid, acetaminophen and phenobarbital in whole blood for routine use.

Methods

Blood samples were extracted with ethylacetate at pH 1.5. After evaporation of the organic solvent, the residue was dissolved in solvent. Spiked blood samples (1-100 mg/kg carisoprodol, meprobamate, salicylic acid, acetaminophen and phenobarbital) were used for the calibration curve. The analysis was performed on a Quatro micro MS/MS (Waters) coupled to an HPLC (Agilent). The separation column was a Persuit C18. The solvent consists of ammonium-acetate buffer pH 8.5 : acetonitril (40 : 60). The masses: 261.1 (carisoprodol), 219.0 (meprobamate), 136.9 (salicylic acid), 149.9 (acetaminophen) and 231.1(phenobarbital) were used for quantification.

Results

Detection limit was < 1 mg/kg and the quantification limit was 1-2 mg/kg for the compounds. The calibration curves were linear in the measuring interval. The linearity was evaluated with polynomial regression. Within day precisions for blood controls spiked at 1 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg were 5-15%. Between day precision for blood controls spiked at 20 mg/kg and 75 mg/kg were <15%. Recovery for spiked blood samples at 5, 25 and 75 mg/kg was > 60%. The laboratory participates in an external quality control program. Some ion suppres-sion was observed for salicylic acid. The other compounds did not show any ion suppression.

Conclusion

A validated method has been described. The method is useful for quantification of carisoprodol, meprobamate, salicylic acid, acetaminophen and phenobarbital in whole blood.

Keywords: **Acidic and neutral drugs, LCMS/MS, Validation, Whole blood**

P82 Simultaneous SPE and LC-APCI-MS Assay for Nicotine, Cotinine, *trans*-3′-Hydroxycotinine and Norcotinine in Plasma

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Nicotine, the major alkaloid in tobacco, is rapidly and extensively metabolized in humans, with substantial inter-subject variability. Nicotine is primarily metabolized by cytochrome P450 (CYP2A6) to cotinine and subsequently hydroxylated to *trans*-3′-hydroxycotinine. This method was designed to simultaneously quantify nicotine, cotinine, *trans*-3′-hydroxycotinine, and norcotinine in human plasma.

Calibrator and quality control samples were prepared in blank plasma. One mL of plasma specimens, quality control (QC), or calibrator samples was fortified with d_4-nicotine and d_4-norcotinine as internal standards, mixed with sodium acetate buffer (pH 5.5), and loaded onto the conditioned SPE columns (Bond Elut Certify). Columns were washed with 0.1 M acetic acid and methanol and dried. Analytes were eluted with methylene chloride:2-propanol:ammonium hydroxide (78:20:2 v/v/v) and added 100 µL of 1% hydrochloric acid/methanol (v/v) prior to evaporation. Extracts were evaporated to dryness under a stream of nitrogen at 40°C and reconstituted in 50 µL of initial mobile phase. 30 µL were injected and analyzed by LC-APCI-MS in the selected ion monitoring (SIM) mode. Chromatographic separation was achieved with a Synergi Polar RP column (150 x 2.0 mm i.d.) using gradient elution with mobile phase A (aqueous 10 mM ammonium acetate) and mobile phase B (acetonitrile), at a flow rate of 300 µL/min. The initial condition of 15% B was increased to 40% at 3.0 min, raised to 90% at 8 min and held for 2 min. The ions for each analyte were monitored in the following elution order (quantitative ions are indicated in parenthesis): *trans*-3′-hydroxycotinine m/z (193), 80, 134; d_4-norcotinine, m/z (167), 84, 139; norcotinine, m/z (163), 80, 135; cotinine, m/z (177), 80, 98; d_4-nicotine, m/z (167), 84, 110; and nicotine, m/z (163), 80, 132.

Eight point calibration curves for all analytes were linear from 2.5 to 500 ng/mL plasma (r^2 >0.99). The limits of detection and quantification were 1.0 and 2.5 ng/mL, respectively, for all analytes. Intra-assay (within-run) precision and accuracy were determined by replicate analysis (n = 5) of QC (8, 80, and 400 ng/mL) samples. Intra-assay precision for all analytes proved to be less than 11.9% and accuracy <15.0% across the linear range of the assay. Inter-assay (between-run) precision and accuracy were assessed with 20 specimen samples at each QC concentration on 4 separate runs and ranged from 4.2 –9.8% and 0.5 – 13.3%, respectively, for all analytes at all QC concentrations. Mean recoveries were between 90.5 – 110.8% at all three concentrations.

A sensitive, and specific LC-MS procedure for the simultaneous determination of nicotine, cotinine, *trans*-3′-hydroxycotinine, and norcotinine in human plasma is presented. SPE was paired with LC/APCI-MS SIM mode to achieve an accurate and precise quantification of nicotine and metabolites. The method should be useful for monitoring tobacco exposure, for nicotine pharmacokinetic studies, and for determining the usefulness of smoking biomarkers, including metabolite ratios.

Key Words: **Nicotine, Nicotine metabolites, LC-APCI-MS assay, Plasma**