



Interpol Review of Toxicology 2016-2019

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ASCLD Forensic Research Committee Future Forensics Subcommittee

Analysis of Toxicology 2016-2019 (Summarized by Alicia Rairden and Ashraf Mozayani)

Toxicological analysis in the past three years has focused on three major components:

1. Surveillance in Toxicology with focus on new standards, uncertainty of measurement, analytical methods for matrices and result interpretation
2. Challenges in selected topics of forensic interests including drug facilitated crime and new psychoactive substances (fentanyl and its derivatives, synthetic cannabinoids)
3. Advances in toxicological analyses from sample preparation to result interpretation

Surveillance in Toxicology

1. Surveillance discussion focuses on new accreditation and quality assurance standards [1] with minimum requirements for validating forensic toxicological methods [2]
 - a. Best practices for measurement and traceability in forensic toxicology, with the goal of establishing reliability and confidence in analytical results, were published by the American Academy of Forensic Sciences [3]
 - b. Several improvements to uncertainty measurements were made, topics of interest include:
 - i. Mistakes in the evaluation of uncertainty from linear calibration in chromatographic literature were highlighted [23]
 - ii. Revised and improved Widemark by recommending the use of Monte Carlo simulation for calculated uncertainty of measurement for ethanol [24]
 - iii. Due to an expanded uncertainty of 19.74%, a study recommended BAC tests near legal limits should be reported as the ethanol concentration obtained and the corresponding uncertainty [28]
 - iv. Freely available software (for the forensic community) was developed to calculate uncertainty, including methodological decision limits, and assess specification compliance [29]
 - v. Major contributors to uncertainty of measurement were found to be precision of method and sample/calibrator preparation in a bottom-up approach uncertainty of measurement calculation of benzoylecgonine and 11-nor- Δ 9 - tetrahydrocannabinol-9-carboxylic acid [32]
 - c. Interference of other volatiles in ethanol analysis was also an area of concern, [71] developed a method utilizing two chromatographic columns of different polarities to identify ethanol in the presence of other interfering volatile substances



2. Toxicological analyses include that of different bodily fluids, the body of knowledge was increased via studies of oral fluid, blood, urine and breath
 - a. Oral fluid
 - i. A good correlation (>80%) between drug recognition experts (DRE) and an on-site oral fluid testing device [74]
 - ii. Generally consistent results were found in the screening test of oral fluid with Aldere DDS[®] 2 compared to blood specimens in six drug categories [75]
 - iii. Utilized according to manufacturer cut-off concentrations, Aldere DDS[®] 2 also found to have high sensitivity (90%), specificity (100%) and accuracy (97%) [78]
 - iv. Oral fluid testing with DrugWipe5S[®] also showed cocaine detection up to at least 4 hours post intake (accuracy 75-98% utilizing legal decision limit of 10 ng/mL) [81]
 1. Authors also found that oral fluid concentration was higher and detection of cocaine had a longer duration compared to plasma utilizing this same decision limit
 - v. Oral fluid not found to be an accurate method for estimating blood concentration of THC [82]
 - b. Blood
 - i. Whole blood samples were utilized for a targeted screening method covering over 467 substances was based upon broadband collision induced dissociation UHPLC-TOF-MS had high sensitivity and broad scope for a single injection [87]
 - c. Urine
 - i. Procedure developed allowing for fast extraction of small sample volume (200 μ L) allowed quantitation of cocaine and its two main metabolites in urine using microextraction by packed sorbent GC/MS [88]
 - d. Breath
 - i. Limit of detection levels in the pg range was found for 28 drugs of abuse (and most of their metabolites) utilizing a method developed and validated by Ullah et. al. [90]
3. Back calculations of BAC require an understanding of alcohol pharmacokinetics
 - a. [91] showed a shift to first order kinetics from zero order kinetics when BAC concentrations fell below 0.19 g/kg and mean elimination rates fell to 0.083 g/kg/hr
4. Analyses of toxicological samples for workplace surveillance also provided insight into sample adulteration, drug testing methods/techniques and testing for intentional versus passive exposure
 - a. Urine adulteration
 - i. Workplace or court surveillance testing require validity of the urine specimen as between 1.9 and 3.81% of samples were found to have been tampered [107]
 - ii. Synthetic urine markers were identified as benzisothiazolinone (BIT) and ethylene glycols (triethylene glycol (E3G) and tetraethylene glycol (E4G)) [109]
 - b. Drug Detection – Hair



- i. Analysis of extraction methods found that a two-step method using methanol and acidified methanol was most efficient while acetonitrile itself was the least efficient extraction method [115]
 - 1. Authors suggest evaluating efficiency and recovery of extraction methods
 - c. Drug Detection – Oral Fluid
 - i. LC-MS/MS method was utilized to rapidly detect and quantify 32 synthetic stimulants and hallucinogens [9]
 - d. Drug Detection – Breath
 - i. Exhaled breath was used to identify THC up to 6 hours after smoking [124]
- 5. Passive or Occupational Exposure
 - a. Ketamine was found to absorb through the skin resulting in occupational exposure in hand contact of ketamine solutions or clinical activity animal body fluid exposure [125]
 - b. Police drug exposure from working environments was unlikely [126]
 - c. Active use exposure to cannabis had distinct indicators [128]
 - d. Two criteria to distinguish between real and passive cocaine users were developed [134]
 - e. Detection window of children hair analysis for drug exposure is difficult to determine as children's hair is more porous, subject to contamination and grows asynchronously [136]

Challenges – Forensic Topics of Interest

- 1. Chemical Warfare Agents
 - a. Development of a sensitive GC-MS/MS method with potential use as a rapid screening tool could determine six nerve agents and their corresponding breakdown products [164]
- 2. Drug Facilitated Crime
 - a. Alcohol and benzodiazepine most prevalent in drug facilitated sexual assault cases wherein victims were aged 16 years or older [169]
 - b. Sensitive method developed to detect drugs in drug facilitated crimes wherein reporting is often delayed [175]
 - c. 5 benzodiazepines in beverage detection and quantification method was developed using liquid-liquid extraction with low temperature partitioning and paper spray mass spectrometry [176]
 - d. GHB concentration in hair samples was found utilizing a method of solid phase extraction and GC/MS-MS [177]
- 3. New Psychoactive Substances
 - a. Fentanyl and derivatives
 - i. Furanylfentanyl concentration ranged from 0.38 to 8.7 ng/mL in associated deaths
 - ii. Carfentanil
 - 1. Most common analogue of fentanyl in Ohio drug overdose deaths in 2017 [217]



2. significantly increased detection in blood in DUID cases from 2016-2017 in Palm Beach County Florida (5% to 38%) [219]
 - iii. Methoxyacetylfentanyl – concentration in related deaths ranged from 0.022 to 0.056 mg/kg [239] and 0.21 to 39.9 ng/mL [240]
 - iv. Cyclopropylfentanyl - concentration in related deaths ranged from 1.1 to 270 ng/g [243] and 5.6 to 82 ng/mL [244]
- b. Synthetic Cannabinoids
- i. Detection
 1. MBMB-CHMICA –the concentration of fatal intoxication ranged from <0.2 ng/mL in post-mortem cases to 5.6 ng/mL in antemortem cases [265]
 2. 5F-MDMB-PICA - 12 phase I metabolites detected of 5F-MDMB-PICA but immunochemical assays were not capable of detecting 5F-MDMB-PICA in urine [269]
 - ii. Stability
 1. 4-chloromethcathinone (4-CMC) concentration dropped by 65% 3 days after measurement when stored at 4°C [322]
 2. 25I-NBOMe concentration dropped 40% at RT [323]
 - iii. Metabolism
 1. detection period is longer in urine; however, metabolites are identified as opposed to the parent synthetic cannabinoid [349]
 - iv. Immunoassay
 1. CUMYL-PEACLONE analyzed by ready to use homogenous enzyme immunoassays showed no positive results in 15 positive authentic urine samples [366]

Advances – Sample to Interpretation

Advances in this section focus on extraction methods, analytical instrumentation, and bodily fluid/secretions matrices for substance testing

1. Extraction methods
 - a. Supported liquid-liquid extraction method from whole blood samples in DUID cases was utilized for opiates, benzodiazepines, and amphetamines [444]
 - b. 24 compounds (including aliphatic and aromatic volatile hydrocarbons) were quantified with headspace solid phase microextraction [461]
 - c. Solid phase microextraction of postmortem blood, coupled with 2D GC/HRTOF-MS can be used for profiling of volatile organic compounds [462]
2. Instrumentation
 - a. Method validated for screening 7185 drugs and metabolites and simultaneous quantification of over 90 drugs with LC-QTOF-MS [507]
 - b. LC-QTOF-MS as a screening method for 320 forensically significant compounds in blood [508]



- c. LC-HRMS with Orbitrap as a targeted screening method for blood/plasma samples for ~700 compounds and data-dependent acquisition for unknowns [514]
- d. Discussion of analytical issues for UHPLC-QTOF with untargeted analytes discussed by [525]
- 3. Bodily fluid/secretions matrices for substance testing
 - a. Skeletal remains
 - i. Bone tissue poorly incorporates, or does not incorporate, drugs with pKa values lower than physiological pH (drug to metabolite ratio in skeletal tissue likely more useful than absolute found concentration) [546]
 - ii. Fluorescence issues in Raman techniques can be resolved by scraping better than chemical bleaching [551]
 - b. Vitreous Humor
 - i. Validated method using liquid-liquid extraction of vitreous humor followed by GC-EI-MS detected methylenedioxyamphetamine derivatives [555]
 - c. Intraosseous Fluid
 - i. 96% correlation between intraosseous fluid and central or cardiac blood were found in ELISA drug screening results; however, lipophilicity and conjugation likely prevent drug classes like oxycodone, tricyclic antidepressants and cannabinoids from being screened positively [558]
- 4. Interpretation
 - a. GHB detection window in urine was extended by 3-4 hours compared to blood, but longer delays in reporting/testing may require analysis of hair or nails [599]

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