INNOVATIONS IN SYSTEMATIC TOXICOLOGICAL ANALYSIS:

AMPHETAMINE-TYPE SUBSTANCES AND DESIGNER ANALOGUES

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Dedication

This effort is dedicated to my grandmother, whose last words to me were:

"I am proud of you."

We love you and miss you.

This is also dedicated to Leigh Ann. I often think of the times we all had while growing up, and can only wonder where you'd be today. You are loved and missed.

Abstract

Recently, several novel technologies have emerged with substantial benefits in toxicological analysis. These include the development of bead-based multiplex immunoassay (Suspension Bead Array, SBA), the use of reduced-volume centrifugal ion-exchange extraction (Spin-SPE), and Ultra-Performance™ liquid chromatographic separation coupled to mass spectrometry (UPLC™/MS^n). This work sought to investigate the efficacy and practicality of these innovative approaches against a benchmark of established methods and instrumentation for the screening and confirmation of amphetamine-type substances.

This study begins with a statistical survey of amphetamine-type substances encountered in an accredited forensic laboratory supporting the Australian Capital Territory and regional New South Wales. Over the 5-year period 2001–2005, it was determined that 6683 case submissions required presumptive screening for amphetamines. Of these cases, 1269 (19.0%) required confirmative analysis of amphetamine-type substances, including amphetamine, methamphetamine, MDA, MDMA, MDEA, ephedrine, pseudoephedrine, and phentermine. Such analytical needs were then used in comparative assessment of the novel and established methodologies, including examination of immunoassay specificity, extraction efficiency, chromatographic resolution, general resource efficiency, and total analysis time.

Development of a bead-based immunoassay platform (SBA) for multiplex amphetamines analysis proved to be a complex task. Efforts to multiplex the amphetamine and methamphetamine immunoassay models into a single assay exhibited a significant degree of non-specific antibody cross-reactivity. However, the merits of the individual bead assays were demonstrated. Upon comparison with commercially available enzyme-linked immunosorbent assays for amphetamine or methamphetamine (ELISA), it was observed that the SBA models exhibited specificity comparable to that of the ELISA assays and linearity over a concentration range of toxicological relevance (0–1000 ng/mL amphetamine or methamphetamine). In addition, the results indicated the practical applicability of the individual SBA assays for an oral fluid matrix, and demonstrated

significant reductions in the volumes of reagents required and length of time of analysis. Additionally, in an optimised multiplex system, the amount of sample required for screening could be reduced as the SBA technology theoretically permits analysis of up to 100 different drugs or metabolites from one volume of sample.

The aspect of forensic sample conservation was further explored with investigation of reduced-volume extraction techniques, such as the application of centrifugal ion-exchange extraction columns (Spin-SPE). Following initial development, the Spin-SPE technique was applied to the isolation of amphetamine-type substances from oral fluid and compared with a mixed-mode SPE method for both extraction and resource efficiency. From the observed results, both extraction methods were demonstrated to be effective in the isolation of amphetamine, methamphetamine, ephedrine, pseudoephedrine, PMA, MDA, MDMA, MDEA, MBDB, and 2CB from an oral fluid matrix with detection by heptafluorobutyric acid derivatisation (HFBTA) and GC/MS. The Spin-SPE model demonstrated comparable efficacy with reduced sample volume (200 μL), as well as significant reductions in the volumes of reagents required for column conditioning, washing, and elution. In addition, the linear working range (0–2000 ng/mL) and sensitivity of the method indicated the potential to further reduce sample volume.

In the confirmative separation and identification of drug compounds, the technological advancement of Ultra-Performance™ liquid chromatography (UPLC™) has recently evolved from efforts to improve LC resolution, sensitivity, and time of analysis. In this research, UPLC™ coupled to mass spectrometry was demonstrated to be capable of rapidly identifying several amphetamine-type substances (phenylethylamine, amphetamine, phentermine, methamphetamine, ephedrine, pseudoephedrine, PMA, 4-MTA, MDA, MDMA, MDEA, MBDB) and ketamine in an analysis time of less than five minutes. In addition, UPLC™/MS demonstrated a resolving power comparable to GC/MS with significantly reduced instrumental analysis time.

This research reveals the promise of these new applications in advancing towards a more efficient and modernised systematic toxicological approach. The continued development and optimisation of SBA multiplex immunoassays will permit customisable systems

capable of simultaneously detecting numerous compounds with antibody-based sensitivity and selectivity. In circumstances where low sample volumes are required for confirmation of drug use, such as in roadside saliva drug testing for driving under the influence offences, reduced-volume Spin-SPE has been demonstrated to be a practical and effective alternative for sample preparation. In addition, a more streamlined procedure is further enhanced with the use of UPLC™ coupled to mass spectrometry for analyte separation and molecular identification.

It is expected that illicit drug use will remain a significant public concern. With the continued desire for more rapid and comprehensive methodologies, further study of these and other innovative technologies will be of considerable future benefit to laboratories such as that serving the Australian Capital Territory region.

Abbreviations

2CB	4-bromo-2,5-dimethoxyphenylethylamine (also BDMPEA)
2CD	4-methyl-2,5-dimethoxyphenylethylamine
2CE	4-ethyl-2,5-dimethoxyphenylethylamine
2CI	4-iodo-2,5-dimethoxyphenylethyl-amine
2C-T-2	2,5-dimethoxy-4-ethylthiophenethylamine
2C-T-7	2,5-dimethoxy-4-propylthiophenethylamine
4-CB	4-carbethoxyhexafluorobutyryl chloride
4-MTA	4-methylthioamphetamine
5-IAP	1-(5-indanyl)-2-aminopropane
6GP-DH	glucose-6-phosphate dehydrogenase
ACT	Australian Capital Territory
ACTGAL	Australian Capital Territory Government Analytical Laboratory
AM	amphetamine
AM-BSA	amphetamine conjugated to bovine serum albumin
APCI	atmospheric pressure chemical ionisation
API	atmospheric pressure ionisation
BDB	benzodioxazolylbutanamine
BDMPEA	4-bromo-2,5-dimethoxyphenylethylamine (also 2CB)
BEH	bridged ethylsiloxane hybrid particles (UPLC™ column bed)
BSA	bovine serum albumin
CE	capillary electrophoresis
CEDIA	cloned enzyme donor immunoassay
CI	chemical ionisation
CID	collision-induced fragmentation
CPR	chlorophenolred
CPRG	chlorophenolred-β-galactoside
CV	coefficient of variation (%)
DAD	diode-array detection
DMA	2,5-dimethoxyamphetamine
DNC	dansyl chloride
DOB	2,5-dimethoxy-4-bromoamphetamine
DOE	2,5-dimethoxy-4-ethylamphetamine
DOM	2,5-dimethoxy-4-methylphenylisopropylamine
EA	enzyme acceptor
ECD	electron capture detection
ED	enzyme donor
EDC	1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride
EI	electron (or electron impact) ionisation
ELISA	enzyme-linked immunosorbent assay

Abbreviations (continued)

EMIT	enzyme-multiplied immunoassay technique
EMV	electron multiplier voltage
EPH	ephedrine
ESI	electrospray ionisation
FD	fluorescence detection
FID	flame ionisation detection
FITC	fluoroscein isothiocyanate
FPIA	fluorescence polarisation immunoassay
GC	gas chromatography
GC/ECD	gas chromatography–electron capture detection
GC/FID	gas chromatography–flame ionisation detection
GC/MS	gas chromatography–mass spectrometry
GC/NPD	gas chromatography–nitrogen–phosphorus detection
GC/PDHID	gas chromatography–pulsed discharge helium ionisation detection
GC/TOFMS	gas chromatography–time-of-flight mass spectrometry
HFB chloride	heptafluoro-*n*-butyryl chloride
HFBTA	heptafluorobutyric acid anhydride
HPLC	high-performance liquid chromatography
HPLC/DAD	high-performance liquid chromatography–diode array detection
HPLC/FD	high-performance liquid chromatography–fluorescence detection
IA	immunoassay
IR	infrared (wavelength)
KIMS	kinetic interaction of microparticles in solution
LC/MS	liquid chromatography (high-performance)–mass spectrometry
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LPME	liquid-phase microextraction
LTPC	*l*-triproyl chloride
MA	methamphetamine
MA-BSA	methamphetamine conjugated to bovine serum albumin
MA-D5	deuterated methamphetamine
MBDB	*N*-methyl-1-(3,4-methyl-enedioxyphenyl)-2-butanamine
MBTFA	*N*-methyl-bis(trifluoroacetamide)
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine

Abbreviations (continued)

MDMA	3,4-methylenedioxymethamphetamine
MDMA-D5	deuterated 3,4-methylenedioxymethamphetamine
MRM	multiple reaction monitoring
MS	mass spectrometry
MSD	mass spectrometry detector/detection
MS/MS	tandem mass spectrometry (liquid chromatography; LC/MS/MS)
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NE	norephedrine
NMR	nuclear magnetic resonance (spectroscopy)
NPD	nitrogen–phosphorus detection
PBS	phosphate buffered saline
PCP	phencyclidine
PDHID	pulsed discharge helium ionisation detection
PE	phycoerythrin
PFBBr	pentafluorobenzyl bromide
PFBC	pentafluorobenzoyl chloride
PFBSC	pentafluorobenzenesulfonyl chloride
PFPA	pentafluorpropionic anhydride
PFTBA	perfluorotributylamine
PM	post-mortem
PMA	*p*-methoxyamphetamine
PMMA	*p*-methoxymethamphetamine
PPA	phenylpropanolamine
PSE	pseudoephedrine
Q1/Q2	first/second qualifier ion (mass spectrometry diagnostic ions)
RIA	radioimmunoassay
RSV	reduced solvent volume
SBA	Suspension Bead Array
SCX	strong cation exchange
SIM	selected ion monitoring
SIR	selected ion recording
S/N	signal to noise ratio (PtP S/N = peak to peak signal to noise)
S-NHS	*N*-hydroxysulfosuccinimide
SPE	solid-phase extraction
Spin-SPE	centrifugal solid-phase extraction
SPME	solid-phase microextraction
SSI	sonic spray ionisation

Abbreviations (continued)

TCAA	trichloroacetic anhydride
TFAA	trifluoroacetic acid anhydride
TIC	total ion chromatogram
TLC	thin-layer chromatography
TMA	3,4,5-trimethoxyamphetamine
TMA-2	2,4,5-trimethoxyamphetamine
TMB	tetramethylbenzidine
TMS	trimethylsilyl
TOFMS	time-of-flight mass spectrometry
UPLC™	Ultra-Performance™ liquid chromatography
UPLC™/MS	Ultra-Performance™ liquid chromatography–mass spectrometry
UV	ultraviolet (wavelength)
UV-Vis	ultraviolet-visible (wavelength)

Publications

Papers

Apollonio, L.G., Whittall, I.R., Pianca, D.J., Kyd, J.M., Maher, W.A. (2007). Matrix effect and cross-reactivity of select amphetamine-type substances, designer analogues, and putrefactive amines using the Bio-Quant Direct ELISA presumptive assays for amphetamine and methamphetamine. *Journal of Analytical Toxicology* 31(4): 208–214.

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Presentations

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