

**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.

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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™/MSⁿ). 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 μ L), 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-dimethoxyphenylethylamine
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	fluorescein 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- <i>n</i> -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	<i>l</i> -triproyl chloride
MA	methamphetamine
MA-BSA	methamphetamine conjugated to bovine serum albumin
MA-D5	deuterated methamphetamine
MBDB	<i>N</i> -methyl-1-(3,4-methyl-enedioxyphenyl)-2-butanamine
MBTFA	<i>N</i> -methyl-bis(trifluoroacetamide)
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethylamphetamine

Abbreviations (continued)

MDMA	3,4-methylenedioxyamphetamine
MDMA-D5	deuterated 3,4-methylenedioxyamphetamine
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
PFBS	pentafluorobenzenesulfonyl chloride
PFPA	pentafluoropropionic anhydride
PFTBA	perfluorotributylamine
PM	post-mortem
PMA	<i>p</i> -methoxyamphetamine
PMMA	<i>p</i> -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	<i>N</i> -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

Conferences and Workshops

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Table of Contents

Chapter 1: Literature Review	26
1.1 PROJECT DESIGN	27
1.2 SYSTEMATIC TOXICOLOGICAL ANALYSIS	28
1.3 MATRICES IN FORENSIC AND POST-MORTEM TOXICOLOGY	31
1.3.1 Conventional matrices	33
1.3.2 Alternative specimens.....	36
1.3.2.1 <i>The “new” significance of oral fluid</i>	37
1.3.3 Special considerations in post-mortem toxicology.....	38
1.3.3.1 <i>Drug distribution and post-mortem drug redistribution</i>	38
1.3.3.2 <i>Endogenous compounds–putrefactive amines</i>	40
1.3.3.3 <i>Other post-mortem considerations</i>	41
1.3.4 Selection of matrices.....	42
1.4 DRUG COMPOUNDS AND SUBSTANCES INCLUDED IN THIS STUDY	43
1.4.1 Amphetamine-type substances and analogues	43
1.4.2 Inclusion of ketamine	50
1.4.3 Selection of compounds	50
1.5 PRESUMPTIVE SCREENING IN FORENSIC/POST-MORTEM TOXICOLOGY	52
1.5.1 Theory and use of immunoassay technology	52
1.5.1.1 <i>ELISA in routine screening and its application in this research</i>	56
1.6 SAMPLE EXTRACTION	58
1.6.1 Liquid–Liquid Extraction (LLE).....	58
1.6.2 Solid-Phase Extraction (SPE)	61
1.6.2.1 <i>Diatomaceous earth extractions in toxicology</i>	62
1.6.2.2 <i>Bonded phase extractions: Hydrophobic interactions–Reversed phase SPE</i>	62
1.6.2.3 <i>Bonded phase extractions: Ion-exchange SPE</i>	63
1.6.2.4 <i>Bonded phase extractions: Copolymeric (mixed-mode) SPE</i>	64
1.6.2.5 <i>Microextraction techniques</i>	65
1.6.2.6 <i>SPE models employed in this research</i>	67
1.7 CONFIRMATION: GC AND LC	68
1.7.1 Chromatography – GC in toxicology	68
1.7.1.1 <i>Derivatisation in GC</i>	71
1.7.1.2 <i>GC/MS in this research</i>	74
1.7.2 Chromatography – LC in toxicology.....	75
1.8 BREATHING NEW LIFE INTO SYSTEMATIC TOXICOLOGICAL ANALYSIS	78
1.8.1. Suspension Bead Array as an innovative multiplex immunoassay	79
1.8.2 Centrifugal reduced-volume SPE (Spin-SPE)	80
1.8.3 UPLC™/MS and UPLC™/MS/MS: Moving LC towards GC resolution	81
1.9 CONTEXT FOR RESEARCH	82
Chapter 2: The Context	83
2.1 INTRODUCTION	84
2.2 EXPERIMENTAL	85
2.2.1 Category definitions.....	85
2.2.2 Laboratory Analysis.....	85
2.3 RESULTS AND DISCUSSION	85

2.3.1 Post-mortem Toxicology.....	85
2.3.1.1 Deaths by Asphyxia	86
2.3.1.2 Homicides.....	87
2.3.1.3 Motor Vehicle Accidents (MVA).....	88
2.3.1.4 Natural Deaths.....	90
2.3.1.5 Overdose Deaths.....	90
2.3.1.6 Deaths Classified as “Other”.....	92
2.3.1.7 Suicides.....	94
2.3.1.8 Deaths of “Unknown” Classification.....	96
2.3.2 Forensic Toxicology Services.....	98
2.3.3 Road Traffic Safety.....	100
2.3.4 Alcohol and Drug Services	102
2.3.5 Illicit Drug Analysis – Forensic Chemistry.....	105
2.4 GENERAL SUMMARY.....	110
2.4.1 Post-mortem toxicology	110
2.4.1.1 Update: Post-mortem toxicology data from 2006	110
2.4.2 Forensic Toxicology Services.....	111
2.4.3 Road Safety	111
2.4.4 Alcohol and Drug Services	111
2.4.5 Forensic Chemistry / Illicit Drug Analysis	112
2.5 CONTEXT FOR RESEARCH	112
Chapter 3: Presumptive Screening – ELISA	113
3.1 INTRODUCTION	114
3.2 EXPERIMENTAL.....	116
3.2.1 Samples and reagents.....	116
3.2.2 Apparatus.....	116
3.3 METHODS.....	117
3.3.1 Standard curve preparation.....	117
3.3.2 Cross-reactivity/Specificity.....	117
3.3.3 Matrix effect.....	117
3.3.4 ELISA procedure	118
3.4 RESULTS.....	118
3.5 DISCUSSION	125
3.6 CONCLUSIONS.....	128
3.7 FUTURE DIRECTIONS.....	128
Chapter 4: Presumptive Screening – SBA	130
4.1 INTRODUCTION	131
4.1.1 Principle of SBA and its application to the toxicological screening of amphetamines	132
4.2 MATERIALS AND METHODS.....	134
4.2.1 Standards and Reagents.....	134
4.2.2 Instrumentation	134
4.3 PROCEDURES.....	135
4.3.1 Testing antibody recognition in the 96-well plate assay model (fluorescence plate reader).....	135
4.3.1.1 Protein preparation	135
4.3.1.2 Assay protocol.....	135

4.3.2 Bead coupling and testing recognition in the bead model (flow cytometry)	136
4.3.2.1 Protocol for protein coupling	136
4.3.2.2 Assay protocol.....	137
4.3.3 Testing the assay model and multiplex analysis (Bio-Plex™ technology).....	138
4.3.3.1 Assay protocol.....	138
4.4 RESULTS AND DISCUSSION	139
4.4.1 Fluorescence determination in the plate ELISA assay model.....	139
4.4.2 Analysis of bead assays using flow cytometry	140
4.4.3 Individual bead assays using the Bio-Plex™	147
4.4.4 Multiplex analysis using the Bio-Plex™.....	149
4.5 CONCLUSIONS.....	153
4.6 NOTES ON THE ORIGINAL ASSAY MODEL	154
4.7 COMPARISON WITH ELISA.....	155
4.8 FUTURE DIRECTIONS.....	158
Chapter 5: Extraction – SPE and Spin-SPE	161
5.1 INTRODUCTION	162
5.1.1 Oral fluid testing and road safety interests in the Australian Capital Territory	162
5.1.2 General applications of SPE.....	163
5.1.3 SPE model for amphetamine-type substances in oral fluid	164
5.1.4 Reduced-volume Spin-SPE	164
5.1.5 Choice of elution reagent for SPE	165
5.1.6 Derivatisation of amphetamine-type substances for analysis using GC/MS	166
5.2 EXAMINATION OF THE MIXED-MODE SPE METHOD	167
5.2.1 Experimental	167
5.2.1.1 Standards and reagents	167
5.2.1.2 Specimens	167
5.2.1.3 Extraction and derivatisation procedure	167
5.2.2 Results and Discussion	171
5.2.2.1 Elution reagent study.....	171
5.2.2.2 Peak response	175
5.2.2.3 Qualifier ion ratios	180
5.2.3 Summary	182
5.3 EXAMINATION OF THE REDUCED VOLUME SPIN-SPE METHOD.....	182
5.3.1 Experimental	182
5.3.1.1 Standards and reagents	182
5.3.1.2 Specimens	182
5.3.1.3 Extraction and derivatisation procedure	183
5.3.1.4 GC/MS instrumentation.....	184
5.3.2 Results and Discussion	184
5.3.2.2 Peak response	184
5.3.2.3 Qualifier ion ratios	188
5.3.3 Summary	190
5.4 CONCLUSIONS: COMPARISON OF MIXED-MODE SPE TO SPIN-SPE	191
5.4.1 Peak response	191
5.4.2 Ion ratios.....	191
5.4.3 Comments on internal standard	192
5.4.4 Resource efficiency of the reduced-volume extraction procedure	192

5.5 FUTURE DIRECTIONS.....	193
Chapter 6: Robustness of the GC/MS Method	196
6.1 INTRODUCTION.....	197
6.2 EXPERIMENTAL.....	199
6.2.1 Chemicals and reagents.....	199
6.2.2 Extraction of oral fluid specimens.....	199
6.2.3 GC/MS instrumentation.....	200
6.2.4 Analysis of tune data.....	200
6.2.5 Internal standard applicability.....	201
6.3 RESULTS AND DISCUSSION.....	201
6.3.1 Examination of tune data.....	201
6.3.1.1 <i>Statistical analysis: All tune data</i>	202
6.3.1.2 <i>Statistical analysis: Omission of poor tune data</i>	202
6.3.1.3 <i>Statistical correlation between parameters</i>	205
6.3.2 Internal standard applicability.....	211
6.3.2.1 <i>Theoretical model</i>	211
6.3.2.2 <i>Example of data from extracted samples</i>	212
6.4 CONCLUSIONS.....	214
6.5 FUTURE DIRECTIONS.....	215
6.6 THE EMERGENCE OF UPLC™: MOVING LC TOWARDS GC RESOLUTION	215
Chapter 7: Confirmation using UPLC™-MSⁿ	217
7.1 A DEMONSTRATION OF THE USE OF ULTRA-PERFORMANCE™ LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (UPLC™/MS) IN THE DETERMINATION OF AMPHETAMINE-TYPE SUBSTANCES AND KETAMINE FOR FORENSIC AND TOXICOLOGICAL ANALYSIS.....	218
7.1.1 Introduction.....	218
7.1.2 Experimental.....	220
7.1.2.1 <i>Standards, reagents, and sample preparation</i>	220
7.1.2.2 <i>UPLC™ Conditions</i>	220
7.1.2.3 <i>MS Conditions</i>	221
7.1.3 Results and Discussion.....	221
7.1.4 Conclusions.....	225
7.2 PRODUCT ION MASS SPECTRA OF AMPHETAMINE-TYPE SUBSTANCES, DESIGNER ANALOGUES, AND KETAMINE USING ULTRA-PERFORMANCE™ LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY (UPLC™/MS/MS).....	226
7.2.1 Introduction.....	226
7.2.2 Experimental.....	229
7.2.2.1 <i>Drug standards</i>	229
7.2.2.2 <i>UPLC™ Conditions</i>	229
7.2.2.3 <i>MS Conditions</i>	229
7.2.3 Results and Discussion.....	230
7.2.4 Conclusions.....	235
7.3 A COMPARISON OF ATMOSPHERIC PRESSURE CHEMICAL IONISATION (APCI) AND ELECTROSPRAY IONISATION (ESI) IN TESTING FOR AMPHETAMINE-TYPE SUBSTANCES AND KETAMINE USING ULTRA-	

PERFORMANCE™ LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY (UPLC™/MS)	236
7.3.1 Introduction	236
7.3.2 Experimental	238
7.3.2.1 <i>Drug standards</i>	238
7.3.2.2 <i>UPLC™ Conditions</i>	238
7.3.2.3 <i>MS Conditions</i>	238
7.3.2.4 <i>Calculating Signal-to-Noise (PtP S/N)</i>	239
7.3.3 Results and Discussion	239
7.3.4 Conclusions	242
7.5 CONCLUSIONS: COMPARING UPLC™/MS TO GC/MS	242
7.6 FUTURE DIRECTIONS	244
Chapter 8: General Discussion and Future Directions	246
8.1 GENERAL DISCUSSION AND FUTURE DIRECTIONS	247
8.1.1 Statistical survey of amphetamine-type substances in the Australian Capital Territory and regional New South Wales 2001–2005	248
8.1.2 Presumptive screening of amphetamine-type substances: ELISA and SBA	248
8.1.3 Sample preparation: mixed-mode SPE and reduced-volume cation exchange Spin- SPE.....	252
8.1.4 Separation and identification of amphetamines: GC/MS and UPLC™/MS ⁿ	254
8.1.5 Closing comments.....	256
Bibliography	257
Appendix (Publications)	288

List of Figures

Figure 1.1	Structural examples of common amphetamine-type substances, designer analogues, and metabolites	32
Figure 1.2	Chemical structures of select putrefactive amines	40
Figure 1.3	The chemical structure of <i>p</i> -methoxyamphetamine (PMA)	44
Figure 1.4	The chemical structure of 4-methylthioamphetamine (4-MTA)	45
Figure 1.5	Chemical structures of the designer drugs TMA-2, DOB, DOM, 5-IAP, and fluoro-methoxy-substituted phenylalkylamines	46
Figure 1.6	Chemical structures of the designer drugs 2CB, 2CI, 2CD, and 2CE	47
Figure 1.7	Chemical structures of the designer drugs 2C-T-7 and 2C-T-2	47
Figure 1.8	Chemical structures of medications that metabolise to methamphetamine and/or amphetamine in the body	48
Figure 1.9	Chemical structures of ketamine and phencyclidine (PCP)	50
Figure 1.10	Chemical structures for amphetamine-type substances and ketamine included in this research	52
Figure 2.1	Total post-mortem cases received at ACTGAL 2001–2005	86
Figure 2.2	Post-mortem asphyxia samples submitted to ACTGAL 2001–2005	87
Figure 2.3	Homicide cases submitted to ACTGAL 2001–2005	87
Figure 2.4	Post-mortem motor vehicle accident samples submitted to ACTGAL 2001–2005	88
Figure 2.5	Post-mortem motor vehicle accident samples with amphetamine-type substances detected	88
Figure 2.6	Annual detection incidence of amphetamine-type substances in post-mortem motor vehicle accident samples	89
Figure 2.7	Post-mortem cases of known natural etiology submitted to ACTGAL 2001–2005	90
Figure 2.8	Post-mortem overdose cases submitted to ACTGAL 2001–2005	91
Figure 2.9	Post-mortem overdose cases with amphetamine-type substances detected	91
Figure 2.10	Post-mortem deaths classified as “Other” submitted to ACTGAL 2001–2005	92
Figure 2.11	Post-mortem cases classified as “Other” with amphetamine-type substances detected	93
Figure 2.12	Detection incidence of amphetamine-type substances in post-mortem cases classified as “Other”	93
Figure 2.13	Post-mortem suicide cases submitted to ACTGAL 2001–2005	94
Figure 2.14	Suicide cases with amphetamine-type substances detected	95
Figure 2.15	Detection incidence of amphetamine-type substances in suicide cases	95
Figure 2.16	“Unknown” etiology post-mortem cases submitted to ACTGAL 2001–2005	96

Figure 2.17	“Unknown” etiology post-mortem cases with amphetamine-type substances detected	97
Figure 2.18	Detection incidence of amphetamine-type substances and ketamine in “Unknown” etiology post-mortem cases	97
Figure 2.19	Forensic toxicology samples submitted to ACTGAL 2001–2005	99
Figure 2.20a	Detection incidence of amphetamine-type substances in samples submitted for forensic toxicological analysis.	99
Figure 2.20b	Detection incidence of MDA, MDMA, phentermine, and ketamine in samples submitted for forensic toxicological analysis	100
Figure 2.21	Annual samples submitted to ACTGAL for road safety analysis 2001–2005	101
Figure 2.22	Number of road safety submissions with full toxicological analysis requested	101
Figure 2.23	Detection incidence of amphetamine-type substances in road safety sample submissions	102
Figure 2.24	Total alcohol and drug services samples submitted to ACTGAL 2001–2005	104
Figure 2.25a	Detection incidence of amphetamine-type substances and benzodiazepines in alcohol and drug services samples	104
Figure 2.25b	Detection incidence of MDA, MDMA, phentermine, and ketamine in alcohol and drug services samples	105
Figure 2.26	Forensic drug chemistry exhibits submitted to ACTGAL 2001–2005	106
Figure 2.27a	Detection incidence of amphetamine-type substances in forensic chemistry exhibits	107
Figure 2.27b	Detection incidence of MDA, MDMA, MDEA, and ketamine in forensic chemistry exhibits	108
Figure 2.27c	Detection incidence of phentermine, BDMPEA (2CB), caffeine, and cocaine in forensic chemistry exhibits	109
Figure 3.1	Standard curve response for amphetamine and methamphetamine using the Bio-Quant Direct ELISA	120
Figure 3.2	Relative cross-reactivity of common amphetamine-type substances, designer analogues, and putrefactive amines	122
Figure 3.3	Comparison of the influence of matrix on the absorbance response of the Bio-Quant Direct ELISA assays	123
Figure 3.4	Comparison of matrix influence on the absorbance response of the Bio-Quant Direct ELISA (concentration curves)	124
Figure 4.1	Assay scheme of Suspension Bead Array technology	133
Figure 4.2	Suspension Bead Array standard curves for amphetamine and methamphetamine	142
Figure 4.3	Reduction of fluorescence in Suspension Bead Array	143
Figure 4.4	Relative cross-reactivities of select amphetamine-type substances for Suspension Bead Array	144
Figure 4.5	Fluorescence microscopy photograph of Suspension Bead Assay	145
Figure 4.6	Effect of an oral fluid matrix on the fluorescence intensity	146

Figure 4.7	Initial assay scheme of Suspension Bead Array technology	154
Figure 5.1	Average GC/MS target ion response for elution reagents used in the mixed-mode solid-phase extraction of select amphetamine-type substances	172
Figure 5.2	Total ion chromatogram for amphetamine-type substances using mixed-mode SPE–GC/MS	173
Figure 5.3	Average GC/MS analyte to internal standard ratio (I.S. ratio) for select elution reagents	175
Figure 5.4	Peak/Ion contribution of MA-D5-HFBTA to <i>m/z</i> 118 using SPE–GC/MS	181
Figure 5.5	Total ion chromatogram for amphetamine-type substances using Spin-SPE–GC/MS	184
Figure 5.6	Peak/Ion contribution of MA-D5-HFBTA to <i>m/z</i> 118 using Spin-SPE–GC/MS	190
Figure 6.1	Cluster graphs for all MS tune data	204
Figure 6.2	Cluster graphs for corrected MS tune data	205
Figure 7.1a	Selected ion recording chromatograms of a polydrug standard containing eight amphetamine-type substances and phenylethylamine putrefactive marker	222
Figure 7.1b	Selected ion recording chromatograms of four amphetamine-type substances, phenylethylamine, and ketamine from extracted whole blood	222
Figure 7.2a	Chromatogram (254 nm) of polydrug reference standard containing MDA, amphetamine, MDMA, methamphetamine, and ketamine	232
Figure 7.2b	Individual chromatograms (254 nm) for PMA, 4-MTA, and MBDB	232
Figure 7.3c	Overlay of chromatograms at 254 nm for MDA, PMA, amphetamine, MDMA, methamphetamine, ketamine, 4-MTA, and MBDB	232
Figure 7.3	Acquired product ion mass spectra for PMA, 4-MTA, MBDB, MDA, amphetamine, MDMA, methamphetamine, and ketamine	233
Figure 7.4	Multiple reaction monitoring chromatograms for MDA, amphetamine, MDMA, methamphetamine, and ketamine	234
Figure 7.5	Ion chromatogram for product ion scan at <i>m/z</i> 122 and acquired mass spectrum for phenylethylamine	235
Figure 7.6	Overlays of extracted ion chromatograms for amphetamine methamphetamine, MDA, MDMA, and ketamine using positive electrospray and atmospheric pressure chemical ionisation	240

List of Tables

Table 3.1	Relative cross-reactivity of common amphetamine-type substances, designer analogues, and putrefactive amines using Bio-Quant Direct ELISA	121
Table 4.1	Mean fluorescence intensities and relative signal reductions for the multiplex Suspension Bead Array analysis of amphetamine and methamphetamine	150
Table 5.1	Selected ion monitoring (SIM) specifications for the extraction and confirmation of amphetamine-type substances from oral fluid using SPE–GC/MS	169
Table 5.2	Instrument and analytical method specifications for the extraction and confirmation of amphetamine-type substances from oral fluid using SPE–GC/MS	170
Table 5.3	Average target ion response and internal standard ratios of five SPE elution reagents for amphetamine-type substances in oral fluid using GC/MS	174
Table 5.4	Average linearity, internal standard ratio, and quantitation of amphetamine-type substances using mixed-mode SPE–GC/MS	176
Table 5.5	Average qualifier ion ratios for select amphetamine-type substances using mixed-mode SPE–GC/MS	180
Table 5.6	Average linearity, internal standard ratio, and quantitation of amphetamine-type substances using Spin-SPE–GC/MS	185
Table 5.7	Average qualifier ion ratios for select amphetamine-type substances using Spin-SPE–GC/MS	189
Table 6.1	Statistical analysis of tune data	203
Table 6.2	Correlation of select tune parameters	207
Table 6.3	Summary of inter-day regression for m/z 69, 219, and 502 abundance and predicted mass abundance at m/z 258, 121, and 135	212
Table 7.1	Retention times and peak-to-peak resolution of ephedrine, phenylethylamine, pseudoephedrine, MDA, amphetamine, MDMA, phentermine, methamphetamine, MDEA, and ketamine	223
Table 7.2	Summary of signal-to-noise ratios observed for positive electrospray and atmospheric pressure chemical ionization modes using ultra-performance™ liquid chromatography-mass spectrometry (UPLC™/MS)	241