Validation of targeted qualitative screen for 134 therapeutic and abused drugs and 7 internal standards by LC MS/MS

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Abstract

This paper describes a liquid chromatography tandem mass spectrometry (LC MS/MS) qualitative screening method for 134 therapeutic and abused drugs and 7 internal standards in whole blood samples. Samples were extracted by protein crash and analyzed on a Waters Acquity I-class Liquid Chromatograph coupled to a Waters TQ-S Tandem Quadrupole Detector. Data acquisition was performed in multiple reaction monitoring (MRM) mode with positive electrospray ionization. Waters TargetLynx™ Application Manager Software was used for sample data processing.

Method validation is the process that is used to verify a method for a particular analysis to ensure that it is appropriate for its anticipated use. Results from method validation are used to judge the quality, reliability and reproducibility of the results. It is an important part of analytical practices (5-6). The validation parameters demonstrated in this study include interference, carryover, ionization suppression/enhancement and limit of detection (LOD). There were no interferences detected in the blood samples, or from the internal standards for the analytes; however, when the samples were spiked with drug mix solutions at high concentrations to determine interference from commonly encountered analytes, there were interferences detected. There was no carryover detected. There were some analytes that

showed ionization suppression or enhancement at their retention times. The LOD study was not completed but an initial trial run was performed and information on further LOD studies is also described.

Introduction

Screening biological samples for a wide range of drugs and toxic compounds is an important task in forensic toxicology. Liquid chromatography–mass spectrometry with tandem mass spectrometry (LC MS/MS) is the most used and effective technique in screening biological samples for drugs. LC MS/MS with MRM offers better sensitivity and selectivity than full scan mode (1-3).

The aim of this study was to validate a LC MS/MS screening method for the determination of 134 therapeutic and abused drugs and 7 internal standards in whole blood samples. The 134 analytes of interest include drugs from various classes: cocaine, amphetamines, opiates, benzodiazepines, fentanyl, and other basic, acidic and neutral drugs. The 7 internal standards include methaqualone-d7, morphine-d6, diazepam-d5, cocaine-d3, amphetamine-d11, fentanyl-d5, and SKF-525A. Seven different internal standards are included so the screening method is useful for the analysis of various classes of drugs at any time.

According to the Scientific Working Group for Forensic Toxicology (SWGTOX), method validation is defined as “the process of performing a set of experiments that reliably estimates the efficacy and reliability of an analytical method or modification to a previously validated method (5).” It must be demonstrated that the method can be used successfully at the level of its intended use. The method’s limitations under normal operating conditions also need to be

identified during validation (4-5). Method validation demonstrates that the method works reliably and is able to provide accurate and consistent results. Method validation is performed when it is necessary to confirm that the method's performance parameters are appropriate for a particular analysis. The parameters that need to be evaluated depend on the method and the situations in which it would be used. Revalidation of methods is performed depending on changes made to the method or laboratory policies (5-6).

Experimental

Method Validation

The method was validated according to the West Virginia Office of Chief Medical Examiner (OCME) Validation SOP, which is based on the SWGTOX Standard Practices for Method Validation in Forensic Toxicology.

Standards

Reference compounds, dissolved in methanol or acetonitrile, were obtained from Cerilliant (Round Rock, Texas), Sigma Aldrich (Saint Louis, Missouri) and Grace (Columbia, Maryland).

Bovine blood and one blank whole human blood sample were obtained from Golden West Biologicals (Temecula, California). A second blank whole human blood sample was obtained from UTAK Laboratories (Valencia, California). Optima grade methanol, acetonitrile, and H_2O were obtained from Fisher Scientific (Pittsburgh, Pennsylvania).

Instrument Parameters

The instrument used was a Waters Acquity I-class Liquid Chromatograph coupled to a Waters TQ-S Tandem Quadrupole Detector. The instrument was operated in ESI+ (electrospray positive mode) and MRM mode. Argon was used as the collision gas. The MRM transitions, cone voltages, collision energies and retention times of the 134 analytes and 7 internal standards are listed in Appendices A and B. Waters TargetLynx™ Application Manager Software was used for sample data processing. Chromatography was performed on a Waters Acquity UPLC HSS C18, 2.1 x 150 mm, 1.8 µm column. The instrument parameters are shown in Table 1. Table 2 shows the inlet method and the linear gradient used.

Table 1. LC MS/MS instrument parameters

Injection Volume	5 µl
Column Temp	40.0 °C
Target sample temp	10.0 °C
Source temp	150 °C
Desolvation temp	350 °C
Cone gas flow	150 L/Hr
Desolvation gas flow	650 L/Hr
Collision gas flow	0.25 ml/Min
Nebuliser gas flow	7.00 bar
Mobile Phase A	Optima grade H_2O with 0.1% formic acid
Mobile Phase B	Optima grade Acetonitrile with 0.1% formic acid

Table 2. Inlet method parameters

Time (min)	Flow rate (µl/min)	% A	% B
Initial	0.450	90.0	10.0
13.00	0.450	28.0	72.0
13.70	0.450	1.0	99.0
14.00	0.450	1.0	99.0
14.10	0.450	90.0	10.0

Extraction Procedure

The extraction used was protein precipitation where 1 mL of ice-cold Acetonitrile: Methanol (85:15) was added to 200 µL sample. The samples were vortex mixed and centrifuged at 32000 x g for 15 minutes. 100 µL of the supernatants were transferred to autosampler vials and 900 µL Optima grade H_2O was added.

Interference Study

SWGTOX describes interferences as "non-targeted analytes (i.e., matrix components, other drugs and metabolites, internal standard, impurities)" that may affect the capability to detect, identify, or quantify the targeted analyte (5). Thus, an interference study is performed to check if there are any other "non-targeted analyte" signals detected, which might lead to false positive results. Interference studies on the matrices, internal standards and analytes were performed to establish that there were no signals detected other than those produced by the target analytes.

Interferences associated with the matrix

An interference study on matrices was performed to ensure that no interferences were detected in negative whole blood samples. Ten negative blood samples (nine human and one bovine) were used in this study. The nine human samples were previously analyzed cases from the WV Office of the Chief Medical Examiner Office Toxicology Laboratory. Details on the blood samples are shown in Table 3. These blood samples had been previously analyzed for drugs by the laboratory and none were detected.

Table 3. Information on the ten negative blood samples

Labeled As:	Description
1	15-0048
2	15-0197
3	15-0203
4	15-0308
5	15-0323
6	15-0511
7	15-0623
8	15-0836
9	15-1126
10	ABP 2000 (bovine blood)

Interferences associated with the internal standards

An interference study on the internal standards was performed, as deuterated drugs can contain the non-labeled drugs as impurities. Seven internal standard solutions were prepared at concentrations shown in Table 4 and the method was tested to ensure that it was free of interference due to internal standards. Bovine blood was spiked with the seven internal standards, extracted and run on the LC MS/MS. The seven internal standards were tested at the concentrations used in routine analysis in the laboratory.

Table 4. Concentration of internal standards used

Internal Standard	Concentration (ng/ml)
Methaqualone-d7	500
Morphine- d6	500
Diazepam- d5	500
Cocaine-d3	50
Amphetamine- d11	5000
Fentanyl-d5	125
SKF-525A	1250

The 134 analytes were divided into three groups according to their expected lower limits observed in casework: the low concentration group (100 ng/mL), the middle concentration group (1000 ng/mL), and the high concentration group (10,000 ng/mL). Analytes in the middle concentration group were further grouped so that each group had 10 to 12 analytes. Bovine blood was spiked with the analytes (concentrations shown in Appendix C, Tables 1 through 5), extracted, and run on the LC MS/MS to ensure that there were no analyte signals detected at the retention times of the internal standards.

Interferences associated with other commonly associated analytes

Seven drug mix solutions (basic mix, opiates mix, benzodiazepines mix, buprenorphine/fentanyl mix, acid/neutral mix, amphetamine mix, and cocaine mix) were prepared in bovine blood, extracted, and run on the LC MS/MS, monitoring the signal of the other analytes to ensure that there was no signal other than for analytes in the mixes. Table 5 shows the analytes present in each drug mix solution and their concentrations.

Table 5. Drug mix solutions used for determining interferences from commonly encountered analytes.

Mix	Analytes and Concentrations
Basic	Amitriptyline, Nortriptyline, Methadone, Sertraline, Paroxetine, Diltiazem, Doxepin, Nordoxepin, Venlafaxine, Cyclobenzaprine, Diphenhydramine, Meperidine, Dextromethorphan, Bupropion, Chlorpheniramine, Fluoxetine, Verapamil/Norverapamil, O-desmethylvenlafaxine, Tramadol, N-desmethyltramadol, Citalopram, Desmethylcitalopram, Doxylamine, Brompheniramine, Clomipramine, Zolpidem, Mirtazapine, Olanzapine, Promethazine (at 5,000 ng/ml)

Opiates	Morphine, Codeine, Hydrocodone, Hydromorphone, Naloxone, Acetylcodeine, Oxycodone, Oxymorphone, Dihydrocodeine, 6-monoacetylmorphine (at 5,000 ng/ml)
	Morphine-d6 (at 500 ng/ml)
Benzodiazepines	Diazepam, Nordiazepam, 7-aminoclonazepam, Temazepam, Alprazolam, Clonazepam, Lorazepam, Midazolam, Chlordiazepoxide, Demoxepam, Oxazepam (at 5,000 ng/ml)
	Diazepam-d5 (at 500 ng/ml)
Buprenorphine/ Fentanyl	Fentanyl, Norfentanyl, Buprenorphine, Norbuprenorphine, Acetyl fentanyl, Oxymorphone (at 5,000 ng/ml)
	Fentanyl-d5 (at 500 ng/ml)
Acid/Neutral	Acetaminophen, Ibuprofen, Butalbital, Phenytoin (at 50,000 ng/mL)
Amphetamine	Phenethylamine, Pseudoephedrine, Amphetamine, MDA, MDMA, Methamphetamine, Phentermine (at 5,000 ng/ml)
	Amphetamine-d11 (at 500 ng/ml)
Cocaine	Cocaine, Ecgonine methyl ester, Benzoylecgonine (at 5,000 ng/ml)
	Cocaine-d3 (at 500 ng/ml)

Carryover Study

According to SWGTOX, carryover is defined as "the appearance of unintended analyte signal in subsequent samples after the analysis of a positive sample." An analyte that is not present in a sample might be inadvertently detected due to carryover from previously run positive samples, creating a false positive result (5). The carryover study was performed to ensure that there were no analytes detected in the negative blood samples injected after spiked blood samples.

Analytes were grouped similarly to the groups in the interference study, with the low concentration (100 ng/mL), the middle concentration group (1000 ng/mL), and the high concentration group (10,000 ng/mL). The seven internal standards were grouped together. Bovine blood was spiked with each analyte in the group at the concentrations listed, extracted

and run on the LC MS/MS. Samples were run in triplicate with a blank extracted matrix run in between. An example sample table is shown in Figure 1. Tables 1 through 5 in Appendix D show the groupings and concentrations.

1	blank
2	5_29_15_carryover_study_blank1
3	5_29_19_carryover_study_LC1A_1
4	5_29_15_carryover_study_blank2
5	5_29_19_carryover_study_LC1A_2
6	5_29_15_carryover_study_blank3
7	5_29_19_carryover_study_LC1A_3
8	5_29_15_carryover_study_blank4
9	5_29_19_carryover_study_HC2A_1
10	5_29_15_carryover_study_blank5
11	5_29_19_carryover_study_HC2A_2
12	5_29_15_carryover_study_blank6
13	5_29_19_carryover_study_HC2A_3
14	5_29_15_carryover_study_blank7

Figure 1. An example of the sample table for the carryover study

Ionization Suppression/Enhancement Study - Post-Column Infusion

Ionization suppression/enhancement is defined as the "direct or indirect alteration or interference in the instrument response due to the presence of co-eluting compounds' (5). Biological samples may contain compounds that co-elute, causing suppression or an enhancement of ionization at a particular analyte's retention time. This may affect parameters like LOD, thus it is important to know if and how ionization suppression or enhancement affects the analytes (4). This study was performed to determine the presence of any suppression or enhancement of the 7 internal standards and the 134 analytes of interest.

Isobars are defined as two species having the same atomic weight. In LC MS/MS, two species may undergo similar fragmentation and produce identical MRMs; therefore, care must be taken to ensure that the presence of an isobar is not mistaken for a compound of interest.

Extractions prepared from ten negative blood samples (9 previously analyzed case samples and one bovine blood) were injected on the LC MS/MS while simultaneously infusing neat solutions of an internal standard mix, low concentration analyte mix, and high concentration analyte mix from the fluidics system of the LC MS/MS one solution at a time. The composition and concentrations of these mixes are shown in Tables 1 through 5 in Appendix E.

Limit of Detection (LOD) Study

The limit of detection is defined as "an estimate of the lowest concentration of an analyte in a sample that can be reliably differentiated from blank matrix and identified by the analytical method" (5). LOD establishes the lower concentration limit for qualitatively evaluating the presence or absence of an analyte in the samples. The LOD was determined by ensuring that all the transitions were present and that peaks were well defined.

An initial trial run was performed to estimate each analyte's LOD. Concentrations tested ranged from 50 ng/mL to 1 ng/mL in whole blood. Again, mixes of the analytes were prepared by combining a maximum of ten analytes. The groupings of the analytes are shown in Table 1 in Appendix F.

Results and Discussion

Interference Study

No interferences were detected from the matrix or internal standards. For interferences associated with other commonly encountered analytes, interferences were detected in the standard mixes. Those interferences are listed in Table 6.

From the results, it is concluded that these interferences occur at 5,000 ng/mL. The deuterated internal standards in the mixes can be excluded as sources of interferences as the results from previous interference experiment demonstrated that there were no interferences. Further studies should be performed to determine which specific analytes are responsible for the interferences and at what concentration of these analytes results in no interferences.

In the cocaine mix, a signal for atropine was detected at 2.91 minutes, the retention time of benzoylecgonine. However, atropine is an isobar to benzoylecgonine, which is present in the cocaine mix, and both atropine ($C_{17}H_{23}NO_3$) and benzoylecgonine ($C_{16}H_{19}NO_4$) have the same molecular weight of 289 g/mol and produce similar MRMs. Because they were separated chromatographically they could be identified correctly and not as interferents.

Table 6. Interferences detected in the drug mix solutions of commonly encountered analytes.

Solution Mix	Interference Detected
Basic Mix	Interference with imipramine was detected
Opiate Mix	No interferences detected
Cocaine Mix	No interferences detected.
Benzodiazepines Mix	Interference with meperidine was detected
Amphetamine Mix	No interferences detected
Buprenorphine/Fentanyl Mix	Interferences with MDMA and MDA were detected
Acid/Neutral mix	No interferences detected

Carryover Study

No carryover was observed in the negative blood samples that were run in between the spiked blood samples.

Ionization Suppression/Enhancement Study - Post-Column Infusion

Table 7 summarizes the ionization suppression and enhancement observed in the blank samples. Figures 2 and 3 show examples of enhancement and suppression for methaqualone-d7 and ecgonine methyl ester, respectively.

Table 7. Summary of results from ionization suppression/enhancement study

Group	Concentration	Sample	Analyte	Suppression/ Enhancement
Internal Standard Mix	-	0203,0308,0323,0511, 0623,0836,1126, Bovine	Methaqualone- d7	Enhancement
LC1A	High (100 ng/mL)	-	-	-
	Low (1 ng/mL)	-	-	-
HC2A	High (10,000 ng/mL)	-	-	-
	Low (100 ng/mL)	-	-	-
MC3A	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC3B	High (1000 ng/mL)	0048,0197,0203,0308, 0323,0511,0623,0836, 1126, Bovine	Tramadol	Suppression
	Low (10 ng/mL)	0197,0203,0308,0323, 0511,0623,0836,1126, Bovine	Tramadol	Suppression
MC3C	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	0048,0197,0203,0308, 0323,0836,1126,	mCPP	Suppression
MC3D	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC3E	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC4A	High (1000 ng/mL)	0197,0203,0308,0323,	Ecgonine methyl	Suppression

		0511, Bovine	ester	
	Low (10 ng/mL)	-	-	-
MC4B	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC4C	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC4D	High (1000 ng/mL)	-	-	-
	Low (10 ng/mL)	-	-	-
MC4E	High (1000 ng/mL)	0048,0197,0203,0308, 0323,0511,0623,0836, 1126, Bovine	Zaleplon	Enhancement
	Low (10 ng/mL)	-	-	-
MC4F	High (1000 ng/mL)	0048,0197,0203,0308, 0323,0511,0623,0836, 1126, Bovine	Venlafaxine	Suppression
	Low (10 ng/mL)	0048,0197,0203,0323, 0511,0836,	Venlafaxine	Suppression

Ionization enhancement observed for methaqualone-d7 has no negative effect on the method since it is an internal standard that is always used at 500 ng/mL. For zaleplon, ionization enhancement was observed at the high concentration. Thus, the observed LOD may be lower than expected. For tramadol, mCPP, ecgonine methyl ester, and venlafaxine, ion suppression was consistently observed at both high and low concentrations. Thus, the observed LOD may be higher than expected.

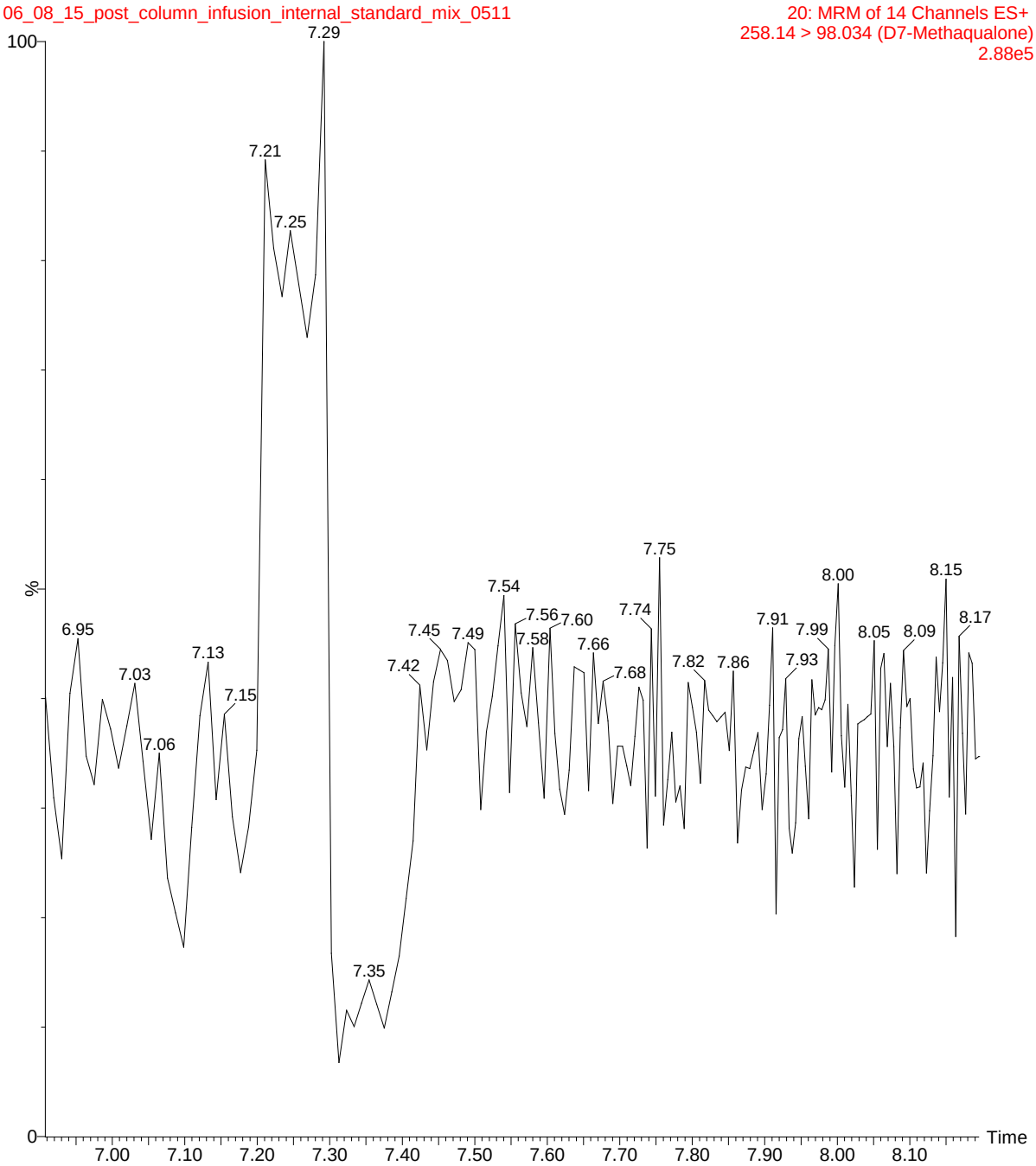

Figure 2. Chromatogram of bovine blood sample 0511 showing ionization enhancement of methaqualone-d7 at 7.29 minutes

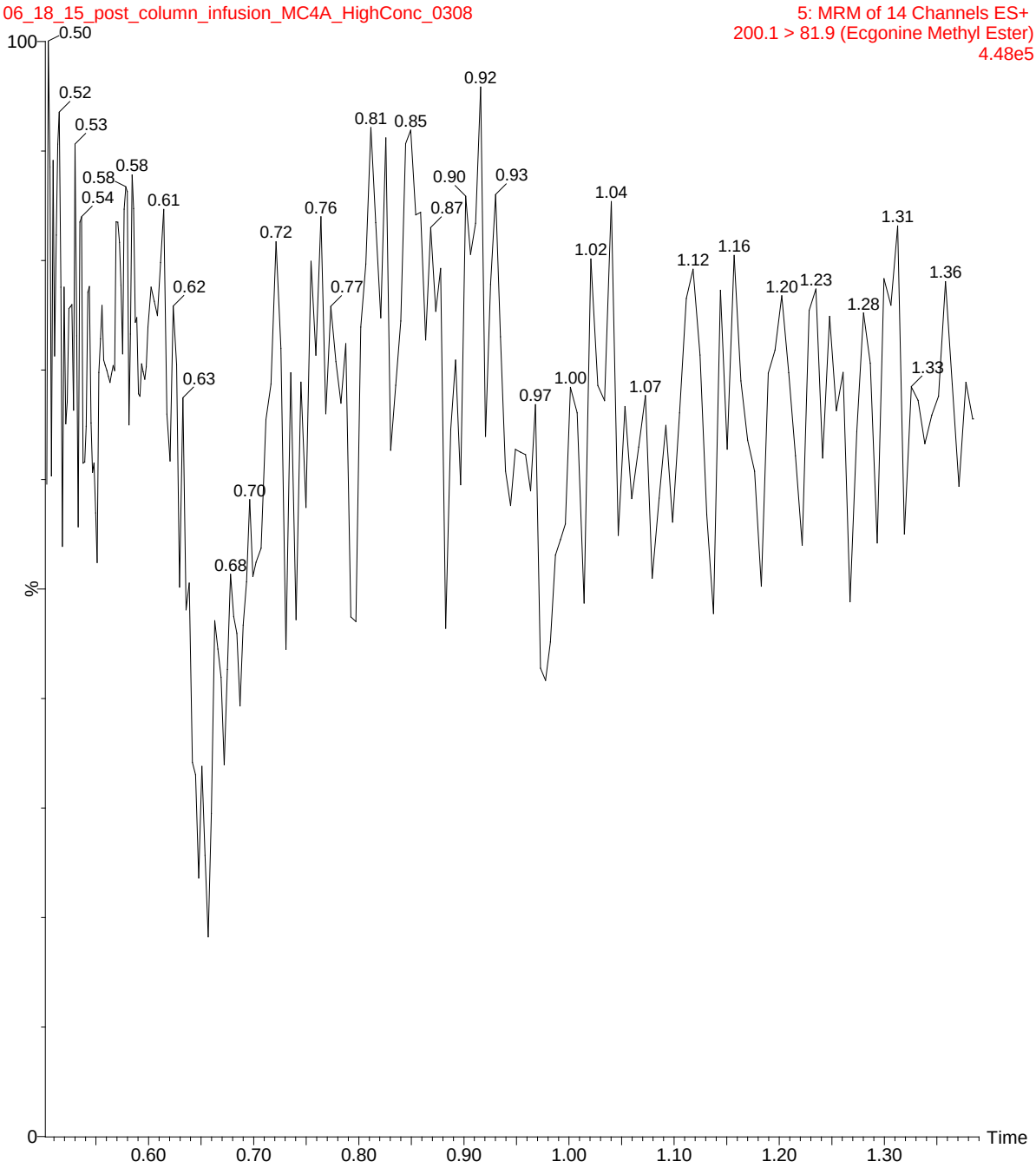

Figure 3. Chromatogram of blood sample 0308 showing ionization suppression of ecgonine methyl ester at 0.70 minutes

Limit of Detection (LOD) Study

Table 8 summarizes the approximated LODs for the 134 analytes determined from the initial trial run. Norfentanyl, a drug typically encountered in casework at very low concentrations, was expected to be detected at a lower concentration, and yet was not detected until 50 ng/mL. Thus, modification of the instrument parameters may be necessary to improve the detection of norfentanyl. The approximated LOD for tramadol and ecgonine methyl ester from the initial trial run was less than 50 ng/mL, and for mCPP it was less than 30 ng/mL. It was anticipated that these drugs would be detected at lower concentrations, but the higher LODs may be explained by the ion suppression that was observed.

Table 8. LOD initial trial results

Group	**Analyte**	**LOD approximation (ng/mL)**	**Group**	**Analyte**	**LOD approximation (ng/mL)**
LC1A	Acetyl Fentanyl	1	MC3E	Thioridazine	30
LC1A	Fentanyl	1	MC3F	mCPP	30
LC1A	Norfentanyl	< 50	MC3F	Trazodone	2 to 3
LC1A	Buprenorphine	6	MC3F	Trifluoperazine	< 50
LC1A	Norbuprenorphine	4 to 5	MC3F	Lidocaine	7
LC1A	Sufentanil	5	MC3F	Diacetylmorphine	8
LC1A	Alfentanil	4	MC4A	Oxymorphone	9 to 10
HC2A	Acetaminophen	< 50	MC4A	Butorphanol	30
HC2A	Carbamazepine	9	MC4A	Dextromethorphan	8
HC2A	Gabapentin	5	MC4A	Propoxyphene	20 to 30
HC2A	Lamotrigine	7	MC4A	Naloxone	9
HC2A	Primidone	7	MC4A	Cocaine	4 to 5
HC2A	Phenytoin	8	MC4A	Benzoylecgonine	5 to 6
HC2A	Levetiracetam	< 50	MC4A	Ecgonine methyl ester	< 50
HC2A	Meprobamate	< 50	MC4A	Methamphetamine	20 to 30
HC2A	Methocarbamol	< 50	MC4A	Amphetamine	< 50

HC2A	Oxcarbazepine	4	MC4B	Pseudoephedrine	<50
MC3A	Oxycodone	8	MC4B	α PVP	4
MC3A	Hydrocodone	7	MC4B	MDMA	6 to 10
MC3A	Hydromorphone	10	MC4B	MDA	20
MC3A	Dihydrocodeine	6	MC4B	Ethylone	5
MC3A	Codeine	10	MC4B	Mephedrone	< 50
MC3A	Acetyl codeine	7	MC4B	Methcathinone	30 to 40
MC3A	Morphine	4	MC4B	Benzylpiperazine	40
MC3A	6MAM	5	MC4B	Phentermine	<50
MC3A	Methadone	< 50	MC4C	Methylphenidate	4
MC3A	EDDP	7	MC4C	Phenmetrazine	< 50
MC3B	Meperidine	1 to 3	MC4C	Oxazepam	5 to 6
MC3B	Tramadol	< 50	MC4C	7aminoclonazepam	20
MC3B	Tapentadol	1 to 4	MC4C	Flunitrazepam	3 to 4
MC3B	Pentazocine	4	MC4C	Triazolam	5
MC3B	MDPV	2 to 4	MC4C	Phenazepam	5 to 6
MC3B	Phenylpropanolamine	< 50	MC4C	Clobazam	20
MC3B	Phendimetrazine	< 50	MC4C	Cyclobenzaprine	< 50
MC3B	Caffeine	< 50	MC4C	Diphenhydramine	40
MC3B	Nicotine	20	MC4D	Chlorpheniramine	10
MC3B	Alprazolam	2 to 4	MC4D	Promethazine	10
MC3C	Diazepam	8	MC4D	Loratadine	40 to 50
MC3C	Nordiazepam	5 to 8	MC4D	Amitriptyline	20
MC3C	Temazepam	8	MC4D	Clomipramine	6
MC3C	Chlordiazepoxide	3 to 6	MC4D	Fluoxetine	<50
MC3C	Demoxepam	8 to 10	MC4D	Bupropion	10
MC3C	Clonazepam	3 to 6	MC4D	Citalopram	3
MC3C	Lorazepam	10	MC4D	Paroxetine	<50
MC3C	Midazolam	20	MC4D	Sertraline	<50
MC3C	Flurazepam	2 to 6	MC4E	Mirtazapine	5
MC3C	Nitrazepam	20	MC4E	Duloxetine	<50
MC3D	Orphenadrine	7 to 10	MC4E	Trimipramine	10
MC3D	Estazolam	< 50	MC4E	Zopiclone	10
MC3D	Brompheniramine	10	MC4E	Zaleplon	<50
MC3D	Meclizine	10	MC4E	Ketamine	6
MC3D	Nortriptyline	4 to 5	MC4E	Verapamil	5
MC3D	Imipramine	5 to 6	MC4E	Diltiazem	2
MC3D	Desipramine	9	MC4E	PCP	40

MC3D	Doxepin	8 to 9	MC4E	Olanzapine	10
MC3D	Amoxepine	< 50	MC4F	Risperidone	20
MC3D	Nefazodone	9	MC4F	Clonidine	10
MC3E	Fluvoxamine	8	MC4F	Haloperidol	1
MC3E	Zolpidem	2 to 3	MC4F	Clozapine	6
MC3E	Atropine	3	MC4F	Loxapine	10 to 20
MC3E	Propranolol	5 to 6	MC4F	Benztropine	7
MC3E	Atenolol	3	MC4F	Fluphenazine	10
MC3E	Metoprolol	3 to 4	MC4F	Doxylamine	2
MC3E	Albuterol	20 to 30	MC4F	Venlafaxine	10
MC3E	Quetiapine	5	MC4F	Hydroxyzine	2
MC3E	Chlorpromazine	8			

Further studies to determine LOD would involve preparing spiked blood solutions of the analytes at narrower concentration ranges to find the lowest concentration at which the analytes are consistently detected. Table 2 in Appendix F shows the regrouping of the analytes according to the new ranges. Blood would then be spiked with the analytes at these concentrations and extracted three times per day on each of three days. The LOD of the analytes would be concluded from the data collected from the nine total runs.

Conclusion

A LC MS/MS qualitative screening method for 134 therapeutic and abused drugs and 7 internal standards in blood samples was validated according to guidelines set forth by SWGTOX. The method produced reliable and reproducible data and thus is a viable method in the forensic toxicology laboratory. Further studies for LOD, as described in the discussion, should be performed to complete the validation.

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Appendix A

MRM transitions, cone voltages, and collision energies of the 134 drugs and 7 internal standards

Compound	Parent	Daughter	Cone Voltage (V)	Collision Energy (eV)
Amphetamine	136.10	91.02	20.00	14.00
	136.10	119.08	20.00	8.00
Amphetamine- d11	147.14	70.01	20.00	32.00
	147.14	97.05	20.00	16.00
	147.14	130.09	20.00	10.00
Phenylpropanolamine	152.04	115.10	15.00	25.00
	152.04	117.05	15.00	16.00
	152.04	134.10	15.00	10.00
Codeine	300.10	152.90	50.00	44.00
	300.10	165.08	50.00	28.00
	300.10	215.10	50.00	38.00
Olanzapine	313.20	84.08	35.00	22.00
	313.20	256.12	35.00	28.00
Naloxone	328.17	212.15	35.00	48.00
	328.17	253.16	35.00	28.00
	328.17	268.31	35.00	20.00
Methamphetamine	150.07	91.07	25.00	18.00
	150.07	119.03	25.00	12.00
Phendimetrazine	192.17	44.93	40.00	28.00
	192.17	117.04	40.00	24.00
	192.17	148.37	40.00	24.00
Hydrocodone	300.10	127.90	55.00	50.00
	300.10	170.90	55.00	44.00
	300.10	199.06	55.00	28.00
6-MAM[1]	328.10	165.08	50.00	46.00
	328.10	193.10	50.00	26.00
	328.10	211.04	50.00	28.00
Phentermine	150.08	91.08	16.00	16.00
	150.08	133.04	16.00	10.00
Mephedrone	178.13	119.05	20.00	24.00
	178.13	145.03	20.00	24.00
	178.13	160.00	20.00	24.00
Lidocaine	235.22	57.99	25.00	40.00
	235.22	86.04	25.00	24.00
Ketamine	238.20	125.10	30.00	26.00
	238.20	220.20	30.00	15.00

[1]6-MAM (6-monoacetylmorphine)

Atropine	290.10	76.97	55.00	56.00
	290.10	92.99	55.00	32.00
	290.10	124.05	55.00	34.00
Acetaminophen	152.07	65.02	30.00	30.00
	152.07	92.83	30.00	24.00
	152.07	110.02	30.00	24.00
Methcathinone	164.03	76.91	25.00	34.00
	164.03	131.11	25.00	24.00
	164.03	145.98	25.00	22.00
Albuterol	240.11	57.05	30.00	34.00
	240.11	120.92	30.00	34.00
	240.11	148.01	30.00	26.00
Atenolol	267.20	74.10	40.00	22.00
	267.20	145.10	40.00	25.00
Oxymorphone	302.10	198.10	35.00	50.00
	302.10	227.10	35.00	32.00
	302.10	242.10	35.00	22.00
Nicotine	163.10	105.96	35.00	22.00
	163.10	116.97	35.00	22.00
	163.10	130.06	35.00	24.00
Benzylpiperazine	177.15	56.00	35.00	28.00
	177.15	84.99	35.00	24.00
	177.15	90.98	35.00	26.00
Ecgonine methyl ester	200.10	81.90	40.00	26.00
	200.10	91.06	40.00	26.00
	200.10	181.90	0.003	40.00
Morphine	286.10	152.80	0.003	55.00
	286.10	165.08	0.003	55.00
	286.10	201.10	0.003	55.00
Morphine-d6	292.19	64.07	0.003	55.00
	292.19	152.94	0.003	55.00
Pseudoephedrine	166.07	117.01	0.003	20.00
	166.07	133.01	0.003	20.00
Levetiracetam	171.09	68.96	0.003	20.00
	171.09	125.96	0.003	20.00
Gabapentin	172.05	54.95	0.003	30.00
	172.05	137.00	0.003	30.00
	172.05	154.11	0.003	30.00
Clonidine	229.98	58.99	0.003	55.00
	229.98	159.86	0.003	55.00
	229.98	212.93	0.003	55.00
Hydromorphone	286.10	152.80	0.003	50.00
	286.10	157.09	0.003	50.00

	286.10	185.04	0.003	50.00
Dihydrocodeine	302.10	128.10	0.003	50.00
	302.10	171.10	0.003	50.00
	302.10	199.09	0.003	50.00
Phenmetrazine	178.05	91.04	30.00	32.00
	178.05	132.55	30.00	26.00
MDA[2]	180.06	105.02	22.00	20.00
	180.06	133.10	22.00	18.00
	180.06	163.10	22.00	10.00
Ethylone	222.15	72.05	25.00	24.00
	222.15	146.18	25.00	24.00
	222.15	174.17	25.00	24.00
Oxycodone	316.10	212.20	40.00	40.00
	316.10	241.10	40.00	34.00
	316.10	256.10	40.00	18.00
MDMA[3]	194.09	105.04	20.00	22.00
	194.09	133.00	20.00	20.00
	194.09	163.04	20.00	14.00
Caffeine	195.10	69.05	30.00	32.00
	195.10	110.00	30.00	24.00
	195.10	138.06	30.00	22.00
Doxylamine	271.20	167.10	30.00	36.00
	271.20	182.10	30.00	15.00
mCPP[4]	197.09	43.98	40.00	22.00
	197.09	118.90	40.00	26.00
	197.09	154.04	40.00	22.00
Primidone	219.20	91.20	29.00	25.00
	219.20	162.20	29.00	12.00
Tramadol	264.11	58.03	34.00	12.00
	264.11	93.30	34.00	15.00
	264.11	246.13	34.00	10.00
Metoprolol	268.29	56.09	30.00	32.00
	268.29	116.17	30.00	22.00
Tapentadol	222.15	46.00	40.00	24.00
	222.15	107.07	40.00	30.00
	222.15	121.06	40.00	22.00
Methylphenidate	234.20	56.10	31.00	40.00
	234.20	84.10	31.00	18.00
Acetylcodeine	342.10	165.07	55.00	60.00
	342.10	197.10	55.00	34.00

[2] MDA (3, 4-Methylenedioxyamphetamine)
[3] MDMA (3, 4-methylenedioxy-methamphetamine)
[4] mCPP (meta-Chlorophenylpiperazine)

	342.10	225.10	55.00	32.00
Diacetylmorphine	370.18	58.03	50.00	38.00
	370.18	165.00	50.00	62.00
	370.18	165.00	50.00	50.00
	370.18	211.02	50.00	40.00
Meprobamate	219.20	97.10	22.00	20.00
	219.20	97.20	22.00	15.00
	219.20	158.20	22.00	8.00
Pentazocine	286.15	69.10	45.00	36.00
	286.15	217.88	45.00	30.00
Loxapine	328.19	84.01	40.00	26.00
	328.19	192.99	40.00	54.00
Trazodone	328.19	271.10	40.00	24.00
	372.15	78.06	55.00	48.00
	372.15	147.93	55.00	38.00
	372.15	176.16	55.00	30.00
α PVP	232.19	91.04	40.00	28.00
	232.19	104.97	40.00	28.00
	232.19	126.07	40.00	28.00
Bupropion	240.15	57.07	20.00	24.00
	240.15	130.95	20.00	36.00
Chlordiazepoxide	300.05	165.07	30.00	55.00
	300.05	227.07	30.00	26.00
	300.05	241.04	30.00	16.00
Zolpidem	308.23	92.08	50.00	62.00
	308.23	235.28	50.00	40.00
	308.23	263.18	50.00	32.00
Clozapine	327.20	84.00	45.00	32.00
	327.20	192.01	45.00	52.00
	327.20	270.11	45.00	30.00
Risperidone	411.20	68.98	40.00	50.00
	411.20	110.04	40.00	46.00
	411.20	191.10	40.00	26.00
Norfentanyl	233.15	56.02	10.00	24.00
	233.15	84.09	10.00	30.00
	233.15	177.16	10.00	14.00
7-aminoclonazepam	286.09	121.07	25.00	20.00
	286.09	222.08	25.00	40.00
	286.09	250.10	25.00	20.00
Acetyl fentanyl	323.18	79.03	10.00	52.00
	323.18	105.09	10.00	36.00
	323.18	188.17	10.00	22.00
Fentanyl-d5	342.22	105.09	42.00	38.00

	342.22	188.16	42.00	20.00
Carbamazepine	237.07	165.04	40.00	42.00
	237.07	179.03	40.00	36.00
	237.07	194.06	40.00	18.00
Nortriptyline	264.31	91.04	25.00	24.00
	264.31	105.04	25.00	24.00
	264.31	233.22	25.00	18.00
Imipramine	281.31	58.04	25.00	35.00
	281.31	86.09	25.00	20.00
	281.31	208.15	25.00	32.00
Duloxetine	298.25	44.05	10.00	16.00
	298.25	154.10	10.00	6.00
Methocarbamol	242.07	56.98	25.00	36.00
	242.07	77.10	25.00	46.00
	242.07	118.10	25.00	20.00
Meperidine	248.21	70.03	40.00	38.00
	248.21	174.16	40.00	24.00
	248.21	220.16	40.00	24.00
Chlorpheniramine	275.13	117.85	30.00	56.00
	275.13	167.06	30.00	54.00
	275.13	230.08	30.00	24.00
MDPV[5]	276.21	126.12	35.00	30.00
	276.21	135.04	35.00	32.00
	276.21	175.12	35.00	22.00
Cocaine	304.09	82.01	40.00	26.00
	304.09	105.02	40.00	26.00
	304.09	182.10	40.00	26.00
Cocaine-d3	307.15	85.08	35.00	32.00
	307.15	104.99	35.00	32.00
PCP[6]	244.24	86.10	15.00	26.00
	244.24	91.05	15.00	26.00
	244.24	159.00	15.00	28.00
Propranolol	260.28	56.09	30.00	32.00
	260.28	116.10	30.00	20.00
Quetiapine	384.20	209.93	45.00	50.00
	384.20	221.05	45.00	42.00
	384.20	253.06	45.00	28.00
Oxcarbazepine	253.01	180.00	35.00	30.00
	253.01	208.06	35.00	24.00
Diphenhydramine	256.22	127.90	15.00	54.00
	256.22	152.04	15.00	46.00
	256.22	167.07	15.00	24.00
Citalopram	325.32	58.04	40.00	35.00

[5] MDPV (methylenedioxypyrovalarone)

[6] PCP (Phencyclidine)

	325.32	109.06	40.00	32.00
	325.32	262.20	40.00	20.00
Flurazepam	388.17	99.97	40.00	36.00
	388.17	108.95	40.00	54.00
	388.17	315.13	40.00	22.00
Phenytoin	253.14	76.99	25.00	52.00
	253.14	104.00	25.00	34.00
	253.14	182.15	25.00	26.00
Cyclobenzaprine	276.08	205.21	30.00	50.00
	276.08	215.10	30.00	50.00
	276.08	216.11	30.00	22.00
Fluvoxamine	319.20	71.20	29.00	17.00
	319.20	200.20	29.00	15.00
Hydroxyzine	375.20	166.00	20.00	35.00
	375.20	173.15	20.00	14.00
	375.20	201.07	20.00	20.00
Lamotrigine	255.94	58.02	60.00	38.00
	255.94	144.89	60.00	50.00
	255.94	210.93	60.00	24.00
Mirtazapine	266.08	71.96	40.00	24.00
	266.08	195.04	40.00	24.00
	266.08	209.10	40.00	24.00
Benzoylecgonine	290.09	77.01	48.00	46.00
	290.09	105.03	48.00	28.00
	290.09	168.08	48.00	16.00
Zopiclone	389.24	112.00	20.00	54.00
	389.24	138.88	20.00	60.00
	389.24	245.11	20.00	28.00
Methaqualone-d7	258.14	70.05	50.00	55.00
	258.14	98.03	50.00	44.00
	258.14	139.09	50.00	30.00
Alprazolam	309.05	204.90	50.00	44.00
	309.05	274.10	50.00	24.00
	309.05	281.05	50.00	28.00
Clomipramine	315.11	57.96	35.00	42.00
	315.11	86.02	35.00	24.00
Triazolam	343.12	239.00	55.00	50.00
	343.12	308.17	55.00	36.00
	343.12	315.08	55.00	32.00
Trifluoperazine	408.12	70.02	40.00	48.00
	408.12	140.35	40.00	52.00
	408.12	141.20	40.00	35.00
Desipramine	267.31	72.10	25.00	18.00
	267.31	208.18	25.00	24.00

Orphenadrine	270.19	140.97	20.00	52.00
	270.19	165.97	20.00	34.00
	270.19	181.07	20.00	24.00
Demoxepam	287.00	104.90	35.00	20.00
	287.00	180.00	35.00	20.00
	287.00	268.90	10.00	25.00
Paroxetine	330.25	44.05	40.00	28.00
	330.25	70.02	40.00	34.00
	330.25	192.21	40.00	24.00
Sufentanil	387.22	110.97	40.00	48.00
	387.22	238.16	40.00	24.00
	387.22	355.25	40.00	24.00
Nordiazepam	271.02	139.90	20.00	26.00
	271.02	165.02	20.00	26.00
	271.02	208.05	20.00	24.00
Clonazepam	316.07	206.90	50.00	26.00
	316.07	214.02	50.00	32.00
	316.07	241.10	50.00	32.00
Lorazepam	321.04	229.09	40.00	32.00
	321.04	275.05	40.00	22.00
	321.04	303.01	40.00	18.00
Fluphenazine	438.22	70.01	55.00	58.00
	438.22	143.12	55.00	40.00
	438.22	171.17	55.00	32.00
Dextromethorphan	272.23	147.13	45.00	32.00
	272.23	171.08	45.00	42.00
	272.23	213.17	45.00	32.00
Midazolam	326.11	222.91	45.00	55.00
	326.11	249.23	45.00	44.00
	326.11	291.09	45.00	28.00
Fentanyl	337.20	79.02	45.00	52.00
	337.20	105.08	45.00	44.00
	337.20	188.10	45.00	22.00
Alfentanil	417.27	98.99	35.00	44.00
	417.27	197.10	35.00	34.00
	417.27	268.19	35.00	24.00
EDDP	278.14	186.03	50.00	40.00
	278.14	234.07	50.00	32.00
	278.14	249.17	50.00	22.00
Promethazine	285.05	70.98	30.00	50.00
	285.05	86.03	30.00	24.00
	285.05	197.95	30.00	30.00
Haloperidol	376.16	95.01	45.00	64.00

	376.16	123.01	45.00	46.00
	376.16	165.06	45.00	26.00
Venlafaxine	278.31	58.05	30.00	24.00
	278.31	121.05	30.00	35.00
	278.31	147.09	30.00	28.00
Brompheniramine	319.14	118.23	25.00	60.00
	319.14	167.03	25.00	52.00
	319.14	274.04	25.00	22.00
LSD[7]	324.20	208.20	40.00	33.00
	324.20	223.10	40.00	28.00
Butorphanol	328.17	55.95	40.00	46.00
	328.17	69.04	40.00	46.00
	328.17	310.27	40.00	22.00
Norbuprenorphine	414.20	83.05	55.00	58.00
	414.20	101.10	55.00	44.00
	414.20	187.10	55.00	52.00
Amitriptyline	278.31	84.15	35.00	30.00
	278.31	91.05	35.00	26.00
	278.31	105.11	35.00	28.00
Nitrazepam	282.09	180.03	50.00	44.00
	282.09	207.23	50.00	34.00
	282.09	236.20	50.00	24.00
Benztropine	308.27	142.09	55.00	40.00
	308.27	151.99	55.00	56.00
	308.27	167.10	55.00	32.00
Prochlorperazine	374.05	70.01	50.00	50.00
	374.05	113.05	50.00	34.00
	374.05	141.12	50.00	24.00
Doxepin	280.31	58.05	35.00	26.00
	280.31	84.14	35.00	28.00
	280.31	107.05	35.00	28.00
Amoxepine	314.07	70.00	50.00	62.00
	314.07	192.85	50.00	56.00
Diltiazem	415.24	108.95	40.00	64.00
	415.24	150.03	40.00	52.00
	415.24	178.02	40.00	34.00
Buprenorphine	468.30	55.10	60.00	56.00
	468.30	83.80	60.00	60.00
	468.30	414.20	60.00	46.00
Diazepam	285.03	154.02	50.00	26.00
	285.03	193.08	50.00	38.00
	285.03	222.07	50.00	38.00
Diazepam-d5	290.14	154.15	50.00	30.00

[7] LSD (Lysergic acid diethylamide)

	290.14	198.12	50.00	34.00
Clobazam	301.08	76.98	40.00	64.00
	301.08	224.04	40.00	36.00
	301.08	259.06	40.00	28.00
Phenazepam	348.96	104.21	40.00	54.00
	348.96	183.97	40.00	30.00
	348.96	206.15	40.00	32.00
Meclizine	391.26	165.91	30.00	40.00
	391.26	201.07	30.00	24.00
Oxazepam	287.08	269.06	30.00	12.00
	287.10	163.00	30.00	30.00
	287.10	241.07	30.00	24.00
Trimipramine	295.16	58.02	35.00	34.00
	295.16	100.02	35.00	24.00
	295.16	192.85	35.00	56.00
Methadone	310.20	56.98	25.00	30.00
	310.20	104.97	25.00	30.00
Fluoxetine	310.21	44.01	25.00	14.00
	310.21	148.13	25.00	8.00
Estazolam	295.02	86.89	30.00	48.00
	295.02	112.02	30.00	54.00
	295.02	117.23	30.00	48.00
	295.02	205.20	30.00	50.00
Sertraline	306.19	129.05	20.00	35.00
	306.19	159.05	20.00	30.00
	306.19	275.12	20.00	14.00
Chlorpromazine	319.10	58.20	45.00	45.00
	319.10	86.10	45.00	30.00
Nefazodone	470.26	56.01	55.00	64.00
	470.26	274.21	55.00	34.00
Temazepam	301.10	177.03	35.00	40.00
	301.10	255.10	35.00	20.00
	301.10	283.07	35.00	12.00
Flunitrazepam	314.04	183.04	50.00	58.00
	314.04	239.15	50.00	44.00
	314.04	268.06	50.00	30.00
SKF-525A	354.15	90.98	40.00	44.00
	354.15	104.98	40.00	30.00
	354.15	167.03	40.00	26.00
Thioridazine	371.23	58.11	35.00	42.00
	371.23	98.07	35.00	36.00
	371.23	126.11	35.00	28.00
Loratidine	383.16	258.21	45.00	62.00
	383.16	267.02	45.00	40.00

	383.16	337.19	45.00	22.00
Zaleplon	306.14	118.69	50.00	48.00
	306.14	236.08	50.00	30.00
	306.14	264.13	50.00	22.00
Propoxyphene	340.18	58.03	20.00	24.00
	340.18	91.10	20.00	36.00
Verapamil	455.38	150.15	50.00	54.00
	455.38	165.06	50.00	36.00
	455.38	303.24	50.00	32.00

Appendix B

Retention Times

Analyte	Retention Time (min)	Analyte	Retention Time (min)
Methaqualone-d7	7.29	Naloxone	1.70
Morphine-d6	1.02	Acetaminophen	1.60
Diazepam-d5	8.65	Cocaine	3.98
Cocaine-d3	4.01	Benzoylecgonine	2.91
Amphetamine-d11	2.08	Ecgonine methyl ester	0.70
Fentanyl-d5	5.34	Methamphetamine	2.37
SKF-525A	8.43	Amphetamine	2.13
Acetyl fentanyl	4.52	Ephedrine/Pseudoephedrine	1.78
Fentanyl	5.34	MDPV	3.95
Norfentanyl	2.97	α PVP	3.79
Buprenorphine	5.81	MDMA	2.42
Norbuprenorphine	4.40	MDA	2.20
Diacetylmorphine	3.69	Ethylone	2.23
Codeine	1.77	Mephedrone	2.73
Acetylcodeine	3.65	Lidocaine	2.91
Morphine	1.02	Phenylpropanolamine	1.52
6-MAM	2.12	Methcathinone	1.78
Oxycodone	2.10	Benzylpiperazine	0.93
Oxymorphone	1.14	Phentermine	2.58
Hydrocodone	2.29	Methylphenidate	3.56
Hydromorphone	1.29	Phendimetrazine	2.33
Dihydrocodeine	1.68	Phenmetrazine	2.27
Methadone	7.03	Caffeine	2.14
EDDP	6.20	Nicotine	0.72
Meperidine	4.08	Alprazolam	7.30
Tramadol	3.48	Diazepam	8.70
Tapentadol	3.65	Nordiazepam	7.27
Butorphanol	4.35	Temazepam	8.04
Dextromethorphan	5.31	Oxazepam	6.99
Pentazocine	4.65	Chlordiazepoxide	4.42
Propoxyphene	6.89	Demoxepam	6.20
Sufentanil	6.48	Clonazepam	7.27
Alfentanil	5.21	7-aminoclonazepam	2.88
Lorazepam	7.26	Fluoxetine	7.17

Flunitrazepam	7.79	Bupropion	4.40
Midazolam	5.39	Citalopram	5.72
Estazolam	6.98	Paroxetine	6.32
Flurazepam	5.62	Sertraline	7.34
Triazolam	7.51	Mirtazapine	3.11
Phenazepam	8.47	Duloxetine	6.73
Clobazam	8.28	Nefazodone	7.37
Nitrazepam	6.74	Trazodone	4.73
Carbamazepine	6.46	mCPP	3.63
Lamotrigine	3.25	Trimipramine	7.10
Primidone	3.38	Fluvoxamine	6.66
Phenytoin	6.52	Zolpidem	4.15
Levetiracetam	1.74	Zopiclone	3.27
Cyclobenzaprine	6.64	Zaleplon	6.41
Meprobamate	4.57	Ketamine	2.89
Orphenadrine	6.28	Atropine	2.81
Methocarbamol	3.96	Verapamil	6.83
Diphenhydramine	5.60	Diltiazem	5.93
Chlorpheniramine	4.07	Propranolol	5.10
Brompheniramine	4.39	Atenolol	1.25
Promethazine	6.16	Metoprolol	3.45
Hydroxyzine	6.68	Albuterol	1.21
Loratadine	7.68	PCP	5.01
Meclizine	9.26	Quetiapine	5.11
Amitriptyline	6.90	Chlorpromazine	7.31
Nortriptyline	6.75	Prochlorperazine	7.02
Imipramine	6.65	Thioridazine	8.11
Desipramine	6.49	Olanzapine	1.79
Doxepin	5.87	Risperidone	4.15
Amoxepine	5.77	Clonidine	1.90
Clomipramine	7.59	Trifluoperazine	7.70
Venlafaxine	4.55	Haloperidol	6.02
Gabapentin	1.84		
Clozapine	4.50		
Loxapine	6.04		
Benztropine	7.05		
Fluphenazine	7.39		
Doxylamine	2.27		
Oxcarbazepine	5.45		
LSD	4.34		

Appendix C

Interference Study Tables

Table 1. Low Concentration (100 ng/ml) group

Low Concentration (100 ng/ml)
Acetyl fentanyl
Fentanyl
Norfentanyl
Buprenorphine
Norbuprenorphine
Sufentanil
Alfentanil
LSD

Table 2. Middle Concentration (1000 ng/ml) group 3

Group	Middle Concentration (1000 ng/ml)	Group	Middle Concentration (1000 ng/ml)
MC3A	Oxycodone	MC3D	Orphenadrine
MC3A	Hydrocodone	MC3D	Estazolam
MC3A	Hydromorphone	MC3D	Brompheniramine
MC3A	Dihydrocodeine	MC3D	Meclizine
MC3A	Codeine	MC3D	Nortriptyline
MC3A	Acetyl Codeine	MC3D	Imipramine
MC3A	Morphine	MC3D	Desipramine
MC3A	6-MAM	MC3D	Doxepin
MC3A	Methadone	MC3D	Amoxepine
MC3A	EDDP	MC3D	Nefazodone
MC3B	Meperidine	MC3D	Trazodone
MC3B	Tramadol	MC3E	Fluvoxamine
MC3B	Tapentadol	MC3E	Zolpidem
MC3B	Pentazocine	MC3E	Atropine
MC3B	MDPV	MC3E	Propranolol
MC3B	Phenylpropanolamine	MC3E	Atenolol
MC3B	Phendimetrazine	MC3E	Metoprolol
MC3B	Caffeine	MC3E	Albuterol
MC3B	Nicotine	MC3E	Quetiapine
MC3B	Alprazolam	MC3E	Chlorpromazine
MC3C	Diazepam	MC3E	Thioridazine
MC3C	Nordiazepam	MC3E	Trifluoperazine
MC3C	Temazepam	MC3E	Lidocaine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC3C	Chlordiazepoxide
MC3C	Demoxepam
MC3C	Clonazepam
MC3C	Lorazepam
MC3C	Midazolam
MC3C	Flurazepam
MC3C	Nitrazepam
MC3C	mCPP

Table 3. Middle Concentration (1000 ng/ml) group 4

Group	Middle Concentration (1000 ng/ml)	Group	Middle Concentration (1000 ng/ml)
MC4A	Oxymorphone	MC4D	Chlorpheniramine
MC4A	Butorphanol	MC4D	Promethazine
MC4A	Dextromethorphan	MC4D	Loratadine
MC4A	Propoxyphene	MC4D	Amitriptyline
MC4A	Naloxone	MC4D	Clomipramine
MC4A	Cocaine	MC4D	Fluoxetine
MC4A	Benzoylecgonine	MC4D	Bupropion
MC4A	Ecgonine methyl ester	MC4D	Citalopram
MC4A	Methamphetamine	MC4D	Paroxetine
MC4A	Amphetamine	MC4D	Sertraline
MC4B	Pseudoephedrine	MC4E	Mirtazapine
MC4B	α PVP	MC4E	Duloxetine
MC4B	MDMA	MC4E	Trimipramine
MC4B	MDA	MC4E	Zopiclone
MC4B	Ethylone	MC4E	Zalepon
MC4B	Mephedrone	MC4E	Ketamine
MC4B	Methcathinone	MC4E	Verapamil
MC4B	Benzylpiperazine	MC4E	Diltiazem
MC4B	Phentermine	MC4E	PCP
MC4C	Methylphenidate	MC4E	Olanzapine
MC4C	Phenmetrazine	MC4F	Risperidone
MC4C	Oxazepam	MC4F	Clonidine
MC4C	7-aminoclonazepam	MC4F	Haloperidol
MC4C	Flunitrazepam	MC4F	Clozapine
MC4C	Triazolam	MC4F	Loxapine
MC4C	Phenazepam	MC4F	Benztropine
MC4C	Clobazam	MC4F	Fluphenazine
MC4C	Cyclobenzaprine	MC4F	Doxylamine
MC4C	Diphenhydramine	MC4F	Venlafaxine
		MC4F	Hydroxyzine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

Table 4. Higher Concentration (10,000 ng/ml) group

High Concentration (10,000 ng/ml)
Acetaminophen
Carbamazepine
Gabapentin
Lamotrigine
Primidone
Phenytoin
Levetiracetam
Meprobamate
Methocarbamol
Oxcarbazepine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

Appendix D

Carryover Study Tables

Table 1. Low Concentration (100 ng/ml) group 1

Group	Lower Concentration (100ng)
LC1A	Acetyl fentanyl
LC1A	Fentanyl
LC1A	Norfentanyl
LC1A	Buprenorphine
LC1A	Norbuprenorphine
LC1A	Sufentanil
LC1A	Alfentanil
LC1A	LSD
LC1A	Diacetylmorphine
LC1A	Prochlorperazine

Table 2. Middle Concentration (1000 ng/ml) group 3

Group	Middle Concentration (1000 ng/ml)	Group	Middle Concentration (1000 ng/ml)
MC3A	Oxycodone	MC3D	Orphenadrine
MC3A	Hydrocodone	MC3D	Estazolam
MC3A	Hydromorphone	MC3D	Brompheniramine
MC3A	Dihydrocodeine	MC3D	Meclizine
MC3A	Codeine	MC3D	Nortriptyline
MC3A	Acetylcodeine	MC3D	Imipramine
MC3A	Morphine	MC3D	Desipramine
MC3A	6-MAM	MC3D	Doxepin
MC3A	Methadone	MC3D	Amoxepine
MC3A	EDDP	MC3D	Nefazodone
MC3B	Meperidine	MC3D	Trazodone
MC3B	Tramadol	MC3E	Fluvoxamine
MC3B	Tapentadol	MC3E	Zolpidem
MC3B	Pentazocine	MC3E	Atropine
MC3B	MDPV	MC3E	Propranolol
MC3B	Phenylpropanolamine	MC3E	Atenolol
MC3B	Phendimetrazine	MC3E	Metoprolol
MC3B	Caffeine	MC3E	Albuterol
MC3B	Nicotine	MC3E	Quetiapine
MC3B	Alprazolam	MC3E	Chlorpromazine
MC3C	Diazepam	MC3E	Thioridazine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC3C	Nordiazepam	MC3E	Trifluoperazine
MC3C	Temazepam	MC3E	Lidocaine
MC3C	Chlordiazepoxide		
MC3C	Demoxepam		
MC3C	Clonazepam		
MC3C	Lorazepam		
MC3C	Midazolam		
MC3C	Flurazepam		
MC3C	Nitrazepam		
MC3C	mCPP		

Table 3. Middle Concentration (1000 ng/ml) group 4

Group	Middle Concentration (1000 ng/ml)	Group	Middle Concentration (1000 ng/ml)
MC4A	Oxymorphone	MC4D	Chlorpheniramine
MC4A	Butorphanol	MC4D	Promethazine
MC4A	Dextromethorphan	MC4D	Loratadine
MC4A	Propoxyphene	MC4D	Amitriptyline
MC4A	Naloxone	MC4D	Clomipramine
MC4A	Cocaine	MC4D	Fluoxetine
MC4A	Benzoylecgonine	MC4D	Bupropion
MC4A	Ecgonine methyl ester	MC4D	Citalopram
MC4A	Methamphetamine	MC4D	Paroxetine
MC4A	Amphetamine	MC4D	Sertraline
MC4B	Pseudoephedrine	MC4E	Mirtazapine
MC4B	α PVP	MC4E	Duloxetine
MC4B	MDMA	MC4E	Trimipramine
MC4B	MDA	MC4E	Zopiclone
MC4B	Ethylone	MC4E	Zalepon
MC4B	Mephedrone	MC4E	Ketamine
MC4B	Methcathinone	MC4E	Verapamil
MC4B	Benzylpiperazine	MC4E	Diltiazem
MC4B	Phentermine	MC4E	PCP
MC4C	Methylphenidate	MC4E	Olanzapine
MC4C	Phenmetrazine	MC4F	Risperidone
MC4C	Oxazepam	MC4F	Clonidine
MC4C	7-aminoclonazepam	MC4F	Haloperidol
MC4C	Flunitrazepam	MC4F	Clozapine
MC4C	Triazolam	MC4F	Loxapine
MC4C	Phenazepam	MC4F	Benztropine
MC4C	Clobazam	MC4F	Fluphenazine
MC4C	Cyclobenzaprine	MC4F	Doxylamine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC4C	Diphenhydramine	MC4F	Venlafaxine
		MC4F	Hydroxyzine

Table 4. Higher Concentration (10,000 ng/ml) group 2

Group	**Higher Concentration (10,000ng)**
HC2A	Acetaminophen
HC2A	Carbamazepine
HC2A	Gabapentin
HC2A	Lamotrigine
HC2A	Primidone
HC2A	Phenytoin
HC2A	Levetiracetam
HC2A	Meprobamate
HC2A	Methocarbamol
HC2A	Oxcarbazepine

Table 5. Middle Concentration (1000 ng/ml) internal standards concentrations

Group	**Middle Concentration (1000 ng/ml) Internal Standards**
*MCIS	Methaqualone-d7
MCIS	Morphine-d6
MCIS	Miazepam-d5
MCIS	Cocaine-d3
MCIS	Amphetamine-d11
MCIS	Fentanyl-d5
MCIS	SKF-525A

*MCIS: Middle Concentration Internal Standards

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

Appendix E

Ionization Suppression/Enhancement Study - Post-Column Infusion Tables

Table 1. Concentrations of internal standards used for neat solutions

Group	Internal Standards	Concentration (ng/ml)
MCIS	d7 Methaqualone	500
MCIS	d6 Morphine	500
MCIS	d5 Diazepam	500
MCIS	d3 Cocaine	50
MCIS	d11 Amphetamine	5000
MCIS	d5 Fentanyl	125
MCIS	SKF-525A	1250

Table 2. Low group 1 used for neat solutions

Group	High Concentration (100 ng/ml) Low Concentration (1 ng/ml)
LC1A	Acetyl fentanyl
LC1A	Fentanyl
LC1A	Norfentanyl
LC1A	Buprenorphine
LC1A	Norbuprenorphine
LC1A	Sufentanil
LC1A	Alfentanil
LC1A	LSD

Table 3. Middle group 3 used for neat solutions

Group	High Concentration (1000 ng/ml) Low Concentration (10 ng/m)	Group	High Concentration (1000 ng/ml) Low Concentration (10 ng/m)
MC4A	Oxymorphone	MC4D	Chlorpheniramine
MC4A	Butorphanol	MC4D	Promethazine
MC4A	Dextromethorphan	MC4D	Loratadine
MC4A	Propoxyphene	MC4D	Amitriptyline
MC4A	Naloxone	MC4D	Clomipramine
MC4A	Cocaine	MC4D	Fluoxetine
MC4A	Benzoylecgonine	MC4D	Bupropion
MC4A	Ecgonine methyl ester	MC4D	Citalopram
MC4A	Methamphetamine	MC4D	Paroxetine
MC4A	Amphetamine	MC4D	Sertraline
MC4B	Pseudoephedrine	MC4E	Mirtazapine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC4B	α PVP	MC4E	Duloxetine
MC4B	MDMA	MC4E	Trimipramine
MC4B	MDA	MC4E	Zopiclone
MC4B	Ethylone	MC4E	Zalepon
MC4B	Mephedrone	MC4E	Ketamine
MC4B	Butylone	MC4E	Verapamil
MC4B	Methcathinone	MC4E	Diltiazem
MC4B	Benzylpiperazine	MC4E	PCP
MC4B	Phentermine	MC4E	Olanzapine
MC4C	Methylphenidate	MC4F	Risperidone
MC4C	Phenmetrazine	MC4F	Clonidine
MC4C	Oxazepam	MC4F	Haloperidol
MC4C	7-aminoclonazepam	MC4F	Clozapine
MC4C	Flunitrazepam	MC4F	Loxapine
MC4C	Triazolam	MC4F	Benztropine
MC4C	Phenazepam	MC4F	Fluphenazine
MC4C	Clobazam	MC4F	Doxylamine
MC4C	Cyclobenzaprine	MC4F	Venlafaxine
MC4C	Diphenhydramine	MC4F	Hydroxyzine

Table 4. Middle group 4 used for neat solutions

Group	**High Concentration (1000 ng/ml) Low Concentration (10 ng/m)**	**Group**	**High Concentration (1000 ng/ml) Low Concentration (10 ng/ml)**
MC4A	Oxymorphone	MC4D	Chlorpheniramine
MC4A	Butorphanol	MC4D	Promethazine
MC4A	Dextromethorphan	MC4D	Loratadine
MC4A	Propoxyphene	MC4D	Amitriptyline
MC4A	Naloxone	MC4D	Clomipramine
MC4A	Cocaine	MC4D	Fluoxetine
MC4A	Benzoylecgonine	MC4D	Bupropion
MC4A	Ecgonine methyl ester	MC4D	Citalopram
MC4A	Methamphetamine	MC4D	Paroxetine
MC4A	Amphetamine	MC4D	Sertraline
MC4B	Pseudoephedrine	MC4E	Mirtazapine
MC4B	α PVP	MC4E	Duloxetine
MC4B	MDMA	MC4E	Trimipramine
MC4B	MDA	MC4E	Zopiclone
MC4B	Ethylone	MC4E	Zaleplon
MC4B	Mephedrone	MC4E	Ketamine
MC4B	Methcathinone	MC4E	Verapamil
MC4B	Benzylpiperazine	MC4E	Diltiazem

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC4B	Phentermine	MC4E	PCP
MC4C	Methylphenidate	MC4E	Olanzapine
MC4C	Phenmetrazine	MC4F	Risperidone
MC4C	Oxazepam	MC4F	Clonidine
MC4C	7-aminoclonazepam	MC4F	Haloperidol
MC4C	Flunitrazepam	MC4F	Clozapine
MC4C	Triazolam	MC4F	Loxapine
MC4C	Phenazepam	MC4F	Benztropine
MC4C	Clobazam	MC4F	Fluphenazine
MC4C	Cyclobenzaprine	MC4F	Doxylamine
MC4C	Diphenhydramine	MC4F	Venlafaxine
		MC4F	Hydroxyzine
		MC4F	Diacetylmorphine
		MC4F	Prochlorperazine

Table 5. High group 2 used for neat solutions

Group	**High Concentration (10,000 ng/ml) Low Concentration (100 ng/ml)**
HC2A	Acetaminophen
HC2A	Carbamazepine
HC2A	Gabapentin
HC2A	Lamotrigine
HC2A	Primidone
HC2A	Phenytoin
HC2A	Levetiracetam
HC2A	Meprobamate
HC2A	Methocarbamol
HC2A	Oxcarbazepine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

Appendix F

Limit of Detection (LOD) Study Tables

Table 1. 134 Analyte groupings for Limit of detection Initial Trial Run

Group	Analytes	Group	Analytes
LC1A	Acetyl fentanyl	MC3F	mCPP
LC1A	Fentanyl	MC3F	Trazodone
LC1A	Norfentanyl	MC3F	Trifluoperazine
LC1A	Buprenorphine	MC3F	Lidocaine
LC1A	Norbuprenorphine	MC3F	Diacetylmorphine
LC1A	Sufentanil	MC4A	Butorphanol
LC1A	Alfentanil	MC4A	Dextromethorphan
HC2A	Acetaminophen	MC4A	Propoxyphene
HC2A	Carbamazepine	MC4A	Naloxone
HC2A	Gabapentin	MC4A	Cocaine
HC2A	Lamotrigine	MC4A	Benzoylecgonine
HC2A	Primidone	MC4A	Ecgonine methyl ester
HC2A	Phenytoin	MC4A	Methamphetamine
HC2A	Levetiracetam	MC4A	Amphetamine
HC2A	Meprobamate	MC4B	Pseudoephedrine
HC2A	Methocarbamol	MC4B	α PVP
HC2A	Oxcarbazepine	MC4B	MDMA
MC3A	Oxycodone	MC4B	MDA
MC3A	Hydrocodone	MC4B	Ethylone
MC3A	Hydromorphone	MC4B	Mephedrone
MC3A	Dihydrocodeine	MC4B	Methcathinone
MC3A	Codeine	MC4B	Benzylpiperazine
MC3A	Acetylcodeine	MC4B	Phentermine
MC3A	Morphine	MC4C	Methylphenidate
MC3A	6-MAM	MC4C	Phenmetrazine
MC3A	Methadone	MC4C	Oxazepam
MC3A	EDDP	MC4C	7-aminoclonazepam
MC3B	Meperidine	MC4C	Flunitrazepam
MC3B	Tramadol	MC4C	Triazolam
MC3B	Tapentadol	MC4C	Phenazepam
MC3B	Pentazocine	MC4C	Clobazam
MC3B	MDPV	MC4C	Cyclobenzaprine
MC3B	Phenylpropanolamine	MC4C	Diphenhydramine

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

MC3B	Phendimetrazine	MC4D	Chlorpheniramine
MC3B	Caffeine	MC4D	Promethazine
MC3B	Nicotine	MC4D	Loratadine
MC3B	Alprazolam	MC4D	Amitriptyline
MC3C	Diazepam	MC4D	Clomipramine
MC3C	Nordiazepam	MC4D	Fluoxetine
MC3C	Temazepam	MC4D	Bupropion
MC3C	Chlordiazepoxide	MC4D	Citalopram
MC3C	Demoxepam	MC4D	Paroxetine
MC3C	Clonazepam	MC4D	Sertraline
MC3C	Lorazepam	MC4E	Mirtazapine
MC3C	Midazolam	MC4E	Duloxetine
MC3C	Flurazepam	MC4E	Trimipramine
MC3C	Nitrazepam	MC4E	Zopiclone
MC3D	Orphenadrine	MC4E	Zaleplon
MC3D	Estazolam	MC4E	Ketamine
MC3D	Brompheniramine	MC4E	Verapamil
MC3D	Meclizine	MC4E	Diltiazem
MC3D	Nortriptyline	MC4E	PCP
MC3D	Imipramine	MC4E	Olanzapine
MC3D	Desipramine	MC4F	Risperidone
MC3D	Doxepin	MC4F	Clonidine
MC3D	Amoxepine	MC4F	Haloperidol
MC3D	Nefazodone	MC4F	Clozapine
MC3E	Fluvoxamine	MC4F	Loxapine
MC3E	Zolpidem	MC4F	Benztropine
MC3E	Atropine	MC4F	Fluphenazine
MC3E	Propranolol	MC4F	Doxylamine
MC3E	Atenolol	MC4F	Venlafaxine
MC3E	Metoprolol	MC4F	Hydroxyzine
MC3E	Albuterol		
MC3E	Quetiapine		
MC3E	Chlorpromazine		
MC3E	Thioridazine		

HC Group Name: High Concentration Group Name
MC Group Name: Middle Concentration Group Name
LC Group Name: Low Concentration Group Name

Table 2. 134 Analyte groupings for actual Limit of detection Study

Group LOD1: 1,5,10 ng/ml			
LOD1A	Acetyl fentanyl	LOD1D	Diacetylmorphine
	Fentanyl		Cocaine
	Buprenorphine		Benzoylecgonine
	Norbuprenorphine		α PVP
	Sufentanil		Ethylone
	Alfentanil		Methylphenidate
	Gabapentin		Oxazepam
	Oxcarbazepine		Flunitrazepam
	Morphine		Triazolam
	6MAM		Phenazepam
LOD1B	Meperidine	LOD1E	Clomipramine
	Tapentadol		Citalopram
	Pentazocine		Mirtazapine
	MDPV		Ketamine
	Alprazolam		Verapamil
	Chlordiazepoxide		Diltiazem
	Clonazepam		Haloperidol
	Flurazepam		Clozapine
	Orphenadrine		Doxylamine
	Nortriptyline		Hydroxyzine
LOD1C	Imipramine		
	Zolpidem		
	Atropine		
	Propranolol		
	Atenolol		
	Metoproplol		
	Quetiapine		
	Chlorpromazine		
	Trazodone		
	Lidocaine		
Group LOD2: : 5,10,15 ng/ml			
LOD2A	Carbamazepine	LOD2C	Nefazodone
	Lamotrigine		Fluvoxamine
	Primidone		Oxymorphone
	Phenytoin		Dextromethorphan
	Oxycodone		Naloxone

LOD Group Name: Limit of Detection Group Name

	Hydrocodone		MDMA
	Hydromorphone		Chlorpheniramine
	Dihydrocodeine		Promethazine
	Codeine		Bupropion
	Acetylcodeine		Olanzapine
LOD2B	EDDP	LOD2D	Clonidine
	Diazepam		Benztropine
	Nordiazepam		Fluphenazine
	Temazepam		Venlafaxine
	Demoxepam		Trimipramine
	Lorazepam		Zopiclone
	Brompheniramine		
	Meclizine		
	Desipramine		
	Doxepin		
Group LOD3: : 50,75,100 ng/ml			
LOD3A	Norfentanyl	LOD3C	Phenmetrazine
	Acetaminophen		Cyclobenzaprine
	Levetiracetam		Diphenhydramine
	Meprobamate		Loratadine
	Methocarbamol		Fluoxetine
	Methadone		Paroxetine
	Tramadol		Sertraline
	Phenylpropanolamine		Duloxetine
	Phendimetrazine		Zaleplon
	Caffeine		PCP
LOD3B	Estazolam		
	Amoxepine		
	Trifluoperazine		
	Ecgonine methyl ester		
	Amphetamine		
	Pseudoephedrine		
	Mephedrone		
	Benzylpiperazine		
	Phentermine		
Group LOD4: 15,20,25 ng/ml			
LOD4A	Nicotine		
	Midazolam		

LOD Group Name: Limit of Detection Group Name

	Nitrazepam
	MDA
	7-aminoclonazepam
	Clobazam
	Amitriptyline
	Risperidone
Group LOD5: 20,25,30 ng/ml	
LOD5A	Albuterol
LOD5A	Propoxyphene
LOD5A	Methamphetamine
Group LOD6: 25,30,35 ng/ml	
LOD6A	Thioridazine
LOD6A	mCPP
Group LOD7: 30,35,40 ng/ml	
LOD7A	Methcathinone
	Prochlorperazine
Group: 10,15,20 ng/ml	
Self	Loxapine
Group: 10,20,30 ng/ml	
Self	Butorphanol
Group: 50, 75, 100 ng/ml	
Self	LSD

LOD Group Name: Limit of Detection Group Name