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

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Abstract

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. 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. Waters TargetLynx™ Application Manager Software was used for sample data processing. 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 described.

Introduction

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

Materials and Methods

- 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.
- Materials include:
  - 134 analytes of interest included drugs from various classes: cocaine, amphetamines, opiates, benzodiazepines, fentanyl, and other basic, acidic and neutral drugs.
  - 7 internal standards include methaqualone-d7, morphine-d6,
  - diazepam-d5, cocaine-d3, amphetamine-d11, fentanyl-d5, and SKF-525A. 7 different internal standards are included so that the screening method can be used concurrently with analyses methods for several drug classes.
  - Bovine blood and two blank whole human blood samples.
  - Optima grade Acetonitrile, Methanol and H_2O.
- The extraction used was protein precipitation, where 1 ml of ice-cold Optima Acetonitrile: Optima Methanol (85:15) was added to 200 µl sample. The samples were vortex mixed and centrifuged at 32000 *x g* for 15 minutes. A 100 µL aliquot of the supernatant was transferred to autosampler vial and 900 µL Optima grade H_2O was added.

Interference Study

- The interference study had 3 parts; first was interferences associated with the matrix. 10 negative blood samples were extracted and injected to ensure that no interferences were observed. Nine were human samples from previously analyzed cases from the WV Office of the Chief Medical Examiner Office Toxicology Laboratory, where drugs and alcohol were not detected following immunoassay and volatiles analysis and the tenth was a bovine blood sample.
- The second was interferences associated with the internal standards. Blank blood was fortified with internal standards to ensure no signals from the analytes of interest were observed and blank blood was fortified with the analytes of interest to ensure no internal standard signals were observed. Seven internal standard solutions were prepared at concentrations shown in Table 1.
- The 134 analytes were divided into 3 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) and run on the LC MS/MS to ensure no signal for the analytes of interest were observed.
- The third was interferences associated with other commonly associated analytes. Seven drug mix solutions (basic, opiates, benzodiazepines, buprenorphine/fentanyl, acid/neutral, amphetamine, and cocaine mix) were prepared in bovine blood, extracted, and run on the LC MS/MS.

Table 1. 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

Carryover Study

- The carryover study was performed to ensure that no analytes were detected in the negative blood samples injected after spiked blood samples.
- The 134 analytes were added 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). The seven internal standards were added at concentrations listed in Table 1.

Ionization Suppression/Enhancement Study - Post-Column Infusion

- Biological specimens may contain compounds that co-elute, causing ionization suppression or enhancement, which may affect parameters such as LOD.
- Extractions of 10 negative blood samples were injected while simultaneously infusing neat solutions of internal standards, low concentration analyte solutions, and high concentration analyte solutions to observe if any ionization suppression or enhancement occurred.

Limit of Detection (LOD) Study

- LOD establishes the lower concentration limit for qualitatively evaluating the presence or absence of an analyte in the samples.
- 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.

Results & Discussion

Interference Study

- No interferences were detected from the matrix or internal standards. Interferences associated with other commonly encountered analytes, as determined using the standard mixes described previously, are listed in Table 3.
- 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.

Table 2. Interferences detected in the drug mix solutions of commonly encountered analytes

Solution Mix	Interference Detected
Basic Mix	Interference with imipramine was detected
Benzodiazepines Mix	Interference with meperidine was detected
Buprenorphine/Fentanyl Mix	Interferences with MDMA and MDA were detected

Carryover Study

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

Limit of Detection (LOD) Study

- Due to time constraints, only the trial LOD study was performed. Further studies to determine LOD would involve preparing spiked blood solutions of the analytes at lower concentrations. These would be extracted three times per day for three days. The LOD of the analytes would be concluded from the data collected from the nine total runs.

Ionization Suppression/Enhancement Study - Post-Column Infusion

- An increase or decrease in analyte signal of ± 25% is considered to be significant ionization suppression or enhancement
- Table 3 summarizes the findings of the post column infusion study for ionization suppression and enhancement.

Table 3. Results from ionization suppression/enhancement study

Analyte	Suppression/ Enhancement
Methaqualone-d7	Enhancement
Zaleplon	Enhancement
Tramadol	Suppression
mCPP	Suppression
Ecgonine methyl ester	Suppression
Venlafaxine	Suppression

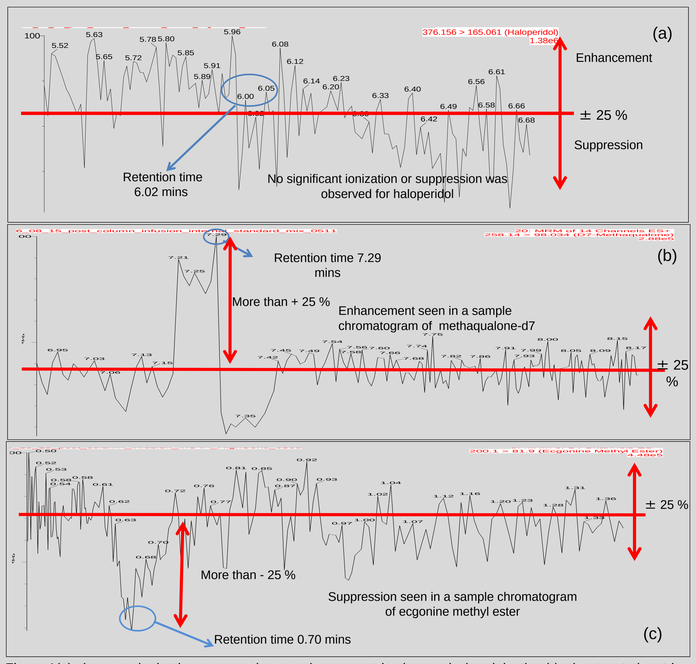

Figure 1(a) shows no ionization suppression or enhancement is observed when injecting blank extracted matrix during infusion of haloperidol. 1(b) shows an example of enhancement seen in a sample chromatogram of methaqualone-d7. 1(c) shows an example of suppression seen in a sample chromatogram of ecgonine methyl ester

Conclusions

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.

References

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Acknowledgements

The authors would like to thank the West Virginia Office of the Chief Medical Examiner Toxicology Lab for allowing the internship that was needed for this project and for the use of their facilities and instruments for the project.