Validation of a GC/MS method for the determination of alkaline drugs in whole blood

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Abstract

Forensic toxicologists analyze drugs and other toxicants found in bodily fluids or tissue to determine if the drugs present in the sample contributed to death or if their presence was relevant in the circumstances surrounding death. Reliable analytical data are required for the correct interpretation and evaluation of toxicological findings, to ensure this data is being produced, methods and instruments need to undergo validation. A gas chromatography-mass spectroscopy (GC/MS) method was optimized and validated for the determination of 31 alkaline drugs in blood, however only six were fully validated (amitriptyline, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), methadone, nortriptyline, paroxetine, and sertraline). The method was validated utilizing the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines for method validation in forensic toxicology.

Introduction

- Forensic toxicologists investigate unnatural deaths including suicide, motor vehicle crashes, homicide, suspicious, and drug-related fatalities or unwitnessed deaths that could be natural [1]
- Concentrations of drugs found in blood can help interpret the cause and manner of death [2,3]
- Reliable analytical data are required for correct interpretation and evaluation [2,4]
- Aim to validate a precise and sensitive GC/MS method for the separation, identification, and quantification of 31 alkaline compounds in whole blood
- SWGTOX guidelines for quantitative method validation [5]:
  - Matrix interference, interference from other commonly encountered analytes, carryover, calibration model, bias, precision, limit of quantitation (LOQ), limit of detection (LOD)

Methods

Alkaline Liquid-Liquid Extraction

- Calibrators
  - 1 mL whole blood fortified with 10, 25, 50, 100, 250, 500, 1000, and 2000 ng/mL
- Controls
  - 1 mL whole blood fortified with 65, 130, and 650 ng/mL
- Addition of internal standard
  - 250 ng SKF-525a added to each calibrator and control
- Alkaline extraction, acidic back extraction, re-extraction
- Collection of organic layer

GC/MS Analysis

- Agilent 7890B GC with a 5977A MS detector and 7693 autosampler
- 2 µL sample injected
- HP-5MS Column (30 m x 0.25 mm x 0.25 µm)
- 100 ºC for 1 min, ramped to 325 ºC at 15 ºC/min and held for 5 min

Methods

Method Validation

- Matrix Interference
  - 17 blank whole blood samples extracted and evaluated without internal standard
- Interference from other commonly encountered analytes
  - Analytes commonly encountered in routine casework evaluated at high therapeutic or lethal concentrations
- Carryover
  - Three extracted negative matrix samples analyzed immediately following a 5000 ng/mL sample of the extracted alkaline drugs of interest
- Calibration Model
  - Statistical tests for model of fit used with acceptable calibrators [5,6]
    - Weighting
      - F-test to evaluate variance at lowest and highest concentrations
        - $p > 0.05$, data homoscedastic
        - $p < 0.05$, data heteroscedastic
    - Weighting Factor ($1/x$ or $1/x^2$)
      - Graph of variance as a function of concentration
    - Model Order (linear or quadratic)
      - Two-way ANOVA test
        - $p > 0.05$, linear model used
        - $p < 0.05$, quadratic model used
- Bias
  - Whole blood spiked at low (75 ng/mL), medium (750 ng/mL), and high (1500 ng/mL) concentrations and performed in triplicate over 5 days [5]
- Precision
  - Evaluated within-run and between-run precision using data generated in the bias study [5]
- LOQ
  - Administratively set as the lowest acceptable non-zero calibrator
- LOD
  - Estimated for analytes following a linear calibration model [5]
  - Defined as the lowest non-zero calibrator for analytes not following a linear calibration model

Results

Table 1. The 31 alkaline compounds evaluated in the study

Analytes
bupropion, meperidine, fluoxetine, diphenhydramine, doxylamine, tramadol, N-desmethyltramadol, chlorpheniramine, EDDP, venlafaxine, brompheniramine, dextromethorphan, methadone, O-desmethylvenlafaxine, amitriptyline, nortriptyline, doxepin, cyclobenzaprine, desmethyldoxepin, mirtazapine, promethazine, sertraline, citalopram, clomipramine, desmethylcitalopram, paroxetine, olanzapine, zolpidem, diltiazem, verapamil, norverapamil

Interference Studies

- No matrix interference or interference from other commonly encountered analytes was observed

Carryover

- No carryover was observed for any of the analytes listed in Table 1

Results

Calibration Model

- Weighting
  - $p < 0.05$ for EDDP, methadone, amitriptyline, nortriptyline, sertraline, and paroxetine
- Weighting Factor ($1/x^2$)

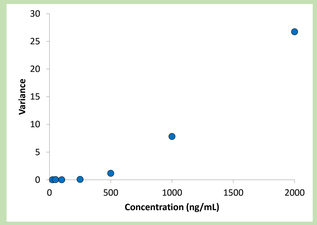

Figure 1. Association between variance and concentration.

- Model Order
  - $p > 0.05$ for EDDP, methadone, amitriptyline, and sertraline (linear model)
  - $p < 0.05$ for nortriptyline and paroxetine (quadratic model)

Bias

- Bias for EDDP, methadone, amitriptyline, nortriptyline, sertraline, and paroxetine were below the maximum acceptable (±20%) bias at each concentration [5]

Precision

- Both within-run and between-run precision for EDDP, methadone, amitriptyline, nortriptyline, sertraline, and paroxetine were below the maximum acceptable precision (±20%) at each concentration [5]

LOQ

- Set as 25 ng/mL for EDDP, methadone, amitriptyline, nortriptyline, and sertraline
- Set as 50 ng/mL for paroxetine

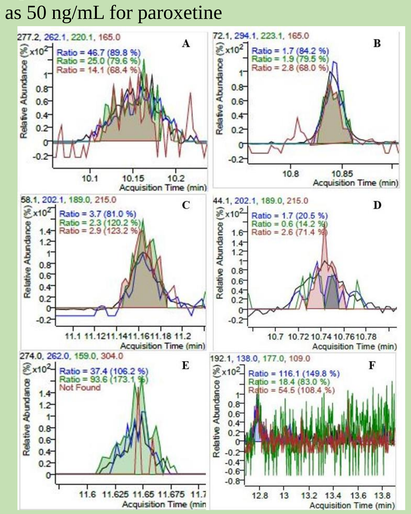

Figure 2. The extracted ion chromatogram for the quantifier (black) and qualifier (blue, green, red) ions for (A) EDDP, (B) methadone, (C) amitriptyline, (D) nortriptyline, and (E) sertraline at 25 ng/mL and for (F) paroxetine at 50 ng/mL.

Results

Limit of Detection

Table 2. LOD was estimated for EDDP, methadone, amitriptyline, and sertraline. LOD was set to the LOQ for nortriptyline and paroxetine.

Analyte	LOD (ng/mL)
EDDP	18
Methadone	6
Amitriptyline	6
Nortriptyline	25
Sertraline	10
Paroxetine	50

Conclusions

- No interference from matrix or other commonly encountered analytes was observed
- No carryover observed at high therapeutic or lethal concentrations
- Linear model with inverse weight by concentration squared ($1/x^2$) with acceptable bias and precision for EDDP, methadone, amitriptyline, and sertraline
- Quadratic model with inverse weight by concentration squared ($1/x^2$) with acceptable bias and precision for nortriptyline and paroxetine
- LOQ set as lowest acceptable calibrator
- LOD estimated for analytes following a linear model
- LOD administratively set as LOQ for analytes following a quadratic model
- The GC/MS method developed at the WV-OCME Toxicology Laboratory has been shown to be reproducible and accurate

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