

Drug Screening in Medical Examiner Casework by High-Resolution Mass Spectrometry (UPLC-MS^E-TOF)

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Postmortem drug findings yield important analytical evidence in medical examiner casework, and chromatography coupled with nominal mass spectrometry (MS) serves as the predominant general unknown screening approach. We report screening by ultra performance liquid chromatography (UPLC) coupled with hybrid quadrupole time-of-flight mass spectrometer (MS^E-TOF), with comparison to previously validated nominal mass UPLC-MS and UPLC-MS-MS methods. UPLC-MS^E-TOF screening for over 950 toxicologically relevant drugs and metabolites was performed in a full-spectrum (*m/z* 50–1,000) mode using an MS^E acquisition of both molecular and fragment ion data at low (6 eV) and ramped (10–40 eV) collision energies. Mass error averaged 1.27 ppm for a large panel of reference drugs and metabolites. The limit of detection by UPLC-MS^E-TOF ranges from 0.5 to 100 ng/mL and compares closely with UPLC-MS-MS. The influence of column recovery and matrix effect on the limit of detection was demonstrated with ion suppression by matrix components correlating closely with early and late eluting reference analytes. Drug and metabolite findings by UPLC-MS^E-TOF were compared with UPLC-MS and UPLC-MS-MS analyses of postmortem blood in 300 medical examiner cases. Positive findings by all methods totaled 1,528, with a detection rate of 57% by UPLC-MS, 72% by UPLC-MS-MS and 80% by combined UPLC-MS and UPLC-MS-MS screening. Compared with nominal mass screening methods, UPLC-MS^E-TOF screening resulted in a 99% detection rate and, in addition, offered the potential for the detection of nontargeted analytes via high-resolution acquisition of molecular and fragment ion data.

Introduction

Analytical toxicology is a foundation of medical examiner investigation as practiced throughout the world. Comprehensive screening, often referred to as general unknown or systematic toxicological analysis, is essential for the comprehensive detection of both suspected and unsuspected drugs and toxins present in body fluids and tissue at the time of death. Nominal mass spectrometry interfaced with gas chromatograph (GC-MS) has served as a primary analytical technology for general unknown screening for many years due to the availability of large mass spectra databases acquired under a standardized 70 eV electron impact ionization (1, 2). Liquid chromatography (LC) coupled with MS technology (LC-MS) has also been reported in drug screening using single-stage (3–8), tandem (8–10) and linear trap (11–16) applications. We have previously compared general unknown screening in blood from a large cohort of postmortem cases using GC-MS, ultra performance liquid chromatography (UPLC)-MS and UPLC-MS-MS methods and have reported the increased detection sensitivity and high degree of specificity achieved with LC-MS methods (8).

Drug screening with high-resolution mass analysis has also been reported in recent years and offers the advantage of sensitive, full-spectrum detection along with the potential for molecular formula and exact mass product ion analysis. Drug screening based on the monoisotopic mass of the protonated molecule, with varying use of retention time and isotopic pattern fit data, has been previously reported for a range of biological matrices (17–24). While screening by intact molecule criteria may be performed even without reference material, the specificity of drug detection is limited by the prevalence of constitutional isomers with the same formula but widely varying structures and functional groups. Tyrkko *et al.* (25) found a 13% incidence of isomers in their in-house toxicology database of 874 substances and demonstrated the importance of fragment ion analysis as qualifier ions in the distinction of isomeric agents. Fragment ions can be generated by collision induced dissociation (CID) using either in-source (26, 27) or collision cell (28–33) methods. Ion acquisition techniques employing collision cell technology allow the optimization of precursor and product ion detection across a wide range of screened substances. Using a data-dependent acquisition technique, single-stage mode surveillance of threshold-level precursor ions directs mass selection for tandem MS-TOF analysis using multiple CID energies. An alternative approach reported initially by Plumb *et al.* (34), called MS^E, employs simultaneous ion acquisitions with low and high CID energy for optimum dual detection and time alignment of precursor and product ions in a single-stage mode. MS^E allows continuous full-spectrum acquisitions with application to targeted and nontargeted screening, and an MS^E-based general unknown drug screening method by UPLC-hybrid quadrupole time-of-flight mass spectrometer (MS^E-TOF) has been developed with a database of precursor ion, fragment ion and chromatographic retention time data (33).

While high-resolution mass analysis coupled with LC holds great promise for general unknown screening in the medical examiner setting, method validation with postmortem specimens and large-case application studies are still needed. We report the application of UPLC-MS^E-TOF in general unknown drug screening for the determination of the cause and manner of death. In this study, we validate postmortem blood screening by UPLC-MS^E-TOF and compare screening performance with nominal mass UPLC-MS and UPLC-MS-MS methods used in our routine medical examiner casework.

Materials and methods

Chemicals and reagents

Reference compounds used in preparing blood pools for quality control and recovery studies were obtained from Cerilliant (Round Rock, TX, USA) and Alltech Applied Science (State College, PA, USA) and stored at –20°C. The internal standard

methapyrilene was obtained from Alltech. Ammonium acetate, polyethylene glycol (PEG 400), ACS grade methanol, dibasic sodium phosphate, monobasic sodium phosphate as well as HPLC grade acetonitrile and methylene chloride were purchased from Fisher Scientific (Fairlawn, NJ, USA). AR grade hydrochloric acid, ammonium hydroxide, acetic acid and spectral-grade hexane were from Mallinckrodt Inc. (Paris, NY, USA). Formic acid and leucine enkephalin were obtained from Sigma Aldrich (St Louis, MO, USA). Deionized water was produced by combined ultrafiltration (AquaPure, Schenectady, NY, USA) and reverse osmosis (Alpha Water Systems, Montague, NJ, USA) treatment.

Mass calibration for UPLC-MS and UPLC-MS-MS analyses were performed with 2 mM ammonium acetate and 50 mg/L PEG 400 in acetonitrile:water (50:50 v/v) and for UPLC-MS-TOF was performed with 5 mM sodium formate solution in propanol:water (90:10 v/v). A lock mass calibrant for UPLC-MS^E-TOF was leucine enkephalin at a stock concentration of 400 mg/L in water with storage of aliquots at -20°C for up to 6 months. Working leucine enkephalin (2 mg/L) was prepared by further dilution of the stock solution with acetonitrile:water (50:50 v/v) containing 0.1% formic acid. A solid phase extraction (SPE) elution solvent with ammonium hydroxide:isopropanol:methylene chloride (2:20:80 v/v/v) was prepared for extraction column elution of basic drugs. For all gradient UPLC analysis, mobile Phase A (5 mM ammonium formate, pH 3.0) and mobile Phase B (0.1% v/v formic acid in acetonitrile) were prepared along with a starting mobile phase mixture solution containing 87% mobile Phase A and 13% mobile Phase B.

Validation samples

Blood bank blood was obtained and analyzed for use as a negative blood pool. Whole blood reference panels were prepared by supplementing negative blood with reference standard material at concentrations ranging from 0.5 to 250 ng/mL. Whole blood Panel A was supplemented with amphetamine, benzoylecgonine, chlorpheniramine, citalopram, cocaethylene, codeine, N-desmethyltramadol, dextromethorphan, diazepam, diphenhydramine, doxapatin, 2-ethylidine-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), ephedrine, hydrocodone, hydroxyalprazolam, imipramine, levetiracetam, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methadone, methamphetamine, mirtazapine, morphine, nordiazepam, oxycodone and oxymorphone. Panel B contained alprazolam, amiodarone, amitriptyline, amoxapine, atropine, bisoprolol, brompheniramine, caffeine, carbamazepine, carisoprodol, clomipramine, clonazepam, clonidine, clozapine, cyproheptadine, desipramine, diltiazem, disopyramide, doxylamine, fluconazole, flunitrazepam, fluoxetine, flurazepam, gabapentin, haloperidol, hydroxyzine, quinidine, and sertraline. Panel C was spiked with ketamine, lamotrigine, lidocaine, lorazepam, loxapine, maprotiline, meperidine, mepivacaine, meprobamate, metoclopramide, metoprolol, midazolam, milnacipran, nadolol, nalorphine, naloxone, naltrexone, nicotine, olanzapine, orphenadrine, papaverine, paroxetine, phencyclidine, pheniramine, phentermine, promethazine and propafenone.

Medical examiner case samples

Postmortem blood used in the comparison of UPLC-MS, UPLC-MS-MS and UPLC-MS^E-TOF screening methods was obtained from 300 consecutive postmortem toxicology cases submitted for testing to the Forensic Toxicology Laboratory at the Albany

Medical Center. The decedent cohort included 210 males with an average age of 45 (range <1–83 years) and 90 females with an average age of 39 (range <1–92 years). Following UPLC-MS and UPLC-MS-MS analyses, blood extracts along with any available demographics and confirmation results were de-identified and recorded in accordance with an Albany Medical College IRB reviewed protocol prior to analysis by UPLC-MS^E-TOF. Detection sensitivity assessment was performed without the inclusion of positive caffeine, nicotine and cotinine findings due to the high frequency of these positive findings and their limited value in the determination of the cause and manner of death.

Analytical methods

Extraction procedure

Blood samples were extracted using Clean Screen extraction columns (ZSDAU020) obtained from United Column Technology (Bristol, PA, USA) and preconditioned sequentially with 3 mL of methanol, 3 mL of water and 1 mL of 0.1 M phosphate buffer (pH 6.0). Methapyrilene at a final concentration of 500 ng/mL was added to 2 mL of postmortem and control blood followed by sonication for 15 min. Following the addition of 4 mL of 0.1 M phosphate buffer, the samples were loaded onto extraction columns at an aspiration rate of 1 mL/min, followed by sequential washing with 2 mL of deionized water, 1 mL of 0.1 M acetic acid and 3 mL of hexane. Acid/neutral analytes were then eluted with ethyl acetate (3 mL) into a collection tube and dried. After a column wash with 2 mL of methanol, basic analytes were eluted with 3 mL of SPE elution solvent (3 mL) into the same collection tube used for acid/neutral analytes with addition of 0.1 N HCl (50 µL) and drying. The dried extracts were reconstituted with 100 µL of starting mobile phase mixture and submitted for analysis.

UPLC-MS and UPLC-MS-MS methods

UPLC-MS and UPLC-MS-MS analysis methods were performed with a Waters Acquity UPLC system (Milford, MA, USA) interfaced with a TQ Detector, using electrospray ionization (ESI) in a positive-ion mode. System software included MassLynx™ (4.1 SCN 714), ChromaLynx™ and TargetLynx™ (Waters). Chromatographic separation was performed with an HSS C₁₈ 1.8 µm (2.1 × 150 mm) column (Waters) maintained at 50°C. A mobile phase gradient from 87% A and 13% B to 5% A and 95% B was used with a 10 µL sample volume, a 400 µL flow rate per minute and a total analytical run time of 15 min. MS conditions for UPLC-MS analysis include capillary voltage (3.5 kV), cone voltage (20–95 V) in 15 V increments (in-source CID), desolvation temperature (400°C), desolvation gas flow (800 L/h), source temperature (150°C), acquisition range (*m/z* 80–650) and scan speed >7,000 atomic mass units per second. An in-source CID mass spectral library of >900 drugs and metabolites was obtained from Waters with additional mass spectra library additions performed in-house. Criteria for a positive drug finding include retention time within 0.3 min and an average library forward fit of ≥650. A contemporaneous analysis of reference material is also performed for all positive drugs and metabolite findings, when available. Additional criteria included a tentative library forward fit of ≥450 to 649 plus identification of the protonated species and two major fragment ions. Tandem MS conditions for UPLC-MS-MS analysis include an ESI positive-ionization mode,

capillary voltage (3.0 kV), desolvation temperature (400°C), desolvation gas flow (800 L/h), source temperature (150°C) and collision gas (argon) pressure closely maintained at 4.5×10^{-3} mBar. Multiple reaction monitoring (MRM) for two transitions ions was performed for 208 targeted drugs and metabolites. Cone voltage and collision energy were optimized in-house for each analyte transition, with a target transition ion ratio established for use in criteria-based identification of the analyte. European Union criteria (35, 36) were used for positive identification, including a transition ion ratio within 20% of the target ratio for transition ratios of ≥ 0.50 , within 25% for transition ratios between 0.20 and < 0.50 , within 30% for transition ratios between 0.10 and < 0.20 and 50% for transition ratios of < 0.10 . Analyte-specific transition ratios are established for each analytical run with contemporaneous analyzed reference material. Mass calibration was performed prior to each analytical run for both screening assays.

UPLC-MS^E-TOF method

For UPLC-MS^E-TOF analysis, analyte separation was also performed using a Waters Acuity UPLC system using the same column type and chromatographic conditions as described above for the nominal mass analysis except for the use of a 5-μL injection volume. UPLC was interfaced by electrospray ion source technology to a hybrid time-of-flight mass spectrometry (Waters Xevo™ G2 QTOF) with a working resolution of $\sim 20\,000$ full-width at half-maximum. Tuning conditions employed cone voltage (20 V), capillary voltage (0.8 kV), source block temperature (120°C) and desolvation gas flow of 600 L/h at 400°C. Waters MassLynx™ software (v4.1) was used for system operation, and data acquisition was performed at a rate of 10 spectra per second in the centroid mode from *m/z* 50 to 1,000 using MS^E functions. Function 1 was acquired at a collision energy of 6 eV and Function 2 ramped from 10 to 40 eV. A second electrospray was used for baffled entry of the reference solution (leucine enkephalin) at a flow rate of 5 μL/min with 25 s samplings for a Function 3 acquisition. Data were analyzed with the ChromaLynx™ software (Waters) with an exact mass database for over 950 toxicologically relevant drugs and metabolites that included protonated molecule, fragment ion and chromatographic retention time data (33). Criteria for a positive drug finding included retention time within 0.3 min, detection under Function 1 acquisition of the protonated species with a measured mass accuracy criteria of within 5 ppm compared with absolute mass and detection under Function 2 acquisition of a qualifier fragment ion with a 5 ppm mass accuracy. A contemporaneous analysis of reference material is also performed for all positive drugs and metabolite findings, when available. Additional criteria used in the evaluation of screening specificity included the identification of two or more qualifier fragment ions with measured mass within the 5 ppm and an average isotope ratio fit for M+, M + 1 and M + 2 isotopes within 10% of the calculated ratio. Salicylate, barbiturates, ibuprophen and acid metabolites such as formic and glycolic acid are not included in either the nominal or high-resolution mass screening methods, as alternative ion source polarity and mobile phase conditions are needed for the detection of these acidic agents.

Validation procedures

Whole blood Panels A, B and C supplemented at drug and metabolite concentrations of 100 ng/mL were extracted and analyzed

in replicate UPLC-MS^E-TOF runs for the assessment of detection reproducibility and for the determination of average mass error for each analyte. The limit of detection sensitivity for UPLC-MS-MS and UPLC-MS^E-TOF analyses was determined with Panel A, B and C drugs and metabolites by replicate extraction and analysis of negative blood supplemented with 1, 10, 50, 100 and 150 ng/mL concentrations of each reference analyte as described in the validation sample section above. The limit of detection was defined as the lowest concentration resulting in a reproducible positive drug finding based on assay criteria.

Column recovery, matrix effects and overall process efficiency were assessed at a concentration of 100 ng/mL for reference analytes. Column recovery was calculated as a percentage by comparing mean precursor ion abundance (Function 1 acquisition) by UPLC-MS^E-TOF analysis for reference analytes spiked into negative blood before extraction with those of samples spiked after extraction of negative blood. Matrix effect was assessed by the percent change in precursor ion abundance of reference analytes spiked after extraction when compared with ion abundance of reference analytes spiked into the mobile phase. Overall process efficiency was determined as a percentage by comparing mean precursor ion abundance after extraction and analysis of spiked blood when compared with ion abundance of reference analytes in the mobile phase.

Results and discussion

Analytical validation

Figure 1 shows a representative postmortem blood analysis report with overlapping total ion chromatograms at low and high CID acquisitions at the top and with corresponding extracted ion chromatograms for the selected positive compound (benzoyllecgonine) at the bottom. All of the positive drugs, metabolites and internal standard are listed in the center of the report with the full drop-down set of benzoyllecgonine data including database retention time and accurate mass of both the protonated species and qualifying fragment ion followed by actual analyte retention time, ion abundance and mass error along with isotope cluster data. Each positive analyte, as well as the internal standard, may be selected during the data review process for a similar display of ion data and chromatograms.

A comparison of the mass resolution for protonated benzoyllecgonine as determined by nominal and high-resolution mass analyses is shown in Figure 2. Superimposed mass scales in daltons for TOF and MS allow a comparison of mass resolution with direct infusions of 250 ng/mL benzoyllecgonine prepared in acetonitrile : water (50 : 50 v/v). The infusion study clearly shows the equivalent accuracy of the average mass determination by both TOF and nominal MS analyses, but also demonstrates the enhanced resolution and precision of mass acquisition and analysis by TOF. The effect of enhanced TOF resolution on mass error and detection reproducibility was further evaluated for representative drugs and metabolites by between-run replicate ($n = 10$) UPLC-MS^E-TOF analysis of whole blood Panels A, B and C with drug and metabolite concentrations of 100 ng/mL. All reference drugs and metabolites were reproducibly detected in the screening method over multiple runs, and Figure 3 displays the average (\pm SD) mass error in ppm for each reference agent. The mean absolute mass error of 1.28

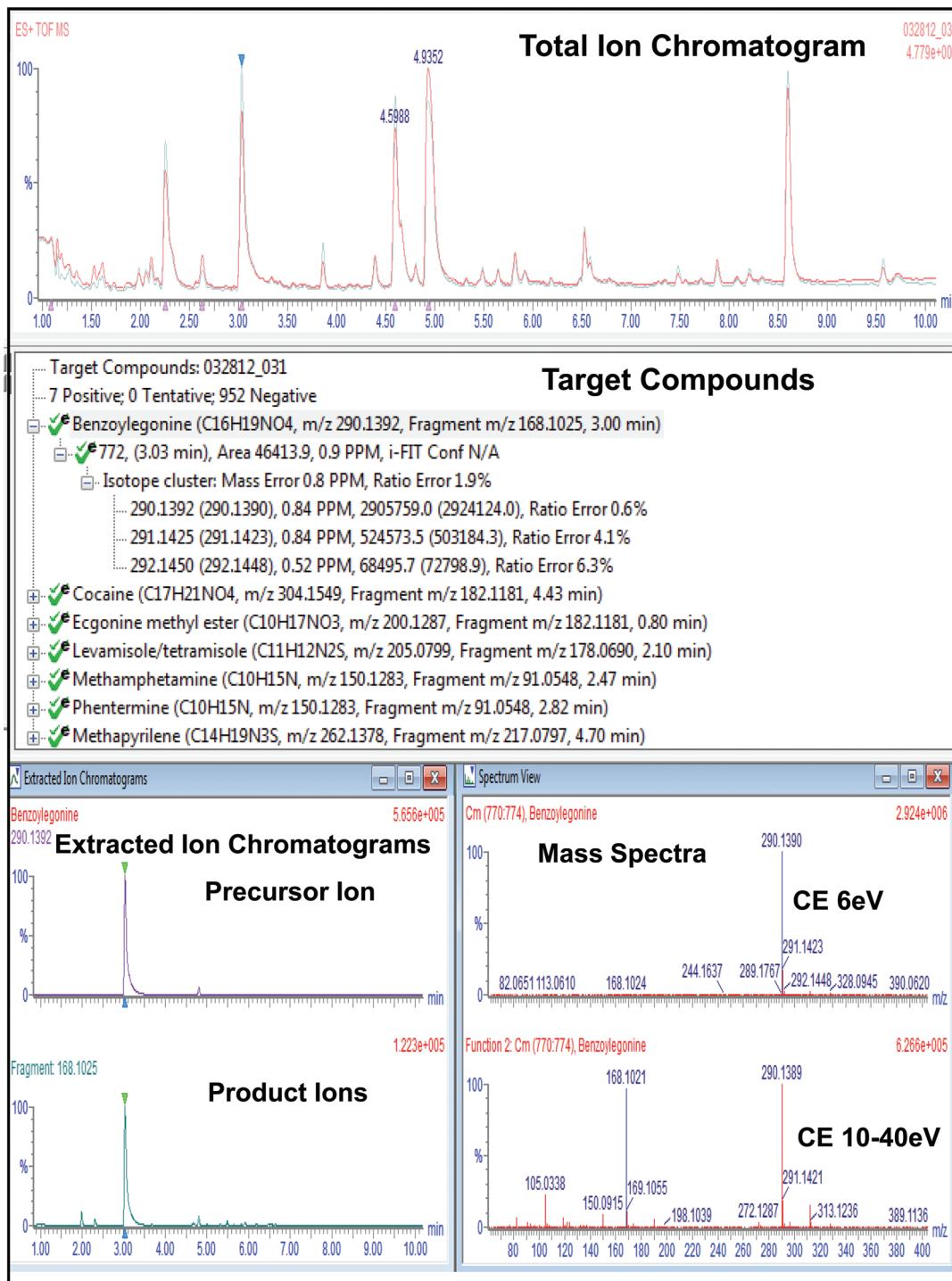


Figure 1. Representative report of UPLC-MS^E-TOF chromatograms, mass spectra and acquisition data for postmortem blood analysis.

ppm with an average standard deviation of 0.91 ppm for absolute mass error was within the method's 5 ppm threshold (red lines) for a positive detection of screened agents.

The limit of detection of the UPLC-MS^E-TOF screening method was also determined for representative drugs and metabolites by replicate analysis of whole blood Panels A, B, and C at progressively lower concentrations (100, 50, 25, 10, 5, 1, 0.5 and

0.1 ng/mL). The assessment of reproducible detection was based on the positive criteria employed in the screening method. A mean limit of detection of 6.3 ng/mL, with a median, SD and range of 1.0, 14 and 0.5–100 ng/mL, respectively, was determined across the 81 reference agents in Panels A, B and C. Figure 4 shows the limit of detection for each of the agents. Figure 4 also shows the results of the same sensitivity assessment

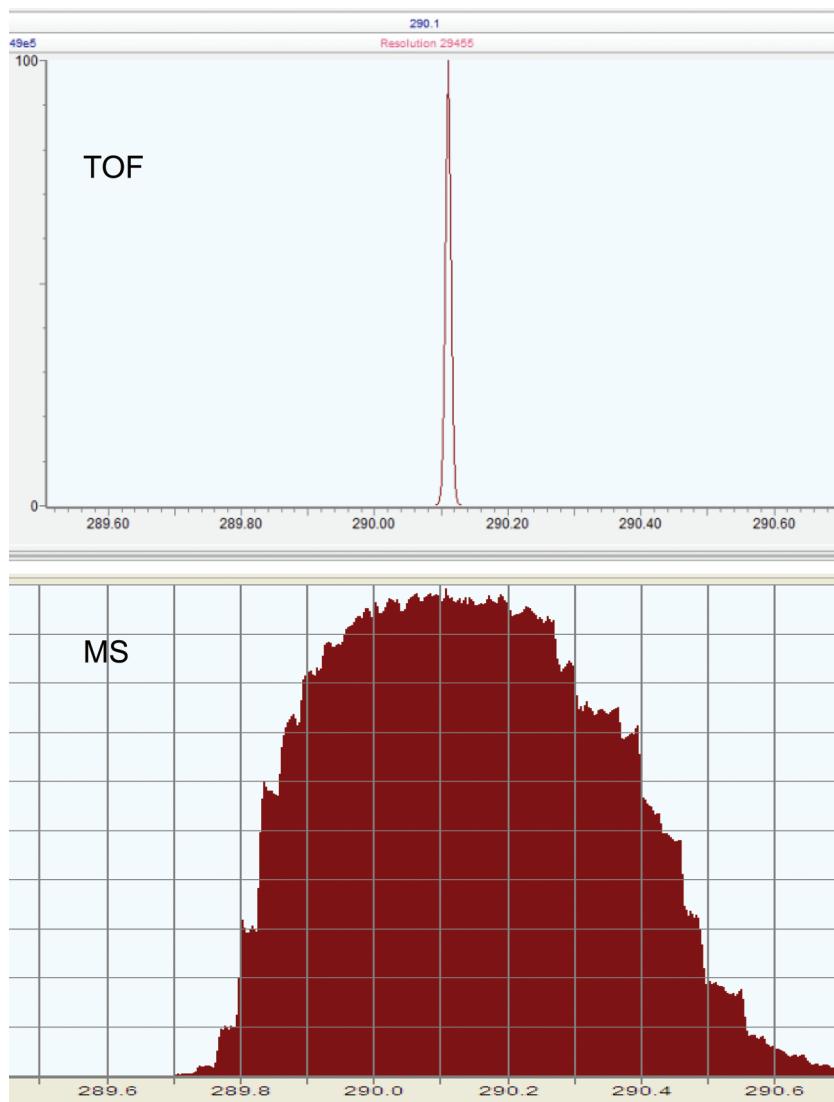


Figure 2. Comparative resolution (precision) of protonated benzoylecgonine mass determination by TOF (top) and nominal MS (bottom) technologies with a direct infusion of 250 ng/mL concentration.

for screening by the nominal mass UPLC–MS–MS method currently used in our forensic practice. A mean limit of detection sensitivity of 7.3 ng/mL was determined by UPLC–MS–MS analysis for the 81 reference agents with a median, SD and range of 1.0, 15 and 0.5–100 ng/mL, respectively. Therefore, UPLC–MS–MS and UPLC–MS^E–TOF compare closely in detection limit. It should be noted that while the detection sensitivity for these reference agents is similar for the two methods, detection by UPLC–MS^E–TOF may be extended to a much larger targeted set of over 900 agents with the potential for further expansion of the targeted screen.

Factors affecting UPLC–MS^E–TOF screening performance were also studied. The combined effect (process efficiency) of solid-phase extraction recovery and matrix effect for Panels A, B, and C reference analytes is shown in Figure 5. Process efficiency averaged 73% across all analytes, with a 100-fold range in efficiency (1.3–130%). A significant number (24 of 81) of the agents had a process efficiency of <60%, with 75% of these

low-process efficiency agents demonstrating a correlating limit of detection above the 1 ng/mL median sensitivity reported above. Process efficiencies determined with gabapentin (4.6%), morphine (21.4%) and oxymorphone (29.5%), for example, had corresponding detection limits of 100, 50 and 50 ng/mL, respectively, all well above the limit of detection for other agents with high process efficiency.

The contribution of column recovery and matrix effects to process efficiency is compared in Figures 6–8 for Panel A, B and C agents. Column recovery and matrix effect influence process efficiency in an analyte-dependent manner. For example, with amiodarone, promethazine, paroxetine, morphine and oxymorphone analysis, column recovery and ion suppression are both significant factors in reducing overall process efficiency. For other agents, either matrix effect (nicotine, fluoxetine and sertraline) or column recovery (nalorphine and levetiracetam) are the predominant factors. It also appears that while ion enhancement of >20% is found for just two reference analytes (caffeine

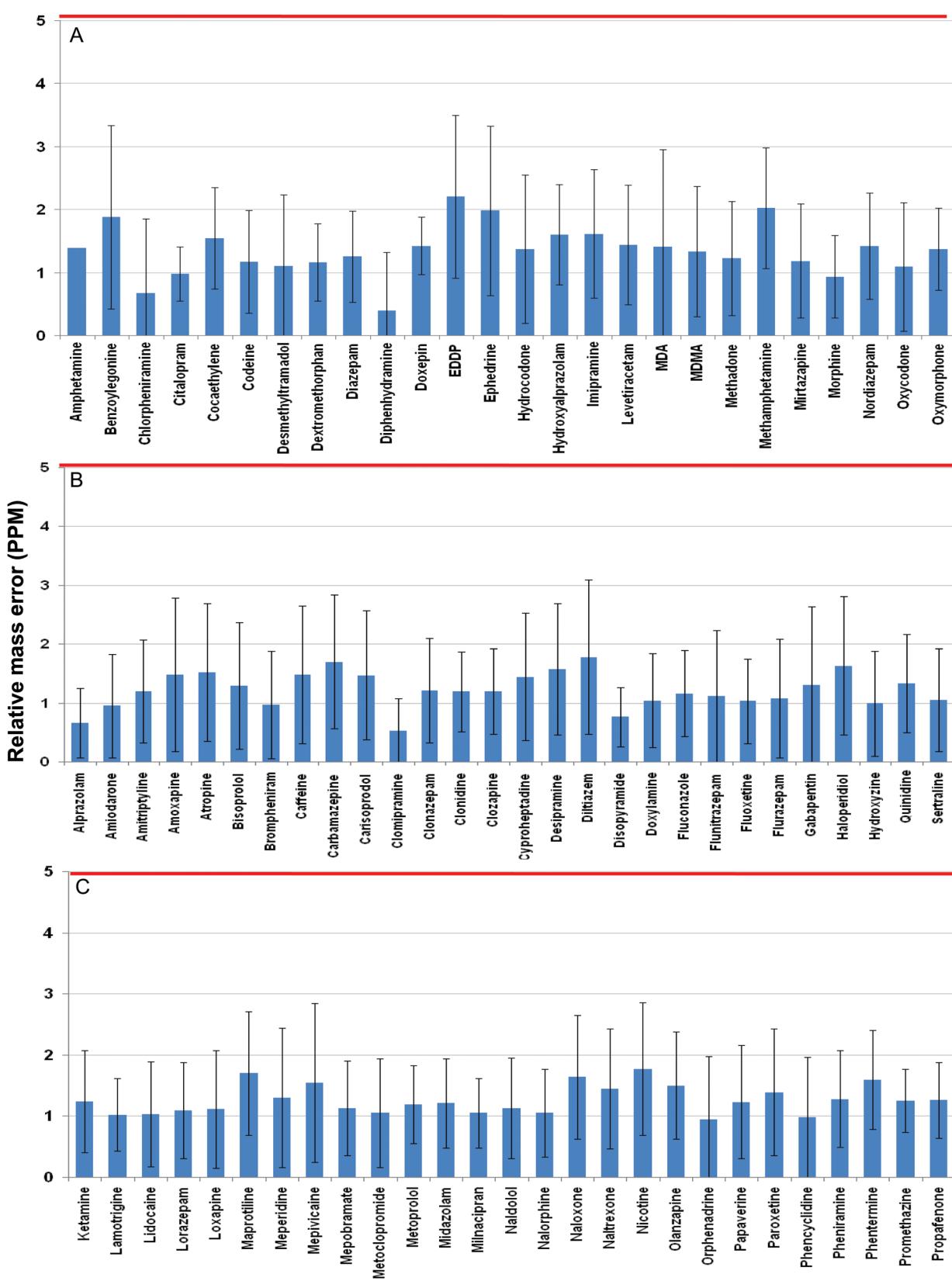


Figure 3. Mean (\pm SD) mass error of panel drugs and metabolites at concentrations of 100 ng/mL with replicate ($n = 10$) between-run analysis of blood extracts by UPLC-MS^E-TOF for Panel A, B and C control pools.

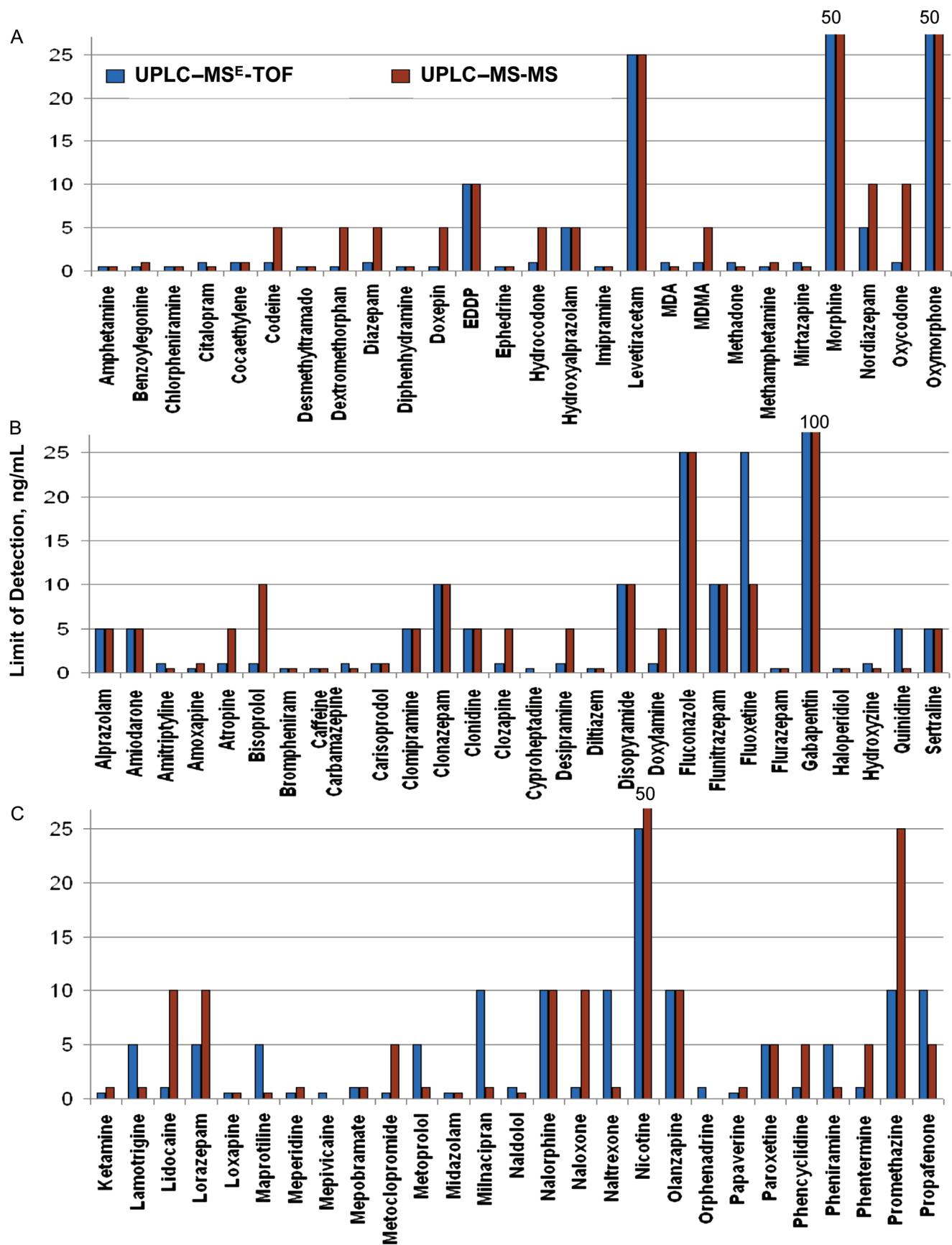


Figure 4. Limit of detection sensitivity for representative drugs and metabolites analysis by both UPLC-MS^E-TOF and UPLC-MS-MS in whole blood Panels A, B and C. Detection limits for UPLC-MS^E-TOF is shown to the left of UPLC-MS-MS for each analyte.

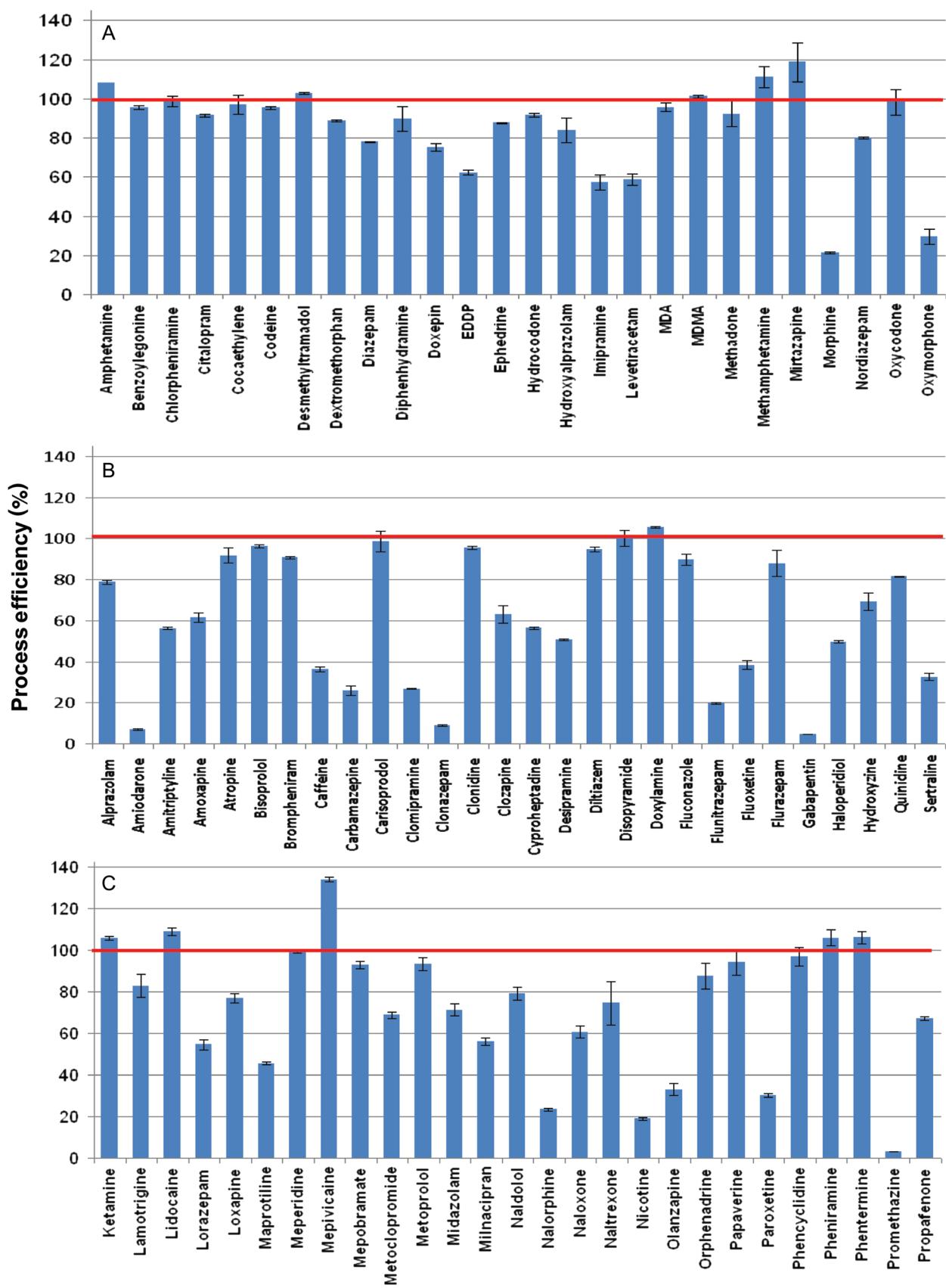


Figure 5. Percent process efficiency of representative drugs and metabolites analyzed by UPLC–MS^E-TOF in whole blood Panels A, B and C.

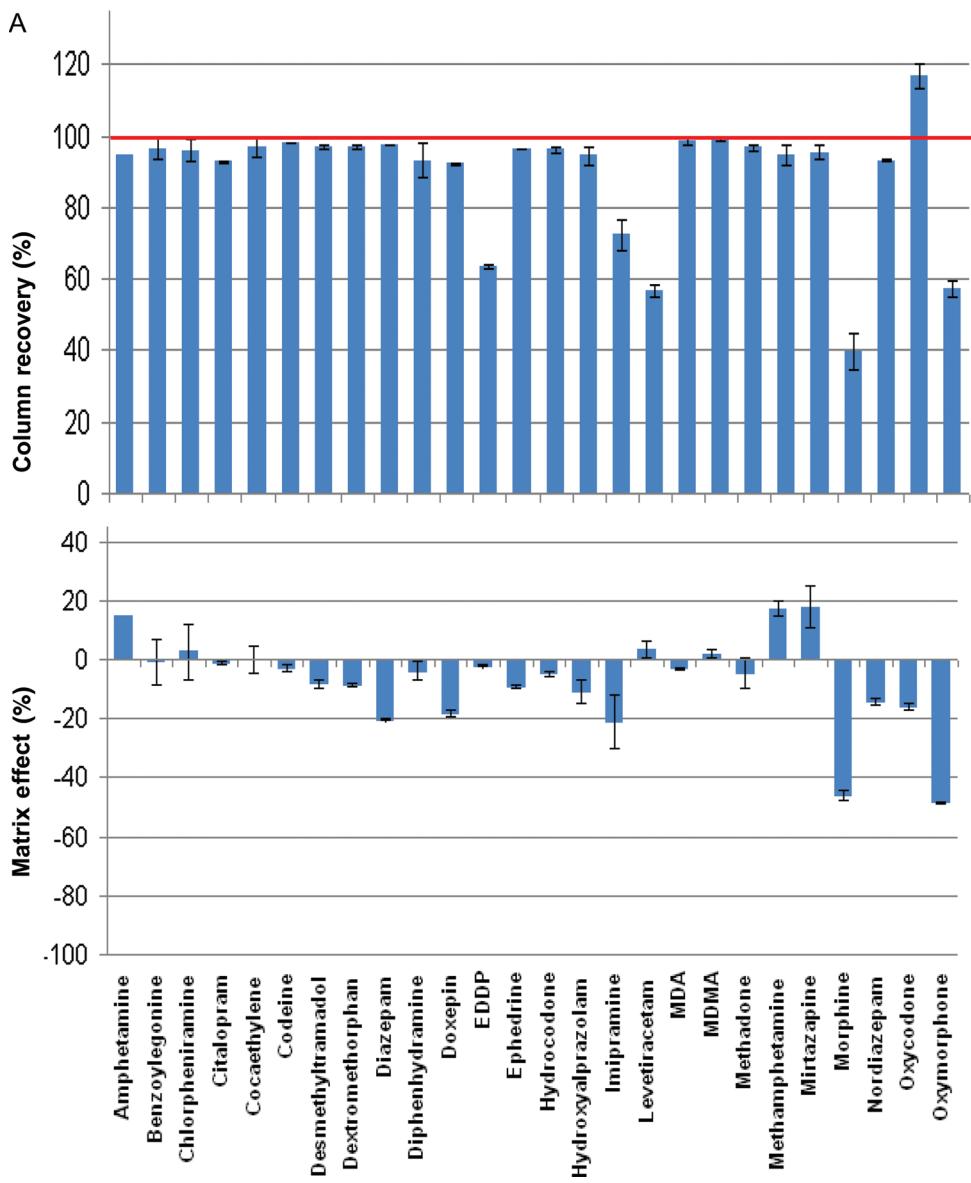


Figure 6. Column recovery (upper) and matrix effect (lower) for Panel A drugs and metabolites analyzed by UPLC-MS^E-TOF.

and mepivacaine), ion suppression is the predominant matrix effect that may occur.

The degree of matrix effect across the 81 reference drugs and metabolites also revealed an interesting relationship with column retention time as shown in Figure 9. Ion suppression occurs with early and late eluting agents in support of the concept of ion suppression at the ion source by co-eluting polar compounds near the void volume as well as by late eluting phospholipids in the extracted sample matrix (37, 38). While mixed mode solid-phase extraction and UPLC separation have been shown to reduce, but not eliminate, the matrix effect (39, 40), others have also shown that matrix effects may variably influence the limit of detection sensitivity of individual analytes (37), making optimization of a general unknown method for each individual screening agent challenging (38). Major factors that influence the limit of detection sensitivity include the selection of

ion source tuning parameters that must be standardized across all agents, the molecular properties of the analyte and the presence of co-eluting agents competing at the source of ion entry. In our study, optimal column recovery combined with limited matrix effect, as observed with the majority of reference analytes, allowed a detection limit as low as 0.5 ng/mL but, even with the range of column recovery and matrix effects, screening sensitivity remained consistently <100 ng/mL as needed in a medical examiner setting.

Method comparison studies

Screening performance in routine medical examiner casework was determined in further validation of the method by comparison of drug and metabolite detection by nominal and high-resolution screening methods in a 300 case cohort. Postmortem

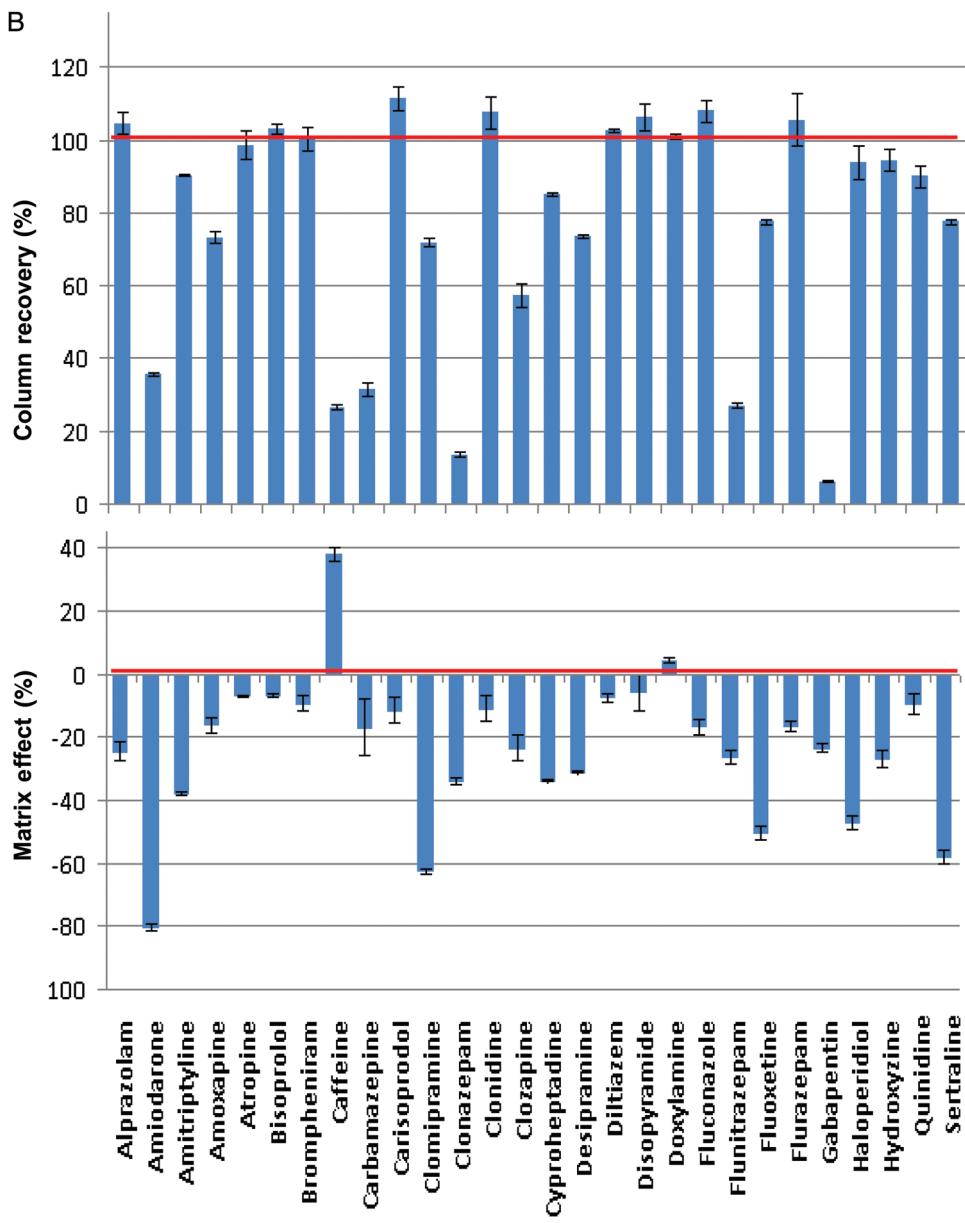


Figure 7. Column recovery (upper) and matrix effect (lower) for Panel B drugs and metabolites analyzed by UPLC-MS^E-TOF.

blood was screened by UPLC-MS, UPLC-MS-MS and UPLC-MS^E-TOF methods, and comparative results are listed by individual drug or metabolite in Table I. Since our routine screening protocol employs screening by both UPLC-MS and UPLC-MS-MS methods, positive findings by this combined screening approach are also shown. A total of 1,528 drugs or metabolites were detected by all methods for 204 drug or metabolite agent types. Table II summarizes the total positive findings and detection rates. Compared with detection rates for UPLC-MS (57%), UPLC-MS-MS (72%) or combined nominal mass screening (80%), the 99% detection rate by UPLC-MS^E-TOF was highest. Since UPLC-MS-MS detection is limited to only 208 targeted analytes, the detection rate for this subset of analytes was also calculated for a more direct comparison of UPLC-MS-MS and UPLC-MS^E-TOF screening performance. A total of 1,163 screen

positive findings for 124 drug or metabolite agent types were determined within this subset of analytes. UPLC-MS-MS and UPLC-MS^E-TOF compared more closely within this subset, with a detection rate of 94.4% by UPLC-MS-MS and 98.5% by UPLC-MS^E-TOF. Even within this targeted set of analytes, however, an additional 48 positive findings were made by UPLC-MS^E-TOF analysis.

In a previous study, we compared GC-MS, UPLC-MS and UPLC-MS-MS screening in postmortem blood and demonstrated a screening specificity of >98% for all methods along with an improved detection rate by combined UPLC-MS and UPLC-MS-MS screening. This led to the replacement of GC-MS with combined UPLC-MS and UPLC-MS-MS screening in our routine practice. In the current study, UPLC-MS^E-TOF detected an additional 302 agents in postmortem blood when compared with

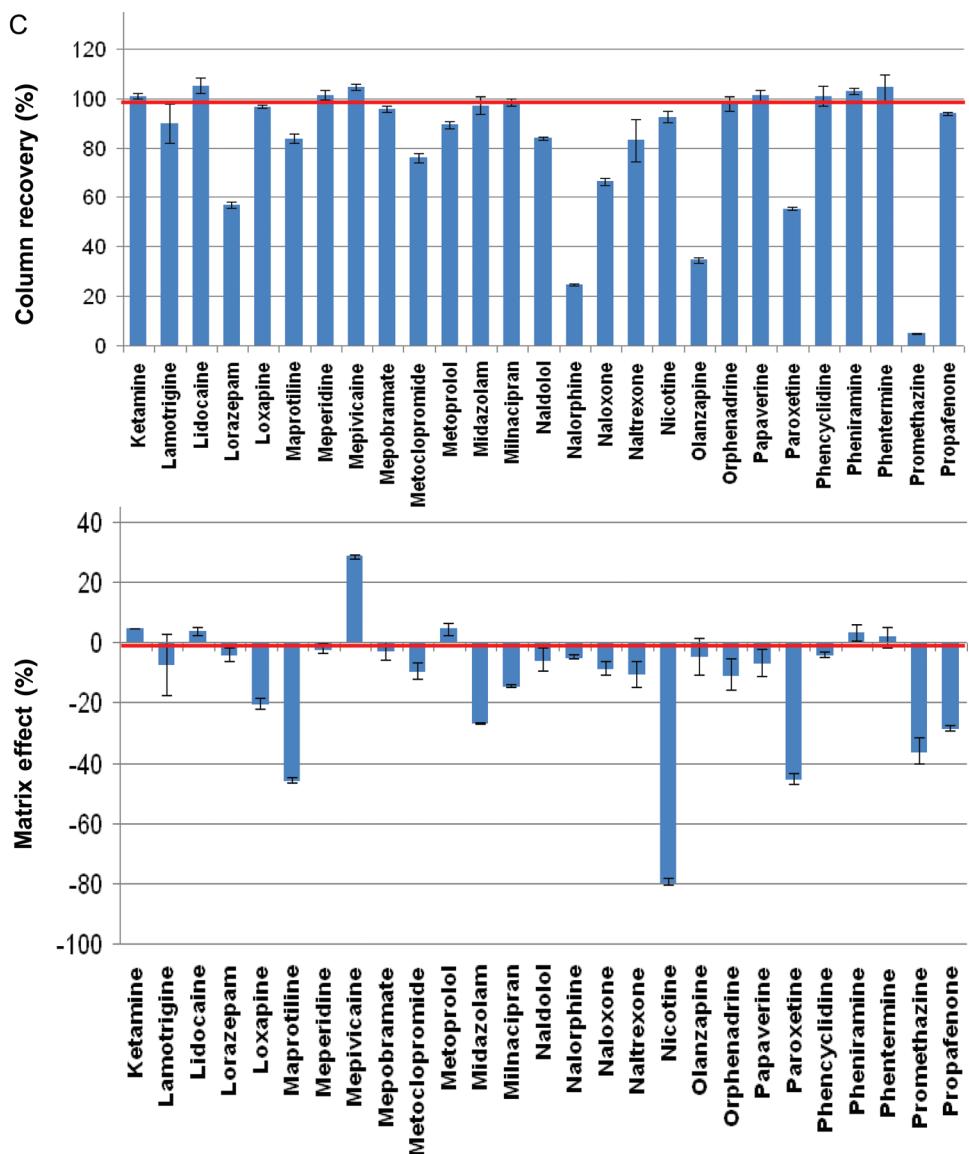


Figure 8. Column recovery (upper) and matrix effect (lower) and for Panel C drugs and metabolites analyzed by UPLC–MS^E-TOF.

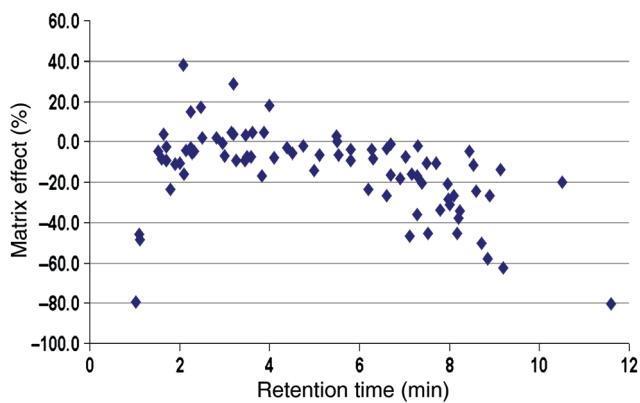


Figure 9. Relationship between percent matrix effect and column retention time with UPLC–MS^E-TOF analysis.

combined UPLC–MS and UPLC–MS-MS screening across all analytes. The majority (73%, 221 agents) of these additional positive findings were, however, metabolites of drugs that were confirmed as positive by both nominal and high-resolution screening methods. The remaining 81 new analyte findings by UPLC–MS^E-TOF were further analyzed for isotope pattern and additional qualifying fragment ions. All the additional new agent findings showed an isotope pattern fit within 10% of the theoretical pattern. For 64 of these new agents, two or more qualifying fragment ions with mass error criteria of <5 ppm were identified. While further confirmatory testing of decedent blood was limited by the confidentiality protocol employed in this study, the potential for a high degree of detection specificity is evident from the method comparison, drug–metabolite relationships and additional isotopic and fragment analyses.

Table I

Specific drug and metabolite positive findings for all, individual and combined screening methods

Drug/metabolite	All methods	UPLC–MS ^E -TOF	UPLC–MS-MS	UPLC–MS	UPLC–MS-MS + UPLC–MS
Acetaminophen	31	29	31	18	31
Allopurinol	2	2	0	1	1
Alprazolam	27	26	27	17	27
Alprazolam, hydroxy	1	1	NI	0	0
Amantadine	3	3	1	3	3
Amiodarone	20	20	20	17	20
Amiodarone, desethyl	4	4	NI	1	1
Amitriptyline	4	4	4	3	4
Amlodipine	7	7	NI	0	0
Amoxapine	1	1	1	0	1
Amoxapine, 8-hydroxy	1	1	NI	1	1
Amphetamine	9	9	9	8	9
Amphetamine, 4-fluoro	1	1	NI	0	0
Amphetamine, ethyl	1	1	NI	0	0
Aprenavir	1	1	NI	1	1
Aripiprazole	2	2	2	1	2
Atazanavir	1	1	NI	0	0
Atenolol	7	7	7	5	7
Atorvastatin	4	4	0	0	0
Atropine	47	47	47	40	47
Benzepril	1	1	NI	0	0
Benzhexol/trihexyphenidyl	2	2	NI	0	0
Benzoylconidine	49	49	49	40	49
Benztropine	2	2	2	1	2
Bisoprolol	1	1	1	1	1
BkMDMA	2	2	NI	2	2
Brompheniramine	1	1	NI	1	1
Brompheniramine, desmethyl	1	1	NI	0	0
Bropropion, hydroxy	10	10	NI	9	9
Bupivacaine	3	3	3	2	3
Buprenorphene, nor	3	2	2	0	2
Buprenorphene	4	4	3	1	3
Bupropion	10	10	9	8	9
Buspirone	1	1	1	1	1
Carbamazepine	7	7	7	7	7
Carbamazepine, 10-hydroxy	4	4	NI	1	1
Carbamazepine, epoxide	4	4	NI	1	1
Carisoprodol	1	1	1	0	1
cathine (norpseudoephedrine)	1	1	NI	1	1
Cathinone, 4-methy-N-ethyl	1	1	NI	0	0
Cetirizine	17	17	NI	9	9
Chlordiazepoxide	1	1	1	0	1
Chlorpheniramine	16	16	16	14	16
Chlorpheniramine, desmethyl	3	3	NI	0	0
Chlorpheniramine, dinor	5	5	NI	0	0
Chlorpheniramine, N-oxide	1	1	NI	0	0
Chlorpromazine	2	2	1	1	1
Ciprofloxacin	3	3	NI	0	0
Citalopram	25	25	25	23	25
Citalopram, n-desmethyl	23	23	NI	11	11
Citalopram, didesmethyl	8	8	NI	2	2
Clarithromycin	1	1	NI	0	0
Clomipramine	1	1	1	1	1
Clomopramine, n-desmethyl	1	1	NI	0	0
Clonazepam	2	2	1	0	1
Clonazepam, 7 amino	17	17	17	9	17
Cloridine	1	0	0	1	1
Cocaethylene	26	26	26	18	26
Cocaine	39	39	38	28	39
Cocaine, hydroxy	8	8	NI	0	0
Cocaine, nor	10	10	NI	0	0
Codeine	12	12	11	9	11
Codeine, acetyl	1	1	NI	0	0
Codeine, nor	4	4	NI	0	0
Cyclobenzaprine	12	12	12	11	12
Desipramine	1	1	1	1	1
Dextromethorphan	10	10	10	8	10
Diaminodiphenylsulfone	1	1	NI	1	1
Diazepam	25	25	25	23	25
Dihydrocodeine	19	18	19	16	19
Diltiazem	12	12	12	10	12

(continued)

Table I Continued

Drug/metabolite	All methods	UPLC–MS ^E -TOF	UPLC–MS-MS	UPLC–MS	UPLC–MS-MS + UPLC–MS
Diltiazem, deacetyl	9	9	NI	0	0
Diltiazem, desmethyl	10	10	NI	0	0
Diphehydramine	46	46	46	36	46
Diphenhydramine, Desmethyl	9	9	NI	0	0
Doxazosin	1	1	NI	1	1
Doxepin	2	2	2	2	2
Doxepin, desmethyl	2	2	NI	0	0
Doxylamine	9	9	9	6	9
Duloxetine	4	4	4	1	4
Egonine methylester	34	34	33	0	33
EDDP	11	11	11	9	11
Ephedrine/pseudoephedrine	9	9	9	6	9
Etomidate	3	3	1	3	3
Famotidine	2	2	NI	0	0
Fentanyl	20	18	20	6	20
Fentanyl, nor	12	12	NI	0	0
Fexofenadine	4	4	NI	4	4
Fluconazole	4	4	4	2	4
Fluoxetine	7	7	7	5	7
Fluoxetine, nor	5	5	NI	2	2
Fluphenazine	1	1	1	0	1
Flurazepam	1	1	1	0	1
Gabapentin	15	15	14	3	14
Gipizide	1	1	NI	1	1
Guaiifenesin	2	2	2	0	2
Haloperidol	3	3	3	2	3
Haloperidol, dihydro	2	2	NI	0	0
Hydrocodone	26	26	25	21	25
Hydromorphone	10	7	10	1	10
Hydroxyzine	25	25	25	17	25
Ifenprodil	1	1	0	1	1
Imipramine	2	2	2	1	2
Irbesartan	1	1	NI	1	1
Ketamine	2	2	2	1	2
letamine, demethyl	1	1	NI	0	0
Labetalol	2	2	2	1	2
Lamotrigine	8	8	7	8	8
Levamisole/tetramisole	42	42	NI	28	28
Levetiracetam	4	4	4	4	4
Levorphanol	2	2	0	0	0
Lidocaine	28	28	27	21	28
Loperamide	4	4	NI	1	1
Loperamide, desmethyl	3	3	NI	0	0
Loratadine	3	3	NI	2	2
Lorazepam	5	5	4	1	4
Losartan	5	5	NI	4	4
Loxapine	1	1	1	1	1
Loxapine, 8-hydroxy	1	1	NI	0	0
mCCP	18	18	NI	8	8
MDA	2	2	2	1	2
MDMA	1	1	1	1	1
Meperidine	1	1	1	1	1
Meperidine, nor	1	1	NI	1	1
Meprobamate	2	2	2	0	2
Methadone	14	14	14	12	14
Methamphetamine	1	1	1	1	1
Methocarbamol	1	1	1	0	1
Methoxymethamphetamine	1	1	NI	0	0
Methylphenidate	2	2	2	2	2
Methylprednisolone	1	1	NI	1	1
Metoclopramide	2	2	2	2	2
Metoprolol	17	17	17	12	17
Metoprolol, hydroxyl	15	15	NI	0	0
Midazolam	7	7	7	3	7
Midazolam, alpha-hydroxy	2	2	NI	0	0
Mirtazepine	5	5	5	4	5
Mirtazepine, hydroxy	3	3	NI	0	0
Mirtazepine, n-desmethyl	4	4	NI	3	3
Morphine	23	21	23	13	23
Morphine, 6-acetyl	8	8	8	4	8
Morphine, dihydro	2	2	NI	0	0
Moxifloxacin	1	1	NI	0	0
Nadolol	3	3	3	3	3

(continued)

Table I Continued

Drug/metabolite	All methods	UPLC-MS ^E -TOF	UPLC-MS-MS	UPLC-MS	UPLC-MS-MS + UPLC-MS
Naloxone	23	23	22	12	22
Naproxen	1	0	1	0	1
Nordiazepam	27	27	27	22	27
Nortriptyline	8	8	8	6	8
Noscapine	2	2	NI	0	0
Oflaxacin	8	8	NI	5	5
Olanzapine	5	5	4	3	4
Olanzapine, N-desmethyl	5	5	NI	0	0
Ondansetron	9	9	NI	0	0
Oxazepam	8	8	8	0	8
Oxcarbazepine	3	3	2	1	2
Oxybutynin	1	1	NI	0	0
Oxycodone	28	28	28	23	28
Oxycodone, nor	17	17	NI	0	0
Oxymorphone	3	3	3	3	3
Papaverine	9	9	9	4	9
Paroxetine	4	4	4	2	4
Perphenazine	1	1	NI	1	1
Pheniramine	2	2	1	2	2
Phenylpropanolamine	3	3	3	1	3
Phenytoin	3	0	3	0	3
Prilocaine	1	1	NI	1	1
Promethazine	6	6	6	1	6
Promethazine, hydroxydesmethyl	5	5	NI	0	0
Promethazine, sulfoxide	6	6	NI	0	0
Propoxyphene	2	2	2	1	2
Propoxyphene, desmethyl	2	2	2	1	2
Propranolol	3	3	3	2	3
Propylhexedrine	1	0	NI	1	1
Protriptyline	1	1	NI	0	0
Quetiapine	15	15	15	14	15
Quinidine/quinidine	13	13	13	7	13
Quinidine, hydroxy	8	8	8	2	8
Ranitidine	4	4	4	2	4
Risperidone	4	4	4	3	4
Risperidone, dihydro	1	1	NI	0	0
Risperidone, hydroxy	6	6	NI	3	3
Rosuvastatin	1	1	NI	0	0
Salbutamol	2	2	2	1	2
Scopolamine	2	2	1	1	1
Sertaline	8	8	7	7	8
Sidenafil	3	3	3	2	3
Sulfamethoxazol	1	1	NI	0	0
Temazepam	11	11	11	6	11
Timolol	1	1	NI	0	0
Topiramate	2	2	2	1	2
Tramadol	14	14	14	8	14
Tramadol, desmethyl	15	15	15	10	15
Tramadol, o-desmethyl	13	13	NI	0	0
Trazadone	25	25	25	22	25
Triancinolone acetonide	1	1	NI	0	0
Trimethoprim	8	8	7	5	7
Venlafaxine	7	7	7	5	7
Venlafaxine, o-desmethyl	7	7	NI	4	4
Warfarin	5	5	5	4	5
Zolpidem, metabolite 1	10	10	NI	0	0
Zolpidem, metabolite 2	2	2	NI	0	0
Zolpidem	10	10	10	7	10
Zopiclone	1	1	1	1	1
Zopiclone, desmethyl	1	1	NI	0	0

NI, analyte not included in an MRM method.

Conclusions

An UPLC-MS^E-TOF method has been validated for use in postmortem drug screening and shows an enhanced drug and metabolite detection sensitivity compared with nominal mass screening methods. Screening by high-resolution mass spectrometry is performed with full-spectrum acquisition in the MS^E mode, combining molecular and fragment ion acquisitions and analysis. The study indicates that screening by UPLC-MS^E-TOF

Table II

Method comparison of screen positive rates for general unknown testing in 300 medical examiner cases

Method(s)	UPLC-MS	UPLC-MS-MS	UPLC-MS + UPLC-MS-MS	UPLC-MS ^E -TOF
Drugs/metabolites detected by all methods (<i>N</i> = 1,528)	870	1,098	1,223	1,510
Percent screen positive rate (%)	57.0	71.9	80.0	98.8

can be performed at an MRM level of sensitivity without restriction on the number of analytes in the targeted screen. An increased number of initial screen findings of therapeutic and illicit drugs will be made in medical examiner casework, thus requiring the availability of confirmatory methods of equivalent or greater sensitivity. UPLC-MS^E-TOF screening also offers the potential for true, nontargeted, general unknown screening through elemental composition and molecular structure analysis of drugs and their fragmentation ions.

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