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Simultaneous Screening and Quantitation of Drugs and Their Metabolites in Postmortem Samples by Liquid Chromatography–High-Resolution Mass Spectrometry: Does It Provide Any Benefits?

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Abstract

Screening of postmortem blood and urine samples is used to identify compounds that may have contributed to an individual's death. Toxicologically significant compounds detected by the screen are then quantitated in blood to determine their likely effect upon death. In most laboratories, this is a two-step process. This study compares an established two-step screening and quantitative processes, utilizing a gas chromatography–mass spectrometry (GC–MS) screen followed by quantitation by GC–MS or high-performance liquid chromatography with diode array detection (HPLC–DAD), with a novel method utilizing liquid chromatography–high-resolution mass spectrometry (LC–HRMS). The LC–HRMS assay is able to screen postmortem blood and urine samples and simultaneously measure the concentration of toxicologically significant compounds in postmortem blood. Screening results of 200 postmortem blood samples and 103 postmortem urine samples by LC–HRMS and GC–MS showed that LC–HRMS detected key compounds in 125% more instances and there was a 60% increase in the number of compounds detected. Quantitative values generated using the LC–HRMS assay were within ±10% of values obtained using the established methods by GC–MS or HPLC–DAD. A retrospective analysis of turnaround times pre- and post-adoption of LC–HRMS showed a decrease for all of the compounds in the analysis, including a 43% reduction for free morphine and codeine, a 50% reduction for amphetamine and a 37% reduction for cocaine. Combining screening and quantitation reduced staffing requirements by 2 days for opiate quantitation and 1 day for most other analytes. The adoption of LC–HRMS also significantly reduced sample volume requirements. These results demonstrate that the adoption of LC–HRMS for simultaneous screening and quantitation delivered significant benefits in comparison to the two-step procedure.

Introduction

Postmortem toxicological analysis of biological samples can contribute to understanding the cause of death. Most laboratories will undertake a two-step process in which samples are first screened to identify compounds that may be present, followed by measuring the concentration of any toxicologically significant compounds. Screening is most commonly performed on postmortem blood and urine samples using mass spectrometry coupled to chromatography, such as gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–tandem mass spectrometry (1–3). Any screening tool used to analyze postmortem samples must be able to identify a wide range of drugs and their metabolites with a high degree of sensitivity and specificity, particularly markers of commonly abused substances, such as cocaine, heroin and amphetamine, and prescribed medication, including antidepressants, antiepileptics, antipsychotics and antihistamines.

In recent times, the emergence of new psychoactive substances (NPSs) has increased the number of substances, for which screening is necessary. Such compounds include fentanyl and its analogs (4–6) and novel benzodiazepines (7). This has necessitated the introduction of new screening techniques and instrumentation to allow the laboratory to keep abreast of changing trends in substance misuse.

High-resolution mass spectrometry (HRMS), most commonly coupled to liquid chromatography (LC–HRMS), has been used in a number of toxicological applications (8) including the screening of biological samples for NPSs (9) and prescription and illicit drugs and their metabolites (10, 11). LC–HRMS is well suited to screen postmortem samples. Its high degree of specificity enables "unknown" screening, in which compounds are identified on the basis of their molecular characteristics rather than by reference to a compound library (12, 13). However, identification is more routinely performed by comparison to a reference library (14). Therefore, LC–HRMS is highly suited to be the basis of a broad screening tool that can detect a wide range of compounds with a high degree of certainty.

Many compounds, once identified in postmortem samples, need to be measured to assess their impact on the cause of death. In most laboratories, compound quantitation in postmortem blood is performed in batch processes separated from the initial screening assay and using a range of analytical

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techniques. If a sample contains multiple drug compounds, several individual quantitative assays may be required to determine the concentration of all relevant analytes in the blood. This process can be time-consuming, labor-intensive and often requires a large sample volume.

In recent times, LC–HRMS has been used to combine screening with quantitation, streamlining laboratory workflows. Methods have been developed which use ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry to screen postmortem blood and urine samples using libraries containing ~2,598 compounds and which can simultaneously quantitate ~90 compounds in blood (15–18). Although the above-mentioned examples have demonstrated the suitability of time-of-flight accurate mass technology for screening and quantitation applications, there have been no studies that examine the benefits that adopting this approach may bring to the laboratory.

This study compares the performance of a two-step screening and quantitation workflow with a novel LC–HRMS assay which can screen for a range of drug compounds and their metabolites in postmortem blood and urine, while simultaneously measuring the concentration of 42 of these compounds. GC–MS screening is compared to screening by LC–HRMS, and the quantitative results obtained using LC–HRMS are compared to quantitative results achieved using well-established stand-alone quantitative assays by GC–MS and HPLC–DAD. A retrospective analysis of turnaround times and staffing requirements pre- and post-introduction of LC–HRMS is performed to determine if the investment in accurate mass technology delivers benefits. The assay itself is the first documented application of Orbitrap™ accurate mass technology for a combined screening and quantitative assay in postmortem blood and uses a protein crash pre-analytical phase with no sample derivatization to simplify analysis in comparison to other similar assays.

Table I. Compounds and Internal Standards for LC–HRMS Quantitation

Chemical class	Compound	Internal standard
Opiates	Morphine	Morphine-d_3
	Morphine-3-β-D-glucuronide	Morphine-3-β-D-glucuronide-d_3
	Morphine-6β-D-glucuronide	Morphine-6-β-D-glucuronide-d_3
	Codeine	Codeine-d_6
	Dihydrocodeine	Dihydrocodeine-d_6
	Hydrocodone	Hydrocodone-d_6
Cocaine and metabolites	Cocaine	Cocaine-d_3
	Benzoylecgonine	Benzoylecgonine-d_3
Methadone	Methadone	(±)-Methadone-d_3
Amphetamines	Amphetamine	(±)-Amphetamine-d_5
	Methamphetamine	(±)-Methamphetamine-d_5
	MDMA	(±)-MDMA-d_5
	MDA	(±)-MDA-d_5
	MDEA	(±)-MDEA-d_5
Benzodiazepines	Diazepam	Diazepam-d_5
	Nordiazepam	Nordiazepam-d_5
	Temazepam	Temazepam-d_5
	Oxazepam	Oxazepam-d_5
	Chlordiazepoxide	Chlordiazepoxide-d_5
	Nitrazepam	Nitrazepam-d_5
	Alprazolam	Alprazolam-d_5
Opioids	Tramadol	^{13}C-Tramadol-d_3
	O-Desmethyltramadol	*O*-Desmethyl-cis-tramadol-d_6
	Oxycodone	Oxycodone-d_6
Antipsychotics	Clozapine	Clozapine-d_4
	N-Desmethylclozapine	Clozapine-d_4
Antidepressants	Amitriptyline	Amitriptyline-d_3
	Nortriptyline	Nortriptyline-d_3
	Citalopram	Citalopram-d_6
	N-Desmethylcitalopram	Citalopram-d_6
	Fluoxetine	Fluoxetine-d_6
	Mirtazapine	Mirtazapine-d_3
	Venlafaxine	Venlafaxine-d_6
	O-Desmethylvenlafaxine	*O*-Desmethylvenlafaxine-d_6
	Sertraline	Sertraline-d_3
Antihistamines	Diphenhydramine	Diphenhydramine-d_3
Antiepileptics	Gabapentin	$^{13}C_3$-Gabapentin
	Pregabalin	$^{13}C_3$-Pregabalin
	Lamotrigine	Levetiracetam-d_6
Others	Propranolol	Propranolol-d_7 (ring-d_7)
	Paracetamol	Paracetamol-d_4
	Ketamine	Ketamine-d_4

Materials and Methods

Chemicals and reagents

All compounds for which quantitation was performed (Table I) were supplied as 1,000 µg/mL certified reference solutions (Cerilliant®, Round Rock, TX, USA). All internal standards were supplied as 100 µg/mL solutions (Cerilliant®). Drug-free lysed horse blood was from TCS Biosciences Ltd (Botolph Claydon, UK). Drug-free urine was from UTAK (Valencia, CA, USA). External quality assurance (EQA) solutions were from LGC Limited (Bury, UK). Formic acid was from VWR (Fontenay-sous-Bois, France), ammonium formate was from Sigma-Aldrich (Gillingham, UK), zinc sulfate was from Merck (Darmstadt, Germany), HPLC-grade methanol and acetonitrile were from Fisher Scientific (Gothenburg, Sweden) and deionized water was from a Milli-Q® system (Millipore, Billerica, MA, USA).

LC–HRMS Assay

Calibrator and internal standard solutions

Calibrator and internal standard solutions were prepared as outlined in Appendix A. The batch-to-batch consistency of calibrator solutions was checked by the assessment of commercial and in-house quality control (QC) solutions coving the full range of analytes and analyte concentrations. QC results achieved using the new calibrators which were within ±2 SDs of the mean QC values achieved using the previous calibrations were deemed to show acceptable performance of the new calibrators. New calibration solutions were also analyzed as samples, with their concentrations measured using the previous calibrators. A calculated value within ±10% of the target value was deemed to be acceptable. New QC materials were assessed prior to routine use by analysis on 20 separate batches, with means and standard deviations for the new QC materials calculated to determine their acceptable performance parameters.

Liquid chromatography

HPLC was performed using an UltiMate 3000 system (Thermo Scientific, Waltham, MA, USA). Injection volume was 5 µL. Chromatographic separation was via a Raptor biphenyl column (2.7 µm, 2.1 × 100 mm, Thames Restek, Saunderton, UK) held at 40°C. Compounds were eluted from the column using gradient separation (eluent A: 0.1% (v/v) formic acid, 2 mmol/L ammonium formate in deionized

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water; eluent B: 0.1% (v/v) formic acid, 2 mmol/L ammonium formate in methanol:acetonitrile (1 + 1), total flow rate 0.4 mL/min). Initial mobile-phase conditions were 10% organic phase which increased to 99% after 7 minutes. 99% organic was maintained for 1 minute, followed by 5 minutes of column re-equilibrium at 10% organic. The total run time was 13 minutes.

Mass spectrometry

Column eluent was analyzed using a Q Exactive Focus mass spectrometer (Thermo Scientific, Waltham, MA, USA) operating in positive ion electrospray mode. Data collection was in full-scan mode (mass resolution of 70,000 covering a mass range of 120–1,000 atomic mass units (amu)) with all ion fragmentation (stepped higher-energy collisional dissociation cell settings of 10, 20 and 30 at a mass resolution of 17,500 covering a mass range of 80–1,000 amu).

Sample preparation

Urine samples were prepared by diluting urine (1 + 9) in 10% methanol solution. Blood samples, QC and calibrator solutions were prepared by dilution 1 + 6 with ice-cold protein crash solution (4 + 1 + 1 internal standard solution, deionized water, 0.1 M zinc sulfate solution). Diluted samples were vortex mixed and then centrifuged (3,000 rpm, 10 minutes), before the supernatant was evaporated to dryness under nitrogen at 40°C. Once dry, the mixture was reconstituted in 250 µL of 10% methanol for analysis.

Screening library

Postmortem blood and urine samples were screened using the Tox Explorer™ library of compounds (Thermo Scientific, Waltham, MA, USA) and interrogated using TraceFinder Forensic 3.3 software (Thermo Scientific, Waltham, MA, USA). The liquid chromatography conditions supplied for use with the Tox Explorer™ library were adapted for use with the UltiMate 3000 system and the Raptor biphenyl column such that the retention times of 60 commonly encountered compounds were within ±10 seconds of the retention time in the Tox Explorer™ library. Thus, for the most commonly encountered compounds, definitive retention times were identified. However, for the less-commonly encountered compounds, suspect screening was performed based upon the retention times in the Tox Explorer™ library. Compounds were identified as being present on the basis of the accurate mass of the parent ion (mass tolerance 5 ppm), accurate mass of at least one fragment ion, correct retention time (±10 seconds) and isotope pattern (fit threshold (%) 70, allowed mass deviation (ppm) 10 and allowed intensity deviation (%) 30). Limit of detection (LOD) was determined for all compounds for which quantitation was also performed and for a selection of compounds in compound classes not covered by those being quantitated. Interference studies to exclude interference from isobaric substances were performed for the compounds in the library (see Appendix B).

Assay validation

The LC–HRMS assay was fully validated in line with the requirements of ISO 15189 by the UK Accreditation Service. This included an assessment of imprecision, bias, recovery, linearity, lower limit of quantitation and detection (LLOQ and LLOD), carryover, ion suppression/enhancement, interference, dilutional integrity and stability. Further information about the validation experiments performed can be seen in Appendix B.

Results comparison

Postmortem blood and urine samples submitted to the Department of Specialised Clinical Chemistry, Sheffield Teaching Hospitals, were analyzed by an established and fully validated GC–MS assay following basic extraction in line with the protocol outlined by Rab et al. (19). Samples were also analyzed using the LC–HRMS assay, and any compounds present in the samples were identified by reference to the Tox Explorer™ library. Identification was on the basis of retention time, accurate mass of parent ion, accurate mass of 1 fragment ion and isotope pattern. Samples containing one of the compounds from Table I were also analyzed using a range of fully validated established quantitative assays (19) to determine the concentration of these compounds in blood. Results from reference screening and quantitation assays were compared to results generated using LC–HRMS using Passing–Bablok regression analysis. An acceptable regression equation was deemed to be an equation with a systematic variation of <15% and proportional variation of <5% of the upper limit of the linear range of the assay. Total morphine (20, 21) was calculated using the following equation: total morphine (mg/L) = free morphine (mg/L) + (morphine-6-glucuronide (mg/L) + morphine-3-glucuronide (mg/L)) × 0.62.

Retrospective results comparison

A search of the laboratory information management system was performed to identify all instances where the compounds or metabolites outlined in Table III were measured in 3 months prior to or after the introduction of the LC–HRMS assay. Median and interquartile range (IQR) turnaround times were calculated, and the results obtained prior to the introduction of LC–HRMS were compared to those obtained following the introduction of LC–HRMS. All data collected were anonymized.

Results

A comparison of the screening results of 200 postmortem blood samples and 103 postmortem urine samples revealed 2,739 instances of a compound in the Tox Explorer™ LC–HRMS library being identified in blood (1,604 instances) or urine (1,135 instances). In comparison, the established GC–MS screen revealed a total number of 1,214 instances of a compound in the GC–MS library being identified in blood (645) and urine (569). This represents a 125% increase in the number of instances of a compound being detected by the LC–HRMS Tox Explorer™ library in comparison to the GC–MS library. The Tox Explorer™ library identified 157 different compounds in the blood and urine samples, whereas the GC–MS assay identified 98 compounds, i.e., a 60% increase in the number of compounds detected by the LC–HRMS screen. Most of the compounds in the library were detected in much more cases by the LC–HRMS screen than the GC–MS screen, indicating the improved sensitivity of the LC–HRMS screen. Of particular significance in this category were alprazolam and alpha-hydroxyalprazolam, gabapentin,

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Table II. Comparative Quantitative Results Generated in Postmortem Blood Samples Using LC–HRMS and a Range of Validated Reference Methods by HPLC–DAD and GC–MS

Compound	Regression equation	Range (*n*)
Morphine	$y = 0.99x$	0.03–12.35 (51)
Total morphine	$y = 1.07x$	0.08–12.82 (60)
Codeine	$y = 0.92x + 0.01$	0.03–2.46 (47)
Dihydrocodeine	$y = x - 0.01$	0.003–9.38 (16)
Hydrocodone	N/A	N/A
Cocaine	$y = 1.03x + 0.02$	0.10–1.12 (23)
Benzoylecgonine	$y = 0.89x + 0.01$	0.03–7.29 (47)
Methadone	$y = 1.09x + 0.01$	0.07–3.60 (16)
Amphetamine	$y = 1.15x - 0.01$	0.06–2.61 (6)
Methamphetamine	$y = 0.99x + 0.06$	0.03–10.67 (8)
MDMA	$y = 1.10x + 0.03$	0.14–6.90 (9)
MDA	$y = 0.97x + 0.06$	0.07–0.61 (8)
MDEA	N/A	N/A
Diazepam	$y = 1.06x + 0.01$	0.03–1.27 (31)
Nordiazepam	$y = 1.07x + 0.01$	0.05–1.67 (31)
Temazepam	$y = 1.03x + 0.02$	0.07–2.62 (8)
Oxazepam	N/A	N/A
Chlordiazepoxide	$y = 1.07x - 0.04$	0.07–1.88 (8)
Nitrazepam	N/A	N/A
Alprazolam	N/A	0.002–0.12 (1)
Tramadol	$y = 0.98x + 0.04$	0.06–5.14 (10)
O-Desmethyltramadol	$y = 0.97x + 0.03$	0.01–2.91 (6)
Oxycodone	$y = 0.88x$	0.05–0.86 (9)
Clozapine	$y = 1.09x + 0.07$	0.13–8.24 (5)
N-Desmethylclozapine	$y = 0.96x - 0.02$	0.11–3.57 (5)
Amitriptyline	$y = 1.10x - 0.02$	0.03–2.15 (17)
Nortriptyline	$y = 1.04x + 0.02$	0.002–3.49 (12)
Citalopram	$y = 1.06x + 0.1$	0.04–5.34 (12)
N-Desmethylcitalopram	N/A	N/A
Fluoxetine	$y = 1.09x - 0.01$	0.02–2.77 (17)
Mirtazapine	$y = 1.09x + 0.01$	0.07–2.03 (20)
Venlafaxine	$y = 1.07x - 0.03$	0.01–3.06 (15)
O-Desmethylvenlafaxine	$y = 1.10x - 0.11$	0.003–3.40 (15)
Sertraline	$y = 1.10x - 0.03$	0.003–3.51 (37)
Diphenhydramine	$y = 1.08x$	0.003–3.62 (9)
Gabapentin	N/A	0.26–59.04 (2)
Pregabalin	$y = 1.02x + 0.06$	0.13–14.49 (4)
Lamotrigine	$y = 1.09x + 0.12$	0.24–24.80 (11)
Propranolol	$y = 0.97x + 0.05$	0.07–6.18 (12)
Paracetamol	$y = 1.08x - 1.23$	0.56–774 (48)
Ketamine	$y = 1.08x + 0.07$	0.07–1.82 (8)

pregabalin, fentanyl and norfentanyl and morphine and its glucuronides. Of the compounds detected by GC–MS that were not detected by LC–HRMS, the most significant compounds of note were salicylate, propofol, tetrahydrocannabinol (THC) and barbiturates.

Quantitative results in postmortem blood, obtained using the LC–HRMS methodology, were compared to the results obtained using established quantitative methodologies by GC–MS or HPLC–DAD. The results are seen in Table II.

Retrospective results comparison

A retrospective evaluation of the turnaround times (point of sample receipt to result authorization) for 26 drugs and metabolites pre- and post-introduction of the LC–HRMS assay gave the results in Table III. This decrease in turnaround time for the compounds measured by the LC–HRMS screen resulted in a median decrease in case turnaround time from specimen reception to report generation of 2 days (9% decrease in turnaround time).

Table III. Turnaround Times for Compounds Measured in Postmortem Blood Samples[a]

Compound	Median pre-LC–HRMS turnaround time (days), (*n*, IQR)	Median LC–HRMS turnaround time (days), (*n*, IQR)	% Difference in turnaround time
Free morphine	21 (106, 16–27)	12 (119, 8–15)	–43
Total morphine	22 (107, 16–33)	13 (144, 9–16)	–41
Codeine	21 (84, 17–23)	12 (103, 7–15)	–43
Dihydrocodeine	20 (17–25, 33)	12 (29, 8–15)	–40
Amphetamine	22 (13, 16–27)	11 (23, 8–15)	–50
Cocaine	19 (102, 14–27)	12 (142, 8–15)	–37
Benzoylecgonine	19 (102, 14–27)	12 (142, 8–15)	–37
Amitriptyline	18 (35, 15–23)	15 (57, 12–16)	–17
Nortriptyline	18 (35, 15–23)	14 (58, 12–16)	–22
Citalopram	20 (33, 15–23)	15 (30, 10–19)	–25
Sertraline	18 (65, 14–23)	16 (48, 13–24)	–9
Venlafaxine	27 (20–29)	16 (12–19, 22)	–41
Mirtazapine	15 (68, 13–20)	14 (80, 8–17)	–7
Diazepam	17 (91, 13–24)	13 (70, 8–16)	–24
Temazepam	36 (5, 35–36)	23 (27, 20–25)	–36
Chlordiazepoxide	26 (15, 22–38)	14 (7, 12–15)	–46
Diphenhydramine	24 (16, 21–33)	17 (10–22)	–29
Propranolol	30 (14, 25–36)	23 (19–27, 30)	–23
Paracetamol	17 (111, 14–22)	16 (155, 14–25)	–6
Tramadol	20 (14–28)	16 (37, 15–22)	–20
O-Desmethyltramadol	20 (14–28)	16 (36, 15–22)	–20
Gabapentin	23 (18–30)	12 (22, 8–18)	–48
Pregabalin	23 (92, 18–31)	13 (103, 8–18)	–43
Alprazolam	48 (2, 42–54)	15 (6, 12–15)	–69
Clozapine	43 (3, 37–45)	25 (4, 24–32)	–42
Oxycodone	29 (4, 27–32)	26 (17, 23–35)	–10

[a]The turnaround times for gabapentin, pregabalin, alprazolam, clozapine and oxycodone pre-LC–HRMS are based upon results from samples referred to other laboratories for analysis.

An assessment of the costs of measuring the analytes in Table III found that the most significant saving was due to the reduction in staff time required to screen the samples and then quantitate significant compounds in the blood. The time involved in setting up and reading a batch of samples is roughly equivalent for both the LC–HRMS and GC–MS screens. However, the quantitation of the analytes in Table III is performed as part of the LC–HRMS screen meaning that the time taken to perform a separate quantitation using the existing method is saved. This time saving equates to 2 days of staff time per batch of opiates and 1 day for assays measuring the other compounds.

Discussion

The timely screening of postmortem samples for a wide range of drugs and their metabolites in postmortem blood and urine is an important step in the investigation of death where drug use may have made a contribution. Any screening tool must be comprehensive, detecting as many compounds as possible. Following the identification of significant compounds, their concentration should be measured in postmortem blood to determine if the compounds identified are likely to have contributed to death.

Time from sample receipt to result is becoming increasingly critical. In England and Wales, the Chief Coroner's guidance stipulates that all inquests should be concluded within 6 months of being informed of death of an individual (22). This has led to a reduction in the stipulated turnaround times for toxicological analysis. Workload has been increasing

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with increased drug and pharmaceutical abuse (23–25), and depression caused by the coronavirus (COVID-19) pandemic (26) is a recognized potential cause of a further increase in workload in both the short and medium terms (27–29). Social and economic effects of the pandemic have led to a number of challenges for the treatment and monitoring of substance misusers (30), who are themselves at increased risk from COVID-19. Faced with these drivers, there is a pressing need to deliver more results faster with the same, or fewer, resources. This demands an innovative solution to increase efficiency and the quality of the results generated.

The LC–HRMS methodology outlined in this study allows an operator to screen postmortem blood and urine samples for drugs and their metabolites. When compared to a reference screening methodology by GC–MS, the LC–HRMS shows superior performance both in the range of compounds detected and in the sensitivity of the assay. The majority of the compounds detected by LC–HRMS but not by GC–MS were available in the GC–MS libraries. This suggests that the difference in the detection rate of the two assays is due to the differing sensitivities of the assays or the selectivity of the extraction procedure for GC–MS.

The ability of the LC–HRMS methodology to quantitate many of the commonly encountered compounds at the same time as the screen has led to a big improvement in efficiency, decreasing the amount of staff time required to screen and quantitate postmortem samples as well as vastly improving turnaround times. This decrease in turnaround time is evident across all compounds, for which the retrospective analysis was performed. However, it is particularly evident for analytes that require complicated solid-phase extraction prior to analysis. This includes the opiates and cocaine and benzoylecgonine. For total morphine, the measurement of morphine glucuronides and free morphine has allowed for the accurate calculation of total morphine concentration and removed the need for time-consuming hydrolysis. This has resulted in a median decrease in turnaround time for these analytes of between 37% and 41%, a decrease of at least 7 days. Compounds that are less commonly encountered, such as chlordiazepoxide, diphenhydramine, venlafaxine, amphetamine and temazepam, also showed significant reductions in turnaround time. This is likely to be due to the fact that batches in which infrequently encountered analytes were measured were run less frequently than those for more common analytes. However, with the LC–HRMS assay, all compounds are quantitated at the same time, removing any delay. In contrast, commonly encountered compounds for which a regularly scheduled quantitative assay was available, such as paracetamol, only showed a small reduction in turnaround time.

A further benefit of the introduction of the LC–HRMS assay was that it allowed the laboratory to bring in-house compounds which were previously sent to other laboratories for analysis. Such compounds include gabapentin, pregabalin, oxycodone, clozapine and alprazolam. When compared to the time taken to refer the samples to another laboratory, a reduction in turnaround time of between 10% and 69% was achieved.

Postmortem samples are precious, and the volume received may be small. Screening of blood and urine samples using the GC–MS assay requires 600 µL of blood or urine, while the quantitative assays require between 100 and 600 µL. This means that, if several compounds are detected by the screen, several milliliters of sample may be required for complete analysis. This may be a problem if limited sample is available. The LC–HRMS assay vastly decreases the amount of sample required to perform the screen and quantitation, with only 50 µL of blood required in most cases. Following the adoption of the LC–HRMS assay, we have been able to perform analysis in cases where, previously, the volume of sample provided would have been insufficient to perform even a basic screen.

The LC–HRMS assay is run in positive electrospray ionization mode. While this allows the user to identify most significant compounds, some compounds that ionize in negative ionization mode may not be detected as molecular ions. This includes compounds such as THC, salicylate and barbiturates. However, these analytes may be identified in positive ionization mode using a range of their adducts or metabolites (31–33). In the future, further work should be performed to enable the assay to detect these compounds, either by inclusion of their adducts and/or metabolites in the positive ionization library or by development of an assay that operates in negative ionization mode. There are also some compounds not currently quantitated by the LC–HRMS methodology which are significant when detected in postmortem samples. Such compounds include fentanyl, zopiclone, olanzapine, quetiapine and etizolam. This assay has the potential to add these compounds to further improve its usefulness for postmortem analysis. Usefulness would also be improved by adapting the assay for other postmortem sample types, such as gastric contents and vitreous fluid.

The screening library used in this case was assessed by comparison to an existing GC–MS methodology. Commonly encountered isobaric interferences were excluded, and LODs were determined for at least one compound in each compound class. However, LODs were not determined for every compound in the library. In the future, detection limits for any outstanding compounds in the library should be determined to further improve upon the understanding of the performance of this assay.

Guidelines for toxicological screening may recommend a screening and confirmatory approach to ensure reliable results (34). In the combined screening and quantitation method described here, reliable results are ensured by the use of four compound identifiers: retention time, accurate mass of parent ion, accurate mass of fragment ions and correct isotope pattern. Further reassurance is given by analyzing blood and urine samples from a single case and comparing the results from both samples to ensure that they match. Results obtained are also compared to case information to determine if they are consistent with any medication history or history of substance misuse. Where only a single sample has been provided for a case, high-throughput immunoassay screening is used to check the results generated by LC–HRMS, and repeat analysis of the sample is performed to exclude the possibility of missampling. This repeat analysis does extend the time taken to complete the analysis of a case. However, as batches are analyzed on a regular basis, any delay is minimized. A disadvantage to the combined screening and quantitation methodology is the reliance upon a single analytical methodology. This means that any deficiencies in the analytical methodology

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chosen are not compensated for by the use of other complementary analytical techniques. However, the high degree of specificity and sensitivity provided by LC–HRMS minimizes the potential for deficiencies when combined screening and quantitation is employed. With the majority of analyses being performed by one instrument type, it is also advisable to have a back-up instrument in case of instrument downtime. With so many of the analytical results coming from the analysis of a single sample in the combined approach, it is vital that the integrity of the sample is maintained throughout the whole of the analytical process. Duplicate analysis, which may be on a separate batch, can be used to mitigate the potential for error.

Conclusion

The comparison between a two-step screening and quantitation procedure and an LC–HRMS assay for the simultaneous screening and quantitation of toxicological compounds shows that the LC–HRMS assay delivered significant benefits. The LC–HRMS screen is superior to the GC–MS screen in both the range of compounds detected and the sensitivity of the assay. The LC–HRMS assay has been shown to give comparable results in postmortem blood in comparison to established stand-alone assays, allowing it to be used for quantitative analysis. A retrospective analysis of turnaround times pre- and post-implementation of the LC–HRMS assay has shown a decrease for all of the compounds captured by the analysis, with decreases in turnaround time of ∼43% for the quantitation of opiates, 50% for amphetamine and 37% for cocaine. The coupling of screening with quantitation has reduced the amount of staff time required to perform the analysis, freeing staff for other activities. It has also allowed full screening and quantitation to be performed on a much smaller sample volume than has been previously required. Given these significant benefits, it is believed that the investment in LC–HRMS for simultaneous screening and quantitation in postmortem samples is worthwhile, allowing the operator to meet the challenges encountered in postmortem toxicological analysis in the current climate.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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