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Fast targeted analysis of 132 acidic and neutral drugs and poisons in whole blood using LC–MS/MS☆

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ABSTRACT

The aim of this study was to develop an LC–MS/MS based screening technique that covers a broad range of acidic and neutral drugs and poisons by combining a small sample volume and efficient extraction technique with simple automated data processing. After protein precipitation of 100 μL of whole blood, 132 common acidic and neutral drugs and poisons including non-steroidal anti-inflammatory drugs, barbiturates, anticonvulsants, antidiabetics, muscle relaxants, diuretics and superwarfarin rodenticides (47 quantitated, 85 reported as detected) were separated using a Shimadzu Prominence HPLC system with a C_{18} separation column (Kinetex XB-C_{18}, 4.6 mm × 150 mm, 5 μm), using gradient elution with a mobile phase of 25 mM ammonium acetate buffer (pH 7.5)/acetonitrile. The drugs were detected using an ABSciex® API 2000 LC–MS/MS system (ESI+ and −, MRM mode, two transitions per analyte). The method was fully validated in accordance with international guidelines. Quantification data obtained using one-point calibration compared favorably to that using multiple calibrants. The presented LC–MS/MS assay has proven to be applicable for determination of the analytes in blood. The fast and reliable extraction method combined with automated processing gives the opportunity for high throughput and fast turnaround times for forensic and clinical toxicology.

1. Introduction

Liquid chromatography (tandem) mass spectrometry (LC–MS/MS) is widely used in clinical and forensic toxicology as it provides the ability for fast and reliable detection and quantification of an extensive range of drugs and poisons. In combination with appropriate sample preparation techniques, small volumes of specimen can be sufficient for sensitive detection of drugs at low concentrations. In forensic toxicology, whole blood is generally the specimen of choice for analysis as plasma is difficult to obtain from decomposed or refrigerated blood. While gas chromatography mass spectrometry (GC–MS) was widely recognized as the gold standard for analytical methods, LC–MS/MS is well suited for analysis of thermo-labile and polar substances as well as exhibiting increased sensitivity which is useful in the development of large multi-analyte procedures. Although liquid chromatography time of flight mass spectrometry is the current state-of-the-art technique, simple LC–MS/MS can be successfully employed to confirm analyte detection and satisfy international guidelines [1,2].

It is widely accepted that multi-analyte procedures are more desirable than single-analyte approaches as they allow for faster turnaround times whilst being more cost-effective. While there has been a steady increase in the number of efficient multi-analyte procedures targeting common prescription drugs such as anti-psychotics [3–6], sometimes in combination with benzodiazepines and other drugs in whole blood [7–9], post-mortem blood [10] or plasma [11–15] there is a lack of suitable LC–MS/MS methods for the detection and quantification of multiple acidic and neutral compounds in whole blood.

While there are some published multi-analyte methods using GC–MS for analysis of acidic drugs in blood such as barbiturates and non-steroidal anti-inflammatory drugs, derivatization is required for analysis [16–19]. In cases where derivatization was not employed the method was restricted to only a handful of analytes [20,21].

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To the authors' knowledge, liquid–liquid extraction (LLE) for several classes of acidic drugs has only been employed in one published method [22], targeting 12 acidic and neutral compounds in whole blood. However, it is generally desirable to combine as many relevant analytes as possible with similar chemical characteristics in one analytical method in order to streamline processes in the laboratory and minimize the specimen volume required for systematic toxicological analysis. There is a limited number of published multi-analyte procedures using post-mortem whole blood that target acidic and neutral compounds employing a variety of solid-phase-extraction (SPE) technologies [8–10,23]. This approach is less economical than other techniques due to the ongoing high material costs such as replacement cartridges and filters. Additionally, problems regarding cartridge blockages are not uncommon, especially if working with post-mortem specimens [24]. Protein-precipitation (PP) is widely used as a time efficient sample clean-up procedure. However, the resulting supernatants frequently require additional filtration in order to obtain extracts that are sufficiently clean to be injected into an LC–MS/MS system [25–27]. There has only been one published method to date comprising five antiarrhythmic drugs and employing simple PP without additional sample clean-up [28]. However, no other drug families were incorporated into this method.

The aim of this study was to develop an LC–MS/MS based screening technique to target a broad range of acidic and neutral drugs and poisons and to complement existing screening methods for basic drugs. Our required turnaround times necessitated the development of a method that would be conducive to the use of small sample volumes and minimal sample preparation, namely an efficient PP extraction technique without the requirement for subsequent clean-up.

2. Materials and methods

2.1. Chemicals and reagents

A total of 132 drugs were used in this study. Acebutolol, amiloride, atenolol, baclofen, chlorthalidone, coumatetralyl, dipyridamole, felodipine, flecainide, frusemide, glibenclamide, indomethacin, labetalol, lysergic acid diethylamide, metformin, methaqualone, methotrexate, metoprolol, mexiletine, naproxen, oxprenolol, pentoxifylline, phenindione, phenobarbitone, phenytoin, pindolol, prazosin hydrochloride, propranolol, quinalbarbitone sodium, ranitidine hydrochloride, salbutamol, sulfamethoxazole, sulindac, sulthiame, timolol, and betaxolol hydrochloride were purchased from Australian Government Analytical Laboratories (Melbourne, Australia). Ipratropium bromide monohydrate was obtained from Boehringer Ingelheim (Sydney, Australia). Flucloxacillin sodium, amlodipine, carbamazepine-10,11-epoxide, celecoxib, desethylamiodarone, diclofenac sodium, etoricoxib, lansoprazole, losartan potassium, mefenamic acid, meloxicam, nefazodone, oxcarbazepine, pantoprazole sodium sesquihydrate, perindoprilat, rosiglitazone maleate, ticlopidine, trimipramine, warfarin, salmeterol xinafoate, and carvedilol were obtained from the Division of Analytical Laboratories, (Sydney, Australia). Glipizide was received from Farmitalia (Sydney, Australia); Pentobarbitone, formoterol, thiopentone and trandolaprilat were purchased from PM Separations (Brisbane, Australia). Agomelatine, amiodarone, barbitone, bisoprolol fumarate, bromadiolone, carbamazepine, cefaclor monohydrate, clopidogrel hydrogen sulphate, dextromethorphan, eprosartan mesylate, fosinoprilat, gabapentin, glimepiride, irbesartan, ketoprofen, lacosamide, lamotrigine, lercanidipine, levetiracetam, meprobamate, methylprednisolone, minoxidil, modafinil, nimodipine, nizatidine, oxybutynin hydrochloride, pioglitazone hydrochloride, pregabalin, quinaprilat, rabeprazole sodium, sitagliptin, sotalol, telmisartan, tirofiban hydrochloride monohydrate, topiramate, valsartan and zonisamide were purchased from the National Institute of Forensic Science (Sydney, Australia). Acenocoumarol, esmolol hydrochloride, flurbiprofen, and ramiprilat were received from Sapphire Bioscience (Sydney, Australia). Acetazolamide, amoxicillin, brodifacoum, bumetanide, chlorophacinone, chlorothiazide, chlorzoxazone, cyclobenzaprine hydrochloride, dicloxacillin, difenacoum, flocoumafen, hydrochlorothiazide, ibuprofen, metronidazole, papaverine hydrochloride, paracetamol, phenoxymethylpenicillin, phenylbutazone, piroxicam, prednisolone, salicylic acid, terbutaline hemisulfate, trazodone, trimethoprim, valproic acid, carisoprodol and indapamide were purchased from Sigma–Aldrich (Sydney, Australia). Trimipramine-d_3 was purchased from Kinesis (Brisbane, Australia) and nimodipine-d_7 was purchased from PM Separations (Brisbane, Australia). Acetonitrile (ACN), methanol (MeOH), hydrochloric acid (HCl), sodium hydroxide (NaOH), dimethylsulfoxide (DMSO) and ammonia were purchased from Merck (Darmstadt, Germany). Ammonium acetate was purchased from Sigma–Aldrich (Sydney, Australia). All chemicals were of analytical grade or better and water was purified using a Merck Millipore Milli-Q Direct-8 Ultrapure Water System (Melbourne, Australia).

2.2. Specimens

Whole blood for validation purposes was obtained from drug-free volunteers (clinical samples). Post-mortem blood samples for validation purposes were submitted to the authors' laboratory for routine toxicological analysis. All clinical and post-mortem samples were collected into 10 mL Sarstedt (Adelaide, Australia) polypropylene tubes containing 1% fluoride-oxalate preservative as per standard collection procedure in the laboratory. The post-mortem blood samples were regarded as drug-free if none of the existing routine tests in the laboratory showed the presence of the studied drugs in any specimen (including blood, liver, and urine). All blood samples were stored at −20 °C for a maximum of one year prior to analysis.

2.3. Apparatus

The LC system consisted of a Shimadzu 20AD HPLC (Melbourne, Australia) which comprised of a degasser, a binary pump and an autosampler. The analytical system consisted of an ABSciex API 2000™ mass spectrometer equipped with a linear accelerator (LINAC™) collision cell quadrupole mass spectrometer (Melbourne, Australia) operated in multiple reaction monitoring (MRM) mode and an electron spray ionization (ESI) TurboIonSpray source.

2.4. HPLC conditions

Gradient elution was performed on a Kinetex XB-C_{18} (4.6 mm × 150 mm, 5 μm particle size) column from Phenomenex (Melbourne, Australia). The mobile phases consisted of 25 mM aqueous ammonium acetate (3.85 g of ammonium acetate dissolved in 2L of deionized water) adjusted to pH 7.5 with ammonia (eluent A) and ACN (eluent B) which were degassed by the integrated Shimadzu 20AD degasser. Gradient elution was programmed as follows: equilibration time (−5.00 to 0.00 min), 20% eluent B; 0.00–9.00 min, 95% eluent B; 9.01–15.00 min, 95% eluent B. The flow rate was 1 mL/min and the column oven was maintained at 60 °C. The autosampler was operated at room temperature and the autosampler needle was rinsed with isopropanol before and after every injection.