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High throughput screening of sub-ppb levels of basic drugs in equine plasma by liquid chromatography–tandem mass spectrometry

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Abstract

This paper describes a high throughput LC–MS–MS method for the screening of 75 basic drugs in equine plasma at sub-ppb levels. The test scope covers diversified classes of drugs including some α- and β-blockers, α- and β-agonists, antihypotensives, antihypertensives, analgesics, antiarrhythmics, antidepressants, antidiabetics, antipsychotics, antiulcers, anxiolytics, bronchodilators, CNS stimulants, decongestants, sedatives, tranquilizers and vasodilators. A plasma sample was first deproteinated by addition of trichloroacetic acid. Basic drugs were then extracted by solid-phase extraction (SPE) using a Bond Elut Certify® cartridge, and analysed by LC–MS–MS in positive electrospray ionization (+ESI) and multiple reaction monitoring (MRM) mode. Liquid chromatography was performed using a short C_8 column (3.3 cm L × 2.1 mm ID with 3 μm particles) to provide fast analysis time. The overall instrument turnaround time was 8 min, inclusive of post-run and equilibration time. No interference from the matrices at the expected retention times of the targeted masses was observed. Over 60% of the drugs studied gave limits of detection (LoD) at or below 25 pg/mL, with some LoDs reaching down to 0.5 pg/mL. The inter-day precision for the relative retention times ranged from 0.01 to 0.54%, and that for the relative peak area ratios (relative to the internal standard) ranged from 4 to 37%. The results indicated that the method has acceptable precision to be used on a day-to-day basis for qualitative identification.

Keywords: Basic drugs; Horse blood; Plasma; High throughput screening; Liquid chromatography–mass spectrometry

1. Introduction

Urine has long been the preferred matrix over blood in equine sports drug testing because a large volume of urine can usually be obtained, and the concentrations of drugs and metabolites are generally much higher. Blood however, offers an advantage over urine in that it can be collected on demand, thus, assuring samples are always available from the subject selected for testing. In some cases, blood may be the only sample available if the horses selected for testing fail to provide a urine sample. In addition, parent drugs can usually be found in blood to serve as good target analytes, whereas in urine, analysts might have to resort to the detection of metabolites, particularly for drugs that are extensively metabolized. The lack of reference materials for some metabolites and unknown drug metabolism in the horse further, complicate the problem. It is therefore, highly desirable if a mass-spectrometry based method for detecting a large variety of drugs in equine blood can be established. Gas chromatography–mass spectrometry (GC–MS) has long been the gold standard for testing of drugs in biological matrices. Unfortunately, it often lacks the required sensitivity for detecting drugs in blood, particularly for many basic drugs with exceptionally low concentrations in blood. The rapid development of liquid chromatography–tandem mass spectrometry (LC–MS–MS) in the past decade has provided analysts with a powerful tool for detecting and confirming the presence of drugs in complex biological matrices. Several workers have reported their applications for the detection of drugs in equine or human plasma samples in recent years [1–6]. However, their test scopes were limited to a single or only a few analytes of the same drug class. Recently, Herrin et al. [7] and Mueller et al. [8] have reported separately the application of a hybrid triple-quadruplole linear ion trap mass spectrometer (QTrap) to comprehensive screening of respectively, over 100 and 300 drugs in human blood samples. The strategy basically employed multiple reaction monitoring

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(MRM) as survey scans for target detection, with automatic triggering of an enhanced product ion (EPI) scan in an information dependent acquisition (IDA) experiment. Drug identification was performed by library search with an in-house MS/MS library of EPI spectra collected at three different collision energies. This approach is quite appealing as both drug screening and identification can be done in the same LC–MS–MS run. However, most of their reported LoDs were only applicable to forensic investigation of toxicology cases, and not for doping control purpose where detection for evidence of prior exposure is often required. This paper describes a high throughput LC–MS–MS method for the simultaneous screening of 75 basic drugs of diversified drug classes in equine plasma at sub-ppb levels.

2. Experimental

2.1. Materials

Anileridine hydrochloride, butorphanol tartrate, cimetidine, clonidine hydrochloride, cocaine hydrochloride, desipramine hydrochloride, droperidol, famotidine, guanabenz acetate, labetalol hydrochloride, lignocaine, mazindol, mephentermine sulphate, methadone hydrochloride, methoxamine hydrochloride, methoxyphenamine hydrochloride, methylphenidate hydrochloride, nadolol, naphazoline hydrochloride, nizatidine, nordazepam, nylidrin hydrochloride, oxycodone hydrochloride, oxymetazoline hydrochloride, oxymorphone, pindolol, prazosin hydrochloride, ranitidine hydrochloride, ritodrine hydrochloride, terbutaline sulphate, tuaminoheptane sulphate, and xylometazoline hydrochloride were obtained from USP (Rockville, MD, USA). Clenbuterol, salmeterol xinafoate, spiperone, and tetrahydrozoline hydrochloride were acquired from Sigma (St. Louis, MO, USA). Benzoylecgonine and *N*-norpropoxyphene maleate were obtained from Alltech (Deerfield, IL, USA). Anhydrous acepromazine, atenolol, haloperidol, nortriptyline hydrochloride, perphenazine, propylhexedrine, sotalol hydrochloride and thebaine were obtained from BP (Middlesex, UK). Potassium losartan and bisoprolol fumarate were obtained from Merck (Darmstadt, Germany), trifluperidol from Janssen Pharmacetica (NJ, USA), benperidol from Janssen-Cilag (Buckinghamshire, UK), and bambuterol hydrochloride from ASTRA (Södertälje, Sweden). Romifidine and telmisartan were from Boehringer Ingelheim (Ingelheim, Germany); hydroxydetomidine hydrochloride and detomidine hydrochloride were from Farmos (Turku, Finland). Buspirone hydrochloride was obtained from Bristol-Myers Squibb (NY, USA), bromocriptine mesylate from Apotex (Auckland, New Zealand), practolol from ICI (Now Zeneca Plc, UK), etafedrine from Merrel Dow Research (OH, USA), carteolol hydrochloride from Otsuka (Tianjin, China), sildenafil citrate from Pfizer (NY, USA), α-hydroxyalprazolam from Cerilliant (Austin, TX, USA), nalbuphine hydrochloride from Research Biochemicals Incorporated (MA, USA), buprenorphine hydrochloride from Schering-Plough (Hull, UK), carvedilol from Roche (Mannheim, Germany), irbesartan from Sanofi (Paris, France), pioglitazone from Takeda Chemical Industries (Osaka, Japan), and esmolol hydrochloride from The Boots (Isando, South Africa). Amisulpride was obtained from Lab Synthelabo (Kuwait), flupentixol from Lundbeck (Lumsas, Denmark), midodrine hydrochloride from Hafslund Nycomed (Linz, Austria), isometheptene mucate from Manx (Kent, UK), repaglinide from Novo Nordisk (Bagsvaerd, Denmark), sulpiride from Sanofi-synthelabo (NY, USA), rilmenidine from Servier (France), and rosiglitazone maleate was from Smithkline Beecham (PA, USA).

Acetic acid, glacial (100%), acetonitrile (LiChrosolv®), ammonium acetate (10 mM, pH 3.8), ammonia solution (25%, GR grade), dichloromethane (GR grade), ethyl acetate (GR grade), isopropanol (GR grade), methanol (LiChrosolv®), potassium hydroxide (pellets), potassium dihydrogen phosphate buffer (0.1 M, pH 6.0) and trichloroacetic acid (GR grade) were obtained from Merck (Darmstadt, Germany). Bond Elut Certify® cartridges (130 mg, 3 mL) were purchased from Varian (CA, USA). Deionized water was generated from an in-house water purification system (Milli-Q, Molsheim, France).

2.2. Sample preparation and extraction procedures

Blood samples were centrifuged at 2100 g for 30 min. The plasma fraction (3 mL) was deproteinated by the addition of trichloroacetic acid (10% in deionized water, w/v, 200 μL). The deproteinated plasma was left standing at room temperature for 10 min and then centrifuged at 2100 g for 10 min. The supernatant was pipetted out and placed in another centrifuge tube. Nadolol (15 ng) was added as an internal standard (I.S.), followed by addition of potassium dihydrogen phosphate buffer (pH 6.0, 0.1 M, 2 mL). The pH was further adjusted, if necessary, to 6.0 using either potassium hydroxide (0.1 M) or hydrochloric acid (0.1 M). The sample was loaded onto a Bond Elut Certify® cartridge that had been pre-conditioned with methanol (2 mL), deionized water (2 mL), and potassium dihydrogen phosphate buffer (pH 6.0, 0.1 M, 2 mL). The cartridge was then washed with phosphate buffer (pH 6.0, 0.1 M, 2 mL), followed by acetic acid (1.0 M, 2 mL); dried for 5 min with nitrogen at 20 psi, and then eluted with dichloromethane/ethyl acetate (4:1, v/v, 3 mL) to collect the neutral and acidic fraction (this fraction can be used for the screening of neutral and acidic drugs if desired). The SPE cartridge was further washed with methanol (2 mL), dried for 5 min with nitrogen at 20 psi, and eluted with ethyl acetate/dichloromethane/isopropanol (5:4:1, v/v/v, 3 mL) containing 2% of concentrated aqueous ammonia to collect the basic fraction. The eluate was then evaporated to dryness under nitrogen at room temperature, and the residue was reconstituted in methanol (50 μL). The content was transferred to a conical insert in a Chrompack autosampler vial for LC–MS–MS analysis.

2.3. Instrumentation

All LC–MS–MS analyses, except those described under "Method Applicability", were performed on an Applied Biosystems 4000 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an Agilent 1100 series HPLC system consisting of a quaternary gradient pump (Agilent Tech-