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Simultaneous determination of eperisone hydrochloride and paracetamol in mouse plasma by high performance liquid chromatography-photodiode array detector

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ABSTRACT

This paper reports the validation of a quantitative high performance liquid chromatography-photodiode array (HPLC-PDA) method for the simultaneous analysis, in mouse plasma, of eperisone hydrochloride and paracetamol by protein precipitation using zinc sulphate–methanol–acetonitrile.

The analytes were resolved on a Gemini C_{18} column (4.6 mm × 250 mm; 5 μm particle size) using a gradient elution mode with a run time of 15 min, comprising re-equilibration, at 60 °C (±1 °C). The method was validated over the concentration range from 0.5 to 25 μg/mL for eperisone hydrochloride and paracetamol, in mouse plasma. Ciprofloxacin was used as Internal Standard.

Results from assay validations show that the method is selective, sensitive and robust. The limit of quantification of the method was 0.5 μg/mL for eperisone hydrochloride and paracetamol, and matrix-matched standard curves showed a good linearity, up to 25 μg/mL with correlation coefficients (r^2) ≥ 0.9891. In the entire analytical range the intra and inter-day precision (RSD%) values were ≤1.15% and ≤1.46% for eperisone hydrochloride, and ≤0.35% and ≤1.65% for paracetamol. For both analytes the intra and inter-day trueness (bias%) values ranged, respectively, from −5.33% to 4.00% and from −11.4% to −4.00%.

The method was successfully tested in pharmacokinetic studies after oral administration in mouse. Furthermore, the application of this method results in a significant reduction in terms of animal number, dosage, and improvement in speed, rate of analysis, and quality of pharmacokinetic parameters related to serial blood sampling.

1. Introduction

Biological fluids such as serum, plasma and urine are very complex matrices that could vary over several orders of magnitude in components concentration. The use of high performance extraction procedures and separation techniques are essential for the correct quali-quantitative determination of specific compounds at low concentration levels. Several papers have been published on this topic [1–3], reporting instrument configurations [3–5], extraction procedures [6], multi-components analyses [7–9], and also using chemometric approach [10].

Often, different active principle associations are used for disease treatment, therefore analytical procedures must be developed and validated in order to maintain (or improve) sensitivity and selectivity, respect to the target compounds.

Recently, eperisone hydrochloride (or (2RS)-1-(4-ethylphenyl)-2-methyl-3-(piperidinyl)-1-propanone hydrochloride, Fig. 1a), a potent new generation antispasmodic agent [11–13] was used in association with paracetamol (or N-(4-hydroxyphenyl)acetamide, Fig. 1b), a centrally and peripherally acting non-opioid analgesic and antipyretic agent, for the treatment of moderate to severe pain. Eperisone hydrochloride shows low bioavailability

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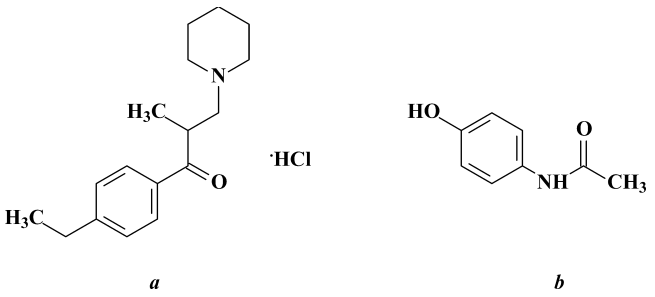

Fig. 1. Chemical structures of eperisone hydrochloride (a), and paracetamol (b).

after oral administration and broad first-pass metabolism [14,15], which leads to low plasma concentration. In order to evaluate biological fluid concentration, a sensitive method for the determination of eperisone in plasma is required. Several papers have reported the determination in pharmaceuticals and biological fluids using classic instrument configuration, such as high performance liquid chromatography (HPLC) [16], but also hyphenated ones, such as gas chromatography–mass spectrometry (GC–MS) [17,18], and high performance liquid chromatography–mass spectrometry (HPLC–MS) [19–21].

On the other hand, paracetamol (acetaminophen) is quickly absorbed from the gastrointestinal tract and is primarily metabolized through conjugation with glucuronic and sulphuric acid in order to form glucuronide (paracetamol-glucuronide, PG) and sulphate (paracetamol-sulphate, PS) derivatives, respectively, and consequently excreted in the urine [22]. Several papers were recently published also for paracetamol, reporting its determination (and/or its metabolite profiles) in different matrices [23–25], its characterization [26], and its selective extraction using magnetic molecularly imprinted polymer (m-MIPs) for solid-phase extraction and sample clean up [27]. Furthermore, only one paper is present in the literature that reports the simultaneous determination of these two drugs in pharmaceuticals [28], but no work considers their simultaneous determination in biological fluids, such as plasma.

In continuation of our studies on method validation for drugs, metabolites, impurities, and particularly, quantitative analyses of drug-associations for clinical purposes [29–34], we report herein a new high performance liquid chromatography-photodiode array (HPLC-PDA) method for the simultaneous determination of eperisone hydrochloride and paracetamol in mouse plasma, and their quantitative evaluation samples after single oral dose of 0.5 mg/kg (eperisone hydrochloride) and 5 mg/kg (paracetamol) of commercially available formulation.

2. Experimental

2.1. Chemicals and reagents

Eperisone hydrochloride (>99% purity index) was purchased from Santa Cruz Biotechnology (Dallas, USA), while paracetamol (98% purity index) was supplied by Sigma-Aldrich (Milan, Italy). Ciprofloxacin (IS, >98% purity index), ammonium acetate (>98% purity index), and acetic acid (to obtain ammonium acetate buffer at pH = 3) were obtained by Fluka Chemie (Buchs, Switzerland).

Methanol and acetonitrile (AcN) (HPLC-grade) were purchased from Carlo Erba Reagenti (Milan, Italy). Water is produced by Millipore Milli-Q Plus water treatment system (Millipore Bedford Corp., Bedford, MA, USA).

The eperisone hydrochloride and paracetamol combination was customized as reported in Supplementary material, Section S.5.

2.2. Experimental animals, drug administration, and serial sampling collection and storage

All experimental animals (C57BL/6JOlaHsd mice, 4 weeks old), supplied by Harlan Laboratories S.r.l. (Udine, Italy), were housed in individually metabolic cages, and all procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with Institutional Animal Ethics Committee (IAEC) of University "*Magna Graecia*" of Catanzaro, Department of Health Sciences (as reported in Supplementary material, Section S.6).

For pharmacokinetic studies anaesthetized animals received orally a drug equivalent dosage of 0.5 mg/kg of eperisone hydrochloride and 5 mg/kg of paracetamol, respectively according to the following therapeutic protocol: group 1 (untreated mice or control, $n = 6$); group 2 (0.5 mg/kg eperisone hydrochloride, $n = 6$); group 3 (5 mg/kg paracetamol, $n = 6$); group 4 (0.5/5 mg/kg eperisone hydrochloride/paracetamol, $n = 6$). The different formulations were dissolved in mineral water and administered *in bolus* (1 mL). The control group received single oral dose of mineral water for pharmacokinetic comparison.

Serial blood samples (200 μL) were drawn from the retro-orbital plexus at 30 min, 1 h, 4 h, 8 h, 16 h and 24 h after oral administration, collected in heparinized polythene tubes, centrifuged for 10 min at 4000 rpm at 4 °C. Plasma was separated and stored at −20 °C until further analysis.

2.3. Plasma sample preparation

A 90 μL aliquot of mouse blank plasma was mixed with a 5 μL aliquot of analytes working solutions and 5 μL aliquot of Internal Standards working solution (at final concentration of 25 μg/mL) and vortexed for 1 min (10% of matrix modification in calibration and QC samples preparation, 5% of matrix modification in real samples analyses).

For protein precipitation, 50 μL of $ZnSO_4$ solution (5%, *p:v*) in water:methanol:acetonitrile were added, stirred, and centrifuged at 12,000 × *g* for 10 min. Then the supernatant, after a filtration on Phenex-PTFE (4 mm, 0.45 μm) syringe filters (Phenomenex, Torrance, CA, USA), was transferred into vials and 20 μL of samples were injected into the HPLC-PDA system.

2.4. Apparatus and chromatographic condition

HPLC analyses were performed on a waters liquid chromatograph equipped with a model 600 solvent pump, and a 2996 PhotoDiode Array Detector. Mobile phase was directly *on-line* degassed by using Degassex, mod. DG-4400 (Phenomenex, Torrance, CA, USA). Empower v.2 Software (Waters Spa, Milford, MA, USA) was used for data acquisition and elaboration.

A Gemini C18 packing column (4.6 mm × 250 mm, 5 μm particle size; Phenomenex, Torrance, CA, USA) was employed for the separation, protected by a disposable Security Guard column (4.0 mm × 3.0 mm, 5 μm particle size; Phenomenex, Torrance, CA, USA) and the column was thermostated at 60 °C (±1 °C) using a Jetstream2 Plus column oven.

For quantitative analyses, selective detection was performed at 259, 245, and 279 nm for eperisone hydrochloride, paracetamol, and ciprofloxacin (IS), respectively (see Supplementary material, Section S.1 for analytes and Internal Standard UV/vis spectra).

Gradient elution mode was performed using a ternary solvent system composed by ammonium acetate buffer (10 mM, pH 3), AcN, and methanol at 1.2 mL/min flow rate, as reported in Table 1.