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Journal of Chromatography B, 816 (2005) 35–40
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Liquid chromatography–electrospray tandem mass spectrometry method for determination of indapamide in serum for single/multiple dose bioequivalence studies of sustained release formulations

Florin Albu[a], Cristina Georgiţă[a], Victor David[b], Andrei Medvedovici[b,*]

[a] *LaborMed Pharma, Splaiul Independentei no. 319, Bucharest (6)-060044, Romania*
[b] *Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, Sos. Panduri, no. 90, Bucharest (5)-050663, Romania*

Received 24 August 2004; accepted 1 November 2004
Available online 18 November 2004

Abstract

Indapamide and internal standard (5-chloro-2-methoxy-*N*-[2-(4-sulphamoylphenyl)ethyl]benzamide) were isolated from plasma by a single step liquid–liquid extraction in *t*-butyl methyl ether. The chromatographic separation was achieved on a reversed-phase C_{18} monolithic column with a mobile phase consisting in a methanol/aqueous 0.1% formic acid mixture and a flow rate of 0.8 ml/min, in isocratic conditions, within 11 min. Target compounds were transferred in an ion trap analyzer via an atmospheric pressure electrospray interface (AP-ESI). The mass analyzer was used in a selected reaction monitoring (SRM) mode, in order to enhance on detection selectivity. Whole method produces quantitation limit for indapamide of 1 ng/ml. Method was successfully applied to assess bioequivalence of two sustained release marketed pharmaceutical formulations of indapamide 1.5 mg coated tablets, carried-out in a single/multiple doses, randomized design.

Keywords: Indapamide; Serum; Liquid–liquid extraction; Liquid chromatography; Electrospray tandem mass spectrometric detection; Bioequivalence; Sustained release formulations

1. Introduction

Indapamide, 4-chloro-*N*-[(2*RS*)-2-methyl-2,3-dihydro-1*H*-indole-1-yl]-3-sulphamoylbenzamide (CAS 26807-65-8) is an antihypertensive agent also acting as a diuretic, belonging to the new indolines class.

Although the assay of indapamide in pharmaceutical formulations and determination of the related substances profile is frequently referred in literature, using spectrometric [1–4], electrometric [5,6] and chromatographic [7–9] methods, only very few HPLC/UV methods were dedicated to its determination in biological fluids [10–15].

Indapamide is marketed as immediate release pharmaceutical formulations containing 1.25 and 2.5 mg active substance per dose and as sustained release coated tablets of 1.5 mg per dose. According to [16], expected maximum plasma concentration after a single intake dose of 2.5 mg indapamide formulation with immediate release should be three to five-folds higher than for a 1.5 mg single dose of a sustained release product (maximum plasma concentrations are reduced from 80 to 100 ng/ml to 10 to 30 ng/ml). In reference [17], a mean maximum blood concentration of 115 ng/ml indapamide was determined after about 2 h for a 2 × 1.25 mg immediate release tablet intake (study was carried out only on male subjects). The final results of the present work are in good agreement with data above cited.

Isolation of indapamide from plasma is a tedious task, because of low concentration levels and matrix induced interferences. More often, liquid–liquid extraction procedures require two or three successive steps (extraction, back-extraction, and re-extraction) [12,15] to eliminate matrix interferences. More over, ethyl acetate and diethyl ether were

* Corresponding author. Tel.: +40 723330423; fax: +40 12213692.
E-mail addresses: avmedved@yahoo.com, qc@labormedpharma.ro (A. Medvedovici).

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doi:10.1016/j.jchromb.2004.11.002

used as extracting phases. Solid phase extraction (SPE) was successfully used to isolate indapamide from plasma samples resulting after 5 mg active substance intake as an immediate release product [11,13]. However, recovery of indapamide strongly depends on the type of the hydrophobic adsorbent, variations from 30 to 100% being observed. It is worthwhile to note that UV detection can be considered as not selective and sensitive enough to assay indapamide in biological fluids. Recently, a SPE/HPLC/(AP-ESI) MS^2 method has been proposed for the assay of 35 diuretics (including indapamide) in urine [18]. The automated SPE approach was developed for doping control purposes. Recoveries for most analytes were greater than 80% for concentrations below 100 ng/ml. The liquid–liquid extraction approach developed within the present paper for isolation of indapamide from plasma samples in the view of bioavailability/bioequivalence (BA/BE) evaluation is characterized by identical recovery and quantitation limits around 1 ng/ml. Costs related to SPE automation as well as cartridge consumption during complete BA/BE studies should be also considered somewhat limitative (note that the multiple use of a SPE cartridge is not recommended and for a study completion, including validation purposes, around 2000 samples are run).

Mass spectrometry combined with capillary gas chromatography was used for the sensitive and selective determination of indapamide and other 17 diuretics in human urine [14]. Isolation from urine was done by liquid–liquid extraction, followed by an additional derivatization procedure, microwave assisted. Mass spectrometric detection interfaced to HPLC by means of AP-ESI was recently reported in literature for monitoring reversed-phase chiral separation of indapamide enantiomers [19].

Therefore, our aim was to combine high separation capabilities of liquid chromatography with the selective/sensitive characteristics of mass spectrometry for determination of indapamide in plasma samples at ng/ml level. Isolation of indapamide was achieved by a single step liquid–liquid extraction in *t*-butyl methyl ether, followed by solvent evaporation, re-dissolution of the residue and injection onto the chromatographic column. The analytical procedure was fully validated and successfully used to assess bioequivalence of two marketed pharmaceutical formulations of 1.5 mg indapamide with sustained release.

2. Experimental

2.1. Instrument

Experiments were performed on Agilent 1100 series LC/MSD system composed of the following modules: degasser (G1379A), quaternary pump (G1311A), thermostated autosampler (G1329A), column thermostat (G1316A), AP-ESI standard interface (G1948A), ion trap mass spectrometric detector SL series (G24450), nitrogen generator (5183-2003). System control and data acquisition were made with the Agilent LC/MSD trap Software version 4.2. incorporating the MSD Trap Control software version 5.1. from Brucker Daltronics. The system was operationally qualified before and after the bioequivalence study. The repeatability ($n = 6$) of the MSD ion trap SL determined for 5 pg of reserpine loaded to interface was characterized during the study by relative standard deviations (R.S.D.%) of 10.9% (before) and 13.8% (after).

2.2. Chromatographic conditions

A monolithic Chromolith Performance RP-18e column (Merck, Germany), 100 mm length and 4.6 mm internal diameter fitted with a Chromolith Guard cartridge RP-18e (10 mm × 4.6 mm) was used. Column was thermostated at 40 °C. Column was validated before and after study completion, by computing the height equivalent to the theoretical plate (HETP) of fluoranthene peak (variation from 7.6 to 11.3 μm was noticed during the study including method validation).

Elution was isocratic, using methanol and aqueous 0.1% (v/v) formic acid as mobile phase constituents, mixed in the volumetric ratio of 42.5:57.5 and a flow rate of 0.8 ml/min. Injection volume was set at 100 μl.

2.3. Interface parameters

The parameters controlling the AP-ESI standard interface were: drying gas flow: 12 l/min; drying gas temperature: 365 °C; pressure of the nebulizer gas: 65 p.s.i.; capillary voltage: 3000 V; high voltage end plate offset: −500 V.

2.4. MSD ion trap SL operational parameters

Ion polarity was positive for both indapamide and internal standard (I.S.). SRM working mode was used. The trap parameters for indapamide were: chromatogram segment: 4–7.4 min; scanning interval: 125–370 *m*/*z*; accumulation time: 200 ms; ion current control: 20,000; eight averaged spectra per data point, isolation mass: 366.0; width: 4; fragmentation amplitude: 1.2 V. The trap parameters for I.S. were: chromatogram segment: 7.4–11 min; scanning interval: 155–375 *m*/*z*; accumulation time: 200 ms; ion current control: 30,000; eight averaged spectra per data point, isolation mass: 369.1; width: 4; fragmentation amplitude: 1.0 V.

2.5. Materials

All solvents were HPLC grade from Merck (Darmstadt, Germany). Formic acid (98–100%) was reagent Ph. Eur. grade, also from Merck. Water for chromatography (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory by means of a TKA Lab HP 6UV/UF instrument and used during experiments. Indapamide and the I.S., 5-chloro-2-methoxy-*N*-[2-(4-