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Turbulent flow and ternary column-switching on-line clean-up system for high-throughput quantification of risperidone and its main metabolite in plasma by LC–MS/MS Application to a bioequivalence study

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Abstract

A high-throughput LC–MS/MS method was developed for the simultaneous determination of Risperidone and 9-OH-risperidone in human plasma. A semi-automated sample preparation procedure was applied, including protein precipitation after addition of ACN, via a robotic system, and subsequent sub-zero temperature extraction of the latter. Injections of the ACN extractants were performed on a turbulent flow ternary column-switching system, consisted of dual extraction columns in parallel for on-line purification of samples and an analytical column. Toggling with the assistance of two valves provided a run cycle time of 3 min and the whole procedure minimized carry-over effect. On-line clean-up procedure along with sub-zero temperature extraction increased sample purification and extended column life. The analytical range of the method was 0.1–200 ng mL^{-1} for both analytes with excellent linearity and very good accuracy and precision. The proposed method was employed in a bioequivalence study after per os administration of a 2 mg tablet of risperidone and allowed the completion of the study (>1400 samples) in only 4 days time.

Keywords: Risperidone; Column-switching; Mass spectrometry; 96-Well; Sub-zero extraction; Bioequivalence

1. Introduction

Risperidone (RIS) is an atypical antipsychotic drug used in the treatment of schizophrenia and other psychotic disorders [1–4]. 9-Hydroxy-risperidone (9-OH-RIS) is the main metabolite of RIS formed in liver by cytochrome P450 isoenzymes [5]. 9-OH-RIS has a similar pharmacological activity as the parent compound and the sum of both molecules constitutes the total active moiety responsible for the pharmacological responses to RIS administration. Typical oral doses of RIS in the treatment of chronic schizophrenia range from 2 to 6 mg per day resulting in plasma levels of 5–100 nM of RIS and 9-OH-RIS [6,7].

Numerous HPLC methods with UV or electrochemical detection [8–13] have been described for the quantitative determination of RIS and its hydroxy-metabolite in human plasma. Such methods usually lack the combined sensitivity and selectivity needed to analyze complex mixtures. Liquid chromatography coupled with atmospheric pressure ionization tandem mass spectrometry is nowadays the method of choice for the quantitative determination of pharmaceutical substances in complex biological matrices. Few methods have been reported for the determination of RIS and 9-OH-RIS that employ mass spectrometric detectors. Three of them [14–16] apply conventional extraction protocols, while recently [17] a LC–MS/MS method using column switching with one extraction column for on-line clean-up has been presented.

Plasma protein precipitation (PPP), liquid–liquid extraction (LLE) and solid-phase extraction (SPE), are the sample preparation techniques most commonly used for processing plasma and tissue samples. These processes are labor-intensive and time-consuming and constitute the rate-limiting step in high-throughput pharmacokinetic studies. The introduction however

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of several liquid workstations for the parallel processing of samples has greatly facilitated this task, resulting in the development of numerous automated methodologies for the determination of drugs and their metabolites in biological fluids during the last few years [18–24]. An alternative approach to automated sample extraction is the use of turbulent flow chromatography (TFC) with column-switching [25–30]. By using this approach sample pre-treatment is minimized. The biological sample can be injected directly onto a narrow-bore large particle size extraction column where the sample matrix is rapidly washed away using a high flow rate aqueous mobile phase while analytes are retained. After the load and wash steps are completed, the composition is changed to high organic solvent for the elution of the analytes in the backflush mode from the extraction column onto an analytical column, under laminar flow rate conditions, and finally into the mass spectrometer for detection. In most cases only a single extraction column is utilized in such procedures [31–34]. These single extraction column systems are simple and quite fast and have been applied to the analyses of numerous clinical samples. However, in such approaches the time for column equilibration between injections is often added to the respective run time. Utilization of dual extraction columns in parallel for purification and an analytical column for analysis results in a system with increased sample throughput.

In the present study we report the development and validation of the first method combining semi-automated sample pre-treatment with turbulent flow chromatography and column-switching for the determination of RIS and 9-OH-RIS. Plasma proteins are initially precipitated with ACN containing the internal standard in order to achieve cleaner extracts and thus increase the life of both extraction and analytical columns. Following the example of a previously presented protocol [35] the resulting samples are injected onto a ternary column on-line system with dual extraction columns. However, the use of a single valve in this protocol (10-port switching valve) resulted in significant carry-over problem. In the current protocol we added a six-port valve and such phenomena along with the appropriate wash solvent selection were minimized. With this system, two on-line processes can be staggered on the two extraction columns. While one column is in the wash, load or equilibration step, the other is in the elution step. Thus, the equilibration time does not add to the run time and the sample throughput is significantly increased. All liquid transfer steps including preparation of calibration standards and quality control samples (QCs), transfer of study samples and addition of ACN containing the internal standard were performed automatically via robotic systems.

The proposed method enabled the automated high-throughput and reliable determination of RIS and 9-OH-RIS in a bioequivalence study after per os administration of a 2 mg tablet in 30 healthy volunteers.

2. Experimental

2.1. Chemicals and reagents

Risperidone, hydroxy-risperidone and the compound R68808 (Fig. 1) used as the internal standard were obtained from

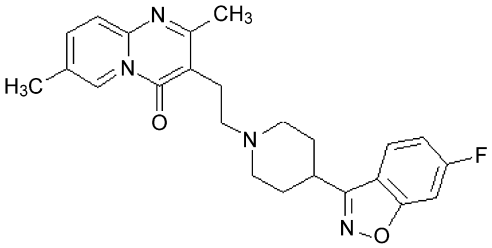

Fig. 1. Molecular structure of IS (R68808).

HELP Pharmaceutical Company (Athens, Greece). Methanol (HPLC grade), Acetonitrile (HPLC grade), were obtained from Sigma–Aldrich (Athens, Greece). Glacial acetic acid (analysis grade) and ammonium acetate (analysis grade) were purchased from Metrolab (Athens, Greece). All aqueous solutions and buffers were prepared using de-ionized and doubly distilled water (resistivity > 18 MΩ) from a Millipore Milli-Q Plus System (Malva, Athens, Greece). Pooled human control plasma (heparinized) was kindly donated from Ippokrateio hospital (Athens, Greece).

2.2. Instrumentation

A PerkinElmer Multiprobe® II HT-EX workstation (PerkinElmer, Downers Grove, IL) equipped with an eight-tip robotic arm coordinated *x*-, *y*- and *z*-axis was employed for transferring the plasma samples from 2 mL eppendorf microfuge tubes (Lab Supplies, Athens, Greece) into 2.2 mL square 96-deep-well plate (Sigma–Aldrich, Athens, Greece) as well as for the addition of ACN containing the internal standard. The workstation was controlled by WinPrep Software. Conductive disposable tip-boxes (1000 μL) were purchased from E&K Scientific Products (Cambell, CA, USA), a tipchute, reagent troughs and a tip flush/wash station were purchased from PerkinElmer. A Tomtec Quadra 96 model 320 robotic liquid-handling system equipped with a 96-tip pipetting head (Bidservice, NJ, USA) was used for transferring the supernatant organic layer, after protein precipitation, into a new 2.2 mL 96-deep-well plate. An Eppendorf 5810 R (Bacakos, Athens, Greece) centrifuge that could accommodate 96-well plate as well as Eppendorf microfuge tubes was also utilized during sample preparation. The HPLC system included two Agilent 1100 series binary pumps, a degasser and a column oven/cooler (Hellamco, Athens, Greece). The CTC PAL autosampler (Hellamco) could accommodate six 96-deep-well plates allowing an automated measurement of a big amount of samples. The PAL 2-Valve drive module consisted of two individually controlled valve drives, a six-port injection valve and a 10-port switching valve. The Oasis HLB extraction columns (S-25 μm, 20 mm × 2.1 mm i.d.) used were from Waters (Milford, MA, USA) while the Cyano (CN) analytical column (S-5 μm, 50 mm × 4.0 mm i.d.) was from YMC (Schermbeck, Germany). A PE Sciex API 3000 triple quadrupole mass spectrometer (Biosolutions, Athens, Greece) interfaced with the HPLC via a turbo ionspray source was used for the mass analysis and detection, operating under Analyst 1.4.1 software. Eppendorf deepwell mats for covering the 96-well plates were purchased