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Liquid chromatographic–mass spectrometric assay for simultaneous pyrimethamine and sulfadoxine determination in human plasma samples

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Abstract

We present a liquid chromatographic–mass spectrometric assay for the simultaneous determination of sulfadoxine and pyrimethamine in human plasma samples. Sample clean-up was achieved by adding acetonitrile for protein precipitation. Gradient elution in only 10 min resulted in high throughput capability. Tandem mass spectrometric detection in multiple reaction monitoring was used for quantification. The developed analytical approach was successfully validated and was applied in the pharmacokinetic evaluation of the bioavailability between two sulfadoxine/pyrimethamine formulations available on the Eastern African market, using a cross-over design.

Keywords: Sulfadoxine; Pyrimethamine; Liquid chromatography; Mass spectrometry

1. Introduction

Background *Plasmodium falciparum* resistance has rendered chloroquine monotherapy ineffective in much of Africa [1]. As the problem of chloroquine resistance in Eastern Africa is worsening, the use of chloroquine as the first-line drug for the treatment of uncomplicated malaria is very much compromised [2]. Therefore, the synergistic combination of sulfadoxine (SD), a long-acting benzene sulphonamide, and the dihydrofolate reductase inhibitor pyrimethamine (PR) became a cheap and effective replacement for chloroquine [3]. In e.g. Tanzania, the pyrimethamine/sulfadoxine combination has recently replaced chloroquine as first-line drug for the treatment of uncomplicated malaria [4]. Due to the low solubility of both these drugs, their effectiveness depends on the bioavailability of both components after oral administration. In that respect, questions have arisen on the quality, and thus bioavailability of the pharmaceutical formulations present on the African market. Poor bioavailability not only compromises the profylaxis of the patient, drug resistance too is of course favoured due to the exposure of the parasite to sublethal concentrations as a result of suboptimal drug regimens and the use of substandard drug formulations [5]. The quality with respect to potency and in vitro dissolution of sulfadoxine/pyrimethamine tablets marketed in Rwanda and Tanzania was previously assessed [4,6]. The latter study revealed a significant in vitro difference in the dissolution properties of two commercially available PM/SD formulations [4]. The study also demonstrated the presence of two commercially available sulfadoxine/pyrimethamine formulations on the Tanzanian market that failed dissolution tests according to the United States Pharmacopoeia (USP) 24 monograph. To determine if the observed in vitro differences were also reflected in the in vivo behaviour of the formulations, the bioavailability needed to be investigated. To that end, a quantitative method for the simultaneous determination of both drugs in human plasma was required.

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Analytical difficulties for the simultaneous determination of the two drugs are linked to their disparate chemical properties (sulfadoxine is both an acid and a weak base, whereas pyrimethamine is a weak base) and to their high concentration ratio (SD/PM) in plasma [7,8]. There are several HPLC–UV methods for simultaneous measurements of SD–PM in serum, plasma, dried whole blood and urine [7–14]. Bonini et al. reported a GC method for the determination of SD and PM in blood and urine [15]. These methods share the disadvantage of time-consuming liquid/liquid extraction procedures, mainly because the amphipathic nature of SD precludes its efficient extraction in an organic solvent at any pH [9–13,15], or solid-phase extraction procedures (SPE) [7,8,14].

Considering all of this, we report on a liquid chromatographic method combined with tandem mass spectrometric detection in human plasma samples. For optimum sensitivity and selectivity, the mass spectrometric analysis was performed in multiple reaction monitoring (MRM) on a triple quadrupole instrument. Due to their high sensitivity and specificity, LC–MS/MS techniques are more and more used in the pharmaceutical industry as the definitive technology for the determination of levels of drugs in biological fluids obtained from pharmacokinetic and toxicological studies [16]. Surprisingly is the fact that no LC–MS(/MS) approach has yet been reported in the target compound analysis of SD and PM. Due to the outstanding improvements in LC–MS/MS, for the majority of applications, sensitivity is most often no longer an issue. On the other hand, analytical challenge shifts towards reproducibility. Moreover, the focus is put on rudimentary, hence rapid sample preparation, necessary in view of the high sample throughput in pharmacokinetic applications.

2. Experimental

2.1. Chemicals

Sulfadoxine was obtained from Indis (Aartselaar, Belgium), while pyrimethamine and sulfamerazine were purchased from Sigma-Aldrich (Bornem, Belgium). Sulfamerazine was chosen as internal standard, because of its structural similarity to and small mass difference with the analytes (Fig. 1).

Stock solutions (pyrimethamine 1.009 mg/mL; sufadoxine 30.07 mg/mL) were prepared by separately dissolving the analytes in 10 mL of 50/50 (v/v) methanol/acetonitrile. 10.17 mg of the internal standard was dissolved in 10 mL of acetonitrile. These solutions were stored at −20 °C. Working standards and quality control standards were diluted in acetonitrile using a Hamilton Digital Diluter (Bonaduz, Switzerland). The concentrations of the working standard solutions were between approximately 0.001 and 0.1 mg/mL for pyrimethamine and between 0.27 and 27 mg/mL for sulfadoxine (6 data points). The internal standard working solution was also prepared in acetonitrile (2 μg sulfamerazine/mL). Quality control solutions were prepared at 2.018, 10.09, 60.54 and 90.81 μg/mL acetonitrile for pyrimethamine and at 0.5412, 2.705, 16.24 and 24.35 mg/mL acetonitrile for sulfadoxine. Spiking of 20 μL of these working standards into 1.98 mL plasma resulted in calibrators at 10.09, 20.18, 40.36, 403.6, 807.2 and 1009 ng/mL plasma for pyrimethamine and at 2.706, 5.412, 10.82, 108.2, 216.5 and 270.6 μg/mL plasma for sulfadoxine and quality control samples at 20.18 (QC1), 100.9 (QC2), 605.4 (QC3) and 908.1 (QC4) ng/mL plasma for pyrimethamine and at 5.412 (QC1), 27.05 (QC2), 162.4 (QC3) and 243.5 (QC4) μg/mL plasma for sulfadoxine. Blank human plasma was used for method development and the preparation of calibrators.

HPLC grade acetonitrile, methanol and formic acid were supplied by Merck (Darmstadt, Germany). A Synergy 185 system (Millipore Corporation, Bedford, MA, USA) was used to generate high-purity water for the preparation of all aqueous solutions.

2.2. Sample preparation

After adding 100 μL of internal standard solution, sample clean-up was achieved by protein precipitation with 1650 μL of acetonitrile added to 250 μL of crude plasma. After thorough mixing and centrifugation (2700 × *g*), the supernatant was decanted and evaporated on a Zymark Turbovap LV evaporator (Zymark Corporation, Hopkinton, MA, USA) at 40 °C. The residue was dissolved in 500 μL of 0.1% (v/v) formic acid in a 15/85 (v/v) acetonitrile/water mixture. After mixing and centrifugation, 10 μL of the supernatant was injected on the column.

2.3. Mobile phases

LC eluents A and B consisted respectively of 0.1% (v/v) formic acid in water and 0.1% (v/v) formic acid in a 80/20 (v/v) acetonitrile/water mixture. Both solvents (A) and (B) were filtered through a 0.45 μm membrane filter.

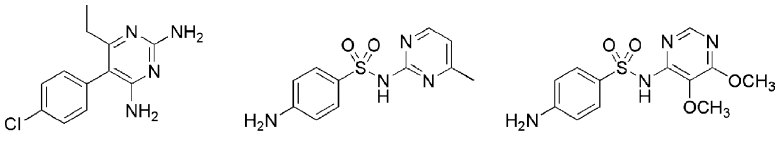

Fig. 1. Chemical structures of analysed compounds.