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Short communication

Capillary zone electrophoresis as a potential technique for the simultaneous determination of sulfadoxine and pyrimethamine in tablet formulations

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ABSTRACT

A novel, simple and rapid capillary zone electrophoresis method with UV detection has been developed for the simultaneous determination of pyrimethamine and sulfadoxine in tablet formulations. The compounds are separated in 6 min in a fused silica capillary, 30 cm long (20 cm to detector) × 50 μm using a 100 mM phosphate buffer pH 7.2 as background electrolyte, a 330 V cm^{-1} electric field and a detection wavelength of 214 nm. Analysis of different tablet formulations has shown a good agreement with the liquid chromatography method described in the United States Pharmacopoeia. Main advantages of the CZE method are the rapid set-up of instrumentation and capillary equilibration, short analysis time and low running cost.

1. Introduction

Because each year, in the malaria-endemic areas of Africa, around 25 millions of pregnant women are at risk of *Plasmodium falciparum* during their pregnancy [1], a preventive antimalaria treatment for pregnant women has been recommended by World Health Organisation. The national policy which has been adopted in Côte d'Ivoire is to use pyrimethamine (PYR) and sulfadoxine (SDX) in combination for this purpose [2]. In this antimalaria chemotherapy, PYR which is a dihydrofolate inhibitor is combined in a synergetic effect with SDX, a sulfonamide which inhibits dihydropteroate synthetase. The two active substances sequentially block the two enzymes involved in the biosynthesis of folic acid within *P. falciparum*. Among separation methods, liquid chromatography (LC) methods have been described for the simultaneous determination of SDX and PYR in tablets [3–6], cleaning validation swabs [7] or human plasma [8–10]. Capillary electrophoresis (CE) methods have been reported for the determination of sulfonamides in food, pharmaceuticals and water [11–13] or for the determination of PYR as drug substance or in drug formulations [14–16] but there are no CE methods reported for a simultaneous determination of SDX and PYR in pharmaceuticals. Since alternative or complementary methods may be of interest to guarantee the quality of the drug or to detect drug adulteration, or counterfeit medicines [17], the possibility of using CE for the simultaneous determination of PYR and SDX in tablets has been investigated.

2. Experimental

2.1. Chemicals

PYR, SDX, procaine hydrochloride (PRO) and phenobarbital (PHE) were from Sigma Aldrich (Saint Quentin Fallavier, France). All other chemicals were of analytical grade from different suppliers. Fansidar® (Roche), Maloxine® (Exphar) and Ubigen SP® (Ubithera) commercial tablet formulations, were purchased from a pharmacy in Côte d'Ivoire. Amalar® (Micro Labs Ltd.), Fansidar® (Roche), Malabase® (Ronak), Maloxine® (Tycol Pharm, London, UK, Britlodge Ltd.; SW Pharma, China) tablets were purchased from a street market in Abidjan. All formulations had a declared content of 500 mg of SDX and 25 mg of PYR per tablet.

2.2. Solutions

2.2.1. Background electrolyte solution

100 mM sodium dihydrogenophosphate aqueous solution adjusted to pH 7.2 with a 0.1 M sodium hydroxide solution and filtered through a 0.45 μm Millipore membrane filter, type HVLP.

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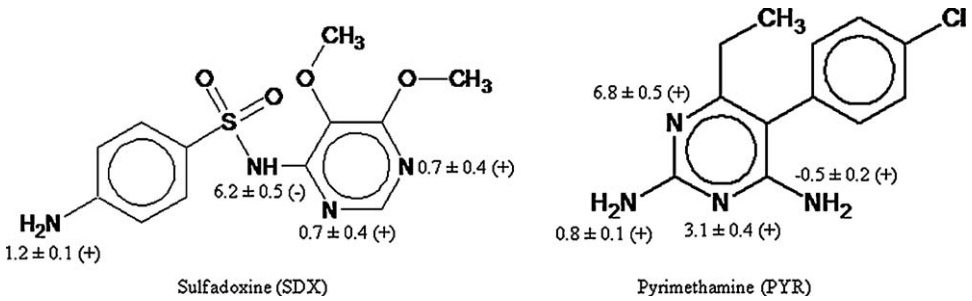

Fig. 1. Chemical structure and pK_as of sulfadoxine (SDX) and pyrimethamine (PYR).

2.2.2. Standard solution

A solution (24.0 mg l^{-1} PYR and 480.0 mg l^{-1} SDX) is prepared in a volumetric flask by sonication of an accurately weighed amount of PYR and SDX in acetonitrile (17% of the flask volume). After addition of 0.1% aqueous phosphoric acid solution to volume, the resulting solution (solution S) is sonicated for 5 min. A volume of 1 ml of internal standard (IS) solution (PRO, 300 mg l^{-1} and PHE, 2520 mg l^{-1} in acetonitrile – 0.1% phosphoric acid solution, 1:1, v/v) is added to 5 ml of solution S. This working standard solution contains PYR, 20 mg l^{-1}; SDX, 400 mg l^{-1}; PRO, 50 mg l^{-1} and PHE, 420 mg l^{-1}.

2.2.3. Test solution

Test solutions corresponding to theoretical PYR, 20 mg l^{-1}; SDX, 400 mg l^{-1} concentrations are prepared from an accurate amount of powdered tablet following the same procedure. After centrifugation of the extract at 5000 rpm for 5 min, 1 ml of the IS solution is added to 5 ml of supernatant. Standard and test solutions are stable for at least 24 h at ambient temperature.

2.3. Apparatus and operating conditions

A Beckman P/ACE MDQ (Fullerton, CA) instrument with a photodiode array detector was used with a fused-silica capillary, 30 cm long (20 cm to the detector), 50 μm internal diameter (TSP, Composite Metal Services, Hallow, Worcs, UK), housed in a cartridge with a 200 μm × 800 μm detector window. Prior to its first use, the capillary was pre-conditioned by washing at 20 psi for 20 min with a 0.1 M sodium hydroxide solution, and then flushed with water for 5 min. Every working day a preconditioning was carried out with 0.1 M sodium hydroxide followed by water and electrolyte buffer at 20 psi for 5 min. Optimised operating conditions for analysis were as follows: capillary rinse, 0.1 M HCl (1 min; 20 psi), 0.1 M NaOH (1 min; 20 psi), electrolyte (2 min; 20 psi); sample introduction (anodic side, 3 s, 0.3 psi); wait, water (0 s); separation, 330 V cm^{-1} with a 0.17 min ramp voltage, cartridge temperature, 25 °C (i = 80 μA); detection at 214 nm.

3. Results and discussion

3.1. Method optimisation

3.1.1. Selection of background electrolyte and separation conditions

The respective pK_a values of 6.8 (+) and 6.2 (−) [18] of PYR and SDX (Fig. 1) allow their separation under cationic (PYR) and anionic (SDX) forms in a pH range of 5–8. Different phosphate buffer solutions were tested in this range. An optimal separation was obtained with a phosphate buffer pH 7.2 at high concentration (100 mM) to improve peak efficiency by a stacking effect. Using a short length capillary (30 cm) to achieve the separation in the shortest time possible and applying a moderate voltage (10 kV) at a 25 °C separation temperature resulted in an acceptable current developed (80 μA). Under these conditions, a minimum of 50 separations can be achieved with the same set of separation vials without noticeable drift of migration times (MTs) due to buffer depletion.

3.1.2. Selection of a sample solvent

Different sample solvents were investigated. Methanol, mixtures of methanol–water or methanol–phosphoric acid used in different ratios, resulted in a tailing peak for PYR. Using the sample solvent described in the USP (0.1% phosphoric acid–acetonitrile, 83:17, v/v) resulted in better peak shapes which can be explained by the stacking effect of acetonitrile in the presence of salt [19].

3.1.3. Selection of internal standards

The use of an IS is needed for quantitative analysis in CE to take into account small variations of the injected volumes due to the injection system. Because there is a disproportion in PYR and SDX, it was decided to use two different ISs for quantification. Among potential candidates, PRO (pK_a 9) and tetracaine (pK_a 8.5) were tested for PYR, and PHE (pK_a 7.4), benzoic acid (pK_a 4.3) and sorbic acid (pK_a 4.76) for SDX. PRO and PHE were selected as they give peaks well resolved (Fig. 2), close to the analytes and do not increase the analysis time.

3.1.4. Selection of detection wavelength

Due to the low concentration of PYR in the tablet extract, detection at a low wavelength is needed for sensitivity. A 214 nm wavelength (which allows the use of an instrument equipped with

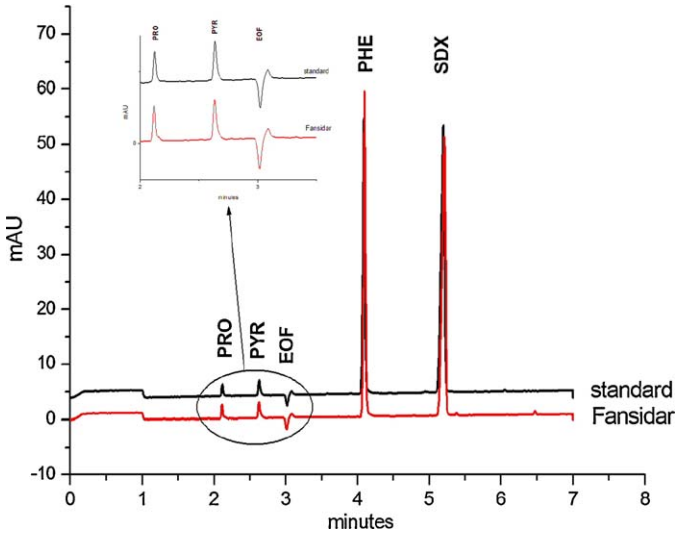

Fig. 2. Electropherograms of a standard solution of PYR and SDX (upper trace) and a test solution of Fansidar (lower trace). Conditions: 100 mM phosphate buffer pH 7.2; capillary, 50 μm I.D. × 30 cm; inj 3 s, 0.3 psi, PYR 20 mg l^{-1}, SDX 400 mg l^{-1}; 10 kV; 25 °C; detection, λ = 214 nm.