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Development of a simple, rapid, and robust liquid chromatographic method for the simultaneous determination of sulfalene, sulfadoxine, and pyrimethamine in tablets

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ABSTRACT

A simple, cost effective, accurate, and precise RP-HPLC method was developed for the simultaneous determination of sulfalene and sulfadoxine in fixed dose dual combinations with pyrimethamine together with their related substances. Proprietary products containing these combinations are often being prescribed in malaria endemic countries. Quantification of the active compounds and impurity profiling was achieved using two standard C_{18} columns with a mobile phase being composed of 60% (v/v) of a 0.05 M KH_2PO_4 buffer solution (pH = 2.6) and 40% (v/v) of methanol, applying an isocratic elution mode and a detection wavelength of 215 nm. The method allows a quick quantitative determination of sulfadoxine and sulfalene and the separation of the respective impurities within a total runtime of approximately 15 min and was validated with respect to specificity, linearity, precision, accuracy, limits of detection and quantification, robustness, and stability of the standard and sample solutions. The method is simpler than the corresponding method described in the International Pharmacopoeia and the United States Pharmacopoeia in terms of being easy to apply, being less time consuming, and utilizing reagents and chemicals which are cost efficient.

1. Introduction

Malaria is a parasitic disease caused by different species of *Plasmodia* which accounts for over 240 million cases and about one million deaths annually [1]. It is a major cause of morbidity and mortality particularly for pregnant women, the foetus, and the newborn. Although various highly effective active pharmaceutical ingredients (APIs) exist for the treatment of this infectious disease, the fixed dose dual combinations of sulfadoxine/pyrimethamine and sulfalene/pyrimethamine, respectively, are still commonly administered. Of note, using the combination of sulfadoxine and pyrimethamine for the intermittent preventive treatment in pregnancy is recommended by the World Health Organization (WHO) in almost all malaria endemic countries [2]. Sulfadoxine (SD), sulfalene (SL), and pyrimethamine (PYR) are well known antimalarial drugs having similar pharmacological activities (for structures see Fig. 1). Sulfalene and sulfadoxine potentiate the effect of pyrimethamine by interfering with the tetrahydrofolate synthase and the dihydrofolate reductase in the malaria parasites [1,3,4], thus they are normally being sold as fixed dose combinations.

Cases of poor-quality, substandard and/or counterfeit products are frequently reported in countries of the developing world [5–9]; some 50% of the global drug market are affected by counterfeit medicines, particularly in low and middle income countries [10]. A recent quality survey performed by the WHO revealed that up to two thirds of the samples collected in sub-Saharan African countries were substandard, characterized by a lower amount of the API, failures in mass uniformity, a higher amount of related substances, or unknown impurities [11]. The use of such antimalarial drugs may result in treatment failures or delays, the development of drug resistances, and adverse effects which hinder the progress in combating malaria in these vulnerable populations [7,8,10].

Abbreviations: KAAD, Catholics Academics Ausländer-Dienst; BfArM, Bundesinstut für Arzneimeittel und Medizinprodukte; TFDA, Tanzania Food and Drugs Authority; QC, quality control; API, active pharmaceutical ingredient; RP-HPLC, reverse phase- high performance liquid chromatography; RSD, relative standard deviation; Ph.Int., International Pharmacopoeia; USP, United States Pharmacopoeia; LOD, Limit of Detection; LOQ, Limit of Quantification; ICH, International Council on Harmonization.

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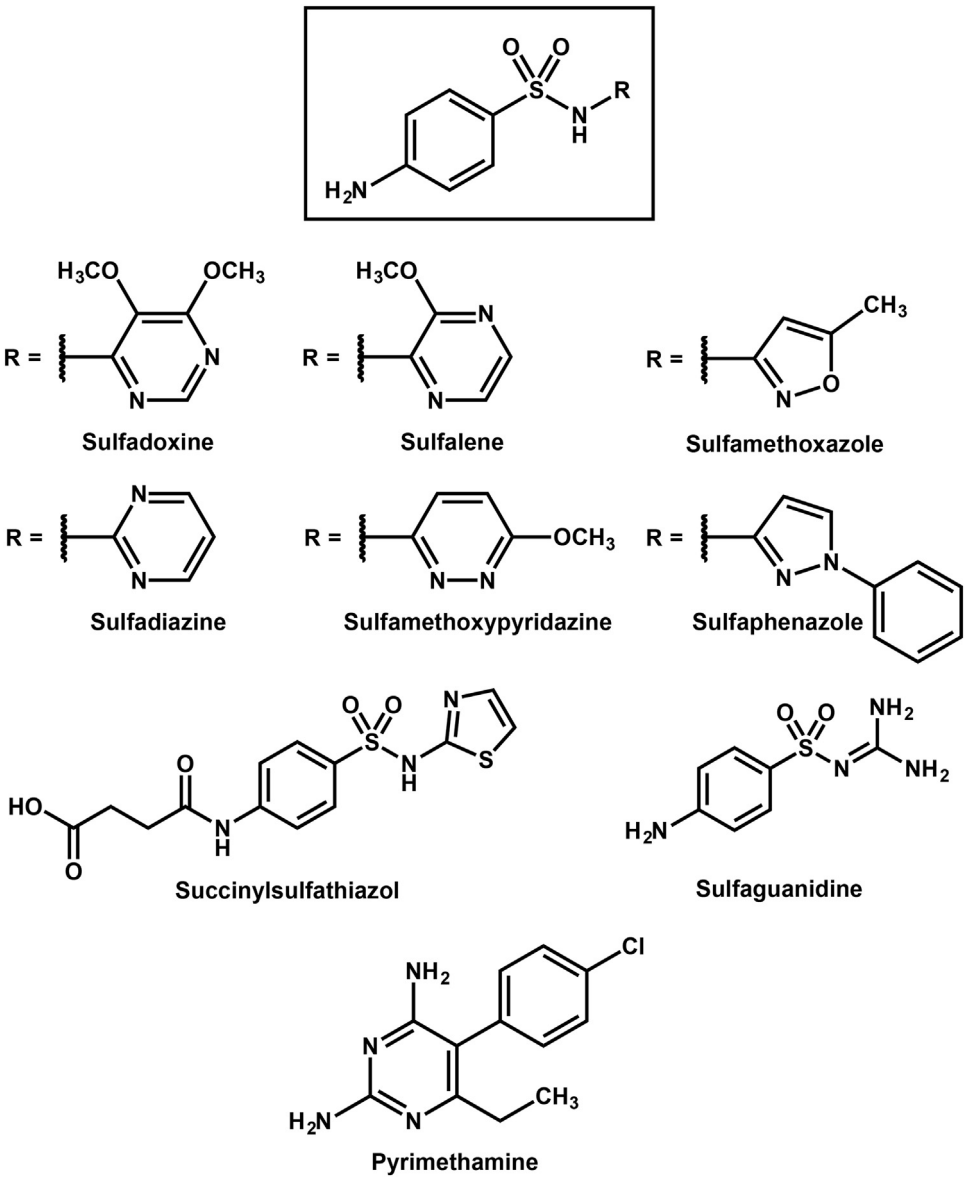

Fig. 1. Chemical structures of sulfadoxine, sulfalene, and pyrimethamine as well as additional sulphonamides.

Some studies reported the quantification of sulfadoxine and pyrimethamine applying UV/Vis spectroscopy or utilizing complexation reagents to produce coloured compounds [12–15]. Capillary zone electrophoresis using a 100 mM phosphate buffer (pH = 7.2) as background electrolyte [16], high performance thin layer chromatography on precoated silica gel plates [17], refractometric and calorimetric methods [18] as well as liquid chromatographic protocols have also been described [1,6,12,19–25]. Monographs of the International or the United States Pharmacopoeia are not suitable for the simultaneous quantification of the three APIs in commercially available fixed dose combinations, nor for characterizing the impurities of the respective compounds. The new method is easy to apply, less time consuming, and requires cheap chemicals and reagents only. Existing methods mostly make use of acetonitrile which is not readily available in resource constrained countries while some have been developed for biological samples only.

Having simple and robust methods available for determining the quality of essential APIs and their formulations is of particular interest for countries with limited financial resources and restricted regulatory infrastructures. The prevalence of counterfeit medicines is considerably high, and quality control laboratories often fail to apply the compendial methods, for example pharmacopoeial monographs, because the chemicals, reagents, and other consumables such as HPLC columns, acetonitrile, or triethylamine are not readily available. Therefore, suitable methods should be designed in a very streamlined and robust manner and shall utilize simple and easily available chemicals, reagents, and equipment only [26]. Of note, methods exhibiting a high grade of ruggedness can preferably be applied in developing countries, where extreme temperature fluctuations due to lacking laboratory air conditioning or very basic instrument setups are commonly encountered. We therefore aimed for the development and validation of a simple, cheap, precise, and accurate HPLC method for the determination of the three active compounds together with their related substances (cf. Fig. 2) in commonly prescribed medicines containing either 500 mg of sulfadoxine and 25 mg of pyrimethamine, or 500 mg of sulfalene and 25 mg of pyrimethamine, respectively. The mobile phase consists of a simple phosphate buffer and methanol, while the stationary phases are two commercially available, inexpensive