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Simultaneous quantification of mefloquine (+)- and (−)-enantiomers and the carboxy metabolite in dried blood spots by liquid chromatography/tandem mass spectrometry☆

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ABSTRACT

Mefloquine (MQ), a racemic mixture of (+)-(11S,12R)- and (−)-(11R,12S)-MQ, has been used for treatment and prophylaxis of malaria for almost 30 years. MQ is metabolized by the cytochrome P450 3A subfamily to 4-carboxymefloquine (CMQ), which shows no antimalarial activity in vitro. Highly stereospecific pharmacokinetics of MQ have been reported, although with contradictory results. This might be due to incorrect assignment of the absolute configuration as shown only recently. Gastrointestinal as well as neuropsychiatric adverse events were described after prophylaxis and treatment with MQ. Data are indicating that the tolerability of the enantiomers may vary considerably. An involvement of the main metabolite CMQ in the development of neuropsychiatric adverse events has also been supposed. Due to these inconsistent results we established a novel liquid chromatography/tandem mass spectrometry (LC–MS/MS) method for the simultaneous quantification of MQ enantiomers and the metabolite CMQ to investigate the attribution of efficacy and adverse effects to the single enantiomers as well as the main metabolite. Separation of the MQ enantiomers was achieved on a quinidine-based zwitterionic chiral stationary phase column, CHIRALPAK® ZWIX(−) (3.0 × 150 mm, 3 μm) in an isocratic run using a pre-mixed eluent consisting of methanol/acetonitrile/water (49:49:2 v/v) with 25 mM formic acid and 12.5 mM ammonium formate. We used stable isotope-labelled analogues as internal standards. The method was validated according to the FDA guidelines. With a linear calibration range from 5 to 2000 nM for the MQ enantiomers and from 13 to 2600 nM for CMQ respectively, the method was successfully applied to dried blood spot (DBS) samples from patients under prophylactic MQ treatment. The method was also applicable for plasma samples.

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1. Introduction

Mefloquine (*rac*-erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol, MQ) has been used for prophylaxis and treatment of malaria since the mid-1980s. MQ is orally administered as a racemic mixture of (+)-(11S,12R)- and (−)-(11R,12S)-MQ (Fig. 1). It is metabolized by cytochrome P450 (CYP) 3A subfamily to 4-carboxymefloquine (CMQ), which shows no antimalarial activity in vitro [1].

Only recently the absolute configurations of the MQ enantiomers have been determined conclusively by Schmidt et al. [2]. Up to then publications on stereospecific pharmacokinetics of MQ were contradictory due to incorrect or incomplete assignment of the enantiomers' configurations [3–8]. In consideration of the absolute configurations described by Schmidt et al. [2] the half-life of the (+)-enantiomer was determined to be more than four times longer than of the (−)-MQ (433 h vs. 94 h) at prophylactic doses as reported by Hellgren et al. [4]. Furthermore, the observed maximal concentration (C_{max}) after the first dose was found to be higher for the (+)-MQ compared to (−)-MQ (0.65 ± 0.16 vs. 0.30 ± 0.06 μM) [4]. At first glance Gimenez et al. [3] determined higher C_{max} values

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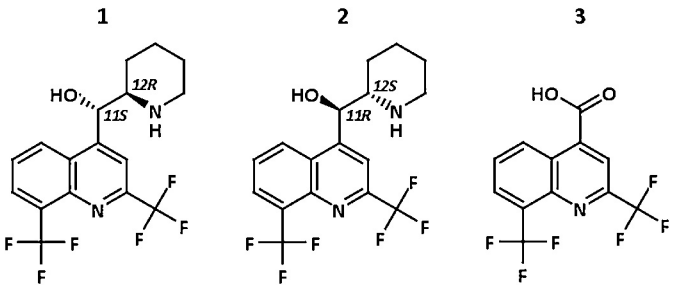

Fig. 1. Chemical structures of (+)-(11S,12R)-MQ (1), (−)-(11R,12S)-MQ (2) and the main metabolite CMQ (3).

and a higher half-life for the (−)-enantiomer. However, from the description of the chemical structure, one can conclude that the assignment of the enantiomers was not correct and their results are in line with the work of Hellgren et al. [4].

Furthermore, MQ has been associated with gastrointestinal adverse events such as nausea and vomiting. Less frequently, but more severely observed were neuropsychiatric symptoms such as anxiety, depression, hallucination and acute psychosis [9]. It has been reported that the enantiomers differ in their biological activities related to CNS side effects [10,11]. The stereo-selective passage of MQ through the blood-brain barrier in rats was investigated by Baudry et al. [8]. They found higher concentrations of the (+)-enantiomer in brain tissue as compared to the antipode. In plasma the inverse ratio between (+)- and (−)-enantiomer was shown, which is contrary to the results in humans as discussed above.

However, Björkman [12] assumed the main metabolite, CMQ to be involved in the development of neuropsychiatric adverse events after MQ medication due to its high plasma levels and long elimination half-life.

On account of these inconsistent results investigations on the attribution of efficacy and adverse effects to the single enantiomers require an analytical method for the enantioselective determination of both MQ enantiomers. Furthermore, the main metabolite should be included in the analysis concept for data completion regarding potential influences on the development of adverse events.

Several HPLC methods for the direct or indirect determination of MQ enantiomers were described [13,14]. The indirect approach utilized derivatizing agents, e.g. 1-(9-fluorenyl)ethyl chloroformate, yielding diastereomeric derivatives of MQ, which differ in their physico-chemical properties allowing a separation on achiral columns [13]. One of the obstacles here was the low sensitivitiy due to low derivatization yields. The direct approaches made use of either chiral mobile phase additives or chiral stationary phases (CSP). Magalhães et al. described an HPLC method using a CHIRALPAK® AD column and UV detection [14]. These chiral separations utilize mobile phases with nonpolar organic solvents only compatible with UV detection and not with electrospray ionization (ESI) mass spectrometry (MS) detection, whereas a better sensitivity is achieved using MS detection.

The cornerstone for the usage of chiral columns with LC–MS-analysis of MQ was laid by Hoffmann et al. introducing a zwitterionic stationary phase, CHIRALPAK® ZWIX, which is compatible ESI-MS owing to the use of polar organic mobile phases and volatile additives such as ammonium formate and formic acid [15,16]. The structure of the CSP is based on a cinchona alkaloid fused with trans-2-aminocyclohexanesulfonic acid (ACHSA) at the C-9-position via a carbamate linkage. E.g. the chiral selector in CHIRALPAK® ZWIX(−) is built by the fusion of quinidine with (R,R)-ACHSA. This structure is covalently bonded onto a 3 μm silica gel (Fig. 2). The separation of chiral analytes is achieved by ion-pairing mediated adsorption processes.

Here we describe for the first time a sensitive LC–MS/MS method for the enantioselective determination of both (+)- and (−)-MQ using a CHIRALPAK® ZWIX(−) column in dried blood samples (DBS). Furthermore, the main metabolite CMQ could be analyzed simultaneously by this method. The method was validated according to FDA guidance [17] and successfully applied to DBS samples from patients under prophylactic MQ treatment. Furthermore, the method was also tested for the use of plasma samples.

2. Material and methods

2.1. Chemicals and materials

MQ hydrochloride was purchased from Sigma–Aldrich (Steinheim, Germany). (+)(11S,12R)-MQ hydrochloride was procured from SynphaBase (Pratteln, Switzerland). CMQ and D_{10}-MQ hydrochloride were obtained from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). D_3CMQ was obtained by chemical synthesis (vide infra). D_5-aniline, chloral hydrate, 1,1,1-trifluoroacetone were purchased from Aldrich (Steinheim, Germany). 2,4-Dimethyl-7-nitro-5-(trifluoromethyl)dibenzothiophenium triflate was obtained from ABCR GmbH &Co. KG (Karlsruhe, Germany). Hydroxylamine hydrochloride was procured by Alfa Aesar GmbH &Co. KG (Karlsruhe, Germany).

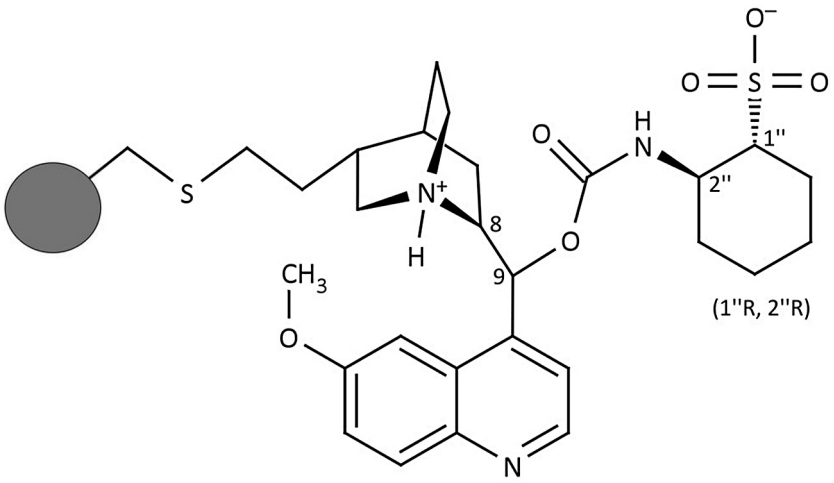

Fig. 2. Chemical structure of the zwitterionic CHIRALPAK® ZWIX(−) stationary phase covalently bonded on 3 μm spherical silica gel.

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