Separation of the Positional Isomer Quinocide from the Anti-Malarial Drug Primaquine Using a Discovery® HS F5 HPLC Column

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Introduction

Malaria is one of the most deadly diseases on earth with an estimated death rate of about 2.7 million people per year. It is especially widespread in Africa where the death toll is highest among children. Malaria is caused by the protozoa Plasmodium vivax and other Plasmodium ssp. The drug primaquine di-phosphate is used for causative treatment of malaria infections. Primaquine (CAS 90-34-6) and its positional isomer quinocide (CAS 525-61-1), are highly toxic substances. Both substances are anti-malarial drugs having a number of side effects.

It has previously been assumed that the main contaminant of primaquine is the enantiomer, and a possible separation of the racemate has been reported (1). In some publications, the separation of the positional isomer from primaquine was mistaken as separation of the stereoisomer. Separation of isomers should be supported by MS analysis. We were able to show by using liquid chromatography-mass spectroscopy (LCMS) that the main contaminant of primaquine is the positional isomer quinocide (2).

The Discovery HS F5 column has unique selectivity for the positional isomers of primaquine and excellent separation characteristics (Figure 1) compared to other stationary phases.

![Figure 1. Separation of Quinocide from Primaquine on Discovery HS F5 Column (567517-U 238074)](image)

Materials and Methods
For HPLC analysis, an Agilent® 1100 chromatograph with diode array detector (DAD) was used. A Discovery HS F5 column, 25 cm x 4.6 mm I.D., 5 µm particle size was utilized to enhance the separation.

Baseline separation was achieved with isocratic conditions at a flow rate of 1.0 ml/min. The mobile phase composition finally chosen was acetonitrile: 20 mM ammonium acetate in distilled water, pH 7.0, 50:50. The analytes were detected at 268 nm with reference at 300 nm. This method resulted in baseline separation of the positional isomer quinocide from the drug primaquine (Figure 1) resulting in a simple and reproducible method.

Conclusions

The anti-malarial drug primaquine is an important human drug, especially in third world countries. Precise quantification of toxic contaminants in this therapeutic agent is therefore of great value. We found that in pharmaceutical samples of primaquine, the concentration of quinocide was as high as 5.1% (2). In the British Pharmacopoeia 2000 (3) and in the European Pharmacopoeia, 2001, 3rd Supplement (4) related substances are allowed to be present in the drug at a maximum of 3%.

The structure of primaquine and quinocide are shown in Figure 2. The Discovery HS F5 column in isocratic mode gave baseline separation of the positional isomers primaquine and quinocide. Separation of positional isomers is supported by co-chromatography of primaquine and quinocide shown in Figure 1 and by previous LC-MS (2). Baseline separation gives the opportunity to perform precise quantification of quinocide in primaquine. Because the quinocide concentration in primaquine samples tends to be high and quinocide is more toxic and less stable than primaquine, some previously published investigations should be reconsidered.

![Figure 2. Structures of Primaquine and Quinocide](image)

Figure 2. Structures of Primaquine and Quinocide

Materials

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References

