Asian Journal of Chemistry Vol. 18, No. 3 (2006), 1705–1709

Reversed-phase HPLC Separation of Quinine and its Diastereoisomer Quinidine in Pharmaceutical Tablets

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In this paper, the effects of mobile phase composition on the retention time and peak shape of quinine and quinidine in tablets are described. The method is reproducible and sensitive and suitable for routine use.

Keys Words: Quinine, Quinidine, HPLC.

INTRODUCTION

Among the alkaloids that are found in cinchona alkaloids, quinine and quinidine are now gaining wider use for treatment of malaria. Quinine (3R, 4S, 8S, 9R) and quinidine (3R, 4S, 8R, 9S) are basic drugs and they are diastereoisomers (Fig. 1); they have different physical properties and different biological effects.

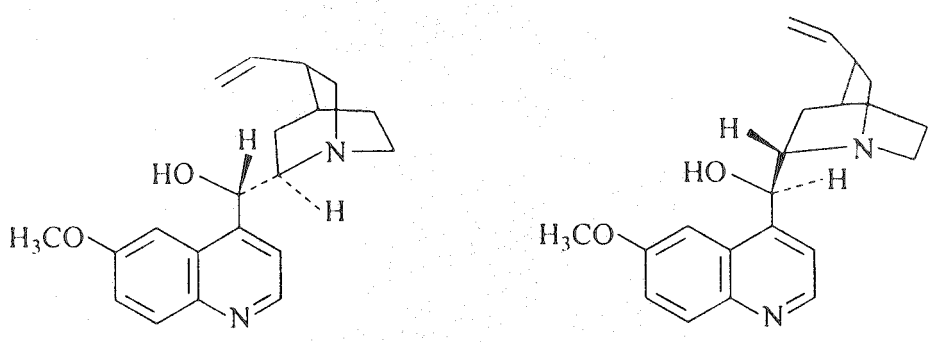

Fig. 1

Quinidine can be prepared from quinine is used as a cardiac antiarrythmic drug in the treatment of atrial fibrillation. About half of the world market for quinine is used for the synthesis of quinidine[1]. Therefore quinidine can be considered as an impurity to quinine. In both the drugs one can be considered as more important of the two drugs in their wide application as catalytic reagents in chiral organic synthesis or to produce high performance liquid chromatography (HLPC) stationary phases (selectors) for chiral separation[2]. Many analytical procedures have been developed for the assessment of quinine and quinidine and their metabolites in human biological fluids[3, 4].

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These include TLC, GC, capillary electrophoresis, capillary chromatography and HPLC. However, reversed phase HPLC has been by far the most widely used at present by pharmaceutical analysts. Since its development, a number of column packing materials have been disigned which operate on this principle.

Among these the most commonly used are the octadecyl alkyl bonded phase silica (ODS). Although silica-based reversed phase column materials have been successful, a number of problems are encountered in their use. One of these problems is the narrow usable pH range (pH = 3–8) in which these materials can usefully be employed.

It is well known that the pH mobile phase may dramatically alter the retention and selectivity of the RP-HPLC separation by influencing analyte polarity and ion-exchange interactions that may be present. The influence of pH is exerted on the charged state of the analyte that may be altered depending on its ionizability and its pK_a relative to the pH of the mobile phase. The change of state of the stationary phase that may be altered due to residual ionizable sites on the support material.

The compounds under study in this work are the basic quinine having two pK_a's 5.07 and 9.7 and quinidine having pK_a's 5.4 and 10. This extends the interaction with stationary phase and makes the analysis more difficult. For basic, nitrogen containing compounds, such as those in which severe peak tailing is often observed, this has been demonstrated to be due to hydrogen-bonding interactrion between the compound nitrogen and column silinol (Si—OH) group. These silinol group interactions interfere with the normal separation procedures and lead to broad asymetrical peaks. Suppression of silinol influences can be achieved by incorporating either with a long chain or a small amine into the aqueous phase[5–8]. The excess of this amine prevents the analytes from interacting with the silinols.

As with any HPLC separation many parameters may be varied during the course of method development. They include organic modifiers (up to three in most instances): flow rate, temperature, eluent pH and buffer concentration.

EXPERIMENTAL

The chromatographic system consisted of a Shimadzu LC 5A pump (Touzart, Matignon, France) connected to a Rheoyden 7520 syringe loading sample injector valve fitted with a 20 μL sample loop, a variable UV detector (UVSPD 6A) and a Shimadzu chart recorder. The column was a 150 × 4.6 mm i.d., packed with hypersil ODS C18, 3 μm particle size (Touzart, Matignon, France). Methanol, acetonitrile and potassium dihydrogenphosphate were of HPLC grade; quinine sulfate (Qn) (Sigma-Aldrich) and quinidine bisulfate (Qd) (Sigma-Aldrich).

Twenty intact tablets of quinine and twenty of quinidine were weighed separately to obtain the average tablet weight of quinine and quinidine. The tablets were crushed and triturated in a mortar until a fine powder was obtained. An amount of the powder equivalent to one tablet was weighed accurately and taken in a 100 mL volumetric flask. The powder was dissolved in mobile phase consisting of ACN : 50 mM phosphate buffer + 0.75% glacial acetic acid + 0.25% TEA (9 : 91); pH adjused to 3 with phosphoric acid and filtered under vacuum through 0.45 millipore filter using an all-glass apparatus and degassed by ultrasonication.

Optimization of mobile phase

Optimization of mobile phase was required to achieve resolution between

quinine and quinidine, when peak symmetry and reasonable analysis time were required. This was accomplished by the effect of acidic pH, percentage organic modifier and other masquing agents (TEA, CH_3COOH).

Preparation of calibration curves

Standard preparations: A stock solution of quinine (100 mg/L) was prepared and linearity over the range of 10–100 μg/mL of quinine was examined. The same procedure was carried out for standard solutions of quinidine; linearity over the range of 10–100 μg/mL of quinidine was also examined.

RESULTS AND DISCUSSION

In setting up the conditions for separation of quinine and its diastereoisomer quinidine, the choice of a detection wavelength was based on the scanned absorption spectrum for both quinine and quinidine. The spectrum was scanned over the range of 200–400 nm and was obtained by measuring the absorption of 10 μg/mL solution of mixture of quinine and quinidine in mobile phase. The spectrum was obtained by using a 1 cm silica cell and the reference cell contained mobile phase; the choice of the wavelength was based on the linearity of Beer's law and as a result a wavelength of 254 nm was chosen.

In optimizing the separation of quinine and quinidine, the effect of the concentration of organic modifier (ACN and MeOH) on the retention time of the two drugs was examined. The next step was to examine the pH in order to improve the resolution and resonable analysis time.

Until now, the range of permissible mobile phase pH had been limited by the chemical or physical stability of HPLC support particle. Despite the fact that many compounds of pharmaceutical interest are basic and ionizable with pK_a values above 8, most HPLC methods on silica-based column are run below pH 7 because of the limited pH range of silica.

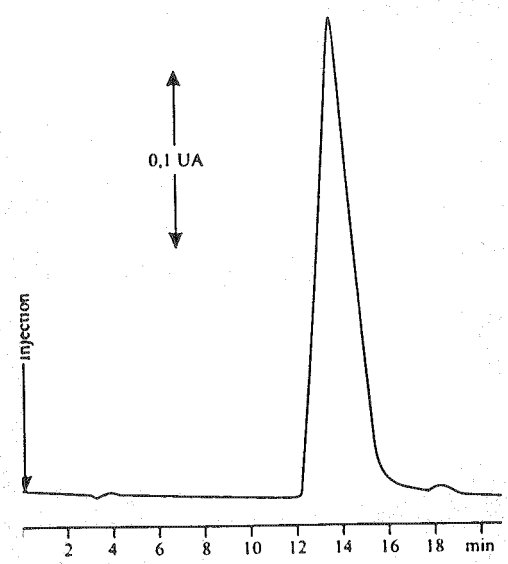

Fig. 2. Chromatogram of quinine, TEA 1%, pH = 2.5

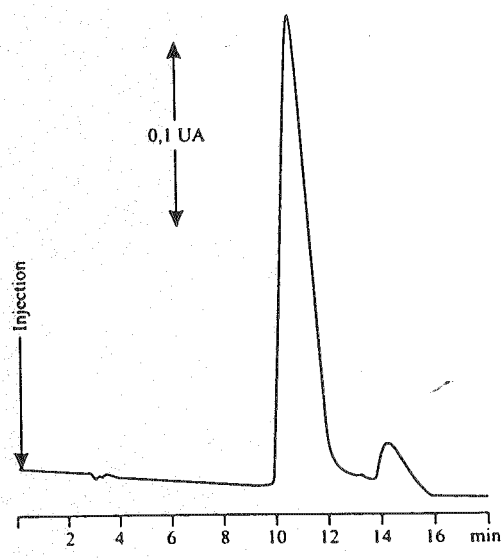

Fig. 3. Chromatogram of quinidine, TEA 1%, pH = 2.5

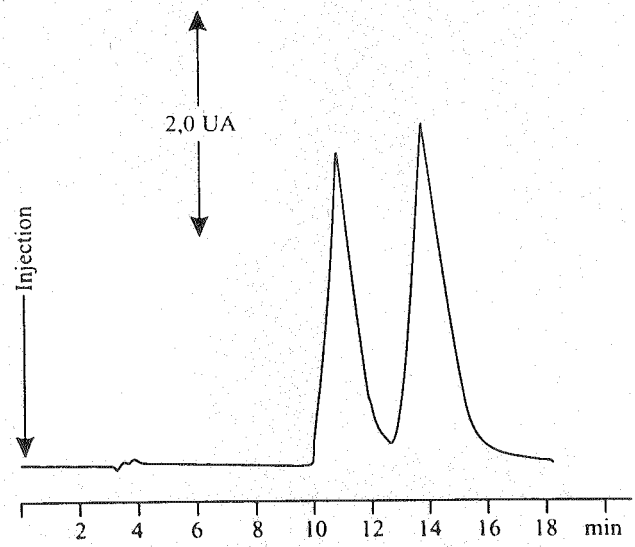

Fig. 4. Chromatogram of separation of quinine and quinidine, TEA 1%, pH = 2.5

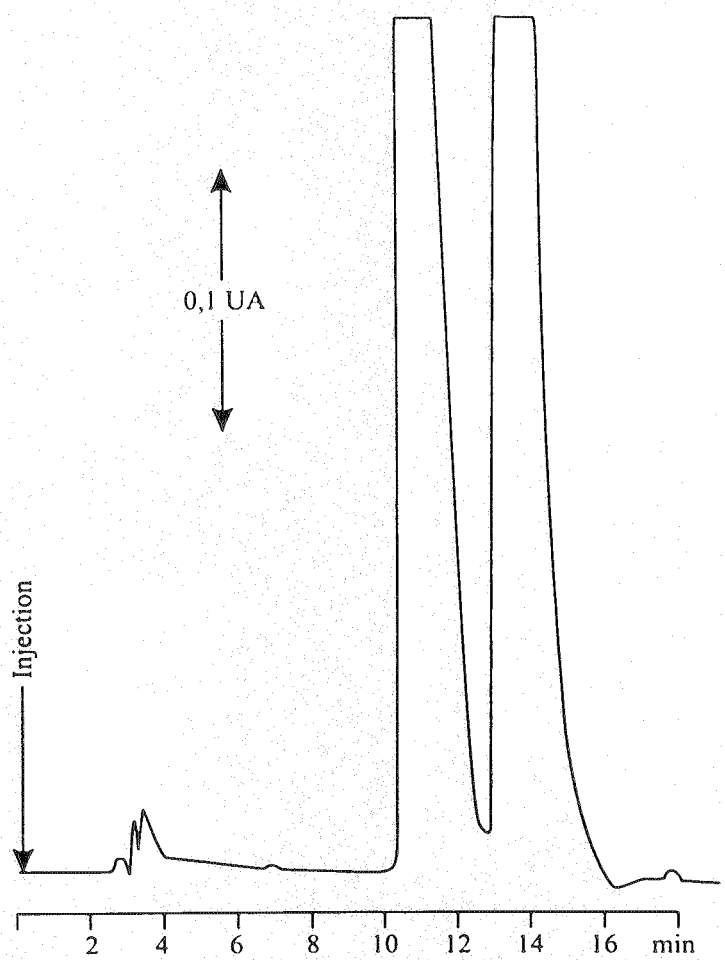

Fig. 5. Chromatogram of separation of quinine and quinidine at high sesitivity, TEA 1%, pH = 2.5

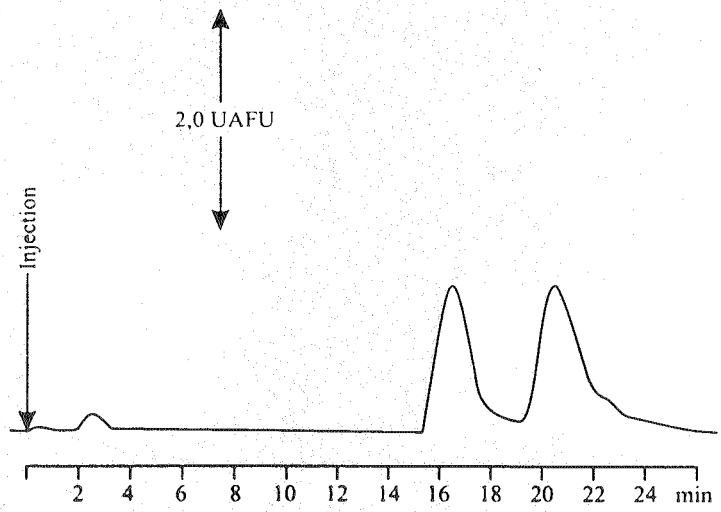

Fig. 6. Chromatogram of separation of quinine and quinidine and the effect of TEA 0.25% and acetic acid 0.75% at pH = 3 (see text)

In this study, pH in the range 2.51–6.06 for both quinine and quinidine was examined and pH 3 was chosen to save column life time. It is reported that peak shape can be improved by adding a small amount of glacial acetic acid and TEA to mobile phase ACN : 50 mM phosphate buffer + 0.75% glacial acetic acid + 0.25% TEA (9 : 91); pH 3 was chosen.

All the optimum conditions were demonstrated on the chromatograms (Figs. 2–6) for quinine and quinidine both singly and as quinine and quinidine mixture.

Linearity over the range 1–100 μg/mL of both quinine and quinidine was examined and determined by plotting the peak heights response *vs.* the concentration of quinine and quinidine and the results showed that the method was linear in accordance with Beer's law over this range and the linearity equations were:

$$Y_Q = 0.309X - 0.44, \quad R = 0.9997 \ (n = 5)$$

$$Y_{QD} = 0.2788X - 0.7225, \quad R = 0.9954 \ (n = 5)$$ (Q = quinine, QD = quinidine).

The reproducibility for replicate injection was good (RSD = 2.35%, n = 10 for quinine at 50 μg/mL and RSD = 1.24%, n = 10 for quinidine at 50 μg/mL).

The absolute limit of detection defined as signal-to-noise of 2 was examined and found to be 5 ng/mL. Reproducibility, which expresses the precision under different conditions, such as different laboratories with different analysts, using separate instrumentation, was also examined and the method was evaluated on the basis of constant retention times obtained.

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(*Received*: 14 April 2005; *Accepted*: 27 February 2006) AJC-4642