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Simultaneous Separation of Quinine and Its Diastereoisomer Quinidine by RP-HPLC

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Reversed-phase high-performance liquid chromatography method was developed for separation of quinine and its diastereoisomer quinidine. The best separation was performed on symmetry C_{18} (150 × 4.6 id, particle size 3.5 µm. Acetonitrile-water-triethylamine-acetic acid 9:90:0.25:0.75 (v/v/v/v) was used as a mobile phase with flow rate 1 mL/min. pH was adjusted to 3.03 with orthophosphoric acid. UV detection was performed at 254 nm. The validation of the method performance characteristics was established to be adequate for intended use .The evaluation of number of parameters ,such as selectivity , linearity was realized with validation manager logician VWR international GmbH, Germany.

Key Words: Symmetry column liquid chromatography, Quinine, Quinidine, Aqueous mobile phase modifier, Triethylamine, Acetic acid.

INTRODUCTION

Cinchona alkaloids are a group of about 35 bases occurring in the bark of *Cinchona* and *Remijia* species which are indigenous to the Andes but have been cultivated commercially in South and Central America, India, Sri Lanka and the East Indies[1,2]. The principal *Cinchona* alkaloids are the quinoline alkaloids quinine, quinidine, cinchonine and cinchonidine. The 'average' bark contains 7-12 % total alkaloids[3], of which quinine accounts for 70-90 %, cinchonidine 1-3 % and quinidine up to 1 %. Bark preparations have been used for the treatment of malaria for centuries and the history of the early uses of quinine and other *Cinchona* alkaloids, together with their isolation and structural determination, have been documented recently[4]. For the last 50 years or so quinine has suffered competition from synthetic antimalarial drugs such as chloroquine. However, resistance of the parasite *Plamodium falciparum* to quinine appears to be significantly less than with chloroquine[5-8], thus quinine is still widely used.

Quinidine, which 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 (3R, 4S, 8S, 9R) is used for the synthesis of quinidine (3R, 4S, 8R, 9S)[3]. An

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important use of the principal alkaloids is their wide application as catalytic reagents in chiral organic synthesis[9,10] or to produce high-performance liquid chromatography (HPLC) stationary phases for chiral separations[11-16].

Reversed-phase high-performance liquid chromatography (RP-HPLC) separations on bonded silica have been by far the most widely used for analysis of the *Cinchona* alkaloids, probably due to their perceived general advantages, including UV transparency and low cost of the typical mobile phases, reproducibility of retention times, compatibility with aqueous samples and high column efficiencies. The application of reversed-phase chromatography to the separation of the alkaloids is not straightforward, however, due to their basic nature and thus possible interaction with silanol groups. Due to steric effects, a considerable number of silanol groups (as many as half[17]) can remain underivatized on bonded RP packings. End-capping procedures, where underivatised silanols are further reacted with a small silylating agent such as trimethylchlorosilane, can remove the influence of some of the more active silanols. However, the total number of reacted silanols is largely unaltered by this procedure[18]. Quinine (Fig. 1) has two basic nitrogen atoms of pK approximately 4.3 and 8.5, (the quinoline nitrogen having the lower basicity) and thus the molecule is likely to be at least partially protonated over the whole pH range of operation of RP columns (pH 2-8). The underivatised column silanol groups have an average pK of 7.1[17] although this average conceals a range of acidities, with some silanols likely to have a pK 3.

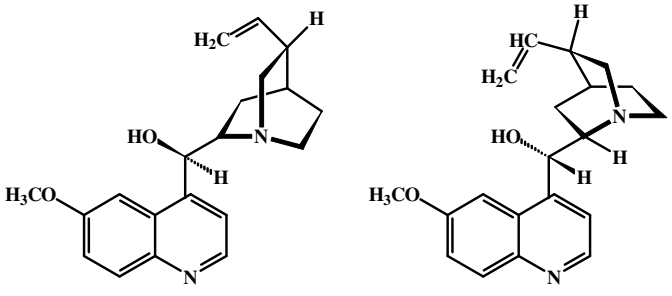

Fig. 1

EXPERIMENTAL

The mobile phases were prepared with water for HPLC (VWR, France), glacial acetic acid 99.9 % triethylamine (Carlo Erba, France). The solvent used acetonitrile was obtained from Carlo Erba, (France). Quinine and quinidine were obtained from Sigma-Aldrich (France).

The powder of quinine and quinidine were dissolved in mobile phase consisting of acetonitrile: 50 mM phosphate buffer + 0.75 % glacial acetic acid + 0.25 % triethylamine (9/ 91); pH adjusted to 3.03 with orthophosphoric acid.

HPLC experiments were performed with a Hitachi-Merck HPLC system which consisted of L-6200 intelligent pump, L-4200 UV-Vis detector, D-2500 Integrator from WVR (Germany), pH meter WTW pH537 Microprocessor

RESULTS AND DISCUSSION

Mobile phase pH is another important consideration, both in terms of optimising peak shape and altering the selectivity of the separation. pH 3 generally gives better peak shapes for basic compounds than pH 7 (accepted as the general high pH stability limit, although silica-based columns claiming stability at considerably higher pH are available). At pH 3, most silanol groups are probably undissociated even though alkaloids are protonated, thus there is limited possibility for ion-exchange effects[19,20].

No systematic comparisons of different buffer salts seem to have been carried out specifically for *Cinchona* alkaloid analysis. In general for basic compounds, potassium seems to be a better choice of buffer cation than sodium, due to peak shape effects caused by the relative strength of ion-exchange interactions[18]; most work has been carried out with potassium phosphate buffers (50 mM KH_2PO_4).

Successful separation of the quinine and quinidine can be obtained even on classical RP-HPLC silicas by incorporation of amines into the mobile phase (1 %, 0.25 % triethylamine and with acetic acid 1 %, 0.75 %) which compete with the analytes for column silanol sites (Fig. 2).

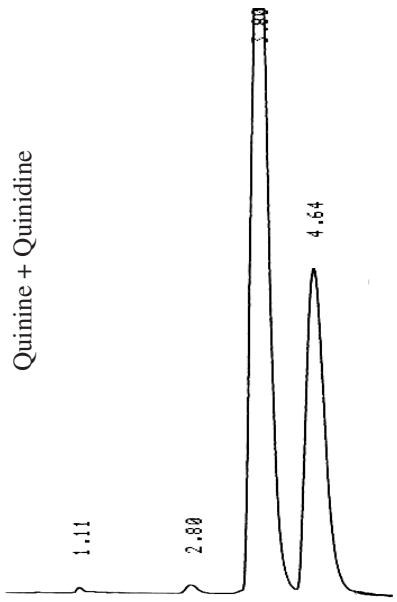

Fig. 2. Analysis of quinine and quinidine. Column C_{18} symmetry (150 × 4.6 ID, 3.5 µm). Mobile phase: acetonitrile/50 mM KH_2PO_4, glacial acetic acid, triethylamine (9/91/0.75/0.25 v/v/v) a pH = 3.03. Detection UV 254 nm, Flow rate 1 mL/min

The validation of the method performance characteristics was established to be adequate for intended use. The evaluation of number of parameters ,such as selectivity, linearity was realized with validation manager logician VWR international GmbH, Germany. Linearity over the range 1-100 μg/mL of both quinine and quinidine was examined and determined by validation manager logician VWR international GmbH, Version: 2.20 serie number: 141000004146VWR International GmH, Germany.

	For quinine:	For quinidine:
Origin:	-4.40000 ± 001	-8.35500 ± 001
Slope:	3.09000 ± 001	2.84617 ± 001
R2:	0.997264	0.993508
RSD:	2.01 %	4.07 %

Conclusion

High-performance liquid chromatography in the reversed-phase mode using UV detection, is still the method of choice for the separation of quinine and quinidine. Good results may be obtained using 'new generation' phases based on pure silicas (symmetry column particle size 3.5 μm) in conjunction with an acidic phosphate buffer (pH 3.03) modified with acetonitrile. Column efficiencies are variable due to the variety of complex solute-stationary phase interactions which can take place.

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