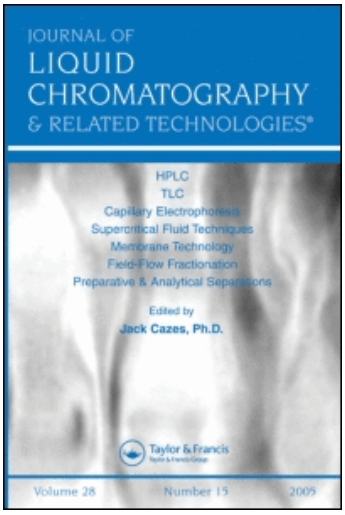

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QUANTITATIVE OVER-PRESSURE LAYER CHROMATOGRAPHY OF QUININE, QUINIDINE, AND HYDROQUINIDINE IN BIOMEDICAL ANALYSIS

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ABSTRACT

An over-pressure layer chromatography (OPLC) method for the separation and quantification of quinine, quinidine and hydroquinidine is discussed in this paper.

After the development with the mobile phase méthanol-acétonitrile-ammonia 0,2M (130 : 68 : 2, v/v), a densitometric detection was performed by the fluorescence emission (excitation wavelength = 348 nm; emission wavelengh = 420 nm). This technique is simple, fast and adapted to small series of determination for the quality control and therapeutic drug monitoring.

1991

INTRODUCTION

Quinine has been used as an anti-malarial drugs since 1633, although its use declined following the introduction of chloroquine in 1934. However, due to the emergence of strains of Plasmodium falciparum malaria resistant to chloroquine and other anti-malarial drugs there has been renewed interest in quinine (1,2,3) -

Cardiac depressants quinidine and hydroquinidine are used widely in the treatment of certain cardiac arrhythmias. Due to the narrow range between effective and toxic concentrations of quinidine (3-5 µg/ml) (4,5 6) there is a need for a rapid, accurate and sensitive

A quantitative method applied in quality control and therapeutic drug monitoring of quinine, quinidine and hydroquinidine is proposed.

Recently, more high-performance liquid chromatographic (HPLC) methods were developed. Separations using adsorption or partition on reversed - phase have been described previously (7,8,9,10,11). Among these methods one was of particular interest (11), being carried out on silica gel.

We attempted to transpose this method to over-pressure layer chromatography (OPLC) - OPLC is an new technique of planar chromatography (12, 13, 14, 15) using a pressurized ultramicro

The solvent layer is completely covered by a membrane under external pressure, so that the vapour phase above the layer is virtually eliminated. Solvent is admitted under pressure by means of

The advantages of OPLC are particularly pronounced shorter migration times and higher separation efficiencies in the case of longer separation distances. In addition, it is possible to adjust the flow rates of the eluents for coping with special separation problems. It was also observed that the velocity of the moving phase is very

This method allows to determine quinine using hydroquinidine as internal standard or to quantify hydroquinidine using quinine or quinidine as internal standard. A fluorimetric detection was conducted after the separation by OPLC.

EXPERIMENTAL

Apparatus.

Samples were automatically streaked with the Linomat IV (Camag, Muttenz, Switzerland).

The over-pressure layer chromatography was carried out with the Chrompres - 25 (Labor MIM, Budapest, Hungary). For qualitative and quantitative evaluations, a densitometer CD- 60 (Desaga, Heidelberg, FRG) was used. Measurements were carried out by UV absorbance at 325 nm and by fluorimetry (λ excitation = 348 nm and λ emission = 420nm).

Materials an Methods

HPTLC aluminium sheets silica gel 60 (without fluorescent indicator pre-coated), 20 x 20 cm, 0.25 mm thick, were supplied by Merk (Darmstadt, FRG). All solvents and reagents were of analytical-reagent grade. The mobile phase was methanol-acetonitrile-ammonia 0.2 M (130 : 68 : 2 , v/v).
After sonication (30 min), the eluent was filtered on 0.45 µm membrane HVLP - Millipore (Saint-Quentin en Yvelines, France).

Quinine, Quinidine were purchased as sulfate salts from Sigma (Saint Louis, MO, USA) and hydroquinidine as hydrochloride salt from Roussel Uclaf (Romainville, France).

Standard Solutions

Solutions of quinine, quinidine and hydroquinidine were prepared in methanol to give concentrations of 4,5,7 and 10 mg. l^{-1}.

Preparation of plasma samples.

In a measuring tube (5 ml) 0.1 ml of plasma, 0.15 ml of methanol, 0.1 ml of 2 M sodium hydroxide and 1.5 ml of a mixture chloroform-isoamyl alcohol (92 : 2,v/v) were introduced.
Treated plasma were then vortex mixed 1 min. and spundowned at 3700 g. The organic phase was dried on anhydrous sodium sulfate and evaporeted. The residue was reconstituted with 0.1 ml of mobile

Chromatography

Before development, a pre-run was conducted with hexane in order to limit the adverse effects of the " disturbing zone " on OPLC (16).
The instrumental conditions were as follow : flow rate, 0.30 ml. min^{-1}; run time, 60 min ; membrane pressure, 25 bars ; inlet pressure, 5 bars.

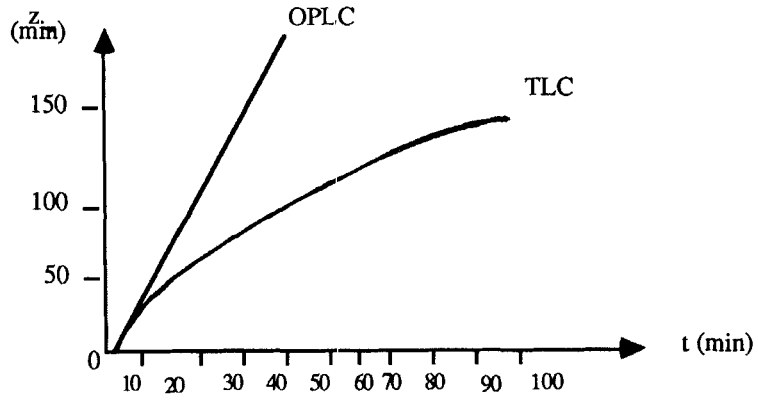


Figure 1:Range of migration of the mobile phase front as a function of time

Densitometric measures

A revelation by spraying phosphoric acid was useful before quantitative analysis. The densitometric detection was performed by measuring the UV absorbance at 325 nm and the fluorescence emission. Fluorescence measurements were carried out using an excitation wavelengh of 348 nm and an emision filter with a cut-off

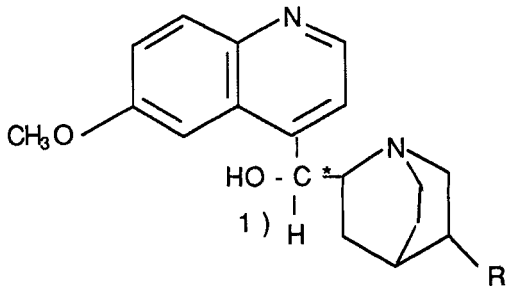
RESULTS AND DISCUSSION

Characteristics of the OPLC method.

The specific properties of OPLC chambers are the result of the elimination of solvent vapours from over the solvent, allowing one to conduct chromatography in a closed system with controlled parameters and with the possibility of influencing the flow rate of the The migration is linear ; the quadratic law of TLC development defined by the equation $Z^2 = kt$ is not valid (Z = distance ; t = time) The migration of the solvent front is described by a simple equation : $Z = kt$ where the velocity constant (k) is a function of the flow velocity of mobile phase, the quality of the sorbent and the dimensions of the bed particles . (Fig. 1).

Table I

Structure of Studied compounds



		R
1)	---QUININE	$CH=CH_2$
	QUINIDINE	$CH=CH_2$
	HYDROQUINIDINE	C_2H_5

This property allows us to separate quinine, quinidine and hydroquinidine with a one hour continuous migration. A separation on 20 cm takes 30 min , so in one hour the development was carried out on 40 cm .

The Rf values of the studied compounds (table I) were 0.14 for hydroquinidine, 0.26 for quinidine and 0.29 for quinine. The elution order of these compounds is identical by OPLC and by HPLC technique (11) . We cannot separate quinine and quinidine by 20 cm development with the advocated eluent solvent. Indeed the migration distances for 20 cm development were calculated and are 2.8 cm for hydroquinidine, 5.25 cm for quinidine and 5.75 cm for quinine.

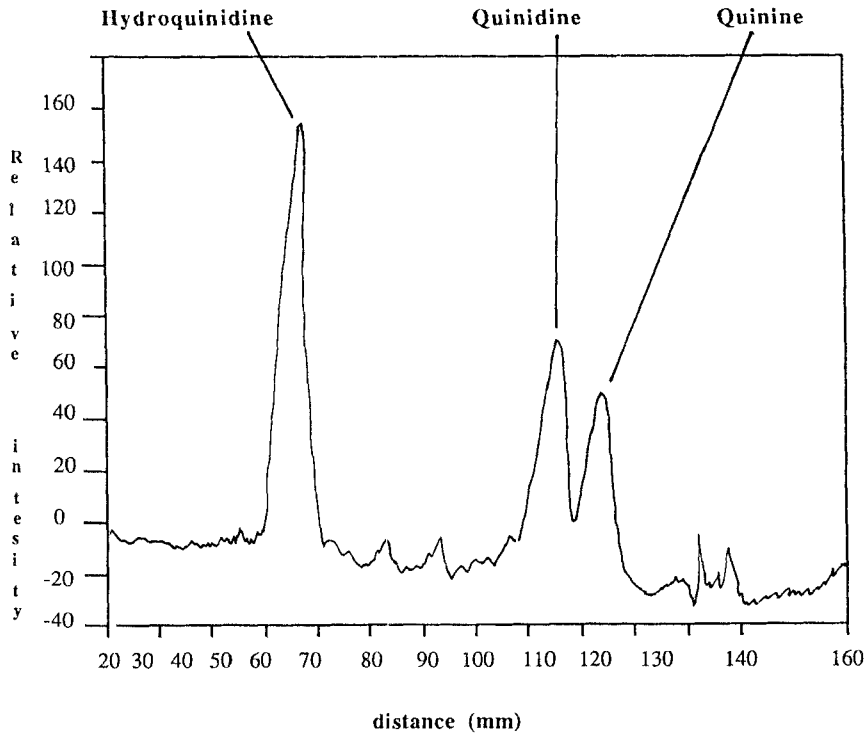
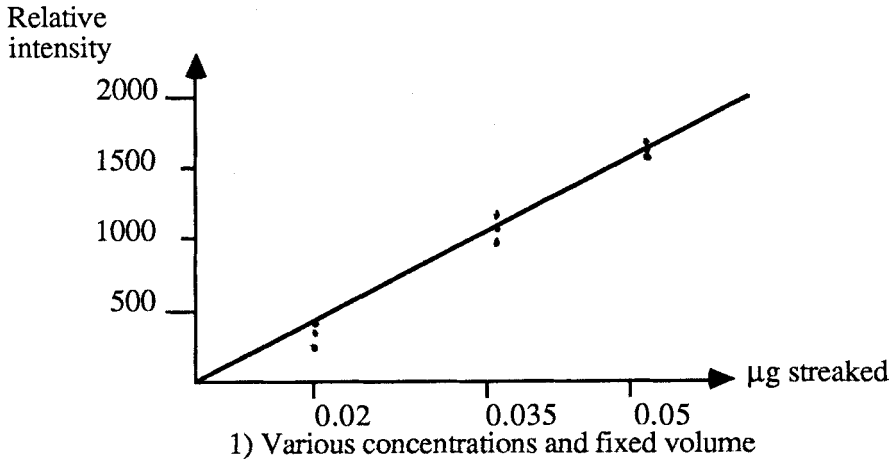
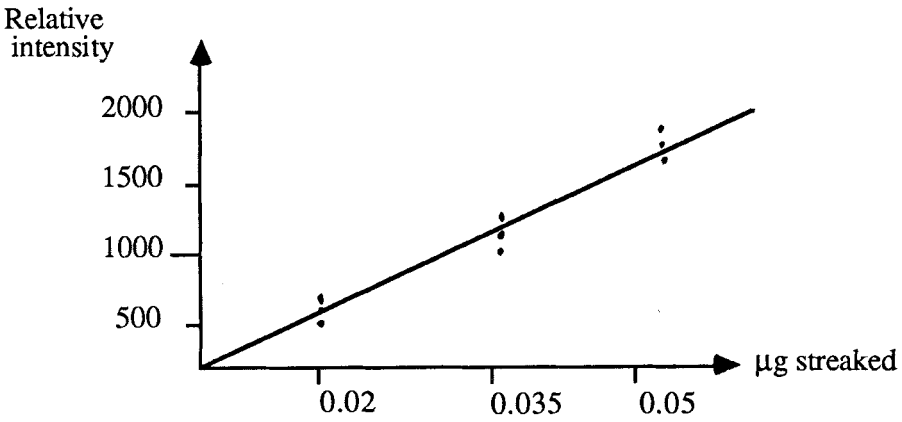


FIGURE 2. Chromatogram of a mixture of quinine, quinidine, and hydroquinidine after fluorimetric detection (40 cm development) 20 ng applied.

The maximum resolution is occured at the flow-rate at which the number of theoretical plates is the greatest. The optimal efficiency or the minimum plate height was obtained at a flow-rate of the solvent of 0.30 ml/min. When the flow-rate was higher than this value, the eluent pomp of the Chrompres-25 undergoes an excessive pressure on account of viscosity and nature of the eluent solvent ; so development must be interrupted. When the flow-rate was lower than 0.30 ml/min the run time was increased but the quality of chromatography was not improved. An inlet pressure of about 5 bars allowed to avoid the demixing problems. Chromatogram obtained after densitometric measurement in fluorescence mode is shown on Fig 2.



1) Various concentrations and fixed volume



2) Changing volumes and fixed concentration

Figure 3

Calibration curves

Determination of compounds .

- Study of the linearity : The linearity has been studied by streaking various concentrations (4 mg. l^{-1}, 7 mg. l^{-1}) of quinine solutions at fixed volume (5 µl) and by applying changing volumes (4 µl, 7 µl, 10 µl) of a fixed concentration (5 mg. l^{-1}) solution. Fluorescence emission detections were determined. It appears that the first method with fixed volume gives best results (r = 0.99729 and F^2_8 = 0.1010). The method with fixed concentration and changing volumes gives a r value = 0.99280 and a F^2_8 value = 0.2498. But for both systems the hypothesis of linearity is acceptable (p = 95 %).
Calibration curves are given Fig. 3.

- Precision and accuracy : Repeatibility tests were carried out by applying ten loadings (6.0 µl each) of hydroquinidine (5 mg. l^{-1} solution) to each HPTLC plate. Measurements were performed by fluorimetric detection.The coefficient of variation for each plate varied from 5 % to 10 %.

The recovery studies of quinine , quinidine and hydroquinidine were carried out by spiking drug-free plasma with a known amount of quinine, quinidine and hydroquinidine. The results demonstrated that

- Study of the detection limit : Calibration curves with standard solutions of quinine, quinidine and hydroquinidine have been carried out by UV absorption at 325 nm and by fluorimetric detection (λ excitation = 348 nm and λ emission = 420 nm). With UV absorption detection, the limit of detection is 0. 1 µg streaked or 1 µg/ml of plasma for quinine and quinidine and 0.05 µg streaked or 0.5 µg/ml of plasma for hydroquinidine.
The fluorescence detection is approximately ten times more sensitive than UV absorption detection .
For quinine and quinidine, the limit of detection is 0.01 µg streaked or 0.1 µg/ml of plasma and for hydroquinidine it is 5 ng streaked or 0.05 µg/ ml of plasma.

This OPLC technique presents the advantage to be simple, fast and adapted to small series of determination. It allows identification and quantification of quinine, quinidine and hydroquinidine for the

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