Journal of Chromatography, 149 (1978) 331–340
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CHROM. 10,832

LIQUID CHROMATOGRAPHY IN THE MONITORING OF PLASMA LEVELS OF ANTIARRHYTHMIC DRUGS

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SUMMARY

High-performance liquid chromatography has been employed in the development of assay methods for six antiarrhythmic drugs, disopyramide, lidocaine, tocainide, procainamide, aprinidine and quinidine. Liquid–solid chromatography has been used and separation times of about 5 min have usually been sufficient. Owing to the capacity of the liquid chromatographic systems, sample preparation has been minimized to a single extraction and direct injection of a considerable part of the extract. The overall time of analysis is very short and the methods are well suited for monitoring of plasma levels of the antiarrhythmic drugs and in some instances (procainamide and disopyramide) also for their main metabolites. UV detection at the optimal wavelength has permitted determinations down to 50 pmole (20 ng) in 1 ml of plasma for the amines with high absorbance.

INTRODUCTION

Many antiarrhythmic drugs have a small therapeutic index and control of the concentration levels in blood plasma is often required.

Chromatographic methods of determination offering sufficient sensitivity and selectivity have been presented in the last 5 years as alternatives to the methods previously employed for antiarrhythmic drugs, usually based on photometric or fluorimetric techniques. Gas chromatographic methods have been applied to disopyramide[1], lidocaine[2], procainamide[3], aprinidine[4] and quinidine[5] and in the recent time liquid chromatography has been applied to the assay of disopyramide[6], lidocaine[7], tocainide[8], procainamide[9–11] and quinidine[12]. In the chromatographic methods presented so far, the sample preparation seems to be too tedious in many instances, which increases the time of analysis unnecessarily.

This paper demonstrates how high-performance liquid chromatography can be applied as a simple but powerful technique in the monitoring of plasma levels for six antiarrhythmic drugs (Fig. 1), disopyramide, lidocaine, tocainide, procainamide, aprinidine and quinidine. In two instances the main metabolites, N-deisopropyldisopyramide and N-acetylprocainamide, were also of interest and they could be assayed simultaneously with the parent drug. The simplicity of the liquid chromatographic methods has been of most concern. The sample preparation is restricted to a

single extraction followed by injection of an aliquot of the extract without any preceding evaporation of solvent. The separation times in the chromatographic systems used, are in many instances only about 5 min, contributing to the short times of analysis, which are of utmost importance in the monitoring of drug plasma levels. Strongly absorbing compounds have been determined down to 50 pmole/ml (20 ng/ml) by means of UV detection.

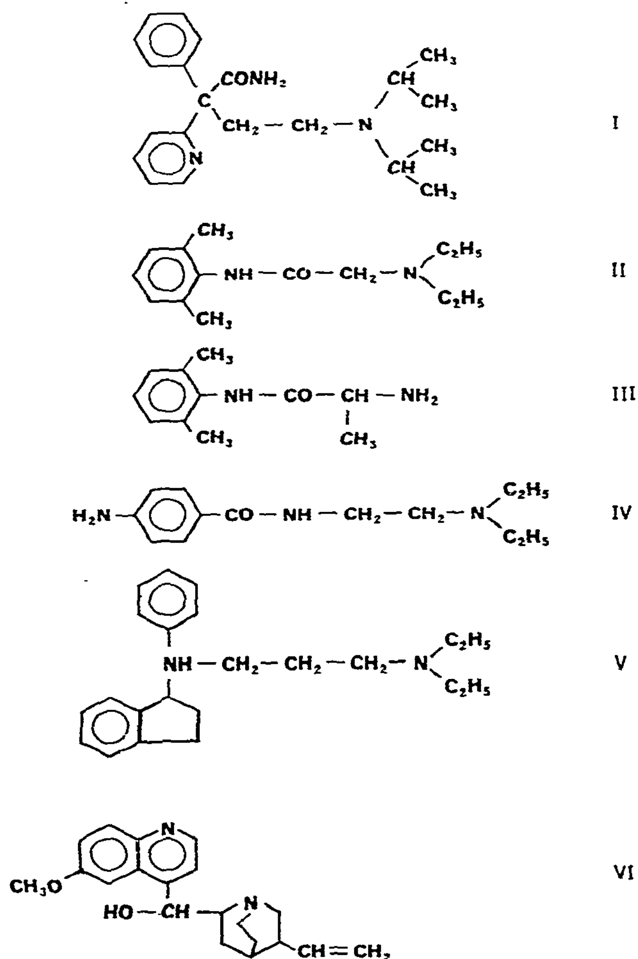

Fig. 1. Chemical structures of antiarrhythmic compounds. I, Disopyramide; II, lidocaine; III, tocainide; IV, procainamide; V, aprinidine; VI, quinidine.

EXPERIMENTAL

Chromatographic apparatus

The liquid chromatograph was a Milton Roy (Philadelphia, Pa., U.S.A.) minipump with a pulse damper (Laboratory Data Control, Riviera Beach, Fla., U.S.A.; 711-47) and a Cecil 212 (Cambridge, Great Britain) UV spectrophotometer

with an 8-μl flow cell or a Waters 440 (Waters Assoc., Milford, Mass., U.S.A.) UV detector. The injector was a Rheodyne (Berkely, Calif., U.S.A.; 70-10) with 20–250-μl loops. The separation column of precision-bore stainless steel (length 150 mm, O.D. 6.35 mm, I.D. 4.5 mm) had end fittings of modified Swagelok connections. Room temperature was used.

Chemicals and packing material

Methylene chloride, 1,2-dichloroethane, 1-butanol, 2-propanol, methanol and 25% ammonia solution (pro analysi grade, E. Merck, Darmstadt, G.F.R.) were used.

All reagent and buffer solutions were prepared with analytical-reagent grade chemicals.

Disopyramide and N-deisopropyldisopyramide (phosphate salts) were supplied by Searle Labs. (Chicago, Ill., U.S.A.).

The drug compounds fulfilled the quality requirements of the Pharmacopoeia Nordica.

The silica particles used in the separation columns were LiChrosorb SI 60 (average diameter 7 μm) (Merck), LiChrosorb SI 100 (10 μm) and Partisil 5 (5 μm) (H. Reeve Angel, Clifton, N.J., U.S.A.). The choice of packing material for the different applications was not based on any comparative studies and was not regarded as important.

Column packing and equilibration

The separation column was packed with 5-, 7- or 10-μm microporous silica particles by the balanced-density slurry technique[13].

The mobile phase, usually at least 50–100 ml, was passed through the system until a constant capacity factor was obtained. The retention time of an unretained compound (t_0) was determined by injection of toluene dissolved in mobile phase. The capacity factor, k' was defined as $(t_R - t_0)\, t_0^{-1}$, where t_R is the retention time of the solute in question.

Determination of distribution constants

The distribution constant (K_D) for each amine between methylene chloride and water was determined by equilibration in centrifuge tubes. As the aqueous phase either sodium hydroxide solution (0.1 M) or phosphate buffer solutions, depending on the distribution properties, were used. After phase separation by centrifugation, the concentration of the amines were determined spectrophotometrically in both phases. The results are given in Table I.

Analytical procedure

The general scheme for the analytical procedures for the six antiarrhythmic drugs in plasma is: (1) A 1-ml volume of plasma (200 μl for procainamide) in a centrifuge tube is made alkaline with 100 μl of sodium hydroxide solution (1 M) —carbonate buffer for aprinidine— and extracted with 1 ml of methylene chloride for 5–10 min (60 min for aprinidine). (2) After centrifugation for 10 min, 250 μl of the organic extract is injected into the chromatographic column.

The chromatographic procedure for each compound is summarized in Table I, together with the distribution constants, recoveries and minimum determinable concentration of the drugs (the level where the standard deviation is $\leqslant$10%).

TABLE I

ANALYTICAL PROCEDURES

Sample: 1 ml of plasma; procainamide, 200 μl. pH at extraction: 13; aprinidine, pH 9. Extraction agent: 1 ml of methylene chloride. Extraction time: 10 min; aprinidine, 60 min. Injection volume: 250 μl of the methylene chloride extract. Chromatographic system: liquid–solid chromatography.

Drug	*Mobile phase component*	*Proportion (v/v)*	*Wavelength (nm)*	*Log K_D**	*Recovery (%)*	*Level (pmole/ml) (S.D. ≤ 10%)*	*Chromatogram shown in Fig. No.*
Disopyramide	$HClO_4$ (1 *M* aq.)	1	265	3.55	100	75	5
N-Deisopropyl-disopyramide	Methanol	9		1.78	100	75	
	Dichloroethane	90					
Lidocaine	$HClO_4$ (1 *M* aq.)	0.3	228	3.50**	100	200	6
	Methanol	4					
	Dichloroethane	95.7					
Tocainide	$HClO_4$ (1 *M* aq.)	0.5	230	0.90	90	250	7
	Methanol	10					
	Dichloroethane	89.5					
Procainamide	Methanol	10	280	0.84	90	200	8
N-Acetyl-procainamide	CH_2Cl_2	20		0.42	90	200	
	CH_2Cl_2 (NH_3)	70					
Aprinidine	NH_4Ac (1 *M* aq.)	1.5	254	≥ 6	100	100	9
	NH_3 (1 *M* aq.)	3.5					
	Methanol	95					
Quinidine***	1-Butanol	10	254	2.00**	100	50	ref. 12
	CH_2Cl_2	70					
	n-Hexane	20					

* Distribution constant; organic phase: methylene chloride.

** Taken from ref. 14.

*** Liquid–liquid chromatographic system; stationary phase: $HClO_4$ (0.2 *M*) + $NaClO_4$ (0.8 *M*); taken from ref. 12.

Quantitative evaluation

In the routine analysis of drug levels in plasma samples, peak heights were measured and the concentrations were obtained from standard graphs constructed by analysing plasma samples spiked with known amounts of the drug. A standard graph was prepared daily. The use of an internal standard was not considered to be necessary for the accuracy and precision of the methods, owing to the normally very simple analytical procedures. The overall time of analysis is thereby reduced.

RESULTS AND DISCUSSION

Sample preparation

In the development of analytical methods for monitoring plasma levels of antiarrhythmic drugs, special care was taken to facilitate the preparation of the samples before the chromatographic separation. From experience, it is obvious that after the introduction of high-performance liquid chromatography the extent of sample preparation such as extraction, back-extraction, re-extraction and evapora-

tion will be decesive for the through-put rather than the chromatographic separation time. This is at least valid when the requirement of the analysis is limited to the plasma concentration of a single drug and in some instances its main metabolite.

Methylene chloride was used as the organic solvent in the extraction, in the methods presented in Table I, as it exhibits a good extraction ability towards amines. The time needed for maximum extraction is often not critical and 5–10 min are generally sufficient. We reported earlier that extended extraction times may be necessary for the quantitative transfer of tricyclic lipophilic amines[15]. Aprinidine showed a similar behaviour and an extraction time of 60 min was required. Further, the rate of extraction was pH dependent and was higher at pH 9 than at pH 12.5 (Fig. 2). This may be due to different degrees of protein precipitation and interactions with the lipoprotein fraction in the plasma. Aprinidine is a very lipophilic compound ($K_D \geqslant 10^6$) and strong binding of compounds of similar character to lipoproteins has been reported earlier[16].

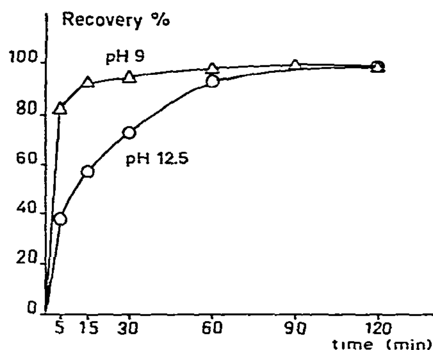

Fig. 2. Extraction rate of aprinidine from plasma samples, measured by liquid chromatography. The revovery was obtained by comparison with an injected standard of aprinidine dissolved in methylene chloride.

Concentration of the organic extract was not necessary in the recommended methods (Table I). Evaporation procedures, in order to increase the sensitivity, are commonly included in bioanalytical methods for drugs, although losses by evaporation of the drug, degradation and adsorption on glass walls may occur[11,15]. Injection of large volumes ($\geqslant$100 μl) of a solvent with properties different to those of the mobile phase into an analytical column may be hazardous and cause peak distortion. In most of the separation systems used in this study, methylene chloride is present as a major component of the mobile phase and, generally peak distortion was not a problem. Interference by negative peaks in the chromatograms caused by changes in absorption or refractive index of the eluent had to be taken into account in a few instances in the choice of chromatographic conditions. The reason for these effects may be differences in the equilibrium water content of the mobile phase and the injected methylene chloride extract.

Chromatographic systems

In liquid chromatography on regular silica, amines can be retained either in

charged form, as ion-pairs, or in uncharged form as bases. Both kinds of separation systems have been used in this paper.

LSC systems with an ion-pairing agent in the mobile phase have frequently been employed as a useful alternative to the regular ion-pair LLC[12,15] in the separation of amines. The separation mode probably involves both adsorption and liquid–liquid distribution of the ion-pairs.

Aqueous perchloric acid was used in this study as the acidifying and ion-pairing agent dissolved in mixtures of chloroalkanes and alcohols as mobile phases. The regulation of the retention in such a separation system by variation of the concentration of perchloric acid and alcohol in the mobile phase was investigated.

In ion-pair LLC, the capacity factor is inversely proportional to the counter-ion concentration in the aqueous phase[17], while in this LSC system an increase of the perchloric acid concentration by a factor 50 gave a decrease of only 2.5-fold in the k' value of lidocaine (Fig. 3). From the same diagram, it appears that for disopyramide and its N-deisopropylderivative there is even an increase in the capacity factors with increasing perchloric acid concentration. These two amines are divalent with $pK'_{H_2A^{+2}} = 2.1$ (in water) and there is probably an increasing proportion of the amines present in divalent form as the perchloric acid concentration increases. A further indication of this is continuous changes in the response from the UV detector due to variation in the proportions between the divalent and monovalent form of the ammonium ion migrating in the mobile phase (Table II).

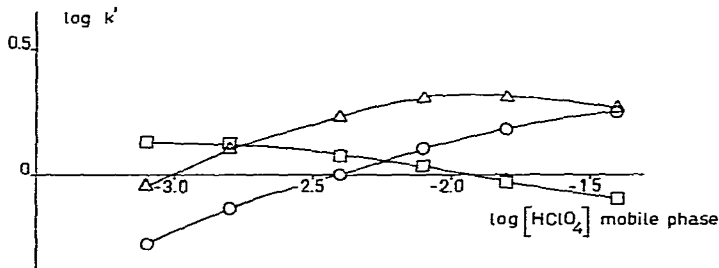

Fig. 3. Capacity factors (k') for the divalent amines disopyramide and N-deisopropyldisopyramide and for the monovalent amine lidocaine in an LSC system. Packing material: LiChrosorb SI 60, 7 μm. Mobile phase: water + 2-propanol + dichloroethane (0.8 + 5 + 84.2, v/v) (+ $HClO_4$ dissolved in the mobile phase). ○, Disopyramide; △, N-deisopropyldisopyramide; □, lidocaine.

TABLE II

MOLAR ABSORPTIVITY OF DISOPYRAMIDE IN MOBILE ORGANIC PHASES WITH DIFFERENT CONCENTRATIONS OF PERCHLORIC ACID

Mobile phase: $HClO_4$ in mixtures of water + 2-propanol + $C_2H_4Cl_2$ (0.8 + 15 + 84.2, v/v).

$HClO_4$ concentration ($\cdot 10^3$) (M)	*Molar absorbance ($\cdot 10^{-3}$)*
0.8	3.9
4	4.1
16	5.0
100	6.4

As the concentration of perchloric acid in the mobile phase had a rather limited effect on the retention, the influence of the alcohol concentration was considerable (Fig. 4). If the 2-propanol concentration in the mobile phase was increased from 5 to 10%, the capacity factor was decreased about 6-fold.

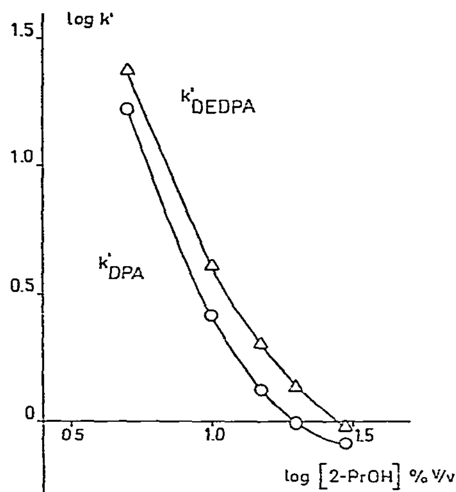

Fig. 4. Capacity factors (k') of disopyramide (DPA) and its N-deisopropyl metabolite (DEDPA) with varying concentration of 2-propanol in an LSC system. Packing material: LiChrosorb SI 60, 7 μm. Mobile phase: 0.2% of 1 M aqueous $HClO_4$ in a mixture of 2-propanol + dichloroethane.

The nature of the alcohol component was found to influence both the column efficiency and the separation factor for the pair disopyramide and its N-deisopropyl derivative. In equimolar concentrations, methanol was superior in both respects while the values for 2-propanol and 1-butanol were almost identical (Table III).

TABLE III

SEPARATION FACTOR (α) AND PLATE NUMBER (N) IN A LIQUID–SOLID SYSTEM WITH DIFFERENT ALCOHOLS OF EQUIMOLAR CONCENTRATION (2 M) IN THE MOBILE PHASE

Packing material: LiChrosorb SI 60, 7 μm. Mobile phase: 0.4% $HClO_4$ (1 M, aq.) in mixtures of alcohol and dichloroethane. Sample: 20 μl of a solution of disopyramide (DPA) and N-deisopropyldisopyramide (DEDPA). $\alpha = k'_{DEDPA}/k'_{DPA}$.

Alcohol	α	N
Methanol	2.3	3300
2-Propanol	1.4	1100
1-Butanol	1.4	1100

These kinds of LSC systems have appeared to be very useful in the routine monitoring of drug plasma levels and have been employed in the methods for determination of disopyramide and its N-deisopropyl metabolite, lidocaine and tocainide in plasma as shown in Figs. 5–7.

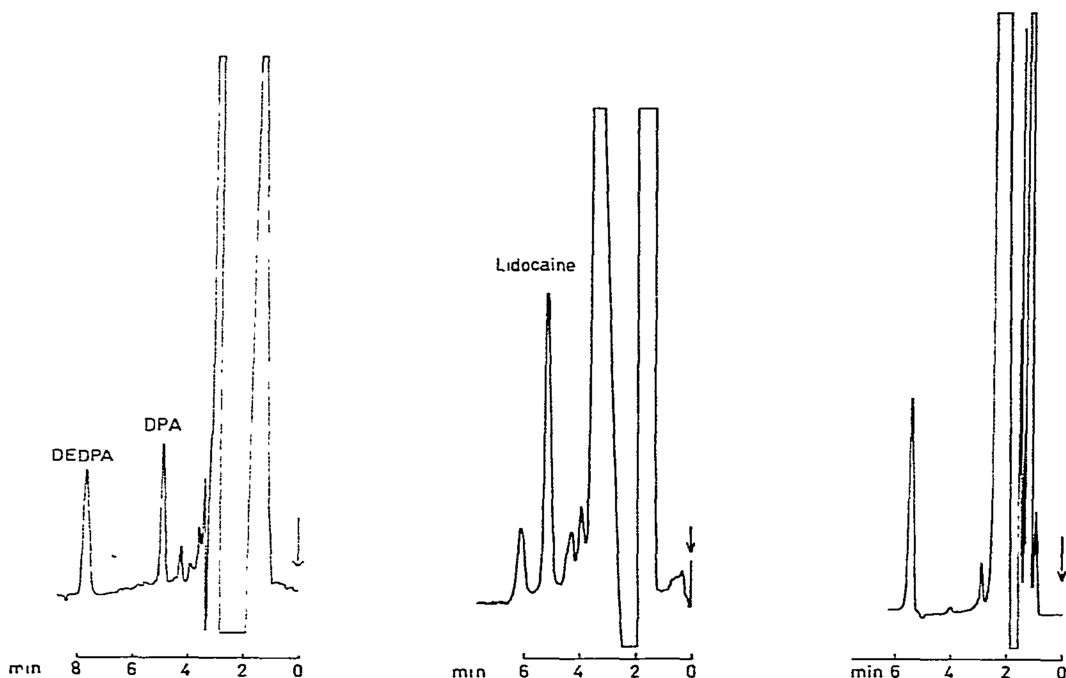

Fig. 5. Disopyramide (DPA) and its N-deisopropyl metabolite (DEDPA) from a plasma sample (LSC system). Packing material: LiChrosorb SI 60, 7 μm. Mobile phase: 1 *M* (aq.) $HClO_4$ + methanol + dichloroethane (1 + 9 + 90, v/v). Mobile phase flow-rate: 1 ml/min. Wavelength: 265 nm. Sensitivity: 0.01 a.u.f.s. Sample: 250 μl of an extract from 1 ml of plasma spiked with 250 pmole/ml (90 ng/ml) of each amine.

Fig. 6. Lidocaine in a plasma sample. Packing material: LiChrosorb SI 100, 10 μm. Mobile phase: 1 *M* (aq.) $HClO_4$ + methanol + dichloroethane (0.3 + 4 + 95.7, v/v). Mobile phase flow-rate: 1 ml/min. Wavelength: 228 nm. Sensitivity: 0.01 a.u.f.s. Sample: 250 μl of an extract from 1 ml of plasma spiked with 1.7 nmole/ml (400 ng/ml) of lidocaine.

Fig. 7. Tocainide in a plasma sample. Packing material: Partisil 5. Mobile phase: 1 *M* (aq.) $HClO_4$ + methanol + dichloroethane (0.5 + 10 + 89.5, v/v). Mobile phase flow-rate: 1.0 ml/min. Wavelength: 230 nm. Sensitivity: 0.05 a.u.f.s. Sample: 150 μl of an extract from 1 ml of plasma spiked with 8 nmole/ml (1.5 μg/ml) of tocainide.

The LSC of amines in the straight-phase mode usually utilizes mobile phases containing a base in order to improve the chromatographic behaviour of the solutes. Such a separation system was employed in the separation of procainamide and its N-acetyl metabolite in plasma samples (Fig. 8). The ammonia solution in the mobile phase can be replaced with an aliphatic amine. A lower column efficiency, however, was then obtained, which is in accordance with observations made in a recent report on the separation of tricyclic amines in reversed-phase chromatography[18].

Aprinidine could be well separated in extracts from plasma samples by LSC using perchloric acid present in the mobile phase. There is, however, a decrease in the detector response of about 6-fold on going from the base form to the ammonium perchlorate form and sufficient sensitivity could not be achieved. Even non-polar mobile phases containing an aliphatic amine gave capacity factors that were too low to permit

separation from co-extracted sample components present in the front of the chromatogram.

The chromatographic system selected for aprinidine (Table I, Fig. 9) was based on suggestions by Wheals[19], who used very polar mobile phases with a major proportion of methanol in aqueous buffer solutions for the separation of amines on silica. In such a chromatographic system, it may be questioned if the silica packing material is still the most polar phase or if there is a reversed-phase separation mechanism. Despite the different character of the mobile phase in this separation system, the methylene chloride extract could be injected into the column without any significant disturbances. A chromatogram from a plasma sample is presented in Fig. 9.

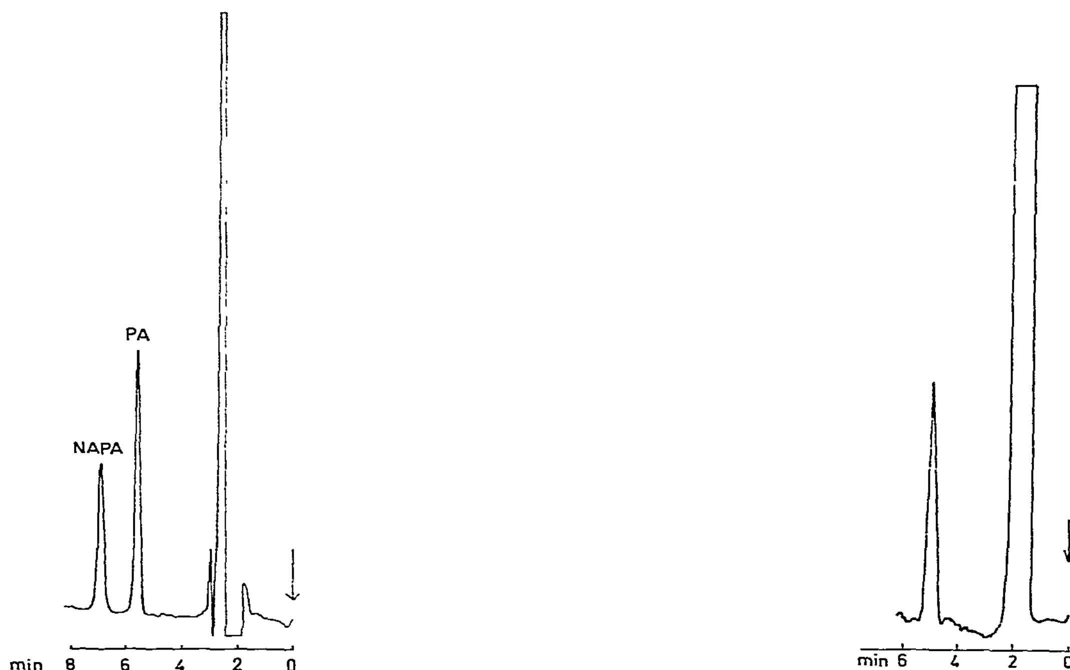

Fig. 8. Procainamide (PA) and N-acetylprocainamide (NAPA) from a plasma sample. Packing material: Partisil 5. Mobile phase: methanol + CH_2Cl_2 (saturated with 25% NH_3 solution) + CH_2Cl_2 (10 + 70 + 20, v/v). Mobile phase flow-rate: 1 ml/min. Wavelength: 280 nm. Sensitivity: 0.05 a.u.f.s. Sample: 250 μl of an extract from 200 μl of plasma spiked with 6 nmole/ml (1.5 μg) of procainamide and N-acetylprocainamide.

Fig. 9. Aprinidine in a plasma sample. Packing material: LiChrosorb SI 60, 7 μm. Mobile phase: 1 *M* (aq.) NH_4Ac + 1 *M* (aq.) NH_3 + methanol (1.5 + 3.5 + 95, v/v). Mobile phase flow-rate: 1 ml/min. Wavelength: 254 nm. Sensitivity: 0.02 a.u.f.s. Sample: 150 μl of an extract from 1 ml of plasma spiked with 1.5 nmole/ml (500 ng/ml) of aprinidine.

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