International Journal of Pharmaceutics, 2 (1979) 155–166

SEPARATION, IDENTIFICATION AND QUANTITATIVE ANALYSIS OF SUBSTITUTED BENZAMIDES IN PHARMACEUTICAL DOSAGE FORMS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY

N. VERBIESE-GENARD, M. HANOCQ, M. van DAMME and L. MOLLE

Laboratoire de Chimie analytique et de Toxicologie, Institut de Pharmacie, Université libre de Bruxelles C.P., 205/1 Campus Plaine, B-1050 Bruxelles (Belgium)

(Received January 13th, 1978)
(Revised version received June 12th, 1978)
(Accepted June 19th, 1978)

SUMMARY

Reverse phase high pressure liquid chromatography has been used to separate, identify and analyse five closely structurally related benzamides: procainamide, sultopride, tiapride, sulpiride and metoclopramide. The influence of various parameters on the separation (solvent composition, pH, type and concentration of the anion modifying the ionic strength of the buffer) has been examined. The results of this study suggest that the separation mode is similar to an ion pair partition chromatography in which the nature of the counter-ion is essential. The method has been applied successfully to the quantitative analysis of some of the above mentioned compounds in pharmaceutical dosage forms.

INTRODUCTION

Until now, the molecules of the substituted benzamide series used in therapeutics have not been adequately studied from the analytical viewpoint. With the exception of Pitel and Luce's studies (1965, 1970) on the spectrophotometric determination of metoclopramide and sulpiride, procainamide has been mostly studied.

Among the proposed methods used to analyze this compound, there are colorimetric techniques based on the well-known Bratton et al.'s reaction (1939), ultraviolet spectrophotometric determination after an organic extraction with an appropriate solvent (Koch-Weser and Klein, 1969; Hamm, 1976), spectrofluorimetric techniques (Stenart and Lotti, 1970) commonly used by Klein and Koch-Weser (1971), partition gas chromatography using ionization flame detection (Atkinson et al., 1972; Frigerio and Pantarotto, 1977) and, more recently, methods utilizing high pressure liquid chromatography. In this latter, analysis was carried out on a C18 reverse phase column using acetonitrile (Adams et al., 1976; Carr et al., 1976; Shukur et al., 1977) or methanol (Rocco et al., 1977) with

sodium acetate buffer (Carr et al., 1976; Rocco et al., 1977; Shukur et al., 1977) or an hydrogen–dihydrogen phosphate buffer (Adams et al., 1976) as solvent. In the present work, a similar technique has been used and has been adapted to the separation, identification and quantitative determination not only of procainamide but also sulpiride, sultopride, tiapride and metoclopramide in pharmaceutical dosage forms.

MATERIALS AND METHODS

Reagents

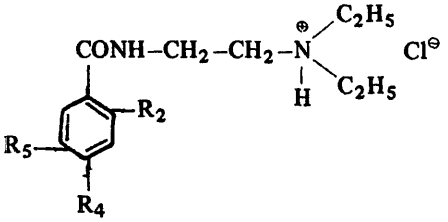
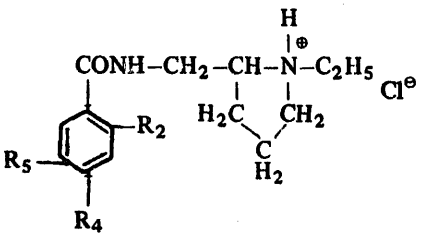
The structures of the five derivatives studied are described in Table 1; these products were purified as hydrochlorides following a technique described elsewhere (Topart et al., 1973). All the reagents were of analytical grade (Merck). The chromatographic mobile phase was a mixture of acetonitrile (Merck-Uvasol) 10% v/v and a phosphate buffer, dipotassium hydrogenphosphate–potassium dihydrogenphosphate, 0.3 M, pH 6.0, 90% v/v, analytical reagent grade (Merck).

Apparatus and procedure

A liquid chromatograph, Perkin Elmer Model 1220, was used. It was equipped with a Model LC 55 spectrophotometer and with a syringe sample-loading injector Rheodyne 7105. The chromatograph was fitted with an octadecyl Sil X I column (0.25 m × 26 mm) of particle size 10 μm. The mobile phase was pumped at a flow rate of 2.0 ml/min and the column was operated at a temperature of 40°C. The detection was made at a wave

TABLE 1

BENZAMIDE STRUCTURES

General structure	Substituents			Compound name (DCI)
	R_2	R_4	R_5	
	H	NH_2	H	Procainamide
	OCH_3	H	SO_2CH_3	Tiapride
	OCH_3	NH_2	Cl	Metoclopramide
	OCH_3	H	SO_2NH_2	Sulpiride
	OCH_3	H	$SO_2C_2H_5$	Sultopride

length of 215 nm, chosen following the results obtained in a previous spectral study (Topart et al., 1973, 1976).

To determine the percentage of tiapride in the injectable solution Tiapridal, 1 ml of the solution was diluted to 100 ml with bidistilled water and 10 μ^1 injected in the chromatograph. Sulpiride and metoclopramide were quantitatively determined in Dogmatil and Primperan syrups, respectively, after dilution of 10 ml of 100 ml with bidistilled water. These solutions were filtered through a micropore membrane and 10 μl injections were made. Quantitative determinations of tiapride, sulpiride and metoclopramide in tablets were carried out following a classical procedure: the average weight of 10 tablets was first determined, they were finely ground and the powder was then extracted with bidistilled water at room temperature except for the metoclopramide which required 50°C. The solutions were then filtered through a micropore membrane and 10 μl injections were made. The results of the determination were calculated for the average weight of one tablet. Quantitation was accomplished by comparison of the peak height (or area) of the drug studied to the peak obtained with the same concentration of the corresponding standard.

RESULTS AND DISCUSSION

A systematic study of the influence of three parameters (ionic strength, pH and acetonitrile percentage) on the retention time led to the definition of optimal experimental conditions for a good paration and identification of the studied molecules (see Fig. 1).

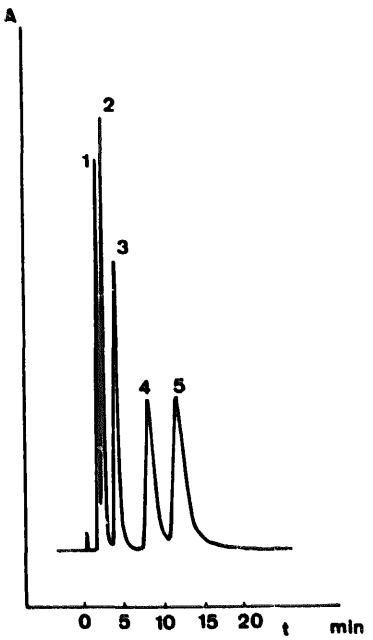


Fig. 1. Five benzamides mixture chromatograms. 1, procainamide; 2, sulpiride; 3, tiapride; 4, sultopride; 5, metoclopramide. Experimental conditions: solvent, acetonitrile–phosphate buffer 0.3 M, pH 6.0, 10:90; flow rate, 2 ml/min; temperature, 40°C.

Choice of the experimental conditions

Influence of the ionic strength on the retention time

While pH (6.0) and acetonitrile percentage (10% v/v) were maintained constant, in a first series of experiments the phosphate buffer molarity was modified by varying the phosphate ions concentrations (see Table 2). If reference is made to the equilibrium:

$$H_2PO_4^- + H_2O \rightleftharpoons HPO_4^{2-} + H_3O^+$$

and the following equations:

$$pK_a = pH - \log \frac{[HPO_4^{2-}]}{[H_2PO_4^-]} \quad (1)$$

$$C_i = [H_2PO_4^-] + [HPO_4^{2-}] \quad (2)$$

$$\mu = \tfrac{1}{2}[[H_2PO_4^-] + 4\,[HPO_4^{2-}] + [OH^-] + [H^+] + [Na^+]] \quad (3)$$

$$pK_a = pK_T + \frac{0.509\,(2\,Z - 1)\sqrt{\mu}}{1 + \sqrt{\mu}} \quad (4)$$

then it is possible to demonstrate that

$$\mu = 1/2\,\frac{C_i}{1 + 10^{pH - pK_a}} + 2\,C_i\left(1 - \frac{1}{1 + 10^{pH - pK_a}}\right) + \frac{1}{2}\,\frac{V_a C_i}{V_a + V_b} + \frac{V_b C_i}{V_a + V_b} \quad (5)$$

where pK_a = apparent pK_a of $H_2PO_4^-$; pK_T = thermodynamic pK_a of $H_2PO_4^-$; Z = charge

TABLE 2

IONIC STRENGTH INFLUENCE ON THE CAPACITY FACTOR k'

Derivatives	μ(M)								
	0.062 [0.05] [a]	0.128		0.271		0.424		0.584	
		[0.1] [a]	b	[0.2] [a]	b	[0.3] [a]	b	[0.4] [a]	b
Procainamide	2.76	2.12	1.56	1.80	1.16	1.66	1.04	1.64	1.02
Sulpiride	3.88	3.08	2.30	2.72	1.58	2.46	1.50	2.50	1.38
Tiapride	6.86	5.46	3.92	4.84	2.72	4.32	2.54	4.46	2.30
Sultopride	13.84	11.38	7.88	10.18	5.36	8.76	5.06	9.10	4.38
Metoclopramide	20.96	16.88	14.84	14.96	8.18	12.78	7.88	13.18	6.84

[a] Buffer ionic strength modified by varying phosphate ions concentrations. The buffer molarity is written [ ].

[b] Ionic strength modified by addition of potassium chloride to the 0.05 M phosphate buffer.

of $H_2PO_4^-$; C_i = total phosphate concentration; V_a = volume of the $H_2PO_4^-$ solution used in the preparation of the buffer; and V_b = volume of the HPO_4^{2-} solution used in the preparation of the buffer. Eqn. 5 can be rewritten as follows:

$$\mu = \frac{C_i}{2}\,\frac{V_a + 2V_b}{V_a + V_b} + 2C_i - \frac{3}{2}\,\frac{C_i}{1 + 10^{pH - pK_T + \frac{1.527\sqrt{\mu}}{1+\sqrt{\mu}}}} \qquad (6)$$

As this is an implicit expression in μ, it leads itself to an iterative calculation procedure to solve for the ionic strength.

In a second series of experiments, the ionic strength was modified by dissolving in the buffer [0.05 M (μ = 0.062 M)] increasing amounts of potassium chloride to obtain the same ionic strengths as in the first series. The pH (6.0) and the acetonitrile percentage (10% v/v) were again kept constant. The results of this second study are presented in Table 2 which shows the variation of the capacity factor k' * as a function of the ionic strength. It is clearly evident that a mobile phase increasing ionic strength induces a corresponding retention time decrease for each benzamide molecule and that the nature of the ion plays an essential role.

Influence of the pH on the retention time

The pH of the buffer was the second parameter studied. It was modified by varying the proportion of the sodium monohydrogen and dihydrogenphosphate; the solvent composition of acetonitrile buffer (10:90) was unchanged. Three pH (5.00, 6.00, 7.00) values were studied and the corresponding ionic strength was calculated following Eqn. 6, they were equal to 0.208 M, 0.271 M and 0.484 M, respectively. The plots presented in Fig. 2 clearly show that an increasing ionic strength produced a decrease of the capacity factor, the pH should considerably influence the chromatographic process. However, in this limited range of acidity, the ionic dissociation of the benzamides investigated was nearly 99% (Hanocq et al., 1973, 1977; van Damme et al., 1976) so that the influence of the pH was negligible. The increasing values of k' could be explained by the variation of the ratio $[H_2PO_4^-]/[HPO_4^{2-}]$: at pH 5.0 this ratio was high whereas it was close to 1.0 at pH 7.0. Again the nature of the anion seemed to be very important; so the hypothesis could be made that it was not the pH which was responsible of the capacity factor decrease but the concentration of the anion $H_2PO_4^-$ which is high at pH 5.0 and very low at pH 7.0.

Influence of the acetonitrile percentage on the retention time

The third and last parameter studied was the acetonitrile percentage varying between 8 and 15% while the pH and the ionic strength were maintained unchanged. Fig. 3,

---

* $k' = \frac{t_r - t_0}{t_0}$

with t_r = retention time of the studied derivative, and t_0 = retention time of a non retained product (in this case the standard solvent retention time (methanol) has been chosen).

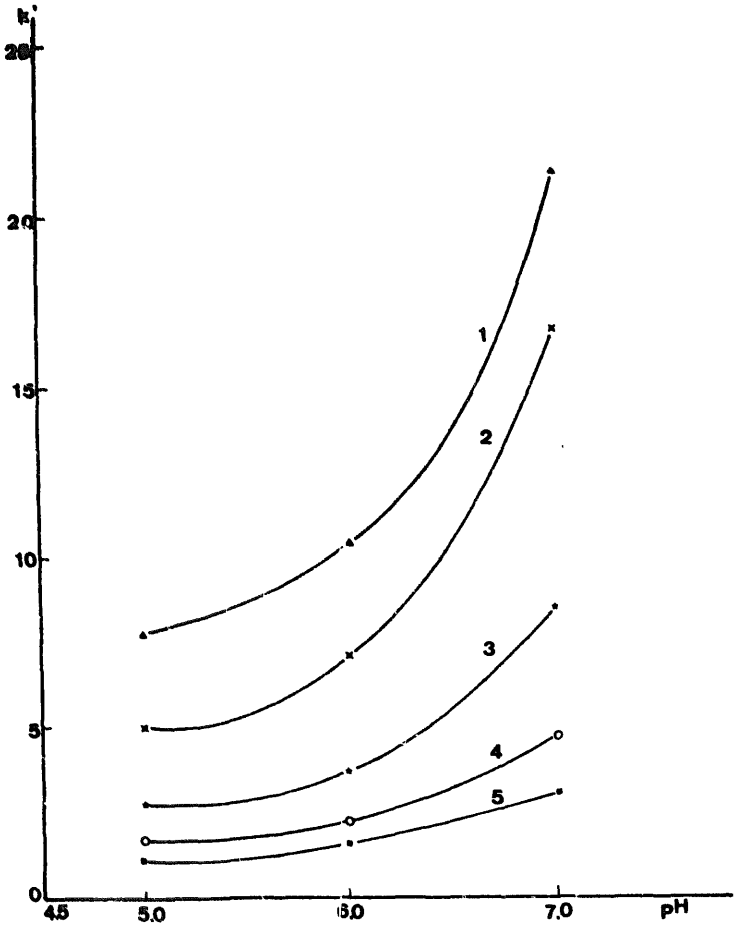


Fig. 2. Buffer pH influence on the capacity factor k'. 1, metoclopramide; 2, sultopride; 3, tiapride; 4, sulpiride; 5, procainamide.

obtained by plotting capacity factor variations against acentonitrile percentage, shows that the retention time decreased when the acetonitrile content increased. When the acetonitrile percentage was 8%, the elution of the benzamides required a long time and the chromatographic peaks were very much enlarged. On the contrary, at 15% acetonitrile, the five compounds were almost not separated so a compromise had to be chosen to have a good separation in a minimum of time.

Interpretation of the partition mechanism

Since the benzamides have an amphipatic structure, it might be suggested that this chromatographic process is similar to an ion-pair partition one. In fact, although these molecules were injected as hydrochlorides, the experiments indicated that they were not eluted by the acetonitrile–water solvent without any excess of counter-ion. Since the solvent had a certain ionic strength due to chloride or dihydrogenphosphate, the elution became possible. These two anions probably formed a pair with the benzamides and thus the chloride would favour a more reapid elution. The observed deceleration of the elution

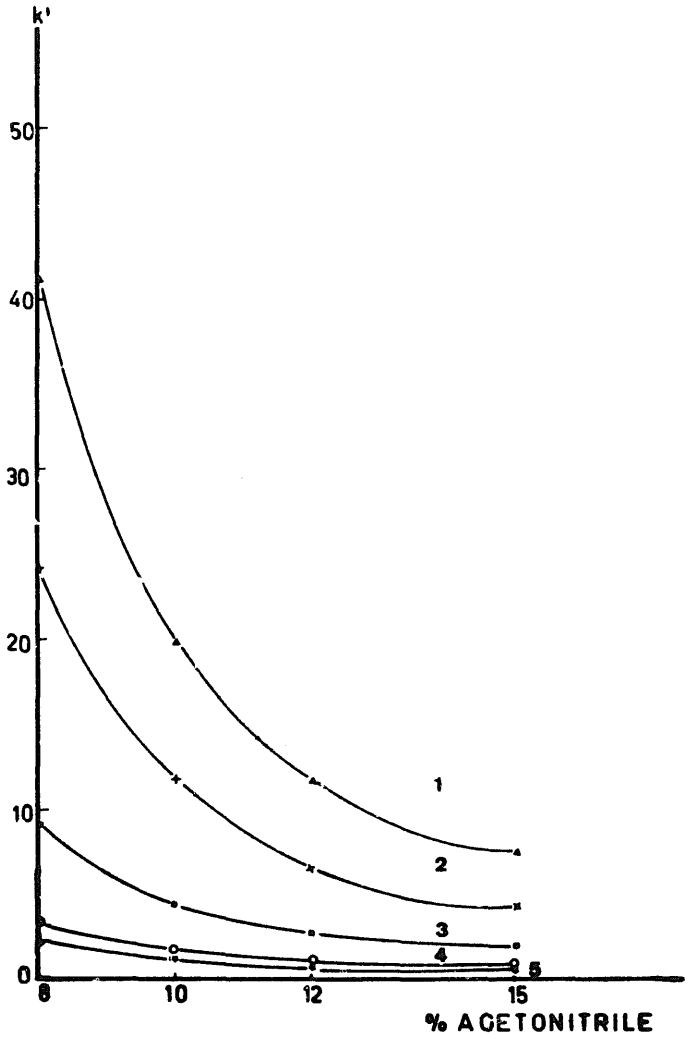


Fig. 3. Influence of the acetonitrile percentage in the buffer solution, on the capacity factor k'. 1, metoclopramide; 2, sultopride; 3, tiapride; 4, sulpiride; 5, procainamide.

when there was exclusively $H_2PO_4^-/HPO_4^{2-}$ buffer, could be explained either by an ion-pair formation competition between these two anions, or by a less important solvation of the monohydrogenphosphate ion-pair in acetonitrile. This assumption would also explain the increase of the capacity factor which occurred with a pH change or when phosphate ions were added in the chloride containing eluent. Indeed, the most rapid elution of the different compounds was obtained with an acetonitrile–potassium chloride (0.128 M), 10:90, system. For example, the procainamide capacity factor became lower than 0.6, whereas it was 1.56 for an identical ionic strength but free from KCl. The results presented in Table 2 demonstrate this hypothesis.

TABLE 3

COMPARISON, AT THREE WAVELENGTHS, OF THE EXTINCTION RATIOS CALCULATED FROM CHROMATOGRAPHIC DATA AND DIRECT SPECTROPHOTOMETRIC STANDARD ABSORBANCES

$\bar{R}$ = mean number of the ratios; N = number of assays; s = standard deviation; CV% = coefficient of variation.

Derivative	Extinction ratios [a]	HPLC				Spectrophotometry			
		$\bar{R}$ (2)	N (3)	s (4)	CV% (5)	$\bar{R}$ (2)	N (3)	s (4)	CV% (5)
Procainamide	I	1.87	9	0.04	2.1	1.75	9	0.04	2.7
	II	0.71	9	0.01	1.6	0.75	9	0.02	2.7
	III	1.32	9	0.02	1.7	1.31	9	0.03	1.9
Sulpiride	I	12.10	9	0.50	3.8	12.3	9	0.2	1.3
	II	1.40	9	0.05	3.8	1.36	9	0.02	1.6
	III	17.00	9	0.2	1.3	16.7	9	0.3	1.6
Tiapride	I	9.90	9	0.10	1.5	10.2	9	0.1	1.0
	II	2.30	9	0.05	2.2	2.24	9	0.04	2.0
	III	22.6	9	0.5	2.3	22.8	9	0.5	2.2
Sultopride	I	7.79	9	0.09	1.2	8.22	9	0.08	1.0
	II	2.77	9	0.06	2.0	2.66	9	0.09	3.3
	III	21.60	9	0.50	2.2	21.80	9	0.7	3.0
Metoclopramide	I	3.90	9	0.1	3.3	4.13	9	0.05	1.1
	II	0.61	9	0.02	3.7	0.62	9	0.01	1.1
	III	2.34	9	0.06	2.6	2.58	9	0.02	0.7

[a] I: $E_{215\ nm}/E_{255\ nm}$
II: $E_{255\ nm}/E_{300\ nm}$
III: $E_{215nm}/E_{300\ nm}$

IDENTIFICATION AND QUANTITATIVE DETERMINATION OF THE BENZAMIDES IN VARIOUS PHARMACEUTICAL FORMS

Although the molecules have been classically identified by comparison of the retention times and volumes with reference solutions, this method has been advantageously completed to use a more specific technique which avoided interferences to some extent.

The same concentration of an analyzed compound was injected three times at different wavelengths (215, 255 and 300 nm) * and extinction ratios were calculated as shown in Table 3. The 'chromatographic extinction' ratio was compared with the ratio classically measured by spectrophotometry in the same solvent and under the same conditions.

* These wavelenghts were selected with reference to the benzamides ultraviolet spectra performed in the same solvent system.

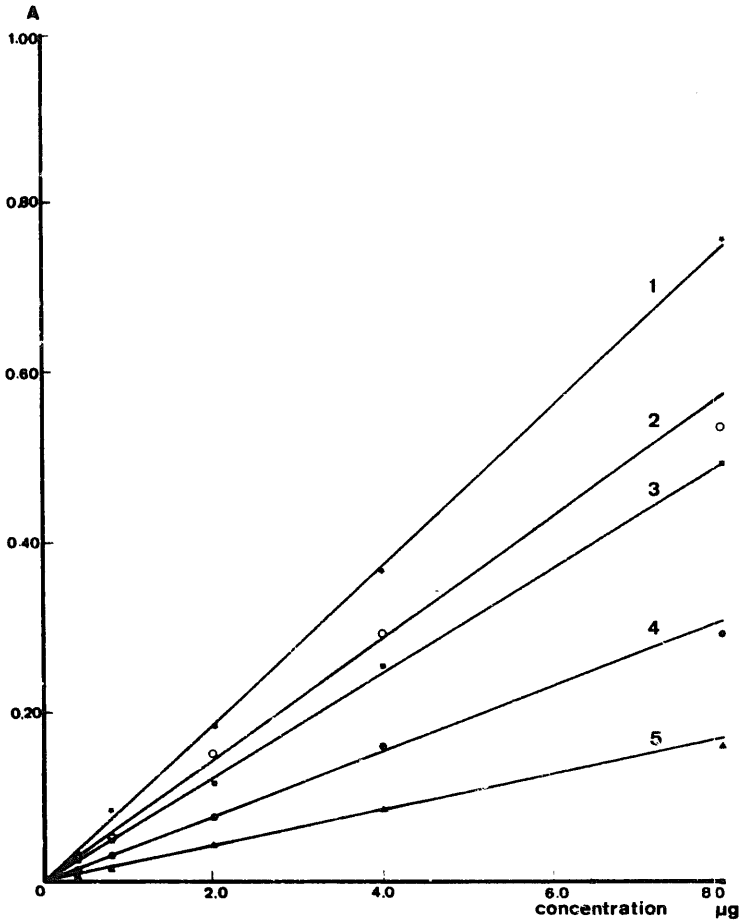


Fig. 4. Peak heights standard calibration curves; 1, sulpiride; 2, tiapride; 3, procainamide; 4, sultopride; 5, metoclopramide.

TABLE 4

QUANTITATIVE DETERMINATION OF TIAPRIDE, SULPIRIDE AND METOCLOPRAMIDE IN SOME PHARMACEUTICAL DOSAGE-FORMS

Drug quantitatively determined	Dosage forms	N [a]	Theoretical	Experimental	s [b]	% Recovery
Tiapride	Injection	7	111.2 mg/2 ml	115.4 mg/2 ml	0.8	104
	Tablets	7	111.2 mg/tab.	114 mg/tab.	3	102
Sulpiride	Syrup	6	25 mg/5 ml	26.1 mg/5 ml	0.4	104
	Tablets	6	200 mg/tab.	196 mg/tab.	5	99
Metoclopramide	Syrup	7	5 mg/5 ml	4.8 mg/5 ml	0.1	96
	Tablets	7	10 mg/tab.	9.7 mg/tab.	0.1	97

[a] Number of assays
[b] Standard deviation

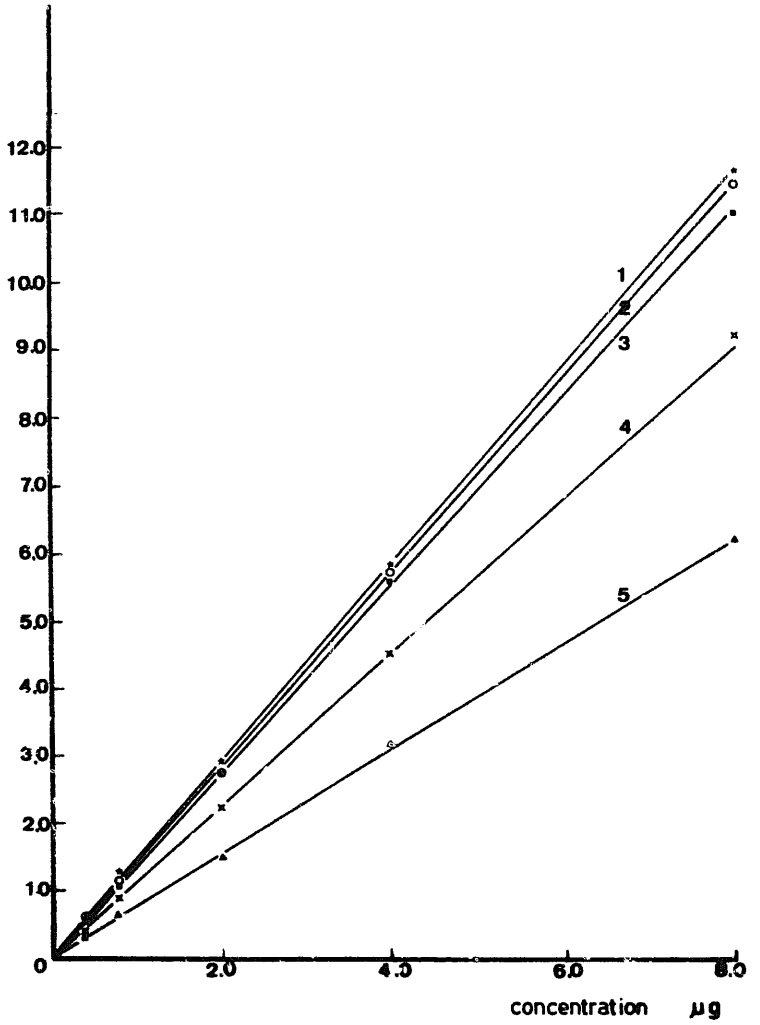


Fig. 5. Peak areas standard calibration curves. 1, sulpiride; 2, sultopride; 3, tiapride; 4, metoclopramide; 5, procainamide.

The five standard calibration curves have been obtained by plotting peak heights or areas against concentration. They were linear between 0.4 and 8.0 µg (see Figs. 4 and 5). The detection limit was 50 µg or less.

By examining Table 4, the precision and reproducibility of the method may be appreciated. The chromatogram given as an example (Fig. 6) shows that the dyestuffs, the sweeteners and other compounds present in the Dogmatil syrup did not interfere with the quantitative determination of sulpiride.

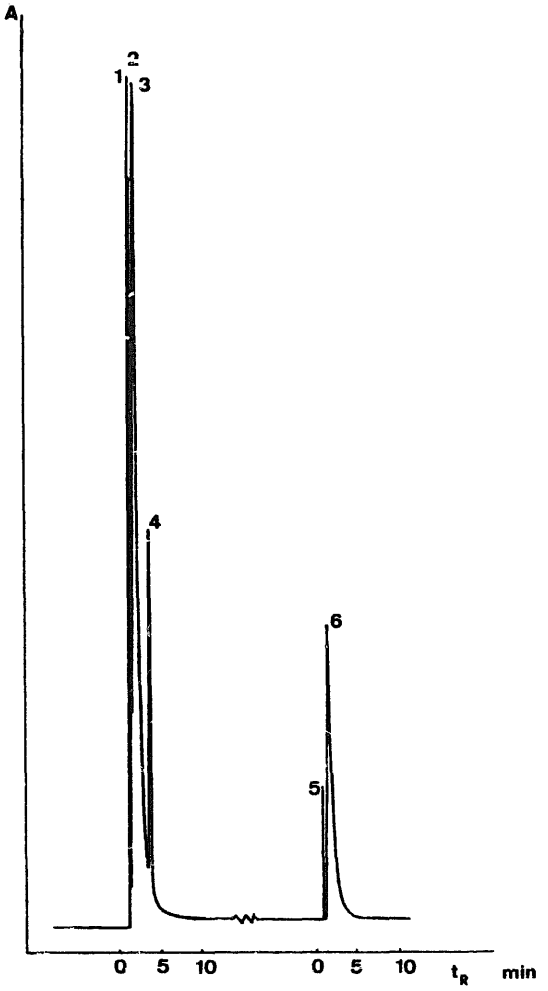


Fig. 6. Illustrative chromatogram of sulpiride quantitative assay in Dogmatil syrup. 1–3, various syrup consituents; 4, sulpiride 5, methanol (standard solvent); 6, sulpiride standard injection.

ACKNOWLEDGEMENTS

Our thanks are due to the Delagrange Laboratory for the supply of some materials and we also thank Simes Co., (Milan), who kindly supplied procainamide hydrochloride. This work was supported by F.R.S.M. (Convention 3.4541.76).

REFERENCES

Adams, R.F., Vandemark, F.L. and Schmidt, G., The simultaneous determination of lidocaine and procainamide in serum by use of high pressure liquid chromatography. Clin. Chim. Acta, 69 (1976) 515–524.

Atkinson, A.J., Parker, M. and Strong, S., Rapid gas chromatographic measurement of plasma procainamide concentration. Clin. Chem., 18 (1972) 643–646.

Bratton, A.C., Marshall, E.K., Babbitt, O.D. and Hendrikson, A.K., A new coupling component for sulfanilamide determination. J. Biol. Chem., 128 (1939) 537–550.

Carr, K., Woolsley, R.L. and Oates, J.A., Simultaneous quantification of procainamide and N-acetyl-procainamide with high performance liquid chromatography, J. Chromatogr., 129 (1976) 363–368.

Frigerio, A. and Pantarotto, C., Quantitative determination of sulpiride in biological samples from rats by gas liquid chromatography and chemical ionisation-mass fragmentography. J. Chromatogr., 130 (1977) 361–367.

Hamm, J.C., Collaborative study of the spectrophotometric determination of procainamide hydrochloride. J. Assoc. Off. Agr. Chem., 59 (1976) 807–810.

Hanocq, M., Topart, J., van Damme, M. and Molle, L., Détermination de constantes d'ionisation de quelques N-(diéthylamino-2-éthyl)benzamides substitués. J. Pharm. Belgique, 28 (1973) 649–662.

Hanocq, M., van Damme, M., Hanus, R. and Molle, L., Détermination spectrophotométrique des constantes d'acidité d'orthobenzamides substitués. Analusis, 5 (1977) 380–384.

Klein, S.W. and Koch-Weser, J., Procainamide dosage schedules, plasma concentration and chemical effects. J. Am. Med. Assoc., 215 (1971) 1454–1458.

Koch-Weser, J. and Klein, S.W., Antiarrhythmia prophylaxis with procainamide in acute myocardial infarction. New Engl. J. Med., 281 (1969) 1253–1260.

Pitel, G. and Luce, Th., Dosage du métoclopramide en milieu biologique. Ann. Pharm. Fr., 23 (1965) 673–681.

Pitel, G. and Luce, Th., Dosage du sulpiride en milieu biologique. Ann. Pharm. Fr., 28 (1970) 409–415.

Rocco, R.M., Abbott, D.C., Giese, R.W. and Karger, B.L., Analysis of procainamide and N-acetyl-procainamide in plasma by high performance liquid chromatography. Clin. Chem., 23 (1977) 705–708.

Shulur, L.R., Powers, J.L., Marques, R.A., Winter, M.E. and Sadee, W., Measurement of procainamide and N-acetylprocainamide in serum by high performance liquid chromatography. Clin. Chem., 23 (1977) 636–638.

Stenart, J.T. and Lotti, D.M., Improved colorimetric determination of primary aromatic amines with 9-chloroacridine: application to some local anesthetics. J. Pharm. Sci., 59 (1970) 838–842.

Topart, J., Hanocq, M., van Damme, M. and Molle, L., Etude spectroscopique de quelques N-(diéthyl-amino-2-éthyl)benzamides substitués. J. Pharm. Belgique, 28 (1973) 633–648.

Topart, J., Hanocq, M., van Damme, M. and Molle, L., Etude spectroscopique de N-(diéthylamino-2 éthyl) et de N-((éthyl-1-pyrrolidyl-2)méthyl)benzamides substitués. Pharm. Acta Helv., 51 (1976) 314–325.

van Damme, M., Hanocq, M., Topart, J. and Molle, L., Détermination des constantes d'ionisation du N-(éthyl-1-pyrrolidyl-2-méthyl)méthoxy-2-sulfamoyle-5 benzamide (sulpiride). Analusis, 4 (1976) 299–307.