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Separation and quantitative determination of homatropine methylbromide and opium alkaloids in admixture in pharmaceutical preparations by gas–liquid and high-performance liquid chromatography

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Summary

Gas–liquid and high-performance liquid chromatographic methods have been developed to quantitate homatropine methylbromide in admixture with 5 opium alkaloids (morphine, codeine, thebaine, papaverine and noscapine) in pharmaceutical preparations.

Silylated morphine, mandelic acid (as the hydrolysis product of homatropine methylbromide), codeine and thebaine were quantitatively determined by gas chromatography using 3% Dexil 300 as partition liquid. Papaverine and noscapine were determined semiquantitatively by the same method.

High-performance liquid chromatographic separation of all the compounds directly was achieved in one step using Nucleosil 5 C8 as the stationary phase and acetonitrile + 0.01 M phosphate buffer pH 5.0 (2:3) as the mobile phase. This method is shown to be suitable to evaluate the content uniformity of tablets containing the 5 opium alkaloids and homatropine methylbromide.

Introduction

Determination of the individual drug substances in multicomponent pharmaceutical preparations by classical methods is time-consuming, labour intensive and often inaccurate because several extractions and separation procedures precede the quantitation of each component. Chromatographic methods, in particular gas–liquid

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(GLC) and high-performance liquid chromatography (HPLC) offer an easier, more accurate approach to the analysis of such preparations.

It is a known phenomenon that the phenolic group present on the morphine molecule causes adsorption on the GLC column leading to non-linear and variable quantitative results. This problem can be partly overcome by careful deactivation of the solid support (Street, 1968) and it can be eliminated completely if the drug is converted into a suitable non-polar derivative before entering the column (Rasmussen, 1976). The results presented here show several problems in complete silylation of a mixture of opium alkaloids and homatropine methylbromide, thus necessitating a 3-step GLC method, in which morphine is isolated and silylated prior to injection, homatropine methylbromide is hydrolyzed according to an earlier described method (Grabowski et al., 1973), and the remaining opium alkaloids are chromatographed without pretreatment.

The pharmaceutical preparations in question contain 5 opium alkaloids besides homatropine methylbromide and for the tablets a method suitable for single-dose determination is necessary to evaluate content uniformity. As the above-mentioned GLC method failed to be sufficiently precise for this purpose a single-step HPLC method was elaborated. The literature on liquid chromatography of opium alkaloids is abundant. Methods using gradient elution (Ziegler et al., 1975) as well as several isocratic methods utilizing either straight-phase (Vincent and Engelke, 1979; Jane, 1975; Hansen et al., 1980) or reversed-phase (Wu and Wittick, 1977; Oliemann et al., 1977; Lurie, 1977; Fehér et al., 1979; Nobuhara et al., 1980) have been published. Only a few of these methods are able to separate all the 5 opium alkaloids in question and none of them are suitable for the mixture of opium alkaloids and the quaternary ammonium, homatropine methylbromide.

This paper presents an isocratic, reversed-phase HPLC method which is sufficiently precise for single-dose determination of the above-mentioned tablets.

Materials and methods

Chemicals

Acetonitrile of HPLC S grade (Rathburn, Walkerburn, U.K.) was used; drug substances were of pharmacopoeial standard; all other reagents were of analytical grade.

Preparations

Tablets: 1 tablet contains 2 mg homatropine methylbromide, 0.21 mg thebaine hydrochloride, 0.38 mg codeine hydrochloride, 0.44 mg papaverine hydrochloride, 2.28 mg noscapine hydrochloride and 6.7 mg morphine hydrochloride.

Injections: 1 ml contains 1.5 mg homatropine methylbromide, 0.84 mg thebaine hydrochloride, 1.5 mg codeine hydrochloride, 1.76 mg papaverine hydrochloride, 9.1 mg noscapine hydrochloride and 26.8 mg morphine hydrochloride.

Gas chromatography

A Carlo Erba Fractovap model GV gas chromatograph equipped with a flame

ionization detector was used. The column was a 1.5 m × 3 mm i.d. glass column packed with acid washed and silanized diatomaceous earth (Chromosorb W, 100–120 mesh, Supelco) coated with 3% carborane methyl silicone (Dexsil 300). Nitrogen was used as the carrier gas and the column was operated at 155°C for homatropine methylbromide, 250°C for morphine hydrochloride, 240°C for codeine hydrochloride and thebaine hydrochloride, and 300°C for noscapine hydrochloride and papaverine hydrochloride. Chromatograms were recorded on a Speedomax model G recorder and integrated by a Carlo Erba model 75 electronic integrator.

Liquid chromatography

A liquid chromatograph consisting of a Haskel model 26980 pump, a Cecil model 212 spectrophotometer detector, and a Valco model CV-6-UHPa-N60 injection valve with a 10-μl loop was used. A column 120 mm × 4.65 mm i.d. (Knauer, Oberursl, G.F.R.) packed with Nucleosil 5 C 8 (Macherey–Nagel, Düren, G.F.R.) as earlier described (Helboe and Thomsen, 1977) was used for preliminary investigations. For the analysis 2 identical columns in series were used.

0.01 M phosphate buffer, pH 5.0, modified with 40% of acetonitrile was used as the mobile phase.

The columns were operated at room temperature using a flow rate of 1 ml/min. The pressure for the single column was 7 M Pa and for the two columns in series 14 M Pa.

Chromatograms were recorded on a Kipp and Zonen model BD 8 recorder and retention times and peak areas were measured by means of a Hewlett-Packard model 3353 A laboratory data system.

Test and standard solutions

Gas chromatography

Internal standard solutions: 2-naphtol: a solution of 24 mg 2-naphtol in 100 ml of anhydrous ether. Nalorphine: a solution of 0.5 g nalorphine hydrochloride in 10 ml of carbon dioxide–free water. Homatropine: a solution prepared by extraction of homatropine base from 16 mg homatropine hydrobromide into 10 ml of chloroform.

Test solution for determination of homatropine methylbromide: to tablet powder —prepared from 10 tablets—equivalent to about 1.75 mg homatropine methylbromide or to 2 ml of the injection is added 20 ml of hydrochloric acid buffer, pH 2.2, (USP XX, 1980) and the solution is extracted with three 20 ml portions of anhydrous ether. The aqueous solution is adjusted to pH 10–11 with 10% sodium hydroxide solution and heated to boiling for 20 min. Following cooling the solution is adjusted to pH 2.2 using hydrochloric acid and extracted with three 20 ml portions of anhydrous ether. To the combined, dried (anhydrous sodium sulphate) and filtered extract is added 5 ml of 2-naphtol internal standard solution and the solution is evaporated to dryness with a stream of nitrogen. The residue is dried over silica gel and dissolved in 200 μl of N,O-bis-trimethylsilylacetamide (BSA) and the solution is allowed to stand at room temperature for 20 min. 0.2 μl is injected on column, using a nitrogen flow rate of 25 ml/min.

Test solution for determination of morphine hydrochloride: to tablet powder—prepared from 10 tablets—equivalent to about 33 mg of morphine hydrochloride or to 2 ml of the injection is added 8 ml of 1 N sodium hydroxide and 1 ml of nalorphine internal standard solution. The solution is extracted with five 15 ml portions of chloroform and the combined chloroform extract is reextracted with a mixture of 5 ml of water and 3 drops of 2 N sodium hydroxide. The chloroform extract is used to prepare the test solution for determination of other opium alkaloids. The combined alkaline solution is neutralized with 2 N hydrochloric acid. 25 ml of chloroform + 2-propanol (3 : 1) and 5 ml of a 4% aqueous solution of sodium hydrogen carbonate is added and the mixture is shaken immediately. Following separation of the organic layer the aqueous solution is extracted with further three 25 ml portions of the chloroform-2-propanol mixture. The combined extracts are dried (anhydrous sodium sulphate) filtered and evaporated on a water-bath. The residue is dried over silica gel and dissolved in 10 ml of dehydrated pyridine. To 200 μl of the pyridine solution is added 200 μl of BSA and 100 μl of trimethyl chlorosilane, (TMCS), and the solution is allowed to stand for 20 min at room temperature. 2 μl is injected on column using a nitrogen flow rate of 25 ml/min.

Test solution for determination of other opium alkaloids (codeine hydrochloride, thebaine hydrochloride, papaverine hydrochloride, and noscapine hydrochloride): the chloroform extract (described under morphine hydrochloride) is dried (anhydrous sodium sulphate), filtered and evaporated to dryness on a water bath. The residue is dissolved in a mixture of 1 ml of chloroform and 1 ml of homatropine internal standard solution. 1 μl is injected on column using a nitrogen flow rate of 80 ml/min.

Liquid chromatography

Tablets: to 1 tablet or to the prescribed amount of tablet powder is added 10 ml of the eluant and the mixture is shaken for 15 min. The suspension is centrifuged at 3000 rpm for 10 min. 10 μl of the clear supernatant is injected.

Injections: 1 ml is diluted to 50 ml with eluent. 10 μl is injected.

Standard solutions: amounts (accurately weighed) of each of the 6 components equivalent to the contents of 10 tablets or 2 ml of injection are dissolved in 100 ml of eluant. 10 μl is injected.

Results and discussion

Gas–liquid chromatography

Preliminary investigations were carried out on 5 columns with different partition liquids: Dexil 300, SE-30, OV-101, OV-1 and OV-11. In the selection of stationary phases important prerequisites were high maximum operating temperature as well as relatively low polarity due to the fact that all the compounds present were polar and of only low volatility.

By using Dexil 300, OV-101, OV-1 and SE-30 all compounds gave a response but homatropine methylbromide and morphine were only partly eluted. Total silylation

was tried by applying various silylating agents: BSA, bis-trimethylsilyl-trifluoroacetamide (BSTFA), BSA + TMCS) (2 : 1), and BSTFA + TMCS (2 : 1), but, as was predictable, only morphine and codeine formed derivatives quantitatively. The peaks of homatropine methylbromide, papaverine and noscapine remained unaffected. Thebaine, however, gave rise to two peaks which might be due to degradation.

Since complete recovery of morphine from the columns could only be assured by silylation and thebaine could be analyzed quantitatively by GLC only without silylation, the separation of morphine from the other compounds prior to chromatography was inevitable. The separation described in this work makes use of the fact that only morphine possesses an acidic (phenolic) group. Nalorphine was chosen as internal standard for the assay of morphine. It differs from morphine only in having an allyl-group instead of a methyl-group, thus their chromatographic characteristics are very similar. In Fig. 1 is shown the chromatogram of the silylated mixture of morphine and nalorphine on Dexil 300.

As mentioned above, homatropine methylbromide was only partly eluted from the column and silylation produced no effect, thus a separation of this compound was also necessary. The method used had been earlier reported by Grabowski et al. (1973), and is based on the formation of mandelic acid by hydrolysis. Also for this compound Dexil 300 was found to be the most suitable stationary phase and in Fig. 2 is shown a chromatogram of the silylated mixture of mandelic acid and the internal standard 2-naphtol.

By applying temperature programming codeine, thebaine, papaverine and noscapine could be well separated on the same Dexil 300 column. The peaks of codeine and thebaine were symmetrical but those of papaverine and noscapine showed some tailing. Therefore, the better reproducible, and for quantitative purpose more reliable, isothermal conditions were preferred for the assay of codeine and thebaine as well as for the semiquantitative estimation of papaverine and noscapine, although a slight tailing of the codeine peak thereby was observed. The chromatogram of codeine, thebaine and the internal standard homatropine is displayed in Fig. 3, and the separation of papaverine and noscapine in Fig. 4.

For the quantitative evaluation of gas chromatograms the internal standard method was used. Detector response linearities of morphine, codeine, thebaine and mandelic acid were checked in concentration ranges from 2.5 mg/ml to 7 mg/ml, from 1.5 mg/ml to 4 mg/ml, from 0.8 mg/ml to 2.5 mg/ml, and from 2 mg/ml to 5 mg/ml, respectively.

Accuracy data, given as recovery results for each of the 4 quantitated components are displayed in Table 1.

Precision data, obtained for each of the 4 quantitated components of the tablets can be seen in Table 2.

Table 3 shows the results of analysis of the injection.

High-performance liquid chromatography

For the separation of mixtures of polar compounds—as in the present example—HPLC offers a convenient alternative to GLC. For the separation of the present

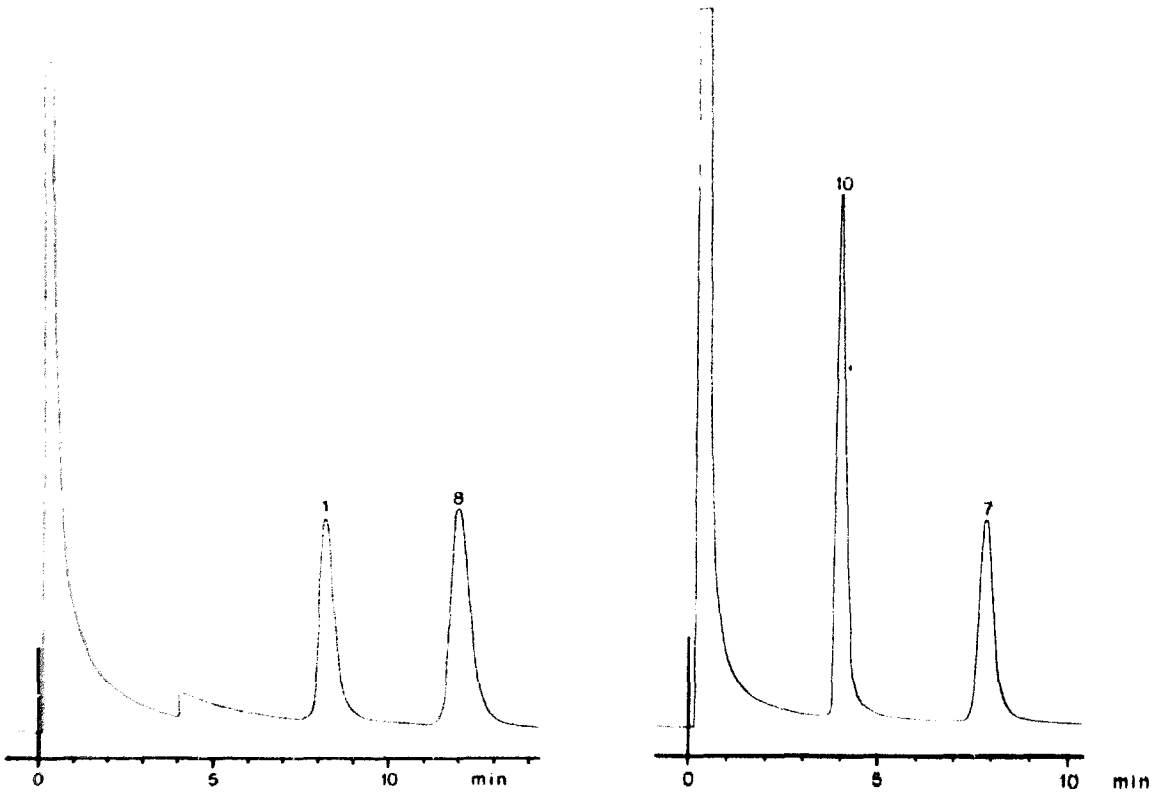

Fig. 1. GLC-chromatogram of a silylated mixture of morphine (1) and nalorphine (8). Column: 1.5 m × 3 mm i.d.; 3% Dexil 300 on Chromosorb W. Oven temperature: 250°C. Nitrogen flow rate 25 ml/min.

Fig. 2. GLC-chromatogram of a silylated mixture of mandelic acid (10) and 2-naphtol (7). Oven temperature: 155°C. Other conditions as in Fig. 1.

mixture also containing the quaternary ammonium compound, homatropine methylbromide the reversed-phase mode is the more suited. Nucleosil 5-C 8 as the stationary phase was preferred and two series of mobile phases were investigated, water modified with methanol and acetonitrile, respectively. By the addition of

TABLE I

Accuracy of the GLC method

Drug substance	Weighed (mg)	Found (mg)	Mean recovery (%)
Morphine hydrochloride	6.70	6.68	99.7
Codeine hydrochloride	0.38	0.38	100.0
Homatropine methylbromide	2.0	1.91	95.5
Thebaine hydrochloride	0.21	0.23	109.0
Papaverine hydrochloride *	0.44		
Noscapine hydrochloride *	2.28		

Recovery experiments were carried out on a laboratory-prepared mixture of the active ingredients without the adjuvants. 5 extracts were analyzed. Each extract was chromatographed 10 times.

* Papaverine and noscapine are estimated semiquantitatively.

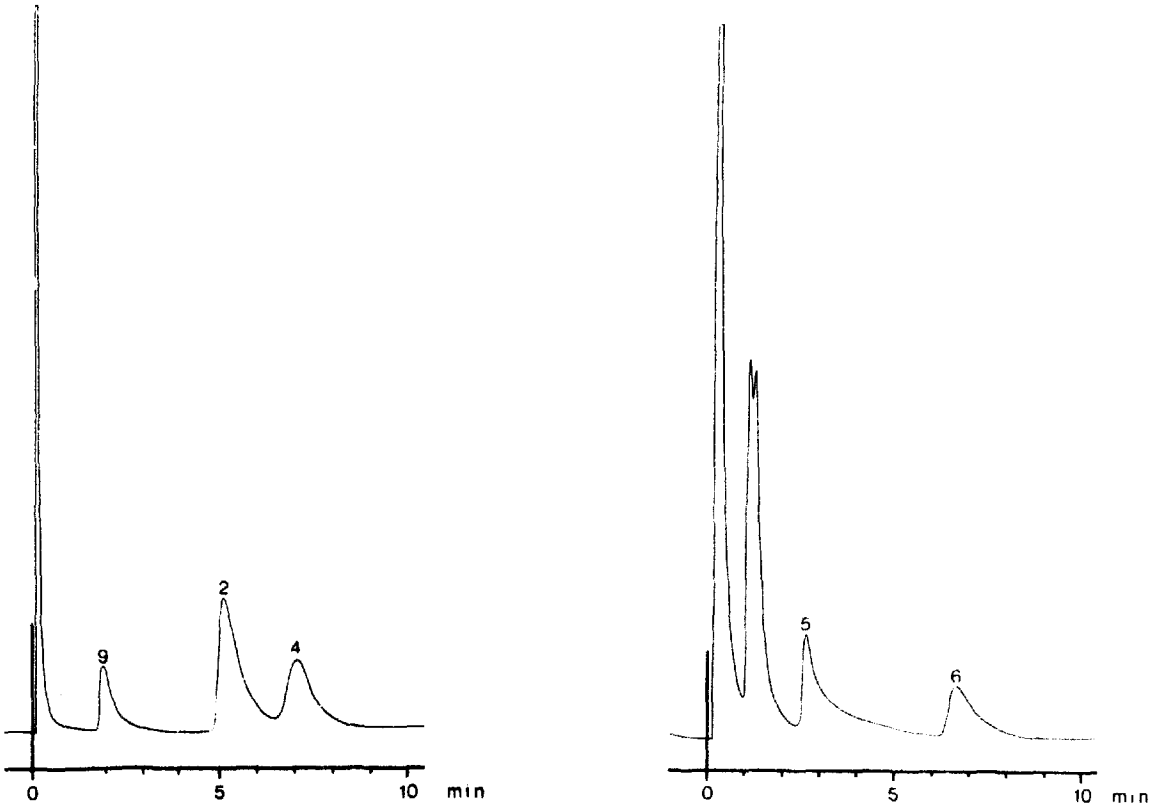

Fig. 3. GLC-chromatogram of a homatropine (9), codeine (2) and thebaine (4) mixture. Oven temperature: 240°C. Nitrogen flow rate: 80 ml/min. Other conditions as in Fig. 1.

Fig. 4. GLC-chromatogram of a papaverine (5) and noscapine (6) mixture. Oven temperature: 300°C. Nitrogen flow rate: 80 ml/min. Other conditions as in Fig. 1.

alkane sulphonates as counter-ions, using methanol as the modifier (55%) an increase in the retention of all compounds was achieved but the selectivity was only affected to a minor extent, thus a sufficient separation of homatropine methylbromide from codeine was not possible. Using acetonitrile as the modifier (40%) it

TABLE 2

Results of analysis of the tablets by GLC

Drug substance	Label claim (mg)	$\bar{x}_5$ (mg)	S_r (%)
Morphine hydrochloride	6.7	6.53	1.8
Codeine hydrochloride	0.38	0.34	2.8
Homatropine methylbromide	2.0	1.87	2.7
Thebaine hydrochloride	0.21	0.23	5.4
Papaverine hydrochloride *	0.44		
Noscapine hydrochloride *	2.28		

The means and the relative standard deviations were determined by the analysis of 5 extracts. Each extract was chromatographed 10 times.

* See Table 1.

TABLE 3

Results of analysis of the injection by GLC

Drug substance	Label claim (mg/ml)	Found (mg/ml)
Morphine hydrochloride	26.8	25.53
Codeine hydrochloride	1.5	1.49
Homatropine methylbromide	1.5	1.41
Thebaine hydrochloride	0.84	0.84
Papaverine hydrochloride *	1.76	
Noscapine hydrochloride *	9.1	

Each of 2 aliquots were chromatographed 5 times.
* See Table 1.

was found that the influence of added alkane sulphonates was much less pronounced and a sufficient separation was not achieved.

As shown also by Nobuhara et al. (1980) variations in pH were found to considerably affect the selectivity. From Fig. 5 it appears that the retention of all 6 components increases when increasing pH. The rate of the change in retention, however, differs for the 6 components leading to alterations in the selectivity. Using methanol as the modifier separation of homatropine methylbromide and codeine is not possible, whereas with acetonitrile an optimal separation of all 6 compounds is achieved at pH 4.5–5.0.

The detection wavelength was selected from the UV-absorption spectra of the 6 components at the same relative concentrations as in the preparations. It was found that a major consideration had to be paid to the 3 components codeine, methylhomatropine and thebaine due to their weak absorptivity at higher wavelengths in the actual concentrations. Thus a low detection wavelength was necessary and 220 nm was selected.

On account of the great water-solubility of all 6 compounds tablets could be extracted simply by shaking whole tablets or tablet powder with a suitable amount of the eluant. A chromatogram of tablet extract is shown in Fig. 6.

For the quantitative evaluation of HPLC chromatograms the external standardization method was used. Detector response linearities of each of the 6 components were established in concentrations ranging between 50 and 150% of those corrresponding to the declared contents of the preparations.

The precision of the HPLC method was investigated by powdering 20 tablets and analyzing amounts of powder corresponding to one tablet. The results appear from Table 4.

In Table 5 are shown results from the analysis of 10 individual tablets. From the table it is possible to evaluate the content uniformity both according to the fixed limit test of the USP (1980) and to the calculated content precision (S_r%). All compounds except papaverine comply with the USP limits and exhibit a content precision within 2.4%. The content of papaverine does not comply with the USP test, the contents of 2 tablets falling outside the 85–115% range; correspondingly the content precision is calculated to 10%.

In Table 6 are shown the results of the analysis of the injection preparation.

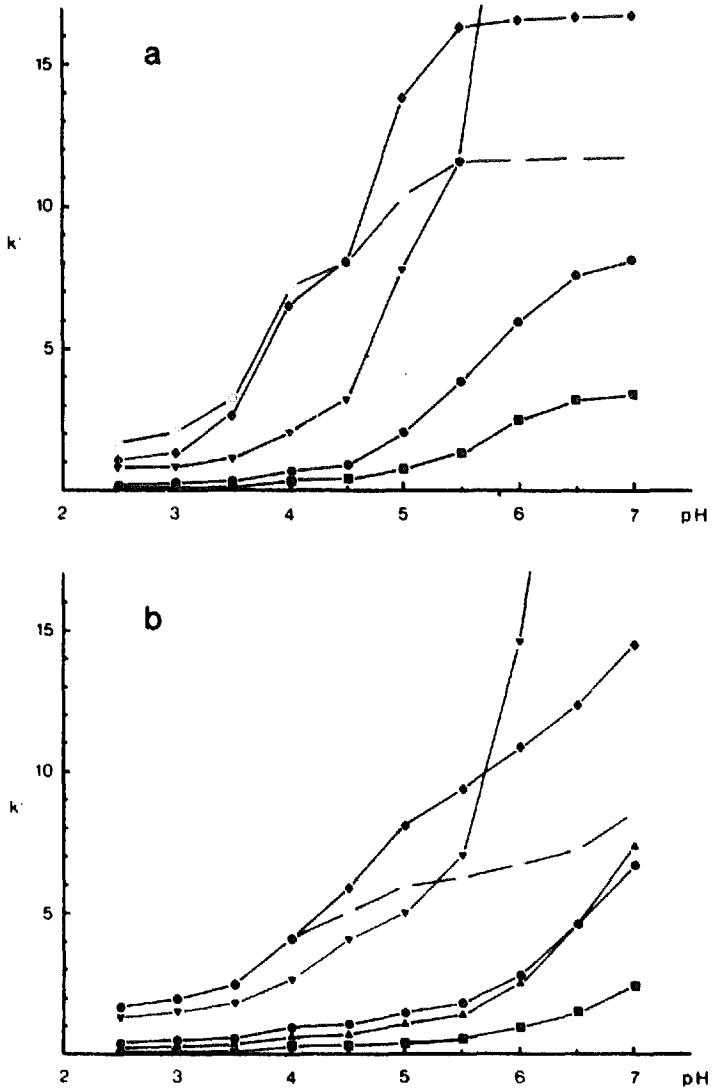

Fig. 5. Influence of buffer pH on the retention times of the following compounds during HPLC using as the modifier either 55% methanol (a) or 40% acetonitrile (b). ■, morphine; ●, in (a) homatropine methylbromide and codeine, in (b) homatropine methylbromide; ▲, in (b) codeine; ▼, thebaine, ○, papaverine; ◆, noscapine. Column: Nucleosil 5 C8 12 cm×4.6 mm i.d. Detection wavelength: 220 nm. Solvent velocity: 1.5 mm/sec. Pressure 7 M Pa.

TABLE 4

Precision of the HPLC method used for the tablets

Drug substance	Label claim (mg)	$\bar{x}_{10}$ (mg)	S_r (%)
Morphine hydrochloride	6.7	6.59	2.1
Codeine hydrochloride	0.38	0.365	3.0
Homatropine methylbromide	2.0	2.014	2.7
Thebaine hydrochloride	0.21	0.206	3.2
Papaverine hydrochloride	0.44	0.438	1.4
Noscapine hydrochloride	2.28	1.909	0.9

The means and the relative standard deviations were determined by the analysis of powdered tablets. Each of the 10 extracts were chromatographed in triplicate.

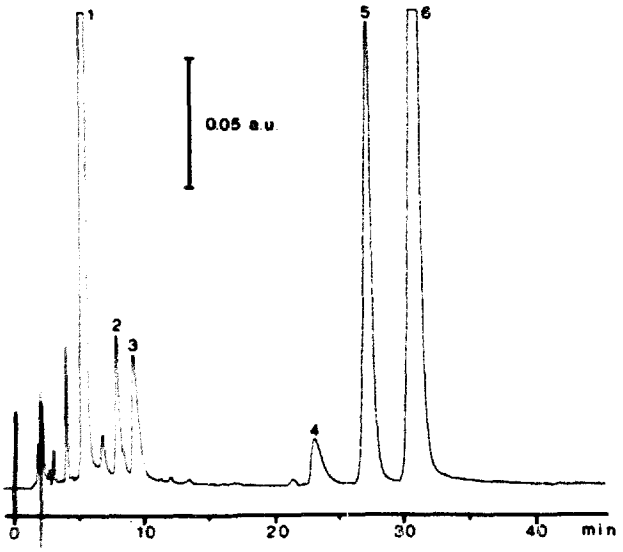

Fig. 6. HPLC-chromatogram of morphine (1), codeine (2), homatropine methylbromide (3), thebaine (4), papaverine (5) and noscapine (6) in tablet extract using two columns (12 cm×4.6 mm i.d.) of Nucleosil 5 C8 in series. Eluant: phosphate buffer (0.01 M, pH 5.0)–acetonitrile (6 : 4). Solvent velocity 1.5 mm/sec. Pressure 14 M P_a. Detection wavelength: 220 nm.

TABLE 5

Results of tablet content uniformity test

Drug substance	10 assays within (mg)	$\bar{x}_{10}$ (mg)	Content precision S_r (%)
Morphine hydrochloride	6.25 –6.67	6.47	0.0
Codeine hydrochloride	0.359–0.404	0.379	2.4
Homatropine methylbromide	1.946–2.134	2.021	1.4
Thebaine hydrochloride	0.200–0.216	0.208	0.0
Papaverine hydrochloride	0.366–0.519	0.439	10.0
Noscapine hydrochloride	1.877–2.046	1.939	2.4

Each extract of 10 individual tablets was chromatographed in triplicate. The content precision is corrected for analytical error (cf. Table 4).

TABLE 6

Results of analysis of the injection by HPLC

Drug substance	Label claim (mg/ml)	Found (mg/ml)
Morphine hydrochloride	26.8	26.65
Codeine hydrochloride	1.5	1.63
Homatropine methylbromide	1.5	1.41
Thebaine hydrochloride	0.84	0.853
Papaverine hydrochloride	1.76	1.72
Noscapine hydrochloride	9.1	8.65

Each of 2 dilutions were chromatographed in triplicate.

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