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The simultaneous determination of active ingredients in cough–cold mixtures by isocratic reversed-phase ion-pair high-performance liquid chromatography

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Abstract: A simple, rapid and accurate method for the simultaneous determination of active ingredients in cough–cold mixtures using isocratic reversed-phase ion-pair high-performance liquid chromatography has been developed. It involves the use of an octadecylsilane column as the stationary phase with methanol, water, tetrahydrofuran, phosphoric acid mixtures as mobile phase including sodium dioctylsulphosuccinate as the ion-pair agent. The pH of the mobile phase was adjusted to 4.6 by means of phosphoric acid and ammonium hydroxide solutions.

The proposed method involves the simple dilution of the samples with the mobile phase and the addition of metoclopramide hydrochloride as the internal standard. The active ingredients under investigation were chlorpheniramine, codeine, diphenhydramine, ephedrine, ethylmorphine, phenylephrine, phenylpropanolamine and pholcodine, which exist as various combinations in cough–cold mixtures.

The optimum composition of the mobile phase and the optimum flow rate were determined and are reported. The method was applied to the determination of active ingredients in seven commercially available cough–cold mixtures.

Keywords: *Isocratic reversed-phase ion-pair high-performance liquid chromatography; cough–cold mixtures; chlorpheniramine; codeine; diphenhydramine; ephedrine; ethylmorphine; phenylephrine; phenylpropanolamine; pholcodine.*

Introduction

Antihistamine, antitussive, and decongestant drugs are used extensively in cough–cold mixtures, two or more of which are often combined in the mixtures. In addition to active ingredients, most cough–cold mixtures contain dye(s), preservative(s), flavour(s), and a sweetening agent. Owing to interferences, no easy conventional methods are available for the simultaneous quantitative determinations of the active ingredients in cough–cold mixtures.

High-performance liquid chromatography (HPLC) has become a powerful tool for the analysis of pharmaceutical products and has been applied to the determination of the

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active components in cough–cold mixtures [1–4]. The use of ion-pair formation to enhance retention time and hence to better resolve the drugs of interest is becoming popular in reversed-phase high-performance liquid chromatography, and many applications have been reported [5–12]. It was the purpose of this study to develop a simple, rapid and accurate HPLC method which enables the determination of the active ingredients in a number of cough–cold mixtures. The active ingredients included in this study were chloropheniramine, codeine, diphenhydramine, ephedrine, ethylmorphine, phenylephrine, phenylpropanolamine and pholcodine.

Experimental

Instrumentation

The liquid chromatograph consisted of a controller (Beckman, model 421A), a solvent pump (Beckman, model 110B), an injection system (Altex, 210 valve), an analytical column Beckman 5 μ Ultrasphere-ODS 250 × 4.6 mm, i.d., a detector (Beckman, model 163 variable wavelength) and an integrator (Beckman, model 427). The column was protected by a guard column packed with the same packing material.

The flow rate was programmed with the first 5.5 min at 1.3 ml min^{-1} and then at 1.6 ml min^{-1} for the remainder of the chromatogram. The UV detector was set at a wavelength of 254 nm.

Chemicals and reagents

All drugs were of Pharmacopoeial or equivalent purity, and were used without further purification.

All organic solvents were HPLC grade and purchased from E. Merck (Darmstadt, FRG). All other reagents were of analytical grade. Water was doubly deionized.

Mobile phase

The mobile phase was prepared by first mixing methanol (715 ml) and tetrahydrofuran (50 ml), both freshly distilled, and then dissolving sodium dioctylsulphosuccinate (5.8 g) in this mixture. Water (234 ml) and phosphoric acid (85%, 1 ml) were then added after all the ion-pair agent had been dissolved. The mixture was then mixed thoroughly and the pH of the solution was adjusted to pH 4.6 by the addition of ammonia.

The mobile phase was then filtered through a 0.45 μm filter (Millipore). Finally, the mobile phase was degassed by suction and sonication, and then it was stored in an air-tight bottle.

Standard solutions

(i) *Internal standard solution.* Stock solution of metoclopramide hydrochloride (0.744 mg ml^{-1}) was prepared by weighing accurately 0.0744 g of the compound and dissolving it in 100 ml of the mobile phase in a volumetric flask.

(ii) *Standard solutions of the drugs.* Stock solution of chlorpheniramine maleate (0.5 mg ml^{-1}) was prepared by weighing 0.05 g of the compound in 100 ml of the mobile phase in a volumetric flask. The standard solutions were prepared by appropriate dilution of the stock solution with the mobile phase.

Similarly, codeine phosphate (0.3 mg ml^{-1}), diphenhydramine hydrochloride (1.5 mg ml^{-1}), ephedrine hydrochloride (2 mg ml^{-1}), ethylmorphine hydrochloride (0.45 mg

ml^{-1}), phenylephrine hydrochloride (0.7 mg ml^{-1}), phenylpropanolamine hydrochloride (2.5 mg ml^{-1}), phenylpropanolamine hydrochloride (2.5 mg ml^{-1}), pholcodine (1.5 mg ml^{-1}) and promethazine hydrochloride (0.3 mg ml^{-1}) stock solutions were prepared by dissolving the appropriate amounts of the respective compounds in 100 ml of the mobile phase.

Sample solutions

All sample solutions were prepared by diluting an appropriate amount of each sample with the mobile phase in a 10-ml volumetric flask, and adding 1 ml of the internal standard solution before diluting to the mark with the mobile phase.

The sample solutions were then filtered by a syringe equipped with a piece of 0.45 μm filter paper.

Determination

The column was equilibrated by mobile phase at a flow rate of 1 ml min^{-1} for 45 min, followed by washing with 50% methanol in water for 30 min. Before storage between daily operation, it was washed with pure methanol for 1 h to ensure long column life.

A sample/standard solution (20 μl) was injected into the system and the signals were recorded as peaks and their areas calculated by the integrator. The analyte peaks were identified by comparing the observed retention times with those of the respective standards.

The calibration graphs were plotted with the peak area ratios of the standards with respect to the area of the internal standard solution against the concentration of the respective standards. The unknown concentration of each component in the sample was determined by interpolation on the appropriate calibration graph.

Results and Discussion

Column and mobile phase selection

Preliminary experiments indicated that qualitative separation of the drugs under study could be obtained using an octadecylsilane (μ Bondapak C_{18}) column and a mobile phase containing methanol, water, tetrahydrofuran and phosphoric acid, with sodium dioctylsulphosuccinate added as the ion-pairing agent as described by Halstead [8]. Thus the column and the mobile phase described above were chosen for the present study. The relative proportions of methanol and tetrahydrofuran were then optimized in order to yield the best peak shapes and the shortest possible retention times for the drugs under study.

Optimization of conditions

Effect of tetrahydrofuran content. Whilst tetrahydrofuran added to the mobile phase reducing tailing [8], its presence in the mobile phase also was found to affect significantly the retention times of the drugs under study, as shown in Table 1. It can be seen that an increase of only 1% of tetrahydrofuran leads to a marked reduction in the retention times of the compounds under study. However, when the retention times are too short, overlapping of peaks of the active components and also overlapping with the peaks of the excipients may occur, which in turn may lead to poor resolution. Hence 5% tetrahydrofuran was chosen and the methanol content was then varied, to give a fine adjustment of the resolution of the peaks.

Table 1
The effect of tetrahydrofuran (THF) content in the mobile phase on the separation of some active ingredients in cough–cold mixtures*

Active ingredients	Retention time (min) THF content, 4%	THF content, 5%
Chlorpheniramine maleate	20.45	8.33
Codeine phosphate	5.44	3.49
Ephedrine hydrochloride	7.07	4.09

*The flow rate was set at 1.2 ml min^{-1}. The mobile phase contained, in addition to tetrahydrofuran, methanol (76.5%, v/v), 85% phosphoric acid (0.1%, v/v) and 5.8 g of sodium dioctylsulphosuccinate, and water was added to make up to 100% (v/v).

Table 2
The effect of methanol content in the mobile phase on the separation of some active ingredients in cough–cold mixtures*

Active ingredients	Methanol content:	Retention time (min) 70%	71.5%	73%	76.5%
Chlorpheniramine maleate		15.56	11.17	10.88	7.58
Codeine phosphate		4.57	3.99	3.86	3.21
Ephedrine hydrochloride		6.12	5.15	4.90	3.75
Phenylephrine hydrochloride		3.92	3.54	3.36	—
Phenylpropanolamine hydrochloride		5.86	5.00	4.77	—
Metoclopramide hydrochloride (internal standard)		7.07	5.96	5.81	—

*The flow rate was set at 1.3 ml min^{-1}. The mobile phase contained, in addition to methanol, tetrahydrofuran (5%, v/v), 85% phosphoric acid (0.1%, v/v) and 5.8 g of sodium dioctylsulphosuccinate, and water was added to make up to 100% (v/v).

Effect of methanol content. Next the effect of methanol was studied by varying its content in the mobile phase in the range of 70–76.5% (v/v), the results are shown in Table 2.

As expected, the retention time of the drugs decreased gradually as the methanol content increased from 70 to 76.5%. With 76.5% of methanol in the mobile phase, the excipients' peaks overlapped seriously with the codeine phosphate peak. However, with 73% of methanol, the peaks for codeine phosphate and ephedrine hydrochloride, phenylephrine hydrochloride and phenylpropanolamine hydrochloride were just resolved. However, with 71.5% methanol present, all the peaks were completely resolved. Also, the chlorpheniramine maleate peak was quite sharp when 73 or 71.5% of methanol was used, whereas with 70% of methanol, the peak was diffuse and broad. Consequently, a methanol concentration of 71.5% was chosen for the mobile phase.

Effect of flow rate. In addition to the composition of the mobile phase, the flow rate also could affect the retention times of the drugs. It can be seen from the data in Table 3 that the retention times of the drugs decrease gradually as the flow rate increased. With the flow rate set at 1.1 ml min^{-1}, the chlorpheniramine peak was broad and came out late in the chromatogram. At a flow rate of 1.5 ml min^{-1}, the peaks of the compounds studied were not completely resolved and the back pressure was high. While using a flow rate of 1.3 ml min^{-1}, the peaks were well separated and the chlorpheniramine peak was quite sharp. Hence the flow rate of 1.3 ml min^{-1} was chosen for analysis. It is anticipated that the chlorpheniramine peak could be improved further by using flow programming.

Table 3
The effect of flow rate on the separation of some active ingredients in cough–cold mixtures*

Active ingredients	Flow rate (ml min^{-1}):	Retention time (min) 1.1	1.3	1.5
Chlorpheniramine maleate		13.22	11.17	9.85
Codeine phosphate		4.58	3.99	3.49
Ephedrine hydrochloride		5.94	5.15	4.51
Phenylephrine hydrochloride		—	3.54	3.00
Phenylpropanolamine hydrochloride		—	5.00	4.26
Metoclopramide hydrochloride (internal standard)		6.98	5.96	5.22

*Mobile phase contained 71.5% (v/v) of methanol, 5% (v/v) of tetrahydrofuran, 0.1% (v/v) of 85% phosphoric acid, 23.4% (v/v) of water and 5.8 g of sodium dioctylsulphosuccinate.

Flow programming. The advantage of flow rate programming over solvent programming is that the former does not disturb the column equilibrium and hence the baseline would not be expected to drift when this technique was applied. The effect of flow rate programming on the retention of chlorpheniramine, ephedrine and codeine was studied and the results are shown in Table 4.

The retention times of the chlorpheniramine peak for programmes 3 and 4 were found to be similar. However, under the high flow rate of 1.7 ml min^{-1} in programme 4, the back pressure detected by the solvent pump was up to 4700 psi, which was approaching the highest acceptable limit for the chromatograph under consideration. On the other hand, using programme 3, the back pressure was about 4000 psi, which was considered to be safe and acceptable. Although the retention time of the chlorpheniramine peak using programme 2 was 10.29 min which was satisfactory, the shorter retention time using programme 3 was preferred.

In summary, the mobile phase chosen for analysis had the following composition:

Methanol	715 ml (71.5%, v/v)
Tetrahydrofuran	50 ml (5%, v/v)
Water	234 ml (23.4%, v/v)
Phosphoric acid (85%)	1 ml (0.1%, v/v)
Sodium dioctylsulphosuccinate	5.8 g
pH	4.6

and the flow rate was programmed as follows:

Run time, min	Flow rate (ml min^{-1})
0–5.5	1.3
5.5 Onward	1.6

A typical chromatogram of chlorpheniramine maleate, codeine, ephedrine and metoclopramide is given in Fig. 1.

Retention times of the active ingredients

The retention times of the active ingredients in common cough–cold mixtures were determined using the proposed method and the results are summarized in Table 5. The pK_a values of the respective compounds [13] are also included for reference.

All of the active ingredients under study are amines and are expected to be protonated in acidic medium. The ion-pairing agent, dioctylsulphosuccinate, acted as the counter ion for the protonated amines with the octyl groups rendering the ion pairs hydrophobic for

Table 4
The retention time of some active ingredients in cough–cold mixtures with and without the application of flow rate programming*

Programme no.	1	2	3	4
Active ingredients		*Retention time (min)*		
Codeine phosphate	3.99	3.97	3.95	3.95
Ephedrine hydrochloride	5.15	5.12	5.05	5.10
Chlorpheniramine hydrochloride	11.17	10.29	9.86	9.77
Programme no.	1	2	3	4
Run time (min)		*Flow rate (ml min^{-1})*		
0–5.5	1.3	1.3	1.3	1.3
5.5 onward	1.3	1.5	1.6	1.7

*The mobile phase as described in Table 3.

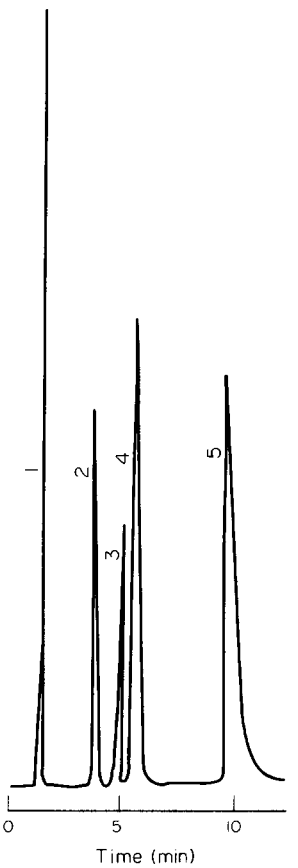

Figure 1
A typical chromatogram of maleate (1), codeine (2), ephedrine (3), metoclopramide (4) and chlorpheniramine (5).

adsorption by the C_{18} column. As a result, the drugs with the higher degree of protonation on the amine group (i.e. greater pK_a) would be expected to have longer retention times. However, results in Table 5 show that the correlation between retention time and pK_a is not very good, which implies that other factors in addition to the pK_a values should be considered.

Table 5
The retention times of some active ingredients in cough–cold mixtures

	Retention time (min)	pK_a
Phenylephrine	3.51	8.9, 10.1 (20°C)
Codeine	3.94	8.2 (20°C)
Ethylmorphine	4.33	8.2 (20°C)
Phenylpropanolamine	4.69	9.4 (20°C)
Ephedrine	5.05	9.6 (25°C)
Metoclopramide (internal standard)	5.86	—
Diphenhydramine	9.43	9.0 (25°C)
Chloropheniramine	9.86	9.1 (25°C)
Pholcodine	13.38	8.0, 9.3 (37°C)
Promethazine	14.31	9.1 (25°C)

The three compounds having the largest retention times, namely, chloropheniramine, pholcodine and promethazine, do not have the largest pK_a values. The high retention times may be ascribed to the fact that all three compounds contain two amine groups which may be protonated.

Both phenylpropanolamine and ephedrine have smaller retention times than expected possibly due to the fact that they contain one hydrophilic hydroxyl group. Furthermore, phenylephrine, having two hydroxyl groups, has the shortest retention time.

Finally, although ethylmorphine and codeine have the same pK_a values, their retention times are different. Codeine, being methylmorphine, is comparatively less hydrophobic than ethylmorphine, and it is not surprising to find its retention time to be slightly shorter.

Calibration graphs

The calibration graphs of the various compounds under study were plotted with the ratio of the peak area of each compound to that of the internal standard, 0.0744 mg ml^{-1} metoclopramide, versus the concentration of the compound.

It was found that all calibration graphs except that for codeine phosphate passed through the origin. Using linear regression analysis, the slope and correlation coefficient of the graphs were calculated and are shown in Table 6.

Hence the determination of active ingredients in various cough–cold mixtures could be readily carried out using the proposed method with concentrations within the linear ranges of these calibration graphs.

Table 6
The slope, correlation coefficient and linear range for the calibration graphs

Standards	Slope (10^{-2} ml mg^{-1})	Correlation coefficient	Response was linear up to (mg ml^{-1})
Chlorpheniramine maleate	766.63	0.99988	0.200
Codeine phosphate	641.92	0.99761	0.122
Diphenhydramine hydrochloride	104.22	0.99995	1.235
Ephedrine hydrochloride	75.28	0.99926	0.954
Ethylmorphine hydrochloride	586.85	0.99975	0.147
Phenylephrine hydrochloride	169.54	0.99957	0.284
Phenylpropanolamine hydrochloride	78.07	0.99972	1.094
Pholcodine	294.02	0.99170	0.500

Table 7
The assay results for the determination of various active ingredients in cough–cold mixtures

Samples	Amount found* (mg/5 ml)	Average† (mg/5 ml)	Label (mg/5 ml)
1. *Wani-Tussin Syrup*			
Chlorpheniramine maleate	0.98 1.00 (2.0) 1.02	1.00 (0)	1
Phenylpropanolamine hydrochloride	24.6 24.5 (0.2) 24.6	24.6 (−1.6)	25
Pholcodine	4.81 4.83 (0.5) 4.78	4.81 (−3.8)	5
2. *Uni-Ephrine Syrup*			
Ephedrine hydrochloride	10.4 10.3 (0.6) 10.3	10.3 (+3.0)	10
Ethylmorphine hydrochloride	1.05 1.04 (0.6) 1.05	1.04 (+4.0)	1
3. *Uni-phen Expectorant*			
Diphenhydramine hydrochloride	13.7 14.1 (1.9) 14.2	14.0 (0)	14
4. *Propahistine Syrup*			
Chlorpheniramine maleate	1.91 2.05 (3.5) 1.98	1.98 (−1.0)	2
Phenylpropanolamine hydrochloride	18.6 18.3 (0.8) 18.4	18.4 (−1.8)	18.75
5. *Uni-Vasin Liquid*			
Chlorpheniramine maleate	3.81 3.93 (1.6) 3.85	3.86 (−3.5)	4
Phenylephrine hydrochloride	4.79 4.89 (1.5) 4.75	4.81 (−3.8)	5
Phenylpropanolamine hydrochloride	5.10 5.08 (3.2) 4.81	5.00 (0)	5
6. *Uni-Pamine C.E. Syrup*			
Chlorpheniramine maleate	1.00 0.93 (3.9) 0.99	0.97 (−3.0)	1
Codeine phosphate	9.50 9.65 (2.4) 9.96	9.70 (−3.0)	10

Table 7 (Continued)

Samples	Amount found* (mg/5 ml)	Average† (mg/5 ml)	Label (mg/5 ml)
Ephedrine hydrochloride	4.81 4.86 (1.9) 4.99	4.89 (−2.2)	5
7. *Wani-cough Syrup*			
Chlorpheniramine maleate	2.07 1.95 (3.4) 1.97	2.00 (0)	2
Codeine phosphate	10.4 10.3 (2.8) 9.9	10.2 (+2.0)	10
Ephedrine hydrochloride	4.78 4.91 (1.6) 4.78	4.82 (−3.6)	5

*The relative standard deviation of the triplicate results in percent is shown in parentheses.
†The relative deviation from label value in percent is shown in parentheses.

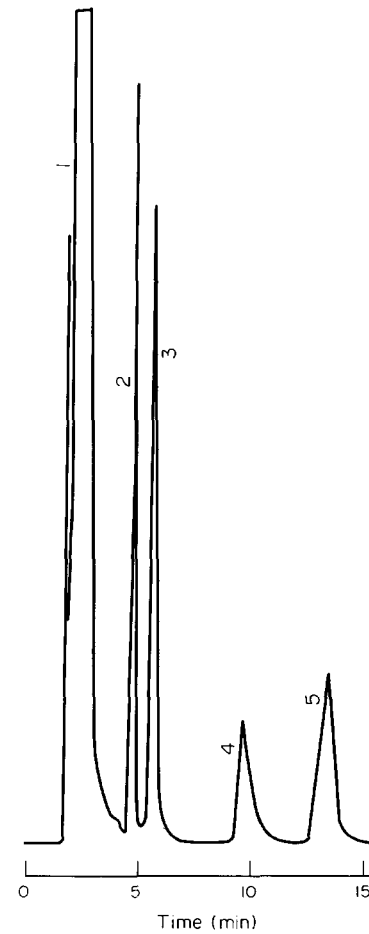

Figure 2
The chromatogram of sample 1, with peaks of excipients (1), phenylpropanolamine (2), metoclopramide (3), chlorpheniramine (4) and pholcodine (5).

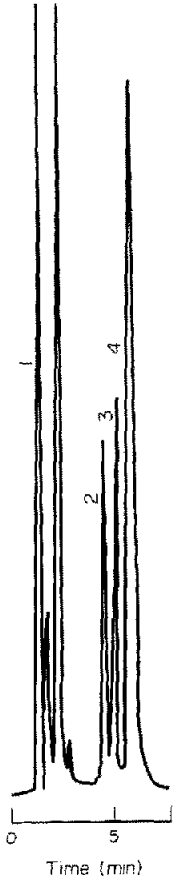

Figure 3
The chromatogram of sample 2, with peaks of excipients (1), ethylmorphine (2), ephedrine (3) and metoclopramide (4).

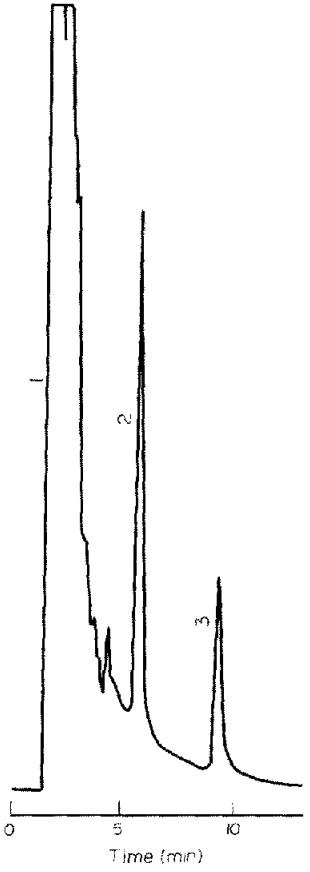

Figure 4
The chromatogram of sample 3, with peaks of excipients (1), metoclopramide (2) and diphenhydramine (3).

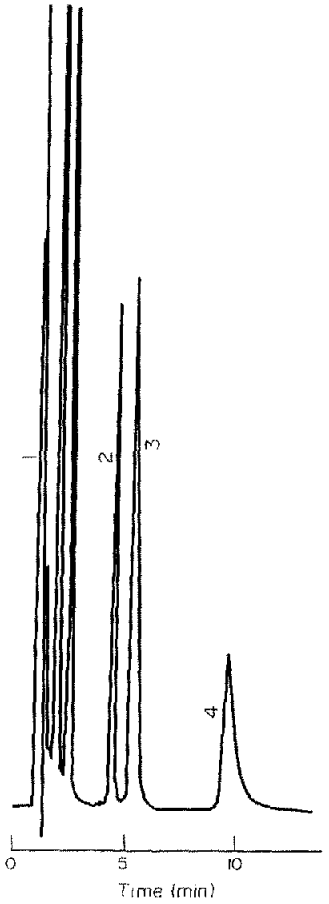

Figure 5
The chromatogram of sample 4, with peaks of excipients (1), phenylpropanolamine (2), metoclopramide (3) and chlorpheniramine (4).

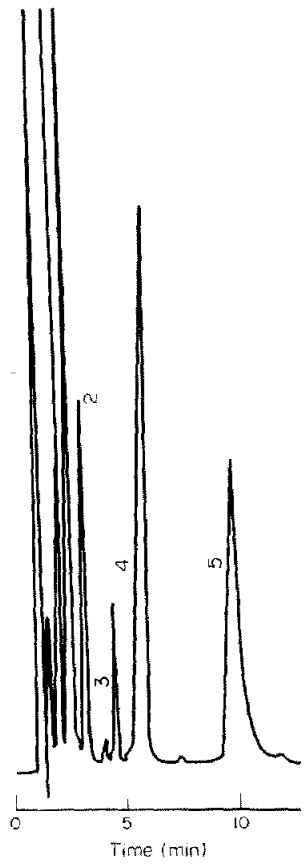

Figure 6
The chromatogram of sample 5, with peaks of excipients (1), phenylephrine (2), and phenylpropanolamine (3), metoclopramide (4) and chlorpheniramine (5).

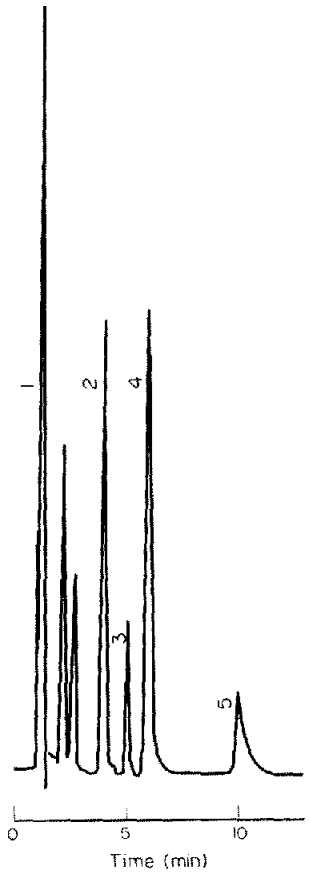

Figure 7
The chromatogram of sample 6, with peaks of excipients (1), codeine (2), ephedrine (3), metoclopramide (4) and chlorpheniramine (5).

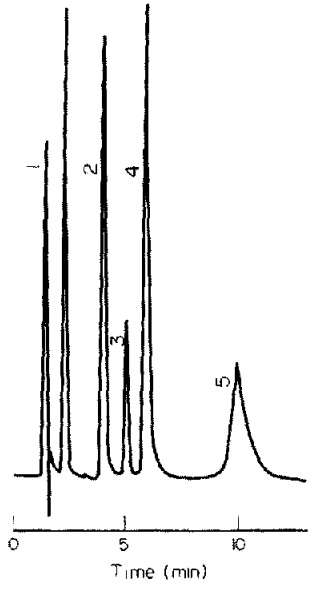

Figure 8
The chromatogram of sample 7, with peaks of excipients (1), codeine (2), ephedrine (3), metoclopramide (4) and chlorpheniramine (5).

In aqueous solution promethazine is degraded by heat and light with the rate of degradation being more rapid in the presence of air or oxygen [13]. It was noticed that promethazine was degraded to give four additional peaks with retention times of 5.05, 5.38, 7.56 and 14.31 min, respectively. As a result, although the determination of promethazine by the proposed method was possible, the actual determination of promethazine can only be carried out using freshly-prepared real samples and standards. As no freshly prepared real sample containing promethazine was available, the cough–cold mixture containing promethazine was excluded in the real sample analysis.

Determination of active ingredients in cough–cold mixtures

The content of chlorpheniramine hydrochloride, codeine phosphate, diphenhydramine hydrochloride, ephedrine hydrochloride, ethylmorphine hydrochloride, phenylephrine hydrochloride, phenylpropanolamine hydrochloride and pholcodine in seven cough–cold mixtures was determined in triplicate by the proposed method. The samples under study contained combinations of several active ingredients which were identified by comparing the retention time of the peaks observed with those obtained from standard solutions containing the respective active ingredients examined under the same conditions. The results were checked with the corresponding label values. The assay results for the samples studied are presented in Table 7 and typical chromatograms shown in Figs 2–8. There was a close agreement between the results obtained from the proposed method and the label values. The relative standard deviations for these determinations were found to be in the range 0.2–3.9%, and the precision may be considered to be good. Thus both the accuracy and precision of the proposed method are satisfactory.

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