

High-performance liquid chromatographic determination of active ingredients in cough–cold syrups with indirect conductometric detection

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Abstract

An HPLC method using indirect conductometric detection is proposed for the simultaneous determination of eight active ingredients in cough–cold syrups. It involves the use of an Ultrasphere 5 μm Spherical 80 Å Pore CN analytical column (250 mm \times 4.6 mm) as the stationary phase with a mixture of water, acetonitrile and ethanol (38:60:2) containing 1 mM perchloric acid as the mobile phase. The active ingredients included bromhexine hydrochloride, chlorpheniramine maleate, codeine phosphate, dextromethorphan hydrobromide, diphenhydramine hydrochloride, ephedrine hydrochloride, papaverine hydrochloride and phenylephrine hydrochloride. Derivatization of the drugs is not required.

The effect of background conductance on detector response, and the factors affecting column separation of the ingredients were studied. The detector responses for all the drugs are similar. The calibration graphs exhibited a wide linear concentration range of 0–500 $\mu\text{g}/\text{ml}$ for a sample size of 20 μl with correlation coefficients of better than 0.999 for all the drugs under study. The relative standard deviation for 10 replicate measurements of the content of each drug in cough–cold syrups was always less than 3%. The method has been applied to determine the active ingredients in a number of cough–cold syrups.

1. Introduction

Cough–cold syrups usually contain a number of active ingredients to cater for the therapeutic needs of cough and common cold. The action, properties, and the structures of these ingredients have been detailed in various references [1–5]. Liquid chromatography (HPLC) is the commonest approach to assay the drugs because the method is accurate, simple and does not

require prior conversion of the drugs to the base form as needed in the gas–liquid chromatographic (GLC) methods. The HPLC methods have been investigated by many workers [6–12], and most of them were based on ion-pair formation, and the detection method typically based on measuring the UV absorbance of the analytes. The methods cannot be applied to drugs without UV absorbing character.

Other analytical techniques such as derivative spectrophotometric methods [13–15] and GLC methods [7,8,16] have also been reported but the

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spectrophotometric methods could only determine two to three components simultaneously, and the GLC methods involve more tedious sample preparation steps than the HPLC methods.

The present work proposes to employ indirect conductometric detection to determine the active ingredients in cough–cold syrups after HPLC separation. The advantages of the proposed detection method are: (1) the method works equally well for drugs without UV absorptivity; (2) the detector responses for all the drugs are similar, and (3) it can reduce the number of dilution steps required in the current HPLC methods. The effect of background conductance on detector response, and the factors affecting column separation of the ingredients were studied. The results of analysis were compared with those obtained by HPLC methods using UV detection [11,12].

2. Experimental

2.1. Apparatus

The HPLC system used consisted of a Waters 510 HPLC pump (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) with an Alltech Free-Flow pulse dampener (Alltech Associates, Deerfield, IL, USA), a Wescan Model 21511001 conductivity detector with Model 24020001 temperature controller and Model 26650051 column compartment (Alltech), a Rheodyne injection valve with a 20- μ l sample loop (Rheodyne, Berkeley, CA, USA), and a Beckman 427 signal integrator (Beckman Instruments, Altex Division, San Ramon, CA, USA). An analytical column from Beckman, Ultrasphere 5 μ m Spherical 80 Å Pore CN analytical column (250 mm \times 4.6 mm) was used. The proposed mobile phase was water–acetonitrile–ethanol (38:60:2) containing 1 mM HClO₄. Other mobile phases with different acetonitrile contents and perchloric acid strength were also used as indicated in the Results and Discussion section.

Instrumental settings were: flow rate, 1 ml/

min; column temperature, 30°C; detector zero suppression, 2; detector range, 1 or 10; and chart speed, 0.5 cm/min.

Peaks were detected as negative changes in conductance, and the detector–integrator connections were reversed in polarity to give positive display of peaks on the integrator.

2.2. Reagents

All the drugs were of BP or USP quality and were used without further purification. HPLC grade solvents were used to prepare the mobile phase. All other chemicals were of analytical reagent grade. Water used was distilled and deionized by passing through Millipore Milli-Q 50 ultra pure water system (Millipore, Molsheim, France).

2.3. Standard solutions

Standard solutions of the drugs used for HPLC measurements were prepared by dissolving the drugs in the mobile phase and diluting to the desired concentrations.

2.4. Determination

Each syrup was diluted with the mobile phase to give a final concentration of the analyte in the range of 5 to 100 μ g per ml, and a single dilution in the ratio of 1 to 25 or 1 to 50 usually sufficed. The solution was homogenized by shaking, filtered and then injected into the chromatograph.

2.5. The counter-check HPLC method

The active ingredients in the cough–cold syrups were counter checked by HPLC methods with UV detection as proposed by Koziol et al. [11] and Chao et al. [12] but with slight modification in the composition of the mobile phase to optimise column separation.

The HPLC system consisted of a Beckman 110B Solvent Delivery Module, a Waters Lambda-Max Model 481 LC UV Detector and a Beckman 427 integrator. The injection port was

a Rheodyne injector with sample loop size of 20 μ l. An Alltech 5 μ m Econosphere C₁₈ stainless steel column (250 mm \times 4.6 mm) was used.

3. Results and discussion

3.1. Characteristics of the chromatogram

Retention of the drugs

The retention times (t_R) and capacity factors of the drugs using different mobile phases are listed in Table 1.

There was baseline resolution of all the drugs with the exception of codeine–ephedrine, where the resolution was about 0.9. In general, all the above drugs could be determined simultaneously in a single chromatographic run, provided that the concentration of each drug is not so high as to produce a very large peak obscuring a neighbouring one.

Solvent peak intensity and analyte concentration

The chromatogram was found to exhibit a negative solvent peak at about 2.5 min. As the

polarity of the conductivity detector was reversed, the solvent peak was actually caused by an increase in conductance of the eluent. The peak area or peak height of the solvent peak was found to be directly proportional to the analyte concentration, and the correlation coefficient was better than 0.99 for both peak area and peak height.

3.2. Effect of the composition of mobile phase on the retention time

Changing the water:acetonitrile ratio

It can be seen from Table 1 that the retention times decreased with increase in acetonitrile content, where there was a parallel decrease in the polarity of the mobile phase. Moreover, the rate of decrease was higher for drugs with higher molecular mass, such as bromhexine and chlorpheniramine, which are also more bulky and less polar in nature, compared with those having lower molecular mass and being more polar, such as ephedrine and phenylephrine. The results indicate that hydrophobic interaction of the drugs with the column was one of the essential

Table 1
Effect of acetonitrile content on the retention times (t_R) of the drugs

Drug	t_R (min) in mobile phase			Capacity factor k' (Mobile phase B)
	A	B	C	
Phenylephrine hydrochloride	11.9	8.0	7.4	2.20
Ephedrine hydrochloride	12.7	9.4	7.6	2.76
Codeine phosphate	14.3	9.0	8.1	2.62
Papaverine hydrochloride	19.6	12.1	8.0	3.84
Dextromethorphan hydrobromide	25.5	13.1	8.7	4.24
Diphenhydramine hydrochloride	26.2	14.2	8.3	4.67
Bromhexine hydrochloride	34.8	17.4	8.8	5.96
Chlorpheniramine maleate	35.7	21.2	14.3	7.48

Mobile phases: (A) water–acetonitrile–ethanol (58:40:2) with 1 mM HClO₄; (B) water–acetonitrile–ethanol (38:60:2) with 1 mM HClO₄; (C) water–acetonitrile–ethanol (18:80:2) with 1 mM HClO₄.

operating mechanism for the above column separation.

The mobile phase B shown in Table 1 was chosen as the solvent system for the proposed method as it gave well resolved peaks within about 20 minutes or less for all the drugs under study. Note that two percent of ethanol was added to the above mobile phase to improve column separation efficiency and to enable better degassing of the solvents.

Changing the concentration of the conducting species

The role of perchloric acid in the mobile phase is to create a conducting background to enable detection. Our study on the retention behaviour of the drugs indicated that the retention time decreased with increase in the concentration of perchloric acid. For example, the retention time of the drugs at 1 mM HClO₄ was found to be only about 0.6 of that at 0.5 mM of the acid. A plot of log *t_R* versus the concentration of perchloric acid, from 0.5 mM to 2.0 mM, was found to give a correlation coefficient (*r*) in the range of $-1 < r < -0.9$ for all the drugs under study.

A similar trend in retention time was also observed when the conducting species used was potassium sulphate.

As a similar trend in retention times was observed for different conducting species, the change in retention was unlikely to be caused by the reaction of the eluent conducting species with the drugs. Although protonation of the drugs may, to a certain extent, increase the polarity of the drugs and hence decrease their hydrophobic interaction with the column, this is unlikely to be significant, as replacement of hydronium ion with potassium ion resulted in similar changes. A more plausible explanation is that the higher concentration of the conducting species results in their higher competing power for the active sites on the column and hence the eluting power for the drugs is greater. Moreover, the higher concentration of the conducting species would also increase the polarity of the column surface and hence reduce the hydrophobic interaction of the column with the drugs, resulting in reduction in retention. The decrease in column capacity due to decrease in resin pore

size as a result of an increase in ionic content of the mobile phase as proposed by some authors [17] may also be another explanation for the observation.

3.3. Detector response

Effect of molecular mass of the drugs

When the drugs were dissolved in the mobile phase and loaded onto the chromatograph, the counter anions of the drugs being very soluble in the mobile phase were little retained and would be eluted from the column, and hence the detector response of each drug was entirely due to the drug irrespective of the counter anion. It follows that in the discussion below, only the

Table 2
Detector response of ingredients in cough-cold syrups with mobile phase B in Table 1

Ingredient ^a	Detector response ^b (<i>A</i> × <i>M</i>)
Bromhexine hydrochloride (376.1)	8.36
Chlorpheniramine maleate (274.8)	10.08
Codeine phosphate (299.4)	8.15
Dextromethorphan hydrobromide (271.4)	7.75
Diphenhydramine hydrochloride (255.4)	8.19
Ephedrine hydrochloride (165.2)	8.04
Papaverine hydrochloride (339.4)	8.52
Phenylephrine hydrochloride (167.2)	8.10
Average	8.4 (R.S.D. 8.6%)

^a Molecular mass (*M*) of the drugs (base form) in parentheses.

^b *A* = Area counts per μg.

respective molecular mass (M) of each drug in the base form was considered.

The area response per μg (A) (arbitrary unit) of each drug multiplied by the respective molecular mass (M), ($A \times M$), is shown in column 2 of Table 2. It can be seen that $A \times M$ is essentially constant and has an average value of 8.4 (R.S.D. 8.6%) and six out of the eight values cluster around the value 8.2. $A \times M$ relates the detector response to the same number of molecules as shown below:

$$\begin{aligned} A \times M &= (\text{Area count}/\mu\text{g}) \times M \\ &= \text{Area count}/(\mu\text{g}/M), \text{ and} \\ \mu\text{g}/M &\propto \text{no. of molecules per } \mu\text{g} \end{aligned}$$

The above result implies that the detector response is directly proportional to the number of molecules, and not dependent on the nature of the analyte.

Effect of the concentration of the conducting species

The peak area response per μg of the drugs eluted with mobile phases having perchloric acid concentrations from 0.5 mM to 2.0 mM were found to be essentially constant and with values

within 10% of those given in Table 2. This observation is consistent with our discussion on detection theory that displacement of the background conducting species was quantitative and proportional to the number of analyte species.

3.4. Detection theory

When a solution of the drug was loaded onto the column, the drug in the protonated form was retained by the column while the counter anion was eluted at almost the same time as the solvent. The eluent conducting species, E, which is HClO_4 in the present system, dissociates to give anions and cations. Under column equilibrium conditions, the background conductance of the eluent, G_B , as indicated by the conductivity detector of the system can be deduced according to Fritz et al. [18,19] as follows:

$$G_B = \frac{(\lambda_{o,E^+} + \lambda_{o,E^-}) C_E I_E}{10^{-3} K} \quad (1)$$

where K is the cell constant, λ_{o,E^+} and λ_{o,E^-} are respectively the limiting ionic conductance of the cation and anion of the eluent conducting species, and C_E and I_E are respectively the

Table 3
Results of recovery test and precision study

Ingredient	% Recovery ^a	Precision ^b (R.S.D., %)
Bromhexine hydrochloride	99.6	2.2
Chlorpheniramine maleate	99.8	2.8
Codeine phosphate	103.5	2.0
Dextromethorphan hydrobromide	99.1	1.9
Diphenhydramine hydrochloride	99.5	1.9
Ephedrine hydrochloride	95.1	1.7
Papaverine hydrochloride	98.2	2.6
Phenylephrine hydrochloride	97.7	1.7

^a Average value of three determinations.

^b The relative standard deviation of ten replicate determinations of the drugs in cough-cold syrups.

Table 4
Assay results of active ingredients in cough–cold syrups

Sample	Ingredient	Label value, mg per 5 ml	Percentage of label claim		
			Proposed method ^a	HPLC with UV detection ^b	
1	Bromhexine hydrochloride	2.5	78 (3.9)	79	
	Codeine phosphate	4.5	100 (0.8)	102	
	Diphenhydramine hydrochloride	4.5	100 (2.5)	101	
	Ephedrine hydrochloride	4.5	104 (1.1)	107	
	Papaverine hydrochloride	1.25	100 (2.0)	100	
	2	Chlorpheniramine maleate	1	100 (5.0)	97
		Dextromethorphan hydrobromide	5	101 (1.3)	101
Phenylephrine hydrochloride		5	121 (0.3)	122	
3		Chlorpheniramine maleate	1	102 (3.1)	95
	Dextromethorphan hydrochloride	5	104 (0.9)	101	
	Phenylephrine hydrochloride	5	99 (0.6)	99	
	4	Codeine phosphate	9	101 (2.6)	100
Ephedrine hydrochloride		7.2	104 (0.5)	109	
5		Chlorpheniramine maleate	4	101 (0.9)	101
	Phenylephrine hydrochloride	5	96 (0.5)	96	
	6	Chlorpheniramine maleate	2	99 (2.2)	101
Ephedrine hydrochloride		5	97 (2.5)	105	
7		Chlorpheniramine maleate	4	100 (0.8)	103
8	Bromhexine hydrochloride	2.5	101 (1.9)	100	
	Codeine phosphate	4.5	99 (1.1)	101	
	Diphenhydramine hydrochloride	7.5	100 (0.8)	100	
	Ephedrine hydrochloride	7.5	101 (1.9)	100	

Table 4 (Continued)

Sample	Ingredient	Label value, mg per 5 ml	Percentage of label claim		
			Proposed method ^a	HPLC with UV detection ^b	
9	Bromhexine hydrochloride	4	99 (0.7)	102	
	Codeine phosphate	4.5	94 (1.1)	101	
	Diphenhydramine hydrochloride	4	100 (2.0)	100	
	Ephedrine hydrochloride	4	101 (0.9)	102	
	Papaverine hydrochloride	1.25	100 (1.2)	102	
	10	Diphenhydramine hydrochloride	14	97 (3.0)	^c

^a Average of three determinations and the relative standard deviation of the triplicate results in percent is shown in parentheses.

^b Average value of two determinations.

^c Sample insufficient for measurement.

concentration of the eluent conducting species and the fraction of these species present in the ionic form.

The drug would displace from the column active sites an equivalent number of eluent cations so as to maintain electroneutrality. The eluted eluent cations would cause an instantaneous increase in detector signal and result in the solvent peak the intensity of which, as described above, was found to be proportional to the analyte concentration. Further, when the sample band passes through the detector, the eluent cation concentration is given by:

$$[E^+]_{\text{At sample elution}} = C_E I_E - C_S I_S \quad (2)$$

where C_S is the concentration of the drug in the sample, and I_S is the degree of ionization of the drug. The conductance measured at the detector during sample elution is therefore given by:

$$G_{\text{Elution}} = \frac{\lambda_{o,E^+} (C_E I_E - C_S I_S)}{10^{-3} K} + \frac{\lambda_{o,E^-} C_E I_E}{10^{-3} K} + \frac{\lambda_{o,S^-} C_S I_S}{10^{-3} K} \quad (3)$$

where λ_{o,S^+} is the limiting ionic conductance of the drug. Note that the first term in Eq. 3 is the conductance due to the eluent cation, whose concentration is given by Eq. 2; the second term is the conductance due to the eluent anion, and the third term is the conductance due to the drug itself. The change in conductance (ΔG) associated with the elution of the sample solute is obtained by subtracting the background conductance (Eq. 1) from the conductance during sample elution (Eq. 3). Assuming $I_S = 1$, and $\lambda_{o,S^+} \ll \lambda_{o,E^+}$, since the drugs are bulky and their conductances are much smaller than that of H^+ , ΔG takes the form:

$$\Delta G = -\frac{\lambda_{o,E^+} C_S}{10^{-3} K} \quad (4)$$

which indicates that (1) the detector response will be proportional to C_S , the concentration of the drug, and the limiting ionic conductance of the eluent cation, and (2) the signal would be a decrease in conductance. This explains the negative conductance signal as well as the linear relationship of the detector response with the drug concentration.

3.5. Calibration graphs

The calibration graphs were obtained by plotting the peak area (integrator count, unit arbitrary) against the corresponding concentrations of the drug in the mobile phase. In quantitative determination of the active ingredients in cough-cold syrups the results were calculated from the linear regression equations of the calibration graphs, which were found to be linear from 0 to 500 $\mu\text{g/ml}$ at least for a sample size of 20 μl , with correlation coefficients of better than 0.999 for all the plots of peak area vs concentration.

The smallest quantity of each drug that can be measured with an accuracy of within $\pm 20\%$ of the actual quantity by the proposed system was about 10 nanograms.

3.6. Recovery and precision

The accuracy of the method was studied by adding known amount of each drug separately to a cough-cold syrup where it was known to be absent, and then determining it using the proposed method. Most drugs have recoveries of around 98–99%. Only codeine and ephedrine have poorer recoveries of 103.5 and 95.1 respectively.

The precision of the method was studied by determining the concentration of each drug in cough-cold syrups ten times by the proposed method.

The results of recovery and precision study as shown in Table 3 indicate that the method is reliable and precise.

3.7. Determination of active ingredients in cough-cold syrups

The contents of the eight drugs in nine cough-cold syrups were determined by the proposed method and the results are presented in Table 4. The chromatograms of two cough-cold syrups are shown in Figs. 1 and 2, respectively. Apart from slightly high deviations from spectrophotometric

detection observed for some of the results of codeine and ephedrine, which were mainly due to their incomplete baseline resolution by the cyano column and their closeness to the excipient peak, there were in general close agreements between the results obtained using the proposed method and those using the HPLC

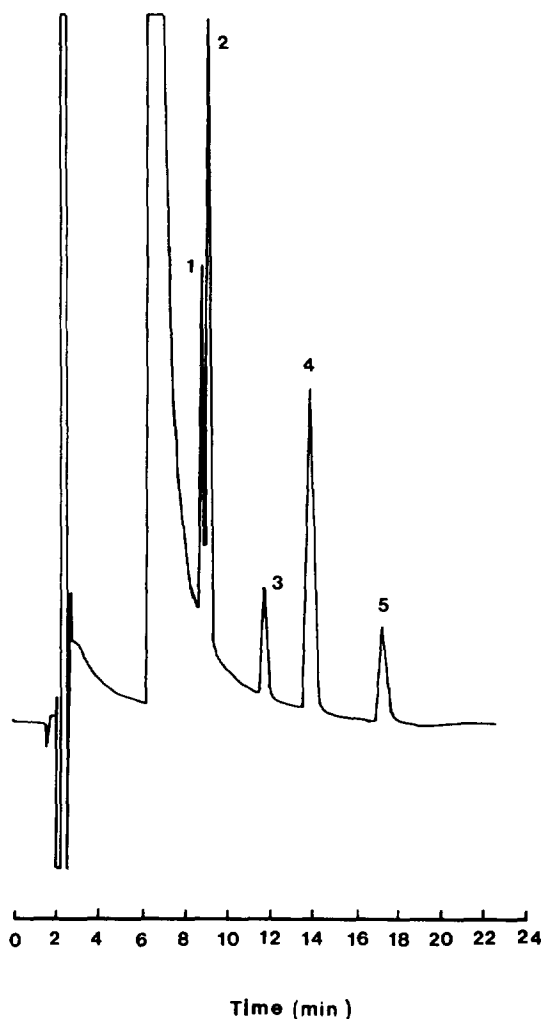


Fig. 1. Indirect conductometric detection of ingredients in cough-cold syrup No. 1. Peaks: 1 = codeine phosphate (0.36 μg); 2 = ephedrine hydrochloride (0.36 μg); 3 = papaverine hydrochloride (0.1 μg); 4 = diphenhydramine hydrochloride (0.36 μg) and 5 = bromhexine hydrochloride (0.2 μg). Excipient peak at about 7 min.



Fig. 2. Indirect conductometric detection of ingredients in cough-cold syrup No. 3. Peaks: 1 = phenylephrine hydrochloride ($0.4 \mu\text{g}$); 2 = dextromethorphan hydrobromide ($0.4 \mu\text{g}$) and 3 = chlorpheniramine maleate ($0.08 \mu\text{g}$). Excipient peak at about 7 min.

method with spectrophotometric detection, the deviations being generally less than 5%.

4. Conclusion

The present study has shown that indirect conductometric detection is a sensitive, reliable

and accurate method for the determination of active ingredients in cough-cold syrups. A similar approach can be applied to determine other drugs in pharmaceutical preparations. The proposed detection method is sensitive, as it can detect the drugs down to nanogram levels, with a wide linear range of detector response, which covers most analytical applications on drugs analysis. The beauty of the method is that it does not rely on any specific functional group for detection, and hence, that drugs and other organic compounds which could not be detected previously because of the lack of specific functional group can now be detected.

The detector responses of all the drugs are similar and are proportional to the number of molecules, which enables the ingredients in many pharmaceutical preparations to be analysed in a single chromatographic run, and dilutions necessary to for the concentrations of the analytes to fall within the detector ranges can be greatly reduced. The method is straightforward and simpler than the commonly used HPLC methods involving ion pairing or derivatization.

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