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Short communication

High-performance liquid chromatographic determination of atropine and atropine-like alkaloids in pharmaceutical preparations with indirect conductometric detection

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Abstract

An HPLC method using indirect conductometric detection is proposed for the determination of atropine and atropine-like alkaloids in pharmaceutical preparations. It involves the use of an Ultrasphere 5 μm CN analytical column (250 mm×4.6 mm) and a mobile phase of water–acetonitrile–tetrahydrofuran (67:30:3, v/v/v), containing 1 m*M* perchloric acid for all alkaloids except hyoscine butylbromide, where in the mobile phase the ratio of water to acetonitrile was 47:50. Derivatisation of the drugs is not required. The detector responses for all the alkaloids are similar. The calibration graphs exhibited wide linear concentration range of 0–500 μg ml^{-1} for a sample size of 20 μl for all the analytes. The method has been applied to determine the drugs in a number of pharmaceutical preparations.

Keywords: Atropine; Homatropine; Hyoscine

1. Introduction

Atropine, hyoscyamine and hyoscine (or scopolamine) are alkaloids obtained from various solanaceous plants [1]. A number of atropine-like compounds have been synthesized, the more common ones being homatropine, hyoscine methobromide and hyoscine butylbromide. The alkaloids have similar structures and are used in pharmaceutical preparations for anticholinergic purposes [1,2].

Microgram quantities of the above alkaloids are commonly used in pharmaceutical preparations, however, quantitation of the drugs is frequently difficult because of their low UV absorptivity. Furthermore, some of them are quaternary ammonium compounds, which render extraction and purification difficult.

Analytical methodologies are available for the quantitation of the alkaloids including spectrophotometry [3–6], fluorometry [7,8], high-performance liquid chromatography [7–10], gas–liquid chromatography [4,5,11,12] and potentiometric titration [2]. The spectrophotometric methods are non-specific and determine total alkaloids in the preparation. Determination of the drugs by GLC requires prior extraction and their conversion to the base form. The procedure is tedious, and column adsorption of the drugs is possible. The potentiometric method is non-specific and not sensitive and is applied mainly to the

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assay of raw materials. Assay by HPLC methods is a good approach, but the drugs exhibit low UV absorptivities above 220 nm, and detection in the UV range of 200–220 nm is easily interfered with by common additives of pharmaceutical preparations. Further, detection at this range limits the choice of HPLC solvents. HPLC methods with fluorimetric detection can lower the detection limit, but they require derivatisation of the drugs. Problems associated with derivatisation, such as completeness of reaction, can complicate the analysis.

The present work proposes to employ indirect conductometric detection for the determination of the alkaloids after HPLC separation. The chromatographic behaviour of the alkaloids was studied. The method has been applied to determine the drugs in various pharmaceutical products and the results are compared with those obtained by an established HPLC method using UV detection [9].

2. Experimental

2.1. Apparatus

The HPLC system used consisted of a Beckman 110B solvent delivery pump with an Alltech free-flow pulse dampener, a Wescan Model 21511001 conductivity detector with Model 24020001 temperature controller and Model 26650051 column compartment, a Rheodyne injection valve with a 20-μl sample loop and a Beckman 427 signal integrator. An analytical column from Beckman Instruments, Ultrasphere 5 μm Spherical 80 Å Pore CN (250 mm×4.6 mm) was used.

Instrumental settings were: flow-rate, 1 ml min^{-1}; column temperature, 30°C; detector zero suppression, 2; detector range, 1 or 10 and chart speed, 0.5 cm min^{-1}.

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 alkaloids used as standards were of BP or USP grade and were used without further purification. HPLC grade solvents were used to prepare the mobile phases. All other chemicals were of analytical reagent grade. Water used was distilled, and deionised by passing through Millipore Milli-Q 50 ultra pure water system.

2.3. Mobile phases and standard solutions

The following mobile phases were used for the assay of pharmaceutical products: (i) water–acetonitrile–tetrahydrofuran (67:30:3, v/v) with 1 m*M* $HClO_4$ for the determination of atropine sulphate, homatropine hydrobromide, hyoscine hydrobromide and hyoscine methobromide and (ii) water–acetonitrile–tetrahydrofuran (47:50:3, v/v) with 1 m*M* $HClO_4$ for hyoscine butylbromide. Other mobile phases with different water acetonitrile ratios and perchloric acid contents as indicated in Table 1 were used to study the effect of the change in the composition of the mobile phase on column separation and detector response.

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

Table 1
The retention times and capacity factors of the alkaloids

	Alkaloid[a]	Mobile phase[b]	Capacity factor k'
1	Atropine sulphate (289.4)	B	4.56
		A	6.12
2	Homatropine hydrobromide (275.4)	B	3.84
3	Hyoscine hydrobromide (303.4)	B	3.92
4	Hyoscine methobromide (318.4)	B	4.52
5	Hyoscine butylbromide (360.5)	D	5.00
		C	6.60

[a] Molecular mass (*M*) of the alkaloid in the base form indicated in parentheses; for hyoscine methobromide and hyoscine butylbromide, the figure in parentheses is the formula mass of the alkaloid cation.

[b] Mobile phases used were 1 m*M* in $HClO_4$ plus (A) water–CH_3CN–THF (77:20:3, v/v); (B) water–CH_3CN–THF (67:33:3, v/v); (C) water–CH_3CN–THF (57:40:3, v/v) and (D) water–CH_3CN–THF (47:50:3, v/v).

2.4. Determination

2.4.1. Liquid samples

They were diluted with the mobile phase to give a final concentration of the analyte in the range of 20–80 μg ml^{-1}. The solution was homogenized by shaking, and was injected into the chromatograph after filtering through 0.2 μm PTFE filters.

2.4.2. Tablets

Twenty tablets were finely powdered. For tablets with analyte of >0.5 mg per tablet, a sample portion equivalent to one tablet was accurately weighed and quantitatively transferred to a flask, into which were pipetted 25 ml of the mobile phase. The flask was stoppered and shaken in an ultrasonic bath for 20 min, with manual shaking at 5-min intervals to avoid coagulation of the powdered sample. Ice was added to the bath to maintain the temperature below 30°C. For tablets with analyte content of <0.5 mg per tablet, a sample portion equivalent to 5 tablets and 10 ml of the mobile phase was used instead and was shaken as above. For both cases, the mixture was then let stand or centrifuged so as to obtain a clear supernatant, which was further diluted with the mobile phase to obtain a solution with analyte concentration in the range of 10–50 μg ml^{-1}. The solution was then filtered through a 0.2-μm PTFE filter and analysed with the liquid chromatograph.

The procedure can be extended to determine content uniformity by using a smaller volume of the mobile phase, down to 2 ml, for extraction so as to achieve sensitivity. The precision and accuracy were found to be similar to those obtained using 10 ml of the mobile phase.

2.4.3. Ointment

A sample with ointment on plastic patch (2.5 cm^2) was transferred to a conical flask and 50 ml of dichloromethane were added. The flask was shaken in an ultrasonic bath for 30 min with the water temperature maintained below 30°C. A 10-ml portion of the dichloromethane extract was evaporated under nitrogen to dryness. The residue was reconstituted with 10 ml of the mobile phase, filtered and analysed with the chromatograph.

3. Results and discussion

3.1. Characteristics of the chromatograms

The proposed chromatographic system gave well defined symmetrical peaks of the alkaloids, and the chromatographic conditions have been optimised such that the retention times were about 15 min to enable fast analysis. The peaks of the alkaloids were well separated from those of the excipients found in common pharmaceutical preparations. The capacity factors of the alkaloids are listed in Table 1.

The chromatograms of the alkaloids were found to have a negative peak, commonly known as the solvent peak, at about 2.6 min. As the polarity of the conductivity detector was reversed, the negative solvent peak indicated an increase in conductance of the eluent. The area of the solvent peak was found to be directly proportional to the analyte concentration.

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

3.2.1. Changing the water–acetonitrile ratio

The retention times for the alkaloids were determined using mobile phases with different water–acetonitrile ratios as shown in Table 1. Perchloric acid was added to the mobile phase to create a conducting background to enable detection. The small and fixed amount of tetrahydrofuran (THF) (i.e., 3%) was added to improve separation efficiency and enable more effective degassing of the mobile phase.

The retention time for hyoscine hydrobromide, which has a lower molecular mass than hyoscine methobromide, was less than that of the latter in the same mobile phase. Further, when the water–acetonitrile ratio was decreased, thus decreasing the polarity of the mobile phase, the retention of both atropine sulphate and hyoscine butylbromide by the column were reduced. The fact that the detector response is directly proportional to the number of analyte species (see Section 3.3) suggests an ion exchange mechanism in column retention. Nevertheless, the increase in column retention with increase in molecular mass also suggests that hydrophobic interaction was an important operating mechanism in

the system apart from dipole–dipole and dipole-induced dipole interactions of the sorbent.

3.2.2. Changing the concentration of the conducting species

The perchloric acid concentration in the mobile phase was found to affect the retention behaviour of the alkaloids under study. A plot of ln k' vs. perchloric acid concentration is shown in Fig. 1, where it can be seen that all the alkaloids were less retained by the column when the concentration of perchloric acid was increased. The substantial change in retention with a small change in acid concentration indicated a weakening of interaction between the column and the analyte, which may be due to an increase in polarity of the column surface as a result of the change in perchloric acid content and the increase in charge of the analyte, both of which would weaken the hydrophobic interaction between the column and the analyte. The greater eluting power of perchloric acid for the alkaloid cations at higher concentration of the acid or the reduction in column capacity as a result of increase in ionic strength of the mobile phase may also help to explain the change in retention of the analyte species [14].

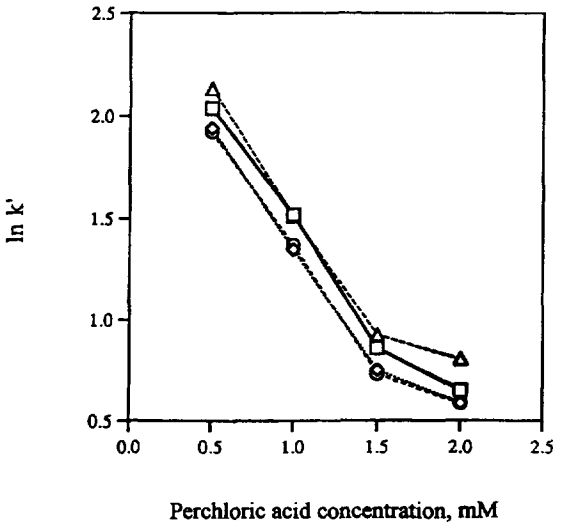

Fig. 1. ln k' vs. perchloric acid concentration for atropine sulphate (-□-), homatropine hydrobromide (· · ◇ · ·), hyoscine hydrobromide (· · ○ · ·) and hyoscine methobromide (· · △ · ·).

3.3. Detector response

3.3.1. Effect of molecular mass of the drug

The detector response of the drugs, in terms of peak area counts per unit mass, in base form or cationic form are listed in Table 2. The mobile phase used was water–CH_3CN–THF (67:30:3, v/v/v) with 1 mM $HClO_4$. Listed in column 3 of Table 2 are the area counts per μg, A, multiplied by the molecular mass M. The mass of the anion was not counted because the detector signal of the drug was not related to the associated anion.

As discussed before, the alkaloids compete with the conducting species for active sites on the column, and hence the occupation of active sites would be related to the number rather than the mass of the analyte species. In fact, AM relates the detector response to the same number of analyte species because,

$$AM = (\text{area counts}/\mu g)M = \text{area counts}/(\mu g/M)$$

As $\mu g/M$ is proportional to the number of molecules of the alkaloid per μg, AM is proportional to the area counts/number of molecules of the alkaloid per μg. It can be seen from Table 2 that a nearly constant value of 13 was obtained, which indicates that the detector response was proportional to the number of the analyte species. This provides evidence that the displacement of the eluent species and the occupation of active sites on the column was proportional to the number rather than the mass of the alkaloid molecule.

3.3.2. Effect of the concentration of the conducting species

The peak area responses per μg of the alkaloid eluted with mobile phases having perchloric acid

Table 2
Detector response of the alkaloids with detector range at 10, and concentrations in the range of 0–100 μg ml^{-1}

Alkaloids	$AM/10^6$[a]
Atropine sulphate	13.08
Homatropine hydrobromide	12.85
Hyoscine hydrobromide	12.47
Hyoscine methobromide	13.88
Average	13.1±0.6

[a] A = area count/μg, and refer to Table 1 for M.

concentrations from 0.5–2.0 m*M* were found to be essentially constant, which, as described above, was due to the fact that the displacement of background conducting species was quantitative and proportional to the concentration of the analyte species.

3.4. Detection theory

When a solution of the analyte, such as atropine sulphate, is loaded onto the column, the bulky organic cation will be retained by the column, and the counter anion, being very soluble in the mobile phase, is little retained and will be coeluted with the solvent. The detection theory for the analyte is the same as that described for the active ingredients in cough–cold syrups reported earlier [13].

The equation relating the concentration of the analyte to the change in conductance is as follows [13],

$$\Delta G = -\frac{\lambda_{o,E^+} C_s}{10^{-3} K} \quad (1)$$

where ΔG is the change in ionic conductance, λ_{o,E^+} the limiting ionic conductance of the cation of the background conducting species, K the cell constant and C_s the concentration of the alkaloid in the

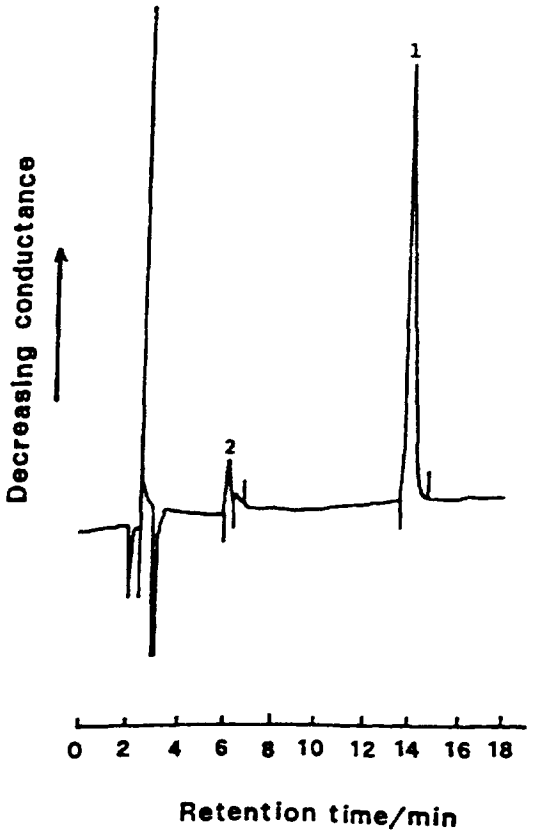

Fig. 2. Indirect conductometric detection of atropine sulphate (0.96 μg) in injection with peaks (1) atropine sulphate (at 13.93 min) and (2) unknown.

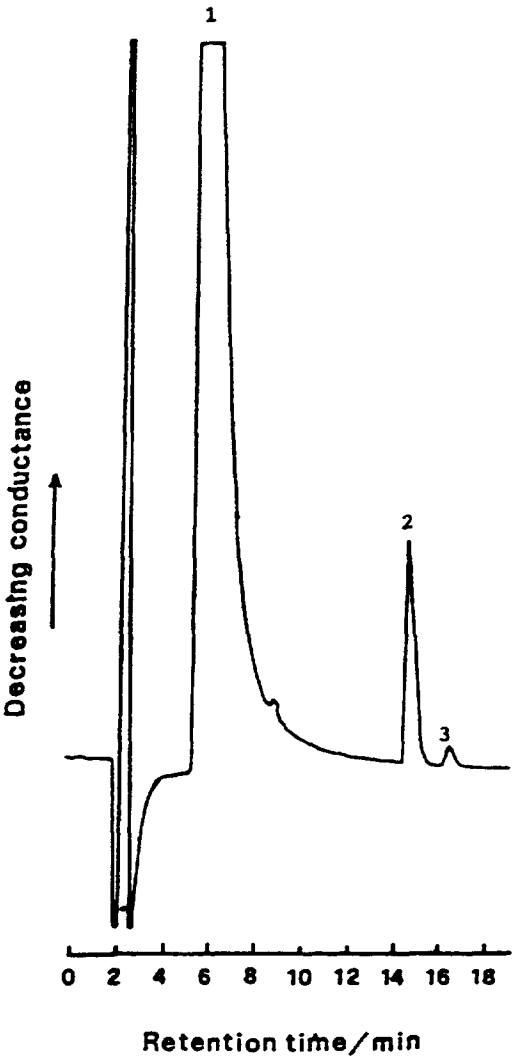

Fig. 3. Indirect conductometric detection of hyoscine butylbromide (0.8 μg) in Buscop liquid with peaks (1) excipient; (2) hyoscine butylbromide (at 14.57 min) and (3) unknown.

solution. Eq. (1) indicates that the detector response will be a decrease in conductance and proportional to the concentration of the analyte.

3.5. Calibration graphs and precision

The calibration graphs were obtained by plotting the peak area (integrator counts, units arbitrary) against the corresponding concentrations of the alkaloid in the mobile phase. In quantitative determination of the alkaloid in pharmaceutical preparations, the results were calculated from the linear regression equations of the calibration graphs, which were found to be linear from 0 to 500 $\mu g\,ml^{-1}$ at least for a sample size of 20 μl, which is equivalent to 0–10 μg of the analyte, with correlation coefficients of better than 0.999. As low as 10 ng of the alkaloid can be measured to within +20% of the actual quantity by the proposed method.

The precision of the method was studied by replicate analyses of the preparations over 4–5 days, and the relative standard deviations (R.S.D.) for 10 replicate injections of different drug preparations were typically 1–2%.

3.6. Assay of pharmaceutical preparations

The contents of atropine sulphate, homatropine hydrobromide, hyoscine hydrobromide, hyoscine methobromide and hyoscine butylbromide in various pharmaceutical preparations were determined by the proposed method. The drugs were found to be well resolved from the excipients and other ingredients, and interferences were not observed. The chromato-

Table 3
Assay results of the drugs in pharmaceutical preparations

	Sample form	Ingredient and label value	Percentage of label claim	
			Proposed method[a]	HPLC with UV detection[b]
1	Eye drops	Atropine sulphate (1%)	100 (0.3)	93
2	Injection	Atropine sulphate (0.6 mg ml^{-1})	98.8 (0.3)	[c]
3	Injection	Atropine sulphate (0.6 mg ml^{-1})	104 (0,g)	111
4	Tablet	Atropine sulphate (0.025 mg/tablet)	96 (4.6)	101
5	Tablet	Atropine sulphate (0.025 mg/tablet)	95 (2.3)	94
6	Eye drops	Homatropine hydrobromide (2%)	103 (1.4)	100
7	Eye drops	Homatropine hydrobromide (2%)	101 (1.3)	104
8	Ointment	Hyoscine (1.5 mg per patch)	101 (1.1)	105
9	Tablet	Hyoscine methobromide (1.0 mg/tablet)	100 (1.9)	104
10	Tablet	Hyoscine butylbromide (10 mg/tablet)	99 (4.6)	102
11	Tablet	Hyoscine butylbromide (10 mg/tablet)	102 (3.2)	100
12	Injection	Hyoscine butylbromide (20 mg ml^{-1})	102 (1.1)	102
13	Buscop liquid	Hyoscine butylbromide (5 mg/5 ml)	100 (1.4)	102

[a] The R.S.D. of results of triplicate analysis in percent shown in parentheses.
[b] Average value of two determinations.
[c] Not determined due to insufficient sample.

grams of some preparations are shown in Figs. 2 and 3. The assay results are shown in Table 3. There was close agreement between the results obtained using the proposed method and the label values. As listed in Table 3, the results were also counter-checked using an established HPLC method with spectrophotometric detection [9] and with slight modification in the mobile phase composition to optimise column separation and depending on the preparation, the detector wavelengths were chosen from 215–250 nm to minimize interference from other additives, where the precision of the results obtained using the counter-check method was in line with those reported in [9]. Micro-quantities of the drugs, down to 0.025 μg per tablet, can be easily determined by the proposed method. The results indicated that the proposed method can be applied to determine the drugs in pharmaceutical preparations.

4. Conclusion

The present work proposes indirect conductometric detection as an alternative approach on HPLC detection. The method can be extended to determine other organic compounds in various sample matrices. The method developed enables easy determination of some of the alkaloids such as hyoscine butylbromide, which could not be easily determined before. The significance of this approach is that it is sensitive and does not require the analyte to have special structural properties, which is quite different from the current methods on the analysis of organic compounds by HPLC.

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