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HIGH-PERFORMANCE ION-PAIR LIQUID CHROMATOGRAPHY

APPLICATION TO DISSOLUTION RATE AND CONTENT UNIFORMITY TESTING OF PHARMACEUTICALS

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SUMMARY

Schill and his group have shown that ion partners with good chromophores can be used to improve detection properties following separation of picrate ion pairs on cellulose columns. We have shown recently that the same concept can be transferred to high–performance liquid chromatographic conditions using small particle silica gel.

In this work these systems were investigated to further substantiate some of the claims previously made concerning the predictability of chromatographic data and the application of these systems.

Good correlations were found between capacity factors calculated from batch experiments and chromatographically determined data. In the course of this study the influence of temperature, pH and concentrations of picric acid [X$^-$] on E_{QX} and on retention data (k', α) were studied. Trinitrobenzenesulfonic acid (TNBS) was tried as a substitute for picric acid due to its better solubility properties. It was found that the picric acid continuously formed in the trinitrobenzenesulfonic acid-stationary phase and not the TNBS is responsible for ion pair formation.

The applications work revealed up to a fiftyfold enhancement in detection limits for several tropa alkaloids in comparison to reversed-phase chromatography (detection at 215 nm). In addition large concentrations of non-complexing compounds can be suppressed by using the picric acid absorption maximum at 345 nm. The method has been applied to content uniformity studies and dissolution rate testing of tablets. The reproducibility of these operations were always below 2% rel. S.D.

INTRODUCTION

Of the alkaloids and other drug substances that have been tested, the tropa alkaloids were always the most difficult to subject to trace analysis, owing to their poor chromophoric activity and lack of suitable sites for chemical derivatization[1].

Some chromatographic work on this group of compounds was published by Stutz and Sass[2] on adsorption systems using a detection wavelength of 254 nm. The detection limits obtained of ca. 1 μg per injection were inadequate for the analysis of the low-dosage pharmaceutical forms encountered with these highly potent species. An improved method based on adsorption chromatography was reported by Verpoorte and Baerheim Svendsen[3]. Honigberg et al.[4] proposed a reversed-phase system on octadecyl- and phenyldichlorosilane-treated supports using methanol–water as the mobile phase.

The use of commercially available small-particle (5 μm) reversed-phase materials such as RP-8 (Merck, Darmstadt, G.F.R.) has permitted the efficient separation of these alkaloids and, with water–acetonitrile–methanol mixtures as the mobile phase, detection at 215 nm resulted in an improvement of up to 10-fold in the detection limits in comparison with adsorption chromatography[4]. Nevertheless, these detection limits were not sufficient for many of the problems encountered and the selectivity of the separation step did not permit the separation of tropa alkaloids from other drug substances in complex drug combinations in a single step. In this paper, it is demonstrated that the enhancement of both sensitivity and selectivity in ion-pair chromatography can be utilized in the analysis of complex pharmaceuticals.

EXPERIMENTAL

Reagents

The drug substances (see Table I) were obtained from Sandoz, Basel, Switzerland. The chromatographic solvents and supports, analytical reagent grade buffers and ion-pair reagents were obtained from Merck, with the exception of picric acid (puriss.) and trinitrobenzenesulphonic acid, which were purchased from Fluka (Buchs, Switzerland). They were used without further purification. The pH of the stationary phase was checked and, if necessary, adjusted with 5 M sodium hydroxide solution.

Apparatus

A Hewlett-Packard Model UFC-1000 liquid chromatograph was used. The injector was a valve Rheodyne Model 70-10 (Rheodyne, Berkeley, Calif., U.S.A.). A Perkin-Elmer Model LC-55 spectrophotometric detector and a W + W Model 600 recorder (Kontron, Zürich, Switzerland) were used. Data processing was carried out with a Hewlett-Packard Laboratory Data System Model 3354. The column loading apparatus is shown in Fig. 1. The column was packed by a dynamic slurry technique. The packing apparatus has been described earlier[5].

Procedure for loading a column

Regeneration. Prior to impregnation, the column has to be regenerated in a stream of hot nitrogen. The upper end of the column is connected via a metal capillary to the nitrogen flask and the capillary at the bottom end is dipped into a test-tube filled with paraffin oil. Nitrogen is then drawn through the column at a pressure of 3–4 bar until residues of mobile phase are removed. The column is then placed for 2 h in an oven at 200° and flushed continously with a gentle stream of nitrogen. After cooling in the stream of nitrogen, the column is prepared for loading according to Fig. 1.

TABLE I

SUBSTANCES TESTED

Compound	Structure	Molecular weight
Hyoscyamine	H_2C——CH——CH_2 / $N \cdot CH_3$ $CH \cdot O \cdot CO \cdot CH(C_6H_5) \cdot CH_2OH$ / H_2C——CH——CH_2	289.36
Scopolamine	CH——CH——CH_2 / O $N \cdot CH_3$ $CH \cdot O \cdot CO \cdot CH(C_6H_5) \cdot CH_2OH$ / CH——CH——CH_2	303.37
Apoatropine	CH——CH——CH_2 / $N \cdot CH_3$ $CH \cdot O \cdot OC \cdot C$ C_6H_5 CH_2 / CH——CH——CH_2	271.35
Scopoline	CH_3N O	155.19
Belladonine	C_6H_5 CO_2——CH CH_2——CH——CH_2 $N \cdot CH_3$ CH_2——CH——CH_2 CO_2——CH CH_2——CH——CH_2 $N \cdot CH_3$ CH_2——CH——CH_2	542.69
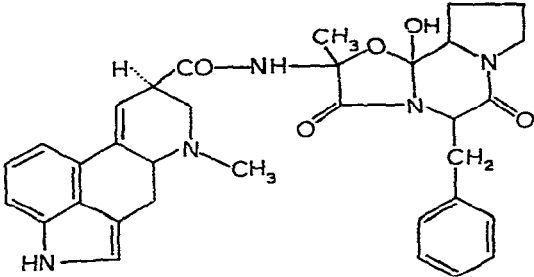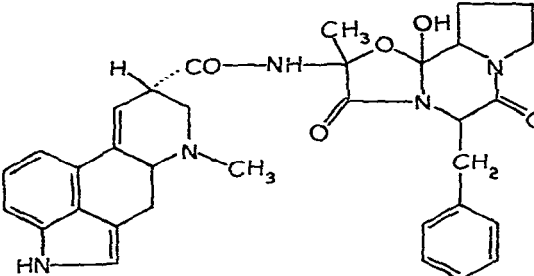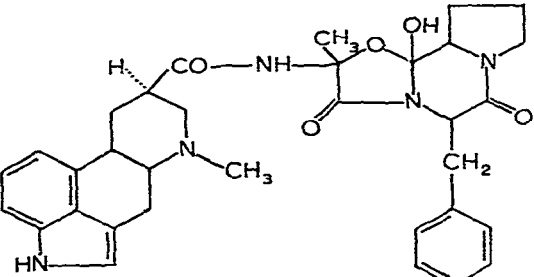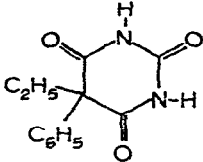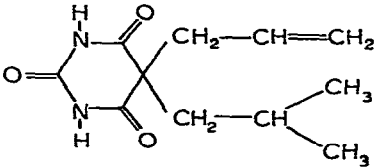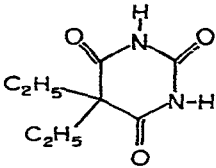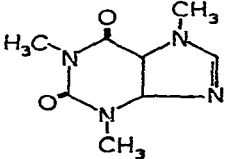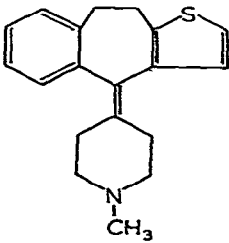
TABLE I (*continued*)

Compound	Structure	Molecular weight
Ergotamine·		581.68
Ergotaminine		581.68
Dihydroergotamine		583.70
Phenobarbital		184.19

TABLE I (*continued*)

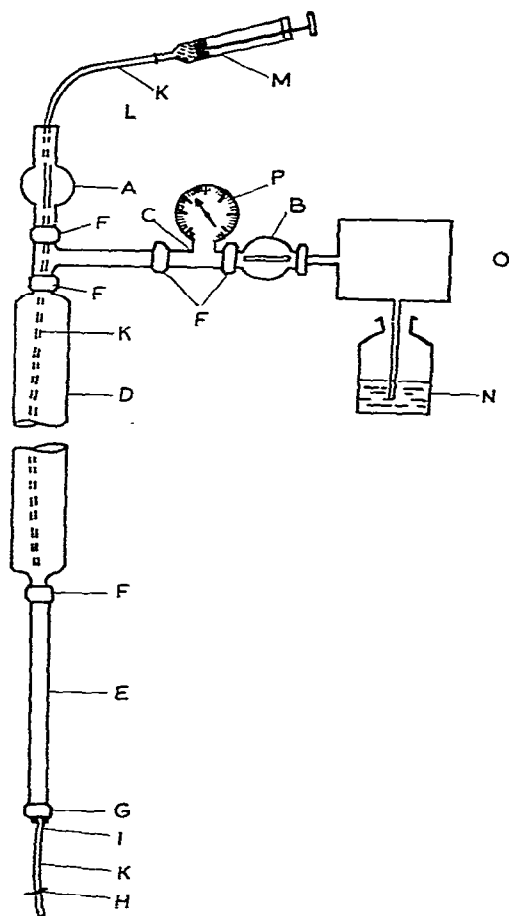| Compound	Structure	Molecular weight
Butalbital		224.25
Barbital		184.19
Caffeine		194.19
Pizotifene		429.53

Fig. 1. Column and column loading apparatus. A, B, Whitey ball valve, Type S5 4354; C, T-piece, 0.6 cm O.D., 0.3 cm I.D.; D, reservoir made of stainless steel, 2.6 cm O.D., 2.0 cm I.D., length 32 cm; E, chromatography column; F, Swagelok connection, 1/4 in.; G, reduction with built-in metal frit (Perkin-Elmer NFCA RU 2.6); H, metal clamp; I, steel capillary, 0.25 mm I.D.; K, polyethylene tubing; L, injection needle; M, 50-ml syringe; N, solvent container; O, Haskel Model 26980 pump (P_{max} = 500 bar); P, manometer, 0–400 bar.

Loading and conditioning. The apparatus for loading the column is shown in Fig. 1. A 50-ml syringe serves to fill the stationary phase into the loading apparatus (tube D). The remainder of the tube is filled with *n*-hexane. The stationary phase consists of an aqueous solution, usually buffered with a citrate buffer to pH 6, and with a picrate ion concentration of 0.06 *M* (unless otherwise mentioned). *n*-Hexane is then pumped carefully with valves A and B open until it overflows in valve A to replace trapped air. Valve A is closed and approximately 20 ml of the stationary phase are pumped at 0.4 ml/min through the column. The column is then disconnected and equilibrated over night at a flow-rate of 0.2 ml/min with mobile phase consisting of chloroform saturated with stationary phase.

Reloading. If a column ceases to function owing to loss of stationary phase, it can be reloaded by washing it with 50 ml of methanol at a flow-rate of 1 ml/min and then going through the regeneration and loading steps as described above.

Procedure for measuring V_s/V_m

Two methods have been employed and the results compared.

Method 1. The column was loaded and equilibriated and the volume of mobile phase (V_m) measured using dodecylbenzene as the non-retained component (retention time t_0). Then the stationary phase was washed off with the solvent used for preparing this stationary phase and the picrate ion concentration was measured photometrically in the washing solution. Knowing the original concentration of the solution used for loading and the amount of picric acid washed off the column, one can calculate the volume of stationary phase (V_s).

Method 2. V_m was determined for a non-impregnated column and then for the impregnated column (V'_m). V_s can then be calculated from the equation

$$V_s = V_m - V'_m$$

In both instances a 25 cm × 3 mm I.D. column packed with SI-1000 (10 μm) was used. The impregnating solution (stationary phase) consisted of 0.05 M picrate ion buffered to pH 6. The flow-rate of mobile phase for measuring t_0 was 0.2 ml/min.

Procedure for measuring extraction constants (E_{QX})

The extraction constants were determined batchwise according to a procedure described by Schill[6] and based on eqn. 2 (see below).

RESULTS AND DISCUSSION

Supports

The influence of the column material and the flow-rates on the stability of the ion-pair chromatographic system has been studied in detail previously[1]. In that work, the Merck silica gel materials SI-100 and SI-1000 were both found to be suitable as supports and, provided that low flow-rates were used (0.2 ml/min), a satisfactory stability of the system was achieved (see Fig. 2). For SI-100, a more favourable k' range was obtained than for SI-1000 (better separation efficiency) but, owing to the stronger retention, the separation times were longer. The good selectivity of ion-pair separation permitted the use of short columns (5–15 cm) packed with 5-μm SI-100 material with separations in a few minutes.

Influence of temperature, pH and picrate concentration

The retention behaviour (capacity factor, k') of ion pairs in a chromatographic system can be represented by the equation

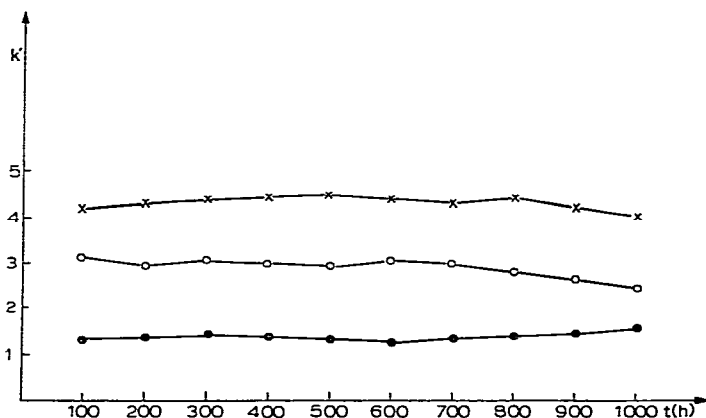$$k' = V_s/V_m \, E_{QX} \, [\text{X}^-] \tag{1}$$

Fig. 2. Stability of the ion-pair system for k' values of hyoscyamine (●), ergotamine (○) and scopol-
amine (×). Column: SI-100, 5 μm, 10 cm length, 3 mm I.D. Stationary phase: picric acid, 0.06 M,
pH 6. Mobile phase: chloroform saturated with stationary phase; flow-rate, 0.2 ml/min.

where [X⁻] is the concentration of the counter ion and E_{QX} is the extraction constant,
defined as

$$E_{QX} = [QX]_{org}/[Q^+]_{aq} \, [X^-]_{aq} \qquad\qquad (2)$$

QX being the ion pair in the organic phase.

From eqn. 1, it can be seen that if V_s and V_m are kept constant, the retention
behaviour can be modified by varying E_{QX} and [X⁻]. The concentration of X⁻, in
this work picrate, can be controlled in the stationary phase and will attain equilibrium
with the mobile phase. Another important factor is the pH, which determines the
concentration of the protonated species Q⁺ and indirectly E_{QX}. The extraction con-
stant E_{QX} is additionally influenced by the temperature of the system.

It was therefore of interest to investigate the influence of the three variables
temperature, pH and [X⁻] on E_{QX} and in turn on k'. For these batch studies, hyos-
cyamine, apoatropine and pizotifene were used at concentrations of 10^{-4} M with
an equal amount of picrate ion. The pH was adjusted to 4 with citrate buffer. Chloro-
form was used to extract the ion pair from the aqueous solutions.

Temperature. The results for temperatures from 5 to 35° are shown in Fig. 3.
A gradual decrease in E_{QX} is observed with an increase in temperature, which,
according to eqn. 1, should result in an increase in k'. This does not, however, occur
in practice, as can be seen in Table II, the retention decreasing at higher temperatures.

pH. The pH range 2–6 was studied, citrate buffers being used for the entire
range and the other conditions being as above. The results are presented in Fig. 4.
E_{QX} reaches a maximum between pH 3 and 4 for the three components tested and
then decreases at different rates with increasing pH. For maximal complex stability,
one should therefore work at pH 3–4. Previous investigations[1] have shown, however,
that with organic solvents such as chloroform and other halogenated solvents,the
solubility of picrate is high in this pH range, which causes serious background
interference. A pH of 5 or 6 was therefore chosen for practical work in chromato-
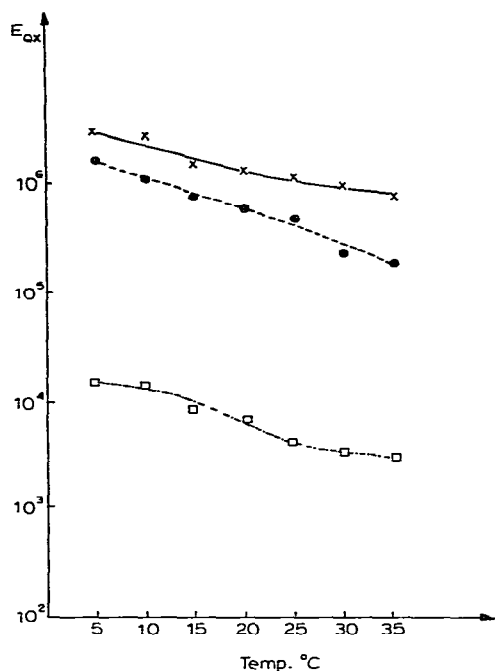graphic systems.

Fig. 3. Variation of E_{QX} as a function of temperature. ●, Apoatropine; □, hyoscyamine; ×, pizotifene.

TABLE II

INFLUENCE OF TEMPERATURE ON RETENTION

SI-100, 5 μm; 12.5 cm × 1.4 mm I.D. column; mobile phase, chloroform saturated with stationary phase.

Compound tested	k'	
	22°	30°
Apoatropine	0.40	0.18
Pizotifene	0.52	0.20
Ergotamine	1.52	0.66
Hyoscyamine	1.0	0.39
Scopolamine	1.68	0.95

Concentration of X^-. The concentration of X^- (picrate) will also influence the retention behaviour. According to eqn. 1, the k' values should decrease with increasing [X^-]. This has been confirmed in an earlier study[1] for hyoscyamine and scopolamine at picrate concentrations in the range $1 \cdot 10^{-2}$ to $5 \cdot 10^{-2}$ M at pH 5 on silica gel 60 and Kieselguhr. Of the substances tested, ergotamine seems to behave abnormally, as can be seen in Fig. 5 for tests carried out on SI-1000. Ergotamine shows little change in retention as a function of [X^-] and in the concentration range $5 \cdot 10^{-3}$ to 10^{-2} a reversal of the separation order between hyoscyamine and ergotamine
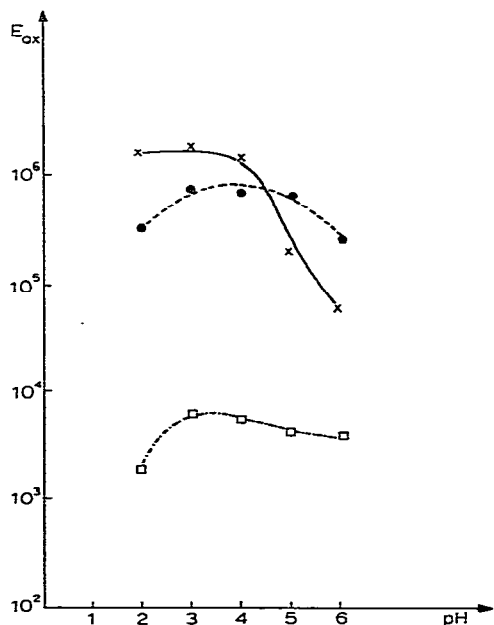
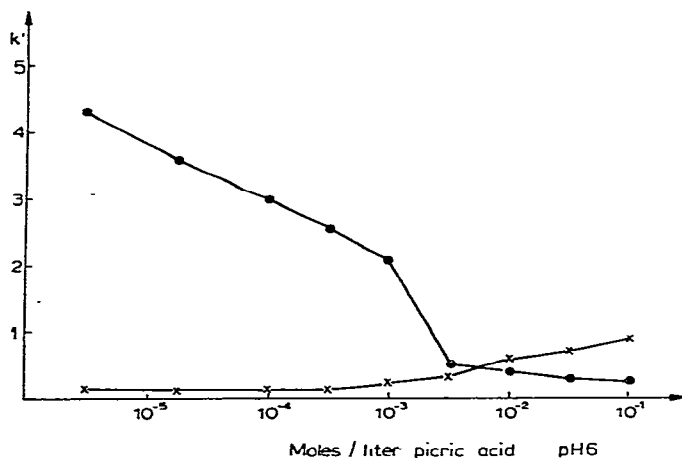Fig. 4. Variation of E_{OX} as a function of pH. Symbols as in Fig. 3.

Fig. 5. k' values as a function of picric acid concentration in the stationary phase. ●, Hyoscyamine; ×, ergotamine. Column: SI-1000, 10 μm. Other conditions as in Fig. 2.

occurs. The steep increase in k' values for hyoscyamine seems hardly attributable to a liquid–liquid partition phenomenon only; at these low picrate concentrations it is possible that adsorption effects participate to an increasing extent.

For practical work, the picrate concentration was kept above 10^{-2} M, as this brings the k' values of all of the compounds of interest into a reasonable range. The chromatographic system is also easier to control and has better stability.

Validity of the retention equation

The validity of eqn. 1 was investigated, using the techniques for measuring V_s/V_m and E_{QX} described under Experimental. The V_s/V_m ratios determined by the two methods agreed within 10%. The experimental values were determined from the chromatogram shown in Fig. 6, and the calculated and experimental k' values are compared in Table III.
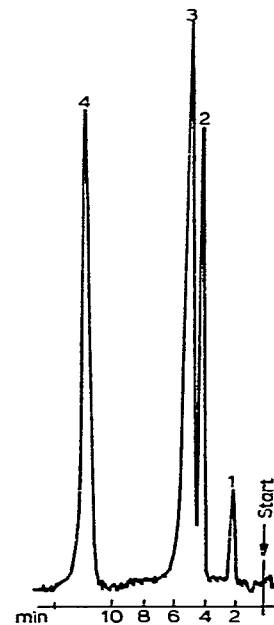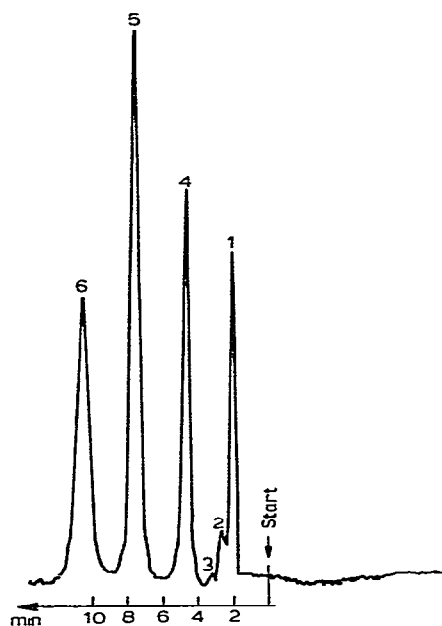

Fig. 6. Typical chromatogram for five ion pairs. (1) t_0 (dodecylbenzene); (2) apoatropine; (3) ergotaminine; (4) hyoscyamine; (5) ergotamine; (6) scopolamine. Conditions as in Fig. 2.

Fig. 7. Chromatogram for (1) t_0, (2) hyoscyamine, (3) ergotamine and (4) scopolamine ion pairs. Stationary phase: TNBS, 0.06 M, pH 6. Other conditions as in Fig. 2.

TABLE III

COMPARISON OF EXPERIMENTAL AND CALCULATED k' VALUES

Compound tested	k'		E_{QX}
	Calculated	Experimental	
Pizotifene	0.22	0.32	$2 \cdot 10^5$
Ergotamine	1.6	2.6	$8.5 \cdot 10^3$
Hyoscyamine	1.80	1.25	$8.9 \cdot 10^3$
Apoatropine	0.11	0.20	$6.6 \cdot 10^5$
Scopolamine	6.9	4.2	$1.9 \cdot 10^3$

Ergotamine behaves rather unusually (see also Fig. 5), but the other data also deviate considerably and one can at best predict the order of separation from such data. It should be borne in mind that eqn. 1 is valid only with the assumption that no adsorption phenomena interfere. Deviations from a true liquid–liquid mechanism may be one reason for the discrepancies.

Tests with trinitrobenzenesulphonic acid

Trinitrobenzenesulphonic acid (TNBS) was investigated as an alternative to picric acid owing to its lower solubility in chloroform and other halogenated solvents. It was added at concentrations of $6 \cdot 10^{-2}$ *M* and at pH 6. A chromatogram is shown in Fig. 7. The stability of such a TNBS system was found to be as good as a picric acid system. An investigation of the resulting ion pairs by spectrophotometry revealed that the picrate ion pair and not a TNBS ion pair is actually being formed and extracted or eluted from the column. Small amounts of picrate ions are constantly being released by the TNBS according to the equation
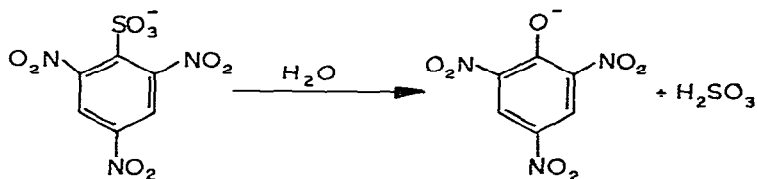
This does not exclude the possibility of using TNBS for ion-pair work; however, as it is difficult to obtain commercial TNBS of uniform purity and the release is harder to control, it is advisable to use picric acid for practical work.

Detection limits and quantitation

One of the prime reasons for choosing picrate ion pairs is the enhancement of detection properties, in addition to the improvement of selectivity. The possibility of working at a detection wavelength of 254 nm also reduces the cost of the detection system. The detection limits for some tropa alkaloids are given in Table IV. The detection limits were taken at a signal-to-noise ratio of 3:1 and compared with detection limits obtained with reversed-phase high-performance liquid chromatography (HPLC) without ion-pair formation and a detection wavelength of 210 nm. Up to an 80-fold decrease in the detection limit can be observed. The detection limits for scopolamine are approximately the same, as in ion-pair chromatography it elutes as the last peak whereas as the most polar component it appears close to t_0 on a reversed-phase column.

Calibration graphs were also constructed. The regression coefficients were 0.9980 or better for an ion-pair concentration range of 0.1–1.0 μg per injection.

The reproducibility in ion-pair chromatography is as good as that in conventional chromatographic techniques. Retention values (k') are reproducible with a relative standard deviation of less than 1 % and the reproducibility of peak areas or peak heights is well below 2 %. Details are given under the appropriate applications below.

APPLICATIONS

As was mentioned in the Introduction, there is a shortage of methods for the selective determination of tropa alkaloids in pharmaceuticals, particularly with mixtures of drug substances (combination drugs). These alkaloids are highly potent drugs and usually present in low dosages in the presence of larger amounts of other

TABLE IV

LIMITS OF DETECTION

Compound tested	Limit of detection (ng per injection)	
	Ion-pair chromatography (detection at 254 nm)	Reversed-phase chromatography (detection at 210 nm)
Apoatropine	5	200
Belladonine	10	100
Hyoscyamine	5	100
Scopolamine	50	50
Scopoline	2.5	—

substances. In addition, they have only a relatively weak chromophore with absorption maxima around 210 and 258 nm. Gas chromatographic techniques, which are about the only ones with adequate sensitivity, are tedious and less universally applicable, as can be seen in the examples.

Analyses of belladonna total alkaloids

Belladonna total alkaloids are plant extracts of tropa alkaloids that are used in many formulations and therefore have to be checked routinely.

Procedure. The alkaloids are extracted in chloroform as ion pairs at pH 6 from $6 \cdot 10^{-2}$ M picrate solution containing the sample. A 20-mg amount of belladonna total alkaloids is dissolved in 20 ml of citrate buffer solution (pH 6). To 5 ml of this solution 1 ml of the $6 \cdot 10^{-2}$ M picrate ion solution is added and the extraction is carried out by shaking for 1 min with 2.0 ml of chloroform. For the chromatographic conditions, see Fig. 8.

Results. The chromatogram of a typical mixture of belladonna total alkaloids is shown in Fig. 8. Belladonine, which could also be present in trace amounts, would appear between hyoscyamine and scopolamine, but it was not detectable in the batches tested.

The results for five batches of belladonna total alkaloids are shown in Table V and are also compared with gas chromatographic (GC) data. The agreement between the different batches is good and so is the agreement between the ion-pair HPLC and GC results.

The well established GC technique suffers, however, from several disadvantages, such as a longer separation time (40 min for GC compared with 12 min for HPLC) and a long sample preparation time. The silylation procedure with methyltrimethylsilylheptafluorobutyramide (MSHFBA) takes $1\frac{1}{2}$ h.

Analysis of combination drugs

Cafergot PB. Cafergot PB (Sandoz) coated tablets are indicated for the treatment of migraine. Each tablet contains 0.125 mg of belladonna total alkaloids, 1 mg of ergotamine tartrate, 100 mg of caffeine and 50 mg of butalbital. The problem is to determine the small amounts of belladonna alkaloids and ergotamine in the presence of butalbital and caffeine.
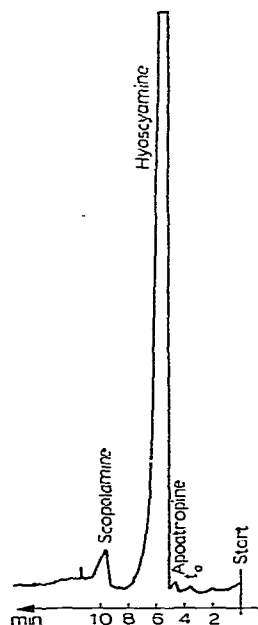
Fig. 8. Chromatogram for Bellafoline (hyoscyamine 7.5 μg, scopolamine 0.37 μg and apoatropine 0.02 μg per 5 μl injection). Column: SI-100, 5 μm, 15 cm length, 3 mm I.D. Mobile phase: chloroform saturated with stationary phase; flow-rate, 0.2 ml/min (Δp = 35 bar). Stationary phase: 0.06 M picric acid, pH 6. Detection at 254 nm (0.2 a.u.f.s.).

TABLE V

COMPARISON OF GC AND HPLC DATA FOR FIVE BATCHES OF BELLAFOLINE

Compound	Method	Concentration (%)				
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
Hyoscyamine	GC	57.4	55.2	54.6	54.3	54.4
	HPLC	55.0	55.7	53.6	55.0	53.5
Scopolamine	GC	3.5	4.3	3.8	3.9	4.5
	HPLC	3.8	4.2	3.6	4.3	4.2
Apoatropine	GC	—	0.3	0.2	0.2	0.2
	HPLC	0.3	0.3	0.3	0.2	0.5
Total alkaloids	GC	60.9	59.8	58.6	58.4	59.0
	HPLC	59.1	60.2	57.5	59.5	58.2

Method. A coated tablet is dissolved in 20 ml of a solution buffered to pH 3 and shaken. After centrifugation, 4 ml of the supernatant solution are mixed with 1 ml of $6 \cdot 10^{-2}$ M picrate solution (pH 6). Extraction is carried out again with 2 ml of chloroform.

Results. A chromatogram of all four components from a tablet, the ergotamine and hyoscyamine as picrate ion pairs and caffeine and butalbital, is shown in Fig. 9. The detection wavelength was 254 nm. For quantitation of the alkaloids, it is recom-
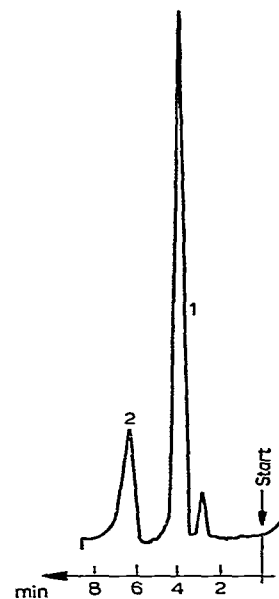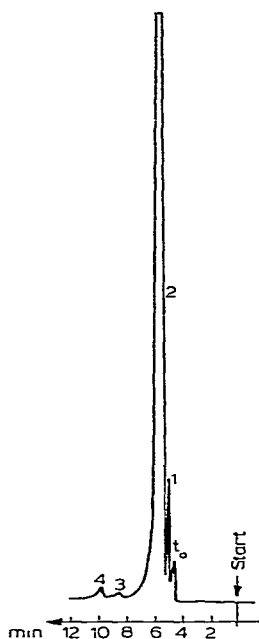
Fig. 9. Separation of components in Cafergot PB. Conditions as in Fig. 8 (1.0 a.u.f.s.). Peaks: (1) butalbital; (2) caffeine; (3) hyoscyamine (0.125 µg); (4) ergotamine (1 µg).

Fig. 10. Separation of components in Cafergot PB. Detection at 345 nm (0.05 a.u.f.s.); other conditions as in Fig. 8. Peaks: (1) hyoscyamine (0.125 µg); (2) ergotamine (1.0 µg).

mended that one should work at the higher maximum of the picrate ion at 345 nm (see Fig. 10), as at this wavelength the large caffeine peak does not interfere. Using a dual detector arrangement, one at 254 nm for butalbital and caffeine and the other at 345 nm for the picrate ion pairs, one can determine all four components in one run. Possible by-products are separated and do not interfere. The quantitation of all four components was effected with a relative standard deviation of better than 1.5%. The ion-pair approach was applied successfully in the content uniformity testing of these tablets (see Table VI)

Plexonal forte. This tablet formulation (Sandoz) has sedative activity and is indicated as a sleep inducer. Each tablet contains 135 mg of barbital, 45 mg of phenobarbital, 75 mg of butalbital, 0.48 mg of dihydroergotamine mesilate and 0.24 mg of scopolamine hydrochloride. The problem is similar to the previous one but even more complex. The two alkaloids have to be determined separately as picrate ion pairs (see Fig. 11) and detected at 345 nm to avoid interference by the barbiturates. The barbiturates can be analysed by gas or liquid chromatography with detection at 254 nm. The experimental procedure is the same as that for Cafergot.

Dissolution rate testing

The measurement of dissolution rates again poses a detection problem for low-dosage tablets because for the initial measurements only a fraction of the drug substance is actually dissolved and available for analysis. It was therefore of particular

TABLE VI

RESULTS OF CONTENT UNIFORMITY TESTING OF CAFERGOT PB COATED TABLETS

Tablet No.	Content of belladonna total alkaloids (%)	Histogram	
		Contents (%)	Number of tablets
1	99.05		
2	101.40	<75	
3	100.31		
4	97.83	75– 77	
5	101.28	77– 79	
6	97.31	79– 81	
7	98.07	81– 83	
8	100.14	83– 85	
9	98.19	85– 87	
10	100.70	87– 89	
11	101.15	89– 91	
12	103.66	91– 93	
13	100.18	93– 95	
14	99.68	95– 97	
15	101.29	97– 99	6
16	101.45	99–101	10
17	98.51	101–103	10
18	99.27	103–105	3
19	100.12	105–107	1
20	102.38	107–109	
21	103.09	109–111	
22	99.56	111–113	
23	100.37	113–115	
24	101.15	115–117	
25	101.89	117–119	
26	101.56	119–121	
27	104.69	121–123	
28	102.38	123–125	
29	105.67		
30	98.23	>125	

Mean   :   100.69
Absolute standard deviation   :   2.01
Relative standard deviation   :   2.00%
Number of tablets   :   30
Relative standard deviation of the method: 1.5%

interest to test the applicability of the ion-pair approach to this type of problem and to take advantage of the detection enhancement effect. Bellergal Retard tablets (Sandoz) were chosen for this example. Each tablet contains 0.6 mg of ergotamine tartrate, 0.2 mg of total belladonna alkaloids and 40 mg of phenobarbital. Of the belladonna alkaloids, hyoscyamine was monitored.

Method. The dissolution tests were carried out according to the U.S. Pharmacopeia by dipping the tablet, contained in a rotating basket (120 rpm), into 100 ml of 0.1 N hydrochloric acid at 37°. Aliquots of 4 ml were taken every hour for analysis. To these 4-ml aliquots, 300 μl of 1 N sodium hydroxide solution and 1 ml of 0.06 M picric acid solution (pH 6) were added and the ion pair extracted by shaking with
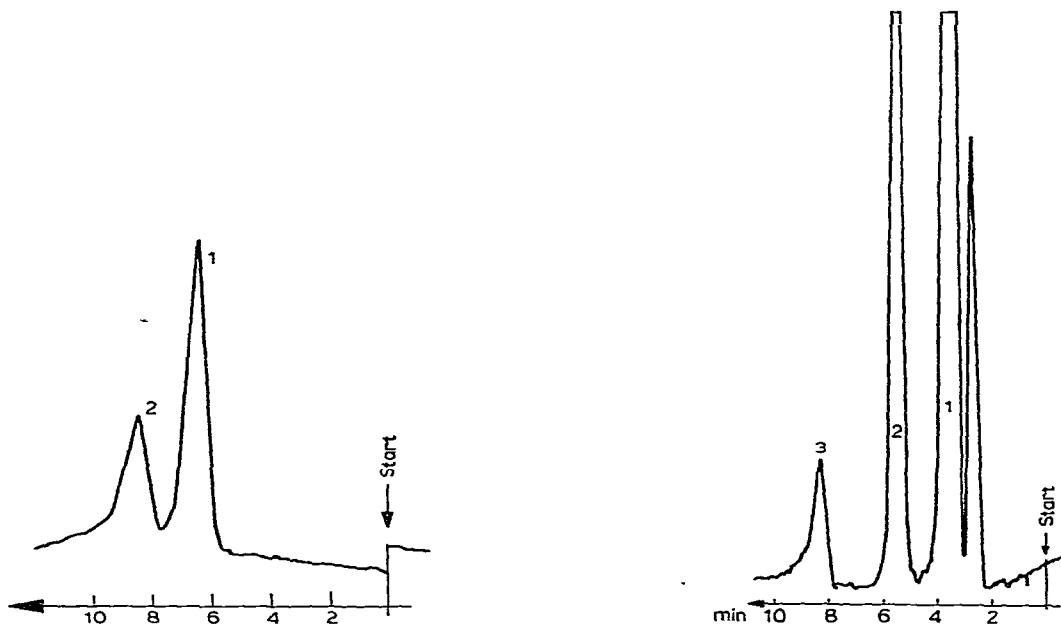
Fig. 11. Separation of (1) dihydroergotamine (0.48 μg per injection) and (2) scopolamine (0.24 μg per injection) as present in Plexonal forte. Detection at 345 nm; other conditions as in Fig. 8.

Fig. 12. Separation of the three major active components in Bellergal Retard: (1) phenobarbital; (2) hyoscyamine; (3) ergotamine. Conditions as in Fig. 8.

2 ml of chloroform for 1 min. Volumes of 10 μl of the organic phase were injected.

Results. A typical chromatogram of the three major components is shown in Fig. 12. After 1 h of the dissolution rate test, the concentration of extracted substances was about 25% for ergotamine and 50% for belladonna alkaloids. This corresponds to about 30 ng of ergotamine tartrate and about 11 ng of belladonna alkaloids per injection. The detection limits are 5 ng per injection for hyoscyamine and about 10 ng per injection for ergotamine; hence in both instances the technique is used close to its limits and it would be difficult to find an alternative method. Scopolamine could not be detected under these conditions unless the injection volume was increased to about 100 μl. A typical dissolution rate curve for Bellergal Retard is shown in Fig. 13.

CONCLUSION

The ion-pair technique with picric acid can be used for the analysis of pharmaceuticals. The chromatographic behaviour of unknown mixtures can be predicted to a limited extent with the use of eqn. 1. The enhancement of the detection limits of up to 50-fold for some of the alkaloids tested makes the technique useful for dissolution rate testing. The sensitivity and the enhanced selectivity permit the simplification of analytical procedures for combination drugs. The GC results compare well with the ion-pair results, but the latter technique is usually preferred because
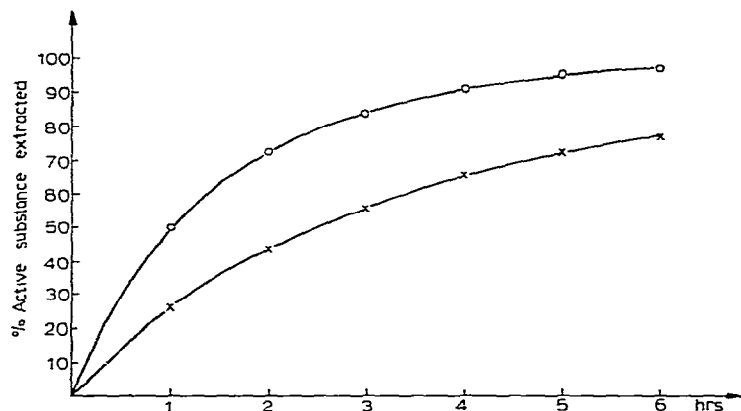
Fig. 13. Dissolution rate curves for hyoscyamine (○) and ergotamine (×) in Bellergal Retard tablets.

of its simplicity and rapidity. The reproducibility of ion-pair chromatography with respect to retention data and peak areas (relative standard deviations less than 2%) makes this approach truly quantitative. A chromatographic system in continuous use can last for several weeks and the column can be reloaded when depleted.

Replacement of picric acid with TNBS cannot be recommended for routine use owing to the irregular quality of the latter.

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