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The simultaneous assay of emetine and cephaeline in ipecacuanha and its preparations by spectrofluorimetry

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Abstract: A rapid assay is described for the simultaneous determination of emetine and cephaeline in ipecacuanha and its preparations, based upon the different fluorescence intensities of the alkaloids at pH 1 and pH 12. The assay involves the measurement of fluorescence at 317 nm of dilutions of the sample in 0.1 M hydrochloric acid (pH 1) and 0.01 M sodium hydroxide (pH 12) with an excitation wavelength of 283 nm. Concentrations of the individual alkaloids are calculated using two simultaneous equations derived from the experimentally determined coefficients of fluorescence of solutions (1 μg/ml) of emetine and cephaeline at pH 1 and pH 12. The procedure, which has been shown to be accurate, precise and sensitive, requires only 1 ml of a liquid sample and less than 0.5 g of powdered root. There was reasonable agreement between the total concentrations of alkaloids in tinctures and liquid extracts of ipecacuanha determined by the spectrofluorimetric method and by the titrimetric procedure of the British Pharmacopoeia. The fluorimetric procedure gave higher levels of alkaloids in the powdered root than did the B.P. method; this difference is explained by incomplete extraction of the alkaloids in the B.P. procedure for powdered ipecacuanha root.

Keywords: *Emetine; cephaeline; ipecacuanha alkaloids; spectrofluorimetry.*

Introduction

The major alkaloids of the underground organs of *Cephaelis* are cephaeline, a phenolic tetrahydroisoquinoline alkaloid, and its *o*-methyl ether, emetine; a number of analytical procedures have been described for the assay of these alkaloids in ipecacuanha and its galenical preparations. The widely used method of the British Pharmacopoeia [1] is based upon acid–alkali titration of the total alkaloids isolated from the sample by solvent extraction, the alkaloid content being expressed as emetine. The numerous extraction and re-extraction stages of the assay of the liquid extracts and tinctures, in particular, render the assay tedious, time-consuming and liable to errors arising from losses. Moreover, it is considered that the yellow colour of the titrated extract and the buffering action of the phenolic alkaloids may mask the end-point of the visual indicator [2].

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Since different species of *Cephaelis* differ not only in their total alkaloid content but also in the relative proportions of the individual alkaloids [3], it is desirable that the assay should determine the ratio of the two alkaloids in addition to their total content. The separate determination of phenolic and non-phenolic alkaloids in ipecacuanha has been achieved by acid–alkali titration or spectrophotometry following selective solvent extraction [4–7], ion-exchange chromatography [8], column chromatography [9–11] and thin-layer chromatography [12, 13], and by high-performance liquid chromatography [14].

The present work was undertaken to develop a simple and rapid procedure for the simultaneous assay of emetine and cephaeline in ipecacuanha. The spectrofluorimetric method that is described utilizes the different fluorescence characteristics of phenolic cephaeline and non-phenolic emetine in aqueous acidic and alkaline solutions.

Experimental

Apparatus

A Perkin–Elmer model 650–40 scanning fluorescence spectrophotometer, equipped with a 150-W xenon lamp and digital display, was used. The fluorescence of solutions in a 1-cm silica quartz cell was measured at the following wavelength and slit settings: 283 nm and 2 nm (excitation); and 317 nm and 8 nm (emission).

Reagents

Emetine hydrochloride (The Wellcome Foundation, London, UK) was assayed by non-aqueous titration [15] and contained 70.0% m/m of emetine base.

Cephaeline hydrochloride (Koch-Light Laboratories Ltd, Colnbrook, UK) contained 77.8% m/m of cephaeline base [15].

Hydrochloric acid (0.1 M and 1.0 M) and sodium hydroxide (0.01 M and 0.1 M) were prepared from AnalaR reagents.

Standard solutions

Standard solutions of emetine (100 μg/ml) and of cephaeline (100 μg/ml) were prepared by diluting to 200 ml with water, accurately weighed quantities of emetine hydrochloride and cephaeline hydrochloride, equivalent to 20 mg of the bases. Two 5-ml aliquots of each solution were transferred to two 500-ml volumetric flasks containing 1 M hydrochloric acid (50 ml) and 0.1 M sodium hydroxide (50 ml), respectively, and the solutions diluted to volume with water. The fluorescence of each solution and of 0.1 M hydrochloric acid and 0.01 M sodium hydroxide blank solutions were measured.

Sample solutions

Tincture of ipecacuanha. One ml of the tincture was diluted to 20 ml with water. The assay was continued as described for the standard solutions by diluting two 5-ml aliquots to 500 ml with 0.1 M hydrochloric acid and 0.01 M sodium hydroxide, respectively.

Ipecacuanha liquid extract. One ml of the liquid extract was diluted to 200 ml with water and the assay was continued as described for the standard solutions by diluting two 5-ml aliquots to 500 ml with 0.1 M hydrochloric acid and 0.01 M sodium hydroxide, respectively.

Powdered ipecacuanha root. A quantity of the powder (300–500 mg), containing approximately 10 mg of the alkaloids, was accurately weighed and transferred to a beaker. The powder was triturated with 2 ml of dimethylsulphoxide and allowed to stand for 30 min with occasional swirling. The contents of the beaker were transferred completely to a 100-ml volumetric flask with four 20-ml volumes of ethanol–0.1 M sulphuric acid (1:3 v/v) and the flask was shaken mechanically for 30 min. The extract was diluted to 100 ml with water, filtered through No. 1 filter paper, and the assay was continued as described for the standard solutions, diluting two 5-ml aliquots to 500 ml with 0.1 M hydrochloric acid and 0.01 M sodium hydroxide, respectively.

Calculations

The fluorescence intensities, corrected for the background fluorescence of the appropriate blank solution, of a standard solution (1 μg/ml) of emetine in 0.1 M hydrochloric acid (F_1) and in 0.01 M sodium hydroxide (F_3) were calculated from the content of the alkaloid base in the salt and the weight of the salt used. Similarly, the fluorescence intensities of solutions (1 μg/ml) of cephaeline in 0.1 M hydrochloric acid (F_2) and in 0.01 M sodium hydroxide (F_4) were calculated. The concentrations of emetine (C_{em}) and of cephaeline (C_{ceph}) in the sample were calculated in terms of %m/v for liquid samples and %m/m for powdered root samples, using the equations:

$$C_{em} = \left(\frac{F_2 \cdot F_{alk} - F_4 \cdot F_{acid}}{F_2 \cdot F_3 - F_1 \cdot F_4}\right) \cdot \frac{D}{10^4 \times V(\text{or } W)}$$

$$C_{ceph} = \left(\frac{F_3 \cdot F_{acid} - F_1 \cdot F_{alk}}{F_2 \cdot F_3 - F_1 \cdot F_4}\right) \cdot \frac{D}{10^4 \times V(\text{or } W)},$$

where F_{acid} and F_{alk} are the fluorescence intensities of the sample solutions diluted to approximately 1 μg/ml with 0.1 M hydrochloric acid and 0.01 M sodium hydroxide, respectively; D is the dilution factor for the samples; V (ml) is the volume of liquid sample used in the assay; and W (g) is the weight of root used in the assay.

Results and Discussion

Development of the method

The change of fluorescence intensity of the phenolic cephaeline and the non-phenolic emetine with variation of pH is shown in Fig. 1. Both compounds show intense, stable fluorescence in acidic solution at their wavelengths of maximum excitation of 283 nm and maximum emission of 317 nm. In alkaline solution, deprotonation of the nitrogen atoms reduces the fluorescence intensity of emetine to about half that of its value in acidic solution, although the wavelengths for maximum fluorescence are unchanged. In contrast, the fluorescence of cephaeline in alkaline solution, like that of many other phenols [16], is almost completely destroyed owing to ionization of the phenolic group, the residual fluorescence being only about 1% of that in acidic solution.

In highly alkaline solution (pH above 13), the fluorescence of emetine is unstable and falls rapidly owing to photodegradation on exposure to high-intensity radiation from the xenon light source. However, in 0.01 M sodium hydroxide (pH 12), and with a narrow excitation slit to give a spectral band width of 2 nm, the fluorescence of emetine is stable

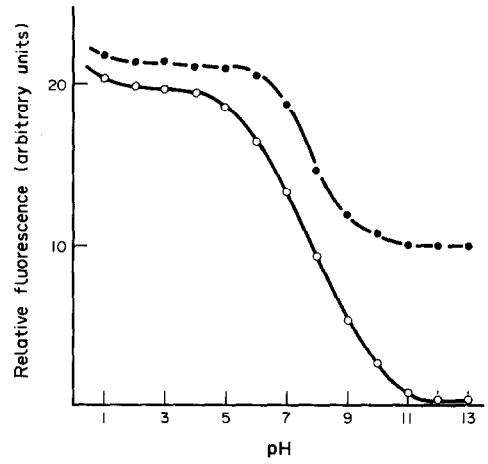

Figure 1
The effect of pH on the fluorescence intensities of solutions (1 μg/ml) of emetine (●) and of cephaeline (○) (λ_{ex} = 283 nm; λ_{em} = 317 nm).

during the measurement; therefore these parameters were selected for the assay. For measurements in acidic solution, 0.1 M hydrochloric acid (pH 1) was chosen to give maximum fluorescence of the alkaloids.

The different fluorescence intensities of emetine and cephaeline at pH 1 and pH 12 at a single pair of wavelengths, 283 nm and 317 nm, permit the concentrations of emetine (C_{em}) and cephaeline (C_{ceph}) in a mixture to be calculated using the classical 'simultaneous equations' procedure of Vierordt, who first applied the procedure to the determination of two absorbing compounds in a single solution by measurement of absorbance at two wavelengths. The construction of the equations is

$$F_{acid} = F_1C_{em} + F_2C_{ceph}$$

$$F_{alk} = F_3C_{em} + F_4C_{ceph},$$

where F_1, F_2, F_3 and F_4 are the experimentally derived coefficients of fluorescence of solutions (1 μg/ml) of emetine at pH 1, cephaeline at pH 1, emetine at pH 12 and cephaeline at pH 12, respectively; and F_{acid} and F_{alk} are the fluorescence intensities of the sample solutions at pH 1 and pH 12, respectively. Solution of the pair of equations and incorporation of dilution factors and sample weights or volumes yield the equations presented above.

Accuracy, precision and sensitivity

The proportionality of the fluorescence intensities of emetine and cephaeline at pH 1 and pH 12 was checked by means of a six-point calibration series of concentrations of 0.4, 0.8, 1.0, 1.2, 1.6 and 2.0 μg/ml. The linear regression data (Table 1) show that the fluorescence intensities of the alkaloids are proportional to their concentration over this range. A concentration of 1 μg/ml of the alkaloids was selected for measurement, both because it provides adequate sensitivity and because the large overall sample dilution (the dilution factors of the tinctures and liquid extracts are 2,000 and 20,000 respectively) yields a solution whose absorbance in a 1-cm cell at the wavelengths of fluorescence measurement (283 nm and 317 nm) is less than 0.03, a condition which avoids the problem of 'inner filter' quenching. The high sensitivity of the fluorescence procedure

Table 1
Calibration and precision data

	Emetine		Cephaeline	
	pH 1	pH 12	pH 1	pH 12
Calibration*				
Slope	21.46	10.52	20.60	0.22
Intercept	0.12	−0.14	0.21	−0.01
Correlation coefficient	0.9998	0.9998	0.9997	0.9991
Concentration range (μg/ml)	0.4–2.0		0.4–2.0	
No. of data points	6		6	
Precision				
Volume of tincture assayed (ml)	1			
No. of replicate results	10			
Concentration (%m/v)	0.055		0.143	
S.D. ($\times 10^{-3}$)	0.2145		1.573	
RSD (%)	0.39		1.10	

* Based on graphs of fluorescence intensity (in arbitrary units) against concentration (μg/ml).

also allows a small sample weight or volume to be used, for example 1 ml of tincture; in contrast, 50 ml is required for the B.P. titrimetric procedure [1].

Also shown (Table 1) are the precision data obtained in 10 replicate determinations of emetine and cephaeline in a sample of tincture of ipecacuanha. The relative standard deviations of 0.39% for emetine and 1.10% for cephaeline indicate excellent precision.

To test the accuracy of the procedure over a wide range of emetine: cephaeline ratios, a number of standard mixtures containing a total concentration of 1 μg/ml and varying in the proportion of the alkaloids from 1:9 to 9:1 were assayed. The results (Table 2) show that good recoveries of both alkaloids were obtained and confirm that the assay is applicable over a wide range of individual concentrations.

Specificity

The wavelengths of maximum excitation and emission and the general shape of the spectra of the sample solutions in 0.1 M hydrochloric acid are identical with those of

Table 2
Recovery of alkaloids in standard mixtures

	Concentration of emetine		Concentration of cephaeline	
Mixture	Added (μg/ml)	Found*	Added (μg/ml)	Found*
1	0.1	99.7	0.9	99.3
2	0.2	98.4	0.8	99.5
3	0.4	97.9	0.6	100.6
4	0.6	99.0	0.4	101.1
5	0.8	97.4	0.2	102.7
6	0.9	102.9	0.1	103.9
	Mean 99.2		Mean 101.2	

* The recovery is expressed as a percentage of the concentration added.

solutions of emetine and cephaeline in 0.1 M hydrochloric acid; this indicates the absence of interference from non-alkaloidal components of the samples. This was confirmed by the observation that in sample solutions which had been made alkaline with ammonium hydroxide, extracted with diethyl ether and then re-acidified with hydrochloric acid to pH 1, there was no fluorescence from the unextracted constituents of the sample.

Authentic specimens of the minor alkaloids of ipecacuanha, psychotrine, (a phenolic alkaloid related to cephaeline) and its non-phenolic methyl ether, *o*-methylpsychotrine (related to emetine) are not available commercially; it has not been possible to investigate the effect of these alkaloids on the accuracy of the assay for emetine and cephaeline. However, the good agreement between the results for emetine and cephaeline by the fluorescence procedure and those for total alkaloids by the titrimetric procedures of the British Pharmacopoeia (Table 3) indicates either that the fluorescence properties of psychotrine and *o*-methylpsychotrine under the conditions of the assay are reasonably similar to those of cephaeline and emetine, respectively, or that the concentrations of the minor alkaloids in the samples are negligible.

Assay results

A number of samples of ipecacuanha powdered root, tincture and liquid extract were assayed for emetine and cephaeline by the fluorescence procedure. For comparison the samples were also assayed for total alkaloids by the titrimetric procedures of the British Pharmacopoeia 1980 [1] and its 1982 Addendum [15], and for non-phenolic alkaloids by the titrimetric procedure of the British Pharmacopoeia 1932 [4, 5]. Good agreement between the results by both procedures was obtained in respect of the concentration of total alkaloids in the liquid samples (Table 3). However, significantly higher (12–18%) concentrations of alkaloids in the roots were obtained by the fluorescence procedure than by the titrimetric procedure. Further investigation showed that the source of the discrepancy arose from the difference between the extraction techniques used to isolate

Table 3
Assay results

	Fluorescence method				B.P. method	
	Emetine* (%)	Cephaeline* (%)	Total* (as emetine)† (%)	Emetine (% of total)	Total* alkaloids‡ (as emetine) (%)	Non-phenolic alkaloids‡ (% of total)
Root	0.88	2.84	3.81	23.1	3.22(3.26)	31.2(30.5)
Root	0.72	2.27	3.06	23.5	2.73(2.74)	29.9(29.9)
Root	0.90	2.64	3.62	24.9	3.08(3.04)	30.7
Tincture	0.056	0.144	0.204	27.5	(0.197)	(32.9)
Tincture	0.052	0.147	0.203	25.3	(0.198)	(33.2)
Tincture	0.070	0.123	0.197	35.3	0.195	39.6
Liquid extract	0.54	1.55	2.14	25.2	2.03(2.06)	32.3(32.5)
Liquid extract	0.57	1.36	1.97	28.8	(2.00)	(34.4)
Liquid extract	0.61	1.33	1.98	30.8	(1.96)	(36.5)
Liquid extract	0.60	1.27	1.91	31.6	1.96	36.3

* Concentration expressed as %m/m for roots or %m/v for liquid samples.
† Presented in this form for comparison with B.P. data.
‡ Values in brackets are the manufacturer's results.

the alkaloids from the powdered root before the analytical measurement. The B.P. assay [15] specifies a simple extraction of the alkaloids from 7.5 g of powdered root (moistened with ammonium hydroxide solution) with 100 ml of diethyl ether, followed by two further 25-ml volumes of ether. The volume of the ether extract recovered by this procedure was found to be only 120–130 ml. Subsequent neutralization of the powder with hydrochloric acid and extraction with 0.1 M hydrochloric acid–methanol (1:3 v/v) produced an extract that when examined by thin-layer chromatography (0.25-mm silica gel G, developed with methanol–chloroform [15:85 v/v]) produced spots which were identical in R_f and colour, when examined under long-wavelength mercury radiation (366 nm), to those of emetine and cephaeline. In addition, the fluorescence spectra of the methanolic extract diluted with 0.1 M hydrochloric acid were identical to those of solutions of emetine and cephaeline in 0.1 M hydrochloric acid; for one sample of the root, the intensity of fluorescence was 10% of that given by the total concentration of alkaloids determined by the B.P. procedure. Thus, the higher concentrations of the alkaloids in the root samples determined by the fluorescence procedure can be explained by the incomplete extraction of the alkaloids by the B.P. procedure.

Discrepancies were also observed between the concentrations of emetine obtained by the fluorescence method and those of non-phenolic alkaloids obtained by titration. The higher values obtained by the latter method are wholly inconsistent with the fluorescence data. The procedure based on simultaneous equations has been used in the assay of emetine to correct for the small contribution of cephaeline to the fluorescence of the sample at pH 12. Even the use of uncorrected values for the fluorescence of samples diluted with 0.01 M sodium hydroxide in order to determine the concentration of emetine does not yield results as high as those given by the titrimetric procedure. Further investigation of the discrepancy in the results would require a complete validation of the 1932 B.P. procedure, particularly in respect of its accuracy and specificity for non-phenolic alkaloids; this task has not been attempted.

Notwithstanding the lack of concordance between the concentration of total alkaloids in the roots assayed by the fluorescence and titrimetric procedures and between the concentrations of emetine determined by fluorescence and of non-phenolic alkaloids obtained by the titrimetric procedure following selective solvent extraction, the major alkaloid in all the samples examined has been shown to be cephaeline, which is present at a concentration approximately twice that of emetine. This indicates that *Cephaelis acuminata* (e.g. Costa Rican or Nicaraguan ipecacuanha) was the source of ipecacuanha in these samples rather than *C. ipecacuanha* (e.g. Rio ipecacuanha) in which the major alkaloid is emetine [3].

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