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SELECTIVE, STABILITY-INDICATING ASSAY OF THE MAJOR IPECACUANHA ALKALOIDS, EMETINE AND CEPHAELINE, IN PHARMACEUTICAL PREPARATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING SPECTROFLUORIMETRIC DETECTION

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SUMMARY

A selective high-performance liquid chromatographic procedure has been developed for the determination of the major Ipecacuanha alkaloids, emetine and cephaeline, in a number of linctus and pastille preparations. The reversed-phase chromatographic procedure uses an octadecyl-bonded column with a mobile phase of aqueous methanol containing an ion-pairing reagent. A spectrofluorimetric detector is used for increased sensitivity and selectivity. Sample preparation is simple, involving either straight dilution for linctus formulations or simple dissolution for pastilles. The procedure has been shown to be stability-indicating. Validation studies, to show that the method is precise, accurate and rectilinear, have been carried out on four linctus formulations and two pastille formulations. The method has been used to determine both emetine and cephaeline at levels as low as 5 μg/g in formulations.

INTRODUCTION

Various methods have been published for the determination of emetine and cephaeline in Ipecacuanha and formulations containing extracts of Ipecacuanha. The official British Pharmacopoeial method for assaying Ipecacuanha, liquid extract of Ipecacuanha and Ipecacuanha tincture involves extraction and titration of the total alkaloids. The method is not selective and is not stability-indicating. The United States Pharmacopoeial assay, based on a method developed by Smith *et al.*[1], requires a determination of the total diethyl ether-soluble alkaloids by extraction and titration, and a separate determination of the cephaeline and emetine by a complex and tedious liquid–liquid partition chromatographic isolation of the individual alkaloids, followed by a spectrophotometric determination. The method is reported to remove minor alkaloids such as emetamine and psychotrine.

More selective procedures, using high-performance liquid chromatography (HPLC) with spectrophotometric detection, have been published by various workers[2–5]. These methods have generally been applied to Ipecacuanha root or liquid extracts in which the alkaloid content is relatively high, between 0.1 and 2%. These

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methods are not sufficiently selective or sensitive when applied to multicomponent formulations containing very low levels of the alkaloids.

In order to improve the limit of determination other workers have used conductance detectors[6], whilst methods involving pre-column derivatisation[7] and post-column derivatisation[8,9] to produce fluorescent compounds have also been described. Crouch *et al.*[10], has recently determined the two alkaloids in biological materials by HPLC using a spectrofluorimetric detector to measure their natural fluorescence.

The current paper describes a selective procedure, using HPLC and a spectrofluorimetric detector, for the rapid, accurate and reproducible determination of emetine and cephaeline at very low levels in a range of pharmaceutical formulations.

EXPERIMENTAL

Chromatographic equipment

A high-performance liquid chromatograph comprising a Waters Assoc. Model 6000A reciprocating pump, a WISP automatic sampler, a Perkin-Elmer LS 4 fluorescence spectrophotometer and an LCI 100 computing integrator was used for routine sample examination.

Chromatographic column

A Waters Assoc. μBondapak C_{18} column, 15 cm × 3.9 mm I.D., was contained in a column oven set at 35°C.

Chromatographic conditions

The pump was set to deliver the mobile phase at 2 ml/min. The fluorescence detector was set with an excitation wavelength of 276 nm, an emission wavelength of 304 nm, a fixed scale value of 0.4 and a response of 4.

Reagents and solutions

Emetine dihydrochloride was supplied by Sigma (Poole, U.K.) and assessed to contain 72% emetine base. Cephealine dihydrobromide was supplied by Sandoz (Basle, Switzerland) and assessed to contain 68% cephaeline base. The other Ipecacuanha related alkaloids were kindly donated by Wellcome Research Lab. (Beckenham, U.K.). All reagents were either HPLC grade or analytical grade and supplied by FSA (Fisons) (Loughborough, U.K.).

The mobile phase was prepared by dissolving 1.0 g of 1-heptanesulphonic acid sodium salt in water; 400 ml of methanol and 1 ml of orthophosphoric acid were added and the solution diluted to 1 l with water. Before use the mobile phase was filtered through a Whatman GF/F filter contained in a Hartley funnel.

Preparation of standard solutions

The required standard solutions were prepared in the following manner.

Internal standard solution: 0.1 g of ethyl 4-hydroxybenzoate was dissolved in 50 ml of acetonitrile and the solution diluted to 500 ml with mobile phase.

Solution of emetine and cephaeline (solution A): 0.01 g of each of emetine dihydrochloride and cephaeline dihydrobromide of known purities were accurately

TABLE I
PREPARATION OF STANDARD SOLUTIONS OF EMETINE AND CEPHAELINE CONTAINING INTERNAL STANDARD

Formulation	*Theoretical Ipecacuanha content (%)**	*Preparation of standard solution*		
		Emetine/cephaeline solution (A) (ml)	*Internal standard (ml)*	*Mobile phase (ml)*
Linctus A and B	0.125	5	10	85
Linctus C	0.3	10	20	70
Linctus D	0.5	20	20	60
Pastilles A and B	0.4	25	50	125

* Expressed as percentage of Ipecacuanha Liquid Extract B.P.

weighed into a 500-ml graduated flask and dissolved in and diluted to volume with mobile phase.

Standard solution of emetine and cephaeline: different strengths of standard solutions were required, depending on the level of Ipecacuanha present in the sample. The standard solutions for the different formulations were prepared as shown in Table I.

Preparation of sample solutions

Sample preparation was dependent on the type of sample being examined *i.e.* liquid or solid, and on the level of Ipecacuanha present. Liquid samples were diluted with mobile phase. Pastilles were dissolved in the mobile phase either with or without gentle heating. The sample solution for the different formulations were prepared as shown in Table II.

Assay procedure

The instrumentation was assembled as previously indicated and the chromatographic column was conditioned by means of a series of injections of the appropriate standard solution of emetine and cephaeline containing internal standard. Before use, and in order to evaluate the chromatographic system, a number of system suitability tests were performed. A standard solution of emetine and cephaeline containing internal standard was injected and the resolution (R) between the cephaeline and internal standard peaks was calculated using the following equation:

TABLE II
PREPARATION OF SAMPLE SOLUTIONS

Formulation	*Sample taken*	*Internal standard (ml)*	*Mobile phase (ml)*
Linctus A and B	10 g	10	80
Linctus C and D	10 g	20	70
Pastilles A and B	10 pastilles	50	150

$$R = \frac{1.18\ (t_2 - t_1)}{W_1 + W_2}$$

where t_1 and t_2 are the retention distances of the peaks and W_1 and W_2 are the widths of the peaks at their half-heights. Typical values for R were in the range 4–6.

At the same time the number of theoretical plates per column (n) was calculated from the cephaeline peak using the following equation:

$$n = 5.54\left(\frac{t}{W}\right)^2$$

where t is the retention distance of the peak, and W = width of peak at its half height. Typical values for n were in the range 1000–2000.

The precision of the chromatographic system was also determined using the percentage relative standard deviation of the ratio of the areas of the cephaeline peak to the area of the internal standard peak and calculated from the result of six successive injections. Typically the percentage relative standard deviation was in the range 0.5–2.

Sample solutions were injected in duplicate with the appropriate standard solution of emetine and cephaeline containing internal standard chromatographed before, after and interspersed with the samples. Volumes of 10 μl were found to be suitable for all samples except Linctus A and B when a volume of 25 μl was used.

Calculations

The areas of emetine, cephaeline and internal standard peaks were measured in the standard and sample chromatograms by integration. Typical retention times of cephaeline and emetine were 1.8 and 3.0 measured relative to the internal standard which was eluted from the column in about 5 min. For each chromatogram the following ratios were calculated:

$$\frac{\text{area of cephaeline peak}}{\text{area of internal standard peak}} \tag{1}$$

$$\frac{\text{area of emetine peak}}{\text{area of internal standard peak}} \tag{2}$$

The mean ratios for cephaeline and emetine in the standard (S_1 and S_2, respectively) and the mean ratios for cephaeline and emetine in the sample (E_1 and E_2, respectively) were calculated.

For the liquid samples the content of cephaeline and emetine was calculated from the following equations:

$$\text{Cephaeline } (\mu\text{g/g}) = \frac{E_1}{S_1} \cdot \frac{W_1}{500} \cdot \frac{\text{Volume of solution A taken (ml)}}{\text{Weight of sample taken (g)}} \cdot P \cdot 10\,000$$

$$\text{Emetine } (\mu\text{g/g}) = \frac{E_2}{S_2} \cdot \frac{W_2}{500} \cdot \frac{\text{Volume of solution A taken (ml)}}{\text{Weight of sample taken (g)}} \cdot F \cdot 10\,000$$

For pastilles the content of cephaeline and emetine was calculated from the following equations:

$$\text{Cephaeline (mg per pastille)} = \frac{E_1}{S_1} \cdot \frac{W_1}{500} \cdot \frac{\text{Volume of solution A taken (ml)}}{\text{Number of pastilles taken}} \cdot \frac{P}{100}$$

$$\text{Emetine (mg per pastille)} = \frac{E_2}{S_2} \cdot \frac{W_2}{500} \cdot \frac{\text{Volume of solution A taken (ml)}}{\text{Number of pastilles taken}} \cdot \frac{F}{100}$$

where W_1 is the weight of cephaeline dihydrobromide (g) taken to prepare solution A, W_2 is the weight of emetine dihydrochloride (g) taken to prepare solution A, P is the percentage of cephaeline in the cephaeline dihydrobromide, F is the percentage of emetine in the emetine dihydrochloride.

METHOD EVALUATION

Selectivity

The use of a spectrofluorimetric detector with variable adjustment of both excitation and emission wavelengths increased the selectivity and the sensitivity of the method. The excitation and emission wavelengths, although not exactly at the maximum values for emetine and cephaeline, were chosen to give a good response for both alkaloids and to allow simultaneous determination of other components present in some of the formulations.

Emetine and cephaeline were separated from each other and from other Ipecacuanha-related alkaloids using the recommended chromatographic conditions. The retention times of the alkaloids, relative to the internal standard are given in Table III.

In order to assess the possibility of interference to emetine and cephaeline from coeluting related alkaloids, diode-array scans were performed on the two alkaloids after chromatographic separation. Because low levels of alkaloids in the formulations precluded accurate spectrophotometeric measurements a sample of Ipecacuanha

TABLE III
RELATIVE RETENTION TIMES OF IPECACUANHA-RELATED ALKALOIDS

Alkaloid	*Relative retention time*
Ethyl 4-hydroxybenzoate (internal standard)	1.0
Cephaeline	1.8
O-methylpsychotrine*	2.1
Didehydroemetine	2.2
Emetamine*	2.7
Tetradehydroemetine*	2.8
Emetine	3.0

* Not detected by fluorimetric detection at recommended wavelengths, retention times measured using a UV detector at 214 nm.

liquid extract was used. The diode-array scans obtained were found to be superimposable with those of a standard solution containing emetine and cephaeline.

The absence of interference in the method (a) from other components of the formulations, (b) from the internal standard and (c) to the internal standard was demonstrated by applying the method (a) to the formulation bases containing no Ipecacuanha liquid extract, (b) to a solution of the internal standard and (c) to samples of the complete formulations without the addition of internal standard, respectively.

Rectilinearity of response

For the chromatographic procedure the relationship between the response, in terms of the ratio of the area of the emetine and cephaeline peaks to that of the internal standard peak, and the amount of each alkaloid chromatographed, was determined. Solutions containing the same concentration of internal standard but different concentrations of emetine and cephaeline were chromatographed. The rectilinearity of concentration–response relationship for emetine and cephaeline is given in Table IV.

Recovery experiments

The accuracy of the method was assessed by applying the method to the appropriate formulation base incorporating accurately weighed amounts of emetine and cephaeline. Recovery experiments were carried out for each formulation and the results obtained are given in Table V.

Precision

The precision of the method was determined by replicate assay of samples with ten complete determinations being performed in each case. The precision results obtained on all the formulations are given in Tables VI and VII.

Stress storage

In order to assess the HPLC procedure as being stability-indicating it was necessary to check the retention times and responses of known degradation products. Schuijt *et al.*[11] had previously shown that on refluxing emetine in water until about

TABLE IV

RECTILINEARITY OF CONCENTRATION–RESPONSE RELATIONSHIP

The estimated regression equations for each analyte are as follows: (1) peak area ratio = 50.8 · (μg of emetine) − 0.03, (2) peak area ratio = 37.6 · (μg of cephaeline) + 0.02. The correlation coefficient for both analytes was 0.999.

Emetine		*Cephaeline*	
μg injected	*Peak area ratio*	*μg injected*	*Peak area ratio*
0.0071	0.319	0.0104	0.413
0.0107	0.525	0.0156	0.599
0.0143	0.704	0.0207	0.801
0.0179	0.876	0.0259	0.992
0.0214	1.063	0.0311	1.180
0.0250	1.235	0.0363	1.389

TABLE V
RECOVERY OF EMETINE AND CEPHAELINE FROM THE FORMULATIONS

Formulation	*Formulation base taken (g)*	*Emetine*			*Cephaeline*		
		Added (mg)	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*
Linctus A	10	0.0713	0.0708	99	0.0756	0.0761	101
Linctus B	10	0.0720	0.0723	100	0.184	0.183	99
Linctus C	10	0.178	0.180	101	0.319	0.317	99
Linctus D	10	0.236	0.238	101	0.613	0.615	100
Pastilles A	20	0.500	0.493	99	0.600	0.614	102

TABLE VI
PRECISION DATA FOR CEPHAELINE ASSAY PROCEDURE

Determination	*Linctus A (μg/g)*	*Linctus B (μg/g)*	*Linctus C (μg/g)*	*Linctus D (μg/g)*	*Pastille A (mg/pastille)*	*Pastille B (mg/pastille)*
1	11.6	11.6	27.9	60.4	0.0101	0.098
2	11.5	11.3	28.3	61.0	0.096	0.097
3	11.5	11.3	27.6	59.2	0.098	0.095
4	11.5	11.4	27.2	59.2	0.099	0.096
5	11.6	11.3	27.8	58.7	0.101	0.098
6	11.5	11.3	28.0	58.5	0.102	0.097
7	11.5	11.4	28.2	59.4	0.098	0.099
8	11.6	11.3	28.2	59.5	0.097	0.100
9	11.5	11.5	28.2	59.0	0.097	0.098
10	11.4	11.0	28.4	57.8	0.097	0.098
Mean	11.5	11.3	28.0	59.3	0.099	0.098
Relative standard deviation (%)	0.5	1.4	1.3	1.5	2.1	1.5

TABLE VII
PRECISION DATA FOR EMETINE ASSAY PROCEDURE

Determination	*Linctus A (μg/g)*	*Linctus B (μg/g)*	*Linctus C (μg/g)*	*Linctus D (μg/g)*	*Pastille A (mg/pastille)*	*Pastille B (mg/pastille)*
1	6.6	6.7	15.8	23.1	0.062	0.057
2	6.4	7.2	15.7	23.1	0.060	0.057
3	6.4	6.9	15.5	21.6	0.060	0.055
4	6.4	7.0	15.2	23.3	0.061	0.054
5	6.5	7.0	15.5	21.8	0.063	0.056
6	6.5	6.9	15.6	22.4	0.065	0.055
7	6.5	6.9	15.7	22.6	0.061	0.056
8	6.7	7.0	15.8	23.0	0.061	0.057
9	6.6	6.9	15.8	22.9	0.061	0.057
10	6.5	7.1	16.1	22.8	0.060	0.056
Mean	6.5	7.0	15.7	22.7	0.061	0.056
Relative standard deviation (%)	1.5	1.9	1.5	2.5	2.6	1.9

15% decomposition of the emetine had occurred the major degradation products were O-methylpsychotrine and didehydroemetine, with small amounts of emetamine and tetradehydroemetine also being produced. The same degradation products were also identified in aqueous solutions of emetine irradiated with UV light at 254 nm, although in this case the degradation was more complex with many additional degradation products also being formed. The recommended HPLC procedure has been shown to be stability-indicating for emetine since it is well separated from its two major degradation products, O-methylpsychotrine and didehydroemetine. The two minor degradation products, emetamine and tetrahydroemetine have no significant fluorescence at the excitation and emission wavelengths recommended and hence do not interfere with the assay. Since cephaeline is very similar to emetine it might be expected to degrade in a similar manner.

In order to show that the assay procedure was stability-indicating for both alkaloids, stress-storage tests were carried out on emetine dihydrochloride, cephaeline dihydrobromide and Ipecacuanha liquid extract solutions in water, 0.1 *M* hydrochloric acid and 0.1 *M* sodium hydroxide solutions. The solutions were heated at 70°C in clear glass vials and sealed with PTFE-lined septa. After two weeks the samples were examined by the recommended HPLC procedure. Emetine and cephaeline were found to degrade readily in aqueous solution, particularly in presence of alkali, as indicated by loss of the analyte when measured by the assay procedure. However, no significant level of degradation products were detected by HPLC. Result of the stress-storage tests are given in Table VIII.

Ruggedness

The ruggedness of the chromatographic system was determined by evaluating alterations to several key parameters. The results of the experiments were used to establish variaton limits for each of the parameters investigated. The parameters investigated included concentration of ion-pair reagent, orthophosphoric acid and methanol in the mobile phase, and column temperature. The following parameter variations could be made for the chromatography to be acceptable to adequately assay samples for emetine and cephaeline.

Concentrations of the mobile phase components were varied as follows; ion-pair

TABLE VIII

RESULTS OF STRESS-STORAGE TESTS ON EMETINE DIHYDROCHLORIDE, CEPHAELINE DIHYDROBROMIDE AND IPECACUANHA LIQUID EXTRACT

Stress-storage test	*Emetine dihydrochloride remaining (%)*	*Cephaeline dihydrobromide remaining (%)*	*Ipecacuanha liquid extract*	
			Cephaeline (%, w/w)*	*Emetine* (%, w/w)*
Two weeks in water in daylight cabinet	96	88	0.70	0.36
Two weeks in water at 70°C	99	62	0.85	0.46
Two weeks in 0.1 *M* hydrochloric acid at 70°C	100	95	1.02	0.50
Two weeks in 0.1 *M* sodium hydroxide at 70°C	11	—**	—**	0.05

* Initial values were: cephaeline, 1.06% (w/w) and emetine, 0.50% (w/w).

** None detected.

reagent: 0.09–0.11%, orthophosphoric acid: 0.05–0.2% and methanol: 36–42%. Within these limits changes of mobile phase composition had little significant effect on the relative separations of the alkaloids and internal standard. Although an increase in column temperature gave a predictable decrease in retention times, temperatures between 30 and 40°C gave satisfactory separations and a column temperature of 35°C was chosen. The use of a controlled temperature improved the precision of the analysis and was essential for automated analysis over a long period of time.

RESULTS AND DISCUSSION

The analytical procedure presented represents a selective, precise, accurate, linear and stability-indicating method for the simultaneous determination of the two major Ipecacuanha alkaloids, emetine and cephaeline, in linctus and pastille formulations.

Selectivity was demonstrated by showing that emetine and cephaeline peaks were free of interference from other Ipecacuanha-related alkaloids and from other components of the formulation bases.

The precision of the method was evaluated for each of the alkaloids in all of the formulations. Relative standard deviations for individual sample preparations were in the range 0.5–2.1% for cephaeline assays and 1.5–2.6% for emetine assays.

The recovery of emetine and cephaeline added to each formulation base was determined. Recoveries of between 99 and 102% were obtained for both alkaloids from all formulations.

The procedure was shown to give a rectilinear response for amounts of emetine injected in the range 0.007–0.025 μg and for cephaeline injected in the range 0.01–0.036 μg.

By showing that known degradation products give no interference with emetine and cephaeline and by examination of stress-stored samples of the alkaloids the procedure has been shown to be stability-indicating.

Although the procedure was developed specifically for the stability-indicating determination of emetine and cephaeline in linctus and pastille formulations it has been used to determine the emetine and cephaeline content of Ipecacuanha liquid extract samples obtained from a number of suppliers. The results obtained are given in Table IX.

TABLE IX
EMETINE AND CEPHAELINE CONTENT OF IPECACUANHA LIQUID EXTRACT SAMPLES

Ipecacuanha liquid extract Sample No.	*Emetine (%, w/w)*	*Cephaeline (%, w/w)*
1	0.57	1.27
2	0.55	1.35
3	0.39	0.98
4	0.50	1.04
5	0.50	1.06
6	0.54	0.80
7	0.55	0.88

Several formulations have been examined for emetine and cephaeline content by the recommended procedure. Each sample was assayed ten times and the results obtained are presented in Tables VI and VII (precision data). Chromatograms of typical linctus and pastille extracts containing internal standard are presented in Figs. 1 and 2, respectively.

Samples of Linctus A after storage for up to six months have been examined for emetine and cephaeline content by the recommended procedure. The results obtained are given in Table X. No significant degradation of the two alkaloids was observed.

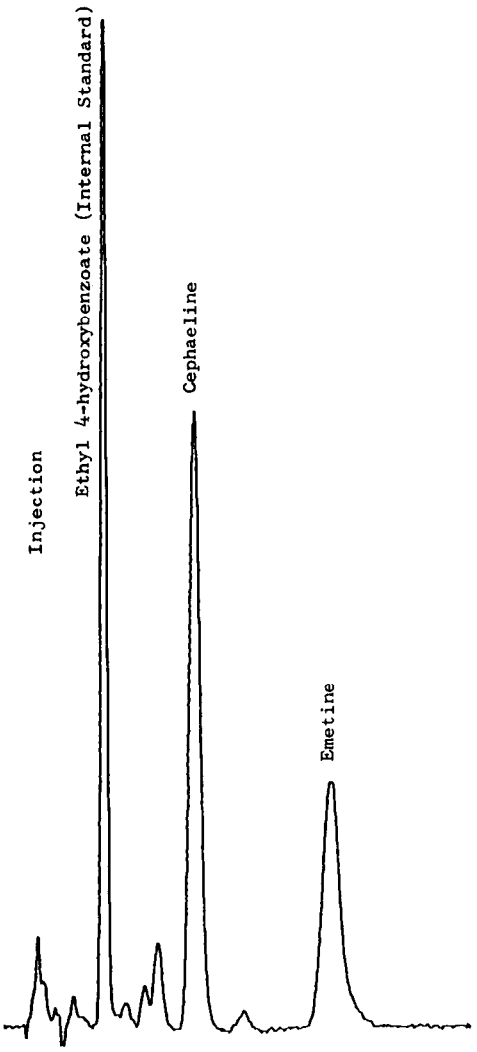

Fig. 1. Chromatogram of linctus solution containing I.S.

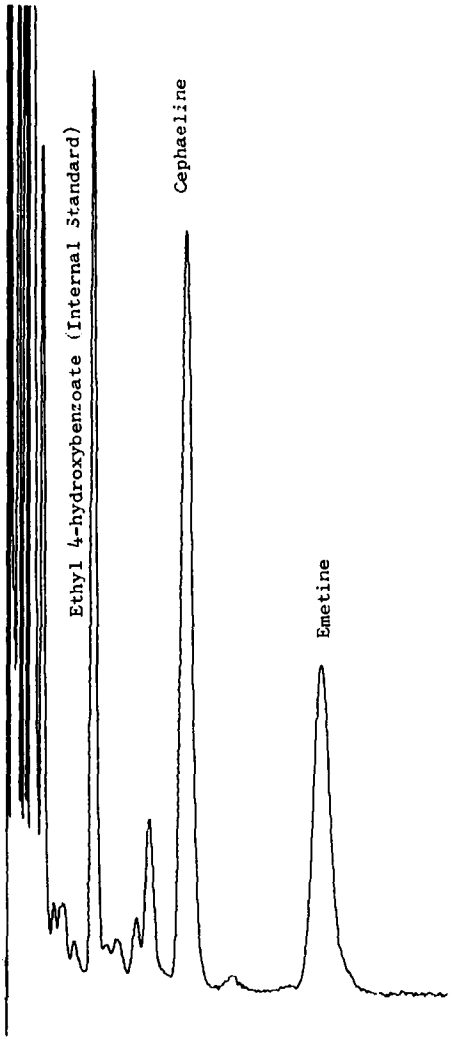

Fig. 2. Chromatogram of pastille extract containing I.S.

TABLE X
EMETINE AND CEPHAELINE CONTENT OF STORED SAMPLES OF LINCTUS A

Linctus A (sample 1)	*Emetine (μg/g)*	*Cephaeline (μg/g)*
Initial	6.6	11.4
One month in daylight cabinet	6.4	11.4
One month at 50°C	6.5	11.2
Three months at 0–5°C	6.4	11.5
Three months at 30°C	6.4	11.4
Three months at 40°C	6.7	11.3
Six months at 22°C	6.5	11.7
Six months at 30°C	6.6	11.8
Six months at 40°C	6.5	11.7

CONCLUSION

The method represents a rugged stability-indicating analytical procedure for the determination of the major Ipecacuanha alkaloids, emetine and cephaeline, in pharmaceutical dosage forms. Selectivity and sensitivity are achieved by the use of a spectrofluorimetric detector. The sample preparation is simple and the analysis time short. The method is amenable to the analysis of a large number of samples and gives precise and accurate results.

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