Journal of Chromatography, 238 (1982) 525–529
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,658

Note

Determination of emetine and cephaeline in Ipecac roots by high-performance liquid chromatography

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(Received December 15th, 1981)

Cephaelis ipecachunha Rich (Rubiaceae), commonly known as Ipecac, has been used[1] as an emetic, a diaphoretic and an expectorant since the 18th century. The importance of Ipecac root increased when Rogers[2] reported the use of emetine, one of the major alkaloids of the Ipecac root, as an excellent remedy for amoebic dysentery and amoebic hepatitis[3]. As the roots are marketed directly and there is the possibility of adulteration, most of the pharmacopoeas[4–6] specifically mention the use of authentic roots with a total alkaloid content of at least 2%. As emetine is the most important of the Ipecac alkaloids, the primary task is to determine the emetine content by an accurate and reproducible method. Although several alkaloids have been isolated[7] from Ipecac roots, it appears that the principal constituents of Indian Ipecac roots are emetine, a non-phenolic alkaloid, and cephaeline, a phenolic alkaloid. The total alkaloid content of Indian roots has been reported to be about 2%, including 1.2–1.3% emetine[8]. Several assay procedures are available for the determination of emetine, such as volumetric[9], colorimetric[10–15], thin-layer chromatographic (TLC)[16,17], polarographic[18], densitometric TLC[19,20], oscillopolarographic[21] and combined methods involving column chromatography and spectrometric methods[22–24]. The most common method is the volumetric method as it does not involve sophisticated instrumentation. However, this method gives only the total alkaloid content.

There have been many reports on the quantitative determination of drugs and drug intermediates by high-performance liquid chromatography (HPLC), such as saponins from *Bupleuri radix* (*Bupleurum falcatum* L.)[25], diosgenin from plants[26], alkaloids from opium[27] and tobacco[28] and phenolic lipids from *Anacardium occidentale*[29] etc. to mention a few. Recently, Verpoorte and Baerheim Svendsen[30] separated 24 alkaloids, including emetine and cephaeline, by HPLC using a silica gel column. Gimet and Filloux[31] have described an HPLC method for the determination of three alkaloids, ephedrine, ethylmorphine and codeine, in syrup samples, which has been extended to the separation of sixteen alkaloids beloning to eight different families. This paper describes a simple and rapid direct isocratic HPLC method for the determination of two major alkaloids, emetine and cephaeline, in Indian Ipecac roots.

0021-9673/82/0000–0000/$02.75

EXPERIMENTAL

Apparatus

A Waters Assoc. liquid chromatograph was used, equipped with a Model 6000A solvent pump, a U6K universal injector, a Model 440 UV absorbance detector an Omniscribe recorder. The chromatograph contained a Waters Assoc. stainless-steel column (300 × 3.9 mm I.D.) packed with μPorasil microparticles (10 μm).

Chemicals and reagents

All solvents used were from BDH (Poole, Great Britain) and were glass distilled. Standard samples of emetine and cephaeline used for the preparation of calibration graphs were isolated from Ipecac roots and purified by standard methods. The purity of each alkaloid was confirmed by TLC on silica gel G with the solvent system benzene–ethyl acetate–diethylamine (7:2:1), HPLC, PMR spectroscopy and preparation of their hydrochlorides.

Preparation of standards

A set of ten standard solutions each for emetine and cephaeline was prepared containing 0.95–0.095 mg/ml and 1.0–0.1 mg/ml, respectively, and were stored in air-tight flasks at 20°C in the dark.

Extraction of alkaloids from Ipecac roots for HPLC analysis

Ipecac roots were dried either at room temperature in air or at 40°C in an air oven and then powdered to 40 mesh. Each of the samples (1 g) was extracted with methanol in a glass percolator for 70 h (5 × 15 ml), and filtered quantitatively using Whatman No. 41 filter-paper and washed with methanol (40°C) until the residue was free from alkaloids. The solution was concentrated *in vacuo* and transferred quantita-

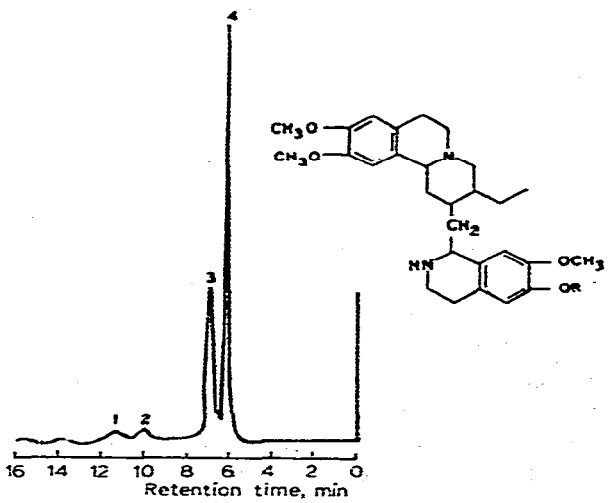

Fig. 1. Representative chromatogram of Ipecac root extract. Peaks: 1, 2 = unknown; 3 = cephaeline, 4 = emetine. Conditions: column, μPorasil (300 × 3.9 mm I.D.); mobile phase, chloroform–methanol–diethylamine (90:10:0.2); flow-rate, 0.5 ml/min; pressure, 150 p.s.i.; detection, UV (280 nm) (1.0 a.u.f.s.); chart speed, 0.5 cm/min; injection volume, 5 μl. Structures: cephaeline, R = H; emetine, R = CH_3.

tively into a volumetric flask using a sample clarification kit consisting of a 10-ml syringe, a Swinney filter holder and millipore filters (0.5 μm). The solution was then made up to a suitable concentration with methanol. A volume of 5 μl of the solution was injected into the chromatograph with a 25-μl Hamilton syringe. A representative chromatogram is shown in Fig. 1.

RESULTS AND DISCUSSION

Separation of emetine and cephaeline was achieved using a μPorasil column (300 × 3.9 mm I.D.) using chloroform–methanol–diethylamine (90:10:0.2) as the mobile phase under isocratic conditions at a flow-rate of 0.5 ml/min and a recorder chart speed 0.5 cm/min. The UV detector was set at 280 nm (1.0 a.u.f.s.). The retention times of emetine and cephaeline were 372 and 420 sec, respectively, and were dependent on the composition of the mobile phase. The determination of emetine and cephaeline was successfully accomplished by comparison with a calibration graph for each alkaloid (Fig. 2) of plotting peak height *versus* amount of compound injected. The relationships were found to be linear over ten measurements at different concen-

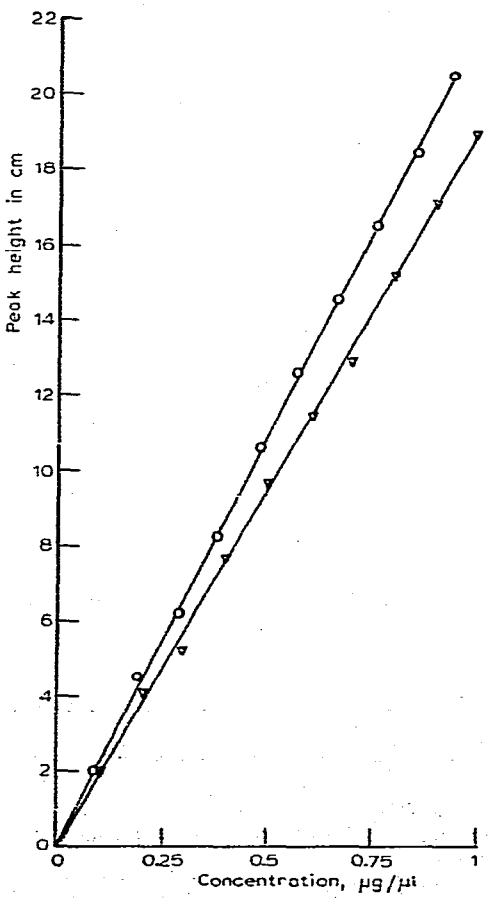

Fig. 2. Calibration graphs for emetine (○) and cephaeline (▽).

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF EMETINE AND CEPHAELINE IN IPECAC ROOTS COLLECTED FROM THE DARJEELING AREA, INDIA

Analysis No.	*Emetine (%)*	*Cephaeline (%)*
1	1.28	0.53
2	1.28	0.53
3	1.30	0.55
4	1.28	0.52
5	1.31	0.55
6	1.25	0.53
7	1.28	0.53
8	1.26	0.50
9	1.31	0.51
10	1.28	0.53
Average	1.28	0.53
Standard deviation	0.02	0.015
Coefficient of variation	1.5	2.8

trations. For analysis 1 g of the sample was used and the method was quantitative and reproducible based on ten measurements. The concentrations of emetine and cephaeline in the samples were found to be 1.28 and 0.53%, respectively. The same results were obtained when more than 1 g of root was used. The coefficient of variation of the method ($n = 10$) was 1.5% for emetine and 2.8% for cephaeline (Table I).

The method is very convenient for the routine simultaneous determination of emetine and cephaeline in Ipecac roots, as the preparation of the sample is easy, the analysis time is short (about 10 min after extraction and preparation of the calibration graphs) and the precision is satisfactory.

ACKNOWLEDGEMENTS

The authors thank Professor B. K. Bachhawat, Director of this Institute, for encouragement, Shri G. C. Subba, Directorate of Cinchona and other Medicinal Plants, Mungpoo, India, for the plant samples and Shri S. K. Roy of this laboratory for technical assistance.

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