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SHORT COMMUNICATION

Quantitative determination of emetine and cephaëline in ipecacuanha root

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Ipecacuanha was introduced into British medicine in 1672 and at the present time two species, *Cephaëlis ipecacuanha* (Brot) A. Rich. (Rio or Brazilian Ipecacuanha) and *Cephaëlis acuminata* Karsten (Cartagena, Nicaragua or Panama Ipecacuanha) are official (British Pharmacopoeia, 1968). The present Pharmacopoeia requires not less than 2% of alkaloid, calculated as emetine; formerly a standard was required for the proportion of non-phenolic alkaloids and this is still of commercial significance.

Volumetric analysis is commonly used for the estimation of the total alkaloid content (British Pharmacopoeia, 1968) but is rather unsatisfactory. It has been claimed (Beckett & Stenlake, 1962) that the phenolic alkaloids exert a buffering action. In addition the yellow colour of the extract tends to mask the end point. Ion-exchange, followed by spectrophotometry, has been applied to ipecacuanha root (Higuchi & Bodin, 1961) and tincture (Kamp, 1957). Kori & Kano (1962) used column partition chromatography for the quantitative separation of the alkaloids. Thin-layer chromatography has previously been applied as a limit test for alkaloids other than emetine (cephaëline, isoemetine, *O*-methylpsychotrine) in emetine hydrochloride (B.P. 1968). It has also been used to identify the constituents of tincture of ipecachuana (Ghosh, Data & Bose, 1968). Such methods have not however been applied to the direct assay of emetine and cephaëline.

This work describes a method for the quantitative estimation of emetine and cephaëline directly on the developed chromatoplates.

Experimental

Materials. Commercial samples of the root of *C. ipecacuanha* and *C. acuminata* were investigated. Pure samples of emetine and cephaëline were used for the preparation of standard solutions of the alkaloids.

Extraction of the root. 1 g of finely powdered root was shaken with 10 ml of a mixture of 3 volumes solvent ether and 1 volume chloroform for 15 min. After standing for 10 min 0·75 ml dilute solution of ammonia was added and the mixture shaken for a further 2 h. The contents of the flask were transferred to a small percolator and the marc percolated with the ether–chloroform mixture until the eluate was free from alkaloid. The extract was evaporated to dryness under reduced pressure and the residue dissolved in absolute ethanol to give 40 ml of solution. This solution was applied to the chromatoplates.

Chromatographic separation. 20 × 20 cm glass plates were coated with a 250 μm layer of Kieselgel G (E. Merck) (aqueous slurry) and dried at 25° for 48 h before use.

An amount of 5 μl of the solution of ipecacuanha alkaloids was applied to the origin together with 5 μl of standard solutions of emetine and cephaëline in ethanol.

The diameter of the zones was 3–7 mm. The loaded plates were developed with a mixture of toluene–benzene–ethyl acetate–diethylamine (35:35:20:10 v/v) for a distance of 12 cm from the origin. The developed plates were dried at 80° for 5 min.

Visualization. Emetine and cephaëline were located by means of their fluorescence in ultraviolet light (366 nm) and by heating at 60° for 20 min after spraying with 0·5% w/v solution of iodine in carbon tetrachloride.

Determination of spot area produced on the chromatoplates was carried out by tracing their outline on 1 cm graph paper and counting squares.

Calibration curves were constructed by plotting the logarithm of the weight of alkaloid applied against the spot area produced by standard solutions of mixtures of emetine and cephaëline developed on the same chromatoplates as the extracts of ipecacuanha root. These were linear over the range 1·5–8 μg.

Total alkaloid content was determined by the method of the B.P. 1968.

Table 1. *Thin-layer chromatography of emetine and cephaëline in ipecacuanha root. Kieselgel G. Toluene–benzene–ethyl acetate–diethylamine* (35:35:20:10 *v/v*)

Alkaloid	Mean Rf value	Ultraviolet light	Iodine reagent: Daylight	Iodine reagent: Ultraviolet light
Emetine	0·54	yellow	yellow	yellow
Cephaëline	0·38	blue	brown	blue

Table 2. *Analysis of the alkaloid content of ipecacuanha root*

Method	Alkaloid	% w/w alkaloid: Rio drug	% w/w alkaloid: Cartagena drug
B.P. 1968	Total	2·49	2·37
TLC	Emetine	1·47* ± 0·16	1·22* ± 0·15
	Cephaëline	0·98* ± 0·13	1·03* ± 0·13
	Total	2·45	2·25

* s.d. on 15 results for each determination.

Results and discussion

Of the various thin layers, alumina gave poor resolution and tailing of the alkaloids; activated silica gel gave good resolution but poor reproducibility. Air-dried plates showed less variation and optimum reproducibility was achieved on plates stored at 25° for 48 h. The solvent system used gave well defined, rounded zones with both alkaloids. Pipetting errors were reduced by applying all solutions with the same micropipette and the use of a constant delivery time for each application.

The results of the chromatographic separation are shown in Table 1.

The total alkaloid of the ipecacuanha samples (B.P. 1968) and the determination of emetine and cephaëline separately are shown and compared in Table 2. The results by the suggested method are in close agreement with those obtained by the official method. Thin-layer chromatography has the advantage that it is less laborious.

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