

## Simultaneous Determination of Emetine and Cephaeline in Ipecac Syrup

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The emetic principles, emetine (EM) and cephaeline (CP), in ipecac syrup were simultaneously analyzed after direct dilution with distilled water, without any other pretreatment. The separation of EM and CP was accomplished by high-performance liquid chromatography (HPLC) with a reversed-phase column, and peaks corresponding to EM and CP were examined by electron ionization-mass spectroscopy (EI-MS). We describe the conditions for the quantitative analysis of EM and CP by HPLC.

**Keywords** ipecac syrup; emetine; cephaeline; high-performance liquid chromatography; emetic; electron ionization-mass spectroscopy

### Introduction

Ipecac has been used as an emetic, an amebacide, and an expectorant in Europe and the U.S.A.<sup>1,2)</sup> Families having children under five years old have often used ipecac syrup as a first-aid medicine when their children had accidentally ingested harmful substances.<sup>3)</sup> In Japan, ipecac has not been used as an emetic, but rather as an expectorant and an amebacide.<sup>4)</sup> In recent years, however, ipecac syrup has been accorded a place in acute medicine, and is beginning to be used in some hospitals in Japan.<sup>5)</sup>

Ipecac contains many alkaloids, for example, emetine (EM), cephaeline (CP), psychotrine, *O*-methylpsychotrine, emetamine, ipecamine, *etc.*,<sup>6)</sup> as shown in Chart 1. Among these alkaloids, EM and CP are the principal ingredients in quantity, and the emetic action is caused by them. The emetic action of CP is twice as strong as that of EM. To evaluate the quality of ipecac, several studies on the analysis of the alkaloids have been reported; thin-layer chromatography (TLC),<sup>7,8)</sup> TLC with densitometry,<sup>9,10)</sup> high-performance liquid chromatography (HPLC),<sup>10,11)</sup> and combined methods involving column chromatography and spectrometric methods.<sup>1)</sup> We have established a simple method for analysis of EM and CP in ipecac syrup. This paper describes a simple and rapid HPLC analysis to evaluate the quantity of ipecac syrup. In order to check the identification and purity of EM and CP peaks separated by HPLC, mass spectroscopy of the two alkaloids was also investigated.

### Experimental

**Authentic Samples** The authentic sample of EM used was purchased from Sigma, and CP was isolated from ipecac powder and purified according to the previous report.<sup>10)</sup> Purity of these alkaloids was confirmed by TLC, electron ionization-mass spectroscopy (EI-MS) and proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectroscopy.

**Materials** Five kinds of commercial ipecac syrup were purchased from the U.S.A. Costa Rican ipecac root, a commercial sample was purchased in Kobe market. Ipecac plants were cultivated in Tsukuba, Japan.

**Preparation of Samples** Two hundred microliters of ipecac syrup was diluted to 4.2 ml with distilled water, and 200  $\mu$ l of chloroquine (6 mg/ml) was added as an internal standard. In order to examine the plant source of ipecac syrup, two kinds of ipecac roots were powdered, and extracted with EtOH-H<sub>2</sub>O (3:1) according to the method described in the U.S.P. The diluted and extracted samples were directly subjected to HPLC analysis.

**HPLC** A Shimadzu liquid chromatograph (model LC-3A) equipped with a variable-wavelength Shimadzu ultraviolet (UV) spectrometer (model SPD-2A) was used. Column: prepacked TSK gel ODS-80TM (5 $\mu$ m, 15 cm  $\times$  4.6 mm). Injection volume: 20  $\mu$ l. Mobile phase and flow rate: 10 mM sodium 1-heptanesulfonate solution adjusted to pH 4 with glacial

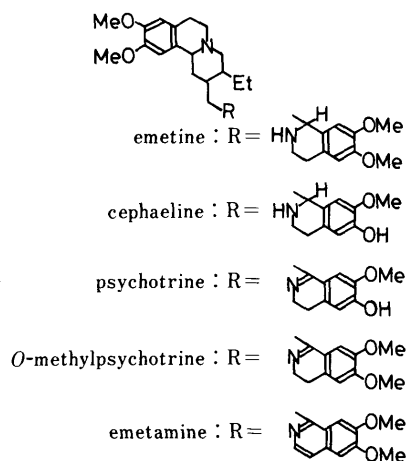


Chart 1. Ipecac Alkaloids

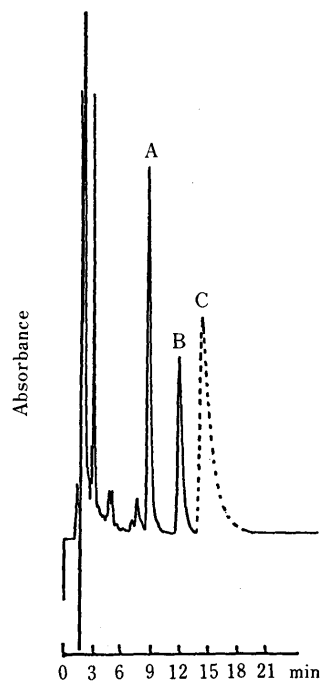


Fig. 1. High-Performance Liquid Chromatogram of Ipecac Syrup

Column, TSK gel ODS 80-TM (4.6 mm i.d.  $\times$  15 cm); mobile phase, 10 mM sodium 1-heptanesulfonate (pH 4 with acetic acid)-MeOH (46:54); flow rate, 1 ml/min; temperature, 25  $^{\circ}$ C; detector, UV at 285 nm. A) CP, B) EM, C) chloroquine (internal standard).

acetic acid-methanol (46:54) at 1 ml/min. Peaks were monitored at 285 nm. For semi-preparative HPLC for EI-MS, a volume of 20  $\mu$ l was injected (10 times). The combined eluates corresponding to each of EM and CP were concentrated and the residues of the EM and CP fractions were extracted with ether and with chloroform, respectively. Each extract was concentrated to dryness and the residues were subjected to EI-MS.

**EI-MS** Measurement conditions: ionizing voltage, 30 eV; ionizing current, 100 A for EM and 300 A for CP; accelerating voltage, 3 kV; ion multiplier, 1 kV; sample temperature, 300 °C; chamber temperature, 200 °C; scan speed, 5 s.

## Results and Discussion

HPLC on a reversed-phase column of TSK gel using 10 mM sodium 1-heptanesulfonate solution-MeOH (46:54) as a mobile phase, as shown in Fig. 1, gave excellent separation of EM and CP.

In order to confirm the identity and the purity of the 2 peaks of EM and CP, semi-preparative HPLC followed by EI-MS was employed. As shown in Figs. 2 and 3, the spectra of the EM and CP isolated by preparative HPLC were found to be identical with those of authentic samples.

It was also confirmed that these peaks separated by HPLC did not contain any impurity. The quantitative analysis of EM and CP was carried out by using chloroquine as an internal standard. As illustrated in Fig. 4, calibration plots of these alkaloids showed good linearity.

Constant weights of EM and CP were added to samples, and the recovery rates were determined (Table I). This method gave good recovery; 99.5% and 105.2% for EM and CP, respectively. Therefore, the present procedure enabled valid quantitative analysis of ipecac syrup.

Previously, we reported the quantitative determination of EM and CP in ipecac powder and roots by using a normal-phase HPLC system.<sup>10)</sup> The method in this report can be applied to not only ipecac syrup but also ipecac powder and root.

By means of this method, we carried out simultaneous determination of the emetic alkaloids in ipecac syrup. Five samples were examined individually five times. The results are summarized in Table II.

The total contents of EM and CP ranged from 153.2 to

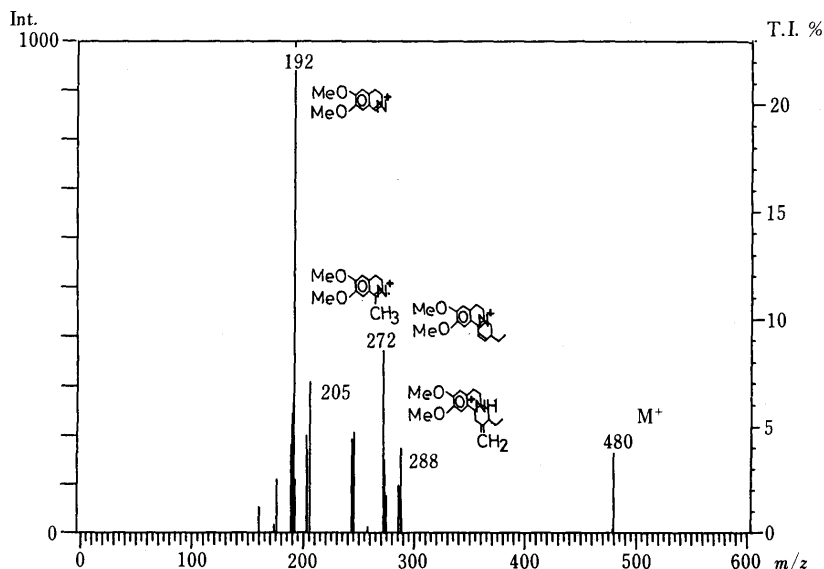


Fig. 2. EI-MS for EM

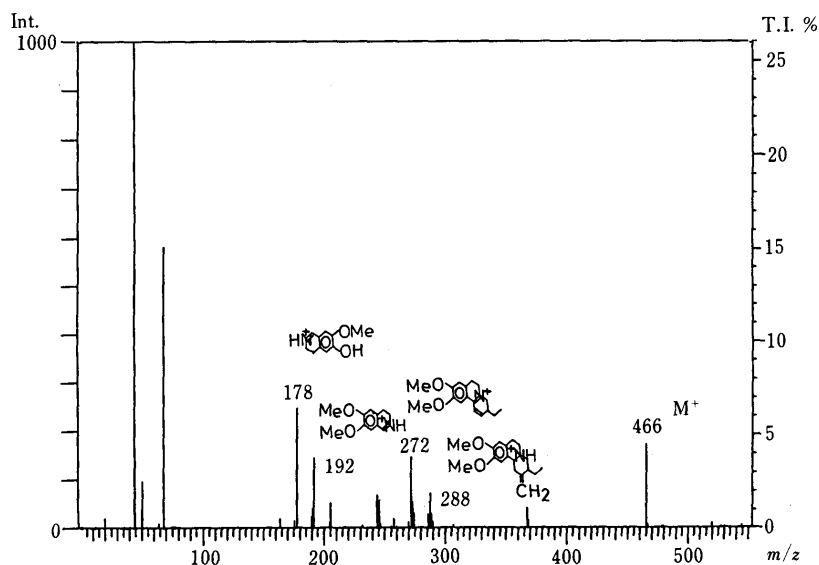


Fig. 3. EI-MS for CP

TABLE I. Recovery of EM and CP from Ipecac Syrup by HPLC

	EM					CP				
	1	2	3	4	5	1	2	3	4	5
Content of alkaloid in ipecac syrup (mg/100 ml)	47.8	45.1	45.4	45.4	42.1	137.1	130.3	135.4	137.4	111.1
Alkaloid added to ipecac syrup (mg/100 ml)	36.2	36.2	36.2	36.2	36.2	34.6	34.6	34.6	34.6	34.6
Alkaloid found in ipecac syrup (mg/100 ml)	80.9	79.3	82.6	82.6	79.3	175.9	171.8	184.2	181.8	153.4
Recovery (%)	96.3	97.5	101.2	101.2	101.3	102.4	104.2	108.4	105.7	105.3
Average (%)			99.5					105.2		

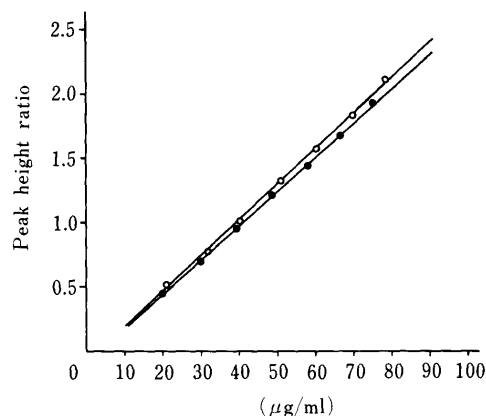


Fig. 4. Calibration Curves of EM and CP

—○—, EM; —●—, CP.

TABLE II. Contents of EM and CP in Ipecac Syrups from the U.S.A. (mg/100 ml)

Sample No.	EM (mg)	CP (mg)	Content ratio (CP/EM)	Total content (mg)
	Mean $\pm$ S.D. <sup>a)</sup>	Mean $\pm$ S.D. <sup>a)</sup>		
1	47.8 $\pm$ 0.3	137.1 $\pm$ 1.1	2.87	184.9
2	45.1 $\pm$ 0.7	130.3 $\pm$ 2.1	2.89	175.4
3	45.4 $\pm$ 0.8	135.4 $\pm$ 2.0	2.98	180.8
4	45.4 $\pm$ 0.5	137.4 $\pm$ 1.8	3.03	182.8
5	42.1 $\pm$ 0.4	111.1 $\pm$ 1.2	2.64	153.2

a) n = 5.

TABLE III. Contents of EM and CP in the Extracts of Ipecac Plant Materials (mg/7 g)<sup>a)</sup>

Material plant	EM content (mg)	CP content (mg)	Content ratio (CP/EM)	Total content (mg)
A <sup>b)</sup>	54.7	103.7	1.90	158.4
B <sup>c)</sup>	49.2	140.8	2.86	190.0

a) Ipecac syrup (100 ml) in U.S.P. includes the extract of 7 g of powdered ipecac. b) Ipecac root from Costa Rica. c) Ipecac root cultivated in Tsukuba, Japan.

184.9 mg/100 ml and the content ratios (CP/EM) were from 2.6 to 3.0. The amount of CP in these ipecac syrups was found to be more than twice that of EM. The levels do not meet the requirements of the U.S.P. standard for ipecac syrup, i.e., "Ipecac Syrup yields, from each 100 ml, not less than 123 mg and not more than 157 mg of the total ether-soluble alkaloids of ipecac. The content of CP varies from an amount equal to, to an amount not more than twice, the content of EM."<sup>1)</sup> Next, the contents of EM and CP in ipecac roots from Costa Rica and cultivated in Tsukuba were determined (Table III).

The content ratio in ipecac roots from Costa Rica was 1.90 and the total content was 158.4 mg/100 ml. Ipecac roots of potted plants cultivated in Tsukuba showed a content ratio of 2.86 and a total content of 190.0 mg/100 ml, i.e., outside the U.S.P. standard. Judging from the results in Table III, the source plant material of ipecac syrups available in the U.S.A. at this time is similar to the ipecac roots grown in Tsukuba.

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