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Journal of Chromatography B, 757 (2001) 197–206

JOURNAL OF CHROMATOGRAPHY B

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High-performance liquid chromatographic assay with fluorescence detection for the determination of cephaeline and emetine in human plasma and urine

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Received 5 December 2000; accepted 16 February 2001

Abstract

A high-performance liquid chromatographic assay method for the quantitation of ipecac alkaloids (cephaeline and emetine) in human plasma and urine is described. Human plasma or urine was extracted with diethylether under alkaline conditions following the addition of an internal standard. Concentrations of alkaloids and internal standard were determined by octadecylsilica chromatographic separation (Symmetry C_{18} columns, plasma analysis; 15 cm×4.6 mm I.D., 5 μm particle size, urine analysis; 7.5 cm×4.6 mm I.D., 5 μm particle size). The mobile phase consisted of buffer (20 mmol/l 1-heptanesulfonic acid sodium salt, adjusted to pH 4.0 with acetic acid)–methanol (51:49, v/v). Eluate fluorescence was monitored at 285/316 nm. The lowest quantitation limits of cephaeline and emetine were 1 and 2.5 ng/ml, respectively, in plasma, and 5 ng/ml in urine. Intra- and inter-day relative standard deviations were below 15%. The assay is sensitive, specific and applicable to pharmacokinetic studies in humans.

Keywords: Cephaeline; Emetine

1. Introduction

Ipecac syrup is an emetic agent prepared mainly from the ethanol extract of *Cephaelis ipecacuanha* (Broterol) A. Richard or *Cephaelis acuminata* Karsten. Ipecac syrup is employed as an initial treatment in a number of countries in cases involving swallowing accidents, including drugs or toxins in children and adults [1,2]. Vomiting usually begins within 30 min following ipecac syrup administration and persists between 30 and 120 min [3,4]. The mechanism of vomit action is believed to involve a twofold process: direct irritation of the gastric mucosa which initiates vomiting, and stimulation of the chemoreceptor trigger zone which aids in the propagation of the emetic response [5].

Cephaeline and emetine are the major pharmacologically active alkaloids in ipecac syrup [2]. Therefore, the development of a chromatographic procedure for the quantitative analysis of these alkaloids in human plasma or urine is necessary for phar-

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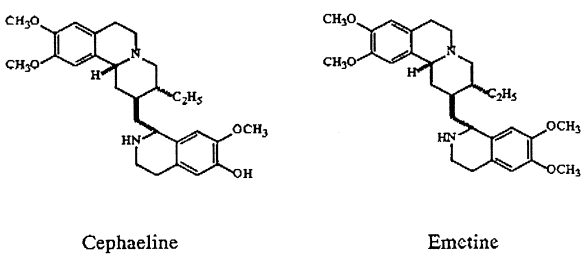
0378-4347/01/$ – see front matter
PII: S0378-4347(01)00170-0

macokinetic studies in clinical trials of ipecac syrup. Little is known about the biodisposition of cephaeline and emetine after oral administration; however, several analytical methods have been published describing high-performance liquid chromatographic (HPLC) approaches [6–8]. These techniques are applicable exclusively to pharmaceutical preparations, moreover, these methods are useful solely in overdose situations. Moran et al. [9] analyzed cephaeline and emetine in blood and urine samples employing a HPLC assay following oral administration of USP ipecac syrup in 10 adult patients. However, the level of biological sample recovered for the assay was not quite low. In this study, greater sensitivity and facilitation of the analytical method were noted when reversed-phase HPLC separation coupled with fluorescence detection is utilized. The methodology for the determination of cephaeline and emetine in human plasma or urine has been validated with respect to the Washington, DC, Conference ''Analytical Methods Validation: Bioequivalence and Pharmacokinetic Studies'' [10]. Subsequently, the technique was applied to the assay of biological samples.

2. Experimental

2.1. Chemicals

Cephaeline and emetine were prepared by Tsumura [11]. The HPLC purities of both compounds with ultraviolet detection were over 99%. Structures are shown in Fig. 1. Yohimbine hydrochloride, 25% ammonia solution, 0.1 and 2 mol/l hydrochloric acid (analytical grade), acetic acid (analytical grade), diethylether (analytical grade), methanol (HPLC grade) and 1-heptanesulfonic acid sodium salt (ion-pair chromatographic grade) were purchased from Wako (Osaka, Japan). High-purity water was prepared as required using a Model DC610 instrument from Organo (Tokyo, Japan).



Cephaeline Emetine

Fig. 1. Chemical structures of cephaeline and emetine.

2.2. Standard solutions

Stock solutions of each reference compound were initially prepared in 0.01 mol/l hydrochloric acid at a concentration of 510 μg/ml. Working standards were derived from the stock solutions by sequential dilution using an identical solvent mixture. Solutions of 5.1, 2.55, 1.275, 0.51, 0.255, 0.1275 and 0.051 μg/ml for plasma sample analysis and of 51, 25.5, 5.1, 2.55, 0.51 and 0.255 μg/ml for analysis of urine were generated. Internal standards (I.S.s) (yohimbine) for plasma and urine analysis were prepared in 0.01 mol/l hydrochloric acid at concentrations of 0.5 and 1 μg/ml, respectively.

2.3. High-performance liquid chromatography

Waters Symmetry C_{18} columns (plasma analysis; 15 cm×4.6 mm I.D., 5 μm particle size, urine analysis; 7.5 cm×4.6 mm I.D., 5 μm particle size) were purchased from Waters (Milford, MA, USA). The chromatographic system consisted of a Shimadzu LC-10AD high-pressure pump (Kyoto, Japan). Jasco fluorescence detectors (plasma analysis; Model FP-920, urine analysis; Model FP-821, Tokyo, Japan) were utilized for the detection of eluted compounds. Both cephaeline and emetine were similarly fluorescent with optimal excitation/emission wavelengths of 287 nm/319 nm and 285 nm/315 nm, respectively in a scanning fluorescence detector (FP-920). This finding was in agreement with previous reports of Hassan [12] and Crouch and Moran [13]. Excitation and emission wavelengths were set to 285 and 316 nm, respectively, according to the report of Crouch and Moran. Excitation and emission slit widths were set to 18 and 10 nm, respectively. Time constant was set at 1.5 s. The temperature of the analytical column was controlled with a Shimadzu OTC-10A. Data acquisition was effected employing Shimadzu C-R4AX CHROMATOPAC. The mobile phase consisted of a

mixture of buffer (20 mmol/l 1-heptanesulfonic acid sodium salt, adjusted to pH 4.0 with acetic acid)–methanol (51:49, v/v). The mobile phase was degassed for approximately 5 min prior to use. Eluting compounds were separated at a flow-rate of 1.0 ml/min and a temperature of 40°C.

2.4. Preparation of calibration standards

Drug-free human plasma was acquired from Cosmo Bio (Tokyo, Japan). The plasma (1 ml) was spiked with 20 μl of working standards (0.051–5.1 μg/ml) in order to provide seven individual concentrations in the range of 1–100 ng/ml. Drug-free human urine was obtained from healthy male volunteers. The urine (1 ml) was spiked with 20 μl of working standards (0.255–51 μg/ml) so as to provide six individual concentrations in the range of 5–1000 ng/ml. A 0.4-ml aliquot of biological sample was mixed with 50 μl of I.S., 50 μl of 0.1 mol/l hydrochloric acid and 2 ml of diethylether. After vortex mixing (2 min) and centrifugation (1600 *g*, 5 min), the organic phase was discarded. This process was repeated twice. Subsequently, 50 μl of 25% ammonia solution and 2 ml of diethylether were added to the aqueous phase. Following further vortex mixing (2 min) and centrifugation (1600 *g*, 5 min), the organic phase was transferred to a glass tube. The organic phase was then evaporated with nitrogen gas. The sample was dissolved in 200 μl of 0.1 mol/l hydrochloric acid. A 100 μl (plasma) or 50 μl (urine) aliquot of the acid phase was injected onto the HPLC system. Peak-area ratios of cephaeline and emetine relative to the I.S.s were calculated by Microsoft Excel (Microsoft, Seattle, WA, USA). A calibration graph of peak-area ratio versus cephaeline and emetine concentrations added to the plasma or urine was then plotted. Each $1/y$ (plasma) or $1/y^2$ (urine) weighted least-squares regression line of best data fit was calculated in order to permit quantitation of drug concentration.

2.5. Extraction yield

The extraction yield from plasma or urine was estimated by comparing the peak area of cephaeline and emetine obtained following extraction of spiked samples with that observed for direct injection without extraction.

2.6. Accuracy and precision

Drug-free human plasma and urine were spiked with standard solutions at concentrations of 1, 2.5, 5, 25, 100 ng/ml and 5, 100, 1000 ng/ml, respectively. An aliquot from each pooled sample was handled by the procedure described above and analyzed on 3 different days in order to evaluate inter-day variations. Intra-day variations were examined in triplicate samples. Accuracy was determined as percentage error [(measured−added)/added]×100 (%). Precision was evaluated by the relative standard deviations (RSDs).

2.7. Freeze–thaw stability

Drug-free human plasma and urine were spiked with standard solutions in order to generate final concentrations of 25 and 100 ng/ml and 100 and 1000 ng/ml, respectively. Aliquots (plasma; 2×0.4 ml, urine; 3×0.4 ml) were immediately taken from each pooled specimen. Subsequently, cephaeline and emetine concentrations were determined. Remaining sample was frozen.

On two separate occasions during the day, the pooled sample was thawed. Aliquots (plasma; 2×0.4 ml, urine; 3×0.4 ml) were taken for assay and the remainder refrozen.

2.8. Storage stability

Drug-free human plasma and urine were spiked with the standard solutions in order to provide final concentrations of 25 and 100 ng/ml and 100 and 1000 ng/ml, respectively. Aliquots (2×0.4 ml) were assayed immediately (day 0). The remaining sample was partitioned and frozen at −20°C after partition (10×1 ml). Subsequently, over a period of up to 90 days, individual aliquots were thawed and 0.4 ml duplicates were assayed against a concurrently prepared standard curve.

2.9. Pharmacokinetics and data analysis

Six healthy male volunteers (phase I study at the Osaka Pharmacology Research Institute) were administered ipecac syrup containing cephaeline (0.843 mg/ml) and emetine (0.503 mg/ml) at doses of 5, 10, 15, 20, 25 and 30 ml. The volunteers received a standardized diet throughout the study period. Prior to and following administration, 150 ml and 50 ml of tap water, respectively, were given to aid in stomach distention. Venous blood samples (5 ml) were obtained prior to dosage and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 12, 48 and 168 h after dosing. Samples were collected in heparinized tubes. Plasma was prepared by centrifugation at 1600 *g* for 10 min. Urine samples were collected initially before drug administration and at intervals of 0–6, 6–12, 12–24 and 24–48 h following dosing. Additionally, urine samples were collected randomly (1, 2, 4, 8 and 12 weeks) after dosing. Plasma and urine specimens were stored at −20°C until analysis.

Pharmacokinetic parameters were determined via non-compartmental procedures. C_{max} represented the maximum concentration detected in plasma, whereas t_{max} denoted the corresponding time. $t_{1/2}$ was defined as the half-life of the terminal phase. Areas under the plasma concentration–time curve ($AUC_{0-168\ h}$) were calculated by the trapezoidal rule.

2.10. Preparation of quality controls

Drug-free human plasma and urine were spiked with standard solutions at final concentrations of 2.5, 25 and 100 ng/ml and 5, 100 and 1000 ng/ml, respectively. Two aliquots (0.4 ml each) were removed from each sample and assayed.

3. Results and discussion

3.1. Chromatographic separation

Typical chromatograms of the extracts from the drug-free samples and the spiked with cephaeline, emetine and internal standard are presented in Fig. 2A and B (plasma) and Fig. 3A and B (urine). Chromatograms are presented in Fig. 2C for plasma (1 h) and Fig. 3C for urine (6–12 h) following the oral administration of 15 ml of ipecac syrup. Ipecac alkaloid (cephaeline and emetine) fluorescence enhanced selectivity and sensitivity. Interference from endogenous substance was not in evidence in either plasma or urine, as illustrated in Figs. 2 and 3.

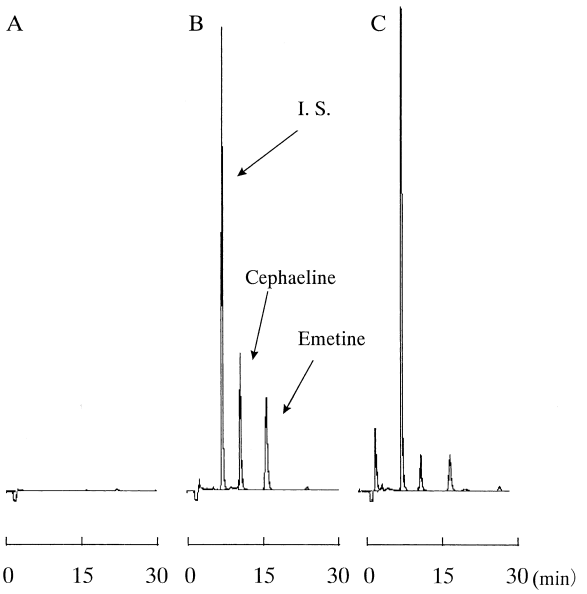


Fig. 2. Chromatograms of extracted (A) drug-free human plasma, (B) drug-free human plasma with 25 ng/ml of both cephaeline and emetine, and internal standard (I.S.), and (C) sample plasma with 8.7 ng/ml of cephaeline and 10.5 ng/ml of emetine, taken 1 h post oral administration of ipecac syrup at 15 ml, spiked with I.S. only.

In plasma, the I.S., cephaeline and emetine eluted at approximately 7, 11 and 16 min, respectively. In urine, the I.S., cephaeline and emetine eluted at approximately 4.5, 7 and 10 min, respectively. Both alkaloids were well separated under established chromatographic conditions.

3.2. Extraction yield

Plasma extraction yields (5 ng/ml, mean±SD, $n=3$) were 84.5±7.8% and 79.4±8.5% for cephaeline and emetine, respectively. Urine extraction yields (50 ng/ml, mean±SD, $n=3$) were 77.6±2.3% and 79.3±10.2%, respectively. Recovery did not attain 100%. It was hypothesized that some alkaloid was lost due to the nature of the liquid–liquid extraction scheme.

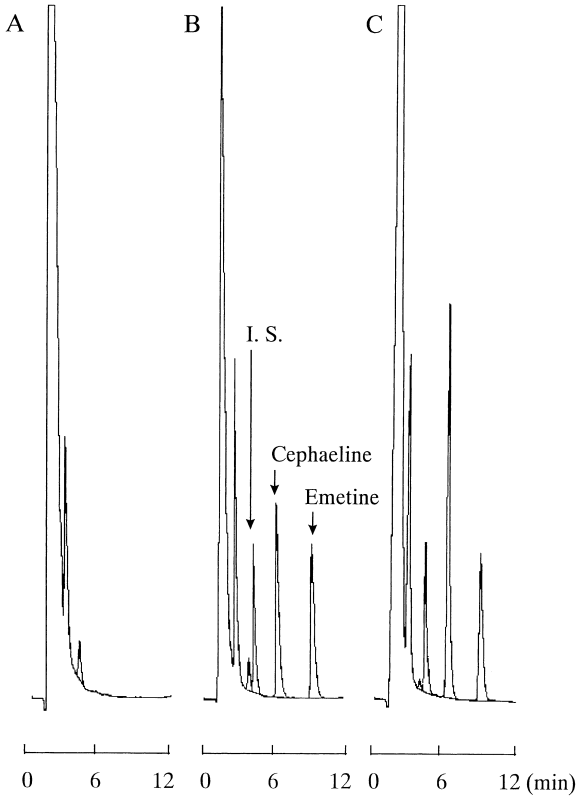


Fig. 3. Chromatograms of extracted (A) drug-free human urine, (B) drug-free human urine with 100 ng/ml of both cephaeline and emetine, and internal standard (I.S.), and (C) sample urine with 144.7 ng/ml of cephaeline and 98.2 ng/ml of emetine, taken 6–12 h post oral administration of ipecac syrup at 15 ml, spiked with I.S. only.

3.3. Linearity of method response

Linearity of response over the concentration range 1–100 ng/ml or 5–1000 ng/ml was demonstrated on the basis of peak-area ratios (cephaeline or emetine/I.S.) versus concentration plots. Values given by $1/y$ (plasma) or $1/y^2$ (urine) were utilized in weighted least-squares linear regression analysis. Linear regression was performed during method validation and sample assay. In all cases, correlation values (r) were greater than 0.995. The mean calibration curves as part of the method validation gave the following equations. Plasma: cephaeline, $y=0.0142x+0.00714$, $r=0.9988$; emetine, $y=0.0134x+0.00783$, $r=0.9977$. Urine: cephaeline, $y=0.0153x-0.000557$, $r=0.9987$; Emetine, $y=0.0134x-0.0224$, $r=0.9989$. Good linearity was observed between the peak-area ratio and concentrations.

3.4. Method accuracy and precision

Plasma concentrations of cephaeline and emetine were determined following spiking at 1, 2.5, 5, 25 and 100 ng/ml using triplicate measurements per concentration for intra-day assay. Single analysis was effected on 3 different days in the case of inter-day assay. Intra- and inter-day parameters calculated from these data are summarized in Tables 1 and 2. At concentrations of 2.5, 5, 25 and 100 ng/ml for each alkaloid, good accuracy and precision were demonstrated by both intra- and inter-day

Table 1
Intra-day variation ($n=3$) in the determination of cephaeline and emetine in human plasma and urine

Nominal concentration (ng/ml)	Precision (RSD, %)		Accuracy[a] (%)	
	Cephaeline	Emetine	Cephaeline	Emetine
Plasma				
1	5.6	14.3	3.3	6.7
2.5	10.6	8.9	0.0	−6.7
5	7.4	9.1	−3.3	−4.0
25	2.9	7.5	2.9	−4.4
100	1.2	2.7	−4.4	−3.0
Urine				
5	2.3	10.3	−4.0	5.3
100	10.8	11.3	−5.1	−2.4
1000	4.1	2.9	−4.0	4.4

[a] Accuracy; error (%)=[(measured−added)/added]×100 (%).

Table 2
Inter-day variation ($n=3$) in the determination of cephaeline and emetine in human plasma and urine

Nominal concentration (ng/ml)	Precision (RSD, %)		Accuracy[a] (%)	
	Cephaeline	Emetine	Cephaeline	Emetine
Plasma				
1	10.0	37.5	0.0	−20.0
2.5	6.0	6.8	1.3	−10.7
5	1.9	5.3	4.0	0.0
25	2.8	5.8	−0.1	−3.3
100	4.9	5.4	0.4	1.0
Urine				
5	9.3	3.8	1.3	4.0
100	4.7	1.5	−3.7	−5.8
1000	2.7	7.6	4.0	−0.1

[a] Accuracy; error (%)=[(measured−added)/added]×100 (%).

assays. Mean concentration occurred within the range 89.3–104.0% of the nominal value with an RSD of less than 15%. Assay of cephaeline concentration of 1 ng/ml proved to be accurate and precise; however, in the case of emetine, the procedure proved less accurate and precise. In this validation, the accuracy and precision were regulated for as follows; the mean value of the accuracy should be for within ±15% of the nominal value except at the limited of quantitation where it should not deviate by more than ±20%. The precision around the mean value should not exceed 15% RSD, except for the limited of quantitation where it should not exceed 20% RSD. On the basis of these findings, the limits of quantitation of cephaeline and emetine for this method were defined as 1 and 2.5 ng/ml, respectively.

Urine concentrations of cephaeline and emetine were determined following spiking at 5, 100 and 1000 ng/ml as per the scheme described above. At concentrations of 5, 100 and 1000 ng/ml for both alkaloids, good accuracy and precision were demonstrated by both intra- and inter-day assays. Accuracy was less than ±6% with an RSD of less than 15% (Tables 1 and 2). On the basis of these findings, the limit of quantitation of cephaeline and emetine for this method was defined as 5 ng/ml.

3.5. Freeze–thaw stability

The repeated thawing and freezing of plasma and urine specimens due to the nature of the experimental procedure were considered. Consequently, the freeze–thaw stability of cephaeline and emetine were investigated. Plasma and urine concentrations determined following up to two freeze–thaw cycles are presented in Table 3. The data suggest that the freeze–thaw process had no significant effect on plasma concentration. In contrast, a second freeze–thaw process in the case of urine revealed an apparent decrease in the determined concentration of 100 ng/ml. As a result, there is a need to partition the study urine samples prior to freezer storage.

3.6. Storage stability

The storage (−20°C) of cephaeline and emetine in human plasma and urine were investigated at concentrations of 25 and 100 ng/ml and 100 and 1000 ng/ml, respectively. Spiked plasma and urine standards were assayed at the time of preparation (day 0) and subsequently on days 30 and 90. These data are summarized in Table 4. The results indicate no apparent time-dependent decrease.

3.7. Pharmacokinetics

Mean cephaeline and emetine plasma concentration–time profiles (0–12 h) for healthy male volunteers following single oral administration of 5, 10, 15, 20, 25 and 30 ml are presented in Fig. 4. Following each oral dosing, cephaeline and emetine were detected in plasma of 4, 4, 5, 6, 4, 6 volunteers and 1, 4, 6, 6, 5, 5 volunteers, respectively. Plasma

Table 3
Freeze–thaw stability of human plasma and urine

Nominal concentration (ng/ml)	Freeze–thaw cycle	Sample concentration[a] (ng/ml)		Deviation from preparation (%)	
		Cephaeline	Emetine	Cephaeline	Emetine
Plasma					
25	–	25.7	26.5	–	–
25	2	24.9	26.8	−3.1	1.3
100	–	102.5	104.9	–	–
100	2	97.9	101.0	−4.4	−3.7
Urine					
100	–	101.5	100.5	–	–
100	1	92.4	92.0	−9.0	−8.5
100	2	77.4	77.1	−23.7	−23.3
1000	–	1058.2	1058.7	–	–
1000	1	976.7	977.8	−7.7	−7.6
1000	2	905.9	905.6	−14.4	−14.5

[a] Data are expressed as the mean (plasma: $n=2$, urine: $n=3$).

cephaeline and emetine were not detected in any samples taken at 168 h post dosing. The pharmacokinetic parameters of both alkaloids are presented in Table 5. Mean t_{max} values tended to be in the range 0.8–2.4 h for cephaeline and 0.3–2.7 h for emetine. Mean C_{max} values of cephaeline and emetine were 1.7–5.6 and 3.6–8.7 ng/ml, respectively. Mean C_{max} values of emetine were higher than those of cephaeline in each group. C_{max} values of cephaeline and emetine were followed by a decrease exhibiting a $t_{1/2}$ of 2.6–18.8 and 9.6–18.0 h, respectively. Concentration profiles of cephaeline and emetine demonstrated a two-peak pattern; however, the mechanism remains unknown. The area under the mean plasma concentration–time profiles ($AUC_{0-168\ h}$) of cephaeline and emetine ranged from 7.0 to 42.7 and 0.9 to 66.8 ng·h/ml, respectively. However, the AUC values of the doses ranging from 5 to 30 ml did not follow a dose-dependent course (cephaeline; $r=0.1122$, emetine; $r=0.2177$). The

Table 4
Plasma and urine storage stability of human plasma and urine at −20°C

Nominal concentration (ng/ml)	Day of assay	Plasma concentration[a] (ng/ml)		Deviation from preparation (%)	
		Cephaeline	Emetine	Cephaeline	Emetine
Plasma					
25	0	25.7	26.5	–	–
25	30	22.6	24.3	−12.1	−8.1
25	90	26.3	24.0	2.3	−9.3
100	0	102.5	104.9	–	–
100	30	97.8	94.4	−4.6	−10.0
100	90	103.9	101.0	1.4	−3.8
Urine					
100	0	93.2	89.6	–	–
100	30	93.9	90.6	0.8	1.1
100	90	102.1	109.2	9.5	21.9
1000	0	928.1	887.7	–	–
1000	30	984.2	944.3	6.0	6.4
1000	90	1020.7	976.5	10.0	10.1

[a] Data are expressed as mean ($n=2$).

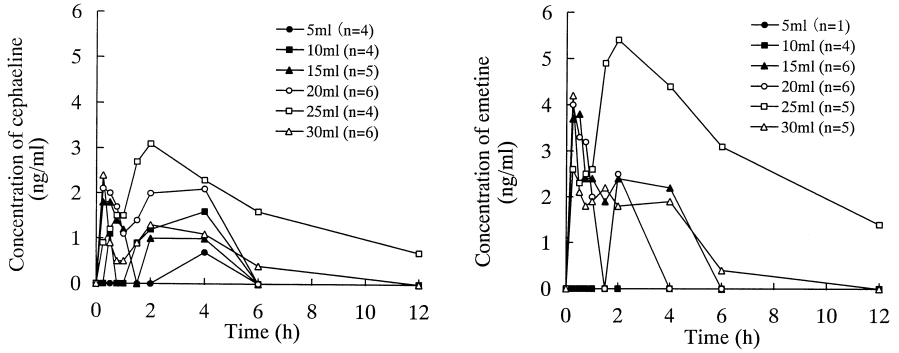


Fig. 4. Mean plasma concentration–time profiles for cephaeline or emetine following single oral administration of ipecac syrup to male healthy volunteers.

reason was believed due to vomiting. In the clinical phase I trial of this drug [14], vomiting, slight diarrhea and headache are evident. Most volunteers exhibited vomiting 1–4 times within 1 h. Cephaeline and emetine levels in vomits per dosage were 9.1–77.3 and 10.1–83.8%, respectively. Consequently, when dosage is corrected for the amount of both alkaloids in vomit, the correlation of AUC versus the dosage for cephaeline and emetine improved to $r=0.5812$ and $r=0.7422$, respectively.

The cumulative urinary excretion amounts of cephaeline and emetine following a single oral administration of 5, 10, 15, 20, 25 and 30 ml is presented in Fig. 5. Cumulative excretion amounts of cephaeline within 48 h following each dosing described above were 28.5±13.8, 99.0±35.0, 108.8±48.4, 207.5±102.1, 350.8±223.2 and 184.5±75.4 μg/ml, respectively. In the case of emetine, cumulative excretion amounts were 27.0±7.2, 75.4±21.8, 71.5±34.6, 148.7±86.3, 229.2±140.5 and 119.3±36.6 μg/ml, respectively. The cumulative excretion amounts of the dosage ranging from 5 to 30 ml did not follow a dose-dependent course as well as AUC. However, when dosage is corrected by the aforementioned method, the correlation of cumulative urinary excretion amount within 48 h versus the dosage of cephaeline and emetine was satisfactory. Values were $r=0.8281$

Table 5
Pharmacokinetic parameters of cephaeline or emetine in human plasma after oral administration of ipecac syrup to male volunteers

Dose (ml)	*n* (detectable)	t_{max} (h)	C_{max} (ng/ml)	AUC_{0-168} (ng·h/ml)	$t_{1/2}$ (h)
Cephaeline					
5	4	2.1±2.2	1.7±0.5	7.9±8.7	18.8±24.6
10	4	1.7±1.7	3.0±1.9	14.0±14.3	7.9±6.3
15	5	0.8±0.7	3.1±2.0	10.0±8.1	8.9±6.4
20	6	1.1±1.4	3.6±2.6	39.6±66.4	11.6±15.0
25	4	2.4±1.1	5.6±2.6	42.7±23.7	8.8±6.2
30	6	1.5±1.5	3.1±3.8	7.0±7.6	2.6±1.4
Emetine					
5	1	0.3	3.6	0.9	
10	4	1.7±1.7	4.5±2.6	12.7±14.5	14.5±21.4
15	6	0.7±0.7	5.8±2.6	14.0±8.3	15.5±16.9
20	6	1.0±1.5	6.0±3.0	44.0±63.1	18.0±24.4
25	5	2.7±1.2	8.7±5.8	66.8±50.7	9.6±4.1
30	5	1.2±1.6	6.2±4.4	13.8±8.5	10.0±10.6

Data are expressed as the mean values±SD for detectable volunteers.

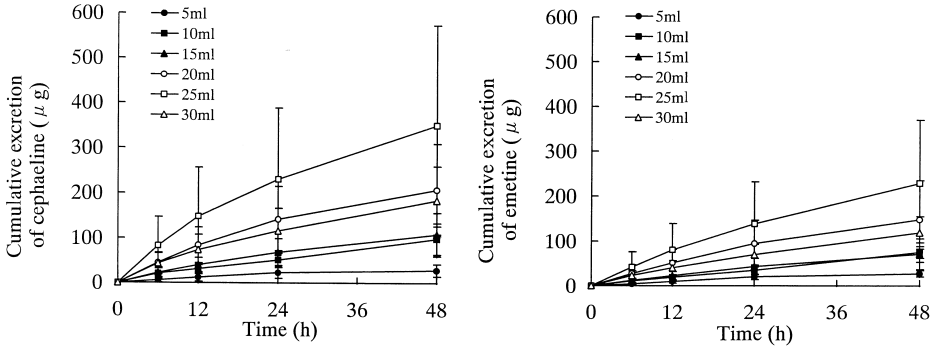


Fig. 5. Cumulative urinary excretion amount of cephaeline or emetine following single oral administration of ipecac syrup to male healthy volunteers. Data are expressed as the mean±SD for six volunteers.

and $r=0.8333$ for cephaeline and emetine, respectively. In any case, cumulative urinary excretion of both alkaloids within 48 h were less than 3%. However, alkaloids were present in urine at 168 h in most volunteers; moreover, in several cases, alkaloids could be detected up to 12 weeks following administration. This finding is in agreement with previous reports indicating that emetine is detectable in urine up to approximately 60 days after administration [15].

3.8. Assay of quality control sample

During the assay of the study samples, duplicate quality controls were prepared at three concentrations and assayed as a check of method performance. On all occasions, accuracy of the quality control standards was within a bylaw value (low; within ±20%, middle or high; within ±15%), the criteria used for the rejection of an assay run.

4. Conclusions

The present study describes a rapid and sensitive method is applicable to the determination of ipecac alkaloids (cephaeline and emetine) in human plasma or urine. The approach entails the utilization of liquid–liquid extraction and HPLC with fluorescence detection. The methodology for the determination of cephaeline and emetine in human plasma or urine has been developed referring to HPLC conditions reported for detection of these alkaloids in biological samples [13] or pharmaceutical preparations [16]. Using 0.4 ml of plasma and urine, the accurate quantitative ranges were determined to 1 (cephaeline) or 2.5 (emetine)–100 ng/ml and 5–1000 ng/ml, respectively based on the method validation. Moreover, the stability of the analyte in biological matrix at storage temperature and freeze–thaw cycles did not exert on the influence of routine drug analysis.

Finally, the present investigation demonstrated suitable applicability of the ipecac alkaloids (cephaeline and emetine) to pharmacokinetic studies.

Acknowledgements

This work was supported by Osaka Pharmacology Research Institute in the form of supplied human biological samples from phase I clinical trials. The authors are grateful to Professor Jyunichi Azuma and his staff.

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