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ORIGINAL PAPER

HPLC–MS analysis of ipecacuanha alkaloids in pharmaceutical relics from eighteenth century

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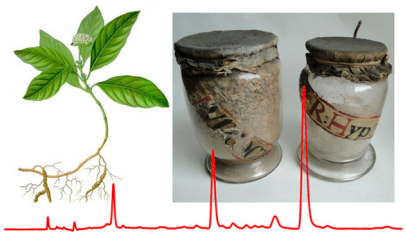
Abstract

The alkaloids in two historical pharmaceutical relics of ipecacuanha, dated to the eighteenth century, were analyzed by HPLC–MS. The conditions of extraction and separation were optimized. Four alkaloids (emetine, cephaeline, protoemetine, and *O*-methylpsychotrine) were found. The content of the main ipecacuanha alkaloids, emetine and cephaeline, in analyzed samples is fairly decreased in comparison with standard current pharmaceutical substance. *O*-Methylpsychotrine has been identified as the emetine decomposition product. The identity of the compounds was confirmed by MS^2, and the ESI^+ fragmentation mechanisms of compounds found have been proposed.

Graphical abstract



Keywords High-performance liquid chromatography · Decomposition · Long-term stability · Mass spectrometry · Emetine · Cephaeline

Introduction

Analysis of historical pharmaceutical relic composition may answer various questions ranging from a pure curiosity, through revealing their true content and purpose, up to the study of their stability and identification of potential degradation products [1]. We have had a unique opportunity to study two historical pharmaceutical relics of ipecacuanha, the one of the drugs which enriched European medicine thanks to the transoceanic expeditions to South America at the beginning of the modern era [2]. Both analyzed relics come from rarely preserved baroque monastic pharmacy of Capuchin order in Prague [3]. The pharmacy was established around the year 1680; its function was terminated at the end of eighteenth century.

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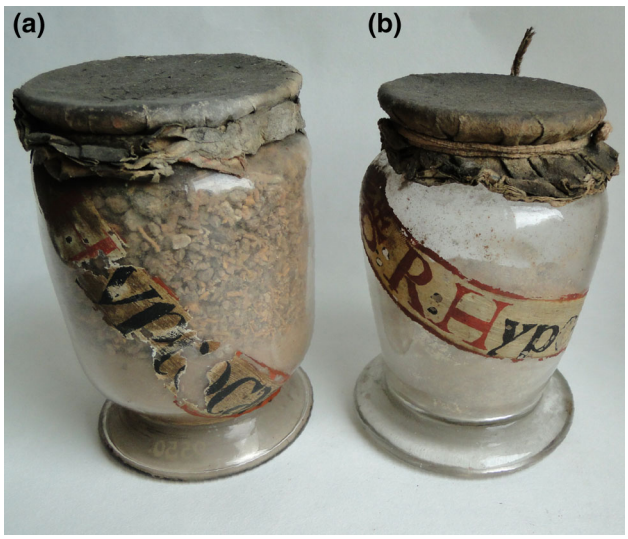


Fig. 1 Baroque pharmaceutical jars provided **a** Historical sample I, root of ipecacuanha (National Museum, inv. no. H2-9556), and **b** Historical sample II, powdered root of ipecacuanha (National Museum, inv. no. H2-9550)

Thereafter the pharmacy was completely untouched to the year 1895, when it was donated to the National Museum in Prague. For that reasons, the pharmacy equipment is preserved in a high baroque state, including almost all the pharmaceuticals. According to the inscriptions on the pharmaceutical glass jars, both studied relics supposedly are ipecacuanha (Fig. 1) and are older than 200 years. Analyzing the content of these jars, we have not only confirmed the authenticity of the content, but also studied its degradation and compared the results obtained with the ones described in the literature. Besides, an optimization of HPLC method and the ESI^{+}–MS^{2} fragmentation mechanisms of the found ipecacuanha alkaloids are presented.

Ipecacuanha consists of the dried roots and rhizomes of either *Cephaëlis ipecacuanha* Brot. or *Cephaëlis acuminata* Karsten [4]. In large doses it is an emetic, and in smaller ones it is an expectorant and a restorative. It is also considered a specific in treatment of dysentery [5]. The drug was introduced in European medicine at the end of the seventeenth century, first to treat dysentery, and later, in the eighteenth century, it was mixed with opium to celebrated Dover's powder which was used widely to treat fevers and agues for the next 200 years [6]. Ipecacuanha principally contains two alkaloids, emetine and cephaeline, which are accompanied by several structurally similar alkaloids [7–9]; the selected structures are depicted in Fig. 2. The treatment with ipecacuanha pharmaceutical substance as well as the pure emetine has been ceased on the grounds of their toxicity, and is gradually replaced by safer and more effective compounds [10]. On the other hand, new curative properties of ipecacuanha alkaloids are intensively studied, e.g., anticancer [11] or antiviral [12] activity.

The influence of light and heat on the stability of ipecacuanha alkaloids is reported in the literature, particularly for injection solution of pure emetine. The oldest works mention coloration of emetine injection solutions kept in diffuse sunlight and only hypothesize decomposition products [13, 14]. Decomposition of emetine hydrochloride injection solutions stored in temperatures 8, 20, 37 °C, and light stability cabinet was studied by Bayraktar-Alpmen [15]. *O*-Methylpsychotrine, psychotrine, cephaeline, emetamine, and rubremetine were found as decomposition products of emetine by thin-layer chromatography. It was also established that an increase of solution pH supported the emetine decomposition. The effect of heating on the stability of alkaloids in the tincture of ipecacuanha root (i.e., in alcoholic extract) was also studied, and decrease of the alkaloid content about 27% was found after 5 h of refluxing [16]. According to Stahl and Schmidt [17], thermolysis of emetine at 220 °C is resulting in the formation of 1-methyl-3,4-dihydro-6,7-dimethoxyisoquinoline, dimethoxyisoquinoline, and 1-methyl-6,7-dimethoxyisoquinoline. Finally, Schuijt et al. [18] thoroughly studied photochemical as well as thermochemical decomposition of emetine and identified ten products. On irradiation of emetine with UV-light, its oxidation products are formed, from which *O*-methylpsychotrine is the most abundant. Based on these findings, stabilization of emetine injection solution has been proposed using cyclodextrins [19] or organic amines [20, 21]. No data on the stability of ipecacuanha root are presented in the literature, only pharmacopeias prescribe that it should be kept in a well-closed container, protected from light.

Results and discussion

Optimization of extraction procedure

Various methods have been proposed for extraction of alkaloids from ipecacuanha [22], employing diethyl ether [23, 24], methanol [25], methanol followed by chloroform re-extraction [7], or binary mixture of diethyl ether and chloroform [26]. Usually the alkaline medium is used as ipecacuanha alkaloids are weak bases. Using standard current pharmaceutical substance of ipecacuanha with known alkaloids content, we tested extraction of alkaloids by methanol or diethyl ether, both with and without addition of aqueous solution of ammonia. The best result is provided by treating the drug with 200-fold volume of diethyl ether and 50-fold volume of 5% aqueous solution of ammonia; the extraction yield of alkaloids is 98.0 ± 3.5%.

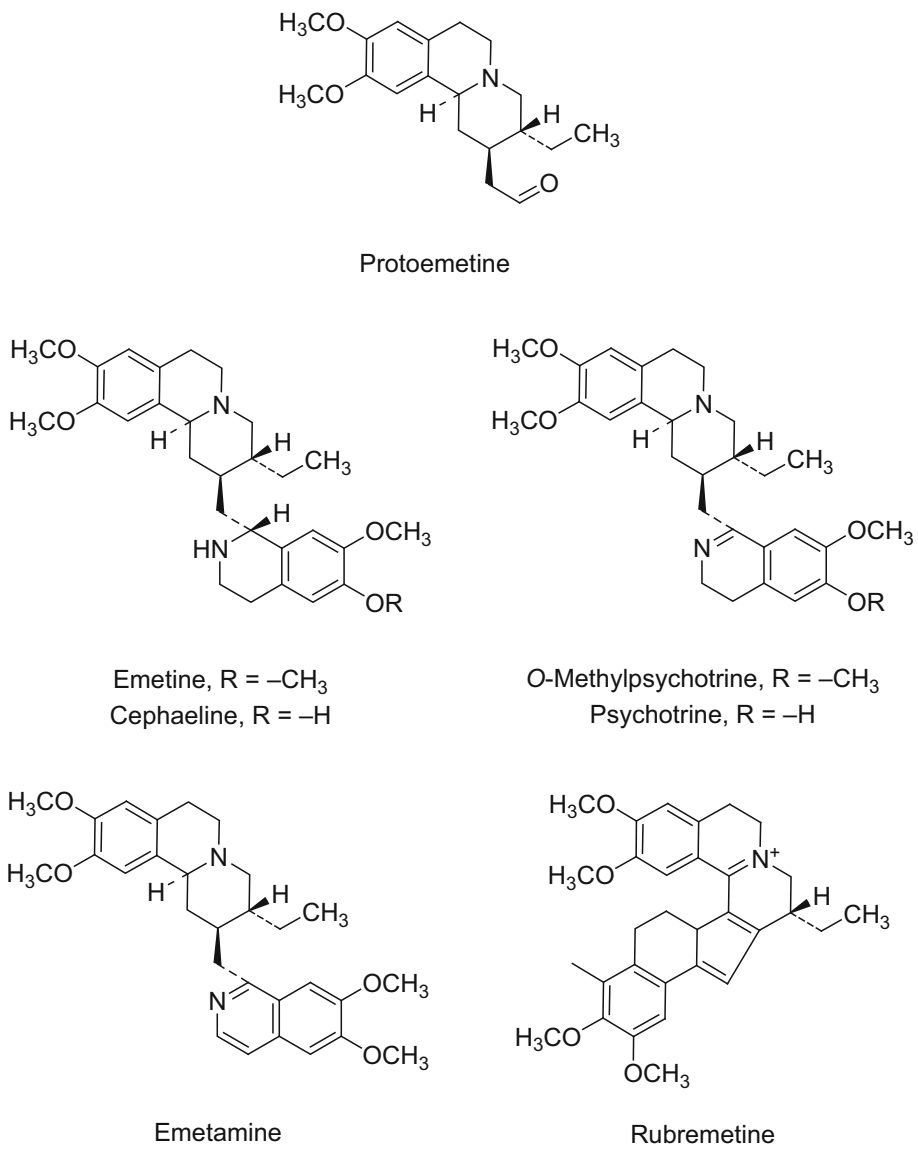


Fig. 2 Chemical structure of emetine and related alkaloids from ipecacuanha

Optimization of separation

High-performance liquid chromatography methods for ipecacuanha alkaloids determination are mainly performed in reverse-phase mode, employing mobile phases based on methanol and aqueous buffers [22, 27] or on acetonitrile and aqueous buffers [28]. We selected method introduced by Elvidge et al. [29, 30] based on RP C-18 column and mobile phase consisting of buffered aqueous sodium 1-hexanesulfonate solution and methanol. To improve the HPLC separation of analytes, we optimized: (1) ratio of both mobile phase constituents in the range of buffer:-methanol (v/v) = 40:60, 50:50, 60:40, and 70:30, and (2) mobile phase flow from 0.1 to 0.3 cm^3/min with step of 0.05 cm^3/min. Finally, isocratic elution by the mixture of 0.02 M sodium 1-hexanesulfonate of pH adjusted to 4.00 and methanol in the ratio 50:50 (v/v), with flow rate of 0.2 cm^3/min, was adopted for analysis.

Application to historical samples

The optimized extraction and separation procedures were applied to the historical samples and (as a control) to standard current pharmaceutical substance of ipecacuanha (Fig. 3). The identification of compounds was based on comparison with standards (for emetine and cephaeline, retention times and UV spectra were observed) and/or the high-resolution tandem mass spectrometry; the quantification was based on calibration dependencies (Table 1). The unlabeled peaks in Fig. 3 were identified as various compounds normally occurring in a plant material. None of these were structurally related to ipecacuanha alkaloids.

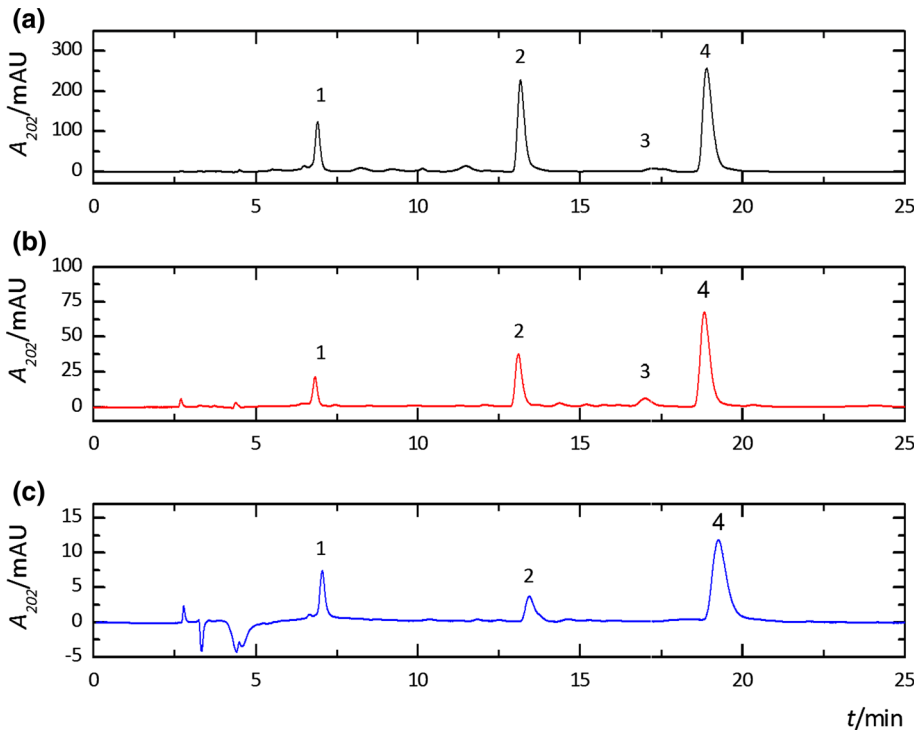


Fig. 3 HPLC–UV chromatograms of the analyzed ipecacuanha samples: **a** standard current pharmaceutical substance, **b** Historical sample I, root of ipecacuanha (National Museum, Inv. No. H2-9556), and (c) Historical sample II, powdered root of ipecacuanha (National Museum, Inv. No. H2-9550). Identified compounds: (1) protoemetine, (2) cephaeline, (3) *O*-methylpsychotrine, and (4) emetine. XBridge® BEH C18 column (150 × 3.0 mm i.d., particle size 2.5 µm; Waters), mobile phase: 0.02 M sodium 1-hexanesulfonate (pH 4.00) and methanol (50:50, v/v), flow rate 0.2 cm^3/min, UV detection at 202 nm

Table 1 Identification and quantification of the compounds in the analyzed ipecacuanha samples (retention time in HPLC, identified compound, relative area of the peak, and concentration in the sample)

t_r/min	Compound	Current substance		Historical sample I		Historical sample II	
		Area/%	Conc./%	Area/%	Conc./%	Area/%	Conc./%
6.9	Protoemetine	11.7	–[a]	8.4	–[a]	14.6	–[a]
13.2	Cephaeline	33.8	0.80 ± 0.10	23.4	0.16 ± 0.02	15.2	0.013 ± 0.004
17.2	*O*-Methylpsychotrine	2.3	–[a]	5.6	–[a]	–[b]	–[b]
19.0	Emetine	52.1	0.89 ± 0.03	62.4	0.29 ± 0.02	70.3	0.05 ± 0.01

[a]Not determined

[b]Peak under limit of detection

The concentration of the main ipecacuanha alkaloids, emetine and cephaeline, in both analyzed historical samples is lower than in standard current pharmaceutical substance. However, the content of alkaloids could only be compared with current drug, because the content in historical samples in the time of their collection is naturally not available. In powdered ipecacuanha (Historical sample II), only 5.6% of standard content of emetine, resp. 1.6% of standard content of cephaeline, has even been found. More than twice higher concentration of *O*-methylpsychotrine in Historical sample I (in comparison with standard current pharmaceutical substance) indicates that it is a degradation product of emetine, which is in accordance with [18]. Unfortunately, the concentration of alkaloids in Historical sample II was so low that *O*-methylpsychotrine peak was under limit of detection.

Protoemetine was found in all analyzed ipecacuanha samples. It has been identified as a precursor of several ipecacuanha alkaloids including cephaeline and emetine [31, 32]. According to the ratio of emetine:cephaeline content (i.e., 2–3:1), both historical samples are indeed *Cephaëlis ipecacuanha* and the standard current pharmaceutical substance is confirmed to be *Cephaëlis acuminata* (the ratio emetine:cephaeline = 1:1) [33].

Mass spectrometry

Mass spectrometry was employed to elucidate or to confirm the identity of compounds found by HPLC–UV in samples. Moreover, the ESI^+–MS^2 spectra of three ipecacuanha alkaloids have been studied in detail to amend information published in the literature.

For protoemetine, the ion m/z = 350.233 was selected as the precursor ion; its MS^2 spectrum is given in Fig. 4a. The mass spectra of protoemetine are slightly mentioned in the literature; only molecular ion without fragmentation has

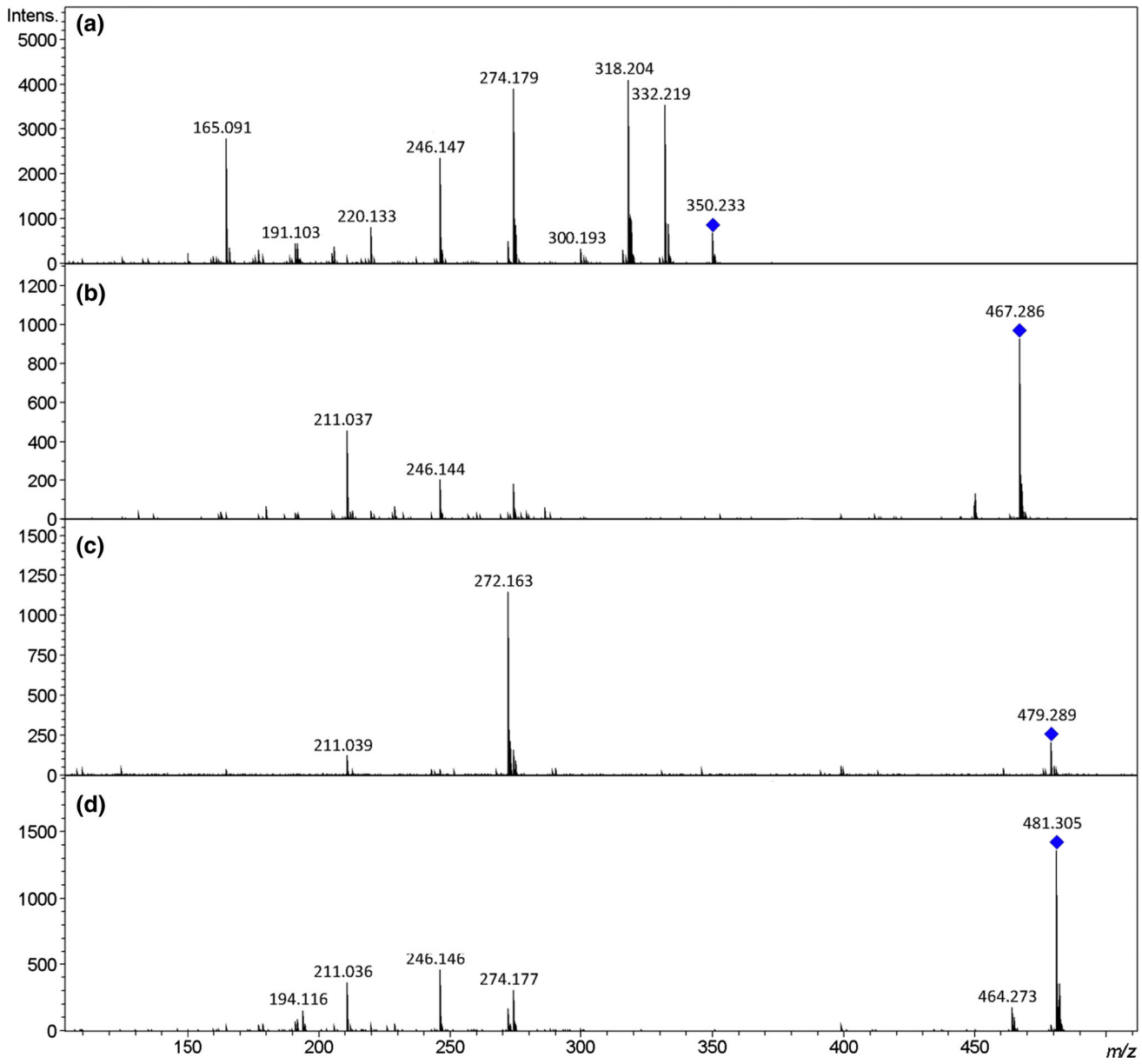


Fig. 4 ESI^+–MS^2 spectra of the product ions of **a** protoemetine, **b** cephaeline, **c** *O*-methylpsychotrine, and **d** emetine

been published, e.g., [32, 34]. The mechanism of protoemetine ESI^+ fragmentation is, therefore, proposed in Fig. 5. Comparing molecular weight of protoemetine with the mass of the precursor ion, the formation of background ion $[M + CH_3OH + H]^+$ of protoemetine with methanol from mobile phase is evident. The main product ion (m/z = 318.205) originates from elimination of a methanol molecule and it is protoemetine plus H^+. Elimination of a water molecule from the ion gives the very unstable ion of m/z = 300.193, as it is obvious by its small intensity in the spectrum. The second most intense product ion of protoemetine with m/z = 274.195 originates by elimination of ethylene group, which increases the stability of this ion as its intensity in spectrum indicates. The fragmentation of this ion by elimination of ethylene group gives rise to the important ion m/z = 246.147, which is common in fragmentation of all ipecacuanha alkaloids. There are two parallel ways of the next fragmentation of this ion. First one produces ion of m/z = 191.103 by elimination of $–NC_3H_2$ group. At the second one, the loss of ethylene group leads to ion m/z = 220.133, and finally the elimination of $–NC_3H_5$ group leads to 1,2-dimethoxy-4-ethenylphenyl ion with m/z = 165.091.

The close structural similarity of emetine and cephaeline leads to parallelism in their mass spectra (Fig. 4b, d). The mechanism of fragmentation of emetine and cephaeline

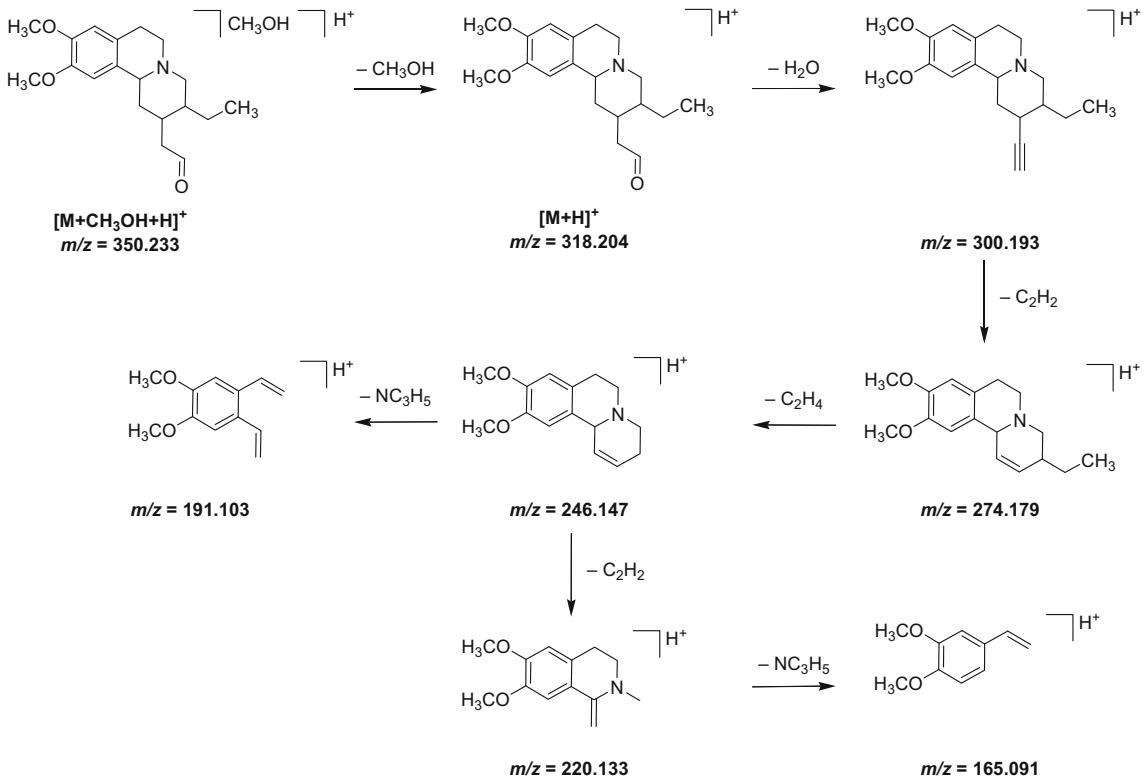


Fig. 5 ESI^+–MS^2 fragmentation of protonated protoemetine

ions is depicted in Fig. 6. At the one mode of fragmentation, the charge localized at the nitrogen atom of secondary amine on isoquinoline moiety is resulting in the cleavage of ammonia molecule. It is followed by elimination of ethyl or methyl group. Second, the fragmentation of bond between both cyclic parts of molecule leads to formation of a pair of ions (**d**, **e** and/or **f**, **g** in Fig. 6). The cleavage of ethyl or methyl group from ions **d** or **g** leads to the formation of the important ion m/z = 246.146, which is common in fragmentation of all ipecacuanha alkaloids. Its next fragmentation was discussed above. The proposed fragmentation is analogous to the interpretation of mass spectra by Budzikiewicz et al. [35], Spiteller and Spiteller-Friedmann [36], and EI ionization published by Thesima et al. [37].

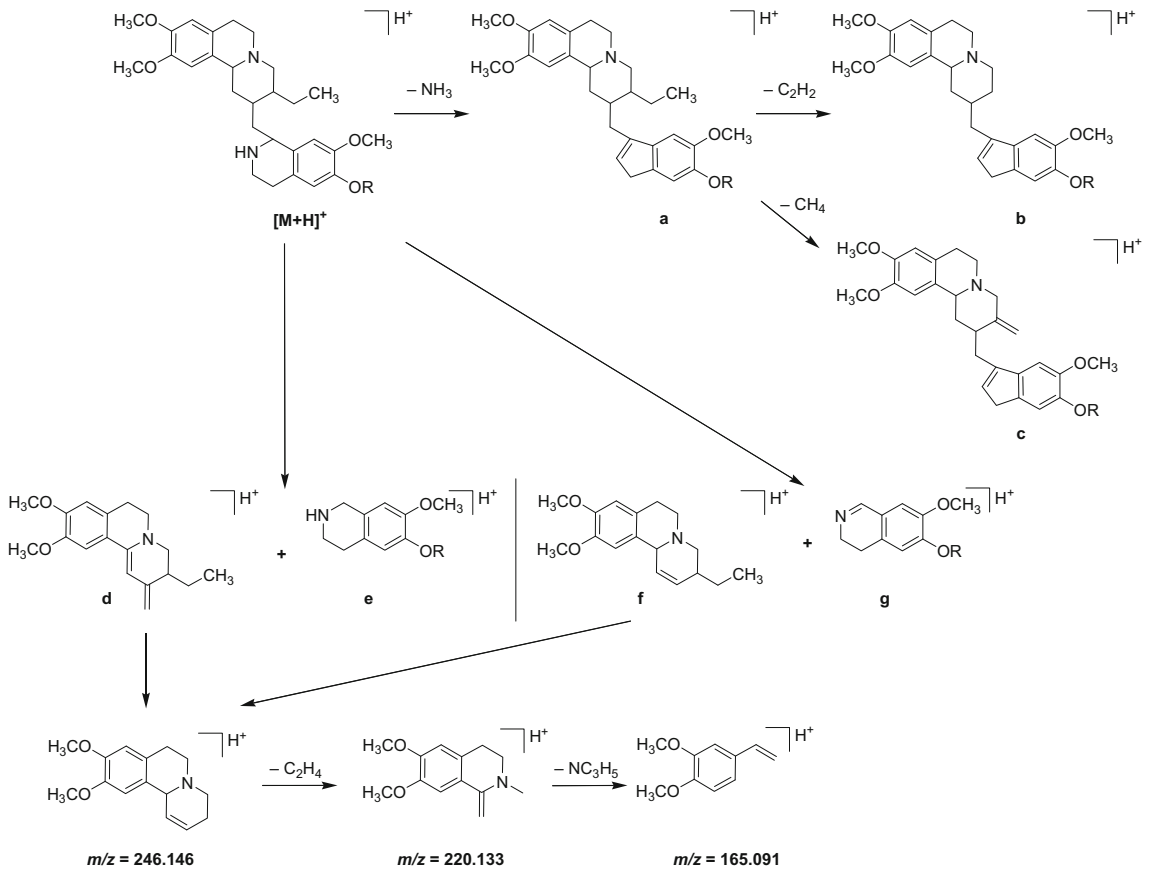
The mass spectrum of the last found alkaloid, *O*-methylpsychotrine, depicted in Fig. 4c, is in full agreement with spectrum and fragmentation pathway published in detail by Schuij et al. [38].

Conclusion

Two historical samples of ipecacuanha, more than 200-year old, were analyzed using HPLC–MS^2. Both the main ipecacuanha alkaloids, emetine and cephaeline, were detectable, but their content is much lower than in standard current pharmaceutical substance of ipecacuanha. Thus, the content of the examined baroque pharmaceutical jars was authenticated. *O*-Methylpsychotrine was found as possible degradation product of emetine. The findings amend published information on stability of ipecacuanha alkaloids. In addition, the detailed ESI^+–MS^2 fragmentation mechanism of protoemetine is proposed, which—to our best knowledge—has not yet been described in the literature.

Experiment

The historical samples of ipecacuanha are coming from collection of old Czech history, National Museum (Prague, The Czech Republic). Both baroque pharmaceutical glass jars are dated to the eighteenth century [3] and are still closed with original leathern lids, fastened by a string (Fig. 1). The first jar (inv. no. H2-9550; here referred as Historical sample I) of height 9.0 cm and diameter 6.5 cm is labeled in Latin 'R. Hypeocan', which on translation means 'root of ipecacuanha.' The second jar (inv. no. H2-9556, here referred as Historical sample II) of the same size is labeled in Latin ' ♃ R: Hypocoaco,' which on translation means 'powdered root of ipecacuanha.' The relevant jar was gently opened, and using glass spoon three samples of the



Compound	*R*	*m/z*							
		$[M+H]^+$	**a**	**b**	**c**	**d**	**e**	**f**	**g**
Cephaeline	–H	467.286	450.264	422.234	–	286.181	180.102	274.177	178.079
Emetine	$–CH_3$	481.305	464.273	436.249	448.248	–	–	274.177	192.102

Fig. 6 $ESI^+–MS^2$ fragmentation of protonated cephaeline or emetine

content were collected: one from the center of the jar content and two from the opposite sides located at the wall of the jar. The collected samples were stored in glass containers in dark. Prior to the analysis, the sample was homogenized in a porcelain mortar and the powder was placed in a desiccator with phosphorus pentoxide as a desiccant on 24 h.

The current pharmaceutical substance of ipecacuanha (the dried root of *Cephaëlis acuminata*) came from pharmacognosy collection of Faculty of Pharmacy, Ankara University, Turkey (inv. no. AEF 27031). The total alkaloid content was determined by potentiometric titration with 0.1 M perchloric acid in glacial acetic acid medium [22] as 1.71 ± 0.94% of dry weight. It was used as a standard in optimization of extraction and separation procedures.

Emetine and cephaeline hydrochlorides, European Pharmacopoeia reference standards, were purchased from Sigma-Aldrich. The other chemicals employed: acetic acid (≥ 99.5%), ammonium hydroxide solution (≥ 25%), chloroform, diethyl ether, methanol, and sodium 1-hexanesulfonate were of HPLC or p.a. grade, and purchased from Sigma-Aldrich.

For extraction of alkaloids, the analyzed powdered sample was weighed about 100 mg to an Erlenmeyer flask. An amount of 5 cm^3 of 5% aqueous solution of ammonia and 20 cm^3 of diethyl ether was added and extraction took 24 h. The organic layer was separated and filtered using 0.2 μm filter (Whatman). An amount of 1 cm^3 of the extract was evaporated at the temperature of 40 °C and redissolved in 1 cm^3 of methanol prior to HPLC analysis.

A liquid chromatograph UHPLC Nexera XR (Shimadzu, Japan) with an internal diode-array detector was used,

Table 2 Figures of merit of HPLC-UV determination of emetine and cephaeline based on area of the peak

Compound	Emetine	Cephaeline
Linear dynamic range/mg dm^{-3}	0.5–15.0	0.5–15.0
Slope of calibration/mAU min mg^{-1} dm^3	126.31 ± 1.89	92.97 ± 0.24
Intercept/mAU min	− 29.3 ± 13.6	− 2.39 ± 1.64
R^2	0.9991	0.9999
Limit of quantification/mg dm^{-3}	1.60	0.31
Limit of detection/mg dm^{-3}	0.48	0.09

followed by a Compact QTOF Bruker mass spectrometer (Bruker, Germany) with ESI ionization. The XBridge® BEH C18 (150 × 3.0 mm i.d., particle size 2.5 μm; Waters) was used. The column temperature was maintained at 40 °C. The mobile phase was composed of methanol and 0.02 M sodium 1-hexanesulfonate of pH 4.00 (adjusted by diluted acetic acid). The volume of injected sample was 5 mm^3. The compounds were detected using diode-array detector working at 202 nm and mass spectrometer. Ionization of the analytes was performed in the positive ion mode at capillary voltage 4.5 kV. The pressure of the nitrogen (nebulizing gas) was set to 0.40 bar. Nitrogen (4.0 dm^3/min) also served as drying gas at 250 °C. Emetine and cephaeline were quantified using calibration dependences of the standards. The calibration was based on area of the peak of HPLC–UV chromatograms, and respective figures of merit of the calibration dependences are given in Table 2.

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