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ORIGINAL PAPER

Laudanum opiatum caesareum: authentication of the composition of a historical pharmaceutical preparation from the eighteenth century using a multianalytical approach

Karel Nesměrák[1] · Martin Štícha[2] · Michal Belianský[1] · Václav Červený[1] · Petr Kozlík[1] · Karel Kudláček[1] · Jana Kunešová[3]

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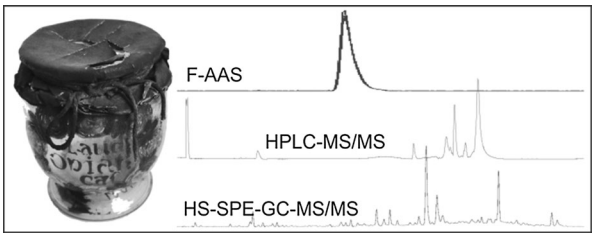
Abstract

The historical relic of pharmaceutical, opium-containing preparation *Laudanum opiatum caesareum* from the eighteenth century was analyzed using a multianalytical approach. Inorganic substances were indirectly determined by atomic absorption spectrometry. HPLC and GC connected with mass spectrometry were used for the detection of organic substances. The volatile organic substances were captured by headspace-solid phase microextraction before analysis. From the results, it was possible to largely authenticate the original recipe, although the results show that the period apothecary changed the recipe by omitting some ingredients and by the addition of an extra ingredient. A total of 59 organic substances were detected, most of which served as markers confirming the presence of ingredients according to the period prescription. Besides, it was possible to confirm the assumption from the previous publication that the calculated ratio of the concentration of noscapine and cotarnine can be used as a marker for the age of the opium-containing preparations. The results also demonstrate that even though more than two centuries have passed since the preparation of the analyzed relic, and the container in which it has been stored was not hermetically sealed, it was possible to detect many volatile substances even after such a long time.

Graphic abstract



Keywords Alkaloids · Authentication · GC–MS · HPLC–MS · Mass spectrometry · Opium · Volatile organic compounds

Introduction

The analysis of the relics of historical pharmaceutical preparations is a very attractive application area of analytical chemistry because it provides an interesting insight into the scientific knowledge and social habits of our ancestors [1]. In principle, each such analysis is a unique case and is always a great challenge for the analyst because on the one hand there is often a very complicated composition of the sample and on the other hand there is a very limited amount

✉ Karel Nesměrák
nesmerak@natur.cuni.cz

[1] Department of Analytical Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

[2] Department of Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

[3] Collection of Old Czech History, Historical Museum, National Museum, Prague, Czech Republic

of sample and its uniqueness. This results in the need to use a multianalytical approach, i.e., a combination of several analytical methods, to obtain qualitative and/or quantitative information about the sample. This is especially true for pharmaceutical preparations from the eighteenth century, a time when very complex mixtures of many substances (which, moreover, were not chemical individuals) were popularly used to treat diseases.

One of the most important groups of drugs used by mankind since time immemorial is analgesics, medicines that are used to relieve pain [2, 3]. Until the discovery of modern analgesics in the late nineteenth century, opium was the main substance for the management of pain. Opium is the dried latex obtained by incision from the unripe capsules of poppy (*Papaver somniferum*), which is produced until today. The main disadvantage of opium, when administered orally, is its very bitter taste. In the sixteenth century, the famous Renaissance physician Theophrastus Bombastus von Hohenheim (1493–1541), called Paracelsus, made an important discovery, that opium (or better to say its alkaloids) dissolves much better in alcohol than in the water used so far [4, 5]. Paracelsus called this alcoholic solution *laudanum* (from the Latin verb *laudare*, to praise) and it became the basis of many analgesic preparations for several more centuries [6]. In addition to this liquid preparation, Paracelsus formulated a famous pill called *Specificum anodynum* (a specific painkiller), later known as *Laudanum Paracelsi* [7, 8]. It was prepared from one ounce (approx. 35 g) of opium, orange and quince juice (six ounces each), cinnamon, and cloves (half an ounce). The mixture was thoroughly homogenized in a mortar and left in a closed glass for a month in a warm place. It was then squeezed and the liquid was mixed with one and a half scruples of musk (one scruple is approx. 1.5 g), four scruples of amber, half an ounce of saffron, one and a half scruples of "Coral Extract" and the same amount of "Pearl Extract". Both these extracts were chemical preparations of highly variable composition, most likely in the case of the first mercuric oxide, in the case of the second salt of antimony acid, but at the same time, in both cases, calcium compounds are also possible. The mixture was again left in a warm place for one month, and finally, one and a half scruples of "gold quintessence" (powdered gold or colloidal gold solution) were added and the preparation was ready. The formulation of Paracelsus' preparations with opium has been subsequently cultivated and many variations arose.

In this paper, we present an authentication study of opium preparation *Laudanum opiatum caesareum*, dated to the second half of the eighteenth century. It is a part of our long-term project focused on the analysis of historical relics of pharmaceutical preparations [9–11]. The first aim of the work was to find markers in the sample that would confirm the period recipe, according to which *Laudanum opiatum caesareum* in question was prepared. The second goal of the work was to determine the content of opium alkaloids in the sample and to verify the possibility of using the calculated concentration ratio of alkaloids noscapine and cotarnine as a marker of the age of preparation, as we suggested recently [10]. For this purpose, a combination of separation methods (HPLC, GC) was used in connection with mass spectrometry to detect organic compounds, whereas atomic absorption spectrometry served for the indirect determination of inorganic substances via elements of their cations.

The analyzed relic of *Laudanum opiatum caesareum* (Fig. 1), which looks like a fine black powder, comes from the exceptionally preserved baroque pharmacy of Capuchin order in Prague, which was opened in 1680 and operated until the end of the eighteenth century [12]. Fortunately, the baroque pharmaceutical preparations in the original containers, which have not been open since the end of the eighteenth century, are preserved in the pharmacy. So the analyzed sample is more than 200 years old.

In the past, pharmaceutical preparations were not prepared with as strict precision as they are today, and the pharmaceutical literature has served the pharmacist only as a possible guide. If one of the ingredients was not available or was too expensive, it was often omitted or alternated. Therefore, a comparison of the actual content of preserved relics of pharmaceutical preparations with literature data is interesting. The analyzed *Laudanum opiatum caesareum* was probably prepared according to the *Dispensatorium pharmaceuticum Austriaco-Viennense* from 1729 [13], which was used in the mentioned Capuchin pharmacy [12]. The *Dispensatorium* also mentions the preparation in question under the synonymous name "Nepenthes", which refers to the ancient word νηπενθής. In Homer's *Odyssey*, this is the name given to a magical drink that comforts all sorrow and


EXTRACTUM SEU
LAUDANUM CÆSAREUM
COMPLETUM, ETIAM NEPENTHES DICTUM.

℞. Extracti opii correcti uncias tres,
Croci unciam unam,
Croci ſolis drachmam unam,
Margaritarum orientalium unciam ſemis,
Unicornu veri
Coralliorum rubrorum ana drachmas duas,
Olei Caryophyllorum
Cinnamomi
Macis
Citri
Succini ana ſcrupulum ſemis,
Ambræ gryſeæ
Moſchi ana drachmam ſemis,
Miſceantur omnia, & fiat, lege artis opiatum ad extracti conſiſtentiam.

Fig. 1 Baroque pharmaceutical jar provided analyzed sample of *Laudanum opiatum caesareum* (National Museum, inv. no. H2-4381) and Latin recipe for its preparation from *Dispensatorium pharmaceuticum Austriaco-Viennense* from 1729 [13]

pain [14]. Based on the mentioned *Dispensatorium* [13], the theoretical content of ingredients in the analyzed preparation is given in Table 1.

As it follows from Table 1, an opium extract was the main component of the analyzed preparation. According to the mentioned *Dispensatorium* [13], the extract was prepared by maceration of crude opium in a mixture of water with alcohol for several days, the resulting liquid was filtered, and the filtrate evaporated to a pasty consistency. Due to the majority of the extract in the analyzed preparation, high content of various opium alkaloids can be expected [10].

Ingredients containing several calcium compounds were the second most represented component in the preparation (in total about 22%). Pearls contain mainly calcium carbonate; similarly, the skeleton of red corals is composed of the same compound [16]. Either fossil bones or narwhal tusk was considered to be the horns of the unicorn, in both cases various types of calcium phosphate come into consideration. Therefore, the relatively high calcium content in the analyzed sample can be assumed. Pearls, corals, and unicorn horns had not therapeutic but, as rare and expensive substances, psychological effect, and they increased the attractiveness of the product for noble and wealthy patients. Moreover, all these ingredients were considered universal antidotes at that time [17].

Saffron is the third most represented ingredient in the analyzed preparation. It consists of the dried threads of *Crocus sativus* and is one of the most famous spices of all time. From a chemical point of view, two carotenoid pigments crocin (CASRN 42553-65-1) and picrocrocin (CASRN 138-55-6) are typical for saffron in particular [18, 19]. Crocin on hydrolysis yields gentiobiose and crocetin (CASRN 27876-94-4), while picrocrocin yields glucose and safranal (CASRN 116-26-7). Safranal is largely responsible for the characteristic odor and together with picrocrocin the taste of saffron.

The fourth place, in terms of content, is occupied by "Crocus solis" (crocus of the sun), which was most often powdered gold, or gold prepared in the form of Purple of Cassius [20, 21].

The main purpose of the other ingredients was to correct the bitter taste of opium and increase the attractiveness of the preparation; this is especially true of ambergris and musk. Ambergris is a pathological product found in the intestines of sperm whales (*Physeter macrocephalus*) or cast by them into the sea [18]. It is a very expensive substance that is used mainly in perfumery. Ambergris contains about 25% of triterpene alcohol ambrein (CASRN 473-03-0) with a typical fragrant odor [22]. Musk is the dried secretion from the preputial follicles of the musk deer (*Moschus moschiferus*), which acts as an important ingredient of many high-class perfumes [18, 23]. The chief constituent of musk is odorous cyclic ketone muskone (CASRN 956-82-1) followed by muscopyridine (CASRN 501-08-6) and other alkaloids and peptides.

The remaining ingredients of the analyzed preparation are essential oils. Clove oil is prepared by the extraction of the dried flower buds of *Syzygium aromaticum* [18, 24, 25]. Clove oil contains 84–95% of phenols (mainly eugenol, CASRN 97-53-0, with about 3% of acetyleugenol), sesquiterpenes (α- and β-caryophyllenes), and small quantities of esters, ketones, and alcohols. Cinnamon oil is distilled from the fresh bark of *Cinnamomum zeylanicum* [18, 24, 26]. It contains about 70% of *trans*-cinnamaldehyde (CASRN 104-55-2), about 4–10% of phenols (chiefly eugenol), and hydrocarbons (α-pinene, CASRN 80-56-8). Nutmeg oil is distilled from the kernels of the seeds of *Myristica fragrans* [18]. It mainly contains 15–26% of α-pinene (CASRN 80-56-8), 13–18% of β-pinene (CASRN 127-91-3), and 5–12% of myristicin (CASRN 607-91-0) [27]. Lemon oil is prepared mainly from *Citrus limon*, and it contains about 95% of terpenes (mainly (+)-limonene, CASRN 5989-27-5), followed by sesquiterpenes, aldehydes, and esters [18].

Table 1 Composition of *Laudanum opiatum caesareum* according to *Dispensatorium pharmaceuticum Austriaco-Viennense* from 1729 [13] in period apothecary units, and recalculated according to [15] on grams and weight percent

Ingredient	Weight				%
	Ounce	Drachm	Scruple	Gramm	
Extract of opium	3.0			105	56.0
Saffron	1.0			35.0	18.7
Crocus of the sun		1.0		4.40	2.3
Pearls	0.5			17.5	9.3
Horn of unicorn		2.0		8.80	4.7
Red corals		2.0		8.80	4.7
Clove oil			0.5	0.75	0.4
Cinnamon oil			0.5	0.75	0.4
Nutmeg oil			0.5	0.75	0.4
Lemon oil			0.5	0.75	0.4
Amber oil			0.5	0.75	0.4
Amber		0.5		2.20	1.2
Musk		0.5		2.20	1.2

Results and discussion

Determination of inorganic compounds

First, the total content of inorganic substances in the analyzed historical relic was determined gravimetrically and (16.4 ± 1.3)% of the non-combustible fraction was found.

Based on the above theoretical composition of the analyzed preparation, the calcium and gold content in the

sample was determined by atomic absorption spectrometry. The calcium content was determined to be (1.8 ± 0.2)%. Considering that calcium carbonate and calcium phosphates contain roughly 40% of calcium, the determined amount corresponds to 4.5% of the content of these compounds in the sample. This sufficiently confirms the content of calcium-containing ingredients in the sample (although it is lower than the theoretical content).

On the other hand, no absorption signal was observed at the wavelength of the analytical line of gold in any spectrum attained with sample solutions. Therefore, the content of gold in the analyzed historical relic is under the limit of detection which is 0.11 mg dm^{-3} of solution (or 0.25 mg g^{-1} of the sample). The theoretical gold content in the sample is 2.3%, which is about a hundred times above the limit of detection. The probable explanation is that the period pharmacist omitted this ingredient.

Screening for markers of ingredients by HPLC–MS

To search the markers of the presumed ingredients of the analyzed historical relic, a method, which we optimized and developed in our previous work [10] for the analysis of opium-containing preparations, was applied. The sample was extracted by 200-fold volume of acetonitrile–water (85:15, v/v) mixture. The analytes were separated using HPLC–MS on XBridge® BEH C18 column under gradient elution with a binary mobile phase of methanol (solvent A) and 0.01 M ammonium acetate buffer pH = 3.00 (solvent B) in both positive and negative ESI modes.

A wide range of substances with different intensities was found in the positive ESI, from which 38 compounds were identified using high-resolution mass spectrometry (Table 2). On the contrary, the negative ESI mode did not prove to be beneficial, only five compounds of high molar masses were proved. It could be hypothesized that they are glycosides (a specific structure could not be elucidated due to low intensity).

Mainly alkaloids naturally occurring in opium were found among the substances identified in the analyzed historical relic, i.e., morphine, codeine, hydrocodone, laudanine, naltrexone, thebaine, papaverine, and noscapine. Two less common isoquinoline alkaloids found in the plant family *Papaveraceae* was also found: bulbocapnine [28] and salutaridine [29]. Hypothetically we can suppose that their concentration in the analyzed relic was increased due to the use of opium extract in its preparation. Decomposition products of papaverine and noscapine were also found in the analyzed historical relic. For papaverine, its oxidation products papaverinol and papaveraldine were identified. For noscapine, also two oxidation products were identified: cotarnine and meconin. This is in agreement with the findings in our previous study [10].

The presence of picrocrocin is a marker that saffron was truly used in the preparation of the analyzed historical relic. Another identified marker is myristicin, demonstrating the use of nutmeg oil in the preparation of the analyzed relic. The presence of 4-aminobenzoic acid is surprising at first glance, but it was recently proved in some plants [30].

Screening of volatile organic compounds by GC–MS

Because substances characteristic for essential oils (except nutmeg oil) were not detected by HPLC–MS, we employed GC–MS analysis in the next step.

First, a simple hexane extract of the analyzed historical relic was analyzed, as non-polar substances (namely ambrein and caryophyllenes) should be detected. The results are summarized in Table 3. All identified compounds are typical of resinous materials from various plants of the plant family *Pinaceae*, especially for larch turpentine [31, 32]. A possible explanation is that the period pharmacist changed the recipe and added this fragrance ingredient. Alternatively, it may be secondary contamination.

Headspace-solid phase microextraction (HS-SPME) was subsequently used to increase the probability of volatile organic compounds capture. The substances were released at 50 °C and sorbed on polydimethylsiloxane fiber. Table 4 summarizes the 16 compounds found.

Most of the identified compounds using HS-SPME can be assigned as the markers of the essential oils in question (Table 4). Remarkable is the absence of compounds that could serve as markers of lemon oil. Three substances found (borneol, dihydrocarveol, and 2,3-pinanediol) are common components in several essential oils [18]. The presence of most of the expected essential oils in the analyzed historical relic was proved, although the markers found are non-specific and individual oils (clove, cinnamon, or nutmeg) cannot be distinguished.

The presence of safranal is the second marker that saffron was truly used in the preparation of the analyzed historical relic (as already found in HPLC–MS). The volatile steroid 18-norestrone methyl ether could hypothetically be a marker for the presence of musk in the analyzed historical relic, but unfortunately, no further information was found in the literature. Finally, the finding of manool is another proof for the presence of resinous materials from the plant of the plant family *Pinaceae* [31, 32].

Quantitation of opium alkaloids

Recently, we proposed the application of the concentration ratio of noscapine and cotarnine as a possible marker of the age of the opium-containing preparations. Therefore, the content of selected opium alkaloids was determined in the

Table 2 Chemical constituents found in the HPLC chromatogram of acetonitrile extract of the analyzed historical relic *Laudanum opiatum caesareum* using mass spectrometry (retention time in HPLC, formula of $[M+H]^+$ ion and its experimental and theoretical *m/z*, identity and CASRN of the substance)

t_r/min	Formula $[M+H]^+$	*m/z*			Identity, CASRN
		Experimental	Theoretical	Δ/ppm	
6.4	$C_7H_8NO_2$	138.0550	138.05500	0.0	4-Aminobenzoic acid, 150-13-0
8.1	$C_7H_{14}NO_2$	144.1025	144.10190	0.1	Unresolved
12.6	$C_{17}H_{20}N_3O$	286.1443	286.14380	−1.9	Morphine, 57-27-2
21.6	$C_{18}H_{22}NO_3$	300.1594	300.15942	−0.1	Codeine, 76-57-3
21.6	$C_{12}H_{16}NO_2$	206.1176	206.11756	−0.1	Cyclohexyl ester of 4-pyridinecarboxylic acid, 163778-04-9
22.4	$C_{20}H_{22}NO_4$	340.1542	340.15430	0.4	Unresolved
22.7	$C_{18}H_{22}NO_3$	300.1590	300.15942	1.5	Hydrocodone, 125-29-1
22.7	$C_{12}H_{14}NO_2$	204.1019	204.10190	−0.2	Unresolved
23.1	$C_8H_{16}NO_6$	222.0971	222.09720	0.3	5-Butyl-1*H*-indole-2,3-dione, 18331-71-0
23.2	$C_{12}H_{12}NO_3$	218.0812	218.08120	0.0	1-(1-Oxobutyl)-1*H*-indole-2,3-dione, 92675-59-7
23.9	$C_8H_{19}O_3$	163.1328	163.13290	0.5	Unresolved
24.0	$C_{19}H_{22}NO_4$	328.1539	328.15430	1.4	Naloxon, 465-65-6
24.0	$C_{20}H_{26}NO_4$	344.1858	344.18560	−0.5	Laudanine, 85-64-3
24.1	$C_{20}H_{24}NO_4$	342.1699	342.17000	0.3	Naltrexone, 16590-41-3
24.2	$C_{10}H_9O_5$	209.0447	209.04440	−1.1	Unresolved
24.6	$C_{16}H_{27}O_7$	331.1750	331.17510	0.4	Picrocrocin, 138-55-6
25.3	$C_{14}H_{24}NO_{10}$	366.1394	366.13950	0.2	Unresolved
25.6	$C_{12}H_{16}NO_4$	238.1081	238.10740	−3.1	Cotarnine, 82-54-2
25.8	$C_{10}H_{22}NO_6$	252.1441	252.14420	0.1	Unresolved
25.8	$C_{19}H_{22}NO_3$	312.1595	312.15940	−0.4	Thebaine, 115-37-7
26.0	$C_{19}H_{20}NO_4$	326.1384	326.13870	1.0	Bulbocapnine, 298-45-3
26.0	$C_{19}H_{22}NO_4$	328.1542	328.15430	0.3	Salutaridine, 1936-18-1
26.2	$C_{21}H_{24}NO_5$	370.1646	370.16490	0.8	Diacetylmorphine, 561-27-3
26.3	$C_{20}H_{22}NO_5$	356.1493	356.14930	−0.1	Papaverinol, 482-76-8
26.4	$C_{12}H_{26}NO_7$	296.1704	196.17040	0.1	Unresolved
26.5	$C_{10}H_{11}O_4$	195.0652	195.06520	−0.2	Meconin, 569-31-3
26.7	$C_{11}H_{20}NO_8$	294.1183	294.11830	0.1	*N*-Acetyl-α-muramic acid, 61633-75-8
26.7	$C_{20}H_{20}NO_5$	354.1336	354.13360	0.1	Papaveraldine, 522-57-6
26.9	$C_{14}H_{30}NO_8$	340.1963	340.19660	0.3	Unresolved
27.2	$C_{22}H_{28}NO_8$	446.1807	446.18090	0.6	Unresolved
27.2	$C_{16}H_{34}NO_9$	384.2226	384.22280	0.4	Unresolved
27.3	$C_{20}H_{22}NO_4$	340.1544	340.15440	−0.3	Papaverine, 58-74-2
27.7	$C_{18}H_{38}NO_{10}$	428.2487	428.24900	0.8	Unresolved
27.8	$C_{14}H_{32}NO$	230.2480	230.24780	−0.7	Unresolved
28.2	$C_{11}H_{13}O_3$	193.0859	193.08590	0.1	Myristicin, 607-91-0
28.3	$C_{22}H_{24}NO_7$	414.1547	414.15470	0.1	Noscapine, 128-62-1
28.3	$C_{14}H_{24}NO_{10}$	366.1392	366.13950	0.7	Unresolved
28.6	$C_{17}H_{28}NO_{12}$	438.1601	438.16060	1.0	Unresolved

analyzed historical relic using HPLC–UV according to our method [10].

The following content was found: (0.085 ± 0.031)% of cotarnine, (0.112 ± 0.070)% of meconin, (0.225 ± 0.068)% of papaverine, (0.038 ± 0.030)% of noscapine. The content of morphine was below the limit of quantitation. Comparing the total amount of opium alkaloids with theoretical and with results of analysis of historical relics of opium-containing pharmaceutical preparations, the relatively low representation of these alkaloids is evident. This confirms that period pharmacists had opium of very different qualities at their disposal. Moreover, decomposition of opium alkaloids may have occurred in the preparation of the

Table 3 Chemical constituents found in the GC chromatogram of hexane extract of the analyzed historical relic *Laudanum opiatum caesareum* using mass spectrometry (retention time in GC, formula of $[M]^{\cdot+}$ ion and its experimental *m/z*, identity and CASRN of the substance)

t_r/min	Formula $[M]^{\cdot+}$	*m/z*	Identity, CASRN
16.246	$C_{20}H_{34}O$	290	13-Epimanool, 1438-62-6
18.062	$C_{20}H_{34}O_2$	306	Larixol, 1438-66-0
18.451	$C_{21}H_{32}O_2$	316	Methyl abietate, 127-25-3
19.015	$C_{20}H_{32}O$	288	Isopimara-7,15-dien-3β-ol, 4752-56-1
20.126	$C_{20}H_{28}O_2$	300	Dehydroabietic acid, 1740-19-8

extract, which was used as an ingredient in the preparation of the analyzed historical relic.

On the other hand, the calculated ratio of the concentration of noscapine and its direct disintegration product cotarnine, which is 0.45, confirms the assumption from our previous publication [10] that this ratio can be used as a marker for the age of the opium-containing preparations. For fresh opium, the ratio is greater than 100 and decreases with time. For preparations more than 200 years after preparation, the ratio is in the range of 0.4–3.8.

Conclusions

Using a multianalytical approach, a partial authentication of the composition of historical pharmaceutical preparation *Laudanum opiatum caesareum*, dated to the second half of the eighteenth century, was achieved. Calcium was detected from the presumed inorganic substances, on the contrary, the expected gold content was not confirmed (probably the period pharmacist omitted this expensive ingredient). Altogether 59 organic substances were identified (in some cases only partially), most of which served as markers for the presence of ingredients according to the period recipe. The presence of opium alkaloids, including the degradation products of papaverine and noscapine, has been demonstrated. It was confirmed that the concentration ratio of noscapine and cotarnine can be used as the marker of the age of the analyzed sample. The use of saffron in the preparation of the analyzed preparation was demonstrated by the identification of specific markers: picrocrocin and safranal. Several markers for essential oils have been identified. Nutmeg oil has been unequivocally confirmed by the presence of myristicin. The presence of clove and cinnamon oils was confirmed using common markers. On the other hand, no marker for expected lemon oil could be found. In contrast to the period prescription, substances were found indicating the use of resinous materials from a plant of the plant family *Pinaceae*. The finding of the volatile steroid 18-norestrone methyl ether is a dubious marker for the presence of musk

Table 4 Chemical constituents found in the GC chromatogram after HS-SPME from the analyzed historical relic *Laudanum opiatum caesareum* using mass spectrometry (retention time in GC, formula of $[M]^{\cdot+}$ ion and its experimental *m/z*, identity and CASRN of the substance, assignation of markers to essential oils in question [18, 24–27])

t_r/min	Formula $[M]^{\cdot+}$	*m/z*	Identity, CASRN	Marker of		
				Clove oil	Cinnamon oil	Nutmeg oil
6.295	$C_{10}H_{16}$	136	α-Pinene, 80-56-8	√	√	√
8.972	$C_{10}H_{16}O$	152	*trans*-2-Caren-4-ol, 4017-82-7	√	√	
9.039	$C_{10}H_{16}O$	152	Camphor, 464-48-2	√		√
9.310	$C_{10}H_{18}O$	154	Borneol, 507-70-0			
9.572	$C_{10}H_{18}O$	154	1,6-Dihydrocarveol, 619-01-2			
9.693	$C_{10}H_{14}O$	150	Safranal, 116-26-7			
11.131	$C_{15}H_{24}$	204	α-Cubebene, 17699-14-8	√		√
11.427	$C_{15}H_{24}$	204	α-Copaene, 3856-25-5	√	√	√
11.555	$C_{15}H_{24}$	204	γ-Gurjunene, 22567-17-5	√		
11.692	$C_{10}H_{18}O_2$	170	2,3-Pinanediol, 53404-49-2			
12.387	$C_{15}H_{24}$	204	γ-Muurolene, 30021-74-0	√	√	
12.590	$C_{15}H_{24}$	204	α-Muurolene, 10208-80-7	√	√	
12.749	$C_{15}H_{24}$	204	γ-Cadinene, 39029-41-9	√	√	
15.227	$C_{18}H_{22}O_2$	270	18-Norestrone methyl ether, 4147-10-8			
15.687	$C_{22}H_{45}NO$	339	4-Octadecylmorpholine, 16528-77-1			
17.127	$C_{20}H_{34}O$	290	Manool, 596-85-0			

in the analyzed historical relic. No markers were found to detect the expected presence of ambergris. Although the analyzed historical relic was over 200 years old at the time of analysis (wherein the apothecary jar has only been closed with a leather cap and storage conditions were not known), several volatile compounds were detected even after such a long time. The findings made it possible to largely confirm the original recipe and at the same time showed that the period apothecary alternated the original recipe on the preparation of analyzed preparation.

Experimental

Sample

The analyzed sample comes from the Collection of Old Czech History, National Museum (Prague, The Czech Republic), inv. no. H2-4381. The baroque pharmaceutical jar is made from clear glass in a shape of a cup of height 60 mm and diameter 50 mm. The jar is labeled in Latin "Laud: Opiat: cæf:", the inscription is placed in a label made of stylized roses (Fig. 1). The original leathern lid, fastened by a string, was gently opened and three portions of a sample of the content were collected using a glass spoon; one from the center of the jar content, and two from the opposite sides located at the wall of the jar. The collected sample was stored in a glass container in a dark. Before the analysis, the sample was homogenized in a porcelain mortar and the powder was placed in a desiccator with phosphorus pentoxide as a desiccant for 24 h.

Chemicals

The certified reference materials of calcium (10.00 ± 0.05 mg dm^{-3} in HNO_3) and gold (10.00 ± 0.05 mg dm^{-3} in HCl) were purchased from Analytika, Czech Republic. The analytical standards of cotarnine, morphine, meconin, papaverine, and noscapine came from collections of the Department of Analytical Chemistry, Faculty of Pharmacy, Charles University. Their identity was confirmed by mass spectrometry and purity was checked by potentiometric titration with 0.1 M perchloric acid in a glacial acetic acid medium [33].

The other chemicals employed were: acetic acid (concentrated, p.a.; Lach-Ner, Czech Republic), acetonitrile HPLC, Macron Fine Chemicals, USA), ammonia (25% aqueous, solution, p.a.; Lach-Ner, Czech Republic), ammonium acetate (p.a.; Lach-Ner, Czech Republic), *n*-hexane (p.a.; Sigma-Aldrich), hydrochloric acid (37%, p.a.; Centralchem, Slovakia), methanol (for HPLC; Honeywell), nitric acid (concentrated, p.a.; Analytika, Czech Republic), perchloric acid (68%, p.a.; Lach-Ner, Czech Republic).

Procedures, instrumentation

Gravimetric determination of the non-combustible fraction of the sample was performed in two replicates. The amount of 25 mg of sample was taken and was ignited in a porcelain crucible at 900 °C to a constant weight.

The resulted dry material from gravimetric determination was used for the determination of calcium and gold by atomic absorption spectrometry. The material was dissolved in aqua regia (mixture of concentrated nitric acid and concentrated hydrochloric acid in ratio of volumes 1:3). A high-resolution continuum-source atomic absorption spectrometer ContrAA® 700 (Analytik Jena, Germany) was used. The resulting net signal of the three central (100–102 from 200) pixels was always evaluated as an average integrated absorbance (3 s integration time) of three replicates at the wavelength of 242.7950 nm for gold and 422.6728 nm for calcium. Whereas acetylene–air flame was used for the atomization of gold, the acetylene–nitrous oxide type of flame was used for the atomization of calcium. No interference was detected for either of the two elements. The nonlinear calibration function made by certified reference standards in the solution of the nearest possible composition was used.

The HPLC–MS was performed using an Agilent 1200 HPLC System with a binary pump model. 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 binary mobile phase of methanol (solvent A) and 0.01 M ammonium acetate buffer pH = 3.00 (solvent B) was used, starting with 10% of A which was maintained constant for 4 min, increased to 80% of A within 10 min, maintained constant for 2 min, returned to 10% of A within 1 min, and finally maintained constant for 13 min. The total time of analysis was 30 min. The flow rate of the mobile phase was 140 mm^3 min^{-1}. About 5 mg of powdered sample was weighed in the test tube, and an amount of 1 cm^3 of a mixture of acetonitrile–water (85:15, v/v) was added. The extraction took 15 min under sonication. The solution was filtered using a 0.2 µm filter (Whatman) and appropriately diluted by mobile phase before HPLC analysis. ESI–MS detection was conducted on a Bruker QqTOF compact instrument operated using Compass otof Control 4.0 (Bruker Daltonics, Germany) software. Compass DataAnalysis 4.4 (Build 200.55.2969) (Bruker Daltonics, Germany) software was used for data processing. ESI–MS data were collected in positive ion mode at scan range from *m/z* 50 to *m/z* 1000. The temperature of the drying gas was set to 220 °C at a 3.0 dm^3 min^{-1} flow rate. The cone voltage was 2800 V. The measured mass spectra were analyzed using software Compass CompoundCrawler 3.0 (Bruker) and compared with databases ChEBI [34], ChemSpider [35], and PubChem [36].

The GC–MS measurements were performed on a GC–MS Shimadzu QP-2010 Instrument. The separation was achieved by Zebron ZB-5 ms capillary column (30 m × 0.25 mm). The oven temperature initially set at 35 °C was increased at a rate of 5.0 °C min^{-1} to 250 °C and then held for 10 min. The injector temperature was 250 °C. The pressure of carrier gas (helium) was 60 kPa. The injection was performed in split mode. The parameters of detection were GC–MS transfer line temperature 230 °C, electron energy 70 eV. The measured mass spectra were compared with NIST/EPA/NIH 02 Mass Spectral Library [37], and only spectra with similarity > 95% were taken into account. The hexane extract was prepared by sonicating 20 mg of the sample in 1 cm^3 of *n*-hexane for 10 min. The solution was filtered using a 0.2 µm filter (Whatman) and 0.8 mm^3 was injected into the GC instrument. Headspace-solid phase microextraction was performed with a manual fiber holder (Supelco, USA). An amount of 20 mg of sample was weighed into a 4 cm^3 vial with the septum. The vial was tempered at 50 °C for 10 min. Then the released compounds were sorbed onto a 100 µm polydimethylsiloxane fiber for 5 min.

A liquid chromatograph UHPLC Nexera XR (Shimadzu, Japan) with an internal diode-array detector was used for quantitation of opium alkaloids using calibration dependences of the standards (the calibration was based on the area of peak of HPLC–UV chromatograms). The details are described in our previous publication [10].

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