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Study of the molecular compositions of ointments from the 18th baroque pharmacy of the Capuchin monastery in Hradčany (Prague, Czech Republic)

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Abstract:	A multi-analytical approach based on four different analytical methods was used for the first time to study six historical ointments from the 18th century belonging to the baroque pharmacy of Capuchin Monastery in Hradčany (Prague, Czech Republic) in order to gain information on the ointment formulation, the presence active substances, and also possible chemical modification deriving from the procedures used for their preparation. All ointments were initially characterized by gas chromatography/mass spectrometry following saponification, extraction and derivatization to identify the main classes of ingredients. The volatile organic compounds emitted by the ointments were then characterized by gas chromatographic analysis following solid-phase microextraction, providing complementary information on the possible more volatile and active ingredients present in the ointments. Finally, the botanical/animal origin of triacylglycerols was investigated by liquid chromatography-high resolution mass spectrometry, while the presence of beeswax was proved by flow injection analysis. The results obtained were used to hypothesize the probable original medical purpose of the ointments by comparing the results with historical recipes and the period literature. The use of this comprehensive multi-analytical approach allowed us to contribute into the knowledge of ancient ointments and to obtain information on the long term stability of the chemical compounds in the ointment formulations.

Cover Letter

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Prague, 27 January 2023

Dear Editor,

On behalf of the authors, it is my pleasure to present to you a manuscript entitled *"Study of the molecular compositions of ointments from the 18th baroque pharmacy of the Capuchin monastery in Hradčany (Prague, Czech Republic)"*, which we hope you will kindly consider for publication in your prestigious and highly respected journal *Microchemical Journal*.

The use of a novel multi-analytical approach based on four different analytical methods (GC-MS, SPME-GC-MS, HPLC-MS, FIA-MS) to study the composition of historical ointments is presented in the manuscript. The main objective of the work is to obtain information on the composition of ointments analyzed, and on the presence of active substances and possible chemical modifications resulting from the procedures used in preparation of ointments in the past.

This newly designed approach has been shown to identify most putative substances or groups of substances in samples analyzed. The findings were compared with the information (mainly recipes) available in the period literature. The results obtained were used to hypothesize the probable original medical purpose of the ointments and, at the same time, it was found that contemporary pharmacists often modified the formulations to suit their needs.

The use of a comprehensive multi-analytical approach allowed us to contribute to the knowledge of ancient ointments and to obtain information on the long-term stability of the chemical compounds in the ointments. Moreover, the proposed multi-analytical procedure can also be used for the analysis of other, not only historical, samples of complex composition.

I confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with its submission to *Microchemical Journal*.

Yours sincerely

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Highlights

- A multi-analytical approach based on four different analytical methods was used for the first time
- The approach is very effective in characterizing organic materials used in the past
- The ointment bases of historical ointment remains from the 18th century were identified
- Contemporary pharmacists often modified the formulations to suit their needs

Study of the molecular compositions of ointments from the 18th baroque pharmacy of the Capuchin monastery in Hradčany (Prague, Czech Republic)

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Abstract

A multi-analytical approach based on four different analytical methods was used for the first time to study six historical ointments from the 18th century belonging to the baroque pharmacy of Capuchin Monastery in Hradčany (Prague, Czech Republic) in order to gain information on the ointment formulation, the presence active substances, and also possible chemical modification deriving from the procedures used for their preparation. All ointments were initially characterized by gas chromatography/mass spectrometry following saponification, extraction and derivatization to identify the main classes of ingredients. The volatile organic compounds emitted by the ointments were then characterized by gas chromatographic analysis following solid-phase microextraction, providing complementary information on the possible more volatile and active ingredients present in the ointments. Finally, the botanical/animal origin of triacylglycerols was investigated by liquid chromatography-high resolution mass spectrometry, while the presence of beeswax was proved by flow injection analysis. The results obtained were used to hypothesize the probable original medical purpose of the ointments by comparing the results with historical recipes and the period literature. The use of this comprehensive multi-analytical approach allowed us to contribute into the knowledge of ancient ointments and to obtain information on the long term stability of the chemical compounds in the ointment formulations.

Keywords: decomposition, degradation, GC-MS, HPLC-MS, FIA-MS, historical ointment

1. Introduction

The study of historical pharmaceutical preparations is a challenging task that requires a complex analytical methodology to obtain a plastic picture of their compositions [1,2]. Historical pharmaceutical preparations, until the early 19th century, when active substances began to be isolated from natural sources [3], were mainly prepared by mixing various natural substances or extracting active ingredients from natural sources using water, ethanol, or other solvents [4–6]. Due to the complexity of the ingredients used in the preparation of historical ointments, their matrices can be quite complex, unlike, for example, injectable preparations, which typically contain far fewer ingredients [7,8].

The pharmaceutical active ingredients in medicinal products are usually analyzed to authenticate or understand the original medicinal purpose [7–9], but in the case of ointments, it is necessary not only to examine the pharmaceutically active ingredients, but also to characterize the composition of the ointment base [1]. The knowledge that can be obtained about the composition of an ointment is very useful for comparing a historical sample with medical prescriptions from the same period, but also for estimating the original purpose of the ointment. The main ingredients of the ointments are usually lipids (triacylglycerols or wax), one or more active pharmaceutical ingredients, and other auxiliary components, such as substances that modify the aesthetic properties of the preparation [10,11].

The complexity of the chemical composition of lipids due to degradation phenomena and ageing processes, together with its variability, makes the analysis of this class of compounds very challenging. In addition, active compounds are often present in the sample in a very limited percentage relative to the ointment base, making them more difficult to detect and characterize. For this reason, multi-analytical methods are required to solve complex ointment compositions. Currently, several studies have been published in the literature focusing on the characterization of historical ointments from the 16th to 18th centuries.

Baeten et al. [12] investigated the composition of materials present in fragments of ceramic vessel from Middelburg castle (Belgium, 16th century) using both spectroscopic and chromatographic methods. Chromatographic analysis indicated the presence of beeswax and a small amount of lipids, while the spectroscopic approach revealed the presence of calcium carboxylate, calcium sulfate (gypsum), lead sulfate and traces of iron.

Riedo et al. [13] reported the application of pyrolysis gas chromatography coupled with mass spectrometry to study four samples of ointment from the 16th century that belong to the Aboca Museum collection (Arezzo, Italy). Both historical and reference modern samples of ointments prepared according to historical recipes were analyzed. Using this approach allowed the authors to characterize the lipidic and resinous compounds present in the samples. The data obtained were further elaborated using multivariate data analysis to define the origin of the lipid materials.

An alternative and more specific approach for the characterization of the lipidic ingredients in historical ointments is high performance liquid chromatography coupled with mass spectrometry. This approach allows the acyl glycerides or esters of a sample to be characterized directly without hydrolysis or saponification, thus obtaining a representative fingerprint of the material that retains all information of its original composition, making it easier to identify its natural origin [14]. This approach was applied by Saliu et al. [15] to analyze another series of ointments from the collection of the Aboca Museum prepared in the 17th century, and a set of ointments belonging to the pharmacy of Real Cartuja de Valldemossa (Palma de Majorca, Spain). The authors

compared the triacylglycerol profiles of historical samples with those of fresh lipidic ingredients and reference ointments prepared according to historical recipes. The distribution of triacylglycerols allowed the authors to determine the origin of vegetable oils and animal fats, showing a predominance of olive oil and lard. The authors also used this approach to evaluate the effects of ageing on fresh lipid materials and demonstrated that this approach is an effective analytical tool not only for the evaluation of the origin of such material but also for understanding the behavior of lipids during long-term storage.

In this paper, we demonstrate the application of a comprehensive multi-analytical strategy that combines complementary approaches to characterize the lipids, resins and active substances in six historical ointments from the 18th century, which originated from the pharmacy of the Capuchin monastery in Hradčany (Prague, Czech Republic). Gas chromatography coupled to mass spectrometry (GC-MS) after saponification, extraction and derivatization with a silylating agent was used as the first method to screen all organic compounds present in the historical ointments and to define and tailoring specific analyses to further characterize of each constituent. The volatile organic compounds emitted by the samples were then analyzed by head space analysis using solid-phase micro extraction (SPME) and GC-MS. This approach allowed us to identify and characterize the most volatile constituents of the analyzed samples that would otherwise be impossible to detect due to their limited abundance relative to the ointment base, typically in excess and more polar. Finally, liquid chromatography coupled with high-resolution mass spectrometry with electrospray (HPLC-MS) and flow injection analysis (FIA-MS) were used to characterize the lipids or waxes and unambiguously identify their natural origin.

2. Materials and methods

2.1. Materials and chemicals

All chemicals were purchased from Carlo Erba Reagents (Italy). Acetonitrile, chloroform, diethyl ether, ethanol, hexane, isopropanol, and methanol as solvents were HPLC or LC-MS grade. Trideacnoic acid, hexadecane (internal standards) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (derivatization agent) were LC-MS grade. The other reagents used, potassium hydroxide, hydrochloric acid and isooctane, were p. a. quality.

2.2. Historical pharmaceutical ointments

In this paper, six historical remains of ointments produced in the 18th century in the pharmacy of the Capuchin monastery in Hradčany, Prague (Czech Republic) were analyzed; for the history of the pharmacy [16]. These remains have been preserved in their original containers (Fig. 1). The theoretical composition of these ointments is given by the *Dispensatorium pharmaceuticum Austriaco-Viennense* of 1729 [17], which was used in the pharmacy at that time. Historical remains of ointments were sampled in 2017 from the original containers, which are now stored at the National Museum in Prague. The sampling was carried out using a glass spoon and three portions were taken from each container (one from the center and two from opposite sides of the container). The samples were stored in the dark at laboratory temperature until analysis in 2019.

Fig. 1 Baroque pharmaceutical jars, which were the source of the analyzed samples: (a) Ointment of celery (National Museum, inv. no. H2-4943), (b) Ointment of fruits (National Museum, inv. no. H2-4946), (c) Ointment of roses (National Museum, inv. no. H2-4948), (d) Ointment of poplar buds (National Museum, inv. no. H2-4949), (e) Ointment by Jacobo de Pinto (National Museum, inv. no. H2-4943), and (f) Fat from wild rabbit (National Museum, inv. no. H2-AC).

Sample H2-4943 comes from a milk glass container with an original leather lid, the container is labeled "Ung: De Apio", which means *Unguentum ex apio* (Ointment of celery). According to [17], the ointment contained 50% celery juice (*Apium graveolens*), 37.5% honey and 12.5% wheat flour (*Triticum aestivum*). The resulting mixture was thickened by heat to a paste-like consistency. The sample was therefore not supposed to contain lipids.

Sample H2-4946 comes from a milk glass container with an original leather lid, the container is labeled "Ung: Pomat:", which means *Unguentum pomatum* (Ointment of fruits). According to [17], the ointment was prepared by boiling 2 lemons (*Citrus limon*), 2 oranges (*Citrus sinensis*) and 4 apples (*Malus domesticus*) in 1260 g of lard. Twenty drops of each of the following ingredients were added to the resulting ointment: distilled benzoin oil (*Styrax*), cinnamon oil (*Cinnamomum verum*), clove oil (*Eugenia caryophyllata*), and rosehip oil (*Rhodiola rosea*).

Sample H2-4948 comes from a milk glass container with an original leather lid, the container is labeled "Ung: Rosat:", which means *Unguentum rosatum simplex* (Ointment of roses). According to [17], 840 g of lard was used to prepare the ointment, which was repeatedly washed with rose water and a few drops of rosehip oil (*Rhodiola rosea*) were added.

Sample H2-4949 comes from a milk glass container with an original leather lid, the container is labeled "Ung: Popul:", which means *Unguentum populeum* (Ointment of poplar buds). According to [17], the ointment contains 14.6% fresh black poplar buds (*Populus nigra*) macerated in 58.5% lard. Subsequently, 4.9% of the black henbane leaves (*Hyoscyamus niger*) are added, followed by 2.4% each of the white poppy leaves (*Arctomecon merriamii*), black nightshade (*Solanum nigrum*), sempervivum (*Greater celandine*), cobweb house-leek (*Sempervivum arachnoideum*), prickly lettuce (*Lactuca serriola*), greater burdock (*Arctium lappa*), wood violet (Viola odorata), navelwort (*Umbilicus rupestris*) and fruits of blackberry (*Rubus*).

Sample H2-9293 comes from a glass container with an original leather lid, the container is labeled "Bals: Jacob: De:", which means Ointment by Jacobo de Pinto recipe. According to [18], the ointment is prepared by mixing frankincense (genus *Boswellia*), myrrh (*Commiphora myrrha*), mastix (*Pistacia lentiscus*), aloe (*Aloe vera*), sarcocolla (*Astragalus sarcocolla*), storax (*Liquidambar orientalis*), benzoe (genus *Styrax*), each ingredient is 1.7% of the total volume of the ointment. In addition, the ointment contains the following ingredients (the content of the ingredient in the total volume of the ointment is indicated in brackets): St. John's wort oil (*Hypericum perforatum*, 62.9%), yellow wax (10.5%), colophony (3.5%), Venetian turpentine (*Larix decidua*, 3.5%), human fat (2.6%), naphtha (2.6%), lavender oil (*Lavandula officinalis*, 1.7%), and juniper berry oil (*Juniperus communis*; 0.4%).

Sample H2-AC comes from a glass container with an original leather lid, the container is labeled "Axung: Cunicul:", which means fat from wild rabbit (*Oryctolagus cuniculus*). In this case, it is not a historical pharmaceutical preparation, but a sample of a pharmaceutical substance.

2.3. GC-MS analysis

The analytical procedure was slightly modified from [19]. 1.5 mg of sample was saponified with 300 µL of 10% potassium hydroxide in ethanol in a Microwave Digestion System Ethos One (Milestone), for 80 min at 80 °C (200 W). Then 1 mL of water was added and the resulting solution was extracted three-times using 400 µL of hexane. The acidic extraction was performed after adding 1 mL of 6 mol L^{-1} hydrochloric acid to the mixture using 400 µL of diethyl ether three times. A volume of 5 µL of internal standard (tridecanoic acid in isooctane, 151 ppm) was added into the 20 µL of neutral or acidic extract. The sample was dried with nitrogen and 20 µL of *N,O*-bis(trimethylsilyl)trifluoracetamide and 50 µL of isooctane were added. Derivatization was carried out at 60 °C for 30 min. An additional 100 µL of isooctane and 10 µL of hexadecane (second internal standard, 143 ppm) were added. A volume of 2 µL of the resulting mixture was injected into the GC-MS system for analysis.

The analysis was carried out using a 6890N gas chromatograph with split/splitless injector connected to a 5973 quadrupole mass detector (Agilent Technologies, USA). An HP-5MS silica column (stationary phase 5% diphenyl + 95% dimethyl-polysiloxane, 30 m length, i.d. 0.25 mm, film layer 0.25 µm; J&W Scientific, Agilent, USA) was used for both GC-MS techniques. The identification was based on the mass spectrum libraries: NIST 1.7 main EI MS library, and WILEY 275 MS library [20]. The gradient program for the GC-MS screening starts at a temperature of 80 °C for 2 min and increases up to 200 °C with a step of 10 °C min^{-1}. Next, the temperature increases up to 330 °C with a step of 30 °C min^{-1} and remains constant for 60 min. The gradient program for SPME-GC-MS starts at 40 °C for 1 minute and then increases up to 280 °C with a step of 10 °C min^{-1}. The final temperature remains constant for 20 min.

2.4. SPME-GC-MS analysis

20 mg of the sample was homogeneously spread on the bottom wall of a 10 mL glass vial. The vial was sealed with a PTFE/silicone septum. Solid-phase microextraction was performed after 30 minutes of stabilization at 60 °C using a Stable Flex Divinylbenzen/Carboxen/PDMS fiber (Supelco, USA). The fiber exposure was performed for 30 min at constant laboratory temperature. The analytes were desorbed in a GC-MS injector in splitless mode. The analyses were carried out using a 6890N gas chromatograph with split/splitless injector connected to a 5973 quadrupole mass detector (Agilent Technologies, USA).

Chromatographic conditions were as follows: initial temperature 40 °C, 1 min isothermal, 5.0 °C min^{-1} up to 250 °C, isothermal 20 min; transfer capillary temperature 280 °C; ion source 250 °C; injection temperature 250 °C; injection operating with a split flow of 50 mL min^{-1} and a splitless time of 0.6 min; electron ionization energy 70 eV; mass range 50−1000 *m/z* for GC-MS screening, or 35−700 *m/z* for SPME-GC-MS. Helium (purity 99.9995%) was used as carrier gas at the flow of 1.2 mL min^{-1}.

2.5 RP-HPLC-MS analysis

Sample preparation is described in [21–23]. 2 mg of sample was extracted with 1 mL of hexane:chloroform mixture (2:3, *v/v*) in an ultrasonic bath for 15 min at 60 °C. A volume of 100 µL was evaporated with a flow of nitrogen and dissolved in the mobile phase (methanol:isopropanol mixture, 90:10, *v/v*) to give a dilution of 1:100 and 1:1000. Presented triacylglycerol profiles involve only triacylglycerols of abundance more than 1.0% in the total ion chromatograms.

RP-HPLC-MS was performed using an Agilent 1260 Infinity instrument connected to the Jet Stream ESI interface of the Q-TOF mass spectrometer (both Agilent Technologies, USA). For RP-HPLC-MS, chromatographic separation was performed using an InfinityLab Poroshell 120 EC-C18 column (3.0×75 mm; i.d. 2,7 µm). The injection volume was 1 µL. The mobile phase consisted of methanol (solvent A) and isopropanol (solvent B). The gradient program started with 90% solvent A for 7.5 min, then decreased to 10% solvent A in 37.5 min, then remained constant for another 15 min and finally increased to 90% in 7.5 min. The flow rate was set at 0.3 mL min^{-1}. The column temperature was 45 °C.

The operating conditions for mass spectrometry were as follows: ESI positive mode; high resolution spectra measured in the range 100−3000 *m/z*; drying gas (N_2 purity >98%): 350 °C and flow 10 L min^{-1}; the nebulizer gas (N_2, purity >98%): 0.024 bar; sheath gas (N_2, >98% purity): 375 °C and flow 11 L min^{-1}. The voltage was: capillary at 4.5 kV; fragmentor at 200 V; noozle at 1000 V, skimmer at 65 V, and octapole RF at 750 V. The collision energy for MS^2 experiments was 50 eV (N_2, purity 99.999%). Data were collected in auto-MS^2 mode at a scan rate 1.06 s^{-1} for MS and 1.08 s^{-1} for MS^2. Only one precursor was acquired per cycle; the relative threshold was 0.010%. Calibration was based on Agilent HP0321 mix standard (Agilent Technologies, USA) dissolved in acetonitrile and water. The MassHunter workstation (B.04.00) was used for data analysis [24]. The relative triacylglycerol representation was expressed as a percentage of the peak area.

2.6 FIA-MS analysis

Flow injection analysis with mass spectrometry was performed using a 1200 Infinity HPLC coupled to a Jet Stream ESI interface with a Quadrupole-Time-of-Flight 6530 Infinity Q-TOF tandem mass spectrometer (Agilent Technologies, USA). The eluents were methanol and isopropanol (90:10); the flow rate was 0.2 mL min^{-1} and the injection volume was 1 µL. Sample pretreatment and theoperating conditions for ESI and MS were the same as for the HPLC. A mixture of methanol and water (85:15, *v/v*) at a flow rate of 0.2 mL min^{-1} was used as the mobile phase for FIA-MS. The ESI in positive mode was used, and high resolution spectra were measured in the range of 100−3200 *m/z*. The collected data for FIA-MS were compared with [25].

3. Results and discussion

3.1. Screening of organic compounds by GC-MS analysis

All ointments analyzed were first characterized by GC-MS analysis following saponification, extraction, and derivatization with *N,O*-bis(trimethylsilyl)trifluoracetamide. The results, which allow the identification of the main classes of ingredients such as vegetable oils, animal fats, waxes and natural resins, are summarized in Table 1.

Table 1

Identification of organic compounds in historical ointments analyzed by GC-MS screening.

Compound, CASRN	Diagnostic ions, *m/z*	Presence in a sample					
		H2-4943	H2-4946	H2-4948	H2-4949	H2-9293	H2-AC
Fatty acids							
Arachidic acid, 506-30-9	384, 269, 311, 295				√	√	√
Azelaic acid, 123-99-9	332, 317	√	√	√	√	√	√
Behenic acid, 112-85-6	412, 397, 339, 323		√	√	√	√	√
Capric acid, 334-48-5	244, 229, 171, 155			√	√	√	√
Caprylic acid, 124-07-2	216, 201, 143, 127			√	√	√	√
Cerotic acid, 506-46-7	468, 453, 395, 379				√		
p-Coumaric acid, 501-98-4	338, 323, 249	√					
Ferulic acid, 1135-24-6	308, 293, 219	√					
Gondoic acid, 5561-99-9	382, 367, 309, 293				√		√
Heneicosylic acid, 2363-71-5	398, 383, 325. 309				√		
14-Hydroxypalmitic acid, 59642-38-5	416, 104	√	√	√	√	√	
15-Hydroxypalmitic acid, 4552-17-4	416, 101	√	√	√	√	√	
Lauric acid, 143-07-7	256, 241, 183, 167			√		√	√
Lignoceric acid, 557-59-5	440, 425, 267, 531		√	√	√	√	
Linoleic acid, 60-33-3	352, 337, 279, 263	√	√	√	√	√	√
γ-Linolenic acid, 506-26-3	350, 335, 277, 261					√	√
Margaric acid, 506-12-7	342, 327, 269, 253		√	√	√	√	√
Myristic acid, 544-63-8	300, 85, 227, 211		√	√	√	√	√
Myristoleic acid, 26444-03-1	288, 273, 215, 199			√	√	√	√
Nonadecylic acid, 646-30-0	370, 355, 297, 281				√	√	√
Oleic acid, 112-80-1	354, 339, 281, 265	√	√	√	√	√	√

Palmitic acid, 57-10-3	328, 313, 255, 339	√	√	√	√	√	√
Palmitoleic acid, 373-49-9	326, 311, 253, 237	√		√	√	√	√
Pelargonic acid, 112-05-0	230, 216, 157, 141			√	√	√	√
Pentadecelyc acid, 1002-84-2	314, 299, 241, 225				√	√	√
Sebacic acid, 111-20-6	346, 331	√	√	√	√	√	√
Stearic acid, 57-11-4	356, 341, 283, 267	√	√	√	√	√	√
Alkanes							
Hentriacontane, 630-04-6	436	√		√	√		
Heptacosane, 593-49-7	380	√	√	√	√	√	√
Hexacosane, 630-01-3	366	√	√	√			
Nonacosane, 630-03-5	408	√	√	√	√	√	
Oktacosane, 630-02-4	394		√				
Pentacosane, 629-99-2	352	√	√	√		√	
Tetracosane, 646-31-1	338		√				
Aliphatic fatty alcohols							
Docosan-1-ol, 661-19-8	384		√				
Dotriacontan-1-ol, 6624-79-9	524	√	√	√	√	√	
Hexacosan-1-ol, 506-52-5	440	√	√	√	√	√	
Oktacosan-1-ol, 557-61-9	468	√	√	√	√	√	
Tetracosan-1-ol, 506-51-4	412	√	√	√		√	
Triacontan-1-ol, 593-50-0	496	√	√	√	√	√	
Terpenic acid							
Abietic acid, 514-10-3	256	√					
Dehydroabietic acid, 1740-19-8	239	√	√		√	√	
Dehydroabietic acid, 1740-19-8	239	√	√		√	√	
15-Hydroxydehydroabietic acid, 54113-95-0	342	√				√	
Isopimaric acid, 5835-26-7	241	√			√	√	
Isopimaric acid, 5835-26-7	241	√			√	√	
7-Oxodehydroabietic acid, 18684-55-4	253	√		√	√	√	
7-Oxodehydroabietic acid, 18684-55-4	253	√		√	√	√	
Pimaric acid, 127-27-5	121, 257, 359	√			√	√	
Sterols							
Cholesterol, 57-88-5	329; 129; 168; 458	√	√	√	√	√	√
7α-Hydroxyandrostendione, 62-84-0	359; 374					√	
3-Hydroxy-2-methoxy-1,3,5(10)-estratrien-17-one O-methyl oxime, 69833-94-9	401; 372					√	
16α-Hydroxytestosterone, 63-01-4	143; 225; 448					√	
Sitosterol, 83-46-5	357; 396	√			√	√	

The fatty acid profiles of all the samples analyzed were characterized by the presence of monocarboxylic fatty acids with an even number of carbon atoms ranging from 8 to 26, together with monocarboxylic fatty acids with an odd number of carbon atoms (9, 15, 17 and 19). While fatty acids with an even number of carbon atoms can be considered ubiquitous in most vegetable oils, the presence of fatty acids with an odd number of carbon atoms, especially C15 and C17, may indicate the presence of animal fats. Sample H2-4943 did not contain any vegetable oil or animal fat; therefore, the presence of fatty acids is lower and at a blank level in respect to other samples analyzed, in agreement with the information reported in [17]. Azelaic and sebacic acids were detected in all samples analyzed. These compounds can be associated with the oxidation of lipid materials containing unsaturated fatty acids. Finally, sample H2-4943 also contains derivatives of cinnamic acid, *p*-coumaric and

ferulic acids that can be associated to celery [26], an ingredient included in the formulation of this historical ointment.

Alkanes with 24–29 and 31 carbon atoms in the chain and alcohols with an even number of carbon atoms in the chain (22–32 atoms) were also identified in the samples analyzed. All of these compounds are generally found in vegetable and animal fats, as well as in beeswax [12].

The presence of fatty alcohols (C24–C36) in samples, together with 14-hydroxypalmitic and 15-hydroxypalmitic acids [25,27] and long chain hydrocarbons, can instead be considered specific of the presence of beeswax [12,28]. The presence of diterpenes or triterpenes can be considered specific to the presence of a natural resin [29–32]. All samples except H2-AC contained 14-hydroxypalmitic and 15-hydroxypalmitic acids that are specific markers for beeswax [25,27]. The markers of beeswax [33] were also detected in sample H2-4949 despite the historical recipe [17]. We can therefore assume that beeswax is a contaminant that probably comes from the leather caps used to seal the ointment containers. Leather was probably impregnated with beeswax for this purpose.

Identifying sterols by GC-MS provided additional information on the possible nature of lipids in the samples analyzed, especially since the plant material may contain campesterol and sitosterol, while the animal material is characterized by the presence of cholesterol [32]. Cholesterol was found in all samples, indicating the presence of animal material, but its presence could also come from secondary contamination during the use of the ointment. Sitosterol was identified in samples H2-4843, H2-4949, and H2-9293. Finally, derivatives of androstenone and estradione were found in sample H2-9293. These sterols could be related to the presence of human fat, which according to [17,34] is one of the constituents of ointment. As these compounds were not found in any of the other samples analyzed, it can be assumed that this is not contamination, but a reflection of the actual human fat content of the ointment.

GC-MS screening also allowed the identification of *Pinaceae* resins via specific markers. Samples H2-4943, H2-4949 and H2-9293 were characterized by the presence of isopimaric and pimaric acids and some oxidation products of abietic acid. According to historic recipe [17], sample H2-4943 should not contain pine resin, but the presence of specific markers, especially from the terpenic acids group, suggests the addition of pine resin during the preparation of the ointment by a period pharmacist. For samples H2-4949 and H2-9293, the presence of derivatives of abietic acid and pimaric acid could be associated with the use of several materials, in particular colophony or Venetian turpentine, which are from the *Pinaceae* family and were used in the ointment formulations [17]. Sample H2-AC cannot, due to of its origin, contain any resins and therefore does not contain substances from the terpenic acid group.

3.2. Characterization of volatile organic compounds by SPME-GC-MS

All ointments analyzed were characterized by SPME-GC-MS analysis to provide complementary information to the GC-MS approach described in previous Section 3.1. The volatile organic compounds identified in the samples analyzed by SPME-GC-MS are listed in alphabetical order in Table 2.

Table 2

Identification of volatile organic compounds in historical ointments analyzed by SPME-GC-MS.

Compound, CASRN	t_r/ min	Presence in a sample					
		H2-4943	H2-4946	H2-4948	H2-4949	H2-9293	H2-AC
Benzaldehyde, 100-52-7	13.39					√	
Benzocycloheptatriene, 264-09-5	20.24				√		
Borneol, 507-70-0	18.19	√	√			√	
5-Butyldihydro-2(3*H*)furanone, 104-50-7	19.66		√	√			√
Butyric acid, 107-92-6	5.04		√				
Camphor, 76-22-2	17.81		√				
(-)-Camphor, 464-48-2	17.79	√					
Capronic acid, 142-62-1	14.87			√			√
Caprylic acid, 127-07-2	18.40		√	√			√
α-Copaene, 3856-25-5	21.42	√					
(*E*)-Car-2-en-4-ol, 4017-82-7	19.05					√	
Decan-2-one, 693-54-9	18.63		√		√		
Decanal, 112-31-2	18.83						√
1.2-Dichlorobenzene, 95-50-1	14.89	√			√	√	
Dihydro-5-pentyl-(3*H*)furanone, 104-61-0	21.18		√	√			√
Dihydro-5-propyl-2(3*H*)-furanone, 105-21-5	17.95			√			
6,6-Dimethylbicyclo[3.1.1]heptan-3-ol, 28664-09-7	17.69				√	√	
Dodecane, 112-40-3	18.72				√		
Eduesma-4(14)-11-diene, 17066-67-0	22.92				√		
Enanthic acid, 111-14-8	16.70		√	√			√
Epizonarene, 41702-63-0	21.80	√					
5-Ethyl-2(5*H*)-furanone, 22122-36-7	15.59						√
Ethylcyclohexane, 1678-91-7	15.60				√		
Heptanal, 111-71-7	11.01		√	√		√	√
Heptanol, 111-70-6	13.88			√	√		√
1-Hexanal, 66-25-1	5.35		√	√	√	√	
D-Limonene, 5989-27-5	15.38		√			√	√
4-Methoxybenzaldehyde, 123-11-5	19.61					√	
Methoxyphenyloxime, 67160-14-9	11.68			√			
2-(1-Methylethylidene)cyclohexanone, 13747-73-4	17.43		√	√			
Methy-3-hydroxystearate, 2420-36-2	21.46						√
1-Methyl-4-(1-methylethenyl)benzene, 1195-32-0	16.72	√			√		
1-Methyl-4-(1-methylethyl)benzene, 99-87-6	15.28		√		√	√	
Naphtalene derivatives	23.04–23.34	√			√		
Nonan-2-one, 821-55-6	16.81		√	√	√		√
Nonanal, 124-19-6	17.05		√	√	√	√	√
Not identified	18.93				√	√	
Not identified	21.63				√		
Not identified	21.88	√					
Not identified	22.75				√		
Oktan-1-ol, 111-87-5	16.37		√	√		√	
Oktan-2-one, 111-13-7	14.47		√			√	√
Oktanal, 124-13-0	14.77		√	√	√	√	√
Pelargonic acid, 112-05-0	19.82		√	√			√
Pentadecane, 692-62-9	18.73						√
Pentylcyclopropane, 2511-91-3	16.39						√
2-Pentylfuran, 3777-69-3	14.47	√			√		
1*R*-α-pinene, 80-56-8	12.39				√		

2-Piperidinmethylamine, 22990-77-8	17.68		√			
Terpinen-4-ol, 562-74-3	18.37	√				
α-Terpineol, 98-55-5	18.60	√				
3-Thujen-2-ol, 3310-03-0	13.15	√			√	√

Several volatile organic compounds identified in the historic ointments samples analyzed are ubiquitous in many natural materials and, therefore, are not specific to the determination of the material origin. Lipids and other auxiliary substances of ointment bases can be indirectly confirmed by the identification of some specific volatile oxidation/degradation products, in particular short aldehydes or alkanes [35,36]. Hexanal, heptanal and nonanal were detected in samples H2-4946, H2-4948 and H2-9293, which according to historical recipe [17] all contained beeswax and/or lipid materials. Caprylic acid was found in H2-4948 and H2-AC samples, both of which are characterized primarily by the presence of lipids in their composition. It may indicate the rancidity of these materials [37].

SPME-GC-MS analysis also allowed the detection of monoterpenes originating from resinous materials. Specifically, α-pinene and 3-thujene-2-ol [38] were both found only in sample H2-4949. According to the historical recipe [17], this ointment should not contain any resins, but the presence of these species can be associated with the buds of poplar used in the formulation. Terpineol was also identified in the sample H2-4943, although its formulation should not contain any resin. These compounds are common in the volatile organic compound profiles of olibanum and labdanum resins [39].

Borneol was identified in samples H2-4943, H2-4946 and H2-9293. This compound may originate from various plant materials, especially citrus fruits [40]. Indeed, citrus fruits were used in the preparation of the sample H2-4946 according to historical recipe [17].

3.3. Characterization of lipids by RP-HPLC-MS

The HPLC-ESI-MS method was used for a detailed characterization of the lipid fraction of the analyzed samples. This approach allows the direct identification of triacylglycerols (TAGs), which provide specific information on the origin of the lipids [15]. Table 3 lists all identified TAGs in the samples analyzed.

Table 3

Identification of triacylglycerols in historical ointments analyzed by RP-HPLC-MS. Abbreviations of fatty acids used: A – arachidic, B – behenic, C – cerotic, Ca – capric, Cy – caprylic, E – eicosatrienoic, G – gondoic, H – heneicosanoic, L – linolic, La – lauric, Li – lignoceric, Ln – γ-linolenic, M – myristic, Ma – margaric, Mm − heptadecenoic, Mo – myristoleic, N – nonadecanoic, O – oleic, P – palmitic, Pa – pentadecanoic, Po − palmitoleic, Pp – pentadecenoic, Rn – ricinoleic, S – stearic.

Analyte number	t_r / min	Abbreviation of TAG	Empirical formula	*m/z*	
				$[M + Na]^+$	Product ions
1	41.55	MMP, LaPP	$C_{47}H_{90}O_6$	773.661	495.440; 517.451; 523.459; 545.451; 551.492; 573.485
2	41.87	MOPo	$C_{51}H_{94}O_6$	825.670	521.456; 543.436; 549.486; 571.465; 575.503; 579.520
3	41.87	LLL	$C_{57}H_{98}O_6$	901.724	599.501; 621.482
4	42.40	MPP	$C_{49}H_{94}O_6$	801.693	523.454; 545.437; 551.496; 573.475

5	42.52	LOP	$C_{55}H_{100}O_6$	879.812	575.501; 577.508; 597.475; 599.494; 601.510; 623.493
6	42.53	MPP, PPaPa	$C_{49}H_{94}O_6$	801.687	523.464; 537.481; 545.456; 551.491; 559.464; 573.464
7	42.60	LLL. LnLnS	$C_{57}H_{98}O_6$	901.776	595.518; 599.488; 601.507; 617.485; 621.463; 623.546
8	42.72	MOP	$C_{51}H_{96}O_6$	827.708	523.467; 545.448; 549.483; 571.461; 577.512; 599.492
9	42.92	MOO	$C_{53}H_{98}O_6$	853.741	549.487; 571.471; 603.529; 625.518
10	43.04	PPPa	$C_{50}H_{96}O_6$	815.708	537.484; 551.499; 559.471; 573.503
11	43.04	PPPo	$C_{51}H_{96}O_6$	827.717	549.479; 551.490; 571.483; 573.484
12	43.30	OOP	$C_{55}H_{102}O_6$	881.762	577.508; 599.491; 603.527; 625.508
13	43.37	OPPo, MOO	$C_{53}H_{98}O_6$	853.731	549.480; 571.485; 575.493; 577.521; 597.468; 599.498
14	43.37	MaPaPa, PPPa	$C_{50}H_{96}O_6$	815.713	523.473; 537.484; 545.499; 551.498; 559.465; 573.482
15	43.62	MPS	$C_{51}H_{98}O_6$	829.725	523.474; 545.441; 551.499; 573.480; 579.537; 601.501
16	43.62	PPP	$C_{51}H_{98}O_6$	829.722	551.500; 573.489
17	43.82	OPP	$C_{53}H_{100}O_6$	855.739	551.503; 573.481; 577.518; 599.501
18	43.88	PPS	$C_{53}H_{102}O_6$	857.799	551.502; 573.485; 579.529; 601.515
19	44.14	OPS	$C_{55}H_{104}O_6$	883.764	577.510; 579.517; 599.492; 601.497; 605.532; 627.515
20	44.36	OOO	$C_{57}H_{104}O_6$	907.765	603.528; 625.509
21	44.42	MaPP, PPaS	$C_{52}H_{100}O_6$	843.739	537.474; 551.494; 559.465; 565.514 579.537; 587.501; 601.524
22	44.62	MaOP, PpSS, MmMaMa	$C_{54}H_{102}O_6$	869.764	563.496; 565.509; 577.513; 579.520; 585.481; 587.498; 591.526; 599.489; 601.503; 607.509; 613.510; 629.529;
23	45.05	OOS	$C_{57}H_{106}O_6$	909.786	603.529; 605.545; 625.516; 627.529
24	45.05	OSS	$C_{57}H_{108}O_6$	911.799	605.535; 607.568; 627.525; 629.540
25	45.05	LSS, OOS	$C_{57}H_{106}O_6$	909.786	603.530; 605.547; 607.550; 625.521; 627.527; 629.539
26	45.21	OPS, PoSS	$C_{55}H_{104}O_6$	883.771	577.510; 579.515; 599.505; 601.491; 605.529; 607.529; 627.533; 629.504
27	45.45	MaPS	$C_{54}H_{104}O_6$	871.775	565.518; 579.528; 587.497; 593.546; 601.511; 615.528
28	45.47	PSS	$C_{55}H_{106}O_6$	885.793	579.531; 601.516; 607.558; 629.537
29	45.47	APPa	$C_{54}H_{104}O_6$	871.770	537.463; 559.480; 593.546; 607.573; 615.531; 629.516
30	45.60	MaOS	$C_{56}H_{106}O_6$	897.796	591.547; 593.548; 605.522; 613.519; 615.525; 627.529
31	46.16	OSS, GPS	$C_{57}H_{108}O_6$	911.808	571.511; 601.503; 605.542; 607.558; 627.525; 629.538; 633.573; 655.559
32	46.22	SSS	$C_{57}H_{110}O_6$	913.823	607.552; 629.551
33	46.39	MaSS	$C_{56}H_{108}O_6$	899.801	593.545; 607.563; 615.540; 629.545
34	46.67	APS	$C_{57}H_{110}O_6$	913.813	579.531; 601.506; 607.562; 629.545; 635.601; 657.580
35	47.05	LiPPa	$C_{58}H_{112}O_6$	927.824	537.473; 559.452; 649.592; 663.597; 671.545; 685.659
36	47.11	LiPPo	$C_{59}H_{112}O_6$	939.840	549.480; 571.465; 661.637; 663.620; 683.632; 685.621
37	47.71	BPS	$C_{59}H_{114}O_6$	941.853	579.530; 601.523; 635.602; 657.569; 663.644; 685.617

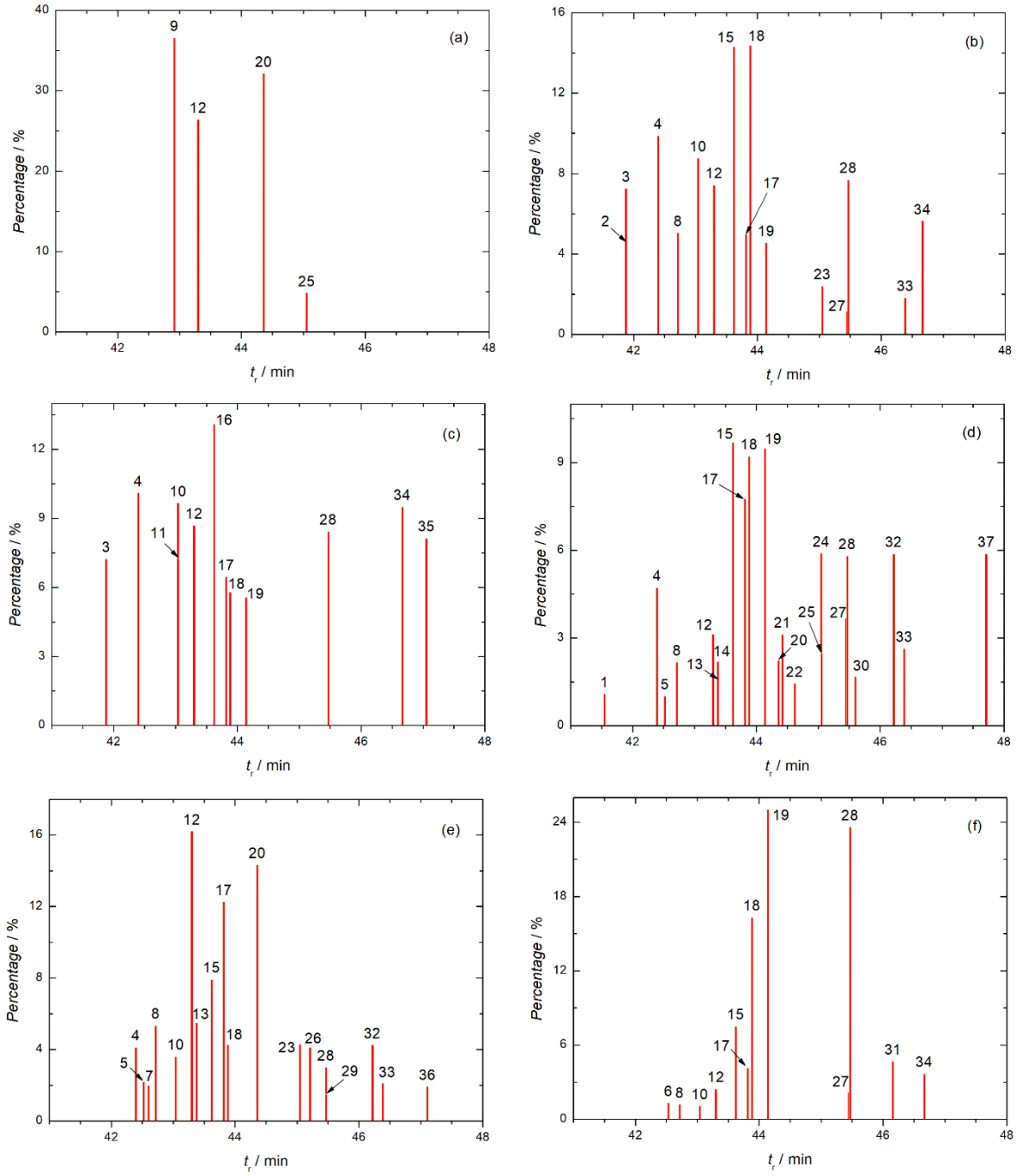


Fig. 2 Triacylglycerol profiles of historical ointments analyzed obtained by RP-HPLC-MS: (a) H2-4943, (b) H2-4948, (c) H2-4946, (d) H2-4949, (e) H2-9293, (f) H2-AC. For the identification of the analytes, see Table 3. Separation using Infinity Lab Poroshell 120 EC-C18 (3.0×75 mm; particle size 2.7 μm) column, column temperature 45 °C. Mobile phase: methanol (solvent A) and isopropanol (solvent B), F = 0.3 mL min^{-1}, for gradient elution see Section 2.5.

For sample H2-4943, the measured TAG profile is characterized by only a few unspecific TAGs (Fig. 2a). This result is consistent with the data known from the historical recipe, which does not indicate the use of lipids in the preparation of this ointment [17].

The TAG profile of sample H2-4946 (Fig. 2c) contains MPP (analyte #4, 10.1%) and PSS (analyte #28, 8.4%), indicating the presence of a lipid of animal origin. Furthermore, the presence of a relatively high abundance of APS (analyte #34, 9.5%) suggests that it is lard [41]. The presence of OOP (analyte #12, 8.7%), OPS (analyte #19, 5.6%) and PSS (analyte #28, 8.4%) in high percentages and the absence of MPS (analyte #15) and MOP (analyte #8) [41–44] further confirm the presence of lard in the sample [15]. This is in complete agreement with the historical recipe [17].

The TAG profile of sample H2-4948 (Fig. 2b) is characterized by the presence of a combination of TAGs that can be attributed to lard and/or ruminant fat. In detail, the presence of lard is indicated by the presence of MPP (analyte #4, 9.9%) and PSS (analyte #28, 7.7%) [41]. Additionally, the presence of lard in the sample is confirmed by the identification of APS (analyte #34, 5.6%) [41] together with OPP (analyte #12, 7.4%), OPS (analyte #19, 4.6%) and PSS (analyte #28, 7.7%) [15]. For MPS (analyte #15) and MOP (analyte #8), which are present in low levels in lard, a high relative concentration was found in the sample (14.3% for MPS and 5.1% for MOP), which suggest the presence of another source of animal fat. In particular, the TAG profile of the sample contains MaSS (analyte #33, 1.8%), a specific marker for ruminant fat [41], and odd-numbered carbon chain of fatty acids (C15 and C17) [32] suggesting also the presence of this lipid material. Finally, the lipid profile of sample H2-4948 also shows the presence of LLL (analyte #3, 7.3%), a TAG typical for vegetable oil. These findings are consistent with the historical recipe [17].

Similarly to the previous sample, the TAG profile of sample H2-4949 (Fig. 2d) is characterized by a combination of animal fat and vegetable oil. In detail, the analysis showed the presence of MPP (analyte #4, 4.7%) and PSS (analyte #28, 5.8%), which are characteristic of animal fat [41]. The high proportion of SSS (analyte #32, 5.9%) [42,43] and MaSS (analyte #33, 2.6%) [41] indicates the presence of ruminants fat. Lard contains the majority of OOP and PSS [15] and lower amounts of MPS and MOP [41–44]: the relative abundances of these substances, together with the absence of APS [41] (analyte #34) allowed to exclude the presence of lard in the sample and confirm the presence of ruminant fat. The TAG profile of sample H2-4949 also highlights the presence of unsaturated TAGs. Notably, the presence of trioleate (OOO, analyte #20, 2.2%) suggests the possible presence of olive oil in the ointment analyzed [15,45].

For sample H2-9393, the TAG profile shows the presence of vegetable oil, ruminant fat and human fat (Fig. 2e). In more detail, the presence of animal fat is highlighted by the identification of MPP (analyte #4, 4.1%) and PSS (analyte #28, 3.0%) [41], which together MaSS (analyte #33, 2.1%) and SSS (analyte #32, 4.3%) indicate the presence of ruminant fat [41–43,46]. The high proportion of OOO (analyte #20, 14.3%) implies the presence of vegetable oil. This could be olive [15,47] or sunflower oil, which may have been used in the preparation of St. John's wort oil [48], one of the components of the ointment. The presence of OOS (analyte #23, 4.3%) indicates the presence of olive oil. The high variability in the relative abundance of some TAGs, such as OOP (analyte #12, 16.2%), LOP (analyte #5, 2.2%) and OOO (analyte #20, 2.24%) could be due to the presence of human fat in the ointment [49].

The last historical sample analyzed is the pharmaceutical substance: fat from wild rabbit. The measured TAG profile of this sample (Fig. 2f) shows the presence of MPP (in the coelution with PPaPa; analyte #6, 1.34%) and PSS (analyte #28, 23.6%). These two TAGs are specific for animal fat. The high proportion of PSS, OOP (analyte #12, 2.5%) and OPS (analyte #19, 25.0%) would suggest the possible presence of lard [41] (but this contradicts the assumption that it is fat from wild rabbit). Interestingly, the TAG profile of the sample

contains a non-negligible percentage of MOP (analyte #8, 1.2%) and MPS (analyte #15, 7.5%), which, on the other hand, negate the presence of lard. In addition, TAGs specific for ruminant fat [41–43,46], such as MaSS, are found at low concentrations. Therefore, this particular TAG profile can be assigned to the fat of *Leporidae* (from which the H2-AC sample originated). These animals are herbivores like ruminants, and therefore, given their similar diet, they could have similar TAG profiles. Finally, the presence of high amounts of TAGs containing fatty acids with an odd number of carbons in the chain (C15, C17 and C19) confirms in fact the presence of herbivore animal fat [32].

3.4. Characterization of beeswax in samples analyzed by FIA-MS

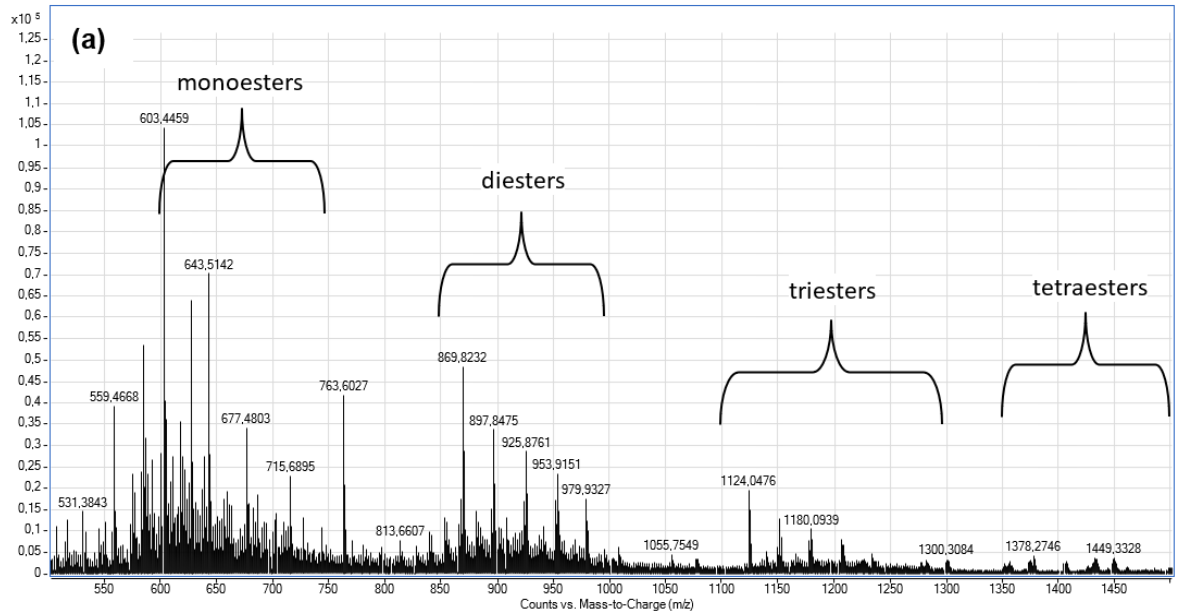
The FIA-MS method was used to characterize the high molecular weight compounds in the samples analyzed of historical ointments that could not be separated by the chromatographic methods used above. The method is particularly effective in identifying beeswax in samples. Beeswax can be identified by characterization of four specific groups of peaks in different characteristic *m/z* ranges [25]. The first group includes palmitin monoesters of fatty alcohols in the *m/z* range 600–750. The second group, in the range the *m/z* range 850–1000, is the characteristic of palmitin and 15-hydroxypalmitin diesters of fatty alcohols. The third and fourth groups, in the *m/z* ranges 1100–1300 and 1350–1500, are characteristics of palmitin and 15-hydroxypalmitin triesters and tetraesters.

The measured mass spectra of samples H2-4943 (Fig. 3a) and H2-4946 (Fig. 3b) demonstrated the presence of beeswax in these ointments, as all four ionic clusters of beeswax esters can be clearly identified. Most ions matched the literature with errors up to 50 ppm (Table S1). This high difference could be associated withthe presence of diglycerides and triglycerides that coelute with beeswax esters due to the absence of chromatographic separation. In the case of sample H2-4943, the historical recipe [17] indicates honey as one of the raw materials, which is consistent with the presence of beeswax in this sample. In the case of sample H2-4946, the presence of beeswax is excluded by the historical recipe [17], but may be secondary contamination from the leather cap treated with beeswax (as discussed in Section 3.1).

The mass spectrum of sample H2-4948 (Fig. 3c) contains ion clusters of mono-, di-, and triesters, whereas the spectra of samples H2-4949 (Fig. 3d) and H2-9293 (Fig. 3e) contain only mono- and diesters. The absence of tri- and tetraesters may be due to possible hydrolysis processes involving the higher molecular weight compounds. According to the period literature [17], only sample H2-9293 should contain beeswax, so the presence of beeswax in the other two samples is most likely to be again due to contamination from the leather lids sealing the containers.

The mass spectrum of sample H2-AC (Fig. 3f) does not contain any clusters of ions characteristic of beeswax.

These results are in agreement with both the GC-MS results (see Section 3.3) and the assumptions of the sample composition from the historical recipes [17].


(a)
x10 5
monoesters
diesters
triesters
tetraesters
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643,5142
559,4668
677,4803
763,6027
715,6895
531,3843
813,6607
869,8232
897,8475
925,8761
953,9151
979,9327
1055,7549
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1180,0939
1300,3084
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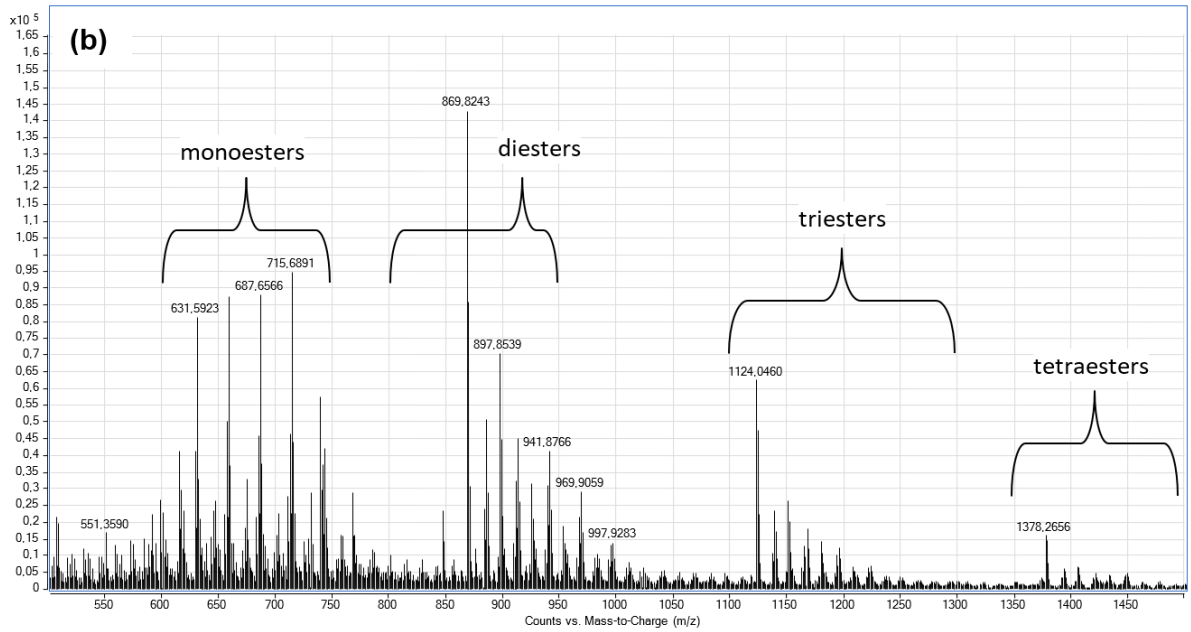
1449,3328
Counts vs. Mass-to-Charge (m/z)


(b)
x10 5
monoesters
diesters
triesters
tetraesters
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715,6891
687,6566
631,5923
897,8539
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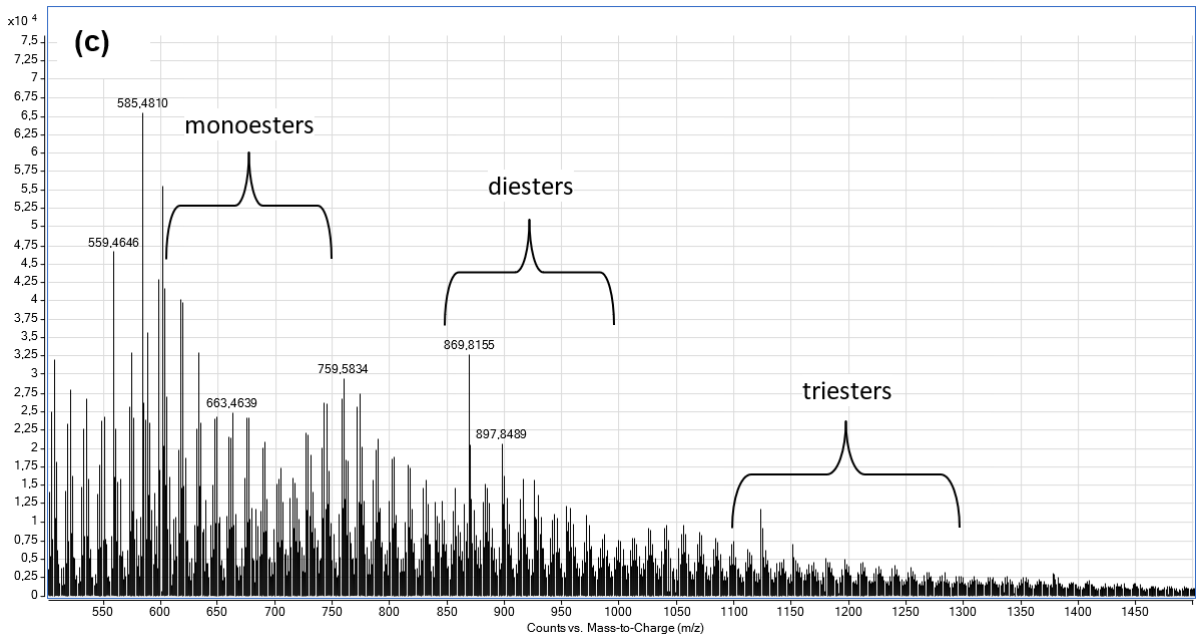
969,9059
997,9283
551,3590
1124,0460
1378,2656
Counts vs. Mass-to-Charge (m/z)


(c)
x10 4
monoesters
diesters
triesters
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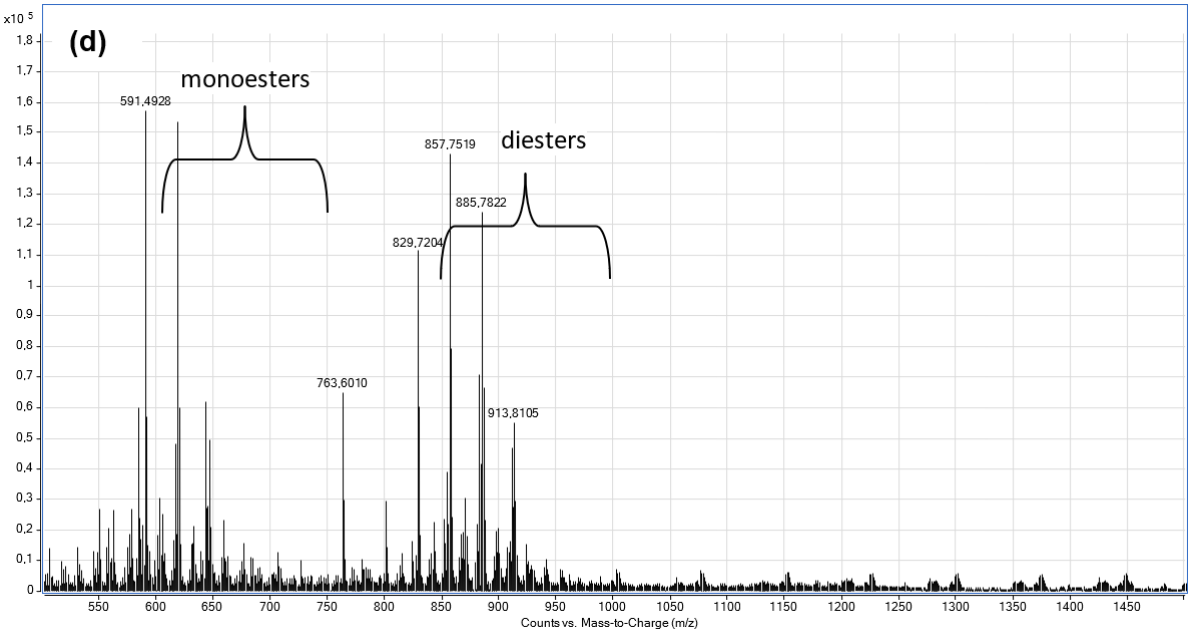
559,4646
663,4639
759,5834
869,8155
897,8489
Counts vs. Mass-to-Charge (m/z)


(d)
x10 5
monoesters
diesters
591,4928
857,7519
885,7822
829,7204
763,6010
913,8105
Counts vs. Mass-to-Charge (m/z)

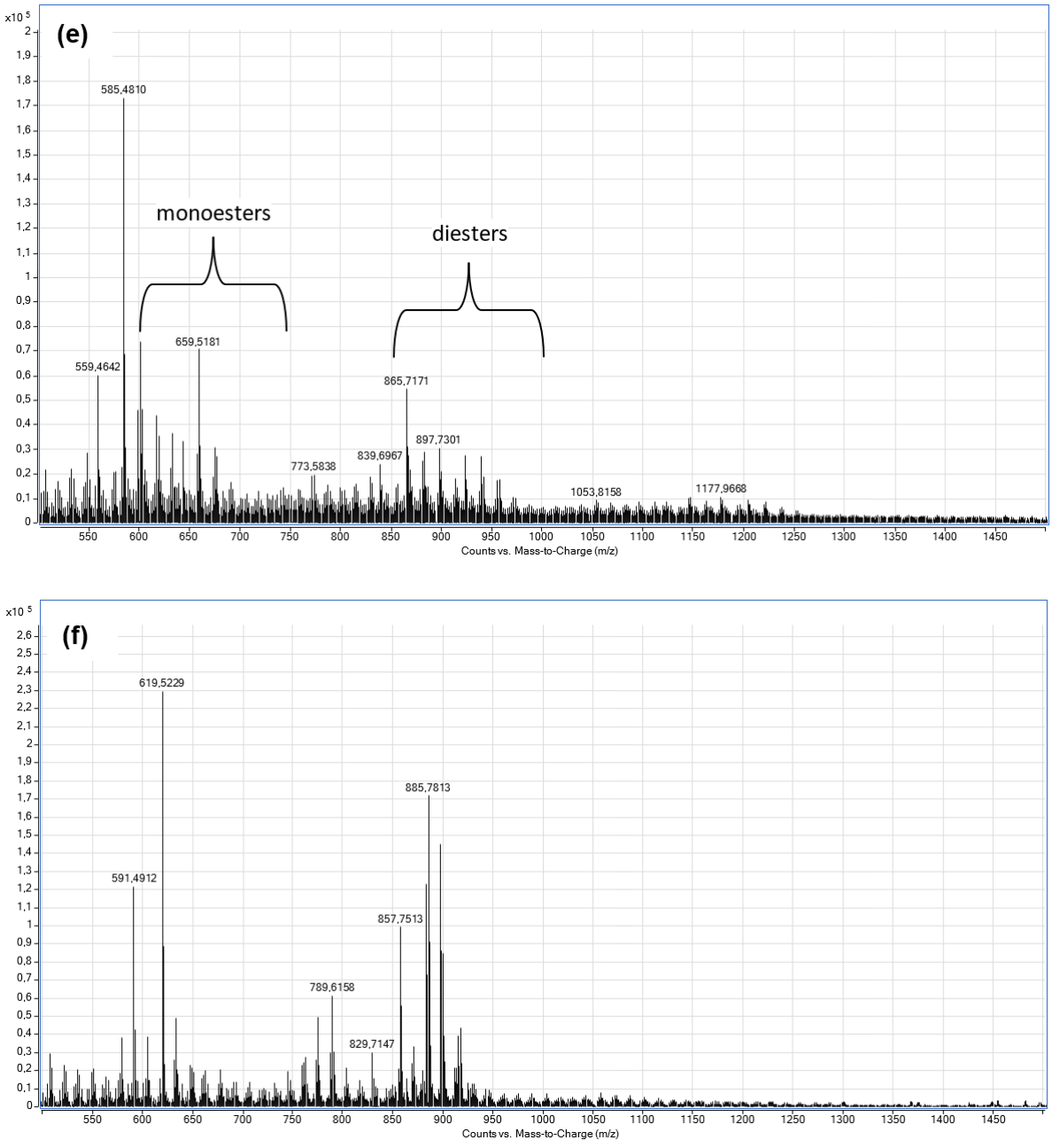


Fig. 3 FIA-MS spectra of the historical ointments analyzed: (a) H2-4943, (b) H2-4946, (c) H2-4948, (d) H2-4949, (e) H2-9293 and (f) H2-AC. Mobile phase: methanol and water (85:15, *v/v*), F = 0.2 mL min^{-1}. Sample injection volume: 1 µL. For information on ionization, see Section 2.6.

4. Conclusions

In this work, a multi-analytical approach based on four different analytical methods was used for the first time to study six historical ointments from the 18th century. The findings were compared with the recipes available in the period literature. This approach was shown to identify most putative substances or groups of substances (Table 4). At the same time, it was found that contemporary pharmacists often modified the formulations to suit their needs.

According to the historical recipe, sample H2-4943 (Ointment of celery) does not contain lipids, which is consistent with the results of the analysis. The plant material (celery) is proven by the presence of *p*-coumaric and ferulic acids. Sitosterol also originates from plant material. Furthermore, the presence of beeswax, which comes from the honey used, is demonstrated. Contrary to the recipe, the sample also contains resins of *Pinaceae*.

Sample H2-4946 (Ointment of fruits) is supposed to be made from lard according to a historical recipe, which was indeed confirmed by the analysis of the TAG profile and cholesterol profile. Contrary to the recipe, beeswax has been identified, which probably comes from contamination like that in the previous case. The identification of borneol and camphor is related to the ingredient of fruits.

According to the historical recipe, the lipid base of sample H2-4948 (Ointment of roses) is lard. This was indeed confirmed by the TAG profile and the detected presence of cholesterol. Contrary to the recipe, the sample also contains ruminant fat (possibly from an alteration of the recipe by a contemporary pharmacist) and beeswax, probably originating from contamination. Rose water contains the fragrance compound 2-phenylethan-1-ol [50], which was not detected in the sample. However, the presence of rose water is indicated by a TAG profile showing the presence of vegetable oil. Although a single resin marker was found, it failed to detect resin in the sample.

In sample H2-4949 (Ointment of poplar buds) animal fat was detected, probably ruminant. It is inconsistent with the historical recipe (it prescribed the use of lard). In addition, compounds derived from plant material (sitosterol, olive oil macerators) were identified. Volatile organic compounds were also identified, the source of which probably is the poplar buds used in the preparation of the ointment. On the other hand, poplar buds are also rich in phenolic compounds and flavonoids [51], the presence of which was not identified in the sample. This sample was also found to be contaminated with beeswax, perhaps from the leather cap enclosing the jar.

According to the TAG profile, sample H2-9293 (Ointment by Jacobo de Pinto) contains vegetable oil and ruminant fat, which also shows sitosterol and cholesterol. These components are consistent with the composition of St John's wort oil, one of the ointment constituents described in the period literature [15]. Although human fat was not detected by the TAG profile, derivatives of androstenone, androstenedione and estratrienone were identified. The presence of human fat in the sample is therefore likely. Beeswax and *Pimaceae* resin were identified in accordance with the historical recipe. Although St John's wort oil represents the majority of the sample, its authentication is difficult. Pharmaceutically active substances, such as hypericin and its derivatives, which are present in *Hypericum officinalis*, are unstable [50].

TAG analysis of the H2-AC sample (wild rabbit fat) and the identified cholesterol prove animal fat. The sample does not contain beeswax, resins, or other specific analytes.

In summary, the multi-analytical approach used has proven to be very effective in characterizing organic materials used in the preparation of ointments in the past. The detailed knowledge of the constituents that can be obtained by this approach is not only very useful for evaluating the best methodology for their further preservation (to avoid their degradation), but also has allowed new insights into medical and pharmaceutical technology in the past.

Table 4

Applicability of the analytical methods used for the identification of different components of the analyzed samples of historical ointments

Sample ID, name	Component	Method proved for identification			
		GC-MS	SPME-GC-MS	RP-HPLC-MS	FIA-MS
H2-4943, *Unguentum ex apio*	Honey / beeswax	√			√
	Plant material	√	√		
	Wheat flour				
H2-4946, Unguentum pomatum	Lard		√	√	
	Plant material		√		
	Vegetable oil			√	
H2-4948, Unguentum rosatum simplex	Lard		√	√	
	Plant material			√	
H2-4949, Unguentum populeum	Lard			√	
	Plant material	√	√	√	
H2-9293, Ointment by Jacobo de Pinto	Beeswax	√			√
	Human fat	√	√	√	
	Plant material	√	√	√	
	Vegetable oil			√	
H2-AC, Axungia cuniculi	Animal fat		√	√	

Supplementary Information

The online version contains supplementary material.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of interests

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