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ORIGINAL PAPER

Authentication of senna extract from the eighteenth century and study of its composition by HPLC–MS

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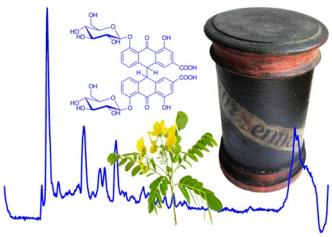
Abstract

The historical relic of medicinal preparation of senna extract, over 200 years old, was analyzed using RP-HPLC connected with ESI^--MS^n. The conditions for extracting the compounds from the sample and separating them by HPLC were optimized. A broad spectrum of glycosides was identified. Both main senna anthraquinone glycosides, sennoside A and sennoside B, were not detected in the sample. Nevertheless, the found presence of other substances typical of senna allows the positive authentication of the sample. Three possible degradation products of sennosides were identified; rhein and two compounds with unresolved structure. Remarkable stability of some glycosides in the historical sample was found. Detailed ESI^--MS^n fragmentation mechanisms of sennoside A and B have been proposed.

Graphic abstract



Keywords Decomposition · Glycosides · High-performance liquid chromatography · Mass spectrometry · Senna

Introduction

Extraction is one of the oldest used methods in the preparation of medicines [1]. Named after the Latin word "extrahere" (i.e., to drag out), it grounds on the separation of pharmacologically active compounds of plant or animal tissues from the ballast matter through the use of selective solvents. Extraction played a crucial role for isolation and discovering various pharmacologically active compounds in the nineteenth century, e.g., morphine from the poppy-opium, colchicine from a crocus plant, or salicylic acid from the bark of the white willow tree. Moreover, access to pure alkaloids and glycosides meant that the dosing of the pharmacologically active ingredients became more accurate and safe. To this day, extraction remains an important method used in the preparation of pharmaceuticals both in industry and in the household (e.g., preparation of infusions from herbs).

So far only analyzes of historical relics of medicinal wines (i.e., alcohol extracts) have been described in the literature [2–4]. In this work, we studied the historical relic of senna extract, preserved from the eighteenth century (Fig. 1). The source of the relic was the collections of the National Museum in Prague, which holds the Baroque Capuchin pharmacy from Prague. This pharmacy was founded in 1680 and is exceptionally preserved in a virtually intact state from the end of the eighteenth century, including period medicinal

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preparations [5]. According to pharmacopoeias which were used by the pharmacists in the mentioned pharmacy, particularly *Dispensatorium pharmaceuticum Austriaco-Viennense* (i.e., Viennese pharmacopoeia issued in 1729 [6]) and *Dispensatorium medico-pharmaceuticum Pragense* (i.e., Prague pharmacopoeia issued in 1739 [7]; Fig. 1), senna extract was prepared by macerating a suitable amount of pure senna leaflets in clean water in a cool place for 24 h. The colored liquid was decanted, the leaflets squeezed out and the combined liquids evaporated at low temperature. Ash obtained by burning the leaflets was added to the residue. The aims of our study were to confirm that analyzed historic relic is indeed senna extract and to study the potential degradation of its major components. In addition, glycosides, which are the major active ingredients of senna, have not been studied in historical pharmaceutical relics [8].

Senna appears to have been medically used as a purgative to treat constipation since the ninth or tenth century [9–11]. To date, the most common use is to administer the decoction prepared by pouring about 3 g of senna with a cup of hot water for 15–20 min. Liquid and dry extracts, tablets, syrups, and other senna preparations are also commercially available.

Senna is a plant drug consist of dried leaflets (or occasionally dried pods) of *Cassia* genus species [12, 13]. There are two main species of the senna plant, one that originated in northeastern Africa, *Cassia senna* L. (synonym *Senna alexandrina* P. Mill.), thus known as Alexandrian or Nubian senna, and *Cassia angustifolia* Vahl, that originated in India and is, therefore, known as Indian senna or Tinnevelly senna. The chemical composition of both senna species is quite similar. The anthraquinone derivatives in senna are responsible for the laxative effect. The chief components are two dianthrone glucosides, sennoside A and sennoside B (Fig. 2). Sennoside A and B are optic isomers distinguishable on the configuration of the C-10 and C-10′, as sennoside A is dextrorotary and sennoside B is meso-form. Their total content in dried senna leaflets varied in the range of 1.5–3.0%. The less abundant anthraquinone derivatives in senna include, in particular, sennosides C and D, palmidin A, aloe-emodin dianthrone diglucoside, aloe-emodin-8-glucoside, rhein-8-glucoside, and emodin-8-sophoroside. In senna also two naphthalene glycosides are present: 6-hydroxymusizin glucoside and tinnevellin glucoside (Fig. 2) [14]. Next, senna contains the yellow flavonol kaempferol, its glucoside kaempferin, and isorhamnetin; also a sterol and its glucoside, mucilage, calcium oxalate, and resin. Senna also contains a number of volatile constituents (mono- and sesquiterpenes, phenylpropanes, fatty acids, and esters) [15], but they are unlikely in senna extract because they leak during its preparation.

Sennosides are light-sensitive and senna must, therefore, be protected from light; otherwise, isomerization of sennoside A to B is accelerated by the light effect [16]. Both compounds are trended to be easily degraded by hydrolysis to form the corresponding aglycones sennidin A and sennidin B. Degradation of both sennosides, including hydrolysis of the *O*-β-linked glucoses and/or reduction of the dianthrone structure with rupture of the 10–10′ bond, can be also caused by a variety of microorganisms enzymes [17]. Moreover, it has been shown that sennosides A and B are not stable in water solutions and that rhein 8-*O*-glucoside and rhein are main degradation products [18, 19]. But as the laxative potency of senna is based on anthraquinone derivatives, this degradation does not alter the efficacy of the senna extract.

Results and discussion

Optimization of extraction and HPLC procedures

Extraction of senna constituents from the sample was the first step of the analysis. Although sennosides and other awaited compounds of interest are readily water-soluble, for analytical purposes an extraction to organic solvents is employed, as it prevents the degradation of these compounds [19–21]. We adopted and modified the method developed by Ohshima et al. [22]. Using current pharmaceutical substance

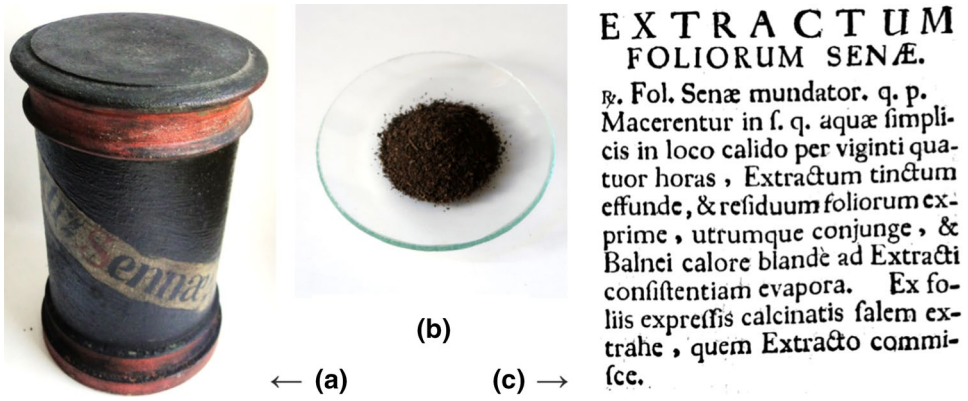

Fig. 1 **a** Baroque pharmaceutical jar provided analyzed sample of senna extract (National Museum, inv. no. H2-4653), **b** the appearance of the representative sample of the jar content, **c** the original Latin recipe of *Extractum foliorum sennæ* from *Dispensatorium medico-pharmaceuticum Pragense* 1739 [7] (for English translation see the text)

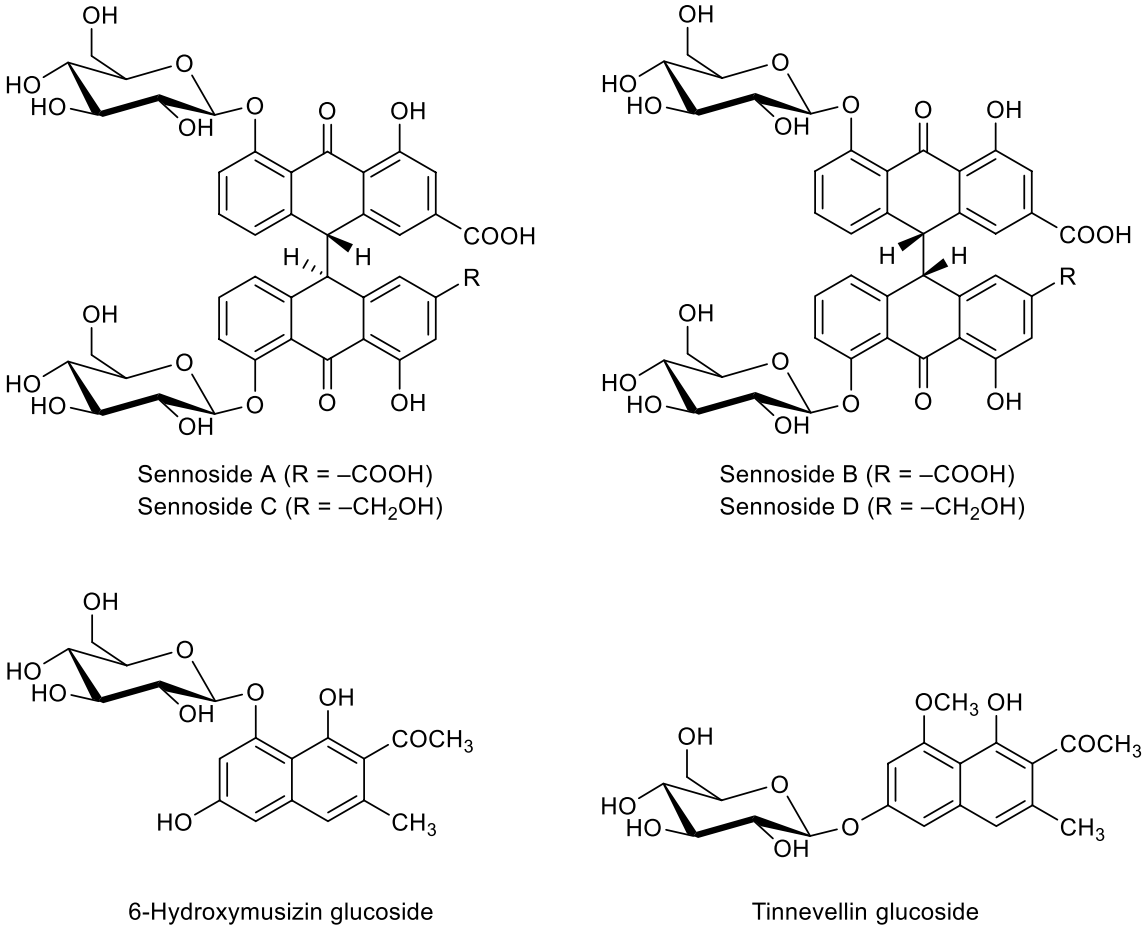

Fig. 2 Chemical structures of the main senna constituents

of senna with established sennosides content of 1.59%, we tested the extraction of senna constituents by pure methanol and methanol–water mixture at various conditions. The best result (highest recovery and reproducibility) was provided by triple extraction of the sample using 70% methanol. The yield of extraction (expressed as a yield of sennoside A) was $95.2 \pm 5.9\%$.

A number of analytical methods have been proposed for determination of constituents in senna drug or preparations, most often by HPLC on reverse stationary phases using mixtures of methanol–water [20] methanol–buffers [18, 21, 23], methanol and water with ion-pairing reagents [24], acetonitrile-buffers [19, 25, 26] or tetrahydrofuran-buffer [22]. With respect to the compatibility of separation with mass spectrometric detection, we have adopted the method introduced by Verma et al. [27] and adjusted by Bala et al. [21]. The separation is based on RP C18 column and mobile phase consisting of methanol and aqueous solution of acetic acid in the isocratic mode. To improve the HPLC separation of constituents of the analyzed historical sample, we optimized: (1) concentration of acetic acid in aqueous part of mobile phase in the range of 0.0–0.5%, (2) replacement of isocratic elution with gradient programs with various profiles. Finally, the optimized gradient elution with a binary mobile phase of methanol (solvent A) and 0.2% aqueous solution of acetic acid (solvent B) was used with a flow rate of 0.2 $cm^3\ min^{-1}$. The separation was starting with 25% A which increased to 65% within 17.5 min, next increased to 80% within 0.5 min, maintained constant for 2 min, returned to 25% within 0.5 min, and finally maintained constant for 4.5 min. The total time of analysis was 25 min.

Analysis of the historical sample

The historical relic of senna extract, reference current pharmaceutical substance of senna, and the mixture of analytical standards of sennoside A and sennoside B were analyzed using optimized extraction and separation procedures (Fig. 3). The identification of compounds was based on the comparison of high-resolution tandem mass spectrometry with literature data (Table 1).

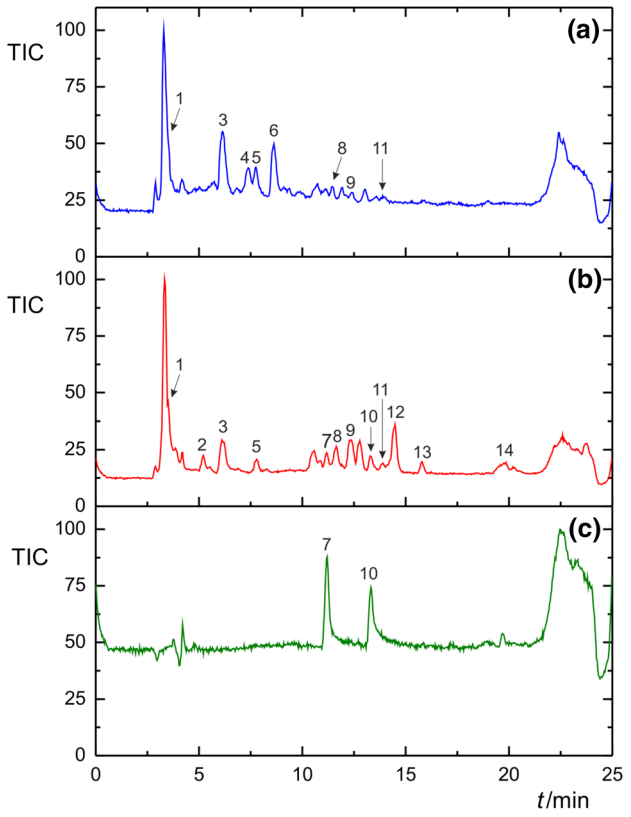

Fig. 3 HPLC–MS chromatograms of compounds in **a** historical relic of senna extract, **b** current pharmaceutical substance of senna, **c** the mixture of standards of sennoside A and sennoside B. For peak identification see Table 1. XBridge® BEH C18 column (150×3.0 mm i.d., particle size 2.5 µm; Waters), gradient elution with methanol and 0.2% aqueous acetic acid (for details see section "Optimization of extraction and HPLC procedures"), flow rate 0.2 $cm^3 min^{-1}$

The obtained results show a broad spectrum of compounds found in the analyzed historical relic of senna extract. Although the two major senna glycosides, sennoside A and sennoside B, were not detected in the sample, the authenticity of the sample, i.e. that it is senna extract, can be deducted on the basis of evidence of other substances typical of senna (Table 1). The comparison of the chromatogram of the analyzed historical relic of senna extract with the chromatogram of the current pharmaceutical substance showed two possible decomposition products, whose composition could not be determined despite all efforts (peaks 4 and 6, the latter could be guessed as epicatechin on the basis of literature [28]). Another degradation product of both sennosides in the historical relic is rhein (peak 1), which is in accordance with the literature [18, 19]. Although both major senna glycosides (i.e., sennoside A and sennoside B) have decomposed, it is remarkable that some glycosides in the sample have remained stable for more than two centuries after its preparation.

Mass spectrometry of sennoside A and sennoside B

The ESI^--MS^n mass spectra of sennoside A and B were studied in detail to enrich the published information in the literature, as their fragmentation pathways are slightly and incompletely described in the literature [35–37].

As was expected, the structural similarity (actually isomerism) of sennoside A and sennoside B resulted to nearly identical fragmentation patterns in their mass spectra, so only ESI^--MS^n of sennoside A is presented in Fig. 4. Both, sennoside A and B, showed the characteristic product ion at $m/z = 699.1350$ ($C_{36}H_{27}O_{15}$), due to the loss of glycoside unit (162.0523 Da). It only was found that in the case of sennoside B the fragmentation starts at lower collision energy, which quite corresponds to the prolongation of the glycosidic bond. The semi-empirical computational approach PM6 was used to calculate bond length changes for the deprotonated molecules. It was confirmed that significant bond elongation may be expected for sennoside B. Similarly, the fragment ion $m/z = 537.0973$ is sennoside aglycon, resulting from the loss of both glycoside units [28].

The mechanism of sennoside A ESI^- fragmentation is proposed in Fig. 5. The construction of the fragmentation scheme is based on semi-empirical computational calculations of optimized configurations, taking into account, in particular, the differences in the length of the bonds and the values of the total electronic energy. Based on these results, a configuration with lower electronic energy can be considered more likely, and it can be assumed that a weaker bond dissociates preferentially. Moreover, the proposed fragmentation scheme is in conformity with literature. Nevertheless, since ions $m/z = 655.1345$, 493.0929, 418.0899, and 195.0455 have not yet been described in the literature, this is an extension of the fragmentation scheme details.

Conclusion

Using RP-HPLC and ESI^--MS^n, the authenticity of a historical relic of the medicinal preparation labeled "Extractum Sennae", over 200 years old, was confirmed. Although both the major anthraquinone glycosides of senna, sennoside A and sennoside B, were not detected in the sample, the proven authentication of senna is demonstrated by the presence of other senna-typical substances. Comparison of the compounds found in the historical sample with the current pharmaceutical substance of senna allows identification of possible degradation products of sennosides: rhein and two other compounds with unresolved structure. It has also been found that, despite the easy degradability of glycosides, some of them remain stable in the analyzed preparation for more than two centuries after its preparation. In addition, the

Table 1 Identification of the compounds in the chromatograms of historical relic of senna extract, current pharmaceutical substance of senna, and the mixture of sennoside A and sennoside B (peak number in Fig. 1, retention time in the chromatogram, *m/z* of $[M–H]^-$ ion, molecular formula, identity and CASRN of the substance, reference to mass spectrum used for confirmation of the substance, and presence of the substance in the analyzed sample)

Peak number	t_r/min	*m/z* of $[M–H]^-$			Formula	Identity, CASRN	References	Presence in	
		Experimental	Theoretical	Δ/ppm				Historical sample	Current substance
1	3.90	283.0252	283.0243	−1.3	$C_{15}H_8O_6$	Rhein, 478-43-3	[28]	Yes	Yes
2	5.02	285.0413	285.0400	2.9	$C_{15}H_{10}O_6$	Luteolin, 491-70-3	[29]	No	Yes
3	6.15	479.0829	479.0826	0.5	$C_{21}H_{20}O_{13}$	Myricetin 3-*O*-glucoside, 19833-12-6	[30]	Yes	Yes
4	7.36	195.0873	–	–	–	Unresolved	–	Yes	No
5	7.72	593.1497	593.1507	−1.5	$C_{27}H_{30}O_{15}$	Kaempferol-*O*-rhamnoside-*O*-hexoside, 2392-95-2	[31]	Yes	Yes
6	8.61	441.0837	441.0822	3.2	$C_{22}H_{18}O_{10}$	Unresolved	–	Yes	No
7	11.19	861.1925	861.1884	−4.3	$C_{42}H_{38}O_{20}$	Sennoside B, 128-57-4	–[a]	< LOD	Yes
8	11.89	463.0889	463.0877	−1.6	$C_{21}H_{20}O_{12}$	Quercetin 3-*O*-glucoside, 482-35-9	[28]	Yes	Yes
9	12.14	609.1457	609.1450	0.6	$C_{27}H_{30}O_{16}$	Rutin, 153-18-4	[28]	Yes	Yes
10	13.33	861.1925	861.1884	−4.3	$C_{42}H_{38}O_{20}$	Sennoside A, 81-27-6	–[a]	< LOD	Yes
11	13.90	593.1497	593.1507	−1.5	$C_{27}H_{30}O_{15}$	Kaempferol-3-*O*-rutinoside, 17650-84-9	[32]	Yes	Yes
12	14.29	447.0947	447.0922	−3.1	$C_{21}H_{20}O_{11}$	Quercetin 3-*O*-rhamnoside, 522-12-3	[28]	No	Yes
12	14.29	393.1204	393.1186	3.2	$C_{19}H_{22}O_9$	6-Hydroxymusizin glucoside, 23566-96-3	[33]	No	Yes
13	15.79	407.1357	407.1343	2.3	$C_{20}H_{24}O_9$	Tinnevellin 8-*O*-glucoside, 80358-06-1	[34]	No	Yes
14	19.58	285.0413	285.0399	2.9	$C_{15}H_{10}O_6$	Kaempferol, 520-18-3	[28]	No	Yes

[a]The detail analysis of MS/MS, see section "Mass spectrometry of sennoside A and sennoside B"

ESI^--MS^n detailed fragmentation mechanisms of sennosides A and B have been proposed.

Experimental

Sample and extraction procedure

The analyzed sample was provided by the Collection of Old Czech History, National Museum (Prague, The Czech Republic). The baroque pharmaceutical jar (inv. no. H2-4653), dated to the eighteenth century [5], is made from wood in a cylindrical shape (height 14.0 cm, diameter 9.0 cm). The jar is marked with Latin inscription "Extr: Sennæ" (Fig. 1a). To collect the sample of jar content, the lid of the jar was gently opened and using glass spoon three samples of the content were collected: one from the center of the jar, and two from the opposite sides located at the wall of the jar. The collected material was stored in a glass container in the 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.

For extraction, an amount of 200 mg of the powdered sample was weighed to the tube. An amount of 5 cm^3 of 70% methanol was added and extraction took 20 min in an ultrasonic bath. After centrifugation (10 min at 5000 rpm), the liquid phase was removed, and 2 cm^3 of 70% methanol were added to the solid phase and the extraction was repeated for 5 min in an ultrasonic bath. This step was once again repeated. The three obtained extracts were combined and made up to a total volume of 10 cm^3 with 70% methanol in a volumetric flask. If needed, the solution was appropriately diluted by mobile phase before HPLC analysis.

Chemicals

The current substance of senna was purchased from a local pharmacy as tea bags with the label "Sennae Folium" (Megafyt Pharma, Czech Republic). The content of sennosides A and B was established by European Pharmacopoeia method

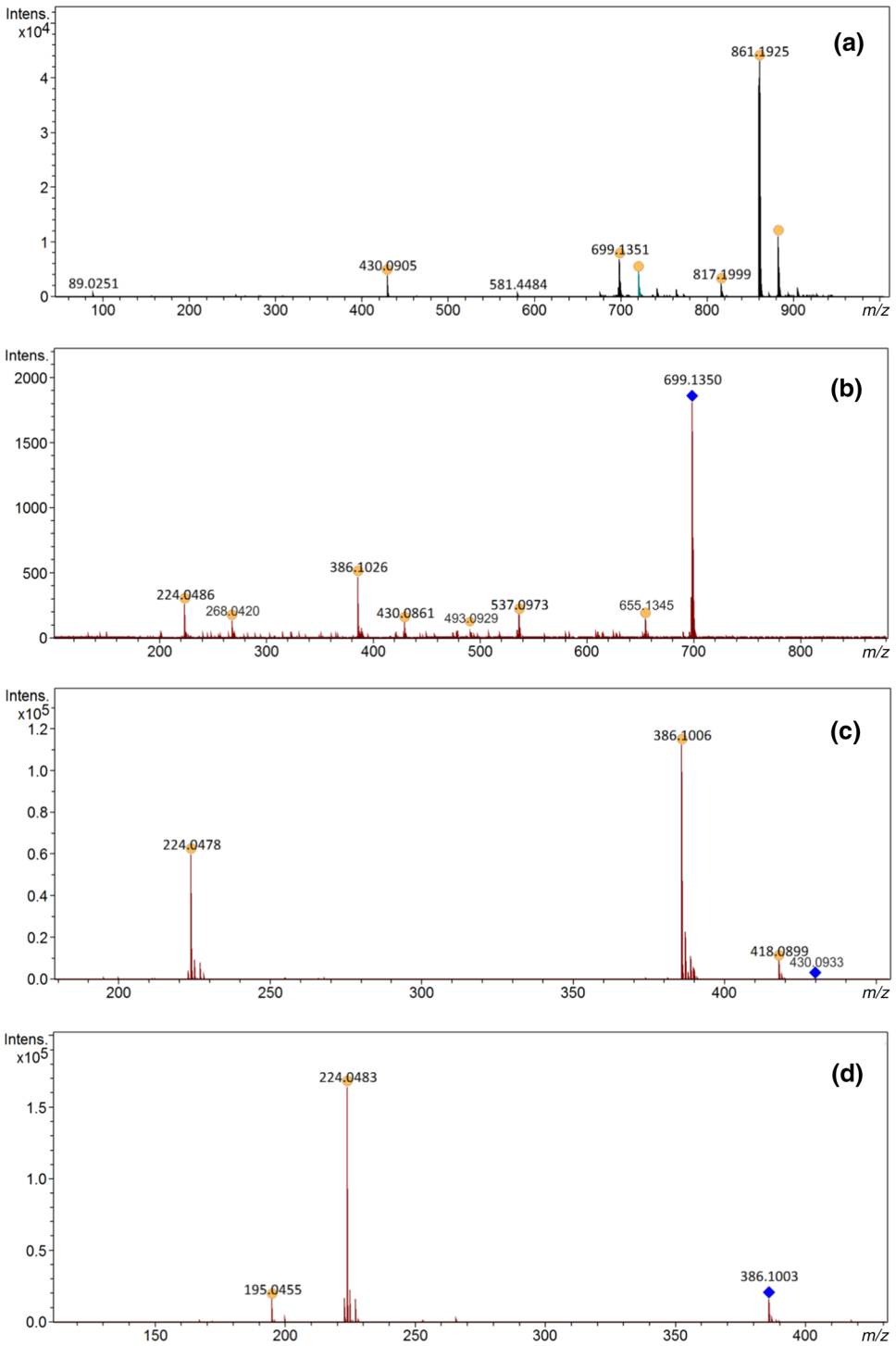

Fig. 4 ESI$^-$ mass spectra of **a** sennoside A parent $[M\text{-}H]^-$ ion of $m/z = 861.1925$, **b** MS^2 spectrum of the ion $m/z = 699.1350$, **c** MS^2 spectrum of parent ion $m/z = 430.0933$, and **d** MS^3 spectrum of parent ion $m/z = 386.1003$ (precursor ion is marked by blue diamond) (color figure online)

[38] to 1.59%. The analytical standards of sennoside A and sennoside B were purchased from Sigma-Aldrich. The other chemicals employed: acetic acid (LC–MS purity), methanol (Hypergrade for LC–MS), and sodium acetate (99.995% trace metals basis) were purchased from Sigma-Aldrich.

Instrumentation

A liquid chromatograph UHPLC Nexera XR (Shimadzu, Japan) connected with a Compact QTOF Bruker mass spectrometer (Bruker, Germany) was used. The XBridge® BEH C18 (150 × 3.0 mm i.d., particle size 2.5 µm; Waters), tempered at 40 °C, was used. The gradient elution with

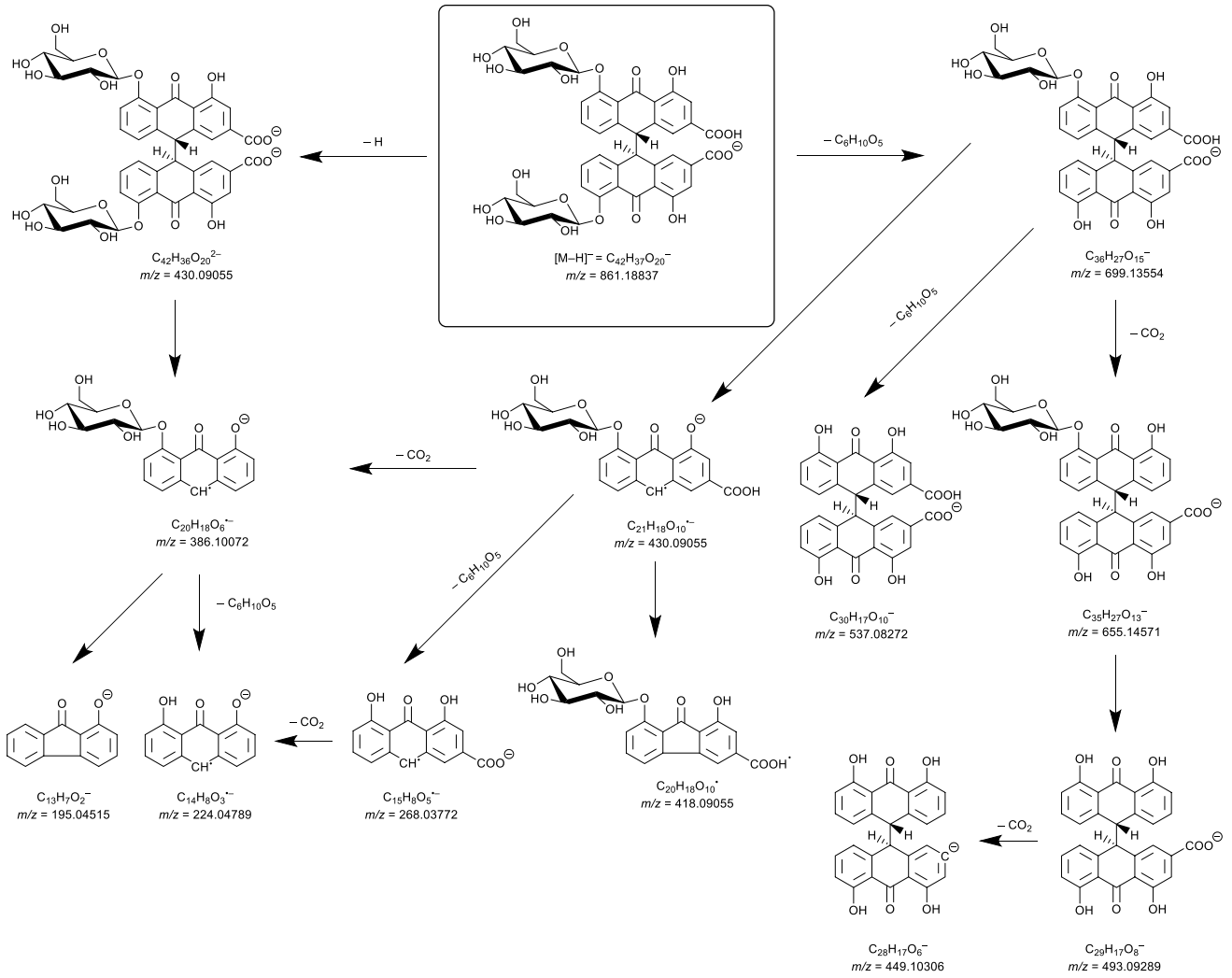

Fig. 5 Proposed ESI$^-$-MS^n fragmentation of sennoside A (the *m/z* values are calculated)

binary mobile phase of methanol and 0.2% aqueous acetic acid was used at a flow rate of 0.2 cm^3 min^{-1} (for gradient program see "Optimization of extraction and HPLC procedures" section). The volume of injected sample was 3 mm^3. Mass spectrometer worked in the scan range of *m/z* = 50–1000. The ionization of the analytes was performed in the negative ion mode at capillary voltage 2.7 kV. The pressure of the nitrogen (nebulizing gas) was set to 0.50 bar. Nitrogen (4.0 dm^3 min^{-1}) also served as drying gas at 250 °C. Sodium acetate served as a standard for calibration of the mass spectrometer. The calibration dependences of sennoside A and sennoside B were based on the area of peak in HPLC–MS chromatograms at *m/z* = 861.1925. The respective figures of merit of the calibration dependences are given in Table 2.

Table 2 Figures of merit of HPLC-MS determination of sennoside A and sennoside B based on the area of the peak at *m/z* = 861.1925

Compound	Sennoside A	Sennoside B
Linear dynamic range/mg dm^{-3}	5–40	5–40
Slope of calibration/10^6 area mg dm^{-3}	1.61 ± 0.17	1.09 ± 0.019
Intercept/10^6 area	6.5 ± 4.2	0.8 ± 0.4
R	0.9881	0.9997
Limit of detection/mg dm^{-3}	0.70	0.07
Limit of quantification/mg dm^{-3}	2.31	0.23

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