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Short communication

Rapid UHPLC determination of polyphenols in aqueous infusions of *Salvia officinalis* L. (sage tea)

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ABSTRACT

Sage tea, the aqueous infusion of dried sage leaves (*Salvia officinalis* L.), is used as a form of food as well as a form of traditional herbal medicine. Several *in vivo* and *in vitro* studies point to sage polyphenols as active principles that may inhibit lipid peroxidation and improve antioxidant defences. This study describes an UHPLC methodology with MS/MS and UV detection, which allows the separation, identification and quantification of the major phenolic constituents in sage tea within 34 min, and was used to characterize 16 commercial brands of sage tea. The quantitatively dominating compounds were either rosmarinic acid (12.2–296 mg/L) or luteolin-7-O-glucoside (37.9–166 mg/L). In general, considerable differences in polyphenolic composition between the brands were detected, leading to the demand for quality standardization and control, especially if these sage teas are to be used for therapeutic purposes.

1. Introduction

Sage (*Salvia officinalis* L.) has been an important medicinal plant since earliest times and is still used in domestic medicine, typically as an herbal tea preparation – an infusion of dried sage leaves with boiling water (sage tea) [1]. Our previous investigations focused on the adverse potential of sage, caused by the presence of the potentially neurotoxic monoterpene compound thujone; however, this compound was found to be below thresholds at normal levels of use [2]. In contrast, this contribution focuses on the polyphenol content. The lack of knowledge on these constituents may be derived from the fact that they are not covered by current regulations. Neither the European Pharmacopoeia monograph [3] nor the ISO 11165 standard [4] on sage demands a minimum content of polyphenols.

Though previous investigations were conducted on alcoholic or organic extracts or on oil isolated by steam distillation [5–12], very little research (only a single specimen [13]) has been conducted on aqueous infusions, which is the form of sage that is normally consumed. Only Fecka and Turek [14] presented a survey of various herb infusions (including sage) with quantitative data of six identified (and one unidentified) polyphenols.

Based on our experience with polyphenol analysis [15–20] and mass spectrometric structure elucidation [21], we have developed a rapid analytical method to characterize and quantify the major polyphenolic sage constituents including hydroxycinnamic acids and their derivates (e.g., caffeic acid or rosmarinic acid), flavon glycosides and phenolic terpenes (e.g., carnosic acid). With this, we will be the first to provide a comprehensive overview of 16 different sage tea brands from the German market.

2. Experimental

2.1. Reagents, standards and sample preparation for LC–MS/MS

All solvents and additives used for the eluents were LC–MS-grade: water (Optigrade, Promochem, Wesel, Germany), acetonitrile (Mallinckrodt Baker, Deventer, The Netherlands), and formic acid (Fluka/Sigma Aldrich, Steinheim, Germany). The standards were: caffeic acid (98%, Sigma/Sigma Aldrich, Steinheim, Germany), rosmarinic acid (96%, Aldrich/Sigma Aldrich,

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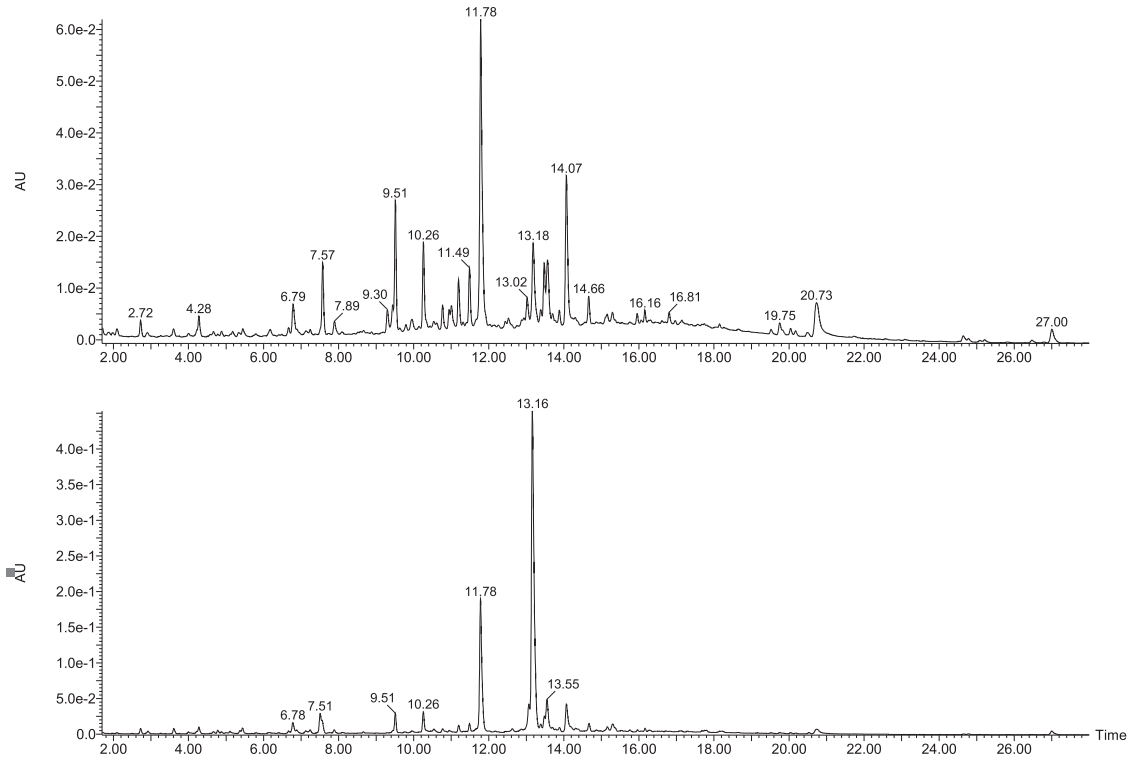

Fig. 1. Representative UV-chromatograms of two sage tea infusions with different concentrations of polyphenols. The peaks can be identified using their retention time listed in Table 1.

Steinheim, Germany), luteolin-7-O-glucuronide and luteolin-7-O-glucoside (isolated from carrot leaves [22]), and carnosic acid (91%, Sigma/Sigma Aldrich).

Infusions were prepared by adding 150 g boiling water to one tea bag or 1.5 g of sage leaves in a 250-mL-Erlenmeyer flask. The infusion was allowed to steep for 15 min. One millilitre of the infusion was filtered through a disposable polyester filter (Chromafil PET-20/25 (Macherey-Nagel, Düren, Germany)) with 0.20 μm pore size and injected into the HPLC system within 20 min.

2.2. Apparatus and software for LC–MS/MS measurement

An Acquity UPLC system by Waters (Milford, MA, USA) consisting of a binary pump (BSM), an autosampler (SM; cooled to 10 °C; injected volume: 3 μL), a column oven (CM) set at 40 °C, a diode array detector (PDA) scanning from 190 to 400 nm (wavelengths for quantification: 273 nm, 320 nm, and 360 nm), and a triple quadrupole mass spectrometer (Acquity TQD) with electrospray interface operating in negative mode was used. An Acquity BEH Shield RP18 column (150 mm × 2.1 mm, 1.7 μm; Waters) was used for separation with water (A) and acetonitrile (B) as eluents, both acidified with 0.1% (v/v) formic acid, at a flow rate of 0.4 mL/min with gradient elution: 0–14 min: 4–27% A; 14–28 min: 27–59.7% A; 28–28.2 min: 59.7–100% A; 28.2–30.5 min: 100% A; 20.5–31 min: 100–4% A; 31–34 min: 4% A (linear solvent composition change between the indicated points).

The mass spectrometer was tuned using a solution of pure rosmarinic acid and luteolin-7-O-glucuronide. The resulting parameters for the measurements were a compromise between the optimal parameters for these compounds: capillary voltage −2.0 kV; cone voltage 30 V; extractor voltage 1.0 V; RF voltage 0.20 V; source temperature 150 °C; desolvation temperature 400 °C; cone gas (nitrogen) flow 50 L/h; desolvation gas (nitrogen) flow 800 L/h. The collision gas (argon) flow in tandem mass spectrometry mode was 0.3 mL/min. The whole system was controlled by MassLynx 4.1 software.

First, peaks with known m/z and fragments from literature were searched using selected ion monitoring and selected reaction monitoring. Further compounds (especially those that gave major signals in the UV chromatogram) were detected using single MS scanning, followed by fragment ion scans. The resulting mass spectra were used to postulate structural assignments.

3. Results

UV-chromatograms of two different *Salvia* infusions are shown in Fig. 1. Fig. 2 shows a comparison of a UV- and a MS-chromatogram of the same sample. The phenolic acids and flavonoids were separated within 15 min. Next, the phenolic triterpenes were eluted. Table 1 lists the detected peaks. The total separation time was 28 min, and the total analysis time was 34 min. All listed peaks showed higher intensity when negatively ionized than when the positive ionization mode was used. In most cases, the identification is confirmed by published data (see references in Section 4 for each compound below). Some peaks could not or not definitely be assigned to known *Salvia* constituents. These cases are discussed below. The detected ions and their fragments and UV maxima (if possible) are also reported in Table 1.

Since reference compounds were not available for most of the detected compounds, flavone glycosides are quantitated as luteolin-7-O-glucuronide (at 348 nm); caffeic acid derivatives are quantitated as rosmarinic acid (at 330 nm); and phenolic triterpenes are quantitated as carnosic acid (at 205 nm). The calibration curves ($1/x$ weighting) were proved to be linear in the ranges of 2.5–500 mg/L with $r^2 = 0.99795$ (luteolin-7-O-glucuronide), 4.0–750 mg/L with $r^2 = 0.99936$ (rosmarinic acid), and 1.5–60 mg/L with $r^2 = 0.99912$ (carnosic acid). In total, 8 flavone glycosides, 2 caffeic acid derivatives and 6 triterpenes were quanti-

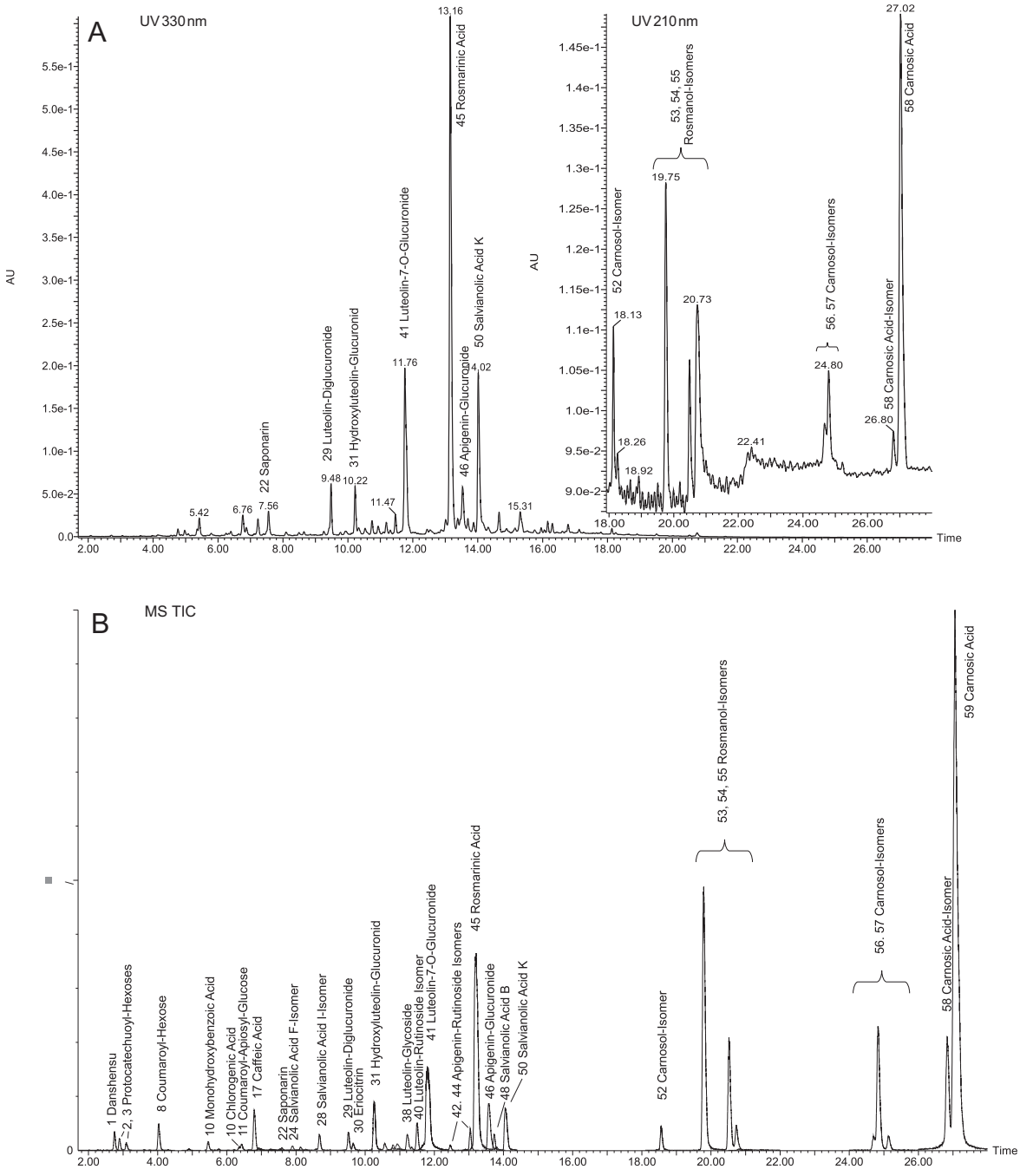

Fig. 2. Comparison of a UV-chromatogram (A) and a MS-chromatogram (B) of the same sample (UV: at 330 nm; inset at 210 nm).

fied, representing the highest UV peaks (see Fig. 2). The determined concentrations are shown in Table 2.

The quantitatively dominating compound is either rosmarinic acid or luteolin-7-O-glucoside. The concentrations range from 12.2 to 296 mg/L (rosmarinic acid) and from 37.9 to 166 mg/L (luteolin-7-O-glucoside). The ratio of these two compounds ranges from 0.3 to 3.0. Among the triterpenes, carnosic acid has the highest concentrations, ranging from 9.1 to 32.9 mg/L. In all but two samples the sum of the concentrations of the caffeic acid derivatives is higher than the sum of the concentrations of the triterpenes.

4. Discussion

The gradient of the HPLC method was optimized to separate as many flavonoids and phenolic acids as possible in a short time. The use of 1.7 μm-particles (UHPLC) enables the separation of the phenolic acids and flavonoids within 15 min.

Some peaks could not unambiguously be assigned to known *Salvia* constituents. The following structural suggestions are derived from their UV and mass spectra, but it must be noted that, for final assignment, a secondary technique, such as NMR, would be necessary.

Hydroxybenzoic acid derivates: Peak 2 and peak 3 at 2.93 min and 3.10 min, respectively, have the same $[M-H]^-$ ion mass of m/z 315 and the same main fragment m/z 153 $[M-162-H]^-$. Peak 3 shows another weak fragment with m/z 109 $[M-162-44-H]^-$, which is not detectable in the mass spectrum of peak 2. This may be due the low concentration of the compound. These mass spectra suggest the presence of protocatechuoyl hexose (or protocatechuic acid hexoside), which has been described in plants, such as those

Table 1
Identification of detected peaks in sage tea infusions.

Class	Peak number	Compound	RT	$[M-H]^-$	Main fragment	Other fragments	UV_{max}
HCA	1	Danshensu	2.78	197	123	135, 151, 179, 109	280
HBA	2	Protocatechuoyl-hexose	2.93	315	153	n.dtc.	n.dtm.
HBA	3	Protocatechuoyl-hexose	3.10	315	153	109	n.dtm.
HBA	4	Protocatechuic acid	3.65	153	153	109	263
HBA	5	Dimethoxybenzoic acid	3.80	181	119	163, 159, 135	263sh
HBA	6	Monohydroxybenzoyl-hexose	4.05	299	137	93	280, 326
Unknown	7	Unknown	4.32	475	253	363, 299, 233	267, 312, 322
HCA	8	Coumaroyl-hexose	4.93	325	163	n.dtc.	282
HCA	9	Caffeoyl-fructosyl-glucose	5.46	503	161	341, 281, 221, 179, 135	n.dtm.
HBA	10	Monohydroxybenzoic acid	5.52	137	93	n.dtc.	n.dtm.
HCA	11	Coumaroyl-apiosyl-glucose isomer	5.80	457	163	295, 251, 205, 191, 187	n.dtm.
HCA	12	Chlorogenic acid isomer	6	353	191	n.dtc.	n.dtm.
HCA	13	Chlorogenic acid	6.42	353	191	n.dtc.	326
HCA	14	Coumaroyl-apiosyl-glucose isomer	6.45	457	163	n.dtc.	n.dtm.
HCA	15	Chlorogenic acid isomer	6.70	353	191	n.dtc.	n.dtm.
Other	16	Methydihydrojasmonic acid isomer	6.85	225	97	147, 135	n.dtm.
HCA	17	Caffeic acid	6.87	179	135	n.dtc.	324
HCA	18	Feruloyl-glucose isomer	6.94	355	235	295, 265, 193, 175, 149, 134	n.dtm.
Other	19	Methydihydrojasmonic acid isomer	7.01	225	97	147, 135	n.dtm.
Unknown	20	Unknown	7.24	461	329	415, 221, 201	325, 294
HCA	21	Salvianolic acid I isomer	7.40	537	491	329, 161,101	287, 325
Fon	22	Apigenin-6-C-glucoside-7-O-glucoside syn. saponarin	7.56	593	353	503, 473, 383	271, 336
HCA	23	Feruloyl-glucose isomer	7.60	355	235	295, 265, 193, 175, 149, 134	n.dtm.
HCA	24	Salvianolic F isomer	8	313	197	153, 121	n.dtm.
HCA	25	Salvianolic acid I isomer	8.10	537	339	161	n.dtm.
HCA	26	Compound 1 from Wang2000	8.15	473	193	175, 235, 297	n.dtm.
HCA	27	Salvianolic acid I isomer	8.34	537	339	179, 165	n.dtm.
HCA	28	Salvianolic acid I isomer	8.74	537	519	161, 341, 179	288, 324
Fon	29	Luteolin-diglucuronide	9.50	637	285	461	254, 265sh, 348
Fva	30	Eriodictyol-rutinosid syn. eriocitrin	9.65	595	287	n.dtc.	283, 324
Fon	31	Hydroxy-luteolin-glucuronide	10.28	477	301	n.dtc.	281, 342
HCA	32	Methylmelitric acid A	10.60	551	551	533	286, 326
Fon	33	Apigenin-diglucuronide	10.78	621	269	445	268, 334
HCA	34	Sagecoumarin	10.80	535	359, 161	281, 493	267, 337
Fon	35	Luteolin-rutinoside isomer	10.95	593	285	n.dtc.	252, 264, 348
HBA	36	Monohydroxy benzoic acid	11	137	93	n.dtc.	n.dtm.
Unknown	37	Salvianolic acid I isomer	11.13	537	493	359, 313, 295	267, 342
Fon	38	Luteolin-7-O-glucoside	11.20	447	285	n.dtc.	265, 345
Fon	39	Luteolin-hexoside	11.41	447	285	n.dtc.	265, 345
Fon	40	Luteolin-rutinoside isomer	11.58	593	285	n.dtc.	252, 264, 348
Fon	41	Luteolin-7-O-glucuronide	11.80	461	285	n.dtc.	254, 267, 344
Fon	42	Apigenin-rutinoside isomer	12.50	577	269	n.dtc.	n.dtm.
Fon	43	Apigenin-hexoside	12.90	431	268	n.dtc.	n.dtm.
Fon	44	Apigenin-rutinoside isomer	13	577	269	n.dtc.	267, 281, 334
HCA	45	Rosmarinic acid	13.24	359	161	197, 179	29, 330
Fon	46	Apigenin-glucuronide	13.56	445	269	n.dtc.	267, 339
Fva	47	Hispiludin-glucuronide	13.60	475	299	n.dtc.	n.dtm.
HCA	48	Salvianolic acid B	13.76	717	519	321	284, 337
HCA	49	Salvianolic acid K isomer	14	555	359	493, 401, 161, 135	288, 324
HCA	50	Salvianolic acid K isomer	14.35	555	359	493, 401, 161, 135	288, 324
Fva	51	Luteolin-rutinoside isomer	15	667	285	n.dtc.	252, 264, 348
PDT	52	Carnosol isomer	18.56	329	211	275, 229, 171	n.dtm.
PDT	53	Rosmanol isomer	19.82	345	301	283, 267, 258, 227	220, 283, 323
PDT	54	Rosmanol isomer	20.54	345	283	267, 253, 227	220, 283, 322
PDT	55	Epirosmanol	20.75	345	283	301, 267, 253, 227	196, 221, 277, 332
PDT	56	Carnosol isomer	24.70	329	285	269, 201	221
PDT	57	Carnosol isomer	24.84	329	285	269, 201	221
PDT	58	Carnosic acid isomer	26.84	331	287	271, 243	222
PDT	59	Carnosic acid	27.08	331	287	271, 215	222, 283

Abbreviations: RT, retention time; HBA, hydroxybenzoic acid and derivates; HCA, hydroxycinnamic acid and derivates; Fon, flavon glycosides; Fva, flavanon glycosides; PDT, phenolic diterpenes; n.dtc., not detected; n.dtm., not determined.

that are member of the *Prunus* genus [23], but never before in *S. officinalis*.

Peak 5 at 3.80 min, with $[M-H]^-$ *m/z* 181, has the molecular mass and some typical fragments (*m/z* 163: $[M-H_2O-H]^-$; *m/z* 119: $[M-H_2O-CO_2-H]^-$) of a dimethoxybenzoic acid. Topcu et al. [24] described 2,4-dimethoxybenzoic acid in *Salvia candidissima*.

Peak 6 at 4.05 min shows a very similar mass spectrum as found in peak 2 and peak 3 at 2.93 min and 3.10 min, respectively, but all *m/z* of peak 6 are 16 units lower compared to the fragments of peak 2 and peak 3, suggesting a monohydroxylbenzoyl hexose (or monohydroxybenoic acid hexoside), which has also never before been described in *S. officinalis*.

Peaks 12, 13, and 15 at 6.00, 6.42 and 6.70 min, respectively, show the characteristic mass spectrum of chlorogenic acid. Peak 13 at 6.42 min was identified as 3-caffeoylquinic acid in comparison to a pure standard.

Peaks 18 and 23, at 6.94 min and 7.60 min, respectively, with $[M-H]^-$ *m/z* 355, show identical mass spectra suggesting feruloyl glucose. Wang et al. [25] described 6-O-(E)-feruloyl-

Table 2
Concentrations (±standard deviation) of phenolics in 16 commercial sage tea infusions (in mg/L).

Compound	Measured as	Brand 1	Brand 2	Brand 3	Brand 4	Brand 5	Brand 6	Brand 7	Brand 8	Brand 9	Brand 10	Brand 11	Brand 12	Brand 13	Brand 14	Brand 15	Brand 16
Saponin	Lg	7.2 ± 0.07	8.2 ± 0.15	9.3 ± 0.18	9.8 ± 0.04	10.5 ± 0.24	3.8 ± 0.77	5.5 ± 0.45	5.2 ± 0.30	5.8 ± 0.22	12.9 ± 0.25	8.6 ± 0.09	7.2 ± 0.74	7.6 ± 0.08	9.8 ± 0.38	6.7 ± 0.45	9.4 ± 1.00
Luteolin-Diglucuronide	Lg	14.4 ± 0.49	26.4 ± 0.40	22.3 ± 0.12	26.0 ± 1.34	20.6 ± 0.79	5.1 ± 1.20	11.2 ± 0.06	10.7 ± 0.76	8.7 ± 2.90	44.0 ± 1.99	19.7 ± 0.75	19.5 ± 2.42	13.7 ± 0.86	14.4 ± 0.88	16.0 ± 2.54	22.2 ± 3.10
Hydroxyluteolin-glucuronide	Lg	9.8 ± 0.10	18.1 ± 0.06	19.4 ± 0.88	22.5 ± 1.75	19.7 ± 0.09	10.3 ± 1.35	11.2 ± 0.94	7.2 ± 0.34	6.7 ± 1.57	31.1 ± 0.69	13.2 ± 0.10	15.2 ± 2.64	12.7 ± 0.17	15.6 ± 0.83	16.4 ± 2.18	20.2 ± 1.65
Apigenin-diglucuronide	Lg	3.9 ± 0.07	5.5 ± 0.16	4.7 ± 0.03	5.4 ± 0.20	4.5 ± 0.08	1.1 ± 0.22	2.4 ± 0.04	2.1 ± 0.14	1.6 ± 0.72	9.1 ± 0.05	5.1 ± 0.18	3.4 ± 0.42	3.1 ± 0.21	3.3 ± 0.34	3.1 ± 0.33	4.4 ± 0.38
Luteolin-7-O-glucoside	Lg	5.8 ± 0.14	4.9 ± 0.02	6.4 ± 0.57	6.0 ± 0.62	5.3 ± 0.19	4.5 ± 0.55	4.1 ± 0.90	4.7 ± 0.11	3.5 ± 0.71	8.4 ± 0.22	6.1 ± 0.47	3.7 ± 0.62	4.8 ± 0.46	7.4 ± 0.25	5.1 ± 0.07	6.0 ± 0.58
Luteolin-rutinoside	Lg	6.3 ± 0.18	8.2 ± 0.13	9.1 ± 0.14	10.7 ± 0.60	10.2 ± 0.47	4.9 ± 0.31	4.6 ± 0.26	5.7 ± 0.61	4.5 ± 0.65	12.2 ± 0.37	6.9 ± 0.03	6.6 ± 1.83	6.8 ± 0.24	7.5 ± 0.33	5.8 ± 1.16	8.8 ± 1.12
Luteolin-7-O-glucuronide	Lg	75.3 ± 2.49	102.4 ± 1.35	97.8 ± 1.61	110.5 ± 5.50	107.8 ± 1.18	60.9 ± 8.19	85.7 ± 6.35	37.9 ± 2.17	40.4 ± 6.55	166.3 ± 1.65	92.4 ± 3.05	60.1 ± 3.05	78.4 ± 8.87	106.6 ± 6.21	62.1 ± 6.02	89.6 ± 10.62
Rosmarinic acid	RA	73.4 ± 1.63	104.9 ± 2.91	161.2 ± 1.10	185.3 ± 4.65	123.2 ± 1.61	178.4 ± 3.51	295.7 ± 9.71	12.2 ± 1.78	30.5 ± 1.00	196.7 ± 1.72	75.7 ± 1.84	57.2 ± 8.35	116.7 ± 4.78	106.4 ± 5.15	126.1 ± 5.05	131.1 ± 14.25
Apigenin-glucuronide	Lg	20.4 ± 0.02	24.1 ± 1.19	22.3 ± 1.19	23.2 ± 0.66	27.1 ± 1.50	15.6 ± 2.04	22.5 ± 2.11	8.6 ± 0.39	11.5 ± 0.17	41.1 ± 1.15	29.4 ± 0.69	13.4 ± 1.17	19.4 ± 0.83	28.0 ± 2.35	13.1 ± 1.01	21.3 ± 1.91
Salvianolic acid K	RA	10.7 ± 1.42	29.4 ± 0.86	47.0 ± 1.19	56.4 ± 2.95	36.2 ± 1.34	22.4 ± 3.34	13.0 ± 0.87	10.8 ± 0.94	6.8 ± 0.18	51.3 ± 0.65	11.1 ± 0.18	32.0 ± 0.84	19.8 ± 2.96	13.9 ± 0.82	30.3 ± 0.47	41.9 ± 3.03
Rosmanol-isomer RT 19.7 min	CA	8.8 ± 1.01	4.0 ± 0.48	7.4 ± 0.55	5.7 ± 0.13	4.6 ± 0.39	3.3 ± 0.32	3.4 ± 0.64	6.8 ± 0.39	8.5 ± 0.76	4.3 ± 0.20	8.9 ± 0.62	6.5 ± 0.32	6.1 ± 0.25	6.9 ± 0.16	6.3 ± 0.21	6.4 ± 0.16
Rosmanol-isomer RT 20.5 min	CA	2.3 ± 0.14	2.3 ± 0.04	2.7 ± 0.38	2.3 ± 0.08	1.7 ± 0.21	2.2 ± 0.04	1.3 ± 0.09	2.0 ± 0.66	2.7 ± 0.11	2.2 ± 0.35	2.9 ± 0.39	2.1 ± 0.44	2.7 ± 0.62	2.5 ± 0.16	2.4 ± 0.26	2.4 ± 0.12
Rosmanol-isomer RT 20.8 min	CA	6.6 ± 0.23	6.4 ± 0.05	6.2 ± 0.20	6.8 ± 0.24	6.3 ± 0.00	6.2 ± 0.09	6.0 ± 0.14	6.0 ± 0.48	6.6 ± 0.19	7.2 ± 0.42	6.7 ± 0.39	6.4 ± 0.26	6.8 ± 0.56	7.1 ± 0.20	6.7 ± 0.28	6.8 ± 0.13
Sum of cornosol-isomers	CA	4.9 ± 0.44	3.2 ± 0.29	3.4 ± 0.19	3.0 ± 0.12	2.9 ± 0.01	2.8 ± 0.42	4.1 ± 0.46	3.4 ± 0.04	4.1 ± 0.22	3.6 ± 0.29	4.1 ± 0.16	5.1 ± 0.19	4.7 ± 0.09	2.5 ± 0.26	4.5 ± 0.42	2.6 ± 0.06
Carnosic acid-isomer RT 26.8 min	CA	0.7 ± 0.06	0.8 ± 0.17	0.7 ± 0.07	0.6 ± 0.10	0.4 ± 0.07	0.4 ± 0.10	0.8 ± 0.14	0.4 ± 0.00	0.5 ± 0.01	0.7 ± 0.09	0.8 ± 0.06	0.6 ± 0.11	0.6 ± 0.09	0.3 ± 0.01	0.7 ± 0.20	0.4 ± 0.10
Carnosic acid	CA	29.7 ± 2.43	17.9 ± 1.26	12.7 ± 1.00	15.8 ± 3.14	12.5 ± 0.70	11.1 ± 3.76	30.1 ± 0.14	17.5 ± 1.63	20.6 ± 0.77	21.8 ± 2.87	16.0 ± 1.27	32.9 ± 3.71	28.4 ± 1.96	9.1 ± 1.20	22.2 ± 0.60	10.2 ± 2.33
Sum of flavone-glycosides	Lg	102.9	134.8	129.4	143.5	145.4	80.3	113.7	51.7	57.7	220.3	130.4	80.7	105.5	144.4	81.9	120.3
Sum of rosmarinic acid derivatives	RA	84.0	134.3	208.2	241.8	159.4	200.8	308.6	23.0	37.3	248.0	86.8	89.2	136.5	120.3	156.4	173.1
Sum of cornosol derivatives	CA	53.1	34.7	33.2	34.2	28.5	26.0	45.6	36.0	43.1	39.8	39.3	53.7	49.3	28.3	42.7	28.7

Abbreviations: Lg: Luteolinglucuronide; RA: Rosmarinic acid; CA: Carnosic acid; RT: retention time.

glucopyranoside in *S. officinalis*. Here, an additional feruloylhexoside is hypothesized.

Hydroxycinnamic acid derivates: Peak 6 at 4.93 min with $[M-H]^-$ *m/z* 325 shows fragments that suggest coumaroyl hexose (or coumaric acid hexoside). Lu and Foo [26] described two coumaric acid glycosides with two sugar moieties, but a monohexoside of coumaric acid has never before been described in *S. officinalis*.

Peak 24 at 8.00 min with $[M-H]^-$ 313 is an isomer of salvianolic acid F, but the mass spectrum is not the same as what is reported in the literature [27,28]. The fragments of 197 and 153 suggest the involvement of protocatechuic acid.

Salvianolic acid I-isomers: There are five peaks with $[M-H]^-$ *m/z* 537, i.e., isomers of salvianolic acid I (peaks 21, 25, 27, 28, and 37; retention times 7.40, 8.10, 8.34, 8.74, 11.13 min, respectively). Liu et al. [27] reported the fragment *m/z* 339. This fragment was only found in the mass spectra of peak 27 and peak 28 at 8.34 min and 8.74 min, respectively. With the exception of the last peak, all the peaks show fragments with *m/z* 179 and/or *m/z* 161, which are typical for molecules containing a caffeic acid moiety. Peak 37 at 11.13 min has no common fragments with the other salvianolic acid I-isomers. The UV spectra of the peak 21 and peak 28 at 7.4 min and 8.74 min, respectively, show maxima at 288 and 324 nm similar to salvianolic acid I [27] in contrast to peak 37 at 11.13 min. Hence, the latter is not considered to be a caffeic acid derivate.

Flavons: Peak 22 at 7.56 min shows a mass spectrum similar to that of saponarin (apigenin-6-C-glucoside-7-O-glucoside). Some flavone-C-glycosides have been described in *Salvia* (for references see Lu and Foo [29]), but saponarin has not been so described.

Two peaks with $[M-H]^-$ *m/z* 593, with the main fragment *m/z* 285, i.e., isomers of luteolin rutinoside, were found. Peak 35 at 10.95 min has a more stable $[M-H]^-$ ion than does peak 40 at 11.58 min. Thus, the single sugar moieties are probably bound in different ways. The same phenomenon applies to the apigenin rutinoside isomers (peak 42 and peak 44) at 12.5 min and 13.0 min, respectively.

Phenolic triterpenes: Three peaks with $[M-H]^-$ *m/z* 329, were found, i.e., isomers of carnosol (peaks 52, 56, and 57; retention times 18.56, 24.70, and 24.84 min, respectively). The fragments *m/z* 285, *m/z* 269, and *m/z* 201 correspond with those found by Cuvelier et al. [30]. These fragments were only found in the mass spectra of the peaks 56 and 57 at 24.70 min and 24.84 min, respectively.

Three peaks with $[M-H]^-$ *m/z* 345 were found, i.e., isomers of rosmanol (peaks 52, 53, and 54; retention times 19.82, 20.54, and 20.75 min). After positive ionization, all the three isomers showed 301 as the main fragment and *m/z* 281 and *m/z* 273 as further fragments in correspondence with Cuvelier et al. [30]. The mass spectra after positive ionization are also similar, but the peak at 19.82 min has a base peak of *m/z* 301, while the other two have a base peak of *m/z* 283, especially at low collision energy. UV spectra of the two earlier eluting peaks (52 and 53) are the same, but peak 54 at 20.75 min has a maximum at 196 nm, which has been reported for epirosmanol [30].

Two peaks with $[M-H]^-$ *m/z* 331 were found, i.e., isomers of carnosic acid (peaks 58 and 59; retention times 26.84 and 27.08 min, respectively). Mass and UV spectra are the same and in correspondence with the literature [30]. The same two isomers are found in the standard of carnosic acid with the later eluting peak being the far dominant peak. So the latter is considered to be carnosic acid.

5. Conclusions

This study introduces an efficient and fast UHPLC-UV-MS method to measure polyphenols in aqueous infusions of sage tea. While the previous research was generally focused on the determining the polyphenols directly present in the herbal material, our study is the first to provide a comprehensive overview about commercial material in the form in which it is actually consumed, i.e., an aqueous infusion. The high variability of possibly beneficiary components in sage suggests the consumer relevance of our research, as neither standardization nor quality control is currently being conducted in this regard.

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