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Quantification of sugars and organic acids in hygroscopic pharmaceutical herbal dry extracts

Marc Schiller[a], Holger von der Heydt[b], Frieder März[b], Peter C. Schmidt[a,*]

[a]*Department of Pharmaceutical Technology, Eberhard-Karls-University Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany*

[b]*Boehringer Ingelheim KG, A Drug Delivery, Binger Strasse 173, 55216 Ingelheim/Rhein, Germany*

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Abstract

Three chromatographic methods have been employed for the determination of hydrophilic compounds, namely carbohydrates and organic acids in herbal dry extracts of *Eschscholtzia californica* Cham. The hydrophilic compounds were separated from the other components of the dry extracts by solid-phase extraction methods, which were optimised with respect to recovery rates. Carbohydrates were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection. Organic acids were analysed by ion-exclusion chromatography with evaporative light scattering detection and gas chromatography–mass spectrometry. Using the latter method, large amounts of glyceric acid were separated from the extracts of *Eschscholtzia californica* Cham. This substance together with sugars may be responsible for the increased hygroscopicity and the poor processing behaviour of the extracts.

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1. Introduction

Herbal dry extracts play an important role in the development and production of solid herbal drug preparations such as tablets and capsules. They are composed of multi-component mixtures containing concomitant substances besides the active ingredients. These concomitant compounds can affect the physicochemical and technological properties as well as the biopharmaceutical parameters of the extracts [1]. These problems are often attributed to the hygroscopicity of some hydrophilic ingredients like carbohydrates and salts of organic acids. In a recent study, some of the dry extracts showed poor processing properties and different water vapour sorption and desorption behaviour on storage. The most conspicuous problem is the cracking phenomenon of film tablets produced from these extracts. Even at moderate relative humidities, the tablets cracked a few days after they were manufactured [2].

The plant *Eschscholtzia californica* Cham. is a traditional herbal remedy of the Indians, used by the natives of California for its analgesic and sedative properties. Pharmacological studies have substantiated these effects. Rolland et al. [3] have shown the anxiolytic and sedative effects of the aqueous extract of *Eschscholtzia californica* Cham. in mice. Al-

*Corresponding author. Tel.: +49-7071-297-2462; fax: +49-7071-295-531.

E-mail address: peter-christian.schmidt@uni-tuebingen.de (P.C. Schmidt).

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though the mechanism of action is not fully understood, the main substances responsible for the sedative effect seem to be flavonoids (e.g. rutin) [4–7] and isoquinoline alkaloids (e.g. californidine and californine) [4,5,8] that are present in the extracts in the range of 1.6–3.7% and 1.8–3.8%, respectively. Besides these so-called active components several other by-products have been found in the aerial parts of *Eschscholtzia californica* Cham. Von der Heydt detected the monosaccharides fructose and glucose, amino acids and chlorophyll in polar extracts [4]. Using a thin-layer chromatographic method Rolland determined malic acid as a compound in ethanolic–aqueous extracts [9].

Carbohydrates are ubiquitous in herbal dry extracts. Due to the absence of a strong chromophore in sugars direct UV detection of carbohydrates is not feasible. The most common method for the quantification of saccharides by HPLC analysis comprises refractive index (RI) detection. Although this method has several drawbacks, it was successfully used to quantify carbohydrates in herbal dry extracts of *Hypericum perforatum* L. [10]. RI detection cannot be used in combination with gradient elution and it also lacks high sensitivity [11]. For this reason a high-performance anion-exchange chromatography system equipped with pulsed amperometric detection (HPAEC–PAD) was used to determine the mono- and disaccharides [12,13]. HPAEC takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. Because of the large amount of other components present in the extract which may interfere with the analytical method, a three-step solid-phase extraction (SPE) column sequence, containing a hydrophobic, an anion-exchange and a cation-exchange column was used to separate the carbohydrates quantitatively [14]. The SPE method was optimised to increase the recovery rate of the carbohydrates to nearly 100%. In addition hydrolysis using trifluoroacetic acid (TFA) was performed to crack possible existing oligo- or polysaccharides [15,16].

The second group of hydrophilic substances that are also ubiquitous in herbal dry extracts are the organic acids, such as citric and malic acid. For the determination and quantification of these two substances, a high-performance ion-exclusion chromatographic method [17,18] was used comprising a separation on an Aminex HPX-87-H (Bio-Rad) column followed by a universal detection principle, the evaporative light scattering detector [19]. Since all components of the mobile phase must be completely volatilised, 0.02 *M* trifluoroacetic acid was used as the eluent. Samples were cleaned up using the SPE method containing a lipophilic and an anion-exchange column, on which the acids were collected and eluted with 0.1 *M* TFA. In addition, the analysis of the organic acids was completed by a gas chromatographic and mass spectrometric (GC–MS) method after silylation of the acids using *N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (BSTFA). First, the acids are separated from neutral or basic compounds. This was achieved by employing anion-exchange followed by organic extraction with ethyl acetate. The major problem, however, is the incomplete extraction of some hydroxyacids (i.e. glyceric acid or citric acid) [20]. Hence, standard substances were treated like the extracts to estimate the content of these acids regardless of their low recovery rates. Using this method other organic acids and by-products could be determined at least qualitatively or semi-quantitatively. The identities of these compounds were confirmed by computerized library matching and by comparison with authentic chemicals.

The aim of this study was to determine groups of hydrophilic substances, which are responsible for the poor tableting behaviour and storage stability of tablets containing some batches of herbal dry extracts. Thus, in this study the hydrophilic components of five batches of herbal dry extracts from *Eschscholtzia californica* Cham. have been separated and quantified. For comparative purposes some batches of the extracts of *Vitis vinifera*, *Xysmalobium undulatum*, *Valeriana officinalis*, *Eleutherococcus senticosus*, *Melissa officinalis*, *Piper methysticum* and *Panax ginseng* were also analysed.

2. Experimental

2.1. Materials

Plant dry extracts from *Eschscholtzia californica* Cham., *Vitis vinifera*, *Xysmalobium undulatum*, *Val-*

eriana officinalis, *Eleutherococcus senticosus*, *Melissa officinalis*, *Piper methysticum* and *Panax ginseng* were produced by Müggenburg Extrakt (Henstedt-Ulzburg, Germany) and delivered by Boehringer Ingelheim (Ingelheim/Rhein, Germany). Water was deionised using a Milli-Q water purifier (Millipore, Eschborn, Germany). Methanol, acetonitrile, ethyl acetate, D-(+)-glucose, D-(−)-fructose, DL-malic acid, L-(+)-tartaric acid and citric acid monohydrate were obtained from Merck (Darmstadt, Germany). Glyceric acid was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) as a 40% (w/w) solution in water. Sucrose was purchased from Südzucker (Mannheim/Ochsenfurt, Germany). The 50% (w/w) NaOH solution was obtained from J.T. Baker (Gross-Gerau, Germany). TFA was supplied by Riedel-de Haen (Seelze, Germany). BSTFA was obtained from Fluka (Buchs, Switzerland) and helium 4.6 from Messer Griesheim (Reutlingen, Germany). Filter papers (diameter 110 mm) were purchased from Schleicher & Schuell (Dassel, Germany). Strong anion-exchange resin (Lewatit Mono Plus M 600) was obtained from Bayer (Leverkusen, Germany). SPE cartridges Isolute 101 (200 mg/6 ml), Isolute C_{18} (EC) (500 mg/6 ml) and Isolute PE-AX (500 mg/3 ml) were supplied by Separtis (Grenzach-Wyhlen, Germany) and the anion- and cation-exchange cartridges Varian Bondelut SAX and SCX (500 mg 3 ml) by Varian (Darmstadt, Germany).

2.2. Analysis of carbohydrates using HPAEC–PAD

2.2.1. Sample preparation

Plant dry extracts (approximately 50 mg, accurately weighed) were suspended in 20.0 ml of water for 20 min using a mechanical stirrer (SM 25, Otto GmbH, Hechingen, Germany) and the resulting suspension was passed through filter paper (diameter 110 mm). For separation of the sugars from other ingredients, a three-step SPE extraction column sequence was used (Fig. 1). A hydrophobic polystyrene/divinylbenzene copolymer cartridge (Isolute 101, 200 mg/6 ml) was preconditioned with 12 ml of methanol followed by 12 ml of water. Anion- and a cation-exchange cartridges (Varian Bondelut SAX and SCX, 500 mg 3 ml) were connected in series and preconditioned with 6 ml of water. After placing the two ion-exchange cartridges under the hydrophobic

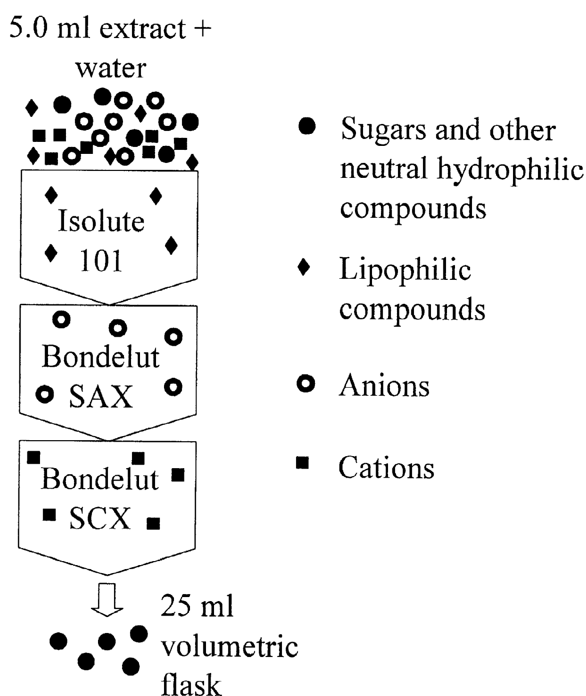

Fig. 1. Separation of carbohydrates from other ingredients of plant dry extracts using a three-step solid-phase extraction column sequence.

one, 5.0 ml of the extract from above were placed on top of the three cartridges and the carbohydrates were eluted with water into a 25-ml volumetric flask (Fig. 1).

2.2.2. Chromatographic conditions

Samples were analysed using a gradient pump GP 40, an electrochemical detector ED 40 (Dionex, Idstein, Germany), an autosampler series 1100 ALS (Agilent, Waldbronn, Germany) and the data system Millennium 32 (Waters, Eschborn, Germany). Carbohydrates were separated on a CarboPac PA-100 (Dionex) pellicular anion-exchange analytical column (250×4 mm) with CarboPac PA-100 guard column (50×4 mm). The carbohydrates were detected by a PAD system in the integrated amperometry mode, using a gold working electrode and an Ag/AgCl reference electrode. The waveforms E_1: 0.05 V for 0.4 s; E_2: 0.75 V for 0.2 s; E_3: −0.15 V for 0.4 s were employed with integration from 0.2 to 0.4 s. Mono- and disaccharides were eluted using an isocratic method (20 m*M* NaOH for 20 min), follow-

ing a rinse step of 5 min with 500 m*M* NaOH. Afterwards, a reconditioning step of 25 min with the mobile phase prior to each injection was carried out to re-equilibrate the column. Eluent flow-rate was 1.0 ml/min. During chromatography the eluent was degassed using helium. The mobile phase was prepared by degassing water, then pipetting in 50% (w/w) NaOH to minimize the amount of carbonate contamination which affects the retention times of carbohydrates. The quantification of the three sugars was carried out using external standard solutions of fructose, glucose and sucrose in combination.

2.2.3. TFA hydrolysis

For the hydrolysis of the carbohydrates, 30 mg extract (accurately weighed), 500 μl water and 500 μl 4 *M* TFA were transferred into an Eppendorf cup and heated for 1 h at 100 °C in a drying oven (Memmert, Schwabach, Germany). Prior to HPLC analysis the hydrolysed samples were diluted with water in a 20-ml volumetric flask and treated with the SPE sequence.

2.3. Analysis of organic acids using ion-exclusion chromatography with evaporative light scattering detection (ELSD)

2.3.1. Sample preparation

Samples of the herbal dry extracts (approximately 300 mg, accurately weighed) were suspended in 25.0 ml water for 20 min using a mechanical stirrer (SM 25, Otto, Hechingen, Germany) and the suspension obtained was passed through filter paper. The hydrophobic and the anion-exchange cartridges were preconditioned as follows. The hydrophobic and end capped C_{18}-cartridge [Isolute C_{18} (EC), 500 mg/6 ml] was rinsed with 3 ml of methanol followed by 3 ml of water for equilibration. The anion-exchange cartridge (Isolute PE-AX, 500 mg/3 ml) was conditioned with 3 ml of water and placed under the hydrophobic one. A 5.0-ml volume of the filtrate from the above sample was placed on top of the two cartridges and the whole system was rinsed with 3 ml of water. The organic acids were eluted from the anion-exchange cartridge into a 10-ml volumetric flask with 0.1 *M* TFA.

2.3.2. Chromatographic conditions

An evaporative light scattering detector Sedex 75 (Sedere, Vitry/Seine, France) with an HPLC pump L-6200A (Merck–Hitachi, Darmstadt, Germany) and an autosampler series 1100 ALS (Agilent) were used in combination with the data system Millennium 32 (Waters) to analyse and quantify the organic acids. The column was an Aminex HPX-87-H strong cation-exchange resin column (300×7,8 mm), fitted with an ion-exclusion Micro-Guard refill cartridge (Bio-Rad Labs., Munich, Germany). The mobile phase was 0.02 *M* TFA. Eluent flow-rate was 0.6 ml/min. Detection with the ELSD system was performed at an optimised drift tube temperature of 60 °C and 3.4 bar nitrogen pressure. The quantification of the organic acids was carried out using external standard solutions of malic, citric and tartaric acid in combination.

2.4. Analysis of organic acids using GC–MS

2.4.1. Sample preparation

A 10.0-g sample of herbal dry extract were suspended in 200.0 ml of water for 20 min using a mechanical stirrer (SM 25, Otto) and filtered through a Büchner-funnel. One hundred ml of the solution was applied onto 200 g strong anion-exchange resin (Lewatit Mono Plus M 600) in a glass column. The neutral and basic substances were washed out using 2×100 ml of water. The organic acids were eluted with 100 ml 0.1 *M* HCl. Afterwards, they were extracted twice with 100 ml ethyl acetate. After evaporation to dryness using a rotatory evaporator (Rotavapor R, Büchi, Konstanz, Germany) the residue was dissolved in 1.0 ml acetonitrile. An aliquot of 100 μl of the solution was mixed with 100 μl BSTFA at room temperature for total silylation of the organic acids. The solution was conditioned to room temperature for approximately 10 min before being analysed using GC–MS.

2.4.2. Chromatographic conditions

The GC–MS analysis was performed using a fused-silica capillary column HP-5MS (25 m×0.25 mm I.D.) with phenyl methyl siloxane stationary phase (thickness 0.35 μm), a gas chromatograph GC 6890 series coupled with a quadrupole mass selective detector 5973 (all from Agilent). Helium at a flow rate of 1.0 ml/min was used as the carrier gas, and 1.0 μl aliquots of samples were injected in splitless mode. After an initial time of 1 min at 50 °C, the

oven temperature was programmed to 200 °C at a rate of 5 °C/min, to 280 °C at a rate of 12 °C/min and finally to 300 °C at a rate of 15 °C/min. The injector temperature was maintained at 250 °C, the ion source temperature was set to 230 °C. The solvent delay was set to 9 min. All mass spectra were acquired in the electron impact (EI) mode at 70 eV. The scanned mass area was 50–550 u. The data were evaluated using an HP Chemstation data system. All mass spectra were compared with the database or with the mass spectra of standard substances to identify nearly all organic acids occurring in plant dry extracts of *Eschscholtzia californica* Cham. Glyceric acid was used as a standard solution which was run through the same sample cleanup.

3. Results and discussion

3.1. Analysis of carbohydrates using HPAEC–PAD

3.1.1. Validation of the method

Using the presented HPLC method, the monosaccharides glucose and fructose as well as the disaccharide sucrose were separated and quantified in plant dry extracts of *Eschscholtzia californica* Cham. and other herbal extracts mentioned earlier. A typical chromatogram is shown in Fig. 2. The calibration of the carbohydrates was performed using combined external standard solutions containing the three sugars at six concentration levels in the range of 0.25–70 mg/l with three injections each. The correlation coefficients were in the range of $R^2 = 0.9998$–0.9999. The recovery rates, determined by addition of the sugars at three concentration levels (50, 100 and 150%), were increased to 96–102% by varying the SPE cartridges and optimising the sample preparation. Thus, the analytical method has been found to have good accuracy and precision.

The hydrophobic cartridge produced from silica-based material could not be used because of the existence of free polar silanol groups, which may attract the polar carbohydrates. Therefore cartridges of a polystyrene–divinylbenzene material (Isolute 101) were used. The limits of quantitation under these conditions were set to 0.15–0.19 μg/ml depending on the different carbohydrates analysed. The detection limits were set at three times the baseline noise. The intermediate precisions of the three sugars, determined by two different co-workers using

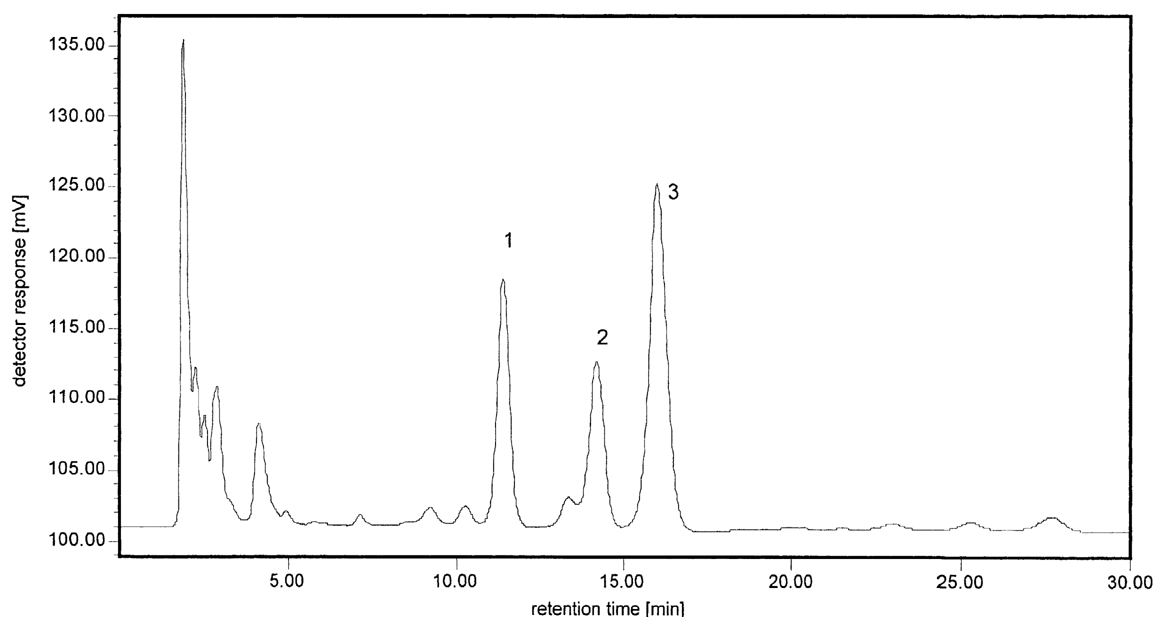

Fig. 2. Separation of 1 = glucose, 2 = fructose and 3 = sucrose from dry extract of *Eschscholtzia californica* Cham. on CarboPac PA-100 (250×4 mm) anion-exchange resin. Conditions: mobile phase 0.02 *M* sodium hydroxide; flow-rate 1.0 ml/min; detector settings as described in the text.

Table 1
Carbohydrate contents in five batches of *Eschscholtzia californica* Cham. dry extracts as determined by HPAEC–PAD

Batch	Content in % (w/w) (±standard deviation) ($n=3$)			
	Glucose	Fructose	Sucrose	Total content of carbohydrates
B1	0.2±0.01	2.0±0.03	0.5±0.08	2.7±0.09
B2	0.9±0.08	3.4±0.02	1.4±0.16	5.6±0.11
B3	1.2±0.03	3.6±0.04	3.8±0.84	8.6±0.86
B4	1.7±0.09	5.1±0.36	3.5±0.09	10.2±0.57
B5	5.3±0.03	6.2±0.04	1.7±0.04	13.3±0.08

Table 3
Contents of monosaccharides in the extracts of *Eschscholtzia californica* Cham. as determined by HPAEC–PAD after TFA hydrolysis

Batch	Content in % (w/w) (content before TFA hydrolysis)				
	Glucose	Fructose	Galactose	Rhamnose	Xylose
B1	1.5 (0.2)	2.3 (2.0)	0.3	0.9	1.1
B2	2.8 (0.9)	3.9 (3.4)	0.1	1.1	1.5
B4	4.7 (1.7)	6.2 (5.1)	0.1	1.2	2.0
B5	7.2 (5.3)	6.5 (6.2)	0.4	1.3	2.1

the same analytical instrument, varied between 2.06% for glucose and 5.24% for sucrose. By using PAD, the oxidation of the surface of the gold working electrode could be decreased. To maintain the robustness of the equipment in the determination of carbohydrates, the electrode was cleaned mechanically before routine measurements were undertaken or after long breaks of analysis.

3.1.2. Sugar analysis

The variations of the content of carbohydrates in five batches of herbal dry extracts of *Eschscholtzia californica* Cham. are shown in Table 1. The qualitative pattern is almost the same but the total content from 2.7 to 13.3% (w/w). The results of the carbohydrate contents in herbal extracts of other plants are shown in Table 2. As can be seen from the table, some batches of extracts of these plants (for instance *Vitis vinifera* or *Xysmalobium undulatum*) contain almost the same amount of sugars as *Eschscholtzia californica* Cham. However, unlike those of *Eschscholtzia californica* Cham., the extracts exhibit little or no problem during production or storage as might have been expected from their hygroscopic property. This indicates that the percentage of free carbohydrates in plant dry extracts does not fully explain the differences in their hygroscopicity. As expected, the content of sugars in the extracts of root drugs (e.g. *Valeriana officinalis* or *Panax ginseng*) is considerably high.

3.1.3. TFA hydrolysis

In order to ensure that there are no large amounts of oligo- or polysaccharides, which could be responsible for the hygroscopic behaviour of the extracts, TFA hydrolysis was carried out to break down the chains into monosaccharides. Table 3 shows the results and the total increase in glucose content is 1.3–3.0% (w/w), which is obtained from hydrolysis of sucrose and flavonoids. The increase in the content of fructose is also a result of sucrose hydrolysis. However, after a short period of time, the fructose content decreases as it is unstable at high temperatures. The monosaccharide rhamnose is released from flavonoids, particularly from the main

Table 2
Carbohydrate contents in dry extracts of different plants as determined by HPAEC–PAD

Plant dry extract (number of batches)	Range of sugar content in % (w/w) ($n=3$)			
	Glucose	Fructose	Sucrose	Total content of carbohydrates
Eschscholtzia californica (5)	0.2–5.3	2.0–6.2	0.5–3.8	2.7–13.3
Valeriana officinalis (5)	1.2–6.3	1.7–7.3	18.7–25.1	26.0–33.8
Vitis vinifera (13)	1.1–6.3	0.3–6.5	0.1–1.5	4.5–14.3
Xysmalobium undulatum (3)	1.0–1.1	1.9–2.0	11.3–11.5	14.3–14-6
Eleutherococcus senticosus (4)	1.0–7.0	4.2–5.8	0.1–2.0	6.6–12.9
Melissa officinalis (2)	5.3–5.4	3.6–3.7	0.2–0.9	9.6–9.8
Piper methysticum (6)	0.3–1.7	0.3–3.0	0.1–0.2	0.6–4.7
Panax ginseng (3)	0.3–0.8	0.9–1.1	6.6–8.5	8.0–10.0

flavonol glycoside rutin. The other two monosaccharides galactose and xylose may have originated from polysaccharides, but the total amount is not so high to influence the hygroscopicity of the extracts. Therefore, further investigations focused on the organic acids or the salts thereof.

3.2. Analysis of organic acids using ion-exclusion chromatography with ELSD

3.2.1. Validation of the method

One drawback of the evaporative light scattering detection method is the non-linear response obtained from the light-scattering detector [21]. The reasons for the sigmoidal calibration curves have been discussed by Mourney and Oppenheimer [22]. According to them, the sigmoidal response is due to the changes in the average diameters of solute particles that occur during nebulisation and desolvation. In the present study, the logarithm of the calibration curves showed correlation coefficients of 0.993 for malic acid and 0.9998 for tartaric acid. The slopes of the response curves are expected to be almost identical for all compounds because of the universal mechanism of detection. Accordingly, the slopes of the logarithmic curves of citric and tartaric acid were almost the same (1.45). The slope of the curve of malic acid, however, was 1.04. This leads to the assumption that malic acid is more volatile than the other two organic acids and thus a portion of it evaporates under the experimental conditions. The calibration of the organic acids was performed using external standard solutions in which all the three acids were present at six concentration levels in the range of 20–1550 mg/l with three injections each. The detection limit was set to three times the baseline noise. The recovery rates, determined by adding the acids at three concentration levels (50, 100 and 150%), were in the range of 96–103%. The limit of quantitation increased to 20 mg/l for citric acid and 60 mg/l for malic acid. Hence, the quantification method was found to be quite sensitive.

3.2.2. Organic acid analysis

Herbal dry extracts of *Eschscholtzia californica* Cham. contain high amounts of cations especially potassium, up to 12% (w/w) [4]. This indicates the presence of counter (negative) ions, which were not found in the group of inorganic anions. The total amount of citric and malic acid in *Eschscholtzia californica* Cham. ranges between 5.8 and 8.0% (w/w) (Table 4). Other acids could not be detected using the specified conditions (Fig. 3). In the extracts of some other plants (e.g. *Vitis vinifera*) another compound, tartaric acid was determined and quantified (Table 5). In an attempt to optimise the method, the tube temperature of the light scattering detector was adjusted to 60 °C. Further decrease in temperature led to unacceptable baseline noise because of the incomplete evaporation of the mobile phase. Thus, with a relatively high temperature, the method suffers a disadvantage as detection of some highly volatile organic acids is not possible. The results of the quantification of the organic acids could not also explain the negative hygroscopic behaviour of the extracts of *Eschscholtzia californica* Cham. Owing to the lack of detecting highly volatile substances, a further method of determination of organic acids, namely GC–MS, was employed.

3.3. Analysis of organic acids using GC–MS

Gas chromatography combined with mass spectrometry provides a suitable analytical tool for the determination of organic acids [23]. In this method, the compounds are first converted into volatile derivatives. Trimethylsilylation is a commonly used method to derivatize polyfunctional acids due to its simplicity, effectiveness, speed of reaction and the low eluting temperatures of unreacted reagent [24]. BSTFA is one reagent used to form trimethylsilyl (TMS) derivatives of acids. Because those derivatives tend to be labile, butyldimethylsilyl ethers are

Table 4
Contents of citric and malic acid in herbal dry extracts of *Eschscholtzia californica* Cham. as determined by ion-exclusion chromatography with ELSD

Batch	Content in % (w/w) (±standard deviation) ($n=3$)	
	Citric acid	Malic acid
B1	3.9±0.10	3.0±0.14
B2	3.5±0.04	2.8±0.08
B3	3.0±0.06	2.9±0.10
B4	1.8±0.04	4.1±0.10
B5	4.1±0.01	3.9±0.09

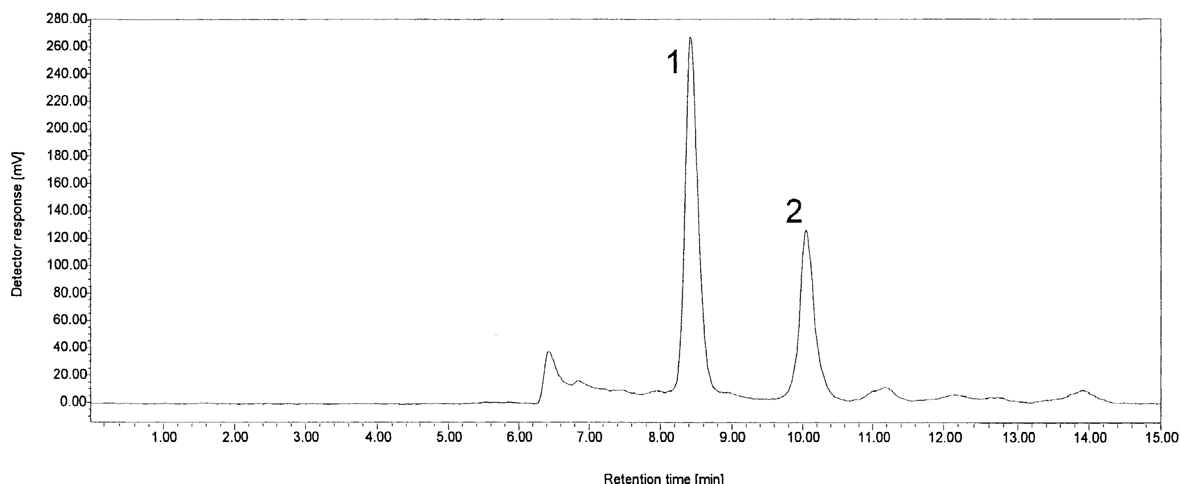

Fig. 3. Separation of 1 = citric acid and 2 = malic acid from dry extract of *Eschscholtzia californica* Cham. on Aminex HPX-87H (300×7.8 mm) cation-exchange resin. Conditions: mobile phase 0.02 *M* trifluoroacetic acid; flow-rate 0.6 ml/min; detector settings as described in the text.

more commonly used. In Fig. 4 a typical chromatogram of the silylated organic acids of batch B4 of *Eschscholtzia californica* Cham. dry extract is shown. Since the recovery rates of the hydrophilic carboxylic acids varied and decreased, estimates of the acid content were made. Table 6 shows the estimated contents of carboxylic acids in batch B4, where a high amount of glyceric acid is noticeable. In Fig. 5 a mass spectrum of trimethylsilylated glyceric acid is shown. The peak at m/z 73 is typical for all silylated substances, as it represents the trimethylsilyl rest of the silylating reagent. 2,3-dihydroxypropionic acid (glyceric acid) is present in the extracts of *Eschscholtzia californica* Cham. in the range of 5.5–12.1% (w/w). Therefore, the large amount of potassium (up to 12%, w/w) present in dry extracts of *Eschscholtzia californica* Cham. [4] could be explained by the existence of an enormous amount of potassium glycerate, which is known to be very hygroscopic. In the dry extracts of the other plants, this substance was not detectable, meaning, even if it was present the content of glyceric acid is below 0.1%. Glycerate is a well-known intermediate product either in the glycolate pathway or in the metabolic pathway involved in serine biosynthesis. This poses the question, why this substance is accumulated especially in aerial parts of *Eschscholtzia californica* Cham. Other compounds that were present in small amounts in the dry extracts include laevulic acid, 3-hydroxypropionic acid, malonic acid, glutaric acid and threonic acid. These acids are not shown in Table 6. Benzoic acid, which was also observed in the blank, seems to be a manufacturing contaminant in the anion-exchange resin. Finally, it

Table 5
Contents of organic acids in dry extracts of different plants as determined by ion-exclusion chromatography with ELSD

Plant dry extract (number of batches)	Range of organic acid content in % (w/w) ($n=3$)		
	Citric acid	Malic acid	Tartaric acid
Eschscholtzia californica (5)	1.8–4.1	2.8–4.1	n.d.
Valeriana officinalis (5)	1.2–1.7	3.0–3.6	n.d.
Vitis vinifera (13)	0.9–1.5	0.4–8.2	2.0–5.7
Xysmalobium undulatum (3)	0.8–0.9	2.0–4.0	n.d.
Eleutherococcus senticosus (4)	0.7–1.0	0.7–0.8	n.d.

n.d. = not detected

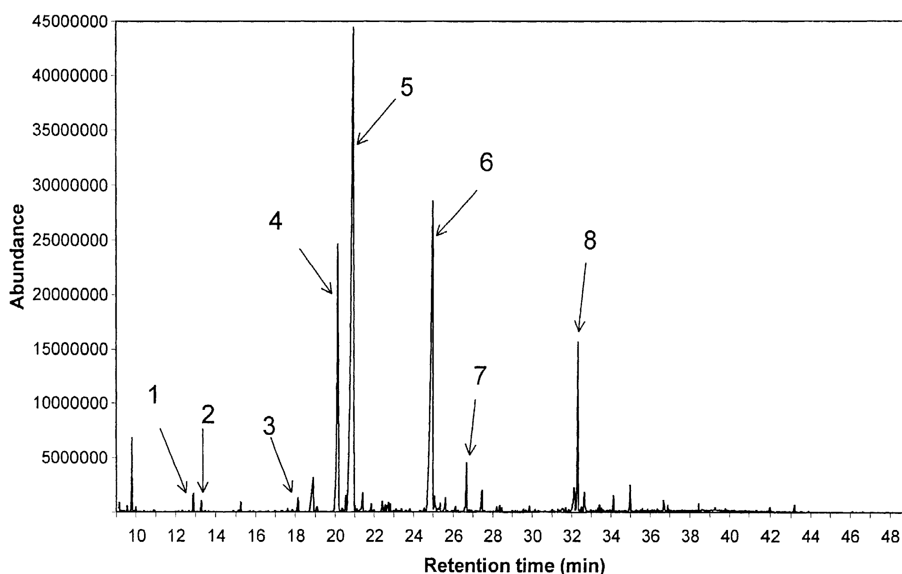

Fig. 4. Separation of 1 = lactic acid, 2 = glycolic acid, 3 = benzoic acid, 4 = succinic acid, 5 = glyceric acid, 6 = malic acid, 7 = threonic acid and 8 = citric acid as trimethylsilyl derivatives from batch B4 dry extract from *Eschscholtzia californica* Cham. on an HP-5MS (25 m × 0.25 mm I.D.) capillary column. Conditions: carrier gas: Helium, flow-rate: 1.0 ml/min, injection volume: 1 μl, splitless mode, oven and detector settings as described in the text.

is worth noting that the amounts of citric and malic acid found in herbal dry extracts from *Eschscholtzia californica* Cham. with the GC–MS method are quite comparable to those of the ion-exchange chromatography–ELSD method (Tables 5 and 6).

4. Conclusions

Using the three chromatographic methods described, various types of carbohydrates and organic acids present in herbal dry extracts of *Eschscholtzia californica* Cham. have been determined and quantified. The anions and particularly the high content of glycerate may be responsible for the hygroscopic properties and hence poor processing behaviour of the extracts as in roller compaction and tableting. The elimination of the anions from the extracts decreased hygroscopicity as reported elsewhere [2]. Extracts from other plants contained no glycerate and film coated tablets produced of these did not show

Table 6
Estimated content of carboxylic acids in batch B4 of *Eschscholtzia californica* Cham. dry extract and the corresponding retention times as determined by GC–MS

	Retention time (min)	Representative masses in the mass spectra	Estimated content of organic acids in % (w/w) Estimated content in %	Standard deviation (SD)
Lactic acid	12.9	117, 133, 191	0.06	0.03
Glycolic acid	13.3	133, 177, 205	0.03	0.01
Benzoic acid	18.1	105, 135, 179, 194	0.28	0.08
Succinic acid	20.2	75, 129, 247	1.23	0.23
Glyceric acid	20.9	103, 133, 189, 292, 307	12.1	1.36
Malic acid	24.9	189, 233, 245, 265, 335	4.94	1.21
Tartaric acid	28.5	189, 292, 305, 333, 423	0.09	0.04
Citric acid	32.3	273, 347, 363, 375, 465	3.19	0.86

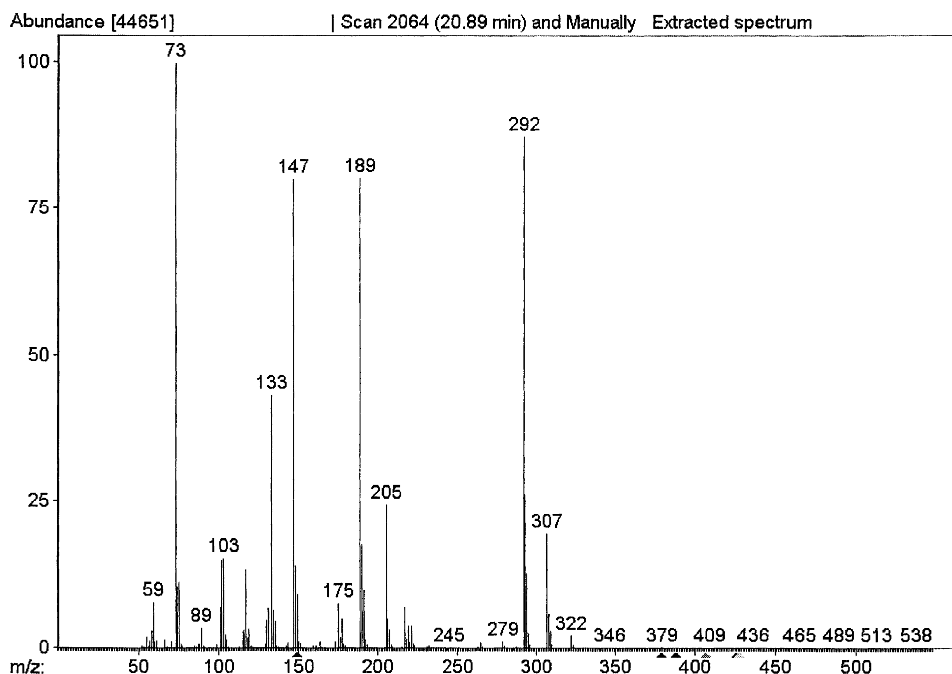

Fig. 5. Mass spectrum of trimethylsilylated glyceric acid.

any cracking tendency as it was observed with dry extracts from *Eschscholtzia californica* Cham.

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References

[1] M. Eder, W. Mehnert, Pharmazie 53 (1998) 285.
[2] M. Schiller, H. von der Heydt, F. März, P.C. Schmidt, S.T.P. Pharma Sci. (2002) in press.
[3] A. Rolland, J. Fleurentin, M.-C. Lanhers, C. Younos, R. Misslin, F. Mortier, J.M. Pelt, Planta Med. 57 (1991) 212.
[4] H. von der Heydt, Ph.D. Thesis, Johannes Gutenberg-University, Mainz, 1994.
[5] M.-A. Beck, Ph.D. Thesis, Philipps-University, Marburg, 1999.
[6] L. Jain, M. Tripathi, V.B. Pandey, G. Rücker, Phytochemistry 41 (1996) 661.
[7] M.-A. Beck, H. Häberlein, Planta Med. 65 (1999) 296.
[8] L. Jain, M. Tripathi, V.B. Pandey, Planta Med. 62 (1996) 188.
[9] A. Rolland, Ph.D. Thesis, University of Metz, Meltz, 1988.
[10] S. von Eggelkraut-Gottanka, S. Abu Abed, W. Müller, P.C. Schmidt, Phytochem. Anal., 13 (2002) 170.
[11] D.A. Martens, W.T. Frankenberger Jr., Chromatographia 29 (1990) 7.
[12] Y.C. Lee, J. Chromatogr. A 720 (1996) 137.
[13] D.C. Johnson, D. Dobberpuhl Rober, R. Roberts, P. Vandeberg, J. Chromatogr. 640 (1993) 79.
[14] T.A. Iuliano, J. AOAC Int. 79 (1996) 1381.
[15] H.J. Blom, H.C. Andersson, D.M. Krasnewich, W.A. Gahl, J. Chromatogr. 533 (1990) 11.
[16] G.A. De Ruiter, H.A. Schols, A.G.J. Voragen, F.M. Rombouts, Anal. Biochem. 207 (1992) 176.
[17] R. Schwarzenbach, J. Chromatogr. 251 (1982) 339.
[18] E. Rajakylä, J. Chromatogr. 218 (1981) 695.
[19] A. Stolyhwo, H. Colin, G. Guiochon, J. Chromatogr. 265 (1983) 1.

[20] Y. Mardens, A. Kumps, C. Planchon, C. Wurth, J. Chromatogr. 577 (1992) 341.
[21] H. Bünger, L. Kaufner, U. Pison, J. Chromatogr. A 870 (2000) 363.
[22] T.H. Mourney, L.E. Oppenheimer, Anal. Chem. 56 (1984) 2427.
[23] E. Jellum, J. Chromatogr. 143 (1977) 427.
[24] R.P. Evershed, in: K. Blau, J. Halket (Eds.), Handbook of Derivatives For Chromatography, Wiley, New York, 1993, p. 51, Chapter 4.