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Journal of Chromatography A, 902 (2000) 369–379

JOURNAL OF CHROMATOGRAPHY A

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Negative atmospheric pressure chemical ionisation low-energy collision activation mass spectrometry for the characterisation of flavonoids in extracts of fresh herbs

Ulla Justesen*

Institute of Food Research and Nutrition, Division of Nutrition, Danish Veterinary and Food Administration, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

Received 7 June 2000; received in revised form 14 August 2000; accepted 14 August 2000

Abstract

The flavonoid composition of commonly eaten fresh herbs such as dill, oregano and parsley was analysed by combined LC, MS and low-energy collision induced dissociation (CID) MS–MS. Negative atmospheric pressure chemical ionisation (APCI) MS and MS–MS were used to provide molecular mass information and product-ion spectra of the glycosyl compounds. The most prominent fragment was found to arise from the aglycone ion, which provides molecular mass information about the glycosyl substituent and the aglycone. Product-ion spectra of the aglycone verified the identity by comparison with product-ion spectra of authentic standards. Methoxylated flavonoids provide characteristic fragmentation, i.e., loss of $\cdot CH_3$, which add to the usefulness of the method for identifying unknown flavonoids. Negative-mode APCI-MS is thus demonstrated to be a good alternative to the commonly employed positive mode operation.

Keywords: Mass spectrometry; Flavonoids

1. Introduction

Natural antioxidants, for example phenolic compounds, constitute a major class of secondary plant metabolites. The phenolic compounds include phenolic acids and flavonoids, and particularly the latter is a highly diverse subgroup. The phenolic acids and flavonoids are mostly present as conjugates in fruits, vegetables and other plant products consumed in a normal diet. We have previously screened fruits, vegetables and beverages for the contents of flavanones, flavones and flavonols, and quantified seven major flavonoids as aglycones after acid hydrolysis [1]. The presence of flavonoids and other phenolic antioxidants as rosmarinic acid have been reported in herbs, e.g., basil, dill, oregano, parsley, rosemary, sage, spearmint and thyme [2–21]. The majority of flavones, flavonols, and flavanones were reported present as glucuronyl or glycosyl conjugates. Mass spectrometric (MS) methods such as electron impact ionisation (EI)-MS [10,22–24], fast atom bombardment (FAB) MS [6–8,11], atmospheric pressure chemical ionisation (APCI) MS [1,3,25–27], electrospray ionisation (ESI) MS [28–30] and particularly liquid-inlet ionisation methods combined with low-energy product-ion scan [22,28,30,31] have proved useful to determine flavo-

*Tel.: +45-3395-6000; fax: +45-3395-6001.
E-mail address: ulj@fdir.dk (U. Justesen).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00861-X

noids in herbs and other foods. The various methods have, with few exceptions [27,31], been applied in the positive-ion mode solely. We find, however, that negative atmospheric pressure ionisation (API) (comprising both APCI and ESI) is excellent for flavonoid analysis, both regarding sensitivity and specific structural information.

In the present study, LC–MS and MS–MS, using negative APCI, were applied for molecular mass and structural information of flavonoid glycosides in herb extracts. Negative APCI was selected in the present study, as it easily provided aglycone fragments by in-source fragmentation of glycosides. These fragments were selected for further characterisation by low-energy collision induced dissociation (CID) MS–MS. Comparison of the aglycone product-ion spectra with the spectra of authentic standards, provided valid identification of the aglycones, present as glycosyl conjugates in herb extracts.

2. Experimental

2.1. Chemicals

Flavonoid standards, acacetin (5,7-dihydroxy-4′-methoxyflavone, M_r 284), apigenin (4′,5,7-trihydroxyflavone, M_r 270), eriodictyol (3′,4′,5,7-tetrahydroxyflavanone, M_r 288) hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone, M_r 302), isorhamnetin (3,4′,5,7-tetrahydroxy-3′-methoxyflavone, M_r 316), kaempferol (3,4′,5,7-tetrahydroxyflavone, M_r 286), luteolin (3′,4′,5,7-tetrahydroxyflavone, M_r 286), morin (2′,4′,3,5,7-pentahydroxyflavonol, M_r 302), naringenin (4′,5,7-trihydroxyflavanone, M_r 272), quercetin (3,3′,4′,5,7-pentahydroxyflavone, M_r 302) and rhamnetin (3,3′,4′,5-tetrahydroxy-7-methoxyflavonol, M_r 316) were purchased from Apin (Oxon, UK) and Sigma (St. Louis, MO, USA). The standards were dissolved in dimethyl sulfoxide (DMSO) to a concentration of approximately 0.1 g/l and kept protected from light at −20°C for up to 3 months.

Working solutions were made up each day by diluting 0.500 ml standard stock solution with 4 ml methanol. Solvents were supplied by Rathburn (Walkerburn, UK).

2.2. Apparatus

The high-performance liquid chromatography (HPLC) system consisted of a Waters system (Milford, MA, USA) 717 autoinjector, 616 pump, and 996 photodiode array (DAD) detection system. UV spectra were recorded from 220 to 450 nm.

Single-stage MS was performed on a VG Platform II single quadrupole (Micromass, Chesire, UK) with an API source using the APCI inlet. Mass spectra were acquired in the negative ion mode, providing deprotonated molecular ions. The instrument was set to scan from 120 to 1000 mass units. The probe and source parameters were: source temperature 80°C, probe temperature 450°C, cone voltage 30 V, and corona discharge 2.7 kV.

Triple-stage MS was performed on a Quattro LC triple quadrupole instrument (Micromass) with a Z-spray API source using the APCI inlet. The parameters were as follows: corona current, cone 30–40 V, source temperature 120°C, probe temperature 400°C, cone gas N_2 120 l/h, desolvation gas N_2 600 l/h. Product-ion scans were obtained, selecting the deprotonated molecular ions for low-energy collisions. Product-ion spectra were recorded in the range 120–1000 u. Furthermore, product-ion scans were recorded of the aglycone fragment ions, obtained by in-source fragmentation at cone 40 V.

The mass spectrometer was connected to the UV cell outlet of the HPLC, using polyether ether ketone (PEEK) tubing.

2.3. Sample preparation

The following fresh herbs were purchased at a local grocery store, freeze–dried and kept at −20°C until use: chives, cress, dill, lovage, mint, oregano, parsley, rosemary, tarragon and thyme.

Herb extracts were produced for determination of flavonoid composition: 0.5 g freeze–dried material was pulverised in a mortar and extracted with 20 ml 62.5% aqueous methanol. After sedimentation, a 2-ml aliquot was filtered before 50 μl injections into the HPLC system for mass spectrometric determination.

2.4. Analytical procedure

Herb extracts were analysed using a Phenomenex

Prodigy (Torrance, CA, USA) RP C_{18} column (250×4.6 mm, 5 μm), with a mobile phase consisting of 30% methanol in water with 1% formic acid (A), and 100% methanol (B). The gradient was 25–40% B in 10 min, 40–43% B in 14 min followed by 43–84% B in 6 min at a flow-rate of 1 ml/min. UV spectra were recorded from 220 to 450 nm.

3. Results and discussion

Negative APCI mass spectra expressed deprotonated molecular ions of the flavonoid glycosides present in the herb extracts. The molecular ions were selected for CID-MS–MS and product ion spectra provided the aglycone as prominent fragment, usually found to represent the base peak. The mass difference between the deprotonated molecular ion and the aglycone fragment determined the molecular mass of the glycosyl or glucuronyl substituent, as the substituent itself does not produce significant ions in negative APCI. In-source fragmentation of the glycosyl compounds provided the aglycones for CID-MS–MS analysis, where the identity of the aglycones was determined by comparison with product-ion spectra of commercial standards (Table 1). The individual compound was thus identified by product ion spectra of the glycoside and of the respective aglycone (Table 2).

Product-ion spectra of commercial standards provided information on specific A- and B-ring fragmentation patterns, shown in Table 1. The A-ring fragments m/z 151 and m/z 107 were produced for all flavone, flavonol and flavanone standards included (exemplified by isorhamnetin in Fig. 1A), except for rhamnetin which has a methoxyl substituent in the 7-position and produce A-ring fragments m/z 165 and m/z 121, and kaempferol, which does not produce significant fragmentation. B-ring fragments were observed for the flavones apigenin (m/z 117) and luteolin (m/z 133), and the flavanones eriodictyol (m/z 135) and naringenin (m/z 119). The flavone acacetin and flavanone hesperetin are B-ring methoxylated and did not provide B-ring fragments.

The methoxylated flavonoids acacetin, isorhamnetin, rhamnetin, and hesperetin provided significant $[M-H-15]\cdot^-$ fragments with a characteristic even m/z value, corresponding to loss of $\cdot CH_3$ from the deprotonated molecular ion (see Table 1). The fragment represent base peak in the product-ion spectra of the B-ring methoxylated flavonoid standards, exemplified by isorhamnetin-spectra in Fig. 1A (collision energy 30 V). In the product-ion spectra of A-ring methoxylated rhamnetin, the base

Table 1
Product-ions of flavone, flavonol and flavanone standard compounds[a]

Flavonoid	OH	OCH_3	$[M-H]^-$	Fragment			Other fragments	CID (eV)
				a	a*	b		
Flavones								
Apigenin	4′,5,7		269 (85)	151 (70)	107 (30)	117 (100)	225 (12), 201 (10), 181 (10), 159 (10), 149 (55)	30
Luteolin	3′,4′,5,7		285 (7)	151 (20)	107 (20)	133 (100)	175 (10), 149 (12)	30
Acacetin	5,7	4′	283 (–)	151 (10)	107 (8)		**268** (100), 240 (10)	30
Flavonols								
Isorhamnetin	3,4′,5,7,	3′	315 (10)	151 (25)	107 (10)		**300** (100), 271 (20), 254 (8)	30
Kaempferol	3,4′,5,7		285 (100)	–	–			30
Morin	2′,3, 4′,5,7		301 ()	151 (100)	107 (8)		163 (20), 125 (50)	20
Quercetin	3,3′,4′,5,7		301 (65)	151 (100)	107 (12)		179 (40), 121 (20)	30
Rhamnetin	3, 3′,4′,5	7	315 (–)	165 (100)	121 (40)		**300** (20), 271 (8)	30
Flavanones								
Eriodictyol	3′,4′,5,7		287 (5)	151 (90)	107 (15)	135 (100)	125 (5)	20
Hesperetin	3′,5,7	4′	301 (60)	151 (30)	(107)		**286** (50), 242 (50), 201/199 (30), 164 (100)	20
Naringenin	4′,5,7		271 (15)	151 (100)	107 (30)	119 (90)	177 (5), 165 (4)	20

[a] The deprotonated molecular ions, produced by negative APCI, was selected for low-energy collision activation at 20–30 eV. A-ring fragments are denoted a and a*, and B-ring fragments b. Other significant fragments are also listed, with relative abundance in parentheses. The specific fragments corresponding to loss of 15 are listed in bold.

Table 2
m/z values of $[M-H]^-$ ions, produced by LC–MS of flavonoid glycosides in herb extracts, product-ions (CID-MS–MS) of $[M-H]^-$ and of the aglycones A^- produced by in-source fragmentation of the flavonoid glycosides

Herb	t_R (min)	M_r	Product ions of $[M-H]^-$	Product ions of A^-	Tentative name
Chives	3.1	463	301	ND	Quercetin-glucoside [14,15]
(*Allium schoenoprasum*)	3.5	477	315	300, 271, 151,107	Isorhamnetin-glucoside
	3.6	447	285	ND	Kaempferol-glucoside [14,15]
Cress	3.2	447?[a]	301	179, 151, 121, 107	Quercetin-glycoside
(*Lepidium sativum*)	3.6	431?[a]	285	–	Kaempferol-glycoside
	3.5	461?[a]	315	300, (152, 108)	Isorhamnetin-glycoside
Dill	5.3	609	301	ND	Quercetin-rhamnoglucoside
(*Anethum graveolens*)	9.4	477	301, 255, 179, 151	179, 151, 121, 107	Quercetin-glucuronide [10]
	12.1	461	285	–	Kaempferol-glucuronide [10]
	12.3	491	315, 300	300, 271, 164, 151, 136, 107	Isorhamnetin-glucuronide [10]
Lovage	5.3	609	301	179, 151, 121, 107	Quercetin-rhamnoglucoside
(*Levisticum officinale*)	6.1	593	285	(175), 151, 133, 107	Luteolin-rhamnoglucoside
Mint	6.1	593	285	175, 151, 133, 107	Luteolin-7-rhamnoglucoside [21]
(*Mentha* var.)	6.2	579	271	ND	Naringenin-rhamnoglucoside
	6.9	609	301	286, 242, 164, 151, 125	Hesperetin-rhamnoglucoside [21]
	7.9	577	269	151, 149, 117, 107	Apigenin-rhamnoglucoside
	8.9	607	299	284, 256, 177, 151, 121	Diosmetin-rhamnosylglucoside (diosmin) [19]
					Rosmarinic acid [20]
	9.1	359	197, 179, 161		Acacetin-acetyl-glucoside-rhamnoglycoside
	14.1	795	283	268, 240, (151)	Acacetin-rhamnoglycoside
	15.0	591	283	268, 240, 151, 107	
Oregano	5.8	799	285	199, 175, 151, 133	Luteolin-glycoside [18]
(*Origanum vulgare*)	8.8	359		197, 179, 161	Rosmarinic acid
	9.2	635	269	ND	Apigenin-acetyl-diglucoside [18]
	15.2	635	593, 299	284, 137	Diosmetin-acetyl-apiosylglucoside [18]
Parsley	8.5	563	269	151, 149, 117, 107	Apigenin-7-apiosylglucoside [19]
(*Petroselinum crispum*)	8.6	593	299	284, 256, (107)	Diosmetin-apiosylglucoside
	10.1	605	563, 269	151, 149, 117, 107	Apigenin-acetyl-apiosylglucoside
	10.1	635	593, 299	284, 256, (107)	Diosmetin-acetyl-apiosylglucoside
Rosemary	8.8	359	197, 179, 161		Rosmarinic acid
(*Rosmarinus officinalis*)	9.2	503	285		Luteolin-acetyl-glucuronide [8]
Tarragon	8.5	607	299	284, (137)	Diosmetin-rhamnosylglucoside
(*Artemisia dranunculus*)					
Thyme	5.9	463	287	151, 135, 107	Eriodictyol-glucuronide
(*Thymus vulgaris*)	6.8	447	285	175, 151, 149, 133, 107	Luteolin-glucoside
	7.7	461	285	175, 151, 149, 133, 107	Luteolin-glucuronide
	8.8	359	197, 179, 161		Rosmarinic acid
	10.0	489	285	175, 151, (149), 133, 107	Luteolin-acetyl-glucoside
	10.0	445	269	151, 149, 117, 107	Apigenin-glucuronide
	12.5	609	285		Luteolin-diglucoside

[a] The molecular mass of the parent glycosides could not be significantly determined. The difference in molecular mass of 146 of the glycosides and the aglycones suggest deoxyhexose conjugates. The short retention time, however, indicates more than one glycosyl conjugate.

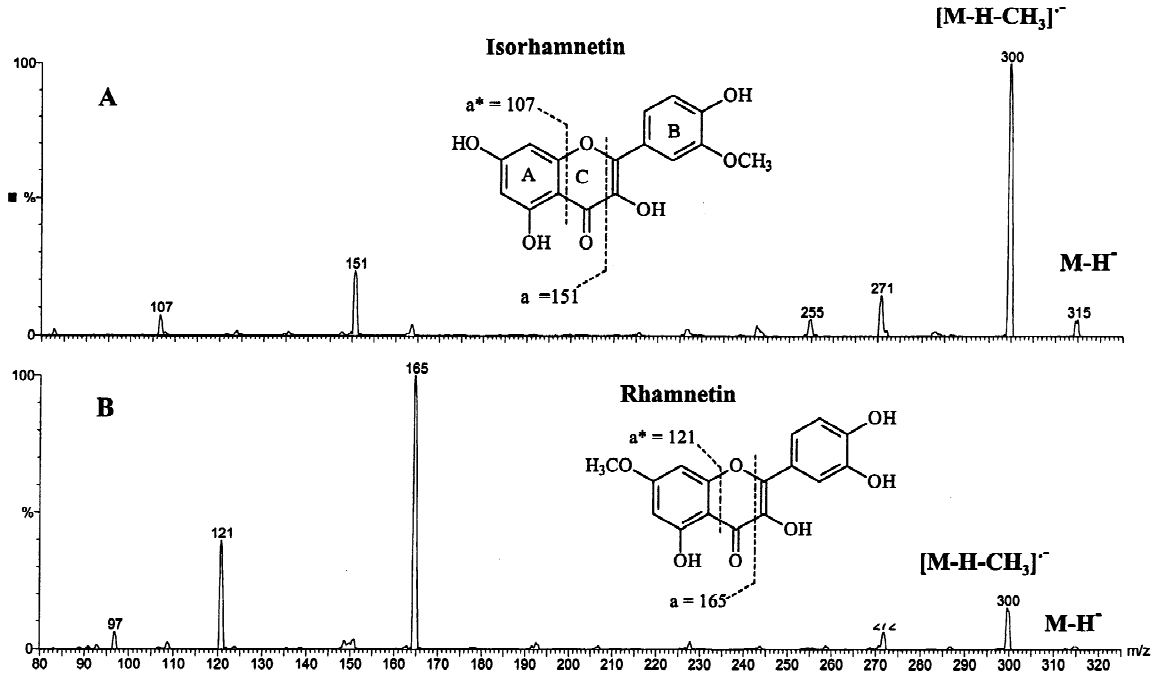

Fig. 1. $[M-H]^-$ product-ion (parent ion m/z 315) spectra at 30 eV of isorhamnetin (A) and rhamnetin (B). Specific A-ring fragments (denoted a and a*) are indicated in the figure.

peak is observed to be A-ring fragment m/z 165 (see Fig. 1B) and not $[M-H-15]\cdot^-$, thus exhibiting different fragmentation pattern from the isomer isorhamnetin at similar CID voltage setting.

Nielsen and Møller [23] first described M−15 fragments corresponding to loss of $\cdot CH_3$ in the electron impact spectra of 6- and 8-methoxy flavonoids. Also, Berahia et al. [24] and Elgamel et al. [32] observed loss of $\cdot CH_3$ radicals from methoxylated flavonoid compounds in low-energy positive ion mass spectra recorded on an electron attachment mass spectrograph. Huck et al. [30] described loss of 15 from the protonated molecular ions in the positive ESI spectra of methoxylated flavones in the flowers of *Primula veris*, but they did not discuss the possibility of the fragmentation being due to loss of a methoxyl radical. Loss of $\cdot CH_3$ has not previously been addressed in negative ion mass spectra obtained by atmospheric pressure ionisation methods. The observation of radical loss from even-electron molecular ions is rather uncommon, and must be closely related to the methoxylated flavonoid structure. The specific fragmentation pattern was used here to identify methoxylated flavonoid constituents in the herb extracts, as described below.

3.1. LC–MS and LC–MS–MS of herb extracts

3.1.1. Chives (Allium schoenoprasum)

Quercetin and kaempferol have previously been reported as constituents of chives [13,14]. The authors do not, however, specify the character of glycosyl constituents. In the present study, glucosides of isorhamnetin, kaempferol and quercetin were found as constituents of chives (Table 2).

3.1.2. Cress (Lepidium sativum)

LC–MS of methanolic cress extract revealed the presence of glycosides of kaempferol and isorhamnetin. Mass spectra of the glycosides provided deprotonated molecular ions at 431 and 461 u, respectively, with significant fragments at m/z 285 and m/z 315. Product-ion spectra of the aglycones corresponded with product ion spectra of kaempferol and isorhamnetin standards. The observed differences in molecular masses of the compounds and the

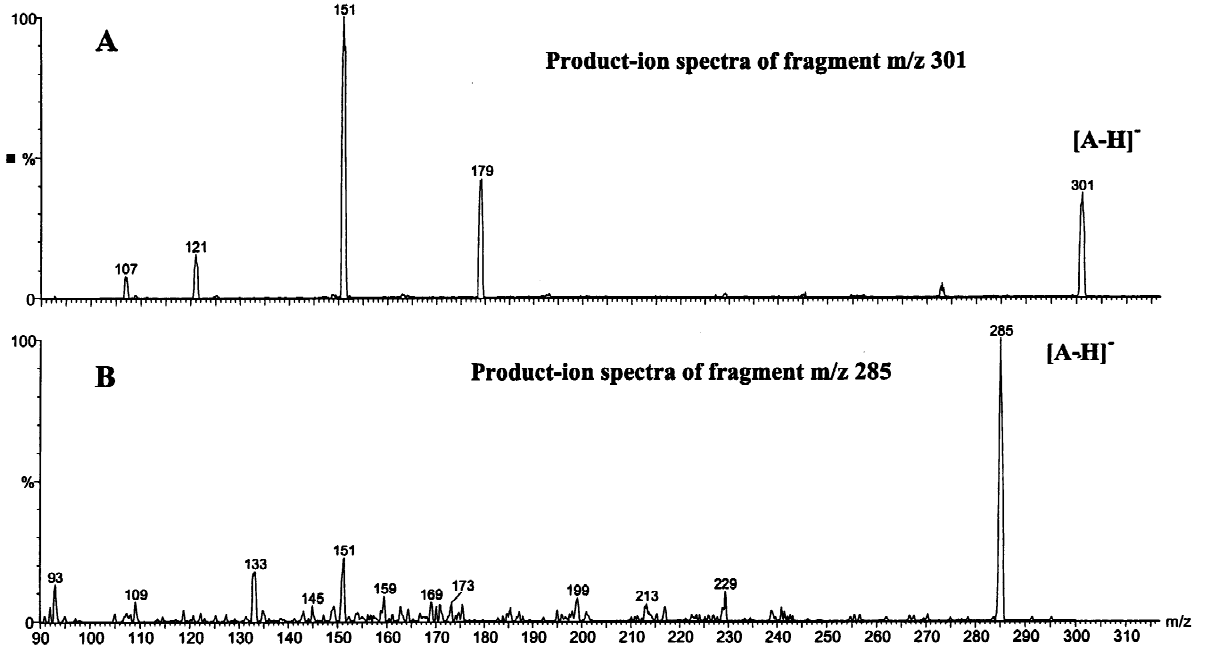

Fig. 2. A^- product-ion (parent ion m/z 301 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (A) and $[A-H]^-$ product-ion (parent ion m/z 285 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (B), determined in lovage extracts.

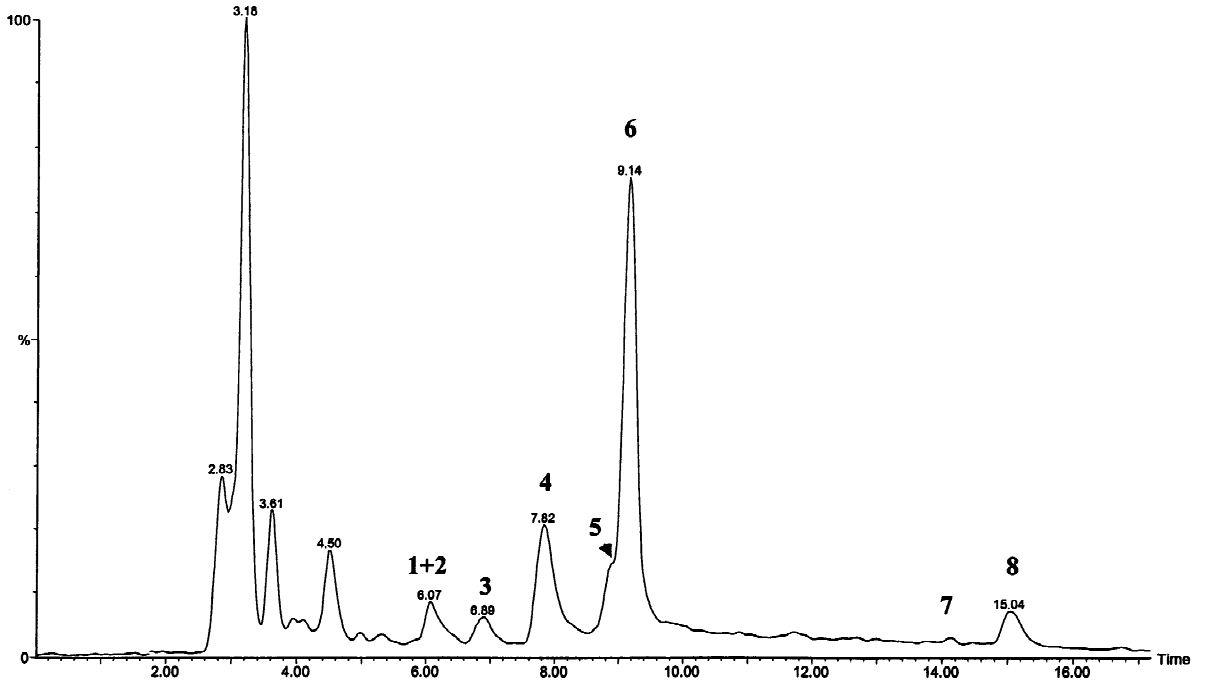

Fig. 3. HPLC–MS total ion chromatogram of mint extracts. Flavonoid components Nos. 1–8 were identified: 1=m/z 593 luteolin-7-rhamnoglucoside, 2=m/z 579 naringenin-7-rhamnoglucoside, 3=m/z 609 hesperetin-7-rhamnoglucoside, 4=m/z 577 apigenin-7-rhamnoglucoside, 5=m/z 607 diosmetin-3-rhamnoglucoside, 6=m/z 359 rosmarinic acid, 7=m/z 795 acacetin-acetyl-glucosyl-rhamnoglucoside, 8=m/z 591 acacetin-rhamnoglucoside.

fragments, suggested the aglycones xylosyl or arabinosyl substituents. No literature references to flavonoid components in cress were found.

3.1.3. Dill (Anethum graveolens)

In dill extracts, isorhamnetin, kaempferol and quercetin were detected as glucuronyl conjugates. Product-ion spectra of the aglycones (m/z 315, 285 and 301) did correspond with spectra of standards (Table 2). Quercetin-rhamnoglucoside (m/z 609) was also detected as a minor components of dill extracts. The results are in agreement with Teuber and Herrmann [9], who report the presence of quercetin and isorhamnetin 3-glucuronides in dill leaves, and kaempferol-3-glucuronide in dill fruits. They also found 3-rhamnoglucosides, 3-glucosides, 3-galactosides, of quercetin and isorhamnetin as minor components of dill leaves.

3.1.4. Lovage (Levisticum officinale)

In leaves of lovage, two flavonoid glycosides were detected. The two components are rhamnoglucosides of luteolin and quercetin, see Fig. 2 for product-ion spectra. Chlorogenic acid (3-caffeoylquinic acid, m/z 353, fragments m/z 191 and m/z 179 in negative ion mode) was also detected. A literature search did not reveal any reference to flavonoids or phenols in lovage.

3.1.5. Mint (Mentha var.)

LC–MS and CID-MS–MS analysis revealed a number of flavonoid constituents in mint extract, see Fig. 3 for total ion chromatogram of mint extract. Product-ion spectra of the component confirmed the presence of hesperetin, luteolin and apigenin (presented in Fig. 4A–C) and naringenin. In addition, two methoxylated flavonoids were detected, m/z 283 present as two glycosyl conjugates and the second m/z 299 present as rhamnoglucoside. The methoxylated aglycones exhibited similar CID fragmentation pattern, i.e., loss of $\cdot CH_3$, as described above for methoxylated flavonoid standards. The product-ion spectra of the aglycone m/z 283 (Fig. 5A) correspond with the product-ion spectra of acacetin (Fig.

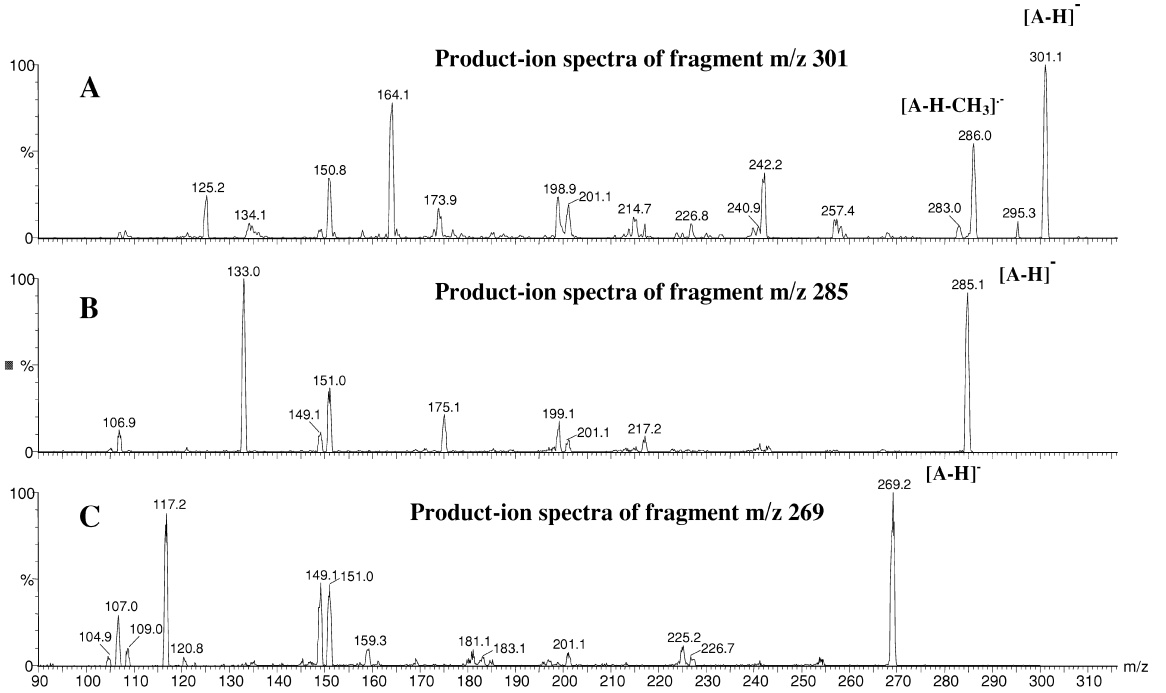

Fig. 4. $[A-H]^-$ product-ion (parent ion m/z 301 produced by in-source fragmentation of $[M-H]^-$) spectra at 20 eV (A), A^- product-ion (parent ion m/z 285 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (B), and $[A-H]^-$ product-ion (parent ion m/z 269 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (C), determined in mint extract.

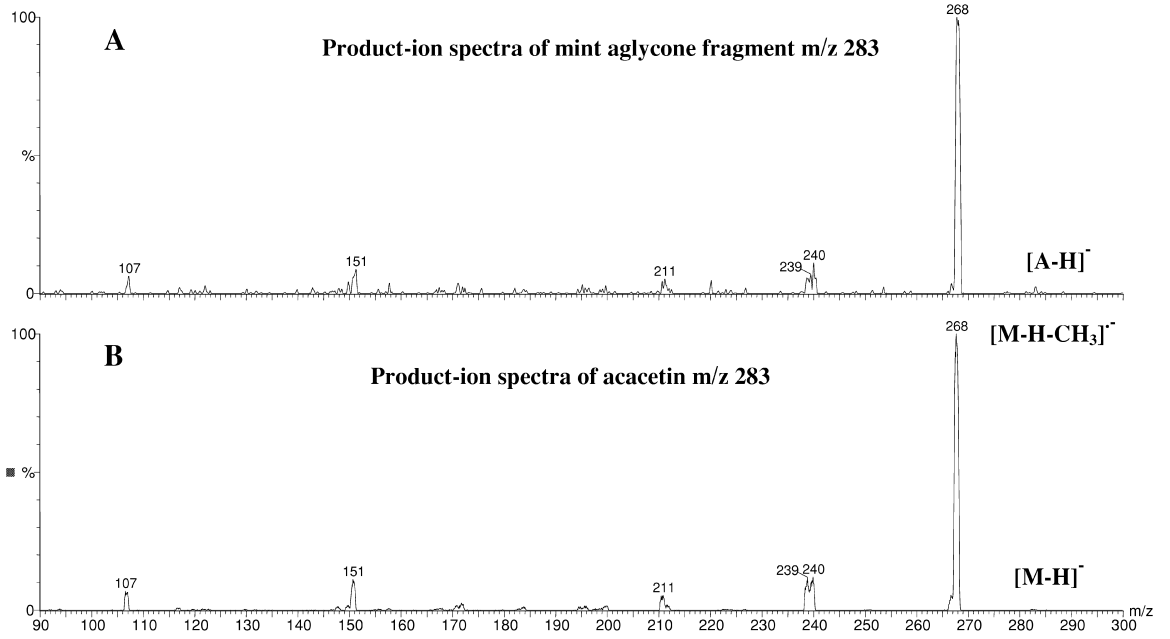

Fig. 5. $[A-H]^-$ product-ion (parent ion m/z 283 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV determined in mint extract (A) and $[M-H]^-$ product-ion (parent ion m/z 283) spectra at 30 eV of acacetin standard (B).

5B), and it is therefore suggested that the dihydroxy-methoxyflavone present in mint extract is acacetin (5,7-dihydroxy-4′-methoxyflavone) and not 5,7-dihydroxy-6-methoxyflavone or 5,6-dihydroxy-7-methoxyflavone, two A-ring methoxylated flavones reported present in *Mentha spicata* by Zaidi et al. [20]. The second methoxylated flavonoid m/z 299 is likely to be diosmetin (3′,5,7-trihydroxy-4′-methoxy-flavone, M_r 300). Product-ion spectra of the glycoside compound m/z 607 and the aglycone fragment m/z 299, are shown in Fig. 6A and B. The compound is suggested to be diosmetin-3-rhamnoglucoside (diosmin) previously reported as constituent of *Mentha spicata* by Tomas et al. [17].

3.1.6. Oregano (Origanum vulgare)

The observations of apigenin, luteolin, and a methoxylated flavonoid aglycone m/z 299, which is suggested to be diosmetin, determined as acetylated glycosyl constituents of oregano extracts, are partly in agreement with El-Ansari et al. [16]. They report the presence of apigenin, luteolin, acacetin, diosmetin and herbacetin (3,4′,5,7,8-pentahydroxy-flavone, M_r 302) as glycosides in fresh material of oregano leaves and stem. El-Ansari et al. [16] do not specify the glycosyl substituents. No traces of acacetin or herbacetin were found in the present study.

3.1.7. Parsley (Petroselinum crispum)

Aapigenin-7-apiosylglucoside, apiin (M_r 564), which is a known component of parsley [17], was detected in parsley extract. A second apigenin-glycoside corresponding to an acetylated apiosylglucoside with deprotonated molecular ion m/z 605 and aglycone fragment m/z 269, was observed (Fig. 7A). In addition, a methoxylated flavonoid was detected in two or possibly three glycosidic forms, see Fig. 7B for ion chromatogram of m/z 299 and Fig. 8A for product-ion spectra of the compound m/z 593. The methoxylated flavonoid aglycone m/z 299 exhibit similar product-ion spectra (Fig. 8B) as the methoxylated flavone diosmetin, observed in mint extracts, most prominent being the significant loss of 15 which corresponds to $\cdot CH_3$. The methoxylated flavone detected in parsley extract is therefore sug-

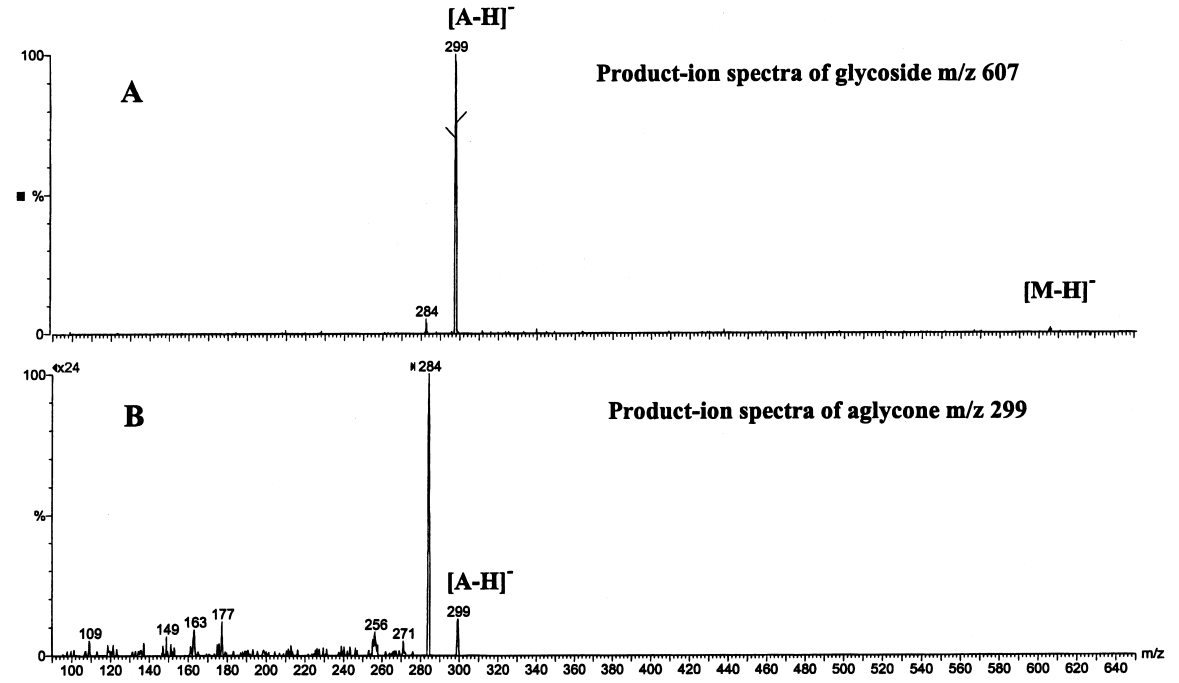

Fig. 6. $[M-H]^-$ product-ion (parent ion m/z 607) spectra at 30 eV (A) and $[A-H]^-$ product-ion (parent ion m/z 299 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (B), determined in mint extracts.

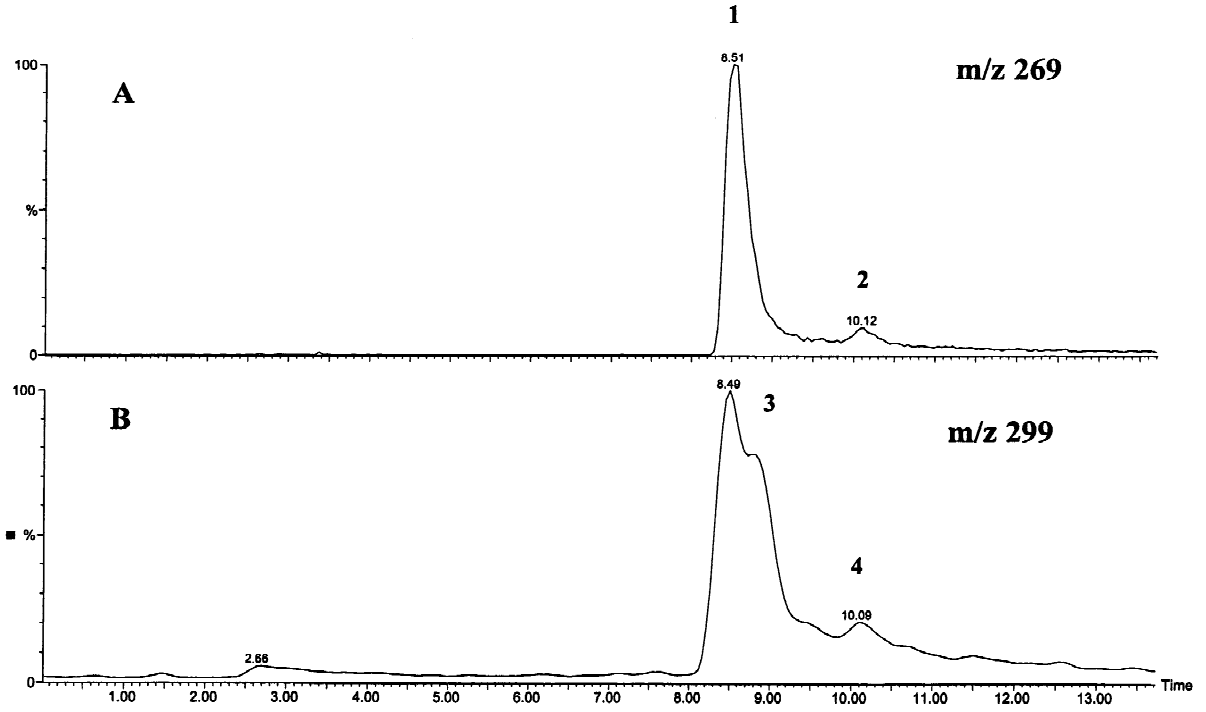

Fig. 7. HPLC–MS ion chromatograms of m/z 269 (A) and m/z 299 (B) in parsley extracts. Flavonoid components Nos. 1–4 were identified: 1=m/z 563 apigenin-7-apiosylglucoside, 2=m/z 605 apigenin-acetyl-apiosylglucoside, 3=m/z 593 diosmetin-apiosylglucoside, 4=m/z 635 diosmetin-acetyl-apiosylglucoside.

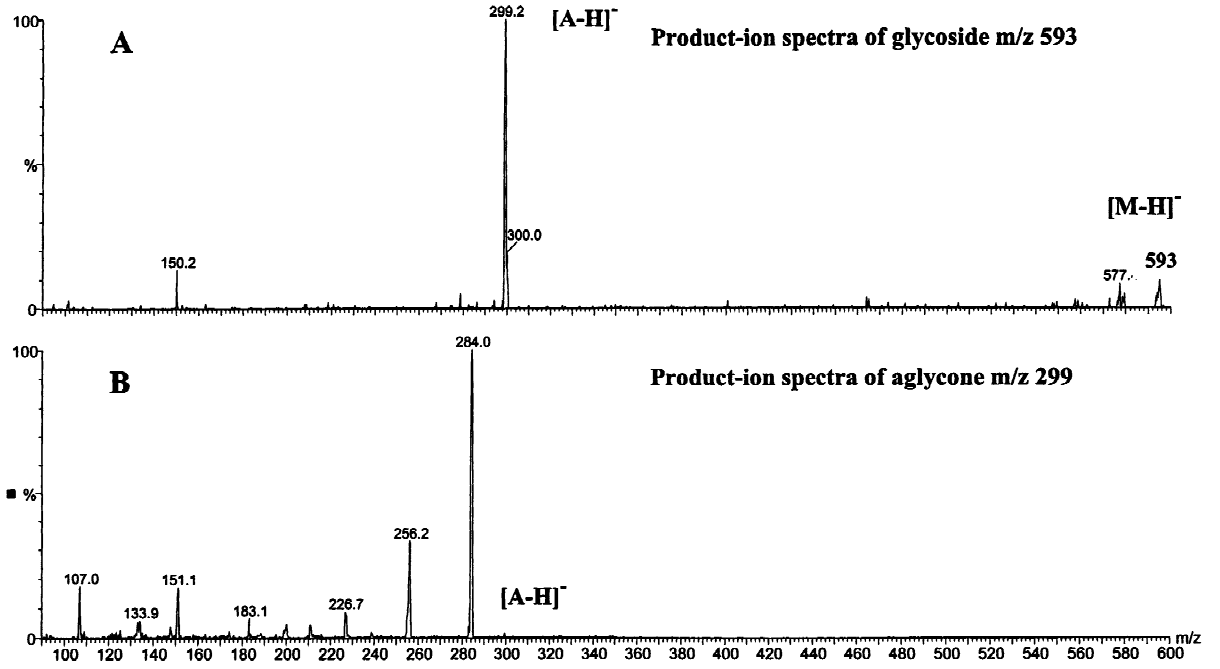

Fig. 8. $[M-H]^-$ product-ion (parent ion m/z 593) spectra at 30 eV (A) and A^- product-ion (parent ion m/z 299 produced by in-source fragmentation of $[M-H]^-$) spectra at 30 eV (B), determined in mint extracts.

gested to be diosmetin. No reference reporting diosmetin in parsley has been found.

3.1.8. Rosemary (Rosmarinus officinalis)

In the present study, a luteolin conjugate, rosmarinic acid, and a number of diterpenes were detected in rosemary extracts. The negative APCI spectra of the luteolin conjugate revealed a deprotonated molecular ion at m/z 503 and fragment m/z 285. The molecular mass of the substituent is 218, suggesting that the compound is a luteolin acetyl-glucuronide.

The composition of rosemary has been extensively described by Cuvelier et al. [3], dividing the 22 identified phenolic compounds in three classes: phenolic acids, diterpenes and flavonoids. Three luteolin glucuronides [luteolin 3′-*O*-β-D-glucuronide, luteolin 3′-*O*-(4″-*O*-acetyl)-β-D-glucuronide, and luteolin 3′-*O*-(4″-*O*-acetyl)-β-D-glucuronide′-*O*-(3″-*O*-acetyl)-β-D-glucuronide], not described by Cuvelier et al. [3], have been reported by Okamura et al. [7]. The luteolin acetyl-glucuronide observed in the present study is therefore suggested to be luteolin 3′-*O*-(3″ or 4″-*O*-acetyl)-β-D-glucuronide. Whether the acetyl substitution is in 3″ or 4″ position, may not be deducted from mass spectrometric determination.

3.1.9. Tarragon (Artemisia dranunculus)

A flavonoid glycoside with deprotonated molecular ion m/z 607 was detected. It exhibit similar chromatographic retention time (8.5 min) and aglycone fragment (m/z 299) as the component in mint extract found to be diosmin. Hoffmann and Herrmann [21] report the presence of quercetin and kaempferol 3-rhamnoglucosides as well as patuletin 3-glucoside and 3-rhamnoglucoside in tarragon. They did not report the presence of diosmin in tarragon.

3.1.10. Thyme (Thymus vulgaris)

The composition of the Spanish *Thymus vulgaris* L. plant has previously been studied by Guillén and Manzanos [10]. They describe a wide range of terpenes, sesquiterpenes, aldehydes, hydrocarbons and identified and unidentified phytosterols in addition to a number of flavonoids, although without

specifying the glycosyl substituent. In the present study, glucuronides of apigenin, eriodictyol, and luteolin, luteolin-glucoside and luteolin-diglucoside were detected, as was rosmarinic acid. No 7-methylapigenin, 7-methylnaringenin, dihydrokaempferol, quercetin or 4′,7-dimethylapigenin, previously reported as constituents of thyme by Guillén and Manzanos [10], were detected in the present study.

4. Conclusions

Negative APCI-MS–MS is an excellent tool for description of flavonoid composition of complex foods as herbs, analysed as crude extracts after chromatographic separation.

Methoxylated flavonoid aglycones exhibited specific fragmentation due to loss of $\cdot CH_3$ radicals from the deprotonated aglycone ions.

The flavonoids were present as glycosides or glucuronides in the herb extract. Quercetin, luteolin, apigenin and kaempferol were the most widespread flavonoids, as they were present in half of the herb samples included in the study. The position of glycosyl or glucuronyl substitution cannot be elucidated by low-energy CID-MS–MS, but complete structures are suggested when supported by literature data. The observed differences between the data obtained in the present study and literature data may reflect variations in composition or levels of the individual compound due to different varieties or growth conditions.

Acknowledgements

The author is grateful to Mrs. Jytte Børup and Mr. Gunnar Rasmussen for skilled technical assistance, and to Dr. Lars Viborg Jørgensen and Mrs. Pia Knuthsen for valuable discussions.

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