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Identification of phenolic compounds in artichoke waste by high-performance liquid chromatography–tandem mass spectrometry

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Abstract

A new fast and efficient method combining liquid chromatography coupled to ionspray mass spectrometry in tandem mode with negative ion detection is described for the qualitative analysis of artichoke waste. Forty-five phenolic compounds were identified on the basis of their mass spectra in full scan mode, mass spectra in different MS–MS modes, and retention times compared with those of available reference substances. The major compounds were found to be both caffeoylquinic and dicaffeoylquinic acids, luteolin glucuronide, luteolin galactoside, quercetin, and some quercetin glycosides.

Keywords: Vegetables; Artichoke waste; *Cynara scolimus*; Phenolic compounds; Flavonoids

1. Introduction

Artichoke (*Cynara scolimus*) is popular for its pleasant bitter taste which is attributed to phytochemicals occurring in the green parts of the plants. The presence of phytochemicals in artichoke has been well documented, the leaves being higher in medicinal value than flowers, with antihepatotoxic, choleretic, diuretic, hypocholesterolemic and antilipidemic properties that are attributed to the phenolic composition [1–4]. Spain is one of the major producers of artichoke in Europe [5], and the canning industry is the most important consumer of this crop. The residues proceeding from this industry can form up to 60% of the harvested plant material, the final management of these wastes representing an additional problem. Until the present, the common disposal of artichoke raw material is as organic mass, animal feed [6], and fuel and fiber production [7]. Nonetheless, owing to the antioxidant capacity of polyphenols and their possible implication in human health in prevention of cancer, cardiovascular diseases and other pathologies [8], artichoke raw ma-

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terial is being subjected to new chemical analysis to determine its phenolic composition.

Some previous work on artichoke analysis was performed by TLC, NMR, GC–MS and HPLC–UV methods, and led to the identification of some caffeoylquinic and dicaffeoylquinic acid derivatives and flavonoids [9–12]. More recently, some minor compounds occurring in leaf artichoke extract have been identified using LC–MS with positive ion detection [13]. The chemical composition of artichoke waste, however, has not been extensively studied. To our knowledge, only one previous study on the analysis of artichoke residue has been done, using a HPLC–UV method, which led to the identification of 10 compounds [14].

In this work, a new and short (23-min consuming time) high-performance liquid chromatography coupled to mass spectrometry in tandem mode method was established for the analysis of phenolic compounds in artichoke residues. The raw material was first subjected to an extraction process followed by a column chromatography fractionation and HPLC analysis of the obtained fractions. For the identification of the different compounds, a first approach included the injection of samples in full scan mode in order to choose the compounds of interest. Then, the selection of MS–MS scan modes was based on the ions obtained in the mass spectra in full scan, and the type of compound tentatively identified. The use of MS–MS scan modes such as constant neutral loss, product ion scan, and precursor ion scan, enables rapid acquisition of useful structural information about the unknown ingredients from a complex matrix. Some examples of the use of MS–MS experiments have been recently reported for qualitative analysis of polyphenolic compounds in plants, such as dried plumbs (*Prunus domestica*) [15], *Erigeron breviscapus* [16] and *Camelia sinensis* [17], but also for the screening of pesticides in environmental samples [18] or characterization of metabolites in drugs [19].

The combination of full scan mode with different MS–MS approaches has not been previously applied to the analysis of artichoke composition, and it has been revealed to be a powerful tool to selectively screen artichoke by-product extracts for the occurrence of phenolics and structurally related substances. In this paper, 45 phenolic compounds were identified in artichoke waste, some of which have been reported to exhibit activity as free radical scavengers and quenchers of reactive oxygen species [20–22]. Twenty-five of the 45 compounds identified have never been reported before in artichoke material, and 33 of them are here described for the first time in artichoke wastes. Although some of the compounds described in artichoke leaf have not been found in this artichoke residue, the 10 compounds previously reported to be present in artichoke waste have been also found in this work. None of the previously reported methods have led to so much chemical information on artichoke composition, which can be useful for both further research and commercial use of this waste.

2. Experimental

2.1. Chemicals and plant extract

Methanol, ethyl acetate and acetonitrile (HPLC grade) were purchased from SDS (Peypin, France), hexane (HPLC grade) and ethanol (analytical grade) from Scharlab (Barcelona, Spain), formic acid (analytical grade) from Probus (Badalona, Spain) and acetic acid (analytical grade) from Merck (Darmstadt, Germany). Ultrapure water (Milli-Q) (Waters, Milford, MA, USA) was used. The phenolic standards were obtained as follows: protocatechuic acid, coumaric acid and quercetin were from Sigma (St. Louis, MO, USA); gallic acid, ferulic acid and caffeic acid were from Fluka (Buchs, Switzerland); chlorogenic acid, hyperoside, quercitrin, isoquercitrin, luteolin, cynaroside, apigenin, naringin, naringenin, prunin, isorhoifolin and rutin were from Extrasynthese (Genay, France). All the standards were prepared as stock solutions at 1 g l^{-1} in methanol. Working standards (1 mg l^{-1}) were made by diluting stock solutions in HPLC mobile phase.

The plant extract investigated here was obtained by extraction of wastes (bracts, receptacles and stems from the fruit) from fresh-handling and industrial canning processing of artichoke hearts with ethanol–water (50:50). The plant material (500 g) was crushed and then extracted three times with 2 l of the solvent mixture by stirring for 4 h at 40 °C. After filtration through a folded filter, the supernatants

were joined and concentrated under reduced pressure until dryness to give 90.42 g of dry extract. The obtained dry extract was ground to powder.

2.2. Extract fractionation

A 20 g amount of the dried and powdered extract was defatted twice with hexane (500 ml each) by sonication at room temperature for 3 min. The obtained suspensions were filtered through a folded filter, and the two solid phases were joined and dried at 40 °C to give 4.02 g of dry extract. This solid was reextracted twice with ethyl acetate (500 ml each) sonicating for 3 min. After filtration through a folded filter, the solvent was concentrated under reduced pressure up to dryness to give 0.75 g of an ethyl acetate clean extract. This extract was chromatographed on Sephadex LH-20 (Amersham Pharmacia Biotech, Buckinghamshire, UK) using methanol at a flow-rate of 5 ml min^{-1} to give 54 fractions. The different fractions were monitored using TLC plates (Alugram Sil G/UV, Macherey–Nagel, Düren, Germany) developing with a mixture of ethyl acetate–water–acetic acid (10:1:1) and under UV visualization at 254 and 360 nm. The similar fractions were joined according to their chromatographic profile thus obtaining seven final subfractions (from A to G), which were concentrated under vacuum at below 40 °C up to dryness (Fig. 1 shows a flow diagram of the extraction and isolation process). The final seven subfractions were reconstituted in water with a 0.1% formic acid–acetonitrile (8:2) solution at the concentration of 1 mg ml^{-1}, filtered through a 0.45 μm PTFE filter (Waters), and kept in a freezer at −20 °C until analysis.

2.3. HPLC conditions

Analyses were performed using an Agilent 1100 (Waldbronn, Germany) quaternary pump equipped

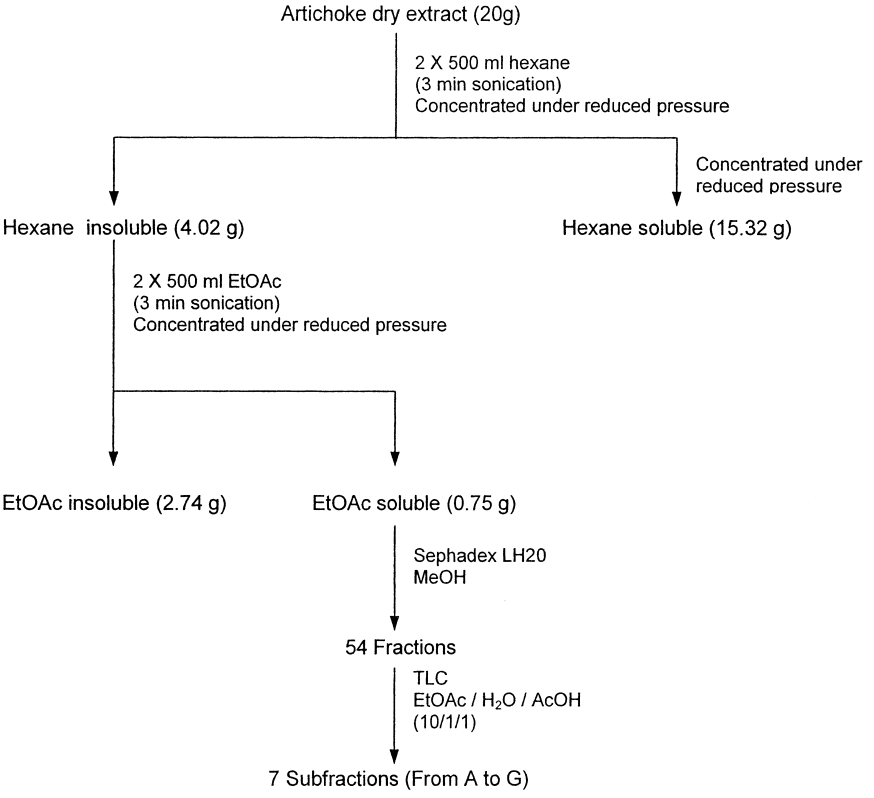


Fig. 1. Flow scheme of the fractionation of the artichoke extract.

with an autosampler and diode array detection (DAD) system. A Chemstation HP Rev. A.08.03 was used for data analysis. A Luna C_{18} Phenomenex (Torrance, CA, USA) column (50×2.1 mm, 3.5 μm) equipped with a Securityguard C_{18} Phenomenex (4× 3 mm I.D.) was used. Gradient elution was done with water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a constant flow-rate of 400 μl min^{-1}. A linear gradient profile with the following proportions of solvent A was applied [*t* (min), %A]: (0, 94), (5, 83.5), (7, 82.5), (12.5, 81.5), (21, 0), (23, 94). The compounds described were monitored at 280, 320 and 365 nm.

2.4. LC–MS–MS system

An API 3000 triple quadrupole mass spectrometer (PE Sciex, Concord, Ontario, Canada) was used to obtain the MS and MS–MS data. All the analyses were performed using the Turbo Ionspray source in negative ion mode with the following settings: capillary voltage −3500 V, nebulizer gas (N_2) 10 (arbitrary units), curtain gas (N_2) 12 (arbitrary units), collision gas (N_2) 10 (arbitrary units), focusing potential −200 V, entrance potential 10 V, drying gas (N_2) was heated to 400 °C and introduced at a flow-rate of 8000 ml min^{-1}. Full scan data acquisition was performed scanning from *m*/*z* 100 to 800 u in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between each scan of 2 ms. To confirm the identity of some of the compounds, precursor ion scan, neutral loss scan, and product ion scan experiments were done. Thus, the main objective for the precursor ion scan experiments was to identify compounds belonging to a group of substances, such as apigenin, luteolin and quercetin derivatives. In neutral loss experiments, the loss of 162 and 176 u corresponding to the loss of a glucose or galactose, and a glucuronic acid for glycoside and glucuronide derivatives, respectively, was studied to confirm their structures. Finally, product ion scan allowed the identification of aglycons by comparison of the MS–MS spectra with those corresponding to the standards after typical fragmentations. MS–MS experiments were produced by collision-activated dissociation (CAD) of selected precursor ions in the collision cell of the triple quadrupole mass spectrometer and mass analyzed using the second analyzer of the instrument. Additional experimental conditions for MS–MS included collision energy (depending on the compound), CAD gas (nitrogen) at 6 (arbitrary units), and scan range, as necessary for the precursor selected. Neutral loss scan of 162 and 176 u experiments were done by scanning within the *m*/*z* range from 200 to 800 u. Precursor ion scan experiments were carried out by scanning Q1 between 300 and 800 u, except for quercetin (between 310 and 800 u). Product ion scan experiments were performed by scanning Q3 between 100 and $[M-H+20]^-$ u. In all the experiments, both quadrupoles (Q1 and Q3) were operated at unit resolution. The MS–MS conditions used in this work were adapted from a previous work dealing with the identification of phenolic compounds in cocoa samples [23].

3. Results and discussion

The seven subfractions obtained from the extraction/clean-up process described above (Section 2.2) were first analyzed by LC–MS in full scan mode for the identification of the phenolic compounds. The examination of the chromatograms in full scan mode revealed the presence of several compounds which were positively identified by comparison with available standards. When this comparison was not possible, MS–MS experiments (product ion scan, neutral loss scan, or precursor ion scan) were carried out in order to confirm the identity of the compound. By means of these experiments, in this work several compounds have been identified in artichoke waste for the first time. Table 1 shows the list of the 45 phenolics identified together with their retention times, the subfraction where they were found, the MS–MS ions used for their identification, and the MS–MS approach applied. Fig. 2 shows the chemical structures of the different compounds identified in this work.

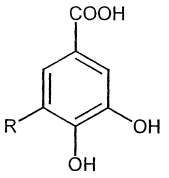
3.1. Full scan mode experiments

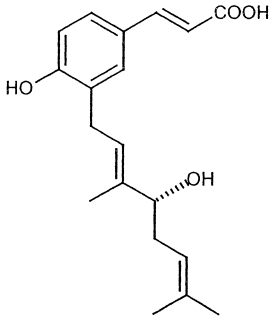
Cynnamic and benzoic acids like gallic, caffeic, protocatechuic, chlorogenic and dicaffeoylquinic acids, as well as some flavonoids, were identified by examination of the chromatograms belonging to the

Table 1
List of the compounds identified in artichoke waste

Number	Compound	Retention time (min)	Subfraction	MS–MS ions	MS–MS approach	Comparison with standard
1	Gallic acid[a,b]	1.20	C	169, 125	FS	Yes
2	Caffeoylquinic acid 1	2.05	C, D	353, 191	FS, NL	No
3	Protocatechuic acid[a,b]	2.20	C, D	153, 109	FS	Yes
4	Caffeoylquinic acid 2	2.70	B, C, D, E, F	353, 191	FS, NL	No
5	Esculin[b]	3.30	B, C	339, 177	FS, NL	No
6	*p*-Coumaric acid-*O*-glucoside 1[a,b]	4.61	F	325, 163, 119	FS, NL	No
7	Chlorogenic acid	5.03	A, B, C, D, E, F	353, 191	FS, NL	Yes
8	Caffeoylquinic acid 3	5.56	A, B, C, D, E, F	353, 191	FS, NL	No
9	Caffeic acid	5.70	C, D, E	179, 135	FS	Yes
10	Dicaffeoylquinic acid 1	6.63	C	515, 353, 191	FS, NL	No
11	*p*-Coumaric acid-*O*-glucoside 2[a,b]	7.36	C, F	325, 163, 119	FS, NL	No
12	Dicaffeoylquinic acid 2	7.33	C, D, E	515, 353, 191	FS, NL	No
13	Dicaffeoylquinic acid 3	8.34	C	515, 353, 191	FS, NL	No
14	Eriodictyol-glucuronide[a,b]	8.74	C	463, 287	FS, NL	No
15	Rutin[a,b] (quercetin-3-*O*-rutinoside)	8.90	C	609, 301	FS, PrIS	Yes
16	Dicaffeoylquinic acid derivative[a,b]	9.00	C, D	677, 515, 353, 191	FS, NL	No
17	Hyperoside[a,b] (quercetin-3-*O*-galactoside)	9.01	C, D, E	463, 301	FS, NL, PrIS	Yes
18	Luteolin-7-*O*-rutinoside[b]	9.10	C	593, 285	FS, PrIS	No
19	Cynaroside[b] (luteolin-7-*O*-glucoside)	9.12	A, D, E, F	447, 285	FS, NL, PrIS	Yes
20	Isoquercitrin[a,b] (quercetin-3-*O*-glucoside)	9.21	C	463, 301	FS, NL, PrIS	Yes
21	Dicaffeoylquinic acid 4	9.29	C	515, 353, 191	FS, NL	No
22	Luteolin-7-*O*-glucuronide[a,b]	9.44	D, E, F	461, 285	FS, NL, PrIS	No
23	Luteolin-7-*O*-galactoside[a,b]	9.78	D, F	447, 285	FS, NL, PrIS	No
24	Naringenin-*O*-hexoside[a,b]	9.78	D	433, 271	FS, NL, PrIS	No
25	Avicularin[a,b] (quercetin-3-*O*-arabinoside)	9.90	C, D, E	433, 301	FS, NL, PrIS	No
26	Isorhoifolin[a,b] (apigenin-7-*O*-rutinoside)	10.34	C	577, 381, 269, 151	FS, PrIS	Yes
27	Dicaffeoylquinic acid 5	10.54	A, C, D, E, F	515, 353, 191	FS, NL	No
28	Quercetin-*O*-pentoside 1[a,b]	10.71	C, D, E	433, 271	FS, NL, PrIS	No
29	Quercitrin[a,b] (quercetin-3-*O*-rhamnoside)	10.86	C, D, E, F	447, 301	FS, NL, PrIS	Yes
30	Luteolin-7-*O*-neohesperidoside[a,b]	10.90	C	593, 285	FS, PrIS	No
31	Apigenin-*O*-glucoside[b]	10.90	D	431, 269	FS, NL, PrIS	No
32	Prunin[a,b] (naringenin-7-*O*-glucoside)	10.94	C, D	433, 271	FS, PrIS	Yes
33	Dicaffeoylquinic acid 6	10.94	D	515, 353, 191	FS, NL	No
34	Naringin[a,b] (naringenin-7-*O*-neohesperidoside)	11.02	C	579, 271	FS, PrIS	Yes
35	Scolimoside[b] (luteolin-7-*O*-rhamnoside)	11.20	D, F	431, 285	FS, PrIS	No
36	Apigenin-7-*O*-glucuronide[a,b]	11.75	C, D	445, 269	FS, NL	No
37	Quercetin-*O*-pentoside 2[a,b]	11.89	C, D, E	447, 301	FS, PrIS	No
38	Dicaffeoylquinic acid 7	12.32	C, D, F	515, 353, 191	FS, NL	No
39	Phloridzin[a,b] (phloretin-2-*O*-glucoside)	12.74	C, D	435, 273, 167	FS, NL	No
40	Feruoylquinic acid-*O*-glucoside[a,b]	14.11	D, E	529, 367, 191	FS, NL	No
41	Luteolin[b]	17.57	C, D, E, F	285, 217, 175, 151	FS, PtIS	Yes
42	Quercetin[b]	17.60	D	301, 151	FS, PtIS	Yes
43	Naringenin[a,b]	18.68	D, E, F	271, 151, 119	FS, PtIS	Yes
44	Apigenin[b]	18.70	C, D, E, F	269, 151, 117	FS, PtIS	Yes
45	Chrysoeriol[a,b]	18.80	D, E	299, 284	FS, PtIS	No

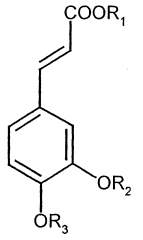
FS, full scan; NL, neutral loss; PrIS, precursor ion scan; PtIS, product ion scan.
[a] Not previously reported in artichoke.
[b] Not previously reported in artichoke waste.



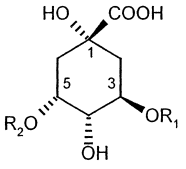


Coumaric acid

Compound	R
Gallic acid	OH
Protocatechuic acid	H



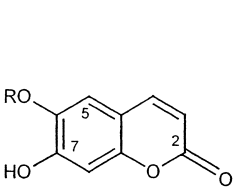
Compound	R_1	R_2	R_3
Caffeic acid	H	H	H
Feruoylquinic acid	H	Me	H
Feruoylquinic acid glucosilated 1	Glu	Me	H
Feruoylquinic acid glucosilated 2	H	Me	Glu

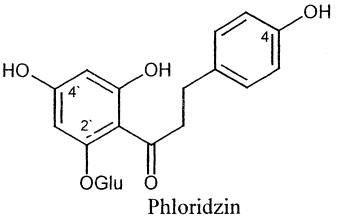


Compound	R_1	R_2
Quinic acid	H	H
Chlorogenic acid	Caffeic acid	H
3, 5 Dicaffeoyl quinic acid	Caffeic acid	Caffeic acid

Fig. 2. Chemical structures of the phenolic compounds found in the artichoke waste.

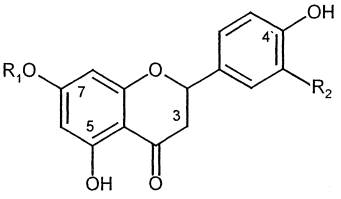
different subfractions in full scan mode. The spectra generated for these compounds in negative ion mode gave the deprotonated molecule $[M-H]^-$ and some fragments even at relatively low declustering potentials (up to −20 V). As an example, loss of CO_2 was observed for gallic, caffeic and protocatechuic acids, and also for *p*-coumaric acid glucoside, giving the $[M-H-44]^-$ as a characteristic ion. Fig. 3





Phloridzin

Compound	R
Esculetin	OH
Esculin	Glu



Compound	R_1	R_2
Prunin	Glucose	H
Naringin	Neohesperidoside (rhamnosyl (α1→2)-glucoside)	H
Naringenin	OH	H
Eriodictyol	OH	OH

Fig. 2. (*continued*)

shows the mass spectra of different groups of compounds identified by full scan mode.

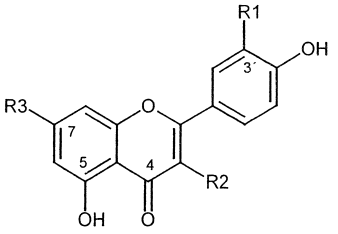
3.2. Neutral loss scan experiments

Experiments in neutral loss scan of 162 and 176 u were carried out in order to identify glucosides or galactosides, and glucuronides, respectively. Likewise, the loss of a caffeic acid unit was also observed using neutral loss scan of 162 u. The results of these experiments are presented in Table 1.

3.2.1. Loss of 162 u

3.2.1.1. Caffeoylquinic acid derivatives

Chlorogenic acid and different isomers of chlorogenic acid were found at different retention times showing the deprotonated molecule $[M-H]^-$ (m/z 353) and the ion corresponding to the deprotonated quinic acid (m/z 191) in full scan mode. Dicaffeoylquinic acid isomers were identified using m/z 515, 353 and 191 ions corresponding to the deprotonated molecule, the loss of a caffeic unit, and the deprotonated quinic acid, respectively. Due to the lack of reference substances (except for chlorogenic acid), isomers of chlorogenic and dicaffeoylquinic acids were confirmed by injection of the subfractions in neutral loss of 162 u (which also corresponds to the loss of a caffeic acid unit). Thus, for example, Fig. 4 shows the chromatogram and some spectra of the different isomers of the chlorogenic (peaks 2, 4, 7 and 8 in Fig. 4B) and dicaffeoylquinic (peaks 10, 12, 13, 21, 27, 33 and 38 in Fig. 4C) acids occurring in subfraction C. Caffeoylquinic and dicaffeoylquinic acids were the main compounds in this artichoke residue extract, and using the proposed extraction/clean-up process, these phenolic substances were mainly concentrated in subfractions C, D and E, which can be regarded as of interest owing to the antioxidant properties of those compounds.



Compound	R_1	R_2	R_3
Rutin	OH	Rutinoside (rhamnosyl (α1→6)-glucoside)	OH
Hyperoside	OH	Galactose	OH
Isoquercitrin	OH	Glucose	OH
Avicularin	OH	Arabinose	OH
Quercitrin	OH	Rhamnose	OH
Quercetin	OH	OH	OH
Luteolin-7-*O*-rutinoside	OH	H	Rutinoside (rhamnosyl (α1→6)-glucoside)
Cynaroside	OH	H	Glucose
Luteolin-7-*O*-glucuronide	OH	H	Glucuronide
Luteolin-7-*O*-galactoside	OH	H	Galactose
Luteolin-7-*O*-neohesperidoside	OH	H	Neohesperidoside (rhamnosyl (α1→2)-glucoside)
Scolimoside	OH	H	Rhamnose
Luteolin	OH	H	H
Isorhoifolin	H	H	Rutinoside (rhamnosyl (α1→6)-glucoside)
Apigenin-7-*O*-glucuronide	H	H	Glucuronide
Apigenin	H	H	H
Chrysoeriol	OMe	H	OH

Fig. 2. (*continued*)

Special attention was paid to the presence of *m*/*z* 677 in neutral loss scan mode of 162 u. The study of the chromatograms of subfractions C and D injected in full scan mode revealed the presence of a dicaffeoylquinic acid derivative at a retention time of 9.0 min. This compound gave the ions at *m*/*z* 677, 515, 353 and 191 corresponding to the loss of either a glucose or caffeoyl unit, and the normal cleavage of the dicaffeoylquinic acid, thus suggesting that it could be either a glucosylated form of the dicaffeoylquinic acid or a tricaffeoylquinic acid.

3.2.1.2. Coumaric acid derivatives

Two coumaric acid derivatives were detected in full scan experiments showing *m*/*z* 325, 163 and 119 at the retention times of 4.61 min in subfraction F, and 7.36 min in different subfractions (from C to F) in decreasing concentrations. The mass spectra of the peaks in full scan mode showed the typical fragmentation of the coumaric acid at *m*/*z* 163 and 119 and the loss of a glucose unit (Fig. 3). Neutral loss scan of 162 u confirmed the loss of a glucose unit, so that both compounds could be tentatively assigned to *p*-coumaric acid glucosides. Since glucosides and galactosides of the same aglycon can be resolved on reversed-phase HPLC, but their retention times are close [24], it is likely that we could confirm that the two *p*-coumaric acid glucosides occurring in the artichoke residue extract are *trans*- or *cis*-*p*-coumaric acid isomer glucosides.

3.2.1.3. Glucosylated feruoylquinic acid

The presence of a peak at the retention time of 14.11 min showing *m*/*z* 529, 367 and 191 in full

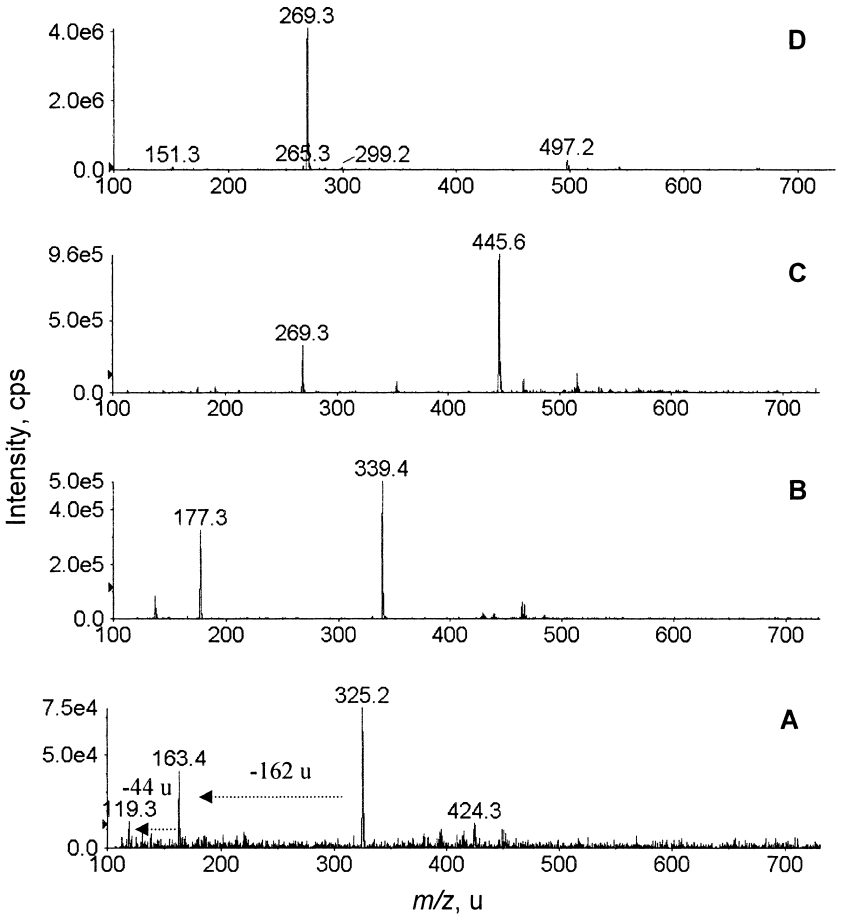


Fig. 3. Mass spectra of the different compounds identified in artichoke waste by full scan mode. (A) *p*-Coumaric acid glucoside; (B) esculin; (C) apigenin-7-*O*-glucuronide; (D) apigenin.

scan mode, could be tentatively assigned to a feruoylquinic acid glucoside (peak 40 in Fig. 5). The loss of the glucose unit was confirmed by a neutral loss scan experiment of 162 u. Nevertheless, no definitive structure assignment could be done in the absence of a standard. This compound appeared mainly in subfraction D, and in a minor concentration in subfraction E.

3.2.1.4. Coumarin derivatives

In the chromatograms of subfractions B and C injected in full scan mode, a peak showing *m*/*z* 339 and 177 was observed at the retention time of 3.3 min, which could be tentatively assigned to esculin, the glucosylated form of esculetin, a dihydroxycoumarin occurring in artichoke [9].

3.2.1.5. Quercetin derivatives

Two glucosides of quercetin, namely hyperoside (quercetin 3-*O*-galactoside) and isoquercitrin (quercetin 3-*O*-glucoside), were identified. Hyperoside was detected at the retention time of 9.01 min in subfractions C, D and E, while isoquercitrin was found in subfraction C at the retention time of 9.21 min, both of them exhibiting a mass spectrum in full scan mode with *m*/*z* 463 and 301. Comparison of the mass spectra and retention times with those of the respective standards confirmed the occurrence of these glycosylated flavonols in this artichoke waste.

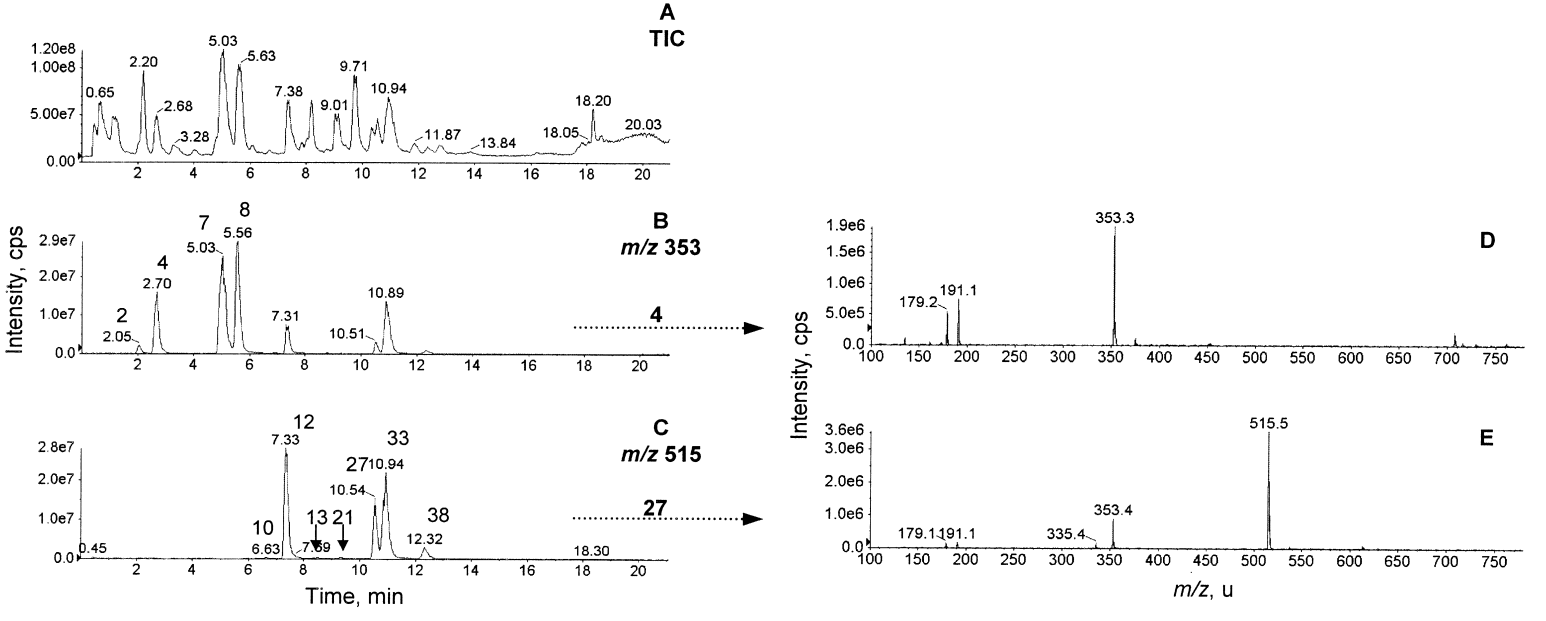


Fig. 4. Chromatograms and mass spectra of caffeoylquinic and dicaffeoylquinic acid isomers occurring in subfraction C. (A) Total ion chromatogram (TIC) in full scan mode; (B) trace chromatogram of m/z 353; peaks: 2, 4 and 8, different isomers of caffeoylquinic acid; peak 7, chlorogenic acid; (C) trace chromatogram of m/z 515; peaks 10, 12, 13, 21, 27, 33 and 38 correspond to different isomers of dicaffeoylquinic acid; (D) mass spectrum of peak 4; (E) mass spectrum of peak 27 (the peak number belongs to the number of the compound listed in Table 1). See Section 2 for HPLC–MS conditions.

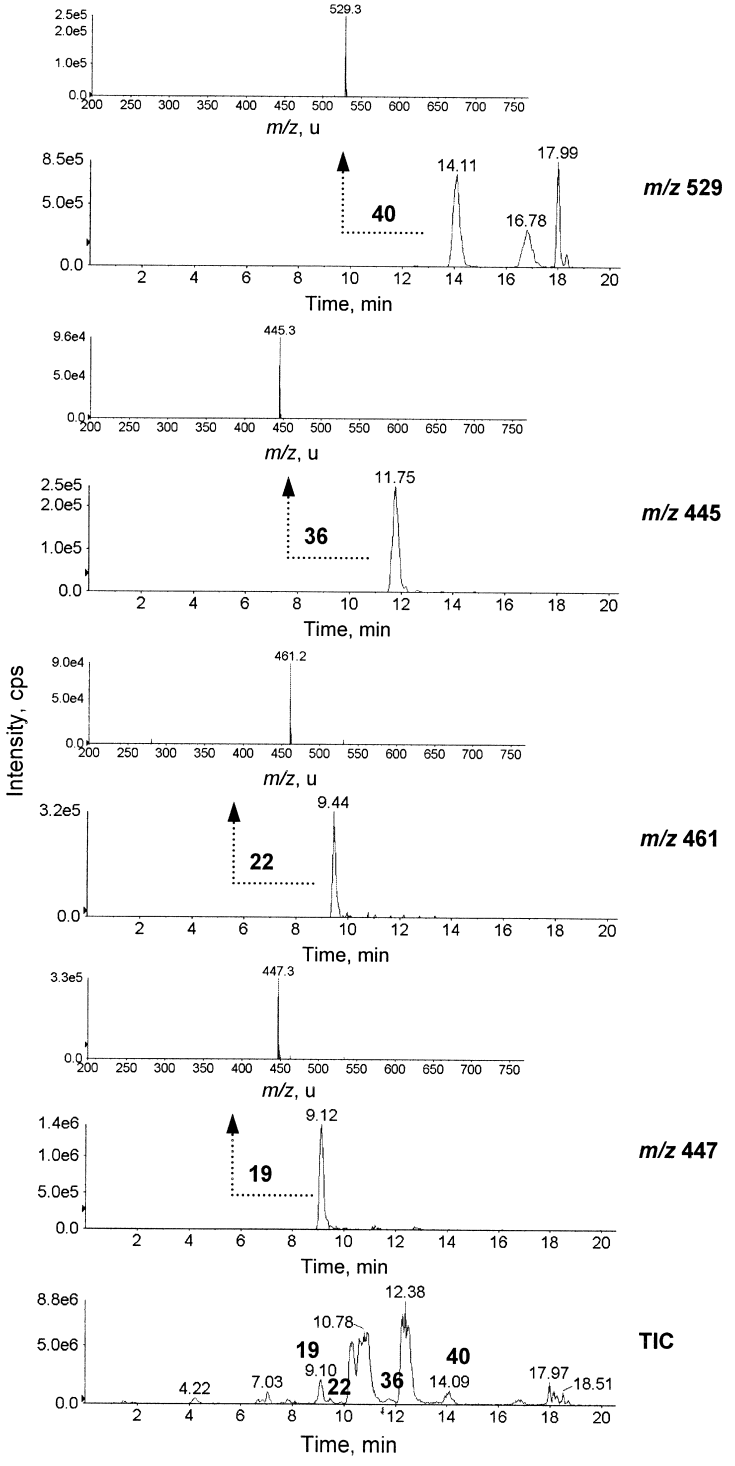


Fig. 5. TIC, trace chromatograms and mass spectra of subfraction D injected in neutral loss scan MS–MS experiments of 162 u for peaks 19 (luteolin-7-*O*-glucoside) and 40 (feruloylquinic acid-glucoside), and of 176 u for peaks 22 (luteolin-7-*O*-glucuronide) and 36 (apigenin-7-*O*-glucuronide). LC–MS–MS conditions are described in the text.

3.2.1.6. Luteolin derivatives

Another family of flavonoid hexosides was also detected. Thus, luteolin-7-*O*-glucoside (cynaroside) was identified by comparison of its retention time (9.12 min) and mass spectrum (peak 22 in Fig. 5) with those of a standard. The presence of a peak with a close retention time (9.78 min) and similar mass spectrum suggested the occurrence of luteolin-7-*O*-galactoside [24], although a definitive assignment could not be performed due to the lack of standard. These two compounds appeared in the middle subfractions, luteolin-7-*O*-glucoside being more abundant in subfraction D, and luteolin-7-*O*-galactoside in subfraction C.

3.2.1.7. Apigenin derivatives

An apigenin glucoside appearing at the retention time of 10.9 min in subfraction D was identified. Its mass spectrum in full scan mode showed the corresponding ion of the apigenin glucoside (m/z 431), and the ion corresponding to the loss of a glucose unit from an apigenin (m/z 269). MS–MS experiments in neutral loss scan of 162 u confirmed the loss of the glucose unit, but the lack of standard did not allow the assignment of the compound.

3.2.1.8. Naringenin derivatives

Another example of the utility of neutral loss scan experiments is that of naringenin hexosides (m/z 433 and 271 in mass spectrum in full scan mode), which to our knowledge have never been identified before in artichoke. The comparison of the retention time and mass spectrum of the peaks with those of the standard prunin (naringenin-7-*O*-glucoside) in both full scan and neutral loss scan modes of m/z 162 u led to the identification of this compound in subfractions C and D. The mass spectra of the peak at the retention time of 9.78 min (m/z 433 and 271 in full scan mode) confirmed the presence of another hexoside derivative of naringenin, but it was not possible to assign its definitive identity owing to the lack of reference substance.

3.2.1.9. Chalcone derivatives

Special attention was paid in the identity of a peak appearing at 12.74 min in the chromatogram of subfraction C in full scan mode and with a mass spectra showing m/z 435 and 273. The loss of a glucose unit was confirmed by neutral loss scan of 162 u. Both experiments suggested the occurrence of phloridzin, a chalcone quite common in apple and apple wastes [25,26], which to our knowledge has not been previously reported either in artichoke or artichoke wastes.

3.2.2. Loss of 176 u

As mentioned above, neutral loss scan of 176 u experiments allowed us to identify some glucuronides in artichoke residues for the first time. Identification of a peak at the retention time of 8.74 min appearing in subfraction C, which exhibited a mass spectrum in full scan mode with m/z 463 and 287, could be tentatively assigned to the eriodyctiol-glucuronide. The loss of 176 u in neutral loss scan mode confirmed the presence of a glucuronide of an aglycon M_r 288 that could correspond to eriodyctiol. Unfortunately, no standard was available, so the identification of the structure is not definitive. Neutral loss scan of 176 u experiments also showed the presence of two peaks at retention times of 9.44 and 11.75 min showing a mass spectrum of m/z 461 and 445, respectively. The peak corresponding to m/z 461 could be attributed to a kaempferol or luteolin glucuronide, so for a more precise identification subfraction C was injected in the product ion scan of m/z 285. The product ion mass spectrum gave m/z 199 and 151 corresponding to a luteolin derivative, as described in a previous work by Sánchez-Rabaneda et al. [23]. On the basis of the MS–MS experiments performed in this work, no kaempferol derivatives were detected in the artichoke residue. The peak at 11.75 min could be attributed to an apigenin glucuronide due to the presence of an ion at m/z 269 which corresponds to the aglycon according to the cleavage of the glucuronide unit. Mass chromatogram and mass spectra concerning the identification of both luteolin-7-*O*-glucuronide and apigenin-7-*O*-glucuronide are given in Fig. 5 (peaks 22 and 36, respectively).

3.3. Precursor ion scan experiments

In order to identify other classes of flavonoids containing sugars such as arabinose or rhamnose, and even for diglycosides such as rutinoside [rhamnosyl ($\alpha 1 \rightarrow 6$)-glucoside] or neohesperidoside

[rhamnosyl (α1→2)-glucoside], precursor ion scan experiments were done.

3.3.1. Quercetin derivatives

As described above, the precursor ion scan experiments allowed the identification of compounds belonging to a concrete group of substances, like quercetin derivatives, using a precursor ion scan of m/z 301. Thus, rutin (a quercetin diglycoside) was identified at the retention time of 8.9 min in subfraction C. The spectrum in full scan mode showed the ions corresponding to the deprotonated molecule (m/z 609) and the loss of a rutinoside unit (m/z 301), while the spectrum in the precursor ion scan mode showed m/z 609. Confirmation was done by comparison of the retention time and spectrum with those of the standard.

Four quercetin pentosides at retention times of 9.9, 10.71, 10.86 and 11.89 min were also detected. The compounds at the retention times of 9.9 and 10.71 min gave the deprotonate $[M-H]^-$ (m/z 433) molecule and the ion corresponding to the deprotonated aglycone $[A-H]^-$ (m/z 301) in full scan mode. One of these compounds could tentatively be assigned as avicularin (quercetin 3-*O*-arabinose). Confirmation was done using the trace of the precursor ion scan of m/z 301. Otherwise, quercitrin and a quercetin-pentoside were detected at the retention times of 10.86 and 11.89 min, respectively, showing the ions m/z 477 and 301 in full scan mode. Confirmation of quercitrin was done by comparison with a standard, but it was not possible in the case of the quercetin-pentoside owing to the lack of standard. To our knowledge, the identification of quercetin pentosides in artichoke material is reported here for the first time.

3.3.2. Luteolin derivatives

Another example of the use of precursor ion scan experiments was for the identification of compounds which contain m/z 285. A peak showing m/z 431 and 285 in full scan mode was observed at a retention time of 11.2 min in subfractions C and D which could be tentatively assigned to scolimoside (luteolin-7-*O*-rhamnoside), a compound previously identified in artichoke extracts [9] but not in artichoke wastes. Experiment in precursor ion scan of m/z 285 confirmed the presence of a compound showing m/z 431. Likewise, two luteolin diglycosides with m/z 593 and 285 at the retention times of 9.1 and 10.9 min were identified in subfraction C. As previously described by Sánchez-Rabaneda et al. [23], flavonol-diglycosides (neohesperidoside, or rutinoside) could be differentiated by the relative abundance of the ions, and also by the fact that neohesperidosides show a more pronounced fragmentation than their rutinoside analogues. Thus, in this case, these two compounds could be tentatively assigned to luteolin-7-*O*-rutinoside (9.10 min) and luteolin-7-*O*-neohesperidoside (10.9 min), although the comparison of their spectra and retention times with those of standards was not possible. This type of experiment also allowed the confirmation of other luteolin derivatives, such as luteolin-7-*O*-glucoside, luteolin-7-*O*-galactoside and luteolin-7-*O*-glucuronide, previously identified by neutral loss scan experiments.

3.3.3. Apigenin derivatives

Furthermore, injection of subfractions in the precursor ion scan of m/z 269 allowed the identification of an apigenin-*O*-glycoside in subfraction C at the retention time of 10.34 min, which is here described for the first time in artichoke. The mass spectrum in full scan mode showed ions with m/z 577 and 269, the deprotonated molecule $[M-H]^-$, and the ion corresponding to the loss of a rutinoside or neohesperidoside unit. The differentiation between these two glycosides could be possible as previously described for the luteolin derivatives. In this case, where only m/z 577, 381, 269 and 151 ions were observed, the compound could be assigned to apigenin-rutinoside (isorhoifolin). Confirmation of isorhoifolin was done by comparison with a standard. Fig. 6 shows the chromatograms and mass spectra concerning the identification of isorhoifolin.

3.3.4. Naringenin derivatives

Another flavonoid linked to a rhamnose-glucose was found in subfraction C at a retention time of 11.02 min, which was identified as naringenin-7-*O*-neohesperidoside (naringin) (m/z 579 and 271 in full scan mode) by comparison of its spectrum and retention time with those of a standard.

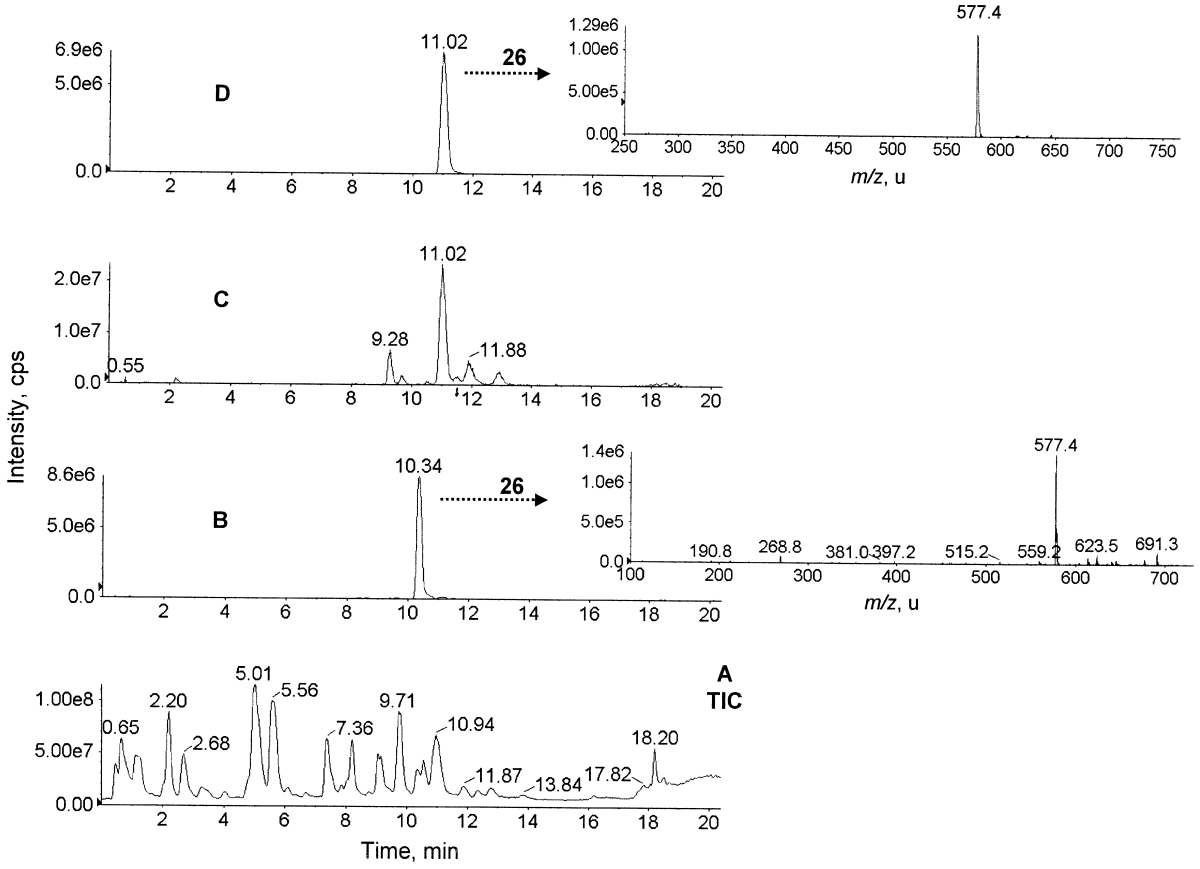


Fig. 6. TIC, trace chromatograms and mass spectra of subfraction C injected in full scan mode, and in precursor ion scan mode of 269 u. (A) TIC in full scan mode; (B) trace chromatogram of m/z 577 in full scan mode and mass spectrum of peak 26 (isorhoifolin); (C) TIC in precursor ion scan mode of 269 u; (D) trace chromatogram of m/z 577 in precursor ion mode of 269 u and mass spectrum of peak 26.

3.4. Product ion scan experiments

With respect to the occurrence of aglycons in artichoke waste, it should be noted that the selected LC–MS–MS conditions used in this work permitted a good chromatographic separation and identification of them. In the chromatograms in full scan mode, some aglycons were observed showing m/z 301, 299, 285, 271 and 269 in subfractions C, D, E and F. In order to confirm the identity of these compounds, subfractions C, D, E and F were injected in the product ion scan mode of m/z 301, 299, 285, 271 and 269 (corresponding to quercetin, chrysoeriol, kaempferol or luteolin, naringenin and apigenin, respectively). These MS–MS experiments caused the cleavage of the flavonoid aglycons into a number of fragments according to strictly fixed pathways, thus confirming the presence of these aglycons, which are here reported for the first time in artichoke waste. These aglycones gave retro Diels-Alder fragmentation as described by Fabre et al. [27] and also confirmed by Sánchez-Rabaneda et al. [23]. As an example, the m/z 151 ion is common for all the aglycones studied, the m/z 117 ion is characteristic for apigenin and its derivatives, whereas the m/z 119 ion is characteristic for naringenin and derivatives. Fig. 7 shows the chromatograms of subfraction E injected in product ion scan mode to illustrate the identification of naringenin.

4. Conclusions

Liquid chromatography coupled to MS–MS offers

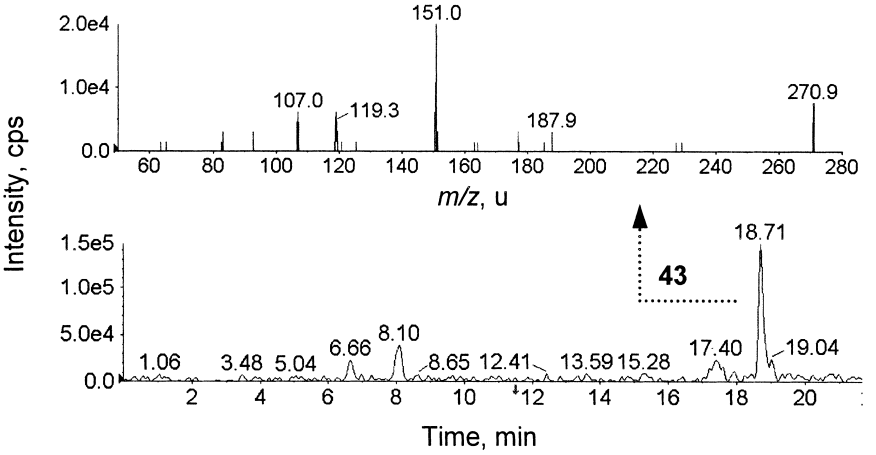


Fig. 7. TIC of subfraction E injected in product ion scan MS–MS experiment of 271 u, and mass spectrum corresponding to peak 43 (naringenin).

a powerful analytical tool due to its selectivity, which enables the use of fast chromatography with low separation efficiency. In the analysis of complex samples, however, the existence of coeluting undetected compounds can lead to an inaccurate method as a consequence of problems with the MS response, due to, for example, ion suppression. Thus, the necessity for an efficient sample purification and chromatographic separation process should not be undervalued.

The LC–MS–MS method here developed was successfully employed to separate and identify both major and minor phenolic compounds from artichoke waste, which allows a better knowledge of both its chemical composition and its potential use as a source of natural antioxidants. The combination of this new chromatographic method and electrospray ionization-MS–MS analysis has also been found to be an excellent tool for the screening of phenolic substances in plants. The small column size (50 mm) together with the low time-consuming method (23 min), shorter than those previously described, permits the injection of a high number of samples and the identification of numerous phenolic compounds in a quick analysis, with a significant reduction in solvent consumption compared with other methods.

The methodology used in this work allowed the positive identification of gallic acid, protocatechuic acid, esculin, two *p*-coumaric acid-glucosides, eryodictiol-glucuronide, rutin, a dicaffeoylquinic acid derivative (dicaffeoylquinic acid-*O*-glucoside or tricaffeoylquinic acid), hyperoside, luteolin-7-*O*-rutinoside, cynaroside, isoquercitrin, luteolin-7-*O*-glucuronide, luteolin-7-*O*-galactoside, naringenin-*O*-hexoside, avicularin, isorhoifolin, two quercetin-*O*-pentosides, quercitrin, apigenin-*O*-glucoside, luteolin-7-*O*-neohesperidoside, prunin, naringin, scolimoside, apigenin-7-*O*-glucuronide, phloridzin, feruoylquinic acid-*O*-glucoside, luteolin, quercetin, naringenin, apigenin and chrysoeriol for the first time in artichoke residues. Caffeic acid and different isomers of both chlorogenic acid and dicaffeoylquinic acid were found to be the major constituents of this residue. The occurrence of all these phenolic compounds in artichoke waste makes this raw material an interesting source of natural antioxidants for the food industry, as well as an ingredient for nutraceuticals or dietary supplements.

Acknowledgements

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