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Determination of flavonoids in a Citrus fruit extract by LC–DAD and LC–MS

Maria de Lourdes Mata Bilbao [a], Cristina Andrés-Lacueva [a], Olga Jáuregui [b], Rosa Maria Lamuela-Raventós [a,*]

[a] Department of Nutrition and Bromatology, CerTA, Faculty of Pharmacy, Barcelona University, Av. Joan XXIII s/n, 08028 Barcelona, Spain
[b] Scientific and Technical Services, CerTA, Faculty of Pharmacy, Barcelona University, Av. Joan XXIII s/n, 08028 Barcelona, Spain

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Abstract

Flavonoids are a group of polyphenolic compounds with health-related properties. *Citrus* fruits are rich in flavonoids and their extracts are being used as functional ingredients for several industrial products. A new high performance liquid chromatography technique with an UV photodiode-array detector was used to analyze flavonoids of an extract of *Citrus* species. To our knowledge this is the first study that reports isoquercitrin presence at a level of 77.3 mg/100 g in a sample made of *Citrus* fruits; four other flavonoids were quantified as rutin (326.59 mg/100 g), naringin (338.36 mg/100 g), quercetin (96.35 mg/100 g) and naringenin (2.35 mg/100 g). Identification was confirmed by a liquid chromatography mass spectrometer system. Method validation was achieved, providing an analytical technique that can be used to detect trace amounts of these compounds in *Citrus* extracts with an extremely rapid sample preparation.

Keywords: Flavonoids; *Citrus*; Chromatography; Mass spectrometry

1. Introduction

Several epidemiologic studies indicate that an increase in the consumption of fruits and vegetables is associated with a decrease in the incidence of cardiovascular disease (CVD) (Bazzano et al., 2002; Kris-Etherton et al., 2002; Liu et al., 2000; Salah et al., 1995).

Flavonoids are widely distributed in fruits, vegetables, fruit juices, cocoa, teas and wines. Most *Citrus* species accumulate substantial quantities of flavonoids during the development of their different organs (Benavente-Garcia, Castillo, & Del Rio, 1993; Castillo, Benavente, & Del Río, 1992; Castillo, Benavente, & Del Rio, 1993). All the flavonoids described in *Citrus* sp. can be classified into these groups: flavanones, flavones, and flavonols. Each species of *Citrus* is characterized (especially in the fruits) by a particular flavanone glycoside pattern which can be separated by HPLC (Macheix, Fleuriet, & Billot, 1990). In grapefruit (*Citrus paradisi*), naringin (naringenin 7-*O*-neohesperidoside) is distinctly dominant, accompanied by narirutin (naringenin 7-*O*-rutinoside). Naringin concentration in whole fruit varies from 170 to 280 mg/100 g. Hesperidin (hesperitin-7-*O*-rutinoside) and neohesperidin (hesperitin-7-*O*-neohesperidoside) are also present in grapefruit juice, but their respective concentrations reach a maximum of 2% of total flavanones (Macheix et al., 1990). Studies on the quantitative distribution of these flavonoids in *Citrus* have shown that the 7-*O*-glycosylflavanones are the most abundant flavonoids in all the species of the genus, whose aglycones are intermediates in the biosynthetic pathway (Benavente-Garcia, Castillo, Sabater, & Del Rio, 1995).

Due to the importance of flavonoids as contributors of beneficial health effects of fruits and vegetables, the identification and/or structural determination of such compounds occurring in plant tissue or other biological systems play an important role in many areas of science. Several publications suggest advances in Citrus flavonoid

* Corresponding author. Tel.: +34 93 4034843; fax: +34 93 4035931.
E-mail address: lamuela@ub.edu (R.M. Lamuela-Raventós).

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determination, especially by HPLC (Kawaii, Tomono, Katase, Ogawaa, & Yano, 1999; Mouly, Gaydou, & Auffray, 1998) in conjunction with diode array detection for their identification and characterization. On the other hand, mass spectrometry (MS) is one of the analytical methods applied to qualitative and quantitative determination of organic compounds. Several studies have reported flavonoid identification in cocoa, tomato, apple and artichoke by applying this technique (Justesen, Knuthsen, & Leth, 1998; Sánchez-Rabaneda et al., 2003; Sánchez-Rabaneda et al., 2003). HPLC coupled with mass spectrometry (HPLC–ESI–MS) has been chosen as a selective technique to analyze citric flavonoids in sour orange extracts (He, Lian, Lin, & Bernart, 1997).

Due to the importance of flavonoids as contributors of beneficial health effects of fruit and vegetables consumption, their identification in plant tissues or other biological systems play an important role in many areas of science. On the other hand, in quality assurance routine analysis the development of rapid methods for adulteration detection of *Citrus* juices is very important. Sample preparation is time consuming, so the simpler the preparation is, the better. Previous works usually applied solid phase or liquid extraction methods. This paper presents a new, direct, optimized and validated method for flavonoids identification and quantification in a *Citrus* fruit extract by HPLC with photo diode array detector with a very rapid sample preparation. Liquid chromatography coupled with mass spectrometry technique was also applied for result confirmation.

2. Materials and methods

2.1. Samples

The *Citrus* fruit extract was composed by four *Citrus* species: grapefruit (*C. paradisi*), bergamot (*C. aurantium*), sweet orange (*C. sinensis*) and tangerine (*C. reticulata*). The sample was maintained at 4 °C and sample dilutions (0.5% and 0.05%) were prepared with water for flavonoid quantification. Prior to injection, sample replicates were filtered with Waters (Milford, MA, USA) 13 mm PTFE 0.45 μm filters.

2.2. Standard and chemicals

Naringin (naringenin-7-*O*-neohesperidoside), quercetin (3,3′,4′,5,7-pentahydroxiflavone) dihydrate, hesperidin (hesperitin-7-*O*-rutinoside) and rutin (quercetin-3-*O*-rutinoside) were purchased from Sigma Chemical CO (St. Louis, USA). Narirutin (naringenin-7-*O*-rutinoside), neohesperidin (hesperitin-7-*O*-neohesperidoside), prunin (naringenin-7-*O*-glucoside), naringenin (4′,5,7-trihidroxyflavanone) were purchased from Extrasynthèse, France. Isoquercitrin, was purchased from Chromadex (LGC Promochem, Spain).

Methanol and acetonitrile were purchased from Scharlau Chemie, S.A., Barcelona (España); formic acid and ethanol were purchased from Sigma Chemical CO and Panreac Química, S.A., respectively, all at HPLC grade. Ultrapure water (Milli-Q) was used.

Standard solutions were made as follows: naringin was dissolved with 2% ethanol/water; isoquercitrin with water at 40 °C and stirring; quercetin and naringenin were dissolved with ethanol, and rutin with methanol. Subsequent dilutions were made with water.

2.3. Equipment

2.3.1. Spectrophotometric analysis

Total amount of polyphenols was measured with an optimized Folin–Ciocalteu method (Vrhovsek, Rigo, Tonon, & Mattivi, 2004) at 765 nm in a Shimadzu UV-160 A spectrophotometer. Sample dilutions of 0.5% with water were made for assay determination. Results were expressed as mg of gallic acid/100 g extract.

2.3.2. HPLC analysis

Flavonoids quantification was performed on a HP (Hewlett–Packard, CA, USA) 1050 gradient liquid chromatograph with DAD 1050 M coupled to a Chemstation HP and using a reverse phase column Luna C18 (2) 5 μm 150 × 2.1 mm (Phenomenex, Torrance, CA, USA). The solvents used were the following: A, 0.1% formic acid in water and B 0.1% formic acid in acetonitrile. Gradients were as follows: 0–35 min 6–50% B, 35–45 min 50–100% B, back to 6% B. Column was equilibrated for 15 min prior to each analysis. The flow rate was 0.4 mL/min; injection volume was 10 μL, and column temperature was at 40 °C. The UV–Vis spectra were recorded from 280 to 400 nm, with detection at 280 and 365 nm.

2.3.3. Method validation

The following parameters for each flavonoid were determined: linearity, precision, accuracy, and sensitivity. Calibration graphs, for linearity determination, were established with five flavonoids solutions, each prepared by duplicate. Concentration range for each flavonoid was as follows: rutin (0.43–2.58 mg/L); isoquercitrin (0.23–0.67 mg/L); naringin (0.44–2.67 mg/L); quercetin (0.27–0.97 mg/L); naringenin (0.1–0.3 mg/L). System precision was performed, injecting six times a standard pool composed by rutin, isoquercitrin, naringin, quercetin and naringenin, and method precision was determined by preparing the sample six times and injecting the replicates into the HPLC. In both cases, relative standard deviation (RSD) was calculated for each flavonoid. Accuracy was determined by adding three known quantities (80%, 100% and 120% of expected value) of each flavonoid to the sample. Three sample replicates were prepared for each case, and percentage of recovery was calculated for each flavonoid. Sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ). LOD was defined as the amount of analyte that gives a peak with a signal-to-noise ratio of 3, whereas LOQ was

the lowest amount of analyte with a signal-to-noise ratio of 10.

2.3.4. LC/MS confirmation analysis

Liquid chromatography (LC)–mass spectrometry (MS) analysis was performed with an Alliance 2690 module from Waters, equipped with an automatic injector. MS was performed using a VG Platform II quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with atmospheric pressure chemical ionization (APCI) ion source (nitrogen flow rate 100 L/h). Working conditions were in APCI negative ion mode and separation was performed using a Xterra Phenyl 5 μm (150 × 2.1 mm) (Waters, Millford USA) column. Mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Separation was carried out in 25 min under the following conditions: gradients starting at 2% B to 8% B in 5 min, to 20% B in 5 min, to 35% B in 5 min, to 60% B in 2 min. The column was equilibrated for 7 min prior to each analysis. Flow rate was 0.6 mL/min and injection volume was 15 μL. Drying nitrogen was heated to 150 °C and introduced to the capillary region at a flow-rate of 200 L/h. Capillary was heated to 400 °C and the cone voltage (CV) held at −30 V. Full data acquisition was performed, scanning form 200 to 700 u in centroid mode and using a cycle time of 2 s and an interscan time of 0.2 s. Peaks were identified on the basis of comparison of retention times, UV–Vis, and MS spectra with standards (naringin, quercetin, rutin, naringenin and isoquercitrin).

3. Results and discussion

3.1. Total polyphenols in Citrus fruit extract

The average content of total polyphenols in the *Citrus* fruit extract evaluated by the FC (Folin–Ciocalteu) assay was 3524 mg gallic acid/100 g extract.

3.2. Sample flavonoids

The sample was composed by four *Citrus* species: *C. paradisi*, *C. aurantium*, *C. reticulata* and *C. sinensis*. Each species of *Citrus* is characterized (especially in the fruits) by a particular flavanone glycoside pattern that can be separated by HPLC (Macheix et al., 1990). It is known that grapefruit and pummelo accumulate naringin as flavanone glycoside in their fruit, leaves and juice, and, to a lesser extent, they also produce narirutin, prunin, hesperidin and neohesperidin (Berhow & Vandercook, 1989; Castillo et al., 1993; Rouseff, Martin, & Youtsey, 1987). On the other hand, it has been demonstrated that sour orange (*Citrus aurantium*) synthesize or accumulate prunin and hesperitin 7-*O*-glucoside during the stage of cell differentiation, which disappears at the end of the linear growth stage during the subsequent maturation period. In sweet orange (*Citrus sinensis*), hesperidin is predominant and in orange juices it is accompanied by narirutin (Leuzzi, Caristi, Panzera, & Licandro, 2000; Macheix et al., 1990; Ooghe, Ooghe, Detavernier, & Huyghebaert, 1994; Pupin, Dennis, & Toledo, 1998).

Nine standards (naringin, quercetin, hesperidin, rutin, narirutin, neohesperidin, prunin, naringenin, isoquercitrin) were chosen for assay performance following data reported in several publications regarding to flavonoids identification in these species by HPLC analysis (Belajova & Suhaj, 2004; Castillo et al., 1993; Mouly, Arzouyan, Gaydou, & Estienne, 1994; Ortuño et al., 1995).

Depending on the fruit development stage, the presence and/or concentrations of flavonoids can be affected. Most *Citrus* species accumulate substantial quantities of flavonoids during their organ development. Ortuño et al. (1995), found in a variety of grapefruit and pummelo, that the highest flavanone levels are detected during the juvenile stages of fruit development. On the other hand, Castillo et al. (1993) demonstrated in *C. aurantium*, that the highest levels of prunin and hesperitin 7-*O*-glucoside are present during fruit development and falls sharply when the corresponding neohesperidosides, naringin and neohesperidin (the most abundant flavanone glycosides in *C. aurantium*) reach their maximum levels.

In several cases, identification of characteristic flavonoids from certain *Citrus* species is used to detect adulteration in industrial products. Flavonoids are very promising for the determination of the authenticity of *Citrus* juices due to their taxonomic specificity (Ooghe et al., 1994; Rouseff et al., 1987). Sweet orange (*C. sinensis*) juices can be adulterated with *Citrus reticulata* (mandarin), *C. paradisi* (grapefruit) and *C. aurantium* (sour orange). The presence of grapefruit and sour orange is identified by the presence of naringin, whereas, the presence of mandarin is detected by a drastic drop of hesperidin/narirutin ratio in *C. sinensis*.

From the nine standards chosen, only five were present in the sample. Chromatograms at two different wavelengths are shown in Fig. 1. Peaks 1, 2, 3, 4 and 5 correspond to rutin, isoquercitrin, naringin, quercetin and naringenin, respectively. Naringin and naringenin reached their maximum at 280 nm, whereas rutin, isoquercitrin and quercetin reached theirs at 365 m. At 280 nm there are two peaks that do not correspond to any of the flavonoids classes that characterize *Citrus* species (flavanones, flavones, and flavonols). These classes obtain their maximum absorption at specific wavelength ranges, flavanones (280–290), flavones (304–350) and flavonols (352–385) (Harborne, Marby, & Mabry, 1975). Peaks 6 and 7 present in Fig. 1 at 280 nm do not correspond to any flavonoid because their maximum absorption wavelength was obtained at 260 nm.

Quantity (mg/100 g) of each flavonoid in the sample was as follows: rutin (326.59), isoquercitrin (77.26), naringin (338.36), quercetin (96.35) and naringenin (2.35). To our knowledge this is the first study that reports the presence of isoquercitrin in a sample made of *C. paradisi*, *C. aurantium*, *C. reticulata* and *C. sinensis*.

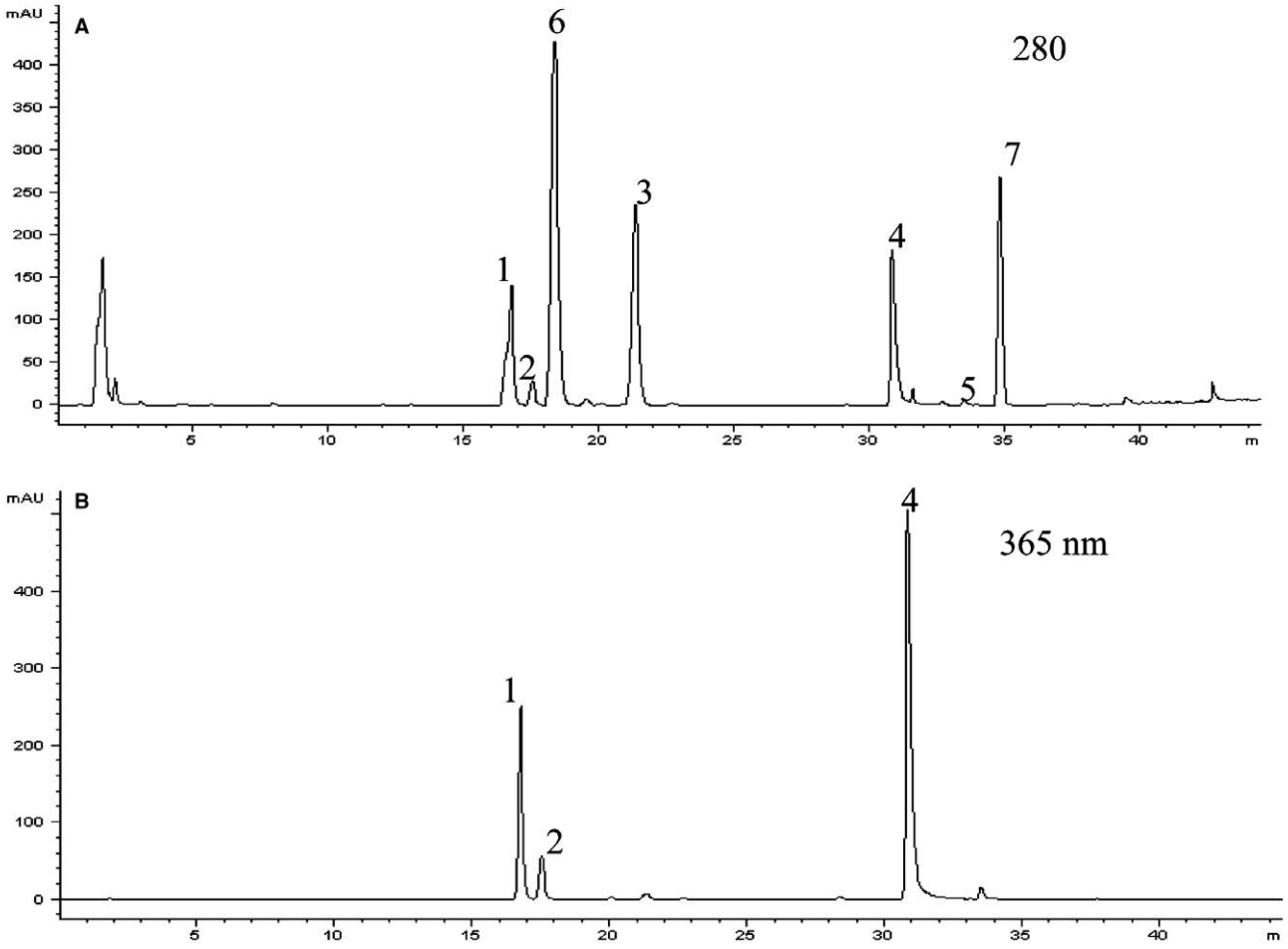

Fig. 1. HPLC–DAD chromatograms corresponding to *Citrus* fruit extract. (A) rutin (1), isoquercitrin (2), naringin (3), quercetin (4) and naringenin (5) recorded at 280 nm. (B) rutin (1), isoquercitrin (2) and quercetin (4) recorded at 365 nm.

3.3. Method validation

Calibration graphs corresponding to rutin, isoquercitrin, naringin, quercetin and naringenin, have shown linear function response, indicated by a $R^2 > 0.99$ value (determination coefficient). RSD values corresponding to system precision and method precision were as follows: rutin (0.49%; 2.23%), isoquercitrin (0.74%; 1.98%), naringin (0.12%; 0.91%), quercetin (0.48%; 9.60%) and naringenin (0.47%; 1.23%). LOD and LOQ for rutin, isoquercitrin, naringin, quercetin and naringenin, values are present in Table 1.

Table 1
Limit of detection (LOD) and limit of quantification (LOQ) of rutin, isoquercitrin, naringin, quercetin and naringenin

Flavonoid	LOD (mg/L)	LOQ (mg/L)	Recovery ($n = 3$) (%)
Rutin	0.04	0.15	93.96 ± 0.41
Isoquercitrin	0.08	0.14	69.28 ± 0.20
Naringin	0.03	0.1	92.51 ± 3.13
Quercetin	0.1	0.28	96.93 ± 2.46
Naringenin	0.014	0.05	95.46 ± 2.78

Recovery values of the five flavonoids identified in the extract.

Accuracy was determined by calculating recovery values for each flavonoid quantified (Table 1). In addition, a *t* Student statistical assay was made (with $n - 1$ degrees of freedom; t_{table} υ: 8, α: 0.05) and there were no significant differences ($P \leqslant 0.05$) with 100% recovery, giving an appropriate accuracy.

3.4. Confirmation analysis

Liquid chromatography coupled to mass spectrometry (LC/MS) technique was applied to confirm the results obtained by LC–DAD analysis. With the advent of atmospheric pressure ionization techniques, electrospray (ESI) and atmospheric pressure chemical ionization (APCI), mass spectrometry has become a powerful analytical tool in phytochemistry due to its sensitivity, rapidity, and low levels of sample consumption (Stobiecki, 2000).

The application of a different column with a different stationary phase proves method selectivity. Fig. 2 represents naringenin ($m/z = 271$) (**A**), naringin ($m/z = 579$) (**B**), rutin ($m/z = 609$) (**C**) quercetin (**D**) ($m/z = 301$) and isoquercitrin (**E**) ($m/z = 463$) mass spectra. As with LC–DAD, five flavonoids were identified, including the presence

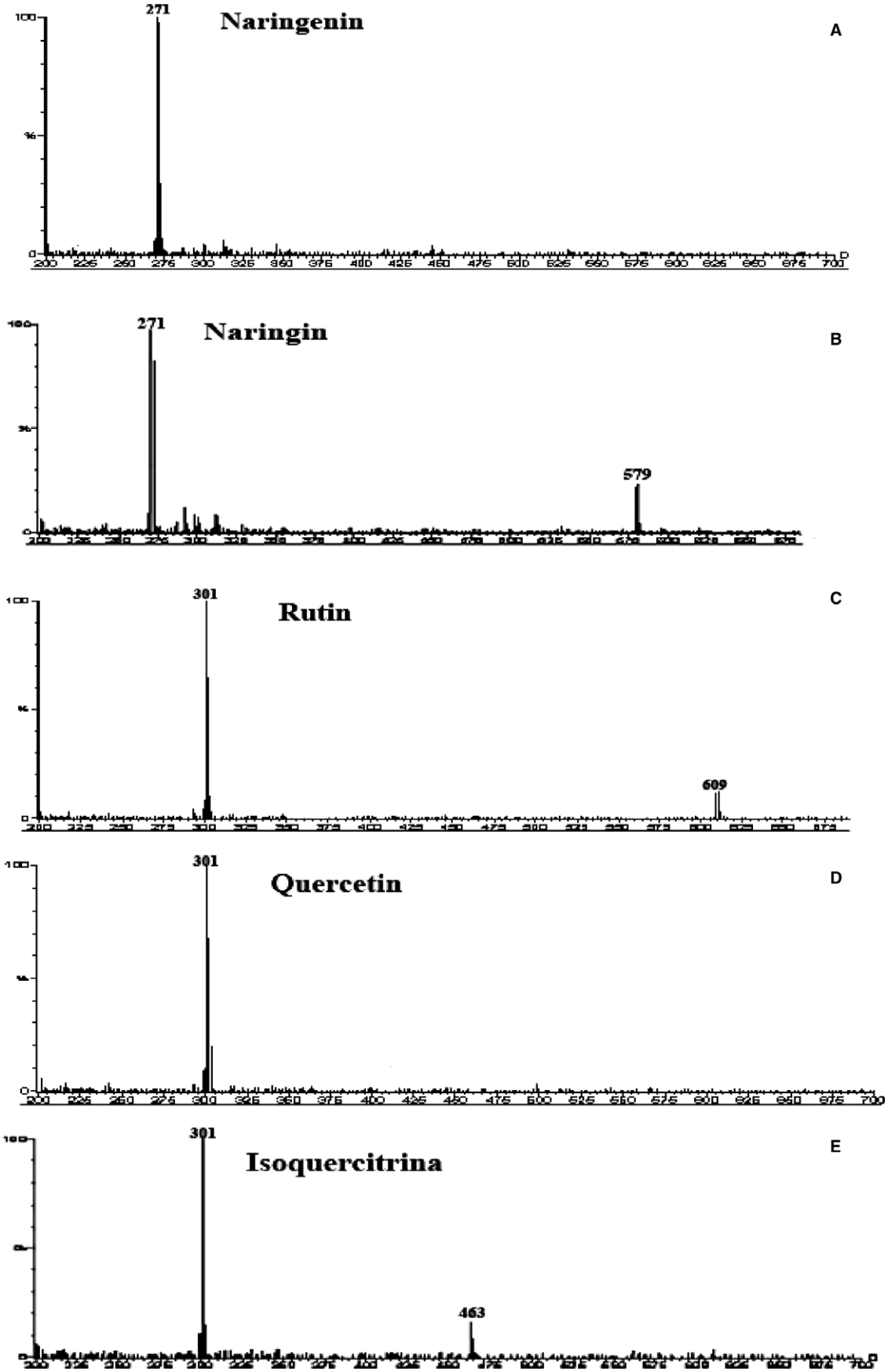

Fig. 2. Mass spectra of naringenin, naringin, rutin, quercetin and isoquercitrin in full scan mode.

of isoquercitrin which, to our knowledge, it has not been reported previously in *Citrus* fruit sample.

4. Conclusion

We have developed a direct and rapid method for quantification of flavonoids in a *Citrus* fruit extract, with a very simple sample treatment. Confirmation of results was achieved by applying LC/MS technique. To our knowledge this is the first study that reports isoquercitrin presence in a sample made of *C. paradisi*, *C. reticulata*, *C. sinensis*, and *C. aurantium.*. The method was validated generating separation and quantification of rutin, isoquercitrin, naringin, quercetin and naringenin in a single run without sample extraction. Due to the rapid sample preparation and simultaneous flavonoid determination, this method can be applied in quality assurance routine analysis for adulteration detection in Citrus concentrates and juices.

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References

Bazzano, L. A., He, J., Ogden, L. G., Loria, C. M., Vupputuri, S., Myers, L., et al. (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults: the first national health and nutrition examination survey epidemiologic follow-up study. *American Journal of Clinical Nutrition, 76*, 93–99.

Belajova, E., & Suhaj, M. (2004). Determination of phenolic constituents in citrus juices: method of high performance liquid chromatography. *Food Chemistry, 86*, 339–343.

Benavente-Garcia, O., Castillo, J., & Del Rio, J. A. (1993). Changes in neodiosmin levels during the development of *Citrus aurantium* leaves and fruits postulation of a neodiosmin biosynthetic pathway. *Journal of Agricultural and Food Chemistry, 41*, 1916–1919.

Benavente-Garcia, O., Castillo, J., Sabater, F., & Del Rio, J. A. (1995). Characterization of a *S*-adenosyl-L-methionine: eriodictyol 4′-*O*-methyltransferase from *Citrus aurantium*. *Plant Physiology Biochemistry, 33*(3), 263–271.

Berhow, M. A., & Vandercook, C. E. (1989). Biosynthesis of naringin and prunin in detached grapefruit. *Phytochemistry, 28*(6), 1627–1730.

Castillo, J., Benavente, O., & Del Río, J. A. (1992). Naringin and neohesperidin levels during development of leaves, flower buds, and fruits of *Citrus aurantium*. *Plant Physiology, 99*, 66–67.

Castillo, J., Benavente, O., & Del Rio, J. A. (1993). Hesperetin 7-*O*-glucoside and prunin in *Citrus* species (*C. aurantium* and *C. paradisi*). A study of their quantitative distribution in immature fruits and as immediate precursors of neohesperidin and naringin in *Citrus aurantium*. *Journal of Agricultural and Food Chemistry, 41*, 1920–1924.

Harborne, J. B., Marby, T. J., & Mabry, H. (1975). *The flavonoids*. London: Chapman and Hall.

He, X. g., Lian, L. z., Lin, L. z., & Bernart, M. W. (1997). High-performance liquid chromatography–electrospray mass spectrometry in phytochemical analysis of sour orange (*Citrus aurantium* L.). *Journal of Chromatography A, 791*, 127–134.

Justesen, U., Knuthsen, P., & Leth, T. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *Journal of Chromatography A, 799*(1–2), 101–110.

Kawaii, S., Tomono, Y., Katase, E., Ogawaa, K., & Yano, M. (1999). HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from *Citrus* juices. *Journal of Agricultural and Food Chemistry, 47*, 128–135.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*(9), 71–88.

Leuzzi, U., Caristi, C., Panzera, V., & Licandro, G. (2000). Flavonoids in pigmented orange juice and second-pressure extracts. *Journal of Agricultural and Food Chemistry, 48*, 5501–5506.

Liu, S., Manson, J. E., Lee, I. M., Stephen, R. C., Hennekens, C. H., Wilett, W. C., et al. (2000). Fruit and vegetable intake and risk of cardiovascular disease: the women's health study. *American Journal of Clinical Nutrition, 72*, 922–928.

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruits flavonoids*. Boca Raton, FL: CRC Press, Inc. pp. 76–80.

Mouly, P., Arzouyan, C., Gaydou, E., & Estienne, J. M. (1994). Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. *Journal of Agricultural and Food Chemistry, 42*, 70–79.

Mouly, P., Gaydou, E. M., & Auffray, A. (1998). Simultaneous separation of flavanone glycosides and polymethoxylated flavones in citrus juices using liquid chromatography. *Journal of Chromatography A, 800*(2), 171–179.

Ooghe, W. C., Ooghe, S. J., Detavernier, C. M., & Huyghebaert, A. (1994). Characterization of orange juice (*Citrus sinensis*) by flavanone glycosides. *Journal of Agricultural and Food Chemistry, 42*, 2183–2190.

Ortuño, A., García-Puig, D., Fuster, M. D., Pérez, M. L., Sabater, F., Porras, I., et al. (1995). Flavanone and nootkatone levels in different varieties of grapefruit and pummelo. *Journal of Agricultural and Food Chemistry, 43*, 1–5.

Pupin, A. M., Dennis, M. J., & Toledo, M. C. F. (1998). Flavanone glycosides in Brazilian orange juice. *Food Chemistry, 61*, 275–280.

Rouseff, R. L, Martin, S., & Youtsey, C. J. (1987). Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in *Citrus*. *Journal of Agricultural and Food Chemistry, 35*, 1027.

Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P., & Riceevans, C. (1995). Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Archives of Biochemistry and Biophysics, 322*(2), 339–346.

Sánchez-Rabaneda, F., Jáuregui, O., Lamuela-Raventós, R. M., Bastida, J., Viladomat, F., & Codina, C. (2003). Identification of phenolic compounds in artichoke waste by high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A, 1008*(1), 57–72.

Sánchez-Rabaneda, F., Jáuregui, O., Casals, I., Andrés-Lacueva, C., Izquierdo-Pulido, M., & Lamuela Reventós, R. M. (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry, 38*, 35–42.

Stobiecki, M. (2000). Application of mass spectrometry for identification and structural studies of flavonoid glycosides. *Phytochemistry, 54*(3), 237–256.

Vrhovsek, U., Rigo, A., Tonon, D., & Mattivi, F. (2004). Quantitation of polyphenols in different apple varieties. *Journal of Agricultural and Food Chemistry, 52*, 6532–6538.