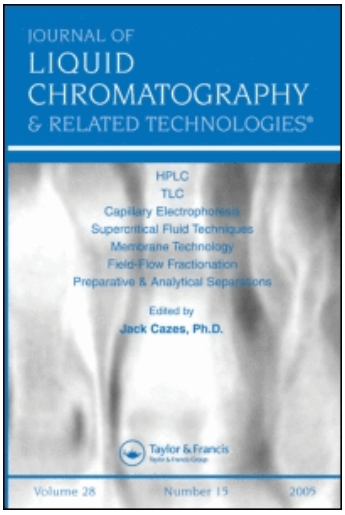

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Determination of Flavanones in Citrus Byproducts and Nutraceutical Products by a Validated RP-HPLC Method

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Abstract: The aim of this work was to develop and validate a specific reversed phase high performance liquid chromatographic method (HPLC) using diode array detection (DAD) for the determination of flavanones, which is the dominant flavonoid class in the genus citrus. The procedures and criteria used for validation of the method including sensitivity, linearity, repeatability, recovery, and robustness were carried out following the EURACHEM guidelines.

The validated RP-HPLC-DAD method was applied for the determination of the mainly flavanone glycosides contained in dried blood orange peel and dried lemon peel, and for the quantitation of flavanone glycosides and hesperetin in nutraceutical products.

Keywords: Citrus byproduct, Flavanones, Flavonoids, HPLC-DAD, Method validation, Nutraceutical tablets

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INTRODUCTION

Byproducts of blood orange fruit and, more in general, of citrus fruits processing industries represent a serious problem, considering that they constitute a severe environmental problem. However food processing industry byproducts can often be upgraded to a higher value as a source of useful products.[1] In the case of citrus, wastes are promising sources of phenolic compounds having beneficial biological activities in humans.[2–4]

Flavanone glycosides are a group of flavonoids distributed in citrus species that exhibit a wide variety of biological effects in mammalian organisms. Flavonoids are common components of the human diet[5] and the interest in these classes of compounds is due to their pharmacological activity as radical scavengers related to their chemical structure.[6] Antioxidant activity of flavonoids has attracted much attention in the prevention of atherosclerosis and thrombosis by protecting low density lipoprotein (LDL) against oxidative damage, as well as by lowering the cytotoxicity of oxidized LDL and platelet aggregation.[7,8] Consequently, consumption of polyphenolic flavonoids in the diet is inversely associated with morbidity and mortality from coronary heart disease.[9–11]

In addition, flavanones, such as hesperidin, hesperetin, and naringin, which are naturally occurring citrus flavonoids, have recently received considerable attention, with particular interest in the use of these phenolic compounds as hypocholesterolemic,[12] as well as anti-cancer compounds.[13] In the last years, much interest has centred on the role of these flavonoids in regards to the above mentioned properties and they have been proposed as possible ingredients of nutraceuticals supplements.

Flavanones are usually identified by standard RP-HPLC techniques. Most LC methods used gradient elution of the mobile phase with detection being carried out by spectrophotometry,[14–16] or photodiode array detection,[15–17] which emerged as the most valuable tool in the preliminary identification of these compounds. More recently, characterization and identification of flavanone glycosides in citrus based products was performed by HPLC-MS,[18] as well as involving the use of capillary electrophoresis.[19]

The aim of this work was the development and the validation of a rapid and reliable reversed phase liquid chromatographic method coupled with photo-array detection (DAD) for the identification and determination of the flavanone glycosides and hesperitin, contained in citrus byproducts, in which the dried peels represented the mix of seeds, pulp, and deoiled flavedo, after essential oil and juice extraction. These citrus byproducts could be considered as a source of flavonoid based ingredients for nutraceutical food and pharmaceutical industries. Furthermore, the validated method was successfully applied to the

simultaneous determination of narirutin, naringin, hesperidin and hesperetin in commercial available nutraceutical products.

EXPERIMENTAL

Materials

All reagents (from Carlo Erba, Milan, Italy) were of analytical reagent grade or HPLC grade, as required. Flavanone glycosides narirutin, hesperidin, naringin, eriocitrin, neoeriocitrin, neohesperidin, and the aglycon hesperetin used as standards were supplied by Extrasynthese (Genay, France). All other chemicals and solvents were of analytical reagent grade and were used without further purification. All sample solutions were filtered through a Nylon 66, 0.45 μm single use membrane filter (Millipore, Bedford, MA, USA). Dried blood orange peel and dried lemon peel were kindly provided by Ortogel SpA (Caltagirone, (CT), Italy). Fresh blood orange and lemon fruits, as well as nutraceutical tablets, were purchased from the local market.

Equipment

The whole HPLC system consisted of a Model SCL-10AVP system controller, equipped with a solvent delivery unit Model LC-10ADVP, an online vacuum membrane degasser Model DGU-14A, a column oven Model CTO-AS10VP, a photodiode array detector UV-Vis Model SPD-10AVP, in conjunction with a LC workstation Model Class VP 5.3 (all from Shimadzu, Milan, Italy). The column employed in the experiments was a Luna 5 μm C18 (2) (250 × 2.0 mm I.D.) from Phenomenex (Torrance, CA, USA). A column Luna C18 (2) 5 μm, 30 × 2.0 mm I.D. guard cartridge system was used to safeguard the analytical column. The temperature of the column oven was maintained at 30°C. The samples were introduced onto the column via a Rheodyne Model 9125 nonmetal (peek) injection valve with a peek 5 μL sample loop.

Chromatographic Conditions

The mobile phase consisted of a gradient of solvents; A was 2-propanol-acetonitrile-water (6:10:84, *v*/*v*/*v*) containing 0.5% formic acid, and solvent B consisting of 2-propanol-acetonitrile (70:30, *v*/*v*). Before use, all mobile phases were filtered with a Nylon 66 membrane filter (0.45 μm, from Millipore, Bedford, MA, USA) and degassed for 10 min in an

ultrasonic bath. After testing different gradients, flow velocities, and column temperatures, the gradient was set as reported in Table 1.

The flow rate was 0.2 mLmin^{-1} and the temperature of the column oven was set at 30°C. The flavanone compounds and hesperetin were detected at 283 nm.

Identification of Constituents and Peak Purity

Assignments of the flavonoid peaks in the HPLC chromatograms of the peel, citrus juices, and nutraceutical extracts were identified on the basis of their retention time (t_R) values and UV spectra by comparison with those of the isolated compound in the standard solution. Peak identity was also confirmed by spiking the extracts with pure standards (standard addition method). The peak purity test was performed using a photodiode array detector coupled to the HPLC system, and comparing the UV spectrum of each peak with those of authentic reference samples. Absorbance spectra were recorded between 200 and 400 nm and collected for all peaks.

Standard Preparation

Stock solutions of the flavanone glycosides were prepared by dissolving a weighted amount of the flavonoid in a small amount of N,N-dimethylformamide (DMF) and diluted with enough methanol to keep the DMF concentration at 10%. The stock solutions were stored at 18°C until used in dark amber glass recipients, and appeared stable during the period of this study. Working standards were prepared fresh daily by making the appropriate dilution with the chromatographic mobile phase.

Table 1. HPLC elution gradient program

Elution time (min)	A (%)	B (%)	Comment
0	100	0	Injection, acquisition start
20	94	6	End first gradient step
26	91	9	End second gradient step
26.1	50	50	Start isocratic step
35	50	50	End isocratic step

Eluent A = 2-propanol-acetonitrile-water (6:10:84, *v*/*v*/*v*) containing 0.5% formic acid.
Eluent B = 2-propanol-acetonitrile (70:30, *v*/*v*).

The diode array detector was set at 283 nm, while UV spectra (DAD) were recorded in the range 200–400 nm. The UV spectra of each peak were computer normalised and the plots were superimposed. Peaks were considered to be homogeneous when there was exact correspondence among the corresponding spectra (match factor >0.995).

Sample Preparation

Extraction of Flavanone Compounds from Citrus Byproducts and Nutraceuticals

Flavanone compounds were extracted from citrus peel powder and nutraceuticals, which were dried at constant weight at 70°C. The dried samples were further ground to a fine powder and extracted three times with a mixture of methanol-DMSO (9:1, *v*/*v*) for 90 min in a ratio of 20 mg of dry weight mL^{-1}. The resulting extracts were concentrated at 240 mbar pressure in a roto-evaporator (Büchi, Switzerland) at 35°C until dryness and then resuspended in methanol to the subsequent HPLC analyses. Dried commercial pellets were powdered and extracted in a similar manner.

Extraction of Flavanone Compounds from Lemon Juice

Samples from lemon juice were prepared by fruits, which were peeled and carefully squeezed by hand to avoid contamination of the juices by components in the peel, then, at 5 mL of juice were added 5 mL of DFA, and the resulting solutions were filtered through a syringe filter (0.45 μm) and aliquots (5 μL) were subjected to HPLC analysis.

Calibration Curves, Limits of Detection and Limits of Quantitation

Quantification was based on external standard method. The assay linearity was determined by analysis of six different concentrations of the standard solutions. Each level of concentration was prepared in triplicate. The standard curves were obtained by plotting peak area (y) vs. nominal concentration x ($\mu g mL^{-1}$) of each compound and were fitted to the linear regression. Concentrations of these marker substances in samples were calculated from this regression analysis.

The limit of detection (LOD) was defined as three times the standard deviation of the blank values (S_b) divided by the slope of the calibration curves, whereas the limit of quantification (LOQ) was defined as $10S_b$ divided by the slope of the calibration curve.[20]

Recovery Tests

The accuracy tests were carried out by spiking known contents of the mixed standard solution into the known concentration of the analyzed samples and assessed by analyzing three different spiking concentrations of analytes in triplicate replications. The percent recoveries for the analytes were defined as mean (found concentration/actual concentration) $\times$ 100.

Repeatability

Measurements of intra- and inter-day variability were utilised to determine the repeatability of the method. The intra-day repeatability was examined on six individual samples in 1 day, and inter-day repeatability was determined for 3 independent days. The relative standard deviation (RSD) was calculated as a measurement of method repeatability.

Robustness

The robustness of the method was evaluated by analyzing the system suitability standard and evaluating system suitability parameter data after varying, individually, the HPLC pump flow rate ($\pm$5%) and column compartment temperature ($\pm$2°C).

RESULTS AND DISCUSSION

Sample Extraction Optimization

In order to obtain quantitative extraction, variables involved in the procedure such as solvent, extraction method, and extraction time were optimized. Pure methanol and methanol-DMSO solutions at different ratios were tried as the extraction solvent. The best solvent was found to be a mixture of methanol-DMSO 9:1 (v/v) that allowed complete extraction of all the flavanone compounds and hesperetin in high yield. Ultrasonic extraction was compared with refluxing. It was found that ultrasonic extraction at room temperature was simpler and more effective for extraction of flavonoids with little impurity. Hence, the ultrasonic bath extraction was chosen as a preferred method. The influence of the extraction time on the efficiency of extraction was also investigated, in which powdered samples were extracted three times with methanol-DMSO 9:1 (v/v) for 30, 60, 90, and 120 min, respectively. The results suggested that

the highest amount of analytes were obtained with the extraction time of 60 min. After extraction, the residue was further extracted with methanol-DMSO 9:1 (v/v) for an additional 60 min, and almost no flavanone glycosides such as narirutin, hesperidin, naringin, eriocitrin, neoeriocitrin, neohesperidin, and the aglycon hesperetin were detected by HPLC. Therefore, the dried peels were further ground to a fine powder and extracted as above reported. Dried commercial pellets were powdered and extracted in a similar manner.

Method Development and Optimization

In a previous work we developed a simple isocratic RP HPLC method for the rapid and direct determination of the citrus flavonoids, neoeriocitrin, narirutin, hesperidin, naringin, neohesperidin, in some commercially available nutraceuticals with good reproducibility and accuracy.[21] However, each isocratic development was unsuitable for the satisfactory separation of these flavanone glycosides with the aglycon hesperetin. The first part of the present study was aimed at developing a chromatographic system capable of eluting and resolving the compounds of our interest by the simultaneous increase of the column peak capacity and the reduction of the analysis time.

The chromatographic conditions described in this method were achieved after investigating different narrow-bore C_{18} or C_8 reversed phase columns and several mobile phases consisting of mixtures of acetonitrile, methanol, or 2-propanol and water in different ratios. Regarding the selection of the reversed phase column and the mobile phase, optimum chromatographic separation of eriocitrin, neoeriocitrin, narirutin, hesperidin, naringin, neohesperidin, and the aglycon hesperetin was achieved using a narrow bore C_{18} reversed phase column and eluting by the step gradient reported in Table 1, in which the strength of the mobile phase was abruptly increased during the elution of the analytes. The addition of formic acid in the solvent system, which suppresses the ionization of phenol groups, lead to better separation of all six flavonoid glycosides and the aglycon hesperetin. The separation was further improved when column temperature was increased to 30°C. The developed method has shown good resolution for all compounds within 30 min and a typical chromatogram is shown in Figure 1 (A). Compound identification was done by match of retention time between the standard solutions and the sample. DAD detection was employed at wavelength range of 200–400 nm to investigate the UV spectra of the seven flavonoids. It was found that 283 nm is the best wavelength for the detection because almost all the investigated constituents have the maximum absorption there. Figure 1 shows the chromatographic profiles of the

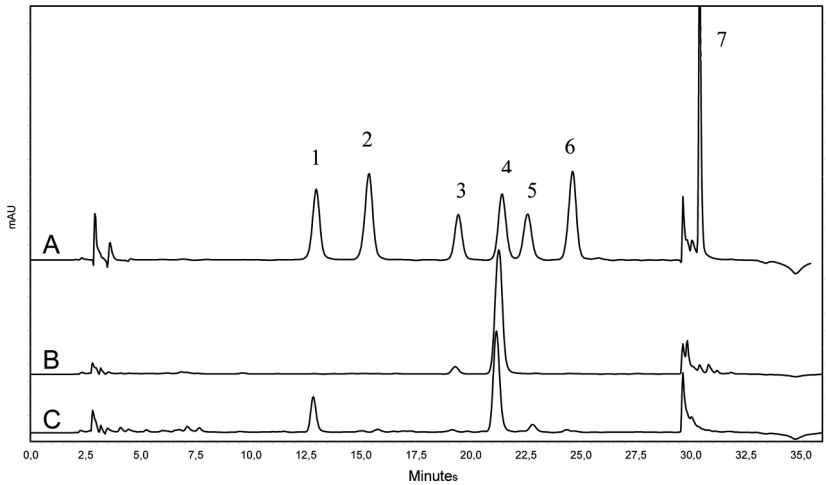

Figure 1. (A) HPLC-DAD analysis of a standard solution of flavanones and hesperitin. Chromatographic conditions as reported in section 2.3. Peak identification: 1) eriocitrin, 2) neoeriocitrin, 3) narirutin, 4) hesperidin, 5) naringin, 6) neoesperidin, 7) hesperetin. (B) Chromatographic profiles of an extract of dried blood orange peel and (C) of an extract of lemon peel.

methanol-DMSO extracts from a dried blood orange peel (B) and a dried lemon peel (C).

Method Validation and Quantitation

The validation process of the optimized HPLC-DAD method was carried out following the EURACHEM guidelines.[20]

A mixture of flavanone glycosides, eriocitrin, neoeriocitrin, narirutin, hesperidin, naringin, neohesperidin, and the aglycon hesperetin, in a standard solution prepared as reported in the experimental part was analyzed six times sequentially at two concentration levels 15 and 35 μgmL^{-1}; this operation was repeated over 3 days. Data regarding R.S.D.s for the peaks area and retention times are summarized in Tables 2 and 3.

Linear regression analysis for all six flavonoid glycosides and the aglycon hesperetin was performed by the external standard method and the obtained results are reported in Table 4 showing a good linear relationship between the peak area (y) and the concentration (x, expressed as μgmL^{-1}) for all the analyzed compounds over the range tested. % R.S.D. values for the slope of the calibration curves for all the flavonoid glycosides and the aglycon hesperetin were ranging from 2.15% to 2.9%. Limits

Table 2. Area precision: Intra-day repeatability (n = 6) and intermediate precision (n = 12) of the method

Analyte	Concentration level (μg/mL)	Intra-day repeatability		Intermediate precision	
		$X_m \pm$ S.D.	R.S.D. (%)	$X_m \pm$ S.D	R.S.D. (%)
Eriocitrin	15	589.32 ± 15.32	2.6	587.57 ± 14.69	2.5
	35	1375.09 ± 15.13	1.1	1368.23 ± 26.00	1.9
Neoeriocitrin	15	688.18 ± 22.02	3.2	685.21 ± 19.87	2.9
	35	1605.75 ± 28.90	1.8	1623.01 ± 27.59	1.7
Narirutin	15	168.01 ± 4.70	2.8	171.15 ± 4.62	2.7
	35	389.01 ± 6.22	1.6	382.30 ± 4.21	1.1
Hesperidin	15	458.67 ± 12.38	2.7	457.71 ± 11.90	2.6
	35	1211.76 ± 16.96	1.4	1215.33 ± 20.66	1.7
Naringin	15	503.45 ± 14.60	2.9	511.54 ± 16.37	3.2
	35	1174.71 ± 21.14	1.8	1167.31 ± 29.18	2.5
Neoesperidin	15	475.12 ± 14.73	3.1	470.75 ± 13.65	2.9
	35	1020.55 ± 17.35	1.7	1018.17 ± 15.27	1.5
Hesperetin	15	355.32 ± 6.40	1.8	356.01 ± 6.76	1.9
	35	989.87 ± 7.92	0.8	999.25 ± 22.98	2.3

of detection (LOD) and quantitation (LOQ), determined, as reported in calibration curves and limit of detection and quantitation, are recorded in Table 4, indicating that the proposed HPLC method was sufficiently sensitive for the determination of flavanone glycosides and hesperetin in citrus byproducts and citrus extracts.

The precision of the extraction procedure was validated by repeating the extraction procedure on the same sample of a dried blood orange peel

Table 3. Intra-day (n = 6) and between day (n = 12) repeatability of retention time

Analyte	Intra-day repeatability		Intermediate precision	
	tr ± S.D.	R.S.D. (%)	tr ± S.D.	R.S.D.(%)
Eriocitrin	12.97 ± 0.15	1.16	12.98 ± 0.11	0.85
Neoeriocitrin	15.55 ± 0.24	1.54	15.42 ± 0.10	0.65
Narirutin	19.49 ± 0.26	1.33	19.51 ± 0.15	0.77
Hesperidin	21.43 ± 0.32	1.49	21.45 ± 0.22	1.02
Naringin	22.75 ± 0.29	1.27	22.53 ± 0.21	0.93
Neoesperidin	24.67 ± 0.37	1.50	24.80 ± 0.27	1.09
Hesperetin	30.47 ± 0.07	0.23	30.45 ± 0.04	0.13

Table 4. Analytical parameters of proposed method

Analyte	Calibration range (μgmL^{-1})	Calibration equation	R^2	LOD (μgmL^{-1})	LOQ (μgmL^{-1})
Eriocitrin	5–800	$y = 40.645x - 0.406$	0.9994	0.05	0.20
Neoeriocitrin	5–800	$y = 9.765x - 0.029$	0.9998	0.08	0.35
Narirutin	10–1600	$y = 49.281x - 0.105$	0.9995	0.05	0.20
Hesperidin	10–1200	$y = 36.554x - 0.588$	0.9999	0.05	0.25
Naringin	10–1200	$y = 37.514x - 0.808$	0.9992	0.08	0.35
Neoesperidin	10–1600	$y = 29.718x - 0.010$	0.9990	0.08	0.35
Hesperetin	10–1200	$y = 104.633x - 0.059$	0.9996	0.05	0.20

extract and a nutraceutical tablet. An aliquot of each extract was then injected and quantified. This parameter was evaluated by repeating the extraction in triplicate on 3 different days with newly prepared mobile phase and samples. The intra- and inter-day % RSD data of the repeated analysis ranged from 2.7 to 3.3, indicating the high level of precision of the method. To ensure the insensitivity of the HPLC method to minor changes in the experimental conditions it is important to demonstrate robustness of the method. None of the alterations, HPLC pump flow rate and column compartment temperature, caused a significant change in resolution between the studied compounds and the impurities, peak area, peak width, or theoretical plates.

Table 5 summarizes the contents of flavanone glycosides found in dried blood orange peel and dried lemon peel sampled from different batches, which were carried out at the same conditions in terms of temperature and drying time. The flavonoid concentrations in dried blood orange peel appear higher than in dried lemon peel and, in all analyzed samples, hesperidin was the major component. The profile of dried blood orange peel sample is characterized by the predominance of the two flavanones hesperidin and narirutin, whereas in the dried lemon peel samples, eriocitrin and hesperidin were the flavanones present. In lemon juice from Sicilian cultivars the hesperidin and eriocitrin concentrations was ranging from 115 to 310 mgL^{-1}, having the same 1:1 ratio in samples with lower overall flavanone glycoside values. These results are in agreement with those recently reported in literature,[22–24] considering that in our case cultivars of the analyzed lemons were not identifiable. However, considering that the highest concentrations of flavanones glycosides in citrus fruit occur in the peel, which represent roughly half of the fruit mass and that in the peel the solubility of hesperidin is nearly 1000 fold greater than its solubility in water,[25] the peel citrus processing byproducts represent a rich source of natural products important to human health and nutrition.[26]

Table 5. Contents of flavanone glycosides found in dried blood orange peel and dried lemon peel

Sample	Eriocitrin (g/kg) ± SD	Narirutin (g/kg) ± SD	Hesperidin (g/kg) ± SD	Naringin (g/kg) ± SD	Hesperidin/ Narirutin
Dbop (sample A)	nq	9.86 ± 0.1	35.67 ± 0.4	nq	3.62
Dbop (sample B)	nq	10.1 ± 0.3	37.59 ± 0.5	nq	3.72
Dbop (sample C)	nq	8.68 ± 0.3	27.90 ± 0.5	nq	3.21
Dbop (sample D)	nq	11.1 ± 0.2	38.82 ± 0.3	nq	3.50
Dlp (sample A)	3.15 ± 0.06	nq	8.92 ± 0.1	1.41 ± 0.05	nq
Dlp (sample B)	2.26 ± 0.02	nq	9.29 ± 0.2	1.48 ± 0.07	nq
Dlp (sample C)	3.32 ± 0.05	nq	9.43 ± 0.3	1.52 ± 0.07	nq
Dlp (sample D)	1.95 ± 0.05	nq	7.49 ± 0.2	1.39 ± 0.05	nq
Dlp (sample E)	3.03 ± 0.03	nq	7.57 ± 0.1	1.40 ± 0.06	nq

Dbop = dried blood orange peel; Dlp = dried lemon peel.
nq = not quantificable.

Table 6. Contents of flavanones found in commercially nutraceutical tablets

Sample	Narirutin (mg/g) ± SD	Hesperidin (mg/g) ± SD	Naringin (mg/g) ± SD	Hesperetin (mg/g) ± SD
Nutraceutical A	2.58 ± 0.06	51.71 ± 1.13	21.85 ± 0.32	293.86 ± 6.11
Nutraceutical B	4.66 ± 0.10	55.68 ± 1.11	8.69 ± 0.12	271.08 ± 5.42
Nutraceutical C	–	106.61 ± 2.01	173.92 ± 3.65	–
Nutraceutical D	–	218.75 ± 4.12	87.50 ± 3.18	43.75 ± 1.42
Nutraceutical E	–	215.87 ± 3.85	88.13 ± 2.76	44.24 ± 1.96

Similarly, flavanones appeared to be the major flavonoids present in the freshly squeezed juices obtained from blood oranges of Moro and Sanguinello varieties,[27] giving a ratio hesperidin/narirutin ranging from 3.4 to 3.7 similar to that found in dried blood orange peel, reported in Table 5.

To determine the recovery and to ensure the validity and reproducibility of the proposed methods, repeated injections (six injections, each of 5 μL) of the same sample of dried blood orange peel and dried lemon peel were used. These samples were prepared by addition of known amounts of standard flavonoids to exact weights of previously assayed citrus dried peel. The overall mean recoveries are reported in Tables 6–8.

Moreover, the developed and validated HPLC-DAD method was applied to identifying and quantifying flavonoids present in commercially available nutraceutical tablets containing narirutin, naringin, hesperidin, and hesperetin. The identification and quantification of the investigated compounds in different commercially nutraceutical tablets are reported in Table 9.

The recoveries of the flavonoids were determined by the method of standards addition as above reported, and the mean recoveries of the flavonoids were 98.1–101.8%, with R.S.D. values ranging from 1.2% to 2.6% ($n = 6$), indicating that the analyzed flavonoids were quantitatively recovered from the analyzed nutraceutical tablets.

Table 7. Recovery of standard addition in dried lemon peel

Analyte	Content (mg/g)	Amount added (mg/g)	Found (mg/g)	Recovery (%)	RSD (%)
Eriocitrin	2.291	0.06	2.350	98.3	2.07
		0.12	2.412	100.8	1.98
		0.18	2.478	103.9	2.27
Hesperidin	9.400	0.25	9.646	98.4	1.89
		0.50	9.889	97.8	2.15
		0.75	10.158	101.1	2.69

Table 8. Recovery of standard addition in dried blood orange peel

Analyte	Content (mg/g)	Amount added (mg/g)	Found (mg/g)	Recovery (%)	RSD (%)
Narirutin	9.982	0.25	10.234	100.8	2.38
		0.50	10.471	97.8	1.46
		0.75	10.725	99.1	2.54
Hesperidin	37.632	0.95	38.586	100.4	1.69
		1.90	39.512	99.1	2.18
		2.85	40.539	102.2	2.73

Table 9. Recovery of standard addition in the commercially nutraceutical tablet 1

	Content (mg/g)	Amount added (mg/g)	Found (mg/g)	Recovery (%)	RSD (%)
Narirutin	2.61	1.30	3.92	100.8	1.97
		2.60	5.27	102.3	2.00
		3.90	6.44	98.2	2.92
Hesperidin	52.52	26.0	78.23	98.9	2.26
		52.0	105.21	101.3	2.94
		78.0	129.94	99.2	2.71
Naringenin	21.93	11.0	32.86	99.4	2.80
		22.0	44.47	102.4	2.67
		33.0	54.63	99.1	2.57
Hesperitin	295.21	147.0	444.59	101.6	2.65
		294.0	584.57	98.5	2.21
		441.0	743.29	101.6	2.69

CONCLUSIONS

The present paper describes a simple and accurate HPLC-DAD method established for the determination of flavanone glycosides in dried blood orange and lemon byproducts. Phenolic compounds contribute to the intake of natural antioxidants in the human diets and agro-industrial byproducts such us dried blood orange peel and dried lemon peel have been explored as a source of natural antioxidants. The fine validation results showed that the proposed method could also be considered as a reliable, convenient, and sensitive quality control supplement for the routine determinations of flavonoids in nutraceutical tablets.

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