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Analytical Methods

Rapid analysis of phenolic acids in beverages by UPLC–MS/MS

Jiří Gruz, Ondřej Novák, Miroslav Strnad *

Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany ASCR, Šlechtitelů 11, CZ-783 71 Olomouc, Czech Republic

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ABSTRACT

A rapid method for qualitative and quantitative analysis of 17 phenolic acids (gallic acid, 3,5-dihydroxybenzoic acid, protocatechuic acid, chlorogenic acid, gentisic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, 3-hydroxybenzoic acid, 4-coumaric acid, sinapic acid, ferulic acid, 3-coumaric acid, 2-coumaric acid, salicylic acid and *trans*-cinnamic acid) in different beverages was developed using ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS). The analytes were detected in multiple reaction monitoring (MRM) mode and quantified using internal standards of deuterium-labelled 4-hydroxybenzoic (2,3,5,6-D4) and salicylic (3,4,5,6-D4) acids. Limits of detection (LODs) ranged from 0.15 to 15 pmol and the response was linear to 1000 pmol injected. Mean method precision of 4.4 RSD% (range, 2.0–9.1%) was obtained, and a mean accuracy (bias) of 1.1% (range, −14.5 to 17.5%). The applicability of this analytical approach was confirmed by the successful analysis of real samples of white wine, grapefruit juice and green tea infusion. Twelve phenolic acids were determined in the analysed beverages, in concentrations ranging from 40.8 to 9046 μg L^{-1} and the results were compared to data from previous studies.

1. Introduction

Phenolic acids (PHAs) are widespread plant secondary metabolites, virtually derived from benzoic and cinnamic acids. They commonly occur as free acids and their esters, glycosides and bound complexes, and they are known to play important roles in plant resistance to pathogens and herbivores, allelopathy, oxidative stress and plant growth regulation. Furthermore, they have been shown to have beneficial effects on human health (Bazzano et al., 2002). Hence, there is considerable interest in PHAs. Most of the prophylactic activities of PHAs have been ascribed to their antioxidant, antimutagenic, antiproliferative and antimicrobial properties (Aziz, Farag, Mousa, & Abo-Zaid, 1998; Birosova, Mikulasova, & Vaverkova, 2005; Kampa et al., 2004; Lodovici, Guglielmi, Meoni, & Dolara, 2001). However, there are considerable uncertainties regarding their bioavailability since, as mentioned, diverse conjugated forms of PHAs are present in plants (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). PHAs also affect the organoleptic properties (flavour, astringency, color) and oxidative stability of plant-derived food products, so they are also continually investigated by food technologists (Luzia et al., 1997).

The analytical methods used for determining PHAs are usually based on reverse-phase high-performance liquid chromatography (HPLC), or another separation technique such as gas chromatography or capillary electrophoresis, followed by ultraviolet (UV), electrochemical (EC), fluorescence (F) or mass spectrometric (MS) detection (Amakura, Okada, Sumiko, & Tonogai, 2000; Ayaz, Hayirlioglu-Ayaz, Gruz, Novak, & Strnad, 2005; Jirovsky, Horakova, Kotoucek, Valentova, & Ulrichova, 2003; Rodriguez-Delgado, Malovana, Perez, Borges, & Montelongo, 2001). Most of these methods require complex extraction, pre-concentration, hydrolysis and/or derivatization procedures to allow quantification but, unfortunately, such steps often lead to the oxidation/degradation of analysed PHAs. It has been reported that adding antioxidants like ascorbic acid to extraction solvents can prevent some of those undesirable effects (Nardini et al., 2002), but there are major uncertainties regarding optimal doses since higher amounts of ascorbic acid seem to have pro-oxidant properties (Nuutila, Kammiovirta, & Oksman-Caldentey, 2002).

"Ultra performance liquid chromatography" (UPLC) is an advanced form of liquid chromatography (LC) in which narrow-bore columns packed with very small particles (1.7 μm) and mobile phase delivery systems operating at high back-pressures are used. The major advantages of UPLC over conventional high-performance liquid chromatography (HPLC) are improved resolution, shorter retention times and higher sensitivity (Yu, Little, Plumb, & Smith, 2006). Coupling UPLC with electrospray ionisation (ESI) tandem mass spectrometry (MS/MS) thus offers a potent alternative to conventional HPLC–MS/MS. The UPLC–MS/MS system has been applied in several recent published analyses of plant/food products, e.g. for determining orientin-derived flavonoids in flowers of *Trollius ledibouri* (Li et al., 2006), and for analysing aristolochic acids (natural nephrotoxic compounds) of *Aristolochia* plants, which are used in Chinese herbal medicine (Jacob, Smith, & Legido-Quigley, 2007).

* Corresponding author. Tel.: +420 585634850; fax: +420 585634870.
E-mail address: strnad@prfholnt.upol.cz (M. Strnad).

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In this paper we present a validated method for the rapid determination of PHAs in different beverages based on UPLC–MS/MS. This is the first time, to our knowledge, that a UPLC-MS/MS method for determining PHAs in beverages or any other food products has been described.

2. Materials and methods

2.1. Chemicals

Standards of gallic, 3,5-dihydroxybenzoic, protocatechuic, chlorogenic (5-caffeoylquinic), gentisic, 4-hydroxybenzoic, caffeic, vanillic, syringic, 3-hydroxybenzoic, 4-coumaric, sinapic, ferulic, 3-coumaric, 2-coumaric, salicylic and *trans*-cinnamic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Deuterium-labeled standards of 4-hydroxybenzoic acid (2,3,5,6-D4) and salicylic acid (3,4,5,6-D4) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). Formic acid and methanol used for preparing mobile phases were purchased from MERCK (Darmstadt, Germany). Deionized water was prepared using a Simplicity 185 system (Millipore, Bedford, MA, USA).

2.2. Preparation of standard solutions

Standard solutions were first prepared in methanol at a concentration of 10^{-3} mol L^{-1} and then serially diluted to working concentrations. Internal standards (IS) of deuterium-labeled 4-hydroxybenzoic and salicylic acids were added to a final concentration of 10^{-5} mol L^{-1} in all standard solutions.

2.3. Sample preparation

White wine (Ryzlink Rynsky, Vino Valtice, Valtice, Czech Republic), grapefruit juice (Grapefruitova Stava, Linea Nivnice, Nivnice, Czech Republic) and leaves of green tea (Gunpowder, Jemca, Jemnice, Czech Republic) were purchased from a retail store in Olomouc (Czech Republic). An infusion of the tea was prepared by incubating 20 mg of dry leaves with 1.5 mL of distilled water in an eppendorf tube for 10 min at 80 °C. IS were added to all beverage samples, as described for the standard solutions. Before use, all solutions were filtered by centrifugation (3500 rpm, 5 min) through 0.2 μm nylon membrane microfilters (Micro-Spin™, Alltech, Deerfield, IL, USA).

2.4. UPLC–MS/MS instrumentation and conditions

UPLC–MS/MS analyses were carried out using an ACQUITY Ultra Performance LC™ system (Waters, Milford, MA, USA) linked simultaneously to both a PDA 2996 photo diode array detector (Waters, Milford, MA, USA) and a Micromass Quattro *micro*™ API benchtop triple quadrupole mass spectrometer (Waters MS Technologies, Manchester, UK), equipped with a Z-spray electrospray ionisation (ESI) source operating in negative mode. MassLynx™ software (version 4.0, Waters, Milford, MA, USA) was used to control the instruments, and for data acquisition and processing.

Sample solutions were injected into a reversed phase column (BEH C_8, 1.7 μm, 2.1 × 150 mm, Waters, Milford, MA), which was maintained at 30 °C. The mobile phase consisted of the following 9.5-min sequence of linear gradients and isocratic flows of solvent B (acetonitrile) balanced with aqueous 7.5 mM HCOOH (solvent A) at a flow rate of 250 μL min^{-1}: 5% B for 0.8 min, 5–10% B over 0.4 min, isocratic 10% B for 0.7 min, 10–15% B over 0.5 min, isocratic 15% B for 1.3 min, 15–21% over 0.3 min, isocratic 21% B for 1.2 min, 21–27% B over 0.5 min, 27–50% B over 2.3 min, 50–100% B over 1 min, and finally 100–5% B over 0.5 min. At the end of this sequence the column was equilibrated under initial conditions for 2.5 min. The pressure ranged from 4000 to 8000 psi during the chromatographic run. The effluent was introduced into a PDA detector (scanning range 210–600 nm, resolution 1.2 nm) and subsequently into an electrospray source (source block temperature 100 °C, desolvation temperature 350 °C, capillary voltage 2.5 kV, cone voltage 25 V). Argon was used as collision gas (collision energy 16 eV) and nitrogen as desolvation gas (500 L h^{-1}). The retention windows used for quantification were as follows: 0.00–3.00 min; 3.00–4.25 min; 4.25–4.70 min; 4.70–5.15 min; 5.15–5.80 min; 5.80–6.30 min; 6.30–7.30 min; 7.30–7.80 min; 7.80–8.90 min; 8.90–10.00 min. Individual ion transitions and dwell times are summarised in Table 1.

2.5. Identification and quantification

PHAs and deuterium-labeled internal standards were detected in multiple reaction monitoring (MRM) mode using mass-to-

Table 1
Retention time, dwell time, MRM transition, fragments, limit of detection (LOD), and linear relation of 17 PHAs

Compound	Time (min)	Dwell time (s)	MRM (*m/z*)	Fragments	LOD (pmol/inj)	Slope	*c*	r^2
Gallic acid	2.59 ± 0.01[a]	0.5	169 > 125		3	0.142	−0.854	0.9990
3,5-Dihydroxybenzoic acid	3.59 ± 0.01	0.5	153 > 109		1	0.236	0.151	1.0000
Protocatechuic acid	3.86 ± 0.01	0.5	153 > 109		1	0.285	0.452	0.9994
Chlorogenic acid	4.49 ± 0.01	0.5	353 > 191	217	1.5	0.296	4.235	0.9983[b]
Gentisic acid	4.79 ± 0.01	0.15	153 > 109		10	0.114	−3.301	0.9969
4-Hydroxybenzoic acid	4.99 ± 0.01	0.15	137 > 93		1	0.671	0.907	0.9999
Caffeic acid	5.31 ± 0.01	0.15	179 > 135	161	3	0.532	−5.687	0.9990
Vanillic acid	5.44 ± 0.01	0.15	167 > 108	152, 123	15	0.056	−0.548	0.9994
Syringic acid	5.55 ± 0.01	0.15	197 > 123	182, 167	15	0.042	0.036	0.9997
3-Hydroxybenzoic acid	5.96 ± 0.00	0.5	137 > 93		1	0.300	0.133	1.0000
4-Coumaric acid	6.62 ± 0.01	0.15	163 > 119		1	1.026	−3.298	0.9979
Sinapic acid	6.92 ± 0.01	0.15	223 > 164	208, 149	10	0.083	−0.836	0.9995
Ferulic acid	7.04 ± 0.02	0.15	193 > 134	178, 149	5	0.322	−0.863	0.9996
3-Coumaric acid	7.57 ± 0.01	0.5	163 > 119		0.15	1.536	1.956	0.9998
2-Coumaric acid	8.13 ± 0.00	0.15	163 > 119		0.75	0.841	−1.485	0.9995
Salicylic acid	8.25 ± 0.02	0.15	137 > 93		1.5	0.764	1.228	0.9987
Trans-cinnamic acid	9.19 ± 0.01	0.5	147 > 103		10	0.051	−0.966	0.9975
4-Hydroxybenzoic acid (IS)[c]	4.95 ± 0.01	0.15	141 > 97					
Salicylic acid (IS)[c]	8.21 ± 0.01	0.15	141 > 97					

[a] Values are means ± SD (n = 6) of inter-day assays.
[b] Calibration range of 5–800 pmol was used.
[c] Internal standards of deuterium-labeled 4-hydroxybenzoic (2,3,5,6-D4) and salicylic (3,4,5,6-D4) acids.

charge (m/z) transitions of precursor and product ions. PHAs were identified by matching retention times and mass spectral data with those of the calibration standards. Analyte concentrations in the samples were derived from analyte:average internal standard peak area ratios, using the following equation:

$$(\text{analyte concentration}) = \text{IS}_c \times \text{slope} \times \text{A} \times 2(\text{IS}_1 + \text{IS}_2)^{-1} + c \quad (1)$$

where IS_c is the concentration of IS in the sample; A is the peak area of the analyte; IS_1 is the peak area of 4-hydroxybenzoic acid (2,3,5,6-D4); IS_2 is the peak area of salicylic acid (3,4,5,6-D4); slope and c are linear regression parameters, as listed in Table 1.

3. Results and discussion

3.1. Optimisation of chromatographic separation and MS/MS conditions

We used similar mobile phases during the UPLC separation optimisation to those we used in a previous study on the HPLC-based separation, characterisation, and quantification of phenolic acids in blueberry fruits (Ayaz et al., 2005), but the previously used phenyl-hexyl stationary phase was replaced by an octyl (C8) phase, due to its commercial availability in UPLC columns. Generally, retention times increased with increases in the HCOOH concentration (and hence reductions in pH) in solvent A, but some analytes were more affected than others, depending on their pK_a values. This phenomenon was successfully exploited to shift the retention time of salicylic acid, the most sensitive acid, away from that of 2-coumaric acid. Apart from this consideration, the HCOOH concentration was kept as low as possible to ensure satisfactory ionisation. With the optimised programme of gradients and isocratic steps we obtained adequate separation of the seventeen standards, including baseline separation of all isomers with 7.5 mM HCOOH (Fig. 1). The separation was six times faster than conventional HPLC separation with the same analytes, and approximately three times faster than HPLC using a high-throughput monolithic column (Ayaz et al., 2005; Castellari, Sartini, Fabiani, Arfelli, & Amati, 2002). Furthermore, it consumed ca. 80% less organic solvent than the corresponding HPLC method.

The MS/MS parameters like ion mode, capillary voltage, cone voltage, collision energy and dwell times were optimised to maximise the overall sensitivity, as estimated from the peak areas of the analytes. The optimised MS/MS parameters and MRM transitions were found to be consistent with those previously described in an analysis of aromatic acid metabolites derived from dietary polyphenols, except that we preferred to use m/z 108 rather than m/z 123 product ions of vanillic acid for quantification (Gonthier, Rios, Verny, Remesy, & Scalbert, 2003). Generally, deprotonated PHAs ($[M-H]^-$) produce a typical fragmentation pattern after collision-induced dissociation, characterised by the loss (−44) of a carboxylic acid group, providing an anion of $[M-H-COO]^-$. In addition, we detected an unusual loss of methyl radicals (−15) from methoxylated derivatives of the PHAs we examined, leading to a very stable anion radical structure of $[M-H-CH_3]^{\cdot -}$. Both types of fragmentation could occur simultaneously, producing a distinct anion radical of $[M-H-COO-CH_3]^{\cdot -}$, as shown for ferulic acid in Fig. 2. In accordance with our findings, Justesen (2001) noted analogous collision-induced fragmentation of deprotonated (poly-)methoxylated flavonoids, distinguished by specific losses of methyl radicals from their respective $[M-H]^-$ anions. Such knowledge of unusual fragmentation patterns could be helpful when monitoring plant extracts for unknown compounds.

Individual MRM transitions used for quantifying PHAs are listed in Table 1. Based on the excellent stability of the retention times (mean of SD, 0.01 min; Table 1), the chromatographic run was split into 10 retention windows with prolonged dwell times, which increased the sensitivity of the analysis.

Due to the high selectivity of MRM mode, the present UPLC–MS/MS method does not require any type of sample pre-concentration prior to injection, although different purification steps, including solid-phase extraction, were often necessary to remove interfering compounds (García, Grande, & Gándara, 2004). This could be considered to be major advantage over conventional methods because pre-concentration steps require additional time to perform and they commonly result in substantial losses of analytes.

3.2. Method validation

After the separation and MS/MS conditions had been optimised, the method was validated as follows. The limits of detection (LODs), based on three times the signal-to-noise ratios, were calculated by repeatedly injecting 5 μL portions of diluted standard solutions. Obtained LODs were in the range of 0.15–15 pmol injected, comparable to those obtained by the conventional HPLC–MS system and an order a magnitude lower than typical HPLC-UV values (Ayaz et al., 2005; García et al., 2004). The wide range of calculated LODs corresponds to the variability in physico-chemical properties of PHAs. In particular, LODs were negatively affected by the presence of methoxy groups in the analyte molecules, which led to extensive fragmentation, as described above. The linearity of the method was studied by injecting standard solutions with PHA

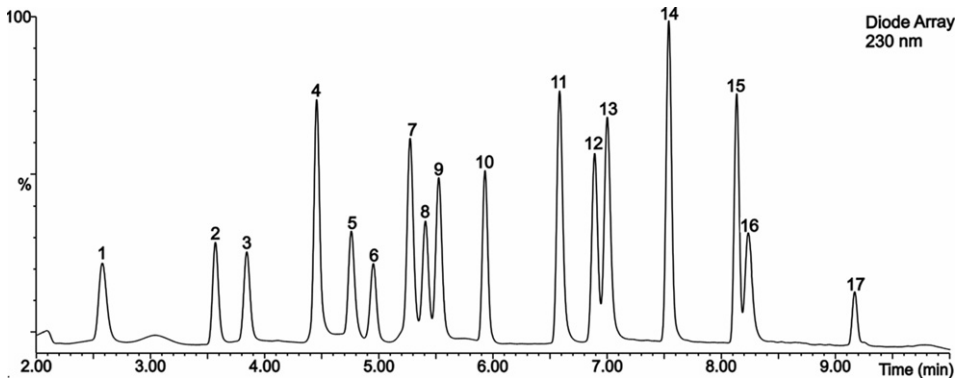

Fig. 1. UPLC–UV chromatogram of a mixture of the following 17 standard PHAs, detected at 230 nm: gallic acid (1), 3,5-dihydroxybenzoic acid (2), protocatechuic acid (3), chlorogenic acid (4), gentisic acid (5), 4-hydroxybenzoic acid (6), caffeic acid (7), vanillic acid (8), syringic acid (9), 3-hydroxybenzoic acid (10), 4-coumaric acid (11), sinapic acid (12), ferulic acid (13), 3-coumaric acid (14), 2-coumaric acid (15), salicylic acid (16) and *trans*-cinnamic acid (17).

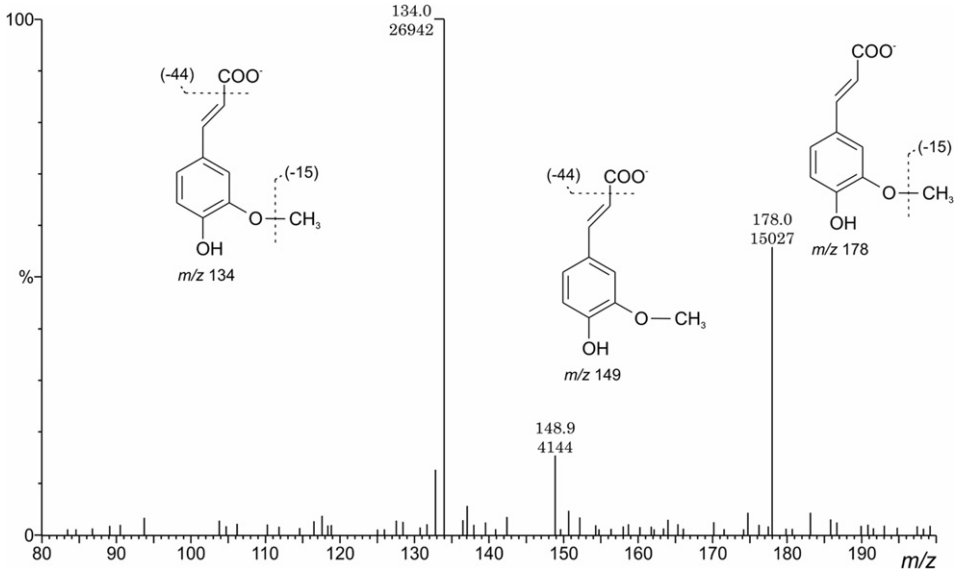

Fig. 2. ESI(−)-MS/MS product-ion spectrum of ferulic acid (*m/z* 193).

levels ranging from 0.1 to 3000 pmol. The response was linear up to 1000 pmol injected for all compounds, except chlorogenic acid, with R^2 values ranging from 0.9969 to 1.0000 (Table 1). In the case of chlorogenic acid, the response was linear in a slightly narrower range of 5–800 pmol injected.

The precision and accuracy of the method were evaluated by spiking green tea infusions with standards at final concentrations of 10 and 100 μmol L^{-1}. Method precision, expressed as relative standard deviation (RSD%), was estimated by measuring six replicates of each concentration. The mean precision was 4.4% (range 2.0–9.1%), and the mean accuracy (expressed as percentage bias) was 1.1%, ranging from −14.5 to 17.5%. In general, the accuracy tended to become worse when lower amounts of standards were added to green tea infusion.

3.3. White wine

Nine PHAs were identified and quantified in wine samples with the amounts ranging from 150 to 5826 μg L^{-1} (Table 2). Similar to results published by Castellari et al. (2002), we have found relatively high concentration (3675 μg L^{-1}) of gallic acid in wine samples. It has been shown that gallic acid can serve as a substrate for *Lactobacillus hilgardii*, the major cause of spoilage in wine, which metabolizes gallic acid into 4-hydroxybenzoic and protocatechuic acids (Alberto, Gomez-Cordoves, & Nadra, 2004). Both of these hydroxybenzoic acids were major components of the PHA complements in the wine samples we analysed, implying that concentration ratios of the mentioned PHAs could be used as markers of the progression of wine spoilage. Other degradation processes, such as the oxidation and browning of white wines, are also correlated with levels of hydroxycinnamic acids and flavanols. This could be related to the presence of caffeic or gallic acids, since concentrations of these substances ⩾40 mg L^{-1} have been shown to strongly inhibit oxidation of monoterpene alcohols, and thus could retard the disappearance of desirable aromas from white wines (Roussis, Soulti, & Tzimas, 2005). We found the total concentration of PHAs to be comparable (15 mg L^{-1}), suggesting that they could function in the proposed way. However, the amount of free PHAs appears to be insufficient to inhibit the growth of lactic acid bacteria in wine, since the growth of various strains of these organisms has been found to be only significantly reduced at PHA concentrations higher than 100 mg L^{-1} (Campos, Couto, & Hogg, 2003).

Table 2
Concentrations of PHAs in commercially available beverages

Compound	White wine (μg/L)	Grapefruit juice (μg/L)	Green tea (μg/L)
Gallic acid	3675 ± 84[a]	nd[b]	9046 ± 272
3,5-Dihydroxybenzoic acid	nd	nd	ta[c]
Protocatechuic acid	2516 ± 85	116 ± 3.4	556 ± 8.5
4-Hydroxybenzoic acid	5826 ± 64	74.3 ± 2.8	758 ± 12
3-Hydroxybenzoic acid	nd	nd	40.8 ± 14
Salicylic acid	335 ± 37	nd	476 ± 34
Chlorogenic acid	nd	847 ± 22	828 ± 93
Gentisic acid	773 ± 25	nd	nd
Vanillic acid	384 ± 51	nd	nd
Caffeic acid	1253 ± 17	233 ± 10	nd
4-Coumaric acid	303 ± 10	68 ± 1.7	127 ± 6.8
Ferulic acid	150 ± 29	153 ± 25	nd
Total	15,215	1491	11,832

[a] Values are means ± SD (n = 3).
[b] Not detected.
[c] Trace amounts.

3.4. Grapefruit juice

Phytochemical and pharmacological properties of grapefruit juice, including flavonoid composition, antimicrobial activity, antioxidant activity and fruit-drug interactions, were intensively studied in recent years. However, no satisfactory data on the content of free phenolic acids in grapefruit juices have been previously published, although Mattila, Hellstrom, and Torronen (2006) have analysed PHAs in grapefruit by HPLC-UV. In our analyses we unambiguously identified and quantified five PHAs in grapefruit juice, in amounts ranging from 68 to 847 μg L^{-1} (Table 2). In accordance with Mattila et al. (2006) these included caffeic, ferulic and 4-coumaric acids (233 μg L^{-1}, 153 μg L^{-1} and 68 μg L^{-1}). In addition, we found chlorogenic acid (847 μg L^{-1}) to be the major phenolic acid, which is consistent with data on various citrus fruits presented by Wang, Chuang, and Ku (2007). Our findings were compared with the cited results solely in terms of PHA profiles, because the cited literature does not provide detailed information on concentrations of free PHAs, or the data were obtained from unlikely processed material, e.g. freeze-dried fruits. However, it should be noted that the concentrations of ferulic and 4-coumaric acids we found in grapefruit juice were similar to previously reported

levels in blood orange juices (Fallico, Lanza, Maccarone, Asmundo, & Rapisarda, 1996).

3.5. Green tea

Eight PHAs were identified in green tea extracts, seven of which were quantified, and found to be present in amounts ranging from 40.8 to 9046 μg L^{-1} (Table 2; Fig. 3). Two of these had been previously detected by Mattila et al. (2006), in green tea extracts at comparable concentrations to those we found (gallic and 4-coumaric acids, at concentrations of 9046 and 127 μg L^{-1}, respectively). However, we also detected chlorogenic, 4-hydroxybenzoic, protocatechuic and salicylic acids at 828 μg L^{-1}, 758 μg L^{-1}, 556 μg L^{-1} and 476 μg L^{-1}, respectively. A particularly interesting compound

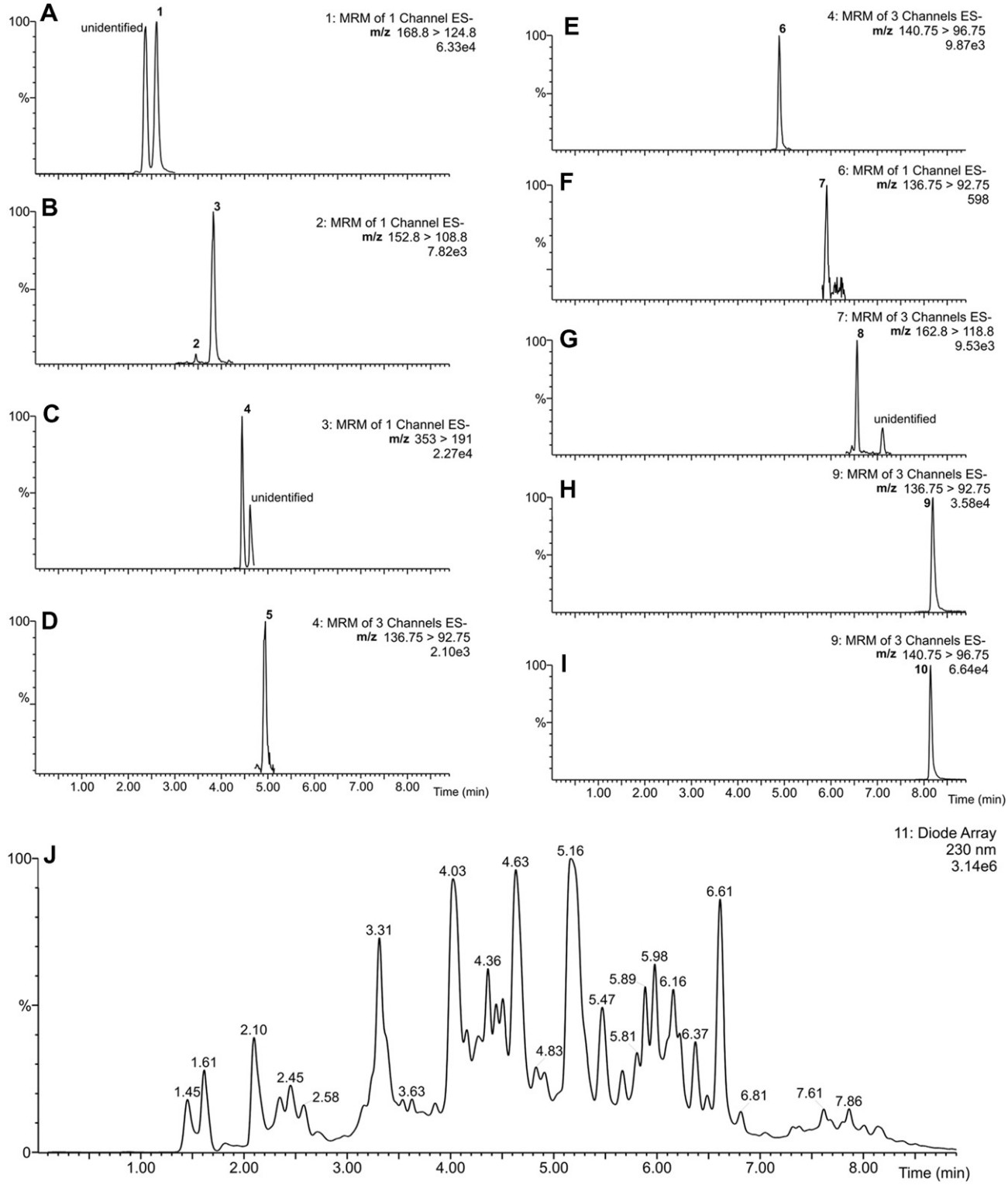

Fig. 3. (A–I) UPLC–MS/MS chromatograms of free phenolic acids in a green tea infusion: gallic acid (1), 3,5-dihydroxybenzoic acid (2), protocatechuic acid (3), chlorogenic acid (4), 4-hydroxybenzoic acid (5), [2,3,5,6-$^{2}H_4$] 4-hydroxybenzoic acid (6), 3-hydroxybenzoic acid (7), 4-coumaric acid (8), salicylic acid (9), [3,4,5,6-$^{2}H_4$] salicylic acid (10). (J) UPLC–UV chromatogram of the green tea infusion detected at 230 nm.

we detected in trace amounts was 3,5-dihydroxybenzoic acid, which is not a natural plant secondary metabolite, so its presence indicates possible contamination by soil microorganisms and/or animal excreta (Ross, Aman, & Kamal-Eldin, 2004).

In contrast to our results, although Rababah, Hettiarachchy, and Horax (2004) detected caffeic, protocatechuic, vanillic and syringic acids in green tea extracts, surprisingly they found no gallic acid, although this compound and its derivatives are regarded as typical phenolic acids of tea leaves (Clifford, Stoupi, & Kuhnert, 2007). This discrepancy may have been due to differences in methodologies and/or the processes used to prepare the tea extracts (Astill, Birch, Dacombe, Humphrey, & Martin, 2001), highlighting a general problem that complicates comparisons of results obtained in different analyses of PHAs in complex biological matrices. For example, the comparison of UPLC–MS/MS and UPLC–UV chromatograms (Fig. 3) of crude green tea extracts suggests that UV detection systems provide insufficient information to discriminate between co-eluted metabolites, and thus cannot provide accurate estimates of the quantities of PHAs in unpurified tea extracts. For these reasons, the purity of quantified peaks should ideally always be checked by a highly selective spectrometric method such as mass spectrometry.

4. Conclusion

We have developed a validated UPLC–MS/MS method for the rapid quantification of PHAs in different beverages. The presented method does not require samples to be preconcentrated/purified due to the high selectivity of MS/MS detection, and thus the manual labour is reduced to pipetting and filtration of liquid samples. The overall time required for chromatographic separation (including sample injection and column equilibration) does not exceed 12 min. The applicability and reliability of this analytical approach was confirmed by method validation and successful analysis of real samples of commercial beverages. In conclusion, the developed method is suitable for routine use in laboratories with access to UPLC–MS/MS equipment and should be utilised for rapidly screening beverages, monitoring of technological processes and analysis of plant extracts.

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