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Analysis of ginkgolides and bilobalide in food products using LC–APCI–MS

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Abstract

A method was developed for the extraction and quantification of five marker compounds characteristic of *Ginkgo biloba*. Five ginkgo terpene trilactones: bilobalide and ginkgolides A, B, C, and J, were selected as marker compounds for this study. Initial studies produced a simple methanol extraction method for determination of gingko markers in solid dietary supplements. Five dietary supplements were analyzed and the results were later compared to the concentrations detected in the analysis of beverages. Beverage samples were prepared by extracting the ginkgo terpene trilactones using an optimized solid phase extraction (SPE) method. The extracts were analyzed using LC–atmospheric pressure chemical ionization (APCI)–MS in the negative ionization mode. The limits of detection of the extraction method ranged from 6.8 to 3.2 ng mL^{-1}. Using the optimized method, 14 drinks and 3 tea products were analyzed. Concentrations of total marker compounds in drinks ranged between 1685 and 21.4 ng mL^{-1} with individual ginkgo terpene trilactones being detected at ppb concentrations. Analysis of brewed tea products presented much higher total marker compound concentrations ranging from 8.12 and 16.6 μg mL^{-1}. Analytical results reproducibility data, and recovery of the SPE method are presented.
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Keywords: *Ginkgo biloba*; Ginkgo; Ginkgolide; Bilobalide; LC–MS; Food analysis

1. Introduction

Over the last 30 years, there has been an increasing awareness of natural herbal remedies and botanical dietary supplements in the US and worldwide. Traditionally, botanicals have been sold as individual dietary supplement products but more recently several end-use products containing botanicals have appeared on the market, including many that would be considered conventional foods. Industry often categorizes these products as "functional foods". A "functional food" is defined as any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains [1]. The U.S. Food and Drug Administration (FDA) does not recognize the term "functional food" and foodstuffs containing botanical ingredients are considered conventional foods. Under current regulations, a botanical ingredient added to conventional foods must be approved by the FDA or meet an exception from the definition of a food additive, such as being generally recognized as safe (GRAS) by qualified experts under the intended conditions of use [2]. Since many dietary supplement products do not fulfill these criteria, they may be subject to regulatory action if GRAS self-determination is not sustainable under the FDA regulations.

Ginkgo biloba is considered the oldest surviving tree species, with geological records indicating this plant has been growing on earth for 150–200 million years [3]. Ginkgo has a long medical history with its use being recorded as early as 2800 BC in the Chinese medical literature [4]. Recently, ginkgo has gained popularity in the US and in 2002 ginkgo was the second highest selling dietary supplement [5]. Ginkgo has been used as a nutritional support for mental alertness, enhanced vitality level, circulatory health and blood vessel health. Adverse events have been reported involving spontaneous bleeding and it has been suggested that anticoagulants and anti-platelet agents [6] may be adversely affected.

When developing methodology for the determination of botanicals it is important to choose appropriate marker compounds as analytes. Marker compounds should be characteristic of the botanical of interest but are not necessarily the active component. Ginkgo extracts are commonly standardized to their flavenol glycoside and terpene trilactone concentrations.

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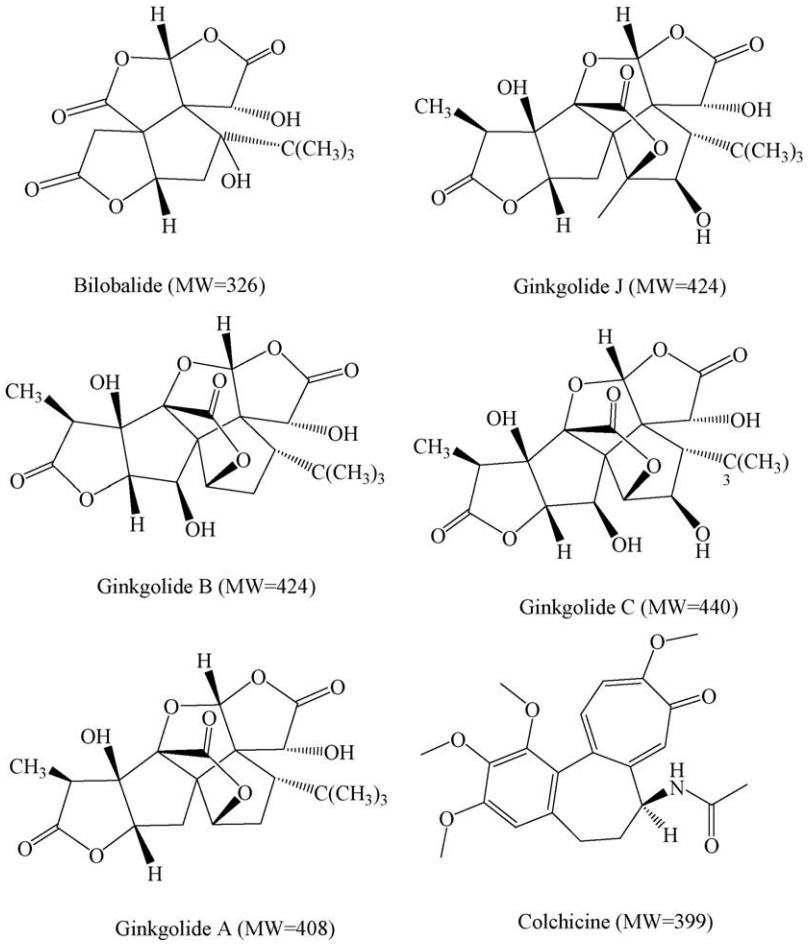

Fig. 1. Structures and molecular weights of ginkgolides A, B, C, and J, bilobalide and colchicine.

Flavenol glycoside compounds are found in many plant species and are not specific to ginkgo. The ginkgo terpene trilactones: bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J are unique to *Ginkgo biloba* and would be more suitable analytical markers (Fig. 1). Several analytical methods for the determination of these compounds have been described in the literature. Methods using GC with FID or MS detection have been reported which require derivatization of the analytes prior to separation [7,8]. LC methods using UV detection have also been reported but these suffer from low sensitivity and lack of specificity [9,10]. Two methods using LC–APCI–MS for the detection of ginkgo terpene trilactones have been reported in the literature [11,12]. In these studies, marker compounds were measured in dietary supplement products and plasma samples. Although the reported methods achieved a high-degree sensitivity they may not have the appropriate level of specificity required for analysis of more complex samples such as foods.

Detection of low levels of phytochemical marker compounds in foods is often complicated by the presence of interferrents in the food matrix. Previous studies on the detection of other botanical ingredients in foods reported very low marker compound concentrations [13,14]. Although the previously reported LC–MS methods achieved high sensitivity, the sample preparation and other analytical conditions may not provide sufficient specificity for detection of the markers at ppb concentrations. Consequently it is important to choose a method of analysis that has the potential to achieve low limits of detection in complicated matrices. Ang and co-workers published a paper on the detection of ginkgo markers in foods using solvent extraction followed by LC–ELSD [15]. The limit of detection reported for drinks was 0.1 $\mu g\,mL^{-1}$, which is not sensitive enough for many drink products. Using LC–MS would decrease the limits of detection and provide mass spectrometric confirmation without excessive sample preparation. Sensitivity and selectivity can be further increased by collecting data in the SIM (select ion monitoring) mode. In this study, SPE and LC–APCI–MS are used to develop a rugged method with high sensitivity, specificity and reproducibility for the determination of ginkgo terpene trilactones in functional beverages.

2. Experimental

2.1. Materials

Analytical standards for ginkgolides A, B, C, and J and bilobalide were purchased from Chromadex Inc. (Santa Ana, CA, USA). All solvents used were HPLC grade or better. Methanol and water were purchased from Burdick and Jackson

Table 1
Gradient elution timetable used for the LC–MS method

Time (min)	% Water	% Methanol
0	70	30
1	70	30
7	55	45
10	55	45
12	30	70
13	30	70
14	70	30
15	70	30

(Muskegon, MI, USA). Acetic acid (99.9%) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Colchicine (95%) was obtained from Sigma (St. Louis, MO) and was used as an internal standard. The solid-phase extraction cartridges used were 500 mg Strata^{TM}-X 33 μm polymeric sorbent (Phenomenex, Torrance CA, USA) and 44 μm Bakerbond 500 mg C18 (J.T. Baker). Tea and drink samples were filtered with Titan 2 30 mm 0.45 μm nylon membrane syringe filters (Sun SRi Wilmington, NC).

2.2. Instrumentation LC–APCI–MS

An Agilent (Palo Alto, CA, USA) 1100 series quadrupole LC–MS with an atmospheric pressure chemical ionization (APCI) interface was used in the negative ionization mode. Data was collected using Chemstation software version A.09.03. A 150 mm × 3.2 mm Hyperclone 5 μm ODS C-18 column (Phenomenex) was used for separation with a flow rate of 0.55 mL min^{-1}. A 30 μL injection was used and the column was held at room temperature. A mobile phase solvent gradient consisting of methanol and water was used (Table 1). The MS signal was collected in the selected ion-monitoring mode (SIM) consisting of the quasi-molecular ions (325, 351, 403, 407,423, 439 *m/z*) of the analytes and internal standard. All quantification was based on a peak area ratio of the SIM signal of the analyte and the internal standard colchicine.

2.3. Standard solutions

Analytical stock standard solutions were made by accurately weighing 1 mg of the analyte and dissolving it in 10 mL of methanol. Retention times and characteristic *m/z* signals were determined by analyzing individual standards in the scan mode of the LC–MS. Stock solutions and calibration standards were refrigerated when not in use.

Mixed calibration standards were made using stock solutions of individual marker compounds. Calibration standards were prepared in 25% methanol and 75% (aqueous 1% acetic acid) solution. Each standard contained the five ginkgo marker compounds and colchicine as the internal standard. The concentrations of each ginkgo marker compound in the ten standards ranged between 6.25 and 0.005 μg mL^{-1}. The internal standard colchicine was added at a concentration of 0.310 μg mL^{-1}. Peak area ratios of analyte to internal standard were used to construct calibration curves. Regression analysis produced calibration curves with good linearity (R^2 values greater than 0.99). Limits of detection (defined as a peak giving a response equal to a blank signal plus three times the standard deviation of the noise) were calculated and are as follows (ng mL^{-1}): bilobalide 4.64, ginkgolide A 3.24, ginkgolide B 1.35, ginkgolide C 3.08, and ginkgolide J 2.5 ng mL^{-1}. Calibration curves consisting of at least five points were constructed daily using freshly prepared standards. The concentration range of the daily calibration lines varied and was determined by the type of sample to be tested. No standard solutions above 6.25 μg mL^{-1} were analyzed.

2.4. Samples

Five types of ginkgo dietary supplement (DS) (3 capsules, 1 tablet and 1 caplet) were obtained from local retailers. Serving sizes ranged from 0.3 to 1.1 g and are listed for each of the supplements in Table 2. Fourteen drink products, which listed *Ginkgo biloba* as an ingredient were purchased from local retailers and internet sources. Compositions of the drink products varied and encompassed fruit-flavored beverages, carbonated

Table 2
Dietary supplement ginkgo marker compound concentrations determined by solvent extraction and LC–MS analysis

Sample (serving size)	Concentration (mg g^{-1})					
	Bilobalide	Ginkgolide J	Ginkgolide C	Ginkgolide A	Ginkgolide B	Total (mg g^{-1})
A (0.30 g)	11.1	2.19	5.40	7.53	4.40	30.62
	8.9	*5.0*	*10.4*	*4.9*	*3.4*	*7.0*
B (0.78 g)	2.99	1.26	2.31	9.99	6.00	22.55
	2.0	*0.9*	*3.5*	*1.8*	*3.9*	*2.2*
C (0.34 g)	3.29	1.13	1.96	6.44	4.21	17.03
	8.0	*2.8*	*9.1*	*6.1*	*6.3*	*6.5*
D (0.45 g)	4.4	1.20	2.11	1.72	1.44	10.87
	4.0	*2.0*	*2.9*	*10.5*	*3.8*	*4.5*
E (1.1 g)	1.91	0.50	1.05	4.45	2.68	10.59
	3.5	*1.2*	*1.8*	*4.7*	*4.9*	*4.0*

Percent relative standard deviation (%R.S.D.) values are given in italics ($n = 3$).

Table 3
Ginkgo marker compound concentrations (ng mL^{-1}) measured in commercially available drink and tea products

	Bilobalide	G-J	G-C	G-A	G-B	Total marker compounds
Drink 1 carbonated drink	120	70.7	61.8	46.5	26.6	325.7
	4.0	*9.0*	*2.7*	*5.8*	*3.4*	
Drink 2 tea drink	26.5	12.0	21.1	nd	12.8	72.4
	2.5	*0.5*	*1.0*		*1.4*	
Drink 3 carbonated soft drink	33.4	12.9	26.9	nd	12.8	86.0
	3.4	*0.8*	*2.8*		*1.1*	
Drink 4 carbonated soft drink	25.7	12.2	22.8	nd	14.4	75.1
	1.9	*1.1*	*1.0*		*2.0*	
Drink 5 fruit flavored drink	nd	12.0	21.3	nd	13.3	46.6
		0.8	*0.8*		*0.6*	
Drink 6 carbonated drink	52.2	55.6	73.7	107	81.3	370.1
	3.2	*10.7*	*2.2*	*8.5*	*4.6*	
Drink 7 carbonated drink	237	98.2	104	60.5	45.6	545.8
	6.0	*13.7*	*3.9*	*17.7*	*12.7*	
Drink 8 tea drink	58.6	28.5	180.7	11.3	29.4	308.5
	5.3	*6.1*	*5.7*	*6.1*	*0.4*	
Drink 9 tea drink	44.8	nd	34.9	20.4	32.7	132.8
	13.0		*3.4*	*4.3*	*0.7*	
Drink 10 fruit flavored drink	nd	nd	43.2	22.9	34.6	100.8
			1.8	*16.6*	*9.5*	
Drink 11 fruit flavored drink	65.6	nd	nd	4.42	20.3	90.3
	5.8			*26.0*	*3.1*	
Drink 12 tea drink	nd	nd	nd	nd	21.4	21.4
					1.1	
Drink 13 fruit flavored drink	34.0	18.1	29.6	6.04	21.3	109.0
	3.0	*1.4*	*0.7*	*3.7*	*0.7*	
Drink 14 fruit flavored drink	432	81.7	195	724	249	1684.5
	4.9	*8.3*	*4.6*	*8.9*	*8.6*	
Tea 1*	2050	290	1620	2790	1360	8120
	3.2	*3.4*	*7.1*	*7.6*	*7.0*	
Tea 2*	2420	500	3360	5270	2620	14160
	5.5	*5.5*	*7.5*	*8.2*	*7.1*	
Tea 3*	3440	380	2370	7100	3300	16600
	8.4	*7.8*	*7.3*	*6.7*	*8.0*	

%R.S.D. values for replicate extractions are given in italics ($n = 3$), nd = not detected.
* Analysis performed on prepared tea samples.

soft drinks and brewed tea drinks. A brief description of each drink product is given in Table 3.

2.5. Sample preparation: dietary supplements

The optimized method of extraction of ginkgo terpene trilactones from dietary supplement capsules and tablets was as follows. Tablets were first ground into a fine powder using a mortar and pestle. For all products, the contents of 10 individual units (tablets or capsules) were combined and mixed well. From this composite, 0.1 g portions were accurately weighed and added to 10 mL of methanol in a 15 mL centrifuge tube. The mixture was shaken for 30 min, centrifuged and the supernatant decanted off. Ten milliliters of methanol was added to the remaining residue and the extraction was repeated two times. The three 10 mL extracts were combined giving a total extract volume of 30 mL. A one-milliliter portion of the extract was taken and diluted to 5 mL prior to analysis.

2.6. Sample preparation: liquid samples

The optimized method of extraction of the marker compounds from liquid samples was as follows. Carbonated beverages were first sonicated for 5 min to remove dissolved gas. Samples were filtered through a nylon syringe filter into a graduated cylinder. The SPE cartridge was preconditioned with methanol and washed with 1% acetic acid solution prior to sample application. An aliquot of brewed tea (25 mL) or other drink product (50 mL) was applied to the cartridge, and the cartridge was washed with 6 mL of a 10% methanol solution. The analytes were eluted with

4 mL of methanol and collected into a 25 mL mixing graduated cylinder. The extract was diluted to 16 mL with 1% acetic acid and mixed well prior to LC–MS analysis.

3. Results and discussion

3.1. Dietary supplement analysis

In order to understand the chemical characteristics of the ginkgo marker compounds, simple extraction studies of solid dietary supplement products were undertaken. The optimal extraction solvent was determined by adding 0.1 g portions of ground dietary supplement to 10 mL aliquots of the following solvents: methanol, acetonitrile, acetone, ethyl acetate, 1% acetic acid, and 2-propanol. The samples were sonicated for 30 min, centrifuged and the supernatant decanted. A 0.250 mL sample of the extract was taken and set aside. The remaining dietary supplement was extracted two more times using the above procedure and the three extracts were combined. The samples of the initial extracts and combined extracts were analyzed by LC–MS. Comparison of concentrations in the initial extracts to the combined extracts showed that for all solvents the three step extraction procedure resulted in significantly greater analyte extraction. Methanol yielded the highest extract concentrations with the lowest variability ($n = 3$). A fourth methanol extraction was performed on the dietary supplement sample and LC–MS analysis did not detect the marker compounds at measurable levels. The effect of extraction time on the three-step extraction was investigated. Extraction times of 5, 10, 15, 30, and 60 min were compared. An extraction time of 30 min provided optimal results and was used for all other extraction studies. A study was done to determine the effect of agitation on recovery. Extractions were performed with either sonication, shaking or no agitation. Extracts produced with no agitation had significantly lower levels of the analytes. Extractions using sonication versus shaking were compared and showed no significant differences in amounts extracted between the techniques. Because shaking is much simpler, this method of agitation was used for the rest of the study. Five dietary supplement products were tested using the optimized extraction procedure. Three separate extractions were done for each product. The extracts were analyzed using the LC–MS and the concentrations and variability of the extractions were calculated. The results of the study are shown in Table 2. Total concentrations of the ginkgo marker compounds varied significantly between products, ranging from 10.6 to 30.6 mg g^{-1}. Reproducibility of the extraction was good with %R.S.D. values for three extractions averaging 4.6%. Using the recommended serving sizes found on the packaging, the average mg of ginkgo marker compounds per serving for each supplement product was calculated to be as follows: $A = 9.18$ mg, $B = 17.6$ mg, $C = 5.78$ mg, $D = 4.91$ mg, and $E = 9.64$ mg. In addition to differences in total marker compound concentration; there are relative concentration differences. For dietary supplement A, bilobalide accounts for 36% of the total marker compound content while in dietary supplement B it accounts for 13.6%. Further differences of these two products can be seen in the ginkgolide A concentration, where supplement A contains 24% and B contains 44%. Phytochemical concentrations are often dependent on growing conditions and similar relative concentration differences of the ginkgo terpene trilactones have been seen in other studies [7,10].

3.2. Solid phase extraction

Solid phase extraction optimization studies were initially done using 50 mL aliquots of 1% acetic acid solutions of ginkgo extract. Initial SPE studies compared Bakerbond C-18 (JT Baker) columns to Strata-X columns (Phenomenex). Three extractions using each of the SPE columns were performed and the recoveries calculated using data obtained from the LC–MS. The differences in recovery between the columns were not statistically significant. The chromatograms obtained using the Strata-X columns had fewer extraneous peaks and appeared to provide superior sample clean up. Because of this, all further method development was done using the Strata-X columns.

Preliminary SPE method development included an aqueous wash step prior to the elution of the analytes. Drink products have complex matrices and sample clean up may be improved

Table 4
Percentage recoveries of ginkgo marker compounds from drinks and teas obtained using the optimized SPE method

	Bilobalide	G-J	G-C	G-A	G-B
Drink					
High concentration (ng mL^{-1})	20.3	19.9	19.9	20.1	19.9
% Recovery ($n = 3$)	86.5	84.7	101	97.7	106
% R.S.D.	2.3	3.1	0.97	7.6	2.9
Low concentration (ng mL^{-1})	5.00	4.97	4.97	5.10	4.97
% Recovery ($n = 3$)	99.7	86.5	107	82.0	104
% R.S.D.	4.3	9.5	3.9	3.1	6.6
Tea					
High concentration (μg mL^{-1})	3.00	2.97	2.97	3.01	2.97
% Recovery ($n = 3$)	87.7	86.6	95.4	104	104
% R.S.D.	2.3	3.1	2.9	4.3	3.2
Low concentration (μg mL^{-1})	1.00	0.990	0.990	1.00	0.990
% Recovery ($n = 3$)	105	83.1	98.6	107	105
% R.S.D.	6.2	8.4	4.5	7.0	7.8

Percent recoveries and %R.S.D. values calculated from three extractions ($n = 3$).

using a wash step with a stronger solvent. The effect of the wash solution composition on recovery was examined. Extractions were performed using wash solutions of 1% acetic acid and 0–20% methanol. Recovery was not reduced until the methanol concentration was above 10%. As a result, a wash solution containing 10% methanol in 1% acetic acid was used for further SPE method development studies.

The recovery of the final method was investigated using a blank drink product (i.e. containing no ginkgo) fortified with the ginkgo marker compound standards. A fruity drink product, which did not list ginkgo as an ingredient (blank sample) was obtained and extracted using the optimized method. The resulting extract was analyzed using LC–MS. The resulting chromatograms yielded no interfering peaks at the retention times of interest. Aliquots of the drink product were fortified in triplicate at 20 and 50 ng mL^{-1}. The exact concentrations of the marker compounds in the fortified samples are shown in Table 4. The recovery and %R.S.D. were calculated (Table 4) for each fortification level. The average recovery for the analytes ranged between 82 and 107% with %R.S.D. values between 1.0 and 9.5%. There was no significant difference between the recoveries at the two concentration levels. The resulting chromatograms showed the extraction procedure provided excellent sample clean-up with good resolution of the peaks of interest (Fig. 2).

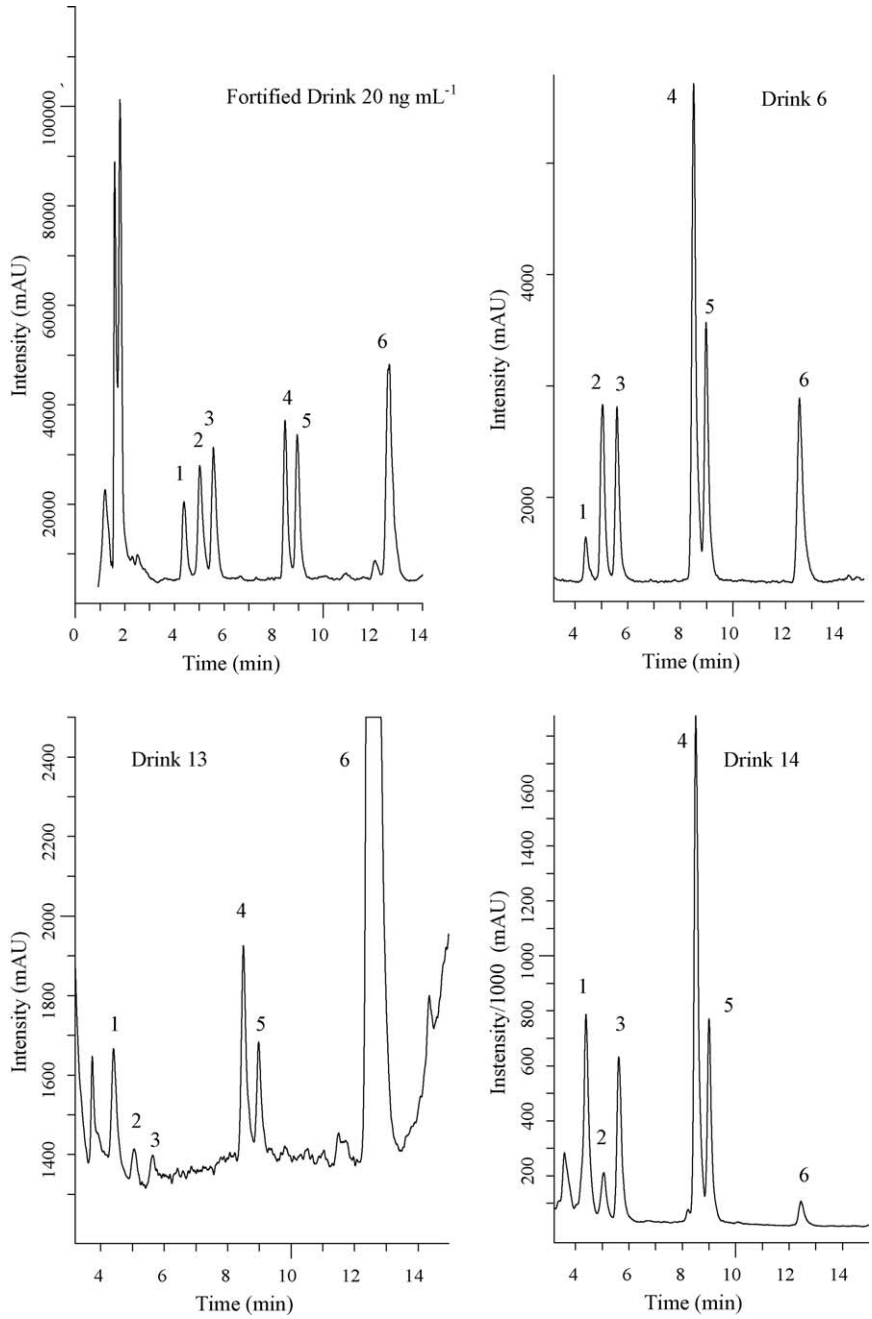

Fig. 2. LC–MS SIM chromatograms of drinks 6, 7, 13 and 14 extracts. Peak identification is as follows: 1 = bilobalide, 2 = ginkgolide-J, 3 = ginkgolide C, 4 = ginkgolide A, 5 = ginkgolide B and 6 = colchicine. Other conditions as specified in Section 2.

The limits of detection of the optimized SPE method from drink products were calculated using the calculated noise in the blank drink extraction chromatograph. Assuming 100% extraction, the limits of detection of the analytes in drinks are as follows (ng mL^{-1}): bilobalide 6.33, ginkgolide A 3.05, ginkgolide B 2.80, ginkgolide C 4.82, and ginkgolide J 4.27 ng mL^{-1}. These numbers are significantly lower than the 0.1 μg mL^{-1} limit of detection using LC–ELSD method, which was previously reported for drink analysis [15].

The SPE recovery study was repeated for tea products. Blank samples of brewed tea were fortified with standards in triplicate at levels of 3 and 1 μg mL^{-1} for each marker compound. Results of the study are given in Table 4. The average percent recovery for the tea samples ranged between 83 and 107% with %R.S.D. values ranging between 2.9 and 8.4%. No significant difference in recovery was found between drinks and teas.

3.3. Drink analysis

Fourteen drink products listing *Ginkgo biloba* as an ingredient were obtained for analysis. The composition of the drink products varied and brief descriptions of each sample are given in Table 3. All of the drink products were filtered through a syringe filter prior to application onto the SPE cartridge. Five of the products tested were carbonated and these were sonicated for 5 min prior to filtration in order to remove carbonation. Three extractions were performed for each drink product. Chromatograms from three of the drink extractions are shown in Fig. 2.

The drink chromatograms illustrate that the optimized SPE method provides good sample cleanup and pre-concentration. Quantification using the SIM signal gives increased sensitivity and selectivity because signals from interferrents and matrix components are reduced. Concentrations of the ginkgo marker compounds in the drink products were calculated using peak area ratio calibration curves and the results are summarized in Table 3. Peak identification was confirmed by the presence of the quasi-molecular ion peak and any fragment ion peaks in the multi-ion SIM signal at the retentions times of the reference standards. An example of the extracted ion signals for Drink 13 is shown in Fig. 3.

Drink product analysis showed significantly lower quantities of ginkgo terpene trilactones compared to those found in dietary supplement products. Examination of the data showed significant differences in marker compound concentrations among drink products. Drink 14 had the highest concentration of ginkgo trilactones with a total concentration of 1685 ng mL^{-1}. Drinks 5 and 12 had concentrations below 50 ng mL^{-1}. Drink products with low analyte concentrations had only some of the marker compounds detected. For drinks 2, 3, 4, 5, and 12 no ginkgolide A was detected and in drinks 9, 10, 11 and 12 no ginkgolide J was detected. All drink products, which claimed to contain *Ginkgo biloba* had measurable quantities of at least one of the marker compounds. The chromatogram obtained for drink 13 (Fig. 3) shows that even at marker compound concentrations as low as 6 ng mL^{-1}, analyte peaks are still clearly discernable. The reproducibility of the extractions from the drink products was calculated. Percent R.S.D. values for the individual marker compounds ranged between 0.5 and 26% and the average %R.S.D. was 5.1%. Ginkgolide A showed the greatest variability of extraction with average %R.S.D. values of 10.8%.

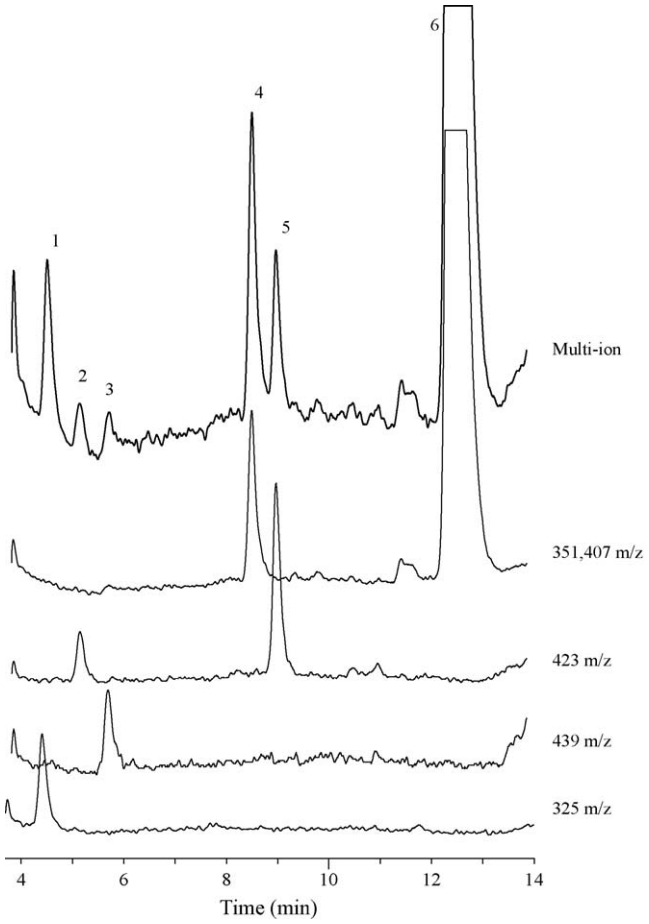

Fig. 3. Extracted ion chromatograms from LC–MS analysis of Drink 13 (chromatograms not to scale). Peak identification as given in Fig. 2. Other conditions as specified in Section 2.

3.4. Tea analysis

Three brands of unprepared ginkgo tea products were obtained from local retailers for analysis. In order to approximate what amount of ginkgo marker compounds would be ingested by the consumer, analysis was performed on prepared tea rather than the dry tea bag contents. The tea samples were prepared by boiling in 240 mL of water in a conical flask. A tea bag was added to the water, gently agitated and allowed to steep for 10 min. The tea bag was removed and the brewed sample was cooled to room temperature. A 10 min brewing time was used because it was the median brewing time suggested on the packages. After cooling, a portion of the tea product was filtered through a syringe filter and extracted using the SPE protocol optimized for the drinks. Because the tea products had significantly higher levels of the marker compounds, the amount of sample applied to the SPE column was reduced

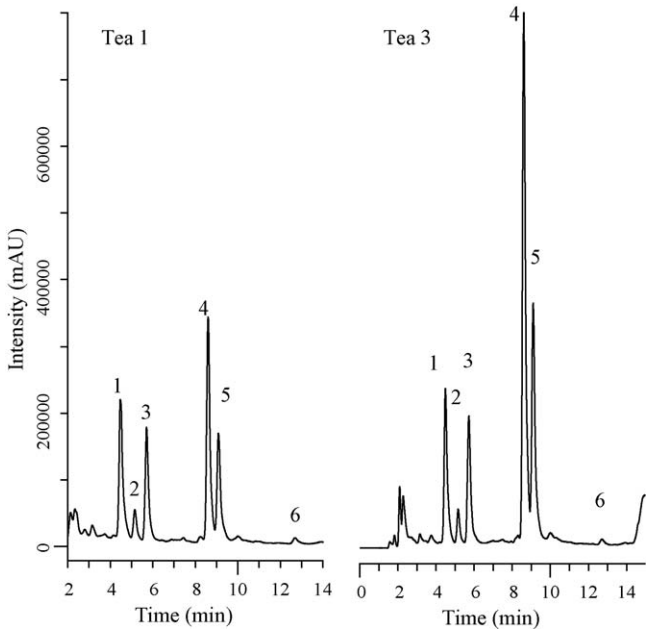

Fig. 4. LC–MS SIM chromatograms of tea product extracts. Peak identification as given in Fig. 2. Other conditions as specified in Section 2.

to 25 mL. Triplicate extractions were done for each tea product and the resulting extracts were analyzed using LC–MS. Chromatograms from the tea product analyses are shown in Fig. 4 and the analytical results are given in Table 3. Analyte concentrations in tea 2 and tea 3 are similar, with average marker compound concentrations of 14.16 and 16.60 μg mL^{-1}. Tea 1 had significantly lower concentrations than the other two teas, with an average marker concentration of 8.12 μg mL^{-1}. Differences in marker compound concentrations among the tea products are not unexpected for two reasons. The supplement fact panels for the products indicate that teas 2 and 3 contain 32 mg of ginkgo leaf extract while tea 1 listed 7.5 mg. The tea products also contained between 290 and 330 mg of ginkgo leaf. Disparity of marker compound concentrations between tea brands has also been seen in the analysis of St John's wort and kava tea products [13,14].

Comparison of the marker compound concentrations detected in the various products shows that dietary supplements have significantly higher levels than tea or drink products. It is also useful to compare the amount of ginkgo marker compounds, which would be found in one serving of a given product. This can be calculated by using a serving size of 240 mL for teas and drinks and the serving sizes listed on the packaging for each of the dietary supplement products. The ginkgo marker compounds measured per serving ranged from 17600–4900 μg for supplements, 3980–1950 μg for teas, and 404–5.2 μg for drinks. The difference in concentrations between product types is similar to the disparity of kava lactone levels found in supplements, teas and drinks in our previous study [13].

4. Conclusions

A robust SPE LC–MS method for the determination of ginkgo terpene trilactones as indicators of *Ginkgo biloba* in drink products has been developed.

The SPE method provided adequate sample clean up and preconcentration for the determination of low analyte concentrations in complex drink matrices. Method validation studies produced analyte recovery between 87 and 107% and %R.S.D. values of 0.97–9.5%. Limits of detection of the drink analysis method were calculated and ranged between 2.8 and 6.3 ng mL^{-1}, which are significantly lower than previously reported methods for drink analysis. Using this method, concentrations of the marker compounds were determined in 14 drink products and 3 teas. Total concentrations of the markers in the drink products varied significantly and ranged from 1684 to 21 ng mL^{-1}. Results of the drink analysis illustrate that many drink products contain ginkgo markers at very low concentrations, approaching the limit of detection. Teas contained two to three orders of magnitude higher concentrations than the drinks tested, ranging between 16.6 and 8.1 μg mL^{-1}. All ginkgo drink products analyzed contained at least one of the marker compounds at measurable concentrations. Many of these samples had concentrations below the limits of detection reported in previous methods. Solid dietary supplement products were analyzed and were found to have significantly higher concentrations compared to teas and drinks.

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