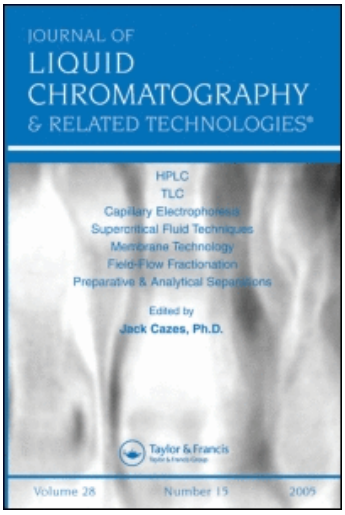

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MSPD Extraction of Phenolic Compounds from Fruit-Green Tea Using Various Non-Polar Sorbents

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ABSTRACT

Phenolic compounds, gallic acid (GA), syringic acid (SyrA), (+)-catechin (C), (−)-epigallocatechin gallate (EGCG), and rutin (R) were determined in fruit-green tea. A sample of fruit-green tea was prepared using matrix solid-phase dispersion (MSPD). A generic MSPD assay was applied using silica based solid supports as MSPD sorbents. Five non-polar solid phases and two elution agents were tested. Extracts were analyzed by HPLC with detection at 280 nm. Gradient elution with a mobile phase, which consisted of methanol and acidic water, (pH of water 2.5 was adjusted with formic acid) was applied.

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Key Words: Matrix solid-phase dispersion; Sample preparation; Phenolic compounds; Tea; Gradient HPLC.

INTRODUCTION

Phenolic compounds are important compounds of many fruits, vegetables, and beverages, which contribute to flavor, color, and secondary properties such as bitterness and astringency.[1] Tea is one of the main sources of phenolic compounds. Recent studies have shown that tea confers great beneficial effects to the health of consumers, including the effects of reduction of cholesterol, depression of hypertension, antioxidation, antimicrobial, protection against cardiovascular disease, and cancer.[2,3] (−)-Epigallocatechin gallate (EGCG) is the major polyphenol component in green tea, and this and other catechins have been shown to have antioxidant activity, and are postulated to have antimutagenic and anticarcinogenic properties.[4–6] Gallic acid (GA) is the most abundant phenolic acid in tea. The amount of GA increases during fermentation owing to its release from catechin gallates.[7]

There are only a few papers dealing with the determination of catechins with GA together. Authors determined catechins and GA in tea infusions,[8] or in tea extracts, after extraction into an organic solvent.[9] The determination of GA, syringic acid (SyrA), (+)-catechin (C), EGCG, and rutin (R) in fruit-green tea has been already published.[10] Phenolic compounds were extracted by liquid–liquid extraction into (a) water or (b) a mixture methanol–water (80 : 20, v/v). SPE was applied in order to clean up the extracts.

The aim of this work was to test the use of Matrix solid-phase dispersion (MSPD) in the analysis of phenolic compounds in green tea and to find optimal conditions for the sample preparation. MSPD is a preparation method that combines both sample homogenization and extraction of the analyzed compounds in one step. The application of MSPD is based on the blending of viscous, solid or semisolid sample with an abrasive solid support material.[11] There are only a few reports dealing with MSPD as a method for extraction of biologically active compounds from plant material.[12–14]

EXPERIMENTAL

Reagents and Chemicals

HPLC grade methanol, *n*-hexane, and dichloromethane were purchased from Merck (Slovakia). Formic acid (p.a.) was supplied by Lachema

(Czech Republic). Standards of SyrA and C were supplied by Fluka (Slovakia). Standards of R and EGCG were obtained from MGP (Czech Republic), and GA from Merck (Slovakia).

Acidic water (pH 2.5) was prepared by adding 1.4 mL of formic acid into 0.5 L of deionized water. Stock solutions (1 mg/mL) of standards were prepared by dissolving 10 mg of the respective compound in 10 mL of methanol. They were stored in a freezer at −20°C. Working solutions were prepared by diluting the stock solutions with a mixture of methanol–acid water pH 2.5, (50 : 50, v/v). Solutions were stored in a refrigerator at 5°C and were permanently stable.

The solid-phase materials used for MSPD were silica based:

- Chromabond C8, 45 μm (Macherey–Nagel, Germany), non-end-capped, 8% C;
- Chromabond C18, 45 μm (Macherey–Nagel, Germany), non-end-capped, 14% C;
- Chromabond C18 ec, 45 μm (Macherey–Nagel, Germany), end-capped, 14% C;

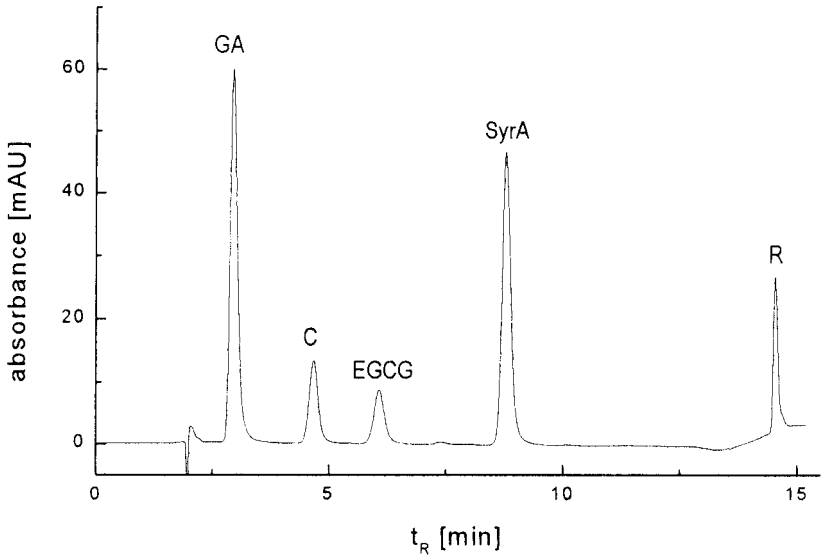

Figure 1. HPLC chromatogram of standard mixture of phenolics. Chromatographic column: Alltima C18. Mobile phase: methanol–water (pH of water 2.5), gradient elution, $F = 0.8$ mL/min. Detection: DAD, 280 nm. Injected volume 20 μL. GA, 10.03 μg/mL; C, 10.57 μg/mL; EGCG, 5.50 μg/mL; SyrA, 8.97 μg/mL; R, 10.00 μg/mL.

- Chromabond C18 HYDRA, 45 μm (Macherey–Nagel, Germany), non-end-capped, 15% C;
- Chromabond C18 f—(fast flow), 100 μm (Macherey–Nagel, Germany), non-end-capped, 14% C.

Solid phases were pre-washed with *n*-hexane, dichloromethane, and methanol; they were dried at 100°C and cooled in a dessicator.

Sample Preparation

Samples of mixed fruit-green tea (Tango) were bought in the Slovak local market. A sample of fruit-green tea was prepared using MSPD. Dry tea was ground to a powder. A 0.5 g amount of the powder was placed in a mortar and mixed with 2 g of previously cleaned sorbent and 1 mL of *n*-hexane. The mixture was homogenized in the mortar for 10 min to obtain a homogenous mixture. The blend was then packed into an 8 mL SPE column. The sample was covered with a paper frit and compressed using a syringe

Table 1. Yields of phenolic compounds extracted from fruit-green tea using MSPD with various sorbents and elution agent MeOH and mixture: MeOH–acid water pH 2.5, (80 : 20, v/v).[a]

Sorbent	Elution agent	GA (mg/g)	C (mg/g)	EGCG (mg/g)	SyrA (mg/g)	R (mg/g)
C8	MeOH	0.52	0.15	6.15	0.02	1.23
	Mixture[a]	0.56	0.50	22.81	0.35	3.22
C18 non-ec.	MeOH	0.57	0.27	5.11	0.02	1.52
	Mixture[a]	1.30	0.40	16.39	0.29	2.22
C18 ec.	MeOH	0.56	0.22	4.81	0.02	1.53
	Mixture[a]	0.78	0.29	2.59	0.03	2.20
C18 HYDRA	MeOH	0.52	0.27	2.56	0.03	1.35
	Mixture[a]	1.48	0.47	40.59	0.29	2.33
C18 f	MeOH	0.53	0.53	1.67	0.02	1.28
	Mixture[a]	0.95	0.95	4.00	0.03	1.97

Note: RSDs ($n = 3$): GA, 2.8–15.4%; C, 1.6–14.1%; EGCG, 8.6–37.3%; SyrA, 3.3–28.9%; R, 1.4–17.1%: MeOH—methanol; C8—Chromabond C8, non-end-capped; C18 non-ec.—Chromabond C18, non-end-capped; C18 ec.—Chromabond C18; end-capped; C18 HYDRA—Chromabond C18 HYDRA, non-end-capped; C18 f—Chromabond C18 f (fast flow), non-end-capped; GA—gallic acid; C—(+)-catechin; EGCG—(−)-epigallocatechin gallate; SyrA—syringic acid; R—rutin.
[a]Methanol : acid water, pH 2.5, (80 : 20, v/v).

plunger. The MSPD column was eluted firstly with 10 mL *n*-hexane and then with 10 mL dichloromethane, to wash out interfering compounds. Then, the column was dried for 5 min under vacuum. Phenolic compounds were eluted directly with 10 mL of the elution mixture methanol–acid water pH 2.5, (80 : 20, v/v). The volume of the eluent was adjusted to 25 mL by acid water (pH 2.5). Eluents were filtered through a nylon microfilter and injected into the HPLC system.

LC Analysis

The HP 1100 system (Hewlett-Packard, Waldbronn, Germany) consisted of a quarternary pump, a degasser, a Rheodyne injector equipped with a 20-μL sample loop, a diode-array detector (DAD), and an HP ChemStation. The chromatographic analysis was performed at ambient temperature onto an Alltima C18 column (150 × 4.6 mm^2 I.D., 5 μm), Alltech (Belgium) with Separon SGX C18 (10 × 4 mm^2 I.D., 7 μm), Watrex (Slovakia) guard column. Mobile phase consisted of methanol and water (pH of water 2.5) with gradient; 0 min 30% methanol, 10 min 40% methanol, 12 min 70% methanol was applied. Flow-rate was maintained at 0.8 mL/min. The UV absorbance was monitored from 200 to 400 nm and the chromatograms were acquired at a wavelength of 280 nm.

RESULTS AND DISCUSSION

A satisfactory resolution was achieved on the HPLC reversed-phase column Alltima C18, using gradient elution with methanol and acid water (pH of water 2.5—adjusted with formic acid). The time of analysis did not exceed 16 min. A chromatogram of standard mixture of phenolic compounds at flow-rate 0.8 mL/min is shown in Fig. 1. The optimum wavelength for detection was 280 nm, at which the best detector response for analytes was obtained.

MSPD was examined as a sample preparation method for the extraction of phenolic compounds from the tea leaves. The performance of MSPD is mainly affected by column packing and the elution procedure. It is important to select an appropriate sorbent that enables the homogenization, disruption of the sample, and acts as a separation material. Five non-polar sorbents were used; C_8 bonded silica and four types of C_{18} bonded silicas with different properties (particle size, carbon load).

The elution profile is another important factor in MSPD procedure. The following elution profile was applied: interferences were washed out with

n-hexane (10 mL) and dichloromethane (10 mL) from the MSPD column; and two elution solvents were tested: (1) methanol and (2) the mixture methanol–acid water pH 2.5, (80 : 20, v/v). Since the solubility of polar phenolic compounds is better in water-containing mixtures than in pure organic solvents, the extraction yields increased when the mixture of methanol and water was used. Therefore, the mixture methanol–water (pH of water 2.5) gave higher extraction yields for each type of sorbent used (Table 1). Also, the mixture methanol–acid water pH 2.5 (80 : 20, v/v) was used for the elution of analytes from the MSPD column.

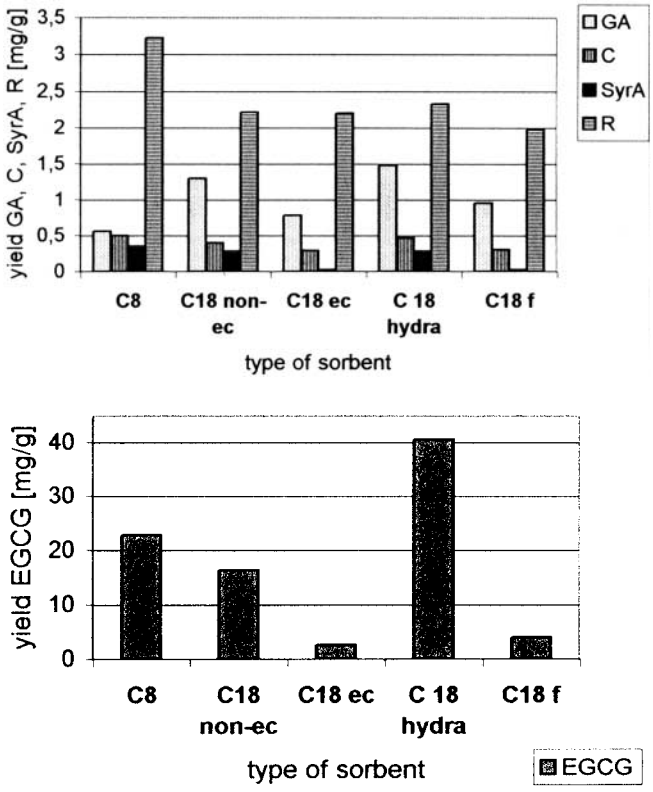

Figure 2. (a) Yields of phenolic compounds from fruit-green tea by MSPD using different sorbents. Elution agent: methanol–water pH 2.5, (80 : 20, v/v). RSDs ($n = 3$): GA, 2.8–10.4%; C, 1.6–14.1%; SyrA, 3.3–12.1%; R, 1.4–5.5%. (b) Yields of EGCG from fruit-green tea by MSPD using different sorbents. Elution agent: methanol–water, pH 2.5, (80 : 20, v/v). RSD ($n = 3$): 9.1–29.1%.

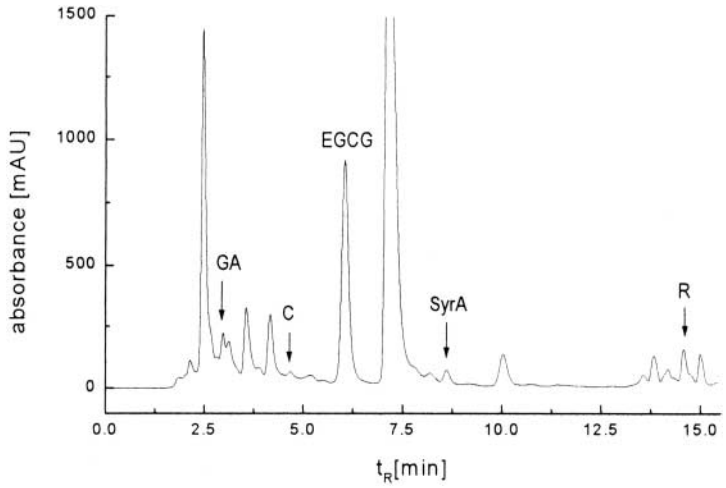

Figure 3. LC-UV chromatogram of fruit-green tea extract after MSPD with Chromabond C8 sorbent and 10 mL methanol–acid water pH 2.5, (80 : 20, v/v) as the elution solvent. Chromatographic conditions: chromatographic column Alltima C18 with the Separon SGX C18 guard column; Mobile phase: MeOH–water (pH 2.5), gradient elution; *F*, 0.8 mL/min; Detection: DAD, 280 nm. Injection volume 20 μL.

As it was mentioned above, different reversed-phase materials were used for the dispersion of the sample material. The results were evaluated for a better elution agent: methanol–acid water, pH 2.5. The results are presented in Fig. 2(a). The highest extraction yields for C, SyrA, and R were obtained using C_8 bonded silica. It was a non-end-capped sorbent with carbon load of 8%. The highest yields for GA were obtained with C18 HYDRA (non-end-capped with carbon load of 15%) with special octadecyl phase for polar analytes, suitable for the isolation of phenols. The comparison of extraction yields of EGCG obtained using different solid phases is illustrated in Fig. 2(b). It is obvious that the highest amount extracted was achieved with C18 HYDRA solid phase.

An LC-UV chromatogram of MSPD extract obtained using C_8 sorbent and methanol–acid water pH 2.5, (80 : 20, v/v) for the elution of phenolic compounds is illustrated in Fig. 3.

CONCLUSION

MSPD was demonstrated as a suitable preparation technique for the isolation of phenolic compounds from tea leaves. It is a simple and comfortable

alternative to LLE and SPE methods. Only a suitable solid and the elution medium need to be chosen.

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