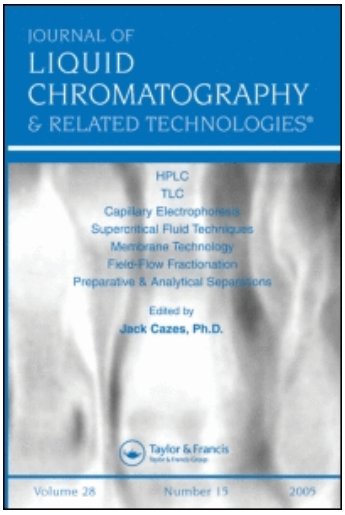

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Miroslava Lachová[a]; Jozef Lehotay[a]; Gabriela Karasová[a]; Ivan Skačáni[a]; D. W. Armstrong[b]
[a] Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Slovak University of Technology, Bratislava, Slovakia [b] Department of Chemistry, Gilman Hall, Iowa State University, Ames, Iowa, USA

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Isolation of L-Theanine from Plant Material using a Molecularly Imprinted Polymer

Miroslava Lachová, Jozef Lehotay, Gabriela Karasová, and Ivan Skačáni
Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Slovak University of Technology, Bratislava, Slovakia

D. W. Armstrong
Department of Chemistry, Gilman Hall, Iowa State University, Ames, Iowa, USA

Abstract: Nylon-6 was used as a molecularly imprinted polymer (MIP). For imprinting L-theanine (5-N-ethylglutamine) in the polymer, 2.0 g of Nylon-6, 0.8 g (MIP 1), and 1.2 g (MIP 2), respectively, of L-theanine templates and 7.2 g of formic acid was used for the phase inversion process in water. The resulting polymers, including the template molecule, were washed with acetic acid solution to extract the template from the polymers. The polymers were investigated as solid phase extraction (SPE) sorbents for the cleanup of water extracts of green tea. Various amounts of the template in the polymerization mixture had no influence on sorptive properties of MIPs in the range under study. Water was found as a suitable solvent for sample loading. The selective binding capacity of MIPs was 3.49 μg/1 g MIP. Water with addition of acetic acid (2% by volume) was used as an elution solvent. The recovery of the MISPE procedure was 87.8% ± 5.5%.

Keywords: Theanine, MISPE, HPLC

INTRODUCTION

Tea is the most popular beverage in the world. There are three main types of tea: green (nonfermented) tea, half-green or Oolong (semifermented) tea, and

Address correspondence to Jozef Lehotay, Faculty of Chemical and Food Technology, Institute of Analytical Chemistry, Slovak University of Technology, Radlinskheo 9, 81237, Bratislava, Slovakia. E-mail: jozef.lehotay@stuba.sk

black (fermented) tea. Tea contains such compounds as polyphenols, caffeine, aminoacids, vitamins, carbohydrates, etc.[1] Many studies have demonstrated the relationship between the green tea quality and the content of amino acids therein.[2]

Theanine (5-N-ethylglutamine) is the most important free amino acid in tea and exists only in the free (non-protein) form.[3] It was first isolated from tea leaves and identified in the late 1940s by Sakato.[4] The other known natural source of theanine is the mushroom *Xerocomus badius*.[5] Theanine accounts for 50% of the total free amino acids in tea. The content of theanine in tea is between 1 and 2% (on average) of the total dry weight.[6]

Besides having a delicate taste, theanine also has many biological effects. For example, it has been reported that theanine can decrease the level of norepinephrine and serotonin in the brain; the intake of theanine by hypertensive rats results in decreased blood pressure.[7] Cooperative effects of antitumor agents for cancer and theanine have also been reported.[8] A number of papers have documented the inhibition of peroxidation of low density lipoproteins (LDL) by tea extract.[9] Another study using electroencephalography (EEG) also showed an inhibiting effect of theanine on caffeine stimulation.[10]

Theanine, like most amino acids, is chiral. It was found that all teas, regardless of the manufacturing process, contained L-theanine and smaller percentages of D-theanine.[3] It has been shown that the enantiomers of theanine have different pharmacokinetics and it is likely that they have different pharmacological effects as well.[11,12]

During the past decade, molecularly imprinted polymers (MIPs) have emerged as attractive and highly accepted synthetic molecular recognition agents. Molecular imprinting (MI) is a technique used for the preparation of polymers with synthetic recognition sites having a predetermined selectivity for a specified analyte. The imprint is obtained by the polymerization of functional and crosslinking monomers in the presence of a template (target) molecule. The resultant imprints possess a steric (size and shape) and chemical (spatial arrangement of complementary functional groups) memory for the template molecule. Removal of the template from the polymer matrix creates vacant recognition sites that enable the polymer to selectively rebind the imprint molecule from a mixture of closely related compounds.[13,14]

A technology, using selective solid phases based on MIPs in molecularly imprinted solid phase extraction (MISPE), is an active area of research. Analytes can adsorb to MIPs via selective and non-selective adsorption. If the analyte associates with the MIP by ionic or hydrophobic interactions, then aqueous solutions of the analyte can be loaded directly. Subsequently, the non-selectively bound components can be removed by washing with an organic solvent, and the analyte of interest retained on the MIP, will switch from non-selective to selective binding.[15] MIP adsorbents have been used in pharmaceutical,[16–18] environmental,[19–21] and food analysis.[22–25]

The aim of this work is to develop a theanine selective MIP adsorbent and to use it in a plant specific (i.e., tea) application.

EXPERIMENTAL

Chemicals and Reagents

L-theanine and DL-theanine (racemic mixture of D-theanine and L-theanine) were prepared according the literature.[26] Acetonitrile, methanol, and ethylamine were obtained from Merck (Germany). Formic acid and acetic acid were supplied from Lachema (Czech Republic). Nylon-6 and L-glutamine were purchased from Aldrich (Germany).

Plant Material

China green tea (Sanny Tea, Slovakia) was purchased from the local market.

HPLC Analysis

An HP 1100 system (Hewlett-Packard, Germany), consisting of a pump with a degasser, a diode-array detector (DAD), a 100 μL injector, and an HP Chem-Station were used. Analyses were carried out on the following analytical columns: Separon SGX C18 (125 × 4 mm, 7 μm) (Waters, USA) and Chirobiotic T (250 × 4.6 mm, 5 μm) (Advanced Separation Technologies Inc., USA), at laboratory temperature. The mobile phase for the Separon SGX column consisted of water and acetonitrile (90:10) at a flow rate of 1.0 mL/min. The mobile phase for the Chirobiotic T column consisted of water and methanol (20:80) at a flow rate of 1.0 mL/min. Diode array detection was used in the range of 190–400 nm and the chromatograms were acquired at wavelengths of 200, 254, and 280 nm.

Polymer Preparation

The molecularly imprinted polymer was prepared according to the method Reddy et al.[27] in the following manner: 2.0 g of Nylon-6 and 0.8 g of L-theanine (template) was dissolved in 7.2 g of formic acid (MIP 1). The solution was incubated at 50°C for 24 hours. After solidification of the Nylon-6 in distilled water at 21°C, the solidified Nylon-6 was thoroughly washed with distilled water to remove the formic acid and the template from the polymer. The polymer was dried, cooled in liquid nitrogen, ground, and passed through a 100 μm sieve. The rest of the template was

removed from the polymer by washing with 2% acetic acid solution at 40°C for 2 days. The polymer of 200 mg was suspended in water and packed in a 3 mL empty polypropylene SPE cartridge provided with frits to secure the packing. The MIP 2 was prepared in the same manner, but with 1.2 g of L-theanine. The reference blank polymer (NIP) was prepared in the same manner as in the case of MIPs, but in the absence of template molecules in the formic acid solution.

Evaluation of MIP

The cartridge capacity of MIP for L-theanine was tested in methanol and water. Prior to applying the solution of L-theanine, the polymer was pre-equilibrated with 5 mL of methanol and, then, with 5 mL of solvent in which the capacity was studied. Aliquots (0.5 mL) of L-theanine solution (1 μg/mL) were applied gradually onto the cartridge until breakthrough was detected. The same solvents and concentration levels were applied on the NIP in the same way.

Then, the performance of the washing solvents was tested. The 200 mg amount of dried polymer was packed into a polypropylene cartridge and prior to use, the cartridge was conditioned with 5 mL of methanol, 5 mL of solvent, which was used in the washing step, and with 5 mL of water. After conditioning of the cartridge, 0.5 mL of L-theanine in water (1 μg/mL) was applied onto a polymer. Methanol, water, methanol with addition of acetic acid (2% by volume of methanol), and water with addition of acetic acid (1, 2, 10% by volume of water) were used as washing solvents. Each effluent was collected, evaporated to dryness (to remove acetic acid), dissolved in a mobile phase, and analyzed by HPLC. The same procedure was performed on NIP, but the amount of the polymer in the cartridge was 500 mg and the amount of L-theanine was lower (0.5 mL of solution of L-theanine in water with concentration of 0.5 μg/mL was applied on NIP cartridge).

In order to study the selectivity of the MIPs, the cartridge capacity in water and methanol particularly for DL-theanine and L-glutamine was determined. The procedure was the same as described for L-theanine. The concentration of DL-theanine and L-glutamine was 1 μg/mL.

Preparation of Plant Material

The tea sample was treated according to the method of Zhu et al.[28] Tea, (0.5 g) which had been grounded in a mill, was steeped at 80°C for 30 min in 100 mL water. After cooling, the sample solution was filtered through microfilters (Watrex, Slovakia, pore size 0.45 μm), prior to the injection into the HPLC system.

MISPE Procedure

A 300 mg quantity (dry weight) of MIP was packed into a polypropylene cartridge. It was conditioned with 10 mL of methanol and 10 mL of water. Of the tea extract, 1 mL was diluted with water at a ratio of: sample/water 1:49. The diluted sample of 0.5 mL was applied onto the conditioned MIP. Elution of theanine was performed with 2 mL of water with addition of acetic acid (2% of volume of water). Elution of other components of the sample was performed with 5 mL of the methanol/acetic acid (98:2, v/v) mixture. All effluents were collected, evaporated to dryness (to remove acetic acid), redissolved in water, and injected into the HPLC system.

The method for isolation of theanine from the sample using MISPE was analogous to the method of isolation of theanine from the sample using SPE with octadecylsilanized silica (C_{18}) as sorbent (Watrex, Slovakia). The procedure was the same as described for MISPE.

RESULTS AND DISCUSSION

Cartridge Capacity

As described previously, the capacity of the MIPs was evaluated in two different solvents, water and methanol. The same measurements were done on the MIP and also on the NIP. The results are presented in Table 1. Specific binding capacity was calculated by subtracting the amount of theanine non-specifically sorbed on the NIP from the amount sorbed on the MIP.

A higher value of the specific binding capacity was obtained using water (porogen) for sample loading. Although specific binding was achieved in water, the value of specific binding capacity is relatively low. L-theanine is a small molecule and there are relatively few recognition sites in polymer. According to results in Table 1, we can suppose that various amounts of templates in the polymerization mixture have no influence on the sorptive properties of MIPs in the range under study.

Washing Step and Elution

After determining the capacity of the MIP 1, an examination of the washing step and elution step were investigated. According to the literature the same solvent used for sample loading can be used in the washing step as well.[24] Water, which was chosen for sample loading, was also tested as a washing solvent. After application of water onto the MIP 1 (Figure 1), small amounts of L-theanine (13%) were washed from the cartridge. This means that recovery is low for this solvent and it could be used as a washing

Table 1. Capacity of the MIP, NIP and specific binding capacity for L-theanine in water and methanol (μg L-theanine/1 g sorbent)

	MIP 1		MIP 2		NIP			
Solvent	Capacity (μg/1 g MIP 1)	RSD (%)	Capacity (μg/1 g MIP 2)	RSD (%)	Capacity (μg/1 g NIP)	RSD (%)	SBC[a] of MIP 1	SBC[a] of MIP 2
Water	3.49	6.6	3.49	6.6	0.85	6.4	2.64	2.64
Methanol	2.29	5.8	2.29	5.8	2.26	5.7	0	0

[a]The specific binding capacity (SBC) was calculated by the deduction of the amount non-specifically adsorbed on NIP from the amount adsorbed on MIP.

RSD values for capacity: n = 3.

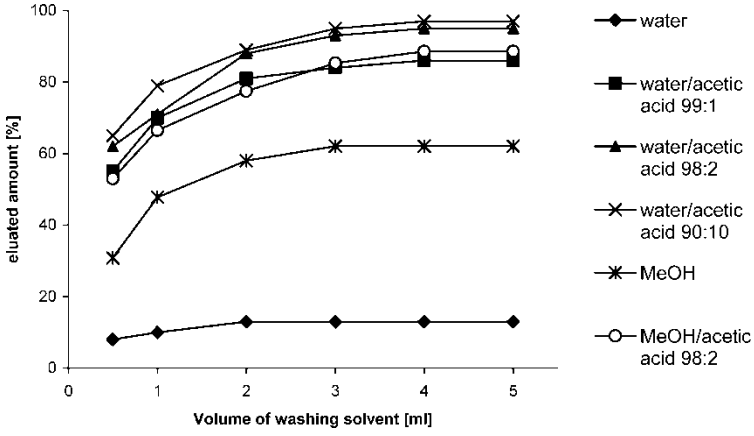

Figure 1. The relationship between volume of washing solvents and eluted amount of L-theanine from MIP 1.

solvent. However, when water was applied onto the NIP, approximately 45% of L-theanine was washed out from the NIP with 5 mL of water (Figure 2). This proves that L-theanine was non-specifically adsorbed on the NIP. After a methanol application onto MIP 1, about 58% of L-theanine was washed from the MIP 1. Methanol probably interferes with hydrogen bonding between the polymer and analyte.

When a small amount of acetic acid was added into water or methanol, almost the total elution of L-theanine was obtained. Therefore, these solvents are suitable for the elution step. Different amounts of acetic acid in

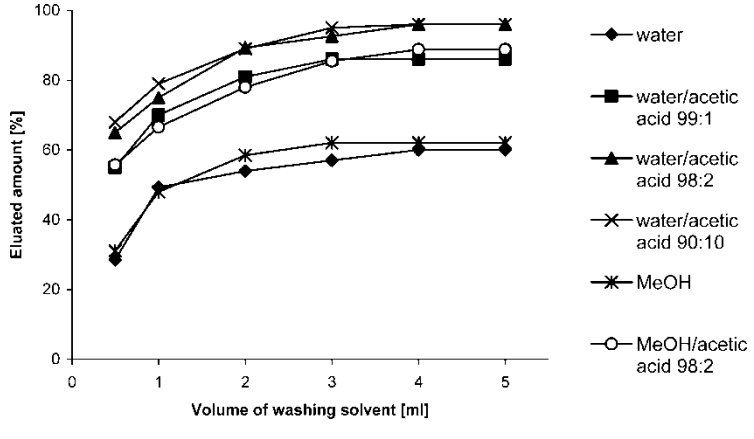

Figure 2. The relationship between volume of washing solvents and eluted amount of L-theanine from NIP.

water were tested (1%, 2%, 10%) and the influence of acetic acid in the recovery was investigated. With an increase of the amount of acetic acid in the water recovery step, the L-theanine recovered increases slightly, but the differences are not considerable. The same influence of acetic acid on the recovery was also observed in the case of the NIP.

Evaluation of MIP and NIP Towards all Analytes

The adsorption capacity and selectivity of the MIPs and NIP for DL-theanine and L-glutamine were investigated. The cartridge capacity in water was evaluated and the results are shown in Table 2. As is obvious from presented results, the specific capacity of DL-theanine is nearly as identical as specific capacity of L-theanine. The value of L-glutamine specific capacity is lower than the value of L-theanine specific capacity, but the difference is relatively small. This can be explained on the basis of the similarity of molecular structures of L-theanine and L-glutamine. This can also indicate non-selective adsorption.

Likewise as above, washing and elution solvents (water, water/acetic acid 98:2, v/v) were tested for DL-theanine and L-glutamine. Of each solvent, 5 mL was applied. The influence of solvent volumes on the recovery of compounds was estimated. Figures 3, 4 show the relationship between the percentage cumulative recovery of compounds and the amount sorbed onto MIP 1, and the volume of washing solvent and elution solvent, respectively.

As is obvious from Figure 3, 5 mL of water washed out an equivalent amount of L-theanine and DL-theanine (13%), whereas the same amount of water washed out 16% of L-glutamine.

A mixture of water/acetic acid (98:2, v/v) was used as the solvent agent for L-, DL-theanine and L-glutamine. Relatively high recoveries were obtained for all analytes –95% for L- and DL-theanine, 90% for L-glutamine (Figure 4). The same solvents were tested on the NIP and the recoveries for all analytes were similar to those obtained on the MIP.

Analysis of Tea Extract

Tea extract was prepared according to the preparation of plant material section. The extract was cleaned using MISPE described in the experimental section. A mixture of methanol/acetic acid (95:5) was used as the washing solvent to remove matrix components. However, this step was integrated into the procedure after the elution of theanine, because the matrix components were more strongly sorbed on the MIP than theanine, (and theanine can aslo elute with the mixture methanol/acetic acid). Elution of theanine was done by means of water with addition of acetic acid (2% by volume).

Table 2. Capacity of the MIP, NIP, and specific binding capacity for L-theanine, D-theanine and L-glutamine in water (μg analyte/1 g sorbent)

	MIP 1		MIP 2		NIP			
Analyte	Capacity (μg/1 g MIP 1)	RSD	Capacity (μg/1 g MIP 2)	RSD	Capacity (μg/1 g NIP)	RSD	SBC[a] of MIP 1	SBC[a] of MIP 2
L-theanine	3.49	6.6	3.49	6.6	0.85	6.4	2.64	2.64
DL-theanine l	3.48	6.9	3.48	6.9	0.85	6.6	2.63	2.63
L-glutamine	3.31	5.9	3.31	5.9	0.82	6.4	2.49	2.49

[a]The specific binding capacity was calculated by the deduction of the amount non-specifically adsorbed on NIP from the amount adsorbed on MIP. RSD values for capacity: n = 3.

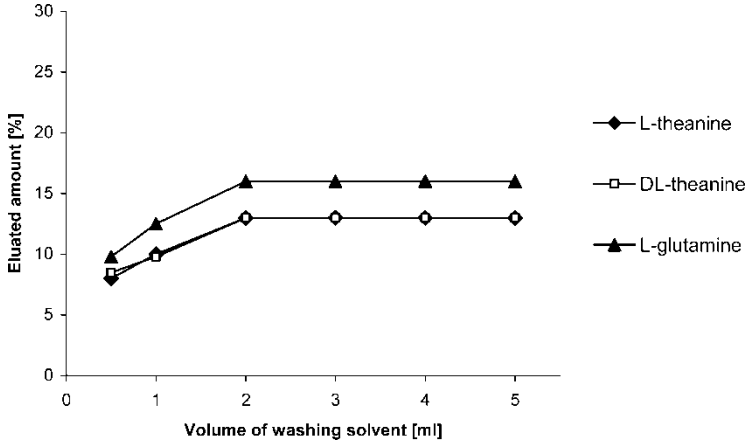

Figure 3. The relationship between volume of washing solvent (water) and eluted amount of L-, DL-theanine and L-glutamine from MIP 1.

This procedure was reproduced using an SPE cartridge with C_{18} as the sorbent. A companion of the SPE procedure on MIP1 and on C_{18} was done and the results are summarized in Table 3. The recovery was calculated as the ratio of the amount of theanine eluted by water/acetic acid (98:2) and the amount applied on the SPE cartridge.

As seen from the presented results, the recognition obtained on MIP and on C_{18} is similar.

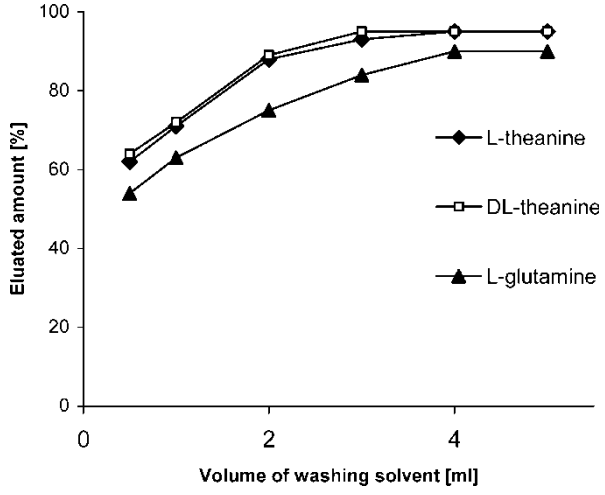

Figure 4. The relationship between volume of elution solvent (water/acetic acid, 98:2, v/v) and eluted amount of L-, DL-theanine and L-glutamine from MIP 1.

Table 3. Analysis of plant material: the amounts of theanine applied on the MIP and C_{18} cartridges, amounts retained on MIP and C18, eluted amounts and percent recoveries of theanine after SPE

	Amount of theanine (μg)					
Sorbent	Applied amount[a]	Adsorbed amount[b]	Eluted with water/ acetic acid (98:2, v/v)	Eluted with methanol/ acetic acid (98:2, v/v)	Recovery[c] (%)	RSD (%)
MIP 1	0.41	0.36	0.36	ND	87.8	5.5
C_{18}	0.41	0.35	0.35	ND	85.4	5.7

RSD values for recovery: n = 3.

[a]Amounts of theanine determined in tea extract before SPE.

[b]Calculated as the difference between amount applied and amount determined in the effluent after sample application.

[c]Calculated as the ratio of amount of theanine eluted by mixture of water/acetic acid (98:2, v/v) and amount applied on the cartridge.

ND = not detected.

The content of theanine in the tea extract was determined by HPLC using the Separon SGX C18 column.

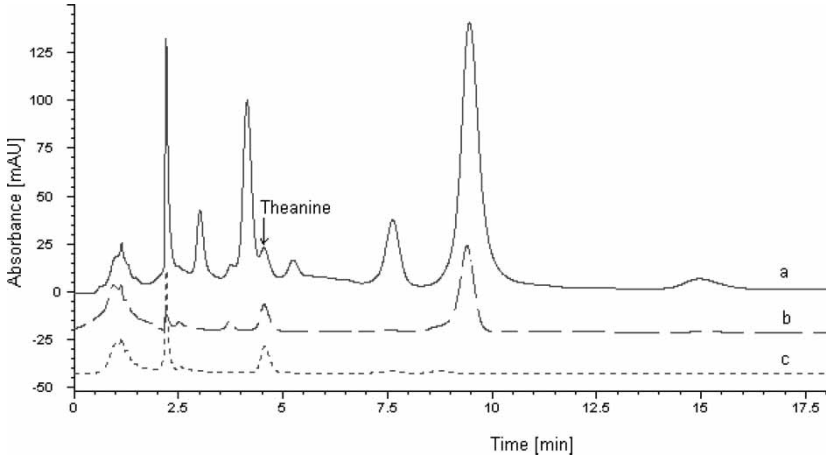

Figure 5. HPLC chromatograms of extract of green tea before SPE (a) after SPE on C_{18} (b) and after MISPE (c) HPLC column: Separon SGX C18. Mobile phase: water: acetonitrile (80:20, v/v), izokratic elution. $F = 1.0$ ml/min. Detection: DAD, 200 nm. Injected volume: 100 μL, LOD: 0.02 μg/ml, LOQ: 0.07 μg/ml.

The efficiency of the different cleanup procedures can be seen in Figure 5.

The tea extract also was analyzed by HPLC using an enantioselective LC column. D-theanine in the extract of green tea was under LOD (LOD: 0.05 μg/mL, LOQ: 0.16 μg/mL).

The recovery of the whole MISPE procedure was 87.8% ± 5.5%.

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REFERENCES

1. Horie, H.; Kohata, K. Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis. J. Chromatogr. A **2000**, *881*, 425–438.
2. Chu, D.C.; Kobayashi, K.; Juneja, L.R.; Yamamoto, T. *Chemistry and Application of Green Tea*; Yamamoto, T., Juneja, L.R., Chu, D.C., and Kim, M., (eds.); CRC Press: Boca Raton, FL, 1997, 8.
3. Ekborg-Ott, K.H.; Taylor, A.; Armstrong, D.W. Varietal differences in the total and enantiomeric composition of theanine in tea. J. Agric. Food Chem. **1997**, *45*, 353–363.

4. Sakato, Y. The chemical constituents of tea; III. A new amide, theanine. Nippon Nogeikagaku Kaishi **1949**, *23*, 262–267.
5. Casimir, J.; Jadot, J. Separation and characterization of N-ethyl-γ-glutamine in *Xerocomus badius* (*Boletus ladius*). Biochim. Biophys. Acta **1960**, *39*, 462–468.
6. Goto, T.; Yoshida, Y.; Amano, I.; Horie, H. Chemical composition of commercially available Japanese green tea. Foods Food Ingred. J. Jpn. **1996**, *170*, 46–51.
7. Yokogoshi, H.; Kato, Y.; Sagesaka, Y.M.; Takihara-Matsuura, T.; Kakuda, T.; Takeucho, N. Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats. Biosci. Biotechnol. Biochem. **1995**, *59*, 615–618.
8. Sugiyama, T.; Sadzuka, Y. Combination of theanine with doxorubicin inhibits hepatic metastasis of M5076 Ovarian Sarcoma. Clin. Cancer Res. **1999**, *5*, 413–416.
9. Kakuda, T. Neuroprotective effects of the green tea components theanine and catechins. Biol. Pharm. Bull. **2002**, *25*, 1513–1518.
10. Kakuda, T.; Nozawa, A.; Unno, T.; Okamura, N.; Okai, O. Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat. Biosci. Biotechnol. Biochem. **2000**, *64*, 287–293.
11. Desai, M.J.; Armstrong, D.W. Analysis of derivatized and underivatized theanine enantiomers by high-performance liquid chromatography/atmospheric pressure ionization-mass spectrometry. Rapid Commun. Mass Spectrom. **2004**, *18*, 251–256.
12. Desai, M.J.; Gill, M.S.; Hsu, W.H.; Armstrong, D.W. Pharmacokinetics of theanine enantiomers in rats. Chirality **2005**, *17*, 154–162.
13. Poole, C.F. New trends in solid-phase extraction. TrAC **2003**, *22* (6), 362–373.
14. Haginaka, J. Selectivity of affinity media in solid-phase extraction of analytes. TrAC. **2005**, *24* (5), 407–415.
15. Ensing, K.; Majors, R.E. Selective sorbents for solid-phase extraction based on molecularly imprinted polymers. LC-GC Europe **2002**, 16–25.
16. Wu, S.G.; Lai, E.P.C.; Mayer, P.M. Molecularly imprinted solid-phase extraction-pulsed elution-mass spectrometry for determination of cephalexin and α-aminocephalosporine antibiobics in human serum. J. Pharm. Biol. Anal. **2004**, *36*, 483–490.
17. Caro, E.; Marcé, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F. Synthesis and application of an oxytetracycline imprinted polymer for the solid-phase extraction of tetracycline antibiotics. Anal. Chim. Acta **2005**, *552*, 81–86.
18. Caro, E.; Marcé, R.M.; Cormack, P.A.G.; Sherrington, D.C.; Borrull, F. Novel enrofloxacin imprinted polymer applied to the solid-phase extraction of fluorinated quinolones from urine and tissue samples. Anal. Chim. Acta **2006**, *562*, 145–151.
19. Zhu, X.; Yang, J.; Su, Q.; Cai, J.; Gao, Y. Selective solid-phase extraction using molecularly imprinted polymer for the analysis of polar organophosphorus pesticides in water and soil samples. J. Chromatogr. A **2005**, *1092*, 161–169.
20. Moullec, S.; Bégos, A.; Pichon, V.; Bellier, B. Selective extraction of organophosphorus nerve agent degradation products by molecularly imprinted solid-phase extraction. J. Chromatogr. A **2006**, *1108*, 7–13.
21. Tarley, C.R.T.; Kubota, L.T. Molecularly imprinted solid-phase extraction of catechol from aqueous effluents for its selective determination by differential pulse voltammetry. Anal. Chim. Acta **2005**, *548*, 11–19.
22. Tamayo, F.G.; Casillas, J.L.; Martin-Esteban, A. Evaluation of new selective molecularly imprinted polymers prepared by precipitation polymerization of the extraction of phenylurea herbicides. J. Chromatogr. A **2005**, *1069*, 173–181.

23. Guzman-Vázquez de Prada, A.; Martinéz-Ruiz, P.; Reviejo, A.J.; Pingarrón, J.M. Solid-phase molecularly imprinted on-line preconcentration and voltammetric determination of sulfamethazine in milk. Anal. Chim. Acta **2005**, *539*, 125–132.
24. Blahová, E.; Lehotay, J.; Skačáni, I. The use of molecularly imprinted polymer for selective extraction of (+)-catechin. J. Liq. Chromatogr. & Rel. Technol. **2004**, *27* (17), 2705–2721.
25. Karasová, G.; Lehotay, J.; Sádecká, J.; Skačáni, I.; Lachová, M. Selective extraction of derivates of p-hydroxybenzoic acid from plant material by using a molecularly imprinted polymer. J. Sepn. Sci. **2005**, *28* (18), 2468–2476.
26. Sakato, Y. Amino acids in tea. Synthesis of theanine from pyrrolidoncarboxylic acid. J. Agric. Chem. Soc. Japan **1951–1952**, *25*, 25–26.
27. Reddy, P.S.; Kobayashi, T.; Fujii, N. Molecular imprinting in hydrogen bonding networks of polyamide nylon for recognition of amino acids. Chem. Lett. **1999**, 293–294.
28. Zhu, X.; Chen, B.; Ma, M.; Luo, X.; Zhang, F.; Yoa, S.; Wan, Z.; Yang, D.; Hang, H. Simultaneous analysis of theanine, chlorogenic acid, purine alkaloids and catechins in tea samples with the help of multi-dimension information of on-line high-performance liquid chromatography/electrospray-mass spectrometry. J. Pharm. Biomed. Anal. **2004**, *34*, 695–704.

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