Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 695–704
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

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

Xiaolan Zhu [a], Bo Chen [a,*], Ming Ma [a], Xubiao Luo [a], Fei Zhang [a], Shouzhuo Yao [a,1], Zutian Wan [b], Dajin Yang [b], Hongwei Hang [b]

[a] *Chemistry and Chemical Engineering, Chemical Research Institute, Hunan Normal University, Changsha 410081, PR China*
[b] *National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing 100021, PR China*

Received 10 July 2003; received in revised form 18 October 2003; accepted 25 October 2003

Abstract

A reverse phase high performance liquid chromatography (RP-HPLC) separation coupled with photo diode array detection (DAD) and electrospray ionization mass spectrometry (ESI–MS) detection was established for the analyzing of multiple bioactive compounds in tea and tea extracts. Theanine, chlorogenic acid, purine alkaloids and catechins were identified with authentic standard compounds and with MS-spectra. The content of theanine and catechins was measured by employing DAD and caffeine, chlorogenic acid, theobromine and theopylline by protonated molecular ion on selective ion recording (SIR) mode. The unity of LC/ESI–MS provides more qualitative and quantitative information comparing with general HPLC in the analysis of multi-components in tea, and complex extraction or sample pretreatment is unnecessary. The chromatogram acquired by using this method can be used as a bioactive components fingerprint for the quality control of tea and its extracts. With the help of multi-dimension information of HPLC–DAD–ESIMS, the compounds owning different chemical structure such as amino acid, catechins, etc. in tea and its extracts could be identified and determined in one run successfully.

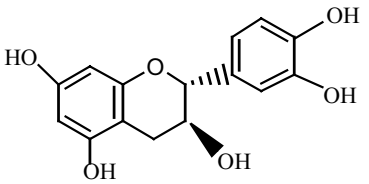
Keywords: Theanine; Chlorogenic acid; Purine alkaloids; Catechins

1. Introduction

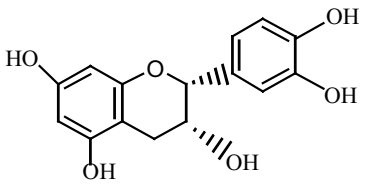
Tea, known as the most popular beverage in the east world, especially enjoyed by the people from China, Korea, Japan, stirs great interests in scientist for its beneficial effects to people's health. Modern phytochemical research shows that tea contains a large number of plant secondary metabolites owning different chemical structures such as amino acids, catechins, purine alkaloids, chlorogenic acid, etc. And each group of compounds has their special biological properties. For example, catechins possess potent antioxidant properties [1–4]; theanine, the characteristic

* Corresponding author. Tel.: +86-731-8865515; fax: +86-731-8865515.
E-mail address: dr-chenpo@vip.com.cn (B. Chen).
[1] Co-corresponding author.

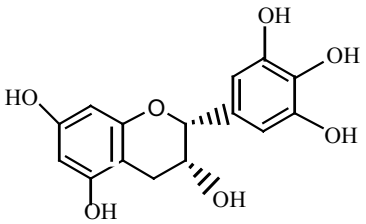
0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0731-7085(03)00605-8



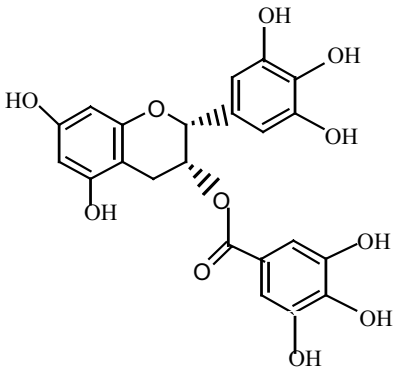
(+)-catechin (C)



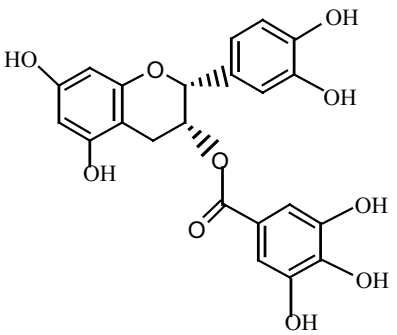
(-)-epicatechin (EC)



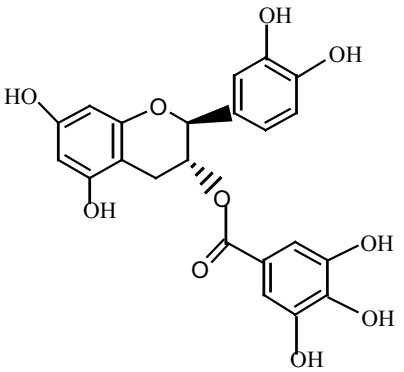
(-)-epigallocatechin (EGC)



(-)-epigallocatechin gallate (EGCG)

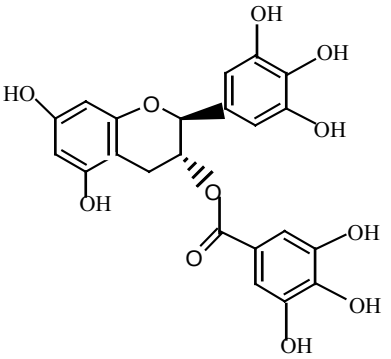


(-)-epicatechin gallate (ECG)

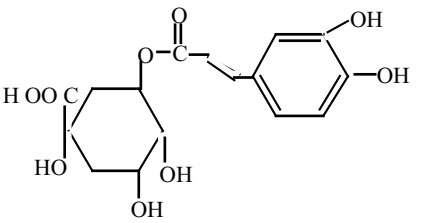


(-)-catechin gallate (CG)

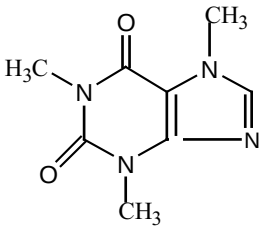
Fig. 1. Chemical structures of the major compounds analyzed in this study.



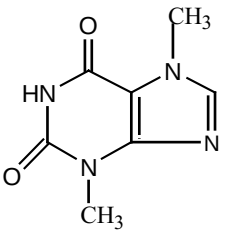
(-)-gallocatechin gallate (GCG)



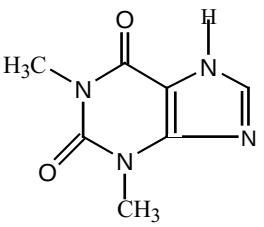
chlorogenic acid



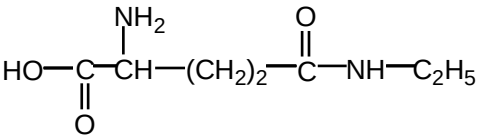
caffeine



theobromine



theophylline



theanine

Fig. 1. (*Continued*).

nonprotein amino acid, has cooperative effects of anti-tumor agents [5,6] and chlorogenic acid has anti-inflammatory effects [7]. Purine alkaloids, according to Rumpler et al.'s research, are effective in the control of body weight [8].

Methods for the identification and determination of each group of compounds are attainable, respectively [9]. Among the different analytical methods, HPLC is the most powerful technology; catechins, purine alkaloids, chlorogenic acid, etc. are generally analyzed by HPLC [10–14]. Tea also contains abundant amino acids such as theanine, aspartic acid, glutamic acid, and glutamine and so on. The content of amino acids, especially, theanine, is also an important quality control index. For the analysis of amino acids, most widely used analytical methods are ion-exchange [15] or ion-pair reversed-phase liquid chromatography with derivative detection [16–18].

Now, the hyphenated techniques for the chemical screening or analyses of phytochemistry have been

widely applied. HPLC coupled to mass spectrometry has become widespread in the plant source screening. And this technique has been also used in tea and its products widely. For example, Zeeb et al. developed a method for direct micro scale determination of 12 catechins in green and black tea, based on liquid chromatography/atmospheric pressure chemical ionization–mass spectrometry (LC–APCI–MS) [19]. Chaimbault et al. successfully determined 20 underivatized amino acids by ion-pairing chromatography and assisted electrospray mass spectrometry [20]. But we noticed that established analytical strategies have only been rarely available for simultaneous analysis of theanine, catechins, purine alkaloids and other substances. This is probably due to the strong polarity of amino acids, which result in their relatively short retention time on RP-C18 column, thereafter they could not be well separated in a single run time.

It is essential to develop a method for the simultaneous analysis of all the components in tea due to their different health effects. The goal of this research is to develop a simple LC/ESI–MS technique with the help of its multi-dimension information for determining the multiple bioactive compounds in tea simultaneously. The chemical structures of analyzed compounds are shown in Fig. 1. Deferent from other reports, theanine, catechines, purine alkaloids and chlorogenic acid etc. can be identified and quantified in one run.

2. Experimental section

2.1. Instrumentation

The HPLC–MS system was from Waters (USA), and consisted of alliance 2695 liquid chromatography system (Milford, MA) equipped with two pumps, a thermostatically controlled column apartment, an autosampler with a 250-μl loop, a 996 photo diode array detector (DAD) and a Micromass ZQ2000 mass spectrometer (Manchester, UK) equipped with an ESI source and a quadrupole analyzer. All the operations and the acquiring of data were controlled by Masslynx$^{3.5}$ software.

2.2. Chemicals

The standards of theobromine (TB), theophylline (TP), caffeine, chlorogenic acid, (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-catechin gallate (CG), (−)-gallocatechin gallate (GCG), (−)-epicatechinn (EC), (+)-catechin (C), epicathin gallate (ECG) were purchased form Sigma (St. Louis, MO, USA). Theanine (>98%) was prepared in our laboratory on Waters Prep LC 4000 system (Waters, USA); its purity was confirmed by ^{1}H NMR, IR, HPLC and MS Spectra. Other amino acids including glutamic acid (Glu), glutamine (Gln), cysteine (Cys), cystine $(Cys)_2$, alanine (Ala), aspartic acid (Asp), leucine (Leu), phenylalanine (Phe), tyrosine (Tyr), arginine (Arg), and valine (Val) were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

HPLC-grade acetonitrile (CH_3CN) purchased from Tedia Company, Inc. (OH, USA), and ultrapure water prepared using a Millipore Milli-Q purification system (Millipore Corp. Bedford, MA) were used as mobile phase of HPLC–MS. Other reagents were of analytical-grade.

2.3. Preparation of standard solutions

Stock solutions of theanine, EGCG, EGC, CG, GCG, EC, C, ECG, TB, TP, caffeine and chlorogenic acid were prepared by dissolving weighted quantities of standard compounds into water. The concentration of each analyte was 10.0 mg/ml. One milliliter aliquots of theanine, EGCG, EGC, CG, GCG, EC, C, ECG was transferred into a 10 ml volumetric flask, and diluted to the volume by adding water to yield the mixed standard solutions, and this solution was diluted step by step to yield series solutions at final concentration of 0.01 0.05, 0.10, 0.20, 0.6, 1.0 mg/ml respectively. Standard mixture solution of TB, TP, caffeine and chlorogenic acid was prepared in the same way as above, the final concentration of each analyte was 0.50, 2.0, 10, 40, 80, 200 μg/ml, respectively, Standard amino acids mixture was used to investigate the possible interference in the analysis of theanine, the concentration of amino acids stocking solutions was 1 mg/ml, and the final concentration of each amino acid in the mixture standard solution was 50 μg/ml. All the stock solutions and working solutions were used within 7 days and was stored in a refrigerator and brought to room temperature before use. These solutions were used to identify and quantify the compounds in tea.

2.4. Preparation of sample solution

Samples of tea such as Lung Ching, Pi Lo Chun, Yun Wu, lipton black tea (purchased from supermarket, changsha, China) and green tea extracts (prepared in our laboratory) were examined by HPLC/ESI–MS. The samples were treated according to Kim et al.'s method [21] with minor modification. Briefly, 0.5 g of tea leaves, which had been grounded in a mill, was steeped at 80 °C for 30 min in 100 ml of water. After cooling for 10 min, solutions of samples were filtered through a 0.45 μm filter; the filtrate was directly analyzed by HPLC/ESI–MS without further treatment. All samples were prepared daily.

2.5. HPLC–DAD–MS conditions

The separation was completed on a 250 mm × 4.6 mm i.d. Spherigel C18 column (Johnsson, Dalian, China), which was packed with 5 μm C18 silica, the operating temperature was maintained at 30 °C. UV spectra were acquired over the range of 195–350 nm. The LC mobile phase consisted of (A) 0.5% formic acid aqueous solution and (B) acetonitrile. The gradient was programmed as follows: within the first 6 min, A maintains at 100%, 6–10 min, solvent B increased linearly from 0% to 12%, and 10–35 min, B linear from 12% to 21%, then 35–40 min, B linear from 21% to 25%, and then 40–50 min, B linear to 100%. The flow rate was 1.0 ml/min and 10 μl was injected. The outlet of the DAD was split, and only 0.2 ml/min portion of the column effluent was delivered into the ion source of MS.

The electrospray ionization source was operated at 105 °C in positive mode to produce $[M+H]^+$ or $[M+Na]^+$ and in negative mode to generate $[M-H]^-$ ions. The desolvation temperature was set at 200 °C, extract voltage was 4 V, desolvation gas and cone gas was set at 250 l/h and 50 l/h respectively. The Full-scan mass spectra was acquired over the range 80–600 amu. Cone voltage was switched to 45 V from 20 V at 16.0 min in the full-scan mode, and the cone voltage for SIR of each content was also different according to the electrical stability of each component, for catechins, cone voltage was set at 45 V, chlorogenic acid at 30 V, amino acids and other compositions at 20 V.

2.6. Comparing method for the determination of theanine

A pre-column *o*-phthaldialdehyde (OPA) derivative RP-HPLC method was used as a comparison method to investigate the validity of the proposed method for the determination of theanine. The HPLC derivatization and separation procedure of amino acid derivatives have been described already in detail [18]; the following is only a brief description of the analytical methodology. For HPLC, A Johnsson 10 A Model HPLC equipment (Dalian, China) was used comprising a binary solvent delivery system, and a fluorescence detector set at 338 nm as excitation wavelength and 425 nm as emission wavelength. Separation of amino acid derivatives was completed on a 250 mm × 4.6 mm i.d. column filled with spherigel ODS 5 μm stationary phase (Johnsson, Dalian, China). For elution of derivatives a linear gradient generated from (A) a mixture of methanol, tetrahydrofuran and aqueous solution containing 50 mM sodium acetate, 50 mM Na_2HPO_4 (pH 7.5) (2:2:96, v/v/v) and (B) a mixture of methanol and water (65:35, v/v) was applied. For the derivatization of amino acids, mixtures of OPA together with 2-mercaptoethanol (2-MCE) in potassium borate buffer (pH 10.4) were used. For calibration and quantification of theanine, a standard solution with lower concentration was prepared.

2.7. Linearity, limit of detection

The standard curves were prepared over 6 different concentrations, five injections were made at each level, and peak areas were plotted against the corresponding concentrations using linear regression to generate the standard curves. The limit of detection (LOD) was evaluated as the mass giving a signal equals to three times of noise (S/N = 3). Limit of quantification (LOQ) are taken as the mass that result in 5% or less relative standard deviation (R.S.D.) upon quantification of the peak area [22]. The result was summarized in Table 1.

2.8. Method validation

A series of sample analyses were performed to validate the performance of the method, precision was evaluated from replicated determinations ($n = 5$) per-

Table 1
Limit of detection, limit of quantification, linear ranges, and relative coefficient of the analytes

Compound	Linear range (μg)	r	LOD (ng)	LOQ (ng)
Theanine[a]	0.102–10.2	0.999	1.7	5.0
TP[b]	0.005–2.00	0.991	0.03	0.09
TB[b]	0.0049–1.96	0.990	0.03	0.08
Caffeine[b]	0.0051–2.04	0.993	0.02	0.06
Chlorogenic acid[b]	0.0049–1.96	0.991	0.20	0.65
EGC[a]	0.098–9.8	0.996	1.2	5.0
C[a]	0.102–10.2	0.997	1.5	6.0
EC[a]	0.101–10.1	0.998	2.0	8.0
EGCG[a]	0.103–10.3	0.997	0.5	3.5
ECG[a]	0.098–9.8	0.998	0.8	4.0
GCG[a]	0.099–9.9	0.998	1.2	5.0
CG[a]	0.102–10.2	0.995	1.5	5.0

[a] Shows the result of using DAD as detector.
[b] Shows the result of employing MS as detector.

Table 2
Contribution of each analyzed compounds in samples (% w/w, $n = 5$)

Compound	Lung Chin	Pi Lo Chun	Yun Wu	Lipton	Extract
Theanine	0.57 ± 0.01	1.81 ± 0.05	1.16 ± 0.03	0.98 ± 0.03	25.77 ± 0.52
TP	ND	ND	ND	ND	0.04 ± 0.002
TB	0.19 ± 0.01	0.23 ± 0.01	0.06 ± 0.003	0.13 ± 0.01	0.35 ± 0.02
Caffeine	3.21 ± 0.12	3.64 ± 0.15	3.83 ± 0.14	2.97 ± 0.18	1.12 ± 0.05
Chlorogenic acid	0.029 ± 0.002	0.16 ± 0.01	ND	0.39 ± 0.02	N.D.
EGC	4.74 ± 0.10	3.65 ± 0.08	7.98 ± 0.17	ND	ND
C	0.65 ± 0.03	0.93 ± 0.03	0.37 ± 0.02	0.55 ± 0.02	ND
EC	3.56 ± 0.07	4.21 ± 0.10	3.88 ± 0.09	1.03 ± 0.04	ND
EGCG	13.62 ± 0.27	10.34 ± 0.31	9.91 ± 0.27	1.04 ± 0.05	ND
ECG	2.95 ± 0.05	4.77 ± 0.11	3.69 ± 0.09	1.98 ± 0.05	ND
GCG	0.07 ± 0.004	1.35 ± 0.07	0.06 ± 0.04	ND	ND
CG	0.97 ± 0.04	0.55 ± 0.03	0.68 ± 0.04	0.03 ± 0.002	ND

Where ND is not detected.

formed on the same day for each samples. The mean assay values and derivation was listed in Table 2. The accuracy was assessed by recovery experiment. The known quantities of 12 standards was added to green tea samples at low, medium and high levels, the recovery was calculated by comparing the found mount of standards to those of added.

3. Results and discussion

3.1. ESI–MS

The analyzed target components in this paper own quite different chemical structures. Hence, for different compounds, the optimum conditions for ionization are not the same. To reduce the fragmentation of the molecules, parameters of each standard for ESI interface were optimized by direct flow injection analysis (FIA) with a microliter injector.

It is been found that amino acids response more intensively in positive ionization mode. The intensity of $[M + H]^+$ of compounds mainly depends on collision induced dissociation (CID) fragmentation voltage, so the cone voltage is the key factor influencing ionization and sensitivity in this program. Amino acids produce the most abundant protonated ions at 20 V of cone voltage, and the intensity of $[M + H]^+$ of amino acid became weak at higher voltage. This is probably due to they are polar compounds and have small molecules, so they are easy to be ionized and are apt to be cracked under higher voltage.

Concerning the purine alkaloids, positive mode was also favorable, and abundant protonated ions were generated at the voltage of 20 V. The intensity of protonated ion decreased with the increasing of the voltage, it was quite weak when the voltage was set at 45 V and no protonated ions at the voltage of 80 V. For chlorogenic acid, negative mode is more preferable, and the most suitable cone voltage is 30 V.

For the MS analyses of catechins, different approaches such as APCI–MS [19] and ESI–MS [23] have been reported. In the present paper, optimization of experimental conditions was carried out in both positive and negative mode at different cone voltage. It was found that the S/N ratio of catechins increased with the voltage increasing, the response sensitivity of catechins are high and the noise is low at the voltage of 80, however the intensity of $[M-H]^-$ ions are very weak in MS-spectrum under this cone voltage. Some catechins even gave no molecular ions. Yet, when the experiment was operated at 30 V, few fragment ions was generated, so only a little structure information can be obtained, catechins ionized at 45 V give both fragmental and molecular ions, which is useful for identification and determination. In positive ionization mode, catechins can generate abundant protonated ions and the characteristic retro Diels–Alder fragment ions at *m/z* 139 simultaneously, which agree with the results described by Zeeb et al. [19] and Poon [24], but the background ions are relatively high due to the presence of formic acid. Therefore, subsequent experiments were performed in negative ionization mode.

Due to the great gap in cone voltage between catechins and other groups, it is difficult to choose a moderate value for all the analyzed targets. Thereafter, in analyzing samples, whole process was divided into two parts in full scan mode. As described in the previous experimental section, 16 min is taken as the time turning point of cone voltage switching. Amino acids were washed out within 16 min, and no target substances eluted at this time.

Considering the constituents which have the same molecule weight, for example, theobromine and theophylline, their $[M+H]^+$ are the same (*m/z* 181), the assignment of the peaks was accomplished by injecting single standard. The ions selected for SIR are listed in Table 3.

Table 3
Retention time, molecular weight and the *m/z* values of selective ions for identification or quantification of amino acids and purine alkaloids

Compound	Retention time	Molecular weight	Selective ions (*m/z*)
Glu	3.43	147	148 (+)
Gln	3.43	146	147 (+)
Tyr	9.80	181	182 (+)
Theanine	5.10	174	175 (+)
Asp	3.43	133	134 (+)
Ala	3.43	89	90 (+)
Cys	3.43	121	122 (+)
$(Cys)_2$	2.94	240	241(+)
Leu	7.84	131	132 (+)
Val	4.42	117	118 (+)
Arg	2.97	174	175 (+)
Phe	13.23	165	166 (+)
TB	14.70	180	181 (+)
TP	16.11	180	181 (+)
Caffeine	19.51	194	195 (+)
Chlorogenic acid	19.53	353	354 (−)

Where + shows positive ionization mode, − shows negative ionization mode.

3.2. HPLC mobile

The complete HPLC separation of catechins in green tea had been achieved on RP-C18 column and by using trifluoroacetic acid (TFA) as modifier of the mobile in general [19,25]. However, it is well know TFA is not widely used in LC–MS because it is difficult to be eliminated from the mass spectrometer. While if no acid was added in the mobile, some of catechins could not be separated well and the shape of peaks is not sharp. In this paper, formic acid was used as modifier of the mobile phase, the retention time of amino acids was shortened compared to using TFA as modifier, yet, the most important amino acid, theanine, can still be separated without interference, and the complete separation of catechins and caffeine could be obtained.

Under the given chromatographic condition, chlorogenic acid is overlapped by caffeine; therefore the quantification with DAD of these two compounds was not suitable. However, measurement of these two compounds can be completed using SIR mode of ESI–MS. Fig. 2 exhibits the SIR chromatogram of chlorogenic acid and caffeine. The results show that compounds differ in molecular mass or spectral fragmentation can

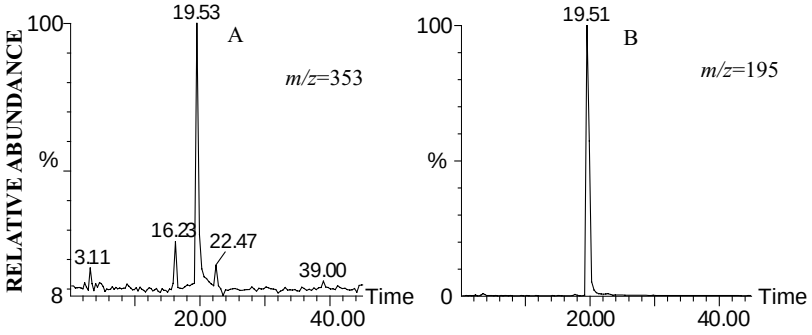


Fig. 2. SIR chromatograms of chlorogenic acid and caffeine. (A) Chlorogenic acid (ESI−), (B) caffeine (ESI+).

be determined. After investigating the response linearity of mixed standards of two compounds, no mutual restraining effects of ionization were found.

3.3. Interferences of amino acids in theanine analysis

Besides theanine, there are a number of amino acids such as phenylalanine, tysosine, and leucine, etc. There are possible interferences of other amino acids in the analysis of theanine. To investigate the veracity of determination of theanine by the proposed method, the elution of other amino acids was studied under proposed conditions. With HPLC–ESI–MS, we can distinguish interferences from other amino acids by using the structural information. The retention times of amino acids are also summarized in Table 3.

It can be seen that amino acids with less polarity elute out slowly than those with strong polarity, less polar compounds such as phenylalanine, tysosine, and leucine, could be well separated. While the polar amino acids with small molecule are washed out very quickly, the retention order partly depends on their isoeletrical point (PI). Those with higher values of PI elute out formerly, and the lower, the latter. The probable reason is that at strong acidic environment, the amino acids with higher PI values exist in the form of double dissociation, which enhances the polarity of amino acids, while those with low PI values dissociate only their $–NH_2$ group, so their polarity is relatively weak, thereafter they elute slowly.

An OPA pre-column derivative RP-HPLC method had been employed as a comparison assay with the present method for the analysis of theanine. The measuring results of theanine in samples display no significant difference.

3.4. Analysis of samples

The contents of caffeine, TB, TP, and chlorogenic acid were measured by using SIR mode. And the determination of theanine and catechins was completed by employing DAD. They show good linearity between peak areas and masses, the regression results, LOD, and LOQ was exhibited in Table 1. It can be seen all the relative coefficients of standard curves are better than 0.990.

The practicability of this method was verified in analyzing tea samples and green tea extracts. Table 2 lists the contribution of 12 components in tea samples and green tea extracts. Fig. 3 shows the UV chromatography of Pi Lo Chun using 200 nm as the detection wavelength, the peak assignments of catechins were accomplished with authentic standards and MS-spectrum, the retention order of catechins quite agrees with the published data [19]. While C eluted out previously to caffeine in the present study. The recovery of target compounds was measured by adding standards to Pi Lo Chun and Yun Wu, each level was analyzed five-fold, the mean recoveries varied form 89.3–104.2% at low level, 93.7–106.4% at medium level, and 93.5–104.8% at high level.

Contrast to Horie and Kohata's review [9], it is unnecessary to remove the polyphenols when analyze purine alkaloids, all the purine alkaloids including theobromine, theophylline, and caffeine can be well separated under the given condition without any interference from catechins, and theobromine can be

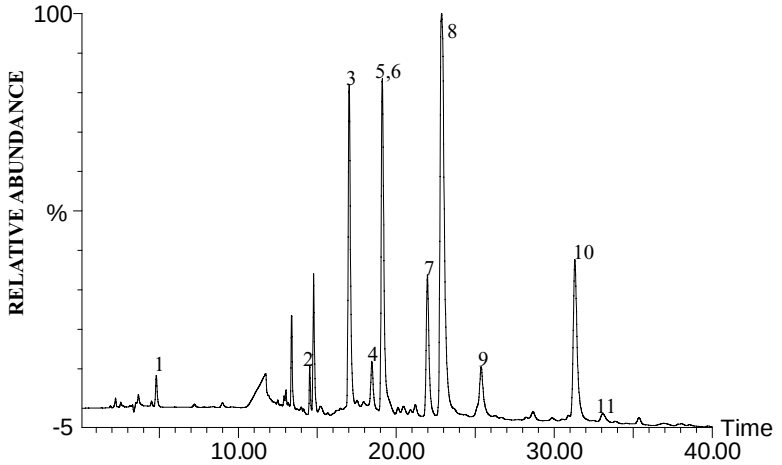


Fig. 3. Chromatogram of DAD detection for tea sample (Pi Lo Chun) analysis. Detection wavelength is 200 nm. Peak identification: (1) theanine, (2) theobromine, (3) EGC, (4) C, (5) caffeine, (6) chlorogenic acid, (7) EC, (8) EGCG, (9) GCG, (10) ECG, and (11) CG.

determined quantitatively. However, no theophylline was detected in teas; it is probably due to the factors such as geographical origins, soils, ages or processing, which can lead to the paucity of under limit of detection.

4. Conclusion

In this paper, the comprehensive contents of tea was analyzed using LC/ESI–MS, high sensitivity and selectivity have been achieved with MS and DAD as the detectors. The unity of LC/ESI–MS provides more qualitative and quantitative information comparing with general HPLC in the analysis of multi-components in tea. And complex extraction or sample pretreatment is unnecessary, the chromatogram acquired by using this method can be served as a bioactive components fingerprint for the quality control of tea and tea extracts. With the help of multi-dimension information of HPLC–DAD–ESI–MS, the compounds owning different chemical structure such as amino acid, catechins, etc. in tea and its extracts could be identified and determined in a single run successfully.

Acknowledgements

This work was supported by the Key Technologies Research and Development Program of the 10th 5-year Plan of the Ministry of Science and Technology of the People's Republic of China and Hunan province (2001BA746C, 2001BA804A21, 2003 AA2Z3515), the Natural Science Foundation of China and Hunan province (02JJY2023).

References

[1] A. Piga, A. Del Caro, G. Corda, J. Agric. Food Chem. 51 (2003) 3675–3681.
[2] L.O. Dragsted, Int. J. Vitam. Nutr. Res. 73 (2003) 112–119.
[3] J.V. Higdon, B. Frei, Crit. Rev. Food Sci. Nutr. 43 (2003) 89–143.
[4] K. Nakagawa, M. Ninomiya, T. Okubo, N. Aoi, L.R. Juneja, M. Kim, K. Yamanaka, T. Miyazawa, J. Agric. Food Chem. 47 (1999) 3967–3973.
[5] T. Sugiyama, Y. Sadzuka, Clin. Cancer Res. 5 (1999) 413–416.
[6] G. Zhang, Y. Miura, K. Yagasaki, Biosci. Biotechnol. Biochem. 66 (2002) 711–716.
[7] D.S. Duarte, M.F. Dolabela, C.E. Salas, D.S. Raslan, A.B. Oliveiras, A. Nenninger, B. Wiedemann, H. Wagner, J. Lombardi, M.T. Lopes, J. Pharm. Pharmacol. 52 (2000) 347–352.
[8] W. Rumpler, J. Seale, B. Clevidence, J. Judd, E. Wiley, S. Yamamoto, T. Komatsu, T. Sawaki, Y. Ishikura, K. Hosoda, J. Nutr. 131 (2001) 2848–2852.
[9] H. Horie, K. Kohata, J. Chromatogr. A 881 (2000) 425–438.
[10] M.S. Bispo, M.C. Veloso, H.L. Pinheiro, R.F. De Oliveira, J.O. Reis, J.B. De Andrade, J. Chromatogr. Sci. 40 (2002) 45–48.
[11] H. Sakakibara, Y. Honda, S. Nakagawa, H. Ashida, K. Kanazawa, J. Agric. Food Chem. 51 (2003) 571–581.

[12] Y.S. Lin, S.S. Wu, J.K. Lin, J. Agric. Food Chem. 51 (2003) 975–980.
[13] B.S. Yu, X.P. Yan, J. Xiong, Q. Xin, Chem. Pharm. Bull. (Tokyo) 51 (2003) 421–424.
[14] M. Sha, A. Cao, S. Yang, Zhong Guo Zhong Yao Za Zhi. 21 (1996) 108–1099, 128.
[15] P. Luo, F. Zhang, R.P. Baldwin, Anal. Chem. 63 (1991) 1702–1707.
[16] J. Saurina, S. Hernandez-Cassou, J. Chromatogr. A 676 (1994) 311–319.
[17] Y. Yokoyama, T.Y. Amaki, S. Horidishi, H. Sato, Anal. Sci. 13 (1997) 963–968.
[18] L. Campanella, G. Crescentini, P. Avino, J. Chromatogr. A 833 (1999) 137–145.
[19] D.J. Zeeb, B.C. Nelson, K. Albert, J.J. Dalluge, Anal. Chem. 72 (2000) 5020–5026.
[20] P. Chaimbault, K. Petritis, C. Elfakir, M. Dreux, J. Chromatogr. A 855 (1999) 191–202.
[21] S.H. Kim, J.D. Park, L.S. Lee, D. Han, Food Sci. Biotechnol. 9 (2000) 214–217.
[22] J.P. Aucamp, Y. Hara, Z. Apostolides, J. Chromatogr. A 876 (2000) 235–242.
[23] J.J. Dalluge, B.C. Nelson, J.B. Thomas, M.J. Welch, L.C. Sander, Rapid Commun. Mass Spectrum. 11 (1997) 1753–1756.
[24] G.K. Poon, J. Chromatogr. A 794 (1998) 63–74.
[25] J.J. Dalluge, B.C. Nelson, J.B. Thomas, L.C. Sander, J. Chromatogr. A 793 (1998) 265–274.