ELSEVIER

Journal of Chromatography A, 794 (1998) 63–74

JOURNAL OF CHROMATOGRAPHY A

Analysis of catechins in tea extracts by liquid chromatography–electrospray ionization mass spectrometry

G.K. Poon*

CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey, UK

Abstract

The analysis of catechin and related polyphenolic compounds in crude tea extracts by direct infusing of the samples into the mass spectrometer is described in this report. The mass spectra of catechin and its analogues all produced strong deprotonated molecules in the negative ionization mode. Further structural elucidation was accomplished by tandem mass spectrometry. This study demonstrates the mass spectrometric capability to analyze different components in crude plant extracts without recourse to liquid chromatographic separation. © 1998 Elsevier Science B.V.

Keywords: Tea; Mass spectrometry; Catechins; Polyphenolic compounds

1. Introduction

Most plants contain antioxidants which are phenolic or polyphenolic compounds. The most commonly occurring phenolic compounds in foods are simple phenols, phenolic acids and flavonoids. Green tea and black tea, which are among the most popular beverages consumed worldwide, contain flavan-3-ols, commonly known as catechins. These compounds are widely distributed in the leaves of green tea, and constitute up to 30% of the dry leaf weight. Much interest has been focused on catechins, not only for their antioxidant activity, but also because of their known antimutagenic and antitumorigenic properties. Thus there are many epidemiological and laboratory studies in progress which demonstrate the inhibitory effects of green tea against cancer.

Some of these studies relate directly to lung cancer attributable to cigarette smoking. For example, the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [1] is a potent tobacco-specific carcinogen formed from the nitrosation of nicotine during tobacco processing and cigarette smoking, and has been shown to induce tumors in the lung, liver, the nasal cavity of rats, and the lung and skin of mice. Catechin (3,3′,4,5,7-pentahydroxyflavan, structure **I**) is nonmutagenic and has been shown to decrease the mutagenicity. When incubated with mouse lung microsomes, green tea and black tea have also been shown to inhibit DNA methylation and DNA binding activity [2]. Other observations include the inhibition of large intestinal cancers induced by 1,2-dimethylhydrazine [3]. Wang et al. have demonstrated that mice with chemically or UV-light induced skin papillomas, when treated with green tea in the drinking water or with i.p. injection of topical application of (−)-epigallocatechin-3-gallate (EGCG, structure **II**), these chemicals not only decreased tumor formation, but also reduced tumor size [4–6]. Further interest centres on the inhibitory activities of (−)-epigallocatechin (EGC, structure **III**) and EGCG against the human immunodeficiency virus-reverse transcriptase (HIV-RT) [7].

*Corresponding author. Present address: Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, USA.

0021-9673/98/$19.00
PII S0021-9673(97)01050-9

EGCG is the most abundant component present in tea extracts and also the most potent chemical of the epicatechin derivatives tested for biological activity. Others include EGC, (+)-catechin, and (−)-epicatechin (EC). As each catechin possesses distinct properties, a simple and rapid method that could be used for analyzing individual catechins in a complex tea mixture would be an advantage. There are several reports on HPLC analysis of catechins [8,9]. However, the structural similarity of the various tea catechins makes the high-performance liquid chromatography (HPLC) analysis of individual catechins difficult. Recently Bailey et al. [10] coupled liquid chromatography with a magnetic sector mass spectrometer using a plasmaspray interface, operated in the positive ionization mode. In the case of plasmaspray ionization, catechin and its analogues all show abundant protonated molecules.

The present report describes a direct infusion of extracted tea samples into the mass spectrometer fitted with either an electrospray ionization interface (ESI-MS), or an ionspray interface (IS), without LC separation. Catechin and its analogues were observed in the mass spectra. Better sensitivity and selectivity were achieved when the mass spectrometer was operated in the negative ionization mode, while further structural confirmation was accomplished by tandem mass spectrometry, utilizing the product-ion and parent-ion scans of the fragment ions characteristic of catechin and gallic acid (3,4,5-trihydroxybenzoic acid, structure **IV**).

2. Experimental

2.1. Chemicals and materials

Gallic acid (97% purity) and (+)-catechin hydrate (98% purity) were purchased from Aldrich Chemical Co., (Poole, UK). (−)-Quinic acid (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid) was purchased from Sigma–Aldrich Chemical Co., (Poole, UK). HPLC grade acetonitrile and methanol was obtained from Romil Chemicals Ltd. (Loughborough, UK).

2.2. Sample preparation

Tea infusions were prepared by boiling the tea samples in water for 10 min, as described by Wellum and Kirby [8]. The extracts were filtered thorough 0.45 μm Millipore filters. The filtrates were purified by Sep-Pak C_{18} cartridges conditioned by methanol–water (50:50, v/v 2 ml), followed by water (3 ml). The compounds of interest were eluted with methanol–water (80:20, v/v).

2.3. Mass spectrometry

Two mass spectrometry systems were used to analyze the tea extracts. One analysis was performed on the Finnigan MAT TSQ 700 triple quadrupole mass spectrometer fitted with an electrospray ionization source (San Jose, CA, USA). The heated capillary and voltage were maintained at 230°C and −4.5 kV. A Harvard syringe pump (Harvard Apparatus Inc., South Natick, MA, USA) was used to infuse the tea extracts into the mass spectrometer at 10 μl/min. Full scan Q_1 mass spectra were acquired with a scan speed of 3 s/scan. Tandem mass spectrometry was performed using argon as the collision gas, operated at 0.45 mtorr and the collision energy set at 40 eV. All mass spectrometry data were acquired in the negative ionization and profile modes, the scan range varied according to the molecular weights of the parent compounds. Profile data for 64 scans were averaged to improve the signal to noise ratio in the spectrum. The DEC 2100 data system was controlled by Finnigan ICIS software (version 7).

The second triple quadrupole mass spectrometer used was Perkin–Elmer Sciex API 300 (Concord, Canada) equipped with a Turbo Ionspray interface. The ionspray and orifice voltage were maintained at −3150 and −33 V, respectively. For tandem mass spectrometry, nitrogen was used as collision gas (solenoid setting at 4), nebulizer gas (solenoid setting at 15) and curtain gas (solenoid setting at 8). Collision energy was set at 21 eV. All mass spectrometry data were acquired in the negative ionization profile mode and processed by the PE Sciex proprietary software program Multiview (version 1.2).

3. Results and discussions

The full scan negative ionization mass spectra of gallic acid, catechin and quinic acid are shown in

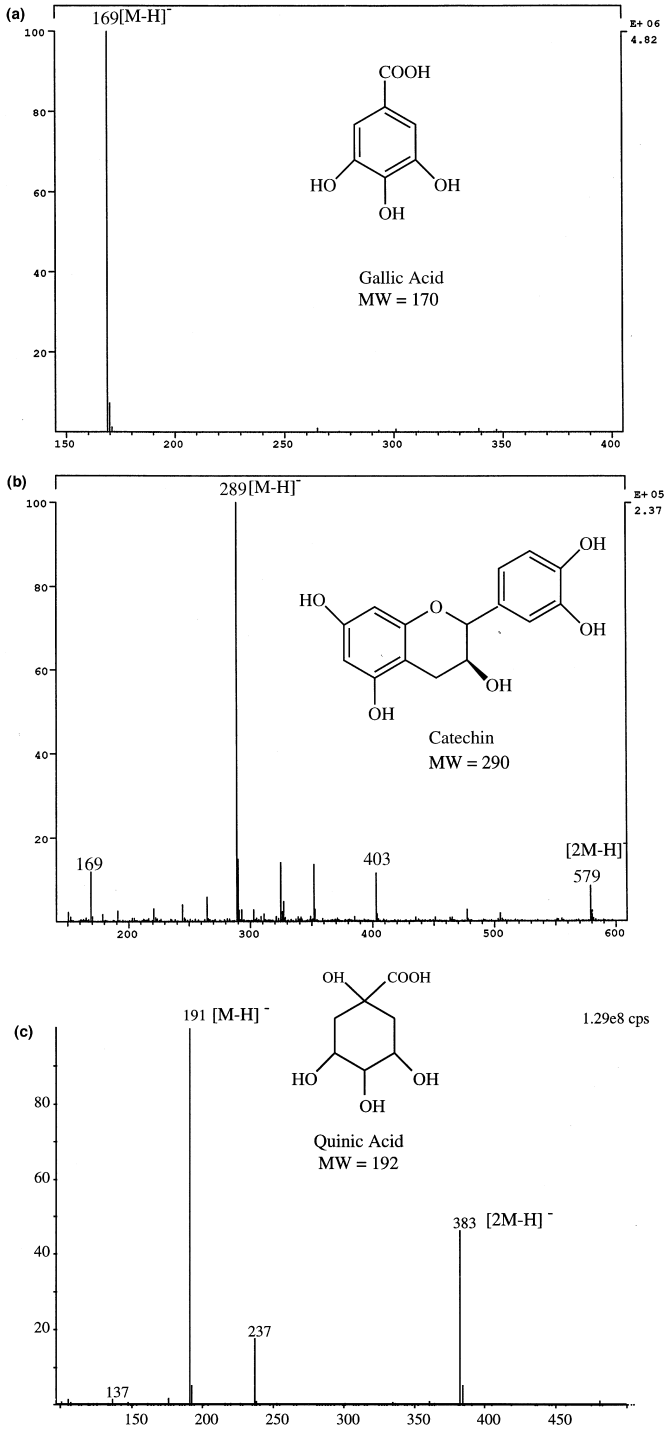


Fig. 1. Negative ion mass spectra of (a) gallic acid, (b) catechin and (c) quinic acid.

Fig. 1a–c. These compounds show the deprotonated molecules ($[M-H]^-$) at m/z 169, 289 and 191, respectively. The signals at m/z 579 (Fig. 1b) and at m/z 383 (Fig. 1c) correspond to the dimer of catechin and quinic acid because these compounds were present at fairly high concentrations in the infusion solutions. Even though infusion of gallic acid, catechin and quinic acid in the presence of methanol–water (50:50, v/v) containing 0.1% formic acid generated protonated molecules at m/z 171, 291 and 193, respectively, other background ions were observed in the mass spectra of gallic acid and quinic acid. Therefore subsequent experiments were performed in the negative ionization mode.

Three individual tea extract samples were infused into the mass spectrometer and their full scan mass spectra were acquired. The first two tea extract samples gave identical mass spectra, as represented

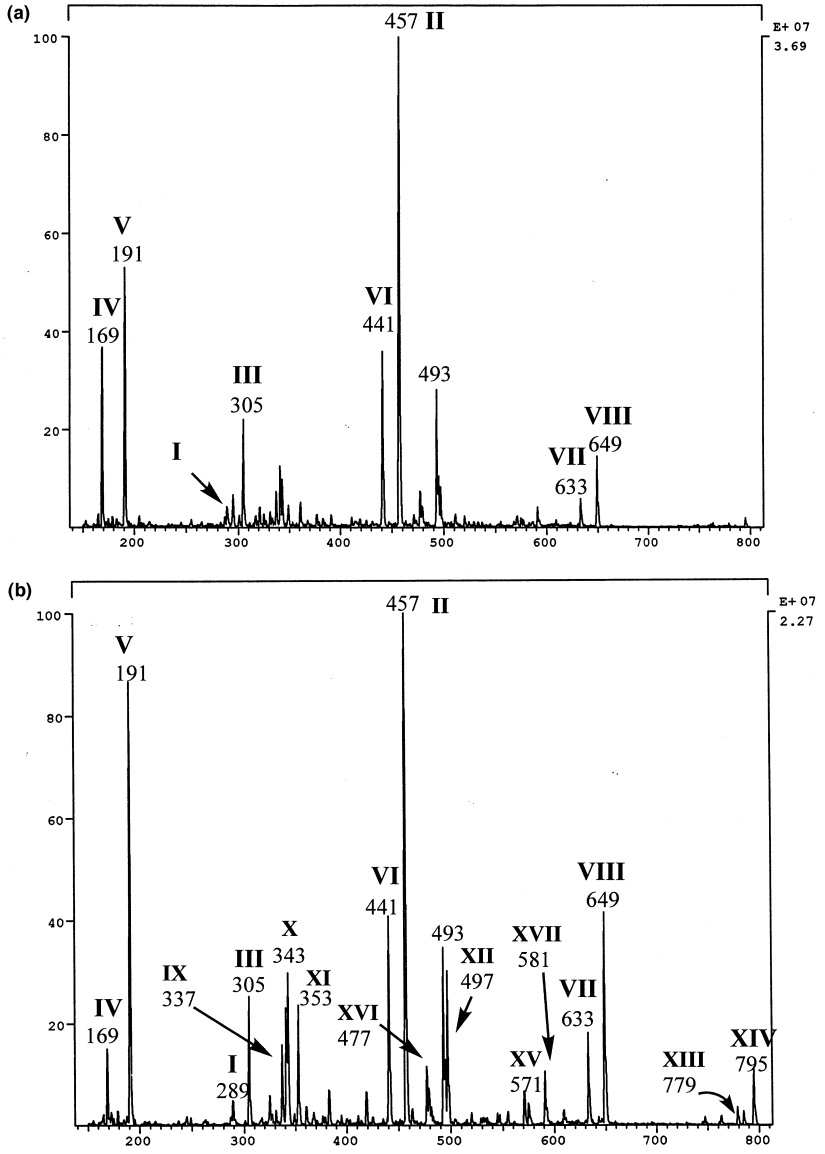


Fig. 2. Negative ion mass spectra of two tea extracts obtained by infusing the samples into the mass spectrometer.

in Fig. 2a. The mass spectrum contains several signals generated from catechins analogues and are labelled **I**–**VIII**. These pseudomolecular ions ([M−H]$^-$) at m/z 169 (**IV**), 191, (**V**), 289 (**I**), 305 (**III**), 441 (**VI**) and 457 (**II**) correspond to gallic acid (structure **IV**), quinic acid (structure **V**), catechin (structure **I**), epigallocatechin (EGC, structure **III**), epicatechin gallate (ECG, structure **VI**) and epigallocatechin gallate (EGCG, structure **II**), respectively. It is possible the signals labelled VII–VIII are also phenolic analogues of catechins. Structural characterization of these catechin analogues signals was achieved by tandem mass spectrometry.

The product-ion mass spectra of compounds **II**, **VI**, **VIII** and **VII** are displayed in Fig. 3a–d. The fragment ion at m/z 169 (derived from gallic acid)

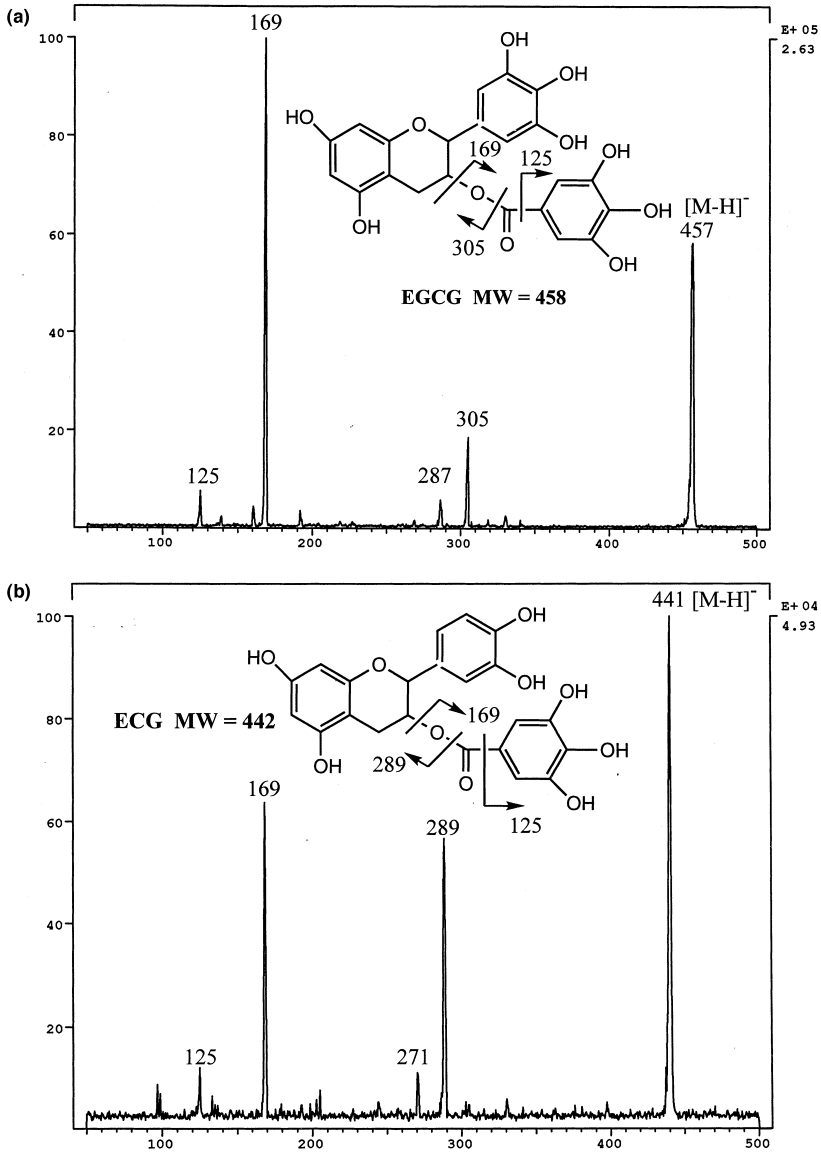


Fig. 3. Product-ion mass spectra of deprotonated molecules: (a) EGCG at [M−H]$^-$ of m/z 457; (b) ECG at [M−H]$^-$ of m/z 441; (c) compound **VIII** at [M−H]$^-$ of m/z 649 and (d) compound **VII** at [M−H]$^-$ of m/z 633.

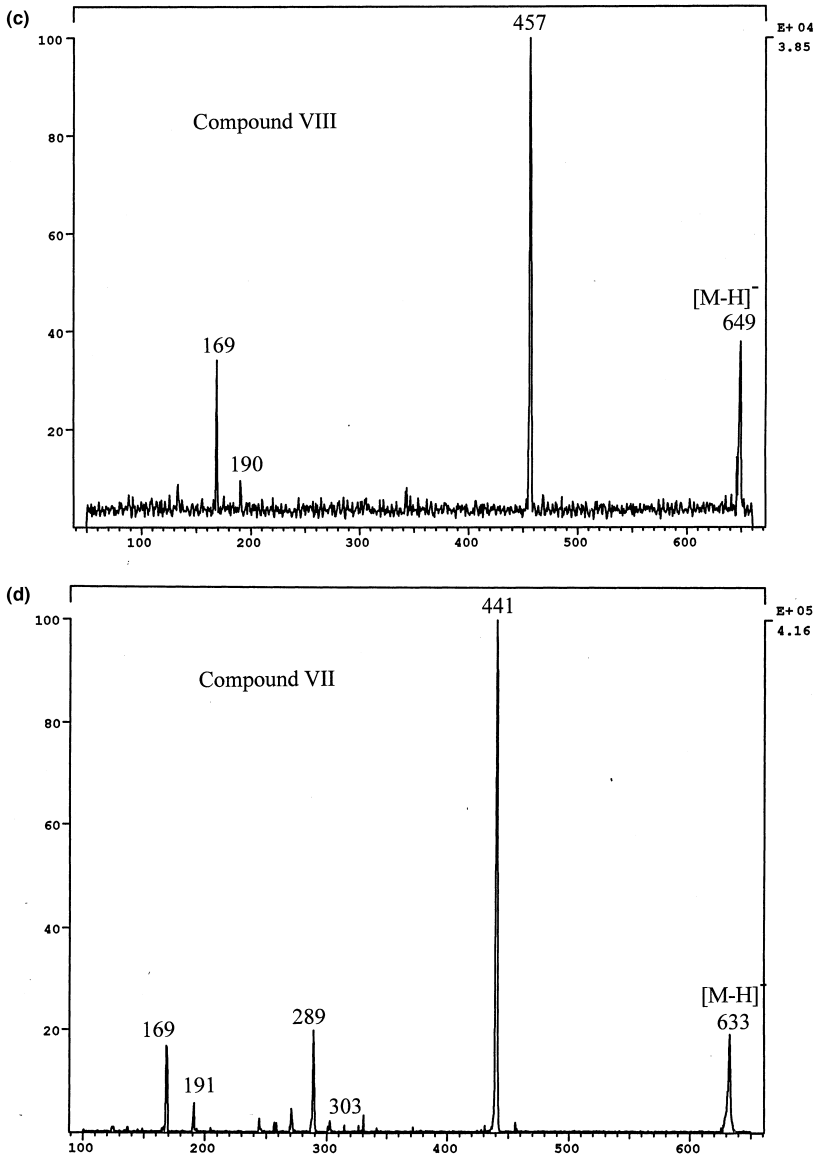


Fig. 3. (*continued*)

predominates in the mass spectra. The product-ion mass spectrum of compound **II** (EGCG) is displayed in Fig. 3a, and consisted of fragment ions at m/z 125 (dihydroxy phenol moiety), 169 (gallic acid), 287 (loss of water from the EGC moiety) and 305 (EGC moiety), which clearly confirms that compound **II** is epigallocatechin-3-gallate. Similarly, the product-ion mass spectrum of compound **VI** (ECG, Fig. 3b) contains four abundant fragment ions at m/z 125 (trihydroxy phenol moiety), 169 (gallic acid), 271 (loss of water from the catechin moiety) and 289 (catechin). The product-ion mass spectrum of compound **VIII** and **VII** are illustrated in Fig. 3c,d. The base peaks at m/z 441 and 457 are 192 mass units less than the pseudomolecular ions (m/z 633 and 649, respectively). It is possible that compounds **VII**

and **VIII** are formed by the addition of quinic acid to ECG and EGCG. Under collision-induced dissociation conditions, quinic acid (m/z 191, structure **V**) was eliminated from the parent compounds to give ECG and EGCG, respectively. Quinic acid is also an abundant ingredient in tea extracts. It was first reported in 1982 by Sakata et al. [11], using ^{13}C Fourier-transform nuclear magnetic resonance spectroscopy. The m/z 191 signal is also clearly observed in the mass spectra of the tea extracts (Fig. 2a,b).

The full scan mass spectrum obtained from the direct infusion of the third tea extract sample is shown in Fig. 2b. Compounds **I**–**VIII** were present in the sample. Additionally, several extra signals at m/z 337, 343, 353, 497, 779, 795, 571, 477 and 581 were detected (labelled as **IX**–**XVII**, respectively). The product-ion mass spectra of compounds **IX**–**XVII** were represented in Fig. 4a–i.

A less intense signal at m/z 337 in the mass spectrum suggests this component could derive from *p*-coumarylquinic acid (structure **IX**, Table 1), a chemical which has previously been detected in tea extracts [10]. The product-ion mass spectrum of m/z 337 displayed fragment ions at m/z 191, 173 and 163, which correspond to quinic acid, trihydroxycyclohexane carboxylic acid and 3-(4-hydroxyphenyl)-1-oxo-2-propenyl moieties (Fig. 4a).

The product-ion mass spectra of compounds **X**–

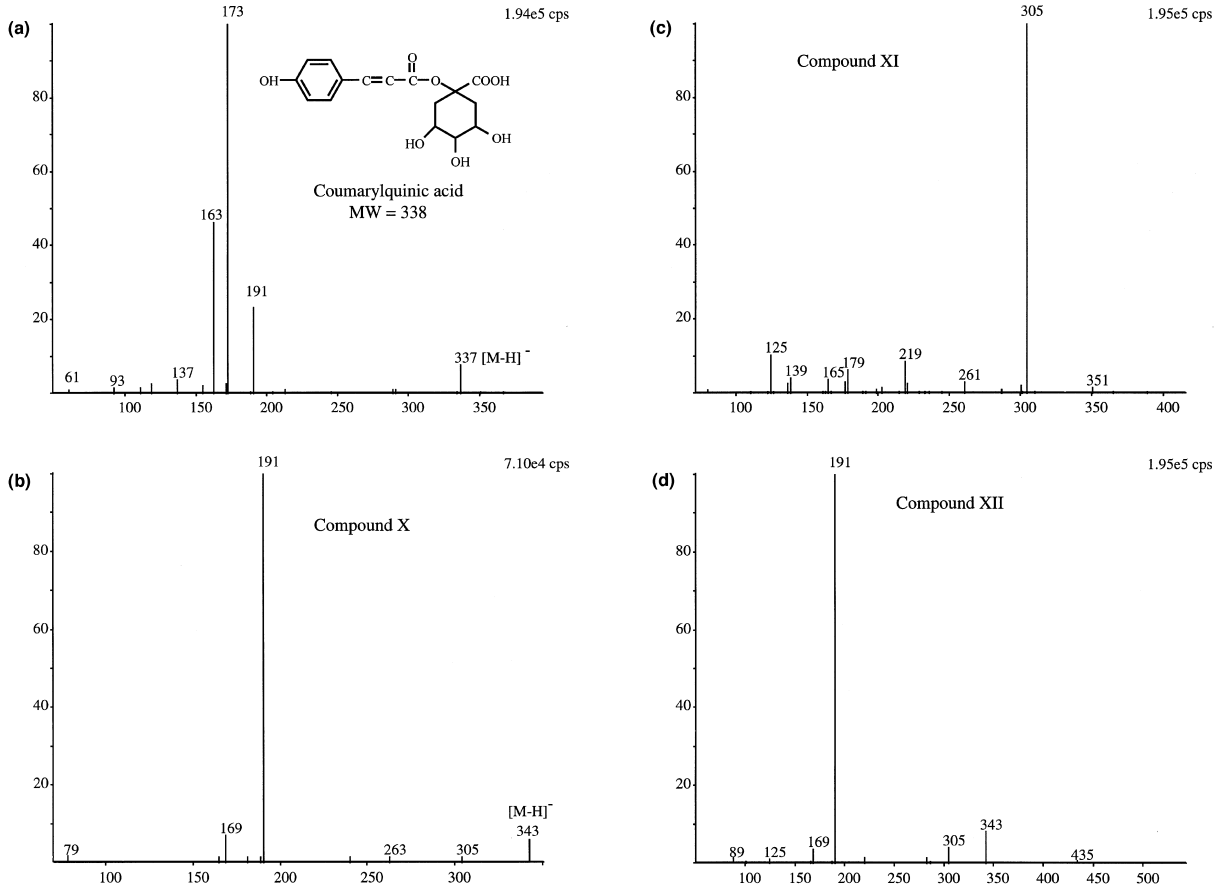


Fig. 4. Product-ion mass spectra of deprotonated molecules: (a) compound **IX** at $[M-H]^-$ of m/z 337; (b) compound **X** at $[M-H]^-$ of m/z 343; (c) compound **XI** at $[M-H]^-$ of m/z 353; (d) compound **XII** at $[M-H]^-$ of m/z 497; (e) compound **XIII** at $[M-H]^-$ of m/z 779; (f) compound **XIV** at $[M-H]^-$ of m/z 795; (g) compound **XV** at $[M-H]^-$ of m/z 571; (h) compound **XVI** at $[M-H]^-$ of m/z 477 and (i) compound **XVII** at $[M-H]^-$ of m/z 581.

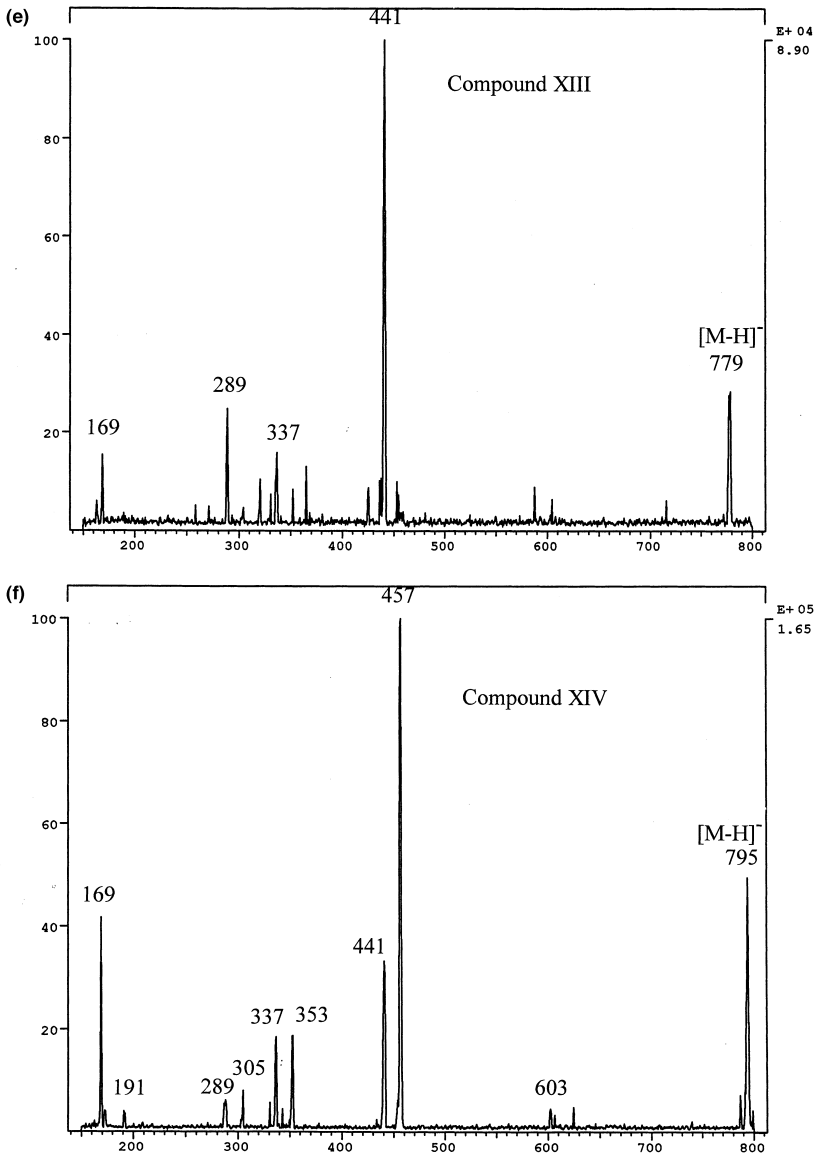


Fig. 4. (*continued*)

XIV mostly contained fragment ions at m/z 169, 191, 305 (Fig. 4b–f) which strongly indicate these components are the analogues of EGC. The fragment ion (m/z 305) in the product-ion mass spectrum of compound **XII** represented a loss of quinic acid. Additionally the product-ion mass spectra of compounds **XIII**, **XIV** and **XVI** displayed a fragment ion at m/z 441 (Fig. 4e,f,h), and the product-ion mass spectra of compounds **XIV**, **XV** and **XVII** were dominated by the fragment ion at m/z 457 (Fig. 4f,g,i), further suggesting that these compounds are analogues of ECG. The precise structures of these compounds have not been characterized. Finally, no product-ion mass spectrum was obtained from the signal m/z 493 observed in the tea extracts (Fig. 2a,b).

Since m/z 169 and 289 are typical fragment ions detected in the mass spectra of catechins, these two

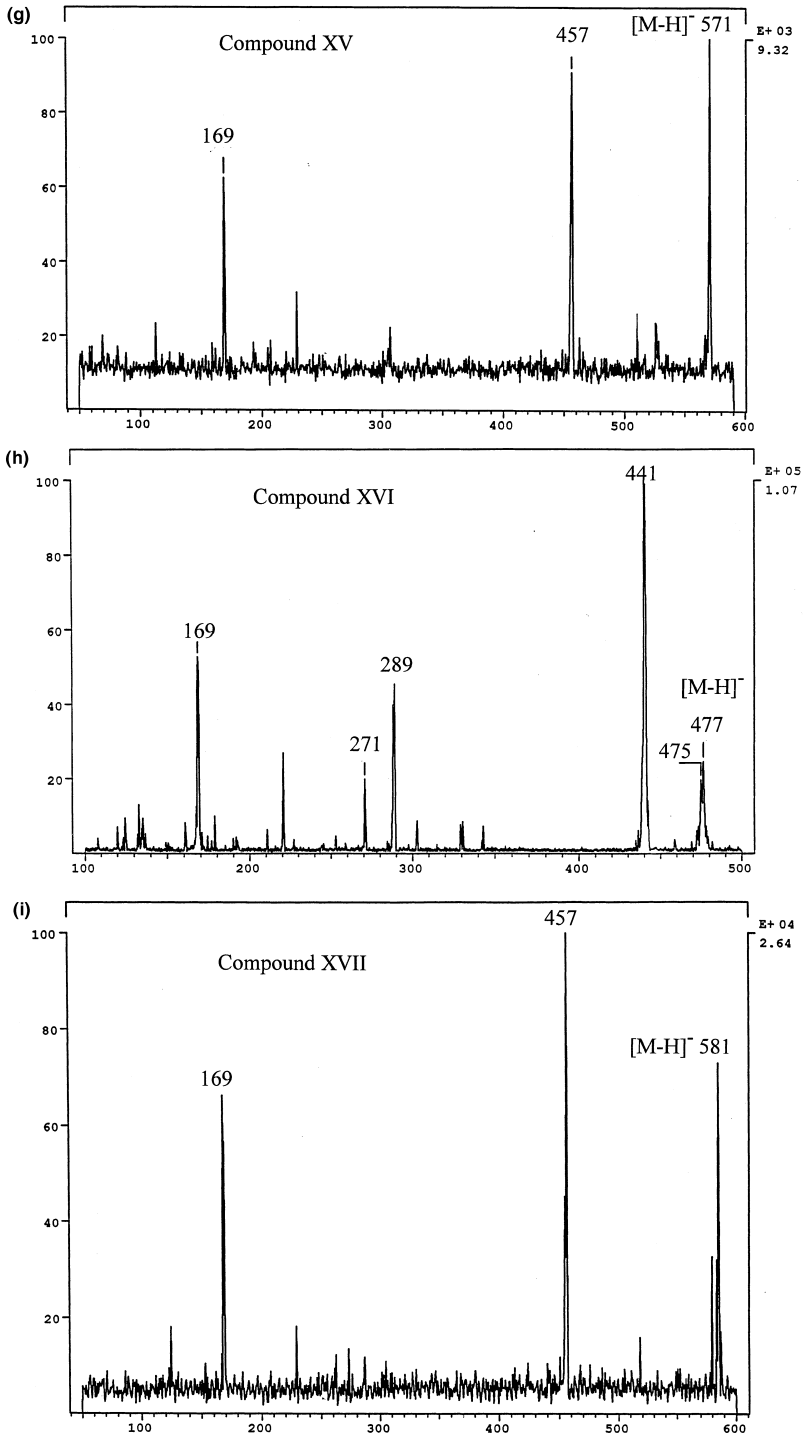
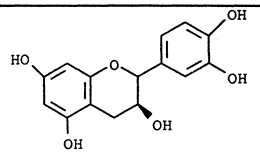
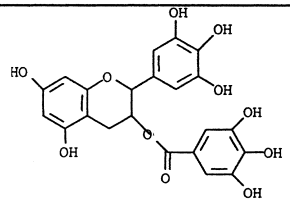
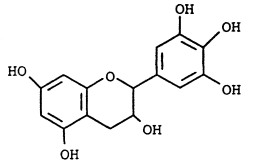
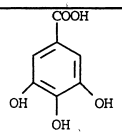
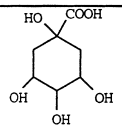
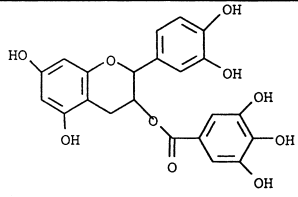
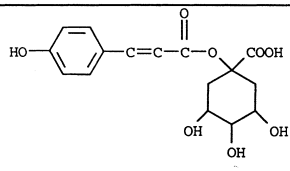


Fig. 4. (*continued*)

Table 1
Structures of catechin and its phenolic analogues

Compound	Abbreviation	$[M-H]^-$	Structure
(+)-Catechin (I)		289	
Epigallocatechin-3-gallate (II)	EGCG	457	
Epigallocatechin (III)	EGC	305	
Gallic acid (IV)		169	
Quinic acid (V)		191	
Epicatechin-3-gallate (VI)	ECG	441	
Coumarylquinic acid (IX)		337	

ions were set as the precursor ions, in the parent ion scanning mode. When the parent ion scans were performed on the tea extract samples, a whole range of catechin analogues were revealed for each ion (Fig. 5a,b). Hence this experiment further confirms compounds **VII**, **VIII**, **XIII** and **XIV** are analogues of catechins. The parent ion scan of m/z 289 revealed other signals such as m/z 493, 555 and 577. These

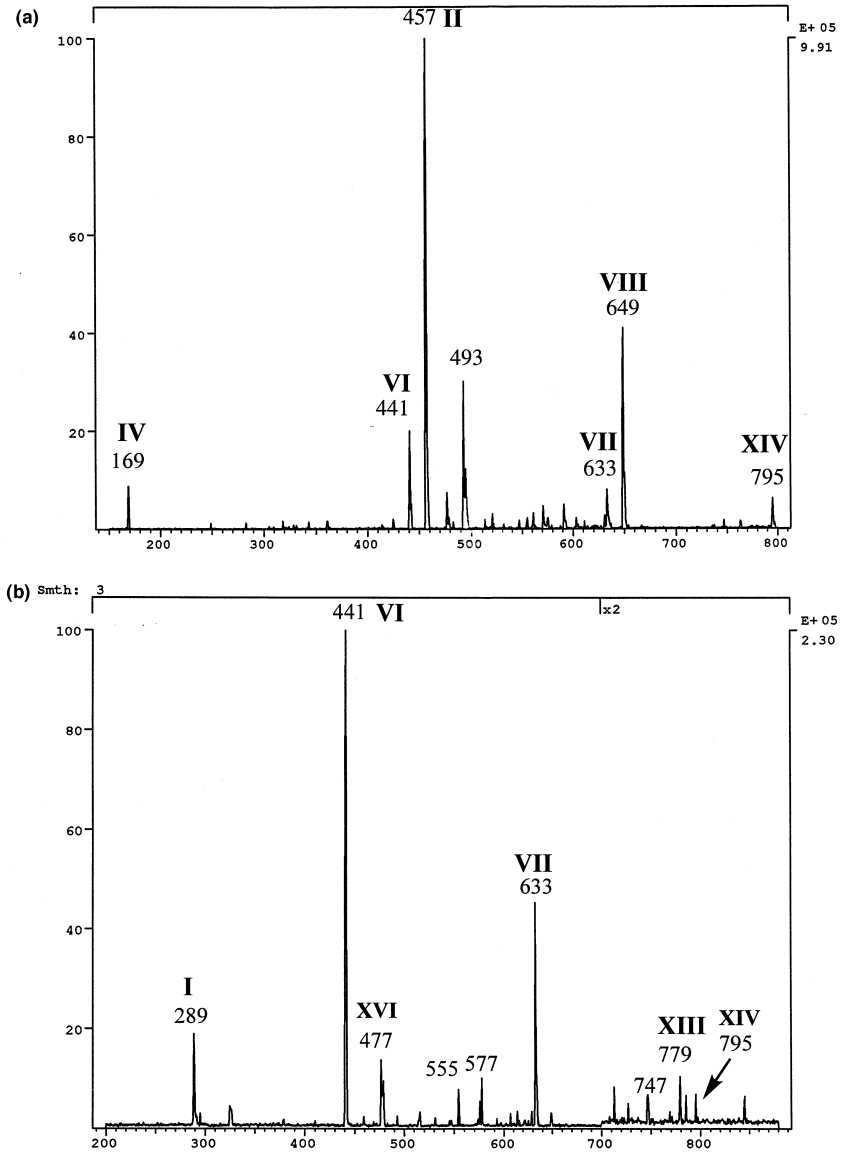


Fig. 5. Parent-ion mass spectra of (a) *m*/*z* 169 and (b) *m*/*z* 289 obtained by infusing the tea extract samples into the mass spectrometer.

components were present in the crude tea extracts (Fig. 2b), but no product-ion mass spectra were available.

4. Conclusion

Two important conclusions can be drawn from the present study. Firstly, this methodology did not require chromatographic method development. Secondly, it demonstrated that the technique is feasible for fast screening of compounds of pharmaceutical interest, after sample purification or extraction. With the introduction of combinatorial chemistry, in which a vast number of compounds will be generated robotically, it is critical for the pharmaceutical industry to establish a high sample throughput program capable of screening large number of

samples. The applications of short LC–MS run times (approximately 2 to 3 min per sample) are well established to support pharmacokinetic studies [12,13]. Recently, Bowers et al. [14] demonstrated it is feasible to interface an automated sample preparation system with the mass spectrometer. Samples were purified on a solid-phase extraction system, the extracts were transferred to a tandem mass spectrometer without the need for an LC analytical column. The quantification limits of some drugs were 50 pg/ml from a 200 μl serum or plasma sample. It is therefore to be expected that flow injection analysis of crude biological extracts will be an acceptable technique in LC–MS in the near future.

Acknowledgements

This work was supported by the Cancer Research Campaign, UK.

References

[1] Z.Y. Wang, J.Y. Hong, M.T. Huang, K.R. Reuhl, A.H. Conney, C.S. Yang, Cancer Res. 52 (1992) 1943.
[2] S.T. Shi, Z.Y. Wang, T.J. Smith, J.Y. Hong, W.F. Chen, C.T. Ho, C.S. Yang, Cancer Res. 54 (1994) 4641.
[3] P. Yin, J. Zhao, S. Cheng, Y. Hara, Q. Zhu, Z. Liu, Cancer Lett. 79 (1994) 33.
[4] Z.Y. Wang, M.T. Huang, C.T. Ho, R. Chang, T. Ferraro, K.R. Reuhl, C.S. Yang, A.H. Conney, Cancer Res. 52 (1992) 6657.
[5] M.T. Huang, C.T. Ho, Z.Y. Wang, T. Ferraro, T. Finnegan-Olive, Y.R. Lou, J.M. Mitchell, J.D. Laskin, H. Newmark, C.S. Yang, A.H. Conney, Carcinogenesis 13 (1992) 947.
[6] G. Hu, C. Han, J. Chen, Nutr. Cancer 24 (1995) 203.
[7] C.W. Chang, F.L. Hsu, J.Y. Lin, J. Biomed. Sci. 1 (1994) 163.
[8] D.A. Wellum, W. Kirby, J. Chromatogr. 206 (1981) 400.
[9] T. Goto, Y. Yoshida, M. Kiso, H. Nagashima, J. Chromatogr. A 749 (1996) 259.
[10] R.G. Bailey, H.E. Nurston, I. McDowell, J. Sci. Food Agric. 66 (1994) 203.
[11] K. Sakata, S. Sakuraba, A. Yagi, D. Ina, T. Hara, T. Takeo, Agric. Biol. Chem. 50 (1986) 1919.
[12] T.R. Covey, E.D. Lee, J.D. Henion, Anal. Chem. 58 (1986) 2453.
[13] J.D. Gilbert, T.V. Olah, A. Barrish, T.F. Greber, Biol. Mass Spectrom. 21 (1992) 341.
[14] G.D. Bowers, C.P. Clegg, S.C. Hughes, A.J. Harker, S. Lambert, LC–GC 15 (1997) 48.