

# Application of liquid chromatography–atmospheric pressure ionization mass spectrometry in natural product analysis

## Evaluation and optimization of electrospray and heated nebulizer interfaces

Shaolian Zhou\*, Matthias Hamburger

*Centre for Natural Product Research, Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge, Singapore 119260, Singapore*

Received 15 March 1996; revised 2 July 1996; accepted 3 July 1996

---

### Abstract

A representative set of 46 secondary metabolites of plant and microbial origin was examined with electrospray and atmospheric pressure chemical ionization MS in both positive and negative ion mode, in order to evaluate the applicability of atmospheric pressure ionization LC–MS as a tool for rapid identification and dereplication of bioactive compounds in the drug screening process. MS characteristics of different compound classes were determined on the basis of quasi-molecular ion and other adduct ion responses observed with the two LC–MS interfaces. Interface parameters and LC mobile phase composition were optimized and an analytical protocol applicable to a wide range of secondary metabolites is proposed.

*Keywords:* Interfaces; Electrospray ionization; Nebulizing interface; Atmosphere pressure chemical ionization; Detection, LC

---

### 1. Introduction

In new drug lead discovery programmes, biological samples still play an important role as source for structurally diverse and unique molecules [1]. However, the success of a high-throughput screening operation critically depends on the implementation of procedures for efficient dereplication of known and/or otherwise uninteresting molecules and the early identification of potentially new leads. Mass spectrometry (MS), with its high sensitivity and capability of providing  $M_r$  and additional structural

information, fulfills many of the requirements of an ideal dereplication technique. With the recent developments in MS interface technology, the prospects for characterization of unknown compounds in complex matrices by means of LC–MS have significantly improved [2,3].

A number of interfaces are of potential interest for dereplication purposes. Among the most commonly used LC–MS interfaces, the particle beam (PB) interface [4] can be operated in both chemical ionization (CI) and electron impact (EI) mode. EI spectra produced by the PB interface are comparable to conventional EI spectra and are therefore searchable thanks to commercially available MS libraries. The major drawback of PB is the lack of sensitivity

---

\*Corresponding author. Present address: Department of Chemistry, University of Tennessee, Knoxville, TN 37919, USA.

for non-volatile compounds. It is thus not suitable for the analysis of polar molecules as encountered in most natural product extracts [2,5]. The thermospray (TSP) interface has been successfully used for the analysis of a wide range of natural products, including polar and non-volatile compounds [2,5–16]. However, good TSP performance critically depends on the operating conditions (vaporizer and source temperature, repeller voltage) which need to be optimized for the analyte of interest [2,5,17]. Thus, the TSP interface is not very suitable in the context of a high-throughput screening programme, where the analytes are typically unknown and of high structural diversity [17]. Also, the high temperature applied during vaporization may lead to decomposition of thermolabile samples [2,5]. The continuous-flow fast atom bombardment (CF-FAB) interface [18] has also found some application in natural product chemistry [19]. Although CF-FAB overcomes some limitations of TSP, its use appears limited due to the difficulties of practical implementation. In the recently introduced electrospray interface (ESI) [20,21], the ions are taken straight from the liquid phase at room temperature and atmospheric pressure. ESI is ideally suited for the analysis of large biomolecules such as proteins, but is also increasingly used for non-volatile molecules in the lower to mid-mass range [22–29]. The heated nebulizer (atmospheric pressure chemical ionization, APCI) is another type of interface operating at atmospheric pressure, where analyte ions are generated via chemical ionization in the gas phase [30]. The temperature control of the APCI desolvation process appears far less critical than in TSP-MS, and a wide range of compounds may possibly be analyzed under the same vaporizer conditions [2,3]. The main application of LC-APCI-MS analysis has been for analysis of drug metabolites in biological samples [31–34]. Despite its inherent advantages over TSP, APCI has not been applied to natural products.

Considering our goal, namely the development of a general LC-MS tool for the analysis of a wide range of natural products in unknown samples, a combination of the ESI and APCI interfaces appeared to hold the best promise. The ionization mechanisms of ESI and APCI differ [35], hence the ionization characteristics for various analytes also

differ. The ESI process is strongly dependent on the solution chemistry, electrophoretic migration and surface behavior of analyte ions [35–39]. The presence of preformed ion species in solution is essential for the formation of gas phase ions. In contrast to ESI, APCI relies more on the gas phase chemistry, e.g., the gas phase acidity or basicity [40]. The analyte is vaporized into the gas stream prior to chemical ionization through the charge transfer or proton transfer from the reagent ions. In LC-MS applications with both atmospheric pressure ionization interfaces, change of LC mobile phase composition may influence the ion formation and response [35,38,41]. We report here on the first systematic optimization of API-MS for natural product analysis and the development of a generally applicable LC-API-MS dereplication protocol. A set of 46 diverse natural products of plant and microbial origin were analyzed under ESI- and APCI-MS condition, both in positive and negative ionization mode (Table 1). MS ionization characteristics and ion response under different instrument conditions and with various mobile phase compositions were examined.

## 2. Experimental

### 2.1. API-MS

All experiments were performed with a Sciex API III<sup>plus</sup> triple quadrupole mass spectrometer coupled with articulated ionspray interface for pneumatically assisted electrospray ionization (ESI) and a heated nebulizer interface for APCI-MS (Sciex, Thornhill, Canada). Major parameters of two interfaces used in this study are listed in Table 2, with exception of those specifically stated in the Section 3 (Results and discussion). The mass ions were recorded in the full scan mode by quadrupole 1. The MS data were averaged from three replicate injections (10  $\mu$ l each).

### 2.2. Liquid chromatography

The HPLC system consisted of a Perkin-Elmer 200 series pump, ISS 200 autosampler and LC-95 UV detector (Perkin-Elmer, Norwalk, CT, USA). Direct flow injection analysis was performed without HPLC columns. The eluent was transferred at the

Table 1  
Natural products investigated in this study

Microbial metabolites	Plant metabolites	
<i>Macrolides</i>	<i>Alkaloids</i>	<i>Purines</i>
Nonactin	Ajmalicine	Adenosine
Oligomycin B	Atropine	Caffeine
Rifampicin	Brucine	
	Papaverine hydrochloride	<i>Amides</i>
<i>Peptides</i>	Quinine	Capsaicin
Actinomycin D	Colchicine	Piperine
Bacitracin	Emetine dihydrochloride	
	Tubocurarine chloride	<i>Phenolics</i>
<i>Aminoglycoside</i>	Vinblastine sulfate	1,8-Dihydroxy anthraquinone
Neomycin trisulfate		Ellagic acid
	<i>Terpenoids</i>	Catechin
<i>Polyether</i>	Abietic acid	Podophyllotoxin
Monensin sodium salt	Caryophyllene epoxide	Xanthotoxin
	Farnesol	Quercetin
<i>Polyene</i>	Picrotoxin	
Amphotericin	$\alpha$ -Santonin	<i>Glycosides</i>
	Stigmasterol	Alonin
<i>Miscellaneous</i>	Taxol	Digitoxin
Benzylpenicillin		Amygdalin
Chlorotetracycline		Chlorogenic acid
Compactin		Glycyrrhizic acid ammonium salt
Erythromycin		Malvine chloride
Novobiocin sodium salt		
Puromycin dihydrochloride		

flow-rate of 1 ml min<sup>-1</sup> directly to the APCI interface while a 1:60 split ratio was used with the ionspray interface (17  $\mu$ l min<sup>-1</sup> into ESI source). Acetonitrile–water (50:50, v/v) mixtures containing various modifiers were used as mobile phases in this study (Table 3).

### 2.3. Samples

All compounds listed in Table 1 were purchased from Fluka (Buchs, Switzerland). Samples were dissolved in HPLC-grade methanol without further purification. The final concentration of the samples

Table 2  
LC-MS conditions used in this study: API-MS settings

Electrospray parameter	Setting	Heated nebulizer parameter	Setting
<i>Electrospray voltage</i>		<i>Discharge current</i>	
Positive ion mode	4.5 kV	Positive ion mode	3 $\mu$ A
Negative ion mode	-4.0 kV	Negative ion mode	-3 $\mu$ A
<i>Orifice voltage</i>		<i>Orifice voltage</i>	
Positive ion mode	75 V	Positive ion mode	75 V
Negative ion mode	-75 V	Negative ion mode	-75 V
Curtain gas flow-rate	0.6 l min <sup>-1</sup>	Curtain gas flow-rate	0.6 l min <sup>-1</sup>
Nebulizer gas pressure	276 kPa (40 p.s.i.)	Nebulizer gas pressure	552 kPa (80 p.s.i.)
Interface heater temperature	60°C	Auxillary gas flow-rate	1.5 l min <sup>-1</sup>
		Vaporizer temperature	450°C

Table 3  
LC-MS conditions used in this study: mobile phase compositions

Mobile phase	Composition for ESI-MS	Composition for APCI-MS
M0	water–acetonitrile (50:50, v/v)	water–acetonitrile (50:50, v/v)
M1	M0+0.1% acetic acid	M0+0.1% acetic acid
M2	M0+0.1% formic acid	M0+0.1% formic acid
M3	M0+0.1% trifluoroacetic acid (TFA)	M0+0.1% trifluoroacetic acid (TFA)
M4	M0+2 mM ammonium acetate	M0+50 mM ammonium acetate
M5	M4+0.1% acetic acid	M4+0.1% acetic acid
M6	M0+0.03% triethylamine (TEA)	M0+0.1% triethylamine (TEA)

for flow injection was  $50 \text{ ng } \mu\text{l}^{-1}$  for microbial metabolites, and  $10 \text{ ng } \mu\text{l}^{-1}$  for plant-derived compounds.

### 3. Results and discussion

Unambiguous identification of the ion species detected by MS is essential for molecular mass determination of an unknown analyte, which in turn is a requirement for natural products database searches. Quasi-molecular ions or other adduct ion species should ideally be of high intensity, allowing for low concentration of analytes to be detected in complex matrices and MS–MS analysis to be performed for further structural information. The nature and intensity of ion species were therefore the main criteria for interface evaluation and optimization.

#### 3.1. Optimization of API interfaces

##### 3.1.1. Electrospray voltage

In ESI, analyte ions move to the droplet surface via electrophoretic migration before being transferred into the gas phase for MS analysis [36–38]. A certain electric field is required to produce fine droplets by which a stable ion signal can be obtained. As the physical position of the spray needle and the pressure of the spray are not analyte-dependent [39], the electrospray voltage setting is the major parameter to be optimized.

Fig. 1a shows the influence of electrospray voltage on the molecular ion response of reserpine with three different solvents: water, water–acetonitrile (50:50) and acetonitrile. A higher proportion of acetonitrile increased the signal intensity. Better spray performance appears to be due to the lower

surface tension of the organic modifier [36–38]. These findings are in accord with data reported by Ikononou et al. [37]. Typical responses for singly and multiply charged  $[\text{M}+\text{H}]^{n+}$  ions are shown in Fig. 1b. Optimal electrospray voltage settings were found to be between 4.0 and 5.0 kV.

##### 3.1.2. Vaporizer temperature of heated nebulizer interface

The heated nebulizer of the APCI interface generates a rapidly evaporating mist of droplets prior to corona discharge ionization. The spray performance is controlled by the vaporizer temperature [39]. Mobile phase composition and flow-rate affect the evaporation of the eluent. The effects of vaporizer temperature and mobile phase composition were investigated with representative thermostable and thermolabile compounds. Data for reserpine are shown in Fig. 2a. The intensity of the  $[\text{M}+\text{H}]^+$  ion signal increases over the temperature range of 300–500°C and with increasing proportion of organic modifier. Low temperature settings (data not shown here) also resulted in high background noise due to incomplete evaporation.

One of the major concerns with the heated nebulizer interface is thermal decomposition of the analyte, although it is believed that the increased speed of the evaporation process at high temperature would prevent decomposition of molecular ion species [39]. The cardenolide glycoside dixitoxin was selected as a representative thermolabile compound. The effect of the vaporizer temperature on the ion intensity of  $[\text{M}+\text{H}]^+$  ( $m/z$  765) and the fragment ions  $m/z$  635, 505 and 375 [successive loss of three digitoxosyl (Dig) moieties] is shown in Fig. 2b. The plot of the intensity ratios of  $[(\text{M}+\text{H})-\text{Dig}]^+ / [\text{M}+\text{H}]^+$ ,  $[(\text{M}+\text{H})-2\text{Dig}]^+ / [\text{M}+\text{H}]^+$  and  $[(\text{M}+\text{H})-3\text{Dig}]^+ / [\text{M}+$

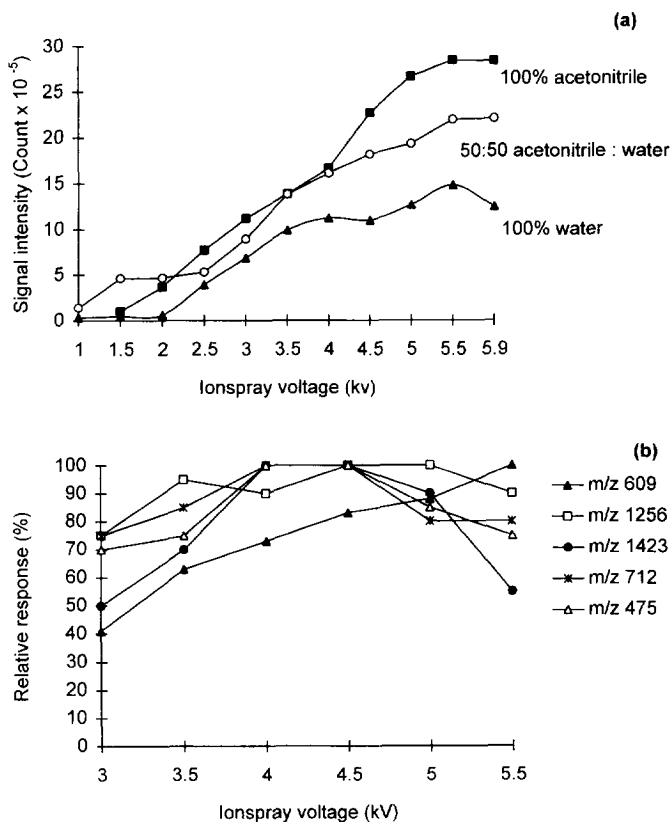


Fig. 1. Effect of electrospray voltage (ISV) on the response of protonated ion species: (a)  $[M+H]^+$  ion of reserpine ( $m/z$  609) in acetonitrile–water mixtures; (b) responses for reserpine  $[M+H]^+$  ( $m/z$  609), actinomycin D  $[M+H]^+$  ( $m/z$  1256), bacitacin  $[M+H]^+$  ( $m/z$  1423),  $[M+2H]^{2+}$  ( $m/z$  712) and  $[M+3H]^{3+}$  ( $m/z$  475) in M1.

$H]^+$  shows rather small changes over a wide temperature range, suggesting that the rate of thermal decomposition is indeed counterbalanced by the time of heat exposure of the analyte. The nature of the fragmentation process (fragmentation of the neutral molecule, the protonated ion or both) was not examined further. In summary, the temperature settings for APCI are much less critical than in TSP. A vaporizer temperature of 450–500°C appears a good compromise for a wide range of molecules.

### 3.1.3. Orifice voltage

Ions generated in the API interfaces need to be transferred through the orifice into the high vacuum stage of the spectrometer [35]. This is achieved by applying a potential difference between the orifice and pre-quadrupole. The potential difference dissociates cluster ions formed in the API ion sources

[35,39] but may also lead to collisionally induced dissociation (CID) of analyte ion species [22,35]. The energy required for efficient transfer of analyte ion and the stability of the charged ion species are sample dependent. The effect of orifice voltage on the total ion current and molecular ion responses was therefore investigated.

A plot of the total ion current of the solvent background ( $m/z$  50 to 1500) vs. orifice voltage is shown in Fig. 3a. The low background at high voltage is due to more efficient cleavage of cluster ions. Plots for quasi-molecular ion species of various natural products are shown in Fig. 3b–d. It appears that the optimum orifice voltage for a given analyte depends on  $M_r$  (Fig. 3b), the charge state (Fig. 3c) and the stability (Fig. 3d). For unknown samples, an initial setting of 55–75 V would seem a good compromise.

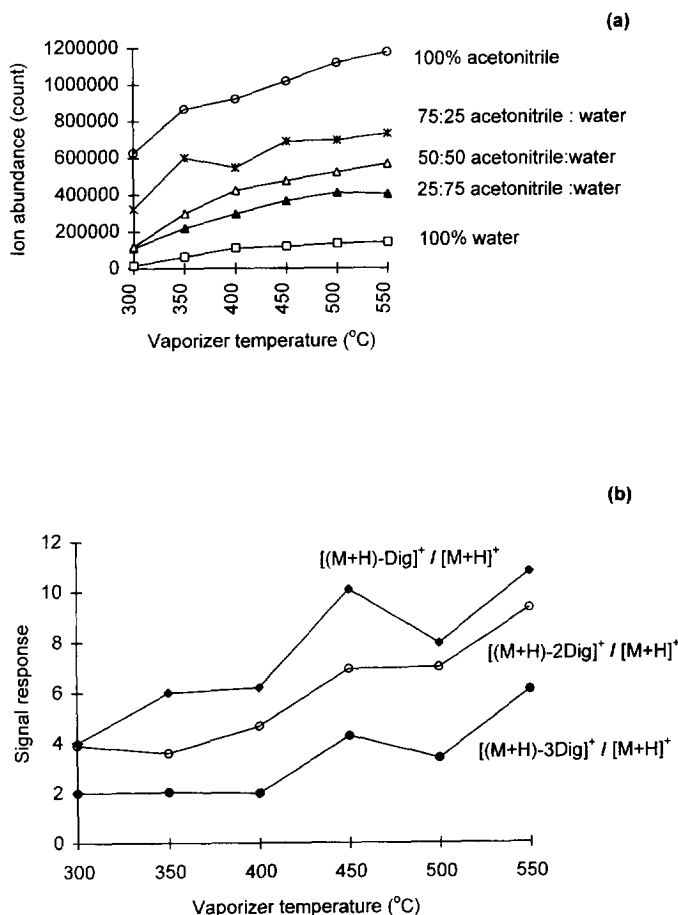


Fig. 2. Response of quasi-molecular ion species  $[M+H]^+$  as a function of vaporizer temperature: (a)  $[M+H]^+$  ion of reserpine ( $m/z$  609) in acetonitrile–water mixtures; (b) ratio plot for  $[M+H]^+$  and fragment ions of digitoxin in M2.

### 3.2. Mobile phase composition

In LC–API–MS, the mobile phase composition requires particular attention. Modifiers added to the eluent for the purpose of optimizing LC separation may affect the ionization process [35,37,38,41]. Likewise, addition of reagents to enhance the MS response may be detrimental to the LC separation [23,35,38].

#### 3.2.1. ESI–MS response vs. mobile phase composition

The effect of the nature and concentration of several modifiers on the ESI response has been reported earlier [37]. It appeared that solution chemistry, ions competition in the droplet surface

and gas phase ion–molecule reaction contribute to signal intensity. Several typical LC mobile phases (M0–M5, see Section 2.2) were used for this more extensive study. Relative intensities of the  $[M+H]^+$  ions for a typical set of structurally diverse compounds are plotted in Fig. 4a.

In general, replacement of M0 by an acidic mobile phase [acetic acid (M1) or formic acid (M2)] led to significant enhancement of the  $[M+H]^+$  ions for the vast majority of compounds tested (Fig. 4a). As reported earlier [38], a concentration of 0.1% formic acid gave the best response, on the other hand, TFA(M3), strongly suppresses the ion signal [38]. For multiply charged ion species, the ratio of  $[M+2H]^{2+}/[M+H]^+$  was significantly increased upon addition of acetic acid or formic acid to M0 (Fig.

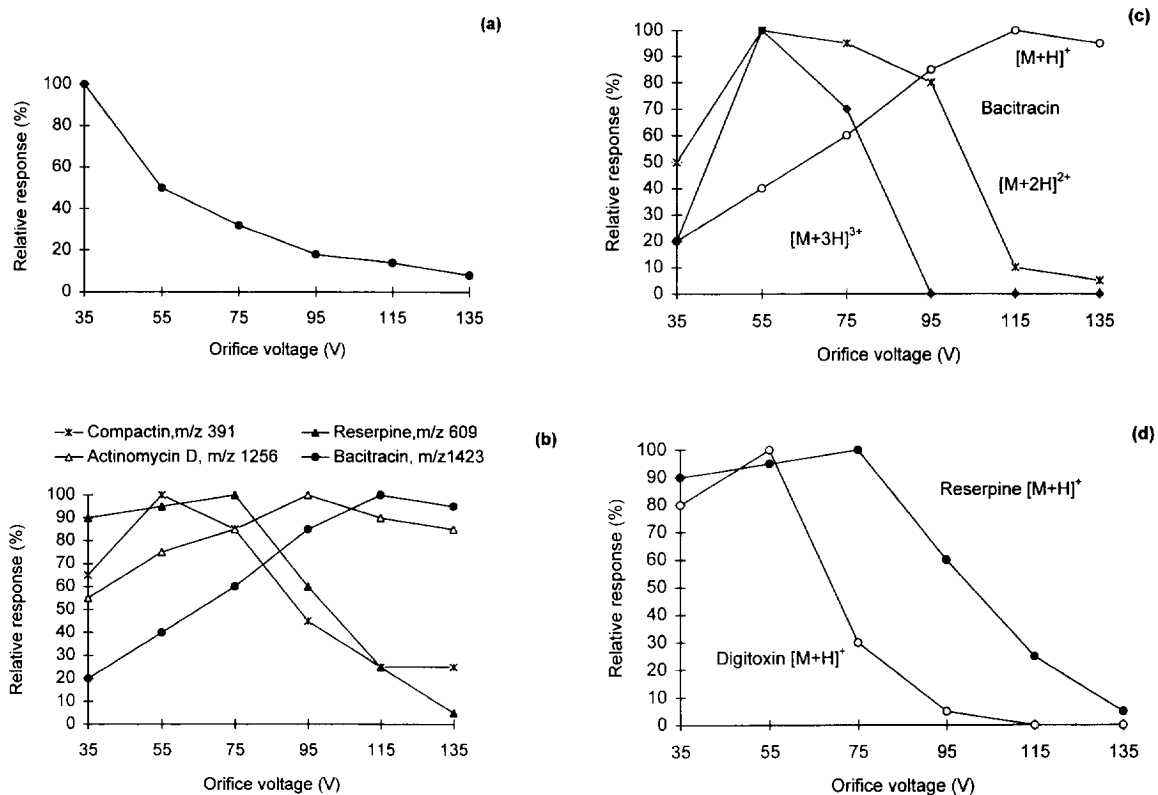


Fig. 3. Effect of the orifice voltage on signal intensity: (a) total ion current of M1 in ESI-MS ( $m/z$  50–1500); (b) ESI-MS response in M1 for  $[M+H]^+$  ions of compactin, reserpine, actinomycin D and bacitracin; (c) ESI-MS of bacitracin in M1: intensity of singly, doubly and triply charged protonated ions; (d) relative  $[M+H]^+$  ion intensity of reserpine and digitoxin in APCI-MS (M0).

4b). The effect of ammonium acetate was more compound-specific. The addition of ammonium acetate decreases the  $[M+H]^+$  ion signal of compounds with low proton affinity to a much higher degree than for ionic compounds and molecules with high proton affinity. For example, the response for the polyphenolic compound quercetin was strongly suppressed in M4. Interestingly, adjustment of the pH to 3.9 (M5) did not revert ion suppression. In this case, the ion competition for the droplet surface appears to be the major factor controlling the ionization process. Compared to M0, M4 significantly enhanced the intensity of  $(M+NH_4)^+$  ions for compounds capable of forming ammonium adducts.

In the negative ion mode, the  $[M-H]^-$  signal was enhanced upon addition of ammonium buffer (M5) (Table 3). Increasing pH through addition of TEA further enhanced the  $[M-H]^-$  response. A more

thorough discussion of negative ion ESI will be published in a forthcoming paper [42].

### 3.2.2. APCI-MS response vs. mobile phase composition

The ionization process of APCI-MS is governed by gas phase ion chemistry mainly in the form of proton transfer reaction [35,39]. Therefore, the ion response is, to large extent, determined by the gas phase basicity or acidity of the analyte and the reactant reagent [35,39,40,43–45]. As the reagent ions are generated from the vaporized mobile phase by corona discharge, the addition of modifiers may change the composition of dominant reactant ions and hence affect the chemical ionization of the analyte [35]. Fig. 5 shows the typical influence of selected modifiers on positive ion responses. The effect of acidic modifiers (M1, M2 and M3) on the

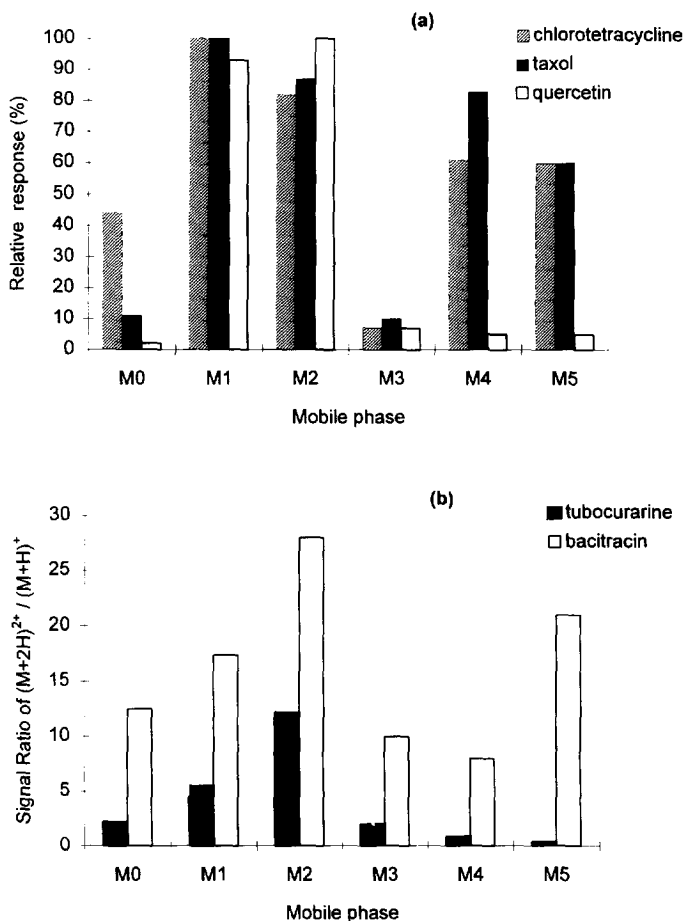


Fig. 4. Effect of mobile phase composition on ion response in ESI-MS: (a) relative response for  $[M+H]^+$  ions of chlorotetracycline ( $m/z$  479), taxol ( $m/z$  855) and quercetin ( $m/z$  303); (b) ratio plots for singly and doubly charged ions ( $[M+2H]^{2+}/[M+H]^+$ ) of bacitracin and tubocurarine.

$[M+H]^+$  ion response (Fig. 5a) was much less pronounced than in ESI-MS. This is not surprising, since the gas phase proton affinity (PA) of acetic acid ( $190.7 \text{ kcal mol}^{-1}$ ;  $1 \text{ cal}=4.184 \text{ J}$ ), formic acid ( $182.8 \text{ kcal mol}^{-1}$ ) and trifluoroacetic acid ( $176.0 \text{ kcal mol}^{-1}$ ) is lower than that of acetonitrile ( $192.8 \text{ kcal mol}^{-1}$ ) [43]. Hence,  $[\text{CH}_3\text{CN}+\text{H}]^+$  ( $m/z$  42) and  $[\text{CH}_3\text{CN}+\text{H}]^+\cdot\text{H}_2\text{O}$  ( $m/z$  60) ions, are still expected to be the dominant reactant ions as observed in the lower mass range. However, presence of  $[\text{NH}_4]^+$  ( $m/z$  18) and  $[\text{NH}_4]^+\cdot\text{CH}_3\text{CN}$  ( $m/z$  59) as the gas phase reactant ions, upon addition of ammonium buffer to the mobile phases (M4 and M5), influenced the ion responses in very different ways for different compound classes.  $[M+H]^+$  sig-

nals of compounds with proton affinity higher than that of  $\text{NH}_3$  ( $205.0 \text{ kcal mol}^{-1}$ ), such as alkaloids and other amine-containing compounds, were not significantly affected. Due to lower proton affinity than ammonia [46], strong suppression of protonated ions was observed with many compounds which contain oxygen as the only heteroatom such as phenolics and glycosides. A possible explanation is that the ammonium ions represent the dominant reactant ion species and that the high proton affinity of ammonia impedes proton transfer to analyte molecules. In chemical ionization, some oxygen-containing compounds such as sugars, tend to form ammonium adduct ion species  $[M+\text{NH}_4]^+$  [47]. For these compounds, the addition of ammonium salt or



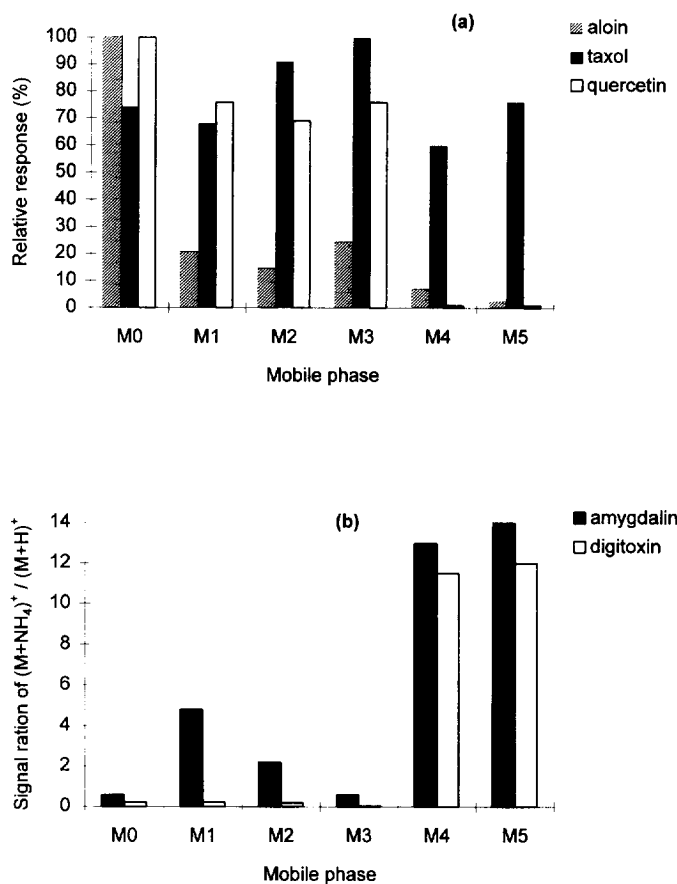


Fig. 5. Effect of mobile phase composition on ion response in APCI-MS: (a) relative response of  $[M+H]^-$  ions of aloin ( $m/z$  349); taxol ( $m/z$  855) and quercetin ( $m/z$  303); (b) relative ion abundance of  $[M+NH_4]^+ / [M+H]^+$ . The concentration of the ammonium buffer in M4 and M5 was 50 mM.

buffer significantly enhanced  $[M+NH_4]^+$  ion signal intensity. The response of two glycosidic compounds is shown in Fig. 5b.

In the negative ion mode, M0 typically produced strong  $[M-H]^-$  analyte ions. The addition of ammonium acetate (M4) had little effect because of the dominating gas phase ionization. However, the presence of free amines (e.g., ammonia or triethylamine) in the solution enhanced the  $[M-H]^-$  ion signal of some acidic compounds, such as chlorogenic acid. Although the gas phase ion reaction is certainly predominant in APCI-MS, it may not be solely responsible for ionization of the analyte. Saikiri and Kambara first reported their findings of direct ion desorption with a modified API interface which was

then named APS-MS [48]. Supporting evidence was also obtained from our own laboratory [49]. A series of experiments were carried out by removing the corona discharge needle and moving the nebulizer close to the orifice (approx. 0.5 cm). Flow injection experiments with reserpine (1 ng  $\mu\text{l}^{-1}$ , 5  $\mu\text{l}$  injection volume, in the mobile phase M2) and chlorogenic acid (10 ng  $\mu\text{l}^{-1}$ , 5  $\mu\text{l}$  injection volume, in the mobile phase M6) gave good response for both compounds, in positive and negative ion mode, respectively.

It can also be assumed that addition of acid or basic modifiers may enhance the ionization of analytes in solution, which should in turn reduce the concentration of neutral sample molecules that could

Table 4

Major quasi-molecular ions and other adduct ions species observed in positive and negative ion ESI- and APCI-MS for compounds listed in Table 1

Compound	Ions intensities <sup>1</sup> in positive ion mode				Ions intensities <sup>1</sup> in negative ion mode			
	Ion species	(m/z)	ESI <sup>2</sup>	APCL <sup>3</sup>	Ion species	(m/z)	ESI <sup>4</sup>	APCI <sup>5</sup>
<i>Macrolides</i>								
Nonactin	[M+H] <sup>+</sup>	(737)	++	+++	[M-H] <sup>-</sup>	(735)	(-)	+
M <sub>n</sub> = 736	[M+NH <sub>4</sub> ] <sup>+</sup>	(754)	+++					
Oligomycin B	[M+H] <sup>+</sup>	(805)	+	+	[M-H] <sup>-</sup>	(803)	(-)	+
M <sub>n</sub>	[MH-H <sub>2</sub> O] <sup>+</sup>	(787)	+	+++				
Rifampicin	[M+NH <sub>4</sub> ] <sup>+</sup>	(822)	+	+				
M <sub>n</sub> = 822	[M+H] <sup>+</sup>	(823)	++	+++	[M-H] <sup>-</sup>	(821)	++	++
<i>Peptides</i>								
Actinomycin D	[M+H] <sup>+</sup>	(1256)	++	++				
	[M+Na] <sup>+</sup>	(1278)	+	(-)				
Bacitracin	[M+H] <sup>+</sup>	(1423)	+	(-)	[M-H] <sup>-</sup>	(1421)	+	(-)
M <sub>n</sub> = 1422	[M+2H] <sup>2+</sup>	(712)	++		[M-2H] <sup>2-</sup>	(710)	+	(-)
	[M+3H] <sup>3+</sup>	(475)	++					
	[M+4H] <sup>4+</sup>	(356)	+					
<i>Aminoglycoside</i>								
Neomycin	[M+H] <sup>+</sup>	(615)	+	(-)				
M <sub>n</sub> = 614	[M+2H] <sup>2+</sup>	(308)	++	(-)				
<i>Polyether</i>								
Monensin	[M+NH <sub>4</sub> ] <sup>+</sup>	(688)	++	++	[M-H] <sup>-</sup>	(669)	++	(-)
M <sub>n</sub> = 670	[M+Na] <sup>+</sup>	(693)	+++	+++				
<i>Polvane</i>								
Amphotericin	[M+H] <sup>+</sup>	(925)	+	(-)				
M <sub>n</sub> = 924								
<i>Miscellaneous microbial metabolites</i>								
Benzylopenicillin	[M+H] <sup>+</sup>	(357)	+++	++				
M <sub>n</sub> = 356								
Chlorotetracycline	[M+H] <sup>+</sup>	(479)	+++	+++	[M-H] <sup>-</sup>	(477)	++	++
M <sub>n</sub> = 478								
Compactin	[M+H] <sup>+</sup>	(391)	++	+++	[M-H] <sup>-</sup>	(389)	(-)	+
M <sub>n</sub> = 390	[M+NH <sub>4</sub> ] <sup>+</sup>	(408)	++	(-)	[M-H-H <sub>2</sub> O] <sup>-</sup>	(371)	(-)	+++
	[M+Na] <sup>+</sup>	(423)	++	(-)				
Erythromycin	[M+H] <sup>+</sup>	(734)	+++	+++	[M-H] <sup>-</sup>	(733)	+	+
M <sub>n</sub> = 390								
Novobiocin	[M+H] <sup>+</sup>	(613)	++	++	[M-H] <sup>-</sup>	(611)	++	(-)
M <sub>n</sub> = 612	[M+Na] <sup>+</sup>	(635)	+					
Puromycin	[M+H] <sup>+</sup>	(472)	++	+++	[M-H] <sup>-</sup>	(470)	++	+++
M <sub>n</sub> = 471	[M+H-H <sub>2</sub> O] <sup>+</sup>	(454)	(-)	+++				
<i>Alkaloids</i>								
Ajmalicine	[M+H] <sup>+</sup>	(353)	+++	+++	[M-H] <sup>-</sup>	(351)	+	+++
M <sub>n</sub> = 352								
Atropine	[M+H] <sup>+</sup>	(291)	+++	+++				
M <sub>n</sub> = 290								
Brucine	[M+H] <sup>+</sup>	(396)	+++	+++				
M <sub>n</sub> = 395								
Papaverine	[M+H] <sup>+</sup>	(341)	+++	+++				
M <sub>n</sub> = 340								
Quinine	[M+H] <sup>+</sup>	(325)	+++	+++	[M-H] <sup>-</sup>	(323)	+	++
M <sub>n</sub> = 324								
Colchicine	[M+H] <sup>+</sup>	(400)	+++	+++	[M-H] <sup>-</sup>	(398)	+	+
M <sub>n</sub> = 399								
Emetine	[M+H] <sup>+</sup>	(481)	++	+++				
M <sub>n</sub> = 480	[M+2H] <sup>2+</sup>	(241)	++	(-)				
Tubocurarine	[M+H] <sup>+</sup>	(699)	+	+++				
M <sub>n</sub> = 698	[M+2H] <sup>2+</sup>	(350)	++	(-)				
Vinblastine	[M+H] <sup>+</sup>	(811)	++	+++				
M <sub>n</sub> = 810	[M+2H] <sup>2+</sup>	(406)	++	(-)				
<i>Purines</i>								
Adenosine	[M+H] <sup>+</sup>	(268)	++	+++	[M-H] <sup>-</sup>	(398)	+	++
M <sub>n</sub> = 267								
Caffeine	[M+H] <sup>+</sup>	(196)	+++	+++				
M <sub>n</sub> = 195								
<i>Amides</i>								
Capsacin	[M+H] <sup>+</sup>	(306)	++	+++	[M-H] <sup>-</sup>	(304)	+	++
M <sub>n</sub> = 305	[2M+H] <sup>+</sup>	(613)	+	(-)				

be desolved into the gas phase. On the other hand, vaporized acidic (e.g., formic acid) or basicity (e.g., ammonia or TEA) reagents in the stream may assist the gas phase ionization. These assumptions, however, have not yet been further investigated.

### 3.3. API-MS characteristics of natural products

The detection and characterization of selected natural products by API-MS has been reported [24–30,32,33]. In this study, a systematic investigation

Table 4. Continued

Compound	Ions intensities <sup>1</sup> in positive ion mode				Ions intensities <sup>1</sup> in negative ion mode			
	Ion species	( <i>m/z</i> )	ESI <sup>2</sup>	APCL <sup>3</sup>	Ion species	( <i>m/z</i> )	ESI <sup>4</sup>	APCI <sup>5</sup>
Piperine	[M+H] <sup>+</sup>	(286)	++	+++	[M-H] <sup>-</sup>	(398)	+	++
<i>M<sub>n</sub></i> =285	[2M+H] <sup>+</sup>	(571)	+	(-)				
<i>Phenolics</i>								
1,8-Dihydroxy anthraquinone	[M+H] <sup>+</sup>	(241)	+	+	[M-H]	(239)	++	+++
<i>M<sub>n</sub></i> =240								
Ellagic acid	[M+H] <sup>+</sup>	(303)	+	+	[M-H]	(301)	++	+++
<i>M<sub>n</sub></i> =302								
Catechin	[M+H] <sup>+</sup>	(291)	++	++	[M-H] <sup>-</sup>	(289)	++	++
<i>M<sub>n</sub></i> =290								
Podophyllotoxin	[M+H] <sup>+</sup>	(415)	++	++	[M-H] <sup>-</sup>	(413)	+	+++
<i>M<sub>n</sub></i> =414	[M+H-H <sub>2</sub> O] <sup>+</sup>	(397)	+	+++	[M+acetate]	(473)	+	++
	[M+NH <sub>4</sub> ] <sup>+</sup>	(432)	+	+				
	[M+Na] <sup>+</sup>	(437)	+	(-)				
Xanthotoxin	[M+H] <sup>-</sup>	(217)	+++	+++				
<i>M<sub>n</sub></i> =216								
Quercetin	[M+H] <sup>-</sup>	(303)	++	+++	[M-H]	(301)	++	++
<i>M<sub>n</sub></i> =302								
<i>Glycosides<sup>5</sup></i>								
Aloin	[M+H] <sup>-</sup>	(419)	+	+	[M-H] <sup>-</sup>	(417)	+	++
<i>M<sub>n</sub></i> =418								
Digitoxin	[M+H] <sup>-</sup>	(765)	+	-+	[M-H]	(763)	++	++
<i>M<sub>n</sub></i> =764	[M+NH <sub>4</sub> ] <sup>+</sup>	(782)	+	+				
	[M+H-gly] <sup>+</sup>	(635)	+	++				
	[M+H-2gly] <sup>-</sup>	(505)	+	-+				
	[M+H-3gly] <sup>-</sup>	(375)	(-)	++				
Amygdalin	[M+H] <sup>+</sup>	(458)	++	++	[M-H] <sup>-</sup>	(456)	++	+++
<i>M<sub>n</sub></i> =457	[M+NH <sub>4</sub> ] <sup>+</sup>	(475)	+	+++				
Chlorogenic acid	[M+H] <sup>+</sup>	(355)	+	+	[M-H] <sup>-</sup>	(353)	++	++
<i>M<sub>n</sub></i> =354					[M-H-gly]	(191)	++	++
Glycyrrhizic acid	[M+H] <sup>+</sup>	(823)	++	(-)	[M-H] <sup>-</sup>	(821)	+	(-)
<i>M<sub>n</sub></i> =822	[M+NH <sub>4</sub> ] <sup>+</sup>	(782)	+	+				
Malvine	[M+H] <sup>+</sup>	(655)	++	++				
<i>M<sub>n</sub></i> =654	[M+H-gly] <sup>-</sup>	(493)	+	+				
<i>Terpenoids</i>								
Abietic acid	[M-H] <sup>+</sup>	(303)	++	+++	[M-H] <sup>-</sup>	(301)	+	++
<i>M<sub>n</sub></i> =302								
Caryophyllene epoxide	[M+H] <sup>+</sup>	(221)	(-)	+++				
<i>M<sub>n</sub></i> =220								
Farnesol								
<i>M<sub>n</sub></i> =222								
Picrotoxin	[M+H] <sup>+</sup>	(603)	+	(-)				
<i>M<sub>n</sub></i> =602								
$\alpha$ -santonin	[M+H] <sup>+</sup>	(247)	+++	+++				
<i>M<sub>n</sub></i> =246	[M+H-H <sub>2</sub> O] <sup>+</sup>	(264)	+	+++				
	[M+NH <sub>4</sub> ] <sup>+</sup>	(264)	+++	(-)				
Stigmasterol								
<i>M<sub>n</sub></i> =412								
Taxol	[M+H] <sup>+</sup>	(854)	++	+++	[M-H] <sup>-</sup>	(852)	++	(-)
<i>M<sub>n</sub></i> =853	[M+NH <sub>4</sub> ] <sup>+</sup>	(871)	++	+				

API-MS conditions as listed in Table 1.

<sup>1</sup> Signal intensity is expressed as: +++ (>1·10<sup>6</sup> counts), ++ (>1·10<sup>5</sup>), + (<1·10<sup>5</sup>), (-) (not observed).

<sup>2</sup> With mobile phase M1.

<sup>3</sup> With mobile phase M0.

<sup>4</sup> With mobile phase M4.

<sup>5</sup> Gly represents various sugar residues (glucosyl, digitoxosyl etc.).

was carried out to compare the mass spectrum of the tested compounds and the results are summarized in Table 4.

### 3.3.1. Microbial metabolites

With ESI-MS, all of the 14 microbial metabolites tested gave protonated ions  $(M+H)^+$ . Multiply charged ions were observed for bacitracin (Fig. 6) and neomycin. Thus, confirmation of  $M_r$  and additional structural information can be obtained for molecules containing more than one amino group amenable to protonation. All oxygen-containing metabolites with no amino groups (nonactin, oligomycin B, monensin, compactin) gave additional  $[M+NH_4]^+$  ions, the intensity of which was significantly enhanced in M4. In the negative ion mode,  $[M-H]^-$  ions were observed for 7 compounds. The peptide antibiotic bacitracin produced a doubly charged negative ion.

With APCI-MS,  $[M+H]^+$  ions were observed for 11 compounds. No molecular ions were obtained for bacitracin, amphotericin and neomycin. This is likely

due to the lability and poor volatility of these compounds and demonstrates the limitations of the heated nebulizer interface.

### 3.3.2. Alkaloids, purines and amides

As a result of their high proton affinity, all the 13 compounds gave strong  $[M+H]^+$  signals in both APCI- and ESI-MS. No fragment ions were observed under the experimental conditions used, except for the purine glycoside adenosine. Doubly charged ions were observed in ESI spectra of alkaloids containing more than one amino group (tubocurarine, vinblastine, emetine). In ESI-MS, the two amides capsaicin and piperin formed the dimer ions  $[2M+H]^+$ . For alkaloids containing acidic functional groups, purines and amides,  $[M-H]^-$  ions could be observed in ESI- and APCI-MS. APCI spectra of ajmalicine in the positive and negative ion modes are shown in Fig. 7.

### 3.3.3. Phenolics and glycosides

The vast majority of compounds investigated formed both positive and negative molecular ions in

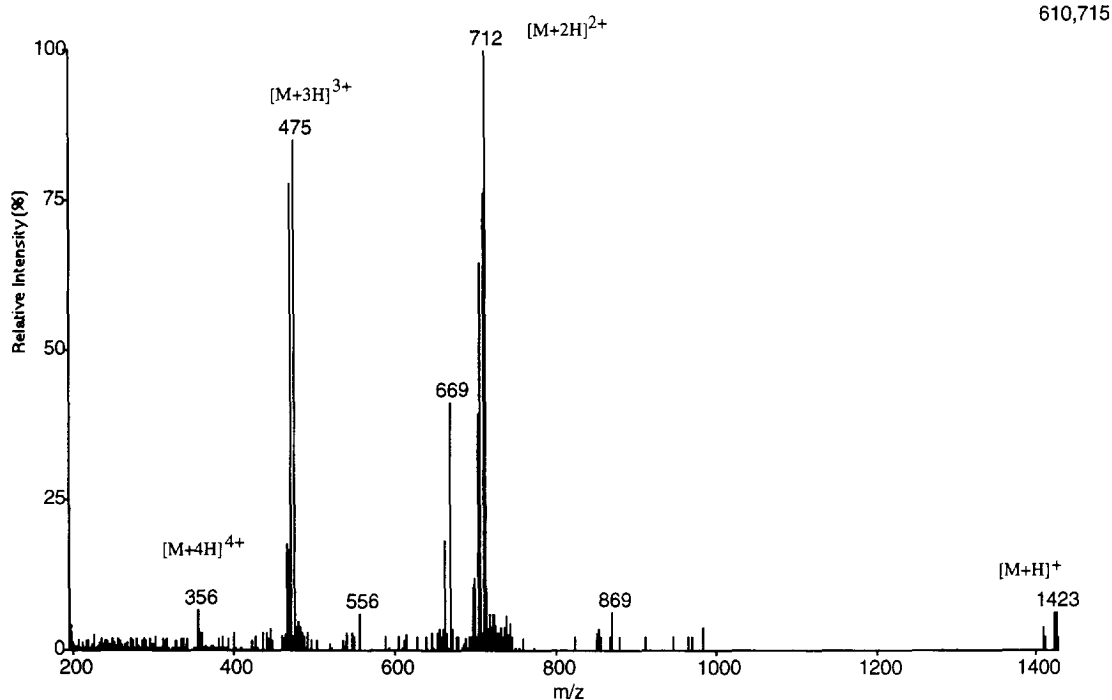


Fig. 6. Positive ion mode ESI-MS of bacitracin in M1.

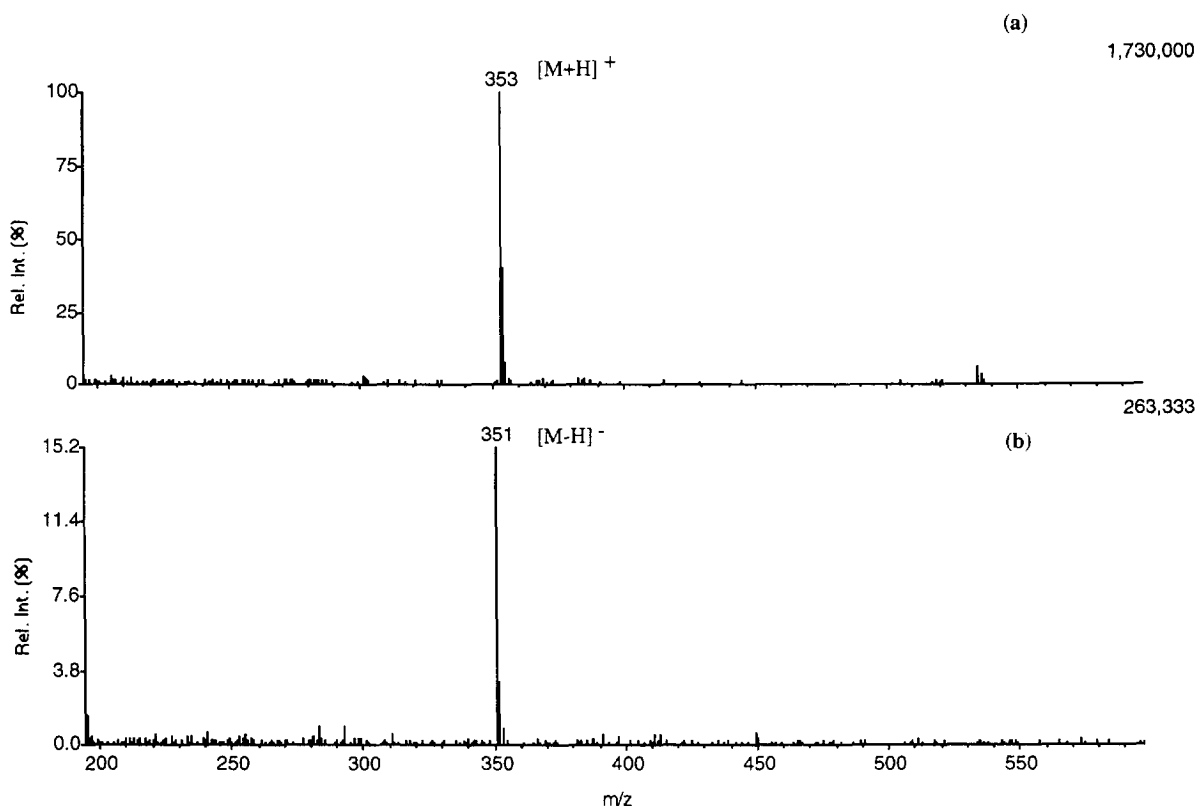


Fig. 7. APCI of ajmalicin in (a) positive and (b) negative ion mode. Both spectra were recorded in M0.

ESI- and APCI-MS. The formation of ammonium and/or sodium adduct ions in several cases provided additional confirmation of the quasi-molecular ions species (Fig. 8). ESI and APCI spectra were characterised by the presence of fragment ions resulting from loss of glycosyl residues (Fig. 9). A characteristic feature of phenolics and glycosides was the strong effect of mobile phase composition on the formation of molecular ion species in ESI-MS (enhancement of  $[M+H]^+$  with M1 and M2, and of  $[M+NH_4]^+$  and  $[M-H]^-$  with M4).

#### 3.3.4. Terpenoids

Four out of seven terpenoids yielded abundant  $[M+H]^+$  ions in the APCI mode. Poorly functionalized terpenoids such as farnesol and stigmasterol (1 hydroxyl group each) could not be ionized due to the lack of functional groups amenable to API ionization.

## 4. Conclusions

A number of conclusions can be drawn from our data:

- (1) For the vast majority of test compounds, reasonably intense molecular ions can be observed with generic interface settings and a very limited number of mobile phases. Not amenable to API are very poorly functionalized molecules, which also tend to be of limited interest as drug leads.
- (2) ESI is more favorable for the analysis of highly polar or ionic compounds. The majority of test compounds yields protonated molecular ions. Since ESI is dominated by liquid phase ionization, a number of ion species, such as singly charged or multiply charged quasi-molecular ions, ammonium and sodium adducts, can be readily detected. In the negative ion mode,  $[M-H]^-$  ions are observed for some compounds. Confirmation of  $M_r$  is thereby facilitated by examining the various ion species. The

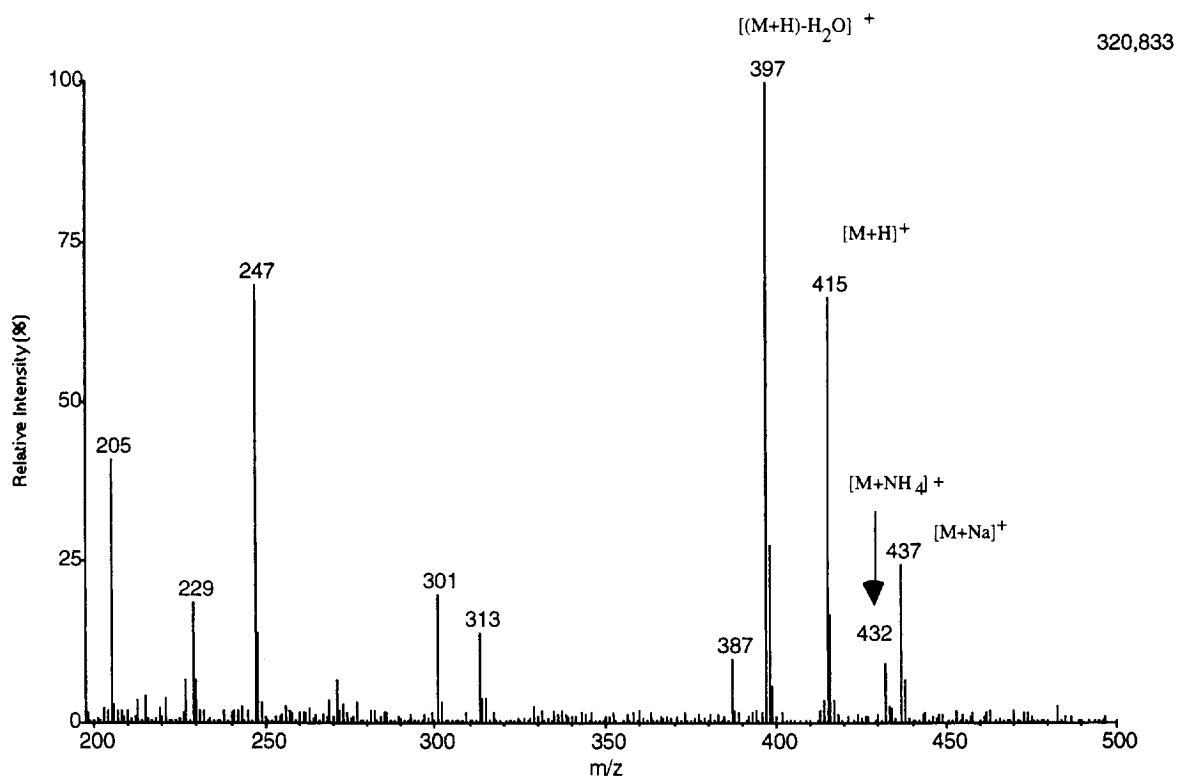


Fig. 8. Positive ion mode ESI-MS of podophyllotoxin in M1.

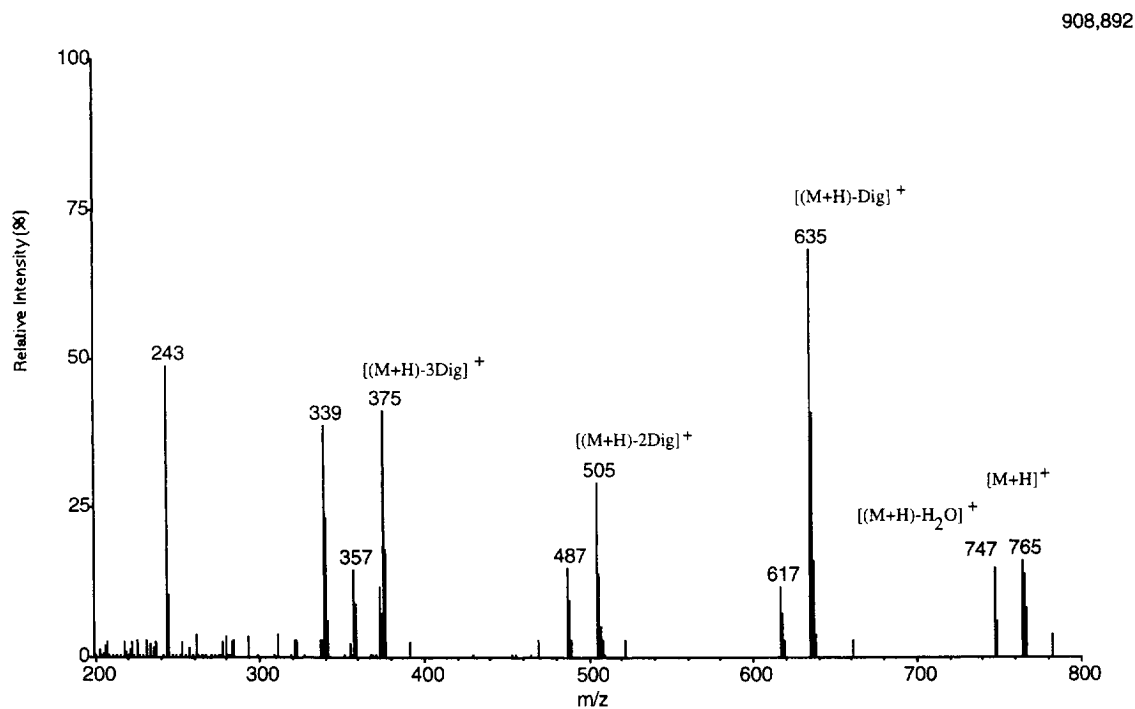


Fig. 9. Positive ion mode APCI-MS of digitoxin. M1 was used as mobile phase.

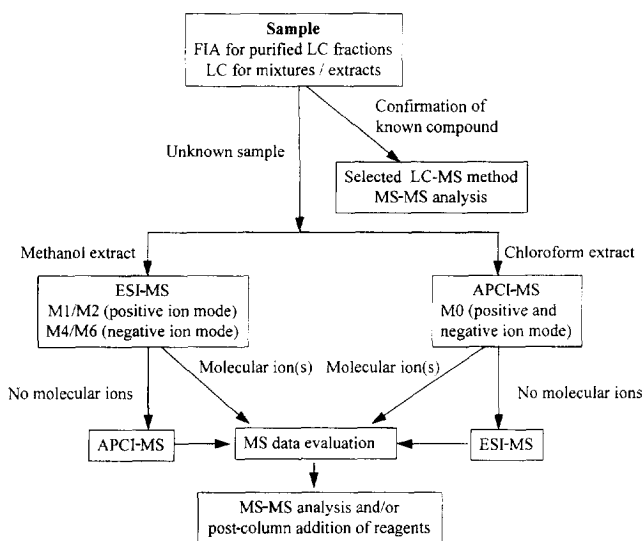


Fig. 10. Protocol for API-MS analysis of natural products.

nature of the quasi-molecular and adduct ions also provides additional structural information valuable for natural product database searches.

(3) With the APCI interface, most compounds produce  $[M+H]^+$  ions and/or  $[M-H]^-$  ions. Exceptions are poorly functionalized and very thermolabile compounds. In terms of sensitivity, APCI produced better response for moderately polar to non-polar compounds than ESI.

Based on our findings, we have developed a generally applicable protocol which is currently applied in our laboratories to all samples submitted for MS analysis (Fig. 10):

(1) Depending on the complexity, samples undergo flow injection analysis (FIA), in case of reasonably pure fractions, or on line LC-MS (in case of complex samples).

(2) If information on the nature of the analytes is available, MS analysis should be carried out using the most suitable interface and mobile phase combinations as described in this publication. MS-MS may provide additional confirmation.

(3) For unknown samples, ESI-MS is the method of choice for polar extracts (e.g., MeOH, EtOH, acetone), while APCI-MS should be used as first choice with moderately polar extracts (e.g.,  $\text{CHCl}_3$ , EtOAc). Samples are analyzed in positive and negative ion mode using the appropriate mobile phases. Post-

column addition of specific ionization reagents and MS-MS analysis may be useful in certain cases for confirmation of the molecular ions and peak purity in LC-MS runs.

(4) The alternative interface is tested in case no clear molecular ions can be obtained.

(5) In a final step, the information obtained from post-column addition of reagents, MS-MS and UV data are combined for natural products database searches.

In a subsequent publication [50], we report on the practical application of API-MS in the analysis of extracts and fractions of plant and microbial origin.

## References

- [1] J.T. Baker, R.P. Borris, B. Carte, G.A. Cordell, D.D. Soejarto, G.M. Cragg, M.P. Gupta, M.M. Iwu, D.R. Madulid and V. E. Tyler, *J. Nat. Prod.*, 58 (1995) 1325.
- [2] J.F. Garcia and D. Barcelo, *J. High Resolut. Chromatogr.*, 16 (1993) 633.
- [3] A.L. Burlingame, R.K. Boyd and S.J. Gaskell, *Anal. Chem.*, 66 (1994) 634R.
- [4] R.C. Willoughby and R.F. Browner, *Anal. Chem.*, 56 (1984) 2626.
- [5] K. Sato, T. Kumazawa and Y. Katsumata, *J. Chromatogr. A*, 674 (1994) 127.
- [6] G.C. Galletti, J. Eagles and F.A. Mellon, *J. Sci. Food Agri.*, 59 (1992) 401.

- [7] S. Steffenrud and G. Maylin, *J. Chromatogr.*, 577 (1992) 221.
- [8] S.P. Auriola, A.-M. Lepist, T. Naaranlahti and S.P. Lapinjoki, *J. Chromatogr.*, 594 (1992) 153.
- [9] M. Hamburger, J.L. Wolfender and K. Hostettmann, *Nat. Toxins*, 1 (1993) 315.
- [10] J.L. Wolfender, M. Hamburger, K. Hostettmann, J.D. Msonthi and S. Mavi, *J. Nat. Prod.*, 56 (1993) 682.
- [11] J.L. Wolfender, M.P. Maillard and K. Hostettmann, *J. Chromatogr.*, 647 (1993) 183.
- [12] J.L. Wolfender and K. Hostettmann, *J. Chromatogr.*, 647 (1993) 191.
- [13] M.P. Maillard and K. Hostettmann, *J. Chromatogr.*, 647 (1993) 137.
- [14] M.P. Maillard, J.L. Wolfender and K. Hostettmann, *J. Chromatogr.*, 647 (1993) 147.
- [15] P. Pietta, R.M. Facino, M. Carini and P. Mauri, *J. Chromatogr. A*, 661 (1994) 121.
- [16] W.A. Korfmacher, J. Bloom, M.I. Churchwell, T.A. Getek, E.B. Hansen, C.L. Holder and K.T. McMabus, *J. Chromatogr. Sci.*, 31 (1993) 498.
- [17] C.E.M. Heeremans, R.A.M. van der Hoeven, W.M.A. Nielsen, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 474 (1989) 149.
- [18] Y. Ito, T. Takeuchi, D. Ishii, M. Goto and T. Mizuno, *J. Chromatogr.*, 346 (1985) 161.
- [19] K.-I. Harada, M. Suzuki and H. Oka, in T. Mastsuo, R. M. Caprioli, M.L. Gross and Y. Seyama (Editors), *Biological Mass Spectrometry: Present and Future*, Wiley, New York, 1994, p. 605.
- [20] M. Yamashita and J.B. Fenn, *J. Phys. Chem.*, 88 (1984) 4671.
- [21] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong and C.M. Whitehouse, *Science*, 60 (1989) 436.
- [22] R.D. Voyksner, *Nature*, 356 (1992) 86.
- [23] R.P. Schneider, M.J. Lynch, J.F. Ericson and H.G. Fouda, *Anal. Chem.*, 63 (1991) 1789.
- [24] W.E. Glassgen, H.U. Seitz and J.W. Metzger, *Biol. Mass Spectrom.*, 21 (1992) 271.
- [25] P.K.S. Blay, P. Thibault, N. Thiberge, B. Kiecken, A. Lebrun and C. Mercure, *Rapid Commun. Mass Spectrom.*, 7 (1993) 628.
- [26] Y. Yoshikawa, K. Ikai, Y. Umeda, A. Ogawa, K. Takesako, I. Kato and H. Naganawa, *J. Antibiot.*, 46 (1993) 1347.
- [27] G.J. Feistner, *Am. Lab.*, September (1994) 32L.
- [28] S. Pleasance, S.W. Ayer, M.V. Laycock and P. Thibault, *Rapid. Commun. Mass Spectrom.*, 6 (1992) 14.
- [29] R.J. Lewis, M.J. Holmes, P.F. Alewood and A. Jones, *Nat. Toxins*, 2 (1994) 56.
- [30] B.A. Thomson and L. Danelewych, *Proc. 31st Ann. Conf. Am. Soc. Mass Spectrom.*, 1983, p. 852.
- [31] J.D. Henion, B.A. Thomson and P.H. Dawson, *Anal. Chem.*, 54 (1982) 451.
- [32] M. Sakairi and H. Kambara, *Anal. Chem.*, 60 (1988) 774.
- [33] H. Kanazawa, Y. Nagata, Y. Matsushima, N. Takai, H. Uchiyama, R. Nishimura and A. Takeuchi, *J. Chromatogr.*, 631 (1993) 215.
- [34] E.C. Huang, T. Wachs, J.J. Conboy, J.D. Henion, *Anal. Chem.*, 6 (1990) 713A.
- [35] A.P. Bruins, *Trends Anal. Chem.*, 13 (1994) 37.
- [36] M.G. Ikonou, A.T. Blades and P. Kebarle, *Anal. Chem.*, 62 (1990) 957.
- [37] M.G. Ikonou, A.T. Blades and P. Kebarle, *Anal. Chem.*, 63 (1991) 1989.
- [38] S. Zhou and M. Hamburger, *Rapid Commun. Mass Spectrom.*, 9 (1995) 1516.
- [39] Anon., *The API book*, PE Scienc, Thornhill, Ontario, Canada, 1992.
- [40] A.G. Harrison, *Chemical Ionization Mass Spectrometry*, 2nd ed., CRC Press, Boca Raton, FL, 1992, pp 32–40.
- [41] G. Hopfgartner, K. Bean and J.D. Henion, *J. Chromatogr.*, 647 (1993) 51.
- [42] S. Zhou and M. Hamburger, in preparation.
- [43] D.H. Aue and M.T. Bowers in M.T. Bowers (Editor), *Gas Phase Ion Chemistry Vol. 2*, Academic Press, New York, 1979, pp. 2–47.
- [44] J. Sunner, G. Nicol and P. Kebarle, *Anal. Chem.*, 60 (1988) 1300.
- [45] J. Sunner, M.G. Ikonou and P. Kebarle, *Anal. Chem.*, 60 (1988) 1308.
- [46] R. Yamdagni and P. Kebarle, *J. Am. Chem. Soc.*, 98 (1976) 1320.
- [47] A.M. Hogg, *Anal. Chem.*, 44 (1972) 227.
- [48] M. Saikiri and H. Kambara, *Anal. Chem.*, 61 (1989) 1159.
- [49] S. Zhou and M. Hamburger, unpublished results.
- [50] S. Zhou and M. Hamburger, in preparation.