Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Chromatography A, 998 (2003) 119–131
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry of the *Alternaria* mycotoxins alternariol and alternariol monomethyl ether in fruit juices and beverages

Benjamin P.-Y. Lau[a,*], Peter M. Scott[a], David A. Lewis[a], Shriniwas R. Kanhere[a], Chantal Cléroux[a], Veronica A. Roscoe[b]

[a] *Food Research Division, Health Canada, Address Locator 2203D, Ottawa, Ontario K1A 0L2, Canada*
[b] *Health Canada, 510 Lagimodière Boulevard, Winnipeg, Manitoba R2 J 3Y1, Canada*

Received 23 October 2002; received in revised form 1 April 2003; accepted 1 April 2003

Abstract

Alternariol (AOH) and alternariol monomethyl ether (AME) are among the main mycotoxins formed in apples and other fruits infected by *Alternaria alternata*. For determination of AOH and AME by LC, apple juice and other fruit beverages were cleaned up on C_{18} and aminopropyl solid-phase extraction columns. Positive and negative ion mass spectra of AOH and AME under electrospray (ESI) and atmospheric pressure chemical ionization (APCI) conditions were obtained. Collision-induced dissociation of the $[M+H]^+$ and $[M-H]^-$ ions for both compounds were also studied. The phenolic anions of both compounds are more stable with less fragmentation. In quantitative analysis, negative ion detection also offers lower background and better sensitivity. Sensitive LC–MS and LC–MS–MS confirmatory procedures based on APCI with negative ion detection were applied to confirm the natural occurrence of AOH in nine samples of apple juice and in single samples of some other clear fruit beverages—grape juice, cranberry nectar, raspberry juice, red wine, and prune nectar (which also contained 1.4 ng AME/ml)—at levels of up to 6 ng AOH/ml. Electrospray LC–MS–MS with negative ion detection and in multiple reaction monitoring mode offers higher sensitivity and specificity. Absolute detection was better than 4 pg per injection for both compounds.
Crown Copyright © 2003 Published by Elsevier Science B.V. All rights reserved.

Keywords: Fruit juices; Food analysis; Mycotoxins; Alternariol; Alternariol monomethyl ether

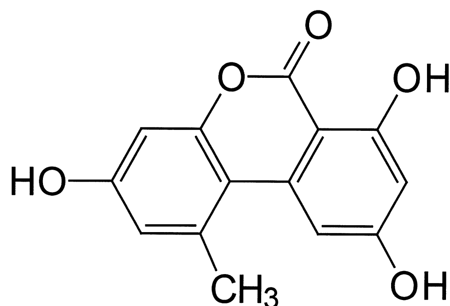
1. Introduction

Fungi of the genus *Alternaria* are commonly parasitic on plants and other organic materials. Many are plant pathogens of field crops while others infect foodstuffs after harvest [1]. *Alternaria alternata* is a frequently occurring species of particular interest to mycotoxicologists because it produces a number of mycotoxins, including alternariol (AOH; 3,7,9-trihydroxy-1-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one) and alternariol monomethyl ether (AME; 3,7-dihydroxy-9-methoxy-1-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one),

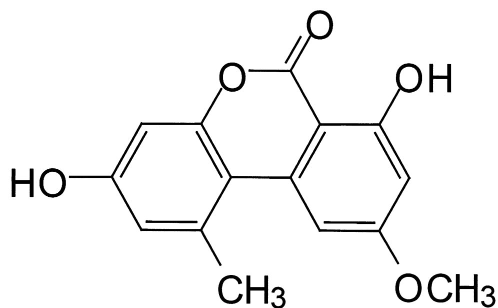
*Corresponding author. Tel.: +1-613-946-6824; fax: +1-613-941-4775.
E-mail address: ben_lau@hc-sc.gc.ca (B.P.-Y. Lau).

0021-9673/03/$ – see front matter Crown Copyright © 2003 Published by Elsevier Science B.V. All rights reserved.
doi:10.1016/S0021-9673(03)00606-X

which were first isolated in 1953 [1–5]. The structures of both compounds are shown in Fig. 1. A culture of *A. alternata* on corn flour has been found to be carcinogenic in rats, and culture extracts were mutagenic in various microbial and cell systems [6–8]. It has been suggested that *A. alternata* might be one of the etiological factors for human esophageal cancer in Linxian, China [8]. AOH and AME are mutagenic, although they are not the only mutagens formed by *A. alternata* [1,7,10–14]. There are also reports of subcutaneous induction of squamous cell carcinoma in mice by human embryo esophageal tissue treated with AOH [9] and of subcutaneous tumorigenicity with NIH/3T3 cells transformed by AME [15].



Alternariol (AOH)
$C_{14}H_{10}O_5$
M.W. 258.23



Alternariol Monomethyl Ether (AME)
$C_{15}H_{12}O_5$
M.W. 272.25

Fig. 1. Structures of *Alternaria* toxins: alternariol (AOH) and alternariol methyl ether (AME). M.W., molecular mass.

Natural occurrence of AOH, AME and in some cases other *Alternaria* toxins has been reported in grains [1,3,4,6–18], sunflower seeds [19,20], oilseed rape [20], pecans [3] and various fruits [4,21–24], including tomatoes, olives, mandarins, melons, peppers, apples and raspberries. As a result of inoculation experiments, potential for their occurrence in other fruits (oranges, lemons and blueberries) has also been demonstrated [4,25].

Surveillance of fruit juices and other fruit products for *Alternaria* toxins are needed to determine the level of human exposure from these foods. Tenuazonic acid has been found occasionally in tomato products [4]. However, the occurrence of AOH in processed fruit products has only recently been reported—in apple juice [26,27], processed tomato products [22] and raspberry drink [22]. AME (mainly traces) has also been detected in apple juice [27].

AOH, AME and other *Alternaria* mycotoxins have been determined by thin-layer chromatography [4,23], gas chromatography [26,28] and liquid chromatography (LC), mainly with ultraviolet detection [26–40], although fluorescence [16,32,36,41,42] and electrochemical detection [43] have also been used. Methods of analysis have recently been reviewed [44]. LC–mass spectrometry (MS) of AOH and AME has only been reported in one laboratory [45,46], which used a particle beam interface and electron impact mode. However, the technique was insufficiently sensitive to detect nanogram amounts of these compounds. We report here on using two atmospheric pressure ionization techniques [atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI)] in LC–MS and LC–MS–MS analyses of AOH and AME and application of these techniques to their confirmation in apple juice and other fruit beverages. Preliminary report have been included in meeting presentations [47,48].

2. Experimental

2.1. Chemicals

AOH and AME were purchased from Sigma (St. Louis, MO, USA). Each was dissolved separately in methanol to make 250 μg/ml stock solutions, from which mixed spiking and standard solutions in methanol and LC mobile phase, respectively, were prepared.

Solvents used were methanol, acetonitrile, dichloromethane, acetone and ethyl acetate (EM Science, all distilled in glass or HPLC grade), glacial acetic acid and 90% formic acid. Water was distilled or deionized (Millipore Milli-Q water system).

Pectinase (from *Aspergillus niger*) was purchased from Sigma.

Solid-phase extraction (SPE) columns were: C_{18}, 3 ml (500 mg), not end-capped (Macherey–Nagel Chromabond or Varian BondElut) and aminopropyl, 3 ml (500 mg) (Chromabond, Macherey–Nagel).

2.2. Sample cleanup

Cleanup of juice and beverage samples was by C_{18} and aminopropyl SPE columns in series, using one of four variations of the method for apple juice analysis published by Delgado et al. [29]. Variation 1 used 5 ml acetonitrile–formic acid (100:1, v/v) instead of 4 ml to elute the toxins from the aminopropyl column. Variation 2 [49,50] increased the water and acetonitrile–water (35:65 instead of 1:3, v/v) wash volumes for the C_{18} column to 6 ml and 2.5 ml, respectively, and the acetone and acetonitrile wash volumes for the aminopropyl column to 3 ml of each. Variation 3 [49,50], for juices containing small amounts of suspended solid, included pretreatment with pectinase at 40 °C, centrifuging and adding the supernatant to the C_{18} SPE column, which was then washed with 5 ml water. The residue was extracted with 0.25 ml acetonitrile–acetic acid (100:1, v/v) (ultrasonic mixing) and then 2.25 ml water. After centrifuging, the supernatant was added to the column; this extraction step was repeated twice, the column was washed with 1.5 ml acetonitrile–water (35:65, v/v) and the toxins eluted with 4 ml acetonitrile–acetic acid (100:1, v/v). After evaporation, the residue was extracted three times with ethyl acetate (ultrasound) before addition to the aminopropyl SPE column. Variation 4 [48] used 0.1 ml methanol plus 0.4 ml ethyl acetate (twice), then ethyl acetate (ultrasound), to extract the residue after the C_{18} cleanup step in variation 2; the acetone and acetonitrile washes of the aminopropyl SPE column were then omitted. Wine was analysed after removal of alcohol by rotary evaporation.

2.3. LC conditions

For LC–MS and LC–MS–MS, a Micromass Quattro II triple quadruple tandem mass spectrometry system (Micromass, Manchester, UK) in conjunction with a HP 1100 LC system (including degasser, binary pumps, autosampler and UV detector) was used. The column was a 5-μm, 250×2.0-mm I.D. Inertsil-ODS2 [Chromatography Sciences (CSC), Montreal, Canada] and the mobile phase was water–methanol–acetonitrile (60:20:20 or 60:30:10, v/v/v) at a flow-rate of 0.25 ml/min. For electrospray experiments, a 2.1 mm I.D.×100 mm Genesis C_{18} column (Jones Chromatography, Hengoed, UK) with 3-μm particle size was used. Pure water (mobile phase A) and methanol (mobile phase B) were the two mobile phases and the flow-rate was reduced to 0.2 ml/min. The initial composition of mobile phase B was 50%. After holding for 1 min after the injection, the percent B was increased to 80% at 10 min and maintained for an additional 6.5 min before returning back to the initial composition. Eight μl of the extract were injected. Detection limits could be improved by increasing the injection volume.

Conventional RPLC with UV detection at 254 nm was also carried out. A 5-μm Inertsil ODS-2 (250×4.6 mm) column (MetaChem Technologies, Torrance, CA, USA) was used with methanol–acetonitrile–1% aqueous orthophosphoric acid (50:20:30, v/v/v) at a flow-rate of 1 ml/min under isocratic conditions [44]; the same solvents were used in a gradient program from 40:20:40 to 20:40:40 (v/v/v) over 10.5 min (flow-rate 0.5 ml/min) with an Ultratechsphere 5-μm ODS (250×3.2 mm) column (HPLC Technology, Macclesfield, UK); and this brand of column (250×4.6 mm) was used at 30 °C with a gradient profile of methanol–0.02% formic acid–water (initially 70:30:0, and finally 70:0:30, v/v/v) at a flow-rate of 0.5 ml/min [45].

2.4. LC–MS

A Micromass Quattro II triple quadrupole tandem mass spectrometer (Micromass, Manchester, UK) was used in all LC–MS and LC–MS–MS experiments. Instrumental control, data acquisition and data processing were performed through MassLynx software version 3.4 (Micromass). Scanning data in both single MS and MS–MS were obtained in continuum mode over the mass range *m/z* 80–350 (single MS) or *m/z* 10–300 (MS–MS). Baseline unit resolution was used in single MS experiments. In the MS–MS mode, the resolution of the first mass analyser was reduced to ~20% valley but remained at baseline unit resolution for the last mass analyser. In order to facilitate better peak integration, mass chromatograms were smoothed once for every two adjacent data points.

2.4.1. APCI conditions

The corona discharge voltage was 2.0 kV, cone voltage was 20 V, probe temperature was 500 °C, source temperature was 150 °C, sheath gas was 250 l/h and the drying gas (nitrogen) was 250 l/h. Using negative ion detection, ions monitored in the single ion monitoring (SIM) mode for AOH and AME were $[AOH–H]^-$ *m/z* 257 and $[AME–H]^-$ *m/z* 271. An on-line UV detector monitoring at 258 nm was connected between the column and the APCI probe.

LC–MS–MS was carried out with APCI in the multiple reaction monitoring (MRM) mode. For negative ion detection, transitions monitored for AOH were *m/z* 257 to 257 [parent ion, collision energy (CE)=5 eV], to 147 (CE=30 eV) and to 215 (CE=25 eV) and for AME were *m/z* 271 to 271 (parent, CE=5 eV), to 228 (CE=27 eV) and to 256 (CE=20 eV). For positive ion detection transitions monitored for AOH were *m/z* 259 $[AOH+H]^+$ to *m/z* 157,161,185, 213 and 244 and for AME were *m/z* 273 $[AME+H]^+$ to *m/z* 199, 212, 227, 230 and 258.

2.4.2. ESI conditions

Capillary voltage was −3 KV, source temperature was 120 °C, nebulizer gas flow-rate was 18 l/h, and the drying gas flow was 400 l/h. Argon was used as the collision gas at 2.3×10^{-3} mbar. Negative ion detection was used in MRM with the following parameters:

Precursor ion	Product ion	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
257	147	0.2	20	32
257	157	0.2	20	25
257	213	0.2	20	25
257	215	0.2	20	25
271	228	0.2	20	27
271	255	0.2	20	30
271	256	0.2	20	20

The resolution for the first quadrupole was set to around 20% valley while base-line unit resolution was maintained for the last quadrupole.

3. Results and discussion

3.1. Method recoveries

Tables 1 and 2 show that different variations in the cleanup procedure all gave good recoveries of AOH and AME from apple juice and other fruit juices and beverages (except citrus juices), as measured by LC with UV detection. Some recovery experiments were made that used LC–MS for the determination step. From apple juice spiked at 2 ng/ml for each toxin, recovery of AOH was 91(±3)% and of AME was

Table 1
Recoveries determined by LC–UV of AOH and AME added to apple juices

Method variation	Conc. added (ng/ml)		Recovery (%)±SD		
	AOH	AME	AOH	AME	*n*
1	12.5	12.5	112±5	87±5	6
2	10	20	79±14	87±16	9,13
2	1.25	1.25	64	118	1
	2.5	2.5	96±21	95±17	17
	5.0	5.0	96±17	85±9	14
3	10	20	84	76	2
3	2.5	2.5	96±18	90±12	4
	5.0	5.0	95±3	78±9	5
4	10	20	87±8	84±6	5
	10	10	94±8	102±13	12

Table 2
Recoveries determined[a] by LC–UV of AOH and AME added at 10 ng/ml to fruit beverages other than apple juice

Beverage	Recovery (%), after correction for blank	
	AOH	AME
Raspberry juice[b,c]	77	90
Raspberry	67	77
Pear juice	59	57
Grape juice (red)	95	81
Grape juice (red)	108	84
Grape juice (white)	95	96
Black currant juice[b,c]	73	86
Black currant	75	75
Cranberry juice[b,c]	94	102
Cranberry nectar	79	88
Red wine[c,d]	67	92
White wine[c, d]	99	95
Prune nectar[b]	134	78
Grapefruit juice[b]	0	30
Orange juice[b]	36	20

[a] Means of duplicate experiments, except for orange juice, analysed with cleanup variation 2.
[b] Analysed by pectinase procedure (variation 3).
[c] Analysed using cleanup variation 4.
[d] Analysed after evaporation of alcohol.

79(±21)% ($n=4$) with cleanup variation 2. From raspberry juice ($n=2$), blackcurrant juice ($n=2$) and prune nectar ($n=1$) spiked at 10 ng/ml for each toxin, recoveries of AOH were 67, 80 and 98%, respectively, and of AME were 88, 86 and 100%, respectively, using a combination of cleanup procedures 3 and 4 for the first two juices and cleanup procedure 3 for the prune nectar. Cranberry juice yielded extracts that were not clean enough for LC–MS determination.

3.2. APCI and ESI mass spectrometry of AOH and AME

Positive ion mass spectra of AOH and AME under both APCI and electrospray conditions are shown in Table 3. Both ionization methods yield predominantly $[M+H]^+$ ions for both compounds (m/z 259 for AOH and m/z 273 for AME). In addition, alkali metal adduct ions, $[M+Na]^+$ (m/z 276 for AOH and m/z 295 for AME) and $[M+K]^+$ (m/z 297 and 311, respectively), are also detected in electrospray mode. In negative ion mode (Table 3) $[M–H]^-$ (m/z 257 for AOH and m/z 271 for AME) are the only major ions observed in both ionization modes.

3.3. Collision-induced dissociation mass spectra of AME and AOH

The collision-induced dissociation (CID) mass spectra of the $[M+H]^+$ and $[M–H]^-$ for both compounds at different collision energies are shown in Fig. 2. The CID mass spectra were identical whether the precursor ions were produced by APCI or ESI.

Fragmentation proposed is that the protonated molecule of AOH ($[M+H]^+$ at m/z 259) loses a neutral molecule of H_2O to form m/z 241; or a methyl radical to form m/z 244; or CH_2CO to form m/z 217; or formic acid to form m/z 213. All these primary product ions in turn can lose CO consecutively. The protonated molecule of AME (m/z 273) can follow similar proposed fragmentation patterns.

Negative ions of the deprotonated molecule ($[M–H]^-$) of AOH and AME follow different pathways under CID conditions. It is proposed that AOH

Table 3
Electrospray (ESI) and atmospheric pressure chemical ionization (APCI) mass spectra of AOH and AME

	Electrospray		APCI	
	Positive ion	Negative ion	Positive ion	Negative ion
AOH (M_r 258)	m/z 259 ($[M+H]^+$), m/z 276 ($M+NH_4^+$), m/z 297 ($[M+K]^+$)	m/z 257 ($[M–H]^-$), m/z 97	m/z 259 ($[M+H]^+$)	m/z 257 ($[M–H]^-$), m/z 229 ($[M–H–CO]^-$)
AME (M_r 272)	m/z 273 ($[M+H]^+$), m/z 295 ($[M+Na]^+$), m/z 311 ($[M+K]^+$)	m/z 271 ($[M–H]^-$), m/z 256	m/z 273 ($[M+H]^+$)	m/z 271 ($[M–H]^-$), m/z 256, m/z 245

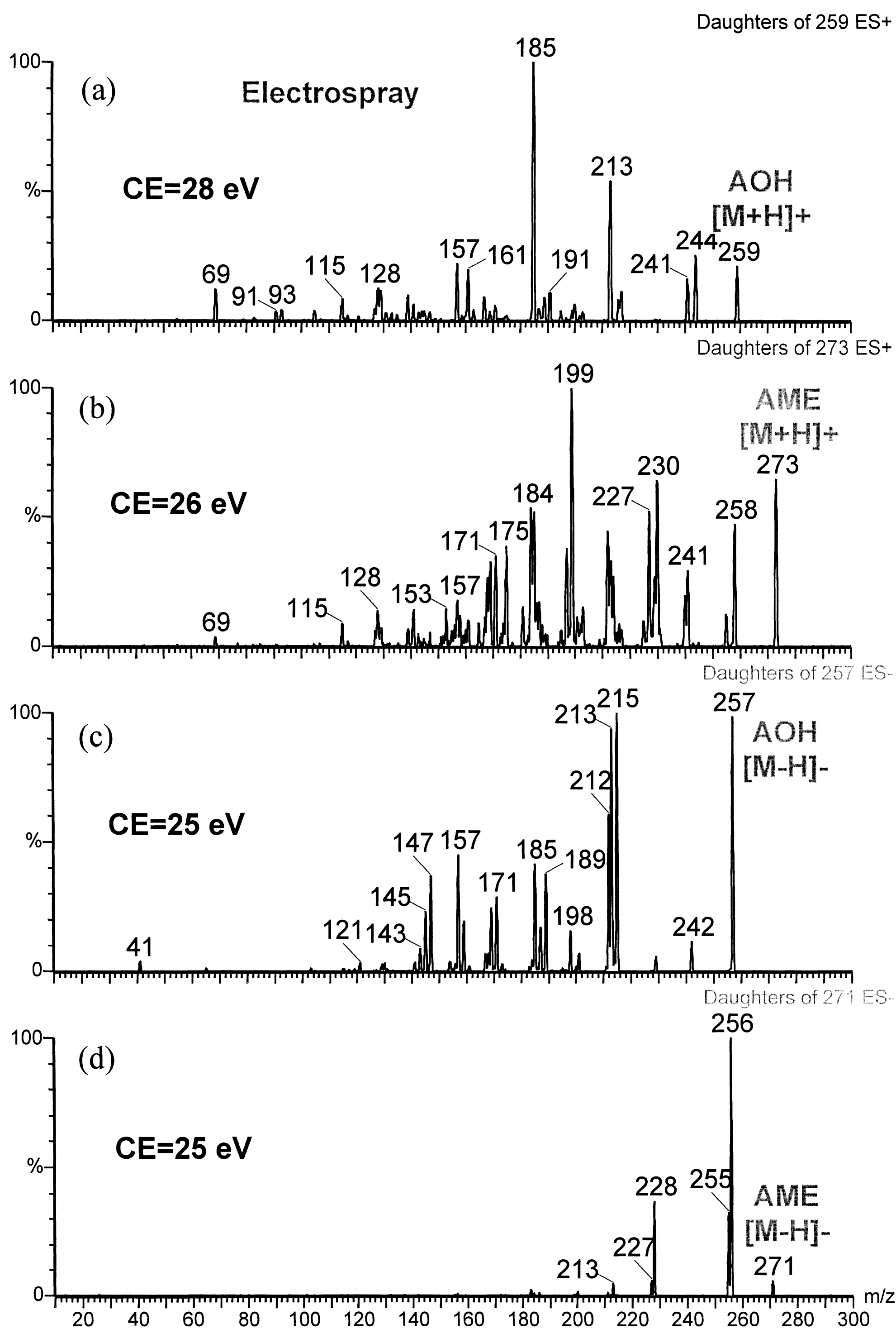


Fig. 2. Collision-induced dissociation (CID) mass spectra of (a) $[M+H]^+$ of AOH; (b) $[M+H]^+$ of AME; (c) $[M-H]^-$ of AOH; and (d) $[M-H]^-$ of AME.

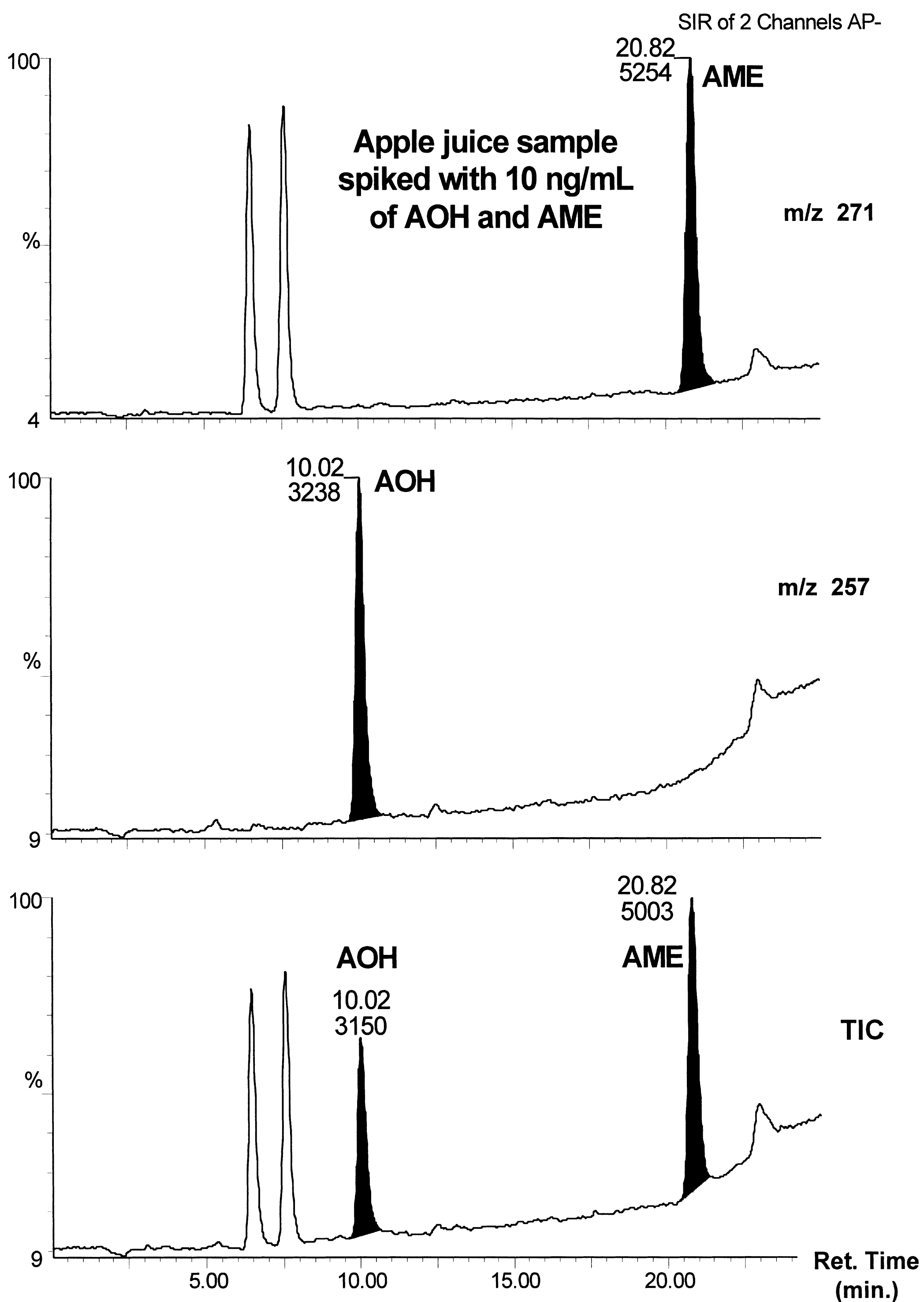


Fig. 3. SIM mass chromatograms from an apple juice sample spiked with 10 ng/ml each of AOH and AME under negative ion APCI.

primarily loses a methyl radical to form m/z 242, Co to form m/z 229, CH_2CO to form m/z 215 or CO_2 to form m/z 213; while it is proposed that AME primarily loses a methyl radical to form m/z 256 (which then loses CO to give m/z 228) and an O radical to form m/z 255. Most fragments are stabilized by the delocalization of the negative charge onto the oxygens through conjugated double bonds.

The MRM for the LC–MS–MS analysis was based on the most dominant product ions of each compound. As one may expect, the production yield of these product ions heavily depends on the collision energy (assuming the collision gas pressure in the CID cell is constant). In order to attain the highest sensitivity, it is important to incorporate these individually optimised collision energies for all precursor→product ion transitions into each MRM cycle. This can be achieved by the acquisition software, which programs the optimal cone voltages and collision energies for each MRM transition.

3.4. LC–MS analysis

During our initial stage of method development, an LC–MS procedure using APCI and negative ion detection was developed. The $[M–H]^-$ ion of both AOH (m/z 257) and AME (m/z 271) were monitored in the SIM mode. The minimum detection limit was estimated to be around 10–20 pg (peak-to-peak $S/N>3$) per injection for both AOH and AME, equivalent to 0.01–0.08 ng/ml in apple juice. Fig. 3 shows the SIM mass chromatograms from an apple juice extract spiked with 10 ng/ml of AOH and AME. Interferences at ~6.5 and ~7.6 min at the AME channel (m/z 271) are typical for apple juice. Other matrices such as orange juice, red wines and prune juice showed higher chemical background and made the low level detections more difficult.

Table 4 shows the comparison of results obtained from both LC–APCI-MS method and LC–UV method in the analysis of AOH and AME in samples of apple juice. In general, results of AOH were lower by LC–MS implying interferences might be present in the LC–UV determinations. Because of the high sensitivity offered by LC–APCI−MS, AME that was not previously found by UV detection in these apple juice samples, could be detected by LC–MS.

3.5. LC–MS–MS analysis

Absolute responses for MRM (LC–MS–MS) analysis decreased roughly by a factor of 10 for AOH and 2 for AME in APCI mode using negative ion detection. As expected, specificity in real−life samples was greatly improved by using tandem mass spectrometry. Fig. 4 shows mass chromatograms from a natural contaminated apple juice sample, which was analysed in LC–APCI-MS–MS mode.

ESI offers much better sensitivity. Minimum detection limits (based on $S/N>3$ determined from raw data) were around 4 pg for both AOH and AME. Fig. 5 shows the mass chromatograms from a pear juice extract spiked with 2 ng/ml of AME and 1 ng/ml of AOH. Ion pairs of m/z 271→256 and

Table 4
Determination of AOH and AME in samples of apple juice by LC–MS

Sample	AOH (ng/ml)		AME (ng/ml)	
	LC–MS	LC–UV	LC–MS	LC–UV
1	1.99	2.7	0.08	ND(<1)
2	0.88	Trace (<1)	Trace	ND(<1)
3	0.88	Trace (<1)	Trace	ND(<1)
4	Trace (0.04)	0.94	Trace (0.03)	ND(<1)
5	2.40	2.2	0.43	ND(<1)
6	0.99	1.9	0.08	ND(<1)
7	1.38	2.5	0.16	ND(<1)
8	0.57	1.2	0.05	ND(<1)
9	0.78	1.4	0.09	ND(<1)
10	Trace (0.04)	1.4	ND (<0.03)	ND(<1)
11	0.88	0.9	0.05	ND(<1)

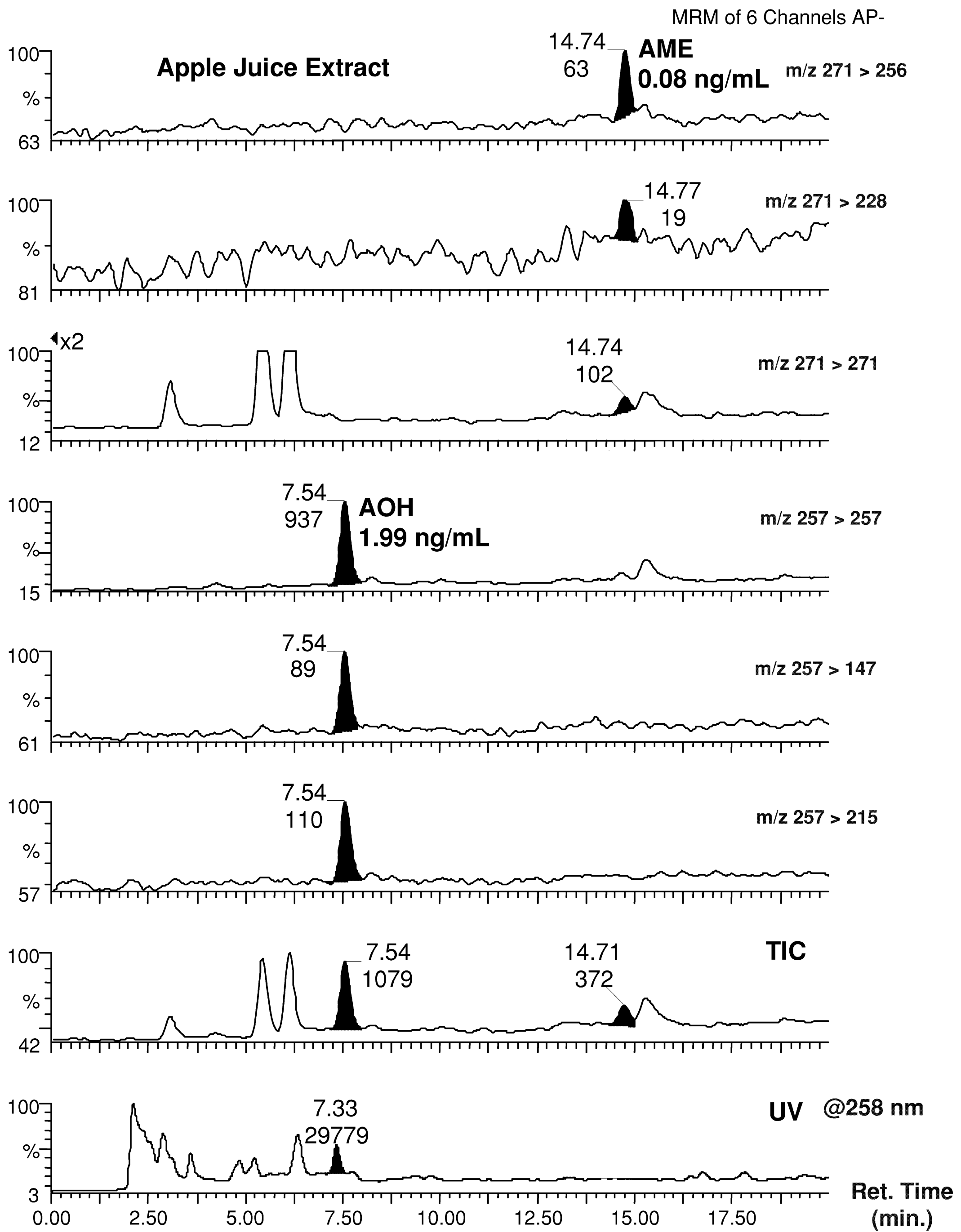


Fig. 4. MRM mass chromatograms from a naturally contaminated apple juice sample under negative ion APCI.

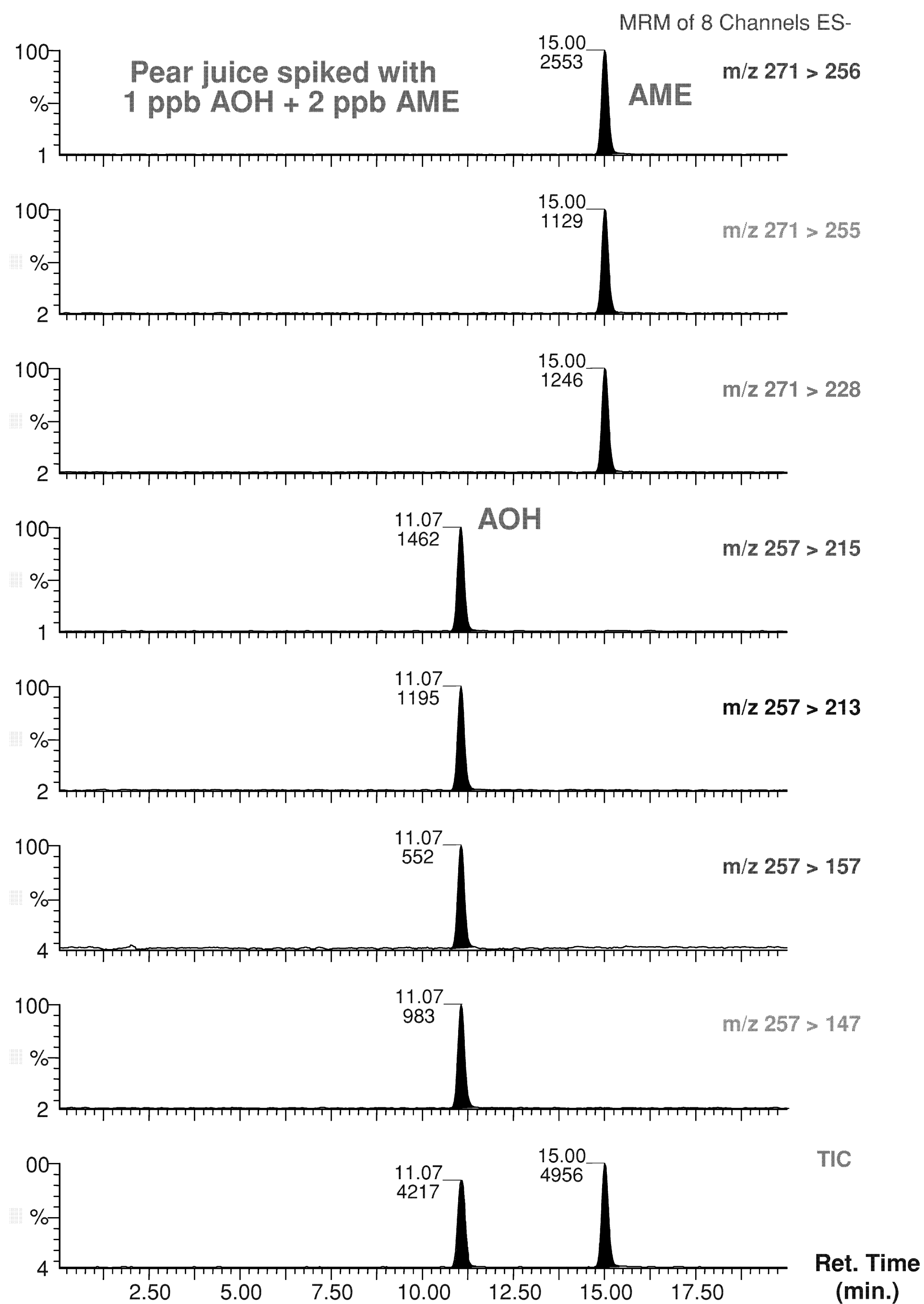


Fig. 5. MRM mass chromatograms from a pear juice sample spiked with 1 ng/ml of AOH and 2 ng/ml of AME under negative ion ESI.

m/*z* 271→228 were chosen as quantitation ions for AME while *m*/*z* 257→215 and *m*/*z* 257→147 were used for AOH. Response curves were linear ($r^2 >$ 0.99) over three orders of magnitude (from 15 to 2000 pg injected). This highly sensitive method has been applied to the confirmation and determination of sub-ng/ml levels of AOH and AME in fruit beverages (such as apple juice, grape juice, cranberry nectar, raspberry juice, prune nectar and red wine). Some matrices occasionally displayed unidentified peaks (at earlier retention times than AOH) in *m*/*z* 257→213 or/and *m*/*z* 257→157 channels but did not interfere with AOH. These two transitions can serve as confirmation.

Table 5 compares LC–MS or LC–MS–MS results to the LC–UV procedure for different fruit beverages analysed for AOH and AME. It is apparent that discrepancies exist between the MS and UV detection methods. However, analyses of cranberry nectar and black currant juice by two analysts using LC–UV detection with different method modifications and LC conditions also yielded considerable variance in results (see Table 5). In all cases, the LC–MS or LC–MS–MS method gave lower values than LC–UV. This brings into question the specificity and accuracy of the UV detection procedure in the analyses of AOH and AME in fruit beverages.

Our later experiments indicated that another Alternaria toxin, altertoxin I (ATX−1) could be successfully incorporated into the analysis (unpublished data) by monitoring the transitions of *m*/*z* 351→297, 351→333, 351→315.

4. Conclusion

Two atmospheric ionization techniques (namely APCI and ESI) were investigated for the LC detection of AOH and AME. Both techniques offer much higher sensitivity and specificity than the conventional UV detection procedure. A combination of electrospray ionization with negative ion detection and tandem mass spectrometry (MS–MS) is the procedure of choice. An absolute instrumental detection limit of low picograms and hence sub-ng/ml amounts of AOH and AME in fruit juice samples can be easily obtained. Simultaneous responses of at least 2 MRM transitions plus the correct retention time for each compound provide unequivocal confirmation of the presence of these mycotoxins in different fruit beverages. The level of confidence will become higher if additional precursor–product ion pairs are monitored and their relative ratios are considered as another positive identification criteria. This technique has overcome previous limitations of other analytical methods, in terms of sensitivity and specificity, to provide useful data on the natural occurrence of these toxins in various fruit products.

Table 5
Analysis of fruit beverages other than apple juice for AOH and AME by LC–MS or LC–MS–MS compared to LC–UV

Beverage	LC–MS[a] or MS–MS[b] detection		LC–UV[c]			
	AOH (ng/ml)	AME (ng/ml)	AOH (ng/ml)		AME (ng/ml)	
Raspberry juice	0.84	ND[d] (<0.12)	–	3.0	–	ND
Cranberry juice	ND (<0.74)	ND (<0.47)	–	ND	–	ND
Cranberry nectar	5.6	0.7	35	1.8	ND	ND
Black currant juice A	ND (<0.31)	ND (<0.20)	–	0.5	–	ND
Black currant juice B	ND (<0.75)	ND (<0.38)	8.8	Trace	ND	ND
Grape juice	1.6	0.23	16.7	10.2	ND	ND
Red wine	1.9[e]	ND (<0.08)	–	2.5[e]	–	ND
Peach	ND	ND	6.7	–	ND	–
Prune nectar	5.5	1.4	–	Int[f]	–	1.4

[a] LC–MS for raspberry, cranberry and black currant B juices, red wine and prune nectar.
[b] LC–MS–MS for cranberry nectar, black currant juice A, grape juice and peach nectar.
[c] Analyses by two analysts using different method modifications and LC conditions.
[d] ND, not detected.
[e] Mean of duplicate experiments.
[f] Interference.

This survey information is too limited so far to comment on the AOH and AME levels in the foods supply.

Acknowledgements

Technical assistance of D. Lavallee in extraction of apple juices is acknowledged.

References

[1] J. Chelkowski, A. Visconti (Eds.), *Alternaria*. Biology, Plant Diseases and Metabolites, Elsevier, Amsterdam, 1992.
[2] H. Raistrick, C.E. Stickings, R. Thomas, Biochem. J. 55 (1953) 421.
[3] A. Bottalico, A. Logrieco, in: K.K. Sinha, D. Bhatnagar (Eds.), Mycotoxins in Agriculture and Food Safety, Marcel Dekker, New York, 1998, p. 65.
[4] A. Visconti, A. Sibilia, in: J.D. Miller, H.L. Trenholm (Eds.), Mycotoxins in Grain. Compounds Other Than Aflatoxins, Eagan Press, St. Paul, MN, 1994, p. 315.
[5] S. Panigrahi, in: J.P.F. D'Mello (Ed.), Handbook of Plant and Fungal Toxicants, CRC Press, Boca Raton, FL, 1997, p. 319.
[6] G. Liu, J. Miao, Z. Liu, G. Yu, Y. Zhen, X. Li, J. Zhang, C. Zhao, H. Hao, Acta Acad. Med. Henan 17 (1982) 5.
[7] Y.Z. Zhen, Y.M. Xu, G.T. Liu, J. Miao, Y.D. Xing, Q.L. Zheng, Y.F. Ma, T. Su, X.L. Wang, L.R. Ruan, J.F. Tian, G. Zhou, S.L. Yang, in: I.K. O'Neil, J. Chen, H. Bartsch (Eds.), Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins, International Agency for Research on Cancer, Lyon, 1991, p. 253.
[8] Z. Dong, G. Liu, Z. Dong, Y. Qian, Y. An, J. Miao, Y. Zhen, Carcinogenesis 8 (1987) 989.
[9] G. Liu, Y. Qian, Z. Peng, W. Dong, Y. Qi, H. Gou, Chin. Med. J. 105 (1992) 394.
[10] T.J. Schrader, W. Cherry, K. Soper, I. Langlois, H.M. Vijay, Teratogen. Carcinogen. Mutagen. 21 (2001) 261.
[11] Y.-H. An, T.-Z. Zhao, J. Miao, G.-T. Liu, Y.-Z. Zheng, Y.-M. Xu, R.L. Van Etten, J. Agric. Food Chem. 37 (1989) 1341.
[12] P.M. Scott, D.R. Stoltz, Mutat. Res. 78 (1980) 33.
[13] M. E Stack, M.J. Prival, Appl. Environ. Microbiol. 52 (1986) 718.
[14] V.M. Davis, E. Stack, Appl. Environ. Microbiol. 60 (1994) 3901.
[15] G.T. Liu, Y.Z. Qian, P. Zhang, Z.M. Dong, Z.Y. Shi, Y.Z. Zhen, J. Miao, Y.M. Xu, in: I.K. O'Neill, J. Chen, H. Bartsch (Eds.), Relevance to Human Cancer of *N*-Nitroso Compounds, Tobacco Smoke and Mycotoxins, International Agency for Research on Cancer, Lyon, 1991, p. 258.
[16] S. Gruber-Schley, A. Thalmann, Landwirtsch. Forschung 41 (1988) 11.
[17] D.J. Webley, K.L. Jackson, J.D. Mullins, A. D Hocking, J.I. Pitt, Aust. J. Agric. Res. 48 (1997) 1249.
[18] F. Li, T. Yoshizawa, J. Agric. Food Chem. 48 (2000) 2920.
[19] S.N. Chulze, A.M. Torres, A.M. Dalcero, M.G. Etcheverry, M.L. Ramirez, M.C. Farnochi, J. Food Prot. 58 (1995) 1133.
[20] S. Nawaz, K.A. Scudamore, S.C. Rainbird, Food Addit. Contam. 14 (1997) 249.
[21] H.A.H. Hasan, Mycopathologia 130 (1995) 171.
[22] H. Giryn, B. Szteke, Rocz. Panstw. Zakl. Hig. 46 (1995) 129.
[23] G. Matysik, H. Giryn, Chromatographia 42 (1996) 555.
[24] I.-M. Chung, H.-I. Ju, S.-C. Sim, S.-B. Paik, S.-H. Yu, Anal. Sci. Technol. 11 (1998) 143.
[25] V.H. Tournas, M.E. Stack, J. Food Prot. 64 (2001) 528.
[26] P.M. Scott, D. Weber, S. R Kanhere, J. Chromatogr. A 765 (1997) 255.
[27] T. Delgado, C. Gómez-Cordovés, J. Chromatogr. A 815 (1998) 93.
[28] P.M. Scott, in: V. Betina (Ed.), Chromatography of Mycotoxins. Techniques and Applications, Elsevier, Amsterdam, 1993, p. 373.
[29] T. Delgado, C. Gómez-Cordovés, P. M Scott, J. Chromatogr. A 731 (1996) 109.
[30] J.E. Schade, A.D. King Jr., J. Food Prot. 47 (1984) 978.
[31] S. Ozcelik, N. Ozcelik, L.R. Beuchat, Int. J. Food Microbiol. 11 (1990) 187.
[32] L.M. Seitz, H.E. Mohr, Anal. Biochem. 70 (1976) 224.
[33] E.G. Heisler, J. Siciliano, E.E. Stinson, S.F. Osman, D.D. Bills, J. Chromatogr. 194 (1980) 89.
[34] M.R. Maas, M.A. Woody, F.S. Chu, J. Food Safety 3 (1981) 39.
[35] M. Wittkowski, W. Baltes, W. Krönert, R. Weber, Z. Lebensm. Unters. Forsch. 177 (1983) 447.
[36] M.E. Stack, P.B. Mislivec, J.A.G. Roach, A.E. Pohland, J. Assoc. Off. Anal. Chem. 68 (1985) 640.
[37] F. Palmisano, P.G. Zambonin, A. Visconti, A. Bottalico, J. Chromatogr. 465 (1989) 305.
[38] A. Visconti, A. Logrieco, A. Bottalico, Food Addit. Contam. 3 (1986) 323.
[39] M. Müller, P. Lepom, Zentralbl. Mikrobiol. 147 (1992) 197.
[40] L. Giacomelli, H. Boggetti, H. Agnelli, J. Anunziata, J.J. Silber, R. Cattana, Anal. Chim. Acta 370 (1998) 79.
[41] E.W. Sydenham, P.G. Thiel, W.F.O. Marasas, J. Agric. Food Chem. 36 (1988) 621.
[42] C.A. Fente, J. Jaimez, B.I. Vázquez, C.M. Franco, A. Cepeda, Analyst 123 (1998) 2277.
[43] F. Palmisano, P.G. Zambonin, A. Visconti, A. Bottalico, Chromatographia 27 (1989) 425.
[44] P.M. Scott, J. AOAC Int. 84 (2001) 1809.
[45] F. Liu, Y. Luo, L.H. Zhang, K.Y. Kuang, S.Y. Shi, Chin. Chem. Lett. 4 (1993) 975.
[46] Y. Luo, F. Liu, J. Feng, L. Zhang, X. Hu, C. Feng, J. Yang, K. Kuang, S. Shi, Chin. J. Chromatogr. 12 (1994) 342.
[47] P.M. Scott, S.R. Kanhere, C. Cléroux, B.P.-Y. Lau, D.A. Lewis, V.A. Roscoe, in: 112th AOAC Int. Annu. Meeting, 13–17 September 1998, Montreal, Canada, 1998, p. 62, Abstracts.

[48] B.P.-Y. Lau, P.M. Scott, D.A. Lewis, S.R. Kanhere, C. Cleroux, V.A. Roscoe, in: Proceedings of 47th ASMS Conference of Mass Spectrometry and Allied Topics, June 14–18, Dallas, TX, 1999, p. 1518, Abstracts.

[49] P.M. Scott, S.R. Kanhere, in: A.E. Pohland, M.W. Trucksess (Eds.), Mycotoxin Protocols, Humana Press, Totowa, NJ, 2000, p. 225.

[50] Determination of Alternariol and Alternariol Methyl Ether in Apple Juice Using Solid-Phase Extraction and High Performance Liquid Chromatography, Laboratory Bulletin WPG-LB-23, Health Protection Branch, Ottawa, 1998.