Assay of Ochratoxin A in Wine and Beer by High-Pressure Liquid Chromatography Photodiode Array and Gas Chromatography Mass Selective Detection

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To routinely assay the concentrations of ochratoxin A (OTA) in wines and beers, two new methods were developed and evaluated. The first utilized solid-phase extraction on a C_{18} cartridge to achieve a 100-fold sample concentration followed by high-performance liquid chromatography on a C_{18} column with gradient elution and quantitation at 333 nm by means of a photodiode array detector. Positive confirmation can be carried out by purity and match-factor analysis as well as peak shift following esterification with BF_3. Total run time is 28 min. The limits of detection (LOD) and quantitation (LOQ) are 0.05 and 0.10 μg/L, respectively. Recovery and imprecision ranged from 83 to 94% and from 4.0 to 8.9%, respectively. With a throughput of 35 assays per working day, this method is ideal for routine OTA analysis. It was used to survey the concentrations of OTA in 942 wines (2 of which gave values between 0.1 and 0.2 μg/L) and 107 beers (2 of which gave values between 0.05 and 0.1 μg/L). OTA was detected more frequently in red than white wines, with the highest incidence in red wines from Spain and Argentina. There was no association between OTA and country of origin or beverage type among the beers analyzed. The second method utilized gas chromatography with mass selective detection monitoring eight specific ions, preceded by extraction in dichloromethane and derivatization with bis[trimethylsilyl]trifluoroacetamide. LOD and LOQ were 0.1 and 2 μg/L, respectively; recovery and imprecision were 69−75 and 9.0−11.1%, respectively. The method is not suitable for routine quantitation but is potentially useful as a confirmatory tool for samples with OTA ≥0.1 μg/L.

Keywords: *Ochratoxin A; wine; beer; high-pressure liquid chromatography photodiode array detector; gas chromatography−mass spectrometry; BSTFA*

INTRODUCTION

Ochratoxin A (OTA), *R*-*N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-phenylalanine (MW 403.18), is a derivative of isocoumarin linked through the carboxyl group to phenylalanine (Figure 1). It is a secondary metabolite produced by the fungi *Penicillium verrucosum* and *Aspergillus ochraceus* that are commonly encountered in food crops grown in semitropical and temperate climates (*1*, *2*). Its generation is dependent upon several factors such as temperature, humidity, and other storage conditions (*2*, *3*). It is stable to heat and other physical processes to which foods are subjected (*4*). It can be absorbed by animals and humans and accumulates in the blood and kidneys (*1*).

OTA has many toxic effects, with renal damage being the most common and serious. Cytotoxicity has been clearly demonstrated in cultured kidney cell lines (*5*). Mechanisms likely to promote nephrotoxicity by OTA include enhanced apoptosis (*6*), interference with mitochondrial respiratory function and pH homeostasis (*7*), and impaired organic anion transport (*8*). Other mechanisms responsible for the toxicity of OTA involve inhibition of tRNA-synthetase, accompanied by reduced protein synthesis, and enhanced lipid peroxidation via the generation of free radicals (*1*).

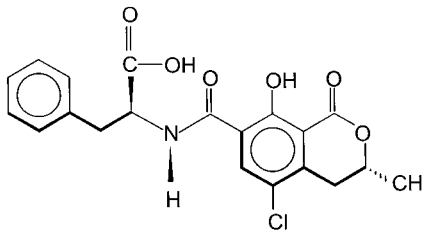

Figure 1. Structure of (−)-ochratoxin A.

Population studies have shown the presence of measurable concentrations of OTA in the blood plasma of many apparently healthy human subjects (*9*, *10*). Higher plasma OTA concentrations were found in proportion to the severity of the disease in patients with end-stage renal failure, nephrosis, and urothelial cancer (*11*). It is widely believed, but not fully established, that OTA may be a prominent etiologic factor in the endemic disease Balkan nephropathy (*1*, *12*, *13*). Animal experiments have shown that OTA can induce the formation of renal tumors, with cytochrome P450-related reactions and DNA adduct generation being potential contributing mechanisms (*14*, *15*). Brain damage caused by OTA has also been demonstrated experimentally (*16*, *17*).

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Anxiety concerning hazards to human health arising from exposure to OTA are enhanced by human and animal studies pointing to the occurrence of measurable concentrations of this compound in the blood serum of healthy humans (*9*, *10*), as well as placental transfer to the fetus and absorption by infants from breast milk (*18*−*20*). In efforts to develop antidotes for overexposure to OTA, aspartame (an analogue of OTA) and choleystyramine (a resin) have shown some promise (*21*−*23*).

Many attempts have been made to quantitate the extent of OTA contamination in commonly consumed foods, especially cereals and fruit (*1*, *24*−*26*). Because they are produced by fermentation of the above, wine (*27*−*31*) and beer (*32*−*34*) have also been widely surveyed for their content of OTA. The levels observed have given rise to some concern, to the point where at least four countries (Finland, The Netherlands, United Kingdom, and Canada) are actively considering the introduction of a maximal allowable limit (MAL) (C. Tricard, Bordeaux, France, personal communication, 1999; *35*). Should this trend accelerate, there will be a universal need for methods that will allow the mass screening of wines and beers and confirmation of unacceptable results. At present, such methods do not exist. High-performance liquid chromatography (HPLC) preceded by extraction of OTA using a commercially available immunoaffinity column (IAC) has been quite popular among reference centers (*30*, *36*, *37*) but is too slow for large sample batches. Capillary electrophoresis with laser-induced fluorescence (*38*) and electrospray tandem mass spectrometry (*39*, *40*) have been employed for various matrices, but they require highly expensive and technically sophisticated equipment that is beyond the scope and means of all but reference and research laboratories and the former has not been applied to the analysis of wines and beers. Other HPLC methods have been reviewed by Valenta (*4*).

Our objective in this work was to develop a method that can be used for quality control purposes and that is specific/selective for OTA, reasonably fast and inexpensive, and has good reproducibility. Two methods for the determination of OTA in wine and beer are described in this paper. The first uses HPLC interfaced to a photodiode array detector (HPLC-PDA) and is suitable for screening. The second employs GC interfaced to a mass selective detector (GC-MSD) and may have a useful role as a confirmatory procedure. These methods were analytically characterized and validated during a period of routine use that enabled us to acquire data on the OTA content of the largest collection of wines and beers yet presented.

MATERIALS AND METHODS

Chemicals and Reagents. The OTA standard used (catalog no. 85, 628-2) and BF_3 dihydrate (catalog no. 35996-3) were obtained from Sigma-Aldrich, Oakville, ON, Canada. The pure standard was in liquid form and was stored in the dark at 4 °C. A stock standard solution (50 ng/mL) was made in 100% acetonitrile. All calibration standards were diluted freshly in acetonitrile from the stock standard to avoid possible degradation at lower concentration levels. Spiking standards were also made in acetonitrile prior to being added to raw wines.

Bis[trimethylsilyl]trifluoroacetamide (BSTFA) (catalog no. 3-3084) was obtained from Supelco, Bellefonte, PA. The He gas was purchased from Praxair, Mississauga, ON, Canada. All solvents and phosphoric acid used were purchased from Caledon Laboratories, Georgetown, ON, Canada. Wines and beers from 21 countries were analyzed; all samples were taken from freshly opened bottles and analyzed within 2−8 h of sampling.

Table 1. Gradient Elution Parameters for the HPLC-PDA Method[a]

time (min)	flow rate (mL/min)	A (%)	B (%)	curve[b]
	0.5	60	40	
5	0.5	40	60	11
15	0.5	40	60	6
20	0.5	60	40	6

[a] See text for experimental details. [b] Defined in the manufacturer's software package.

HPLC-PDA. This method utilizes a Waters System (616 quaternary pump, 500S controller, 717 autosampler, and in-line sparger) interfaced to a Waters 996 PDA detector (all from Waters Inc., Mississauga, ON). A 100 mL aliquot of wine or degassed beer is passed through a 3 mL solid-phase extraction (SPE) column packed with 50 mg of octadecyl C_{18} (catalog no. 76606-04) purchased from J. T. Baker, Phillipsburg, NJ, preconditioned with 1 mL of 100% ethanol followed by 1 mL of 10% (v/v) ethanol in water. The cartridge is dried on a vacuum manifold under a gentle stream of nitrogen for 5 min, and OTA is eluted with 1 mL of dichloromethane. This procedure represents a 100-fold concentration of the sample. The eluate is reduced to dryness under nitrogen at a flow rate of 10 mL/min for 5 min, and the residue is dissolved with 1.0 mL of EtOH/H_2O (1:1 by volume). The simultaneous extraction of 24 samples was possible using a Visiprep solid-phase vacuum manifold (catalog no. 57250-U) obtained from Supelco, Oakville, ON, Canada. Beer samples were degassed in an ultrasonic bath at room temperature for 30 min before extraction on an SPE C_{18} cartridge.

An aliquot of the extract (100 μL) is injected into a reversed-phase Nucleosil C_{18} 5 μm stainless steel column (250 × 4 mm), catalog no. 720014.4, preceded by a KS Nucleosil 120 μm C_{18} (20 × 4 mm) guard column, catalog no. 721312 (all purchased from Caledon Laboratories, Georgetown, ON, Canada) and eluted with a gradient comprising 0.2 mL (3 mM) H_3PO_4 in 1 L of H_2O, pH 3.1 (pump A) and 0.2 mL (3 mM) H_3PO_4 in 1 L of acetonitrile, pH 3.4 (pump B). The gradient conditions are described in Table 1. Detection with PDA is routinely accomplished by monitoring the absorbance signal at 333 nm and deriving the area under the curve (AUC).

Calibration and Quality Control. A five-point calibration prepared by adding different volumes of stock standard solution to an OTA-free sample of the appropriate matrix is used for the analysis of wine and beer with concentrations ranging from 0.5 to 10 μg/L. These amounts take into account the 100-fold concentration of OTA in the wine extract as a consequence of SPE. The AUC of the sample is read against the curve constructed from the AUC of the five standards using a least-squares fit.

A spiked wine sample with OTA at the LOQ (0.1 μg/L) and a blank (the same wine without OTA) are analyzed after every five samples. A reagent blank is analyzed at the beginning of each run. The presence of OTA in concentrations >0.1 μg/L is confirmed by esterification with BF_3/ethanol. In brief, a mixture of 100 μL of ethanol, 20 μL of BF_3, and 10 μL of sample is heated for 1 h at 50 °C, after which time it is thoroughly dried under a stream of nitrogen. The residue is dissolved in water/acetonitrile (50:50 v/v) and vortexed for 30 s before being analyzed by HPLC. The positive confirmation of OTA was shown by the disappearance of the OTA peak at the retention time (RT) of 7.66 min and the appearance of the ester peak at the RT of 21.81 min (Figure 2).

GC-MSD. This method utilizes a 6890 GC equipped with a 7673A autosampler, interfaced to a 5973 MSD, all purchased from Hewlett-Packard, Mississauga, ON, Canada. A 200 μL wine aliquot is extracted twice with 0.5 mL of dichloromethane in a 1 mL vial, vortexed for 1 min each time, and centrifuged. The organic layers (bottom) are combined and evaporated to dryness under nitrogen at a flow rate of 10 mL/min. The dry extract is derivatized with 100 μL of ethyl acetate/BSTFA (1:

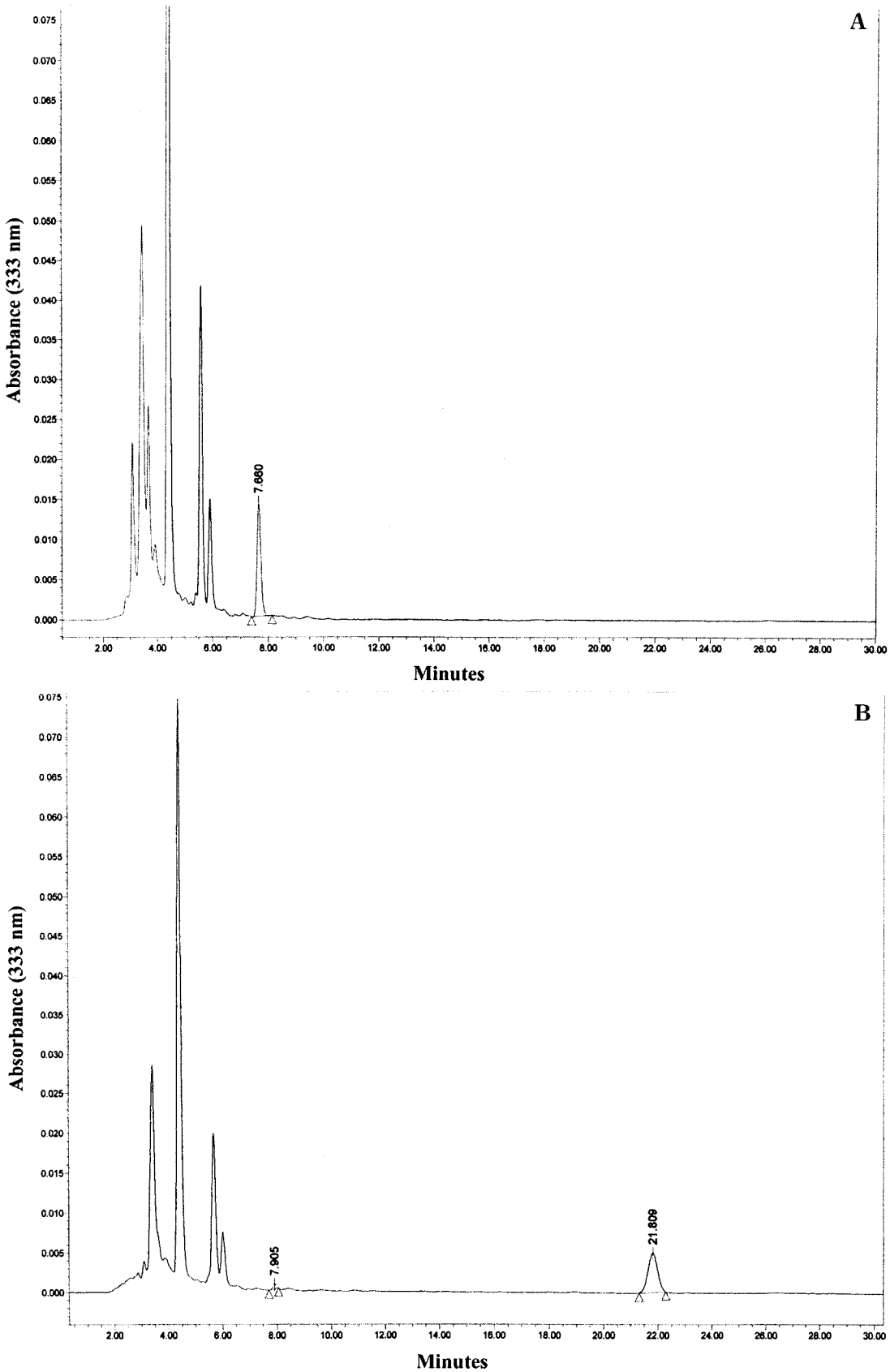

Figure 2. Chromatogram of red wine spiked with 1 μg/L OTA before (A) and after (B) conversion with ethanol/BF_3 to the ethyl ester. Note nearly complete conversion of the former to the latter. The HPLC conditions were as in the final method apart from isocratic elution to permit earlier elution of both peaks of interest (flow, 0.7 mL/min; 40% solvent A/60% solvent B).

1) for 2 h at 70 °C. A 2 μL aliquot of the derivatized extract is injected via a splitless injector onto a DB-5MS column, 15 m × 0.25 mm i.d. × 0.25 μm film thickness, obtained from J&W Scientific, Folsom, CA.

The GC oven program is initially set at 150 °C and increased at a rate of 8 °C/min to 220 °C. After 5 min, the temperature is increased at a rate of 25 °C/min for 5 min to a maximum baking temperature of 290 °C. Ultrahigh purity He with in-

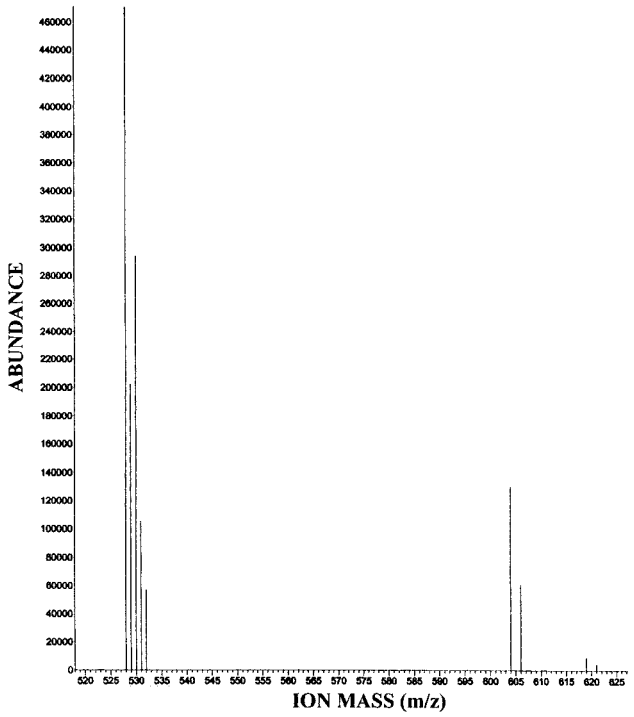

Figure 3. Ion spectrum of OTA (BSTFA derivative).

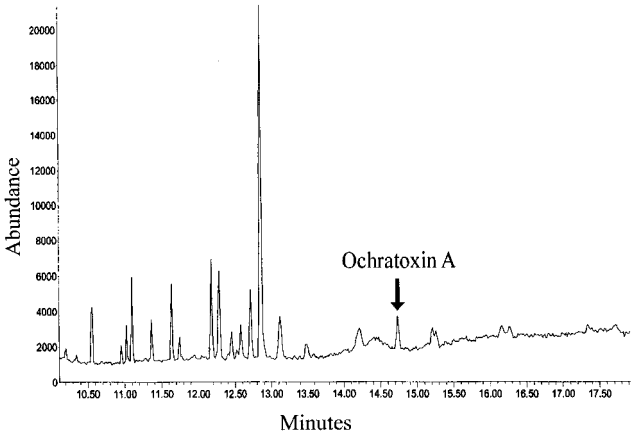

Figure 4. GC elution of OTA as measured by total ion abundance (MS).

line Supelpure moisture trap and hydrocarbon trap (Supelco Canada, Mississauga, ON, Canada) is used as a carrier gas. The carrier gas line is set at 48 psi, column head pressure at 10.9 psi, and total flow at 3.7 mL/min. The injector is in splitless mode. Selective ion monitoring (SIM) is performed at ions 528, 529, 530, 531, 532, 604, 606, and 619 (Figure 3). To achieve a lower detection limit, the sum of all eight ions is used for quantification. The injection port and detector temperatures are set at 260 and 290 °C, respectively. The OTA silyl derivative eluted at 14.52 min with a total analysis time of 16.0 min (Figure 4).

Quality Control. With every six injections, a 0.2 μg/L standard and a wine blank are run. Standard curves are not prepared as the method is unsuitable for quantitation at lower levels.

RESULTS

Characteristics of HPLC-PDA Method. Parts A and B of Figure 5 demonstrate the satisfactory chromatographic resolution accomplished for OTA in white and red wines, respectively. OTA eluted at ~19.2 min in a total run time of 28 min. Quantitation of OTA in both wine matrices was excellent, with linear calibration curves over a range of 0.1−10 μg/L ($r^2 = 1.000$). The limits of detection (LOD) and quantitation (LOQ) were 0.05 and 0.10 μg/L, respectively. In accordance with IUPAC recommendations (*41*), the LOD is based on a signal-to-noise ratio of 3:1. The LOQ was determined by analyzing a wine low in OTA (0.5 μg/L) six times. The standard deviation (SD) of the replicates, multiplied by 3, was taken as the LOQ. The average percent recovery at four different concentrations (spiked at levels from 0.1 to 2 μg/L) and coefficient of variation (CV) (imprecision = SD/mean × 100) ranged from 83 to 94% and from 4.0 to 8.9%, respectively, on the basis of six replicate analyses for each data point. Match-factor spectral analysis of each peak assigned a value between 0 and 100 for comparison between the spectrum of the peak and that of the pure compound. Spectra with match-factor values >90% were acceptable. Purity checks were also employed at the inflection points and apex of each peak. Peaks with purity >90% were considered to be consistent with the presence of OTA.

Characteristics of GC-MSD Method. Resolution of OTA in white wine using the GC-MSD method was excellent (Figure 4). Background noise causing a very high baseline in red wine prevented this method from attaining satisfactory LOD and LOQ. In an attempt to improve the sensitivity of the method and eliminate interferences, two other SPE cartridges were tested, C8 and CN, as well as a liquid−liquid extraction with ethyl acetate/methylene chloride (1:1 by volume). None of these modifications proved to be useful. Linearity ranged from 0.2 to 10 μg/L ($r^2 = 0.999$). The LOD and LOQ were 0.1 and 1 μg/L, respectively. Average percent recovery and CV at two concentrations (2 and 10 μg/L) ranged from 69 to 75% and from 9.0 to 11%, respectively.

Survey of OTA in Commercial Wines. A total of 942 wines were analyzed for OTA on the same day as they were opened. Their characteristics are presented in Table 2. In 3.9% of the white and 16.2% of the red wines, OTA was detectable in a concentration >0.05 μg/L; however, only in one red Australian wine (0.20 μg/L) and one red Spanish wine (0.15 μg/L) did the value exceed 0.1 μg/L (the method LOQ) as shown in Table 3. Values between 0.05 and 0.1 μg/L could be clearly detected but not accurately quantitated. The incidence of OTA in European red wines (Table 2) was quite variable. Spain (33%), Portugal (22%), and Central Europe (22%) were highest; Greece (17%) and Italy (16%) were lower. Evidence for a North−South gradient in wine OTA concentrations is not apparent from these data. Red wines from Argentina (29%) had a higher incidence of detectable OTA content than red wines from most other countries. A rather low incidence was noted in wines from North America.

Survey of OTA in Commercial Beers. One hundred and seven products from 11 countries were classified into 5 major regions as presented in Table 4, which also places them in three color categories. In only two samples did OTA exceed 0.05 μg/L, and none surpassed 0.1 μg/L.

DISCUSSION

Evaluation of Methods. The characteristics of the two methods that we developed for the analysis of OTA in wines and beers are compared with those of six representative recent HPLC methods that have been applied to the same two beverages (Table 5). In the case

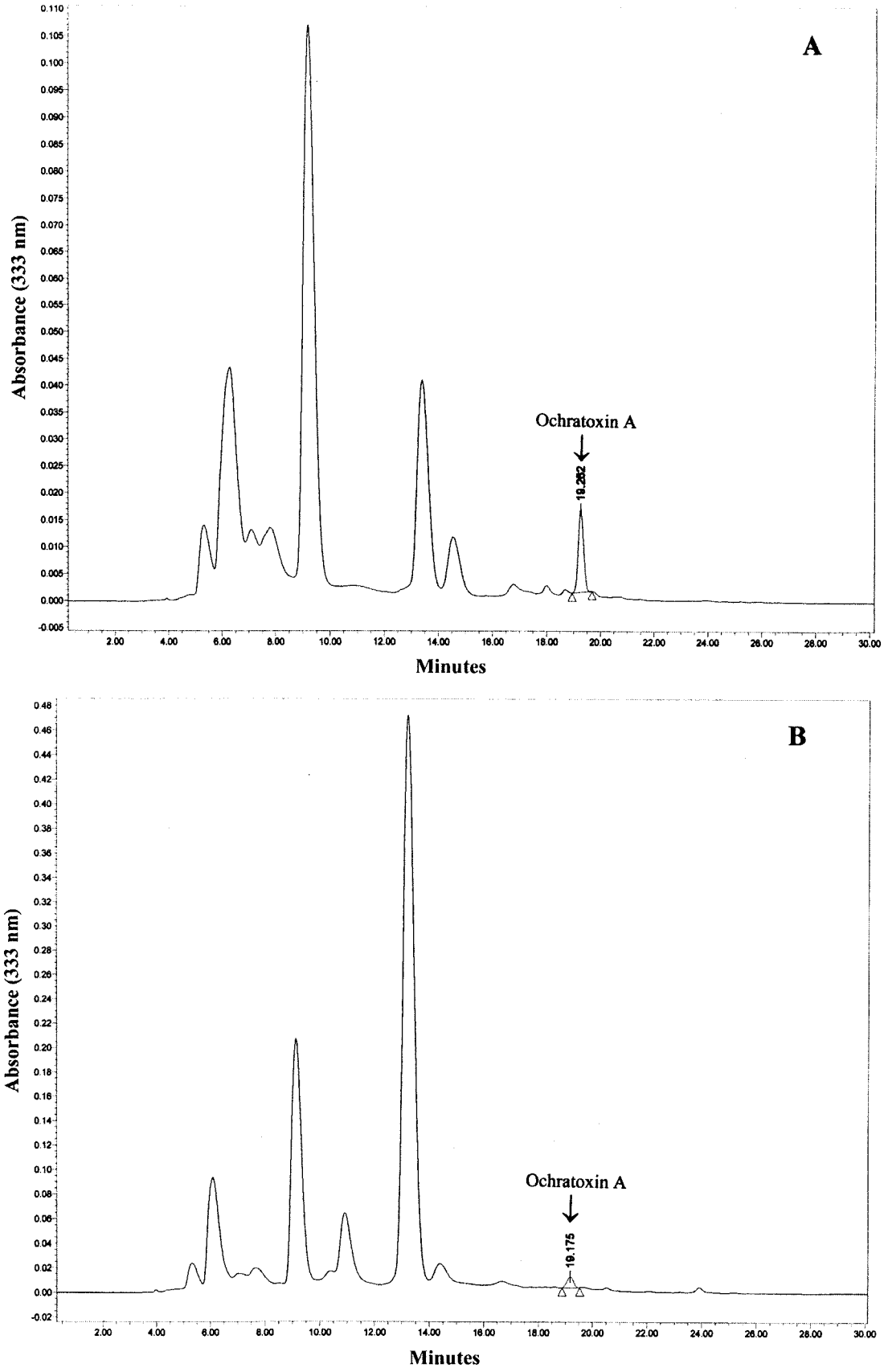

Figure 5. Resolution of OTA by HPLC in white wine (1 μg/L; A) and red wine (1 μg/L; B). The scales were chosen to include all identifiable peaks. With low concentrations of OTA close to the LOQ, its peak can be magnified manyfold by appropriate scale selection.

of two of the latter, some information (LOD or CV) is not provided. The HPLC method that we have developed matches the other HPLC techniques in recovery and imprecision but is less sensitive with regard to the LOD. The other authors did not give the LOQ for their methods; that of ours was 0.1 μg/L. This is below the

Table 2. Description of Commercial Wines Analyzed in This Survey and Number (Percent) with OTA Concentration >0.05 μg/L

	white wines			red wines		
country or region	total	no. >0.05	%	total	no. >0.05	%
Argentina	14	2	14	17	5	29
Australia	11	1	9	45	9	20
Canada	42	0	0	54	3	5.5
British Columbia	10	0	0	18	1	5.5
Ontario	32	0	0	36	2	5.5
Chile	20	0	0	42	8	19
Central Europe	25	3	12	27	6	22
France	33	2	6	59	6	10
Greece[a]	16	2	12.5	23	4	17
Germany	16	1	6	5	0	0
Italy	36	3	8	101	16	16
New Zealand	29	0	0	10	1	10
Portugal	14	0	0	23	5	22
South Africa	18	0	0	13	2	15
Spain	6	0	0	36	12	33
United States	40	0	0	71	8	11
California	32	0	0	58	8	14
Oregon/Washington	8	0	0	13	0	0
total	362	14	3.9	580	96	16.6

[a] Includes wines from Cyprus.

Table 3. Distribution of Ochratoxin A (Micrograms per Liter) by Beverage Category Measured by HPLC-PDA Method

		ochratoxin A		
beverage	no.	<0.050	0.051−0.100[a]	>0.100
white wine	362	348 (96.1%)	14 (3.9%)	0
red wine	580	484 (83.4%)	94 (16.2%)	2 (0.34%)
beer	107	105 (98.1%)	2 (1.9%)	0

[a] Samples above the LOD (0.05 μg/L) but below the LOQ (0.100 μg/L).

Table 4. Characteristics of 107 Beers Analyzed for OTA in This Survey

feature	no.
country/region	
Canada	56
United Kingdom	21
Europe	17
United States	7
Orient	6
color	
pale	57
medium	24
dark	26

MAL for OTA in wine and beer set by Finland (0.5 μg/L) and The Netherlands (0.3 μg/L); it is expected that the European Union will eventually settle on a MAL at the higher of these concentrations (C. Tricard, personal communication, 1999; *35*). That being so, the lower sensitivity of our method that can accurately quantitate to as low as one-fifth of the expected MAL is not disadvantageous. The method is cheap; the SPE cartridges cost only U.S. $1.00. The IAC costs U.S. $8 and can be used only once; moreover, use of these columns is labor-intensive, and a typical reference laboratory can complete only 30 assays per week (Health Canada, Health Protection Branch, Food Chemistry Laboratory, personal communication, 2001), a workload similar to that reported for an HPLC method using solvent extraction followed by SPE (*31*). However, we should mention that some authorities claim a much higher throughput with this method. With our HPLC method, one technician can complete 35 analyses in a single working day.

Another advantage inherent in our use of PDA with a sophisticated software package allowing purity and match-factor analyses is the specificity that it provides, which is strengthened by the use of BF_3 to esterify OTA and shift its spectral peak. Despite using IAC, Nakajima et al. (*33*) adopted a similar approach employing a methylation technique. This emphasizes the fact that commercial IACs are of variable affinity, and even those from different batches by the same manufacturer may differ in their retention of OTA and exclusion of interfering substances. By contrast with absorption spectra, fluorescence properties cannot be reliably used for positive identification.

The GC-MSD method has lower sensitivity, poorer recovery, and a level of imprecision higher than that of other methods. It is not recommended for screening or quantitation, and we have not investigated possible improvements beyond those already described. However, the unique mass spectrum of OTA (Figure 3) together with the ability of the present method to provide reliable positive identification above a concentration of 0.1 μg/L suggests that it may be used as a confirmatory tool by those laboratories using HPLC with fluorescence detection that happen to possess GC-MSD instrumentation and not a PDA system.

OTA Concentrations of Wine and Beer. Zimmerli and Dick (*27*) first reported the presence of OTA in 123 commercial wines with the following median concentrations; white, <3 ng/L; rosé, 19 ng/L; red, 13 ng/L; dessert, 337 ng/L. They also found a North−South gradient among European wines. In the same year, Majerus and Otteneder (*28*) analyzed 144 wines and found that OTA was present in 34% of white wines (median = 0.07 mg/L; maximum = 1.2 mg/L) and 45% of red wines (median = 0.2 mg/L; maximum = 7.0 mg/L), concentrations that were several orders of magnitude higher than those reported by the first authors. In this and in a subsequent paper (*42*), they confirmed that

Table 5. Comparison of Analytical Characteristics of Present Methods and of Previously Published Methods Utilizing Liquid Chromatography for Alcoholic Beverages

technique[a]	matrix	LOD	recovery (%)	CV (%)	reference
SPE-HPLC-fluor	wine	0.01 μg/L	66−93		Ospital et al. (*29*)
IAC-HPLC-fluor	beer	1 ng/L	87	0.8−3.7	Nakajima et al. (*33*)
IAC-HPLC-fluor	wine	0.01 μg/L	88−103	0.2−9.7	Visconti et al. (*36*)
IAC-HPLC-fluor	beer	0.01 μg/L	94−100	3.3−5.7	Visconti et al. (*34*)
SPE-PC-HPLC-fluor	wine		>80	<10	Jornet et al. (*45*)
SE-SPE-HPLC-fluor	wine	0.02 μg/L	87−107	8.9	Festas et al. (*31*)
SPE-HPLC-PDA	wine and beer	0.05 μg/L	83−94	4.0−8.9	this work
SE-DER-GC-MSD	wine and beer	0.1 μg/L	69−75	9.0−11.1	this work

[a] Abbreviations: IAC, immunoaffinity column; fluor, fluorescence; SE, solvent extraction; SPE, solid-phase extraction; PC, preconcentration; DER, derivatization.

OTA concentrations were higher in wines from southern European than from northern European countries.

Ospital et al. (*29*) analyzed a total of 29 wines, with the ranges of OTA in white, rosé, and red wines being 0.01−0.02, 0.01−0.11, and 0.01−0.027 ng/L, respectively. In a survey of 194 wines from Spain and 73 from other European countries, Burdaspal and Legarda (*30*) detected OTA in 65.2% of the white wines (mean = 0.020 ng/L), 90.6% of the rosé wines (mean = 0.031 ng/L), and 92.3% of the red wines (mean = 0.054 ng/L) analyzed. Visconti et al. (*36*) reported much higher concentrations of OTA in Italian wines, 40 of which were commercial and 15 homemade. As many as 11% of white, 75% of rosé, and 95% of red wines had concentrations >0.2 μg/L. By contrast, Festas et al. (*31*) were unable to detect OTA (LOD = 0.02 μg/L) in 64 Portuguese wines.

The remarkable divergence of results evident from the above surveys is puzzling and emphasizes that we simply have no idea what concentrations of OTA are present in wine produced for public consumption. Differences in methodology seem to be unlikely, because IAC extraction followed by HPLC was the dominant technique employed in these investigations. By virtue of its scope and scale, the present study merits serious attention in any debate surrounding the issue of what limits (MAL) should be set for OTA in wine. From the viewpoints of both the percentage of wines containing detectable amounts and the actual concentrations of OTA, our results fall approximately midway between the extremes described above; we could find no evidence of a North−South gradient in OTA concentrations. Low levels are quite common; high concentrations are very rare, with only 2 of the 942 samples analyzed lying between 0.1 and 0.2 μg/L. Using the principle of Good Manufacturing Practice that we have previously applied to the contamination of wines by pesticides (*43*, *44*), an MAL for OTA of 0.2 μg/L is easily achievable and is unlikely to represent a long-term health hazard on the basis of the very large body of experimental observations presently recorded.

The OTA status of commercial beers is also subject to considerable confusion. Of 35 German beers tested, OTA ranged from 0.1 to 0.2 ng/L in 9 (*32*). OTA was detected in 95.5% of Japanese beers and in 91.5% of non-Japanese beers at mean concentrations of 12.5 and 10.1 ng/L, respectively (*33*). Of 61 beers analyzed by Visconti et al. (*34*), 50% were positive for OTA, with a mean value of 0.035 μg/L (range = 0.010−0.135 μg/L). No differences were observed between pale and dark beers. Our results are consistent with those of the last-mentioned group, taking into account the fact that their LOD was 0.01 μg/L, and support the MAL of 0.2 μg/L for OTA in beer set by the government of Italy (*34*).

Conclusion. The simplicity of sample preparation, low cost, and acceptable recovery combined with the unique specificity of the PDA detector make the HPLC-PDA method suitable for routine detection and assay of OTA in red and white wines. On the basis of its performance characteristics as described above, this method can quantitate the anticipated European Community MAL for OTA in alcoholic beverages of 0.5 μg/L. The GC-MSD method, because of its excellent specificity combined with nonideal sensitivity and recovery, is best used as a confirmation tool.

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