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Short communication

Correlation between different clean-up methods and analytical techniques performances to detect Ochratoxin A in wine

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ABSTRACT

Three different clean-up methods and two analytical techniques were compared to determine Ochratoxin A (OTA) in wines. The first clean-up used a MycoSep column, the second an immunoaffinity column (IAC) and the third consisted in a liquid–liquid extraction (LLE) using dichloromethane in acid conditions. Meanwhile, two different OTA determination techniques were also evaluated: a HPLC analysis using a fluorescence detector and an enzyme-linked immunosorbent assays (ELISA) method.

Correlations between clean-up methods and analytical techniques to determine OTA in wine were made evaluating linearity, accuracy and precision.

Both the two first clean-up methods (solid-phase extraction, SPE) showed a good linear fit (r^2 = about 0.9999), followed by LLE. The use of immunoaffinity columns showed the best recoveries, even if also the SPE with MycoSep showed good recoveries while the LLE recoveries were the worst ones. The HPLC analysis showed good precision and accuracy, while ELISA method, even with a sufficient linearity, generally underestimated OTA content in wines.

1. Introduction

Ochratoxin A (OTA) consists of a chlorine-containing dihydroisocumarin linked through the 7-carbonyl group to 1-β-phenylalanine. OTA was discovered in 1965 as a secondary metabolite of *Aspergillus ochraceus* strains [1]. In the following years, several other *Aspergillus* and *Penicillium* species were described as producers of this toxin [2]. *A. ochraceus* and *Penicillium verrucosum* are considered the main OTA-producing species [3].

Within *Aspergillus* section *Nigri* group, *A. carbonarius* was predominantly responsible for the production of OTA in grapes and wine [4–6]. After cereals, wine is considered a major source of daily OTA intake.

OTA is receiving increasing attention worldwide because of the hazard for human and animal health. In 1993, the International Agency for Research on Cancer (IARC) classified OTA into group 2B as a possible human carcinogenic substance [7].

Maximum level for OTA in dried vine fruits (raisin, currants and sultanas) is 10 μg/kg according to Commission Regulation (EC) No. 1881/2006 (Official Journal of the European Union, 2006) [8]. In the European Union, currently maximum permitted levels of 2 μg L^{-1} have been established for OTA in wines and grape must based drinks (Official Journal of the European Union, 2005) [9].

The basic steps of OTA analysis include sampling, extraction of the toxin from the matrix, purification of the extract (clean-up) and concentration, separation, detection, quantification and confirmation of positive findings. Clean-up and concentration are usually necessary when low detection limits are required [10]. Clean-up can be carried out by liquid–liquid partitioning using aqueous Na-bicarbonate or by solid-phase extraction (SPE) [11], but sometimes the cleaning effect is not suitable for the complexity of the matrices.

Ospital et al. [12] obtained satisfactory results in term of recovery and sensitivity, operating a sample clean-up with silica gel SPE cartridges. Among recent improvements the application of a molecularly imprinted SPE method is notable [13]. One of the main advantages is that the polymer can be reused instead of immunoaffinity columns (IACs). Saez et al. [14] developed a polyethylene glycol based extraction method which is relatively simple, rapid and does not require the use of organic solvents, while Gonzalez-Penas et al. [15] optimised a micro-extraction method which was suggested to be an inexpensive alternative to immunoaffinity columns.

Monoclonal antibody based immunoaffinity columns were developed to substitute the traditional solvent clean-up [16]. The main advantage of these columns is that OTA is bound specifically to the antibody and the matrix interferences can be removed

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nearly completely. Furthermore, IACs give an optimal performance in terms of precision and accuracy within a wide range of concentrations and they also reduce the use of dangerous solvents [17]. Nowadays different types of immunoaffinity columns are commercially available for the analysis of OTA: OchraTest (Vicam, USA), Ochraprep (Rhone-Diagnostic Technologies, UK), RIDA Ochratoxin (R-Biopharm, Germany) and OchraStar™ Immunoaffinity Columns (Romer Labs Diagnostic GmbH, Austria). Castellari et al. [18] compared three immunoaffinity clean-up procedures, two using the direct application of wine on different types of IACs and the other using a previous chloroform extraction before clean-up on IACs according to the method proposed by Zimmerli and Dick [19]. All of these procedures gave comparable results in terms of recovery and precision, limit of detection and quantification for OTA in wine. Also repeatability was improved and the time of analysis was reduced if compared with the reference procedure involving a preliminary extraction of OTA with chloroform.

More recently Siantar et al. [20] compared the performance of IA and SPE columns discovering that IACs give higher recoveries compared to C18 or cross-linked polymer-based SPE columns.

The detection and quantification of OTA can be carried out by conventional reversed-phase HPLC or enzyme-linked immunosorbent assays (ELISA), obtaining good and bad recoveries and detection limits depending on sample matrix complexity. Chromatographic separation has been normally performed using RP-C18 columns and isocratic elution with diluted acidified acetonitrile [11] while the analysis includes HPLC with fluorescence detection [12]. Further improvements as Brera et al. [21] developed an automated HPLC method for OTA determination in wines, while Dall'Asta et al. [22] developed a simple reversed-phase HPLC technique which can be applied directly to wine samples without extraction or clean-up.

Leitner et al. [23] compared different analytical methods for OTA determination in wine and found that SPE combined with HPLC–tandem mass spectrometric (MS–MS) detection and immunoaffinity clean-up combined with HPLC–fluorescent detection offered good comparable results. A stable isotope dilution assay using HPLC–MS–MS has also been developed recently; this technique is relatively expensive but provides excellent accuracy [24,25]. Moreover, chromatographic techniques and immunochemical methods have also been developed for rapid screening of OTA in different food commodities [26–28]. The combination of IACs and ELISA detection of OTA in wines was found to be effective and in compliance with the 2 $\mu g\,L^{-1}$ allowable maximum level established by the European Union. In a inter-laboratory survey, ELISA was successfully used to determine OTA content in wines finding a comparable amount to that one obtained by using HPLC [29].

Currently, the method recommended for OTA determination in wines and beer (European Standard prEN 14133) uses IACs columns to clean-up OTA after dilution of the samples in an aqueous solution of polyethylene glycol and $NaHCO_3$ and the samples are analyzed by HPLC with fluorescent detection [17].

The aim of this study was a comparative evaluation of three clean-up and two different analytical methods for the determination of OTA in wine.

2. Material and methods

2.1. Samples

Thirty-seven samples of Montepulciano d'Abruzzo red wines were analyzed. These wines had different OTA content: 10 wines did not contain OTA while the others resulted from spiking in triplicate the same wine not containing OTA, with different concentration of OTA (1.11, 1.31, 1.51, 1.81, 2.01, 3.01, 4.02, 6.03, 8.04 $\mu g\,L^{-1}$). The choice of the number and the range of the spiking levels was made taking into account to have more data around the legal limit. Each sample was analyzed in order to compare the different types of clean-up and analytical technique.

2.2. Apparatus

LC system: PU-980 pump (Jasco International, Tokyo, Japan) connected to FP-1520 fluorescence detector (Jasco International, Tokyo, Japan). Sample injection was made with a 7725 valve (Rheodyne, Cotati, CA, USA) equipped with a 100 μL loop.

Chromatographic column: Inertsil RP-ODS-2 (GL Science, Tokyo, Japan) column (250 mm × 4.0 μm I.D., 5 μm) was used. The column was protected by an inline C18 Security Guard (4.0 × 3 mm I.D., 5 μm) cartridge system (Phenomenex, Torrance, CA, USA). The column was kept at 35 °C using a heater 7980 (Jones Chromatography, Hengeod, UK).

Data collection system: Chromatographic data acquisition and handling were made with the Borwin 1.5 software (JMBS Developments, Grenoble, France).

2.3. Chemicals and materials

OTA standard: A stock solution of OTA (1000 $\mu g\,L^{-1}$) dissolved in benzene–acetic acid (99:1, v/v) was furnished by Rhone Diagnostic Technologies (Glasgow, UK). The purity of this standard was checked by UV spectrophotometer at 333 nm in benzene–acetic acid (99:1), considering a molar adsorption coefficient (ε) of 5550 $M^{-1}\,cm^{-1}$.

OTA standard solutions: The working standard solutions (ranging from 0.05 to 20 $\mu g\,L^{-1}$) were prepared by evaporating under nitrogen the stock solution and dissolving the residue in an appropriate volume of mobile phase.

Mobile phase: Water–acetonitrile–acetic acid (49.5/49.5/1, v/v/v). Acetonitrile, methanol, water and acetic acid were furnished by Merck (Darmstadt, Germany). The mobile phase was daily prepared and filtered (0.22 μm) before use.

Extraction cartridges: OchraStar™ immunoaffinity columns were taken from Romer Labs Diagnostic GmbH (Austria) while MycoSep® 229 Columns were taken from Tecna S.r.l., Trieste, Italy.

Kit I'screen OCHRA ELISA: A quantitative immunoassay for the detection of OTA was obtained from Tecna S.r.l., Trieste, Italy.

Phosphate-buffered saline (PBS): The buffered saline solution was prepared as detailed by Castellari et al. [18], using potassium chloride, potassium dihydrogenphosphate anhydrous disodium hydrogenphosphate and sodium chloride in distilled water. The pH of PBS was adjusted to 7.4. All buffer salts were purchased from Carlo Erba (Milan, Italy).

Water: Purified distilled, deionized water produced by a Milli-Q purification system (Millipore, Bedford, MA) whose resistivity was 18.2 MΩ cm at 25 °C.

Sodium hydroxide 2 M and ammonium acetate solution 0.2 M were prepared in distilled water (Merck, Darmstadt, Germany). Methanol/acetic acid 98/2 (v/v) (Merck, Darmstadt, Germany). Chloridric acid 1 M – Carlo Erba (Milan, Italy). Dichloromethane – Merck (Darmstadt, Germany). Sodium bicarbonate solution 0.13 M was prepared in distilled water (Merck, Darmstadt, Germany). Glacial acetic acid – Merck (Darmstadt, Germany). Acetonitrile – Merck (Darmstadt, Germany).

2.4. OTA extraction and clean-up

Extraction and sample clean-up were performed using three different methods. These were evaluated for linearity and repeatability.

The first method used was a solid-phase extraction. 5 mL of wine were mixed with 0.2 mL of glacial acetic acid and 15 mL of acetonitrile; the solution was handily shaken for 30 s and 5 mL of this solution were transferred into a glass tube; forcing the extract to be filtered upwards through the packing material of the column (MycoSep). The interferences were adhered to the chemical packing in the column and the purified extract passed through the membrane (about 1 mL of filtered extract); 400 μL of purified extract were transferred into a vial; the sample was evaporated under nitrogen at 65 °C; once completely dry, the sample was redissolved in 200 μL of sodium bicarbonate. The dilution factor was 2. Further dilutions were carried out to obtain enough volume for HPLC analysis.

The second method used a direct clean-up on OchraStar™ immunoaffinity columns. Each immunoaffinity column was at first washed with 5 mL of PBS at pH 7.4 before use. Then 10 mL of wine adjusted to pH 7.8 using 2 M sodium hydroxide were diluted with 10 mL of PBS. 4 mL of this solution were applied directly to the IAC, at flow-rate of about 1–2 drops s^{-1}. After the diluted extract had completely passed through, the column was washed with 10 mL of PBS at flow-rate of 3–4 drops s^{-1}. Column was successively washed with 2 × 10 mL of 0.2 M ammonium acetate solution at flow-rate of 3–4 drops s^{-1}. Any liquid left was removed from the column applying a slight negative pressure from below meanwhile avoiding column drying. The next step was the collection of OTA elute. For the elution of OTA, 2 mL of methanol/acetic acid 98/2 (v/v) solution were applied to the column in several small portions. The methanol was left on the column for a short period of time before letting it run off. Then the column was dried under a gentle stream of air. The elute containing OTA was collected and mixed with 2 mL of mobile phase before HPLC analysis.

The third method consisted in a liquid–liquid clean-up (LLE). 5 mL of chloridric acid 1 M were added to 5 mL of the wine sample and 10 mL of dichloromethane were also added. The solution was shacked for 15 min on a low speed shaker (400 rpm) and centrifuged at 2200 × *g*. Two phases resulted: organic (bottom) and aqueous (up) phase. 5 mL of the organic phase were taken and 2.5 mL of the sodium bicarbonate solution (0.13 M) were added; the solution was shacked 15 min with a low speed shaker (400 rpm) and finally centrifuged at 2200 × *g*. The aqueous phase was taken and to separate it from any residual organic phase was centrifuged again for 15 min at 2200 × *g*. The aqueous phase was diluted 1/1 (v/v) with the sodium bicarbonate solution. The dilution factor was 2. Further dilutions were carried out to obtain enough volume for HPLC analysis.

2.5. Liquid chromatography

The extracts were analyzed by a reversed-phase HPLC using water–acetonitrile–acetic acid (49.5/49.5/1, v/v/v) as mobile phase and an isocratic mode at 0.75 mL min^{-1}. Detection was made working at an excitation wavelength of 333 nm and an emission wavelength of 460 nm.

For the quantitative analysis a calibration curve was created by injecting seven solutions containing known amounts of the pure standard ranging from 0.05 to 20 μg L^{-1} of OTA.

2.6. Kit I'screen OCHRA ELISA

The assay was performed in polystyrene micro-wells which had been coated with antibodies (IgG) and anti-IgG of rabbit. OTA standard solution or sample, the enzyme conjugate Ochratoxin-HRP and the specific antibody anti-Ochratoxin A were added to the micro-wells. During the incubation, free Ochratoxin-A molecules and Ochratoxin-HRP competed for the anti-Ochratoxin antibodies binding sites. The anti-Ochratoxin antibodies were simultaneously bound to the solid phase. Any unbound enzyme Ochratoxin-HRP was then removed in a washing step. The bound enzyme (HRP) activity was determined by adding a fixed amount of a chromogenic substrate: the enzyme converted the colorless chromogen into a blue product and the addition of the stop reagent led to a color change from blue to yellow. The absorbance was measured by a microplate reader at 450 nm. The color development was inversely proportional to the OTA concentration in the sample. The detection limit of the Kit I'screen OCHRA in wine and grapes was 0.1 ppb [30]. The calculation of results was based on the calculation of the mean absorbance of blank, standards and samples. The mean absorbance value of each standard and sample, subtracted of the mean absorbance value for the blank, was divided by the mean absorbance of maximum binding (B_0) and multiplied by 100. The maximum binding was thus made equal to 100% and the absorbance values was quoted in percentage. Actually data were processed using a software provided by the producer of the Kit I'screen OCHRA.

$$\frac{\text{Absorbance of standard (or sample)}}{\text{absorbance of maximum binding}} \times 100 = \frac{B}{B_0}\,(\%)$$

The B/B_0 (%) values calculated for each standard against the OTA standards concentration were entered in a semi-logarithmic system of coordinates and the curve was marked. The B/B_0 value was interpolated for each sample to the corresponding concentration on the calibration curve. The concentration of OTA in the samples was obtained from the calibration curve multiplied by the dilution factor, that for wine was 2.

The ELISA analysis was performed only on samples previously cleaned up with LLE and SPE (MycoSep) to limit the variability linked with the use of a kit from a different lot, considering that one kit let only 90 determinations. LLE is traditionally used in the ELISA determination [31] while between the two SPE clean-up methods here evaluated, MycoSep extraction was considered to be the more interesting choice than immunoaffinity extraction, because of its lower costs and good selectivity. Moreover, other authors [29] had previously considered the immunoaffinity clean-up before ELISA and HPLC analysis.

2.7. Statistical parameters

The limits of detection (LODs) were calculated using the following relation:

$$\text{LOD} = [Y_{\text{bl}} + (K \times S_{\text{bl}})]\frac{1}{b}.$$

Being Y_{bl} (area of the blank) and b the respective intercept and the slope of a curve made by analyzing samples of wines. K is a factor

Table 1
Regression equation and correlation coefficients of the methods investigated.

Clean-up	Quantification	Regression equation	r^2
SPE (MycoSep)	HPLC	$74314x + 812.37$	0.9997
SPE (IAC)	HPLC	$72621x + 772.08$	0.9999
LLE	HPLC	$77198x + 1051$	0.9964
SPE (MycoSep)	ELISA	$0.1383 \times \exp(1.1864 \times \exp(-1.9932x))$	0.9565
LLE	ELISA	$0.0739 \times \exp(2.1872 \times \exp(-4.0474x))$	0.9906

Table 2
Comparison of different clean-up and determination methods in terms of accuracy and precision.

	Spiked concn ($\mu g\,L^{-1}$)	Mean recovery (%)	Standard deviation	RSD (%)
SPE (MycoSep) HPLC	1.11	84	3.06	3.6
	1.31	94	3.51	3.6
	1.51	87	4.30	5.0
	1.81	98	6.66	6.9
	2.01	109	3.73	3.4
	3.01	112	9.77	8.7
	4.02	107	2.26	2.1
	6.03	112	5.44	4.8
	8.04	126	13.79	10.9
SPE (IAC) HPLC	1.11	96	3.69	3.9
	1.31	88	2.82	3.2
	1.51	88	5.30	6.0
	1.81	97	1.71	1.8
	2.01	95	6.52	6.8
	3.01	103	7.72	7.5
	4.02	107	10.52	9.9
	6.03	98	9.00	9.2
	8.04	115	13.21	11.4
LLE HPLC	1.11	82	0.51	0.6
	1.31	66	1.98	3.0
	1.51	64	4.90	7.7
	1.81	65	3.37	5.2
	2.01	70	1.94	2.8
	3.01	74	6.21	8.4
	4.02	86	1.69	2.0
	6.03	82	1.69	2.1
	8.04	96	14.70	15.3
SPE (MycoSep) ELISA	1.11	96	6.40	6.6
	1.31	70	3.13	4.5
	1.51	66	7.09	10.7
	1.81	60	6.23	10.6
	2.01	72	1.91	2.6
	3.01	67	8.36	12.5
	4.02	68	10.50	15.4
	6.03	80	12.16	15.2
	8.04	72	21.71	30.2
LLE ELISA	1.11	82	8.47	10.3
	1.31	94	9.16	9.7
	1.51	77	9.77	12.8
	1.81	78	2.82	3.6
	2.01	89	8.61	9.7
	3.01	72	1.17	1.6
	4.02	75	5.99	8.0
	6.03	54	0.62	1.1
	8.04	52	2.84	5.4

Table 3
Regression equation and correlation coefficients of the three clean-up analyzed by HPLC.

Clean-up method	Regression equation	r^2
SPE (IAC) vs SPE (MycoSep)	$1.1067x - 0.1045$	0.9897
SPE (IAC) vs LLE	$0.8485x - 0.2033$	0.9901
SPE (MycoSep) vs LLE	$0.7632x - 0.1120$	0.9914

of 3. S_{bl} (standard deviation of the blank) is the intercept of the curve obtained, representing the standard deviations for each concentration level versus the concentration. The recovery has been determined by comparing the peak area of OTA obtained from the wine spiked samples and from the calibration standards [32]. The precision of a method is calculated as the standard deviation from a series of replicates. Here, the overall precision was estimated from the calibration graph as the standard deviation of differences between predicted and experimental responses for all standards and indicated as s_R. The accuracy measures how different is the calculated concentration from the actual value and gives the sum of systematic and random error in the prediction of the concentration of unknown samples. In this context, the accuracy indicates the ability of an analytical method for estimating the actual concentration of the samples. To measure the accuracy of the method, the error in the prediction of the analyte concentration in validation samples is commonly used.

Table 4
Regression equation and correlation coefficients of the two clean-up analyzed by HPLC and ELISA quantification methods.

Determination methods	Regression equation	r^2
HPLC vs ELISA (SPE)	$0.5624x + 0.2838$	0.8676
HPLC vs ELISA (LLE)	$0.4551x + 0.9336$	0.8994
ELISA (SPE vs LLE)	$0.5700x + 0.8070$	0.8754

3. Results and discussion

3.1. Linearity

Linearity of response (peak area versus the injected analyte amount) was obtained by injecting in HPLC different concentrations of analyte ranging from 1.11 to 8.04 $\mu g\,L^{-1}$ and measuring fluorescence signal as previously reported. Only SPE (MycoSep) and LLE extracted samples were also analyzed by ELISA method. Linearity of response was determined using the difference of absorbance

Table 5
Some analytical figures of merit for clean-up and detection methods.

Clean-up methods	Detection methods	LODs ($\mu g\,L^{-1}$)	Precision (s_R) ($\mu g\,L^{-1}$)	Accuracy (overall prediction error) (%)
SPE (immunoaffinity)	Fluorimeter	0.0106	0.48697	13.233
SPE (MycoSep)	Fluorimeter	0.0109	0.74723	20.306
LLE (chloridric acid and dichloromethane)	Fluorimeter	0.0136	0.68927	18.7311
SPE (MycoSep)	ELISA	0.1000	1.11273	30.2386
LLE (chloridric acid and dichloromethane)	ELISA	0.1000	1.51491	41.1678

versus concentration in the case of LLE samples analyzed by ELISA method.

To construct a regression curve and calculate the correlation coefficient, measurements were done in triplicate at each concentration (Table 1).

The immunoaffinity clean-up showed the best linear fit ($r^2 = 0.9999$), followed by SPE (MycoSep) ($r^2 = 0.9997$) and LLE. ELISA method showed very bad results with a linear regression; therefore after several attempts a Gompertz equation was used to obtain a quite good fitting of data with SPE clean-up ($r^2 = 0.9565$) and good fitting of data with LLE ($r^2 = 0.9906$).

3.2. Recoveries and precision

The best recoveries were obtained with IACs (88–115%) both at high and low concentrations of OTA even if also SPE (MycoSep) showed very good recoveries (84–126%), while LLE obtained lower recoveries (64–96%), especially for OTA concentration near the legal limit (Table 2), as referred in other works [15].

In HPLC analysis, the precision was good resulting less than 10% in all the three clean-up methods apart from samples spiked with the highest amount of OTA.

Using ELISA method the samples cleaned up with MycoSep and the ones extracted with LLE showed lower recoveries and precision (Table 2).

Particularly, LLE showed the best recoveries (72–94%) spiking samples with an amount of OTA around the legal limit while results became worse at maximum levels of Ochratoxin A. Using SPE MycoSep, similar recoveries were obtained (60–96%), even if the technique showed lower recoveries at lower concentrations. Precision is not so good because the variation coefficients (CV) go from 1.1 to 12.8% for LLE and from 2.6 to 30.2% for SPE MycoSep.

Considering the HPLC analysis, the three clean-up methods (Table 3) showed a good correlation. The two SPE clean-up gave comparable results while LLE underestimated OTA concentrations.

Comparing the samples cleaned up with two different methods of analysis, ELISA analysis (Table 4) gave lower results than HPLC one, apart from a quite good correlation between the two analytical methods.

Table 5 shows the comparison of analytical figures of merit among the different analytical methodologies to determine OTA used in this study.

The results above discussed proved that the different tested clean-up procedures are reliable at the same level while ELISA method gave a lower determination instead of what evidenced by other Authors [33]. Nevertheless, ELISA method could be used to carry out a screening of OTA contamination in wine samples, because of its lower costs and its easy application, while HPLC technique ensures more accuracy, higher precision and recoveries.

References

[1] K.J. Van der Merwe, P.S. Steyn, L. Fourie, D.B. Scott, J.J. Theron, Nature 205 (1965) 1112.
[2] J. Varga, E. Kevei, E. Rinyu, J. Teren, Z. Kozakiewicz, Appl. Environ. Microbiol. 62 (1996) 4461.
[3] J.I. Pitt, A.D. Hocking, Fungi and Food Spoilage, 2nd edition, Blackie Academic and Professional, London, 1997.
[4] M.R. Bragulat, M.L. Abarca, F.J. Cabanes, Int. J. Food Microbiol. 71 (2001) 139.
[5] P. Battilani, A. Pietri, Eur. J. Plant Pathol. 108 (2002) 639.
[6] P. Battilani, N. Magan, A. Logrieco, Int. J. Food Microbiol. 111 (2006) S2.
[7] IARC (International Agency for Research on Cancer), Issue 56, IARC Monographs on evaluation of carcinogenic risks to humans: some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins, Lyon, 1993, p. 489.
[8] Official Journal of the European Union, 20/12/2006, L364, 5.
[9] Official Journal of the European Union, 28/01/2005, L25, 3.
[10] I. Festas, P. Herbert, L. Santos, M. Cabral, P. Barros, A. Alves, Am. J. Enol. Viticult. 51 (2000) 150.
[11] H. Valenta, J. Chromatogr. A 815 (1998) 75.
[12] M. Ospital, J.M. Cazabeil, A.M. Betbeder, C. Tricard, E. Creppy, B. Medina, Rev. Franç. OEnol. 169 (1998) 16.
[13] N.M. Maier, G. Buttinger, S. Welhartizki, E. Gavioli, W. Lindner, J. Chromatogr. B 804 (2004) 103.
[14] J.M. Saez, A. Medina, J.V. Gimeno-Adelantado, R. Mateo, M. Jimenez, J. Chromatogr. A 1029 (2004) 125.
[15] E. Gonzalez-Penas, C. Leache, M. Viscarret, A. Perez de Obanos, C. Araguas, A. Lopez de Cerain, J. Chromatogr. A 1025 (2004) 163.
[16] M. Sharman, S. Macdonald, J. Gilbert, J. Chromatogr. A 603 (1992) 285.
[17] A. Visconti, M. Pascale, G. Centone, J. Chromatogr. A 864 (1999) 89.
[18] M. Castellari, S. Fabbri, A. Fabiani, A. Amati, S. Galassi, J. Chromatogr. A 888 (1999) 129.
[19] B. Zimmerli, R. Dick, J. Chromatogr. B 666 (1995) 85.
[20] D.P. Siantar, C.A. Halverson, C. Kirmiz, G.F. Peterson, N.R. Hill, S.M. Dugar, Am. J. Enol. Viticult. 54 (2003) 170.
[21] C. Brera, S. Grossi, B. De Santis, A. Miraglia, J. Liq. Chromatogr. Related Technol. 26 (2003) 119.
[22] C. Dall'Asta, G. Galaverna, A. Dossena, R. Marchelli, J. Chromatogr. A 1024 (2004) 275.
[23] A. Leitner, P. Zoller, A. Paolillo, J. Stroka, A. Papadopoulou-Bouraoui, S. Jaborek, Anal. Chim. Acta 453 (2002) 33.
[24] G.S. Shephard, A. Fabiani, S. Stockenstrom, N. Mshicileli, V. Sewram, J. Agric. Food Chem. 51 (2003) 1102.
[25] M. Lindenmeier, P. Schieberle, M. Rychlik, J. Chromatogr. A 1023 (2004) 57.
[26] I. Barna-Vetrò, I. Solti, J. Teren, A. Gyongyosi, E. Szabò, A. Wolfling, J. Agric. Food Chem. 44 (1996) 4071.
[27] F.Y. Yu, T.F. Chi, B.H. Liu, C.C. Su, J. Agric. Food Chem. 53 (2005) 6947.
[28] Z. Zheng, J. Hanneken, D. Houchins, R.S. King, P. Lee, J.L. Richard, Mycopathologia 159 (2005) 265.
[29] C.A.R. Da Rocha, C.S. de Souza, V. VITITECH America Latina, Santiago, Chile, 2005.
[30] C. Brera, L. Bentivoglio, V. Minardi, F. Debegnach, M. Paleologo Oriundi, Valutazione delle prestazioni di un kit ELISA. Rapporti ISTISAN 05/42, 2004, p. 298.
[31] N. Ramakrishna, J. Lacey, A.A. Candlish, J.E. Smith, L.A. Goodbrand, J. AOAC 73 (1990) 71.
[32] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Chichester, 1984.
[33] J.G. Wilkes, J.B. Sutherland, J. Chromatogr. B 717 (1998) 135.