Title: Coacervative extraction of Ochratoxin A in wines prior to liquid chromatography/fluorescence determination

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Abstract

Coacervates made up of reverse micelles of decanoic acid were assessed as a new strategy for the simplification of wine sample treatment in the determination of Ochratoxin A (OTA). Simultaneous extraction/concentration of this contaminant was based on both hydrophobic and hydrogen bond OTA:coacervate interactions. Parameters affecting extraction efficiency and concentration factors were studied. Concentration of decanoic acid and THF were the most influential parameters, being 0.5% of acid and 5% of THF the selected ones. The procedure was very robust, so that the extractions were not influenced by the pH and the nature or concentration of matrix components. OTA recoveries from different types of wines (white, rosé and red) ranged between 85 and 100% and the actual concentration factors varied from 105 to 125 for sample volumes of 15 mL. The detection limits for OTA, after liquid chromatography/fluorimetry (LC/FL) analysis of the coacervate (20 μL), were 4.5 ng L^{-1} in white and rosé wines and 15 ng L^{-1} in red wines, values which were far below the threshold limit established for OTA by EU directives (2.0 μg L^{-1}). No clean-up of the extracts was required for any of the samples analysed. The overall sample treatment took about 15-20 min and several samples could be simultaneously treated using conventional lab equipment. The precision of the method, expressed as relative standard deviation, was about 5%. The approach developed was successfully applied to the determination of OTA in different wine samples from the South of Spain. The concentrations found ranged between 0.015 and 0.091 μg L^{-1}.

Keywords: Ochratoxin A; Wine; Coacervate; Reverse micelles; Mycotoxins

1. Introduction

The control of food contaminants, like mycotoxins, has become an issue putting a strong demand for analytical methods that permit their rapid determination at the strict regulation limits established. Mycotoxins are toxic secondary metabolites produced at few amounts by various fungi growing in a wide variety of foods. Among them, ochratoxin A (OTA) is widespread in cereals and derived cereal products, dried fruits, grape-based beverages, coffee, etc. According to a 2002 report on the assessment of dietary intake of OTA by European people, wine resulted in one of the main dietary source (10-20%) [1]. Occurrence of OTA in wines depends on both enviromental and manufacturing conditions and is linked to the action of several fungi species, namely *Aspergillus* (96% of the cases) and *Penicillium* (4%) [2]. OTA is a nephrotoxic substance that causes non-reversible disturbances in kidneys and it has immunosuppressive and neurotoxic properties [3]. The International Agency for Research on Cancer (IARC) has considered it as a possible carcinogen to humans (Group 2B). Because of this, there is a growing need for monitoring OTA in food, including wine, for which the EU has set a maximum permitted level of 2.0 μg L^{-1} [4].

Analytical methods for the determination of OTA in wines are generally based in reverse-phase liquid chromatography coupled to fluorescence detection (LC/FL), although other detection methods, such as photodiode array (PDA) [5] or tandem mass spectrometry (MS/MS) [6,7], and other techniques (e.g. capillary electrophoresis with diode array detection [8]) have also been proposed. Due to the complexity of the matrices, sample preparation usually consists of several steps including clean-up, extraction and concentration.

The most frequently used concentration/clean-up technique is solid-phase extraction (SPE) [7,9,10,13]. Immunosorbents or immunoaffinity columns (IAC) [7,10,13], which offer high selectivity, have been the sorbents more extensively used. In fact, IAC is recommended by the Office International de la Vigne et du Vin (OIV) [11] and the Association of Official Analytical Chemists (AOAC) [12]. However there are important disadvantages associated to IAC; immunosorbents are not recyclable, have a limited storage time and, in some cases, show cross-reactivity with Ochratoxin C [13]. Recently, alternative clean-up/ concentration procedures have been reported, which include the use of molecular imprinted polymers (MIPs) [14,15], solid-phase microextraction (SPME) [16] and liquid-liquid microextraction with a porous hollow fiber (LPME) [17]. With regard to MIPs major disadvantages are the high cost and time to produce them. On the other hand, SPME presents several specific drawbacks, such as the high extraction time necessary (60 minutes) and the low robustness of the technique regarding the sample pH [16]. The application of LPME to OTA extraction also requires long extraction times (2 hours) to get concentration factors around 90 and the use of additional clean-up/concentration steps to get adequate selectivity/sensitivity, which stretch even more the analysis time [17].

Because of sample preparation is the bottleneck in wine OTA control and the main reason for low sample throughput, the introduction of new approaches to extract/concentrate this contaminant is desirable and this issue demands research. In this context, this paper investigates the potential of coacervates to simplify sample treatment for OTA determination in wine.

Coacervates constitute a valuable strategy to replace organic solvents in analytical extraction processes. According to the IUPAC [18], coacervation is defined as the separation into two liquid phases (coacervate and equilibrium solution) in colloidal systems. The phenomenon occurs under the action of a dehydrating agent, namely changes in the temperature or the pH of the colloidal solution, or the addition of an electrolyte or a non-solvent for the macromolecule. After separation, the coacervate, a very low volume liquid phase, contains most of the colloidal component. A number of macromolecules have been known to undergo coacervation (i.e. proteins, polyssacharides, drugs, etc [19]), however, the use of coacervates in analytical extractions has largely involved the use of surfactants at levels above their critical aggregation concentration [20-22]. To date, the coacervation of a variety of surfactant aggregates (i.e. non-ionic [23], zwitterionic [24], cationic [25] and anionic [26] aqueous micelles, reverse micelles [27] and vesicles [28]) has successfully been used for the extraction of pollutants from environmental samples [29-32]. A major benefit of the special structure of supramolecular-based coacervates is their high solvation properties for a variety of organic compounds which make them suitable to extract solutes in a wide polarity range.

In this paper, coacervates made up of decanoic acid reverse micelles [27] were assessed for the extraction/concentration of OTA prior to LC/FL determination. The aim was to develop a simple and rapid method for the routine control of this contaminant in wine. The selection of this coacervate was based on both its potential for extracting OTA efficiently and its low volume. Parameters affecting extraction efficiency and concentration factors were optimised and the applicability of the proposed method to the determination of OTA in different red, white and rosé wines from the South of Spain was assessed.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent-grade and were used as supplied. Decanoic acid was purchased from Fluka (Madrid, Spain). Tetrahydrofuran (THF), HPLC-grade acetonitrile, methanol and acetic acid glacial were supplied by Panreac (Sevilla, Spain). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Ochratoxin A (OTA) was obtained from Sigma (St. Louis, MO, USA). A stock standard solution of 10 mg L^{-1} of OTA was prepared in methanol and stored under dark conditions at -20ºC. Working solutions were prepared by dilution of the stock solution with

methanol.

2.2. Apparatus

The liquid chromatographic system used (Spectra System SCM1000, ThermoQuest, San Jose, CA, USA) consisted of a P2000 binary pump and a FL3000 fluorescence detector. In all experiments a PEEK Rheodyne 7125NS injection valve with a 20 µL sample loop was used (ThermoQuest, San Jose, CA, USA). The stationary-phase column was a Hypersil ODS C_8 (5 µm 150 x 4.6 mm) from Analisis Vinicos (Tomelloso, Spain). A Mixtasel Selecta centrifuge was employed for sample preparation.

2.3. Determination of OTA in wine samples

2.3.1. Sample collection and preservation.

Wine samples were purchased in supermarkets from Córdoba (Spain) and were stored at 4ºC until their analysis. Red wines were microfiltered through 0.45 µm nylon filter membranes (Análisis Vínicos S.L. Tomelloso, Spain) to remove suspended solids. All selected wines were manufactured from grapes which had grown in plantations from the South of Spain.

2.3.2. Coacervate-based extraction.

Decanoic acid (80 mg) was dissolved in THF (0.8 mL) in a specially designed glass tube with a narrow neck (tube: 30 mm high, 34 mm i.d.; neck; 70 mm high, ~7.5 mm i.d.). Then, 15 mL of wine sample (pH 3-3.5) were added. Immediately, the coacervate phase separated from the bulk solution. The mixture was stirred (5 min, 800 rpm) to favour OTA extraction and then centrifuged at 3500 rpm for 10 min to accelerate the complete separation of the coacervate phase. Then the volume of the coacervate, which was standing at the narrow neck of the glass tube, was measured with a digital calliper, and 20 µL were withdrawn with a microsyringe and injected into the LC-FL system.

2.3.3. Liquid chromatography/Fluorimetry

Quantification of OTA and separation from the matrix components was carried out by liquid chromatography-fluorimetry. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both containing 1% acetic acid. The elution program was: linear gradient from 60% to 50% in A for 15 min and then isocratic conditions (50% A and 50% B) for 20 min. The flow-rate was 1 mL min^{-1}. OTA was monitored at λ_{ex} 334 nm and λ_{em} 460 nm. Quantification was performed by measuring peak areas. Calibration curves for OTA in methanol were linear in the range 2-5000 µg L^{-1}.

3. Results and discussion

3.1. Decanoic acid reversed micelle coacervate-based extraction of OTA

3.1.1. Coacervate description

Decanoic acid dissolves in THF forming reverse micelles. The addition of water to this binary system causes partial desolvation of the micellar aggregates which makes easier micelle-micelle interaction. As a result, big supramolecular aggregates are formed that separate from the bulk solution as an immiscible liquid phase named coacervate [27]. So, water, a non-solvent for the decanoic acid, is the inductor agent of the coacervation. At a microscopic level, the structure of the coacervate consists of spherical droplets made up of a variable number of reverse micelles that are dispersed in the water:THF continuous phase. Because of reverse micelles are produced from the protonated decanoic acid form (pK_a 4.8±0.2), pH values below 4 are required for the formation of the coacervate.

Figure 1A shows the relative concentration of the three coacervate components at which coacervation is produced in the bulk solution. Beyond the boundaries of the coacervation region, the decanoic acid precipitates or solubilizes in the THF:water mixture. Supramolecular aggregates in the coacervate provide two types of interactions for extraction of solutes, namely van der Waals interactions in the decanoic acid hydrocarbon chain and hydrogen bonds in the micellar core, so a number of analytes can be extracted efficiently with this solvent. The volume of coacervate is a function of both, the decanoic acid concentration and the percentage of THF, and a mathematical expression has previously been derived that permits to know *a priori* the volume of coacervate that can be obtained under given experimental conditions [32].

3.1.2. Wine as an inductor agent of the coacervation

Wine samples have a high water content, 0.87 g mL^{-1} [33], so they were expected to induce the coacervation of reverse micelles of decanoic acid. Figure 1B shows a typical phase diagram obtained from ternary mixtures made up of decanoic acid:THF and wine. All the samples investigated, including white, rosé and red wines, behaved similarly. The phase diagrams always showed three regions, which were a function of the relative THF:wine percentage and corresponded to regions where the decanoic acid was precipitated, coacervated or solubilized. The boundaries in the phase diagrams moved towards lower THF percentages compared with those obtained in ternary mixtures containing distilled water (compare Figures 1 A and B). This behaviour was the result of the higher solubilization capability of the wine sample for decanoic acid owing to the presence of ethanol in its composition (11-13%, v/v, [33]). It was checked that

other major components of wines (proteins, 0.74 mg mL^{-1}; carbohydrates, 27.41 mg mL^{-1}; condensed tannins, 0.6471 and 0.0085 mg mL^{-1} for red and white wine, respectively) [33,34] did not have any influence on phase diagrams. Although the region encompassed by coacervates was slightly smaller in the presence of wine, this behaviour had not analytical consequences since the coacervating region was wide enough and extractions are usually carried out at coacervate compositions near the lower phase boundary in order to use the minimal amount of THF.

In red wine samples, a reddish precipitate, which was standing at the bottom of the coacervate as a very thin layer, was extracted. This precipitate was caused by the condensed tannins present in red wines, their content being especially high (0.6471 mg mL^{-1}) compared to that of white wines (0.0085 mg mL^{-1}). Condensed tannins have been reported to bond to proteins and form large colloidal particles [35]. These macromolecules were agglutinated by the reverse micelles and extracted by the coacervate, but they did not interfere in the recovery of OTA.

3.1.3. Optimisation

Optimisation studies were carried out by extracting red and white samples (10-40 mL) fortified with 1.5 μg L^{-1} of OTA under a variety of experimental conditions (0.1–3% decanoic acid; 1.5-30% THF; pH 0.5-~3.5; stirring time 0-60 min). Experiments were made in triplicate. Selection of the optimal conditions was based on the recoveries (R) and actual concentration factors (ACF) obtained for OTA. Phase volume ratios (PVR) were calculated as the ratio of sample volume over coacervate volume, so they represented the maximum concentration factors that could be obtained under given experimental conditions.

Ochratoxin A is a hydrophobic compound (its octanol-water partition coefficient; log K_{ow}, is 4.74) and, because of their pK_a values (4.4 for the acid group and 7.1 for the alcohol one), OTA is neutral at pH values below 4, at which the coacervate is produced. Consequently, the expected driving forces for the extraction were Van der Waals interactions between the hydrocarbon chains of the decanoic acid and the OTA aromatic framework, and hydrogen bonds, on account of the acceptor and donor groups of the analyte.

A. Phase volume ratios

The volume of coacervate obtained, and consequently the sample/coacervate volume ratio (PVR), was mainly dependent on the amount of decanoic acid and THF added to the wine samples on account of they are major components of the coacervate. Water did not incorporate to the extractant phase in a significant proportion due to its non-solvent character for the reverse micelles.

The volume of coacervate was linearly dependent on the amount of surfactant used (Figure 2A), independently of the type of wine or percentage of THF investigated. The corresponding equations for white and red wine (10% THF) were $y = 32 \pm 26 + 1.86 \pm 0.05x$ and $y = 38 \pm 38 + 1.86 \pm 0.07x$, respectively, where y was the volume of coacervate in μL and x the amount of decanoic acid in mg. The correlation coefficients (r^2) were 0.997 and 0.996. The relationship between the coacervate volume and the THF percentage was exponential (Figure 2B). The corresponding equations (decanoic acid = 200 mg) were $y = 240 \pm 8\ e^{0.045 \pm 0.002x}$ (r^2=0.98) and $y = 237 \pm 7\ e^{0.045 \pm 0.002x}$ (r^2=0.990) for white and red wine respectively, where *y* was the volume of the coacervate in μL and *x* the percentage of THF. The pH of wine samples (range 0.5-3.5) had only minor influence on the volume of the coacervate. This volume kept constant for pH values above 2 and then decreased for stronger acidic conditions in percentages of 5 and 27% from pH 2 to 1.75 and 0.5, respectively.

According to these results, the highest phase volume ratios will be obtained using low amounts of decanoic acid and THF and forming the coacervate under strong acidic conditions. So, recommended coacervate compositions for extraction are those near the lower boundary in the phase diagrams (Figure 1B) provided that they give good extraction efficiencies for OTA. On the other hand, strong acidic conditions are not recommended for OTA extraction on account of the acid hydrolysis it can undergo.

B. Recoveries and actual concentrations factors

Recoveries (R) for OTA were investigated using fortified red and white wines (1.5 μg L^{-1} of OTA) and the corresponding actual concentration factors (ACF) were calculated (0.01*R(%)*PVR) by measuring the respective coacervate volumes with a digital calliper.

Decanoic acid concentration was the most influential parameter on recoveries; they were higher than 85% at surfactant concentrations as low as 0.5% and significantly decreased at lower concentrations (Table 1). Although quantitative extractions were obtained for decanoic acid above 2%, a concentration of 0.5% was selected as optimal on the basis that it provided the best possible ACF for OTA at recovery values high enough for its reproducible quantification. In order to estimate the effect of wine matrix components on OTA recoveries, the extraction of OTA from distilled water as a function of decanoic acid concentration was investigated. The results obtained are included in Table 1. Matrix components decreased recoveries at the lowest decanoic acid concentrations studied (e.g. below 0.3%) and scarcely influenced them as the concentration increased, which reinforces the use of the selected concentration (i.e. 0.5%).

With regard to the influence of THF, maximal extraction efficiencies were obtained in the range 3-12%, beyond it, recoveries progressively decreased (Table 2). According to previous studies [27], decanoic acid incorporates progressively to the coacervate from the bulk solution at low THF concentrations, so the

low recovery obtained at 1.5% THF was in agreement with the fact that only a fraction of the surfactant was incorporated to the coacervate. On the other hand, the solubility of OTA in the bulk solution increased as the THF concentration did, which resulted in decreased partition coefficients for THF percentages above 15%. We selected 5% THF, which gave recoveries around 90-95% and actual concentration factors about 120.

The pH of wine samples did not affect recoveries in the range 2-3.5, but it caused a slightly decreased at lower pHs (e.g. recovery was 80% at pH 0.5). Because of most of wine samples have pHs around 3-3.5, it was not necessary to adjust them before extraction. Extraction equilibrium conditions were rapidly reached; maximal recoveries were achieved after 1 min of stirring the wine samples at 700 rpm.

The volume of wine sample to analyse was selected to get around 100 μL of coacervate per sample, which permitted 2-3 different chromatographic runs in a reliable way (20 μL each injection). So, a volume of wine sample of 15 mL was chosen, which provided volumes of coacervate around 120-130 μL.

3.2. Analytical performance

Calibration curves for OTA were run using standard solutions prepared in methanol. No differences in peak areas or retention times were observed for the analytes injected in organic solvent or coacervates. The correlation between peak areas and OTA concentration (2-5000 $\mu g\ L^{-1}$) was determined by linear regression and was 0.99998, indicating a very good fit. The slope of the calibration curve was 102.6±0.2 L μg^{-1} (n =7). The instrumental detection limit was calculated from blank determinations by using a signal-to-noise ratio of 3 and it was 0.6 $\mu g\ L^{-1}$. The detection limit of the method was calculated by analysing white, rosé and red wines under the proposed experimental conditions (section 2.3). Samples were selected to give OTA concentrations below the detection limit. The values of LOD found were 4.5 ng L^{-1} for white and rosé wines and 15 ng L^{-1} for the red ones, on account of the higher noise value in these more complex samples.

The possible interference of matrix components that could elute with OTA was assessed by comparison of the slopes of the calibration curves (n=7) obtained from standards in distilled water with those obtained from two red and two white wines, fortified with known amounts of OTA (0.03–0.1 $\mu g\ L^{-1}$), and run using the whole procedure. The slopes of the calibration curves were 14.8±0.8 ng L^{-1} in water and 10.4±0.5 and 11.2±0.7 ng L^{-1} in white and red wine, respectively. Differences in both types of calibration curves were only due to the different ACF reached in water (~145) and in wines (105-125), so matrix components were not expected to interfere in OTA determination, although the greater background noise produced in red wine matrices increased the detection limit three-fold.

The precision of the method was evaluated by the extraction of eleven independent fortified samples using red (n=4), white (n=4) and rosé (n=3) wines. The value, expressed as relative standard deviation (RSD), was about 5%.

3.3. Analysis of wine samples.

Red, white and rosé wines from different origin were analysed in order to prove the suitability of the proposed method for the routine control of OTA. Table 3 shows the concentrations found as well as the recoveries obtained after spiking the samples with variable amounts of this contaminant. Values for concentrations of OTA and for recoveries were expressed as the mean value of three independent determinations, besides their corresponding standard deviations. Recoveries ranged between 85 and 100%, 91 and 99% and 85 and 94% for red, white and rosé wine respectively, with relative standard deviations ranging from 1 to 7%.

OTA was quantified in four of the samples analysed (3 white wines and 1 rosé one), their concentration ranging between 0.015 and 0.091 $\mu g\ L^{-1}$. These concentrations were far below the threshold limit set for OTA in wines by EU directives (2.0 $\mu g\ L^{-1}$) [4].

Chromatograms obtained from (A) an OTA standard solution in methanol (7.5 $\mu g\ L^{-1}$), (B) a white wine sample naturally contaminated with OTA (0.063 $\mu g\ L^{-1}$), (C) a non-contaminated ([OTA] < L.O.D.) red wine sample and (D) a spiked (0.1 $\mu g\ L^{-1}$) red wine sample, are shown in Figure 3. No interference from matrix components was detected for any of the samples analysed.

Conclusions

Coacervates made up of reverse micelles of decanoic acid in THF have been proven to be a valuable tool for the extraction and concentration of OTA from wines (white, red and rosé), offering a simple, cheap and rapid alternative for the usually tedious and time-consuming sample treatment methods. The extraction process is robust (extractions were not significantly influenced by the pH and the nature or concentration of the matrix components), simple (sample pre-treatment only involved the microfiltration of red wines to remove suspended solids) and rapid (the complete extraction procedure took about 20 minutes and several samples were simultaneously extracted). It requires low volume sample (15 mL wine), features low cost (conventional equipment in labs is used for extraction) and achieves actual concentration factors around 105-125 for the target compound, which results in detection limits of 4.5 ng L^{-1} for white and rosé wines, and 15 ng L^{-1} for the red ones. Thus, the method developed can be used for the routine control of OTA in wines below the tolerance level permitted by the European Directives (2ug/L) [4].

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Figure captions

Figure 1. Diagrams of phase boundaries corresponding to the following ternary systems: (A) tetrahydrofuran-decanoic acid-water and (B) tetrahydrofuran-decanoic acid-wine sample.

Figure 2. Volume of coacervate (μL) as a function of: (A) the decanoic acid amount (10% THF) and (B) the tetrahydrofuran percentage (decanoic acid 200 mg), for both white and red wines.

Figure 3. LC/Fluorescence chromatograms obtained from (A) an OTA standard (7.5 μg L^{-1}) in methanol; (B) a white wine sample naturally contaminated with OTA (0.063 μg L^{-1}); (C) a non-contaminated ([OTA] < L.O.D.) red wine sample; and (D) a spiked (0.1 μg L^{-1}) red wine sample.

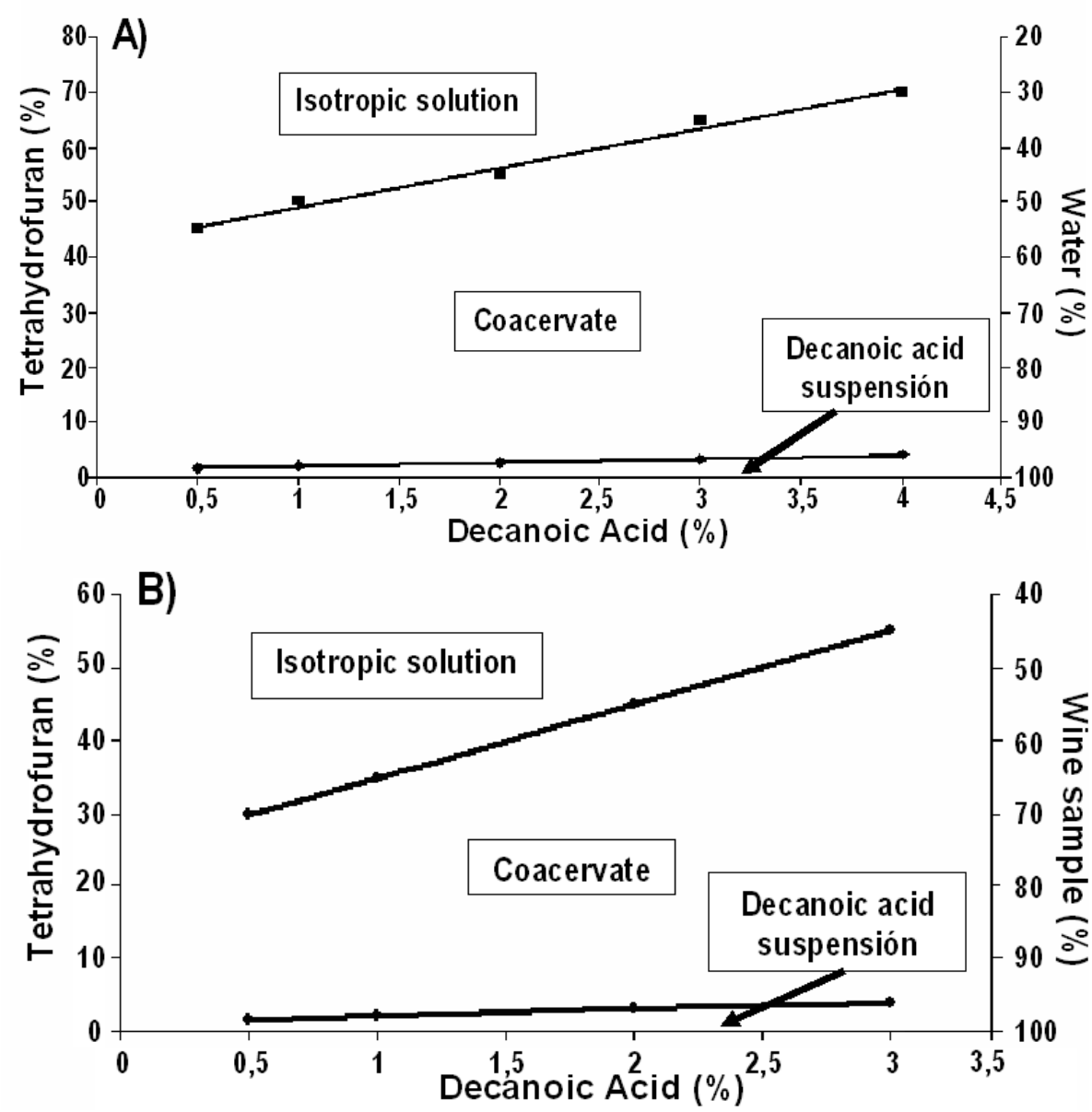
A)
Isotropic solution
Coacervate
Decanoic acid suspensión
Tetrahydrofuran (%)
Water (%)
Decanoic Acid (%)
B)
Isotropic solution
Coacervate
Decanoic acid suspensión
Tetrahydrofuran (%)
Wine sample (%)
Decanoic Acid (%)

Figure 1

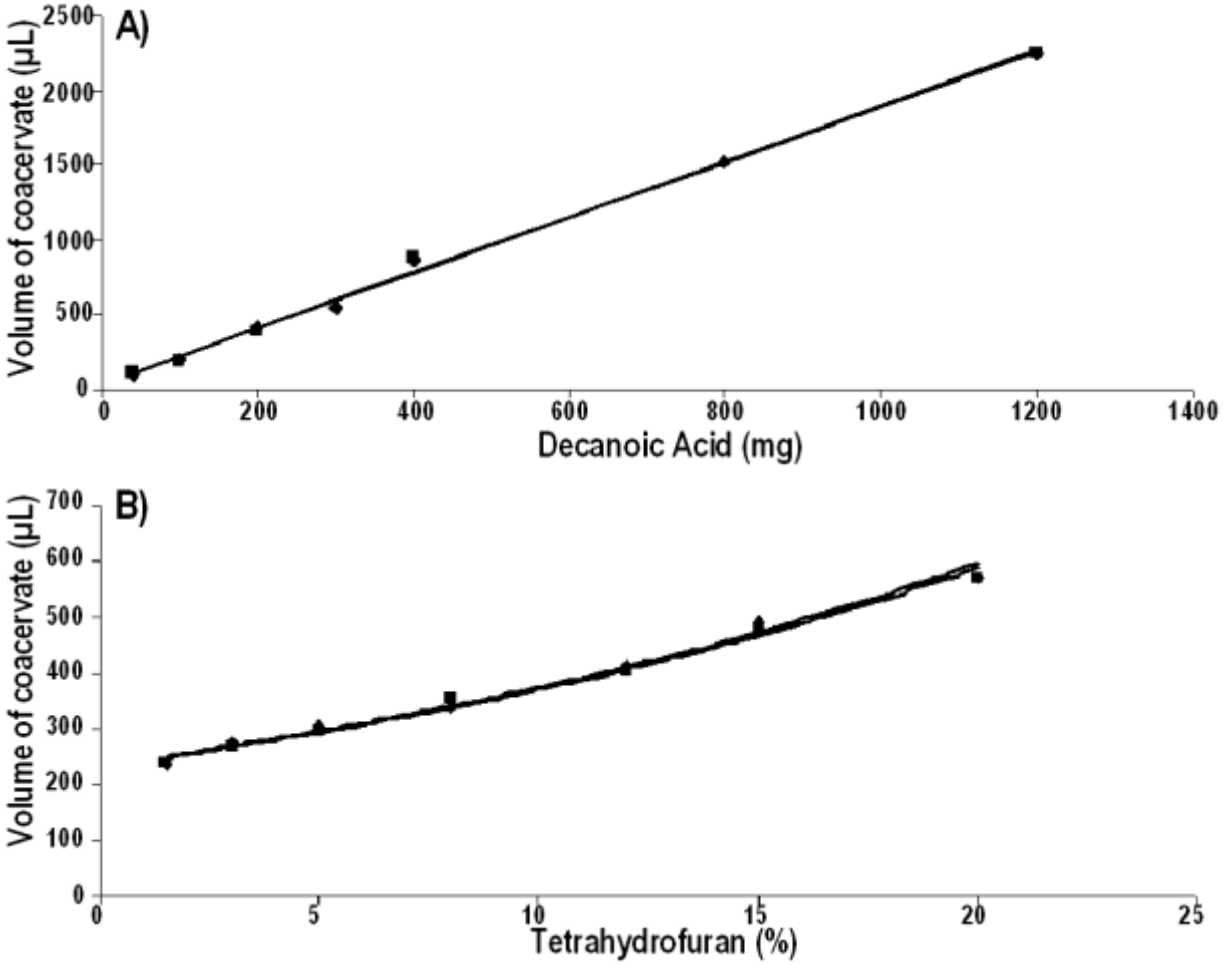
A)
Volume of coacervate (µL)
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
1400
Decanoic Acid (mg)
B)
Volume of coacervate (µL)
700
600
500
400
300
200
100
0
0
5
10
15
20
25
Tetrahydrofuran (%)

Figure 2

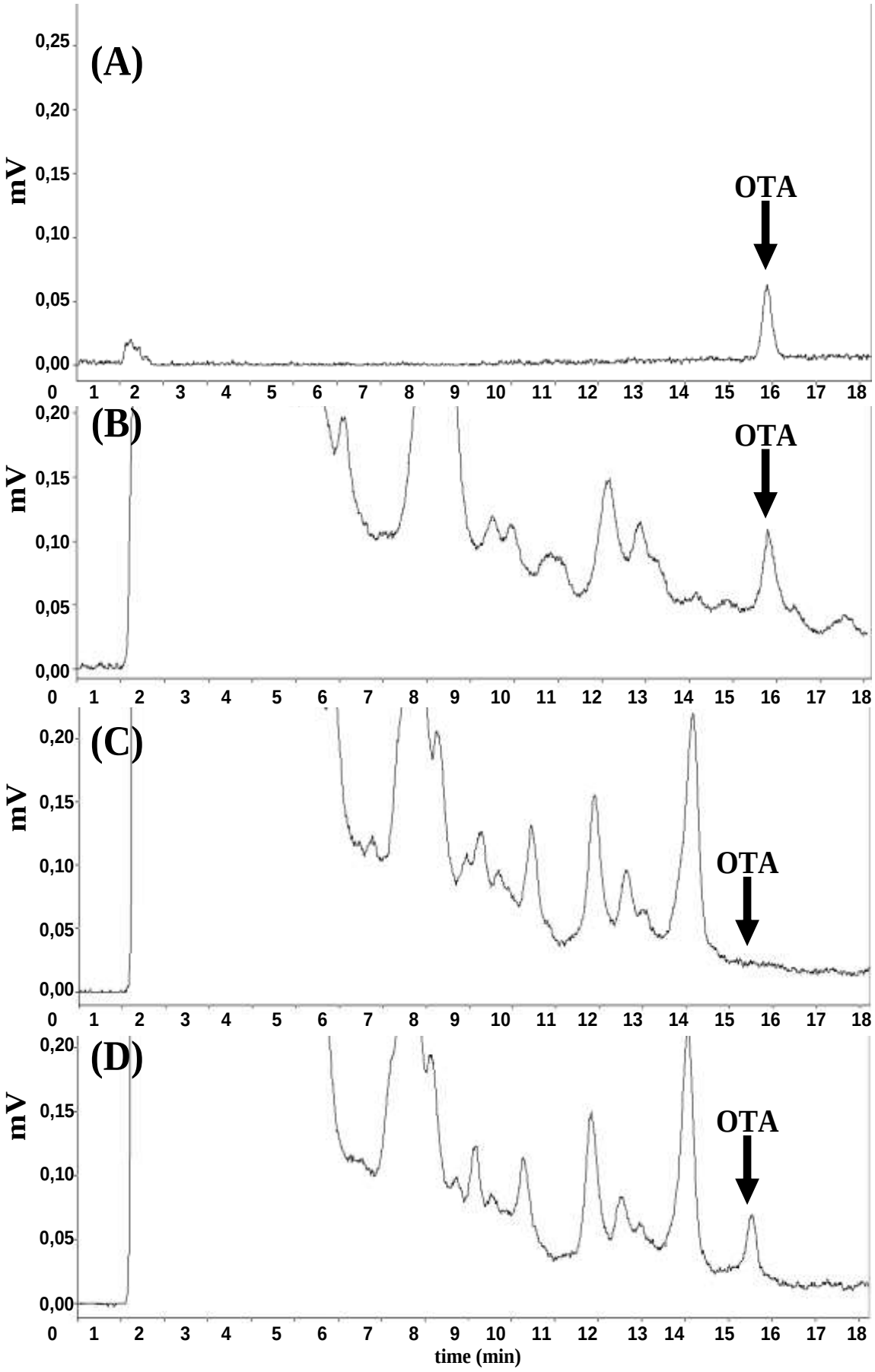
(A)
(B)
(C)
(D)
OTA
mV
0,25
0,20
0,15
0,10
0,05
0,00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
time (min)

Figure 3

Table 1. Mean percent recoveries and standard deviations and actual concentration factors obtained for Ochratoxin A in red and white wine and water using different decanoic acid concentrations

	Red Wine		White Wine		Water	
Decanoic acid (%)	[a]R ± [b]S (%)	[c]ACF	[a]R ± [b]S (%)	[c]ACF	[a]R ± [b]S (%)	[c]ACF
0.1	50±10	193	40±2	152	60±5	317
0.25	76±2	184	71±1	160	80±2	169
0.5	88±4	114	88±1	96	90±1	95
1	92±1	42	91±1	46	93±4	52
2	97±2	24	97±2	25	100±2	26
3	98±1	17	98±1	18	100±1	18

[a]recoveries
[b]standard deviation; n=3
[c]actual concentration factors; THF=10%

Table 2. Mean percent recoveries and standard deviations and actual concentration factors obtained for Ochratoxin A in red and white wine and water using different tetrahydrofuran percentages

	Red Wine		White Wine		Water	
THF (%)	[a]R ± [b]S (%)	[c]ACF	[a]R ± [b]S (%)	[c]ACF	[a]R ± [b]S (%)	[c]ACF
1.5	77±3	129	82±1	139	91±4	153
3	90±2	135	91±7	132	96±2	146
5	95±2	124	90±2	117	98±1	141
7.5	92±1	103	88±3	106	95±2	105
10	91±4	91	88±4	84	93±5	89
15	78±8	65	82±6	66	88±6	72
20	64±3	55	57±3	40	93±7	47
25	36±4	28	53±4	36	87±3	37
30	17±6	38	30±4	31	85±5	28

[a]recoveries
[b]standard deviation; n=3
[c]actual concentration factors; Decanoic acid=0.5%

Table 3. Mean concentrations and recoveries and the respective standard deviations obtained for OTA in the analysis of red, white and rosé wines using the proposed method

Concentrations ± [a]S (µg L^{-1}) and recoveries ± [a]S (%)					
Red Wines		White Wines		Rosé Wines	
Córdoba 1	<L.O.D.[i]	Montilla 1	<L.O.D.[i]	La Mancha 2	0.091±0.002
Spiked sample[b]	90±3	Spiked sample[e]	96±1	Spiked sample[b]	91±7
Córdoba 2	<L.O.D.[i]	Montilla 2	0.063±0.002	Valdepeñas 3	<L.O.D.[i]
Spiked sample[c]	89±4	Spiked sample[f]	96±3	Spiked sample[d]	85±4
Granada 1	<L.O.D.[i]	Montilla 3	0.016±0.002	La Mancha 3	<L.O.D.[i]
Spiked sample[d]	100±3	Spiked sample[g]	97±5	Spiked sample[b]	88±3
Valdepeñas 1	<L.O.D.[i]	Valdepeñas 2	<L.O.D.[i]	Valdepeñas 4	<L.O.Q.[j]
Spiked sample[b]	85±4	Spiked sample[g]	91±3	Spiked sample[b]	87±1
La Mancha 1	<L.O.Q.[j]	Huelva 1	0.015±0.001	La Mancha 5	<L.O.D.[i]
Spiked sample[b]	92±7	Spiked sample[h]	99±2	Spiked sample[b]	94±4

[a]Standard deviation; n=3

[b](0.1 µg L^{-1}) ; [c](0.0855 µg L^{-1}) ; [d](0.4 µg L^{-1}); [e](0.055 µg L^{-1}); [f](0.07 µg L^{-1}); [g](0.025 µg L^{-1}); [h](0.04 µg L^{-1})

[i](Lower than the detection limit); [j](Lower than the quantification limit)

Decanoic acid=0.5%; Tetrahydrofuran=5%; Sample volume=15 mL