Chromatographic Analysis of Water and Wine Samples for Phenolic Compounds Released from Food-Contact Epoxy Resins

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Abstract

Food-contact epoxy resins can release phenolic compounds such as phenol, *m*-cresol, bisphenol F, bisphenol A, 4-*tert*-butylphenol, bisphenol F diglycidyl ether (BFDGE), and bisphenol A diglycidyl ether (BADGE) into foodstuffs. A validated high-performance liquid chromatographic method with fluorometric detection is described for the simultaneous analysis of these compounds in wine and mineral water. Sample preparation by solid–liquid extraction enables detection limits of 2.5 µg/L in wine and 0.25 µg/L in mineral water to be achieved. Recovery rates are close to 100%, except for BFDGE and BADGE (around 60% in wine and 75% in mineral water).

Introduction

Certain types of epoxy resin are used to line the interior of wine storage vats and water towers to ensure water-tightness and to line the interior of drinking water pipes as part of their renovation.

Like all materials that are intended to come into contact with foodstuffs, epoxy resins are regulated. In the United States, the Food and Drug Administration has drawn up a list of substances that are permitted for use in coating formulation based on epoxy resin and has defined restrictions on their use (1). In Europe, such materials must meet the food-contact criteria set out in the European Framework Directives 89/109/CEE (2) and 90/128/CEE (3), indicating that they must not release into foodstuffs a quantity of constituents likely to present a hazard to human health or to lead to an unacceptable modification of the composition of the foodstuffs or their organoleptic character.

To prevent problems with foodstuff modification or toxic accidents, limits for the specific migration into the foodstuff or food-simulant as well as maximum residue limits in the material have been established for certain constituents of food-contact epoxy resins.

For many years, we have studied the migration of these resin constituents into simulants and foodstuffs such as water and wine (4). In 1987, we developed a high-performance liquid chromatographic (HPLC) method with fluorometric detection for the analysis of phenol (tolerable daily intake, 1.5 mg/kg [5]), which is a residue of bisphenol A (BPA) synthesis (a constituent monomer of these resins), in wine (6).

Other researchers (7–9) have used an identical HPLC method to analyze other phenolic constituents of epoxy resins in simulants or extracted polymer, such as bisphenol A diglycidyl ether (BADGE; specific migration limit, 3 mg/L [3]; considered a potent allergen) (10,11), bisphenol F diglycidyl ether (BFDGE), BPA (specific migration limit, 0.02 mg/L; maximum residue limit in the material, 1 mg/L [3]), and bisphenol F (BPF).

To check the possible migration of the different phenolic compounds into foodstuffs, either normally (BPF, BPA, BFDGE, BADGE) or accidentally (phenol, *m*-cresol, 4-*tert*-butylphenol) present in food-contact epoxy resins, we have developed an HPLC method with fluorometric detection for the simultaneous analysis of these compounds. The matrices studied were mineral water and wine. Fluorometric detection was chosen after a study showed it to be twice as sensitive as ultaviolet detection.

Sample preparation by solid–liquid extraction using a C_{18} silica cartridge enabled the constituents of wine interfering with the analysis of the studied compounds to be eliminated and detection limits to be well below the specific migration and maximum residue limits described in the European regulations.

Experimental

Apparatus

A Spectra System P 1000XR (Thermo Separation Products, Les Ulis, France) with a Rheodyne model 7725 (Cotati, CA) valve injection system was used (sample loop, 20 or 50 µL), as well as a Shimadzu (Kyoto, Japan) RF 530 fluorometric detector and a Spectranet PC 1000 program (Thermo Separation Products).

Chromatographic conditions

A LiChrospher 100 RP-18 column (Merck, Darmstadt, Germany) (250 mm × 4-mm i.d., 5-µm film thickness)

protected by a LiChrocart RP-18 guard column (Merck) (5-µm film thickness) was maintained at 20°C in a Lisa 30 oven (Thermo Separation Products). Isocratic acetonitrile–water was used as the mobile phase at a ratio of 20:80 for 10 min, followed by a linear gradient of over 50 min up to 70:30. The flow rate was 1 mL/min. Fluorometric detection was used with excitation at 275 nm and emission at 300 nm (optimum wavelengths for each of the compounds studied).

Reagents

For the mobile phase, Acetonitrile LiChrosolv was obtained from Merck. Water was purified using a Milli-Q system (Millipore, St. Quentin des Yvellines, France).

The following were dissolved in methanol (Carlo Erba, Milan, Italy) at 2 g/L to provide seven stock solutions: phenol (Prolabo, Paris, France, 99% pure), *m*-cresol (Prolabo, 99% pure), BPF (Ciba-Geigy, Rueil Malmaison, France, 99% pure), BPA (Merck, 98% pure), 4-*tert*-butylphenol (Aldrich, St. Quentin Fallavier, France, 99% pure), BFDGE (Ciba-Geigy, 99% pure), and BADGE (Ciba-Geigy, 99% pure).

The stock solutions were diluted with distilled water, mineral water, or wine for the working solutions. Methanol and tetrahydrofuran (SDS, Peypin, France) were used for the sample extraction.

Samples

The study was performed on three diverse wines (different levels of polyphenols, alcohol, sugars, pH, ionic strength, etc.): a sweet red wine, *Maury* (Pyrénées Orientales, France, 1995, 16% [v/v] alcohol); a high-tannin red wine stored in oak casks, *Château Ripeau* (Gironde, France, 1990, 13% [v/v] alcohol); and a dry white wine, *Abbaye de Valmagne* (Hérault, France, 1994, 12% [v/v] alcohol). A mineral water called *Vittel* that was contained in glass bottles was also studied.

Table I. Repeatability, Detection Limits, and Quantitation Limits of the Analytical Method

Phenolic compound	Concentration (mg/L)	Average unit area	Standard deviation (σ)	Variation coefficient (%)	Quantitation limit (µg/L)	Detection limit (µg/L)
Phenol	0.01	3491	551	15.8	20	4
	0.02	5511	322	5.8		
	0.05	13615	390	2.9		
	5	1485015	12640	0.8		
m-Cresol	0.01	3791	840	22.1	50	5
	0.02	5883	1037	17.6		
	0.05	16219	459	2.8		
	5	1829206	7895	0.4		
BPF	0.01	1628	164	10.0	50	10
	0.02	2963	297	10.0		
	0.05	8123	572	7.0		
	5	919650	22615	2.5		
BPA	0.01	2073	262	12.7	20	10
	0.02	2693	236	8.8		
	0.05	7283	642	8.8		
	5	882519	26712	3.0		
4-*tert*-Butylphenol	0.01	3443	302	8.8	10	4
	0.02	5572	454	8.1		
	0.05	14603	1206	8.3		
	5	1658913	13848	0.8		
BFDGE*	0.01	1473	262	17.8	50	8
	0.02	3065	474	15.5		
	0.05	8126	188	2.3		
	5	798211	6847	0.9		
BADGE	0.01	3131	427	13.6	50	4
	0.02	5885	952	16.2		
	0.05	15647	194	1.2		
	5	1504711	17460	1.2		

* Isomer showing the greatest response factor.

Sample preparation

For the purification and concentration of the samples, three types of C_{18} silica cartridges were tested: Sep-Pak classic (360 mg) (Waters, Milford, MA), Sep-Pak plus (840 mg) (Waters), and Bond Elut (6 mL/500 mg) (Varian, Harbor City, CA). All were conditioned through rapid washing with 5 mL distilled water, 5 mL methanol, then 5 mL distilled water.

Results and Discussion

Validation of the phenolic compounds analysis method

Linearity

One milliliter of each of the stock solutions was placed in a 100-mL volumetric flask; the volume was completed with distilled water. The resulting 20-mg/L solution was diluted with distilled water to yield working solutions at 10, 5, 1, 0.1, 0.05, 0.02, and 0.01 mg/L. Calibration lines were plotted using these eight concentration levels, and each concentration was assayed in triplicate (different working solutions).

The linearity in detector response (peak areas) with concentration was verified for each compound. The correlation coefficients of peak area to concentration were between 0.9998 and 1.0000.

Repeatability

Repeatability was verified at four concentrations: 0.01, 0.02, 0.05, and 5 mg/L. Each concentration was assayed six times (different working solutions). The results, expressed as standard deviation and coefficient of variance, are given in Table I.

Detection and quantitation limits

The detection and quantitation limits were estimated during the repeatability studies. They were calculated according to the following criteria (12):

Detection limit: $Sx \geq 3\sigma$

Quantitation limit: $Sx \geq 10\sigma$

Sx represents the net signal (area) generated by analyte x, and σ is the standard deviation of the net signal. The detection and quantitation limits for the different compounds are presented in Table I. It was verified that a sample loop of 50 µL yielded detection limits 2.5 times lower than a 20-µL loop.

Validation of the preparation method for wine samples

Specificity

Given the wide range of phenolic compounds in wines, it was necessary to purify the samples and verify the specificity of the analyses. For this, three types of C_{18} cartridges were tested. The best results were obtained with the Bond Elut (Varian) cartridge, which most effectively eliminated the wine constituents that were interfering with the detection of the compounds under study. This type of cartridge was therefore retained for the study.

The optimal extraction conditions were found to be as follows. Phenolic-fortified wine (5 mL) was filtered on a C_{18} Bond Elut cartridge with a flow rate of 1.5 mL/min, and the cartridge was washed with 5 mL of distilled water. The phenolic compounds were then eluted with 2 mL of 4% tetrahydrofuran in methanol, which corresponds to a 25x concentration.

The specificity of the proposed method was studied on the three wines. The chromatogram of a wine fortified with 1 mg/L total phenolics is given as an example in Figure 1. The proposed method is specific to all the studied phenolic compounds. In the case of the red wine *Château Ripeau*, however, the presence of an interfering peak affected the analysis of BPF.

To guarantee the success of the method on a wine that has possibly been contaminated through contact with an epoxy resin, it would be necessary to analyze a reference sample (i.e., one that had never come into contact with an epoxy lining) to verify the absence of interfering compounds, especially because certain wines may normally contain small amounts of phenol. Implication of an epoxy resin in the contamination of a wine sample is possible, therefore, only if several phenol compounds of polymeric origin are found in the sample.

Accuracy

The accuracy of the proposed method, expressed in terms of percent recovery, was studied using the most complex red wine (*Château Ripeau*) and the dry white wine, which were fortified with different quantities of the compounds studied:

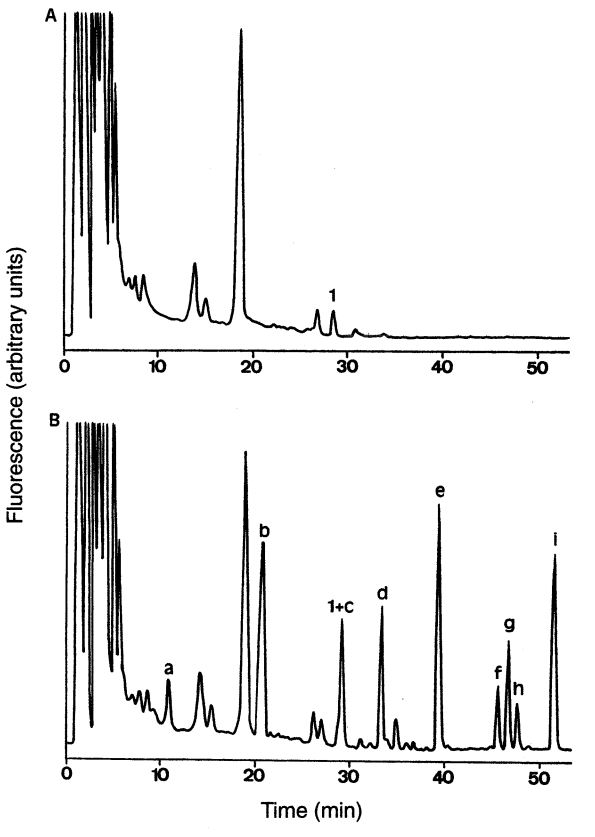

Figure 1. HPLC chromatograms of samples of red wine *Château Ripeau*. (A) Reference. (B) Fortified with a total concentration of 1 mg/L of the following phenolic compounds: (a) phenol, (b) *m*-cresol, (c) BPF, (d) BPA, (e) 4-*tert*-butylphenol, (f,g, and h) BFDGE and (i) BADGE. All were obtained after 25x concentration during extraction. BPF interfered with a wine peak, denoted 1.

5, 20, and 100 µg/L. Each of the three concentrations was analyzed six times (different fortified samples). Given the 25*x* concentration of these samples during extraction, these values were above the limits of detection determined in distilled water. The percent recoveries are given in Table II.

The percent recoveries obtained show the accuracy of the method in the cases of phenol, *m*-cresol, BPF, BPA, and 4-*tert*-butylphenol at all concentrations tested. For BFDGE and BADGE, the accuracy was lower (60% recovery), which can be explained by a strong adsorption of these two compounds to the extraction phase.

The utilization of a different solid extraction phase, which would allow better recovery of BFDGE and BADGE, was not possible because the elution of naturally occurring wine phenolics interfered with the other phenolic compounds studied.

A study on the choice of eluent was carried out. Among the different solvents (acetonitrile, chloroform, and methanol) and solvent mixtures (methanol–dichloromethane [50:50], methanol–tetrahydrofuran [98:2], and methanol–tetrahydrofuran [96:4]) that were tested, the methanol–tetrahydrofuran (96:4) mixture gave the best recovery of BADGE and BFDGE.

Purification and concentration trials on volumes of wine greater than 5 mL showed a lower percent recovery for all the compounds studied; the alcohol contained in the wine flushed the compounds that were fixed on the solid extraction phase.

Linearity

At the same time that the percent recoveries were determined, linearity in detector response (peak area) as a function of concentration was verified for each compound over a concentration range of 5–100 µg/L. The correlation coefficients were between 0.9992 and 0.9999.

Detection limits

The detection limits are given in Table II.

A 25*x* concentration of the phenolic-fortified wines enabled a detection limit of 2.5 µg/L to be achieved with a sample loop of 20 µL. This limit is below the specific migration and maximum residue limits described in the European regulations.

Validation of the preparation method for mineral water samples

The problem of specificity did not occur in the study of mineral water (no interference with the peaks of the phenolic compounds studied). The samples were concentrated using an extraction cartridge. The optimal extraction conditions were

Table II. Accuracy and Detection Limits of the Preparation Method for Wine Samples

Phenolic compound	Concentration (µg/L)	% Recovery (standard deviation) *Château Ripeau* Red wine	% Recovery (standard deviation) White wine	Detection limit (µg/L) *Château Ripeau* Red wine	Detection limit (µg/L) White wine
Phenol	5	115 (12.90)	112 (19.81)	2.5	2.5
	20	102 (4.32)	89 (6.42)		
	100	112 (2.61)	102 (3.39)		
m-Cresol	5	86 (22.45)	96 (21.02)	4	3
	20	95 (10.13)	102 (14.31)		
	100	104 (4.69)	99 (2.96)		
BPF	5	–	109 (22.10)	–	5
	20	–	85 (8.90)		
	100	–	95 (2.52)		
BPA	5	115 (16.87)	92 (17.03)	5	5
	20	103 (5.47)	100 (10.10)		
	100	85 (5.02)	103 (4.21)		
4-*tert*-Butylphenol	5	97 (13.58)	91 (23.32)	2.5	2.5
	20	99 (6.31)	97 (8.56)		
	100	94 (2.99)	101 (4.81)		
BFDGE*	5	51 (23.56)	54 (19.45)	5	5
	20	64 (10.36)	65 (12.51)		
	100	51 (5.98)	55 (3.20)		
BADGE	5	44 (21.63)	71 (21.14)	4	4
	20	62 (15.12)	55 (9.20)		
	100	52 (3.21)	58 (4.52)		

* Isomer showing the greatest response factor.

found to be as follows. Fortified mineral water (50 mL) was filtered on a C_{18} Bond Elut cartridge with a flow rate of 1.5 mL/min, and the cartridge was washed with 5 mL distilled water. The phenolic compounds were then eluted with 2 mL of 4% tetrahydrofuran in methanol, which corresponds to a 25*x* concentration.

Accuracy

The accuracy of the proposed method was studied by fortifying mineral water with different amounts of the compounds studied: 0.5, 1, and 4 µg/L. Each of the three concentrations was analyzed six times (different fortified samples). Given the 25*x* concentration of these samples during extraction, these values were above the limits of detection determined in distilled water. The percent recoveries are given in Table III.

The percent recoveries obtained show the accuracy of the method in the case of *m*-cresol, BPF, BPA, and 4-*tert*-butylphenol at all of the concentrations tested. For BFDGE and BADGE, the accuracy was again reduced, although higher than that obtained for wine (75% recovery). Concentration tests on volumes of mineral water greater than 50 mL revealed a decrease in percent recovery for all the compounds tested.

Table III. Accuracy and Detection Limit of the Preparation Method for Mineral Water Samples

Phenolic compound	Concentration (µg/L)	% Recovery (standard deviation)	Detection limit (µg/L)
Phenol	0.5	33 (10.56)	0.70
	1	38 (5.80)	
	4	43 (1.02)	
m-Cresol	0.5	81 (13.82)	0.40
	1	92 (4.92)	
	4	93 (3.27)	
BPF	0.5	87 (11.11)	0.50
	1	94 (6.73)	
	4	91 (2.04)	
BPA	0.5	107 (15.08)	0.50
	1	93 (9.99)	
	4	93 (6.02)	
4-*tert*-Butylphenol	0.5	92 (13.11)	0.25
	1	90 (7.82)	
	4	102 (5.36)	
BFDGE*	0.5	76 (10.18)	0.50
	1	68 (9.32)	
	4	74 (3.01)	
BADGE	0.5	74 (12.99)	0.40
	1	68 (6.34)	
	4	77 (4.09)	

* Isomer showing the greatest response factor.

Linearity

At the same time that the percent recoveries were determined, linearity in detector response (peak area) as a function of concentration was verified for each compound over a concentration range of 0.5–4 µg/L. The correlation coefficients were between 0.9994 and 0.9999.

Detection limit

The detection limits are given in Table III.

A 25*x* concentration of fortified mineral water enabled a detection limit of 0.25 µg/L to be attained with a sample loop of 20 µL. This limit is much lower than the specific migration and maximum residue limits described in the European regulations.

Conclusion

By detecting very small quantities of phenolic compounds arising from epoxy resins, the proposed HPLC method is economical and rapid to operate and could be used as part of the sanitary control of beverages placed in contact with these resins.

In cases of accidental contamination by the epoxy resin, the simultaneous analysis of these compounds will enable the resin to be implicated with certainty.

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