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Comparison of extraction techniques and mass spectrometric ionization modes in the analysis of wine volatile carbonyls

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ABSTRACT

This work presents a comparative study of the analytical characteristics of two methods for the analysis of carbonyl compounds in wine, both based on the derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). In the first method derivatives are formed in the solid phase extraction (SPE) cartridge in which the analytes have been previously isolated, while in the second method derivatives are formed in a solid phase microextraction (SPME) fibre saturated with vapors of the reagent and exposed to the sample headspace. In both cases detection has been carried out by electron impact (EI) or negative chemical ionization (NCI) mass spectrometry. The possibility of determining haloanisols simultaneously has been also considered.

The method based on SPE presents, in general, better analytical properties than the SPME one. Although linearity was satisfactory for both methods ($R^2 > 0.99$), repeatability of the SPE method (RSD < 10%) was better than that obtained with SPME (9% < RSD < 20%). Detection limits obtained with EI are better for the SPE method except for trihaloanisols, while with NCI detection limits for both strategies are comparable, although the SPME strategy presents worse results for ketones and methional. Detection limits are always lower with NCI, being the improvement most notable for SPME.

Recovery experiments show that in the case of SPE, uncertainties are lower than 12% in all cases, while with the SPME method the imprecision plus the existence of matrix effects make the global uncertainty to be higher than 15%.

1. Introduction

Carbonyl compounds are compounds of interest due to their aromatic [1], environmental, biological and technological relevance [2–6]. The presence of carbonyl compounds is associated with oxidation and fermentation processes in food and drinks [1], with lipid peroxidation in biological systems [7], with olefin ozonolysis, and with photochemical hydrocarbon reactions in the atmosphere [8]. The direct determination of carbonyls in complex matrixes is difficult due to the reactivity of the carbonyl group, particularly aldehyde, to many chromatographic phases, and to the low specificity of their mass spectra [9–10]. In the case of wine, these difficulties are aggravated by interactions with matrix components and by the major presence of carbonyls such as acetaldehyde (more than 300 mg L^{-1}) and pyruvic acid (more than 500 mg L^{-1}) [1,11–14]. For these reasons, the strategies for determining carbonyls usually are based on derivatization of the carbonyl group [6,9,10,15–19]. The most common strategy is derivatization with O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA). The oximes formed with PFBHA have relatively specific mass spectra and high sensitivity in different detection systems, such as electron capture detection (ECD), electron impact mass spectrometry (EI-MS), and negative-ion chemical ionization mass spectrometry (NCI-MS) [4,16,18]. Other reagents have been used, such as 2,4-dinitrophenylhydrazine, cysteamine, and pentafluorophenyl-hydrazine, in the determination of carbonylic compounds [7,10,13,14,16,18].

Other aroma-related compounds closely associated with wine defects are the haloanisols. These compounds have a strong impact on wine quality [20] and their presence is related to microbiological contamination arising mainly from the cork. From a functional point of view, these compounds are similar to the pentafluorobenzyl-oximes, which makes that they could be determined using the same detection system used for the PFBHA-derivatives of the carbonyls, and hence, that “a priori” the simultaneous determination of carbonyls and haloanisols should be possible.

The methods most often used for the analysis of carbonyls in wine are solid phase extraction (SPE) [16–17] and solid phase microextraction (SPME) [18,21,22], in both cases using derivatiza-

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tion with PFBHA. In the case of SPE, Ferreira et al. [16] proposed a method of analysis in which derivatization is performed directly in the same solid phase extraction cartridge in which the compound was extracted. This method allows quantitative analysis of the aldehydes sensorially most significant in wine. In the case of SPME, Wang et al. [18] developed a general strategy for the analysis of carbonyl compounds based on the simultaneous headspace extraction-derivatization on a SPME fibre. The method was applied to the fully automated determination of wine carbonyls. The same strategy was used by Schmarr et al. [21,22] to exhaustively characterize the volatile carbonyls present in wine by using bidimensional gas chromatography and mass spectrometry. One drawback of both methods for the determination of certain carbonyl compounds is the ubiquity of some of the compounds in blanks. The haloanisols have also been determined in wine with both SPE [23,24] and SPME [20,25] methods, fundamentally using GC–MS as the quantification technique.

The comparison of SPE and SPME strategies is not easy at present. On the one hand, SPME has a series of obvious advantages, such as ease of automatization, simple management, and the absence of any need for organic solvents. As a result of these advantages, SPME is gaining ground with respect to other strategies that also are consolidated but require more manual labor and better knowledge of the functioning of chromatographic systems, such as SPE. Nevertheless, the greater tendency of SPME to give matrix-dependent signals in complex systems [26–28] and the problems associated with irregular fibre behavior [29] should be seriously considered and weighed when comparing the two techniques.

For this reason, the primary objective of the present study was to make an in-depth comparison of the analytical characteristics of the SPE-based and SPME-based strategies in the simultaneous determination of carbonyl compounds and haloanisols in a complex sample, such as wine. The comparative study will also include a comparison of the modes of ionization, electron impact (EI) versus negative-ion chemical ionization (NCI).

2. Experimental

2.1. Materials

Isobutyraldehyde 99%, 2-methylbutanal 95%, 3-methylbutanal (isovaleraldehyde) 97%, (*E*)-2-hexenal 98%, (*E*)-2-octenal 98%, (*E*)-2-nonenal 97%, phenylacetaldehyde >90%, methional, 2-methylpentanal 98% and hexachlorobenzene 99% were purchased from Aldrich–Spain (Madrid, Spain). (*E*)-2-heptenal 98%, 2,4-dichloroanisol (>97%, DCA), 2,4,6-tricholoroanisol (99%, TCA), 2,3,6-tricholoroanisol (99%, 2,3,6-TCA), 2,4,6-tribromoanisol (99%, TBA) and O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (99%, PFBHA), were purchased from Fluka-Spain (Madrid). 1-octen-3-one was supplied by Lancaster Synthesis 97% (Eastgate, England). 3-Methyl-2,4-nonadione >97% was a gift from Takasago International Chemicals-Europe (Murcia-Spain).

Dichloromethane (HPLC quality) was from Fisher Chemicals (Leicester, UK), methanol (HPLC grade), *n*-hexane Unisolv for traze analysis, and diethylether Pro Analyst were supplied by Merck (Darmstadt, Germany), *n*-pentane for GC-analysis >99% was purchased from Fluka. Absolute ethanol and sodium hydrogencarbonate, both ARG quality, were from Panreac (Barcelona, Spain), sulfuric acid (95–97%, synthesis grade) was from Scharlau (Barcelona, Spain). Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA).

LiChrolut-EN resins (styrene–vinylbenzene, divinylbenzene polymer), prepacked in a 200 mg cartridge (3 mL total volume) were obtained from Merck. PDMS–DVB (65 μm) SPME fibres were purchased from Supelco-Spain (Madrid, Spain).

Semiautomated solid phase extraction was carried out with a VAC ELUT 20 station system from Varian (Walnut Creek, CA, USA).

Wines for the validation study were four reds and four whites with alcoholic degrees comprised between 11.0% and 14.5% (v/v) and pHs ranging from 3.2 to 3.8; all of them were dry table wines with ages between 1 and 5 years old.

2.2. Methods

2.2.1. Solid phase extraction (SPE)

The method proposed by Ferreira et al. [16] was used. Ten milliliters of wine, containing 20 $\mu g\,L^{-1}$ of 2-methylpentanal and 5 $\mu g\,L^{-1}$ of 2,6-dichloroanisol as surrogated standards, were loaded onto a 200 mg LiChrolut-EN solid phase extraction cartridge (previously conditioned with 4 mL of dichloromethane, 4 mL of methanol and 4 mL of a 12% ethanol (v/v) aqueous solution). Acetaldehyde and some other major wine carbonyl compounds were removed by cleanup with 10 mL of an aqueous solution containing 1% $NaHCO_3$. Carbonyls retained in the cartridge were directly derivatized by passing 2 mL of an aqueous solution of PFBHA (5 $mg\,mL^{-1}$), and letting the cartridge imbibed in the reagent for 15 min at room temperature (25 °C). Excess of reagent was removed with 10 mL of 0.05 M sulfuric acid. Derivatized analytes were finally eluted with 2 mL of hexane–10% of diethylether, containing 300 $\mu g\,L^{-1}$ of hexachlorobenzene in the case of SPE-EI analysis or 500 $\mu g\,L^{-1}$ of 2,3,6-trichloroanisol in the case of SPE-NCI analysis, as internal standards. This volume was collected in a 2 mL autosampler vial and analyzed by injecting 2 μL of the extract in the chromatographic system: GC-EI-MS or GC-NCI-MS.

2.2.2. Solid phase microextraction (SPME)

The general strategy proposed by Wang et al. [30] and Vesely et al. [31] has been applied after some modifications described below. According to the method proposed by Wang 250 μL of wine are added to a 20 mL standard headspace vial that contained 5 mL of brine; then 30 μL of a solution of 2,3-DCA and 2-methylpentanal (500 $\mu g\,L^{-1}$) were added into the vial as internal standards. The PDMS/DVB SPME fibre was then placed in the headspace of the PFBHA solution (500 μL of the PFBHA 6 $\mu g\,mL^{-1}$ solution in 10 mL of deionized water) for 15 min at 50 °C. The SPME fibre loaded with PFBHA was then exposed to the headspace of the sample for 20 min at 50 °C. In both cases agitation speed was 500 rpm. Finally, the fibre containing the PFB-oximes is desorbed directly in the injection port of the chromatographic system for their determination by GC-EI-MS or GC-NCI-MS. Total automation of the procedure was achieved using a CTC CombiPal autosampler (Zwingen, Switzerland), which was programmed using the CycleComposer with macroeditor software and equipped with sample trays, a temperature controlled agitator tray and a fibre-conditioning device.

2.3. GC–MS conditions

The apparatus was a Shimadzu QP-2010 gas chromatograph with a quadrupole mass spectrometric detection system. The injector was a standard split/splitless.

In the case of SPE, splitless mode injection was used at a temperature of 250 °C with a pulse of pressure of 467 kPa during the 1.5 min splitless time. The carrier gas was He at a constant linear velocity of 35 $cm\,s^{-1}$ (≈0.62 $mL\,min^{-1}$) during the run. The flow during the splitless time (1.5 min) was 2.69 $mL\,min^{-1}$. The column was a Factor Four capillary column VF-35MS from Varian, 20 m × 0.15 mm I.D., with 0.15 μm film thickness. The chromatographic oven was held at 45 °C for 2 min, then raised to 200 °C at 10 °C min^{-1}, then to 320 °C at 10 °C min^{-1} and finally the temperature was held at 320 °C for 3 min. The temperature of the ion source was 220 °C and the interface was kept at 250 °C.

Table 1
Masses of the ions selected for the determination of the analytes considered in the study.

Analyte	*m/z*	
	EI	NCI
Isobutyraldehyde	195, 250[a]	178, 217[a]
2,6-DCA	133, 176[a]	35[a], 174
2-Methylbutanal	239[a], 253	178, 231[a]
Isovaleraldehyde	239[a], 266	178, 231[a]
2-Methylpentanal	238, 253[a]	245, 275[a]
TCA	195, 210[a]	35
2,3,6-TCA	195, 210[a]	35
(*E*)-2-Hexenal	250[a], 293	243[a], 273
1-Octen-3-one	140[a], 321	140
(*E*)-2-Heptenal	250[a], 307	257[a], 287
Octanal	239	273, 303
Methional	252[a], 299	249[a], 279
(*E*)-2-Octenal	250[a], 321	271, 301[a]
Nonanal	239	287, 317
TBA	329, 346[a]	79[a], 81
Phenylacetaldehyde	297[a], 315	204[a], 295
(*E*)-2-Nonenal	250[a], 335	285[a], 315
Decanal	239	331
HCB[b]	284[a], 249	284[a], 35
3-Methyl-2,4-nonadione	363[a], 294	379[a], 274

[a] *m/z* of the ion used for quantification.
[b] HCB was finally not used in the analyses carried out with the NCI ion source since it caused interference problems with (*E*)-2-nonenal.

Two different ion sources were used: electronic impact (EI) ion source at 70 keV and the negative chemical ionization (NCI) using methane at 3 bar as reagent gas. The mass analyzer was operated in single ion monitoring (SIM) mode and the selected ions per each analyte are shown in Table 1.

In the case of SPME, the fibre containing the PFB-oximes was desorbed in the injection port of the GC–MS system in splitless mode for 2.5 min at 250 °C. The carrier gas was He programmed at a constant flow of 0.82 mL min^{-1}. The rest of conditions were similar to the ones described before.

2.4. Validation

Method linearity was studied by standard addition to red and white wines as well as by the derivatization of known amounts of analytes in synthetic wine (aqueous solution 12% ethanol (v/v), containing 5 g L^{-1} of tartaric acid and buffered at pH 3.5), according to each method. For those analytes whose two isomeric oximes appear separated in the chromatogram [6], the summed area of both peaks was considered in their quantification. Reproducibility was evaluated by the replicated analysis of eight different wines (four white and four red wines) on different days. In order to evaluate the existence of matrix effects, and to determine the degree of recovery of the method, an experiment of standard recovery was carried out on eight wines (four whites and four reds) spiked or not with known concentrations of the analytes. Detection limits were defined as the concentration giving a peak height three times the signal-to-noise ratio.

3. Results

3.1. Blanks

In previous studies by Ferreira et al. [16,32] and Schmarr et al. [21,22], certain analytes have been reported to be ubiquitous in blanks when PFBHA is used as the derivatization reagent, independently of the derivatization strategy followed. The results obtained using new reagents of the maximum purity available, following rigorous water purification and sorbent media protocols, and conducting the study in an isolated laboratory, are summarized in Table 2. As can be observed, the signal of the blanks is still a problem, not only for octanal, nonanal and decanal, as has been reported previously [32], but also for 4- and 5-carbon atom aldehydes. It is important to note that, with the exception of 2-methylbutanal and isovaleraldehyde, there were more blank problems with blanks using the SPME method than with the SPE method, in contrast with the claims of Schmarr et al. [22]. These authors argued that since the SPME method requires less sample manipulation, the problems with blanks would be reduced. However, results suggest that contamination is not related to sample manipulation, but that has a complex origin. The widespread use of some of the analytes in household cleaning product formulations would explain an environmental origin. Apart from that, the simple exposure of the SPME fibre to the reagent headspace already reveals the presence of the oximes of analytes, suggesting that the reagent itself is a source of contamination. As has been reported [32], all attempts at reagent purification were futile. In fact, the analysis of octanal, nonanal and decanal within the necessary work range was impossible because the blanks obtained for these compounds were too high. In the case of other compounds found in blanks, analysis was possible but the limits of detection were affected.

Table 2
Signals of analytes found in the analysis of blank solutions. Results are expressed as the mass of analyte (in μg L^{-1}) in wine, producing a signal equivalent to that found in the blank samples.

Analytes	SPE	SD	SPME	SD
Isobutyraldehyde	2.3	0.4	3.5	0.6
2-Methylbutanal	3.1	0.5	0.4	0.1
Isovaleraldehyde	1.6	0.3	0.9	0.4
TCA	–	–	–	–
(*E*)-2-Hexenal	–	–	0.14	0.02
1-Octen-3-one	–	–	–	–
(*E*)-2-Heptenal	–	–	0.10	0.03
Octanal	3.8	0.3	5.9	1.3
Methional	–	–	–	–
(*E*)-2-Octenal	–		0.2	0.10
Nonanal	9.9	1.3	11.1	3.4
TBA	–	–	–	–
Phenylacetaldehyde	–	–	–	–
Decanal	6.0	0.9	6.9	1.3
(*E*)-2-Nonenal	0.1	0.02	0.3	0.09
3-Methyl-2,4-nonadione	–	–	–	–

3.2. Linearity

The linearity of the two methods (SPE and SPME) using both detection systems (EI and NCI) was studied in synthetic wine and in two wine samples, a white wine and a red wine, using six levels of concentration and replicates. The linearity was satisfactory, with coefficients of determination of more than 0.99 in almost all cases. No differences could be established between the two methods. It is noteworthy that no signal was obtained for methional using the SPE method on the synthetic wine matrix [6] and that no signal could be obtained for 3-methyl-2,4-nonadione using the SPME method on any sample at any level of concentration.

3.3. Precision

The precision of both methods was evaluated as repeatability by replicated analyses of wines at three concentration levels: unspiked wine samples and the corresponding spiked samples at two different concentration levels (low and high). The results obtained with NCI as the ionization source are shown in Table 3. In addition to results for each concentration level, the table gives itemized for the three concentration levels and an average repeatability, on which comparative statistical analyses were made. In this case, the

Table 3
Repeatability of the methods, expressed as RSD (%). Data are the average RSD (%) obtained in the duplicated analysis of four red and four white wines at three different concentration levels.

Analytes	SPE-NCI				SPME-NCI				F^a	F^b (95%)
	Unspiked	Low level	High level	Total	Unspiked	Low level	High level	Total		
Isobutyraldehyde	7.1	8.3	10.3	*8.6*	11.8	10.6	4.6	*9.5*	1.2	2.1
2-Methylbutanal	6.2	7.8	7.8	*7.3*	6.5	6.0	3.1	*5.4*	1.8	2.1
Isovaleraldehyde	9.3	9.8	4.1	*8.2*	17.7	6.8	11.5	*12.8*	**2.5**	2.1
TCA[c]	–	7.7	9.1	*6.9*	–	10.4	19.3	*12.7*	**3.4**	2.5
(*E*)-2-Hexenal	7.6	6.0	9.3	*7.7*	11.8	5.3	9.3	*9.2*	1.4	2.1
1-Octen-3-one	8.9	8.0	9.2	*8.7*	19.0	9.6	18.4	*16.3*	**3.5**	2.1
(*E*)-2-Heptenal	9.1	6.9	7.9	*8.0*	13.2	15.3	7.2	*12.4*	**2.4**	2.1
Methional	8.0	8.5	7.4	*8.0*	7.1	16.9	9.7	*11.2*	2.0	2.1
(*E*)-2-Octenal	4.8	6.2	7.3	*6.2*	19.6	23.5	7.3	*18.1*	**8.6**	2.1
TBA[c]	–	7.2	6.8	*5.7*	–	7.2	28.8	*17.1*	**8.9**	2.5
Phenylacetaldehyde	7.8	9.1	4.3	*7.4*	17.1	1.6	12.1	*12.1*	**2.7**	2.1
(*E*)-2-Nonenal	7.5	8.9	7.3	*7.9*	17.0	20.7	16.0	*18.0*	**5.2**	2.1
3-Methyl-2,4-nonadione[c,d]	–	7.7	9.5	*7.1*	–	–	–			

The italic values only represent the total repeatability of the method, they do not have any special significance.
[a] *F* quotient to compare the total RSD (%) obtained with the two methods. Significant differences are shown in bold.
[b] Critical *F* for the 95% confidence level.
[c] Not found in the unspiked sample.
[d] Not signal obtained in the SPME method.

repeatability obtained with the SPE method (RSD < 10%) was clearly better than the repeatability obtained with SPME (9% < RSD < 20%), the difference being significant in most cases.

3.4. Limits of detection

The limits of detection in the red wines and white wines, calculated as the concentration that generated a signal of three times the signal-noise ratio, were determined in wine samples containing low levels of analytes, or in spiked samples with low levels of these components, as applicable. The results are shown in Table 4. The limits of detection shown in the table do not take into account the contribution of the blank previously shown in Table 2. In the comparison of SPE with SPME, leaving aside 3-methyl-2,4-nonadione which did not produce a signal in the SPME system, the limits obtained by SPE with electron impact were better, except for isobutyraldehyde, TCA, and TBA. The advantage of SPE was particularly important in the case of phenylacetaldehyde, 1-octen-3-one, and methional (the limits of detection were 6, 4 and 2 times lower, respectively, with SPE than with SPME). In contrast, the limits of detection of isobutyraldehyde, TCA, and TBA were lower with the SPME method, the advantage being greater for TBA (the limits of detection were up to 8 times lower with SPME than with SPE). In the case of the NCI determination mode, the SPE method was still better for phenylacetaldehyde, methional, and 1-octen-3-one (7–13, 2–3 and 2 times lower, respectively, with SPE than with SPME), whereas the SPME method resulted in lower limits of detection for TCA and TBA, as well as (*E*)-2-hexenal and (*E*)-2-heptenal, in contrast with the findings obtained with the EI ionization source. The fact that the analytes that did not undergo derivatization (TCA and TBA) were determined more easily by SPME (Table 4), and that the ketones (1-octen-3-one and 3-methyl-2,4-nonadione) were determined by SPE with a higher sensitivity, suggests that the SPME strategy achieved a higher neat preconcentration, at least for the most volatile analytes, but that the oximation reaction was more difficult in the fibre.

Nonetheless, since the limit of detection in the SPE strategy can be easily improved without altering the analytical characteristics of the method by increasing the injection volume or by off-column preconcentration of the extract, the limits of detection of the SPE method were recalculated for the injection of a10-fold more concentrated red wine extract. Results are also shown in Table 4 and demonstrate that in the case of electron impact ionization, SPE was the most sensitive strategy in all cases, with limits of detection well below 0.1 $\mu g\,L^{-1}$ in all cases except phenylacetaldehyde. In the case of NCI ionization, the SPE strategy continued to be more sensitive

Table 4
Detection limits of the methods, expressed in $\mu g\,L^{-1}$.

Analytes	Red wine						White wine			
	EI			NCI			EI		NCI	
	SPE		SPME	SPE		SPME	SPE	SPME	SPE	SPME
	Crude	Concentrated		Crude	Concentrated					
Isobutyraldehyde	0.186	0.035	0.143	0.097	0.021	0.031	0.148	0.124	0.109	0.033
2-Methylbutanal	0.082	0.043	0.057	0.039	0.024	0.006	0.063	0.080	0.050	0.008
Isovaleraldehyde	0.065	0.057	0.112	0.031	0.019	0.042	0.039	0.098	0.028	0.055
TCA	0.095	0.023	0.063	0.027	0.025	0.017	0.106	0.059	0.029	0.020
(*E*)-2-Hexenal	0.072	0.001	0.091	0.009	0.025	0.004	0.055	0.083	0.010	0.002
1-Octen-3-one	0.051	0.008	0.204	0.011	0.003	0.023	0.035	0.194	0.012	0.025
(*E*)-2-Heptenal	0.049	–[a]	0.084	0.057	0.073	0.008	0.067	0.097	0.039	0.008
Methional	0.07	0.004	0.144	0.036	0.018	0.088	0.079	0.173	0.028	0.096
(*E*)-2-Octenal	0.098	0.021	0.189	0.012	0.003	0.021	0.092	0.136	0.019	0.018
TBA	0.587	0.046	0.076	0.013	0.002	0.002	0.501	0.063	0.010	0.002
Phenylacetaldehyde	0.202	0.159	1.119	0.042	0.026	0.356	0.155	1.052	0.036	0.477
(*E*)-2-Nonenal	0.082	0.005	0.118	0.012	0.004	0.019	0.066	0.127	0.020	0.015
3-Methyl-2,4-nonadione[b]	0.134	0.008		0.038	0.025		0.163		0.033	

[a] No calculated. Signal presented a strong interference for this compound.
[b] No signal obtained for this compound by SPME.

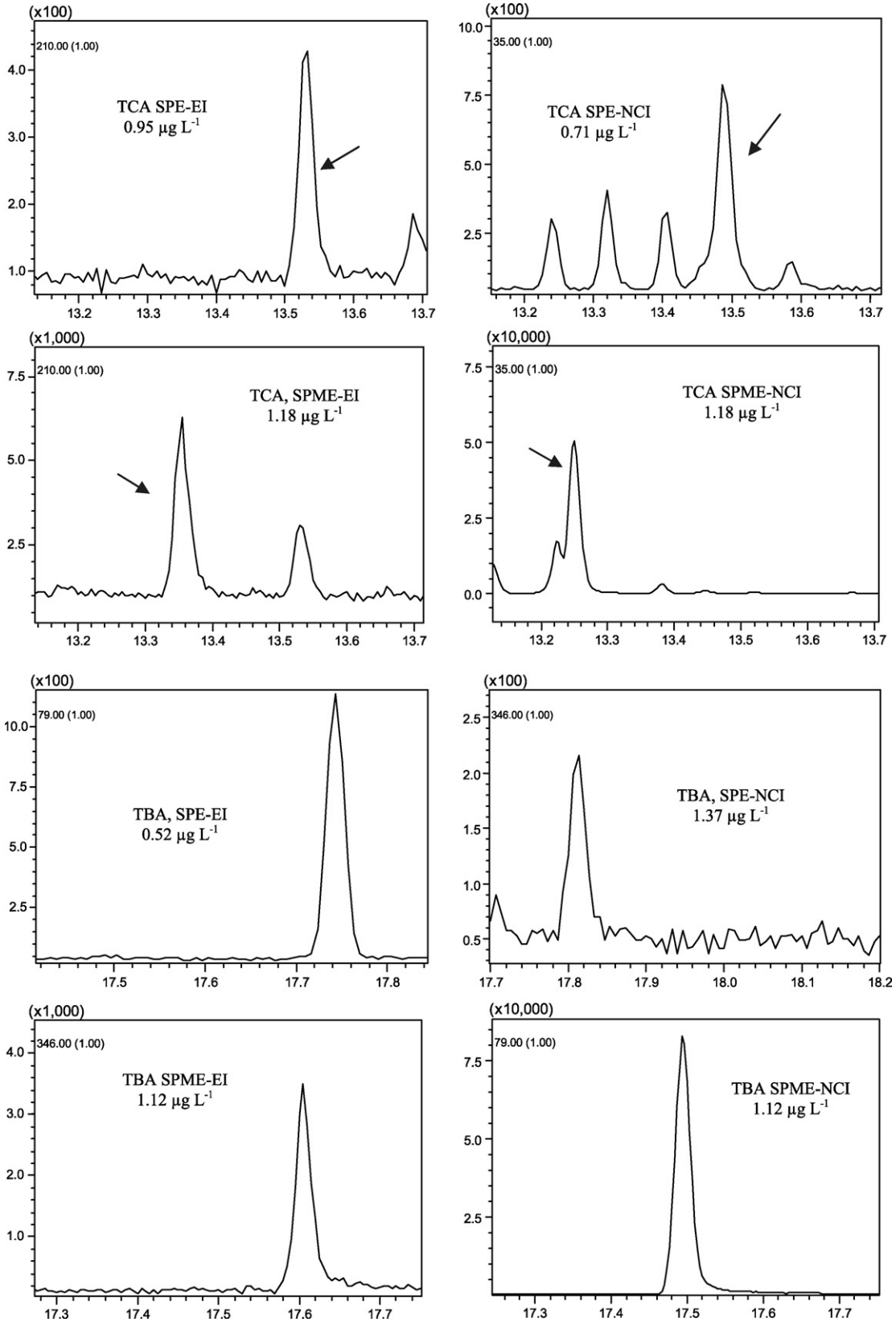

Fig. 1. Typical chromatograms obtained in the SPE-EI-MS, SPE-NCI-MS, SPME-EI-MS and SPME-NCI-MS analysis of wine carbonyl compounds and haloanisols.

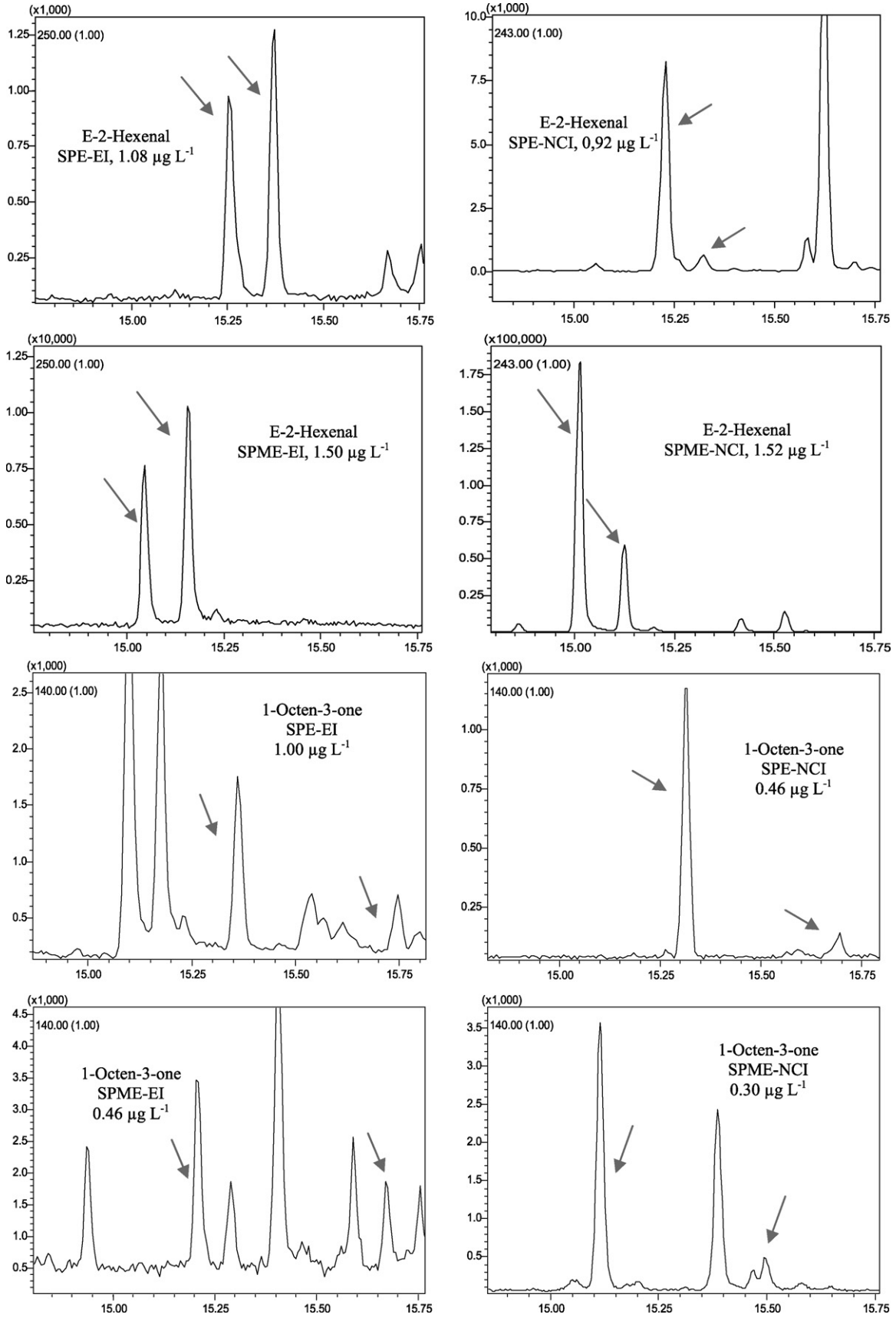

Fig. 1. (*Continued*)

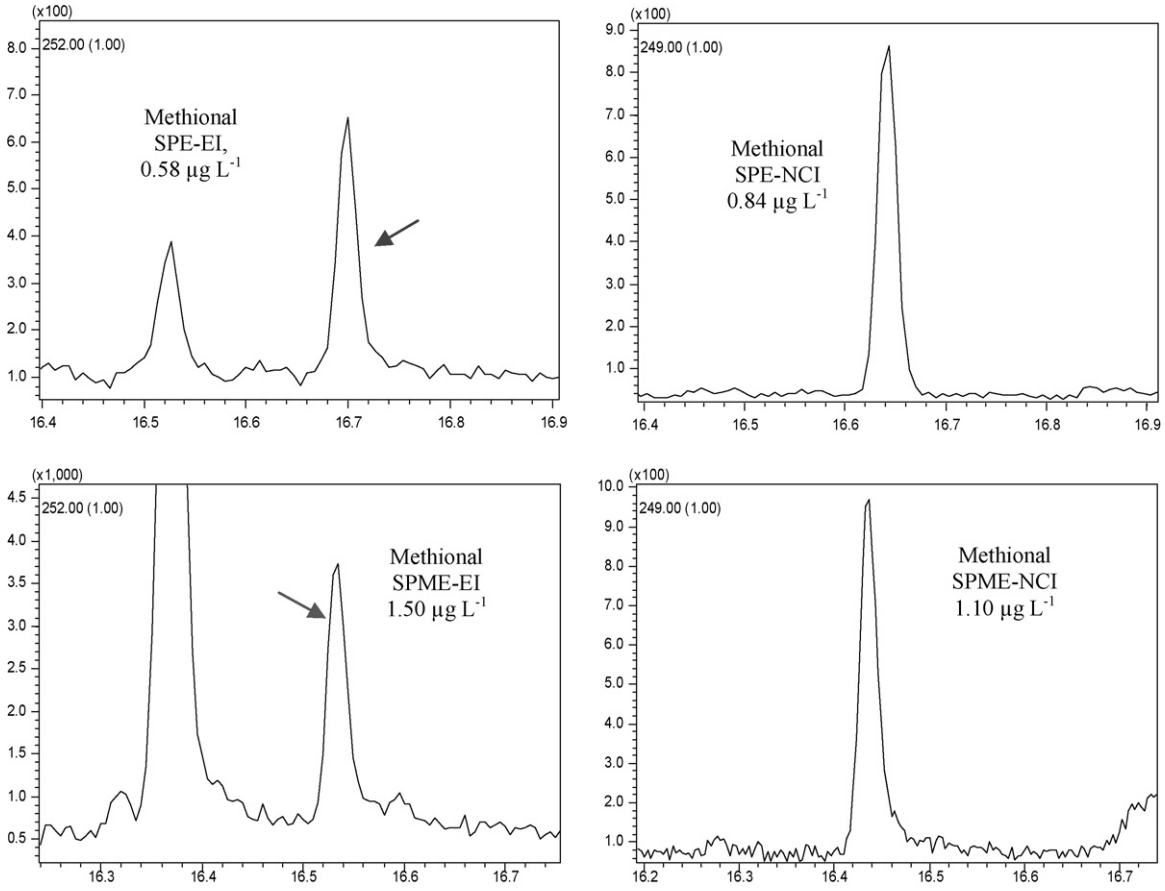

Fig. 1. (*Continued*).

than SPME, except for 2-methylbutanal, TCA, (*E*)-2-hexenal, and (*E*)-2-heptenal.

Comparing the limits of detection observed with the two ionization modes, in all the cases (except (*E*)-2-heptenal in red wine and in SPE) the limits of detection were better with the NCI ionization mode, as expected. The advantages were particularly noteworthy in the case of TBA, due its high electron capture capacity. The sensitivity was improved by a factor of almost 50. It also is noteworthy that the advantages linked to the use of NCI were particularly important with the SPME strategy, which can be attributed to the lower levels of interferences found with this technique, as can be seen in the typical chromatograms shown in Fig. 1. In conclusion, the use of NCI was advantageous, particularly with SPME, and the limits of detection were generally better with the SPE strategy in all the cases in which oximes are formed, particularly for ketones and compounds that are not very volatile.

3.5. Matrix effects

A standard recovery study was made in which eight wines (four red and four white wines) were analyzed using both methods. The increment in the signal obtained in the spiked wines was compared with the signal generated by the same amount of analytes added to a synthetic wine. The results are presented in Table 5 for SPE and in Table 6 for SPME. The data shown are the mean and standard deviation of the recoveries found in the four white and four red wines of the eight wines studied. A t parameter was calculated to evaluate the possible existence of significant differences in recovery levels for red versus white wines and a second *t* parameter was calculated to evaluate whether the average recovery (for red and white wines) was significantly different from 100%.

In the case of the SPE method, differences in levels of recovery between red and white wines were only observed in the case of methional, for which the recovery in red wine was significantly higher. In the cases of isovaleraldehyde and phenylacetaldehyde, higher recoveries were obtained in white wines, although the differences were not significant at the 95% level. In the rest of the cases, the differences in recovery related to the color of the wine were less important. With regard to average recoveries, satisfactory values were obtained for (*E*)-2-hexenal, 1-octen-3-one, (*E*)-2-octenal, phenylacetaldehyde, and (*E*)-2-nonenal. In all these cases, the recoveries did not differ significantly from 100, although in the case of (*E*)-2-nonenal, the high standard deviation of the average recovery indicated that this component experiences important matrix effects. For the rest of the cases, the average recovery values obtained differed significantly from 100, although the standard deviations of recovery were reasonable, except for 3-methyl-2,4-nonadione. These results suggest that calibration can be performed in synthetic medium in the case of (*E*)-2-hexenal, 1-octen-3-one, (*E*)-2-octenal, and (*E*)-2-nonenal, whereas it is advisable to make separate calibrations in red wine and white wine in the case of methional, isovaleraldehyde, and phenylacetaldehyde. In the rest of the cases, calibration should be done in wine. The method can provide estimates with uncertainties of better than 12% for all the compounds studied except (*E*)-2-nonenal and 3-methyl-2,4-nonadione, for which the uncertainty is superior to 20%. It is interesting to note that these results were obtained using as internal standard (IS) 2,3,6-trichloroanisol, a compound that is not derivatized. In fact, the use of 2-methylpentanal as an IS added at the beginning of the process produces inferior results in terms of reproducibility and recovery (data not shown); because of this, 2-methylpentanal

Table 5
Average recoveries with their standard deviations obtained in the SPE method (NCI as ion source) and statistical tests for checking matrix effects. Results are the average of the recoveries found in the analysis of four red and four white wines. Significant differences are shown in bold.

Analytes	White wine	SD	Red wine	SD	%R Mean	SD	t_{mean}[a]	$t(95)$[b]	t_{100}[c]	$t(95)$[d]
Isobutyraldehyde	70.2	13.9	60.7	8.3	65.4	11.4	1.18	2.45	**8.55**	2.36
2-Methylbutanal	110.7	1.2	111.8	4.7	111.3	3.4	0.45	2.45	**9.28**	2.36
Isovaleraldehyde	147	14.7	130.2	2.5	138.6	10.5	2.25	2.45	**10.35**	2.36
TCA	94.5	11.1	87	6.5	90.8	9.1	1.17	2.45	**2.88**	2.36
(*E*)-2-Hexenal	96.4	6.5	93.9	5.3	95.2	5.9	0.60	2.45	2.31	2.36
1-Octen-3-one	99.6	8.4	96.2	13.2	97.9	11.1	0.43	2.45	0.54	2.36
(*E*)-2-Heptenal	89.4	3.2	90.7	11.7	90.1	8.6	0.21	2.45	**3.28**	2.36
Methional[e]	79.6	8.0	99.8	8.2	89.7	8.1	**3.51**	2.45		
(*E*)-2-Octenal	100.3	12.4	90.1	11.5	95.2	12.0	1.21	2.45	1.14	2.36
TBA	76.7	6.5	72.3	8.0	74.5	7.3	0.85	2.45	**9.90**	2.36
Phenylacetaldehyde	101.1	13.5	84.1	5.3	92.6	10.2	2.35	2.45	2.04	2.36
(*E*)-2-Nonenal	85.2	22.6	98.8	21.6	92.0	22.1	0.87	2.45	1.02	2.36
3-Methyl-2,4-nonadione	51.3	17.7	62.6	28.3	57.0	23.6	0.68	2.45	**5.16**	2.36

[a] *t* experimental value (95% significance) for the comparison of the average recoveries in white and red wines.
[b] *t* critical parameter value (95% significance) for the comparison of the average recoveries in white and red wines.
[c] *t* experimental value (95% significance) for the comparison of the average percentage of recovery versus 100%.
[d] *t* critical parameter value (95% significance) for the comparison of the average percentage of recovery versus 100%.
[e] In the case of methional the comparison is not possible because of no signal for this compound was obtained in synthetic wine.

Table 6
Average recoveries with their standard deviation obtained in the SPME method (NCI as ion source) and statistical tests for checking matrix effects. Results are the average of the recoveries found in the analysis of four red and four white wines. Significant differences are shown in bold.

Analytes	White wine	SD	Red wine	SD	%*R* mean	S	t_{mean}[a]	$t(95)$[b]	t_{100}[c]	$t(95)$[d]
Isobutyraldehyde	104.6	16.4	119.6	18.3	112.1	17.4	1.22	2.45	1.97	2.36
2-Methylbutanal	108.5	7.5	118.7	9.1	113.6	8.3	1.73	2.45	**4.61**	2.36
Isovaleraldehyde	90.1	4.4	71.1	22.5	80.6	16.2	1.66	2.45	**3.38**	2.36
TCA	112	37.2	97.6	13.1	104.8	27.9	0.73	2.45	0.49	2.36
(*E*)-2-Hexenal	95.4	23.5	93.4	3.0	94.4	16.8	0.17	2.45	0.95	2.36
1-Octen-3-one	672.4	23.3	646.1	14.0	659.3	19.2	1.94	2.45	**82.30**	2.36
(*E*)-2-Heptenal	83.4	5.1	82.9	7.4	83.15	6.4	0.11	2.45	**7.50**	2.36
Methional[e]	55.1	24.5	106.1	10.0	80.6	18.7	**3.85**	2.45		
(*E*)-2-Octenal	100.3	19.6	77.5	8.5	88.9	15.1	2.13	2.45	2.08	2.36
TBA	100.4	12.6	102.6	8.4	101.5	10.7	0.29	2.45	0.40	2.36
Phenylacetaldehyde	107.2	19.1	124.7	17.5	116.0	18.3	1.35	2.45	**2.46**	2.36
(*E*)-2-Nonenal	167.8	86.6	95.2	23.7	131.5	63.5	1.62	2.45	1.40	2.36

[a] *t* experimental value (95% significance) for the comparison of the average recoveries in white and red wines.
[b] *t* critical parameter value (95% significance) for the comparison of the average recoveries in white and red wines.
[c] *t* experimental value (95% significance) for the comparison of the average percentage of recovery versus 100%.
[d] *t* critical parameter value (95% significance) for the comparison of the average percentage of recovery versus 100%.
[e] In the case of methional there is not comparison to 100% because of the abnormal behavior of this compound in synthetic wine.

was used as surrogated standard, and not IS, in the final procedure.

In the case of the SPME method, differences in recovery in relation to wine color were significant only for methional, which was recovered from red wine in significantly larger amounts. This finding confirms that methional establishes strong interactions in the matrix of white wine. For the rest of the analytes studied, wine color was not a determinant in recovery, although the lack of significance may be attributable to a high uncertainty. Average recovery values were significantly different from 100 in the cases of 2-methylbutanal, isovaleraldehyde, 1-octen-3-one, (*E*)-2-octenal, and phenylacetaldehyde, which is why calibration must unavoidably be performed on wine in these cases. It was interesting that 1-octen-3-one, for reasons not easily explained, produced a very low signal in synthetic medium. The standard deviation of the average recovery showed that the SPME strategy only allowed the determination of 2-methylbutanal, (*E*)-2-heptenal and TBA with less than 15% uncertainty. This parameter peaked up to 27.9% and 63.5% in the cases of TCA and (*E*)-2-nonenal, respectively. Although part of the uncertainty is due to the low repeatability of the procedure, as was shown in Table 3, data in Table 6 suggest that the SPME method suffers important matrix effects, in spite of the facts that the matrix is strongly diluted in the procedure and that an internal standard (2-methylpentanal) relatively similar to the analytes is used.

4. Conclusions

This study shows that the SPE-based method has better general analytical properties than the SPME-based procedure. Although the linearity of SPE and SPME is comparable, the repeatability of SPE is significantly better. The limits of detection for chloroanisols were better using the SPME method, whereas the limits of detection of other compounds, particularly ketones and methional, were better with SPE. NCI detection yielded better results in both SPE and SPME, but it produced more improvement with SPME. Recovery studies showed that SPE achieved uncertainties of less than 12% in most cases, while the SPME method showed matrix effects and uncertainties that were generally higher than 15%.

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