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Simple strategy for the optimization of solid-phase extraction procedures through the use of solid–liquid distribution coefficients Application to the determination of aliphatic lactones in wine

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Abstract

A practical strategy for the optimization of solid-phase extraction (SPE) systems is presented. Critical SPE volumes (sample loaded, rinsing and elution solvent) are calculated from solid–liquid extraction coefficients and from basic bed parameters determined in simple experiments, using the Lövkist Jonsson model and other expressions derived from the general theory of chromatography. The agreement between calculated and measured volumes is satisfactory, which makes it possible to consider different sorbents and rinsing and elution solvents in the SPE optimization with a relatively low experimental effort. The strategy has been successfully applied to the optimization of a SPE method directed to the selective extraction of aliphatic lactones from wine. Six different reversed-phase sorbents were studied and the one showing maximum extraction selectivity was selected. Wine (50 ml) is extracted in a 200 mg cartridge filled with Bond Elut-ENV resins. Interferences are removed with 20 ml of methanol–water (40:60) with 1% $NaHCO_3$. Elution is carried out with 1.8 ml of dichloromethane. The extract is concentrated to 0.15 ml and analyzed by GC–ion trap MS. Eight odor-active aliphatic γ and δ lactones (with 8–12 C atoms) from wine are recovered ($R > 75\%$) in an extract free from wine major volatiles. Detection limits are in the 40–300 ng/l range, well below the odor detection threshold of these compounds. Linearity ($r^2 > 0.996$) and precision (average R.S.D. 3.5%) are satisfactory in all cases. The levels in wine of some of these lactones (γ-octa, undeca and dodecalactones) are reported by first time and results demonstrate that can be present at concentrations above or close to their corresponding odor thresholds.

Keywords: Optimization; Solid-phase extraction; Solid–liquid distribution coefficients; Wine; Food and lysis; Lactones; Volatile organic compounds

1. Introduction

Solid-phase extraction (SPE) is widely used in analytical laboratories for either sample extraction or sample clean up procedures. In spite of its importance, trial-and-error procedures still constitute the general approach to SPE method development [1]. This strategy requires a large number of systematic experiments to check the usefulness of a given phase for the isolation of a given analyte, which seriously limits the number of extracting phases and of rinsing solvents that the researchers consider in the development of the analytical method. As the number of phases offered today is extremely high, particularly in the case of reversed-phase extraction modes, the developed methods are, very often, far from the actual optimum solution.

On the other hand, the theoretical framework for more sophisticated and efficient optimization strategies is well known and has been recently reviewed [1–4]. The relationship between the breakthrough volume and the retention factor and the chromatographic efficiency of the SPE bed is derived from the general theory of frontal chromatography [5–8]; or from more complex models for systems with low plate numbers [9]. Such relationships state that the critical SPE parameters (breakthrough volume, volume of rinsing solvent, elution volume) depend on the kinetic properties of the SPE bed, on its holdup volume and on the retention factor for the analyte. For similar sampling devices, the retention factor becomes the dominant term [1], and its measurement the key problem. Unfortunately, the effort required to measure retention factors following most of the methods proposed in the literature [10–16] precludes their use in normal laboratory optimization. Other promising strategies based on the solvation properties of analytes and sorbents

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[12,14,16–20] require a big database that, at present, is not available.

An indirect measure of the retention factor is the solid–liquid distribution coefficient. This parameter can be easily measured and can provide useful information about the behavior of analytes and interferences in a SPE system [21,22]. Furthermore, retention properties of analytes in a series of different SPE sorbents can be determined with little effort, which facilitates sorbent and solvent selection. In previous papers [22,23], solid–liquid distribution coefficients were applied to estimate the maximum volumes of sample which can be loaded in a SPE system to ensure that breakthrough volumes of analytes are not exceeded. In this paper the use of solid–liquid distribution coefficients is extended to estimate the rinsing and elution volumes of a SPE procedure. This strategy allows for designing optimum SPE systems, taking into account not only the maximum volume of sample that can be loaded on a given SPE bed, but also the conditions (nature and volumes of rinsing and elution solvents) leading to a complete isolation of the analytes from their interferences.

As an application, an optimal SPE method for the quantitative determination of C_8–C_{12} wine aliphatic lactones has been developed. The presence of some of these important odorants in wine is well documented [24–26], but at present, there are no available quantitative data for some of them such as γ-octa-, undeca- and dodecalactones. The developed SPE isolation method, whose parameters have been directly derived using the general strategy proposed in the paper, allows preparation of an extract in which wine major volatile compounds have been depleted. The GC–MS determination of the analytes in this extract is straightforward.

2. Proposed model

2.1. Key parameters defining the SPE operation

(a) System parameters (objectives)
   1. Nature of sorbent and of rinsing and elution solvents.
   2. Dimensions of the bed (V_M or bed holdup volume and N, number of plates of the bed).
   3. Volume of sample to load, V_L; volume of rinsing solvent, V_{RS}; and volume of elution solvent, V_E.

(b) Critical parameters (optimization parameters)
   1. Maximum volume of sample and of rinsing solvent that can be passed through the SPE bed without losses of analyte, $V_L^{max} + V_{RS}^{max}$.
   2. Minimum volume of rinsing solvent that should be passed to eliminate completely interferences, V_{RS}^{min}.
   3. Minimum volume of elution solvent that should be passed to elute completely the analyte, V_E^{min}.
   4. Maximum volume of elution solvent that can be passed without eluting additional interferences, V_E^{max}.

The isolation will be successful if a sufficient sample volume can be loaded and at least one of the two following conditions is fulfilled:

1. $V_{RS}^{min} < V_{RS}^{max}$.
2. $V_E^{min} < V_E^{max}$.

2.2. Basic equations

The two equations that relate the breakthrough volumes, V_B, to the basic properties of analytes and SPE beds are as follows:

$$V_B = (1 + k_s)V_M\left(1 - \frac{2.3}{\sqrt{N}}\right) \quad (1)$$

$$V_B = \frac{1}{\sqrt{a_0 + a_1/N + a_2/N^2}}(1 + k_s)V_M \quad (2)$$

where N is the number of plates, V_M the holdup or dead volume, and k_s is the chromatographic retention factor of the analyte in the liquid sample loaded onto the SPE bed. Eq. (1) gives the breakthrough volume at the 1% breakthrough level and applies to systems in which the conditions of linear chromatography apply and the plate number is large enough. Eq. (2) was proposed by Lövkvist and Jönsson and applies to systems with a small number of plates. The coefficients a_0, a_1 and a_2 are characteristic of the breakthrough level. The values for a_0, a_1 and a_2 can be found in [9].

In a similar form, V_E, the elution volume required for the quantitative recovery (99%) of an analyte is given by the following equation:

$$V_E = (1 + k_E)V_M\left(1 + \frac{2.3}{\sqrt{N}}\right) \quad (3)$$

where k_E is the retention factor of the analyte in the elution solvent.

The relationship between the retention factor and the solid–liquid distribution coefficient, K, is

$$k = \frac{C_s}{C_l}\frac{m_s}{V_l} = K\phi \quad (4)$$

where C_s is the concentration (per mass of sorbent) of analyte in the sorbent, m_s the mass of sorbent in the SPE bed, C_l the concentration of analyte in the liquid sample in contact with the sorbent, and V_l is the volume of liquid in the SPE bed.

2.3. Modeling critical parameters

The critical parameters for a given SPE system can be estimated from data on solid–liquid distribution coefficients, V_M, Φ and N.

V_L^{max} is whatever sample volume smaller than or equal to V_{B1} and higher than or equal to V_L, where V_{B1} is the breakthrough volume of analyte in the liquid sample matrix, calculated through Eqs. (1) and (2) and V_L is the volume of

sample loaded; V_{RS}^{max} can be then estimated with the following equation:

$$V_{RS}^{max} = V_{B2}\left(1 - \frac{V_L^{max}}{V_{B1}}\right) \quad (5)$$

where V_{B2} is the breakthrough volume of analyte in the rinsing solvent, equally calculated through Eqs. (1) and (2). $V_{RS}^{min} = V_{E1}$ and $V_E^{min} = V_{E2}$, where V_{E1} and V_{E2} are the elution volume of the interference and of the analyte in the rinsing and elution solvents, respectively. These elution volumes are calculated through Eq. (3). Finally, $V_E^{max} = V_{B3}$, where V_{B3} is the breakthrough volume of the interference in the elution solvent.

2.4. Algorithm for SPE method optimization

The three-step proposed algorithm is shown in Fig. 1.

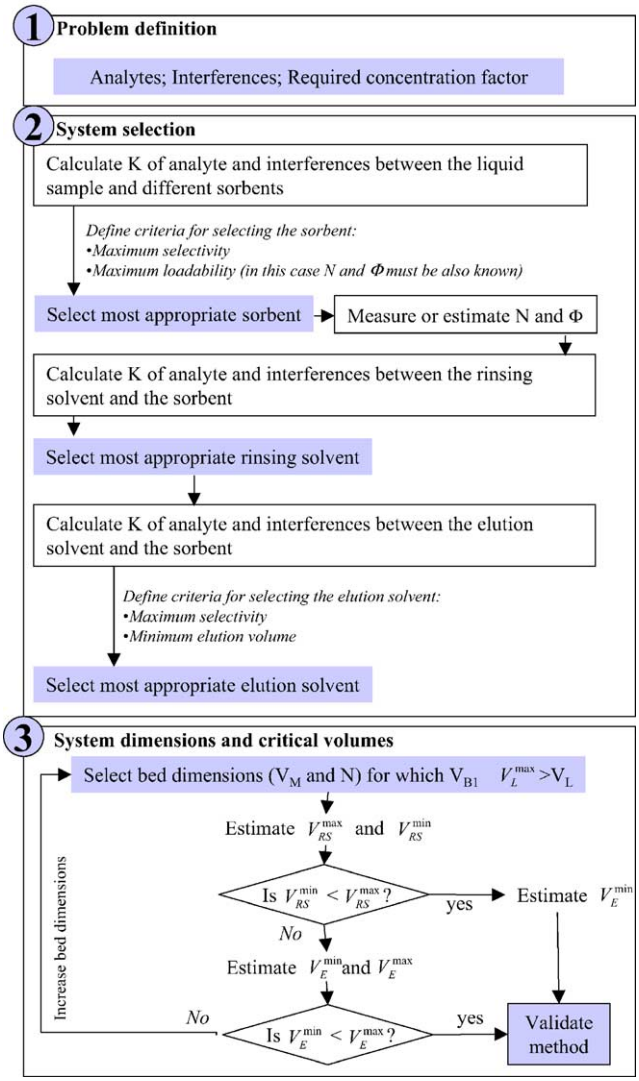

Fig. 1. Proposed algorithm for SPE method optimization.

3. Experimental

3.1. Solvents, sorbents and standards

Dichloromethane (HPLC quality) was from Fisher Scientific (Loughborough, UK), methanol (HPLC grade) was purchased from Lab-Scan (Dublin, Ireland), diethyl ether (analytical reagent grade) from Fluka (Buchs, Switzerland), and pentane 95% (Pestipur grade from SDS (Peypen, France). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Bond Elut LMS, C_{18} and ENV were from Varian (Harbor City, CA, USA), LiChrolut-EN was from Merck (Darmstadt, Germany), Isolute ENV+ was from IST (Mid Glamorgan, UK) and Discovery C_{18} was from Supelco (Bellefonte, PA, USA).

The chemical standards used for quantitative analysis were purchased from Aldrich–España (Madrid, Spain), Fluka or Lancaster. The internal standard solution contained 2-octanol (sol A at 1000 μg/ml in ethanol and solution B at 49 μg/ml in dichloromethane).

3.2. Determination of the distribution coefficients of solid–liquid systems

An exact mass of the sorbent (0.12 g) was placed inside a glass vial, together with a volume (50 ml in the case of wine and rinsing solvents and 2 ml in the case of elution solvents) of solvent or wine sample containing 2 mg/l of the selected compounds (10 mg/l in the case of elution solvents). The vials were shaken softly for 24 h. After this, and in the case of non-organic solvents, 10 ml of the liquid were transferred to a 15 ml centrifuge tube containing 3.3 g $(NH_4)_2SO_4$, plus 20 μl of the Internal Standard solution A and 0.5 ml of dichloromethane. The tubes were closed, shaken gently for 45 min, centrifuged, and the organic phases analyzed by GC–flame ionization detection (FID) (wine major compounds: acetaldehyde, ethyl acetate, isoamyl alcohol, β-phenylethanol, hexanol, hexanoic acid and diethyl succinate) or GC–MS (rest of compounds). In the case of elution solvents, the organic phases were directly analyzed after the addition of the internal standard (solution B). Relative areas were interpolated in calibration graphs, built by the analysis of solutions (wine, rinsing solvents or elution solvents) containing known amounts of volatile compounds. All the experiments were duplicated.

3.3. Determination of phase ratios, Φ, holdup volumes, V_M, and number of plates, N, of the SPE systems

Phase ratio and holdup volumes were directly measured by weighting the chromatographic beds before (m_0) and after (m_1) the addition of the necessary amount of mobile phase (whose density, ρ, was calculated) to form the bed, and after (m_2) the expulsion of interstitial liquid by a flow of air.

- Volume of mobile phase in pores: $(m_2 - m_0)/\rho \approx V_p$ (pore volume).

- Volume of interstitial mobile phase: $(m_1 - m_2)/\rho \approx V_M$ (holdup volume).
- The phase ratio, Φ, is then $\Phi = m_0/(V_M + V_p)$.

The number of plates, N, was estimated from the breakthrough curves built in frontal elution experiments as described below. The graphic procedure for its determination is described in [5].

3.4. Determination of breakthrough volumes and breakthrough curves

Two hundred milligrams cartridges were conditioned with 2 ml of methanol and 4 ml of water. The sample (wine spiked with selected compounds) was then passed through the bed at 2 ml/min. The eluent was collected in 10 ml (0–100 first ml), 20 ml (100–400 second ml), or 50 ml (400–700 ml) fractions. The fractions were then extracted with dichloromethane and analyzed by GC–FID or GC–MS as has been previously explained. Breakthrough volumes were determined as the volumes at which a given percent of mass of analyte is eluted out of the column.

In the case of rinsing and elution solvents, analytes were placed directly into the cartridge by applying 20 μl of a standard solution in ethanol. For rinsing solvents, fractions were 2 ml (0–10 ml), 5 ml (10–50) or 10 ml (50–150 ml) in volume. For elution solvents, fractions were 0.2 ml (0–2 ml) or 0.5 ml in volume. Fractions were analyzed by GC–FID of GC–MS.

3.5. Proposed method for the analysis of higher aliphatic lactones in wine

Prepacked cartridges (3 ml total volume) filled with 200 mg Bond Elut-ENV resins were placed in the extraction system (Vac Elut 20 station from Varian, CA, USA) and conditioned by rinsing with 2 ml of methanol and 4 ml of water. Fifty milliliters of wine were passed through the SPE cartridge at 2 ml/min. The bed was then washed with 5 ml of water, and the interferences were removed with 20 ml of a mixture of methanol-water 40:60 (v/v) enriched with 1% (w/v) $NaHCO_3$. The cleaned cartridge was dried by letting air pass through (30 min). Analytes were recovered by elution with 1.8 ml of dichloromethane. This volume, placed in a centrifuge test tube was spiked with 100 μl of the internal standard solution B, and was further concentrated to 0.15 ml in a water bath at 47 °C. The volume was finally transferred to a micro-vial, sealed and stored at −20 °C until analysis.

Calibration graphs were prepared by the GC–MS analysis of dichloromethane solutions containing known amounts of the standards and of the internal standard.

3.6. Method validation

Method reproducibility, linearity and existence of matrix effects were studied following standard procedures.

Table 1
Chemical standards and MS fragments used for quantitative analysis

Analyte	Supplier	Purity (%)	Quantitative fragments *m/z*
trans-Whiskylactone	Aldrich	98	99
cis-Whiskylactone	Aldrich	98	99
γ-Octalactone	Aldrich	97	85
γ-Nonalactone	Aldrich	97	85
γ-Decalactone	Fluka	>97	85
δ-Decalactone	Lancaster	>97	99
γ-Undecalactone	Fluka	>97	85
γ-Dodecalactone	Aldrich	97	85

3.7. GC–FID

A Hewlett-Packard 5890 Series II fitted to a 7673 HP autosampler was used. The column was a DB-20 (50 m × 0.32 mm and 0.5 μm film thickness) from J&W (Folsom, CA, USA) and was preceded by a 2 m × 0.53 mm uncoated precolumn. The initial temperature was 40 °C held for 5 min and then raised at 5 °C/min up to 190°. The carrier gas was H_2 at 3 ml/min. The injection was performed in splitless mode, the splitless time was 120 s, the split flow was 30 ml/min and the injection volume was 3 μl.

3.8. GC–MS

A Varian CP-3800 gas chromatograph fitted to a Saturn 2000 ion trap mass spectrometer from Varian was used. The column was a DB-WAXetr, 60 m × 0.25 mm and 0.25 μm film thickness from J&W preceded by a 2 m × 0.53 mm uncoated precolumn. The carrier gas was He at 1 ml/min (electronic flow control mode). The initial column temperature was 40 °C, held 5 min, and then raised to 200 °C at 2 °C/min. The transfer line temperature was 220 °C. The injection was carried out automatically in a programmed temperature vaporizer (PTV) injector in splitless mode. During splitless time, a pressure pulse of 40.0 psi (1 psi = 6894.76 Pa)was applied to force flow to 2.5 ml/min. The volume injected was 1 μl. A *m/z* 35–200 mass range was recorded in full scan mode, and the extracted ion chromatogams described in Table 1 were taken for quantitation. The area of the corresponding ionic peaks was normalized by that of the internal standard and was interpolated in a calibration graph built by the analysis of standard solutions in dichloromethane. The results were corrected by the corresponding recovery given in Table 7.

4. Results and discussion

4.1. Relationship between K, V_B, V_{RS} and V_E

The first question that should be addressed is if solid–liquid distribution coefficients can be used to calculate breakthrough, rinsing and elution volumes. In a previous

Table 2
Solid–liquid distribution coefficients and calculated and measured breakthrough volumes (5% level) of a selected group of compounds between wine and two sorbents

	Bond Elut-ENV			LiChrolut-EN		
	K	V_{B1} calculated[a]	V_{B1} measured	K	V_{B1} calculated[a]	V_{B1} measured
Acetaldehyde	1	0	0	3	0	0
Ethyl acetate	10	1	5	35	2	5
Isoamyl alcohol	32	2	5	88	6	5
cis-3-Hexenol	79	5	10	247	16	15
β-Phenylethanol	108	7	15	441	28	30
Hexanol	198	12	30	607	39	40
Hexanoic acid	227	14	35	954	61	50
Diethyl succinate	599	37	40	1337	86	80
Ethyl furoate	647	39	80	1375	88	90
Ethyl butyrate	1200	73	95	2235	143	150
Ethyl vanillate	1423	87	115	2930	188	200
4-Vinylguaiacol	1649	100	110	3391	218	210
Ethyl 2-methylbutyrate	2000	122	165	3235	208	200
trans-Whiskylactone	2003	122	160	4240	272	300
cis-Whiskylactone	2073	126	160	5274	338	300
Ethyl 3-methylbutyrate	2618	159	165	3430	220	220
γ-Nonalactone	2696	164	190	2871	184	200
Isoamyl acetate	2994	182	190	4357	280	250
Eugenol	3380	205	200	9508	610	600
δ-Decalactone	3536	215	220	10000	641	650
Octanoic acid	4348	264	300	9367	601	600
Ethyl octanoate	7055	429	450	10932	701	650
2-Isobutyl-3-methoxypyrazine	7558	459	450	17096	1096	>700
β-Damascenone	37307	2267	>700	60000	3848	>700

[a] The following data were used. In the case of Bond Elut-ENV: $\Phi = 0.45$, $V_M = 0.18$, $N = 7$. In the case of LiChrolut-EN: $\Phi = 0.57$, $V_M = 0.15$, $N = 7$. In both cases, the mass of sorbent was 200 mg packed in a 3 ml standard SPE reservoir.

paper [22], a good relationship between K and V_B was obtained. Here, we have studied such relationship for a larger number of analytes and for two new-generation polymeric sorbents. The results of such study are given in Table 2. The K of all these compounds were determined in a single experiment, and the corresponding breakthrough volumes were calculated following Eq. (2) and measured as explained in the experimental section. As can be seen, the agreement between calculated and measured V_B volumes is fairly good (for the regression of calculated versus measured $r^2 = 0.99$ in both cases, with slopes 0.99 ± 0.05 and 1.02 ± 0.03, respectively) which can be considered satisfactory for the purpose of method optimization.

The calculation of V_B from K requires the determination of some important additional parameters, such as the phase ratio as defined in Eq. (4), the number of plates of the SPE bed, and the holdup volume of the system. The exact measure of all these parameters is difficult, but good approximated holdup volumes and phase ratios can be obtained by the simple method described in the experimental section. The footnote of Table 2 shows those measured values. The determination of the number of plates of the system is a little bit more time-consuming, but our data suggest that similar sorbents have a roughly similar number of plates. In the case of the sorbents studied in Table 2, the number of plates vary from 4 to 15 plates for a 1 cm long bed for the different analytes and sorbents, with 7 plates/cm as average. Differences between sorbents are not very large and, therefore, an exact knowledge of N should not be necessary for comparative purposes.

Solid–liquid distribution coefficients can also be used to calculate rinsing and elution volumes as is shown in Table 3. In this case, the liquid–solid distribution coefficients have been determined for a small group of compounds and a sorbent system (200 mg Bond Elut-ENV from Varian) in two different solvents. The first one is a water–methanol (60:40, v/v) mixture which could be a rinsing solvent. The second one is pentane, typically used in the elution step. The corresponding rinsing or elution volumes have been calculated from K using Eq. (3) and correspond to the concepts called V_{RS}^{min} or V_E^{min}. Breakthrough volumes have been calculated with Eq. (2) and in this context correspond to the concept of V_{B2} in the case of the rinsing solvent or V_E^{max} in the case of the elution solvent.

The values used for N, Φ and V_M were those previously determined. Results in Table 3 show that the similarity between the calculated and measured values is acceptable ($r^2 > 0.99$ in the case of the methanol–water rinsing solvent and >0.97 in the case of pentane; slopes are 1.00 ± 0.04, 0.95 ± 0.05, 1.05 ± 0.09 and 1.27 ± 0.12, respectively), which reinforces the use of K as a practical tool for SPE method optimization and also suggests that the simplified method to calculate bed parameters (N, Φ and V_M) give results accurate enough for our purposes. The poorer results

Table 3
Solid–liquid distribution coefficients and calculated and measured volumes of rinsing and elution solvent of a selected group of volatile compounds between Bond Elut-ENV and two different solvent systems[a]

	K	Methanol–water 40%				K	Pentane 100%			
		Rinsing volumes (V_{RS}^{min})		Breakthrough volumes (V_{B2})			Elution volumes (V_{E}^{min})		Breakthrough volumes (V_{E}^{max})	
		Calculated	Measured	Calculated	Measured		Calculated	Measured	Calculated	Measured
Isoamyl alcohol	25	4	<5	2	<2	1.8	0.6	0.9	0.2	0.2
Phenol	35	6	5	2	<2	14	2.5	2.5	1.0	0.8
cis-3-Hexenol	42	7	5	3	<2	3.1	0.8	0.8	0.3	0.2
β-Phenylethanol	95	15	15	6	4	8.3	1.6	1.5	0.6	0.4
Guaiacol	100	15	10	6	5	6	1.2	1.2	0.5	0.4
Hexanol	160	25	25	10	9	2.4	0.7	0.7	0.3	0.2
Hexanoic acid	200	31	30	12	12	24	4.0	4.2	1.6	1.2
Ethyl furoate	460	70	70	28	25	3.3	0.8	0.9	0.3	0.2
4-Vinylguaiacol	580	88	80	35	35	3.9	0.9	1.1	0.4	0.4
Diethyl succinate	360	55	50	22	25	3	0.8	0.7	0.3	0.2
Eugenol	370	56	60	23	25	4.7	1.0	1.1	0.4	0.2
Ethyl vanillate	490	75	70	30	25	22	3.7	3.4	1.5	1.2
trans-Whiskylactone	870	132	130	53	50	1.2	0.5	0.9	0.2	0.2
Ethyl 2-methylbutyrate	720	109	110	44	45	0.6	0.4	0.7	0.2	0.2
β-Damascenone	1530	232	>150	93	100	0.9	0.5	0.7	0.2	0.2

[a] The following data were used: $\Phi = 0.45$, $V_M = 0.18$, $N = 7$. In all cases, the mass of sorbent was 200 mg packed in a 3 ml standard SPE reservoir.

of the modeling with pentane, are partly due to difficulty in measuring small elution and breakthrough volumes and to possible particle swelling when organic solvents are used.

4.2. Use of the model

Data in Table 3 also show that the selectivity of both the rinsing and the elution solvents can be adequately exploited to achieve a complete separation between groups of compounds or between analytes and interferences. For instance, rinsing the SPE cartridge with 5 ml of the methanol–water mixture would remove 99% of isoamyl alcohol (since $V_{RS}^{min} = 4$ ml) without appreciable losses of most of the other analytes (since $V_{B2} > 4$ ml in all cases except for the three first compounds). On the other hand, β-damascenone could be eluted from the SPE bed with a small volume of elution solvent ($V_{E}^{min} = 0.5$–0.7 ml) while most of the hexanoic acid would remain in the column ($V_{E}^{max} = 1.2$–1.6 ml). As an example, data in Tables 2 and 3 can be used to show how the 3rd part (see Fig. 1) of the proposed model works. Let us take as analytes β-damascenone and eugenol, and as interferences isoamyl alcohol and hexanoic acid. Let the concentration factor to be 100, which implies that the volume of sample which must be processed should be 100 times higher than the minimum volume which can be handled (0.1 ml), i.e., 10 ml. Bond Elut-ENV resins will be considered in the example. The high K of our analytes (>35,000 and 3380) indicate that a small 50 mg bed packed in a 1 ml standard reservoir (internal diameter 5.5 mm; $V_M = 0.045$ ml, $N = 7$) should provide breakthrough volumes (V_{B1} calculated through Eq. (1)) higher than 500 ml (β-damascenone) and 51.5 ml (eugenol). Fixing V_L as 20 ml and V_L^{max} as 30 ml, the following estimations can be made:

(i) V_{B2} (Eq. (2)) = 23.3 ml (β-damascenone) and 5.67 ml (eugenol).
(ii) V_{RS}^{max} (Eq. (5)) = 21.6 ml (β-damascenone) and 2.37 ml (eugenol).
(iii) V_{RS}^{min} (Eq. (3)) = 1.03 ml (isoamyl alcohol) and 7.7 ml (hexanoic acid).
(iv) V_{E}^{min} (Eq. (3)) = 0.12 ml (β-damascenone) and 0.26 ml (eugenol).
(v) V_{E}^{max} (Eq. (2)) = 0.06 ml (isoamyl alcohol) and 0.40 ml (hexanoic acid).

These data suggest that β-damascenone can be easily isolated from both interferences by rinsing with 8 ml of the water–ethanol mixture. On the contrary, this procedure will not work for eugenol, since its V_{RS}^{max} is exceeded. However, it can also be seen that a procedure combining the selectivity of rinsing and elution steps could be successful. A rinsing with 2 ml of the water–ethanol mixture would eliminate isoamyl alcohol, and a selective elution with 0.4 ml of pentane should prevent the elution of hexanoic acid. The experimental results obtained (*R*: β-damascenone = 95%; eugenol = 85%; isoamyl alcohol = 1%; hexanoicacid = 2%), confirmed the results.

4.3. Application: development of an optimized SPE–GC–MS method for the determination of aliphatic lactones from wine

The objective of the isolation method is to provide extracts coming from at least 50 ml of wine (desired concentration factor: 200–500) containing more than 80% of wine aliphatic lactones, and the minimum possible amount of wine major compounds (isoamyl alcohol, β-phenylethanol

Table 4
Average solid–liquid distribution coefficients of analytes and potential interferences between wine and different solid sorbents

	IST-ENV	LiChrolut-EN	Bond Elut-ENV	Bond Elut-LMS	VAR-C18	DSC-C18
Major interferences						
Isoamyl Alcohol	63	88	31	31	3	10
β-Phenylethanol	366	441	108	98	9	15
Hexanoic acid	657	954	227	229	27	33
Analytes						
trans-Whiskylactone	4259	4240	2003	1476	98	87
cis-Whiskylactone	5126	5274	2073	1569	78	62
γ-Octalactone	2240	2178	1912	1205	69	70
γ-Nonalactone	2630	2871	2696	2317	99	164
γ-Decalactone	12000	10000	7882	10003	410	435
δ-Decalactone	3130	3950	3536	3162	308	348
γ-Undecalactone	5785	4390	5257	4879	793	805
γ-Dodecalactone	2480	2671	2567	2223	1905	1379
Selectivity (vs. isoamyl alcohol)						
Average α	74.7	50.5	113	108	157	41.9
Minimum α	35.6	24.8	61.7	38.9	23.0	6.2
Selectivity (vs. β-phenylethanol)						
Average α	12.9	10.1	32.3	34.2	52.2	27.9
Minimum α	6.1	4.9	17.7	12.3	7.7	4.1
Selectivity (vs. hexanoic acid)						
Average α	7.2	4.7	15.4	14.6	17.4	12.7
Minimum α	3.4	2.3	8.4	5.3	2.6	1.9

Study of the selectivity provided by different sorbents.

and fatty acids). According to the proposed model (Fig. 1, step 2) and in order to select the best sorbent, the *K* of analytes and potential interferences between wine and different solid sorbents were determined. The results of such experiment are shown in Table 4. Results in the table clearly show that organic polymeric resins are far more efficient than silica based sorbents. Among the organic resins, LiChrolut-EN from Merck and IST-ENV from IST have an extraordinary extraction capacity and are, therefore, highly recommended sorbents for the extraction of polar compounds from wine or for the production of non-selective extracts. However, when it comes to selectivity, data at the bottom of Table 4 show that Bond Elut-ENV from Varian present maximum selectivity versus the three major compounds which, in wine, constitute ubiquitous interferences. These last resins have been accordingly selected for the subsequent method optimization.

The following step was to identify the rinsing solvent which also provides better selectivity. This was done by comparing the *K* between the selected resins and different water–methanol or water–acetone mixtures for a selected group of analytes. The results of this study are shown in Table 5. As can be seen, the best selectivity is achieved with the methanol-water (40:60) enriched with $NaHCO_3$ which was selected as rinsing solvent. A similar study was also carried out to choose the best elution solvent. Different elution solvent or solvent mixtures were tested (pentane; dichloromethane; and the mixtures pentane-dichloromethane (9/1) and pentane–diethyl ether (9/1)). Such results (data not shown) indicated that none of the solvent tested could provide enough selectivity to isolate the analytes from the interferences. Consequently, dichloromethane which showed maximum strength, was selected as elution solvent.

As for the dimensions of the system, the following calculations can be done:

1. A 200 mg cartridge seems to be a good choice, since the estimated breakthrough volume for the least retained analyte (γ-octalactone) is 116 ml. In this case, $V_L = 50$ ml and V_L^{max} can be fixed at 60 ml.
2. For such analyte $V_{B2} = 43$ (Table 5), and $V_{RS}^{max} = 21$ ml (Eq. (5)). This volume is bigger than V_{RS}^{min} of interferences, which implies that a complete separation should be possible. The volume of rinsing solvent can be fixed as 20 ml.
3. The final elution volume was experimentally fixed as 1.8 ml of dichloromethane.

Fig. 2 shows the chromatogram obtained in the GC–MS analysis of a wine spiked with 10 μg/l of analytes following the proposed procedure. The selectivity provided by the developed isolation procedure is enough to get a good MS signal. The reproducibility of the proposed method is given in Table 6. Average R.S.D. values range from 2.4 to 5.4, with 3.5 as typical value, which can be considered satisfactory for the levels at which the compounds are found in wine. The linearity obtained in the GC–MS analysis of dichloromethane solutions (concentration referred to analyte content in wine) is given in Table 6 and can also be seen that is satisfactory, and covers the normal range of

Table 5
Solid–liquid distribution coefficients of several analytes between Bond Elut-ENV resins and different rinsing mixtures

	Methanol 40%, K	Methanol 30%, K	Methanol 20%, K	Acetone 20%, K	Methanol 40% + 1% $NaHCO_3$		
					K	V_{RS}^{min}	V_{B2}
Major interferences							
Isoamyl alcohol	25	60	120	25	22	4	
β-Phenylethanol	95	245	350	99	89	14	
Hexanoic acid	200	510	890	310	10	2	
Analytes							
trans-Whiskylactone	870	3700	5264	510	760		46
cis-Whiskylactone	910	3750	5420	509	782		48
γ-Octalactone	754	1882	2460	315	708		43
γ-Nonalactone	840	3318	4960	509	758		46
γ-Decalactone	1480	5730	7193	903	1207		73
δ-Decalactone	1230	4712	7002	696	1098		67
γ-Undecalactone	1406	5430	7660	841	1205		73
γ-Dodecalactone	1089	4356	6428	670	907		55
Selectivity							
Average α	10.1	15.1	12.8	4.3	23.0		
Minimum α	3.8	3.7	2.8	1.0	8.0		

Elution (V_R^{min}) and breakthrough volumes (V_{B2}) calculated for the 40% methanol/water–$NaHCO_3$ mixture (for a 200 mg cartridge).

occurrence of these compounds in wine. Recovery experiments were also carried out on two different wines and the results of the experiment are given in Table 7. Results in that table show that recoveries are relatively constant, do not depend on the wine and at range from 77 to 86%. The major part of such losses take place during the concentration of the extract and its subsequent transference to the vial, as it was demonstrated by evaporating standard solutions (data not shown). Method detection limits were estimated by the analysis of real samples and the results, shown in Table 7, corresponds to the concentration at which the signal-to-noise ratio becomes 3. In all cases, this detection limit is well below the odor threshold of the compound (shown in Table 8), which guarantees that the method can be used to determine these odor active compounds in wine.

Results from the analysis of these compounds in wine are presented in Table 8. To our knowledge, it is the first time that the wine content in γ-octalactone, γ-undecalactone and γ-dodecalactone is reported, data in the table show that, leaving aside whiskylactones, which are well-known compounds released from oak wood, γ-nonalactone, γ-decalactone and γ-dodecalactone can be present at concentrations above their corresponding odor thresholds, although this only happens in aged red wines. γ-Octalactone can also be present at a

Table 7
Overall method recovery and method detection limits

	Recovery (%)		Average recovery (%)	DL
	Red wine	White wine		
trans-Whiskylactone	85.4 ± 2.4	86.0 ± 0.4	85.7	2.25
cis-Whiskylactone	83.6 ± 5.6	82.5 ± 1.3	83.1	0.47
γ-Octalactone	78.9 ± 0.6	80.1 ± 3.5	79.5	0.14
γ-Nonalactone	84.6 ± 2.5	79.4 ± 2.7	82.0	0.60
γ-Decalactone	79.8 ± 4.8	75.0 ± 2.7	77.4	0.22
δ-Decalactone	87.5 ± 6.1	84.9 ± 1.6	86.2	0.58
γ-Undecalactone	81.4 ± 1.4	81.0 ± 2.8	81.2	0.11
γ-Dodecalactone	81.2 ± 1.0	82.3 ± 4.7	81.8	0.30

DL: detection limits in μg/l.

Table 6
Method reproducibility and GC–MS linearity

	Red wine		White wine		Average R.S.D. (%)	Linearity	
	Mean (μg/l)	R.S.D. (%)	Mean (μg/l)	R.S.D. (%)		r^2	Range (μg/l)
trans-Whiskylactone	5.86	5.04	6.77	0.68	3.6	0.9974	1.0–200
cis-Whiskylactone	8.38	7.31	12.5	2.20	5.4	0.9974	1.0–200
γ-Octalactone	5.36	1.42	5.91	4.62	3.4	0.9968	0.1–20.8
γ-Nonalactone	20.3	2.82	13.5	4.24	3.6	0.9930	1.0–60
γ-Decalactone	5.83	6.09	6.8	2.67	4.7	0.9978	0.2–20.8
δ-Decalactone	8.21	7.46	1.38	0.81	5.3	0.9922	0.4–41.4
γ-Undecalactone	6.02	2.05	6.85	2.75	2.4	0.9977	0.1–20.9
γ-Dodecalactone	5.19	2.44	6.29	2.75	2.6	0.9959	0.2–20.3

Reproducibility was measured as R.S.D. (%) for the triplicate analysis of two different spiked wine samples. Linearity was measured with standard solutions.

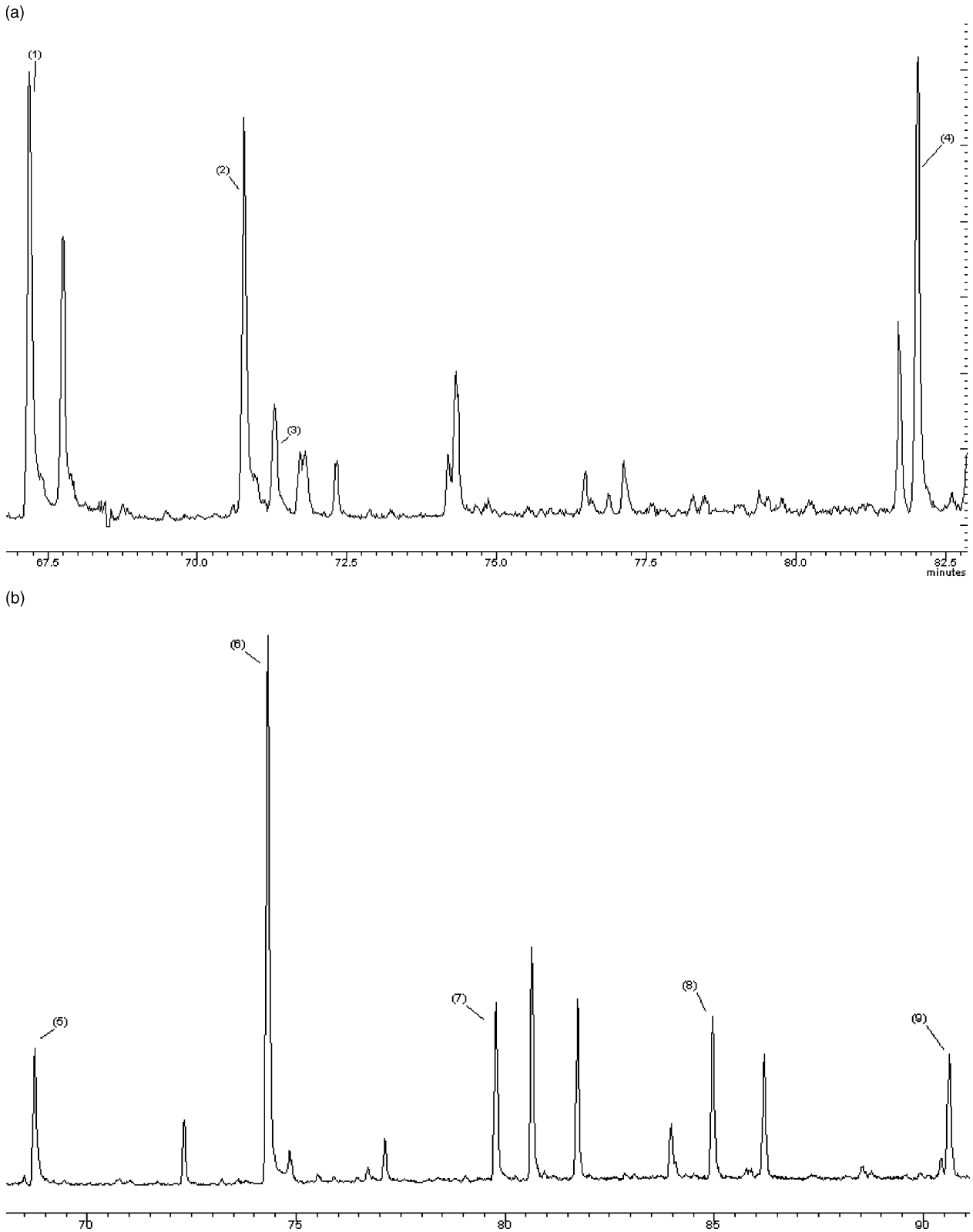

Fig. 2. (a) Ion chromatogram (*m*/*z* 99) from a dichlomethane extract obtained from a red wine spiked with 10 μg/ml of analytes. Peak identification: 1, *trans*-whiskylactone; 2, *cis*-whiskylactone; 3, δ-octalactone; 4, δ-decalactone. (b) Ion chromatogram (*m*/*z* 85) from a dichlomethane extract obtained from a red wine spiked with 10 μg/ml of analytes. Peak identification: 5, γ-octalactone; 6, γ-nonalactone; 7, γ-decalactone; 8, γ-undecalactone; 9, γ-dodecalactone.

Table 8
Quantitative analysis of wines (all data given in μg/l)

	Threshold (μg/l) [27]	Aged red ($n = 5$)			White wine ($n = 4$)			Young red ($n = 4$)		
		Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum
trans-Whiskylactone	69	74.5	22.6	158	0.9	0.0	3.7	1.5	1.0	2.4
cis-Whiskylactone	28	205	64.9	386	1.6	0.0	6.5	0.0	0.0	0.0
γ-Octalactone	7	2.3	1.4	4.6	0.0	0.0	0.0	1.4	1.3	1.6
γ-Nonalactone	25	13.4	3.7	27.0	5.9	2.2	9.6	10.2	6.1	16.4
γ-Decalactone	0.7	0.5	0.0	1.5	0.1	0.0	0.4	0.2	0.1	0.3
δ-Decalactone	100	0.7	0.0	3.3	13.2	0.0	52.7	0.0	0.0	0.0
γ-Undecalactone	60	1.2	0.0	5.7	0.0	0.0	0.0	0.0	0.0	0.0
γ-Dodecalactone	7	4.6	0.7	17.7	0.0	0.0	0.0	1.6	0.4	2.5

concentration relatively close to its odor threshold in some of these wines. The table shows that, in general, red wines are richer than whites in these compounds which suggests that these compounds can be important odorants of aged red wines.

5. Conclusion

Solid–liquid distribution coefficients can be used to predict and model retention, rinsing and elution properties of SPE beds with a relatively low effort and a reasonable accuracy. This can help to optimize SPE isolation strategies, as it has been shown for aliphatic lactones from wine. In this case, the SPE method has allowed to get clean extracts free from wine major compounds, which facilitates the accurate GC–MS analysis of analytes present at trace levels.

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