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Fast fractionation of complex organic extracts by normal-phase chromatography on a solid-phase extraction polymeric sorbent Optimization of a method to fractionate wine flavor extracts

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Abstract

Some properties of LiChrolut-EN resins as normal-phase sorbent have been studied. Retention factors using pentane as solvent range from less than 2 (ethyl esters) to more than 56 (fatty acids and vanillin). All retention factors were smaller than 2 with dichloromethane. The efficiency of the bed was between 4 and 13 plates per cm. A method for the fast fractionation of wine flavor extracts has been further developed. Wine (75 ml) is extracted on a 0.5 g LiChrolut-EN bed. Volatile compounds are recovered in 5 ml of dichloromethane and the extract is further concentrated to 0.1 ml. Recoveries of the extraction procedure are above 85% for all compounds less polar than isoamyl alcohol. This extract is fractionated on a bed (5.0 cm height, 0.6 cm internal diameter) packed with 0.55 g of LiChrolut-EN resins. A first fraction is collected by the elution with 4 ml of pentane. A second one with 6 ml of a mixture pentane/dichloromethane (9:1) and a final fraction with 4 ml of dichloromethane. The first fraction is enriched in ethyl esters and some other non-polar compounds. The second fraction concentrates the alcohols and some volatile phenols, while the third is enriched in fatty acids, vanillin derivatives and some lactones. The recovery in the fractionation is complete. The profile obtained in the fractionation is very stable, and becomes distorted only when the column is loaded with an extract containing 80 mg of major volatiles (coming from more than 150 ml of wine). The fractionation of extracts from different wines showed that the performance of the process does not depend on the composition of the extract. Twenty-seven out of 32 studied compounds eluted reproducibly mainly in one fraction. The results suggest that the method can be applied as an aid for qualitative or quantitative analysis to any kind of organic extract as an alternative to liquid chromatography on silica-gel.

Keywords: Fractionation; Wine; Flavor analysis; Solid-phase extraction; Polymeric sorbents; Styrene–divinylbenzene

1. Introduction

The analysis of trace organic components requires highly selective and efficient enrichment steps since, very often, the selectivity and sensitivity provided by the gas chromatography–mass spectrometry (GC–MS) system are not enough. One of the most important enrichment strategies is based on the fractionation of organic extracts by normal-phase chromatography on silica [1]. The unique selectivity provided by silica, together with its excellent chromatographic properties, explain why column liquid chromatography

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on silica-gel is still frequently used as enrichment or cleanup step in very different fields of organic analysis. In environmental analysis, for instance, it constitutes the basis of several routine methods for the cleanup and separation of organochlorine compounds [2–5]. In flavor analysis it is frequently used for a class separation of flavor compounds since it was first proposed in 1968 [6]. Common applications include the fractionation of extracts for qualitative analysis [7,8] or for the quantitative determination of trace components [9,10]. Silica has, however, several limitations. These are the need to control its activity, its relatively low sample capacity, high analysis times, high solvent consumption, and the occurrence of irreversible adsorption and catalytic degradation of sensitive analytes. While some of these problems can be overcome by the use of high pressure systems [11,12], some others may require the use of alternative sorbents [13,14]. In the case of flavor chemistry, for instance, researchers use columns containing more than 10 g of silica for class separation of complex extracts, which takes several hours [7,8,10]. A micromethod proposed in 1996 [9] carries out the prefractionation in a 0.5 g silica prepacked cartridge in less than 30 min, but the amount of sample that can be loaded is very small, and mass overload occurs if the method is applied to extracts enriched in polar compounds highly retained in silica.

Polymeric sorbents, such as styrene–divinylbenzene copolymers, were introduced in the seventies for the extraction of organic compounds from water [15] and as gas chromatography stationary phases. The first polymers suffered from several limitations which prevent their use in all those cases in which silica-derived sorbents worked properly. They were used mainly for the extraction of polar compounds from aqueous media [15] or for the extraction of non-polar compounds from hydroalcoholic media such as wine [16–19].

However, it has not been until 1995 that polymeric sorbents have become popular as solid-phase extraction sorbents [20]. Since then, different manufacturers are offering a range of products with improved extraction and chromatographic properties, and they are today a frequent choice for reversed-mode extraction, particularly for polar analytes [21]. Being completely organic materials, the number and activity of active sites are highly reduced in comparison to those found in silica [22]. In addition, they have a greater sample capacity. All these characteristics, together with the observation that polymeric sorbents show, in general, a certain ability to retain compounds in normal mode [17,23], have aroused our interest in these materials in the search of substitutes for silica as stationary phase in the fractionation of organic extracts.

The main aims of the present paper are to determine the general applicability of a new generation styrene–divinylbenzene polymer to the fractionation of organic extracts and to develop a specific method for the fractionation of extracts from wine or other alcoholic beverages.

2. Material and methods

2.1. Materials

2.1.1. Solvents

Dichloromethane HPLC-quality was purchased from Fischer Scientific (Loughborough, UK), methanol HPLC-grade was from Lab-Scan (Dublin, Republic of Ireland), and pentane 95% “Pestipur” was purchased from SDS (Peypen, France). Water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). The solid sorbent was LiChrolut-EN resins purchased from Merck (Darmstadt, Germany). The pure chemical standards used to identify and quantify the aroma compounds were purchased from Aldrich (Madrid, Spain), Sigma (St. Louis, USA), Fluka (Buchs, Switzerland), Poly Sciences (Niles, USA), Lancaster (Strasbourg, France), ChemService (West Chester, USA), Interchim (Motluçon, France). β-Damascenone was a gift from Firmenich (Geneva, Switzerland). All the wines used in the development of this work were dry wines from different areas of Spain. The internal standard solution was 2-octanol in dichloromethane (1200 μg/ml).

2.2. Preparation of extracts from wine

An extraction procedure providing an extract containing nearly 100% of volatile compounds from wine less polar than isoamyl alcohol has been developed. Seventy-five milliliter of wine were passed through a SPE bed formed by 0.5 g of LiChrolut-EN resins packed in a 3-ml standard SPE reservoir. The SPE bed was previously washed with 10 ml of

dichloromethane, dried with air, and further conditioned, first with 5 ml of methanol and, finally with 10 ml of a 10% water–ethanol mixture. The wine was percolated at 5 ml/min with the help of vacuum. The extraction was carried out in an automated SPE station from Varian. After the sample was loaded, the SPE bed was rinsed with 10 ml of water. The bed was then dried and volatile compounds were finally eluted with 5 ml of dichloromethane. The extract was concentrated, first in a microKuderna-Danish concentrator up to 0.5 ml and finally the volume was reduced to 0.1 ml under a stream of 99.999% nitrogen. A recovery experiment was carried out to measure the amount of volatile material recovered in this procedure.

2.3. Fractionation of the extracts

The chromatographic bed was formed in a 1-ml (5.5 cm length, 0.6 mm internal diameter) standard SPE polypropylene reservoir supplied by Supelco-España (Madrid, Spain). A frit was introduced at the bottom of the reservoir and 0.55 g of dry LiChrolut-EN resins were then introduced into the reservoir to form the bed (5.0 cm height). The resins were washed with 5 ml of methanol and 5 ml of dichloromethane. After this, the bed was dried by applying vacuum. An extract from wine obtained as described previously (around 0.1 ml) was then carefully applied. The extract was applied drop by drop, letting them evaporate before applying the next drop to avoid the extract penetrating into the bed. After this, a first fraction was recovered by elution with 4 ml of pentane. A small volume of air was passed through the column to force the pentane to run out of the column. A second fraction was obtained by applying 6 ml of a mixture pentane/dichloromethane (9:1) in a similar way. The third fraction was obtained with 4 ml of dichloromethane. If necessary, the fractions were concentrated in a microKuderna-Danish concentrator up to a final volume of 0.5 ml. Eight microliter of the internal standard solution were added to each fraction.

2.4. Optimization and validation of the fractionation method

2.4.1. Optimization

Different sorbent masses (from 0.20 to 0.55 g) and solvent mixtures (pentane, diethyl ether and dichloromethane) were tested with both synthetic solutions and wine extracts. In all cases, fractions were collected from the eluate and, after the addition of the internal standard (2-octanol) and a concentration by solvent evaporation up to 0.5 ml, the fractions were analyzed by GC–MS. The chromatograms reconstructed from the analysis of the fractions were used to determine retention volumes and chromatographic efficiency of the LiChrolut-EN beds. Dead volumes were measured in a HPLC system, in which the LiChrolut-EN bed was the column.

2.4.2. Maximum mass load

In order to determine the maximum mass of extract that can be loaded onto the SPE column, volumes of extract, obtained in all cases as described previously but coming from increasing volumes of an aged red wine (75, 100, 150 and 200 ml), were loaded on the SPE column, fractionated following the proposed procedure, concentrated, and analyzed by GC–MS.

2.4.3. Recovery

The exact mass of volatile compound found in each of the fractions obtained in the previous experiment, was compared with the mass present in the original extract. Masses were determined by means of calibration graphs built with dichloromethane solutions containing known amounts of volatile compounds and a fixed mass of the internal standard.

2.4.4. Reproducibility and matrix effects

Five different wines (white wine from Macabeo, 13% ethanol; white wine from Chardonnay, 12% ethanol; young Grenache red wine, 13% ethanol; 5-year-old wine from Rioja, 12.4% ethanol; sweet white wine from the Canary Islands, 11.5% ethanol and 30 g/l sugar) were extracted as described before. The extracts were fractionated following the proposed procedure, and the fractions, concentrated and analyzed by GC–MS.

2.5. Gas chromatography–mass spectrometry conditions

A Star 3400CX gas chromatograph fitted to a Saturn 4 electronic impact ion trap mass spectrometer from Varian was used. The column was a DB-WAX from J&W (Folsom, USA), 60 m × 0.25 mm with

0.5 μm film thickness, and was preceded by a 3 m × 0.32 mm uncoated (deactivated, intermediate polarity) precolumn. The carrier was He at 1 ml/min. The chromatographic oven was initially 40 °C for 5 min, and then was raised to 230 °C at 2 °C/min. A 1093 septum-equipped programmable injector (SPI) injector from Varian was used. The initial temperature of this injector was 30 °C for 0.5 min, and was then raised to 230 °C at 200 °C/min. A 35–200 *m/z* mass range was recorded, and the ion peaks described in Table 1 were taken for quantitation. Identification of compounds was carried out by comparison of chromatographic and mass spectral data with those of authentic standards.

3. Results and discussion

3.1. LiChrolut-EN resins as normal-phase sorbents

LiChrolut-EN resins were selected because they have shown an excellent ability to retain volatile compounds [24]. When a solution containing a mixture of volatiles with different structures and polarities was chromatographied on a small bed (3.5 cm height, 0.6 cm internal diameter) of LiChrolut-EN resins using pentane as elution solvent, the results obtained were those shown in Fig. 1 and in Table 1. Retention factors for ethyl esters of fatty acids were in all cases around 1 and these compound elute the first. On the other hand, fatty acids and phenolic aldehydes, such as vanillin, were so strongly retained that they could not be eluted with pentane even after more than 56 dead volumes were passed through the column. Fortunately, these compounds could be easily recovered in a small volume (3 ml) of dichloromethane. The rest of studied compounds showed an intermediate retention between these two extremes. The elution order is then as follows:

ethyl esters < β-damascenone < diethyl succinate
< benzaldehyde < c-3-hexenol = alcohols
< furfural < guaiacol ≪ fatty acids
= vanillin derivatives

Compared to silica, the elution order can be considered quite similar, although aromatic structures are delayed and alcohols are less retained in this polymeric sorbent.

As for the chromatographic efficiency of the bed, it can be seen in Fig. 1 that, using a purely manual technique for the introduction of the sample, the bed provided from 4 to 13 theoretical plates per cm. This figure is slightly worse than that provided by the silica-gel 60 beds typically used for the fractionation of organic extracts, as expected from the difference in bed particle sizes. However, on the whole, the chromatographic properties of this sorbent seem to be adequate for the purpose of the fractionation of organic extracts. The elution profile shown in Fig. 1 indicates that, with a short bed (3–5 cm), two or three different fractions could be obtained with pentane, plus at least one more with dichloromethane. This result encouraged further research to develop a method for the fast fractionation of wine flavor extracts.

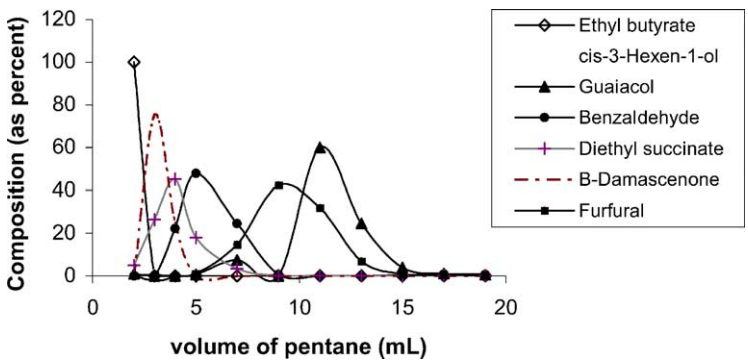

Fig. 1. Reconstructed chromatogram obtained from the analysis of the fractions eluted from a column (3.5 cm height, 0.6 cm internal diameter) packed with 0.3 g of LiChrolut-EN resins and using pentane as elution solvent.

Table 1
Quantitative *m/z* fragments used in the GC–MS analysis of the fractions

Compounds	*m/z*	Recovery (%)	$K_{pentane}$	$K_{dichloromethane}$
Ethyl butyrate	TIC	95	<2	<2
Ethyl isobutyrate	TIC	92	<2	<2
Ethyl 2-metylbutyrate	57 + 74	98	<2	<2
Ethyl hexanoate	TIC	100	<2	<2
Ethyl octanoate	TIC	102	<2	<2
Ethyl decanoate	157 + 200	101	<2	<2
Isoamyl acetate	70	99	<2	<2
Hexyl acetate	56	100	<2	<2
Isobutanol	TIC	42	18	<2
1-Hexanol	69	97	19	<2
cis-3-Hexenol	TIC	101	21	<2
Isoamyl alcohol	TIC	67	19	<2
β-Phenylethanol	TIC	88	48	<2
Guaiacol	109 + 124	91	35	<2
4-Ethylguaiacol	137	99	19	<2
4-Vinylguaiacol	137 + 150	100	47	<2
Furfural	95	40	25	<2
5-Methylfurfural	109	96	31	<2
Benzaldehyde	77 + 105	94	13	<2
Ethyl lactate	TIC	48	19	<2
Diethyl succinate	TIC	99	10	<2
Unknown norisoprenoid	192		7	<2
β-Damascenone	121	100	7	<2
(*E*)-Whiskeylactone	99	101	48	<2
(*Z*)-Whiskeylactone	99	102	48	<2
Hexanoic acid	TIC	92	>56	<2
Octanoic acid	TIC	101	>56	<2
Decanoic acid	129	98	>56	<2
Benzyl alcohol	108	89	>56	<2
γ-Nonalactone	85	101	>56	<2
4-Ethylphenol	107 + 122	96	>56	<2
Vanillin	151 + 152	94	>56	<2
Methyl vanillate	151 + 182	101	>56	<2
Acetovanillone	151 + 166	102	>56	<2

Recovery of volatile compounds in the proposed method of extraction. Retention factors of volatile compounds in LiChrolut-EN resins obtained using pentane and dichloromethane as elution solvents.

3.2. Wine extraction

The developed extraction method makes use also of LiChrolut-EN resins. In the proposed conditions, the recovery of most of the volatile compounds from wine is almost total, as it can be seen in Table 1. Only some highly soluble compounds, such as isobutanol, ethyl lactate or furfural, are not completely extracted. Recoveries for major wine volatiles such as isoamyl alcohol and β-phenylethanol are 67 and 88%, respectively. This implies that an extract from 75 ml of wine contains between 15 mg (white wines) and 50 mg (aged red wines) of major wine volatiles (fusel alcohols, ethyl lactate and diethyl succinate) and that after concentration, the extract can be a solution of minor volatiles in fusel alcohols containing a low proportion of dichloromethane.

3.3. Optimization of the fractionation method

Wine extracts were fractionated in short beds of LiChrolut-EN resins using different solvents or solvent mixtures. The study indicated that the best results are obtained if a series of solvents of increasing strength is used. The series pentane–pentane/dichloromethane (9:1)–dichloromethane showed the best results, since

alcohols were concentrated in the second fraction, while fatty acids were found in the third. When diethyl ether was used instead of dichloromethane, the second fraction contained both acids and alcohols. The fractionation of the volatile compounds present in the wine extract using the series pentane–pentane/dichloromethane (9:1)–dichloromethane is shown in Table 2. This table confirms some of the observations previously made. Pentane only elutes ethyl esters and some other non-polar compounds, the mixture pentane/dichloromethane (9:1) elutes alcohols, phenols and some other compounds of intermediate polarity, while the third fraction will contain vanillin derivatives and acids. It can also be observed that some compounds, such as β-phenylethanol, are poorly chromatographied and appear in several fractions. The results also suggest that an optimal fractionation will be obtained eluting a first fraction with 4 ml of pentane, the second with 6 ml of the mixture pentane/dichloromethane (9:1) and a last fraction with 4 ml of dichloromethane.

4. Method validation

The critical quality parameters in a fractionation method are the recoveries obtained in the process, the precision in the composition of the fractions and the robustness of the separation. Recoveries are shown in Table 3. It can be seen that in all cases they are near

Table 2
Composition (as percent of the total area eluted) of fractions eluted using the series of solvents: pentane–pentane/dichloromethane (9:1)–dichloromethane

Compounds	Pentane (ml)			Pentane/dichloromethane (9:1) (ml)						Dichloromethane (ml)		
	0–3	3–4	4–5	0–3	3–4	4–5	5–6	6–7	7–8	0–2	2–3	3–4
Ethyl butyrate	100	0	0	0	0	0	0	0	0	0	0	0
Ethyl 2-methylbutyrate	90	10	0	0	0	0	0	0	0	0	0	0
Ethyl hexanoate	85	15	0	0	0	0	0	0	0	0	0	0
Ethyl octanoate	44	56	0	0	0	0	0	0	0	0	0	0
Ethyl decanoate	50	45	5	0	0	0	0	0	0	0	0	0
Isoamyl acetate	91	9	0	0	0	0	0	0	0	0	0	0
Isobutanol	0	0	5	56	39	0	0	0	0	0	0	0
1-Hexanol	0	0	0	37	63	0	0	0	0	0	0	0
cis-3-Hexen-1-ol	0	0	0	41	55	3	1	0	0	0	0	0
Isoamyl alcohol	1	1	2	47	47	3	0	0	0	0	0	0
β-Phenylethanol	0	0	0	1	11	6	11	5	27	25	12	0
Guaiacol	0	0	0	17	64	6	2	0	10	0	0	0
4-Ethylguaiacol	1	0	0	0	86	7	3	1	1	1	0	0
4-Vinylguaiacol	0	0	0	0	100	0	0	0	0	0	0	0
Furfural	4	0	1	34	53	4	1	1	1	1	0	0
5-Methylfurfural	0	0	0	4	96	0	0	0	0	0	0	0
Benzaldehyde	12	4	4	0	13	4	4	4	4	3	48	0
Ethyl lactate	1	2	7	67	22	2	1	0	0	0	0	0
Diethyl succinate	13	2	5	58	23	1	0	0	0	0	0	0
Unknown norisoprenoid	0	0	0	0	0	19	0	0	41	0	39	0
(*E*)-Whiskeylactone	0	0	0	0	0	0	0	0	61	0	39	0
(*Z*)-Whiskeylactone	2	0	0	0	75	11	6	1	4	0	0	0
γ-Nonalactone	0	0	0	0	0	0	0	0	37	63	0	0
Hexanoic acid	0	0	0	0	0	0	0	0	21	0	79	0
Octanoic acid	0	0	0	0	0	0	0	0	0	100	0	0
Decanoic acid	0	0	0	0	0	0	0	0	0	0	100	0
Benzyl alcohol	0	0	0	0	1	1	2	1	27	49	19	0
4-Ethylphenol	0	0	0	0	0	0	0	0	0	35	65	0
Vanillin	0	0	0	0	0	0	0	0	0	13	87	0
Methyl vanillate	0	0	0	0	0	0	0	0	0	3	97	0

The chromatographic bed was 5 cm × 0.6 mm internal diameter packed with 0.55 g of LiChrolut-EN resins.

Table 3
Recoveries obtained in the fractionation procedure

Compounds	Recovery	±s
Ethyl butyrate	106	8
Ethyl isobutyrate	94	7
Ethyl 2-metylbutyrate	100	3
Ethyl hexanoate	102	5
Ethyl octanoate	103	8
Ethyl decanoate	99	4
Isoamyl acetate	103	6
Isobutanol	95	10
1-Hexanol	97	3
cis-3-Hexenol	97	2
Isoamyl alcohol	98	3
β-Phenylethanol	104	7
Guaiacol	95	10
4-Ethylguaiacol	101	5
Furfural	100	4
5-Methylfurfural	94	7
Ethyl lactate	96	9
Diethyl succinate	103	9
β-Damascenone	99	6
(*E*)-Whiskylactone	101	2
(*Z*)-Whiskylactone	102	5
Hexanoic acid	105	4
Octanoic acid	106	11
Decanoic acid	95	9
Benzyl alcohol	102	8
4-Ethylphenol	103	6
Vanillin	105	7
Methyl vanillate	96	7
Acetovanillone	104	4

Results indicate the quotient (as percent) between the summation of the masses found in the three fractions and the mass of compound originally present in the extract. Results are the average of four experiments in which different masses of extract were loaded onto the cartridge.

100% and they did not change with the mass of extract fractionated. This means that neither irreversible sorption nor catalytic degradation of compounds in the sorbent take place, and ensures that the method can be used as an aid for both qualitative or quantitative analysis.

With regard to the robustness of the separation, it is particularly important to ensure that small variations in the composition of the extract to be fractionated do not change the pattern of the fractionation. This effect is more likely to happen if the amount of extract loaded is very close to the point of mass saturation. In order to study both the maximum mass of extract that can be loaded and the robustness of the separation, two different experiences were carried out. In the first one, increasing volumes of an extract from the same aged red wine were fractionated following the proposed procedure. In a second experience, extracts from different wines were fractionated.

The effect of the mass of the extract on the profile of the fractionation is shown in Table 4. It can be observed that increasing the mass by a factor 2 (loading about 80 mg of major volatiles) does not bring about important changes in the pattern of fractionation. Fraction one becomes slightly enriched with alcohols (11–12%) and diethyl esters (31%), while the effect on fractions 2 and 3 is almost negligible. On the contrary, the fractionation of a mass of extract coming from 200 ml of wine (containing an estimated amount of 106 mg of major volatiles) provokes, the elution of a part of the acids in the second fraction. This results made us think that, if the bed is loaded with an extract coming from 75 ml of wine (which will have a maximum amount of 50 mg of major volatiles), the pattern of fractionation should be independent from the absolute composition of the extract.

This was confirmed by the fractionation of extracts obtained from different wines. The results of that experiment can be seen in Table 5, while typical GC–MS chromatograms corresponding to the three fractions are shown in Fig. 2. Table 5 gives the average distribution (as percent) of the compounds in the three fractions, and the standard deviation of such values. The first fraction (Fig. 2A) contains nearly 100% of the least polar compounds of the aroma and is virtually free from the rest of compounds. It only contains a small percentage of isobutanol, (*E*)-whiskeylactone and of β-damascenone. The second fraction (Fig. 2B) is enriched in alcohols and phenols, and is virtually free from acids and ethyl esters. Finally, fraction 3 contains fatty acids, vanillin derivatives and is free from alcohols, which are major compounds in all the extracts from alcoholic beverages. There are several compounds that do not elute in a single fraction. 4-Vinylguaicol and whiskey lactones appear in similar proportions in fractions 2 and 3. In these three cases, there is a large imprecision in the exact proportion eluting in a given fraction. Three more compounds: β-phenyl ethanol, guaiacol and ethyl lactate can also be found at significant concentrations in fractions 2 and 3, but in these three other cases, particularly in the case of ethyl lactate, the fractionation profile

Table 4
Effect of the mass of extract (as volume of wine from which it comes from) on the profile of the fractions obtained following the proposed procedure

Compounds	75 ml wine (40 mg of major volatiles)			100 ml wine (53 mg of major volatiles)			150 ml wine (80 mg of major volatiles)			200 ml wine (106 mg of major volatiles)		
	F1	F2	F3	F1	F2	F3	F1	F2	F3	F1	F2	F3
Ethyl butyrate	94	6	0	97	3	0	98	2	0	90	10	0
Ethyl isobutyrate	100	0	0	100	0	0	100	0	0	100	0	0
Ethyl 2-methylbutyrate	100	0	0	100	0	0	100	0	0	98	2	0
Ethyl hexanoate	96	4	0	99	1	0	100	0	0	97	3	0
Ethyl octanoate	93	7	0	99	1	0	99	1	0	96	3	0
Ethyl decanoate	93	7	0	98	2	0	99	1	0	97	3	0
Isoamyl acetate	92	8	0	98	2	0	99	1	0	95	5	0
Hexyl acetate	88	12	0	96	4	0	100	0	0	94	6	0
Isobutanol	6	92	2	8	92	0	6	94	0	17	83	0
1-Hexanol	0	95	5	5	94	1	12	87	1	16	84	0
cis-3-Hexen-1-ol	1	94	5	8	91	1	12	87	1	14	86	1
Isoamyl alcohol	0	97	3	5	94	1	11	88	1	15	85	0
β-Phenylethanol	1	35	64	3	53	43	6	51	43	2	53	45
Guaiacol	0	88	12	0	94	6	0	93	7	0	100	0
4-Ethylguaiacol	0	94	6	2	97	1	4	95	1	3	68	30
4-Vinylguaiacol	9	60	31	6	37	57	16	31	53	4	47	48
Furfural	0	94	6	0	99	1	0	97	3	0	99	0
5-Methylfurfural	0	88	12	0	96	4	0	93	7	0	99	1
Benzaldehyde	1	96	3	6	88	6	6	92	2	6	89	5
Ethyl lactate	0	97	3	10	90	0	21	79	0	22	78	0
Diethyl succinate	0	94	6	15	77	7	31	62	7	26	68	6
Unknown norisoprenoid	0	100	0	0	100	0	0	100	0	40	60	0
β-Damascenone	0	100	0	0	100	0	0	100	0	43	57	0
(*Z*)-Whiskeylactone	0	73	27	0	88	12	1	84	15	2	94	4
(*E*)-Whiskeylactone	0	85	15	1	93	6	3	90	7	3	96	1
γ-Nonalactone	0	100	0	0	100	0	0	100	0	0	100	0
Hexanoic acid	0	0	100	0	0	100	0	0	100	0	20	80
Octanoic acid	0	0	100	0	0	100	0	1	99	1	33	66
Decanoic acid	0	0	100	0	0	100	0	0	100	0	33	67
Benzyl alcohol	0	1	99	0	2	98	0	2	98	0	4	96
4-Ethylphenol	0	1	99	0	0	100	0	0	100	0	1	99
Vanillin	0	0	100	0	0	100	0	0	100	0	0	100
Methyl vanillate	0	0	100	0	0	100	0	0	100	0	0	100

The chromatographic bed was 5 cm × 0.6 mm internal diameter packed with 0.55 g of LiChrolut-EN resins. The wine was an aged red wine containing 267 mg/l isoamyl alcohol, 104 mg/l isobutanol, 95 mg/l β-phenyl ethanol, 260 mg/l ethyl lactate, 72 mg/l diethyl succinate and 26 mg/l fatty acids (C4–C12) and their ethyl esters. F1: first fraction, F2: second fraction, F3: third fraction.

is highly repetitive and not dependent on the composition of the extract. Leaving aside these cases, the profile obtained in the fractionation is very stable and independent on the exact composition of the fractionated extract. The proposed procedure can be used, therefore, not only as a tool to simplify the chromatogram and help with peak identification, but as an aid in the development of quantitative procedures.

In conclusion, the proposed procedure allows for a fast, reproducible, and robust fractionation of flavor extracts. The fractionation of a given extract can be carried out in 20 min (8 min for the application of the extract and 12 min for fraction collection) and only standard SPE material is needed. The dilution induced by the process is not very high and, in any case, the solvents used are easy to evaporate. Although the retention and selectivity properties of this sorbent are

Table 5
Reproducibility of the proposed method of fractionation

Compounds	F1		F2		F3	
	$\bar{X}$ (%)	s (%)	$\bar{X}$ (%)	s (%)	$\bar{X}$ (%)	s (%)
Ethyl butyrate	96	2	4	2	0	0
Ethyl isobutyrate	100	0	0	0	0	0
Ethyl 2-methylbutyrate	100	1	0	0	0	0
Ethyl hexanoate	97	2	3	2	0	0
Ethyl octanoate	96	2	4	3	0	0
Ethyl decanoate	96	3	4	3	0	0
Isoamyl acetate	95	4	5	4	0	0
Isobutanol	6	1	93	2	1	1
1-Hexanol	0	0	95	3	4	3
cis-3-Hexen-1-ol	0	0	96	3	4	3
Isoamyl alcohol	0	0	96	2	4	2
β-Phenyl ethanol	2	1	15	12	84	12
Guaiacol	0	0	85	10	13	10
4-Ethylguaiacol	0	0	95	6	4	6
4-Vinylguaiacol	0	1	30	44	70	49
Furfural	0	0	96	4	3	46
5-Methylfurfural	0	0	93	6	6	6
Benzaldehyde	3	6	97	6	0	0
Ethyl lactate	0	0	86	3	13	3
Diethyl succinate	3	3	96	3	1	0
Unknown norisoprenoid	4	7	96	7	0	0
β-Damascenone	8	13	92	13	0	0
(*E*)-Whiskeylactone	2	6	60	29	38	23
(*Z*)-Whiskeylactone	0	0	64	16	36	16
Hexanoic acid	0	0	0	0	100	0
Octanoic acid	0	0	0	0	100	0
Decanoic acid	0	0	0	0	100	0
Benzyl alcohol	0	0	0	0	100	0
4-Ethylphenol	0	0	0	0	100	0
Vanillin	0	0	0	0	100	0
Methyl vanillate	0	0	0	0	100	0
Acetovanillone	0	0	0	0	100	0

Average composition and standard deviation (as percent of the total mass eluted) of the three fractions obtained in the fractionation of five different wines following the proposed method. F1: first fraction, F2: second fraction, F3: third fraction.

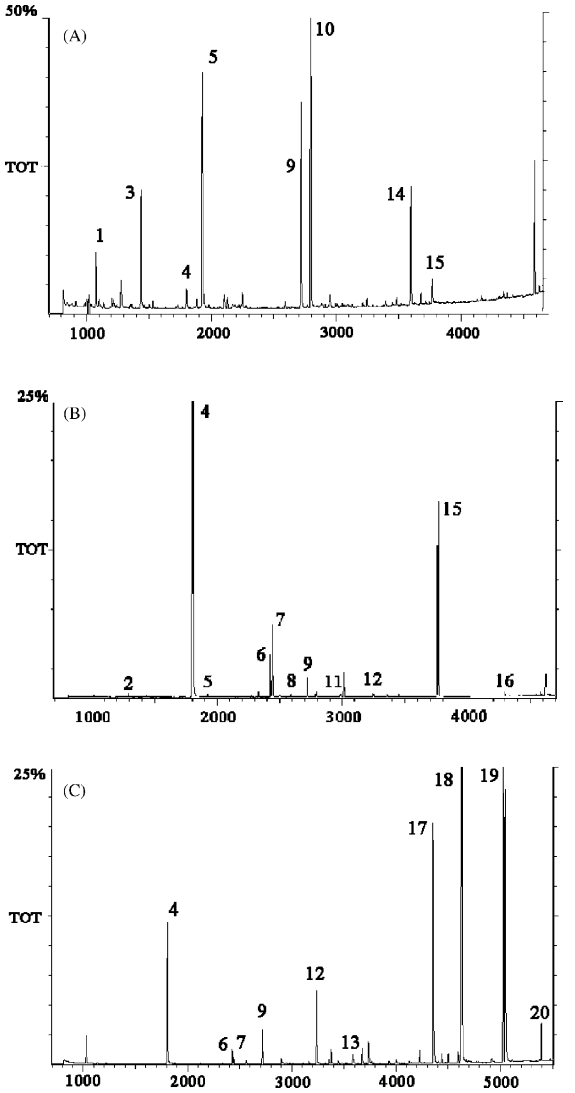

Fig. 2. Chromatograms from the GC–MS analysis of the three fractions obtained in the fractionation of a Spanish aged red wine: (A) first fraction; (B) second fraction; (C) third fraction. Peak identification: 1, ethyl butyrate; 2, isobutanol; 3, isoamyl acetate; 4, isoamyl alcohol; 5, ethyl hexanoate; 6, ethyl lactate; 7, 1-hexanol; 8, *cis*-3-hexen-1-ol; 9, 2-octanol (IS); 10, ethyl octanoate; 11, furfural; 12, benzaldehyde; 13, butyric acid; 14, ethyl decanoate; 15, diethyl succinate; 16, β-damascenone; 17, hexanoic acid; 18, β-phenylethanol; 19, octanoic acid; 20, decanoic acid.

worse than those of bare silica, its better simplicity and reproducibility, together with a decreased risk of irreversible adsorption or degradation of labile solutes, on the whole makes it possible to consider the proposed method to be a valid alternative to classical fractionation on silica. In addition, a comparison of the maximum masses of extract which can be loaded, shows that the sample capacity of these resins (80 mg of major wine volatiles) is at least eight times higher than that of silica (less than 10 mg of major wine volatiles) [9]. Although in the present work the interest has been focused on the fractionation of flavor extracts, it is thought that the proposed method will be of general applicability in the analysis of complex organic samples.

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