Dispersive liquid-liquid microextraction and gas chromatography accurate mass spectrometry for extraction and non-targeted profiling of volatile and semi-volatile compounds in grape marc distillates

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Abstract

The suitability of dispersive liquid-liquid microextraction (DLLME) and gas chromatography accurate mass spectrometry (GC-MS), based on a time-of-flight (TOF) MS analyzer and using electron ionization (EI), for the characterization of volatile and semi-volatile profiles of grape marc distillates (grappa) are evaluated. DLLME conditions are optimized with a selection of compounds, from different chemical families, present in the distillate spirit. Under final working conditions, 2.5 mL of sample and 0.5 mL of organic solvents are consumed in the sample preparation process. The absolute extraction efficiencies ranged from 30 to 100%, depending on the compound. For the same sample volume, DLLME provided higher responses than solid-phase microextraction (SPME) for most of the model compounds. The GC-EI-TOF-MS records of grappa samples were processed using a data mining non-targeted search algorithm. In this way, chromatographic peaks and accurate EI-MS spectra of sample components were linked. The identities of more than 140 of these components are proposed from comparison of their accurate spectra with those in a low resolution EI-MS database, accurate masses of most intense fragment ions of known structure, and available chromatographic retention index. The use of chromatographic and spectral data, associated to the set of components mined from different grappa samples, for multivariate analysis purposes is also illustrated in the study.

Keywords: Volatile compounds profiling; Grape marc distillates; Dispersive liquid-liquid microextraction; Gas chromatography time-of-flight mass spectrometry

1. Introduction

Grape marc distillates (usually named as *grappa*) are alcoholic beverages elaborated in several European wine producing countries [1]. Production of quality distillates has a significant economic interest, as complementary activity to wine elaboration [1]. Grappa contains hundreds of volatile and semi-volatile species, either coming from seeds and peels of grape marc or generated during the fermentation of the pomace [2]. These compounds are responsible for the organoleptic properties, and thus the quality, of the spirits. In addition, they serve as markers of grape variety and maturation state at harvest, geographic origin, storage conditions of grape pomace and /or distillation techniques [3, 4]. Most of grappa volatile components are amenable to gas chromatography (GC) analysis. In fact, the combination of GC with mass spectrometry (MS) is the preferred technique for their characterization [5, 6].

Solid-phase microextraction (SPME) is the most utilized sample preparation technique for GC determination of volatile and semi-volatile compounds in distillate samples [7-9]. SPME conditions frequently involve the use of mixed-sorbents fibers and headspace (HS) extractions, at temperatures in the range between 25 and 55 °C, during a period of 15-60 min [5,7-9]. The applicability to a large number of compounds, the null consumption of organic solvents and automation easiness, if a dedicated SPME autosampler is available, are the principal advantages of the SPME technique [10]. However, less volatile and high water soluble compounds, still amenable to GC analysis, are difficult to extract in the HS SPME mode. Consequently, there is a demand of alternative sample preparation approaches enable to couple with the extraction of a wide set of volatile, semi-volatile, polar and non-polar organic compounds from distillates samples. Simplified methods combining low cost, reduced solvent consumption, high enrichment factors and sample throughput, without the need of dedicated instrumentation, have gained interest. Dispersive liquid-liquid microextraction (DLLME) fits most of the above premises being a technique in constant

evolution, with an increasing number of application fields, from its introduction by Rezaee et al. [11]. DLLME has been used for the extraction of insecticides in honey liqueur [12] and, more recently, for the extraction of phthalate esters in distillates [13]. However, to the best of our knowledge, its suitability for profiling the volatile and semi-volatile compounds of distillate spirits has not been explored yet.

Chemical profiling of minor volatile and semi-volatile compounds in distillates, without a foregoing selection of the compounds of interest, is a characteristic example of non-targeted analysis. Responses for chromatographic entities can be used for geographic and botanic origin discrimination purposes [14, 15]. The accurate mass and full scan high sensitivity features of time-of-flight (TOF) MS systems, following GC separation, have been demonstrated as exceptionally useful for target and non-targeted determination of a very broad range of minor compounds (natural products and contaminants) in complex matrices [10, 16-20], with relevant advantages in terms of sensitivity and selectivity versus unit mass resolution techniques, such as GC quadrupole MS. In combination with electron ionization (EI), GC-TOF-MS provides characteristic fingerprints for any compound recovered from the spirits during sample preparation. The larger the number of compounds, the higher the latent information existing in the full scan GC-EI-TOF-MS records. On the other hand, managing such high number of chromatographic peaks (quite often partially overlapped) and ions requires the use of semi-automated software tools in order to drawn information of usefulness for characterization and/or discrimination of distillate samples.

The aim of this work was to investigate the suitability of the DLLME sample preparation technique, followed by GC-TOF-MS analysis, for the profiling of volatile and semi-volatile organic compounds in grappa distillate samples. Following optimization of DLLME conditions, the responses measured for a selection of compounds were compared to those provided by SPME, as an indirect indicative of the efficiency of the proposed extraction process. Thereafter, identification of major and minor compounds

in the DLLME GC-TOF-MS records was carried out. Finally, a preliminary evaluation of chromatographic profiles to discriminate different distillates by using the *molecular features* extracted from samples is presented.

2. Experimental

2.1. Solvents and sorbents

Methanol and acetonitrile (HPLC grade) were obtained from Merck (Darmstadt, Germany). Pesticide grade acetone, chloroform ($CHCl_3$) and carbon tetrachloride (CCl_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride (NaCl) was acquired from Merck. A standard of 4-decanol (97%) was purchased from Alfa Aesar (Kandel, Germany). This compound was employed as internal surrogate (IS), added to grappa distillates samples before starting the sample preparation process, in some of the performed extractions. A mixture of n-alkanes (C_8-C_{40}) in dichloromethane, provided by Supelco (Bellefonte, PA, USA), was used to calculate the linear retention index (LRI) of compounds in GC-TOF-MS records. Ultrapure water was obtained from a Milli-Q Gradient A-10 system (Millipore, Bedford, MA, USA).

A manual SPME holder and fibers coated with divinylbenzene-carboxen-poly (dimethylsiloxane) (DVB/CAR/PDMS, 50/30 μm film thickness, 1 cm length) and PDMS/DVB (65 μm film thickness, 1 cm length) were obtained from Supelco (Bellefone, PA, USA).

2.2. Samples and sample preparation

Grappa samples were either provided by wine makers from Galicia (North West Spain) or purchased in retail markets. Information regarding the grape variety and distillation technique (direct or steam distillation) was obtained from producers and/or labels on commercial samples. After reception, grappas were stored at room temperature, in capped glass bottles, protected from light with aluminum foil. Table S1 shows the features of the distillates used in the current research.

Under optimized conditions, DLLME extractions were performed in 10 mL volume, conical-shaped bottom glass tubes containing a 2.5 mL aliquot of grappa spiked with the IS (0.1 mL of a 10 μg mL^{-1} acetone solution), 6.5 mL of ultrapure water and 1 g of NaCl. Then, 0.5 mL of the extraction solution, consisting in 0.4 mL of acetonitrile and 0.1 mL of $CHCl_3$, was added using a gas tight syringe. Tubes were capped, shaken for 1 min and centrifuged for 5 min (room temperature) at 3500 rpm. After removing most of the upper aqueous phase, the $CHCl_3$ settled extract (0.047 ± 0.002 mL) was recovered and transferred to an insert for injection in the GC-QTOF-MS system.

SPME extractions were carried out in 20 mL volume glass vessels containing the same sample solution (2.5 mL of distillate, 6.5 mL of water and 1 g of NaCl) as DLLME tubes, plus a stir bar. Tubes were closed with a Teflon-lined septum and an aluminum cap. Extractions were carried at under different conditions (fiber coating, sampling mode and temperature) for 30 min. The SPME fibers were thermally desorbed in the hot splitless injector of the GC-MS system.

2.3. Determination conditions

Compounds were determined using a GC-QTOF-MS instrument, comprised of a 7890A gas chromatograph and a 7200 QTOF mass spectrometer, both acquired from Agilent (Wilmington, DE, USA). The QTOF system was equipped with an electron ionization (EI) source and operated in the single MS mode, at 2 GHz acquisition frequency. Accurate EI-MS scan spectra were recorded every 0.2 s (2715 transients per spectrum), in the centroid mode, between 40 and 650 *m/z* units. The mass axis of the TOF mass analyzer was re-calibrated every three injections using a commercial solution of perfluorotributylamine (PFTBA). Under the above conditions, mass resolution varied from 4600, at *m/z* 69, to 9100, at *m/z* 414. The transfer line and the EI source were set at 250 and 230°C, respectively. The *Mass Hunter* software (B.08 version, Agilent) was employed to control all instrumental parameters in the GC-TOF-

MS. The *Find by Molecular Feature (FMF)* function, integrated in the above software package, was used to mine the different *molecular features* (species) from raw GC-TOF-MS records.

Compounds were separated in a DB-WAXETR column (30m × 0.25 mm i.d., 0.5 µm Carbowax-type film thickness) acquired from Agilent. Helium was used as carrier gas at a constant flow of 1.2 mL min^{-1}. The column temperature was programed as follows: 60°C (1 min), rated at 4°C min^{-1} to 220°C, and finally at 20°C min^{-1} to 240°C with a hold time of 5 min. The total analysis time was 47 min. Injections (2 µL) were made in the splitless mode with the injection chamber set at 260°C. The splitless time and the split flow were 1 min and 60 mL min^{-1}, respectively. The solvent delay was fixed at 5.5 min. One injection of organic solvent ($CHCl_3$) was made between replicates of samples to prevent column contamination with low volatile species. SPME fibers were desorbed for 2 min, with the injection chamber maintained at 260 ºC.

2.4. Compounds identification

A non-targeted search of compounds existing in the GC-EI-TOF-MS chromatograms of distillates was carried out using the FMF function. This algorithm groups those ions appearing at the same retention time, considering also that mass differences among them are compatible with a given empirical formula. The software reports a list of molecular entities for which retention time, deconvoluted spectrum and chromatographic signal intensity (area or height) are available. Spectra from molecular entities can be compared with any available EI-MS database. In our case, the low resolution NIST 2017 library was used.

The *Mass Profiler Professional* (version 14.8) software, provided by Agilent, was employed to manage the information (spectra, retention times and response intensities) corresponding to compounds mined from GC-TOF-MS records. This software integrates different chemometric tools for the treatment of data associated to molecular

entities extracted from raw GC-EI-TOF-MS records, either as non-identified species (the option used in this study) or after confirming the identities of these compounds.

3. Results and discussion

3.1. Optimization of DLLME conditions

Grappa distillates is a complex matrix with hundreds of compounds amenable to GC separation, but displaying different behaviors during sample extraction. In order to obtain a combination of experimental variables which provide the highest possible extraction yields for most sample components, a group of 17 compounds with different chemical functionalities (aldehydes, ketones, esters, alcohols and alkanes), structures (saturated, unsaturated, aromatic and polycyclic species), volatilities and polarities, as well as displaying different response ranges in distillates, was selected. Most of them have been employed in previous studies dealing with characterization of grappa distillates [9].The list of compounds, together with the employed quantification ions, their octanol-water (Log K_{ow}) partition coefficients and retention times (RT) are compiled in Table 1. Retention times ranged from 7 to 36 min and Log K_{ow} from 0.8 to 4.3. Selective responses for this set of compounds was obtained considering a *m/z* window of 0.010 Da centered in the m/z values of their quantification ions. Then, the influence of different DLLME parameters on their extraction efficiency was evaluated. Optimization experiments were performed using aliquots from a pooled sample of different grappas. Unless otherwise stated, extraction conditions were optimized using a univariate approach. The preliminary evaluation of the repeatability of the analytical procedure (sample extraction plus GC-QTOF-MS determination, n= 6 replicates) showed RSDs in the range from 2% to 16%, without using IS correction. The standard deviations of *m/z* values for their quantification ions (Table 1) remained below 0.005 Da.

3.1.1. Selection of extractant and disperser solvents

$CHCl_3$ and CCl_4 were evaluated as extractant solvents due to their short retention times in capillary columns considering also previous DLLME applications, and the easiness of phases separation after compounds extraction [10, 11, 21]. Less volatile solvents, such as toluene 1-octanol or 1-undecanol, also used in previous DLLME applications [22], were not investigated because of their potential co-elution with distillate components. Fig. 1 shows the responses of target compounds obtained with the above extractants (0.1 mL of $CHCl_3$ or CCl_4 combined with 0.9 mL of acetone as dispersant). Depicted data are normalized to those measured for $CHCl_3$, without any correction for differences between the volumes of the final extract. As observed, $CHCl_3$ rendered the highest responses for all the model compounds, with statistically significant differences (95% confidence level) except for eugenol. Thus, this solvent was selected as extractant.

Methanol, acetone and acetonitrile were evaluated as disperser solvents by the addition of 1 mL of the binary mixture, consisted in 0.90 mL of each disperser and 0.1 mL of $CHCl_3$. The results showed significant differences depending on the experimental conditions, for most of the compounds (Fig. S1). Globally, acetonitrile achieved equivalent (isoamyl alcohol, alpha-terpinol, beta-ionone and 4-ethylphenol) of higher responses (rest of model compounds) than acetone. On the other hand, five of the compounds presented much lower signals when methanol was employed as dispersant. So, acetonitrile was used as dispersant solvent in further experiments.

3.1.2. Selection of sample volume

DLLME experiments were performed (n=3) introducing different volumes of sample (from 0.5 to 2.5 mL) in the glass extraction tubes. Then, the required volume of ultrapure water was added to made up the aqueous phase to 9 mL. The higher the sample intake, the larger the concentration of aroma compounds in the extraction solution. On the other hand, the percentage of ethanol (42% in the undiluted pooled

distillate) also increased reducing the affinity of the compounds by the chlorinated solvent. Fig. S2 summarizes the obtained results as relative responses normalized to those measured for the 2.5 mL sample. Linear increase in the responses measured for selected compounds with the sample intake were observed. The only exception was the isoamyl alcohol, which showed better results for 2 mL of sample. Thus, taking into account the achieved results and with the aim to attain a higher sensitivity for the majority of compounds, 2.5 mL sample volume was selected for further studies.

3.1.3. Ionic strength effect

The addition of NaCl is a common practice to enhance the extractability of high and medium polarity, non-ionic, compounds from aqueous solutions in organic solvents. On the other hand, less polar analytes might suffer a significant reduction in their extraction efficiencies due to slower mass transfer kinetics, related to the increased viscosity of the sample. In the specific case of DLLME, the increase in the ionic strength of the aqueous sample also reduces the solubility of the extractant solvent, slightly increasing the volume of the recovered phase.

The effect of NaCl in the responses of compounds compiled in Table 1 was investigated at three different levels (0, 0.50 and 1.0 g). Fig. 2 shows the obtained responses, as normalized values to those measured for 0 g NaCl. Six of the most polar selected compounds, particularly those with an alcohol functionality, undergo considerable increases (i.e. more than 100 % for isoamyl alcohol) in analytical responses when NaCl amount was increased up to 1.0 g. For a second group of compounds, with a more lipophilic behavior (i.e. ethyl hexanoate, TDN, etc.) a steady decrease of their responses with the amount of NaCl was observed. Finally, responses of alpha-terpineol and 4-ethylguaiacol remained practically unaffected.

In general, polar compounds (which were positively affected by the addition of sodium chloride) displayed relative low chromatographic responses in comparison to more

lipophilic species present in the distillates, such as esters. Thus, further extractions were performed adding 1 g of NaCl to diluted grappa samples.

3.1.4. Selection of dispersant and extractant volumes

The effects of both parameters in the yield of the extraction were evaluated simultaneously. To this end, four different volumes of acetonitrile (from 0.5, 1.0, 1.5 and 2 mL) were combined with two different volumes of $CHCl_3$ (0.1 and 0.15 mL). For the lowest dispersant level, the volume of the settled chloroform phase accounted for 47 and 101 microliters, respectively. These volumes decreased in a 10 % extend when the dispersant volume rise up to 2 mL.

Whatever the extractant amount, increasing the dispersant volume turned in lower responses since the solubility of the compounds in the hydro-alcoholic phase increases. Regarding the amount of extractant, two different trends were noticed. For most compounds, increasing the extractant volume led to a reduction in their responses (as a consequence of a more diluted extract) as illustrated in Fig. 3A for isoamyl acetate. On the other hand, a few compounds (i.e. isoamyl alcohol) presented very similar responses for the two volumes of $CHCl_3$, Fig. 3B. In this case, the extra dilution of the CCl_3H extract is balanced with the increase in the yield of the DLLME extraction. Taking into account the above comments, 0.5 mL of the extraction solution, consisted of 0.4 mL of acetonitrile and 0.1 mL of $CHCl_3$, was selected.

3.1.5. Extraction and centrifugation time

The first variable was evaluated at different levels from 1 to 5 min, and the second one between 3 and 15 min. In all the experiments, the closed extraction tubes were centrifuged at 3500 rpm. None of the above parameters affected the DLLME efficiency (data not shown), thus extraction and centrifugation times were maintained at the lowest evaluated levels: 1 and 3 min, respectively

3.2. Efficiency of DLLME sample preparation

The extraction efficiency (EE, %) of the DLLME process was evaluated following an indirect method, using liquid-liquid extraction (LLE) of aqueous solutions of grappa distillate (2.5 mL of distillate, 6.5 mL of water and 1 g of NaCl) with 2 mL $CHCl_3$. EEs were calculated as: EE(%) = (Ans – Aes)/Ans)*100, being Aes and Ans the responses for each compound in the LLE extracts from hydro-alcoholic solutions previously submitted (Aes), and not submitted (Ans) to DLLME concentration, respectively. EEs (%) varied between 31 and 100%, with 11 out of 17 species presenting EEs above 86%, Table 2. Considering a volume of distillate of 2.5 mL and 0.047 mL as the average volume of the settled $CHCl_3$ phase, enrichment factors up to 52 times were obtained, Table 2.

In order to obtain a further evidence of the concentration capabilities of the developed DLLME methodology, responses for the set of model compounds were compared with those obtained using SPME extraction. Following previous literature methods [9, 23, 24], SPME extractions were performed under three different conditions: direct SPME at room temperature (20 ºC), HS SPME at room temperature and HS SPME with samples thermostated at 50 ºC. Obtained results (peak area for each compound, n=3 replicates) were normalized to those achieved by DLLME under optimized conditions. Relative efficiencies provided by the SPME technique varied depending on the considered compound and the experimental SPME conditions, Table 2. TDN was systematically better extracted by SPME. On the other hand, whatever the tested SPME conditions, 12 out of 17 compounds rendered relative SPME responses below 100%. So, they are better extracted with the DLLME technique. The extraction repeatability (see SD values in Table 2) was similar for both techniques.

The obvious limitations of the DLLME technique are (1) the use of chlorinated solvents in the extraction process and (2) the automation difficulties. On the other hand, DLLME offered a much higher sample throughput since many samples can be simultaneously

extracted; moreover, extracts can be handled with a conventional GC autosampler for liquid samples.

3.3. Profiling of volatile compounds in grappa distillates

GC-EI-TOF-MS records of grappa distillates contain thousands of ions (mostly fragment ions) belonging to hundreds of compounds, displaying a broad range of signal intensities and, quite often, overlapped peaks. Thus, the use of automated data mining strategies is compulsory for the characterization of compounds appearing in different samples, and for the further comparison of these samples, following a metabolomics-like approach. The workflow proposed to reach both targets is depicted in Fig. 4.

3.3.1. Mining and identification of distillate components

The FMF algoritm was used to assign a chromatographic signal (retention time and intensity) and an accurate MS spectrum to each species (component) in the GC-EI-QTOF-MS chromatograms. Threshold values of 0.05% and 5% (as relative intensities to the highest peak in each chromatogram and to the base ion in the spectrum of each compound, respectively) were set. To prevent bias of *m/z* ratios of fragment ions due to too weak or too intense (saturating) peaks, it is important to define the regions where the spectral information is obtained. For non-saturating peaks, the average spectrum in the region above 20% of the apex was used. For saturating species, the spectrum was averaged in the regions below 20% of saturation.

Around 200-250 components were found depending on the grappa sample. Fig. 5A and 5B show the raw chromatographic data and the signals of the *molecular features* (11 components) mined in a given region of the chromatogram (c.a. 0.5 min). The spectrum assigned to one of these entities is shown in Fig. 5C. As appreciated in Fig. 5D, the raw spectrum in the region of this compound contains ions from different species; thus, the latter is of little interest for sample characterization/classification purposes.

The identities of compounds isolated in the previous step were proposed from comparison with the NIST low -resolution database, Fig. 4. Several requirements were established for a tentative identification [16]. First, the match between the low resolution spectrum of the candidate species, in the NIST library, and the accurate experimental one must stay above 80%. Second, *m/z* values for, at least, three intense ions in the experimental spectrum fit the calculated ones for fragments, with a known empirical formula, in the low resolution spectrum of the candidate, within a maximum error of 10 mDa. Third, when available, the LRI values of *molecular features* and candidate compounds, using the same or an equivalent coating GC column, must be coherent. Fig. 5E shows the low resolution NIST spectrum of benzyl alcohol, with calculated *m/z* values of several fragments. The difference with values in experimental spectrum assigned to this species (Fig. 4C) remained below 2 mDa.

Taking into account the above criteria, the identities of more than 140 compounds were tentatively assigned. Table S2 summarizes some relevant chromatographic and spectral data for these compounds. The largest group (c.a. 60 species) corresponds to free carboxylic acids and their esters with the major alcohols formed during fermentation of grape pomace. The list of acetals, alcohols, aldehydes and ketones, which are directly correlated with the aroma of distillates is also relevant. Likewise, more than 30 terpenes (from monoterpenes to sesquiterpenes) and 18 aromatic (benzene derivatives), which have been correlated with grape variety, are also identified [2, 9, 25], Table S2. Obviously, final identification of these compounds requires confirmation against authentic standards (in this research just the identities of around 20 compounds, highlighted in bold in Table S2, was confirmed). After this step, a customized database library, with retention times and accurate EI-MS spectra, can be created with the *Mass Hunter* software. Thereafter, this database will allow their target search in new samples [26].

3.3.2. Characterization of grappa samples from deconvoluted components

The workflow in Fig. 4 also shows the use of deconvoluted component data for the characterization of grappa samples. To this end, the *Mass Profiler Professional (MPP)* software was used to align compounds from different chromatographic injections, and to compare the fragment ions in their deconvoluted spectra. The maximum variations for retention time and *m/z* values were set at 0.1 and 10 mDa, respectively. After peak alignment, the same software permits to see changes in the responses of each compound among the different considered samples, which might be useful to detect discriminating species and/or to detect undesirable components, from the point of view of sensorial properties, in a particular sample, figure not shown.

Data of aligned components can be processed using multivariate chemometric tools (integrated in the above software package) useful for exploration and/or classification purposes. As example, the principal component analysis (PCA) plot corresponding to the processed grappas (11 different samples, extracted and analysed in triplicate) is shown in Fig. 6. For PCA analysis, responses for components mined in every triplicate of each sample were first divided by that of the IS and then, normalized to the average component response in each chromatogram. A few components, with large, saturating chromatographic peaks (i.e. the ethyl esters of C8, C10 and C12 carboxylic acids) were not employed for the PCA analysis, which was based on around 170 species.

When considering the 11 different distillates, the sample from Cantabria (code S11, Table S1) was well separated from those elaborated with different Galician grape varieties (codes S1 to S10), Fig. 6A. On the other hand, samples from Torrontés (S5 and S6) and Treixadura (S7 and S8) varieties are poorly discriminated, Fig. 6A. After repeating the PCA analysis with the 10 samples from Galicia, a clear separation among the four different grape varieties (Torrontés, Albariño, Treixadura and Albariño) was observed, Fig. 6 B. Obviously, the number of distillates, from each of the four grape

marc Galician varieties, used in the current study is too low for a reliable PCA study; however, the workflow and the projections depicted in Fig. 4 and Fig. 6, respectively, serve to illustrate the way to handle the accurate spectral data together with retention times of deconvoluted components for chemometric studies.

4. Conclusions

DLLME offers interesting features for the extraction of volatile and semi-volatile compounds from grape marc distillates such as low solvent consumption, fast extraction times, compatibility with GC analysis and acceptable yields for a large range of species from different chemical families. The combination of data mining strategies with the unique fingerprint information contained in the GC accurate EI-MS records of distillates permitted the extraction of a set of valuable data (retention time, intensity and fragment ions) to feed metabolomics software, aiming to classify and/or to discriminate distillate samples. Independently of the sample preparation technique, such methodology is of inherent interest in the spirit industry, and in any other field where GC amenable compounds can be useful for characterization purposes (i.e. alcoholic and non-alcoholic beverages, food and cosmetic industry). Given the universal acceptance of EI for ionization of volatile and semi-volatile species in GC-MS, the development of accurate EI-MS spectral libraries is required to fully exploit the potential offered by GC hyphenated to EI accurate MS.

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Table 1. Summary of model compounds considered during optimization of DLLME conditions.

Compound	Molecular formula	CAS number	Chemical class	Retention time (min)	Quantification ion (m/z)	Log K_{ow}
Isoamyl acetate	$C_7H_{14}O_2$	123-92-2	Ester	6.84	55.0560	1.53
Isoamyl alcohol	$C_5H_{12}O$	123-51-3	Alcohol	9.01	70.0782	1.09
Ethyl hexanoate	$C_8H_{16}O_2$	123-66-0	Ester	9.62	88.0534	2.31
2-Furaldehyde	$C_5H_4O_2$	98-01-1	Aldehyde	16.77	95.0130	0.83
Benzaldehyde	C_7H_6O	100-52-7	Benzene derivative	18.59	105.0339	1.69
Isoamyl octanoate	$C_{13}H_{26}O_2$	2035-99-6	Ester	22.21	70.0794	4.46
Alpha-terpineol	$C_{10}H_{18}O$	98-55-5	Monoterpene	23.49	93.0704	2.20
1,1,5-Trimethyl-1,2-dihydronaphthalene (TDN)	$C_{13}H_{16}$	30364-38-6	C13-Norisoprenoid	24.87	157.1016	4.25
Methyl salicylate	$C_8H_8O_3$	119-36-8	Benzene derivative	25.85	120.0222	2.32
Ethyl phenyl acetate	$C_{10}H_{12}O_2$	101-97-3	Benzene derivative	25.98	91.0555	2.11
Benzyl alcohol	C_7H_8O	100-51-6	Benzene derivative	28.55	108.0575	1.21
Butanedioic acid, ethyl isoamyl	$C_8H_{14}O_4$	28024-16-0	Ester	28.87	101.0231	1.86
Phenyl ethyl alcohol	$C_8H_{10}O$	60-12-8	Alcohol	29.48	91.0553	1.49
Beta-ionone	$C_{13}H_{20}O$	14901-07-6	C13-Norisoprenoid	29.95	177.1279	3.28
4-Ethylguaiacol	$C_9H_{12}O_2$	2785-89-9	Phenol	32.46	137.0593	2.47
Eugenol	$C_{10}H_{12}O_2$	97-53-0	Phenol	35.76	164.0829	2.61
4-Ethylphenol	$C_8H_{10}O$	123-07-9	Phenol	36.04	107.0489	2.63

Table 2. Calculated extraction efficiencies (EEs, %) and enrichment factors (EFs) of the optimized DLLME method. SPME relative efficiencies versus DLLME extraction. Values for n=3 replicates.

Compound	DLLME performance		[a]SPME relative extraction efficiencies (%) ± SD					
	EEs (%) ± SD	[b]EFs	Direct SPME PDMS/DVB	Direct SPME DVB/CAR/PDMS	HS SPME PDMS/DVB	HS SPME DVB/CAR/PDMS	HS SPME,50 ºC, PDMS/DVB	HS SPME, 50 ºC, DVB/CAR/PDMS
Isoamyl acetate	95 ± 8	49	12 ± 1	8.5 ± 0.2	27 ± 3	29 ± 3	15 ± 4	7.3 ± 0.2
Isoamyl alcohol	38 ± 1	20	1.0 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.3 ± 0.2	2.6 ± 0.2
Ethyl hexanoate	100 ± 14	52	18 ± 1	24 ± 2	21 ± 2	28 ± 5	8 ± 1	10 ± 3
2-Furaldehyde	32 ± 1	17	4.3 ± 0.1	74 ± 3	4.6 ± 0.3	53 ± 2	2.7 ± 0.1	45 ± 2
Benzaldehyde	72 ± 2	37	71 ± 6	171 ± 6	74 ± 1	157 ± 11	49 ± 1	93 ± 1
Isoamyl octanoate	100 ± 3	52	46 ± 2	57 ± 3	65 ± 3	85 ± 2	116 ± 1	131 ± 5
Alpha-terpineol	91 ± 2	47	11 ± 1	14 ± 1	5.4 ± 0.6	6.4 ± 0.4	70 ± 6	80 ± 1
1,1,5-Trimethyl-1,2-dihydronaphthalene (TDN)	100 ± 5	52	112 ± 3	140 ± 21	201 ± 10	332 ± 15	300 ± 22	355 ± 23
Methyl salicylate	95 ± 2	49	58 ± 3	142 ± 3	3 5± 2	46 ± 2	21 ± 1	65 ± 5
Ethyl phenyl acetate	97 ± 3	50	35 ± 1	55 ± 5	14 ± 1	19 ± 2	12 ± 1	17 ± 1
Benzyl alcohol	31 ± 3	16	8 ± 1	21 ± 2	5.3 ± 0.4	8.2 ± 0.2	2.6 ± 0.3	9.7 ± 0.5
Butanedioic acid, ethyl isoamyl	100 ± 2	52	29 ± 1	37 ± 4	3.3 ± 0.1	3.3 ± 0.2	11 ± 1	12 ± 1
Phenyl ethyl alcohol	42 ± 3	22	8.3 ± 0.3	17 ± 1	4.1 ± 0.1	6.1 ± 0.3	4.5 ± 0.3	6 ± 1
Beta-ionone	100 ± 5	52	80 ± 2	127 ± 5	14.3 ± 0.5	127 ± 7	48 ± 1	53 ± 2
4-Ethylguaiacol	86 ± 5	45	16 ± 1	31 ± 2	3.3 ± 0.2	4.3 ± 0.5	7.2 ± 0.3	12 ± 2
Eugenol	100 ± 8	52	13 ± 1	15 ± 2	2.1 ± 0.1	3.6 ± 0.2	37 ± 6	31 ± 5
4-Ethyl phenol	74 ± 9	38	19 ± 1	37 ± 5	4.3 ± 0.4	4.3 ± 0.2	7.1 ± 0.1	10 ± 1

[a] Relative responses provided by SPME, under investigated conditions, versus those attained by DLLME.
[b] Average EFs considering 2.5 mL of sample intake and 0.047 mL as the volume of the settle chloroform extract.

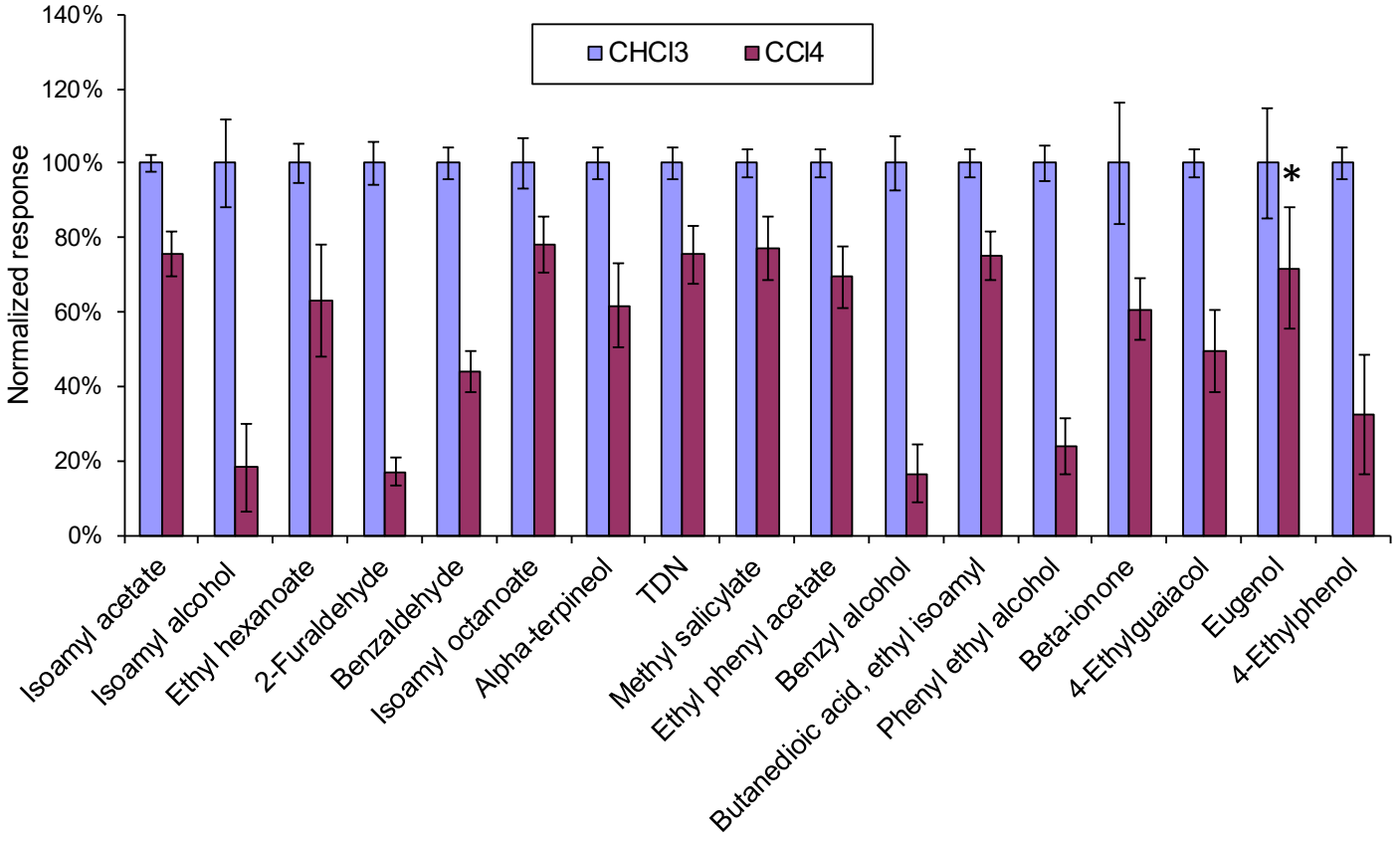

Fig. 1. Effect of the extractant solvent in the performance of DLLME. Normalized responses to $CHCl_3$, n=3 replicates. Compounds marked with asterisk present non-significant changes in their extraction efficiency (95 % confidence level).

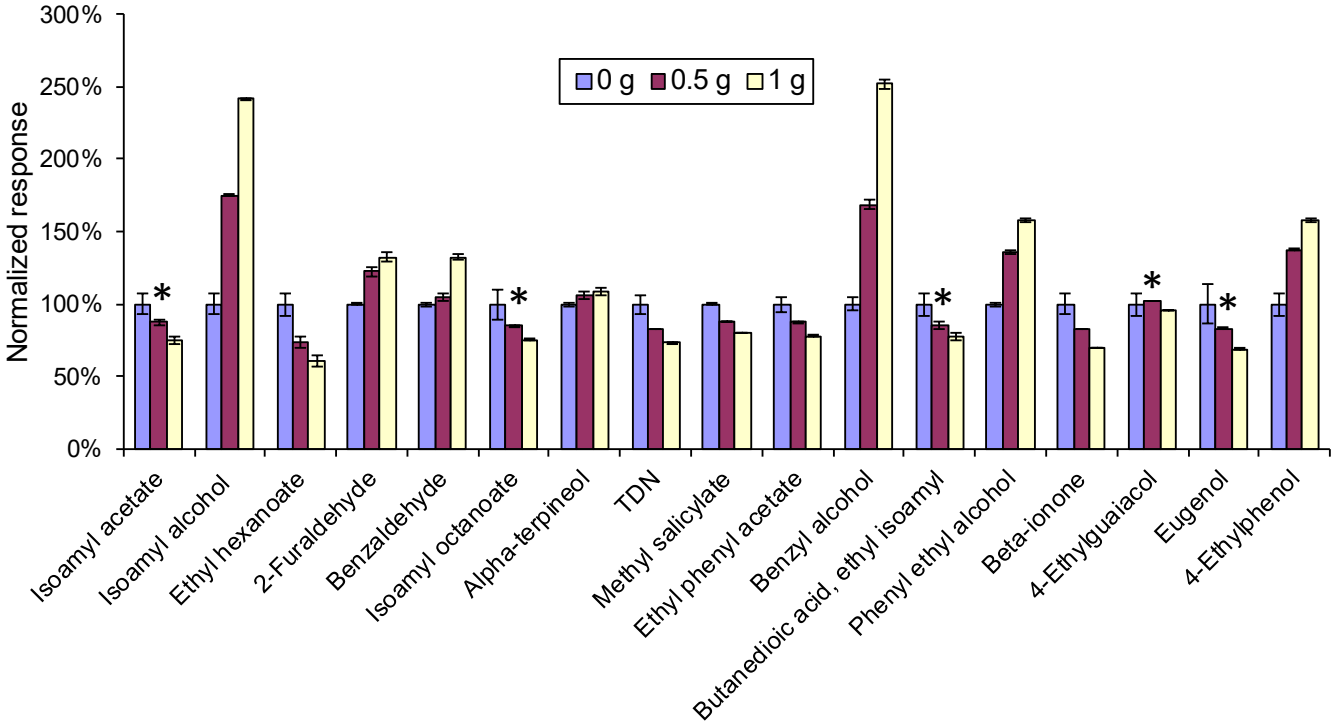

Fig. 2. Influence of NaCl addition in the efficiency of DLLME extraction, n=3 replicates. An asterisk is employed to indicate when extraction yields are similar (95% confidence level) to those attained without NaCl addition.

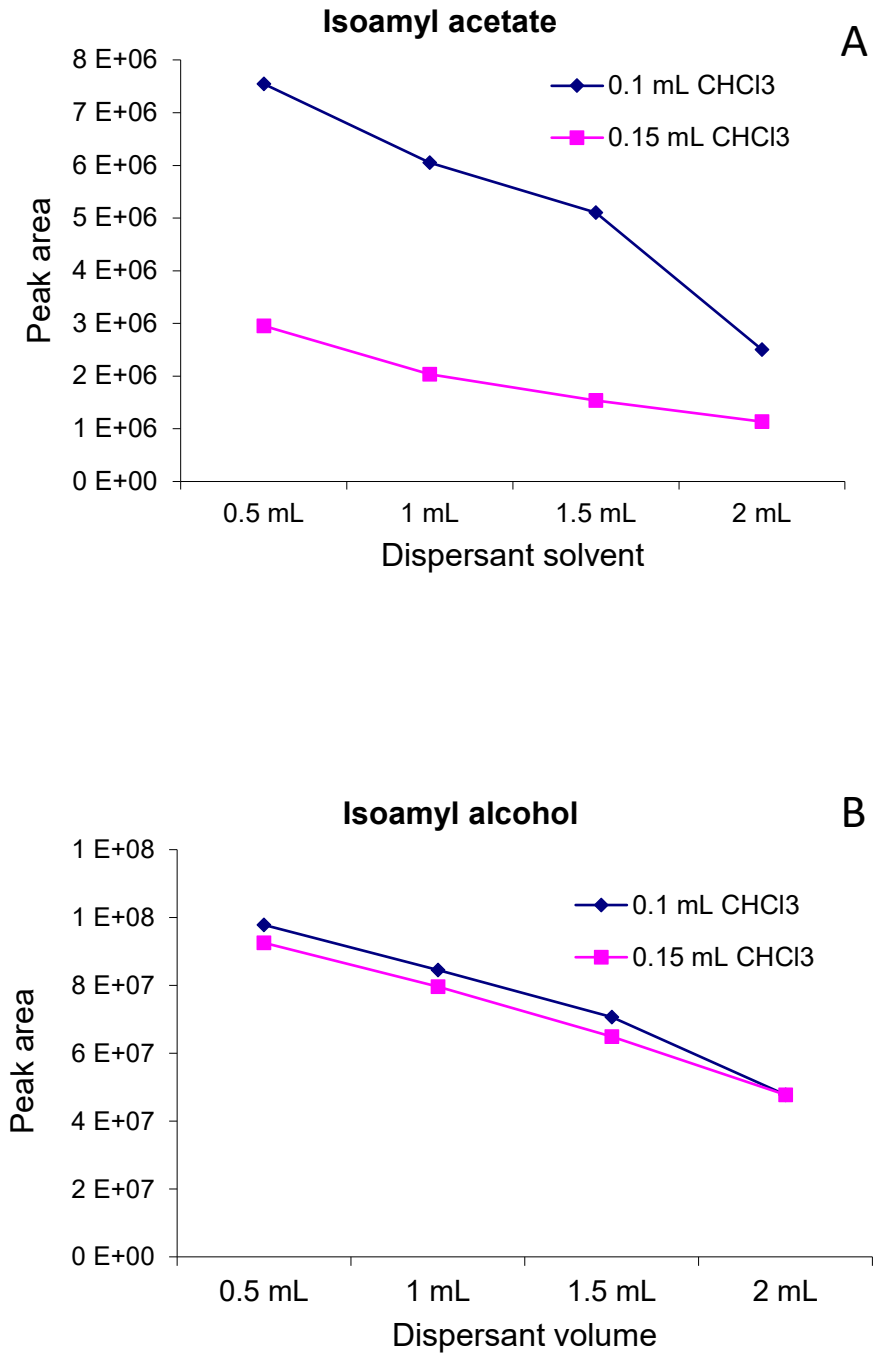

Fig. 3. Comparison of responses (peak areas) as function of dispersant (acetonitrile) and extractant ($CHCl_3$) solvent volumes. Average values for duplicate extractions.

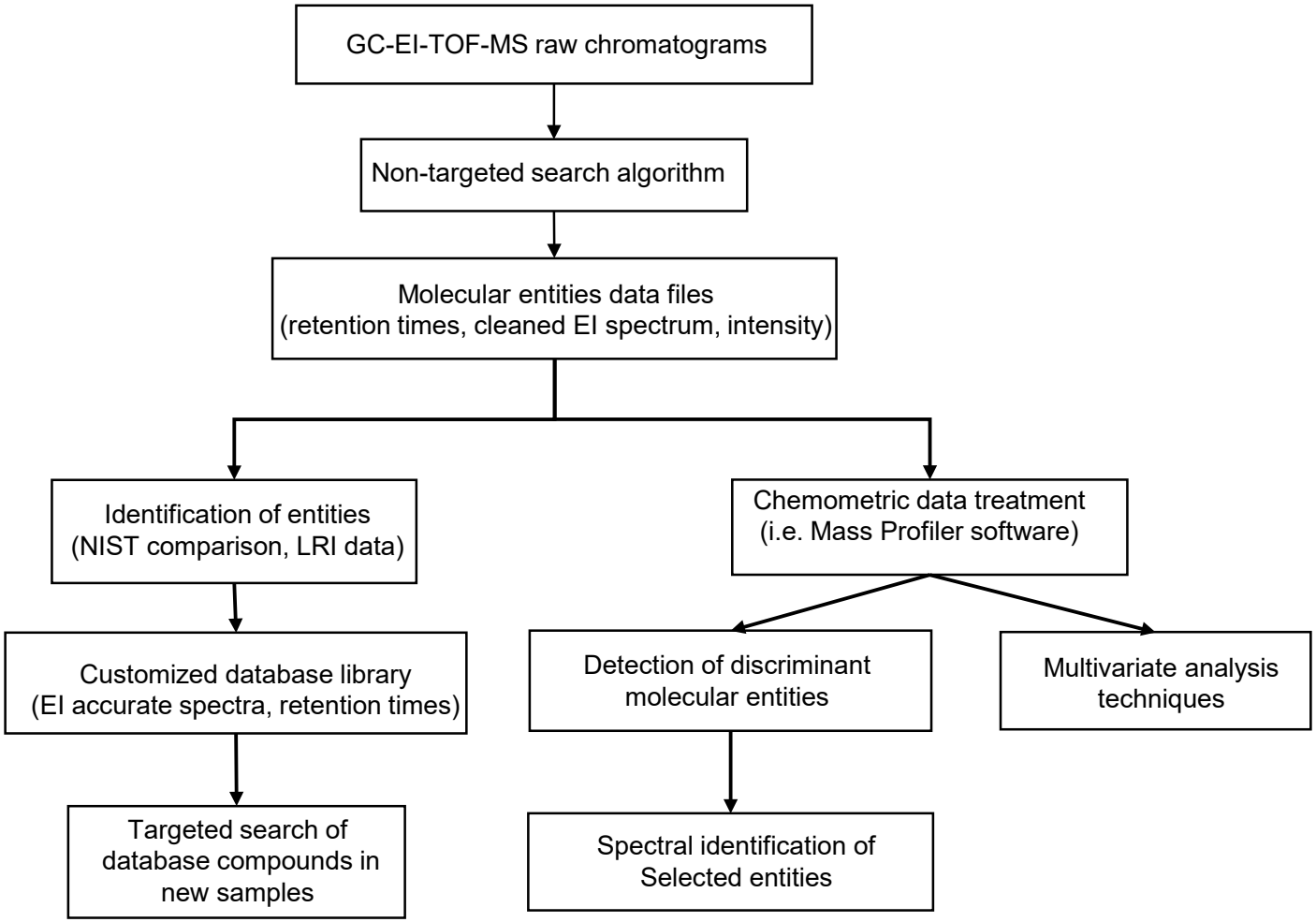

Fig. 4. Workflow scheme followed for data mining, and samples characterization, from GC-EI-TOF-MS raw chromatograms.

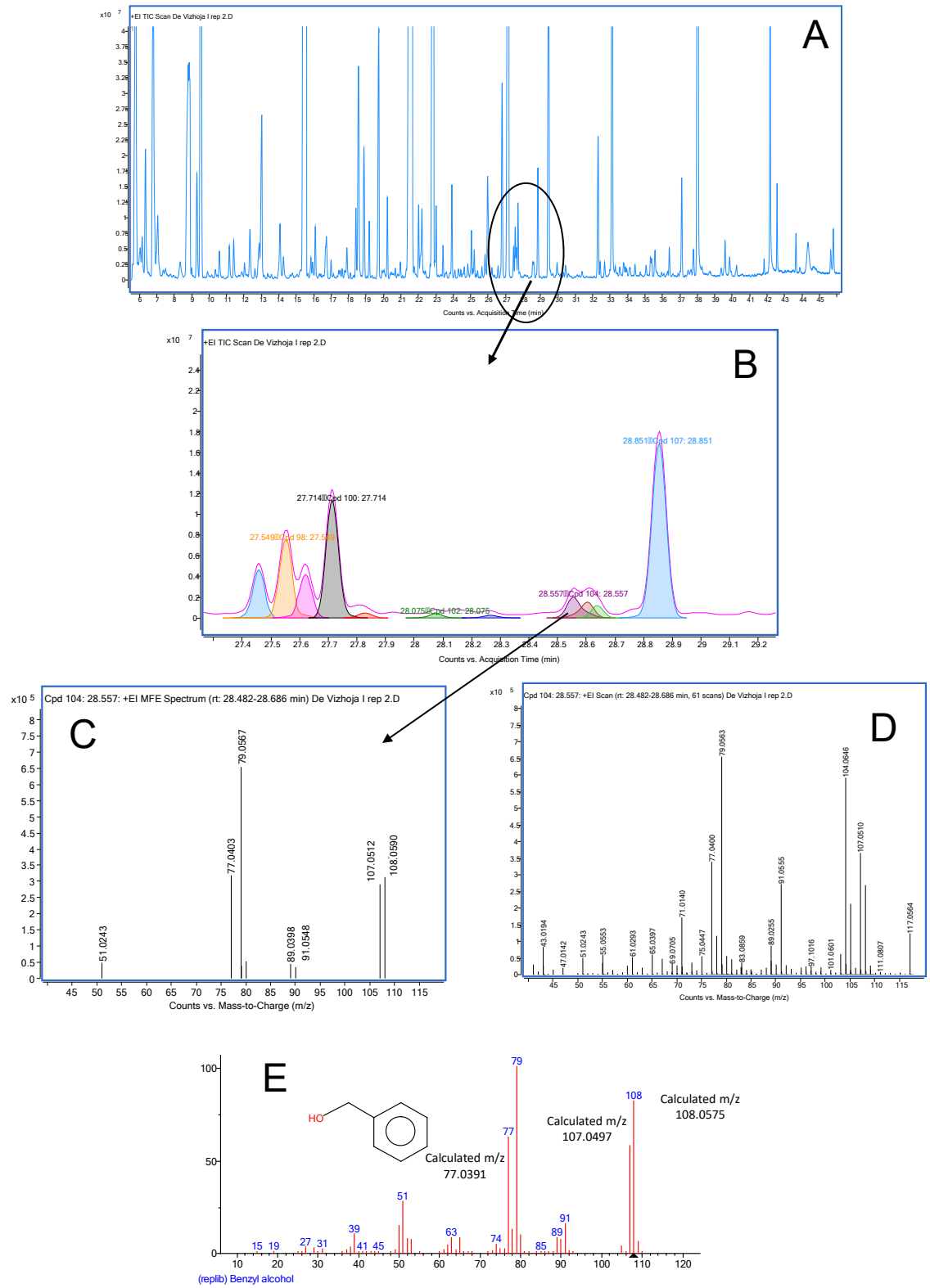

Fig. 5. A, raw GC-EI-TOF-MS spectra of a grappa sample. B, detail of *molecular features* (components) mined from the raw chromatogram. C, cleaned spectrum of the compound at retention time 28.55 min. D, raw spectra at the same retention time. E, NIST spectrum of benzyl alcohol with calculated *m/z* values of known fragment ions.

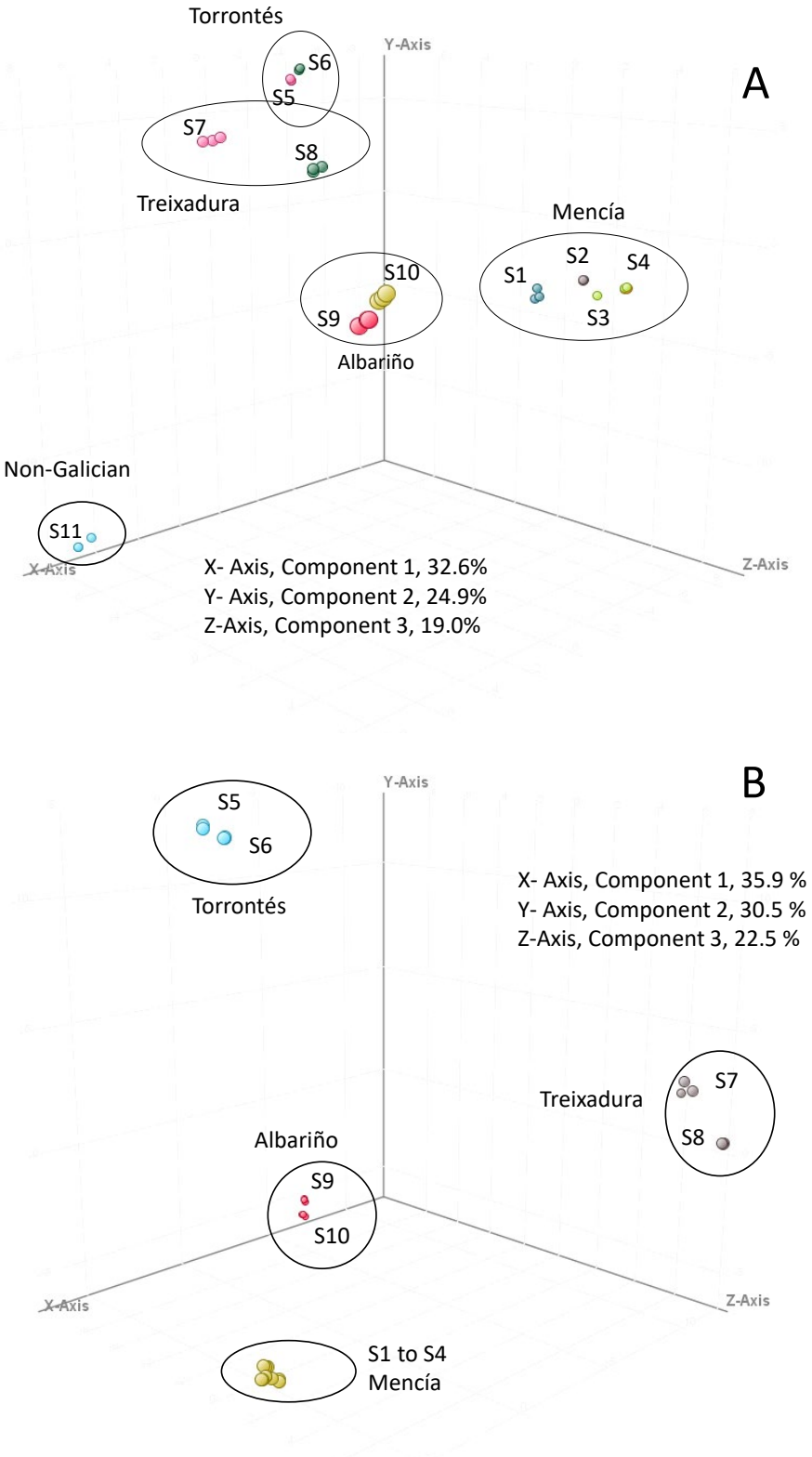

Fig. 6. PCA plots of grappa samples obtained from non-targeted data mined compounds. Each sample processed in triplicate. A, plot for the set of 11 grappa samples. B, PCA plot obtained after removing the grappa sample not produced using Galician grape marc varieties.

Supplementary information to manuscript:

Dispersive liquid-liquid microextraction and gas chromatography accurate mass spectrometry for extraction and non-targeted profiling of volatile and semi-volatile compounds in grape marc distillates

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Table S1. Characteristics of the employed grappa samples.

Code	Grape variety	Grape type	Distillation technique	Ethanol content (%)	Geographic origin
S1	*Mencía*	Red	Direct	40	Galicia
S2	*Mencía*	Red	Direct	40	Galicia
S3	*Mencía*	Red	Steam distillation	45	Galicia
S4	*Mencía (90%)*	Red	Direct	41	Galicia
S5	*Torrontés*	White	Direct	62	Galicia
S6	*Torrontés*	White	Direct	53	Galicia
S7	*Treixadura*	White	Steam distillation	43	Galicia
S8	*Treixadura*	White	Steam distillation	40	Galicia
S9	*Albariño*	White	Steam distillation	40	Galicia
S10	*Albariño*	White	Steam distillation	40	Galicia
S11	*Unknown*	Unknown	Unknown	40	Cantabria (Liebana)

Table S2. Database of compounds tentatively identified in grappa distillate samples.

Compound	**Retention time (min)**	**Base peak (m/z)**	**Ion 1 (*m/z*)**	**Ion 2 (*m/z*)**	**CAS number**	**LRI Experimental**	**LRI Chemspider**	Chemical class
Pentane, 1,1, diethoxy-	5.74	47.0144	75.0453	103.0769	3658-79-5	1143	1135	Acetal
Isopentanal, diethyl acetal	5.81	103.0770	47.0145	75.0453	3842-03-3	1152	n.a.	Acetal
Acetaldehyde ethyl amyl acetal	6.38	45.0355	73.0664	71.0871	13442-89-2	1106	1109	Acetal
Hexanal, diethyl acetal	9.33	103.0757	75.0442	129.1275	3358-93-3	1242	1230	Acetal
Propionaldehyde, 3-ethoxy-, diethyl acetal	11.41	103.0753	87.0804	59.0494	7789-92-6	1318	1297	Acetal
Heptanal, diethyl acetal	12.04	103.0753	75.0439	143.1430	688-82-4	1340	1332	Acetal
Acetaldehyde, phenyl-, diethyl acetal	23.96	103.0747	75.0442	47.0136	6314-97-2	1746	1711	Acetal
1-Butanol	5.99	56.0632	43.0556	41.0400	71-36-3	1132	1145	Alcohol
Isoamyl alcohol	9.01	70.0785	55.0557	42.0477	123-51-3	1229	1237	Alcohol
4-Ethyl cyclohexanol	11.14	81.0335	97.0283	53.0390	4534-74-1	1309	n.a	Alcohol
2-Heptanol	11.93	45.0341	83.0852	55.0545	6033-23-4	1336	1335	Alcohol
1-Hexanol	13.06	56.0638	69.0713	41.0404	111-27-3	1375	1372	Alcohol
2-Methyl cyclopentanol	14.69	57.0338	67.0542	82.0768	24070-77-7	1430	n.a.	Alcohol
1-Octen-3-ol	16.01	57.0339	43.0184	67.0541	3391-86-4	1473	1448	Alcohol
1,10-Decanediol	17.46	67.0540	55.0543	82.0770	112-47-0	1521	n.a	Alcohol
Beta-Linalool	18.91	93.0700	71.0493	69.0707	78-70-6	1569	n.a.	Alcohol
1-Octanol	19.24	55.0556	69.0707	56.0632	111-87-5	1580	1576	Alcohol
E-2-octadecadecen-1-ol	35.34	96.0568	61.0285	84.0570	111-62-6	2195	n.a.	Alcohol
Hexanal	6.06	56.0629	67.0543	72.0569	66-25-1	1100	1120	Aldehyde
Nonanal	14.28	67.0542	81.0698	70.0775	124-19-6	1416	1408	Aldehyde
2-Furaldehyde	16.77	95.0130	96.0203	59.0492	98-01-1	1499	1454	Aldehyde
Benzaldehyde	18.59	105.0339	77.0384	51.0233	100-52-7	1559	1555	Aldehyde
Styrene	10.38	104.0619	78.0462	103.0541	100-42-5	1282	1263	Aromatic
o-Cymene	10.67	119.0584	134.1088	57.0695	527-84-4	1292	1287	Aromatic
Ethyl benzoate	22.74	105.0342	77.0386	122.0364	93-89-0	1703	1706	Aromatic
Acetoxyacetic acid, 3-ethylphenyl ester	25.17	122.1091	107.0856	101.0234	355699	1790	n.a.	Aromatic
Anethole	25.17	148.0886	159.1185	147.0810	104-46-1	1790	1817	Aromatic
Methyl salicylate	25.85	120.0222	92.0267	152.0475	119-36-8	1815	1787	Aromatic

Ethyl phenylacetate	25.98	91.0555	129.0566	65.0387	101-97-3	1820	1783	Aromatic
Ethyl salicylate	26.75	120.0225	92.0268	166.0632	118-61-6	1849	1828	Aromatic
Phenyl ethyl acetate	26.82	104.0639	91.0543	78.0463	103-45-7	1852	n.a.	Aromatic
Benzene, 4-ethyl-1,2-dimethoxy-	28.43	151.0752	166.0988	95.0491	5888-51-7	1912	1875	Aromatic
Benzyl alcohol	28.55	108.0584	107.0507	79.0564	100-51-6	1917	1884	Aromatic
Ethyl hydrocinnamate	28.65	104.0619	91.0540	107.0492	2021-28-5	1921	1914	Aromatic
Butylated hydroxytoluene	29.18	205.1587	182.1300	220.1819	128-37-0	1942	1927	Aromatic
Phenylethyl alcohol	29.48	91.0553	122.0723	65.0385	60-12-8	1954	1939	Aromatic
Ethylguaiacol	32.46	137.0593	152.0827	85.0281	2785-89-9	2073	n.a.	Aromatic
Propylguaiacol	34.37	137.0597	79.0541	98.9838	2785-87-7	2154	2089	Aromatic
Ethyl cinnamate	34.82	131.0508	103.0540	147.0439	103-36-6	2173	2135	Aromatic
Eugenol	35.76	164.0829	204.1868	161.1320	579-60-2	2213	2117	Aromatic
4-Ethylphenol	36.04	107.0489	122.0720	77.0383	123-07-9	2226	2226	Aromatic
Acetic acid	16.65	60.0207	43.0185	44.9977	64-19-7	1495	1484	Carboxylic acid
Alpha-hydroxyisocaproic acid	18.94	69.0697	93.0696	71.0490	498-36-2	1570	n.a.	Carboxylic acid
Hexanoic acid	27.84	60.0222	73.0298	87.0445	142-62-1	1890	1861	Carboxylic acid
Heptanoic acid	30.53	60.0210	73.0254	87.0440	111-14-8	1995	1997	Carboxylic acid
Octanoic acid	33.25	60.0225	73.0303	101.0617	124-07-2	2106	2091	Carboxylic acid
14-Pentadecenoic acid	35.08	96.0568	67.0542	61.0286	17351-34-7	2184	n.a.	Carboxylic acid
Nonanoic acid	35.66	73.0298	60.0220	115.0767	112-05-0	2209	2194	Carboxylic acid
Decanoic acid	38.019	73.0299	60.0221	115.0756	334-48-5	2315	2294	Carboxylic acid
9-Decenoic acid	39.45	55.0544	73.0285	69.0697	14436-32-9	2381	2348	Carboxylic acid
17-Octadecynoic acid	40.65	67.0541	81.0696	95.0851	34450-18-5	>2400	n.a.	Carboxylic acid
Manoyl oxide	39.96	257.2290	275.2382	81.0704	596-84-9	>2400	n.a.	Diterpene
Ethyl butanoate	5.27	43.0560	71.0501	88.0527	105-54-4	n.a.	1043	Ester
Ethyl 2-methylbutanoate	5.5	57.0702	102.0677	74.0363	7452-79-1	n.a.	n.a.	Ester
Ethyl isovalerate	5.75	57.0704	88.0521	61.0287	108-64-5	1148	1131	Ester
Isoamyl acetate	6.84	55.0560	70.0791	87.0448	123-92-2	1137	1130	Ester
Pentanoic acid, ethyl ester	7.08	57.0701	88.0519	73.0284	539-82-2	1148	1131	Ester
Ethyl (Z)-2-butenoate	7.82	69.0336	99.0440	41.0393	6776-19-8	1182	n.a.	Ester
Hexanoic acid, ethyl ester	9.62	88.0534	61.0296	73.0300	123-66-0	1253	1232	Ester
Hexyl ethanoate	10.6	56.0623	69.0698	61.0285	142-92-7	1290	1285	Ester
Ethyl heptanoate	12.355	88.0529	61.0293	73.0293	106-30-9	1351	1337	Ester
Ethyl lactate	12.9	45.0352	75.0076	117.0911	97-64-3	1369	1356	Ester

Methyl octanoate	14.077	74.0367	87.0442	67.0544	111-11-5	1409	1374	Ester
Isoamyl hexanoate	16.15	70.0787	55.0554	99.0805	2198-61-0	1478	1469	Ester
Isobutyl lactate	16.48	45.0340	57.0699	85.0641	585-24-0	1489	1455	Ester
Ethyl 7-octenoate	17.01	96.0570	55.0542	124.0880	35194-38-8	1507	n.a.	Ester
Ethyl 2-hydroxy-4-methylpentanoate	18.15	76.0155	45.0342	87.0804	10348-47-7	1544	1545	Ester
Ethyl nonanoate	18.45	88.0516	73.0284	61.0285	123-29-5	1554	1553	Ester
Butyl octanoate	18.85	57.0703	127.1122	56.0624	589-75-3	1567	1601	Ester
Ethyl trans-2-octenoate	19.03	70.0765	113.0958	85.1007	7367-82-0	1573	n.a.	Ester
Isopenthyl methoxyacetate	19.75	55.0548	70.0777	45.0342	282411 (NIST)	1597	n.a.	Ester
Ethyl malonate	19.93	115.0390	133.0495	43.0185	105-53-3	1603	1580	Ester
Decanoic acid, methyl ester	20.22	74.0374	87.0449	143.1070	110-42-9	1613	1597	Ester
Ethyl diethoxyacetate	21.3	103.0752	75.0439	185.1902	6065-82-3	1651	n.a.	Ester
Isoamyl octanoate	22.21	70.0794	55.0559	127.1136	2035-99-6	1684	1673	Ester
Butanedioic acid, diethyl exter	22.85	101.0251	129.0565	73.0294	123-25-1	1706	1675	Ester
Ethyl trans-4-decenoate	23.11	55.0555	73.0299	61.0297	76649-16-6	1716	n.a.	Ester
Propyl decanoate	23.834	61.0287	173.1540	155.1434	30673-60-0	1742	1729	Ester
Ethyl undecanoate	24.32	88.0519	73.0285	61.0286	627-90-7	1759	1732	Ester
Isobutyl decanoate	24.67	56.0633	155.1442	129.0913	30673-38-2	1772	1751	Ester
Isoamyl nonoate	24.81	70.0775	55.0544	141.1273	7770-70-6	1777	n.a	Ester
Methyl dodecanoate	26.038	74.0367	87.0446	143.1069	111-82-0	1822	n.a.	Ester
Hexyl octanoate	26.19	145.1219	56.0623	127.1118	1117-55-1	1828	1804	Ester
Pentadecanoic acid, 3-methylbutyl ester	27.71	70.0791	55.0554	71.0850	2306-91-4	1885	n.a.	Ester
Ethyl 3-hydroxyhexanoate	28.7	117.0548	71.0127	89.0237	2305-25-1	1923	n.a.	Ester
Butanedioic acid, ethyl isoamyl	28.879	101.0231	129.0544	71.0851	28024-16-0	1930	1901	Ester
Methyl 9-oxoesterate	29.05	70.0773	55.0544	157.1012	1842-70-2	1937	n.a.	Ester
Propyl dodecanoate	29.31	61.0284	201.1850	72.0283	3681-78-5	1947	1930	Ester
Isobutyl dodecanoate	30.03	56.0623	201.1850	183.1746	37811-72-6	1975	1964	Ester
Hexyl decanoate	31.42	173.1539	56.0622	87.0440	30673-36-0	2031	2011	Ester
Ethyl tetradecanoate	32.46	88.0516	73.0283	101.0594	124-06-1	2073	2050	Ester
Isoamyl laurate	32.821	70.0790	55.0550	183.1748	6309-51-9	2088	2059	Ester
Ethyl 9-tetradecenoate	33.35	55.0545	69.0699	83.0847	336608 (NIST)	2110	n.a.	Ester
Ethyl 3-hydroxytridecanoate	33.91	117.0548	71.0129	89.0236	107141-15-1	2134	n.a.	Ester

Ethyl pentadecanoate	34.75	88.0519	73.0285	101.0597	41114-00-5	2170	2179	Ester
Z-10-tetradecenyl acetate	35.44	55.0554	69.0707	83.0862	112-53-8	2199	2128	Ester
Methyl hexadecanoate	36.32	74.0362	87.0440	143.1066	112-39-0	2239	2213	Ester
Ethyl hexadecanoate	37.35	88.0516	73.0283	101.0594	628-97-7	2285	2288	Ester
Farnesol acetate	37.51	69.0696	93.0692	107.0848	29548-30-9	2292	2250	Ester
Isoamyl nonoate	37.62	70.0772	43.0547	211.2054	7779-70-6	1777	n.a.	Ester
Ethyl heptadecanoate	39.33	88.0517	70.0412	101.0595	14010-23-2	2375	2340	Ester
Octanoic acid, 2-phenylethyl ester	40.26	104.0624	57.0699	78.0459	309657	>2400	2376	Ester
2-Heptanone	8.29	58.0417	71.0490	99.0803	110-43-0	1204	1182	Ketone
6-Methyl-5-heptene-2-one	12.64	108.0933	55.0543	93.0696	110-93-0	1360	1338	Ketone
2-Nonanone	14.12	58.0417	43.0187	71.0492	821-55-6	1411	1403	Ketone
2-Decanone	17.28	58.0415	71.0489	43.0184	693-54-9	1515	1508	Ketone
2-Undecanone	20.38	58.0415	71.0490	85.0645	112-12-9	1619	1615	Ketone
Trans-beta-damascenone	26.89	121.1012	69.0336	190.1356	23726-93-4	1854	n.a.	Ketone/C13-Norisoprenoid
Beta-ionone	29.95	177.1300	115.0550	117.0699	14901-07-6	1972	1964	Ketone/C13-Norisoprenoid
2-Pentadecanone	31.73	58.0416	59.0491	71.0489	2345-28-0	2044	2028	Ketone
3-Ethyl-2-methyl-1-heptene	17.81	84.0928	69.0698	41.0392	19780-60-0	1533	n.a.	MonoTerpene
Beta-Bourbonene	18.01	69.0699	45.0342	81.0697	5208-59-3	1539	n.a.	MonoTerpene
(-)-4-Terpineol	20.69	71.0491	93.0697	111.0803	20126-76-5	1630	n.a.	Monoterpene
Hotrienol	20.84	71.0491	82.0776	67.0542	29957-43-5	1635	1614	Monoterpene
Alfa-terpineol	23.49	93.0704	59.0499	121.1017	98-55-5	1729	n.a.	Monoterpene
Citronellol	25.25	67.0552	81.0704	95.0857	1117-61-9	1793	n.a.	Monoterpene
Geraniol	27.56	69.0697	41.0393	93.0696	106-24-1	1879	1879	Monoterpene
Eucalyptol	9.078	81.0700	93.0701	139.1121	470-82-6	1283	1230	Monoterpene
2-Naphthol,1,2,3,4,4a,5,6,7-octahydro-4a-methyl	28.6	104.0619	79.0541	107.0492	91253-94-0	1919	n.a.	Naphthalene
TDN (1,1,5-Trimethyl-1,2-dihydronaphthalene)	24.87	157.1016	142.0777	172.1248	357258	1779	n.a.	C13-Norisoprenoid
2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane	26.59	81.0336	67.0543	190.1355	110823-68-2	1843	n.a.	Norisoprene
Ylangene	16.95	105.0698	119.0854	161.1324	14912-44-8	1505	n.a.	Sesquiterpene
beta-Caryophyllene	20.41	58.0415	71.049	43.0184	87-44-5	1620	n.a.	Sesquiterpene
(-)-Aristolene	20.59	105.0698	161.1326	119.0855	6831-16-9	1626	n.a.	Sesquiterpene
Gamma-Muurolene	21.14	79.0542	94.0774	161.1327	24959-83-9	1646	n.a.	Sesquiterpene
Alpha-Muurolene	23.2	105.0699	161.1328	91.0542	31983-22-9	1719	n.a.	Sesquiterpene

alpha-Acorenol	23.6	93.0697	137.0791	108.0697	374180 (NIST)	1733	n.a.	Sesquiterpene
Isoledene	23.76	161.1329	204.1878	119.0857	156108 (NIST)	1739	n.a.	Sesquiterpene
Naphthalene, 1,2,4a,5,6,8a,-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	24.078	105.0699	79.0542	161.1327	483-75-0	1751	n.a.	Sesquiterpene
Beta-cadinene	24.99	119.0857	161.1330	134.1089	523-47-7	1783	n.a.	Sesquiterpene
Cedr-8(15)-ene	25.09	55.0555	161.1337	69.0704	546-28-1	1787	n.a.	Sesquiterpene
alpha-curcumene	25.43	105.0698	132.0932	73.0346	644-30-4	1799	1769	Sesquiterpene
Selina-3,7(11)-diene	25.65	161.1327	105.0698	91.0541	6813-21-4	1807	n.a.	Sesquiterpene
Alpha-calacorene	29.4	157.1013	142.0775	200.1563	21391-99-1	1951	1916	Sesquiterpene
Gamma-Gurjunene	31.02	161.1325	105.0696	119.0853	22567-17-5	2015	n.a.	Sesquiterpene
Cubedol	32.97	161.1326	119.0855	179.1433	374159 (NIST)	2094	n.a.	Sesquiterpene
1,2-Naphthoquinone, 5-isopropyl-3,8-dimethyl-	33.05	185.1327	200.1563	143.0858	5574-34-5	2098	n.a.	Sesquiterpene
Viridiflorol	34.93	95.0852	161.1324	56.062	122173 (NIST)	2177	n.a.	Sesquiterpene
Alpha-cadinol	35.97	95.0851	121.1003	161.1324	481-34-5	2223	n.a.	Sesquiterpene
Naphthalene, 4-isopropyl-1,6-dimethyl	36.9	183.1172	168.0934	198.1405	483-78-3	2265	2242	Sesquiterpene
2,3-Dihydrofarnesol	37.77	81.0698	69.0698	95.0853	51411-24-6	2304	2273	Sesquiterpene
Juniper camphor	38.59	203.1440	189.1634	105.0695	473-04-1	2341	n.a.	Sesquiterpene
Farnesol	39.62	69.0707	81.0698	41.0394	4602-84-0	2389	2361	Sesquiterpene
Dimethyldisulphide	5.92	93.9915	78.9671	44.9797	624-92-0	1160	n.a.	Others
2-Methyldihydro-3(2H)-thiophenone	18.74	60.0031	116.0288	85.0644	13679-85-1	1563	1557	Others
5-Butyl-4-methyldihydro-2(3H)-furanone	32.12	99.0802	71.0489	55.0543	39212-23-2	2060	n.a.	Others

Identities of compounds in bold was confirmed using authentic standards.

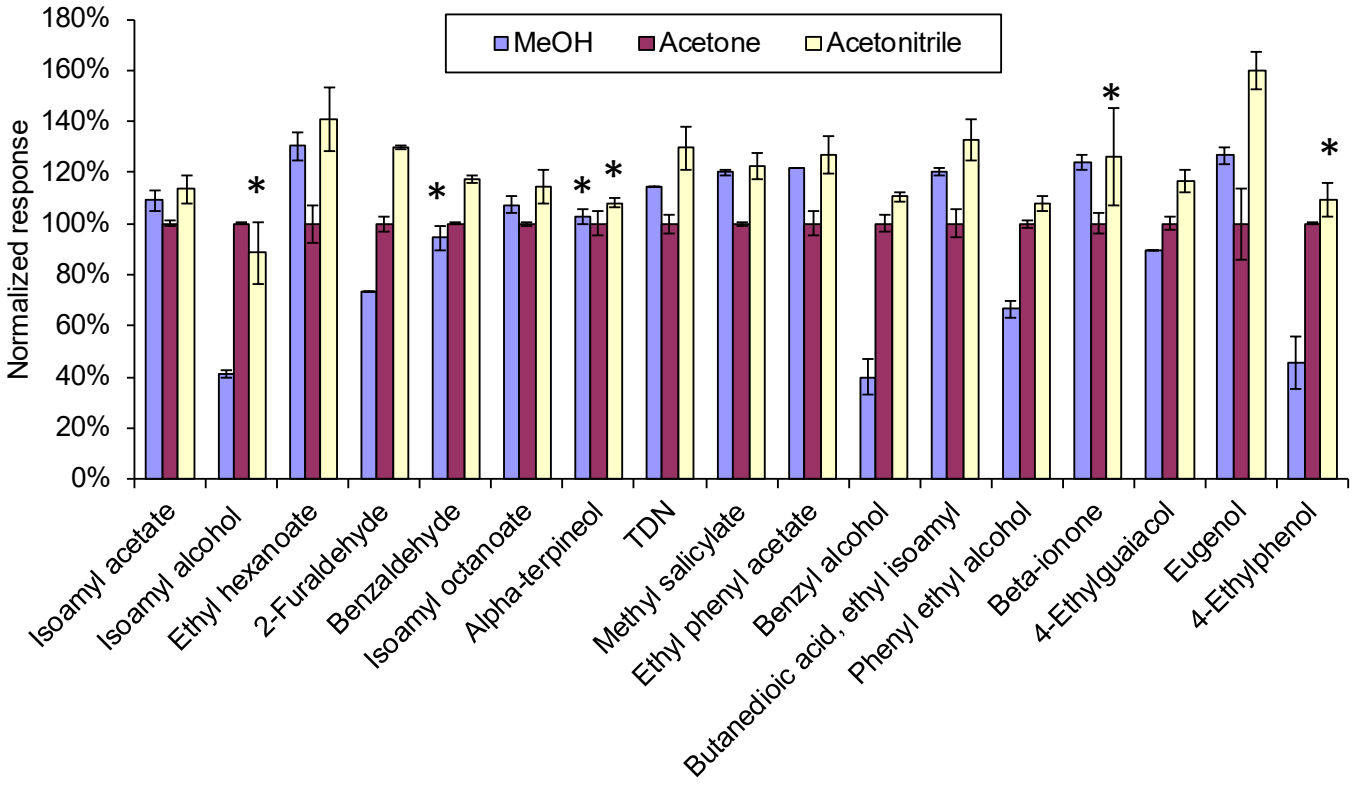

Fig. S1. Effect of the dispersant solvent (1 mL) in the normalized responses of model compounds. Acetone was used as model solvent for normalization of the responses, n=3 replicates. Asterisks are used to point out those situations where the DLLME responses are equivalent (95% confidence level) to those attained using acetone as dispersant.

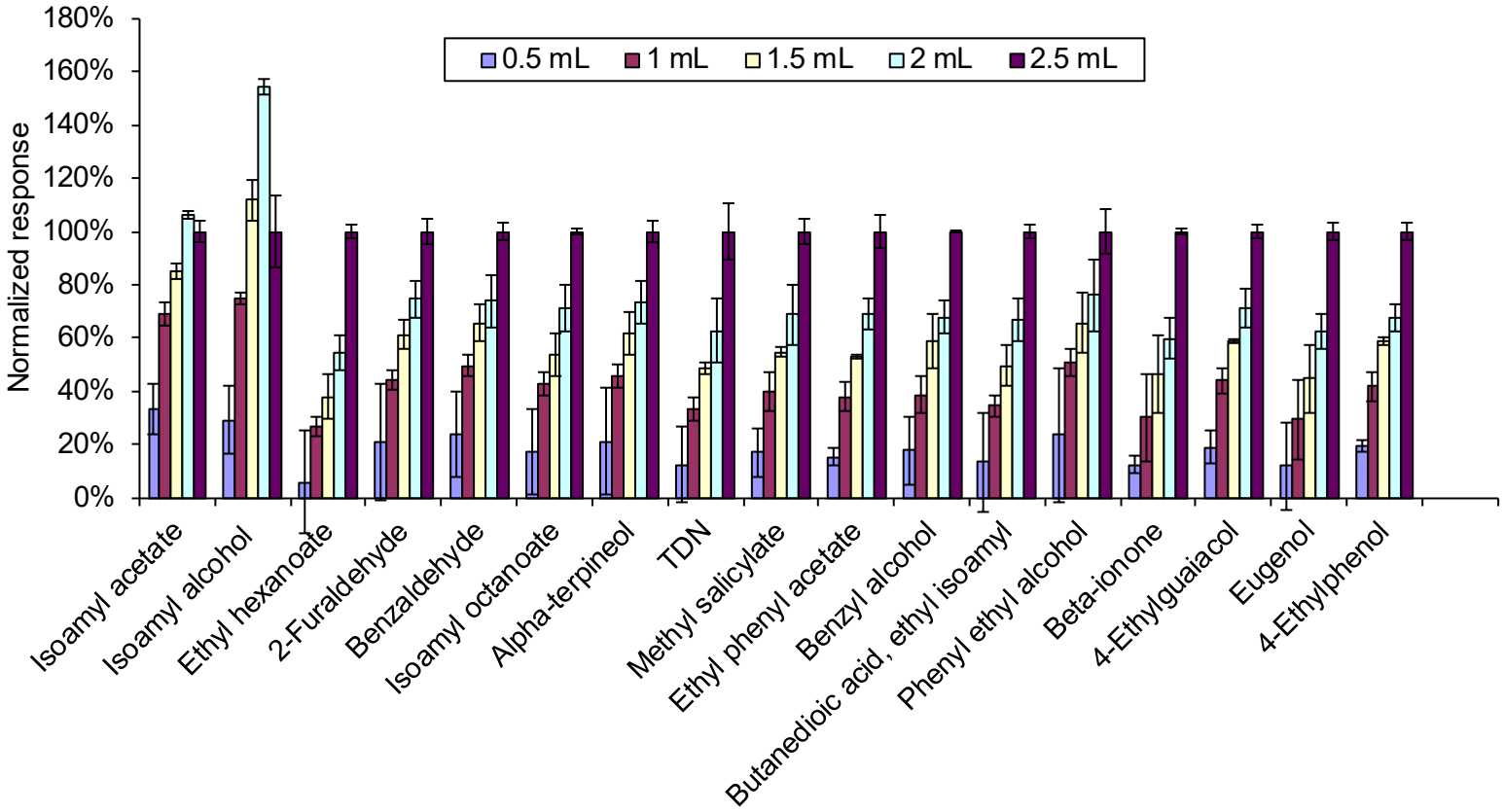

Fig. S2. Normalized responses as function of distillate volume in the DLLME vessel. Final sample volume is adjusted to 9 mL using ultrapure water in all cases, n= 3 replicates.