Method development for multiple partition coefficients determination to understand headspace aroma distribution of complex mixture

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Introduction

Sensory perception is directly related to compounds volatilization from the matrix to the atmosphere. From a physico-chemical point of view, this release may be described by partition coefficients, which correspond to the volatile concentration ratio between the liquid and gaseous phase. Partition coefficients may be determined thanks to Phase Ratio Variation (PRV) method which is based on the fact that, in a closed system, the headspace volatile concentration changes as a function of liquid phase volume (Ettre et al., 1993). This method is generally applied using HS-GC-FID.

For the analysis of various heavy compounds, some authors proposed a design for low-pressure gas chromatography coupled to mass spectrometry (LP-GC-MS), consisting in combining a micro-bore column to a mega-bore one, resulting in faster analysis and a better chromatographic resolution and sensitivity (de Zeeuw et al., 2000).

The goal of this work is to develop and optimize a new approach using multiple partition coefficients calculation in order to study potential modifications of headspace aroma distribution.

Experimental

The impact of various operating conditions on partition coefficients of esters and higher alcohols in headspace analysis was evaluated (Table 1). Five parameters were considered: (i) the time to achieve thermodynamic equilibrium in the gas phase (from 0 to 2880 minutes); (ii) the filling rate of the syringe (250, 500 and 750 µL/s); (iii) the gas injection rate (250, 500 and 750 µL/s); (iv) the volume ratio between the gas and liquid phase, from 227 and 10.4, corresponding to a liquid volume from 50 µL to 5 mL; and (v) the type of analytical column used (micro-bore BP21 capillary column (50 m x 0.32 mm ID, film thickness, 0.25 µm, SGE) or a mega-bore BP21 capillary column with low-pressure (30 m × 0.53 mm, film thickness, 0.5 µm, SGE, connected with a Siltite µ-union (SGE) to a 7 m x 0.25 mm ID deactivated column (SGE) at the inlet end). Each of the above mentioned parameters was optimized one at a time.

For esters and higher alcohols, the equilibrium time was evaluated in diluted alcohol solutions containing the mix of esters and higher alcohols, all at the average concentrations found in red wine (Table 1). All the solutions were prepared at room temperature (20°C); the vials were filled with 1 mL of each solution, and loaded on a tray cooler at 20°C. The headspace was analyzed at 15 different times, from 0 to 2880 minutes, and the surface area of each compound of interest was evaluated for each time.

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For the syringe filling rate, gas injection rate and the type of analytical column, vials were filled with 1mL of a solution containing the higher alcohols and the esters prepared at room temperature (20°C).

Table 1: Ethyl ester, acetate, and higher alcohol concentrations used for method development.

Ethyl Esters and Acetates (µg/L)													*Higher Alcohols (mg/L)*				
C_3C_2	C_4C_2	C_6C_2	C_8C_2	$2MeC_3C_2$	(2S)-$2MeC_4C_2$	(2S)- and (2R)-$2OH4MeC_5C_2$	C_2C_4	C_2C_6	C_2iC_4	C_2iC_5	$3OHC_4C_2$	$3MeC_4C_2$	2MB	3MB	2MP	P	B
150	200	200	200	250	50	400	10	2	50	250	300	50	50	200	100	30	4

C_3C_2, ethyl propanoate; C_4C_2, ethyl butanoate; C_6C_2, ethyl hexanoate; C_8C_2, ethyl octanoate; $2MeC_3C_2$, ethyl 2-methylpropanoate; S-$2MeC_4C_2$, S-ethyl 2-methylbutanoate; $2OH4MeC_5C_2$, ethyl 2-hydroxy-4-methylpentanoate; C_2C_4, butyl acetate; C_2C_6, hexyl acetate; C_2iC_4, 2-methylpropyl acetate; C_2iC_5, 3-methylbutyl acetate; $3OHC_4C_2$, ethyl 3-hydroxybutanoate; $3MeC_4C_2$, ethyl 3-methylbutanoate; 2MB, 2-methylbutan-1-ol; 3MB, 3-methylbutan-1-ol; 2MP, 2-methylpropan-1-ol; P, propan-1-ol; B, butan-1-ol.

Partition coefficients were determined according to the "Phase Ratio Variation" method developed by Ettre et al. (1993), by plotting the inverse of the chromatographic areas against the phase ratio β, in order to obtain values for a and b. Glass vials (22.8 mL, Chromoptic, France) were filled with 6 amounts of volatiles solutions in diluted alcohol solution or in dearomatised red wine (0.05, 0.1, 0.5, 1, 1.5 and 2 mL), with phase ratios from 227 to 10.4 (according to the liquid samples volumes).

Results and discussion

Method development

All the conditions tested and optimized did not allow the detection of all molecules involved in this study, as hexyl acetate and ethyl 3-hydroxybutanoate were not detected. For this last one, in view of its Log P value (0.31), this compound could have a high affinity to the matrix (diluted alcohol solution) and thus be retained in this one. Moreover, it could be hypothesized that these compounds were added at concentrations lower than their limits of detection. For hexyl acetate, this last hypothesis could also be related to its concentration in the matrix (2 µg/L). Chromatographic conditions, and more precisely the phase of the column used (BP21 capillary column, SGE, Nitroterephthalic acid modified polyethylene glycol) did not allow the separation of the 2- and 3-methylbutan-1-ol. The use of other types of columns, such as the CP-Wax 57 (50 m × 0.32 mm i.d.; film thickness, 0.25 µm; Agilent) allowed the separation of these two molecules, but not the detection of most esters used for the aromatic reconstitutions, with only 5 esters being detected. Consequently, the 2- and 3-methylbutan-1-ol were studied as a single peak area for the optimization and the validation of the method. In conclusion, except for ethyl 3-hydroxybutanoate and hexyl acetate, the optimization and validation of the method were realized for the 12 other ethyl esters and acetate, and for the 5 higher alcohols (with the 2- and 3-methylbutan-1-ol as the same peak area).

Optimization of the parameters showed that the thermodynamic equilibrium was achieved after 300 min in diluted alcohol solution for all tested compounds. The optimum syringe filling rate was determined at 750 µL/s and the gas injection rate at 500 µL/s. The investigation of the volume ratios between the gas and liquid phase highlighted that at a volume phase higher than 2 mL, no variation in the peak areas of esters and alcohols in headspace was observed. In conclusion, the volumes used for the partition coefficient determination ranged from 0.05 to 2 mL of liquid phase for ethyl esters and acetates and for higher alcohols. The use of a mega-bore column connected to a micro-bore column at the inlet end allowed to find more esters in the headspace, but also a better resolution for the chromatographic peaks. This type of chromatographic column assembly was called "low-pressure chromatography". In view of these results, but also because low-pressure mega-bore capillary column gave greater sample loadability and ruggedness, all the chromatographic analysis were performed using this column coupled to low-pressure injection (static headspace low pressure gas chromatography coupled to mass spectrometry).

Application of the new SHS-LP-GC/MS method

The new SHS-LP-GC/MS method developed and optimized in this work was used to calculate partition coefficients of various ethyl esters and acetates but also higher alcohols. Partition coefficients for 9 esters were calculated in diluted alcohol solution alone or supplemented with average concentrations of 5 higher alcohols.

As shown in Figure 1, in diluted alcohol solution, the addition of higher alcohols led to a significant decrease of the partition coefficients for esters ($p = 0.05$), except for ethyl propanoate ($p > 0.05$). Partition coefficients for higher alcohols were also calculated at average concentrations found in red wines in dilute alcohol solution alone or supplemented with a pool of 13 esters at average levels. Unlike the effects observed on esters, partition coefficients of higher alcohols were not impacted by the addition of these last ones ($p > 0.05$) (results not shown).

As the partition coefficient represents the distribution of molecules between gas and liquid phases, a decrease of this parameter indicates a decrease of the volatilization in the gas phase. These results therefore indicated that the addition of higher alcohols led to a decrease of esters concentrations in the gas phase. These observations could be explained by the fact that the addition of these 5 higher alcohols was added in the dilute alcohol solution at concentrations not included in the infinite dilution region. In the present study, the 5 higher alcohols were added at molar fractions from $4.7.10^{-3}$ to $5.7.10^{-1}$. Alessi et al. (1991) have introduced the concept of "infinite dilution" which correspond to the conditions "were the addition of an infinitesimal amount of the component 1 does not modified the thermodynamic behavior of the mixture, that is like the component 2 does not notice the addition of the component 1". It was also defined that the range of infinite dilution in mixture started at a mole fraction less than 10^{-4} (Alessi et al., 1991). These data could explain why in our context the addition of higher alcohols, at concentrations higher than the infinite dilution area, modified esters volatility.

Previous sensory analyses have demonstrated that the addition of higher alcohols led to an increase of the olfactory thresholds of the pool of 13 esters, as well as a decrease of the perception of fruity notes and increase the perception of butyric and solvent notes (Cameleyre et al., 2015). The diminution of the volatility of esters (responsible of the fruity character in red wines) in the presence of higher alcohols is a physicochemical fact, which is totally in agreement with the olfactory decrease of fruity perception as a consequence of the addition of higher alcohols.

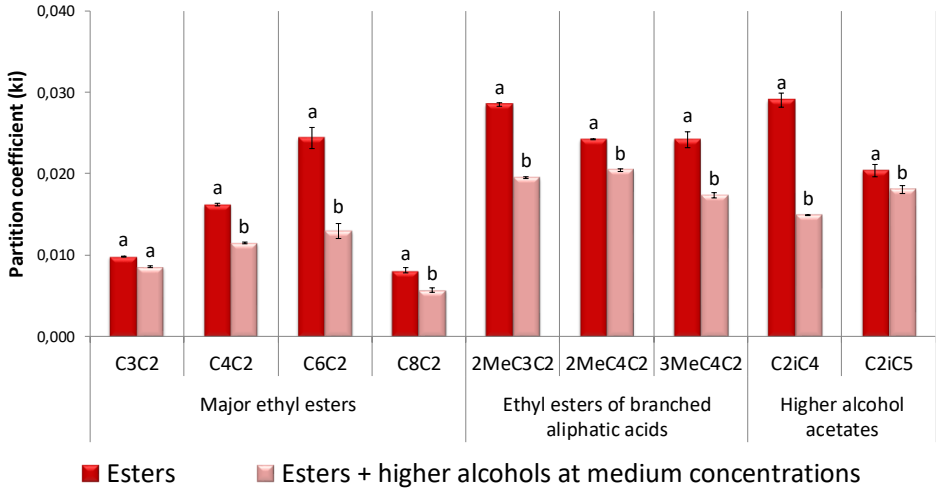

Figure 1: Impact of 5 higher alcohols found in red wines on partition coefficient of ethyl esters and acetates in dilute alcohol solution (12% v/v.)

A new approach to calculate multiple partition coefficients in complex mixture has been developed and particularly validated regarding esters and higher alcohols. This approach used a combination of different methods usually found for headspace analysis and characterization on the one hand (static headspace) and for pesticide analysis on the other hand (low-pressure gas chromatography). This methodology consisted in analyzing vial headspace at the thermodynamic equilibrium using a short guard capillary column connected to an analytical mega-bore column finishing at the MS detector. The association of these two techniques led to an increase of the injection volume and to the detection of more compounds compared to a simple micro-bore column, and additionally to a decrease of the run time.

Thanks to this method, it was possible to calculate partition coefficients for a multi-components mixture, and it was highlighted that the addition of higher alcohols in different matrices led to a decrease of the release of esters in the headspace.

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