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Development of a membraneless extraction module for the extraction of volatile compounds: Application in the chromatographic analysis of vicinal diketones in beer

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ABSTRACT

A membraneless extraction module (MLEM) for the sample preparation of volatile compounds and its use for the chromatographic analysis of vicinal diketones in beer are reported. The extraction process is based on the same principles of gas diffusion (GD) and pervaporation (PV); however it does not use a membrane. This module has a lower chamber where the sample continuously flows, while volatile compounds evaporate to the headspace. Inside the module there is a suspended small reactor, where a small volume of a suitable acceptor solution is placed. This extraction module was tested in the determination of vicinal diketones (VDKs) in beer (CV = 5%; LOD = 4 $\mu g\,L^{-1}$), showing applicability with real samples. Several parameters of the extraction process, such as temperature, sample flow and extraction time, were studied and optimized. This module proved to be a good tool for the sampling of volatile compounds, since the extraction is made without using a membrane avoiding all the robustness problems related with its use.

1. Introduction

In this work, the main vicinal diketones (VDKs) present in beer, diacetyl (DC) and pentane-2,3-dione (PD), were determined using a novel MLEM and *o*-phenylenediamine (OPDA) as the derivatizing agent (Fig. 1) [1–3]. These compounds are very important for the beer industry since they have intense butter like aroma (especially DC), becoming unpleasant in beer, even at very low content. The analytical control of DC concentration is one of the most important analyses done in the beer industry: it is important to ensure the final product quality and also to determine the end point of fermentation [4–6].

Continuous GD is especially useful in the on-line determination of volatile compounds, as it can increase the selectivity of a particular analytical method by avoiding sample matrix effects [7]. In a GD flow system the donor (sample) stream is separated from the acceptor stream by a membrane. The analyte is transferred through the membrane and captured by a suitably formulated acceptor solution [8,9]. However, the applicability of GD to complex samples is limited because of problems related with the membrane use, namely pore clogging by suspended particles or components of high molecular weight and deterioration by the continuous contact with the sample. Therefore, normally, cleanup steps and membrane changes are mandatory after – sometimes brief – periods of utilization. In order to avoid such problems related to the sample contact, PV presents itself as a solution. Analytical PV can be defined as the integration of evaporation and GD in a single module. Volatile substances present in a heated donor-phase diffuse through a porous membrane, and then vapor condenses on the surface of the cool acceptor stream on the other membrane's side [10]. The phenomenon is called 'pervaporation' because, due to the air gap between sample and membrane, initially there is an analyte phase change, from liquid to vapor, and then its diffusion through the membrane [11]. With the presence of an air gap between the membrane and the donor chamber, the sample does not contact the membrane. Consequently membrane clogging and/or deterioration are avoided. However the use of PV has some drawbacks in comparison with GD: lower throughput rate, slower mass transfer through the membrane and less sensitivity.

The GD/PV systems are valuable tools in the analytical extraction of volatile compounds, but they are not very robust processes since the membrane's characteristics (e.g. sensitivity) can vary with time and so it has to be replaced frequently. Therefore the development of continuous extraction systems without membrane seems to be an interesting alternative [12]. Recently, there has been research on the extraction of volatiles without the use of a membrane [13–17].

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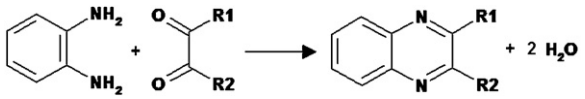

Fig. 1. Derivatizing reaction of VDKs with OPDA.

The purpose of this work was the development of a GD MLEM for the sample preparation of volatile and semi-volatile compounds. As can be seen in Fig. 2 this module contains a lower chamber – fitted with inlet and outlet orifices for the continuous flow of sample (i.e. donor) – an upper chamber and a reactor (filled with an acceptor solution) suspended in a framed support which allows free gas circulation. To perform extraction: the module is closed, the headspace quickly becomes saturated with the volatile analyte and then mass transfer into the acceptor liquid occurs.

2. Material and methods

2.1. Chemicals and samples

If not stated otherwise, all reagents are p.a. grade and were used without further purification. Acetonitrile (HPLC grade), all VDKs and OPDA were purchased from Merck (Darmstadt, Germany). Ultra-pure water from a Millipore Simplicity 185 water purification system (Millipore, Billerica, U.S.A.) was used.

Stock standard solutions (0.1 mol dm^{-3}) of each VDK were prepared in ultra-pure water and stored at 4 °C. These solutions were kept for one month. 10^{-3} mol dm^{-3} solutions of each VDK were prepared daily from the above mentioned stock solutions.

Phosphate buffer 0.1 mol dm^{-3}, pH 7.0 was prepared with disodium hydrogen phosphate (Merck) and the pH adjusted with 1 mol dm^{-3} hydrochloric acid (Merck). The derivatizing solution, 0.01% OPDA in phosphate buffer [3], was daily prepared and kept in the dark.

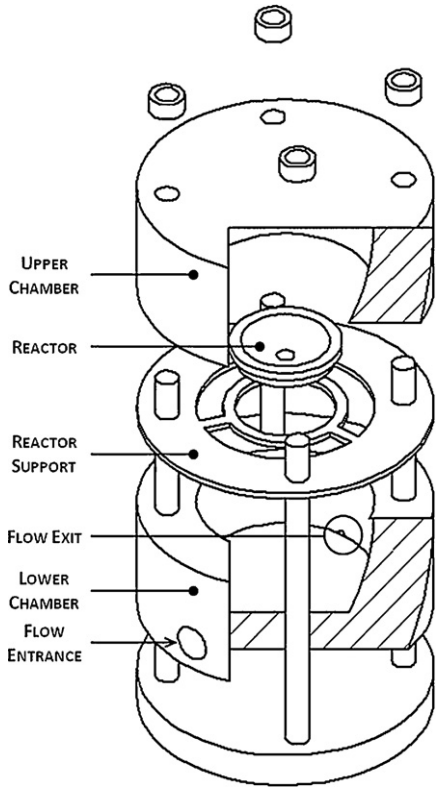

Fig. 2. MLEM drawing, exploded view.

Beers were supplied by a local brewery and kept at 4 °C; they were lagers with an alcohol content of 5.4%.

2.2. HPLC/DAD analysis

The HPLC system (Jasco Corporation, Tokyo, Japan) is composed by a low pressure quarternary gradient unit (model LG-1580-04) with an in-line degasser (model DG-1580-54) and an auto-sampler (model AS-950). The system is equipped with a photodiode array detector (model MD-1510 UV/vis multiwavelength detector). Separations were achieved on a Varian (Palo Alto, U.S.A.) Chromsep HPLC column, reverse phase (RP) C18 column (250 mm × 4.6 mm, 5 μm) in isocratic conditions, 50% acetonitrile and 50% acetate buffer 0.04 mol dm^{-3} pH 4.5 for 15 min. The flow rate was 0.8 mL min^{-1} and the wavelength used for quinoxalines detection was 315 nm. 100 μL of sample were injected into the chromatographic column kept at room temperature. Analytes were identified by comparing their retention times and UV-Vis spectra with standards.

2.3. Extraction system

A Gilson Minipulls II peristaltic pump was used (Gilson, Inc., Midleton, U.S.A.) for the sample flow. Tygon tubing (also from Gilson) with different internal diameters was used in the pump head. PTFE tubing with 0.8 mm internal diameter was used for the connections with a homemade perspex extraction module. The extraction module temperature control was performed with a dry heating block (Termobloc TD 150 P3 from Falc Instruments, Lurano, Italy). The sample was pre-heated in a 20 cm PTFE tubing loop, before arriving at the module.

2.4. Chromatographic determination of VDKs after derivatization with OPDA

The chromatographic conditions used in this work were optimized in a previous work [18]. The derivatization reaction is specific for α-dicarbonyl compounds. The separation of the resulting quinoxalines is achieved in a RP column with 0.8 mL min^{-1} of 50:50 acetonitrile and acetate buffer 0.04 mol dm^{-3} pH 4.5 for 15 min. The retention times of the resulting quinoxalines from the derivatization of DC (2,3-dimethylquinoxaline) and PD (2-ethyl-3-methylquinoxaline) are around 6 and 9 min, respectively (Fig. 3).

3. Results and discussion

3.1. General MLEM working mechanism

In Fig. 2 a detailed schematic representation of the MLEM is shown. The extraction procedure is straightforward. After degasification by magnetic agitation for about 10 min, the beer sample continuously flows through a 20 cm long PTFE tubing, where is pre-heated, and subsequently enters the lower chamber. A rigorous volume (typically 300 μL) of the derivatizing solution is placed, using a micropipette, in the reactor suspended inside the module.

The MLEM is closed by securing the upper chamber and then heated in a thermal unit. The volatile VDKs diffuse from the sample to the module headspace being then absorbed by the acceptor solution. After some time the acceptor solution is collected with a micropipette and injected in a chromatographic system.

Mass transfer, from the sample to the acceptor solution, is enhanced and accelerated by the presence of a derivatizing substance. Having a derivatization reaction where an analyte (x) reacts with a derivatizing agent (y) producing a stable product (z) and considering a steady state in the donor's flow and that the mass analyte content present in the headspace not significant ($x_{donor} \gg x_{headspace}$

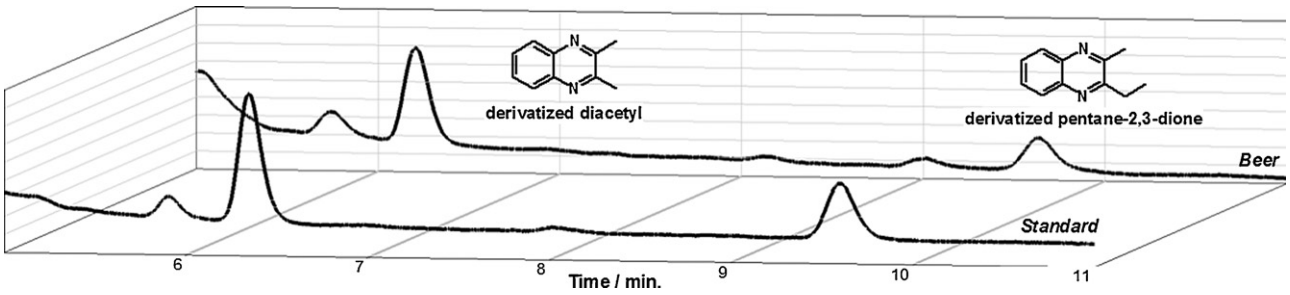

Fig. 3. Typical chromatograms obtained by the reported procedure, derivatized DC and PD peaks appear with a retention time of 6 and 9 min, respectively.

and $x_{acceptor} \gg x_{headspace}$) the distribution coefficient (D) will be the following:

$$D_{acceptor/donor} = \frac{[x_{acceptor}] + [z_{acceptor}]}{x_{donor}} \quad (1)$$

Assuming activity coefficients of 1, i.e. considering that the activities values are equal to the concentrations (a common simplification), the equilibrium constant of the derivatization reaction ($K_{derivatization}$) is described as:

$$K_{derivatization} = \frac{[z]}{[x] \cdot [y]} \quad (2)$$

Eq. (1) can be linked with Eq. (2), as follows:

$$D_{acceptor/donor} = \frac{[x_{acceptor}](1 + [y_{acceptor}] \cdot K_{derivatization})}{[x_{donor}]} \quad (3)$$

$[x_{acceptor}]/[x_{donor}]$ is the liquid/liquid partition coefficient that in this case is 1 since the acceptor and donor solvents are the same, *ergo*:

$$D_{acceptor/donor} = 1 + [y_{acceptor}] \cdot K_{derivatization} \quad (4)$$

This means that with a high value of $K_{derivatization}$ – which is normal in these types of reactions – considerable enrichment factors can be obtained and that is something noteworthy of attention [19]. Of course, one must take into account that these are theoretical conjectures for a state of equilibrium. Kinetic parameters are not taken here into consideration, mathematics involved are not trivial and will be addressed in the near future. However these assumptions provide important information on the way the MLEM works.

One can notice that this extraction system has some resemblances to headspace solid phase microextraction (SPME) [20], which to some extent is true. Still important differences are present particularly the extracting element's physical state, in this case it is liquid which can simplify the experimental connection between extraction and the instrumental analysis; per example, the acceptor solution can be directly injected into a liquid chromatographic system, it can be directly analyzed by spectrophotometry or voltammetry, among others.

3.2. Extraction optimization using the MLEM

Studies were made for the extraction process optimization. The following parameters were evaluated: (a) extraction time; (b) extraction temperature; (c) presence of OPDA in the reactor; (d) and the sample flow rate in the lower chamber.

(a) Extraction time – Although there is a continuous sample flow in the MLEM's lower chamber, the acceptor solution in the reactor is static. Therefore the concentration of derivatized VDKs in the reactor increases with the extraction time used (Fig. 4). For beer samples an extraction time of 10 min was a good compromise between sensitivity and extraction speediness.

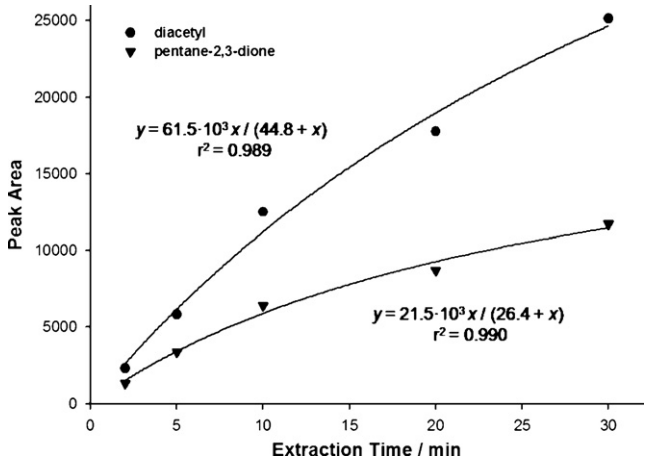

Fig. 4. DC and PD peak area variation with the extraction time. A hyperbola, a typically used function for situations of saturation, is used to explain the obtained results.

(b) Extraction temperature – Since the extraction is based on the analyte volatilization, temperature is obviously a very important parameter to be controlled. The temperature's influence of the heating element encircling the module was studied between 60 and 90 °C and results are presented in Fig. 5. As expected, for higher temperatures extraction is more efficient, due to an increase of volatilization. For this reason 90 °C was set as the extraction temperature.

(c) Presence of OPDA in the MLEM's reactor – The derivatization agent presence in the reactor was tested to evaluate its contribution to the VDKs' extraction. These tests were accomplished comparing the extraction with and without OPDA in the reactor (in the latter situation, the solution was after submitted to derivatization in order to be detectable by HPLC/DAD). The obtained results can be seen in Fig. 6 and show that the presence

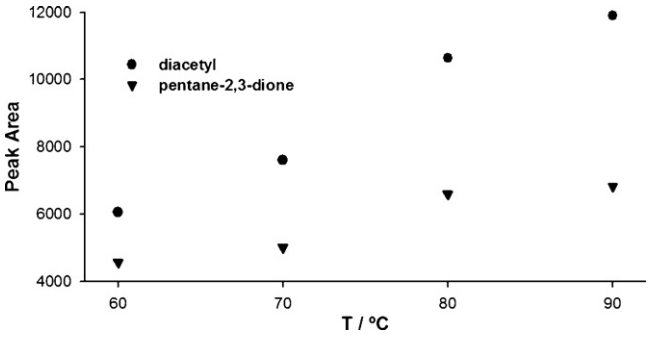

Fig. 5. DC and PD peak area variation with the extraction temperature.

Table 1
Method's features.

VDKs	C.V. ($n=5$)	$y=ax+b$ ($n=6$)	r^2	Linear range ($\mu g\,L^{-1}$)	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
DC	4%	$y=93x+199$	0.9989	10–120	3	10
PD	5%	$y=53x+100$	0.9987	12–120	4	12

Table 2
Method application to beer samples.

	DC ($\mu g\,L^{-1}$)			PD ($\mu g\,L^{-1}$)		
	EBC method 9.24.2	MLEM (HPLC/UV)[a]	MLEM (HPLC/UV)[b]	EBC method 9.24.2	MLEM (HPLC/UV)[a]	MLEM (HPLC/UV)[b]
Beer 1	16 ± 3	17 ± 1	16 ± 2	16 ± 3	17 ± 2	16 ± 3
Beer 2	93 ± 14	93 ± 9	95 ± 10	80 ± 12	67 ± 7	66 ± 7
Beer 3	31 ± 5	30 ± 3	29 ± 3	13 ± 2	12 ± 1	11 ± 1
Beer 4	18 ± 3	16 ± 2	18 ± 2	17 ± 2	20 ± 2	20 ± 2
Beer 5	26 ± 5	27 ± 3	28 ± 3	16 ± 3	16 ± 2	16 ± 2

[a] Without an internal standard.
[b] With an internal standard.

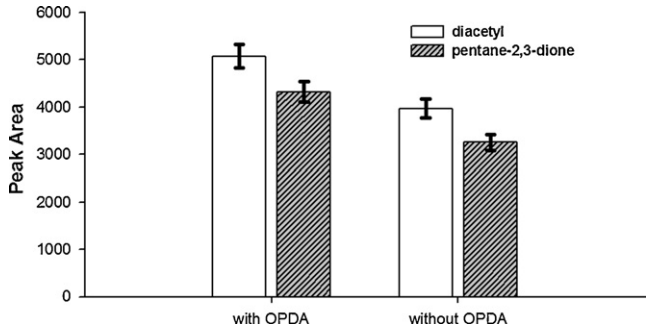

Fig. 6. Influence of OPDA presence in the MLEM's reactor during the extraction of DC and PD.

of OPDA in the reactor besides providing the VDKs' derivatization also has a positive influence on the extraction efficiency and, coherently to what was stated before, on the mass transfer's kinetics.

(d) Sample flow rate in the MLEM's lower chamber – An important parameter that could influence the extraction is the sample flow rate in the lower chamber. As can be seen in Fig. 7, the increase in the sample flow rate does not drastically influence the final output. This result can be interpreted assuming a rapidly obtained gas-liquid equilibrium described by Henry's Law.

3.3. Method's features

The method's features were evaluated using beer samples with standard additions of DC and PD. The figures of merit in terms of linearity, linear range, repeatability ($n=5$) and limits of detection and quantification (three and ten times the standard deviation of the intercept/slope) are summarized in Table 1.

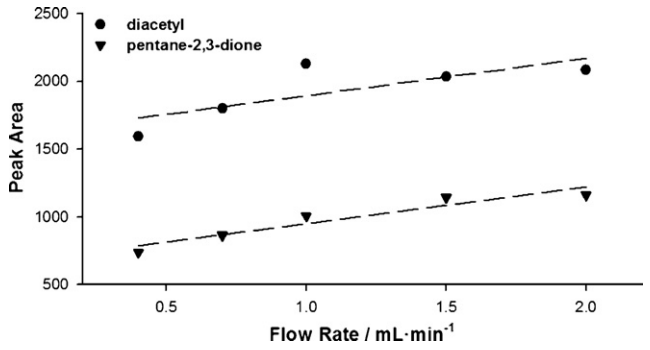

Fig. 7. DC and PD peak area variation with the sample flow in MLEM's lower chamber.

3.4. Application and validation

In order to test the method's accuracy and fitness in the analysis of beer, the method was applied in the determination of both DC and PD in bottled beer samples, which were also analyzed by the reference methodology (EBC method 9.24.2) [21], an analysis by headspace GC-ECD with an overall time of at least 1.5 h, in a certified food control laboratory. Samples were analyzed as previously described. Standard additions method (direct addition to the beer sample) with and without the use of an internal standard (hexane-2,3-dione) was used for quantification. In Table 2 results obtained are compared with the reference values. The experimental results fit very well the reference values, confirming the accuracy of the proposed method.

4. Conclusion

A MLEM for the quick and simple extraction of volatile compounds was reported in this work. This module shown evidence to be a valuable tool: it is very simple to operate and clean up and avoids all the problems related with the use of membranes. Therefore it is a good alternative to classical GD or PV systems. Furthermore, the MLEM is very versatile: it can be used with many types of samples and for a large number of volatile compounds. Moreover, due to the acceptor solution's small volume, considerable enrichment factors can be obtained in a short time. This makes the module useful for the preparation of samples for chromatographic analysis, among others.

The applicability and the good performance of the module were proved with the results obtained in the HPLC/UV determination of the VDKs in beer. After all the optimization studies, the extraction conditions established were the following: extraction temperature of 90 °C, extraction time of 10 min and sample flow rate in the module's lower chamber of 1.0 ml min^{-1}. The methodology shown to be precise and accurate; and when compared to the reference methodology overall analysis is decreased from 1.5 h to only 20 min.

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