Analusis, **2000**, *28*, 163-167

Simple analysis of odorous fatty acids in distillery effluents by capillary electrophoresis

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Abstract. The separation of short-chain carboxylic acids using capillary electrophoresis was already presented in several previous studies. As this method appears as a simple and fast alternative to more classical chromatographic approaches, this paper describes its application to the analysis of malodorous fatty acids in distillery effluents. The operating conditions were modified in order to improve the sensitivity. A 100 µm ID capillary combined with injection by electromigration led to detection limits in aqueous samples from 10 to 45 $\mu g.L^{-1}$. The ability of this method to analyse air samples was also examined. The detection limits obtained are in the order of $\mu g.m^{-3}$. This methodology was then applied to monitor the olfactory pollution of a distillery. The results obtained enabled a modification of the process to be proposed in order to reduce the malodorous emissions.

Key words. Short-chain fatty acids – capillary electrophoresis – distillery effluents.

Introduction

Short-chain fatty acids are mainly produced by food and beverage industries during fermentation processes. For example, volatile acidity, which gives bad taste to wine, may appear during the vinification process, especially during storage. This is due to alcohol oxidation by lactic or acetic bacteria [1,2]. The volatile acids formed are acetic, propionic, butyric and valeric acids [1,2]. These fatty acids are malodorous at relatively low contents in ambiant air, *i.e.* the perception levels of acetic, propionic, butyric and valeric acids are respectively 2, 0.109, 0.014 and 0.020 $mg.m^{-3}$ [3]. Consequently, these compounds may contribute greatly to the unpleasant odor generated by distilleries where by-products of the wine industry are used as raw materials.

In order to reduce the olfactory pollution in such activities, the compounds responsible for the malodorous emissions must be monitored. Therefore, 4 fatty acids (acetic, propionic, butyric and valeric acids) were chosen as odor markers and analysed in liquid effluents of the main steps of the process. According to the numerous analysis expected, a simple, cheap and fast method was requested. The analytical techniques generally used for the determination of carboxylic acids involve gas chromatography [4,5]. However, the polar acids are not suitable for direct GC injection. Therefore, a preliminary derivatization step is needed, using, for example, esterification by pentafluorobenzyl bromide (PFBBr) or methylation catalyzed by trifluoroboron (BF_3) [5,6]. These reactions are performed in organic media and thus require preliminary solvent or solid-phase extractions [7]. These procedures are long and complex to carry out. A more simple and specific approach may consist in performing a separation by ionic chromatography [8]. In this case direct injections of aqueous samples are allowed, but a possible damage of the column can occur after numerous analysis of complex matrices such as distillery effluents. Indeed, this kind of samples are constituted of a wide diversity of organic compounds such as alcohols, carbohydrates, ketones, aldehydes, esters and acids. Thus, capillary zone electrophoresis appears as an interesting alternative method. As example, Roldan-Assad *et al.* developed the separation of linear saturated fatty acids in their free form from C2 to C14 in less than 10 min [9]. The solubility of the most apolar compounds in the water-based electrolyte was improved by adding cyclodextrins and methanol. An indirect UV detection led to minimum detectable concentrations of the order of 0.2 – 0.5 $mg.L^{-1}$. Another study also showed good performances of CZE for the analysis of organic acids in biological matrices with limited sample preparation [10].

According to these encouraging results, this paper described the development of a CZE method for the fast analysis of short-chain fatty acids in distillery effluents. The performances were defined according to different experimental conditions in order to improve the sensitivity. The possibility of analysing gaseous samples was also examined. The methodology was applied to the characterization of liquid effluents of a distillery located in the south-east of France. The results allowed to highlight the main cause of the malodorous emissions and therefore to envisage an appropriate action to reduce the olfactory pollution.

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Received October 1, 1999; revised February 11, 2000; accepted February 11, 2000.

Article available at http://analusis.edpsciences.org or http://dx.doi.org/10.1051/analusis:2000111

Material and methods

Reagents

Individual stock solutions (1 g.L^{-1}) of the acids under investigation were prepared in methanol (Carlo Erba RS for HPLC, 99.9 %).

The model compounds are: acetic acid (Merck Suprapur 96 %), propionic acid (Prolabo Rectapur, 99 %), butyric acid (Prolabo Rectapur, 99 %), valeric acid (Merck For synthesis, 99 %), caproic, isobutyric, isovaleric and oenanthic acids (Fluka, > 98 %).

Working solutions were prepared by dilution in pure water (MilliQ, Millipore) of the stock solutions.

The electrolyte used was a 7.5 10^{-3} mol.L^{-1} aqueous solution of Na_2HPO_4, 12 H_2O (Acros, 98 %) containing an electroosmotic flow modifier OFM-OH 10^{-3} mol.L^{-1} which is a cationic surfactant with OH^- as counter-ions (Waters). The pH is 10.2. This solution can be stored several days at 4 °C and re-used without significant change in compound migration.

Capillary electrophoresis

A Quanta 4000 capillary electrophoresis (Waters), equipped with a Millenium 2010 software (Waters) was used. A direct UV detection was carried out at 185 nm (Hg lamp with relevant filters). Two different fused silica capillaries were tested: 80 cm × 75 µm ID and 80 cm × 100 µm ID. In both cases, the applied voltage for compound migration was 25 kV. Two different injection modes were compared: hydrostatic mode for 45 s, and electromigration mode at 5 kV for 45 s.

Generation of the gaseous atmosphere and gas sampling

The ability of the method to analyse gaseous samples was checked by using a synthetic atmosphere containing controlled amounts of the most volatile model compounds (C2 to C5). This atmosphere was realized by diluting a mixture of the pure different acids in an air stream (flow-rate: 5 L.min^{-1}). At low concentration levels, a methanolic solution of the acidic compounds was used. The quantity of the liquid solution introduced in the air stream was controlled by an automatic syringe dispenser. The experimental device is described in figure 1.

The gaseous sample hence generated was pumped through a 100 mL aqueous basic solution (NaOH 10^{-2} mol.L^{-1}) where the acids are solubilized. The sampling flow-rate was fixed at 1 L.min^{-1} for 4 h. The basic solution was directly analysed by capillary electrophoresis in the conditions described above.

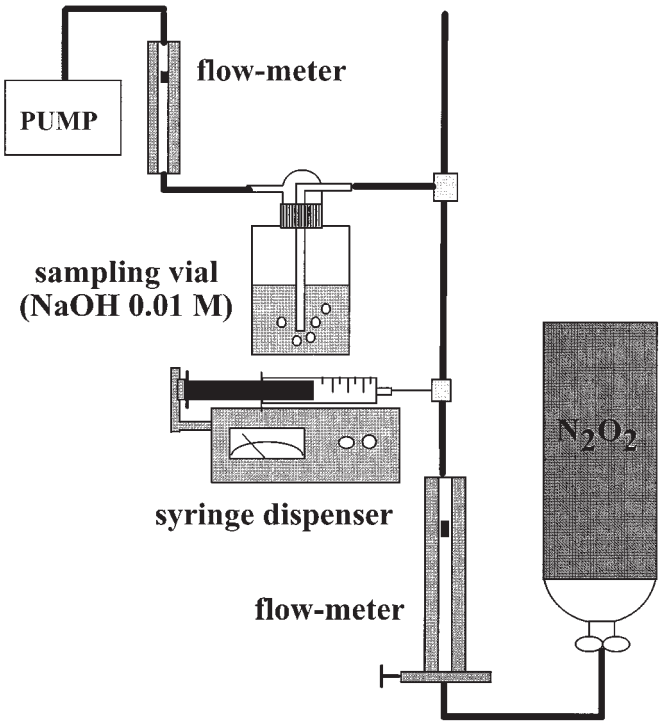

Fig. 1. Gas generation device and air sampling procedure.

Results and discussion

Performances of the method

The performances of the method were defined by using standard aqueous solutions of the model acidic compounds. They were then extended to the analysis of gaseous atmospheres after checking the gas generating device as well as quantitativity and repeatability of the sampling method.

Under standard experimental conditions, *i.e.* 75 µm ID capillary and hydrostatic injection, the detection limits in aqueous samples range from 0.22 to 0.38 mg.L^{-1} (Tab. I). This corresponds to the minimum concentrations stated by Roldan-Assad *et al*. [9]. Sensitivity can be increased in a 3 order of magnitude by using a 100 µm ID capillary. It can be also noted that the peak shape obtained with the 100 µm ID capillary is poor compared to the 75 µm ID one, due to the greatest quantity injected. However, the resolution remains satisfactory. A further improvement (× 15) can be performed by using injection by electromigration (Fig. 2). The repeatability is 2,4 % according to 12 measurements, and the calibration curves are linear from 1 mg.L^{-1} to at least 50 mg.L^{-1}.

For the concentration levels generally found in liquid distillery effluents, it may not be necessary to use the electromigration injection. Moreover, this mode can affect the representativity of the injected aliquot by favouring the

Table I. Migration times (tm) and detection limits (DL) in water and air samples corresponding to the different experimental conditions tested.

	75 µm ID CAPILLARY Hydrostatic			*100 µm ID CAPILLARY Hydrostatic*		*Electromigration*	
ACIDS	tm (min)	D.L. (mg L^{-1})	D.L. (mg m^{-3})	tm (min)	D.L. (mg L^{-1})	D.L. (mg m^{-3})	D.L. (mg L^{-1})	D.L. (mg m^{-3})
acetic	6.3	0.27	0.060	5.3	0.12	0.050	0.017	0.007
propionic	6.8	0.22	0.050	5.7	0.06	0.020	0.010	0.003
butyric	7.2	0.22	0.050	6.0	0.08	0.014	0.014	0.002
valeric	7.5	0.25	0.060	6.3	0.06	0.011	0.011	0.002
caproic	7.8	0.32	–	6.6	0.07	–	0.014	–
oenantic	8.3	0.38	–	7.0	0.22	–	0.045	–
MEAN		**0.28**	**0.055**		**0.10**	**0.024**	**0.019**	**0.004**

introduction of the most mobile species that could be present in such complex matrices.

Considering gas sampling, recovery yields are satisfactory (up to 80 %) for all the volatile acids studied in the individual concentration range from 1 $mg.m^{-3}$ to 30 $mg.m^{-3}$ (Tab. II). Above this concentration level, the gas generation is not quantitative for the less volatile compounds (butyric and valeric acids). The mean repeatability of the overall procedure including gas generation and sampling is 5 % and was evaluated for 5 different experimentations by using 3.4 $mg.m^{-3}$ individual concentrations (Tab. II).

The NaOH solution was then directly analysed by capillary electrophoresis without any pre- treatment. In this case, the electromigration injection mode can be very relevant. Indeed, the concentrations to be measured in air are low, according to the odour perception levels ($\mu g.m^{-3}$). Moreover, the matrix effects in the basic sampling solution are negligible compared to those occuring in liquid effluents. Table I shows that the mean detection limit is around 4 $\mu g.m^{-3}$.

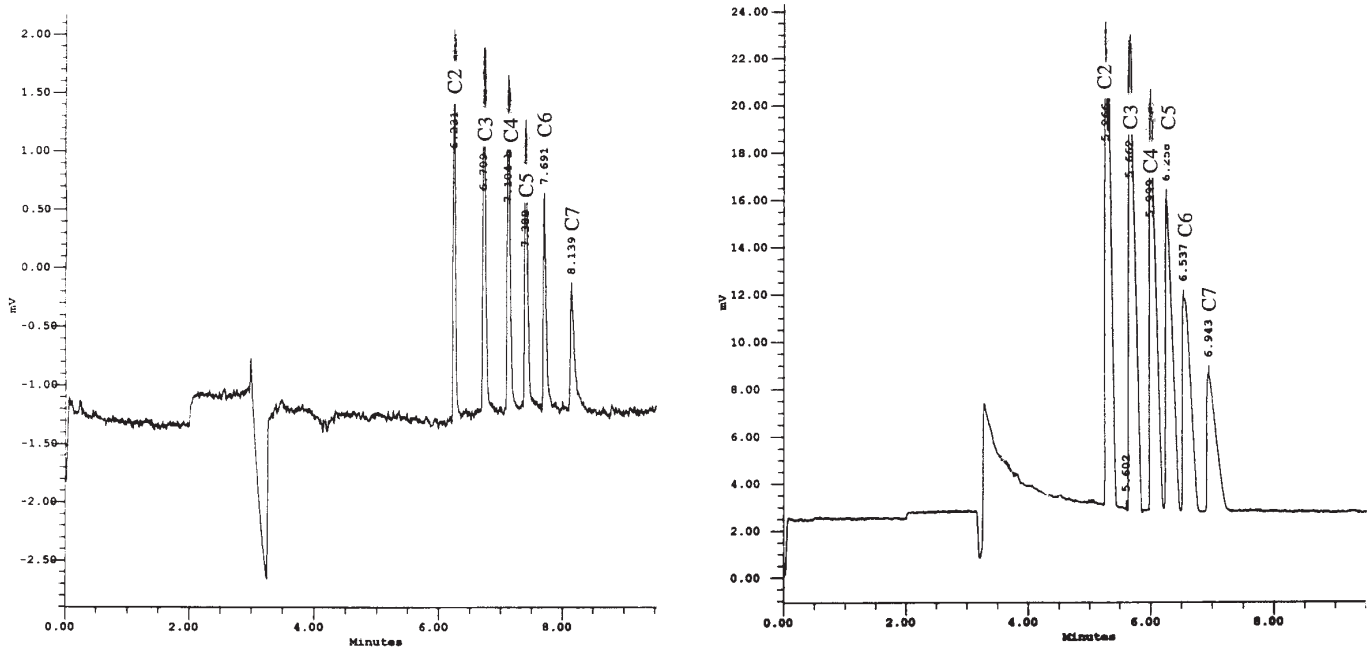

Fig. 2. Electropherograms of a standard solution of fatty acids (10 mg L^{-1} each). C2: acetic acid; C3: propionic acid; C4: butyric acid; C5: valeric acid; C6: caproic acid; C7: oenantic acid.
Left: 75 µm ID capillary and hydrosatic injection - Right: 100 µm ID capillary and electromigration injection (5 kV, 45 s).

Table II. Reliability of the gas generation device and recovery yields of the gas sampling method.

ACIDS	*RECOVERY YIELDS (%)* 1 mg m^{-3}	3.4 mg m^{-3}	32 mg m^{-3}	*Repeatability*
acetic	95	91	95	2 %
propionic	89	90	91	3.5 %
butyric	99	92	82	8 %
valeric	93	85	75	7 %
MEAN	**94 %**	**89.5 %**	**85.7 %**	**5 %**

This could be decreased to 1 µg.m^{-3} by performing the sampling at 1.5 L.min^{-1} during 5 h in a 50 mL NaOH solution.

However, this method has some limits because it does not permit the quantitative analysis of isobutyric, isovaleric and isocaproic acids due to their co-elution with butyric, valeric and caproic acids respectively. The resolution could not be improved even by decreasing the voltage or adding an organic solvent (acetonitrile). However, these acids are rarely present in the effluents studied here. So, the methodology was not further optimized for their analysis.

Application to the analysis of distillery effluents

The method performed was applied to the analysis of the effluents of a distillery where the olfactory pollution was particularly important. The objective was to identify the main origin of the odour in order to propose a solution for reducing the malodorous emissions.

In this aim, short-chain fatty acids were chosen as odour markers and were analysed in different liquid effluents corresponding to the main steps of the distillery process (Fig. 3).

"Vinasses" were analysed according to the methodology previously performed. No specific pre-treatment was required, except for sample dilution in pure water. It can be observed that both fresh and old "vinasses" contain high concentrations of fatty acids (Tab. III). Moreover, the concentrations of the most odorous compounds increase significantly along the treatment chain (from tartric extraction to aero-refrigeration): 500 % increasing for butyric acid and 800 % for valeric acid. This indicates that the reactions of fermentation are not definitely blocked and can be reactivated during the process according to favourable parameters such as temperature, residence time, organic charge...

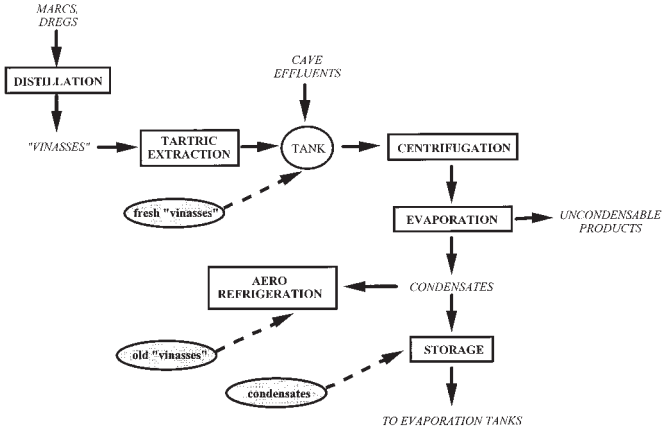

Fig. 3. Scheme of the distillery process and localization of the sampling points.

In order to estimate the impact of fatty acids on the odorous emissions, the purgeable fraction of the "vinasses" was characterized. 1 L of fresh "vinasses" was purged with compressed air at a flow-rate of 1 L.min^{-1} during 5 h. The fatty

Table III. Results obtained for the analysis of "vinasses" and condensates.

	"VINASSES" *Old*	*Fresh*		*CONDENSATES* *Continuous working*	*After 72 h storage*
ACIDS	liquid phase (g L^{-1})	liquid phase (g L^{-1})	gas phase (mg m^{-3})	liquid phase (g L^{-1})	liquid phase (g L^{-1})
acetic	2.00	2.00	13.3	0.646	0.095
propionic	2.90	2.50	9.0	0.028	0.075
butyric	0.75	0.15	0.6	0.013	0.150
valeric	0.40	0.05	4.2	nd*	0.115

*not detected

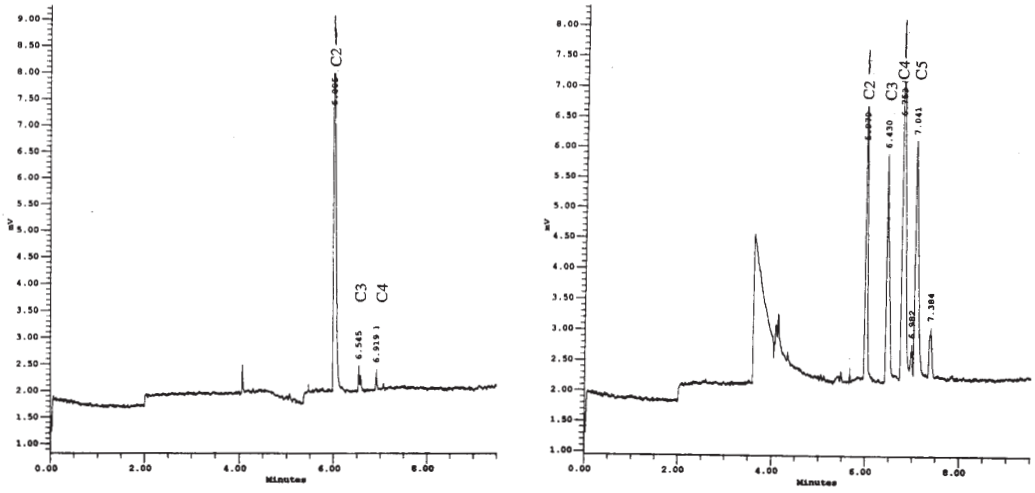

Fig. 4. Electropherograms of the condensates. C2: acetic acid; C3: propionic acid; C4: butyric acid; C5: valeric acid. Left: condensates produced during continuous working (dilution 20) - Right: condensates after a 72 h storage (dilution 5).

acids hence volatilized were solubilized in a 100 mL NaOH solution which was analysed by capillary electrophoresis. Results are given in table III. Even if this procedure enhances the compound volatilization compared to the real phenomenon which occured in the distillery, the gaseous concentrations measured indicate that the perception levels are largely exceeded. This also confirms that fatty acids are good odour markers in such industrial activity.

Condensates were analysed in the same way as "vinasses". Only dilutions in pure water were required before analysis. Two determinations were carried out: the first one corresponds to condensates produced during the continuous work of the distillery (week), the second one corresponds to condensates stored during 72 h (process stopped during the week-end) (Fig. 4). As it was stated for the "vinasses", the concentrations of propionic, butyric and valeric acids increased dramatically (Tab. III). This explains that the complaints of the neighbouring inhabitants mainly occur during week-ends and public holiday. In this case, a resumption of the fermentation process, essentially due to the long storage time of the effluent, could be assumed.

Conclusion

Capillary electrophoresis was successfully applied to a simple, fast and reliable analysis of short-chain fatty acids in distillery effluents. The procedure is easy to perform and does not need particular pre-treatment of the complex liquid effluents. The method was also adapted to the analysis of gaseous effluents with detection limits relevant to odour perception levels.

As application, a sampling campaign was realized in a distillery located in the south-east of France. This showed that the olfactory pollution often generated by this kind of activity is essentially due to a resumption of fermentation during the process and the storage period of the effluents. Therefore, the solution envisaged to reduce the malodorous emissions consists in modifying the process in order to avoid fermentations. The feasability and the efficiency of this treatment were checked in the laboratory by using the analytical technique presented here. The results and the application in situ will be published elsewhere.

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