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SEMIAUTOMATIC FLOW-INJECTION METHOD FOR THE DETERMINATION OF VOLATILE ACIDITY IN WINES

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A flow-injection (FI) method based on analytical pervaporation was assessed for its routine use in the determination of volatile acidity in winery laboratories. The new method was compared with both the official method and the Mathieu method, which is most often used in Spanish wineries, by testing 30 different wines, including young and aged, and sweet and dry wines from Montilla-Moriles *appellation d´origine*. The robustness of the new method was established and then all three methods were studied in terms of range of linearity and regression of the calibration curve, repeatability, reproducibility, sensitivity, detection and quantification limits (LOD and LQ, respectively) and time of analysis. The FI method surpasses the Mathieu method in reproducibility and both the Mathieu and official methods in LOD and LQ and sensitivity; it also requires less personnel involvement and shorter analysis time. The statistical criteria established by the "Office International de la Vigne et du Vin" were applied to the data and the results obtained indicated that the differences between the analytical parameters of the 3 methods are not significant and can be applied indistinctly. The correlation of the methods was studied by taking them 2 by 2, and the corresponding equations, coefficients and deviations confirmed the statistical results. Thus, the new method can be used in winery laboratories with clear advantages over its 2 counterparts (the routine and official methods).

Introduction

Pasteur was the first to observe the importance of volatile acidity on the quality of wines by demonstrating that volatile acids present in wine are quantitatively removed after several distillation steps and water additions for maintaining a constant volume.[1] Later, Jaulmes developed several titration methods to determine this parameter and studied their principles and causes of error.[1] These methods can be divided into 2 groups: distillation and steam dragging methods. The former can be developed in 2 ways depending on whether water is added or not for maintaining a constant volume in the distillation flask; the second is the basis of the official method established by the European Union (EU).[2]

Although both methods are time-consuming and error-prone, they are routinely used in wineries laboratories, causing an overload problem because of their slowness. The analytical literature contains few reports on the determination of volatile acidity in wines, and none on circumventing the shortcomings associated to conventional methods.

The method for the determination of volatile acidity proposed by Tubino and Barros, based on flow injection (FI)[3], coupled with gas-diffusion and conductimetric detection, was only sensitive enough for application to vinegar.[4] The non-linearity of the calibration graph makes the method very imprecise.[5]

A recent contribution to the determination of this parameter, also based on flow-injection, was provided by Su et al.,[6] who used of a membrane-based separation module and a bulk acoustic wave impedance sensor. Although the method seemed promising, the time required for sample pretreatment (20 min boiling for CO_2 removal, oxidation of SO_2 and filtration) and the need of special skills make it useless for routine application.

Other contributions rely on the use of complicated chemometric algorithms to improve the results provided by imprecise analytical methods.[7,8]

The present situation and the necessity for fast, inexpensive, simple and precise methods for decreasing and avoiding the overload in winery laboratories prompted us to develop a method based on FI coupled with analytical pervaporation[9-12] which overcame the drawbacks of the in used methods.[13]

Analytical pervaporation is a membrane-based technique for the removal of volatile analytes or their volatile derivatives from the sample matrix, either solid or liquid. It is defined as the integration of evaporation and gas-diffusion in a single module. The volatile substances present in a heated donor phase evaporate to an air-gap on the sample-surface and diffuse through a porous membrane to an acceptor phase (gas or liquid) at the other side of the membrane. The most important characteristic of pervaporation, as compared with gas-diffusion, is the presence of a constant-volume air-gap between the sample in the donor chamber and the membrane, which hinders any contact between them, thus avoiding clogging and/or deterioration of the membrane.

Based on the FI-pervaporation coupling, the method proposed by one of the authors enabled the sequential determination of total and volatile acidity in wines with a precision, expressed as relative standard deviation (RSD) of 5% and an analysis time of 12 min per sample.[13] This promising method was used as starting point, with the aim of proposing it as an advantageous alternative to be used in winery laboratories. Two standard methods, the official EU method[2] and that developed by Mathieu[1], were selected for exhaustive comparison of the results through the statistical criteria established by the "Office international de la vigne et du vin", OIV.[14]

Experimental

Apparatus

The experimental setup used for the determination of volatile acidity is shown in Fig. 1. A four-channel Gilson Minipuls-3 peristaltic pump (Villiers le Bel, France), fitted with a rate selector, two Rheodyne 5041 injection valves (Elkay, Galway, Ireland), one of them used as selecting valve, and PTFE tubing of 0.5 mm i.d. (Scharlau, Barcelona, Spain) were used to construct the manifold. A Philips PU8625 UV-visible spectrophotometer (Philips, Cambridge, U.K.), equipped with a Hellma 138-QS flow-cell (Hellma, Jamaica, NY), and a Knauer recorder (Scharlau) were also used.

A Selecta Tectrom bio water bath (Barcelona, Spain), a pervaporation module, described elsewhere[10-13] and PTFE membranes of 47 mm diameter and 1.5 mm thickness (Trace,

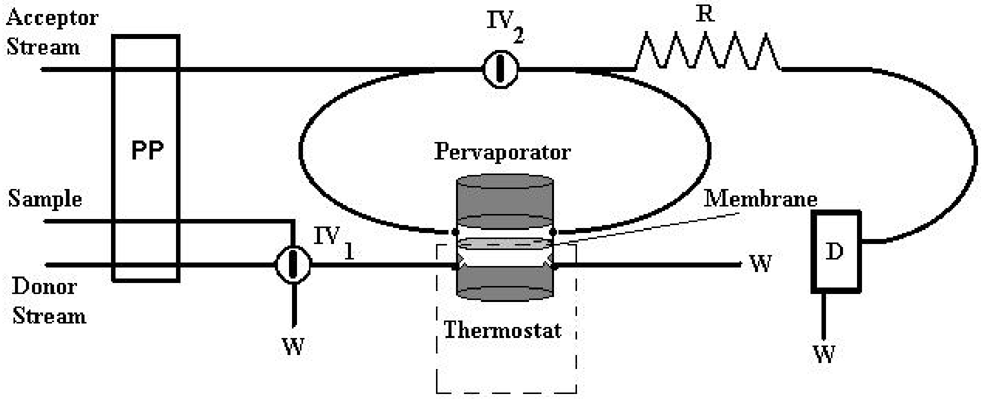

Figure 1. Flow injection manifold for the determination of volatile acidity in wines. PP denotes peristaltic pump; IV, injection valve; R, reactor; D, detector and W, waste.

Braunschweig, Germany) were used. An Eyela A-3S vacuum pump (Tokio Rikakikika, Co. Ltd) and an Ultrasons ultrasonic bath (Selecta, Barcelona, Spain) were used for CO_2 removal.

Reference measurements were developed using a Gibertini DE distillation unit (Milan, Italy) based on water steam dragging. Conventional laboratory glasware was used for development of the Mathieu method. Measurements of free and bound SO_2 were made with a Crison Compact Titrator D, mod. D-33 (Crison, Barcelona, Spain).

Reagents and solutions

The reagents used for calibration were glacial acetic acid, tartaric acid (Merck, Darmstadt, Germany) and absolute ethanol of analytical reagent grade (Panreac, Barcelona, Spain). bromocresol purple $5x10^{-4}$ M was used as indicator in a potassium dihydrogen phosphate buffer of pH 6.3 (both from Merck), which constituted the acceptor solution on the FI method. Sodium hydroxide, iodine, potassium iodide, hydrochloric acid, phenolphthalein and starch were used for developing the official and standard methods.

Procedures

In *Mathieu procedure,* 10 ml of the target wine is distilled until 6 ml of distillate is collected; then, 6 ml of water is added to the distillation flask. The process is repeated 3 more times so that 24 ml of distillate is finally in the collection flask. Approximately 10/11 of the total acetic acid present in the wine is collected in this way. The solution is then titrated with 0.1 M sodium hydroxide after addition of 2 drops of ethanol solution of phenolphthalein. After this, the solution is acidified with HCl and titrated with 0.05M iodine for SO_2 correction in the distillate.

The *official method*, based on dragging with water vapour, requires an apparatus for this first step which fulfils the established regulations. After removing CO_2, 20 ml of the target wine is poured, into the de-bubbler and 0.5 g of tartaric acid is added. The dragging step is developed until 250 ml of the condensate is collected. Then, the solution is titrated with 0.1 M NaOH after addition of 2 drops of ethanol solution of phenolphthalein, and later acidified with HCl and titrated with 0.05M iodine for SO_2 correction in the distillate.

The *proposed FI-pervaporation method* requires the manifold shown in Fig. 1, where a 2 ml of sample is inserted via injection valve IV_1 into a water carrier stream (flow-rate 0.8 ml min^{-1}) which leads the plug to the lower chamber of the pervaporator and then to waste W_1. Meanwhile, valve IV_2 is in the filling position, the content of its loop is static for its enrichment in the pervaporated species and the stream of the acceptor, indicator solution (flow-rate 0.8 ml min^{-1}) is going to the detector thus establishing the baseline. After 4 min from injection, valve IV_2 is switched to the load position and the stream of the acceptor solutions drives the content of the loop to the photometric detector for monitoring, at 590 nm, the change of the indicator caused by the analyte. A calibration graph was required for interpolation of the datum from the sample, which was obtained as average of triplicate injection.

Results and discussion

Robustness study

The FI method was checked and a study of robustness involving variables such as pH, temperature and flow-rate was conducted.

Study of pH

As established under "Reagents", the pH of the acceptor solution into which the volatile acids were colleted was 6.3. Change of ±0.1 units produced a change of the analytical signal (absorbance) of 13%; meanwhile with the change of ±0.2 units the signal error is 30%. Nevertheless, the pH of the acceptor was kept stable during a working day by the presence of a buffer system. At the start of each working day, the pH of this solution was checked and adjusted if necessary.

Table 1. Features of the compared methods

Parameters[a]	Methods		
	Official	Mathieu	FI-pervaporation
Repeatability (g/l)	0.033	0.038	0.038
S_r (g/l)	0.0118	0.0136	0.0136
Reproducibility (g/l)	0.042	0.049	0.046
S_R (g/l)	0.0149	0.0176	0.0162
$Fobs^r$	-	1.34	1.32
$Fobs^R$	-	1.39	1.18
$F_{1-\alpha}$	-	1.84	1.84
$S_{y,x}$ (g/l)	0.0134[b]	0.0171	0.0167
S_b (g/l)	0.0117[b]	0.0067	0.0064
LOD (g/l)	0.041	0.056	0.032
LQ (g/l)	0.084	0.100	0.086
Sample throughput (h^{-1})	5	8	10
Equation	y=0.993x+0.017	Y=0.814x+0.019	y=0.864x-0.009

[a] S_r and S_R are the deviation of repeatability and reproducibility, respectively. $S_{y,x}$ and S_b are residuals and slope deviations. $Fobs = S_r^2 / S_{ref}^2$

[b] Referenced to standard samples

Study of temperature

Temperature is a critical parameter as it determines the volatility of the target compounds and thus their transfer through the membrane after evaporation. Changes in temperature of ±1 ºC involved an error of 6%, with an almost linear variation as a change of ±2 ºC associated an error of 13%; thus surpassing the allowed deviation for wines with high volatile acidity. This high influence of changes in temperature hindered the use of a water thermostat and constant water evaporation made it necessary to refill the bath, with changes in temperature as a result. In addition, the loss of water changed the height of the pervaporator immersed in the hot water. Water was exchanged by polyetilenglycol, thus circumventing the error associated to the use of the former.

Study of the flow-rate

Changes in the optimum flow-rate (0.8 ml min^{-1}), either in the acceptor or donor stream, of ±0.1 ml min^{-1} produced a change of the analytical signal of about 5%. To avoid this error, the flexible tubes of the peristaltic pump were kept in optimal conditions, so that periodical exchange of these tubes every 15 working days was mandatory.

Preparation of the standards

Standards within a wide range of concentrations were prepared by dilution of the appropriate amount of glacial acetic acid in bi-distilled water. These standards were appropriate for wines with low and medium ethanol content (10-15 %). For long aged wines (ethanol content about 20 %) the large amount of ethanol pervaporated with the acid compounds change the absorbance spectrum of the indicator, so the volatile acidity thus determined did not coincide with that obtained by the official method. The addition of 15% ethanol to the standards was necessary for the determination of the volatile acidity in high alcohol-content wines.

The addition of tartatic acid to the standards had a not significant change on the analytical signal they provided.

Assessment of the proposed method

Thirty different wines, including young and aged, sweet and dry wines, from the *appellation d´origine* Montilla-Moriles (Spain) were used for the assessment study. Data of volatile acidity were the average of 3 determinations. All 30 wines were analysed by the 3 methods and the outlier values were deleted by applying the Grubbs test. Proper storage for wine stability was used during the study to avoid changes of the target parameter while the 3 methods were applied.

The protocol for assessment consisted of studying the analytical parameters, linearity range and regression of the calibration curve, repeatability, reproducibility, detection and quantification limits and analysis time by all 3 methods (i.e., the official, Mathieu and FI methods). Then, correlation between each 2 methods was assessed.

Repeatability (r)

The deviation, S_r, for a routine method to be accepted by the OIV must be <0.04 g acetic acid l^{-1} wine. As shown in Table 1, all 3 methods fulfil the requirement. The Snedecor test was applied in order to establish if the differences between repeatability of the methods were significant. The $Fobs=S_r^2 / S_{ref}^2$ was compared with the $F_{1-\alpha}$ obtained from Snedecor tables for $\alpha=0.05$ (P=95%).

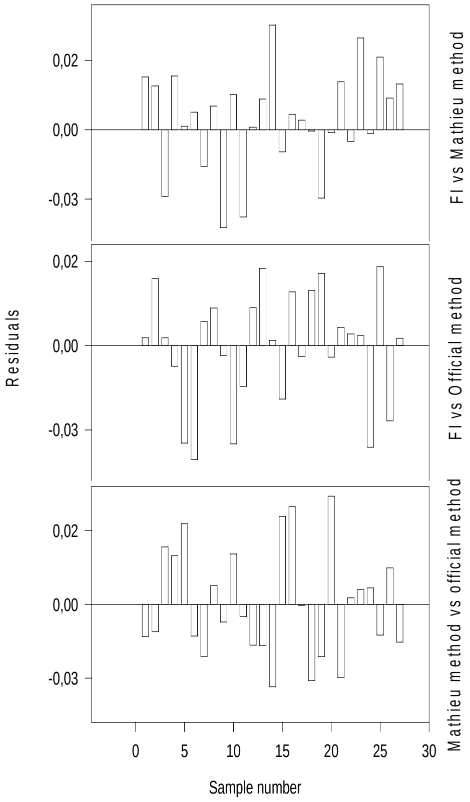

Figure 2. Comparison of residuals from the 3 methods studied.

Table 1 shows that $Fobs<F_{1-\alpha}$, therefore, the repeatabilities of the FI and Mathieu methods are similar to each other and to the official method.

Reproducibility (R)

The deviation in the reproducibility of a method, as established by the OIV, must be<0.08 g acetic acid l^{-1} wine.[14] Table 1 shows the R values obtained and the results of application of the Snedecor test. The reproducibility of the FI method was better than that of the Mathieu method, but both are within the accepted value and similar to that of the official method.

Detection (LOD) and quantification (LQ) limits

The usual volatile acidity values in wine range between 0.4 and 0.6 g acetic acid per liter of wine. As exception, both some young wines and long biological aged wines (*"fino"*) can present volatile acidities of 0.15 g acetic acid l^{-1}. Table 1 shows that the best LOD corresponds to the FI method and that both the official and the FI methods present similar LOQs. All 3 three methods exceeded the need to determine volatile acidity in wines.

Sensitivity

As established by International Union of Pure and Applied Chemistry (IUPAC), this parameter, expressed by the slope of the calibration line, exhibits the highest value for the official method. The proposed FI method is between the official and the Mathieu method.

Linearity

The linearity of the methods was studied by comparing the results obtained from 30 samples of different wines previously analysed by the official method. The linearity of the latter was established by standard solutions of acetic acid of 0.20, 0.30, 0.45, 0.60 and 0.80 g l^{-1}, with an excellent correlation of 0.999 between the concentration of the standards and that found by the method. The equation of the corresponding lines for the 3 methods, as well as the correlation coefficients, the residual deviations, $S_{y,x}$, and those of the slope, S_b (table 1) show that the best $S_{y,x}$ deviation corresponds to the official method followed by the FI method. For the S_b deviation, the best values are presented by the FI method.

Analysis time

The time required to develop each method was calculated as the average of all the steps involved. Table 1 shows that the FI method is the fastest; the value for the Mathieu method was obtained by using a batch or 4 in-series microdistillations which requires 30 min for the analysis.

Comparison of the methods

The methods were compared 2 by 2, using 2-dimensional diagrams (Table 1), where the correlation equations, coefficients and deviations of the slopes and intercepts are also shown. In the Mathieu-official method comparison, the intercept differs from the unit. This fact indicates a systematic error of the Mathieu method with respect to the official method, which is in agreement with the literature,[1] as the distillation method only removed 91% (10/11) of the total volatile acid compounds present in wine.

Similar results are obtained when the FI method is compared with the official method. The reasons for this behaviour are similar: The target compounds in the FI methods are not completely removed; on the contrary, an equilibrium is established between the amount of volatile acids in the donor solution, the air gap and the acceptor solution, which is displaced to the last by the high temperature to which the donor chamber is subjected. There is a close-to-one correlation between the Mathieu and FI methods for these reasons.

The residuals from the methods studied were also obtained to guarantee the data obtained in the regression analyses. Figure 2 indicates that the regression values do not differ significantly from those obtained experimentally.

Conclusions

The developed study has allowed:

(a) To check the Mathieu method, usually used in our laboratory, and compare it with the official method; thus assessing its performance and the goodness of its analytical parameters.

(b) To assess a new, continuous method, that has demonstrated to be better than the Mathieu method and similar but faster than the official method.

(c) In addition to its rapidity, one of the main advantages of the new method is its capability for automation. The use of active interfaces for controlling the peristaltic pump and the injection and auxiliary valves is an easy task, as is the use of a passive interface for collecting the data from the spectrophotometer.

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References

(1) Ribereau-Gayon J., Peinaud E., Sudraud P., *Traité d´oenologie. Sciencies et techniques du vin. Tome 1- Analyse et controle des vins.* Dunod 1976.

(2) Official Journal of European Community, L 272, October 1990.

(3) Valcárcel M., Luque de Castro M. D., *Flow Injection Analysis: Principles and Applications*, Ellis Horwood, Chichester, 1987.

(4) Tubino M., Barros FG., *J. Assoc. Off. Anal. Chem.*, 1991, **74**, 346.

(5) Barros FG., Tubino M., *Analyst*, 1992, **117**, 917

(6) Su XL., Nie LH., Yao SZ., *Fresenius´J. Anal. Chem.*, 1998, **360**, 272.

(7) Sing ASC., Smadja J., Gaydou EM., Conan JY., *Analusis*. 1994, **22**, 332

(8) García-Jares C., Medina B., *Analyst*, 1995, **120**, 1891

(9) M.D. Luque de Castro and I. Papaefstathiou, *Encyclopedia of Environmental Analysis and Remediation*, John Wiley & Sons, Inc. Chichester, 1998, p. 3462

(10) M.D. Luque de Castro, Papaefstathiou I., Trends in Anal. Chem., 1998, **17**, 41.

(11) M.D. Luque de Castro, M.M. Jiménez-Carmona, Química Analítica, 1999, **18**, 263.

(12) J. Amador-Hernandez, M.D. Luque de Castro, Food Chem., 2000, **68**, 387

(13) E. Mataix, M.D. Luque de Castro. Anal. Chim. Acta 381, (1999), 23

(14) *Recueil des méthodes internationales d`anlyse des vins et des mouts. Complement nº1 á l`edition officielle de juin 1990.* Office International de la vigne et du vin.