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SEQUENTIAL SPECTROPHOTOMETRIC DETERMINATION OF METHANOL AND IRON IN VINEGAR BY A FLOW INJECTION-PERVAPORATION METHOD

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An easily automatable sequential flow-injection-pervaporation method is proposed for the photometric determination of methanol and iron in vinegar. The method is based on separation of the methanol from the sample matrix by pervaporation followed by its oxidation to formaldehyde with permanganate, decolouration of the latter with $S_2O_5^{-2}$ and subsequent reaction of formaldehyde with p-rosaniline to yield a violet reaction product with maximum absorption at 567 nm. Iron is determined by an existing method based on reaction with thiocyanate in acidic medium and monitoring at 508 nm. After optimisation by either the univariate or multivariate approaches, as required, the linear range was established for methanol (4-1000 mg L^{-1}) and iron (0.18-20 mg L^{-1}); The proposed method was then compared with reference methods for methanol and iron in terms of repeatability (2.452 mg L^{-1} and 0.245 mg L^{-1}, respectively), reproducibility (4.435 mg L^{-1} and 0.356 mg L^{-1}, respectively), detection limit (LOD=82 and 0.234 mg L^{-1}, respectively) and traceability. The throughput was nine samples per hour.

Keywords: vinegar, pervaporation, flow injection, spectrophotometry, methanol, iron

Introduction

Methanol and iron are two key parameters to be monitored in vinegar. Methanol is always present in vinegar; it is not formed by the acetification process but exclusively by enzymic hydrolysis of the methoxyl groups of pectins during wine fermentation [1]. The methanol content depends on the extent to which solids from grapes, especially the skin, which has a high pectin content, are macerated. Vinegar from red wines has a higher concentration of methanol than white wines. The toxicity of methanol is well-known -following ingestion, it is oxidized, producing formaldehyde and formic acid, both of which are toxic to the central nervous system. Formaldehyde causes deterioration of the optical nerve, causing blindness. The dangeous levels is LD_{50}=350 mg Kg^{-1} [1]. The presence of iron in vinegars is mainly a result both of contamination by contact with ferrous materials during manufacture, and the iron in the wine from which the vinegar has been made. The iron content, which depend on the matrix, can cause hazes or even serious alteration of appearance and colour as a result of interaction with phenol compounds.

The official method for the analysis of methanol content of vinegar is gas chromatography using a split-type injector and a flame ionisation detector [2]. A usual method consists of oxidation of the analyte to formaldehyde by potassium permanganate in phosphoric medium and spectrophotometric monitoring at 575 nm of the product from the specific reaction of formaldehyde with chromotropic acid [3]. The official method for the determination of iron in vinegar is direct measurement by flame atomic absorption spectrometry (FAAS) [4]; another method is based on reaction with potassium thiocyanate and monitoring of the reaction product at 508 nm. The same procedure is used for wines [5].

No simultaneous, sequential, or individual flow-injection method for determination of both methanol and iron in vinegar has been reported in the analytical literature. Some references can be found on the determination of iron in wines, and some of them use flow injection in an attempt of automation. Neira et al. [6] developed a method for online sample preparation by use of sequential-injection analysis; which was based on complexation of iron with 1,10-phenanthroline and photometric detection at 520 nm. Pulido-Tofino et al. [7] used a fluorescent sensor to determine iron-pyoverdin immobilised on controlled-pore glass that reacted selectively with Fe(III), reducing its fluorescence emission. Cladera et al. [8] proposed a method based on the catalytic effect of the iron(III)-ethylenediaminetetracetic acid complex on the oxidation of hydroxylamine by dissolved oxygen with spectrophotometric detection. Only two methods using flow injection for determination of methanol in wine can be found in the analytical literature. One recent method is based on the use of a pervaporation module for the

separation of methanol and other volatile species from wine before their individual separation and determination by GC-FID [9]. For this purpose, the upper chamber of the pervaporation unit is located in the loop of an HPLC injection valve and the pervaporated species were transported by an He stream to the chromatograph by changing the valve to the injection position. The other method is based on the use of an alcohol oxidase electrode [10] located in a continuous-flow system.

The aim of this research was the development of a flow-injection method for sequential determination of methanol and iron in vinegar, to enable fast and inexpensive determination. No similar sequential or simultaneous, methods for these species have previously been reported.

Experimental

Apparatus and instruments

The manifold used is depicted in Fig. 1. It was built using two four-channel Gilson Minipuls-3 peristaltic pumps (Villiers le Bel, France) fitted with rate selectors, three Rheodyne 5041 injection valves (Elkay, Galway, Ireland), one of them used as a selecting valve, PTFE tubing of 0.5 mm i.d. (Análisis Vínicos, Tomelloso, Spain) and a Teflon minicolumn of 5 mm i.d. and 6 cm length filled with active carbon. A Cary 50 Conc spectrophotometer from Varian (Mulgrave, Australia) equipped with a Hellma 138-QS flow-cell (Hellma, Jamaica, NY) and connected to a computer with Cary WinUV v.2.0 ® (Varian) software for data collection and treatment was also used.

An SBS model TFB-1 Selecta (Barcelona, Spain) thermostat, a laboratory-made pervaporation module, described elsewhere [11-13] and PTFE membranes of 47 mm diameter and 1,5 mm thickness (Trace, Braunschweig, Germany) were used. Statistical treatments were made using Statgraphics™ 2.1 plus for windows.

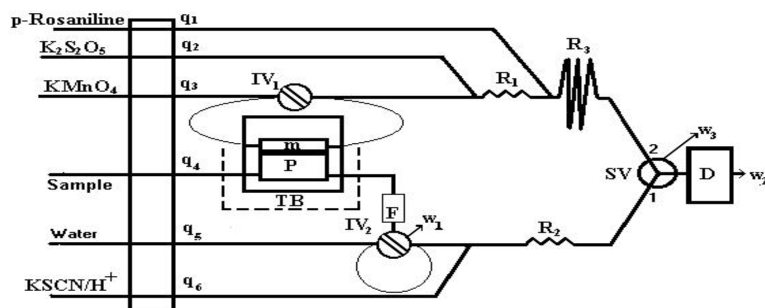

Figure 1. Manifold for the determination of methanol and iron. PP= peristaltic pump, IV= injection valve, SV= selecting valve, R= reactor, D= detector, w= waste, q= flow-rate, m=membrane, TB= thermostatic bath, P= pervaporation module, F= carbon filter.

A Varian 3900 Gas-chromatograph with flame ionisation detector (FID) with a Chrompack capillary column CP-wax-57 CB (50 m 0.25 mm i.d.), both from Varian, and connected to a computer with Star chromatography workstation v.5.52 ® (Varian) software for data collection and treatment was used to obtain methanol reference data.

A Distillatore Elettronico Enochimico (Gibertini, Milan, Italy) based on water steam dragging was used for methanol distillation.

Reagents and solutions

Standard solutions for calibration, optimisation and characterisation studies of the methods were prepared from methanol (Merck, Darmstadt, Germany) and iron (III) nitrate (Panreac, Barcelona, Spain).

Proposed method

The acceptor stream in the pervaporation module was an aqueous solution of 5 g L^{-1} potassium permanganate and 50% (v/v) phosphoric acid, both from Panreac. $K_2S_2O_5$ (Panreac) solution (20 g L^{-1}) was used to decolour surplus permanganate and 0.3 g L^{-1} p-rosaniline (Panreac) solution was also used for methanol determination. Hydrochloric acid (5% v/v) and potassium thiocyanate solution (45 g L^{-1}), both from Panreac, was used for iron determination. Activated carbon from Aldrich (Steinheim, Germany) was used to decolour the sample before filling the injection valve and a 3% hydrogen peroxide solution was used for sample pretreatment (Fe^{+2} to Fe^{+3} transformation).

Reference methods

Hydrochloric acid (37,5%), hydrogen peroxide solution (3%) and potassium thiocyanate solution (200 g L^{-1}), all from Panreac, were used for iron determination. The reference method of methanol used 4-methyl-2-pentanol (Merck) as internal standard; this compound (1110.0 mg, accuracy of 0.1 mg) was dissolved in 5% aqueous-ethanol and the solution was then diluted to 1 L in a volumetric flask. The methanol reference solution was prepared by dissolving methanol (50.0 mg, accuracy of 0.1 mg) in 5% aqueous ethanol (1L); 1 mL internal standard solution was added to 10 mL of the methanol reference solution. NaOH solution (Panreac; 40% m/v) was also used.

Procedures

Reference method for methanol [2]

The sample was neutralised with a 40% (m/v) sodium hydroxide solution. The internal standard solution (1 mL) was added to the neutralised sample (10 mL) and 1 μL of the mixture was injected into the chromatograph. Injector and detector temperatures were 250ºC. The oven temperature was maintained at 50ºC for 6 min, then programmed at 8ºC min^{-1} to 70ºC, which was held for 14 min, then programmed at 8ºC min^{-1} to 210ºC, which was held for 16 min. The carrier gas flow was 10 mL min^{-1}.

Reference method for iron [5]
Hydrochloric acid (1 mL), H_2O_2 (5 drops), and potassium thiocyanate (1 mL) were added to the sample (10 mL) and the absorbance was measured at 508 nm. The spectrophotometer was adjusted to zero by using an acidified sample of 10 mL wine diluted with 1 mL of distilled water as a blank solution to compensate the influence of the colour of the wine.

Table 1. Results of the optimisation study

Variable	Tested range	Optimum value
Chemical		
$KMnO_4$ (g L^{-1})	1-20	5
H_3PO_4 (% v/v)	1-60	50
p-Rosaniline (g L^{-1})	0.1-1	0.3
$K_2S_2O_5$ (g L^{-1})	5-50	20
KSCN (g L^{-1})	10-50	45
HCl (% v/v)	1-15	5
Flow Injection		
$q_1, q_2, q_3=q_4, q_5, q_6$ (mL min^{-1})	0.4-2.0	0.8; 0.6; 1.4; 1.25; 0.4
IV_2 (µL)	50-500	300
R_1, R_2, R_3 (cm)	50-200	75; 150; 100
Pervaporation		
T (ºC)	60-90	85
t (min)	1-8	5

Proposed method
The sample, previously treated with H_2O_2 solution (5 drops), was introduced by aspiration into the dynamic manifold shown in Fig. 1 and pumped into the donor chamber of the pervaporation unit. The methanol was pervaporated and collected in the acceptor solution containing potassium permanganate and phosphoric acid, valve IV_1 remaining in the filling position for acceptance of the volatile fraction into a static acceptor solution. At the same time the sample stream leaving the donor chamber was decoloured by passage through an active carbon minicolumn and directed to valve IV_2, which was in its filling position. The selecting valve (SV) was in position (1), thus establishing the baseline for the determination of iron. Three minutes after introduction of the sample into the donor chamber, the content of IV_2 was injected into an acid stream of potassium thiocyanate and the reaction product monitored at 508 nm. SV was then switched to position (2) to establish the baseline for the determination of methanol. After an interval for sufficient enrichment of the static solution with the pervaporated species

(5 min from sample introduction), valve IV_1 was switched to the injection position and the plug merged with a $K_2S_2O_5$ stream to decolour the excess of permanganate, and then with a p-rosaniline stream to yield a violet product which was monitored at 567 nm. An aqueous stream was introduced between analyses in order to clean the donor chamber of the pervaporation unit.

Results and discussion

Optimisation of the variables affecting each individual method was performed by use of both univariate and multivariate approaches, as required, depending on the interdependence of the variables. The range over which the variables were studied and the optimum values found are given in Table 1.

Optimisation of the method for the determination of methanol

Chemical variables

A multivariate approach was used for optimisation of the concentration of $KMnO_4$ and H_3PO_4 within the ranges 1-20 g L^{-1} and 1-60% (v/v), respectively; the results obtained are plotted in Fig. 2(a). The signal increased when the concentrations of H_3PO_4 and $KMnO_4$ were increased but the former had a greater effect. Concentrations of 5 g L^{-1} potassium permanganate and 50% (v/v) phosphoric acid were selected as optimum. Concentrations higher than 60% phosphoric acid were not tested because of deterioration of the pumping tubes. Because the P-value in the ANOVA table was <0.01%, there was a statistically significant relationship between the variables at 99% confidence level. The equation of the fitted model is: $A = -0.493 + 0.010 [KMnO_4] + 0.030 [H_3PO_4]$. The other chemical variables were studied by use of the univariate method.

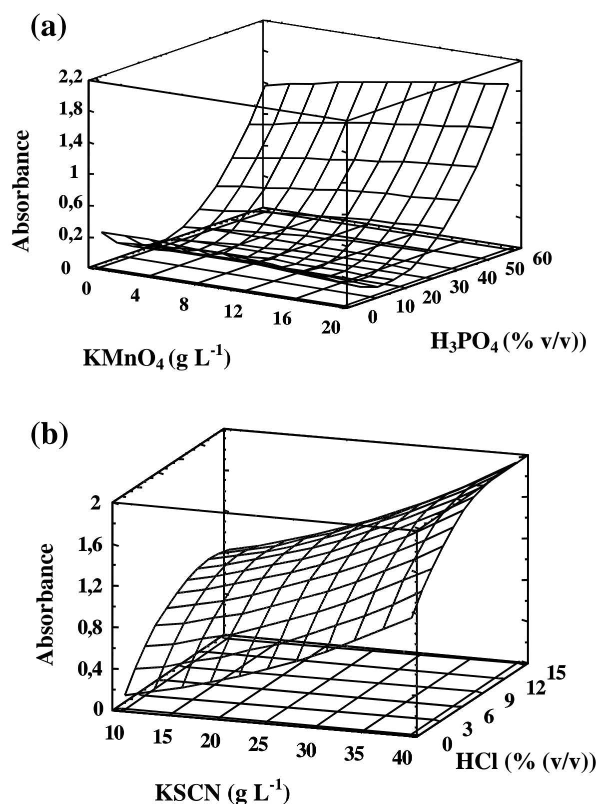

Figure 2. Response surface of the multivariate analysis of: (a) evolution of absorbance versus $KMnO_4$ (g L^{-1}) and H_3PO_4 (%(v/v)) concentrations in the acceptor stream of the pervaporation unit (b) evolution of absorbance versus KSCN (g L^{-1}) and HCl (%(v/v)) concentrations.

Increasing the concentration of p-rosaniline increased the analytical signal, which levelled off at p-rosaniline concentration of 0.3 g L^{-1}. The concentration of $K_2S_2O_5$ used to decolour the acceptor solution before mixing with the p-rosaniline solution was also optimised. A 40 g L^{-1} solution was sufficient for this purpose.

Flow injection and pervaporation variables

Flow-rates q_3 and q_4, in Fig. 1 (corresponding to the acceptor and donor stream, respectively, reaching the pervaporator) were set at the same value in order to prevent membrane deformation. A flow-rate of 1.4 mL min^{-1} was selected as a compromise between sensitivity and sampling-rate and the sample was introduced in a continuous way in order to increase sensitivity.

Reactors R_1 and R_3 had the function of ensuring proper mixing of the chemical reagents and pervaporated analyte before reaching the spectrophotometer. The length required for this function were 75 and 150 cm, respectively.

Because of the low methanol content of the sample, the efficiency of the pervaporation was favoured by stopping the acceptor solution during this step, thus achieving a higher enrichment by a mass-transfer closer to equilibrium. The pervaporation time, during which the acceptor solution remained static, was tested between 1-8 min; the analytical signal increased as the time was increased, because efficient pervaporation was favoured. A value of 5 min was chosen as a compromise between sensitivity and sampling rate.

Increasing the temperature had a predictable positive effect on pervaporation and on the analytical signal as a consequence. The signal obtained at 85ºC was lower than that at 90ºC, but the reproducibility was better (2.35 mg L^{-1} at 85ºC compared with 3.03 mg L^{-1} at 90ºC). For this reason, the temperature of the thermostat was set at 85ºC.

Optimisation of the method for the determination of iron

Preliminary attempts to use a mono-channel manifold with the reagent solution acting as the carrier into which the sample was injected resulted in a calibration plot with a narrow linear range, because of the low dispersion of the injected plug into the reagent stream. For this reason, a water stream was used into which the sample, coming from the low chamber of the pervaporation unit, was injected and merged with the potassium thiocyanate solution as shown in Fig 1. Several conditions were optimised using this manifold.

Chemical variables

The concentrations of both thiocyanate and hydrochloric acid were optimised in the range 10-50 g L^{-1} and 1-15% (v/v), respectively, using a multivariate approach. Figure 2(b) shows a plot of the absorbance against both thiocyanate and hydrochloric concentrations.

Because the P-value in the ANOVA table is less than 0.01, there is a statistically significant relationship between the variables at 99% confidence level. The equation of the fitted model is: A= -0.107 + 0.045 [HCl] + 0.035 [KSCN]. The optimum values were those providing the highest signal with the lowest reagent consumption.

Flow injection variables

The length of reactor R_2 was varied between 50 and 200 cm and the highest transient peak was obtained when the length was 100 cm, as a result of appropriate mixing of the merged streams with minimum dispersion.

The flow-rates of channels q_5 and q_6 were tested in the range 0.4-2.0 mL min^{-1}; the optimum values were 1.25 and 0.4 $mLmin^{-1}$, respectively.

The transient signal provided by the system increased as the injection volume was increased from 50 to 300 μL and then became constant.

Characterisation of the method

Calibration plots

In a first stage, five individual standard solutions for each analyte were prepared containing concentrations between 0 and 1000 mg L^{-1} for methanol and between 0 and 20 mg L^{-1} for iron. Both sets of solutions were injected in duplicate and the range of linear dependence of response on concentration was found for each analyte. Subsequently new calibration plots were obtained by use of standards containing both analytes. The regression equations were Y=0.104 X +0.009, r^2=0.998 and Y=5.5x10^{-3} X +0.003, r^2=0.987 for iron and methanol, respectively.

Different iron-methanol ratios were tested to check for the absence of mutual effects. Iron did not interfere in the determination of methanol, because of the involatile nature of the former, which also ensures its concentration is unaltered during heating the sample in the pervaporation module or filtration through the active carbon micro-column. No statistically significant difference between the results of the individual and joint calibrations.

Assessment of the proposed method

Thirty different vinegars in different stages of fermentation were used in the assessment study. Each result for methanol or iron content was the average from three determinations and outlier values were deleted by applying the Grubs test [14]. The procedure for assessment consisted of studying analytical parameters such as linear range, traceability by the reference method, repeatability, reproducibility, detection and quantification limits and sample throughput. A robustness study was also developed.

*Repeatability (r).*The F-test was used to establish if the difference between the repeatability of the proposed and reference methods was significant. With this aim, the $F_{obs}=S_r^2 / S_{ref}^2$ was compared with the $F_{1-\alpha}$ obtained from F tables for α=0.05

(P=95%). As is apparent from Table 2, $Fobs^r$ was always less than $F_{1-\alpha}$, so the repeatability was similar for the flow-injection and reference method.

*Reproducibility (R) (30 days).*The R values and the results from the application of the F-test, (Table 2), show that the reproducibility for both FI determinations are statistically equal to those of the reference methods because $Fobs^R < F_{1-\alpha}$.

Table 2. Analytical characteristics of the proposed method as compared with the reference method

Characteristic	Reference methods		FI method	
	Iron	Methanol	Iron	Methanol
Repeatability (mg L^{-1})	0.212	1.364	0.245	2.452
S_r (mg L^{-1})	0.078	0.433	0.097	0.580
Reproducibility (mg L^{-1})	0.342	2.347	0.356	4.435
S_R (mg L^{-1})	0.084	0.675	0.108	0.897
$Fobs^r$			1.55	1.79
$Fobs^R$			1.65	1.77
$F_{1-\alpha}$ (n=30)	1.84		1.84	
LOD (mg L^{-1})	0.184	4	0.234	82
Sample throughput (h^{-1})	15 [a]	3 [a]	9 (30)[b]	

S_r and S_R are the deviations of repeatability and reproducibility, respectively
$Fobs = S^2 / S_{ref}^2$
[a] In batches of 4 samples
[b] Individual determination of iron

Detection limits (LOD). In Table 2 the LOD of the reference methods are compared with those of the proposed flow-injection method. For iron the LOD of the reference and flow-injection methods are similar; although this is not true for methanol, the LOD of the proposed method for this analyte is much lower than its usual concentration in vinegars.

*Traceability.*The traceability of the method was studied by comparing the results obtained from 30 samples of different vinegars analysed by both the reference and proposed methods. Figure 3 shows regression plots of results from the flow injection and reference methods for methanol (a) and iron (b). The regression equations are y= 0.990 x + 4,986 (r^2=0.991) and y= 1,05 x –

0.044 (r^2= 0.996), respectively. Both plots are indicative of good correlation between the data from the proposed method and their reference counter-parts. Confidence limits of 95% are shown in Fig. 3 by dotted lines. For both iron and methanol traceability was assured by use of the t-test.

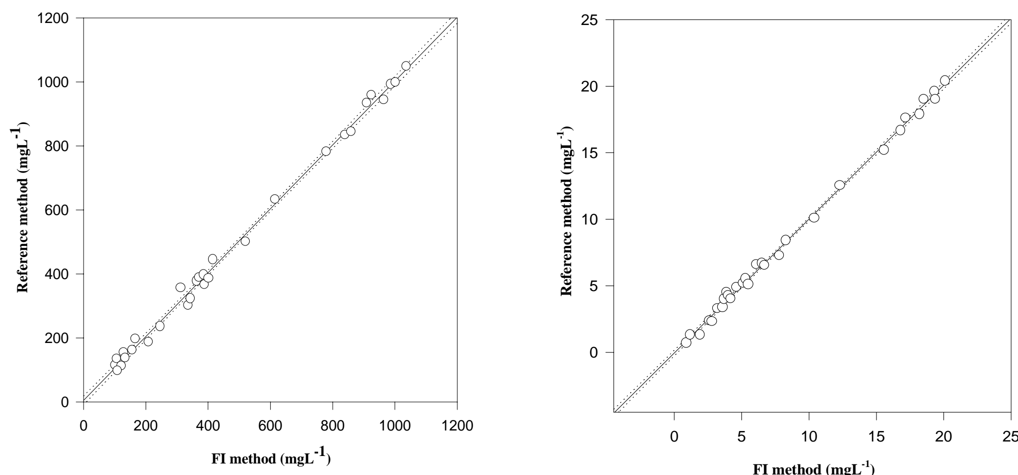

Figure 3. Correlation graph of the reference method with the FI method for (a) methanol and (b) iron. Interval of confidence: 95%.

*Sample throughput.*The results in Table 2 show that the flow-injection method is higher than that for the reference method for individual determinations. The proposed sequential method (nine determinations per hour) cannot be compared with an official sequential counterpart because of the lack of the latter.

Robustness study. Robustness was studied by use of the the Younden-Steiner procedure [15]. The most significant variables of the system (namely, flow-rate, temperature, pervaporation time and concentrations of potassium permanganate, phosphoric acid, p-rosaniline, potassium pyro-sulphite, potassium thiocyanate and hydrochloric acid) were modified by ±10% from their optimum values. Errors were always less than 10% except for phosphoric acid, which gave errors of 18%.

Conclusions

The proposed method enables sequential determination of iron and methanol in vinegar. The method is simple, presents good correlation with the reference methods and can be easily implemented in a winery for quality control of the final product, thus constituting an alternative to the chromatographic analysis usually required. The method is robust and has a sample throughput higher than that of the reference methods. An additional advantage of the method reported here is its easy automation. This method constitutes a unique sequential application of flow injection for determination in vinegar and offers the advantage of determining two parameters using the same manifold, thus reducing time and costs. This is the first time that a continuous simultaneous method for determining iron and methanol is proposed and assessed for its routine use in wineries.

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