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A simple strategy for determining ethanol in all types of alcoholic beverages based on its on-line liquid–liquid extraction with chloroform, using a flow injection system and Fourier transform infrared spectrometric detection in the mid-IR

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Abstract

In this work, a simple strategy for the determination of ethanol in all types of alcoholic beverages using Fourier transform infrared spectrometric detection has been developed. The methodological proposal includes the quantitative on-line liquid–liquid extraction of ethanol with chloroform, through a sandwich type cell equipped with a PTFE membrane, using a two-channel manifold; and direct measurement of the analyte in the organic phase, by means of Fourier transform infrared spectrometry. The quantification was carried out measuring the ethanol absorbance at 877 cm^{-1}, corrected by means of a baseline established between 844 and 929 cm^{-1}. The procedure, which does not require any sample pretreatment (except for the simple degassing of beer and gassy wine samples, and a simple dilution of spirits with water), was applied to determine ethanol in different alcoholic beverages such as beers, wines and spirits. The results obtained highly agree with those obtained by a derivative FTIR spectrometric procedure, and by head space-gas chromatography with FID detection. The proposed method is simple, fast, precise and accurate. Moreover, it can be easily adapted to any infrared spectrometer equipped with a standard transmission IR cell, and provides attractive analytical features, which are comparable to, or better than those offered by other published methods. In consequence, it represents a valid alternative for the determination of ethanol in alcoholic beverages, and could be suitable for the routine control analysis.

Keywords: Ethanol; Alcoholic beverages; Flow analysis; FTIR; On-line liquid–liquid extraction

1. Introduction

The determination of ethanol in alcoholic beverages is a very important task, due to its social and economical implications, particularly in relation to the taxes imposed in different countries to its use. This type of analysis is carried out in many laboratories, not only by producers, but also in government and customs laboratories. Alcoholic beverages may be defined as those with ethanol content less than 60% (v/v); and can be classified into two types: fermented liquors and distilled liquors, or spirits. The current official methods for determining alcoholic content are based on physical measurements carried out after a previous distillation of the sample, to separate the alcohol [1,2] and also by simple volumetric redox titrations [3]. However, all these procedures are tedious and time consuming. Hence, some attempts have been made to simplify the available methods to determine ethanol, and to develop new strategies that can be used with low cost instrumentation and without complex sample pretreatment. In this way, recently, a flow analysis-pervaporation method for the determination of ethanol in beverages using density measurements was described, reporting a sample throughput of 15 samples h^{-1} [4]. In addition, various instrumental methods have been proposed in order to provide a direct determination of ethanol, based on gas chromatography (GC) [5,6], liquid chro-

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matography [7,8], head space-GC-FID [9] and low resolution pulsed nuclear magnetic resonance spectroscopy [10], among others. However, most of them require a relative expensive instrumentation, and most of the time the high cost of acquisition and maintenance do not justify the investment. On the other hand, for the analysis of ethanol in alcohol free and low alcohol beers, enzymatic procedures using different detection techniques such as fluorimetry or spectrophotometry, provide very sensitive and accurate methods [11–14].

Infrared (IR) spectrometry in the mid-infrared (MIR) and the near-infrared (NIR) provides interesting possibilities for the direct determination of ethanol in beverages. By using the transmission mode, NIR Spectroscopy offers the possibility to carry out the determination employing ordinary glass or quartz cells [15,16]. On the other hand, in the mid range, it is necessary the use of cells equipped with water-resistant windows and very small pathlengths ($b \leq 50\ \mu m$), in order to reduce the high absorption of IR radiation by water [17,18]. However, in all instances, special attention has to be paid to the presence of sugars in the sample, which shows an important interfering effect on the analytical bands of ethanol. In order to solve this analytical problem, different strategies have been proposed, based on the use of derivative spectroscopy, matrix simulation, a simple proportional equation approach, etc. Recently, a comparison and joint use of NIR spectroscopy and Fourier transform mid-IR Spectroscopy for the direct determination of several endological parameters in wines, including alcoholic degree, using multivariate calibrations, was published [19]. With the purpose to definitively avoid the interfering effect of sugars, two approaches have been proposed, which involve the analyte:u matrix separation by means of a liquid–liquid extraction or by using a simple vaporization approach.

Tipparat et al. [20] proposed a simple procedure based on the off-line extraction of ethanol with chloroform and the further injection of the organic extract into a $CHCl_3$ carrier of a flow injection (FI)-NIR system. In this case, the quantification was carried out using the ethanol bands at 2305 or 2636 nm. The method was applied to the determination of the alcoholic degree only in distilled liquors, reporting analytical figures of merit (limit of detection: 1% (v/v), dynamic range: 20–50% (v/v), precision (RSD): 3–4%) which are poor compared to those described for other infrared spectrometric methods. Furthermore, recovery values between 84 and 122% were reported for colored samples, thus indicating the presence of matrix interfering effects in the analysis of these types of samples. In addition, the efficiency of the extraction process carried out in a batch mode is reduced (about 20%).

The second strategy, proposed by Pérez-Ponce et al. [21], is based on the direct injection of untreated samples into a volatilizer reactor heated at 80–90 °C. The gas phase generated is transported to the IR gas cell by means of a gas carrier, where the absorption FTIR spectrum is acquired. The ethanol quantification is carried out using the analytical band at 1050 cm^{-1} in the mid-IR. The main drawbacks of this method are the possible co-volatilization of some matrix components, even thought researchers did not report interfering effects in the analysis of real samples and the requirement of a multiple-pass IR gas cell, which is expensive and is not available for most laboratories.

Fourier transform infrared (FTIR) is a fast analytical technique and a very useful tool for the quantitative analysis of complex samples, without requiring complex sample preparation. Moreover, as it has been clearly demonstrated in the last 15 years, it is a very useful detector in flow analysis systems [22,23]. The problems related to the direct determination of ethanol from hydro-alcoholic samples in the mid-IR can be easily avoided changing the solvent to one transparent. So far, it is important to highlight that chloroform presents interesting transparency windows in the mid-IR, where ethanol shows its more intense absorption bands. All of this makes this approach likely to be developed in the mid-IR. In the best knowledge of the authors, up to this moment, the determination of ethanol via its on-line extraction with chloroform, and by using FTIR spectrometry in the MIR has not been reported.

The main aims of this work were: (i) to design and develop a simple, sensitive and selective, low-cost and accessible flow injection (FI)-FTIR spectrometric procedure for the direct determination of ethanol in all types of alcoholic beverages; (ii) to test the analytical quality of the 877 cm^{-1} band for the designed application. The recommended procedure allows: (i) the on-line quantitative extraction of ethanol with chloroform; (ii) the method automation; (iii) the analyte separation from the matrix, allowing to easily eliminate sugars and other potential matrix interfering compounds; (iv) the change of solvent (water/chloroform), allowing the use of greater pathlengths and the employment of the 877 cm^{-1} band. All these conditions provide selectivity and sensibility to the FTIR spectrometric detection and thus, attractive analytical features and accuracy to the methodological proposal.

2. Experimental

2.1. Instrumentation and manifold

All the spectral measurements were carried out with a Perkin-Elmer (Norwalk, CT, USA) Spectrum 2000 series FTIR spectrometer, using a nominal resolution of 2 cm^{-1}. The instrument was equipped with a DGTS detector, a Perkin-Elmer MIR source, a KBr beamsplitter, and a Wilmad (New Jersey, USA) standard rectangular IR transmission cell (IRFC) with 38 mm × 19 mm × 2 mm ZnSe windows. The IRFC was used as a flow cell, fixing the pathlength at 0.5 mm with a PTFE spacer. Spectrum 2000 and Time resolved infrared (TR-IR) softwares, from Perkin-Elmer, were used to control the instrument, to acquire and store data, and also for processing the analytical results.

Fig. 1A depicts the schematic diagram of the FI–FTIR system used in this work, which incorporates two four-channel Ismatec (Glattbrugg, Switzerland) IPC peristaltic pumps (P_1 and P_2) furnished with Viton® tubes; three propulsion channels: one for the carrier (C_{Car}), one for samples and standards (C_{Sam}), and another for the extracting solution (C_{Ext}); an extraction coil (R); a Reodyne (Alltech, Waukegan, USA) injection valve (IV), a liquid–liquid extraction cell (EC) and a FTIR spec-

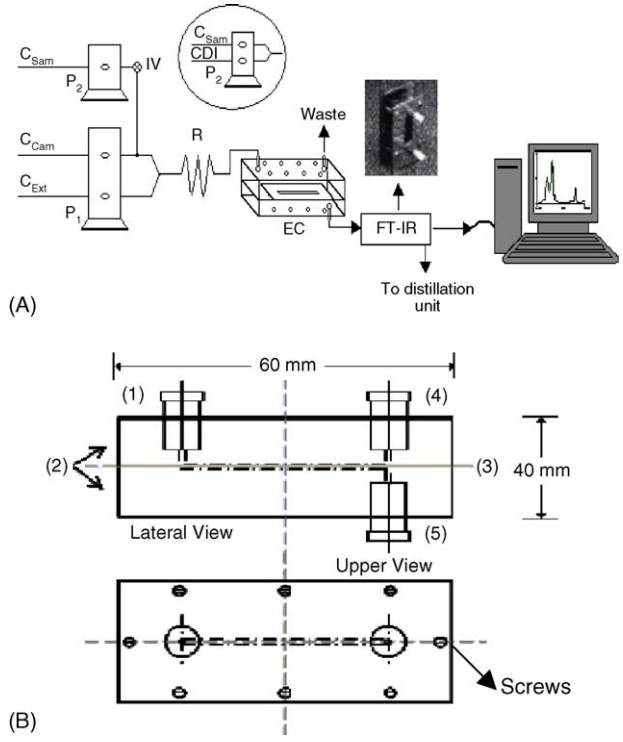

Fig. 1. (A) Schematic diagram of the FI–FTIR system. P_1, P_2: peristaltic pumps, C_{Car}: carrier (DI), C_{Sam} (samples or standards), C_{Ext}: extracting solution (pure and dried chloroform), IV: manual injection valve, R: extraction coil, EC: liquid–liquid extraction cell, IRFC: infrared flow cell, detector: FTIR spectrometer. Inset: double channel manifold with a Y-shaped merging zone proposed for the on-line dilution of samples with high ethanol content (C_{DI}: distilled water channel). (B) Liquid–liquid extraction cell (EC): (1) entrance of the hydro-alcoholic/chloroform mixture, (2) nylon blocks, (3) PTFE membrane, (4) exit for the organic phase (to IRFC), (5) exit for the aqueous phase (to waste), and (o) screws to properly adjust the membrane between the nylon blocks. For further details and operating procedure, see Table 1 and text.

trometer. Alternatively, for the on-line dilution of spirit samples, a double channel system with a Y-shaped merging zone can be incorporated. The proposed system was mainly assembled from commercial accessories and equipment, except for the EC, which was homemade (see Fig. 1B). This cell is a "sandwich type" unit, and represents a modification of the separator proposed by Kubàn [24]. The EC was constructed from two nylon blocks (60 mm × 20 mm) and a PTFE membrane TF-450 of 0.45 μm pore size (Gelman Sciences, MI, USA). The internal volume of the cell is around 140 μl and the effective area of the membrane is 76 mm^2. The EC includes an entrance for the resulting mixture produced in R between the hydro-alcoholic solution and chloroform, and two exits: one for the organic phase that goes to the IRFC, and another for the aqueous phase that goes straight to the waste. In the same way, the design includes eight screws to properly adjust the membrane between the nylon blocks. The organic phase is collected at the exit of the IRFC, and it is distilled in a vacuum evaporator for its further recycling.

2.2. Reagents, standards and samples

Double de-ionized water of 18 MΩ cm^{-1} specific resistivity, obtained in a Milli-Q Plus, Millipore System – referred as DI in the text – was used to prepare all the solutions and to rinse the previously cleaned laboratory material.

Chloroform HPLC grade, without ethanol, from Mallinckrodt (Paris, France) was used as extracting solution. However, prior the analysis, the organic solvent was dried for several hours over $CaCl_2$ anhydrous BDH (Poole; England). During the analysis, the dried solvent was introduced into a closed amber-glass bottle containing powdered molecular sieve, type 5 Å. Analytical, reagent grade, absolute ethanol (99.6%, v/v) from T.J. Baker, Xalostoc, Mexico was employed to prepare the standards, which were daily prepared by diluting the alcohol with DI. Different commercial alcoholic beverages, from beers to spirits, were purchased in local liquor-stores and analyzed by the proposed method.

2.3. General procedure

Initially, samples and reagents were fed through their respective lines at room temperature as indicated in Fig. 1, under the operating conditions given in Table 1. The peristaltic pump (P_1) was on during the analysis to propel continuously the carrier (C_{Car}) and the extracting agent (C_{Ext}), while P_2 was turned on only to fill the injection loop.

The FI–FTIR procedure ran through a cycle of four sequences, as follows. First, the injection valve was switched to the injection position, in order to introduce the sample (stan-

Table 1
Operating conditions of the FI–FTIR system

Parameter	Value
FTIR	
Radiation source	Perkin-Elmer MID-IR source
Detector	DGTS
Beamsplitter	KBr
Spectral range	1200–800 cm^{-1}
Measurement criterion	Analytical band: 877 cm^{-1}, absorbance at 877 cm^{-1}, corrected by means of a baseline established between 844 and 929 cm^{-1}
Nominal resolution	2 cm^{-1}
IR flow cell (IRFC)	Standard rectangular transmission cell (Wilmad), pathlength: 0.5 mm (PTFE spacer), windows: ZnSe with rectangular geometry (38 mm × 219 mm × 2 mm)
FI	
Carrier composition (C_{Car})	Distilled water (DI)
Carrier flow rate (Q_{Car})	0.325 ml min^{-1}
Injection volume (V_i)	1.0 ml
Samples composition (C_{Sam})	Alcoholic beverages
Standards composition (C_{Sam})	Aqueous standard solutions of ethanol [Ethanol]: 0.05–15% (v/v)
Extracting agent composition (C_{Ext})	Pure and dried chloroform
Chloroform flow rate (Q_{Ext})	0.325 ml min^{-1}
Extraction coil (R)	PTFE (100 cm, 0.8 mm i.d.)
Liquid–liquid extraction cell (EC)	See Sections 2.1 and 2.3

dard) into the carrier (sequence 1). In this way, the hydro-alcoholic solution got mixed with the organic solvent in R, where ethanol was quantitatively extracted into the organic phase. Then, on its way to the detector, the organic and aqueous phase mixture made contact with the PTFE membrane in the extraction cell. In this process (sequence 2), the organic phase crossed the membrane and flowed continuously to the IRFC, while the aqueous phase went straight to waste (see Fig. 1). At this point (sequence 3), the FTIR spectra of the resulting solution of ethanol in chloroform were acquired and stored as a function of time. Finally, the absorbance peak height at 877 cm^{-1} corrected by means of a baseline established between 844 and 929 cm^{-1} was evaluated from the flow injection (FI) recording.

Wine samples were analyzed directly without any pretreatment, while beers and gassy wines were degassed by using an ultrasonic water-bath prior to their introduction in the system. On the other hand, samples with ethanol concentration higher than 15.0% (v/v) required to be previously diluted off-line with DI in an adequate proportion. However, this dilution step can be carried out on-line by means of the two-channel system described previously in Section 2.1.

The data obtained from samples were interpolated on the corresponding calibration graph, which was constructed from aqueous standard solutions of ethanol (0.05–15%, v/v) injected and treated in the same way than samples.

2.3.1. Reference procedures

In order to check the accuracy of the proposed method, ethanol was determined in a series of samples (see Section 2.3) by two alternatives methods.

Derivative FI FTIR. Beers and gassy wines were degassed and then introduced directly into the transmission micro flow cell and the corresponding interferogram was recorded accumulating 10 scans at a nominal resolution of 4 cm^{-1}, using a pathlength of 0.029 mm. The first order derivative spectrum was then established with a derivative window of 13 points and the concentration of ethanol in the sample was determined by measuring the absolute value between the peak at 1052 cm^{-1} and the valley at 1040 cm^{-1}, using aqueous solutions of ethanol as standards. For the analysis of spirits a previous dilution of 5 ml of sample to a final volume of 25 ml with DI was required [18].

HS-GC-FID. Samples were also analyzed by a head space-gas chromatography with flame ionozation detection (HS-GC-FID) method developed in our laboratory [25]. Samples (0.5 ml) were placed into the vials and closed with crimps using aluminium caps with butyl rubber septa and put into the HS sampler. Vials were then mixed and kept at 60 °C for 30 min. Thereafter, the HS sample was automatically transferred to the GC column across the transfer-line heated at 90 °C. A fused silica capilary column (Chrompack: 30 mm × 0.25 mm i.d., film thickness 0.25 mm) was used and the temperature programm was set at 40 (4 min) and to 220 °C (2 min) at 15 °C/min, respectively. Helio was used as a carrier gas with linear velocity set at 15 cm/s and the temperature of injector and detector was fixed at 220 and 300 °C, respectively.

3. Results and discussion

3.1. FTIR spectra of ethanol, chloroform, and ethanol standard solutions

The FTIR spectra obtained in film of pure chloroform and absolute ethanol indicate that the organic solvent presents a wide transparency interval between 1200 and 800 cm^{-1}; while the alcohol shows three bands in the same spectral range: an intense band at 1048 cm^{-1}, and two other bands of medium intensity, centered at 1086 and 877 cm^{-1}, respectively (see Fig. 2A). These bands are due to vibrational transitions of the –C–O–H system: C–O stretching vibration (*p*-OH) and –O–H bending vibration out of plane [26]. On the other hand, Fig. 2B–D compares the FTIR spectra obtained in bath, corresponding to standard solutions of ethanol (1 and 10%, v/v) prepared in chloroform (Fig. 2B), with those obtained from aqueous solution, where ethanol was extracted with chloroform using off-line (Fig. 2C) and on-line (Fig. 2D) strategies, respectively. These spectra show basically the same behavior, but they put in evidence three differences with respect to the one obtained form absolute ethanol in film (Fig. 2A). The absorption maximum of the intense band shows a little shift to 1046 cm^{-1}, and presents a shoulder at 1020–1030 cm^{-1}. In addition, the less intense band (1086 cm^{-1}) tends to disappear, looking as a shoulder of the first one. This behavior is probably due to intermolecular interactions between the analyte and solvent. However, the last effect is only observed when using high path-lengths (*b*) values. For $b \leq 0.25$ mm, the 1086 and 1046 cm^{-1} bands appears clearly separate (see Fig. 2E). On the contrary, the 877 cm^{-1} band does not present any sort of alterations. At this point, it is worthwhile to highlight the quality of the blank, which was obtained from chloroform in all cases, and by using a pathway of 0.5 mm, thus indicating the chloroform transparency in the selected spectral range. At the same time, it is important to underline the poor extraction efficiency obtained through the off-line process.

The 1046 and 1086 cm^{-1} bands have been extensively used for the direct determination of ethanol in aqueous solutions [17,18,27,28], while the use of the 877 cm^{-1} band has only been reported in gas phase [29]. This fact is due to the strong absorption that water shows at wave numbers lower than 900 cm^{-1}. On the other hand, methanol, which can be present in some beverages at low concentrations, shows a very intense absorption band at 1030 cm^{-1} (see Fig. 2F); representing an interfering matrix component when the analysis is carried out at the 1046 cm^{-1} band.

Finally, Fig. 3 shows, in a comparative way, the FTIR spectra obtained from real samples (beer, red wine, white rhum and whisky). At the 1046 cm^{-1} band, slightly differences were observed between different samples in the shoulder at 1020–1030 cm^{-1}; thus indicating the presence of interfering matrix effects. Again, the 877 cm^{-1} band does not present any sort of alterations. It is exactly the same in aqueous standards, samples, and sample solutions fortified with ethanol. Based on these preliminary results, the band at 877 cm^{-1} was selected as the analytical band, and the peak height at 877 cm^{-1}, corrected

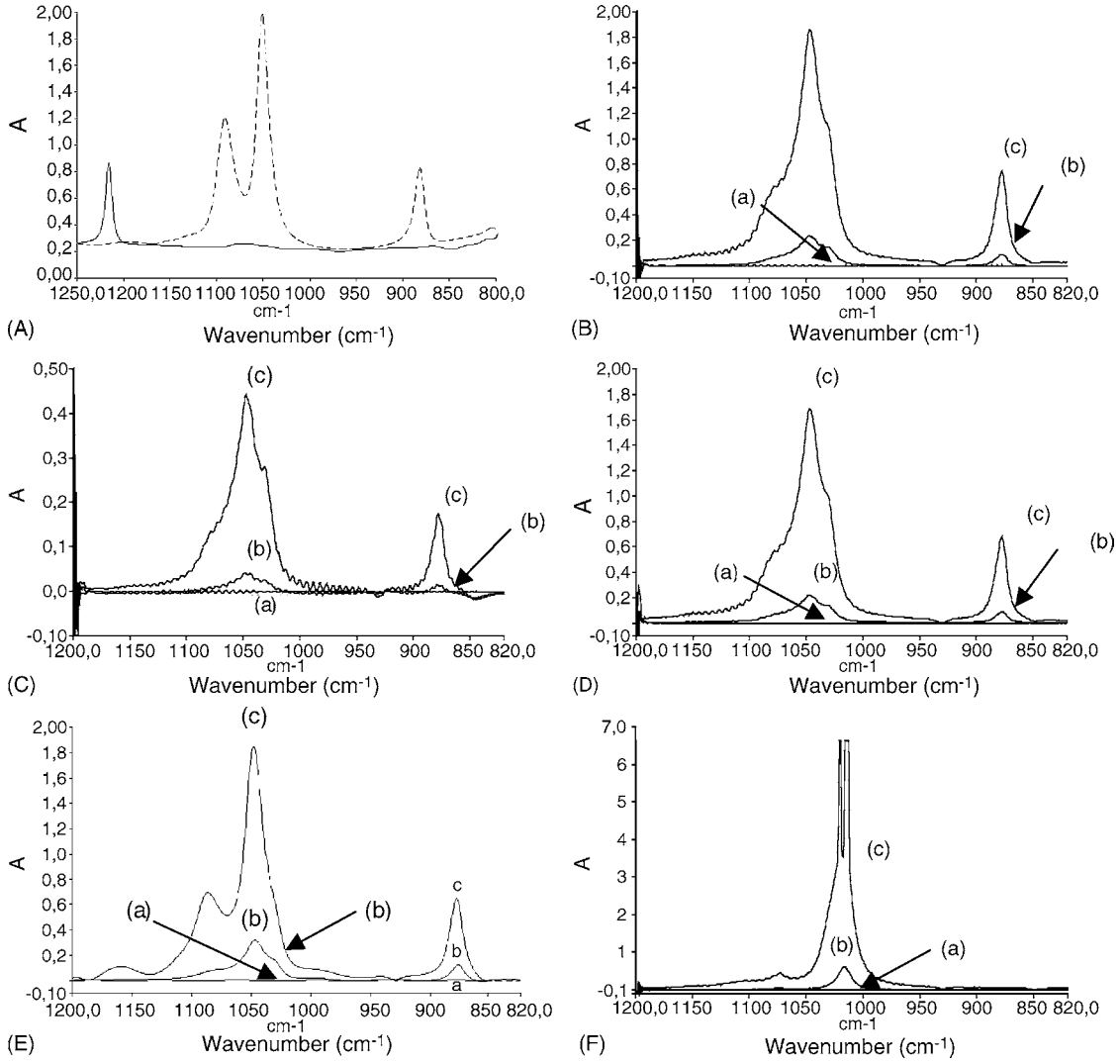

Fig. 2. FTIR spectra of pure chloroform, absolute ethanol and ethanol standard solutions. (A) Spectra obtained in film corresponding to: (—) pure chloroform, (- - -) absolute ethanol. (B) Spectra obtained in batch from solutions of ethanol in chloroform. (C) Spectra (in batch) corresponding to the organic extract obtained by off-line extraction (1:1) of ethanol with chloroform from aqueous solutions of ethanol. (D) Spectra obtained from aqueous standards of ethanol in the proposed system (V_i = 1.0 ml). (E) Spectra obtained from aqueous standards of ethanol in the proposed system (pathlength (b) = 0.25 mm, [Ethanol]: (a) 0% (v/v) (DI), (b) 2% (v/v) and (c) 20% (v/v); V_i = 1 ml). (F) Spectra obtained from aqueous standards of methanol in the proposed system (V_i = 1.0 ml). For (B), (C), (D) and (F): b = 0.5 mm, [Alcohol] = (a) 0% (v/v), (b) 1% (v/v) and (c) 10% (v/v). In all cases the spectra were obtained by accumulating three scans and using pure and dried chloroform as reference.

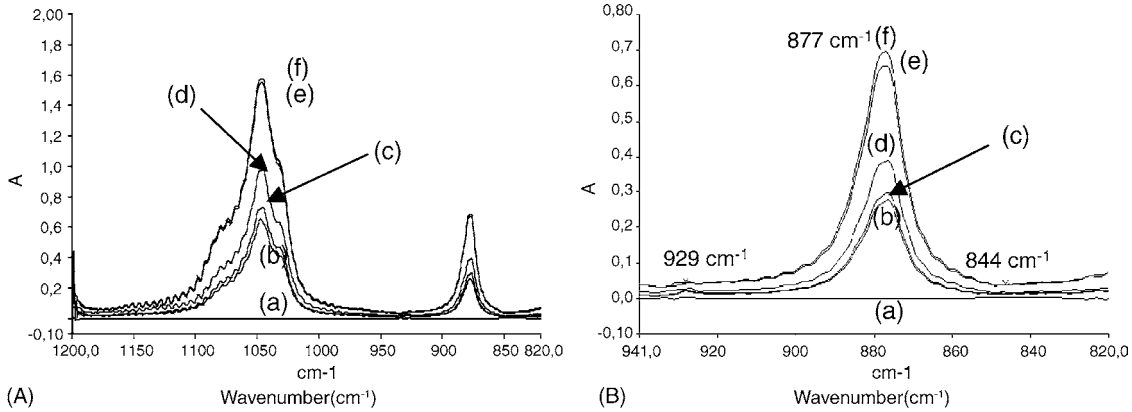

Fig. 3. FTIR spectra of different commercial alcoholic beverages. (A) Amplification of the 1046 cm^{-1} band. (B) Amplification of the 877 cm^{-1} band. Samples: (a) DI, (b) beer (4.5%, v/v), (c) aqueous standard of ethanol (5.0%, v/v), (d) red wine (12.5%, v/v) diluted 50% (v/v) with DI, (e) white rhum (40%, v/v) diluted 25% (v/v) with DI, (f) whisky (43%, v/v) diluted 25% (v/v) with DI.

by means of a base line established between 844 and 929 cm^{-1}, was selected as the measurement criterion for further use.

The proposed FI–FTIR system is very simple, and provides an adequate balance among analytical signal, sensitility, reproducibility, sample and chloroform consumption, and sample throughput. It was developed using identical flow rates for the carrier (Q_{Car}) and the extracting solution (Q_{Ext}). The maximum total flow rate for the mixture of the hydro-alcoholic solution and chloroform ($Q_T = Q_{Sam} + Q_{Car}$) tolerated by the system is 0.70 ml min^{-1}. For higher flow rates, an important fraction of the organic phase flows directly to waste, because the residence time, or contact time, between the hydro-alcoholic solution/chloroform mixture and the PTFE membrane is too short. Under these conditions, the organic phase cannot across the membrane and goes straight away to waste. On the other hand, the use of lower flow rates increase the analysis time. Based on these observations, Q_T was fixed at 0.65 ml min^{-1} ($Q_{Sam} = Q_{Car} = 0.325$ ml min^{-1}).

3.2. Effect of instrumental-spectroscopic parameters

Initially, the influence of the instrumental parameters such as nominal resolution, number of scans, and background conditions on the analytical signal was studied.

The effect of the number of scans used to establish the background and to obtain each spectrum was tested from 1 to 25 scans. Regarding the background definition, three scans are sufficient to establish an appropriate clean, clear, stable and flat background. A greater number of scans do not improve the stability of the reference blank spectrum, and increase the time of analysis. The influence of the number of scans employed to obtain each spectrum was evaluated initially, introducing the sample into the system in a continuous mode. The obtained results clearly indicate that this parameter does not have a significant effect on the quality of the analytical signal, certainly due to the very clean background offered by chloroform in the selected spectral range.

Regarding the nominal resolution (R_N), it is well known that this parameter significantly affects the shape of the FTIR absorption bands [30,31]. An increase in this parameter causes the depression and the broadening of the analytical band, but at the same time, greatly decreases the time required for the spectrum acquisition. However, in a FI system, this parameter is closely related to the number of scans which can be made in a fixed period of time [21]. Fortunately, the last effect does not have a significant influence on the analytical signal due to the high transparency of chloroform.

3.3. Effect of the injection volume

The effect of the injection volume (V_i) on the analytical signal of ethanol was studied in the range 0.25–5.0 ml, fixing the nominal resolution (R_N) at 2 cm^{-1}. The analytical signal increased with V_i up to 0.75 ml, but over 1.0 ml the signal reached a plateau, indicating a condition of continuous introduction of the sample, as can be seen in Fig. 4. Under these conditions, the measure-

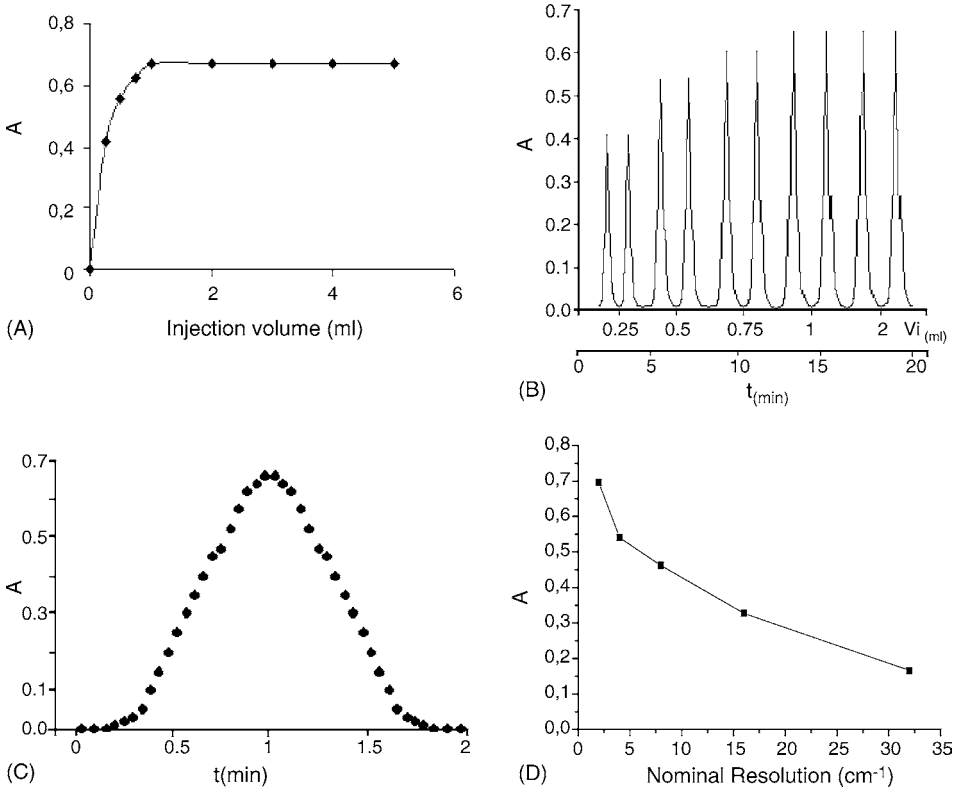

Fig. 4. (A) Influence of the injection volume on the analytical signal. (B) Effect of the injection volume on the FI recording. (C) Construction of a typical transient signal corresponding to an injection volume of 1.0 ml. (D) Effect of the nominal resolution on the analytical signal (V_i = 1 ml). In all experiences an aqueous solution of ethanol 10% (v/v) was used.

ments are carried out in a relatively fast way, obtaining clear and well defined transient signal and achieving the best sensitivity, as can be seen in Fig. 4B and C. Finally, Fig. 4D shows the effect of the nominal resolution on the analytical signal. An increase in this parameter, which varies from 2 to 32 cm^{-1}, clearly decreases the analytical response. Based on these results, a nominal resolution of 2 cm^{-1} and a sample volume of 1.0 ml were selected for further studies.

3.4. The efficiency of the on-line extraction process

In the configuration used in this work, the interaction between the hydro-alcoholic solution and the organic phase takes place in the extraction coil. Thus, the influence of the extraction coil length (L) on the efficiency of the extraction process was evaluated in the range 0–250 cm (see Fig. 5). The analytical response increased with L up to 75 cm. Thereafter, a plateau was reached, therefore indicating that the extraction process had led the maximum efficiency. Based on these results, an extraction coil length of 100 cm was fixed for further studies.

Under the experimental conditions indicated in Table 1 (Q_{Sam} = 0.325 ml min^{-1}; Q_{Ext} = 0.325 ml min^{-1}, $Q_T = Q_{Sam} + Q_{Ext}$ = 0.65 ml min^{-1}) the efficiency of the on-line extraction process was estimated at 98%, through the relation between the corrected absorbance at 877 cm^{-1} obtained from an ethanol solution of 10% (v/v) in chloroform (in batch), and the one corresponding to an aqueous solution of the same concentration, inserted into the proposed system (see Fig. 2B and D).

These results are very different from those reported by Tipparat et al. [20]. In that work, samples/standard aliquots of 20 ml each were "equilibrated off-line" with 10 ml of dried chloroform for 1 min in a separator funnel. Nevertheless, researchers do not report the efficiency of the extraction. In a preliminary test, the influence of the sample-extracting solution volume ratio (V_{Sam}/V_{Ext} = 2, 1 and 0.5; with an extraction time of 1 min) on the off-line extraction efficiency was checked. In all cases the extraction process was highly reproducible, but the efficiency was poor, only about 20%. The enormous difference in the extraction behavior observed in the off-line and on-line extraction strategies necessarily has to be attributed to the different dispersion and interaction pattern developed between the hydro-alcoholic and organic phase in both modalities.

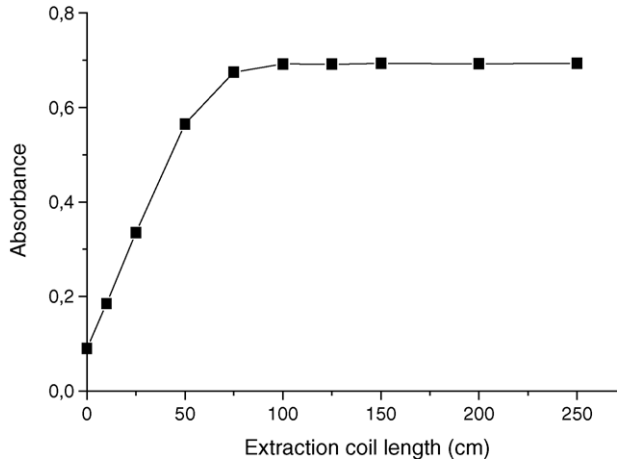

Fig. 5. Effect of the extraction coil length on the analytical signal of ethanol ([Ethanol] = 10% (v/v), V_i = 1.0 ml). Other experimental conditions as indicated in Table 1.

3.5. Analytical features

Under the experimental conditions indicated in Table 1, the analytical signal increased linearly with the ethanol concentration up to 15.0% (v/v). The equation describing the simple calibration line was $A_{(877\ cm^{-1})} = 7 \times 10^{-5} + 0.0692$[Ethanol] with r = 0.9997, where A_c is the absorbance at 877 cm^{-1} corrected by means of a baseline established between 844 and 929 cm^{-1} and [Ethanol] corresponds to the ethanol concentration expressed in % (v/v).

The precision of the procedure was estimated by measuring five replicates of beer, red wine and whisky samples with labeled ethanol contents of 4.5, 12.5 and 40% (v/v), respectively. The corresponding relative standard deviations were 1.3% (0.300 ± 0.004), 0.9% (0.869 ± 0.008), and 0.8% (0.714 ± 0.006), respectively. On the other hand, the real detection and quantification limits for ethanol, defined as three and ten times the standard deviation of the blank (3σ, 10σ), were 0.03 and 0.1% (v/v) respectively, while the sample frequency of the proposed method was 25 h^{-1}.

The analytical sensitivity, the dynamic range, as well as the limits of detection and quantification of the analytical system are directly related with the pathlength (b). Hence, the selection of this parameter involves a compromise between sensitivity and dynamic range. The ethanol concentration of the most popular commercial alcoholic beverages (standard beers, wines and spirits) ranges from 3 to 45% (v/v). Thus, in order to offer a versatile procedure for the analysis of these kinds of samples, the pathlength was fixed at 0.50 mm. Under this condition, normal and low-alcohol beers and wines can be directly analyzed, while samples with ethanol content higher than 15% (v/v), must be adequately diluted off-line or on-line (see Section 2.1) with DI prior to their introduction into the system. Alternatively, these kinds of samples could be analyzed directly using a lower pathlength.

3.6. Effect of matrix

One of the major advantages provided by the proposed method is related to the on-line ethanol extraction, since the analyte separation from most of the potential matrix interfering compounds, such as sugars, is produced in this process. In order to corroborate this fact, a series of aqueous standard solutions containing 10% (v/v) of ethanol and different concentrations of sugars ([Maltose] = 0–60 g l^{-1}; [Saccharose] = 0–250 g l^{-1}, [Glucose] = 0–250 g l^{-1} and [Fructose] = 0–250 g l^{-1}) were analyzed. The results obtained indicated that the analytical signal is completely independent of the concentration of sugars, thus indicating the total separation during the on-line extraction process.

In addition, with the purpose of studying the possible matrix effect in the analysis of real samples, different standard addition

Table 2
Standard addition calibrations

Sample	Equation	$[Ethanol]_{SCC}$[a]	$[Ethanol]_{SAC}$[b]
Normal beer	$A_c = 0.1800 + 0.06950[Ethanol]$ ($r = 0.99987$)	5.18 ± 0.09	5.2 ± 0.1
White wine	$A_c = 0.1675 + 0.06910[Ethanol]$ ($r = 9.99968$)	9.65 ± 0.08	9.7 ± 0.1
Red wine	$A_c = 0.2206 + 0.06990[Ethanol]$ ($r = 0.99969$)	12.70 ± 0.09	12.6 ± 0.1
Dark rhum	$A_c = 0.1390 + 0.06920[Ethanol]$ ($r = 0.99958$)	40.1 ± 0.2	40.2 ± 0.3
Scotch whisky	$A_c = 0.1490 + 0.0680[Ethanol]$ ($r = 0.99958$)	42.9 ± 0.4	42.7 ± 0.5
SCC[c]	$A_c = 7 \times 10^{-5} + 0.06920[Ethanol]$ ($r = 0.99987$)		

[a] $[Ethanol]_{SCC}$ represent the ethanol concentration found by means of a simple calibration mode.
[b] $[Ethanol]_{SAC}$ corresponds to the ethanol concentration obtained through the standard addition calibration.
[c] Equation corresponding to the simple calibration curve ([Ethanol] = 0–15%, v/v).

graphs were made. For this purpose, different volumes ranging from 0 to 30 ml of an aqueous standard of ethanol 40% (v/v) were added to 50.0, 25.0 and 5.0 ml of untreated samples of beer, wine (red and white) and spirit (dark rhum and whisky), respectively, diluting in all cases to a final volume of 100 ml with DI. The results obtained indicated that the standard addition curves did not show a significant difference in their slopes ($p < 0.0005$) with respect to the simple calibration line, which denoted the link of no physical or chemical interference matrix effects. On the other hand, the ethanol content obtained using both calibration modes did not show significant differences either, and is quite similar to those reported by the producer, denoting the absence of any kind of interferences, as can be seen in Table 2.

In order to obtain a preliminary estimation about the accuracy of the proposed method, the solutions used for the standard addition experiences were also analyzed under the optic of recovery studies. In all cases the recoveries ranged from 97.1 to 103.4%, again demonstrating the general reliability of the method.

Table 3
Analysis of various alcoholic beverages

Sample	Reference values			FI–FTIR[a]
	Labeled amount	HS-GC-FID	Derivative FTIR	
Ethanol concentration (%, v/v)				
Beer-1	3.0	3.2	3.05	3.10 ± 0.04 (1.3)
Beer-2	3.0	2.9	3.10	3.05 ± 0.07 (2.3)
Beer-3	5.0	5.3	5.15	5.20 ± 0.09 (1.7)
Beer-4	5.0	5.1	5.05	4.95 ± 0.06 (1.2)
Beer-5	4.5	4.6	4.55	4.60 ± 0.06 (1.3)
Beer-6	4.5	4.7	4.80	4.75 ± 0.05 (1.0)
Beer-7	7.2	7.1	7.30	7.33 ± 0.06 (0.8)
Beer-9	<0.5	–	–	0.35 ± 0.01 (2.8)
Beer-10	0.5	–	–	0.45 ± 0.02 (4.4)
White rhum-1	38.0	38.2	37.9	37.8 ± 0.3 (0.8)
White rhum-2	38.0	38.2	38.0	38.1 ± 0.4 (1.0)
Dark rhum-1[b]	40.0	40.4	40.1	40.2 ± 0.4 (1.0)
Dark rhum-2[b]	40.0	40.0	40.4	40.3 ± 0.3 (0.7)
White wine-1	9.5	9.8	9.7	9.7 ± 0.1 (1.0)
White wine-2	11.5	11.7	11.4	11.6 ± 0.1 (0.9)
White wine-3	12.5	12.2	12.3	12.30 ± 0.09 (0.7)
Red wine-1	12.5	12.8	12.6	12.70 ± 0.09 (0.7)
Red wine-2	12.5	12.7	12.8	12.7 ± 0.1 (0.7)
Red wine-3	12.0	11.8	12.0	11.9 ± 0.1 (0.7)
Rose wine-1	12.5	12.4	12.5	12.60 ± 0.09 (0.7)
Rose wine-2	12.5	12.6	12.7	12.6 ± 0.1 (0.8)
Rose wine-3	12.0	11.9	12.0	12.07 ± 0.08 (0.7)
Whisky-1[b]	40.0	40.4	40.5	40.2 ± 0.4 (1.0)
Whisky-2[b]	40.0	39.6	40.1	39.7 ± 0.3 (0.8)
Whisky-3[b]	43.0	43.3	43.1	42.8 ± 0.5 (1.2)
Whiskey-1[b]	43.0	42.6	43.0	43.2 ± 0.4 (1.0)
Whiskey-2[b]	43.0	44.0	43.3	43.1 ± 0.4 (1.0)
Vodka-1[b]	37.5	37.8	37.6	37.7 ± 0.3 (0.8)
Vodka-2[b]	37.5	37.3	37.4	37.5 ± 0.4 (1.0)
Gin[b]	47.3	47.6	47.5	47.0 ± 0.5 (0.9)
Spirit[b]	40.0	40.2	40.1	40.3 ± 0.2 (0.5)

[a] Mean ± S.D. of five independent measurements, and the corresponding relative standard deviation (R.S.D.).
[b] Samples with ethanol content higher than 15% (v/v) were adequately diluted off line with DI prior the analysis.

3.7. Application of the proposed method to the analysis of real samples

Samples described in Section 2.2 were analyzed by the proposed method. The results obtained are summarized in Table 3, and as it can be seen, the values found agree well with those reported by the producer and with those obtained by a head space-gas chromatography with FID detection method developed in our laboratory [25] and by an alternative derivative FTIR procedure (see Section 2.3.1) [18]. The results obtained show that the proposed FI–FTIR determination of ethanol provides a fast, precise, and accurate means for the analysis of ethanol in alcoholic beverages.

The regression found between the values obtained by the proposed method and those reported by the producers was $Y = 0.118 + 0.998$ with $r = 0.9999$. In the same way, the regression between values obtained by the proposed method (Y) and those obtained by two different alternative procedures (X: HP-GC-FID and derivative FTIR) provides the following regression equations: $Y = 0.081 + 0.9946$ with $r = 0.9997$ and $Y = 0.051 + 0.9965$ with $r = 0.9998$, respectively; which demonstrate that the method does not require a blank correction, because the intercept is statically equal to 0. Results also indicate that the method is free of constant relative errors, because the slope is statistically equal to 1 [32].

4. General comments and conclusions

The proposed method allows the direct determination of ethanol in all types of alcoholic beverages, without any sample pre-treatment, neither requiring complex spectral treatment or corrections, nor sophisticated and expensive IR cells, providing sugar-free measurements. The methodological proposal is simple and easily adaptable to any FTIR spectrometer equipped with a conventional and standard transmission IR cell.

Compared with previously reported procedures involving IR measurements, the recommended procedure provides attractive analytical features (see Section 3.5), which are comparable or better than those offered by other published methods [15–20,26,27], including the on-line vapor phase generation-FTIR [21]; which is one of the most attractive approaches developed for determining the alcoholic degree. On the other hand, a real sample throughput of $25\,h^{-1}$, lower that the nominal sampling frequency reported in other procedures, can be considered as really appropriate for the designed application. The proposed method is simple, fast, precise and accurate. In consequence, it represents a valid alternative for the determination of ethanol in alcoholic beverages, and could be suitable for the routine control analysis.

Under the recommended experimental conditions ($Q_{CHCl_3} = 0.32\,ml\,min^{-1}$), the consumption of chloroform is near to $20\,ml\,h^{-1}$. Taking into account the sample frequency of the procedure, it can be considered as a very low consumption. Additionally, the solvent used can be easily recycled through its distillation. In that sense, certain researchers have proposed manifolds including distillation units, allowing the on-line recycling of the organic solvent [33].

With regards to the PTFE membrane, where the separation from the aqueous phase to the organic phase takes place, it is important to remark that the membrane life time is quite long; during the development of this work – preliminary tests, optimization, tuning of the system, and samples analysis – it was not necessary to change it. This is due to the fact that the involved chemical system (ethanol–water–chloroform) is not aggressive.

Concerning the analytical band, as it was stated along this text (see Section 3.1), the use of the intense band at $1046\,cm^{-1}$ was discharged. The results found using this band were bad, especially for distilled liquors spirits (results not shown). In contrast, the $877\,cm^{-1}$ band, less intense than the previous one, is located in a region where chloroform is highly transparent and is not affected by the methanol presence. It is exactly the same in aqueous standards, samples, and ethanol strengthened samples. In addition, the recovery and standard addition studies indicated that is interference-free. Finally, the problem stemming from its slightest sensitivity is made up for its easiness, by using higher pathlengths. As a consequence, the $877\,cm^{-1}$ band seems ideal for ethanol quantification in these sorts of samples.

The excellent figures of merit, the absence of problems related to water background and the removal of interfering species open an enormous window of applications for the analysis of ethanol in very complex matrices. New studies are under way in order to: (i) determinate ethanol in other matrices and samples, and (ii) develop new and alternative strategies for the calibration of the analytical system.

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