

Quantitative analysis of methanol, an adulterant in alcoholic beverages, using attenuated total reflectance spectroscopy

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To quantify methanol, a spurious adulterant in alcoholic beverages, attenuated total reflectance (ATR) technique is used. Synthetic samples (100) are prepared for developing a suitable calibration model. Spectra are recorded for all samples in wavelength region 4000-600 cm^{-1} using Bio-Rad 175 C FTS spectrophotometer in ATR mode. Calibration equation is obtained using partial least squares (PLS) method. Correlation coefficient and root mean square error values for methanol are R^2_{cal} : 0.9860, R^2_{val} : 0.9839 and RMSEC: 0.0470, RMSEV: 0.0505. Spectral measurement is done for 10 samples and their methanol concentration (0.5-2.5%) is predicted using the developed calibration model. The reliability and repeatability of spectroscopic technique may be appreciated by the agreement between ATR predicted results to those of actual values.

Keywords: Attenuated total reflectance, Calibration, Correlation coefficient, Partial least squares, Root mean square error, Methanol

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Introduction

Various adulterants and the different techniques for determining these adulterants are reported¹⁻⁷. Methanol poisoning cases are also investigated⁸⁻¹⁰. In an analysis¹¹, around 64 percent of alcoholic beverages tested were falsified and few were found with methanol higher than the permitted level. Hence it is essential to prevent/monitor adulteration.

Determination of methanol is carried out using FTIR spectroscopy^{12,13}, vapour-phase FTIR spectroscopy¹⁴ and second derivative NIR spectroscopy¹⁵. Even though determination of methanol in the presence of ethanol is studied using GC, FTIR-GC and MS, attempts regarding adulterant (methanol) determination in alcoholic drinks are a few^{10,16}. In this study, an attempt has been made to quantify methanol in synthetic samples using Attenuated total reflectance (ATR) technique and then extending the focus to work on real alcoholic beverages. ATR technique is used to overcome the problem of strong absorbance in mid IR region.

Materials and Methods

Highly pure methanol (99.8%) and ethanol

(99.8%), obtained from the MERCK, were used for preparing samples in the following concentration: methanol, 0.5-2.5; and ethanol, 10-20%. For small variability, two different batches were used. Samples were prepared in double distill water. Bio-Rad 175C FTS spectrophotometer with resolution 4 cm^{-1} in the wavelength region 400-4000 cm^{-1} was used for experiments. Horizontal type ATR accessory with Zinc Selenide (ZnSe) crystal was employed for spectral measurements. Spectra were collected in the wavelength region 600-4000 cm^{-1} . With air as background, 64 scans were performed for obtaining every spectrum.

Results and Discussion

ATR spectrum of synthetic sample (Fig. 1) shows peaks at 2984 cm^{-1} and 2906 cm^{-1} , which is due to C-H stretch present in both methanol and ethanol. Absorption peak at 1640 cm^{-1} is associated to OH deformation. C-OH deformation causes a peak at 1452 cm^{-1} . Two closely resolved peaks, present at 1084 cm^{-1} and 1045 cm^{-1} , are attributed to the C-O stretch of ethanol and methanol respectively. Concentration variation of the constituents was observed easily from the overlaid spectra of synthetic samples (Fig. 2).

Partial least squares (PLSs) regression method, based on principal component analysis, is used for

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performing calibration (Table 1). Prior to calibration, all the spectra are mean centered. Since the major absorption of the components of interest lies in the region 1750-900 cm^{-1} , this wavelength region is chosen with 426 data points for developing calibration model.

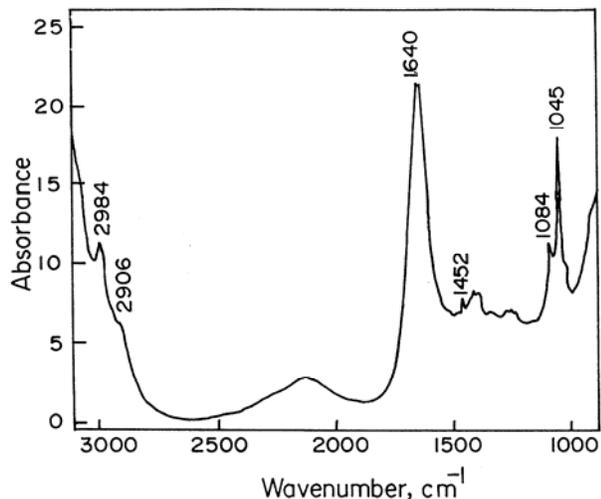


Fig. 1—ATR spectrum of methanol and ethanol present in synthetic sample

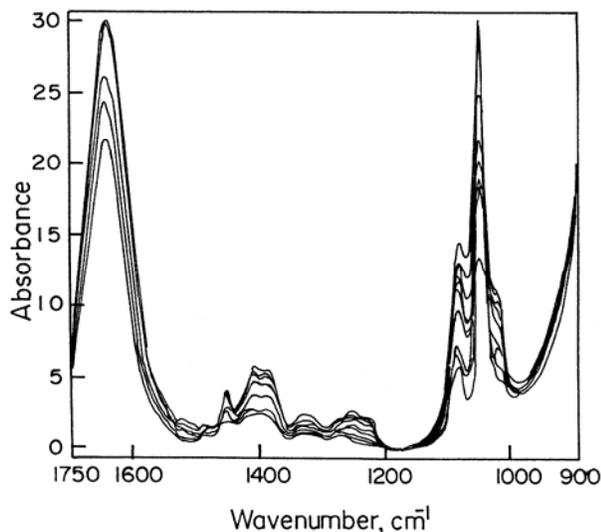


Fig. 2—Overlaid spectra of synthetic samples in 1750-900 cm^{-1} region

PLS method uses both the spectral response and respective reference data for the examined samples to determine PLS factors on the data set. Validation process is accomplished through “cross-validation”, i.e., removing one sample from calibration set at a time and using it for prediction. Actual vs predicted concentration values of methanol plot shows the effectiveness of calibration (Fig. 3). First two principal components (PCs) of methanol for the calibration set indicate that there exist two distinct groups of excipients (Fig. 4). These groups may be due to the presence of two different batches of constituents used for sample preparation. Eigen values for methanol of three PCs are: I, 66; II, 23; and III, 5%.

PLS loading of calibration set samples is useful to decide the optimum number of factors, which are essential for developing calibration; also, they provide information about the contribution of every factor for analysis. PLS loading of first score resembles the raw spectrum. At higher scores, less useful information will be found and the noise dominates. Information about the contribution of different peaks or wavelengths towards analysis is also observable from the

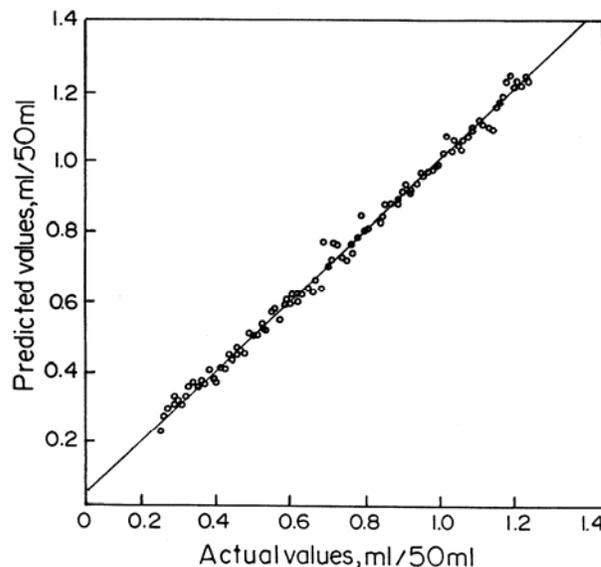


Fig. 3—Actual vs predicted values of methanol in calibration set

Table 1—Calibration statistics of methanol and ethanol in synthetic samples

Analyte	Wavelength region, cm^{-1}	PLS factors	Calibration		Validation	
			R^2	RMSEC	R^2	RMSEV
Methanol	900-1750	3	0.9860	0.0470	0.9839	0.0505
Ethanol	900-1750	4	0.9923	0.1791	0.9898	0.2066

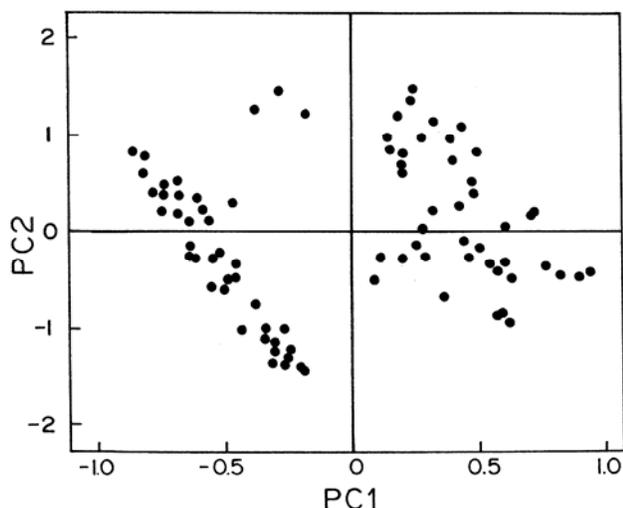


Fig. 4—Score plot PC 1 vs PC 2 for methanol in synthetic samples

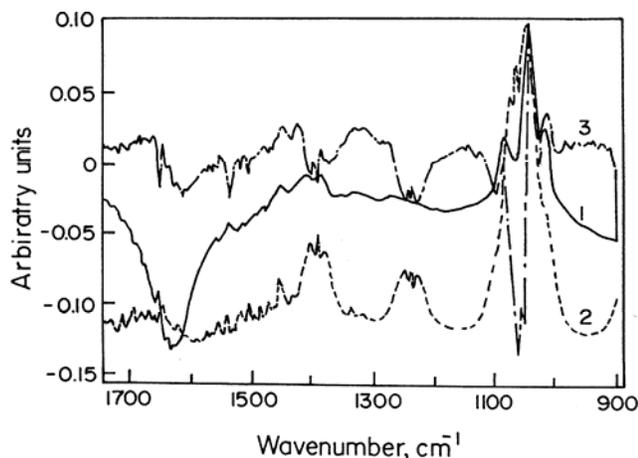


Fig. 5—PLS loading of first three scores of methanol

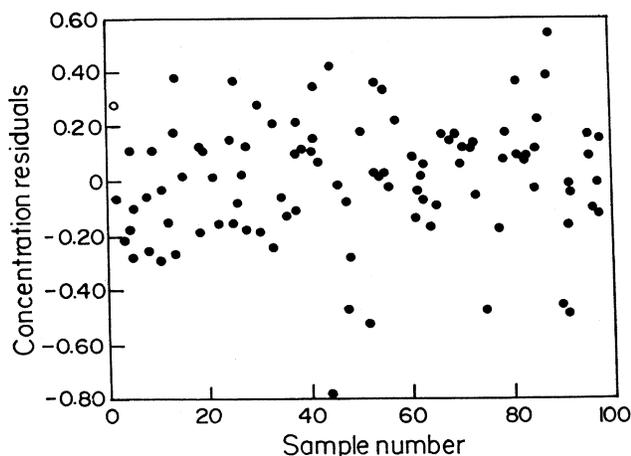


Fig. 6—Concentration residual plot for methanol in calibration set

Table 2—Actual and predicted values of methanol (ml/50ml) in prediction set

Actual values	Predicted values	Bias
0.270	0.334	0.064
0.330	0.372	0.042
0.360	0.403	0.043
0.390	0.404	0.014
0.460	0.461	0.001
0.630	0.613	0.017
0.790	0.966	0.176
0.910	0.893	0.017
1.130	1.162	0.032
1.170	1.174	0.004

$$R^2 = 0.9879, \text{RMSEP} = 0.0636$$

loading. PLS loading for first three factors of methanol (Fig. 5) shows that the maximum contribution for analysis is from the first three scores and the optimum factor for developing calibration is three.

Most of the concentration residual values (CRVs) of methanol (Fig. 6) lie close to zero with a variation of ± 0.4 . One or two CRVs in the plot that lie far from the rest of the set may be considered as outliers. Outliers may be due to the error occurred during experiments. The uniform distribution of CRV reveals the effective sample preparation. Developed calibration model is ensured for its effectiveness through validation process. Once after assuring the reliability of calibration method, a set of 10 samples with unknown methanol concentration is used for prediction. Experiments are carried out for this set of samples in a similar way as that of calibration set. Spectroscopic results lie close to the actual concentration of methanol (Table 2). Prediction statistics, correlation coefficient 0.9879 and RMSEP 0.0636, depict accuracy of the method employed.

Conclusions

An efficient calibration model is developed for quantifying methanol with 90 synthetic samples using attenuated total reflectance technique. The calibration statistics obtained for methanol (R^2_{cal} : 0.9860, R^2_{val} : 0.9839, and RMSEC: 0.0470, RMSEV: 0.0505) is satisfactory. With the developed calibration method, a set of 10 samples is used for prediction of methanol concentration. ATR predicted results are in good agreement with the actual concentration values of methanol. Hence, the developed calibration with ATR method may very well be an effective and efficient

alternate analytical technique for methanol determination in alcoholic beverages.

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