Determination of ethanol in alcoholic drinks using Raman spectrometry

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The aim of this work was to test the applicability of 'Mira DS' handheld Raman spectrometer in food analysis; namely, for the determination of ethanol in selected alcoholic drinks, such as plum brandy, vodka, two types of beer, white rum, and white wine. (Standard) Raman spectra of low-chain aliphatic alcohols and ethyl acetate were used to reveal and identify potentially interfering substances. The results obtained have shown good agreement between the ethanol content determined and the amount declared by producers. However, it was also found out that the portable device tested is not very suitable for monitoring ethanol in beer due to the presence of intensively fluorescent constituents; possible solutions of this problem being discussed and some prospects outlined.

Introduction

Raman spectrometry (RS) represents a spectroscopic technique, which allows one rapid interpretation and highly sensitive structural identification of chemical substances thanks to their unique vibrational characteristics [1].

In brief, a monochromatic radiation exposed upon a molecule and this light will interact in three principal ways: it may be (i) reflected, (ii) absorbed or (iii) scattered in some manner. In the case of RS, the molecule is irradiated with a coherent source, typically a laser. Most of the radiation is elastically scattered, which is known as 'Rayleigh scatter(ing)'. However, a minor portion of such an irradiation is scattered inelastically (so-called 'Raman scatter') being composed of Stokes and anti-Stokes parts; see Fig. 1 overleaf.

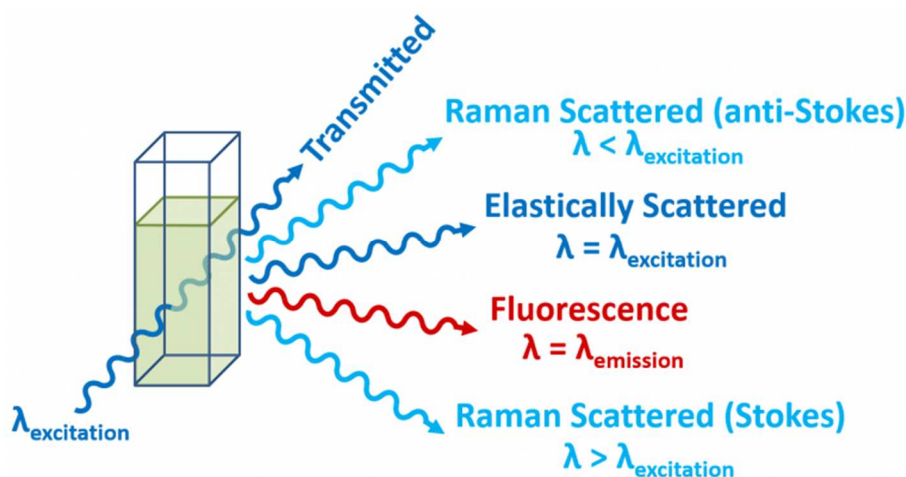* Corresponding author, ✉ milan.sys@upce.cz

Fig. 1 Different interaction types of monochromatic radiation with a molecule (scheme taken and rearranged from [2])

The latter of the two parts is of particular interest for analytical applications because it contains the key-information about the molecular structure [3] and represents the above-mentioned Raman scatter(ing) named after its inventor, famous Indian physicist Sir Raman, who was awarded for this discovery the Nobel Prize in Physics in 1930 [4].

RS detects vibrations involving changes in polarisability, whereas related infrared spectrometry (IRS) detects vibrations involving alterations in the dipole moment. RS thus measures relative frequencies at which a sample scatters radiation, unlike IR spectroscopy that measures the absolute frequencies at which a sample absorbs radiation. Infrared spectrometry, including the Fourier transform variant, is thus sensitive to vibrations and polar bonds of hetero-nuclear functional groups; e.g., –OH, –NH and C=O, whereas Raman spectrometry is sensitive to homo-nuclear molecular bonds, and is therefore capable of distinguishing sensitively between the C−C, C=C and C≡C bonds [5].

In any case, however, RS cannot be considered as universal analytical tool because of certain limitations. Mainly, gaseous substances, most of the non-metallic elements and metals, biatomic molecules with ionic polar bonds, as well as highly fluorescent compounds, cannot be identified [6]. Otherwise, numerous organic compounds in the solid state, in the form of pellets and powders, are analysable by RS [7]. And finally, Unlike IRS, Raman spectrometry can identify and determine organic compounds in aqueous solutions [8].

An effective miniaturisation makes RS attractive for field analysis (see e.g. [9]). Then, RS is frequently used in forensic science [10,11] and clinical or pharmaceutical analysis [12,13]; in all cases for substances providing a structural fingerprint by which the individual organic substances are differentiated.

Concerning the analysis of alcoholic drinks and, in particular, highly alcoholic spirits, it is worthy of mentioning the recent "Methanol affair" taking place from September 2012 to January in 2013, occurring in the Czech Republic and Poland, and associated with a serial methanol poisoning. As a consequence of these sad and severe incidents, 47 citizens of the Czech Republic and 3 people from southern Poland died, when many others had to be hospitalized; a number of the latter having suffered from partial or even total eye damage [14]. In such situations, it is necessary to offer a convenient analytical tool that can immediately provide the desirable information — fingerprint — about the actual composition of marketed alcoholic drinks (including some illegal products).

One of the choices for this purpose is RS; best, in the form of an instrument available in simplified portable construction, like a 'Mira DS' hand-held Raman spectrometer (illustrated in Fig. 2), developed recently from the previous type 'Mira P' [15], and now conquering the market in Czech Republic.

In this work, the 'Mira' device has been tested primarily for monitoring of ethanol and, more specifically, whether or not one can achieve its reliable quantification in different types of alcoholic drinks.

Fig. 2 'Mira DS' apparatus with accessories (reproduced with permission [15])

Experimental

Chemicals and Reagents

Commercially available solutions of 99.5% methanol, 96% ethanol, 99.5% *n*-propanol, 99.7% isopropanol from Lach-Ner (Neratovice, Czech Republic) and 99.8% ethyl acetate from Sigma-Aldrich were used for preparation of their 20% standard solutions and a series of calibration solutions of 5–40% (v/v) ethanol for determining its content in samples.

All the solutions mentioned were optionally diluted using demineralized water (with purity corresponding to an electric resistivity >18.3 MΩ·cm) made in a Milli-Q Millipore purification system (Burlington, Massachusetts; USA).

Instrumentation

A 'Mira DS' handheld Raman analyser (see again Fig. 2 and also [15]) from Metrohm (Czech division, Prague; Czech Republic) was used for all Raman spectrometric measurements.

Method and measurements

Raman spectra of standards, calibrations series of ethanol and selected samples of alcoholic drinks were measured in spectral range of 400–2300 cm^{-1}. Laser output power at the sample was set to 50 mW. The other parameters were as follows: laser wavelength, 785.0 nm; resolution (FWHM), 8 cm^{-1}; and spot size, 0.04 mm with 2.5 mm in raster size.

Sample preparation for analysis

A set of different alcoholic drinks (Table 1) purchased in common stores served for analysis. Samples of plum brandy (home-made "slivovice"), white rum (of the Caribbean type made from sugarcane) and vodka had to be diluted twice by water due to a high ethanol content.

Samples of beer were deaerated using an ultrasonic bath at laboratory conditions for 10 min. Then, a volume of 1.5 mL of so pre-treated samples was pipetted by automatic adjustable transferpette (Labsystems, Finland) into special vials configured for the 'Mira' device(s).

Analyses and statistical evaluation of the data

All analyses of selected standard solutions and of the samples of spirits, wine and beer were always made in three replicates ($n = 3$), if not stated otherwise. The final results calculated from the individual measurements by the software of the 'Mira DS' device are presented as the confidence intervals, $\bar{x} \pm st_{1-\alpha}$, where $\bar{x}$ is the arithmetic mean, s the standard deviation, and $t_{1-\alpha}$ the critical value of Student t-distribution for three replicates at a significance level of $\alpha = 0.05$ (i.e., with 95% probability).

Results and Discussion

Raman spectra of 20% (v/v) ethanol (EtOH) together with methanol (MeOH), *n*-propanol (NPA), isopropanol (IPA), and ethyl acetate (EtOAc) — all as aqueous solutions — were measured to determine positions of specific bands with wavenumbers (in cm^{-1}). As shown in Fig. 3, the standards investigated provide minimally one intensive band with a maximum of 880, 1018, 637, 817 and 860 cm^{-1} for EtOH, MeOH, EtOAc, IPA and NPA, respectively.
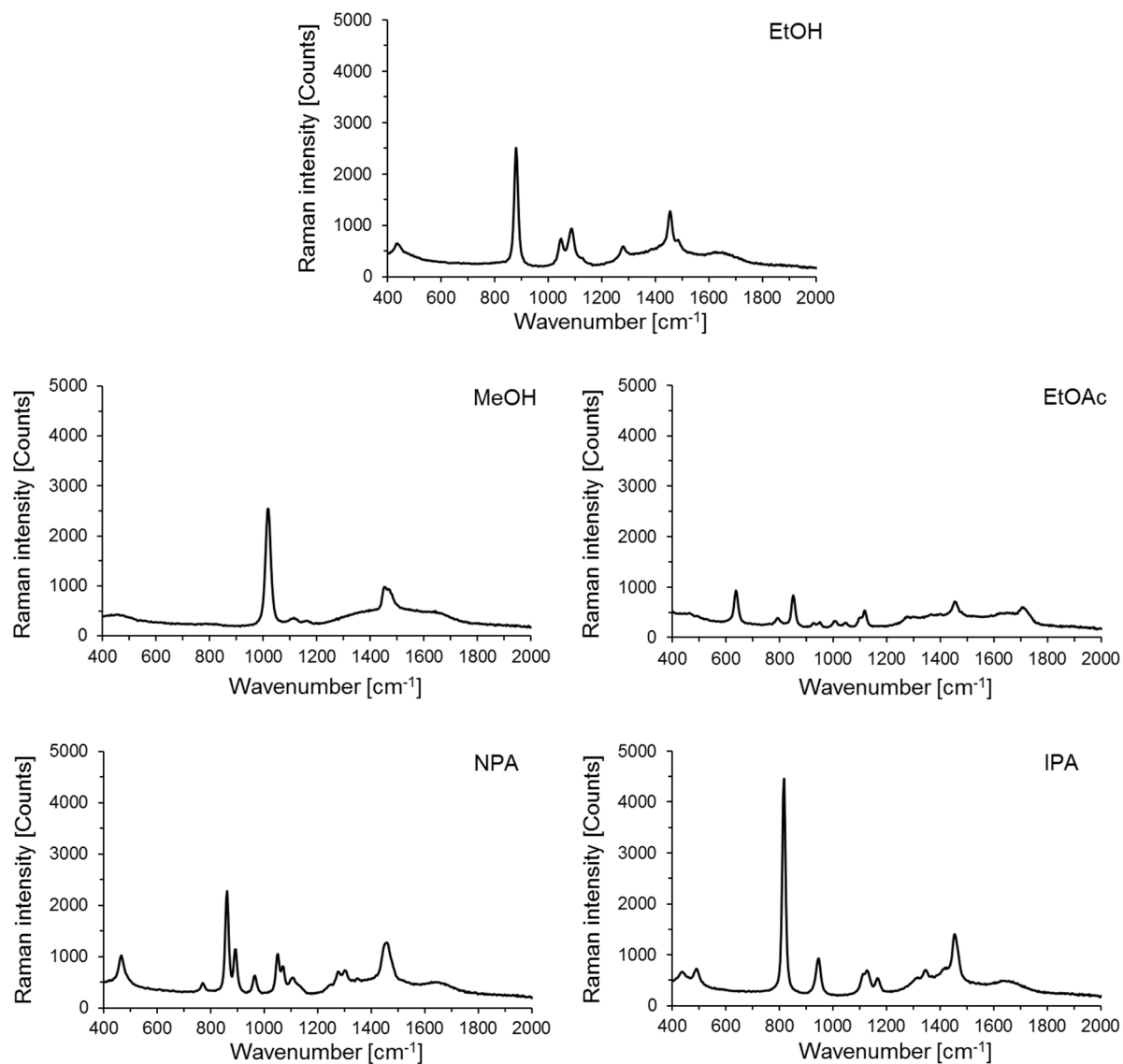

Fig. 3 Raman spectra of 20% standards of ethanol and of potentially interfering substances (for specification, see abbreviations in right upper corners)

It is interesting to notice that the main Raman bands for EtOH and MeOH were located sufficiently far away one from the other so that they can be recognised even in the respective mixtures.

The band at wavenumber of 880 cm^{-1} for EtOH was then chosen for analytical purposes. A univariate calibration model was constructed by using the band heights at 880 cm^{-1} of the individual standard solutions for ethanol, which is depicted in Fig. 4 by the insert in upper right corner.

A satisfactory linear relation between the Raman band height and the ethanol content can be described by regression equation: $y = 0.129x − 0.0861$ and characterized by the correlation coefficient of $r^2 = 0.9951$ obtained for the concentration range of 5–40% EtOH. A very low value of the intercept, −0.0861, suggest one that the standard addition method could also be used; nevertheless, in this work, the calibration curve method was chosen for the determinations of ethanol in all the samples of alcoholic drinks.
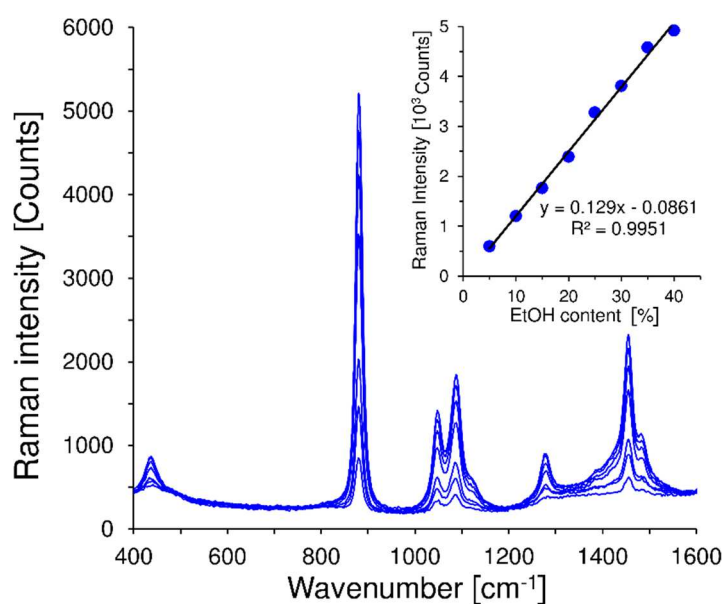

Fig. 4 Raman spectra of 5, 10, 15, 20, 25, 30, 35 and 40% (v/v) standard solutions of ethanol with the corresponding calibration curve

Several types of different alcoholic drinks were selected (Table 1) to show the capabilities of the 'Mira DS' spectrometer in quantitative analysis. Fig. 5 demonstrates that the ethanol content in the samples, such as highly alcoholic spirits and white wine, can be fairly determined because the corresponding Raman spectra are properly drawn and the bands of interest easily evaluable. An increase of the base-line (at a wavenumber of 950 cm^{-1}) was found for the sample of white wine, which can be attributed to a more complex sample matrix. Also, it was confirmed that these samples had not contained any measurable portion of MeOH as no detectable band of this highly toxic substance at a wavenumber of 1018 cm^{-1} was observed.
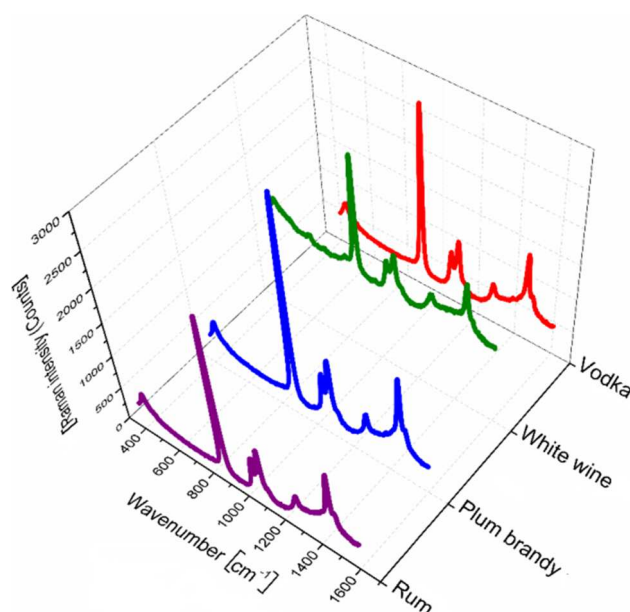
Fig. 5   3D-diagram with the Raman spectra of white wine and twice diluted samples of white rum, plum brandy, and vodka

On the contrary, it is also necessary to admit that assays with beer samples have revealed one distinct limitation of the 'Mira' spectrometers being evident as large overlapped bands that could not be evaluated (not shown). According to the literature [16,17], these broad signals probably correspond to the presence of intensively fluorescent compounds (like riboflavin, polyphenols or numerous flavouring substances) whose secondary light emission may completely drown less intense Raman scattering. Hence, in general, highly fluorescent compounds have to be considered as serious interfering species and the analysis of beer by Raman spectrometry with simplified 'Mira' device still represents a challenge that shall be taken up and subsequently solved.

Table 1   Analysis of selected alcoholic drinks – the results

Sample / specification	Producer / Distributor	Declared content	Found content
Beer / Speciál 24°	Primátor Náchod	10.5	(not found)
Beer / Birell*	Plzeňský Prazdroj	0.5	(not found)
Vodka / jemná	Stock Plzeň-Božkov	37.5	37.1 ± 0.7
White rum / Liberté	LIDL Česká republika	37.5	36.2 ± 0.8
White wine / Pálava	Znovín Znojmo	11.5	11.8 ± 0.3
Plum brandy / slivovice	(private source)	(unknown)	44.6 ± 1.1

Values (v/v percentage) given as confidence intervals $\bar{x} \pm st_{1-\alpha}$, where $\bar{x}$ is the arithmetic mean, s the standard deviation, and $t_{1-\alpha}$ the critical values (2.353) of Student's t-distribution for 3 repetitions of each analysis at $\alpha = 0.05$, respectively. Note: *) trademark for non-alcoholic beer.

Table 1 surveys the results of analyses of selected alcoholic drinks, representing three common categories: beer, wines, and spirits. As can be seen, except for beer, a very good agreement has been achieved between the data from analysis by RS and the declared contents of ethanol.

Conclusions

A 'Mira DS' handheld Raman spectrometer developed for identification of selected organic compounds has been tested to determine ethanol in the samples of alcoholic drinks.

The results obtained in this work have shown that this portable analyser is suitable for analysis of traditional highly alcoholic spirits with content of ethanol about 40% (v/v), for example Caribbean rum, vodka, and plum brandy. Apparently, the device is applicable also to analyse white wines containing ethanol above 10% (v/v). However, as confirmed experimentally, beer usually exhibits a more complex matrix than those of wines and spirits [17] and, therefore, their analysis using Raman spectroscopy still represents a problem due to the presence of intensively fluorescent compounds. Possible suppressing of their undesirable signals could be accomplished via the use of a laser with lesser power or with the aid of special pre-treatment of the samples based on effective removal of intense fluorescence-releasing compounds; for instance, by means of chemical derivatisation.

It can be concluded that 'Mira DS' Raman analyser represents a perspective analytical tool for quick, rapid, but still reliable analysis of alcoholic drinks with higher contents of ethanol. Especially, one-purpose field analyses would be the right applications of this portable and simply-to-use instrument in comparison with gas chromatographs whose performance for the determination of ethanol is surely more effective, but their applicability out of laboratories is hitherto quite rare (including some efforts to propose and develop miniaturised systems for analytical separations in the GC-ECD or GC-PID regime [18]).

Finally, in prospect, there is also one very interesting option of how to use the 'Mira DS' spectrometer in daily practice. It is inspired by existence of various databases of Raman spectra offered by renowned distributors or institutions (see e.g. [19]) and concerns an idea to build up the own library of "reference Raman spectra". Such a collection, as an integral part of the software, would comprise a set of RS-records of various alcohol drinks that are officially marketed in a given country, region, or even in an otherwise specific locality. Then, by sole comparing the reference RS-spectrum of an alcoholic drink with those obtained in terrain, one could readily check the quality of the respective product, thus ensuring the control of its proper composition and, eventually, revealing possible illegal goods. (That such an approach can be utilised for the recognition and authentication of typical commercial products, it is well documented in a recent study with a set of

Scotch whisky – see [20] and also Fig. 6. The corresponding records confirm that RS faithfully reflects some nuances in the final composition of the individual samples of whisky given by their different production and maturation process.)
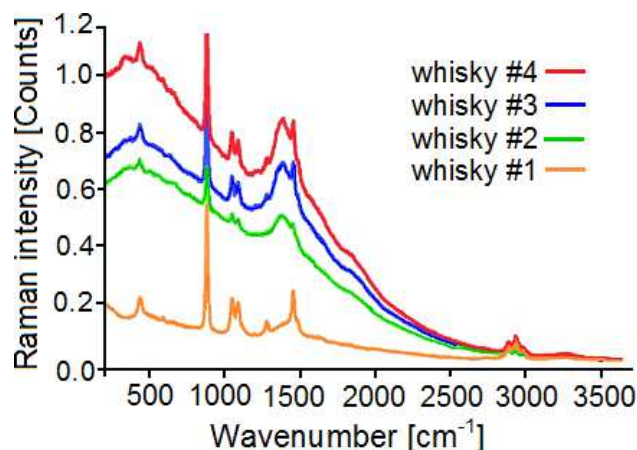

Fig. 6   Raman spectra of four different samples of single malt Scotch whisky (redrawn and rearranged from [20])

Acknowledgements

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