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Spectroscopic Determination of Methanol Content in Alcoholic Drinks

H. Vaskova

Abstract— The aim of this paper is to introduce an innovative method for measuring the concentration of methanol in alcoholic beverages. The novel approach consists in using Raman spectroscopic data for methanol content detection. Raman spectroscopy is a powerful method for material identification giving information about the structure of examined sample. This analytical method enables rapid, noncontact and non-destructive analysis that can be performed through the glass. The method for quantitative determination of methanol in alcoholic beverages has been developed on the basis of diverse Raman spectra for methanol and ethanol, their mathematical processing and using PLS regression method for the calibration and prediction model of methanol. Development of this new method is related to the massive methanol poisonings which occurred in the Czech Republic in September 2012. A large amount of harmful toxic alcoholic drinks containing methanol in quantities many times over legal limit was illegally distributed. This event led up to serious problems with poisoned people and the losses in lives. However, it can be assumed the problems may occur in the future since about a third of the defective alcohol has not been traced. The detection limit of the method lies below the permitted and safe amount of methanol in the beverages regulated by the European Parliament and the Council.

***Keywords*—** Alcoholic drinks, concentration evaluation, ethanol, methanol, Raman spectroscopy.

I. Introduction

SERIES of poisoning caused by high level of methanol in alcoholic beverages appeared in the Czech Republic at the beginning of September 2012. The race against time begun as the Czech Republic started to solve problems with harmful alcohol, increasing numbers of poisoned people and the losses in lives. According to the Ministry of Health one hundred and twenty seven people were poisoned, forty two of them died after consuming dangerous alcohol since last autumn up to the April 2013 [1].

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Counterfeiting is a recent increasing growing problem comprising various products and fields. Counterfeit products use material(s) with similar physical characteristics to the genuine product making it difficult to distinguish between them. Hazardous to health are then counterfeit medicines, food or drinks.

Poisonings by toxic chemicals as methanol occur all over the world. Health problems connected to mass methanol poisoning have been observed in recent years also in Estonia (2001), Norway (2002), Tunisia (2003), China (2004), India (2009) or Turkey (2011) [2].

The reason of many poisonings is the ingestion of methanol itself or ingestion of alcoholic beverages containing high quantities of methanol. Alcoholic beverages are popular in many countries and often consumed. Due to its effects on human organism the popularity is linked to the content of ethanol. There have been reported cases of spread of methanol poisonings when the less expensive and easy to obtain methanol was substituted for ethanol in alcoholic beverages. This way of counterfeiting alcoholic drinks was the reason of massive methanol poisoning in the Czech Republic.

The ingestion of drinks with the nonqualified raw materials containing high levels of methanol can cause serious health difficulties – metabolic problems, blindness, permanent neurological damage or even death. This is a great risk for consumers of alcoholic beverages taken from unknown sources.

Workers of regional health authorities and Health Station in Prague made a total of 27314 controls through the autumn 2012. 2023 samples of alcoholic beverages were analyzed, even bottles from official distilleries. Sixty one samples did not regard the content of methanol. [1] Seven samples showed high toxicity. They contained over 500000 milligrams per one litre of absolute alcohol that means methanol represented over 50% of all alcohol in the bottle [3].

Problem is that not all the dangerous beverages are already captured. This affair can last to the future and after-effects may occur even in several or many years, when someone gets to the bottles with defective alcohol. To taste, smell or appearance methanol cannot be recognized from ethanol. The lethal oral dose for human is not precisely defined; estimations are from 30 to 200 ml of methanol [4].

The most common method for laboratory proving of methanol content are gas chromatography – mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), chromotropic acid colorimetric method (recommended as

official methods by ISO and AOAC and ISO), Fourier transform infrared spectrometry (FTIR) and other methods [5, 6, 7, 8].

Methanol itself is rather not harmless, but in the organism is transformed into highly toxic formic acid. The effects of intoxication are rapid hence it is necessary to recognize the methanol intoxication in time. This is essential assumption for successful treatment. In large-scale poisoning, it is important to suppress the serious consequences. Therefore, there should be an accurate and rapid method for early diagnosis of high number of samples enabling appropriate treatment of patients. Authors of [9] introduce new rapid method for direct determination of formate, the toxic enzymatic metabolite of methanol in human organism, in blood serum samples by capillary electrophoresis with contactless conductometric detection. The authors claim the developed method presents the fastest test currently available to detect formate in blood samples.

Interest in the problematic of detection methanol in beverages has serious reasons that are connected to human health and lives. In the situation when a large quantity of harmful toxic alcohol containing excess of methanol is spread in population, it is advantageous to have a reliable and rapid method for methanol identification and quantification. These features provide Raman spectroscopy a modern analytical method for identification of various types of substances and materials. Raman spectroscopy was used for developing a quantitative determining of methanol in alcoholic beverages.

II. Methanol and Ethanol

Methanol CH_3OH is the simplest aliphatic alcohol. It is a colourless liquid freely miscible with water and other alcohols, transparent, volatile and highly flammable. Methanol is produced from the distillation of wood. This simple alcohol is constituent of a many commercially available solvents which find extensive applications both in industries and household. Methanol is used in the industrial production of many synthetic organic compounds, finds utilization e.g. in the field of fuels. In [10] gasoline-methanol mixtures were examined to perform test on measuring emissions resulting in findings that important reduction of emissions was noted while the percentage of the methanol was increased. Methanol is well absorbed by inhalation and by oral exposure. Poisonings resulted by absorption through the skin and inhalation of air containing methanol betimes happen, but much more disastrous methanol intoxications are related to ingestion of methanol itself or methanol containing beverages.

Confusion of methanol with ethanol CH_3CH_2OH, the alcohol complex with similar properties can have fatal consequences. Recognition cannot be clearly done by smell or taste.

Ethanol is a psychoactive drug and its intoxicating properties are known from ancient times. Besides its presence in alcoholic beverages ethanol is also used also as a solvent, as a source of fuel or, e. g. in thermometers.

In alcoholic drinks significantly prevails ethanol, which is primarily metabolized. Several thousand methanol poisoning happen every year all around the world and many of them end in death [11, 12]. Chemical structures of both simple alcohols are displayed in Fig. 1.

Fig. 1 Structure of methanol CH_3OH (left) and ethanol CH_3CH_2OH (right).

A. *Ethanol Intoxication*

A human body operates with alcohol as a poison. Toxins are generally disposed in the body in the liver. Ethanol is after ingestion exposed to the enzyme alcohol dehydrogenase and is oxidized to acetaldehyde, which is then changed to acetate. The process of ethanol conversion is showed in Fig. 2. These reactions produce a large amount of energy. Drinking alcohol makes people feel they are getting warm, but the released energy in the form of heat is dissipated from the body. Both methanol and ethanol are hydrophilic molecules, i.e. they are soluble in water and thus can be easily excreted by urine.

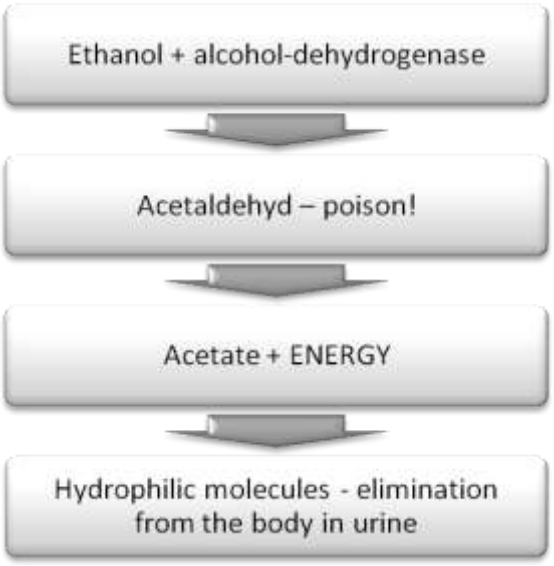

Fig. 2 The process of ethanol conversion in human body

B. *Methanol Intoxication*

Methanol itself is not harmful, but in the organism is converted into highly toxic enzymatic metabolites. Methanol is

also exposed to alcohol dehydrogenase which creates a dangerous formaldehyde, formic acid and formate. These metabolites are dangerous especially for the neural system, optic nerve and tissues. The process of conversion ethanol is showed in Fig. 3.

Methanol intoxication methanol is difficult to diagnose since the initial symptoms are not specific. Between 12 and 24 hours after methanol ingestion is typical that major part of methanol in blood is converted to formate, esters of formic acid. This transformation causes headache, dizziness, abdominal discomfort, blurred vision or complete loss of vision, hyperventilation. These symptoms can lead toserious damage to the optical nerve, respiratory failure, renal failure, coma, cerebral edema or death from cardio-respiratory arrest if not treated. [13, 14, 15]

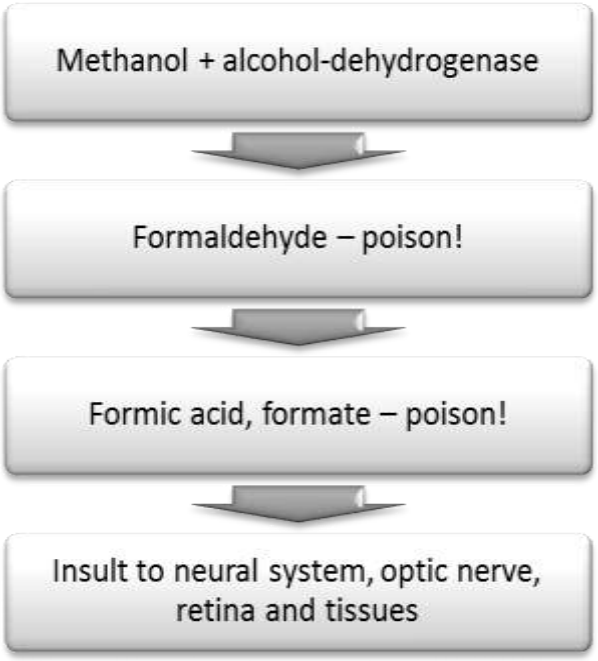

Fig. 3 The process of methanol conversion in human body

C. Treatment

The aim of methanol poisoning treatment is to quickly reach preferential ethanol metabolizing by alcohol dehydrogenase. Therefore the first recommended step is to drink alcohol beverage (cca 2 dcl of drink with 40% vol. of alcohol - ethanol) and immediately seek medical help. The quantity of this enzyme in cells is not infinite. Alcohol dehydrogenase was manufactured by human body to defend against ethanol therefore it is better arranged for this simple alcohol. Drinking ethanol occupies enzymes and methanol is thus unable to convert the dangerous formaldehyde and is dismissed as harmless urine out of the body. Methanol poisoning can be treated by fomepizole [16].

Harmful dose of methanol cannot be clearly described in regards to aspects such as age, weight, mixture with ethanol etc. It ranges from units of ml to about 80 - 100 ml, which causes death.

D. Limits of Methanol in Distillates

Limits of methanol in distillates in the Czech Republic are governed by Regulation (EC) No 1334/2008 of the European Parliament and of the Council. The permitted and safe limit for methanol in spirits and fruit spirits is to 12 grams per 1 litre of pure ethanol [17]. On the basis of known concentrations of the substances for the purposes of the experimental part of this research this value can be recalculated. The limit concentration is then 0.756% of methanol in ethanol.

III. METHANOL DETERMINATION

A. Raman spectroscopy

Raman spectroscopy is an analytical tool that becomes a valuable part of laboratories around the world in recent years. In principle, Raman spectroscopy as a vibrational spectroscopic method has the potential to answer a number of questions related to chemical details of molecular structure what makes this technique definitely proper for material identification. Raman spectroscopy provides very specific chemical „fingerprint“ of every single chemical substance in the form of the Raman spectrum.

Raman effect, the fundamental principle of Raman spectroscopy is known since thirties of the 20th century. The effect remained for a long time only on a theoretical level and practically was marginalized because of low sensitivity and difficulties with overcoming fluorescence phenomena. However, renaissance of Raman spectroscopy is coming even in the last decade hand in hand with technical advancements and latest developments in the field of new extremely sensitive detection devices, efficient filters for filtering Raman scattered light from the Rayleigh scattering, and also innovative laser technology designs [18]. The number of application in scientific and technical fields is perpetually growing [19, 20].

As is known, molecules have an ability to absorb or emit photons – an electromagnetic radiation with specific energy and to change their own energy due to this way. Raman spectroscopy is based on the characteristic vibrations of molecules of investigated sample caused by monochromatic light of laser. After irradiation of the sample by monochromatic light we can observe scattered light with a predominant representation of laser wavelength, but there are also other slightly changed wavelengths characteristic for the substance. Let follow the analogy with human fingerprints: every single human being on Earth have different set of fingerprints by which can be identified. Every single chemical substance has its own specific “fingerprint” its unique Raman spectrum – the key for the material identification. Diagram of the measurement is shown in Fig.4.

Raman spectroscopy brings advantages of non-destructiveness, non-contactless of the measuring. It is not necessary to come into direct contact with hazardous toxic substances. Analyses are rapid Raman spectrum can be

acquired within seconds. Another great benefit especially for application in beverages is the ability to measure samples through transparent packaging materials - glass, plastic, what is safe, convenient and prompt. Raman spectroscopy allows to measure samples of all states of mater and different forms.

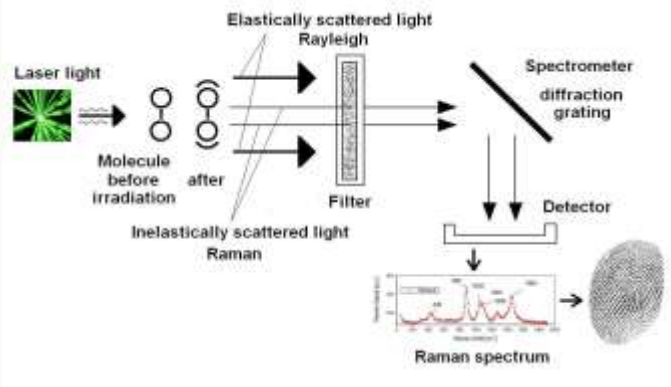

Fig. 4 The sample is irradiated by laser, molecule vibrates, filter eliminates intense Rayleigh scattered light, the grating disperses light onto a CCD detector do generate Raman spectrum which gives information about a molecule bonding and provides a chemical fingerprint for identification.

B. Spectral Data Fitting

Coming out of the quantum theory, transitions between energy levels in molecules after absorbing or emitting energy and related lifetimes it is proper to use for fitting Raman spectral data different functions. Gaussian (1) is usually used for solids, Lorentzian (2) for gasses.

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (1)$$

$$L(x) = \frac{1}{\pi} \frac{\frac{1}{2}\Gamma}{(x-x_0)^2 + (\frac{1}{2}\Gamma)^2} \quad (2)$$

Where x_0 represent the centre, parameter Γ specifies the width. In the case of methanol – a liquid a combination of these functions the Gaussian-Lorentzian profile is an appropriate solution. Spectral line shapes has features of both Gaussian and Lorentzian character. The Gaussian-Lorentzian is represented

$$A * G + (1 - A) * L \quad (3)$$

Where A is a variable parameter in the fit being the fraction of Gaussian character ($0 \leq A \leq 1$).

IV. Experimental part

Firstly a set of samples was prepared to verify the applicability of Raman spectroscopy to measure the methanol content in alcoholic beverages. Then was necessary to find appropriate measurement parameters, measure the samples, process the results and create calibration curves. On the basis of calibration curves was created a model of procedure for experimental detection of the hazardous methanol in alcoholic beverages.

A. Samples

Pure methanol and pure 100% ethanol were used for the set of mixtures. Fifteen mixtures were prepared in concentration range 0.05% to 50% solution of methanol in ethanol. Concentrations close to the limit value of the relevant legislation were chosen with slight intervals.

Mixtures were analyzed both directly under the Raman microscope and in glass vials. Advantage of vials consists in preventing evaporation of the two components, which is relatively fast. Thus the concentration difference between the actual and prepared concentration emerging already in short period of several seconds was reduced.

B. Raman spectroscopy

InVia Basis Raman microscope from Renishaw was used for recording Raman spectra methanol and ethanol mixtures. The Raman instrument uses two lasers as light sources: argon ion laser with the excitation wavelength 514nm and maximum output power of 20 mW and 785 nm and NIR diode laser with maximum output power 300mW. Both were tested but more precise results were obtained using NIR laser.

A Leica DM 2500 confocal microscope with the resolution up to 2µm was coupled to the Raman spectrometer. All measurements were collected at 5x - 20x magnification, with 10 seconds exposure time and 10 accumulations. The samples were firstly scanned in range 100 to 3200 cm^{-1} with 2 cm^{-1} spectral resolution. After determining the principle peaks the spectral range was reduced approximately to the area 800 - 1300 cm^{-1}.

V. Results

Raman spectra of pure methanol and ethanol are displayed in Fig. 5. In Fig. 6 is shown detail on two dominant peaks, one for each substance. Main attention was paid to these two

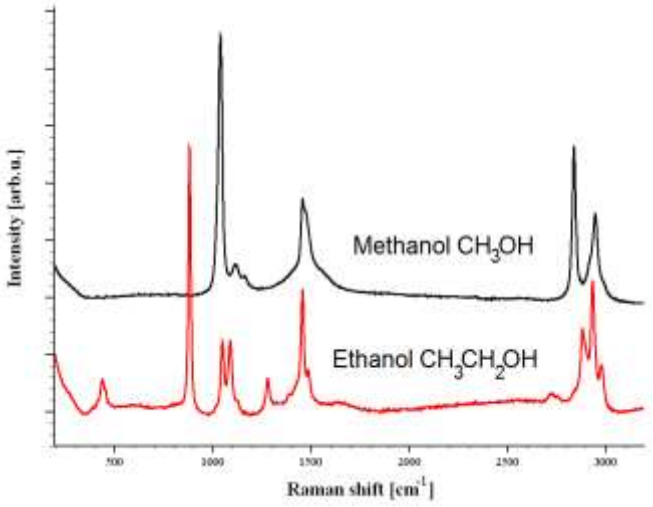

Fig. 5 Raman spectra of pure methanol and 100% ethanol

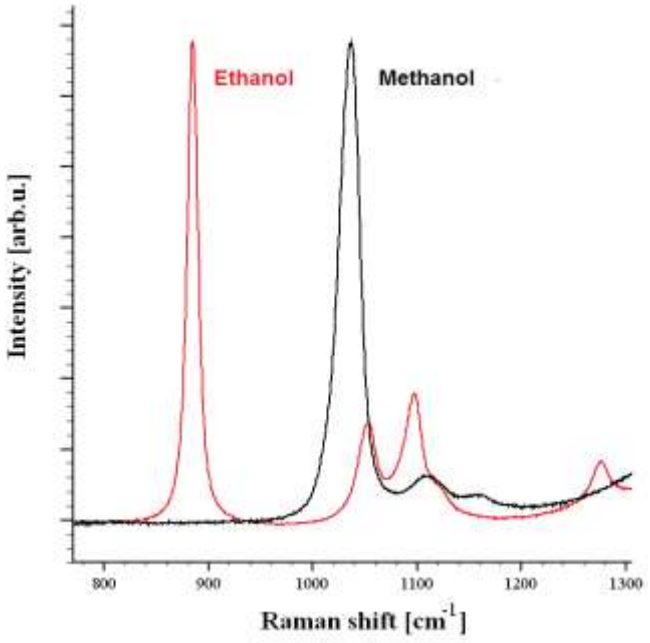

Fig. 6 Raman spectra of pure methanol and ethanol – dominant peaks

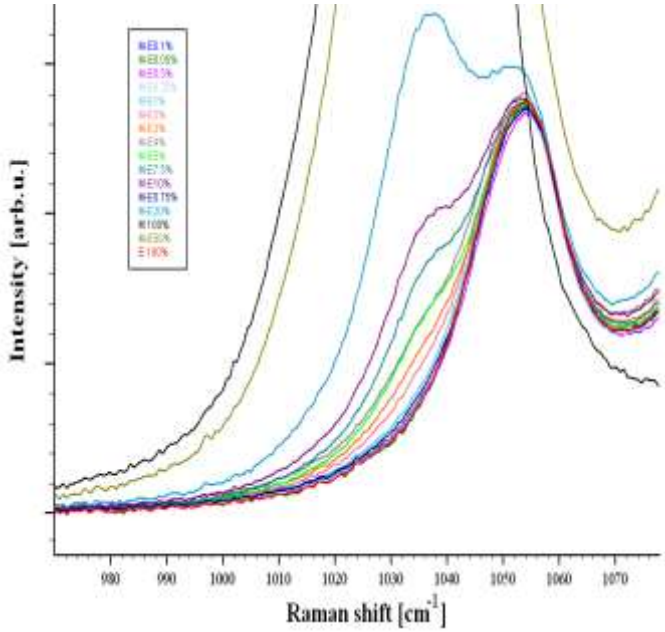

Fig. 8 Focus on a peak 1035 $cm^{-1}$of methanol

strong Raman peaks: 1035 cm^{-1} for methanol corresponding to C – O stretching and 881 cm^{-1} for ethanol corresponding to C – C stretching. Other Raman peaks and their assignments are presented in Table 1.

Pure methanol and ethanol and all mixtures were analyzed under the same conditions. The obtained spectra are shown in Fig.7. Fig. 8 offers the focus on a range of interest i.e. Raman shifts around 1035 cm^{-1}. Baseline corrections were applied on acquired spectra before processing. All spectra were subjected to normalization according corresponding ethanol peak at 881 cm^{-1}. Spectrum of normalized pure ethanol was subtracted from all the resulting spectra what allowed focusing on methanol C – O stretching peak at 1035 cm^{-1}. Subtracted data are shown in Fig 9.

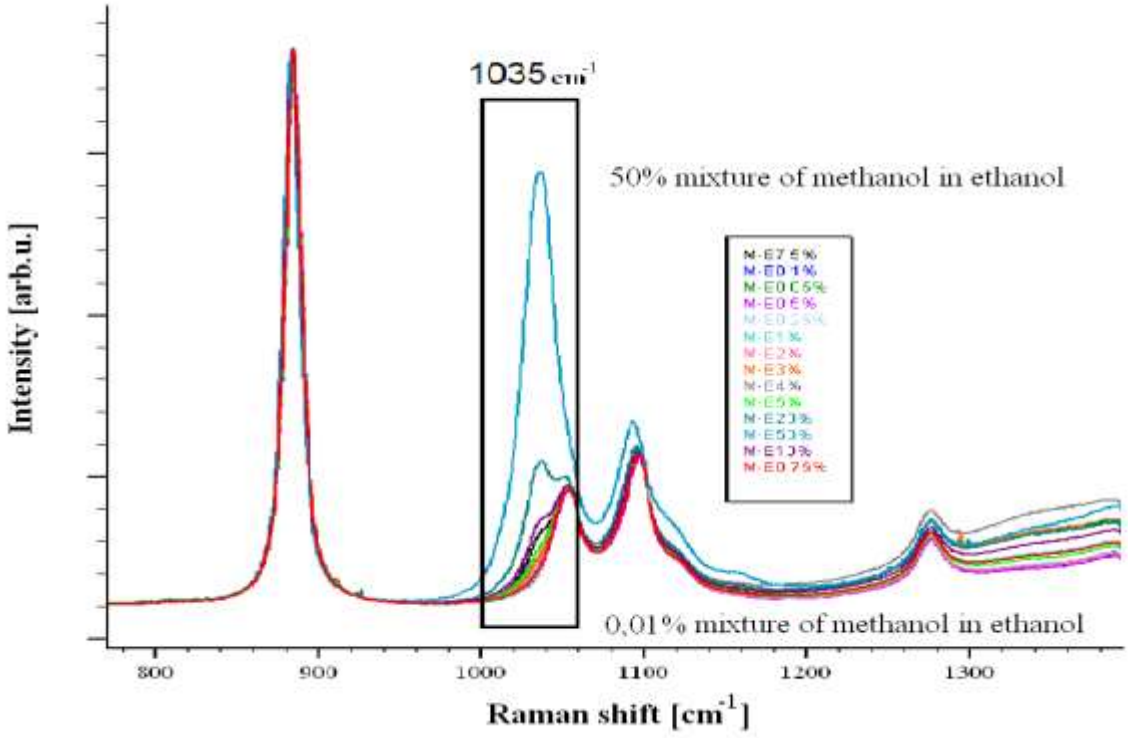

Fig. 7 Raman spectra of mixtures of methanol and ethanol

Table 1 Raman peaks of ethanol and methanol assignment [21, 22]

RAMAN PEAK [cm^{-1}]			
MEASURED	**LITERATURE**	**ASSIGNMENT**	**INTENSITY**
ETHANOL			
884	**883**	**C-C stretching**	**vs**
1052	1054	C-O stretching	S
1097	1096	CH3 rocking	s
1455	1454	CH3 bending	m
1482	1479	CH3 bending	vw
2883	2878	CH2 and CH3 stretching	vs
2934	2929	CH2 and CH3 stretching	vs
2980	2972	CH2 and CH3 stretching	vs
METHANOL			
1035	**1033**	**C -O stretching**	**vs**
1112	1106	CH3 rocking	w
1162	1149	CH3 bending	s, sh
1461	1448	-	s
2839	2832	C-H sym stretching	vs
2948	2940	C -H antisym stretching	vs

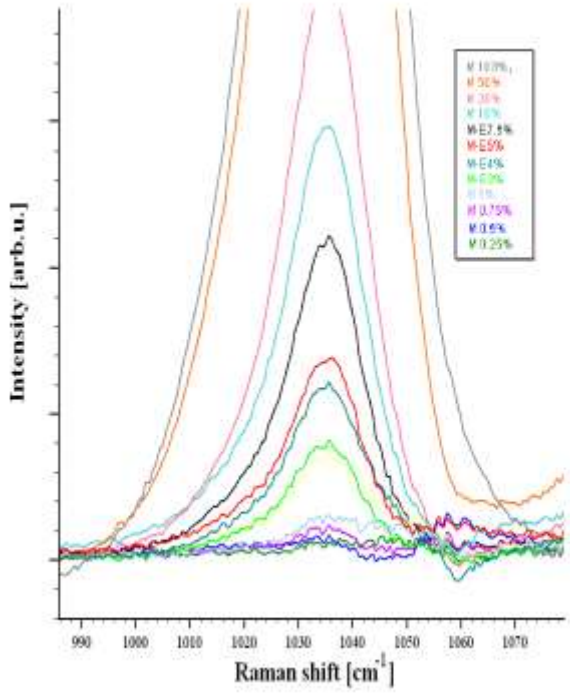

Fig. 9 Methanol peak at 1035 cm^{-1} of mixtures after ethanol spectrum subtraction

Data for calibration curve were acquired by fitting the methanol peak at 1035 cm^{-1} using Gaussian-Lorentzian profile. Obtained peak intensities were subjected to the partial least square (PLS) regression resulting in a linear dependence with coefficient of determination 0,9902, i.e. the calibration curve of methanol serving as prediction model for methanol, as depicted in Fig. 10.

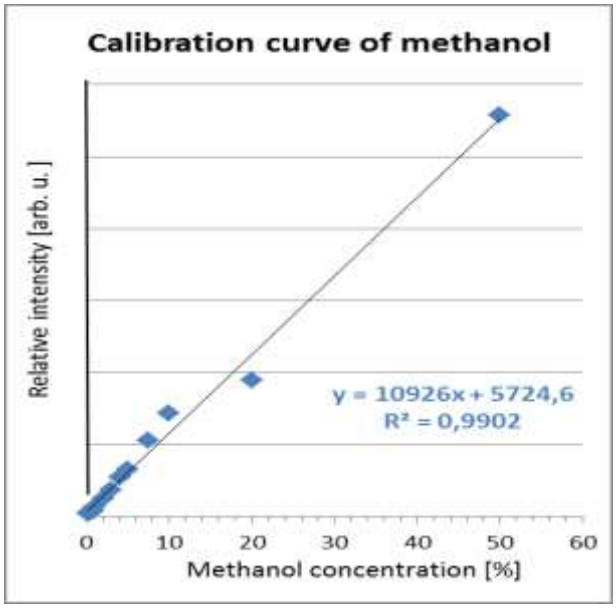

Fig. 10 Calibration curve of methanol

The detection limit for the method was 0,25% of methanol in ethanol corresponding to 3,96 grams of methanol per 1 litre of pure ethanol. Lower concentrations were not detected on Raman spectra.

The process of methanol determination was verified on ten additional mixtures with known concentrations, the standard deviation was at average 0.1%. In following figures Fig. 11 and Fig. 12 are shown spectra of three of these values and of ethanol.

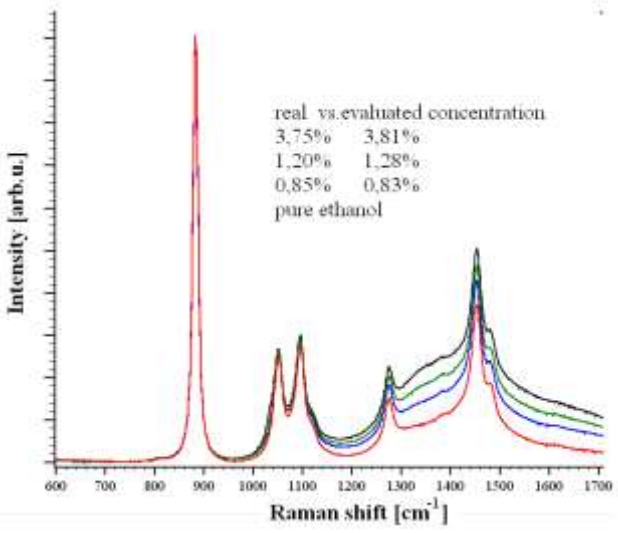

Fig. 11 Some of additional samples

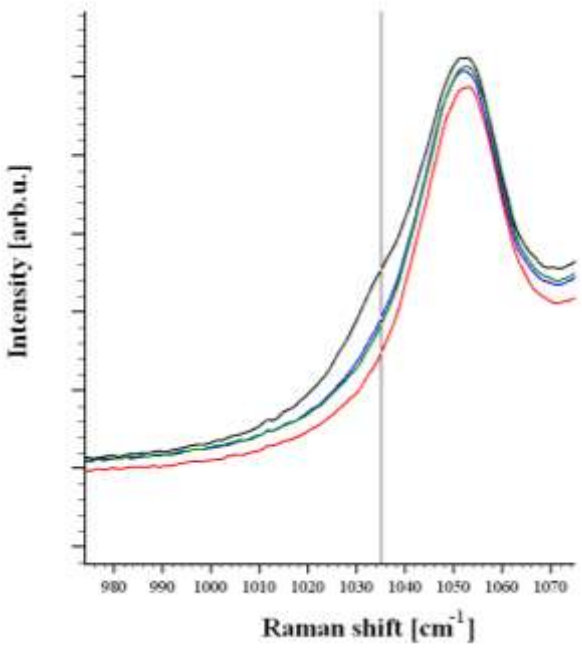

Fig. 12 Additional samples – focus on 1035 cm^{-1} peak

A. Alcoholic drinks

In distilled beverages such as vodka, rum, whiskey, gin, brandy etc. ethanol is produced by means of fermenting fruit or grain. These drinks containing alcohol – alcoholic beverages are mixtures mainly of water and ethanol and also variety substances affecting taste, aroma or colour. The chemical composition can be complex.

The process of measuring the methanol content by Rama spectroscopy in alcoholic beverages is based on the obtained data and model dependences. Sample of alcoholic beverage is analyzed due Raman microscope, a set of procedures is applied (as baseline correction, normalization, etc.) to acquire methanol peak intensity from the resulting spectrum by fitting. Experimental data are evaluated according the calibration curve for methanol.

The method was meanwhile applied on alcoholic beverages mainly from most trusted sources or distilled beverages, detrimental one not among them has not yet been revealed. In Fig. 13 Raman spectra of whiskey, gin, plum brandy and apricot brandy and pure ethanol are displayed. Ethanol is prevalent in all these spectra, other compounds are represented in small extend and do not take effect in spectrum. Different case is a cherry brandy, as shown in Fig. 14.

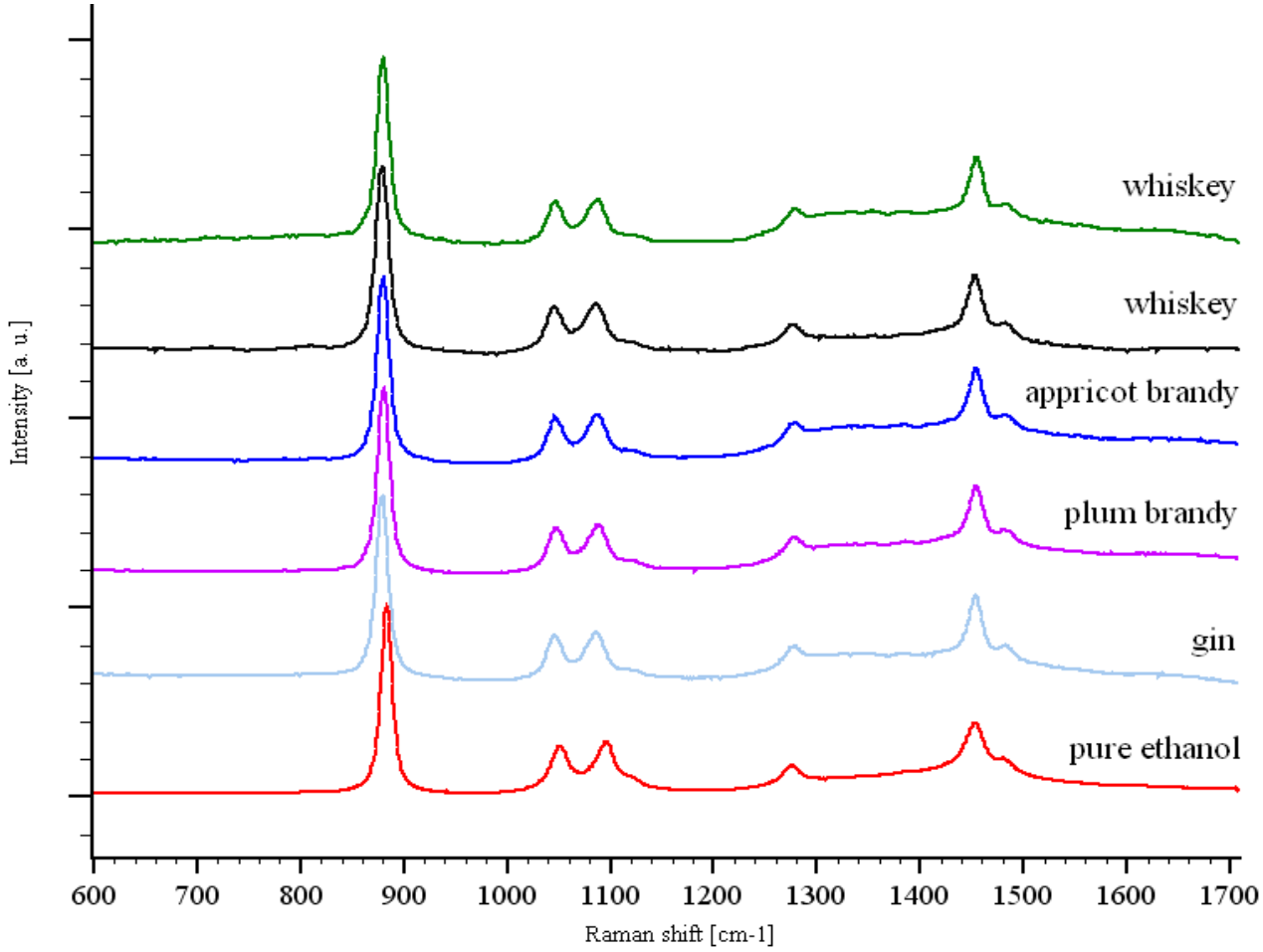

Fig. 13 Raman spectra of alcoholic drinks

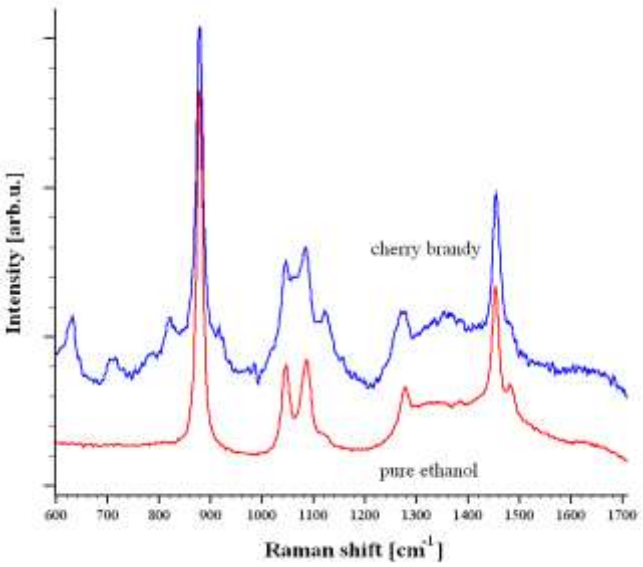

Fig. 14 Raman spectrum of cherry brandy and pure ethanol

VI. Conclusion

Raman spectroscopy was performed as an innovative method for measuring methanol content in alcoholic beverages. The method was developed in connection with the recent methanol affair in the Czech Republic in autumn 2012. Benefits of the method utilizing Raman spectroscopy are rapidity of the measurement, noncontact and non-destructive analysis that can be performed through the glass bottles since the glass does not affect the Raman spectrum of beverage. With knowledge of the prediction model of methanol and implementation of all the mathematical procedures with the measured spectra there can be quite accurately determine whether the alcoholic beverage is defective and health-threatening or not.

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