CHARLES UNIVERSITY

Faculty of Science

Study programme: Analytical chemistry

Anton Korban, M.Sc.

Development and metrological evaluation of gas chromatographic methods for quality and safety control of alcoholic products

Vývoj a metrologické hodnocení metod plynové chromatografie pro kontrolu kvality a bezpečnosti alkoholických výrobků

Doctoral thesis

Supervisor: Assoc. Prof. Radomír Čabala, Ph.D.

Prague, 2022

Prohlašuji, že jsem tuto závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

I hereby declare that I have written this dissertation independently, using only the mentioned and duly cited sources and literature, and that the work has not been used in another university study programme or to obtain the same or another academic title.

In Prague on _____   Anton Korban, M.Sc.

Abstract

Dissertation is dealing with the development of methods of quality control of alcoholic products, particularly, with quantification of volatile congeners. Previously suggested "Ethanol as internal standard (IS)" method was compared with existing analytical methods of internal and external standardisation. After satisfactory results the method was validated in one laboratory and then an inter-laboratory study which included nine laboratories from four countries was conducted. It was shown that the method usage on GC-FID is beneficial due to the absence of necessity of internal standard compound addition into tested sample, and absence of tedious sample alcohol by volume (ABV) or density measurements.

The algorithm of the method usage on GC-MS instruments was also suggested and developed. It was found out that MS detector sensitivity should be reduced in order to register ethanol signal successfully. For this aim it was suggested to employ less abundant 47 m/z ions during ethanol elution. These ions correspond to non-fragmented ethanol molecules containing one heavy isotope, mainly ^{13}C. According to the obtained results the suggested methodology allowed employing the "Ethanol as IS" method on GC-MS instruments with metrological properties better or similar as for the traditional IS method. The method was also tested by analysing 36 real alcoholic samples.

The variation of IS method calibration factors was also studied. Calibration factors were obtained for both suggested and traditional IS methods under different instrument conditions and solution properties. Possible reasons of non-linearity of GC-MS calibration factors were discussed.

Abstrakt

Disertační práce se zabývá vývojem metod kontroly kvality alkoholických výrobků, zejména kvantifikací těkavých kongenerů. Dříve navržená metoda „Ethanol jako interní standard (IS)" byla porovnána s existujícími analytickými metodami interní a externí standardizace. Po uspokojivých výsledcích byla metoda validována v jedné laboratoři a následně byla provedena mezilaboratorní studie, která zahrnovala devět laboratoří ze čtyř zemí. Ukázalo se, že použití metody na GC-FID je výhodné z důvodu absence nutnosti přidávání vnitřního standardu do testovaného vzorku a absence zdlouhavého měření objemu vzorku alkoholu (ABV) nebo hustoty.

Byl také navržen a vyvinut postup použití metody na přístrojích GC-MS. Bylo zjištěno, že pro úspěšnou registraci etanolového signálu by měla být snížena citlivost MS detektoru. Za tímto účelem bylo navrženo použít méně zastoupené ionty 47 m/z během eluce ethanolu. Tyto ionty odpovídají nefragmentovaným molekulám ethanolu obsahujícím jeden těžký izotop, hlavně ^{13}C. Podle získaných výsledků navržená metodika umožnila použití metody „Ethanol as IS" na GC-MS přístrojích s metrologickými vlastnostmi lepšími nebo podobnými jako u tradiční metody IS. Metoda byla také testována na analýze 36 realných vzorků alkoholu.

Byla rovněž studována variace kalibračních faktorů kdy tyto byly získány pro navrhované i tradiční metody IS za různých instrumentálních podmínek a vlastností roztoků. Byly diskutovány možné příčiny nelinearity kalibračních faktorů u GC-MS.

Keywords

Alcoholic products; calibration factors; congeners; gas chromatography; internal standardisation; validation.

Klíčová slova

Alkoholické produkty; kalibrační faktory; plynová chromatografie; těkavé látky; vnitřní standardizace; validace.

Abbreviations

AA – absolute alcohol

ABV – alcohol by volume

AOAC – Association of Official Agricultural Chemists

ES – external standard

FID – flame ionisation detector

GC – gas chromatography

IR – infrared

IS – internal standard

LOQ – limit of quantification

MS – mass spectrometry

RF – response factor

RJ – Rudolf Jelínek

RRF – relative response factor

RSD – relative standard deviation

SIM – single ion monitoring

Contents

Introduction

Analytical chemistry with its qualitative and quantitative analysis plays important role in the quality control of production used in various scientific, manufacturing, and medical spheres. This work is dealing with a certain field of a quality and safety control of alcoholic products.

Millions of litres of alcoholic products are produced and consumed all over the world every year. Every alcoholic product, i.e., either ready to use alcoholic drink or intermediate distillation product, or pure ethyl alcohol contains various amounts of different volatile compounds or congeners. These are mainly simple alcohols, esters, and aldehydes. Volatile compounds content is strictly controlled by corresponding international laws and standards because besides influencing organoleptic properties, some volatile impurities pose a threat to human health owing to their toxicity (e.g., acetaldehyde and methanol) [1-8]. Therefore, quantification of volatile compounds is an important goal for every alcoholic drinks producer.

Today's worldwide practice of volatile compounds quantification consists of gas chromatographic (GC) analysis which employs methods of internal or external standardisations. The existing and prescribed GC methods have certain limitations that may challenge their adequate routine usage. To overcome these limitations and to improve the whole analysis of alcoholic products quality control the "Ethanol as Internal Standard" method was suggested [9]. The method was created to make the whole analysis process faster, cheaper, and more accurate. The hypothesis was that the employment of a major volatile compound or a solvent may be successfully used for the quality control analysis, particularly in case of alcoholic products.

Till now GC coupled to flame ionization detector (FID) acts as the most spread instrumentation for the selected analysis due to the fact of being relatively cheap and easy to use, accurate and repeatable to a high extent [10]. However, despite of having lots of advantages qualitative data about the examined sample is hard to obtain when FID is used. More or less accurate identification of GC-FID signals is possible when comparing their retention times with those of purchased standards. Oppositely to GC-FID, GC coupled to mass spectrometer (MS) opened great perspectives for complex mixtures analysis [11-13]. GC-MS spectral data allow confident identification of individual components which may be very useful for the analysis of elite distillates where the profile of volatile congeners is a key parameter of odour and taste [14]. Besides the fact that only GC-FID is currently mentioned

in legislation GC-MS is actively used by scientists and distilleries worldwide. Moreover, when employed in the selected ion monitoring (SIM) mode, GC-MS analysis is characterized with much lower detection and quantification limits compared with GC-FID. The abovementioned points let us to understanding why GC-MS instrumentation is useful for the analysis of alcoholic products. Consequently, a raised question was whether the suggested "Ethanol as Internal Standard" method may be used on GC-MS instruments, too. However, while being a more sensitive instrumentation GC-MS often becomes limited when analysing compounds in high concentrations that lead MS detector to saturation, to impossibility of peak integration and its subsequent quantification [15]. As the suggested method requires major compound peak integration, procedure of ethanol registration should have been developed and studied.

To sum up, this doctoral thesis deals with the development of gas chromatographic methods of quality control of alcoholic products. More specific goals were as following:

1. To conduct single and interlaboratory test of the suggested "Ethanol as Internal Standard" method on GC-FID instruments and reveal its properties;
2. To find a way of ethanol registration on GC-MS for further application of the abovementioned method on this instrumentation;
3. To conduct studies of calibration factors stability while changing volatile compounds concentrations and matrix composition.

The achieved results which are presented in this dissertation are easily applicable for their direct usage in the routine practice of distilleries and testing laboratories.

1. Literature review

1.1 Traditional methods of alcoholic products quality control

Quality control of an alcoholic product includes various tests: determination of alcoholic strength by volume, determination of total dry extract, determination of volatile substances and others [1]. However, two control tests may be set as main ones: determination of strength and volatile compounds. These parameters drastically influence the product's quality.

Determination of alcoholic strength or ethanol by volume content may be performed in different ways. Thus, there are lots of methods and measuring instruments based on the solution physical or optical density [1], based on IR [16, 17] or other optical spectra [18-20]. GC methods of ethanol quantification are employed as well [21-24].

When speaking about volatile congeners, GC methods step forward being the most universal, fast and beneficial. GC-FID allows adequate separation and subsequent determination of compounds; the sensitivity of this analysis is high as well as the dynamic linearity range that allows quantification of volatile compounds at various concentrations. GC-FID determination of volatile compounds in alcoholic products is described in the European legislation 2870/2000 [1], American AOAC standards [4-5] and in legislation of other countries [2, 3, 6, 7]. Besides this, GC-MS analysis is highly spread among scientists and researchers [11-15].

Quantification in GC can be achieved by usage of various methods. They are: external standard, internal standard, method of standard addition, normalization etc. As for the determination of volatile congeners in alcoholic products, their quantification is traditionally performed with the internal standard (IS) method, rarely with external standard (ES) method.

External standard method is well known to all analytical chemists while being the basic calibration approach. The calibration consists in measuring series of prepared or purchased standards of analysed compound in different concentrations. Afterwards, the calibration graph describing the dependence of detector response on component amount may be plotted. The regression is usually created with the corresponding software by the usage of the least squares method. The slope of the obtained curve represents the response factor (RF) of the studied i-th compound that may be also calculated as:

$$RF_i = \frac{\sum_{l=1}^{M} \sum_{j=1}^{N} \frac{C_{ijl}^{st}}{A_{ijl}^{st}}}{N \cdot M}, \qquad (1^{*})$$

where C_{ijl}^{st} is the concentration of the i-th analysed compound whilst the j-th parallel measurement of the l-th concentration level; A_{ijl}^{st} is the detector response for the i-th analysed compound whilst the j-th parallel measurement of the l-th concentration level. In the equation (1) it is supposed that number of parallel repeatable measurements N for each of M concentration levels is the same.

Concentration of the analysed i-th compound in the tested sample may be then calculated as:

$$C_i = RF_i \cdot \frac{\sum_{j=1}^{N} A_{ij}}{N} \qquad (2)$$

where A_{ij} is the detector response for the i-th compound whilst j-th repeatable measurement of the tested sample.

It should be noted here, that ES method may be employed only under the same instrumental and experimental conditions. Temporal fluctuations of GC system parameters such as temperature, pressure or split ratio will certainly influence the RF value and will raise a need in new calibration. Also, ES method can be burdened with an additional error due to the variations in injected sample volume caused by bubbles or drops inside the injecting microsyringe.

The abovementioned limitations of the ES method are solved by the IS method. This method employs the manual addition of the internal standard compound which is originally absent in the tested sample. Internal standard compound is added both to the calibration and tested samples, ideally in equal amounts. As for the alcoholic products quality control 1- or 3-pentanol is traditionally used as IS compounds, though employment of various different compounds can be met in literature.

Instrument calibration according to the IS method is done by calculating of relative response factors (RRF). These factors represent the ratio of response factors for i-th analysed compound and internal standard and may be calculated according to the following formula:

$$RRF_i^{IS} = \frac{RF_i}{RF_{IS}} = \frac{C_i^{st}}{A_i^{st}} \cdot \frac{A_{IS}^{st}}{C_{IS}^{st}}, \qquad (3)$$

---

where C_i^{st} and C_{IS}^{st} are concentrations of the *i*-th analysed compound and internal standard, correspondingly; A_i^{st} and A_{IS}^{st} are the detector responses for the *i*-th analysed compound and internal standard, correspondingly.

In contrast to ES method in case of the analysis of alcoholic products, IS method could employ a so-called single point calibration. Single- or one- point calibration method consists in measuring only one calibration solution to determine calibration factors. However, the linearity of the employed detector should then be checked in order to state that RRF factor will be the same for different concentrations of analyte.

While being based on the ratio of RF values RRF factors are supposed not to be affected by temporal fluctuations of experimental parameters, either by bubbles in the syringe or changes of the injection volume or split ratio, because amounts of analyte and internal standard are increased or decreased by the same value.

Quantitative analysis of the tested sample is then performed according to the following equation:

$$C_i = RRF_i^{IS} \cdot \frac{A_i}{A_{IS}} \cdot C_{IS} \ , \tag{4}$$

where C_{IS} is the concentration of IS compound in the tested sample.

1.2 Existing problems

As for today existing methods of quality control of alcoholic products have certain limitations and disadvantages. If to speak about the ES method, its main disadvantage lies in the fact of a "fragile" calibration that is sensitive even to minor system changes. In addition, the calibration process is relatively complicated due to the necessity of preparation/purchasing of series of standards which requires financial, labour and time resources.

While employing more stable RRF values IS method main limitation consists in the necessity of IS compound manual addition into every tested sample. Besides complications connected with the preparation of standard solutions, manual addition procedure brings additional error and uncertainty in the resulting value.

Also it should be mentioned, that according to the legislation of many countries, volatile compounds concentration in alcoholic products must be finally expressed in mg/L of absolute alcohol (AA) or analogous units (g/L AA, g/hL AA etc.). These units represent congener

content recalculated hypothetically to pure 100% ethyl alcohol. When using ES method, concentration obtained by the equation (2) has usually mg/L units. To convert these units to the required ones the following equation is used:

$$c_i\left(\frac{mg}{L}\,\text{AA}\right) = \frac{c_i\left(\frac{mg}{L}\right)\cdot 100\%}{\text{ABV}(\%)}, \qquad (5)$$

where ABV is alcohol by volume value or strength.

European regulation declares usage of mg/kg units during calibration, thus subsequent conversion to mg/L AA units is done as follows:

$$c_i\left(\frac{mg}{L}\,\text{AA}\right) = \frac{c_i\left(\frac{mg}{kg}\right)\cdot \rho_{test}\cdot 100\%}{\text{ABV}(\%)}, \qquad (6)$$

where ρ_{test} is density of the measured sample, mg/L.

As it can be seen from equations (5, 6) to present concentrations in the required mg/L AA units, one should undertake measurement of ABV of the sample and sample density. These measurements require additional labour and time resources and act as an additional source of error as well.

1.3 "Ethanol as Internal Standard" method

To overcome the existing limitations while quality control of alcoholic products the "Ethanol as Internal Standard" method was suggested. The idea of the method was first mentioned in 2003 [9], though consistent investigations were carried out after 2017.

In its classical interpretation IS compound must be originally absent in the tested sample and its added amounts should be close to those of analytes. The "Ethanol as IS" method doesn't follow these basic rules and employs ethanol which is the main volatile organic compound of any alcoholic product as an IS compound. Ethanol content is usually 100-1000 times higher than that of any analysed compound. For example, GC-FID chromatogram of an ordinary whiskey is presented in Figure 1 in a semi-logarithmic scale (the response is in logarithmic scale, the time scale is linear).
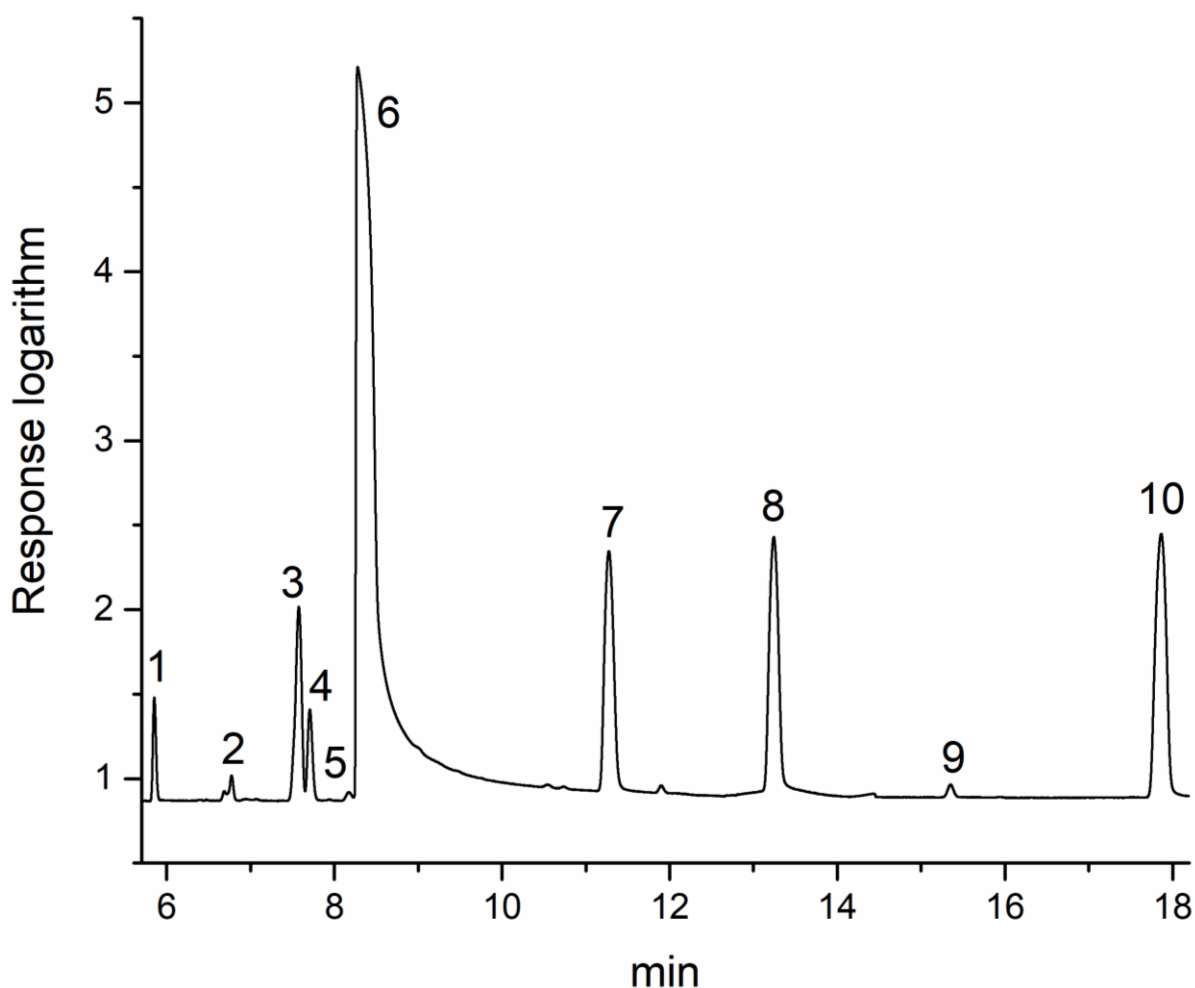
Figure 1. GC-FID chromatogram of a whiskey sample in a semi-logarithmic scale.
1 – acetaldehyde; 2 – methyl acetate; 3 – ethyl acetate; 4 – methanol; 5 – 2-propanol;
6 – ethanol; 7 – 1-propanol; 8 – isobutanol; 9 – 1-butanol; 10 – isoamylol.

In full analogy with the traditional IS method, calibration according to the suggested method is done by calculation RRF factors for i-th volatile congener to ethanol – the selected IS compound:

$$RRF_i^{Eth} = \frac{RF_i}{RF_{Eth}} = \frac{C_i^{st}}{A_i^{st}} \cdot \frac{A_{Eth}^{st}}{C_{Eth}^{st}}. \tag{7}$$

The key moment here is that volatile compounds and ethanol concentrations are presented directly in required mg/L AA units. For instance, it's very easy to recalculate concentrations from mg/L or mg/kg units to mg/L AA, because, as a rule, ethanol content in the calibration matrix is well-known. Extra attention should be paid here to ethanol – the selected internal standard compound. Trying to convert ethanol concentration to mg/L AA units one will

always get the same value of ethanol density which is 789300 mg/L. And this will work for 1% ethanol-water solution as well as for 40% or 96%, or any other ethanol-containing solution. Ethanol concentration in mg/L AA (or similar units like g/L AA, g/hL AA etc.) is equal to ethanol density which is known. Thus, the Equation (7) may be rewritten as:

$$RRF_i^{Eth} = \frac{RF_i}{RF_{Eth}} = \frac{C_i^{st}}{A_i^{st}} \cdot \frac{A_{Eth}^{st}}{789300} \ . \tag{7.1}$$

When standard solution is measured N times under the repeatability conditions, final RRF value is determined according to the following equation:

$$RRF_i^{Eth} = \frac{C_i^{st}}{789300} \cdot \frac{\sum_{j=1}^{N} \frac{A_{ij}^{st}}{A_{Eth,j}^{st}} \cdot}{\sum_{j=1}^{N} \left(\frac{A_{ij}^{st}}{A_{Eth,j}^{st}}\right)^2} \ , \tag{7.2}$$

where A_{ij}^{st} and $A_{Eth,j}^{st}$ are the j-th value of peak area of the i-th volatile compound and ethanol, correspondingly.

Quantification in the tested sample is then done analogously to the Equation (4):

$$C_i = RRF_i^{Eth} \cdot \frac{A_i}{A_{Eth}} \cdot 789300 \ . \tag{8}$$

Equation (8) states that in order to calculate volatile congener's concentration in any ethanol-containing product in the required mg/L AA units one should use pre-determined calibration factors and peak areas of the congener and ethanol obtained during GC measurement. Consequently, there is no need to:

- prepare IS solution, calculate it's concentration and add it to the tested sample;
- measure ABV of the sample;
- measure density of the sample.

The whole analysis thus is changed towards the shortest possible. No sample pre-treatment needed – tested sample is measured directly by GC instrument (if no containing sugars or other non-volatile compounds that should not be injected into the GC system). The advantages of the suggested method are obvious. Elimination of steps and procedures makes the whole analysis faster, cheaper and more robust.

The method has no trick or fraud. Its usage is possible due to the fact all volatile components concentrations must be presented in mg/L units recalculated to 100% ethanol. Under these

14

conditions calculating RRF values of i-th compound to ethanol by using required mg/L AA units allows direct GC determination of i-th compound. By the words "direct GC determination" the procedure of sample GC measurement without any pre-treatment is meant.

It should be noted here that mg/L AA units give user no information about real content of volatile congeners and ethanol. Oppositely mg/L or mg/kg units, mg/L AA units remain the same when the sample is diluted, for instance, with water, because both congeners and ethanol concentrations are decreased in the same manner. However, ABV determination is the second required test and all modern distilleries and testing laboratories establish it in a very short period of time with a specific instrumentation. With the knowledge of concentration in mg/L AA units and ABV of the tested sample one can easily calculate concentration in mg/L units, if necessary (see Eq. (5)).

2. "Ethanol as IS" method application on GC-FID

2.1 Comparison with other methods (Supplement 1)

As mentioned in chapter 1.1, ES and IS belongs to traditionally used analytical methods of quality control of alcoholic products. Supplement 1 presents a work dealing with the comparison of metrological properties of the "Ethanol as IS" method with the traditional IS and ES methods.

A set of 6 standard solutions of 9 volatile compounds in concentrations ranging from 1 to 6000 mg/L AA was prepared gravimetrically and analysed with these three analytical methods with GC-FID in three laboratories in Belarus and Czech Republic.

The obtained results showed that the suggested "Ethanol as IS" and traditional IS methods have similar inter-laboratory precision expressed as a repeatability limit. For both methods repeatability limits were not exceeding 10%; individual values for certain congeners were extremely close for both methods. ES method showed much worse repeatability limits of around 30%. The analogous results were obtained for biases, uncertainties and limits of quantification. Two internal standard methods showed similar metrological properties that were twice or even more times better than that of the external standard method.

The results of the work allowed to state that metrological properties of the "Ethanol as IS" method are similar to that of the traditional IS method. ES showed much worse results thus indicating its routine usage as pointless.

2.2 Single laboratory validation (Supplement 2)

In accordance with the requirements of ISO/IEC 17025 the method must be validated before its application in a routine laboratory practice. The suggested method was not an exception.

The traditional IS method, which is described in commission regulation EC 2870/2000 [1], was validated according to the inter-laboratory experiment with corresponding results being published. Supplement 2 presents the results of a single-laboratory validation of the suggested method. Validation included manual preparation of a set of seven standard solutions of volatile compounds in full compliance with the legislation [1]. Each sample was measured 30 times under repeatability conditions. The following metrological properties were evaluated:

- FID linearity to each of nine analysed volatile congeners characterised with coefficients of determination R^2;

- limits of quantification expressed in mg/L AA;

- relative biases expressed in %;

- repeatability limits expressed in %;

- reproducibility (within-laboratory precision) limits expressed in %.

The obtained results were as following. Flame ionization detector response was linearly correlated with assigned concentrations at a range of 2 to 5000 mg/L AA with coefficients of determination R^2 higher than 0.995 for all analysed components. Repeatability (RSD$_r$ ≤ 4.5%; RSD$_r$ ≤ 2.0%), reproducibility (RSD$_R$ ≤ 5.0%; RSD$_R$ ≤ 2.0%), and trueness (relative bias ≤ 2.6%; relative bias ≤ 1.4%) were obtained for low (10–25 mg/L AA for methanol and 2-10 mg/L AA for other volatiles) and high (25–5000 mg/L AA for methanol and 10–5000 mg/L AA for other volatiles) ranges of concentrations, correspondingly. According to the obtained validation results the method showed satisfactory metrological properties.

2.3 Interlaboratory validation (Supplement 3)

Supplement 3 presents further investigation that included an interlaboratory study of the "Ethanol as IS" method. Nine laboratories from four countries (Belarus, Czech Republic, Russia and Turkey) were supplied with standard solutions containing nine analysed congeners in different concentrations. 3-pentanol was also added to standard solutions as a traditional IS compound to compare two methods. The interlaboratory study was then conducted and evaluated according to the ISO 5725 standards. The studied parameters were:

- within-laboratory precision expressed in %;

- between-laboratory precision expressed in %;

- reproducibility expressed in %;

- biases expressed in %;

- limits of quantification expressed in mg/L AA;

- linearity characterised with coefficients of determination R^2;

- uncertainty expressed in %.

The within-laboratory precision varied between 0.4% and 7.5% for all samples and compounds, showing a sufficiently high repeatability of the suggested method. The between-laboratory precision was found to vary within a satisfactory range of 0.5-10.0%. Precision of the method

was well within the range predicted by the Horwitz equation for all analytes. The analysis of trueness showed that the bias of the method is insignificant at the significance level α = 5%. The determined concentrations of the analytes compared well to the gravimetrical values thus showing very satisfactory accuracy of the method. The average expanded uncertainty *U* (P=0.95, k=2) was 7.3% not exceeding 9.5%. Other parameters, such as linearity and limits of quantification correlated well with a single laboratory validation (chapter 2.2).

The results of the interlaboratory study confirmed that "Ethanol as Internal Standard" method is accurate and precise, and is suggested to be used as a standard reference method for the analysis of volatile compounds in alcoholic products.

2.4 Practical implementation in the routine practice of the distillery

"Rudolf Jelinek" (RJ) is one of the most famous and popular spirit producers in Czech Republic. It is famous all over the world mainly for plum and pear distillates. Mr. Vladimir Darebnik who is responsible for the quality control analysis at the distillery was interested in the practical implementation of the suggested method.

The "Ethanol as IS" method was validated in the RJ laboratory in full analogue with the approach described in chapter 2.2. A set of 6 standard solutions with 10 volatile congeners' concentrations from 80 to 5000 mg/L AA was prepared. One of these solutions was used for the calibration as stated in the EU regulation. The rest was used to establish linearity and accuracy of the method.

The repeatability of the method was evaluated by calculating relative standard deviation (RSD) values. The average RSD among three repeatable measurements of the same solution was found to be 1.3% with maximum of 3.5%. This indicated a good repeatability of the method.

The linearity of the detector response was found to be outstandingly excellent at the whole studied range of volatile congeners' concentrations as the approximation coefficients R^2 were not lower than 0.99986.

The trueness of the method was evaluated by calculating the biases while measuring the rest of prepared standard solutions. The average bias was found to be 0.7% with the maximum of 1.4%. These results also can be estimated as excellent. In addition, a set of distillate samples produced by RJ was measured with the suggested method. The obtained concentrations were

compared with previously obtained results according to the traditional IS method and according to the results presented by accredited testing laboratory.

The fact of the absence of any additional manual operations when using the suggested method was rated highly. This is very useful in the manufacturing environments when time and labour demanding analytical methods are not applicable and welcomed. Taking into account successful validation and technical advantages of the method it was successfully introduced into the routine practice. As for today "Ethanol as IS" method is employed at the RJ distillery for quality control of output products. A grateful letter RJ to Prof. Zima, the dean of the Faculty of Science of Charles University can be found in Supplementary 7.

2.5 Conclusions to the Chapter 2

"Ethanol as IS" method was suggested as a novel and non-traditional approach of volatile compounds determination in alcoholic products by GC-FID. The method was compared with existing traditional external and internal standards methods, and the analysis of obtained data demonstrated that metrological properties of the suggested method are similar or better. Furthermore, the method was validated according to the required procedures in one and then in nine laboratories by the interlaboratory experiment. The results of this experiment repeatedly proved the method's satisfactory metrological properties. At the same time, from the practical point of view, there is no necessity in internal standard compound addition, measurement of the sample ABV or density. The absence of these procedures makes the analysis simpler, faster and decreases the number of factors influencing the uncertainty of final results.

Considering the abovementioned facts, the "Ethanol as IS" method is recommended for the routine usage in distilleries and testing laboratories.

3. "Ethanol as IS" method application on GC-MS

3.1 Aims and challenges

GC coupled to mass spectrometry (MS) detector is an extremely powerful instrument for the analysis of complex mixtures. Oppositely to GC-FID mass spectral data from GC-MS allows reliable qualitative analysis of the measured sample. Some compounds that cannot be analysed by GC-FID such as water, ammonia, hydrogen sulphide may be easily determined by GC-MS. Also GC-MS is often capable of recognising merged peaks with similar retention times. Moreover, when employed in single ion monitoring (SIM) mode GC-MS instrument is characterised with much better sensitivity if to compare with GC-FID. Higher sensitivity may be very useful for the analysis of elite alcoholic drinks, where various volatile congeners are presented in small concentrations. Logically, a question was raised about the opportunity of the "Ethanol as IS" method usage on GC-MS.

A problem encountered was the impossibility of ethanol registration in GC-MS scan mode. While ethanol elution time the detector was saturated with ions to a high extent and this led to the impossibility of its registration. It is clear that the suggested method couldn't be used under these conditions because knowledge of ethanol peak area is essential.

To solve this problem two possible solutions were suggested. The first one is based on reduction of the detector sensitivity by voltage of MS electron multiplier during the ethanol elution time window. The second one consists in the selection of a less abundant quantifier ethanol ion, e.g., m/z 47, which corresponds to the unfragmented ethanol molecule containing one of the heavy isotopes of carbon, oxygen or hydrogen.

3.2 First successful application (Supplement 4)

Supplement 4 represents the work dedicated to the first attempt of the "Ethanol as IS" method usage on GC-MS instrument. In the presented work the suggested method application on GC-MS was compared with its usage on GC-FID. GCMS-QP2010 from Shimadzu (employed instrument) was capable of simultaneous measurements on FID and MS detectors. For this aim, Y-shape splitter was used to divide column effluent to two detectors.

As in case of previous works a set of standard solutions of volatile compounds in water-ethanol matrix was prepared gravimetrically. Calibration of the instrument was done in full accordance with previous works and analogously to the European legislation [1].

GC-MS measurements were performed in SIM mode thus allowing evaluation of sensitivity of the instrument. During ethanol elution time window only 47 m/z ions were registered. This allowed ethanol registration without MS detector saturation. The second suggested way of ethanol peak determination on GC-MS instruments consisted in electron multiplier voltage decreasing from 0.96 to 0.6 kV in the corresponding time window. However, this procedure is more complicated from practical point of view. In spite of the possibility of ethanol registration, this approach revealed non-linear signal dependence on ethanol concentration.

RRF factors obtained for the "Ethanol as IS" method differed greatly for two used detectors. Thus, RRF values obtained for FID ranged from 0.8 to 2.4 which is common. Oppositely, RRF values obtained for the MS detector ranged from 0.006 to 0.02. This phenomenon is caused by the fact detector response to ethanol was artificially decreased (see Eq. (7)).

Eventually, the approach employed in the work demonstrated the usage of two very unusual solutions for analytical chemistry. First one was the usage of a main component as an internal standard compound. The second one was the intended decrease of detector sensitivity. Despite of these uncommon steps, the experimental results showed that repeatability and reproducibility of the method employed at MS detector is the same as for FID. Moreover, as expected, LOQ values for MS detector were lower than for FID.

Two whiskey samples were measured in order to check out if the results obtained with different detectors would differ significantly. To evaluate, if the difference between the results was significant, Student's t-Test of independent samples was used. It was found that there was no statistical evidence that associated populations means, obtained by two methods, differ significantly for all compounds ($P = 5\%$).

The results presented in the paper suggest further favourable perspectives for the method's usage in GC-MS analysis of alcoholic products. Further step was to compare the suggested and traditional IS methods.

3.3 Comparison with the traditional IS method (Supplement 5)

In the work presented in Supplement 5 two IS methods were employed simultaneously for the analysis of 36 real samples of alcoholic products. 33 samples were purchased from commercially available sources. The list of types of purchased and analysed spirits included bourbon, calvados, cognac, gin, grappa, liqueur, metaxa, port wine, rum, sake, tequila, vodka, whiskey, and various fruit distillates. Three homemade fruit distillates were produced by

fermentation of pulpy fruits, or their musts were obtained from local spirit makers. The concentrations of volatile compounds in analysed samples varied from 1 to 13500 mg/L AA, the declared ABV value of analysed samples varied from 15 to 81%. All samples were spiked with 1-pentanol (traditional IS compound) and measured directly by GC-MS. The chromatograms were then processed with two methods and obtained results were compared.

Repeatability was evaluated by RSD calculation. According to the obtained results both methods had similar RSD values with an average value of 2.5% for congeners concentrations less than 50 mg/L AA and of 2.0% for higher concentrations.

To compare trueness of the methods recoveries were evaluated. For this one alcoholic sample was spiked with standard solutions containing all analysed volatile compounds at concentrations of 50, 500 and 5000 mg/L AA. Selected spirit (cherry distillate) initially contained all 11 studied analysed volatile compounds in various concentrations. The original unspiked sample was used as a reference. According to the obtained results recovery of the suggested "Ethanol as IS" method (98.1 ± 3.3%) was slightly better than that of the traditional one (98.0 ± 5.8%).

The results of the experiment thus prove that developed "Ethanol as IS" method is true, precise and reliable when employed on GC–MS instruments.

3.4 Investigation of calibration factors stability (Supplement 6)

Calibration of equipment is a very time consuming and expensive process and, therefore, needs to be as simple as possible. An analyst must be able to discover conditions under which the relative response factors remain constant and repeatable, and to what extent they can minimize fluctuations and drift to optimize the experimental parameters. The action of RRFs on flame-ionization detectors (FID) has already been studied. That's why further research was directed towards study of Relative Response Factors variation when suggested and traditional IS methods are employed.

The experiment was done in the following way. Twelve standard solutions were prepared in 1 of 3 possible matrices (20, 40, and 96 % ABV) with volatile compounds in 1 of 4 concentrations (250, 500, 1000, and 5000 mg/L AA). Ten analysed volatile compounds were selected among compounds that must be determined according to the EU regulation [1]. RRF values were calculated for both methods according to the traditionally employed equations (3) and (7). Each standard solution was measured 4 times under repeatability conditions.

RRF variation was then estimated by RSDs at different levels. Firstly, repeatability within the same solution was evaluated, afterwards – within the same matrix. Eventual RSD represented RRF variation within all three matrices in four different concentrations each.

According to the obtained results, within-sample repeatability of RRF values was 0.9 ±0.3 % and 0.6 ±0.2 % for the "Ethanol as IS" and traditional methods, correspondingly. The within-matrix repeatability is presented in Table 1 for two concentration ranges.

Table 1. The obtained within-matrix repeatability of RRF of two IS methods.

Ethanol content in matrix	IS ethanol	IS 1-pentanol	IS ethanol	IS 1-pentanol
	Concentration range			
	250 – 1000 mg/L AA		250 – 5000 mg/L AA	
20 %	6.9 ±0.7	5.7 ±0.5	10.1 ±1.3	10.2 ±1.7
40 %	1.3 ±0.4	1.9 ±0.6	3.5 ±1.2	3.2 ±1.2
96 %	0.9 ±0.4	0.9 ±0.4	10.1 ±0.6	0.9 ±0.5

RRF variation within all three matrices in four different concentrations was found to be 40% and 8% for the "Ethanol as IS" and traditional IS methods, correspondingly. Thus, according to the experimental results RRFs calculated for standard IS compound are significantly more stable than those for ethanol.

The reasons for higher instability of ethanol RRF values were also reviewed. It was suggested that injection and detection processes are probably responsible for the observed effect. To minimize the variability of RRFs during injection of alcoholic products it is suggested to use liners of bigger internal volumes, reduce injector temperature, and to inject smaller sample volumes. Samples with low ABV values contain high amounts of water and thus tend to produce a much larger vapour phase. For ethanol MS detection the influence of sample injection volume on RRF values was observed, especially a nonlinear relationship between the ethanol peak area and its quantity. This means that samples should not be injected in different volumes.

For optimizing the use of ethanol as an IS with GC-MS, additional studies should be conducted so that RRF behaviour can be better predicted. This would enable the development of a more reliable application model for this type of instrumentation.

3.5 Conclusions to the Chapter 3

Employment of the "Ethanol as IS" method on GC-MS is perspective due to the fact of powerful capabilities of these instruments while alcoholic products analysing. The problem encountered was the impossibility of ethanol registration while usage of traditional scan or SIM methods. It was suggested to use 47 m/z ions for ethanol registration. Firstly, the developed approach was compared with "Ethanol as IS" usage on GC-FID instruments. It was shown that metrological properties of the "Ethanol as IS" method on GC-MS are not worse when compared to GC-FID. Some parameters, as, for instance, limits of quantifications were even better when GC-MS was used.

The method was also compared with the traditional IS method by analysing a big set of 36 real alcoholic products both from commercial and household sources. The concentration values obtained with two methods had no significant difference. At the same time, precision and accuracy of the suggested method was slightly better, thus indicating capability of the "Ethanol as IS" method usage on GC-MS.

However, study related to the variation of calibration factors revealed that RRF values obtained for the "Ethanol as IS" method are quite more fragile and tend to change when the system parameters are changed. This fact brings up the necessity of further investigations in order to avoid possible errors caused by the method's usage.

4. Conclusions

The dissertation aims and results are reliable to the quality control of alcoholic products, i.e. any ethanol-containing sample that can be analysed by GC system coupled either to FID or MS detector. Determination of volatile congeners was the studied quality control test. To overcome the existing limitations of external and internal standard methods the "Ethanol as IS" method was suggested. It consists in using ethanol – the main volatile organic compound of any alcoholic product as an IS compound.

The suggested method was firstly studied at GC-FID systems. It was successfully validated in one laboratory; inter-laboratory validation was carried out as well. The method showed metrological properties close to those of the traditional IS method. At the same time the method requires no preparation and addition of IS compound solution into tested sample, measurement of the tested sample ABV value or its density. The absence of these procedures makes it attractive for everyday routine usage in distilleries and accredited testing analytical laboratories.

Due to the high popularity and abilities of GC-MS instruments, the usage of the suggested method on this instrument was tested and studied as well. It was shown that the usage of M+1 ion allows detection of major component of the sample without MS detector saturation. The suggested approach of the "Ethanol as IS" method usage on GC-MS led to the same or better metrological properties if to compare with the traditional IS method. This fact was proved by the measurement of a big set of 36 different real alcoholic product samples.

Variation of RRF values for the suggested and traditional IS methods was also studied. It was shown that the "Ethanol as IS" method is characterised with much bigger variation of calibration factors if compared with the traditional method. This variation is probably caused by negative effects occurring in injection and detection ports of GC-MS instrument. Additional studies should be carried out in order to reveal the best conditions of the method usage on GC-MS instrumentation.

The results of the research clearly indicate the necessity of implementation of developed "Ethanol as internal standard" method into the routine practice of alcoholic products quality control. While being unusual from the traditional analytical chemistry point of view this method is an extremely powerful tool for the analysis of alcoholic products.

References

1. European Commission. (2002). *Commission regulation (EC) No 2870/2000*. http://faolex.fao.org/docs/pdf/eur35725.pdf

2. *OIV. (1987). OIV-MA-BS-14: R2009. Determination of the principal volatile substances of spirit drinks of viti-vinicultural origin*. (1987).

3. *OIV. (2009). OIV-MA-BS-20: R2009. Propanol-2-ol Determination by gas chromatography*.

4. AOAC. (2005). *AOAC Official Method 972.10 Alcohols (Higher) and Ethyl Acetate in Distilled Liquors*.

5. AOAC. (2005). *AOAC Official Method 972.11 Methanol in Distilled Liquors. GC method.*

6. National Standards of People's Republic of China. (2016). *General Standard for Beverage: GBT 10789-2015*.

7. Food safety and standard authority of India ministry of health and family warfare. (2015). *Manual of Methods of Analysis of Foods: Lab Manual 13*.

8. Pharmacopoeia, E. (2013). *European Pharmacopoeia Vol 2, 7 ed.*

9. Charapitsa, S. (2003, May 9-14). Main component (solvent) as internal standard for chromatographic quantitative analysis of impurities. Pittcon International Conference, Orlando, USA.

10. Wiśniewska, P., Śliwińska, M., Dymerski, T., Wardencki, W., & Namieśnik, J. (2015). The Analysis of Vodka: A Review Paper. *Food Analytical Methods*, *8*(8), 2000–2010. https://doi.org/10.1007/s12161-015-0089-7

11. Špánik, I., Čirka, L., & Májek, P. (2015). Classification of wine distillates using multivariate statistical methods based on their direct GC-MS analysis. *Chemical Papers*, *69*(3), 395–401. https://doi.org/10.1515/chempap-2015-0043

12. Coldea, T. E., Socaciu, C., Moldovan, Z., & Mudura, E. (2014). Minor volatile compounds in traditional homemade fruit brandies from Transylvania-Romania, as determined by GC-MS analysis. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(2), 530–537. https://doi.org/10.1583/nbha4229607

13. Ledauphin, J., Milbeau, C. L. E., Barillier, D., & Hennequin, D. (2010). Differences in the volatile compositions of French labeled brandies (Armagnac, Calvados, Cognac, and Mirabelle) using GC-MS and PLS-DA. *Journal of Agricultural and Food Chemistry*, *58*(13), 7782–7793. https://doi.org/10.1021/jf9045667

14. Ferrari, G., Lablanquie, O., Cantagrel, R., Ledauphin, J., Payot, T., Fournier, N., & Guichard, E. (2004). Determination of Key Odorant Compounds in Freshly Distilled Cognac Using GC-O, GC-MS, and Sensory Evaluation. *Journal of Agricultural and Food Chemistry*, *52*(18), 5670–5676. https://doi.org/10.1021/jf049512d

15. Panosyan, A. G., Mamikonyan, G. v., Torosyan, M., Gabrielyan, E. S., Mkhitaryan, S. A., Tirakyan, M. R., & Ovanesyan, A. (2001). Determination of the composition of volatiles in cognac (brandy) by headspace gas chromatography-mass spectrometry. *Journal of Analytical Chemistry*, *56*(10), 945–952. https://doi.org/10.1023/A:1012365629636

16. Lachenmeier, D. W., Godelmann, R., Steiner, M., Ansay, B., Weigel, J., & Krieg, G. (2010). Rapid and mobile determination of alcoholic strength in wine, beer and spirits using a flow-through infrared sensor. *Chemistry Central Journal*, *4*(1). https://doi.org/10.1186/1752-153X-4-5

17. Tipparat, P., Lapanantnoppakhun, S., Jakmunee, J., & Grudpan, K. (2001). Determination of ethanol in liquor by near-infrared spectrophotometry with flow injection. *Talanta*, *53*(6), 1199–1204. https://doi.org/10.1016/S0039-9140(00)00610-X

18. Boyaci, I. H., Genis, H. E., Guven, B., Tamer, U., & Alper, N. (2012). A novel method for quantification of ethanol and methanol in distilled alcoholic beverages using Raman spectroscopy. *Journal of Raman Spectroscopy*, *43*(8), 1171–1176. https://doi.org/10.1002/jrs.3159

19. Nordon, A., Mills, A., Burn, R. T., Cusick, F. M., & Littlejohn, D. (2005). Comparison of non-invasive NIR and Raman spectrometries for determination of alcohol content of spirits. *Analytica Chimica Acta*, *548*(1–2), 148–158. https://doi.org/10.1016/j.aca.2005.05.067

20. Stupak, M., Kocourek, V., Kolouchova, I., & Hajslova, J. (2017). Rapid approach for the determination of alcoholic strength and overall quality check of various spirit drinks and wines using GC–MS. *Food Control*, *80*, 307–313. https://doi.org/10.1016/j.foodcont.2017.05.008

21. Buckey, G. K., & Mundy, A. P. (1993). Determination of ethanol in beer by gas chromatography (direct injection) – collaborative trial. *Journal of the Institute of Brewing*, *99*, 381–384. https://doi.org/10.1002/j.2050-0416.1993.tb01176.x

22. Wang, M., Choong, Y., Su, N., & Lee, M. (2003). A Rapid Method for Determination of Ethanol in Alcoholic Beverages Using Capillary Gas Chromatography. *Journal of Food and Drug Analysis*, *11*(2), 133–140. https://doi.org/10.1016/S0003-2670(00)88521-9

23. Tiscione, N. B., Alford, I., Yeatman, D. T., & Shan, X. (2011). Ethanol analysis by headspace gas chromatography with simultaneous flame-ionization and mass spectrometry detection. *Journal of Analytical Toxicology*, *35*(7), 501–511. https://doi.org/10.1093/anatox/35.7.501

24. Destanoğlu, O., & Ateş, İ. (2019). Determination and evaluation of methanol, ethanol and higher alcohols in legally and illegally produced alcoholic beverages. *Journal of the Turkish Chemical Society, Section A: Chemistry*, *6*(1), 21–32. https://doi.org/10.18596/jotcsa.481384

List of publications and conference papers

Publications:

1. Charapitsa, S., Sytova, S., Korban, A., Boyarin, N., Shestakovich, I. & Cabala, R. (2018). The establishment of metrological characteristics of the method "Ethanol as Internal Standard" for the direct determination of volatile compounds in alcoholic products. *Journal of Chemical Metrology*. *12*(1), 59-69. https://doi.org/10.25135/jcm.14.18.02.063

2. Charapitsa, S., Sytova, S. Korban, A. & Sobolenko, L. (2019). Single-Laboratory Validation of a Gas Chromatographic Method of Direct Determination of Volatile Compounds in Spirit Drinks: Need for an Improved Interlaboratory Study. *Journal of AOAC International*. *102*(2), 1-6. https://doi.org/10.5740/jaoacint.18-0258

3. Charapitsa, S., Sytova, S., Korban, A. et al. (2019). Interlaboratory study of ethanol usage as an internal standard in direct determination of volatile compounds in alcoholic products. *BIO Web of Conferences*. 15. https://doi.org/10.1051/bioconf/20191502030

4. Cherepitsa, S.V., Sytova, S.N., Egorov, V.V., Leshchev, S.M., Korban, A.L., Sobolenko, L.N., Milochkin D.A., Ustyugov, V.S., Korobov, V.A. & Ismagilov, D.R. (2019). Validation of the Method of Direct Determination of the Quantitative Content of Volatile Components in Alcohol Containing Products. *Beer and Beverages – Pivo i Napitki*. 4, 41-45. https://doi.org/10.24411/2072-9650-2019-10005

5. Korban, A, Charapitsa, S, Cabala, R, Lidia & S, Sytova, S. (2020). The perspectives of ethanol usage as an internal standard for the quantification of volatile compounds in alcoholic products by GC-MS. *Journal of Mass Spectrometry*. 55. https://doi.org/10.1002/jms.4493

6. Charapitsa, S., Sytova, S., Korban, A., Sobolenko, L., Egorov, V., Cabala, R., Yilmaztekin, M. & Cabaroglu, T. (2020). Interlaboratory study of the method for direct determination of volatile compounds in alcoholic products using ethanol as internal standard. *Journal of Belarusian State University. Chemistry*. 1. 74-87. https://doi.org/10.33581/2520-257X-2020-1-74-87

7. Korban, A., Charapitsa, S., Cabala, R., Sobolenko, L., Egorov, V. & Sytova, S. (2020). Advanced GC-MS method for quality and safety control of alcoholic products. *Food Chemistry*. 338, 1-4. https://doi.org/10.1016/j.foodchem.2020.128107

8. Korban, A., Čabala, R., Egorov, V., Bosáková, Z., & Charapitsa, S. (2022). Evaluation of the variation in relative response factors of GC-MS analysis with the internal standard methods: Application for the alcoholic products quality control. *Talanta*, 246. https://doi.org/10.1016/j.talanta.2022.123518

9. Korban, A., Čabala, R., Egorov, V., Charapitsa, S., Bosáková, Z., & Sytova, S. (2022). Fluctuation of internal standard method calibration factors based on a Taguchi designed experiment while alcoholic products analysis with GC – MS. *Monatshefte Für Chemie - Chemical Monthly*, (0123456789). https://doi.org/10.1007/s00706-022-02942-8

Conference papers:

1. Korban, A. & Makhomet, A. (2016, September 19-21). Expansion of the new method "Ethanol as Internal Standard" for direct determination of volatile compounds in the spirit products. 12th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

2. Charapitsa, S., Sytova, S., Korban, A. et al. (2017, April 4-5). Novel method for direct determination of the volatile compounds in alcohol products. International "Metrology-2017" conference, Minsk, Belarus.

3. Charapitsa, S., Sytova, S., Korban, A. et al. (2017, April 4-5). Prospects for creating standard solutions of volatile compounds in alcohol-containing products. International "Metrology-2017" conference, Minsk, Belarus.

4. Charapitsa, S., Sytova, S. & Korban, A. (2017, Mai 19-20). Correct quantification of complex water-ethanol mixtures. 5th Republican "Analytical Chemistry of RB - 2017" conference, Minsk, Belarus.

5. Charapitsa, S., Sytova, S. & Korban, A. (2017, Mai 19-20). The validation of the method of direct quantification of volatile compounds in alcoholic products with the usage ethanol as an internal standard. 5th Republican "Analytical Chemistry of RB - 2017" conference, Minsk, Belarus.

6. Korban, A. (2017, September 21-22). The numerical method for calculation of the quantitative composition of complex water-ethanol solutions. 13th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

7. Charapitsa, S., Sytova, S., Korban, A. et al. (2017, July 17-20). On-line calculator for direct determination of volatile compounds in alcohol products. 10th "In Vino Analytica Scientia" symposium, Salamanca, Spain.

8. Charapitsa, S., Sytova, S., Korban, A. et al. (2017, July 17-20). Novel method "Ethanol as Internal Standard" for direct determination of volatile compounds in spirit drinks. 10th "In Vino Analytica Scientia" symposium, Salamanca, Spain.

9. Korban, A., Charapitsa & S., Sytova, S. (2018, March 20-23). The establishment of metrological characteristics of three analytical methods during the quantification of volatile compounds in alcoholic products. 61th International "Open Readings 2018" Conference for Students of Physics and Natural Sciences, Vilnius, Lithuania.

10. Korban, A. & Sabalenka, L. (2018, April 23-25). The establishment of metrological characteristics of analytical methods concenrning the quantification of volatile compounds in alcoholic products. 10th All-Ukrainian scientific "Chemical Karazinski Readings - 2018" conference for students and Ph.D. students, Kharkiv, Ukraine.

11. Sabalenka, L. & Korban, A. (2018, April 23-25). Standard samples of mass concentrations of volatile impurities in alcohol products. 10th All-Ukrainian scientific "Chemical Karazinski Readings - 2018" conference for students and Ph.D. students, Kharkiv, Ukraine.

12. Sobolenko, L. & Korban, A. (2018, October 4-5). Development of standard solutions of volatile compounds in ethanol-containing products. 17th international scientific and practical "Innovative technologies in the food industry" conference, Minsk, Belarus.

13. Charapitsa, S., Sytova, S., Korban, A. & Sabalenka, L. (2018, Mai 14-18). On-line calculator for the correct quantification of volatile compounds in alcoholic products. 4th scientific and practical "Web Conf 2018" conference, Minsk, Belarus.

14. Charapitsa, S., Sytova, S., Korban, A. & Sobolenko, L. (2018, Mai 23-24). Belarusian software for the correct quantification of volatile compounds in alcoholic products. 10th international scientific and practical "Information technologies in industry, logistics and social sphere" conference, Minsk, Belarus.

15. Korban, A. (2018, September 20-21). A novel way of establishing the quantitative composition of gravimetrically prepared standard solutions of volatile compounds in water-ethanol matrix. 14th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

16. Korban A. (2019, May 25). Breaking the wall of quality control of alcoholic products. Final of the "Falling Walls Lab" contest organised by the German Academic Exchange service (DAAD), Minsk, Belarus.

17. Korban, A. & Sobolenko, L. (2019, September 19-20). The Possibility to Employ Ethanol as an Internal Standard in Quantification of Volatile Compounds in Alcoholic Products with GC-MS. 15th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

18. Korban, A. & Sabalenka, L. (2020, April 21-23). Testing of the advanced internal standard method for quality control of alcoholic products with GC-MS. 12th All-Ukrainian scientific "Chemical Karazinski Readings - 2020" conference for students and Ph.D. students, Kharkiv, Ukraine.

19. Korban, A. (2020, April 21-23). Investigation of the chemical stability of acetaldehyde in water-ethanol matrix. 12th All-Ukrainian scientific "Chemical Karazinski Readings - 2020" conference for students and Ph.D. students, Kharkiv, Ukraine.

20. Korban, A. (2020, September 17-18). Advanced GC-MS method for quality and safety control of alcoholic beverages. 16th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

21. Korban, A. (2020, September 6). On guard of quality control of alcoholic products. National Czech Republic Final of Fame Lab International contest, Prague, Czech Republic.

22. Korban, A. (2021, September 19-17). The perspectives of Relative Response Factors tabulation while alcoholic products analysis with GC-MS. 17th International Students "Modern Analytical Chemistry" Conference, Prague, Czech Republic.

23. Korban, A. (2021, September 27-28). Fluctuation of internal standard method calibration factors using GC-MS. 1st International Meeting for young analytical chemists (IMYAC), Krakow, Poland.

24. Korban, A. (2022, June 18-23). The Development of Methods for Quality Control of Alcoholic Products by GC-MS. International Chromatographic Conference "HPLC 2022", San Diego, USA.