GUIDE

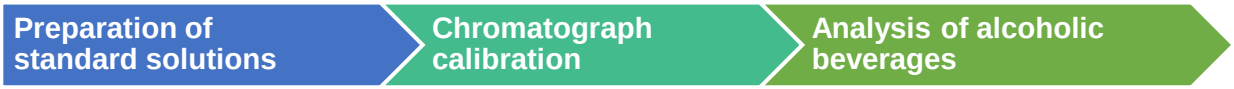
METHOD FOR THE DETERMINATION OF VOLATILE COMPOUNDS IN SPIRIT DRINKS AND WINE USING CONTAINED ETHANOL AS A REFERENCE STANDARD

Part 1

Preparation of Interlaboratory Study of a Gas Chromatographic Method for the Analysis of Volatile Congener Content in Alcoholic Beverages using Ethanol as a Reference Substance

Part 2

Procedure: the Creation of the Calibration Method and the Analysis of Alcoholic Beverages using Ethanol as Reference Substance



Version EIS_TIS_2-150

2026

Contents

Part 1

Preparation of Interlaboratory Study of Gas Chromatographic Method for the Analysis of Volatile Congener Content in Alcoholic Beverages using Ethanol as a Reference Substance

Characteristic of the Method

Scope

This method is suitable for the determination of the following compounds by gas chromatography in spirit drinks and wine: ethanal (acetaldehyde), ethyl ethanoate (ethyl acetate), 1,1-diethoxyethane (acetal), methanol (methyl alcohol), butan-2-ol (sec-butanol), propan-1-ol (n-propanol), 2-methylpropan-1-ol (isobutyl alcohol), butan-1-ol (n-butanol), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol) with analyte content ranging from 5 to 1500 g per 100 L anhydrous ethanol (g/100 L AA).

Definitions

Congeners are volatile compounds formed along with ethanol during fermentation and maturation of wine, spirits of wine origin and other alcoholic beverages.

Principle

Congeners in spirit drinks and wine are determined by direct injection of the beverage or its distillate, into a gas chromatography (GC) system. The congeners are separated by temperature programming on a suitable column and are detected using a flame ionisation detector (FID). Ethyl alcohol contained in beverage is used as a reference substance the same as internal standard (IS). The concentration of each congener is determined with respect to the reference substance ethanol from relative response factors (RRF), which are obtained during calibration under the same chromatographic conditions as those of the beverage analysis.

Distinguishing Features of the Method

The method OENO-SCMA 24-756 uses reference substance ethanol as IS (method EIS) in calculations of RRF and congener concentrations, that is differ from the methods with traditional IS (methods TIS). When the use the method EIS, there is no need to add into samples any further special compound IS as in methods TIS. When analysing alcoholic beverage samples using this method, there is no need to measure the strength and density of the beverage test samples. The results are expressed in units of g/100 L AA. Chromatograph is calibrated using standard solutions of the volatile compounds being determined, in which the compound content is expressed in g/100 L of AA. The method includes quality control measures that allow the user to select the chromatographic system and the most appropriate sample analysis conditions.

Preparation of Interlaboratory Study

Purpose

An Interlaboratory Study is proposed for the gas chromatography (GC) method for determining volatile compounds in wine, spirits of wine origin and other alcoholic beverages (OENO-SCMA 24-756 Et5_2026). It is proposed that 20-30 laboratories from around the world participate in the testing.

Preparation of Alcoholic Beverage Samples

Samples can be purchased at retail stores from various parts of Europe. The samples include brandy, kirsch, grappa, wine distillate, rum and whiskey, with and without fixed levels of volatile components. The test portion can be an original sample of the spirit drinks and wine itself or the its distillate obtained during distillation according to the OIV-MA-AS312-01 or OIV-MA-BS-02: R2009 method.

Alcoholic beverage samples are prepared and sent to participants as 12 blind duplicates (field duplicates) or split level samples for the determination of 1,1-diethoxyethane (acetal), 2-methylbutan-1-ol (active amyl alcohol), 3-methylbutan-1-ol (isoamyl alcohol), methanol (methyl alcohol), ethyl acetate (ethyl acetate), butan-1-ol (n-butanol), butan-2-ol (sec-butanol), 2-methylpropan-1-ol (isobutyl alcohol), propan-1-ol (n-propanol) and ethanal (acetaldehyde).

Blind duplicate is a hidden duplicate sample. It means that the samples are collected under identical conditions, but they are given different names to prevent laboratories from identifying which samples are duplicates. It is used to check repeatability. **Field duplicate** is simply a duplicate sample. The samples are collected under identical conditions, and this is not hidden. It is also used to check repeatability. **Split-level sample** is a sample taken with only slightly different results and sent for measurement. However, any artificial attempt to bring the results closer together will be visible, because the difference is small, but still present. If someone within one laboratory thinks that two samples are blind duplicates and decides to artificially bring them closer together to obtain better repeatability values, a significant error will occur, since other laboratories will not attempt this.

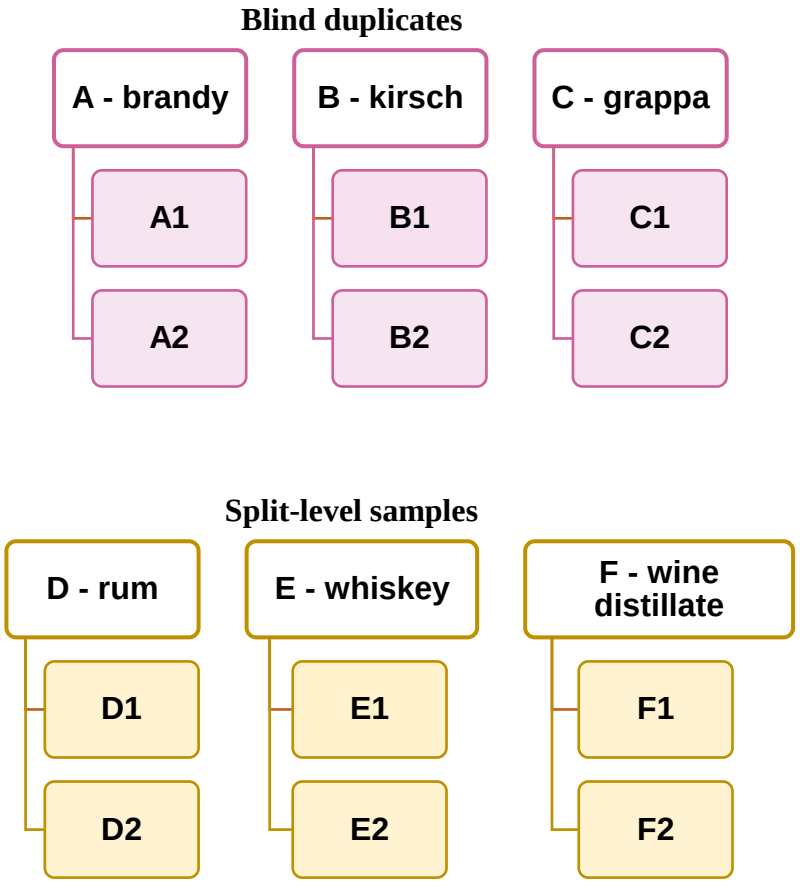
Samples A, B and C are prepared and dispensed into darkened 15 mL vials in 15 mL portions by an external contractor. A, B and C are blind duplicate samples of brandy, kirsch and grappa.

Split level samples D, E and F are prepared by the coordinating laboratory. Sample D1 is used as purchased. Sample D2 is fortified by adding 25 mL of a solution containing all analytes (at 250-500 g/100 L AA) and then made up to 2.5 L with solution D1 in a volumetric flask. Both samples D1 and D2 are stored at <5°C before being dispensed into darkened

15 mL vials.

Sample E1 is used as purchased. Sample E2 is fortified by adding 25 mL of a solution containing all analytes (at 75-500 g/100 L AA) and then made up to 2.5 L with solution E1 in a volumetric flask. Both samples E1 and E2 are stored at <5°C before being dispensed into darkened 15 mL vials.

Sample F is a wine distillate sample. F1 is used as purchased. Sample F2 is fortified by adding 25 mL of a solution containing all analytes (at 75-250 g/100 L AA) and then made up to 2.5 L with solution F1 in a volumetric flask. Both samples F1 and F2 are stored at <5°C before being dispensed into darkened 15 mL vials.



All prepared test materials are labelled with the coordinating laboratory's own codes.

Pre-Trial Studies

Prior to the interlaboratory trial, a method validation is conducted. Initially, the method is evaluated in-house by the coordinating laboratory and then tested externally by two expert

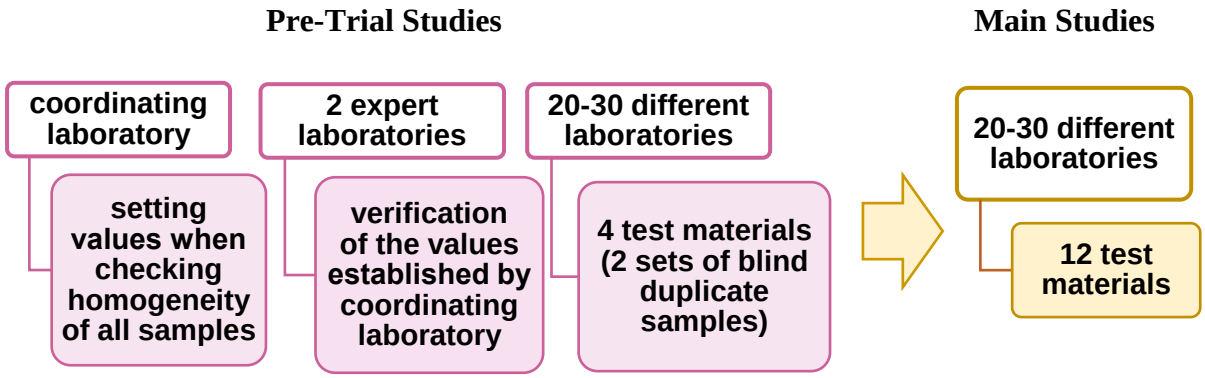
laboratories. The expert laboratories validate the method and verify the coordinating laboratory's assigned values for the analysed material during a homogeneity check. Participants are then allowed to familiarize themselves with the method in "preliminary studies."

For further testing, each participant is sent a detailed method protocol, along with 4 test materials (2 sets of blind duplicate samples) for analysis. Participants are informed that sample strength measurements are not required and that results must be reported in g/100 L AA. They are also required to provide copies of their chromatograms.

The results and chromatograms received from participants are then carefully reviewed by the coordinating laboratory to assess the proper performance of their analytical systems and to ensure quality assurance criteria are met. This assessment is communicated to the participants so that their analyses can be improved when necessary. At this stage, any necessary method adjustments are made and distributed to the participants prior to the main trials.

Main Study

For the main test, participants are sent 12 test materials, a method protocol, an instruction sheet, and an acceptance confirmation form. Participants are sent 12 samples, consisting of 3 sets of blind duplicates and 3 split samples. Participants are asked to ensure that each test material is analysed only once, but the average value of 2-3 gas chromatographic injections is reported. Participants are instructed to store samples at 4°C prior to analysis and to provide copies of their chromatograms in the appendices to their results. Results should be reported in units of g/100 L AA.



Processing of Results

Homogeneity testing

Homogeneity is assessed using internationally agreed-upon procedures. Five randomly selected containers are analysed by the method and then injected in duplicate. Homogeneity is assessed using a one-way analysis of variance and, if necessary, a subsequent F-analysis.

Stability testing

Samples are analysed three times separately throughout the study: during homogeneity testing, before the start of the interlaboratory test and at the end of the interlaboratory test. For each set of test materials, duplicate sample are analysed using the method under study.

Statistical Analysis of Results

Study results are examined for outliers ($p < 0.025$) using the Cochran and Grubbs tests, stepwise, following the procedures described in the internationally agreed Protocol for the Design, Conduct, and Interpretation of Method Performance Studies.

Repeatability and Reproducibility

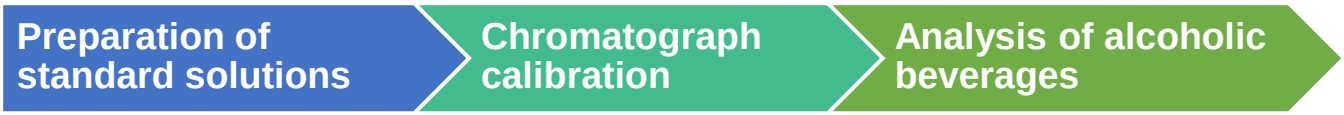
Repeatability and reproducibility, as defined in the Protocol for the Design, Conduct, and Interpretation of Method Performance Studies, are calculated based on the results retained after outlier removal.

Comparison of Method Results

To compare the results obtained using the new OENO-SCMA 24-756 Et5_2026 method with the OIV-MA-BS-14 method, a traditional internal standard can be added to standard solutions and alcoholic beverage samples.

Part 2

Procedure:
the Creation of the Calibration Method and the Analysis of Alcoholic Beverages using Ethanol as Reference Substance



Preparation of Standard Solutions

Equipment and Laboratory Glassware

- Analytical balance, capable of measuring to four decimal places
- Pycnometer or alcoholometer
- Thermometer
- 1-liter volumetric flask with ground glass stopper
- 100-mililiter volumetric flasks with ground glass stopper
- 500-militer graduated cylinder
- Pipette dispensers with variable volume from 100 to 1000 mL

Reagents, Materials and Conditions

Use only reagents of a purity greater than 97.0 %, with certificate of purity, free from other volatile compounds at test dilution and only water of at least grade 3 as defined in ISO 3696. Acetal and acetaldehyde must be stored in the dark at ≤ 5.0 °C, all other reagents should be stored according to the supplier's instructions.

- Ethanol absolute (CAS 64-17-5)
- Methanol (CAS 67-56-1)
- Propan-1-ol (CAS 71-23-8)
- 2-Methylpropan-1-ol (CAS 78-33-1)
- 2-Methylbutan-1-ol (CAS 137-32-6)
- 3-Methylbutan-1-ol (CAS 123-51-3)
- Ethyl acetate (CAS 141-78-6)
- Butan-1-ol (CAS 71-36-3)
- Butan-2-ol (CAS 78-92-2)
- Acetaldehyde (CAS 75-07-0)
- Acetal (CAS 105-57-7)
- Acceptable internal standards: pentan-3-ol (CAS 584-02-1), pentan-1-ol (CAS 71-41-0), 4-methylpentan-1-ol (CAS 626-89-1) or methylnonanoate (CAS 1731-84-6).

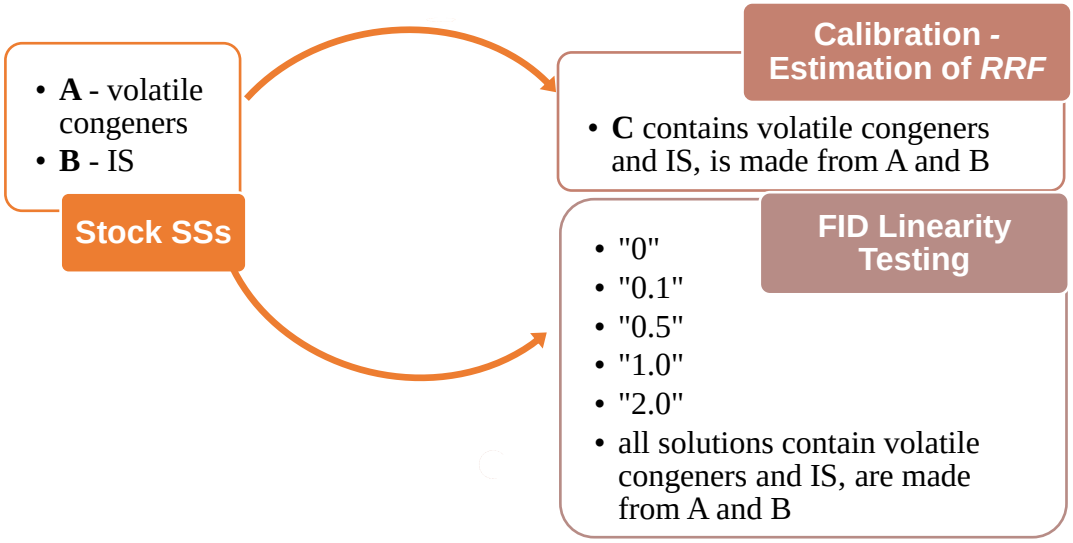
Preparation of 40 % v/v Ethanol Solution in Water (WES)

To prepare a 400 mL/L ethanol solution in water (WES), measure 400 mL of absolute ethanol using a graduated cylinder or measuring flask of the appropriate volume and pour it into a 1-liter volumetric flask. Bring the solution volume up to the 1-liter mark by adding distilled water and mix thoroughly. After preparing the WES, accurately determine the volumetric ethanol content of the prepared solution (*ABV*, %) and the density of the WES (ρ_{WES} , g/L) using standard methods.

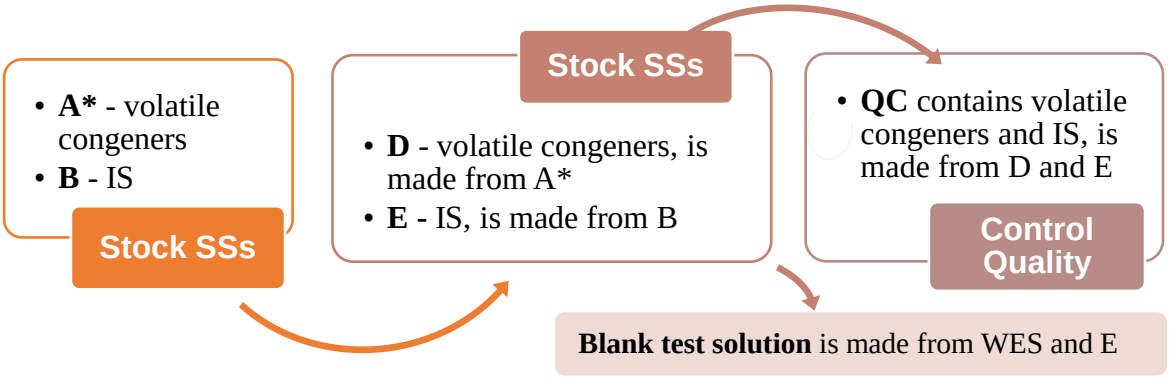
Standard Solutions (SSs)

To work according to the both methods EIS and TIS, the following solutions of volatile compounds and internal standard are prepared:

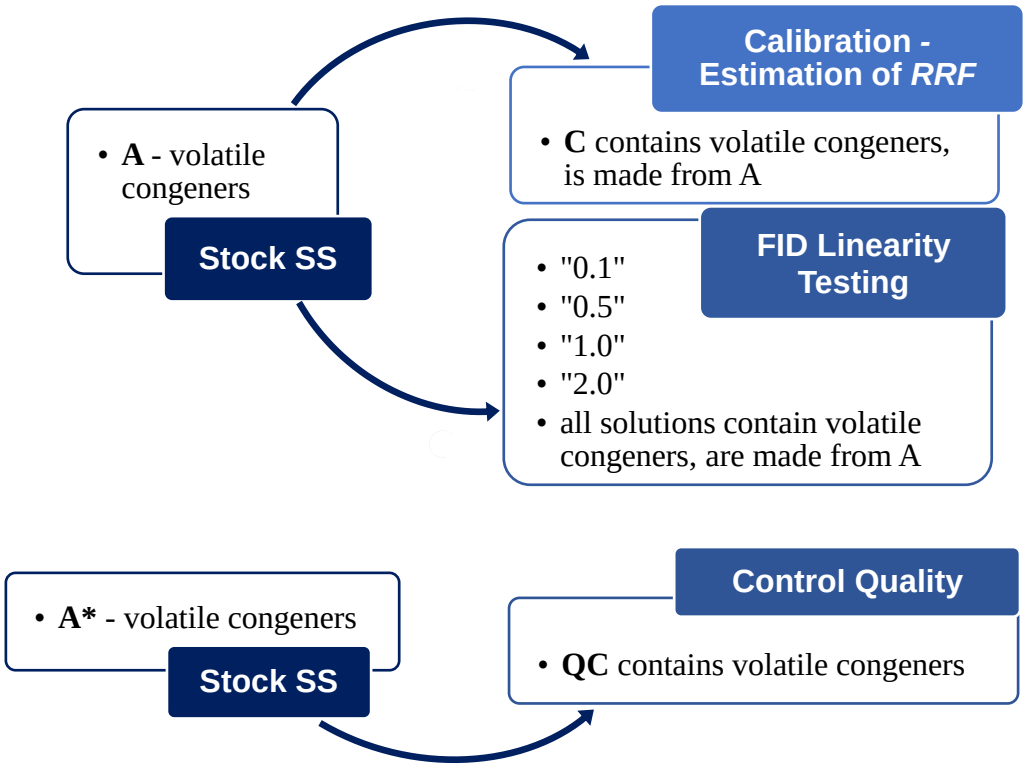
- stock standard solutions (A, A*, B, D, E) – concentrated solutions not subjected to chromatography,
- 1 standard solution C for chromatograph calibration and establishing the *RRF*,
- standard solutions (“0”, “0.1”, “0.5”, “1.0”, “2.0”) for the control of the linearity of the flame ionisation detector (FID) response,
- 1 standard solution QC for checking the quality of the calibration,
- solution for blank test contained IS



In order to maintain analytical continuity and an effective quality control, prepare a quality control standard QC using the previously prepared standard A or, preferably, prepare a control standard as indicated for standard A*, but using different batches or suppliers of reagents.



It is necessary to note that to work according to method EIS only, the solutions B, D, E and special blank test solution contained IS are not need. The scheme of mandatory solutions for EIS method without TIS is represented below and the described in short guides [1, 2]:



Preparation and storage of standard solutions: the calibration ranges should be adapted to the nature of the different types of products analysed by each laboratory. All standard solutions must be stored at ≤ 5.0 °C and be prepared freshly on a monthly basis. Masses of components and solutions should be recorded to the nearest 0.1 mg.

Preparation of Standard Solution A

Pipette the following reagents (Table 1) into a 100 mL volumetric flask, containing approximately 60.0 mL 40% v/v ethanol solution (WES) to minimise component evaporation, make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added m_A^i and WES m_A^{WES} and the total final weight of contents in the Table 1. Approximate mass concentration of reagents in standard solution A is about 7,500 g/100 L AA.

NOTE – It is preferable to add acetal and acetaldehyde last in order to minimise losses through evaporation. The solutions may be prepared individually, and the final solution and dilutions prepared subsequently.

Table 1

Reagent *i*	Reagent purity P_i^i, %	Volume, mL	Approximate mass, mg	The exact value of the mass m_A^i and m_A^{WES}, mg
Methanol		3.0	3000.0	
Propan-1-ol		3.0	3000.0	
2-methylpropan-1-ol		3.0	3000.0	
2-methylbutan-1-ol		3.0	3000.0	
3-methylbutan-1-ol		3.0	3000.0	
Ethyl acetate		3.0	3000.0	
Butan-1-ol		3.0	3000.0	
Butan-2-ol		3.0	3000.0	
Acetaldehyde		3.0	3000.0	
Acetal		3.0	3000.0	
WES		60.0 10.0	60,000 10,000	

Mass of the flask, mg	
Total final weight of contents, mg	

Preparation of Standard Solution B

Pipette 3.0 mL of reagent of IS into a 100 mL volumetric flask, containing approximately 60.0 mL WES, make up to volume with WES and mix thoroughly. Record the weight of the flask, the weight of IS m_B^{IS} and WES m_B^{WES} and the total final weight of contents. Approximate mass concentration of IS in standard solution B is about 7,500 g/100 L AA.

Preparation of Standard Solution C

Pipette 1 mL standard solution A and 1 mL solution B into a 100 mL volumetric flask containing approximately 60.0 mL of 40% v/v ethanol solution (WES), make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added A, B and WES (m_C^A, m_C^B and m_C^{WES}) and the total final weight of contents in the Table 2. Approximate mass concentration of reagents in standard solution C is about 75 g/100 L AA.

Table 2

Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	1.0	1000.0		m_C^A
Standard Solution B	1.0	1000.0		m_C^B
WES	60.0 38.0	60,000 38,000		m_C^{WES}

Mass of the flask, mg	
Total final weight of contents, mg	

Preparation of Standard Solutions "0", "0.1", "0.5", "1.0", "2.0" used to check the Linearity of FID Response

Into separate 100 mL volumetric flasks, containing approximately 60.0 mL ethanol, pipette 0, 0.1, 0.5, 1.0, 2.0 mL standard solution A and 1 mL solution B into and make up to volume with ethanol solution (WES) and mix thoroughly (Table 3). Record the weight of the flask, each component added A, B and WES (m_{SS}^A, m_{SS}^B and m_{SS}^{WES}) and the total final weight of contents in the Table 3. Approximate mass concentration values of reagents in the standard solutions (SS) are about 0, 7.5, 37.5, 75.0, 150 g/100 L AA.

Table 3

Solution "0" - approximate concentration of volatile components 0 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	0.0	0.0		m_0^A
Standard Solution B	1.0	1000.0		m_0^B
WES	60.0 39.0	60,000 39,000		m_0^{WES}

Mass of the flask, mg	

Total final weight of contents, mg	

Solution "0.1" - approximate concentration of volatile components 7.5 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	0.1	100.0		$m_{0.1}^{A}$
Standard Solution B	1.0	1000.0		$m_{0.1}^{B}$
WES	60.0 38.9	60,000 38,900		$m_{0.1}^{WES}$

Mass of the flask, mg	
Total final weight of contents, mg	

Solution "0.5" - approximate concentration of volatile components 37.5 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	0.5	500.0		$m_{0.5}^{A}$
Standard Solution B	1.0	1000.0		$m_{0.5}^{B}$
WES	60.0 38.5	60,000 38,500		$m_{0.5}^{WES}$

Mass of the flask, mg	
Total final weight of contents, mg	

Solution "1.0" - approximate concentration of volatile components 75 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	1.0	1000.0		$m_{1.0}^{A}$
Standard Solution B	1.0	1000.0		$m_{1.0}^{B}$
WES	60.0 38.0	60,000 38,000		$m_{1.0}^{WES}$

Mass of the flask, mg
Total final weight of contents, mg

Solution "2.0" - approximate concentration of volatile components 150 g/100 L AA				
Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution A	2.0	2000.0		$m_{2.0}^{A}$
Standard Solution B	1.0	1000.0		$m_{2.0}^{B}$
WES	60.0 37.0	60,000 37,000		$m_{2.0}^{WES}$

Mass of the flask, mg
Total final weight of contents, mg

Preparation of Standard Solution D

In order to maintain analytical continuity and an effective quality control, prepare a quality control standard using the previously prepared solution A or, preferably, prepare a solution A* as indicated for standard A, but using different batches or suppliers of reagents. Pipette 1 mL standard solution A (or A*) into a 100 mL volumetric flask containing approximately 60.0 mL of 40% v/v ethanol solution (WES), make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added A and WES (m_D^A, m_D^{WES}) and the total final weight of contents. Approximate mass concentration of reagents in standard solution D is about 75.0 g/100 L AA.

Preparation of Standard Solution E

Pipette 10 mL standard solution B into a 100 mL volumetric flask containing approximately 60.0 mL of 40% v/v ethanol solution (WES), make up to volume with WES and mix thoroughly. Record the weight of the flask, each component added B and WES (m_E^B, m_E^{WES}) and the total final weight of contents. Approximate concentration of reagents in solution E is about 750 g/100 L AA.

Preparation of Standard Solution QC

In order to maintain analytical continuity and an effective quality control, prepare a quality control standard using the previously prepared standard A or, preferably, prepare a control standard as indicated for standard A*, but using different batches or suppliers of reagents. Pipette 9 mL solution D and 1 mL solution E into vessel and mix thoroughly. Record the weight of the flask, each component added D and E (m_{QC}^D, m_{QC}^E) and the total final weight of contents. Approximate concentration of reagents in solution QC is about 67.5 g/100 L AA.

Table 4

Solution	Volume, mL	Approximate mass, mg	The exact value of the mass, mg	Symbol
Standard Solution D	9.0	9000.0		m_{QC}^{D}
Standard Solution E	1.0	1000.0		m_{QC}^{E}

Mass of the flask, mg	
Total final weight of contents, mg	

Preparation of Solution BT for Blank Test

Pipette 9 mL WES and 1 mL solution E into appropriate vessel and mix thoroughly. Record the weight of the vessel, each component added E and WES (m_{BT}^{E} , m_{BT}^{WES}) and the total final weight of contents.

It is necessary to note that to work according to method EIS without solutions B, D and E the preparation of the special solution BT for blank test is not need. In this case, WES is used for blank test [1, 2].

Calculation of Approximate Values of Volatile Compound Concentration in Standard Solutions

To calculate the "certified" values of component concentrations C^i in standard solutions, it is necessary to accurately determine the masses of the substances using an analytical balance and record them in the tables 1-4. ***We can use the web-calculator or in Excel template for automatic calculation "certified" values of mass concentration of volatile compounds and IS in standard solutions.***

The new method of internal standard with using ethanol as reference substance (method EIS) differ on the methods of traditional internal standard (methods TIS). In the EIS method, the concentrations of substances in standard solutions are calculated in units of g/100 L of anhydrous alcohol (g/100 L AA).

Zero-order approximation (0_ap). In the zero approximation (0_ap), the calculations assume that ethanol reagent and WES consist only of water and ethanol, and that other compounds such as volatile congeners are absent, as are their fractions.

The mass part W_i^i and W_{IS}^{IS} of the main compound *i* and IS in their initial reagents in units of mg/mg are calculated using the formulas:

$$W_i^i = \frac{P_i^i}{100\ \%}, \quad (1)$$

$$W_{IS}^{IS} = \frac{P_{IS}^{IS}}{100\ \%}, \quad (2)$$

where P_i^i and P_{IS}^{IS} are the purity of the main substance *i* and IS in their initial reagents being determined, specified in the manufacturer's data sheet for the corresponding volatile compound *i* and IS, in % by mass.

In the EIS method, the mass part W_{WES}^{Eth} of ethyl alcohol in WES in mg/mg of the solution is calculated using the formula:

$$W_{WES}^{Eth} = \frac{\rho_{Eth}}{\rho_{WES}} \cdot \frac{ABV}{100}, \quad (3)$$

where *ABV* is the volume content of ethyl alcohol in the WES, %; ρ_{WES} is the density of WES, g/L under normal conditions; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, ρ_{Eth} = 789.270 g/L.

In the EIS method, the mass part W_A^{Eth} of ethyl alcohol in solution A and the mass part W_B^{Eth} of ethyl alcohol in solution B in mg/mg of the solution are calculated using the formulas:

$$W_A^{Eth} = \frac{W_{WES}^{Eth} \cdot m_A^{WES}}{m_A^{WES} + \sum_i m_A^i}, \quad (4)$$

$$W_B^{Eth} = \frac{W_{WES}^{Eth} \cdot m_B^{WES}}{m_B^{WES} + m_B^{IS}}, \quad (5)$$

where m_A^i is the mass of initial substance of the volatile compound *i* being determined, introduced into solution A during its preparation, mg; m_A^{WES} is the mass of WES added into solution A during its preparation, mg; m_B^{IS} is the mass of initial substance of IS being determined, introduced into solution B during its preparation, mg; m_B^{WES} is the mass of WES added into solution B during its preparation, mg.

In the EIS method, the mass parts $W_A^i(n\_\text{ap})$ and $W_B^i(n\_\text{ap})$ of the volatile component *i* in solutions A and B in mg/mg of the solution, to *n*-order approximation, are calculated using the formulas:

$$W_A^i(n\_\text{ap}) = \frac{W_i^i \cdot m_A^i + W_{WES}^i(n\_\text{ap}) \cdot m_A^{WES}}{m_A^{WES} + \sum_i m_A^i}, \quad (6)$$

$$W_B^i(n\_\text{ap}) = \frac{W_{WES}^i(n\_\text{ap}) \cdot m_B^{WES}}{m_B^{WES} + m_B^{IS}}, \quad (7)$$

where n is the number of approximation order; $W_{WES}^i(n\_\text{ap})$ is the mass part of volatile compound i in WES in n-order approximation, mg/mg of the mixture.

In zero approximation, ethanol reagent and WES doesn`t contain volatile congeners $W_{WES}^i(0\_\text{ap}) = 0$ mg/mg. Therefore $W_B^i(0\_\text{ap}) = 0$ mg/mg and:

$$W_A^i(0\_\text{ap}) = \frac{W_i^i \cdot m_A^i}{m_A^{WES} + \sum_i m_A^i}. \quad (8)$$

In the EIS method, the mass concentration $C_C^i(n\_\text{ap})$ of compound i (or IS) in standard solution C in units of g/100 L AA in n-order approximation is calculated using the formula:

$$C_C^i(n\_\text{ap}) = \frac{W_A^i(n\_\text{ap}) \cdot m_C^A + W_B^i(n\_\text{ap}) \cdot m_C^B + W_{WES}^i(n\_\text{ap}) \cdot m_C^{WES}}{W_A^{Eth} \cdot m_C^A + W_B^{Eth} \cdot m_C^B + W_{WES}^{Eth} \cdot m_C^{WES}} \cdot \rho_{Eth}, \quad (9)$$

where m_C^A is the mass of solution A added into solution C during its preparation, mg; m_C^{WES} is the mass of WES added into solution C during its preparation, mg; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, $\rho_{Eth} = 78927\,\text{g/100 L}$.

In the EIS method, in zero approximation $W_B^i(0\_\text{ap}) = 0$ mg/mg and $W_{WES}^i(0\_\text{ap}) = 0$ mg/mg. Therefore:

$$C_C^i(0\_\text{ap}) = \frac{W_i^i \cdot m_A^i \cdot m_C^A}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot \left(W_A^{Eth} \cdot m_C^A + W_B^{Eth} \cdot m_C^B + W_{WES}^{Eth} \cdot m_C^{WES}\right)} \cdot \rho_{Eth}, \quad (10)$$

where $C_C^i(0\_\text{ap})$ is the mass concentration of compound i in standard solution C in zero approximation, g/100 L AA; $\rho_{Eth} = 78927\,\text{g/100 L}$.

As zero approximation, the compound concentration values in solution A $C_A^i(0\_\text{ap})$ and other standard solutions (SS) $C_{SS}^i(0\_\text{ap})$ in g/100 L AA can be calculated in the EIS method using the formulas:

$$C_A^i(0\_\text{ap}) = \frac{W_i^i \cdot m_A^i}{W_{WES}^{Eth} \cdot m_A^{WES}} \cdot \rho_{Eth}, \quad (11)$$

$$C_{SS}^i(0\_\text{ap}) = \frac{W_i^i \cdot m_A^i \cdot m_{SS}^A}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot \left(W_A^{Eth} \cdot m_{SS}^A + W_B^{Eth} \cdot m_{SS}^B + W_{WES}^{Eth} \cdot m_{SS}^{WES}\right)} \cdot \rho_{Eth}, \quad (12)$$

$$C_D^i(0\_\text{ap}) = \frac{W_i^i \cdot m_A^i \cdot m_D^A}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot \left(W_A^{Eth} \cdot m_D^A + W_{WES}^{Eth} \cdot m_D^{WES}\right)} \cdot \rho_{Eth}, \quad (13)$$

where m_{SS}^A is the mass of solution A added into solution SS during its preparation, mg; m_{SS}^{WES} is the mass of WES added into solution SS during its preparation, mg; SS – standard solutions such as "0", "0.1", "0.5", "1.0", "2.0"; m_D^A is the mass of solution A added into solution D during its preparation, mg; m_D^{WES} is the mass of WES added into solution D during its preparation, mg; ρ_{Eth} is the density of anhydrous alcohol at temperature of 20 °C, $\rho_{Eth} = 78927\,\text{g/100 L}$.

Concentration of congeners in the solution of blank test $C_{BT}^i(0\_\text{ap}) = 0$ such as in B and E.

As zero approximation, the volatile compound concentration values in quality control solution QC $C_{QC}^i(0\_\text{ap})$ in g/100 L AA can be calculated in the EIS method using the formula:

$$C_{QC}^i(0\_\text{ap}) = \frac{\frac{W_A^i(0\_\text{ap}) \cdot m_D^A \cdot m_{QC}^D}{m_D^A + m_D^{WES}} \cdot \rho_{Eth}}{\frac{W_A^{Eth} \cdot m_D^A + W_{WES}^{Eth} \cdot m_D^{WES}}{m_D^A + m_D^{WES}} \cdot m_{QC}^D + \frac{W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}}{m_E^B + m_E^{WES}} \cdot m_{QC}^E}, \quad (14)$$

where m_{QC}^D is the mass of solution D added into solution QC during its preparation, mg; m_{QC}^E is the mass of solution E added into solution QC during its preparation, mg; m_E^{WES} is the mass of WES added into solution E during its preparation, mg; m_E^B is the mass of solution B added into solution E during its preparation, mg.

The mass concentration values of IS C_E^{IS}, C_C^{IS}, C_{SS}^{IS}, C_{QC}^{IS} and C_{BT}^{IS} in standard solutions B, E, C, SS ("0", "0.1", "0.5", "1.0", "2.0"), QC and solution BT for blank test in units of g/100 L AA can be calculated using the formulas:

$$C_B^{IS} = \frac{W_{IS}^{IS} \cdot m_B^{IS}}{W_{WES}^{Eth} \cdot m_B^{WES}} \cdot \rho_{Eth}, \quad (15)$$

$$C_E^{IS} = \frac{W_{IS}^{IS} \cdot m_B^{IS} \cdot m_E^B \cdot \rho_{Eth}}{\left(m_B^{WES} + m_B^{IS}\right) \cdot \left(W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}\right)}, \quad (16)$$

$$C_{C(SS)}^{IS} = \frac{W_{IS}^{IS} \cdot m_B^{IS} \cdot m_{C(SS)}^B \cdot \rho_{Eth}}{\left(m_B^{WES} + m_B^{IS}\right) \cdot \left(W_A^{Eth} \cdot m_{C(SS)}^A + W_B^{Eth} \cdot m_{C(SS)}^B + W_{WES}^{Eth} \cdot m_{C(SS)}^{WES}\right)}, \quad (17)$$

$$C_{QC}^{IS} = \frac{W_{IS}^{IS} \cdot m_B^{IS} \cdot m_E^B \cdot m_{QC}^E / \left(\left(m_B^{WES} + m_B^{IS}\right) \cdot \left(m_E^B + m_E^{WES}\right)\right)}{\frac{W_A^{Eth} \cdot m_D^A + W_{WES}^{Eth} \cdot m_D^{WES}}{m_D^A + m_D^{WES}} \cdot m_{QC}^D + \frac{W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}}{m_E^B + m_E^{WES}} \cdot m_{QC}^E} \cdot \rho_{Eth}, \quad (18)$$

$$C_{BT}^{IS} = \frac{\frac{W_{IS}^{IS} \cdot m_B^{IS}}{m_B^{IS} + m_B^{WES}} \cdot \frac{m_E^B}{m_E^B + m_E^{WES}} \cdot m_{BT}^E \cdot \rho_{Eth}}{\frac{W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}}{m_E^B + m_E^{WES}} \cdot m_{BT}^E + W_{WES}^{Eth} \cdot m_{BT}^{WES}}, \quad (19)$$

where m_{BT}^E is the mass of solution E added into blank test solution during its preparation, mg; m_{BT}^{WES} is the mass of WES added into blank test solution during its preparation, mg.

When the method TIS is used, the concentration values of IS in standard solutions and blank test solution are calculated in μg/g using the formulas:

$$\tilde{C}_B^{IS} = \frac{W_{IS}^{IS} \cdot m_B^{IS}}{m_B^{WES} + m_B^{IS}} \cdot 10^6, \quad (20)$$

$$\tilde{C}_E^{IS} = \frac{\tilde{C}_B^{IS} \cdot m_E^B}{m_E^B + m_E^{WES}}, \quad (21)$$

$$\tilde{C}_{C(SS)}^{IS} = \frac{\tilde{C}_B^{IS} \cdot m_{C(SS)}^B}{m_{C(SS)}^A + m_{C(SS)}^B + m_{C(SS)}^{WES}}, \quad (22)$$

$$\tilde{C}_{QC}^{IS} = \frac{\tilde{C}_E^{IS} \cdot m_{QC}^E}{m_{QC}^D + m_{QC}^E}, \quad (23)$$

$$\tilde{C}_{BT}^{IS} = \frac{\tilde{C}_{E}^{IS} \cdot m_{BT}^{E}}{m_{BT}^{WES} + m_{BT}^{E}}. \quad (24)$$

where $\tilde{C}_{B}^{IS}$ is the concentration of IS in solution B, µg/g; $\tilde{C}_{E}^{IS}$ is the concentration of IS in solution E, µg/g; $\tilde{C}_{C(SS)}^{IS}$ is the concentration of IS in solution C (or SS such as "0", "0.1", "0.5", "1.0", "2.0"), µg/g; $\tilde{C}_{QC}^{IS}$ is the concentration of IS in solution QC, µg/g; $\tilde{C}_{BT}^{IS}$ is the concentration of IS in solution QC, µg/g.

In zero approximation $W_{WES}^{i}(0\_ap) = 0$ mg/mg and therefore the volatile compound concentration values are calculated for the standard solutions according to the formulas:

$$\tilde{C}_{A}^{i}(0\_ap) = W_{A}^{i}(0\_ap) \cdot 10^{6} = \frac{W_{i}^{i} \cdot m_{A}^{i}}{m_{A}^{WES} + \sum_{i} m_{A}^{i}} \cdot 10^{6}, \quad (25)$$

$$\tilde{C}_{C(SS)}^{i}(0\_ap) = \frac{\tilde{C}_{A}^{i}(0\_ap) \cdot m_{C(SS)}^{A}}{m_{C(SS)}^{A} + m_{C(SS)}^{B} + m_{C(SS)}^{WES}} = \frac{W_{i}^{i} \cdot m_{A}^{i} \cdot m_{C(SS)}^{A} \cdot 10^{6}}{\left(m_{A}^{WES} + \sum_{i} m_{A}^{i}\right) \cdot \left(m_{C(SS)}^{A} + m_{C(SS)}^{B} + m_{C(SS)}^{WES}\right)}, \quad (26)$$

$$\tilde{C}_{D}^{i}(0\_ap) = \frac{\tilde{C}_{A}^{i}(0\_ap) \cdot m_{D}^{A}}{m_{D}^{A} + m_{D}^{WES}} = \frac{W_{i}^{i} \cdot m_{A}^{i} \cdot m_{D}^{A} \cdot 10^{6}}{\left(m_{A}^{WES} + \sum_{i} m_{A}^{i}\right) \cdot \left(m_{D}^{A} + m_{D}^{WES}\right)}, \quad (27)$$

$$\tilde{C}_{B(E)}^{i}(0\_ap) = 0, \quad (28)$$

$$\tilde{C}_{QC}^{i}(0\_ap) = \frac{\tilde{C}_{D}^{i}(0\_ap) \cdot m_{QC}^{D}}{m_{QC}^{D} + m_{QC}^{E}}, \quad (29)$$

where $\tilde{C}_{A}^{i}$ is the concentration of compound *i* in solution A, µg/g; $\tilde{C}_{C(SS)}^{i}$ is the concentration of compound *i* in C (or SS such as "0", "0.1", "0.5", "1.0", "2.0"), µg/g; $\tilde{C}_{D}^{i}$, $\tilde{C}_{B(E)}^{i}$ and $\tilde{C}_{QC}^{i}$ are the concentration values of compound *i* (or IS) in solutions D, B (or E) and QC, µg/g.

The first-order and second-order approximations are the calculations taking into account the impurity content in the WES. The first and second approximations allow to obtain the most accurate values for the concentration of volatile compounds and values of *RRF* in the case of impure WES.

Initially, the compound concentrations in solution C are calculated as zero-order approximation.

Then, the WES (or blank test solution) and standard solution C are chromatographed, the peaks of volatile compounds and ethanol are integrated and obtained values of peak area are recorded. If the chromatograms of WES (or blank test solution) contain only ethanol peaks and do not contain the peaks of volatile congeners, then apply only zero approximation calculations. Perform calculations in the first and second approximations in the case of impure WES. This allows for more accurate values for component concentrations in standard solutions and values of *RRF*.

Chromatograph Calibration

Equipment and GC Conditions

- A temperature programmed gas chromatograph fitted with a flame ionisation detector (FID), S/SL injector and integrator or other data handling system capable of measuring peak areas
- Gas chromatographic column(s), capable of separating the analytes such that the minimum resolution between the individual components (other than 2-methylbutan-1-ol and 3-methylbutan-1-ol) is, as a guide, at least 1.3, if a simple visual examination of the chromatogram is not sufficient
- Vial crimper, chromatographic vials, septa and caps for them

NOTE – The following columns and GC conditions are given as suitable examples (Table 5):

Table 5

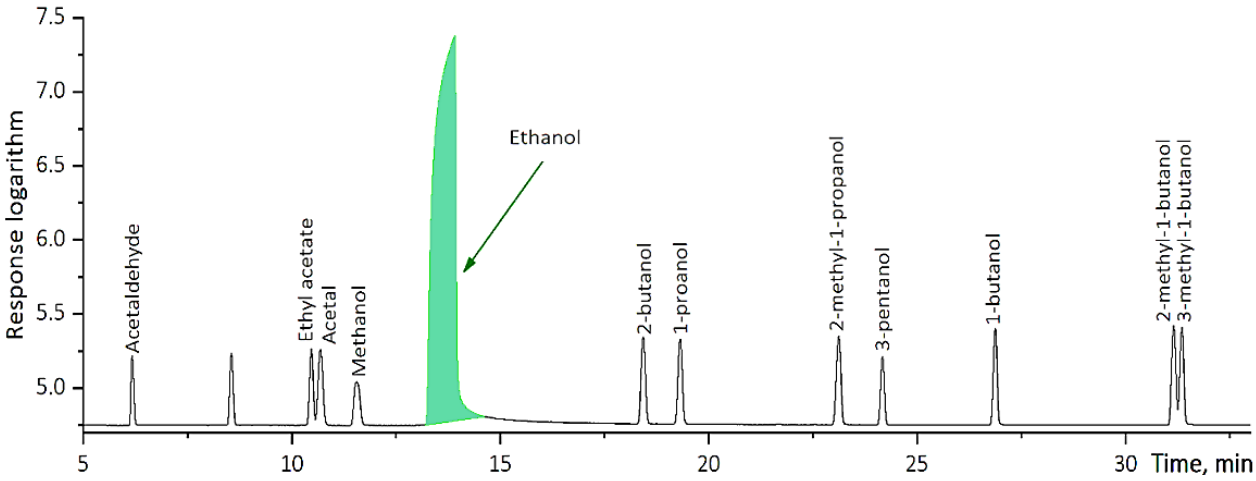
1	A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 µm film thickness. (Columns are connected using press-fit connectors.)
	Carrier gas and pressure: Helium (135 kPa)
	Column temperature:35 °C for 17 min., 35 °C to 70 °C at 12 °C/min., hold at 70 C for 25 min.
	Injector temperature: 150 °C
	Detector temperature: 250 °C
	Injection volume: 1 µL, split 20 to 100:1
2	A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol). (Retention gap is connected using a press-fit connector.)
	Carrier gas and pressure: Helium (65 kPa)
	Column temperature: 35 °C for 10 min, 35 °C to 110 °C at 5 °C/min, 110 °C to 190 °C at 30 °C/min, hold at 190 °C for 2 min.
	Injector temperature: 260 °C
	Detector temperature: 300 °C
	Injection volume: 1 µL, split 55:1

Blank test, Chromatography of WES

Acquire 2-3 chromatograms of 40% v/v ethanol solution (WES) in the case the using EIS method only or special solution BT in the case the using of the both EIS and TIS methods. Measure peak areas for volatile compounds and ethanol and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline.

Preliminary test, Chromatography of Standard Solution C

Inject standard solution C into chromatograph to ensure that all of the analytes are separated with a minimum resolution of 1.3 (except 2-methyl-1-butanol and 3-methyl-1-butanol). Measure peak areas for volatile compounds and ethanol and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline. The calibration solution C is chromatographed at least twice. The example of chromatogram is presented below:



Calculation of Calibration Coefficients RRF and Certified Values of Volatile Compound Concentration in Standard Solutions

Calibration characteristics are named relative response factors (*RRF*) expressing the dependence of the peak area ratio of volatile compound *i* and internal standard (ethyl alcohol or traditional IS) on the concentration ratios of the volatile compound *i* and internal standard (ethyl alcohol or traditional IS) are established using standard solution C.

When the **method EIS** is used, initially, the calibration coefficients are calculated as zero approximation using the formula:

$$RRF_i^{Eth}(0\_\text{ap}) = \frac{C_C^i(0\_\text{ap})}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{Eth}\right)}{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{Eth}\right)^2}, \quad (30)$$

where $RRF_i^{Eth}(0\_\text{ap})$ is the relative response factor of the compound *i* to ethyl alcohol in zero approximation; $C_C^i(0\_\text{ap})$ is the value of concentration of compound *i* in standard solution C in zero approximation (formula 10), g/100 L AA; $A_{C,j}^i$ is the detector response value to the compound *i*, obtained as a result of the measurement *j* of solution C, in peak area units; $A_{C,j}^{Eth}$ is the detector response to ethyl alcohol obtained as a result of the measurement *j* of solution C, units of peak area; N_C is the number of measurements of solution C ($N_C \geq 2$).

The first and second approximations are calculations taking into account the impurity content in the WES (if it is necessary) and allow to obtain the most accurate values for the concentration of volatile compounds and values of *RRF*.

In the first approximation, the mass concentration $C_{WES}^i(1\_\text{ap})$ of the compound *i* in the WES in g/100 L AA and the mass fraction $W_{WES}^i(1\_\text{ap})$ of the compound *i* in the WES in mg/mg of solution are calculated using the formulas (31), (32) and (34) in the case of chromatography of the special BT solution with the addition of IS. If the chromatograms of WES without the addition of the solution E are recorded as the blank test, then the calculations are performed using formulas (33) and (34):

$$C_{BT}^i(1\_\text{ap}) = RRF_i^{Eth}(0\_\text{ap}) \cdot \rho_{Eth} \cdot \frac{1}{N_{BT}} \sum_{j=1}^{N_{BT}} \frac{A_{BT,j}^i}{A_{BT,j}^{Eth}}, \quad (31)$$

$$C_{WES}^i(1\_\text{ap}) = \frac{C_{BT}^i(1\_\text{ap})}{0.997}, \quad (32)$$

$$C_{WES}^i(1\_\text{ap}) = RRF_i^{Eth}(0\_\text{ap}) \cdot \rho_{Eth} \cdot \frac{1}{N_{WES}} \sum_{j=1}^{N_{WES}} \frac{A_{WES,j}^i}{A_{WES,j}^{Eth}}, \quad (33)$$

$$W_{WES}^i(1\_\text{ap}) = \frac{C_{WES}^i(1\_\text{ap}) \cdot ABV}{\rho_{WES} \cdot 100}, \quad (34)$$

where $A_{BT,j}^i$ is the detector response value to the compound *i*, obtained as a result of the measurement *j* of solution BT, in peak area units; $A_{BT,j}^{Eth}$ is the detector response to ethyl alcohol obtained as a result of the measurement *j* of solution BT, units of peak area; N_{BT} is

the number of measurements of solution BT, $N_{BT} \geq 2$; $A^{i}_{WES,j}$ is the detector response value to the compound *i*, obtained as a result of the measurement *j* of WES, in peak area units; $A^{Eth}_{WES,j}$ is the detector response to ethyl alcohol obtained as a result of the measurement *j* of WES, units of peak area; N_{WES} is the number of measurements of WES, $N_{WES} \geq 2$; $\rho_{Eth} = 78927$ g/100 L ; $\rho_{Eth} = 78927$ g/100 L ; *ABV* is the volume content of ethyl alcohol in the WES, %; ρ_{WES} is the density of WES in units of g/100 L, ρ_{WES} [g/100 L] = ρ_{WES} [g/L]·100.

In the first approximation, the mass parts $W^{i}_{A}(1\_\text{ap})$ and $W^{i}_{B}(1\_\text{ap})$ of the volatile component *i* in solutions A and B in mg/mg of the solution are calculated using the formulas:

$$W^{i}_{A}(1\_\text{ap}) = \frac{W^{i}_{i} \cdot m^{i}_{A} + W^{i}_{WES}(1\_\text{ap}) \cdot m^{WES}_{A}}{m^{WES}_{A} + \sum_{i} m^{i}_{A}}, \quad (6`)$$

$$W^{i}_{B}(1\_\text{ap}) = \frac{W^{i}_{WES}(1\_\text{ap}) \cdot m^{WES}_{B}}{m^{WES}_{B} + m^{IS}_{B}}. \quad (7`)$$

In the first approximation, the mass concentration $C^{i}_{C}(1\_\text{ap})$ of compound *i* in standard solution C in units of g/100 L AA is calculated using the formula:

$$C^{i}_{C}(1\_\text{ap}) = \frac{W^{i}_{A}(1\_\text{ap}) \cdot m^{A}_{C} + W^{i}_{B}(1\_\text{ap}) \cdot m^{B}_{C} + W^{i}_{WES}(1\_\text{ap}) \cdot m^{WES}_{C}}{W^{Eth}_{A} \cdot m^{A}_{C} + W^{Eth}_{B} \cdot m^{B}_{C} + W^{Eth}_{WES} \cdot m^{WES}_{C}} \cdot \rho_{Eth}. \quad (9`)$$

The calibration coefficient $RRF^{Eth}_{i}(1\_\text{ap})$ of the volatile component *i* is calculated as first approximation using the formula:

$$RRF^{Eth}_{i}(1\_\text{ap}) = \frac{C^{i}_{C}(1\_\text{ap})}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_C}\left(A^{i}_{C,j} / A^{Eth}_{C,j}\right)}{\sum_{j=1}^{N_C}\left(A^{i}_{C,j} / A^{Eth}_{C,j}\right)^{2}}. \quad (30`)$$

In the second approximation, the mass concentration $C^{i}_{WES}(2\_\text{ap})$ of the compound *i* in the WES in g/100 L AA and the mass fraction $W^{i}_{WES}(2\_\text{ap})$ of the compound *i* in the WES in mg/mg of solution are calculated using the formulas using the formulas (31`), (32`) and (34`) in the case of chromatography of the special BT solution with the addition of IS. If the chromatograms of WES without the addition of the solution E are recorded as the blank test, then the calculations are performed using formulas (33`) and (34`):

$$C_{BT}^{i}(2\_\text{ap}) = RRF_{i}^{Eth}(1\_\text{ap}) \cdot \rho_{Eth} \cdot \frac{1}{N_{BT}} \sum_{j=1}^{N_{BT}} \frac{A_{BT,j}^{i}}{A_{BT,j}^{Eth}}, \quad (31`)$$

$$C_{WES}^{i}(2\_\text{ap}) = \frac{C_{BT}^{i}(2\_\text{ap})}{0.997}, \quad (32`)$$

$$C_{WES}^{i}(2\_\text{ap}) = RRF_{i}^{Eth}(1\_\text{ap}) \cdot \rho_{Eth} \cdot \frac{1}{N_{WES}} \sum_{j=1}^{N_{WES}} \frac{A_{WES,j}^{i}}{A_{WES,j}^{Eth}}, \quad (33`)$$

$$W_{WES}^{i}(2\_\text{ap}) = \frac{C_{WES}^{i}(2\_\text{ap}) \cdot ABV}{\rho_{WES} \cdot 100}. \quad (34`)$$

In the second approximation, the mass parts $W_{A}^{i}(2\_\text{ap})$ and $W_{B}^{i}(1\_\text{ap})$ of the volatile component *i* in solutions A and B in mg/mg of the solution are calculated using the formulas:

$$W_{A}^{i}(2\_\text{ap}) = \frac{W_{i}^{i} \cdot m_{A}^{i} + W_{WES}^{i}(2\_\text{ap}) \cdot m_{A}^{WES}}{m_{A}^{WES} + \sum_{i} m_{A}^{i}}. \quad (6``)$$

$$W_{B}^{i}(2\_\text{ap}) = \frac{W_{WES}^{i}(2\_\text{ap}) \cdot m_{B}^{WES}}{m_{B}^{WES} + m_{B}^{IS}}. \quad (7``)$$

In the second approximation, the mass concentration $C_{C}^{i}(2\_\text{ap})$ of compound *i* in standard solution C in units of g/100 L AA is calculated using the formula:

$$C_{C}^{i}(2\_\text{ap}) = \frac{W_{A}^{i}(2\_\text{ap}) \cdot m_{C}^{A} + W_{B}^{i}(2\_\text{ap}) \cdot m_{C}^{B} + W_{WES}^{i}(2\_\text{ap}) \cdot m_{C}^{WES}}{W_{A}^{Eth} \cdot m_{C}^{A} + W_{B}^{Eth} \cdot m_{C}^{B} + W_{WES}^{Eth} \cdot m_{C}^{WES}} \cdot \rho_{Eth}. \quad (9``)$$

The most exact concentration values $C_{A}^{i}(2\_\text{ap})$, $C_{B}^{i}(2\_\text{ap})$, $C_{D}^{i}(2\_\text{ap})$, $C_{E}^{i}(2\_\text{ap})$, $C_{SS}^{i}(2\_\text{ap})$ and $C_{QC}^{i}(2\_\text{ap})$ of volatile compound *i* in solutions A, B, D, E, SS such as “0”, “0.1”, “0.5”, “1.0”, “2.0”, quality control solution QC in units of g/100 L AA are calculated as the second-order approximation using the formulas:

$$C_{A}^{i}(2\_\text{ap}) = \frac{W_{i}^{i} \cdot m_{A}^{i} + W_{WES}^{i}(2\_\text{ap}) \cdot m_{A}^{WES}}{W_{WES}^{Eth} \cdot m_{A}^{WES}} \cdot \rho_{Eth}, \quad (35)$$

$$C_{B}^{i}(2\_\text{ap}) = \frac{W_{WES}^{i}(2\_\text{ap}) \cdot m_{B}^{WES}}{W_{WES}^{Eth} \cdot m_{B}^{WES}} \cdot \rho_{Eth}, \quad (36)$$

$$C_D^i(2\_\text{ap}) = \frac{W_A^i(2\_\text{ap}) \cdot m_D^A + W_{WES}^i(2\_\text{ap}) \cdot m_D^{WES}}{W_A^{Eth} \cdot m_D^A + W_{WES}^{Eth} \cdot m_D^{WES}} \cdot \rho_{Eth}, \quad (37)$$

$$C_E^i(2\_\text{ap}) = \frac{W_B^i(2\_\text{ap}) \cdot m_E^B + W_{WES}^i(2\_\text{ap}) \cdot m_E^{WES}}{W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}} \cdot \rho_{Eth}, \quad (38)$$

$$C_{SS}^i(2\_\text{ap}) = \frac{W_A^i(2\_\text{ap}) \cdot m_{SS}^A + W_B^i(2\_\text{ap}) \cdot m_{SS}^B + W_{WES}^i(2\_\text{ap}) \cdot m_{SS}^{WES}}{W_A^{Eth} \cdot m_{SS}^A + W_B^{Eth} \cdot m_{SS}^B + W_{WES}^{Eth} \cdot m_{SS}^{WES}} \cdot \rho_{Eth}, \quad (39)$$

$$C_{QC}^i(2\_\text{ap}) = \frac{\frac{W_A^i(2\_\text{ap}) \cdot m_D^A + W_{WES}^i(2\_\text{ap}) \cdot m_D^{WES}}{m_D^A + m_D^{WES}} m_{QC}^D + \frac{W_B^i(2\_\text{ap}) \cdot m_E^B + W_{WES}^i(2\_\text{ap}) \cdot m_E^{WES}}{m_E^B + m_E^{WES}} m_{QC}^E}{\frac{W_A^{Eth} \cdot m_D^A + W_{WES}^{Eth} \cdot m_D^{WES}}{m_D^A + m_D^{WES}} \cdot m_{QC}^D + \frac{W_B^{Eth} \cdot m_E^B + W_{WES}^{Eth} \cdot m_E^{WES}}{m_E^B + m_E^{WES}} \cdot m_{QC}^E} \rho_{Eth}. \quad (40)$$

The calibration coefficient $RRF_i^{Eth}(2\_\text{ap})$ of volatile compound *i* is calculated as second approximation using the formula:

$$RRF_i^{Eth}(2\_\text{ap}) = \frac{C_C^i(2\_\text{ap})}{\rho_{Eth}} \cdot \frac{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{Eth}\right)}{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{Eth}\right)^2}. \quad (30``)$$

When **the method TIS** is used, the calibration coefficients are calculated using the formula:

$$RRF_i^{IS}(0\_\text{ap}) = \frac{\tilde{C}_C^i(0\_\text{ap})}{\tilde{C}_C^{IS}} \cdot \frac{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{IS}\right)}{\sum_{j=1}^{N_C}\left(A_{C,j}^i / A_{C,j}^{IS}\right)^2}, \quad (41)$$

where $RRF_i^{IS}(0\_\text{ap})$ is the relative response factor of the compound *i* to IS in the zero approximation; $\tilde{C}_C^{IS}$ is the value of concentration of IS in solution C (formula 22), in µg/g; $\tilde{C}_C^i(0\_\text{ap})$ is the value of concentration of compound *i* in solution C as the zero approximation (formula 26), in µg/g; $A_{C,j}^i$ and $A_{C,j}^{IS}$ are the detector responses to compound *i* and IS obtained as a result of the measurement *j* of solution C, units of peak area; N_C is the number of measurements of solution C ($N_C \geq 2$).

The first and second approximations are calculations taking into account the impurity content

in the WES (if it is necessary) and allow to obtain the most accurate values for the concentration of volatile compounds and values of *RRF*.

In the first approximation, the concentration values $\tilde{C}^i_{WES}(1\_\text{ap})$, $\tilde{C}^i_A(1\_\text{ap})$, $\tilde{C}^i_B(1\_\text{ap})$, $\tilde{C}^i_C(1\_\text{ap})$ for all volatile compounds in WES and standard solutions A, B, C are calculated using the formulas:

$$\tilde{C}^i_{WES}(1\_\text{ap}) = RRF^{IS}_i(0\_\text{ap}) \cdot \frac{m^E_{BT}}{m^{WES}_{BT}} \cdot \tilde{C}^{IS}_E \cdot \frac{1}{N_{BT}} \sum_{j=1}^{N_{BT}} \frac{A^i_{BT,j}}{A^{IS}_{BT,j}}, \tag{42}$$

$$\tilde{C}^i_A(1\_\text{ap}) = \frac{\tilde{C}^i_{WES}(1\_\text{ap}) \cdot m^{WES}_A + W^i_i \cdot 10^6 \cdot m^i_A}{m^{WES}_A + \sum_i m^i_A}, \tag{43}$$

$$\tilde{C}^i_B(1\_\text{ap}) = \frac{\tilde{C}^i_{WES}(1\_\text{ap}) \cdot m^{WES}_B}{m^{WES}_B + m^{IS}_B}, \tag{44}$$

$$\tilde{C}^i_C(1\_\text{ap}) = \frac{\tilde{C}^i_A(1\_\text{ap}) \cdot m^A_C + \tilde{C}^i_B(1\_\text{ap}) \cdot m^B_C + \tilde{C}^i_{WES}(1\_\text{ap}) \cdot m^{WES}_C}{m^A_C + m^B_C + m^{WES}_C}, \tag{45}$$

where $\tilde{C}^{IS}_E$ is the value of concentration of IS in solution E (formula 21), in μg/g; m^E_{BT} is the mass of solution E added into solution BT, mg; m^{WES}_{BT} is the mass of WES added into solution BT, mg; $A^i_{BT,j}$ is the detector response value to the compound *i*, obtained as a result of the measurement *j* of solution BT, in peak area units; $A^{IS}_{BT,j}$ is the detector response to IS obtained as a result of the measurement *j* of solution BT, units of peak area; N_{BT} is the number of measurements of solution BT, $N_{BT} \geq 2$.

Then, calibration coefficients $RRF^{IS}_i(1\_\text{ap})$ in the first approximation are calculated using the formula:

$$RRF^{IS}_i(1\_\text{ap}) = \frac{\tilde{C}^i_C(1\_\text{ap})}{\tilde{C}^{IS}_C} \cdot \frac{\sum_{j=1}^{N_C}\left(A^i_{C,j} / A^{IS}_{C,j}\right)}{\sum_{j=1}^{N_C}\left(A^i_{C,j} / A^{IS}_{C,j}\right)^2}. \tag{41`}$$

In the second approximation, the concentration values $\tilde{C}^i_{WES}(2\_\text{ap})$, $\tilde{C}^i_A(2\_\text{ap})$, $\tilde{C}^i_B(2\_\text{ap})$, $\tilde{C}^i_C(2\_\text{ap})$, $\tilde{C}^i_{SS}(2\_\text{ap})$, $\tilde{C}^i_{D(E)}(2\_\text{ap})$, $\tilde{C}^i_{QC}(2\_\text{ap})$ for all volatile compounds

in WES and standard solutions A, B, C and other standard solutions, in μg/g, are calculated using the formulas:

$$\tilde{C}^{i}_{WES}(2\_\text{ap}) = RRF_i^{IS}(1\_\text{ap}) \cdot \frac{m^{E}_{BT}}{m^{WES}_{BT}} \cdot \tilde{C}^{IS}_{E} \cdot \frac{1}{N_{BT}} \sum_{j=1}^{N_{BT}} \frac{A^{i}_{BT,j}}{A^{IS}_{BT,j}}, \tag{42`}$$

$$\tilde{C}^{i}_{A}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{WES}(2\_\text{ap}) \cdot m^{WES}_{A} + W^{i}_{i} \cdot 10^{6} \cdot m^{i}_{A}}{m^{WES}_{A} + \sum_{i} m^{i}_{A}}, \tag{43`}$$

$$\tilde{C}^{i}_{B}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{WES}(2\_\text{ap}) \cdot m^{WES}_{B}}{m^{WES}_{B} + m^{IS}_{B}}, \tag{44`}$$

$$\tilde{C}^{i}_{C}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{A}(2\_\text{ap}) \cdot m^{A}_{C} + \tilde{C}^{i}_{B}(2\_\text{ap}) \cdot m^{B}_{C} + \tilde{C}^{i}_{WES}(2\_\text{ap}) \cdot m^{WES}_{C}}{m^{A}_{C} + m^{B}_{C} + m^{WES}_{C}}, \tag{45`}$$

$$\tilde{C}^{i}_{SS}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{A}(2\_\text{ap}) \cdot m^{A}_{SS} + \tilde{C}^{i}_{B}(2\_\text{ap}) \cdot m^{B}_{SS} + \tilde{C}^{i}_{WES}(2\_\text{ap}) \cdot m^{WES}_{SS}}{m^{A}_{SS} + m^{B}_{SS} + m^{WES}_{SS}}, \tag{46}$$

$$\tilde{C}^{i}_{D(E)}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{A(B)}(2\_\text{ap}) \cdot m^{A(B)}_{D(E)} + \tilde{C}^{i}_{WES}(2\_\text{ap}) \cdot m^{WES}_{D(E)}}{m^{A(B)}_{D(E)} + m^{WES}_{D(E)}}, \tag{47}$$

$$\tilde{C}^{i}_{QC}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{D}(2\_\text{ap}) \cdot m^{D}_{QC} + \tilde{C}^{i}_{E}(2\_\text{ap}) \cdot m^{E}_{QC}}{m^{D}_{QC} + m^{E}_{QC}}, \tag{48}$$

As second approximation, calibration coefficients $RRF_i^{IS}(2\_\text{ap})$ are calculated using the formula:

$$RRF_i^{IS}(2\_\text{ap}) = \frac{\tilde{C}^{i}_{C}(2\_\text{ap})}{\tilde{C}^{IS}_{C}} \cdot \frac{\sum_{j=1}^{N_C} \left(A^{i}_{C,j} / A^{IS}_{C,j} \right)}{\sum_{j=1}^{N_C} \left(A^{i}_{C,j} / A^{IS}_{C,j} \right)^2}. \tag{41``}$$

Thus, if WES contains only ethanol and water without impurity congeners, the values of calibration coefficients RRF_i^{Eth} and RRF_i^{IS} are calculated as zero approximation according the formulas (30) and (41). The most exactly values of RRF_i^{Eth} and RRF_i^{IS} in the case impure WES can be calculated using formulas (30``) and (41``) as second approximation.

The Testing of FID Response Linearity

The calibration should be checked using the following procedure. Inject three times standard solutions "0", "0.1", "0.5", "1.0", "2.0" for the testing of FID response linearity into chromatograph. Measure peak areas for volatile compounds and internal standard (ethanol or traditional IS) and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline.

When **the method EIS** is used, plot the following relationships using obtained data for SS "0", "0.1", "0.5", "1.0", "2.0":

- *x*-axis*:* certified concentration (g/100 L AA) of the volatile compound *i* in linearity standard solution / density of anhydrous ethanol (78927 g/100 L) C_{SS}^{i} / ρ_{Eth};
- *y*-axis: the detector response to the volatile compound *i* (peak area), obtained during measurement of linearity standard solution / detector response to the ethanol (peak area), obtained during measurement of linearity standard solution $A_{SS,j}^{i} / A_{SS,j}^{Eth}$.

Concentration C_{SS}^{i} in units of g/100 L AA of volatile compound *i* in SS is from formula (12) in the case WES without impurities and is from formula (39) in the case WES with impurities of volatile congeners. Concentration of ethanol in solutions in units of g/100 L AA is equal to density of anhydrous ethanol 78927 g/100 L, therefore $C_{SS}^{Eth} / \rho_{Eth} = 1$.

When **the method TIS** is used, plot the following relationships using obtained data for SS "0", "0.1", "0.5", "1.0", "2.0":

- *x*-axis*:* certified concentration (µg/g) of the volatile compound *i* / certified concentration (µg/g) of traditional IS in linearity standard solution $\tilde{C}_{SS}^{i} / \tilde{C}_{SS}^{IS}$;
- *y*-axis: the detector response to the volatile compound *i* (peak area), obtained during measurement of linearity standard solution / detector response to the traditional IS (peak area), obtained during measurement of linearity standard solution $A_{SS,j}^{i} / A_{SS,j}^{IS}$.

Concentration $\tilde{C}_{SS}^{i}$ in units of µg/g of volatile compound *i* in SS is from formula (26) in the case WES without impurities and is from formula (46) in the case WES with impurities of volatile congeners.

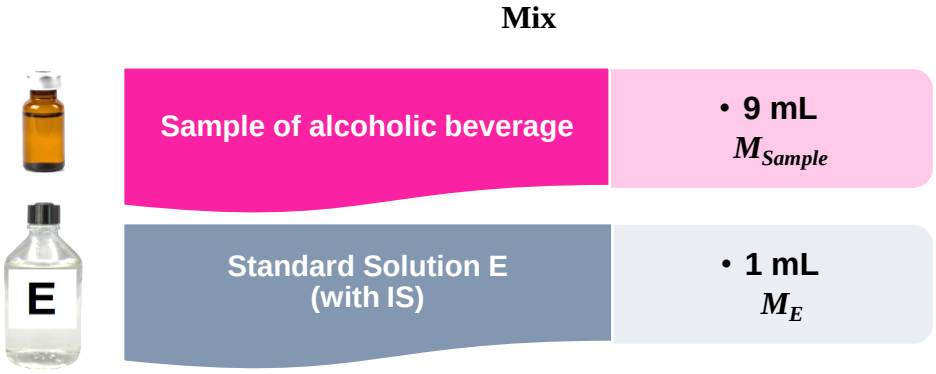
The linearity of the FID response is verified by measuring all standard linearity solutions at least every six months, as well as during column replacement and after chromatograph repair. When verifying the linearity of the detector response, it is recommended to use linear regression without an intercept. The Pearson linear correlation coefficient or the R^2 determination coefficient is established using the gas chromatograph software.

A linear plots should be obtained with a correlation coefficients of at least 0.99.

Analysis of alcoholic beverages

Preparation of Beverage Samples

Pipette 9 mL beverage sample and 1 mL solution E into vessel and mix thoroughly. Record the weight of the vessel, the mass of beverage sample M_{Sample} and solution E M_E and the total final weight of contents.



It is necessary to note that no special preparation of the alcoholic beverage sample is required for working with the EIS method alone without a TIS [1, 2]. The beverage sample or distillate itself is injected directly into the chromatograph.

Determination of volatile compound concentration in beverage samples

It is advisable to store the samples at less than 5.0 °C prior to analysis in order to minimise any volatile losses.

Inject standard solution C and 2 samples of QC standard solution into chromatograph. Follow with unknown beverage samples inserting one QC standard every 10 samples to ensure analytical stability. Inject one standard solution C after every 5 beverage samples.

Chromatography beverage samples at least two times. Measure peak areas for volatile

compounds (congeners) and internal standard (ethanol or traditional IS) and record the peak area values. When integrating the ethanol peak, it is recommended to use a valley-to-valley baseline.

When the **method EIS** is used, calculate the concentration values of congeners in the samples in g/100 L AA using the formula:

$$C^{i}_{Sample} = RRF_{i}^{Eth} \cdot \rho_{Eth} \cdot \frac{1}{N} \sum_{j=1}^{N} \frac{A^{i}_{j}}{A^{Eth}_{j}}, \tag{49}$$

where C^{i}_{Sample} is the mean of congener *i* concentration in the sample, g/100 L AA; RRF_{i}^{Eth} is the relative response factor of the compound *i* to ethyl alcohol; ρ_{Eth} = 78927 g/100 L; A^{i}_{j} is the detector response to the compound *i*, obtained as a result of the measurement *j* of the sample, in peak area units; A^{Eth}_{j} is the detector response to ethyl alcohol obtained as a result of the measurement *j* of the sample, units of peak area; *N* is the number of measurements of the sample ($N \geq 2$).

When the **method TIS** is used, calculate the concentration values of congeners in the samples in µg/g using the formula:

$$\tilde{C}^{i}_{Sample} = RRF_{i}^{IS} \cdot \frac{M_{E}}{M_{Sample}} \cdot \tilde{C}^{IS}_{E} \cdot \frac{1}{N} \sum_{j=1}^{N} \frac{A^{i}_{j}}{A^{IS}_{j}}, \tag{50}$$

where $\tilde{C}^{i}_{Sample}$ is the mean of congener *i* concentration in the sample, µg/g; RRF_{i}^{IS} is the relative response factor of the compound *i* to IS; $\tilde{C}^{IS}$ is the IS concentration in the sample, µg/g; M_{Sample} is the mass of beverage sample, mg; M_{E} is the mass of solution E, mg; A^{i}_{j} is the detector response to the compound *i*, obtained as a result of the measurement *j* of the sample, in peak area units; A^{IS}_{j} is the detector response to IS obtained as a result of the measurement *j* of the sample, units of peak area; *N* is the number of measurements of the sample ($N \geq 2$).

To express congener concentrations in units of g/100 L AA according to the method TIS, it is necessary to accurately determine the density of alcoholic beverage ρ_{Sample} and ethyl alcohol content by volume in the beverage ABV_{Sample}. The formula for converting concentration values from µg/g units to g/100 L AA is:

$$C^{i}_{Sample}[\text{g/100 L AA}] = \frac{\tilde{C}^{i}_{Sample}[\mu\text{g/g}] \cdot \rho_{Sample}[\text{g/L}]}{ABV_{Sample}[\%] \cdot 100}. \tag{51}$$

Final Presentation of Results

Results of the congener concentration determination in beverage samples are presented with an accuracy of 3 significant digits and no more than one decimal place, for example, 11.4 g/100 L AA or 0.5 g/100 L AA or 874 g/100 L AA.

Relative standard deviation RSD_i in % and standard deviation SD_i in g/100 L AA are calculated using formulas:

$$RSD_i = \sqrt{\sum_{j=1}^{N} \frac{\left(\left(A_j^i / A_j^{Eth} \right) / \left\langle A_j^i / A_j^{Eth} \right\rangle - 1 \right)^2}{(N-1)}} \cdot 100\%, \tag{52}$$

$$\left\langle A_j^i / A_j^{Eth} \right\rangle = \frac{1}{N} \left(\sum_{j=1}^{N} \frac{A_j^i}{A_j^{Eth}} \right), \tag{53}$$

$$SD_i[\text{g/100 L AA}] = \frac{RSD_i}{100\%} \cdot C_{Sample}^i[\text{g/100 L AA}], \tag{54}$$

where C_{Sample}^i is the mean of congener *i* concentration in the sample, g/100 L AA; A_j^i is the detector response to the compound *i*, obtained as a result of the measurement *j* of the sample, in peak area units; A_j^{Eth} is the detector response to ethyl alcohol obtained as a result of the measurement *j* of the sample, units of peak area; $\left\langle A_j^i / A_j^{Eth} \right\rangle$ is the mean of peak area ratio; *N* is the number of measurements of the sample ($N \geq 2$).

Quality Assurance and Quality Control

Accuracy is the closeness of the results obtained by the analytical method to the true values. Accuracy can be assessed by analysing a sample of known concentration $C^i(certified)$, for example, standard solution QC (formulas 14, 23 as zero approximation or 40, 48 as second approximation) or any other standard solution except for C and comparing the measured value $C^i(measured)$ to the true value $C^i(certified)$.

Using equation (49)-(51) above, calculate the means of compound concentration $C^i(measured)$ for all congeners in the quality control standard solution QC by the method EIS or TIS. Then, calculate the percentage deviation of the target value (*bias*) for all compounds in QC standard solution using formula:

$$bias_i = \frac{C^i(measured) - C^i(certified)}{C^i(certified)} \cdot 100\ \%. \quad (55)$$

If the analytical results are within ±10% of their true values for each congener, the analysis can proceed. Otherwise, an investigation should be conducted to determine the cause of the discrepancy and appropriate measures should be taken to correct it.

Reference:

[1] – Guide "Method for the Determination of Volatile Compounds in Spirit Drinks and Wine using contained Ethanol as a Reference Standard". *Version 1-1500. – only method EIS (Ethanol as IS) for concentration up to 1500 g/100 L AA*

[2] – Guide "Method for the Determination of Volatile Compounds in Spirit Drinks and Wine using contained Ethanol as a Reference Standard". *Version 1-150. – only method EIS (Ethanol as IS) for concentration up to 150 g/100 L AA*