GUIDE

Calculation of Compound Concentrations in Standard Solutions and Calibration Factors

during preparation the solutions in the accordance of the new OIV method OENO-SCMA 24-756 Et5_2026, using contained ethanol as a reference standard, (EIS method) and the OIV-MA-BS-14 method (TIS method)

Version Calc_SS-TIS_EIS-150

2026

Contents

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).

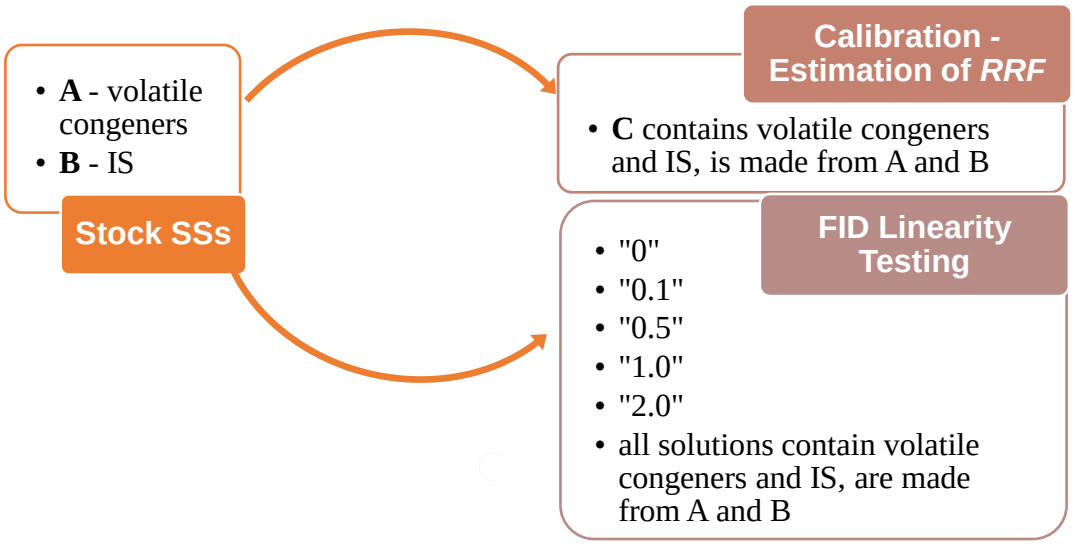
Preparation of 40 % v/v Ethanol Solution in Water (WES)

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.

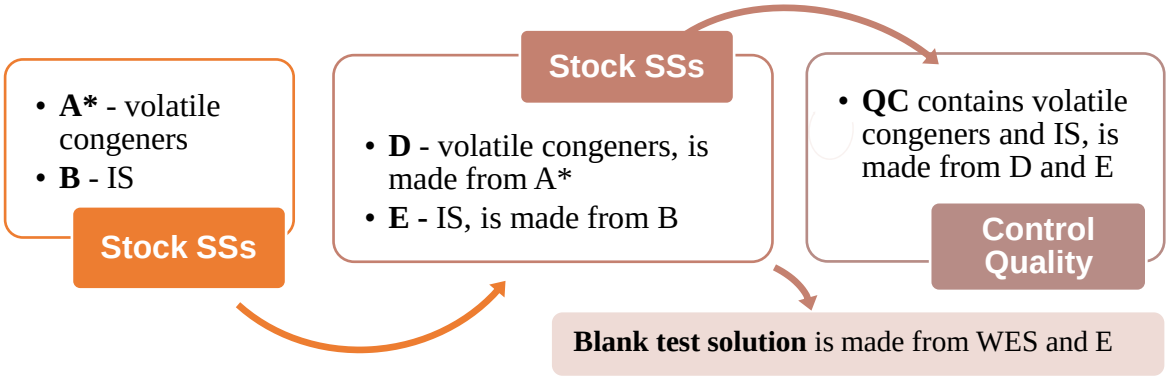
Standard Solutions (SSs)

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

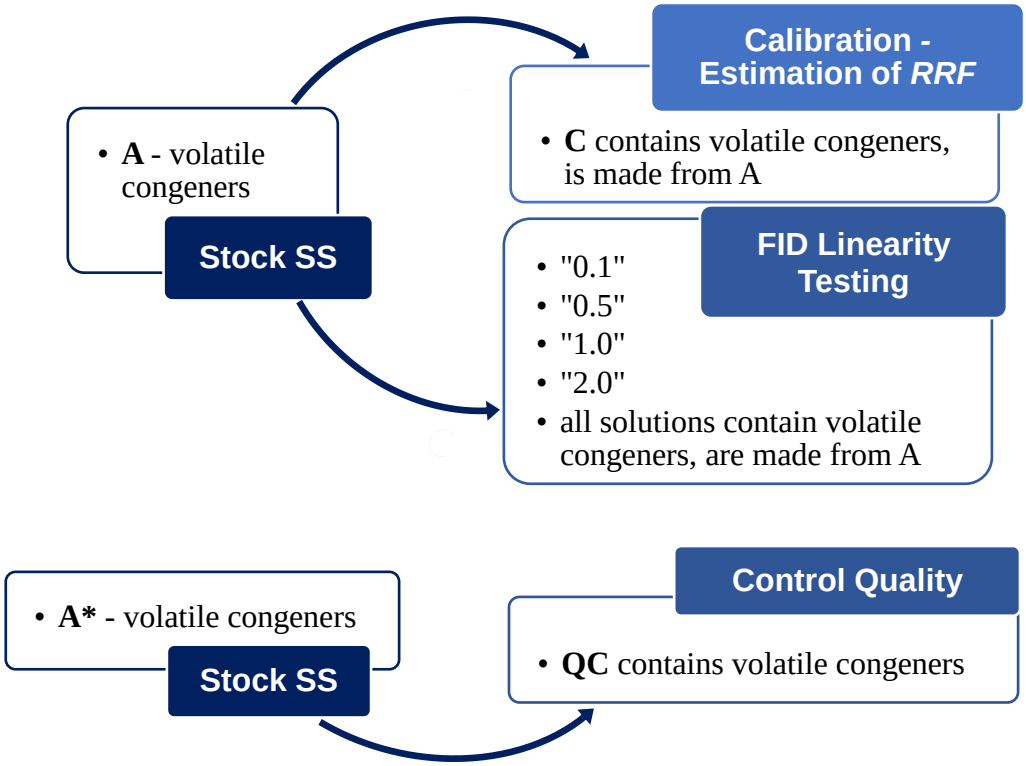
- stock standard solutions (A, A*, B, D, E) – concentrated solutions not subjected to chromatography,
- 1 standard solution C for chromatograph calibration and establishing the *RRF*,
- standard solutions (“0”, “0.1”, “0.5”, “1.0”, “2.0”) for the control of the linearity of the flame ionisation detector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

Into separate 100 mL volumetric flasks, containing approximately 60.0 mL of WES, 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 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 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 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 required. In this case, WES is used for blank test [1, 2].

Approximate Concentrations

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. ***You can use the free web-application "Calculator of volatile compound concentrations in standard solutions prepared in the accordance of OIV methods" for automatic calculation the mass concentration values of volatile compounds and IS in standard solutions and RRF values.***

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:

$$W_i^i = \frac{P_i^i}{100\ \%}, \tag{1}$$

$$W_{IS}^{IS} = \frac{P_{IS}^{IS}}{100\ \%}, \tag{2}$$

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:

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

where *ABV* is the volume content of ethanol in the WES, %; ρ_{WES} is the WES density, g/L under normal conditions; ρ_{Eth} is anhydrous ethanol density at 20 °C, ρ_{Eth} = 789.27 g/L.

In the EIS method, the mass part W_A^{Eth} of ethanol in solution A and the mass part W_B^{Eth} of ethanol 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 WES mass of added into solution D during its preparation, mg; ρ_{Eth} is anhydrous alcohol density at 20 °C, $\rho_{Eth} = 78927\,\text{g/100 L}$.

Concentration of congeners in blank test solution of $C_{BT}^{i}(0\_\text{ap}) = 0$ 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:

$$\tilde{C}_{B}^{IS} = \frac{W_{IS}^{IS} \cdot m_{B}^{IS}}{m_{B}^{WES} + m_{B}^{IS}} \cdot 10^{6}, \tag{20}$$

$$\tilde{C}_{E}^{IS} = \frac{\tilde{C}_{B}^{IS} \cdot m_{E}^{B}}{m_{E}^{B} + m_{E}^{WES}}, \tag{21}$$

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

$$\tilde{C}_{QC}^{IS} = \frac{\tilde{C}_{E}^{IS} \cdot m_{QC}^{E}}{m_{QC}^{D} + m_{QC}^{E}}, \tag{23}$$

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

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

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

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

$$\tilde{C}_D^i(0\_\text{ap}) = \frac{\tilde{C}_A^i(0\_\text{ap}) \cdot m_D^A}{m_D^A + m_D^{WES}} = \frac{W_i^i \cdot m_A^i \cdot m_D^A \cdot 10^6}{\left(m_A^{WES} + \sum_i m_A^i\right) \cdot \left(m_D^A + m_D^{WES}\right)}, \quad (27)$$

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

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

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 "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.

If it is necessary to convert the concentration values of compounds in a sample S between μg/g units and g/100 L AA, the formulas are:

$$C_S^i[\text{g/100 L AA}] = \frac{\tilde{C}_S^i[\mu\text{g/g}] \cdot \rho_S[\text{g/L}]}{ABV_S[\%] \cdot 100} = \frac{\tilde{C}_S^i[\mu\text{g/g}] \cdot \rho_{Eth}[\text{g/L}]}{W_S^{Eth}[mg/mg] \cdot 10^4}$$

$$\tilde{C}_S^i\left[\frac{\mu g}{g}\right] = \frac{C_S^i\left[\frac{\text{g}}{\text{100L AA}}\right] \cdot ABV_S[\%] \cdot 100}{\rho_S[\text{g/L}]} = \frac{C_S^i\left[\frac{\text{g}}{\text{100L AA}}\right] \cdot W_S^{Eth}\left[\frac{mg}{mg}\right] \cdot 10^4}{\rho_{Eth}[\text{g/L}]}$$

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*.

Exact concentrations and RRF

Equipment for Gas Chromatography (GC) and GC Conditions

- A temperature programmed gas chromatograph equipped 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

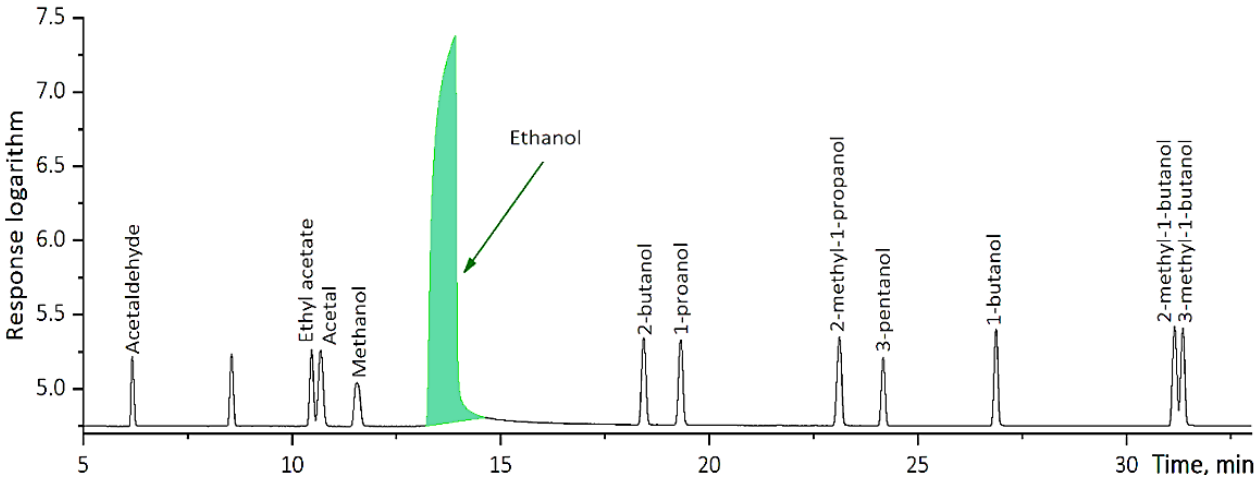
1	A retention gap 1 m x 0.32 mm i.d. connected to a CP-WAX 57 CB column 50 m x 0.32 mm i.d. 0.2 µm film thickness (stabilised polyethylene glycol) followed by a Carbowax 400 column 50 m x 0.32 mm i.d. 0.2 µm film thickness. (Columns are connected using press-fit connectors.)
	Carrier gas and pressure: 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 Exact 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 ethanol 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 ethanol 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 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 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:

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

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

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

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

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 $RRF_i^{IS}(1\_\text{ap})$ in the first approximation are calculated using the formula:

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

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:

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

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

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

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

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

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

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

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

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

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.