ООО “Новые аналитические системы”
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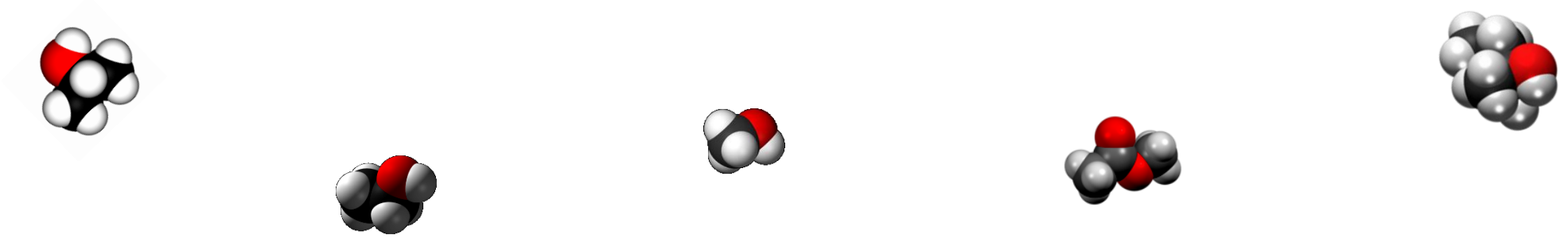
The Creation Of The Calibration Methods And The Analysis Of Alcoholic Drinks Using 3-Pentanol Or Ethanol As Internal Standards (ISTD) in Agilent ChemStation B.04.03



Minsk, 2025

1. The creation of calibration methods

1.1. Acquire the chromatograms of standard solution for calibration (SScalibr).

1.2. Click “Method” in the main menu and press “Load Method…” in the list.

1.3. Select any method and then save the method with new name: “Traditional ISTD” in the case of the using 3-pentanol as internal standard and “Ethanol ISTD” in the case of the using ethanol as internal standard.

1.4. Set and integrate the peaks of the volatiles compounds in the chromatograms.

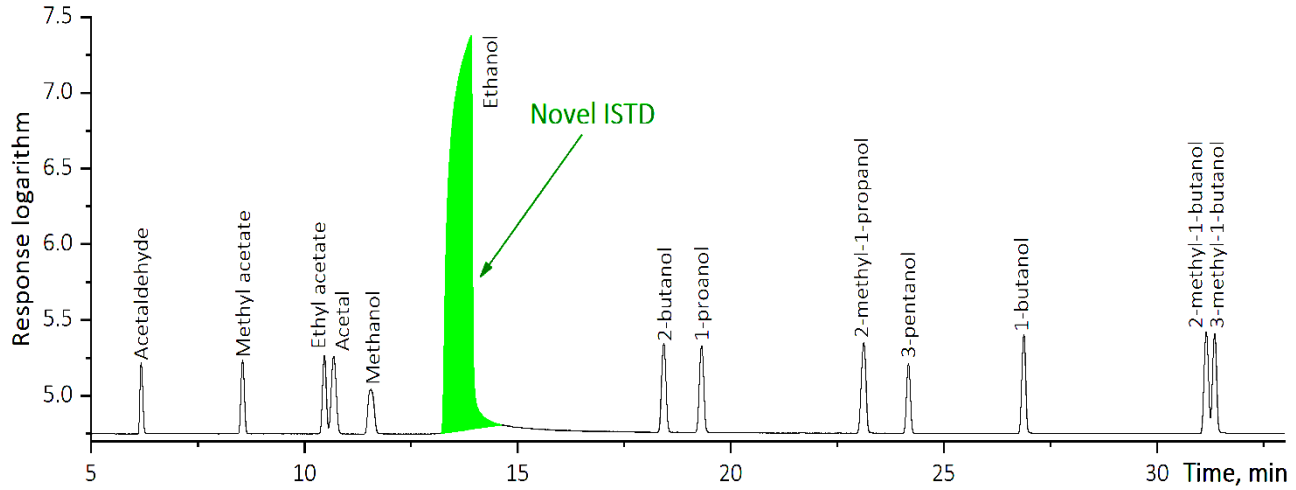
Standard solution
for calibration (SScalibr)

C [µg/g]

Recalculation

C [mg/L AA]

Chromatograms



$$C[\text{mg/L AA}] = C[\mu\text{g/g}] \cdot \rho[\text{g/L}]/(10 \cdot ABV)$$

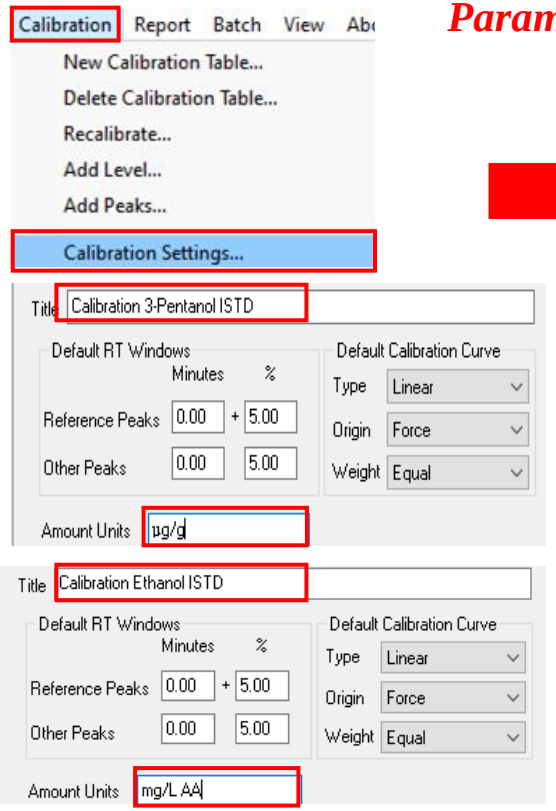
$C_{ethanol} = \rho_{ethanol} = 789270$ mg/L AA

1. The creation of calibration methods

1.5. Fill the input lines in dialog window “Calibration Setting”. Fill in the cell “Amount Units”: “µg/g” in the case of “Traditional ISTD” project and “mg/L AA” in the case of “Ethanol ISTD” project.

1.6. Input the names of the compounds in “Compound” column and the certified values of compound concentration in “Amt[µg/g]” or “Amt[mg/L AA]” column. It will be named these concentration values of compound i (i = 1, …, 13) in SScalibr as (µg/g) in the case of 3-pentanol as ISTD and (mg/L AA) in the case of ethanol as ISTD. Concentration of ethanol in mg/L AA is equal to ethanol density (= 789270 mg/L).

Parameters



Calibration tables

Calibration Table

Enter | Delete | Insert... | Print | OK

#	RT	Compound	ISTD	Lvl	Amt[µg/g]
1	6.2	acetaldehyde	No	1	180.68
2	8.6	methyl acetate	No	1	287.96
3	10.5	ethyl acetate	No	1	260.11
4	10.7	acetal	No	1	233.31
5	11.6	methanol	No	1	203.11
6	13.9	ethanol	No	1	333000.00
7	18.4	2-butanol	No	1	210.07
8	19.3	n-propanol	No	1	204.23
9	23.1	2-methyl-1-propanol	No	1	200.14
10	24.2	3-pentanol	Ye	1	226.16
11	26.9	n-butanol	No / Yes	1	209.60
12	31.2	2-methyl-1-butanol	Yes	1	216.87
13	31.3	3-methyl-1-butanol	No	1	207.88

Calibration Table

Enter | Delete | Insert... | Print | OK | Help

#	RT	Signal	Compound	ISTD	Lvl	Amt[mg/L AA]
1	6.181	FID1 A	acetaldehyde	No	1	428.250
2	8.554	FID1 A	methyl acetate	No	1	682.510
3	10.471	FID1 A	ethyl acetate	No	1	616.510
4	10.693	FID1 A	acetal	No	1	552.980
5	11.557	FID1 A	methanol	No	1	481.410
6	13.912	FID1 A	ethanol	Ye / No / Yes	1	789270.000
7	18.434	FID1 A	2-butanol	No	1	497.890
8	19.318	FID1 A	n-propanol	No	1	484.060
9	23.118	FID1 A	2-methyl-1-propan	No	1	474.370
10	24.168	FID1 A	3-pentanol	No	1	536.030
11	26.872	FID1 A	n-butanol	No	1	496.770
12	31.155	FID1 A	2-methyl-1-butano	No	1	514.010
13	31.348	FID1 A	3-methyl-1-butano	No	1	492.710

$$C[\text{mg/L AA}] = C[\mu\text{g/g}] \cdot \rho[\text{g/L}]/(10 \cdot ABV)$$

$$C_{ethanol} = \rho_{ethanol} = 789270 \text{ mg/L AA}$$

The calibration tables in the methods: "Traditional ISTD" (3-pentanol as ISTD) and "Ethanol ISTD"

#	RT	Compound	ISTD	Implem.#	Amt[µg/g]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	180.68	2.82e-4	640847
				2	180.68	2.86e-4	630730
				3	180.68	2.84e-4	637261
2	8.6	methyl acetate	No	1	287.96	3.67e-4	785668
				2	287.96	3.69e-4	779607
				3	287.96	3.62e-4	795822
3	10.5	ethyl acetate	No	1	260.11	4.64e-4	561140
				2	260.11	4.64e-4	560645
				3	260.11	4.57e-4	568818
4	10.7	acetal	No	1	233.31	5.56e-4	419903
				2	233.31	5.60e-4	416793
				3	233.31	5.49e-4	425099
5	11.6	methanol	No	1	203.11	3.37e-4	602347
				2	203.11	3.39e-4	599041
				3	203.11	3.35e-4	606212
6	13.9	ethanol	No	2	333000.00	0.649	513446
				3	333000.00	0.639	520885
				1	333000.00	0.644	517252
7	18.4	2-butanol	No	1	210.07	6.40e-4	328216
				2	210.07	6.48e-4	324187
				3	210.07	6.38e-4	329344
8	19.3	n-propanol	No	1	204.23	6.11e-4	334346
				2	204.23	6.14e-4	332515
				3	204.23	6.03e-4	338490
9	23.1	2-methyl-1-propanol	No	1	200.14	7.06e-4	283679
				2	200.14	7.16e-4	279593
				3	200.14	7.02e-4	285178
10	24.2	3-pentanol	Yes	1	226.16	7.60e-4	297519
				2	226.16	7.68e-4	294411
				3	226.16	7.54e-4	299922
11	26.9	n-butanol	No	1	209.60	6.87e-4	304936
				2	209.60	6.93e-4	302559
				3	209.60	6.83e-4	306882
12	31.2	2-methyl-1-butanol	No	1	216.87	7.71e-4	281380
				2	216.87	7.75e-4	279748
				3	216.87	7.67e-4	282590
13	31.3	3-methyl-1-butanol	No	1	207.88	7.30e-4	284585
				2	207.88	7.32e-4	283981
				3	207.88	7.25e-4	286813

#	RT	Compound	ISTD	Implem.#	Amt[mg/L AA]	Area	Rsp.Factor
1	6.2	acetaldehyde	No	1	428.25	2.82e-4	1518944
				2	428.25	2.86e-4	1494965
				3	428.25	2.84e-4	1510443
2	8.5	methyl acetate	No	1	682.51	3.67e-4	1862156
				2	682.51	3.69e-4	1847790
				3	682.51	3.62e-4	1886223
3	10.5	ethyl acetate	No	1	616.51	4.64e-4	1329996
				2	616.51	4.64e-4	1328834
				3	616.51	4.57e-4	1348206
4	10.7	acetal	No	1	552.98	5.56e-4	995234
				2	552.98	5.60e-4	987862
				3	552.98	5.49e-4	1007548
5	11.6	methanol	No	1	481.41	3.37e-4	1427679
				2	481.41	3.39e-4	1419844
				3	481.41	3.35e-4	1436840
6	13.9	ethanol	Yes	1	789270.00	0.644	1225966
				2	789270.00	0.649	1216959
				3	789270.00	0.639	1234591
7	18.4	2-butanol	No	1	497.89	6.40e-4	777910
				2	497.89	6.48e-4	768361
				3	497.89	6.38e-4	780582
8	19.3	n-propanol	No	1	484.06	6.11e-4	792457
				2	484.06	6.14e-4	788117
				3	484.06	6.03e-4	802278
9	23.1	2-methyl-1-propanol	No	1	474.37	7.06e-4	672372
				2	474.37	7.16e-4	662689
				3	474.37	7.02e-4	675926
10	24.2	3-pentanol	No	1	536.03	7.60e-4	705161
				2	536.03	7.68e-4	697794
				3	536.03	7.54e-4	710856
11	26.9	n-butanol	No	1	496.77	6.87e-4	722724
				2	496.77	6.93e-4	717091
				3	496.77	6.83e-4	727336
12	31.2	2-methyl-1-butanol	No	1	514.01	7.71e-4	666907
				2	514.01	7.75e-4	663039
				3	514.01	7.67e-4	669775
13	31.3	3-methyl-1-butanol	No	1	492.71	7.30e-4	674513
				2	492.71	7.32e-4	673081
				3	492.71	7.25e-4	679794

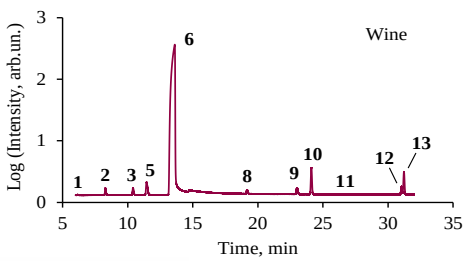
2. Analysis of alcoholic beverages

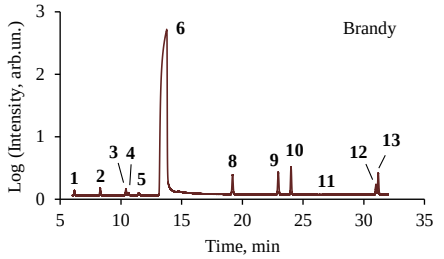
2.1. Acquire the chromatograms of samples of alcoholic beverages. Peaks of compounds are identified and integrated, including the ethanol peaks.

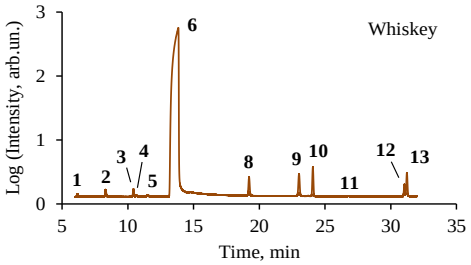
2.2. Click “Report” in the main menu and press “Specify Report…”. Set in “Quantitative Results”: “Calculate” – “ISTDed on” – “Area”.

Samples of alcoholic beverages

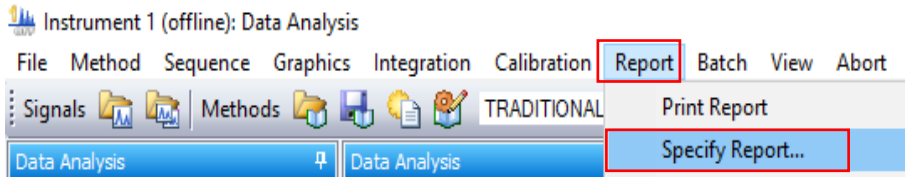
Chromatograms

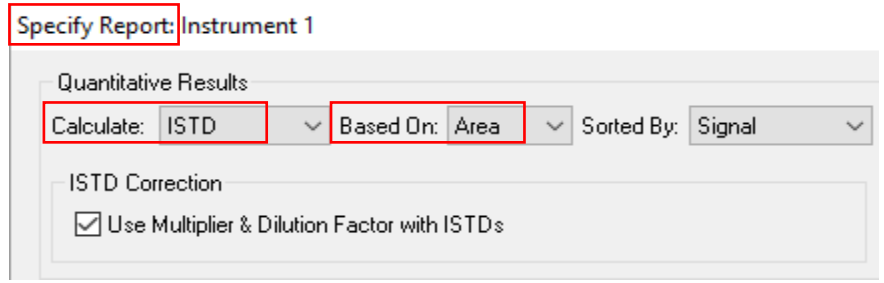






Specify Report





Report (traditional internal standard)

```
Sorted By                :      Signal
Calib. Data Modified     :      Thursday, December 26, 2024 7:15:13 PM
Multiplier               :      1.0000
Dilution                 :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #      [µg/g]
----|-------------|-------------------
  1    227.80000    3-pentanol
```

RRF

```
Signal 1: FID1 A, Front Signal

RetTime  Type  ISTD    Area     Amt/Area    Amount    Grp   Name
 [min]         used   [V*s]      ratio     [µg/g]
-------|------|----|----------|----------|----------|--|------------------
   6.185 BB         1 9.02712e-5   2.14029   58.17736    acetaldehyde
   8.534 BB         1 1.48048e-5   2.64738   11.80194    methyl acetate
  10.446 BB         1 8.49700e-4   1.89561  485.00482    ethyl acetate
  10.668 BB         1 1.39654e-4   1.41479   59.49469    acetal
  11.523 BB         1 4.37130e-3   2.02682 2667.82824    methanol
  13.930 BB         1 7.71661e-1   1.73975 4.04246e5     ethanol
  18.434            1      -         -         -        2-butanol
  19.271 BB         1 4.53447e-4   1.12726  153.91588    n-propanol
  23.052 BB         1 3.04911e-4 9.51327e-1   87.34440    2-methyl-1-propanol
  24.102 BB    I    1 7.56521e-4   1.00000  227.80000    3-pentanol
  26.802 BB         1 9.14371e-5   1.02527   28.22876    n-butanol
  31.091 BB         1 1.38481e-4 9.46042e-1   39.44860    2-methyl-1-butanol
  31.284 BB         1 6.80836e-4 9.59114e-1  196.62790    3-methyl-1-butanol
```

$$C^{measured}[\mu g/g] = C_{3\text{-pentanol}} \cdot RRF^{3\text{-pentanol}} \cdot \frac{A}{A_{3-\text{pentanol}}}$$

Report (ethanol as internal standard)

```
Sorted By                :      Signal
Calib. Data Modified     :      Tuesday, December 24, 2024 7:10:13 PM
Multiplier               :      1.0000
Dilution                 :      1.0000
Use Multiplier & Dilution Factor with ISTDs
Sample ISTD Information:
ISTD  ISTD Amount   Name
  #    [mg/L AA]
----|-------------|-------------------
  1   7.89270e5    ethanol
```

RRF

```
Signal 1: FID1 A, Front Signal

RetTime  Type  ISTD    Area     Amt/Area    Amount    Grp   Name
 [min]         used   [V*s]      ratio    [mg/L AA]
-------|------|----|----------|----------|----------|--|------------------
   6.185 BB         1 9.02712e-5   1.23025  113.59049    acetaldehyde
   8.534 BB         1 1.48048e-5   1.52169   23.04247    methyl acetate
  10.446 BB         1 8.49700e-4   1.08959  946.95446    ethyl acetate
  10.668 BB         1 1.39654e-4 8.13209e-1  116.15915    acetal
  11.523 BB         1 4.37130e-3   1.16502 5208.86353    methanol
  13.930 BB    I    1 7.71661e-1   1.00000 7.89270e5     ethanol
  18.431            1      -         -         -        2-butanol
  19.271 BB         1 4.53447e-4 6.47943e-1  300.51277    n-propanol
  23.052 BB         1 3.04911e-4 5.46821e-1  170.53630    2-methyl-1-propanol
  24.102 BB         1 7.56521e-4 5.74786e-1  444.76045    3-pentanol
  26.802 BB         1 9.14371e-5 5.89298e-1   55.11329    n-butanol
  31.091 BB         1 1.38481e-4 5.43773e-1   77.02034    2-methyl-1-butanol
  31.284 BB         1 6.80836e-4 5.51296e-1  383.90693    3-methyl-1-butanol
```

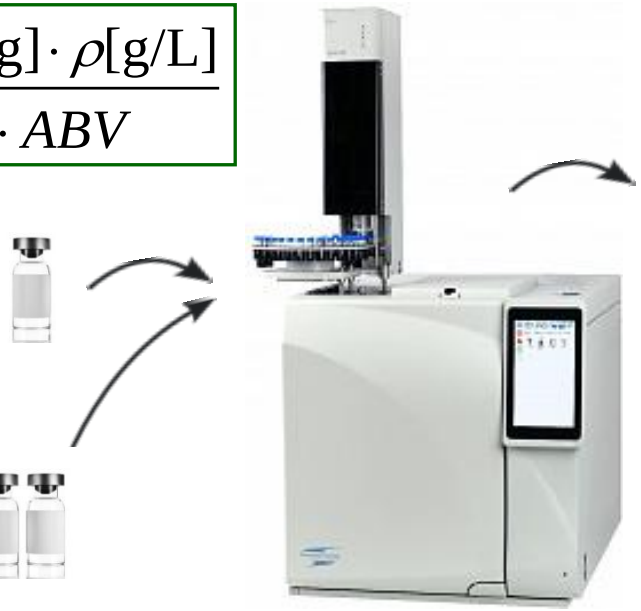
$$C^{measured}[\text{mg/L AA}] = \rho_{ethanol} \cdot RRF^{ethanol} \cdot \frac{A}{A_{ethanol}}$$

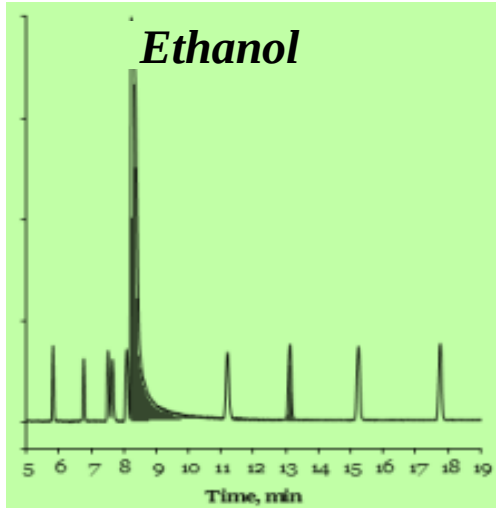
Single-level calibration with internal standard – ethanol

1. Certified values of concentration **in mg/L AA** (per anhydrous alcohol)

$$C[\text{mg/L AA}] = \frac{C[\mu\text{g/g}] \cdot \rho[\text{g/L}]}{10 \cdot ABV}$$

One calibration solution (SS-C) for $RRF^{ethanol}$ determination





Samples of alcoholic beverages

2. Calculation of the relative response factor $RRF^{ethanol}$

Calibration with SS-C using ethanol as internal standard

$$RRF^{ethanol} = \frac{C^{certified}}{\rho_{ethanol}} / \frac{A}{A_{ethanol}}$$

$\rho_{ethanol}$ **= 789270 mg/L**

no need to add an internal standard substance

3. Calculation of compound concentration in the products

$$C^{measured}[\text{mg/L AA}] = \rho_{ethanol} \cdot RRF^{ethanol} \cdot \frac{A}{A_{ethanol}}$$

The concentration of volatile compounds is calculated in mg/L AA
There is **no need to determine the strength** of the alcoholic beverage samples.