УДК 543

On the problems with quantitative GC analysis

Decker S.[1], Schubert L.[1], Schröter F.[1], Rüsch gen. Klaas M.[2], Roessner F.[1]

[1]*Carl von Ossietzky University Oldenburg, Oldenburg, Germany*
[2]*University of Applied Sciences Emden Leer, Emden, Germany*

Поступила в редакцию 15.11.2013 г.

Abstract

Gas chromatography is widely used to analyze volatile compounds. However, quantitative analysis needs special consideration. Depending on the containing functional groups and heteroatoms the signal intensity of a flame ionization detector differs. Response factors that consider this can be determined experimentally or calculated via an increment system. A comparison between experimental determined and calculated response factors was done for different compounds. Furthermore, a method for quantitative analysis using a mass selective detector is proposed. A calibration for every substance in the certain concentration range needs to be done since a prediction of the response behavior is impossible.

Keywords: effective carbon number, response factor, gas chromatography, GC-MS, GC-FID, MSD, FID, ECN

Газовая хроматография широко используется в анализе летучих соединений. Однако количественное определение веществ нуждается в специальном рассмотрении. Отмечено, что интенсивность сигнала пламенно-ионизационного детектора меняется в зависимости от содержания функциональных групп и гетероатомов. Коэффициенты чувствительности, рассматриваемые в данной работе, могут быть определены экспериментально или рассчитаны с помощью системы приращений. Представлено сравнение экспериментальных и расчетных коэффициентов чувствительности (факторов отклика, градуировочных коэффициентов) для различных соединений. Предложен способ количественного анализа с помощью масс-селективного детектора. Показано, что необходима калибровка для всех веществ в определенном диапазоне концентраций, что связано с невозможностью предсказания величины отклика.

Ключевые слова: эффективное число атомов углерода (ECN), коэффициент чувствительности, фактор отклика, градуировочный коэффициент, газовая хроматография, ГХ-МС, ГХ-ПИД, масс-селективный детектор (MSD), пламенно-ионизационный детектор (FID)

Introduction

Gas chromatography (GC) is a standard technique both for offline and online analysis, as used in catalysis research. It can be combined with a wide range of detectors and thus be tuned to the specific analytical problem. Whereas qualitative analysis is well established quantitative analysis is under debate in recent literature [1].

The flame ionization detector (FID) detects ions formed during combustion of hydrocarbons in an oxyhydrogen flame. Hydrocarbons have molar response factors that are equal to the number of carbon atoms in the molecule. Whereas molecules that contain heteroatoms tend to have lower response factors. Thus the signal of an unsaturated

hydrocarbon or a hydrocarbon with heteroatoms can be multiplied with a factor. The approach of effective carbon numbers (ECN) is used to calculate this factor. By that it is possible to compare this recalculated signal with the signal of the corresponding aliphatic hydrocarbon.

In 1985 Scanlon and Willis [2] defined the ECN as:

$$ECN_i = \frac{ECN_{RS}}{RF_{molar}} = \frac{ECN_{RS}}{\left(\frac{M_{RS} \cdot A_{RS} \cdot m_i}{M_i \cdot A_i \cdot m_{RS}}\right)},$$

$$RF_{molar} = \frac{ECN_{RS}}{ECN_i} = \frac{M_{RS} \cdot A_{RS} \cdot m_i}{M_i \cdot A_i \cdot m_{RS}},$$

ECN_{RS} - Effective Carbon-Atom Number of the reference substance; RF_{molar} - Relative molar response factor; M_i, M_{RS} - Molar mass of the analyte and the reference substance; m_i, m_{RS} - Mass of the analyte and the reference substance; A_i, A_{RS} - Peak area of the analyte and the reference substance.

The response factor (RF) that is normalized to the aliphatic equivalent is:

$$RF_i = \frac{N_i}{ECN_i},$$

RF - Carbon content specific response factor; N - Number of carbon atoms in the molecule; ECN_i - Effective Carbon-Atom Number of the analyte.

The ECN_i can be calculated from the relative detector signal, the molar mass M_i of the analyte and a reference substance:

$$ECN_i = N_{RS} \cdot \frac{M_i}{M_{RS}} \cdot f_i = N_{RS} \cdot \frac{M_i}{M_{RS}} \cdot \frac{A_i \cdot m_{RS}}{A_{RS} \cdot m_i},$$

N_{RS} - Number of carbon atoms in the reference substance. $N_{RS} = ECN_{RS}$ for aliphatic hydrocarbons; f_i - Response factor of the analyte relative to the reference substance.

The relative response factor f_i is determined by the quotient of the slope of the calibration lines of the analyte and the reference substance:

$$f_i = \frac{A_i \cdot m_{RS}}{A_{RS} \cdot m_i} = \frac{slope\ (i)}{slope\ (RS)},$$

Thus, the ECN can be experimentally determined using the following equation:

$$ECN_{i,experimental} = N_{RS} \cdot \frac{M_i}{M_{RS}} \cdot \frac{slope\ (i)}{slope\ (RS)},$$

Using the molar response factor the molar amount of a substance x_i can be estimated with good accuracy.

$$x_i = \frac{RF_{molar,i} \cdot A_i}{\sum(RF_{molar,i} \cdot A_i)} = \frac{\frac{A_i}{ECN_i}}{\sum\left(\frac{A_i}{ECN_i}\right)},$$

Additionally, it is possible to determine the injected mass of an unknown substance even though the pure substance is not available. Fig. 1 illustrates this procedure. Only a calibration line of an aliphatic hydrocarbon is needed to determine the detector sensitivity. Using the response factor the peak area of the analyte ($A_{analyte}$) is compared with the peak area of the reference substance. To do this $A_{analyte}$ needs to be multiplied with the analyte's response factor. Because a calibration line of the reference substance is available the corresponding mass can be concluded.

However, if the analyte is not available as pure substance, it is impossible to figure out its ECN. Sternberg et al. developed an ECN increment system in 1962 [3].The contribution to the ECN depends on the functional group the corresponding atom can be assigned to (see table 1).

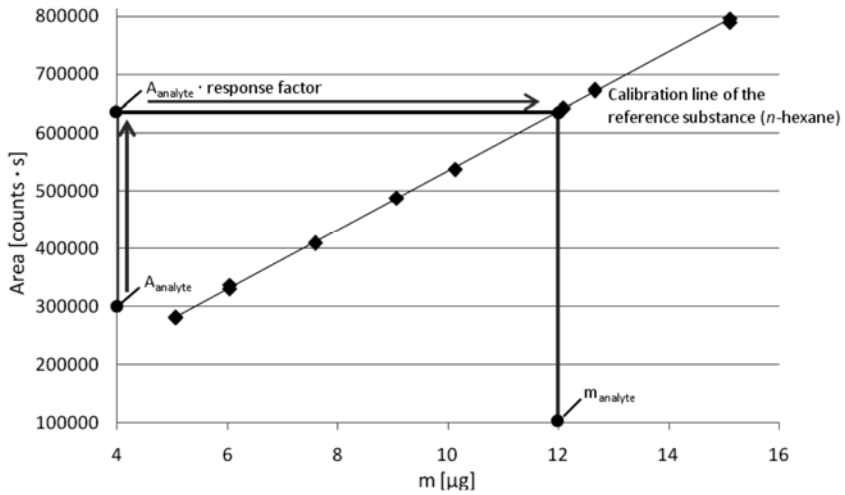

Fig. 1. Determination of injected mass of an analyte using its response factor and a calibration line of a reference substance

Table 1. Contributions to the effective carbon number [2, 4]

Atom	Type	ECN contribution
C	Aliphatic	1.00
C	Aromatic	1.00
C	Olefinic	0.95
C	Acetylenic	1.30
C	Carbonyl	0.00
C	Carboxyl	0.00
C	Nitrile	0.30
O	Ether	- 1.00
O	Primary alcohol	- 0.60
O	Secondary alcohol	- 0.75
O	Tertiary alcohol	- 0.25
O	Ester	- 0.25
O	Carbonyl	0.00
N	Primary amine	- 0.60
N	Secondary amine	- 0.75
N	Tertiary amine	- 0.25

GC is often combined with mass selective analysis (GC-MS) and can be used to identify volatile organic and inorganic compounds. In the mass selective detector (MSD) the substance is ionized, most commonly using electron impact ionization (EI):

$$M + e^- \rightarrow M^{+\cdot} + 2e^-,$$

M - Molecule; $M^{+\cdot}$ - Molecule ion; e^- - Electron.

The resulting ions can be separated by the ratio of mass to charge (m/z), most commonly by using a quadrupole mass spectrometer. A continuous spectrum equally to a gas-chromatogram, the Total Ion Chromatogram (TIC) and an ion chromatogram are

obtained. The ion chromatogram in figure 2 shows the characteristic fragmentation of methanol. The M^+ signal is the highest obtained mass in the ion chromatogram, e.g. m/z = 32, whereas m/z = 33 is a satellite peak due to naturally occurring ^{13}C content in the sample. The base peak ion in methanol is the m/z = 31, which can be attributed to a H_2COH^+ fragment and is characteristic for primary alcohols.

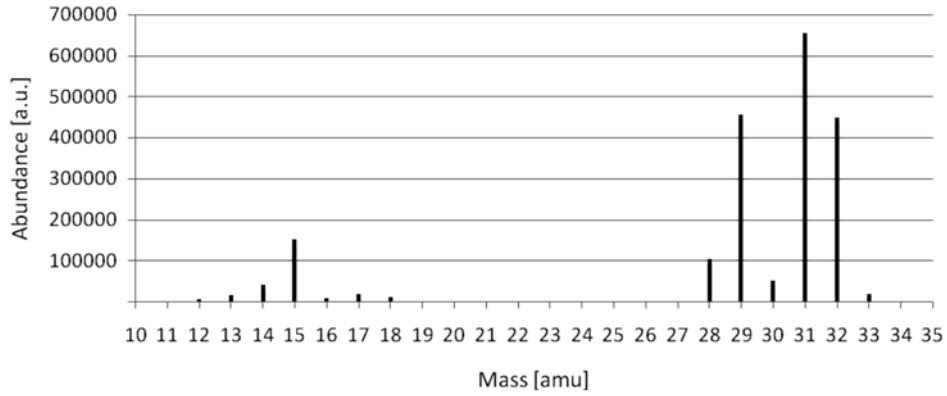

Fig. 2. Ion chromatogram, M^+ =32, fragmentation pattern of methanol, split 1:10

Modern database software can compare the resulting M^+ and fragmentation pattern with a database and identify the substance directly.

The benefits in qualitative analysis are obvious though quantitative analysis is cumbersome. Henkel [5] developed a method for quantitation in routine GC-MS analysis and it will be shown that it is necessary to derive the calibration factors for each compound in the expected concentration range independently.

In this work the ECNs and RFs of different substances with different functional groups were experimentally determined and compared to the results obtained by Sternberg's increment system. Moreover, we investigated whether a prediction of the responsive behavior is not only possible for a FID but also for a MSD.

Experimental

For determination of response behavior the chemicals in table 2 were used. Their purity was verified by GC analysis.

Table 2. Purities of the used chemicals

Substance	Purity [Mass-%]	Source
1	*2*	*3*
Acetone	99.98	VWR BDH Prolabo
Acetonitrile	100.00	VWR BDH Prolabo
Butanal	87.99	Riedel-de Haën
1-Butanol	99.90	BASF AG
2-Butanone	99.86	Roth
m-Cresol	99.97	Merck
Cyclohexane	99.95	Fischer Scientific UK Ltd.
Cyclohexene	99.74	J. T. Baker B. V.
Ethanol	100.00	VWR BDH Prolabo
2-Ethylhexanol	99.28	Fluka A
n-Hexane	99.63	Acros Organics
1-Hexanol	98.01	Sigma-Aldrich

1	*2*	*3*
1-Hexene	99.95	Merck-Schuchardt
Methanol	100.00	VWR BDH Prolabo
n-Pentane	99.55	LAB-SCAN analytical sciences
1-Pentanal	96.48	Sigma-Aldrich
Pentanenitrile	98.00	GFS Chemicals
1-Pentanol	98.78	Sigma-Aldrich
2-Pentanon	97.60	Sigma-Aldrich
1-Pentene	76.40	Sigma-Aldrich
1-Propanol	95.50	Roth
2-Propanol	99.99	Sigma-Aldrich
Propionitrile	99.98	Sigma-Aldrich
Tetrahydrofuran	99.99	Fischer-Scientific
Toluene	99.87	VWR BDH Prolabo
Valeric acid	98.54	Sigma-Aldrich

For GC-FID analysis two solutions with different concentrations of the analyte and the reference substance were analyzed using a HP 5890 Series 2 gaschromatograph with a HP 7673 automatic liquid sampler. The mass fraction ω of these substances in the solvent varied between 0.0014 and 0.0250. Different volumes of 0.4 µL, 0.6 µL, 0.8 µL and 1.0 µL were injected twice into the GC. The GC parameters are listed in table 3.

Table 3. GC-FID parameters

Gaschromatograph		**Column**	
Inlet temperature	290 °C	Name	Optima-5 MS
Detector temperature	300 °C	Stationary phase	5% diphenyl /
Carrier gas flow (H_2)	1.1 - 1.3 mL/min		95% dimethylpolysiloxane
Split ratio	1:43 - 1:50	Polarity	Nonpolar
Makeup flow rate (N_2)	26 mL/min	Inner diameter	0.25 mm
Hydrogen flow rate	32 mL/min	Film thickness	0.25 µm
Air flow rate	413 mL/min	Length	30 m
Injected volume	0.4 µL, 0.6 µL, 0.8 µL, 1.0 µL		

Table 4. GC-MS parameters

Gaschromatograph		**Column**	
Inlet temperature	230 °C	Name	Optima-1 MS Accent
Detector temperature	150 °C	Stationary phase	100%
Detector source temp.	230 °C		dimethylpolysiloxane
Carrier gas flow (H_2)	1.0 mL/min	Polarity	Nonpolar
Split ratio	1:10/1:100	Inner diameter	0.25 mm
Mode	Electron impact	Film thickness	0.50 µm
Scan range	12-550 amu @ 2.72 scans/sec	Length	60 m
Injected volume	0.02 µL-0.1 µL		

GC-MS analysis was done on a HP 6890 GC equipped with a HP 5973 MSD. The exact parameters can be found in table 4. For the determination of response factors alcohols in the range of C1 - C6 and a broad range of C5 compound classes were injected by hand

(see table 2). Volumes of 0.02 µL, 0.04 µL, 0.06 µL, 0.08 µL and 0.1 µL were injected at least six times per concentration and compound. Split ratios of 1:10 and 1:100 were applied to determine the dynamic range of the MSD. To quantitate the data an ion extraction was performed (see table 5) and an average area was calculated for each point.

Table 5. GC-MS analysis, extracted ions for selected compounds

Compound	Extracted ion	Compound	Extracted ion
Methanol	31	Pentanal	44
Ethanol	31	Pentanenitrile	41
Propanol	31	1-Pentene	42
Butanol	31	*n*-Pentane	43
Pentanol	31	Pentanone	43
Hexanol	31	Valeric acid	60

Results and discussion

For the GC-FID analysis the peak area was plotted against the injected mass m_i of the analyte. The slope was obtained by linear regression.

$$m_i = V_{inj} \cdot \rho_{sol} \cdot \omega_i \,,$$

$$\omega_i = \frac{m_i}{m_i + m_{sol}} \,,$$

V_{inj} - Injected volume; ρ_{sol} - Density of the solution; ω_i - Mass fraction of the analyte.

The experimental ECN and RF were determined by this equation:

$$ECN_{i,experimental} = N_{RS} \cdot \frac{M_i}{M_{RS}} \cdot \frac{slope\ (i)}{slope\ (RS)} \,,$$

$$RF_i = \frac{N_i}{ECN_i} \,,$$

At first, the purity of the chemicals was approximately determined by the respective peak area. This is afflicted with a systematic error, if the substances that are responsible for the impurities are not isomers. This would result in different response factors. Because the substances usually have a purity of more than 99 % this error can be neglected.

In contrast to the described behavior of a FID, a MSD does not show a linear but a 2nd order polynomial response curve (see figure 3 for methanol). A strong characteristic ion needs to be found and extracted from the total ion chromatogram (TIC) (see figure 2, table 5). The obtained chromatogram shows only the selected ion and the area is calculated by integration. The resulting data is fitted and from the polynomial regression the coefficients A and B could be derived.

These can then be transposed to give the amount of compound (n_i) from a detected area.

$$A_i = A_{MS,i} \cdot n_i^2 + B_{MS,i} \cdot n_i + C \,,$$

A_I - Area of compound I; $A_{MS,i}$ - Calibration factor A for compound I; $B_{MS,i}$ - Calibration factor A for compound I; C - Constant (neglected); n_i - Amount of compound i.

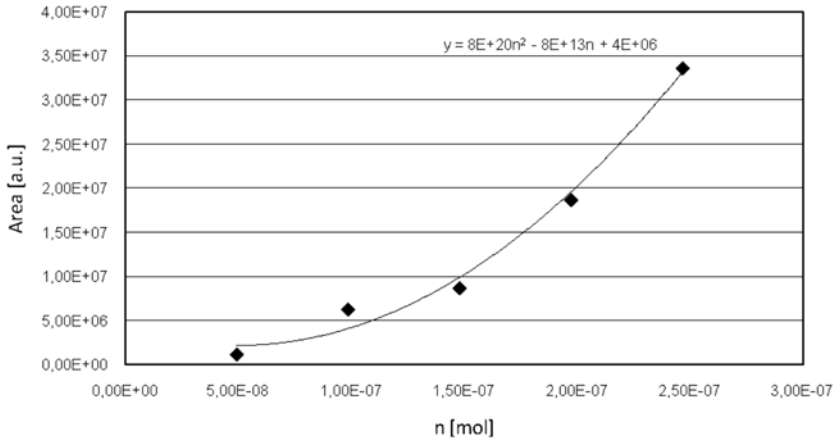

Fig. 3. Response behavior of methanol in MSD, split 1:10,
full line represents polynomial regression

As it can be seen in figure 4, a homologous behavior in most classes of substances could not be observed. In this example of C1 - C6 alcohols from C3 the graphs seemed consistent, but methanol and ethanol showed strong aberrations. A variation in sensitivity was also observable when comparing different classes of molecules as shown in figure 5. The response signal differed in absolute numbers and in slope. Furthermore, the MSD showed a very narrow dynamic range. This can be seen, by comparison of pentanol in figure 4 and figure 5. At a lower concentration (split 1:100) the graph was almost linear but at a 10^1 concentration (split 1:10) the graph is polynomial. In contrast, the dynamic range of an FID reaches over 10^6 concentration units.

Response factors or increment systems for homologous compound classes could not be derived and thus the response of an individual compound needs to be calibrated in the expected molar range.

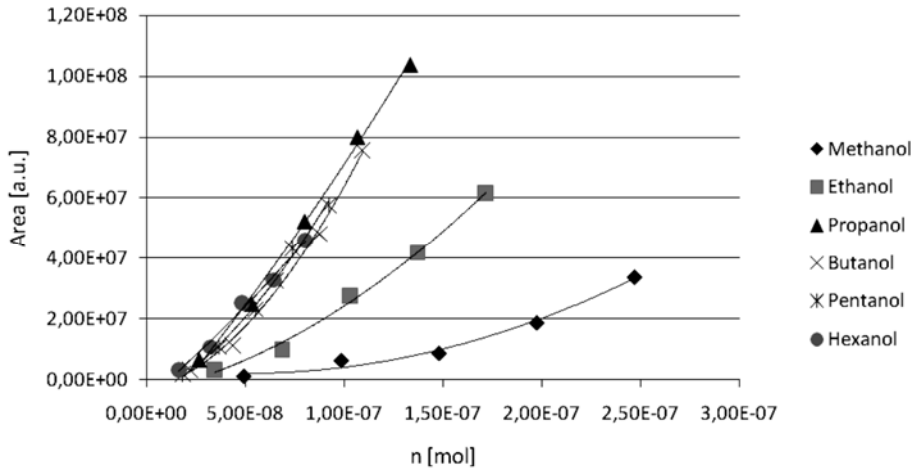

Fig. 4. Response behavior of homologous alcohols in MSD, Split 1:10

Table 6. Effective Carbon Number and Response Factor: Experimental Determination and calculation via literature ECN increment system [4]

	Number C-Atoms	C	C-O	C=O	C ≡N	C-N	Slope$_i$	Coefficient of determination	ECN$_i$ experi.	ECN$_i$, literature	ΔECN$_i$	RF$_i$ experi.	RF$_i$ literature	ΔRF$_i$
n-Hexane	6	6					64.571	0.9980		6				
1-Hexene	6	6					67.470	0.9975	6.10	6	0.10	0.98	1.00	-0.02
Methanol	1		1				20.894	0.9966	0.69	0.55	0.14	1.45	1.82	-0.37
Acetone	3	2		1			31.062	0.9984	1.95	2	-0.05	1.54	1.50	0.04
n-Hexane	6	6					112.423	0.9984		6				
n-Pentane	5	5					109.988	0.9997	4.92	5	-0.08	1.02	1.00	0.02
Cyclohexane	6	6					118.010	0.9993	6.15	6	0.15	0.98	1.00	-0.02
Cyclohexene	6	6					117.351	0.9988	5.97	6	-0.03	1.01	1.00	0.01
2-Ethylhexanol	8	7	1				91.715	0.9990	7.40	7.55	-0.15	1.08	1.06	0.02
n-Hexane	6	6					51.119	0.9997		6				
THF	4	2	2				31.524	0.9981	3.10	3.10	0	1.29	1.29	0
n-Hexane	6	6					50.294	0.9977		6				
Propionitrile	3	2			1		30.760	0.9965	3.10	2.30	0.02	1.29	1.30	-0.01
n-Hexane	6	6					47.921	0.9986		6				
Acetonitrile	2	1			1		22.503	0.9990	1.34	1.30	0.04	1.49	1.54	-0.05
2-Propanol	3	2	1				25.324	0.9986	2.21	2.55	-0.34	1.36	1.18	0.18
n-Hexane	6	6					45.906	0.9950		6				
Toluene	7	7					50.099	0.9966	7.00	7.00	0	1.00	1.00	0
n-Hexane	6	6					48.993	0.9959		6				
2-Butanone	4	3		1			31.147	0.9983	3.19	3.00	0.19	1.25	1.33	-0.08
Butanal	4	3		1			30.659	0.9995	3.14	3.00	0.14	1.27	1.33	-0.06
Dodecane	12	12					57.457	0.9989		12				
ε-Caprolactone	6	4	1	1			31.747	0.9968	4.44	4.55	-0.11	1.35	1.32	0.03
n-Hexane	6						36.325	0.9983		6				
Triethylamine	6	3				3	25.249	0.9981	4.90	5.75	0.85	1.22	1.04	0.18
Dioxane	4		4				12.058	0.9966	2.04	2.20	-0.16	1.96	1.82	0.14

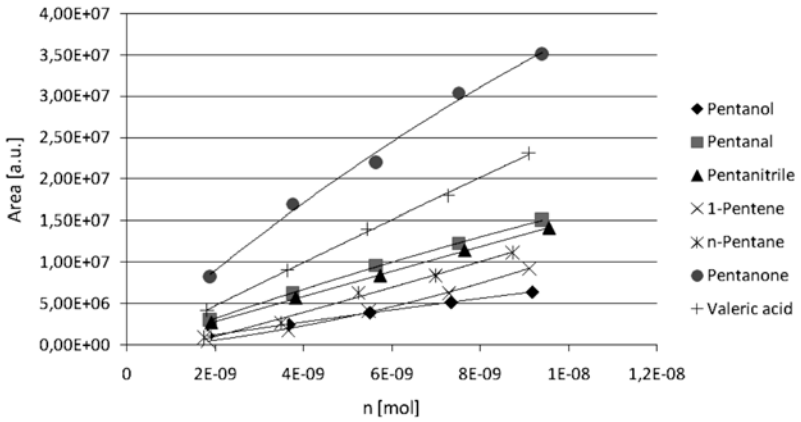

Fig. 5. Response behavior of C5 compounds in MSD, split 1:100

Conclusion

Although both techniques have their individual advantages it can be concluded, that for routine quantitative analysis the FID is much better suited. It was shown, that the experimentally determined ECN are in good agreement with the calculated values for the most molecule types. However, the deviations for alcohols and amines cannot be neglected. The dynamic range of an FID is very high and by calibration of one compound or internal standard (ISTD) the transformation via the ECN concept for uncalibrated compounds is possible and easy. In contrast, the MSD has to be calibrated for every compound in the expected concentration range. A prediction of response behavior cannot be done. The qualitative analysis of unknown compounds on the other hand is very easily done with modern GC-MS systems and database software. Both techniques can be efficiently combined to solve complex analytical problems.

References

1. Trunschke A., Frank B., Xie Z.-L. Higher Alcohol Synthesis: Product Analysis Using the Concept of Effective Carbon Numbers, Chem. Ing. Tech., 2013, 85 (8), p. 1290-1293.

2. Faiola L., Erickson M.H., Fricaud V.L., Jobson B.T., VanReken T.M. Quantification of bionic volatile organic compounds with a flame ionization detector using the effective carbon number concept, Atmospheric Measurement Techniques, 2012, 5, p. 1911-1923.

3. Holm T. Aspects of the mechanism of the flame ionization detector, Journal of Chromatography A, 1999, 842, p. 221-227.

4. Sternberg J.C., Gallaway W.S., Jones D.T.L. Gas Chromatography, Academic Press, New York, 1962, p. 261.

5. Henkel R. The influence of ammonia on Fischer-Tropsch synthesis and formation of N-containing compounds, PhD thesis, Oldenburg, 2013.

Sebastian Decker - M. Sc., PhD student, Department of Chemistry, Carl von Ossietzky University Oldenburg, Oldenburg, Germany

Lennart Schubert - Dipl.-Chem., PhD student, Department of Chemistry, Carl von Ossietzky University Oldenburg, Oldenburg, Germany

Frank Schröter - B. Sc., Master student, Department of Chemistry, Carl von Ossietzky University Oldenburg, Oldenburg, Germany

Mark Rüsch gen. Klaas - Prof. Dr., EUTEC, University of Applied Sciences Emden Leer, Emden, Germany, e-mail: ruesch.gen.klaas@hs-emden-leer.de

Frank Rößner - Prof. Dr. Dr. h.c., Department of Chemistry, Carl von Ossietzky University of Oldenburg, Oldenburg, Germany, e-mail: frank.roessner@uni-oldenburg.de