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Group method approach to the estimation of response factors of unavailable substances in quantitative gas chromatography

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Abstract

The method's accuracy of a compound quantitation by chromatography depends on the calibration procedure with a pure standard of the target analyte, if the latter is unavailable uncertainty is unavoidable. The group method is a different approach in GC quantitative analysis that shows a practicable way for avoiding this uncertainty and accurately quantify a mixture containing one or more unavailable components. This paper is concerned with the definition of the group method quantitative parameters, the application procedures for their calculation, the determination of the quantitative proportion of a group of unavailable components of a mixture and the partial or total quantitation of the latter. The paper also describes the steps for carrying out the so-called group-correlation method in the determination of the response factors of unavailable compounds, which belong to a homologous series. The GC experimental corroboration of the group method approach employing model mixtures of compounds is also presented.

Keywords: Group method; Response factors; Gas chromatography; Group-correlation method; Fatty acids

1. Introduction

The basis for the accurate quantitative calculation by gas chromatography (GC) is the response factor, which can be found by absolute or relative calibration techniques [1]. If the pure substance is unavailable, it is not possible to calculate its response factor by conventional quantitative GC methods.

Very often, in practical chromatography, one can find a situation where the mixture matter of determination contains certain components that are unavailable, and therefore its accurate quantitative estimation becomes rather difficult or impossible [2,3].

There are some theoretical approaches by which the flame ionization detection (FID) response factors could be predicted in cases of unavailability of pure substances [4–7]. The accuracy of these theoretical methods in comparison with direct experimental determination of response factor lie in their predictive ability.

The gas density balance (GDB), that was first designed and built by Martin and James [8,9], had been employed as a calibration detector for the experimental determination of response factors [10–12]. The GDB's set up allows its operation in parallel with the detector to be calibrated, meaning that the components from the mixture are simul-

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taneously detected by both detectors, which is the main drawback of the method.

Another alternative for the experimental calculation of the FID response factors of unavailable compounds is the so-called linear relationship method published by Janik [13,14]. The method calculates response factors by the numerical or graphical solution of a system of linear equations of the following type:

$$\sum_{i=1}^{n} f_i A_i = A_r W/m_r$$

(equation for one analysis), where f_i and A_i are the response factor and the net response of each component of the mixture to be determined, A_r and m_r are the response and the mass of the reference compound added to the mixture and W is the mass of the mixture.

According to our practical experience, in many cases Janik's method does not offer an accurate result for the response factor, due to the quantitative variability caused by the sample introduction technique.

A different solution for the practical determination of the response factors is the presented group method, which can be employed for the partial or total quantitation of a mixture in whose composition are one or several unavailable (meaning that the compound is not commercially accessible or it is difficult to obtain at all) analytes that cannot be determined by the conventional methods of GC quantitative analysis.

2. Method basis

The group method is related to the group concept, which is based on the additive character of the analytical property of the substance (a_i), the chromatographic signal (s_i) and the response (R_i). The analytical property of a particular substance is a function of its nature and quantitative proportion ($a_i = k_i C_i$); the signal is the reaction of the detector sensor to the analytical property ($s_i = k_s a_i$), and the response is the output quantity that represents the detection system reaction to a signal ($R_i = k_R s_i$) where k_i, k_s, k_R are proportionality constants [15].

Considering the additive character of the parameters a_i, s_i and R_i it is possible to define a hypothetical chromatographic band composed of **n** separated components of an analysed mixture no matter their elution order, and accordingly the related analytical property of a group band:

$$\left(a_g = \sum_{i=1}^{n} a_i = k_{g,i} \sum_{i=1}^{n} C_i \right)$$

a group signal:

$$\left(s_g = \sum_{i=1}^{n} s_i = k_{g,s} \sum_{i=1}^{n} a_i \right)$$

and a group response:

$$\left(R_g = \sum_{i=1}^{n} R_i = k_{g,R} \sum_{i=1}^{n} s_i \right)$$

respectively, where $k_{g,i}$, $k_{g,s}$ and $k_{g,R}$ are proportionality constants related to the group concept (Fig. 1).

The practical basis of the group method lies in the

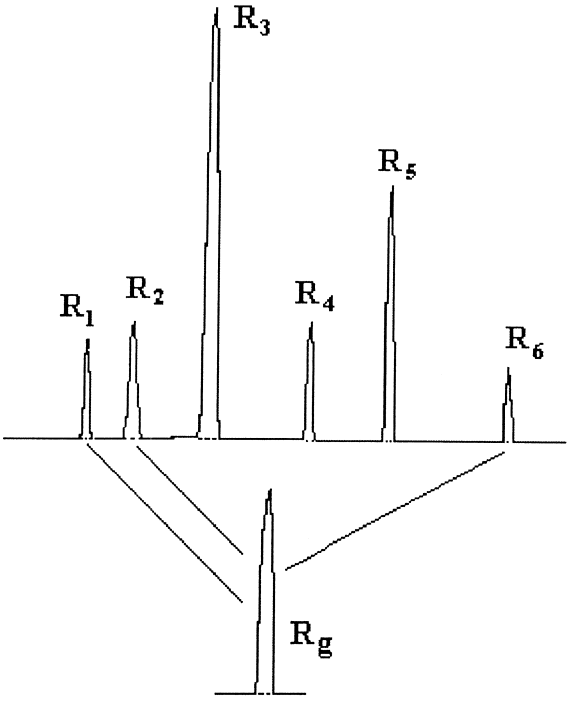

Fig. 1. Group band illustration.

possibility of obtaining the defined Group-standard mixture and measuring experimentally the quantitative group-parameters of **n** unavailable components of a mixture to be object of determination.

3. Quantitative parameters

This theoretical part applies to mass-sensitive detection methods specifically to the FID, which is one of the most widely employed.

3.1. The specific and relative molar response and the related molar response factor

3.1.1. For a single mixture component

The specific molar response (R_i^{mol}) for any component of a mixture composed of **n** different components is defined as the change of the net response to analyte **i** corresponding to a change of the molar concentration of the analyte in the column effluent:

$$R_i^{mol} = \frac{dR_i}{dN_i} \tag{1}$$

and the net detector response (R_i) is related to the analyte peak area (A_i) by:

$$A_i = k\int_{t_1}^{t_2} R_i\, dt = kR_i \tag{2}$$

where k is a proportionality constant related to the detector-amplifier employed.

From Eq. (2), it can be obtained:

$$R_i = \frac{A_i}{k} \tag{3}$$

The instant molar concentration of analyte **i** in the column effluent is:

$$N_i = \frac{dn_i}{dV_M} \tag{4}$$

where n_i = moles of **i** and V_M = volume of the mobile phase.

Since:

$$V_M = F dt \tag{5}$$

then

$$N_i = \frac{dn_i}{F dt} \tag{6}$$

where F = volumetric flow of the mobile phase.

The integration of Eq. (6) gives:

$$N_i = \frac{n_i}{F} \tag{7}$$

and taking into account Eqs. (1)–(7), the definition of the specific molar response for the analyte **i** can be obtained:

$$R_i^{mol} = \frac{dR_i}{dN_i} = \frac{R_i}{N_i} = \frac{\int_{t_1}^{t_2} R_i\, dt}{\int_{t_1}^{t_2} N_i\, dt} = \frac{F\int_{t_1}^{t_2} R_i\, dt}{n_i} = \frac{FA_i}{kn_i} \tag{8}$$

The relative molar response of any analyte is defined as:

$$R_{i,r}^{mol} = \frac{R_i^{mol}}{R_r^{mol}} \tag{9}$$

where R_r^{mol} is the specific molar response of the internal reference substance **r**.

Using Eq. (8), $R_{i,r}^{mol}$ can be rewritten:

$$R_{i,r}^{mol} = \frac{FA_i/kn_i}{FA_r/kn_r} = \frac{n_r A_i}{A_r n_i} \tag{10}$$

where n_r and A_r are the moles and the net response of the component **r** respectively.

By definition the molar response factor (f_i^n) is:

$$f_i^n = \frac{1}{R_{i,r}^{mol}} = \frac{A_r n_i}{n_r A_i} \tag{11}$$

*3.1.2. For a group of **n** components of a mixture*

Eq. (8) defined for a single component of a mixture, can be extended to a group band of **n** analytes of a mixture, in the following way:

$$R_g^{mol} = \frac{\sum_{i=1}^{n} R_i}{\sum_{i=1}^{n} N_i} = \frac{\int_{t_1}^{t_m} R_i\, dt}{\int_{t_1}^{t_m} N_i\, dt} = \frac{F\sum_{i=1}^{n} A_i}{k\sum_{i=1}^{n} n_i} \tag{12}$$

which can be rewritten in short form as:

$$R_g^{mol} = \frac{FA_\Sigma}{kn_\Sigma} \tag{13}$$

Here:

$$\int_{t_1}^{t_m} R_i \, dt = \int_{t_1}^{t_2} R_1 \, dt + \int_{t_3}^{t_4} R_2 \, dt + \cdots + \int_{t_{m-1}}^{t_m} R_n \, dt, \int_{t_1}^{t_m} N_i \, dt = \int_{t_1}^{t_2} N_1 \, dt + \int_{t_3}^{t_4} N_2 \, dt + \cdots + \int_{t_{m-1}}^{t_m} N_n \, dt,$$

$$A_\Sigma = \sum_{i=1}^{n} A_i \quad \text{and} \quad n_\Sigma = \sum_{i=1}^{n} n_i$$

According to the relative molar response definition given by Eq. (9) and considering Eq. (13), the relative molar response for a group band ($R_{g,r}^{\text{mol}}$) is given by:

$$R_{g,r}^{\text{mol}} = \frac{R_g^{\text{mol}}}{R_r^{\text{mol}}} = \frac{(F/k)\sum_{i=1}^{n} A_i \Big/ \sum_{i=1}^{n} n_i}{(F/k)A_r n_r} = \frac{n_r A_\Sigma}{A_r n_\Sigma} \tag{14}$$

Taking into account the definition Eqs. (11) and (14), the molar response factor (F_g^n) for a group band can be expressed as:

$$F_g^n = \frac{1}{R_{g,r}^{\text{mol}}} = \frac{A_r \sum_{i=1}^{n} n_i}{n_r \sum_{i=1}^{n} A_i} = \frac{A_r n_\Sigma}{n_r A_\Sigma} \tag{15}$$

3.2. The specific and relative mass response and the related mass response factor

3.2.1. For a single mixture component

The specific mass response (R_i^m) for any component **i** of a mixture, is defined as the change of the detector net response to analyte **i** corresponding to a change of the mass concentration of analyte **i** in the column effluent that is:

$$R_i^m = \frac{dR_i}{dC_i} \tag{16}$$

The instant mass concentration of analyte **i** in the column effluent may be expressed as:

$$C_i = \frac{dm_i}{dV_M} \tag{17}$$

Using Eq. (5) and integrating it is obtained that:

$$C_i = \frac{m_i}{F} \tag{18}$$

Taking into account Eqs. (2), (3), (17) and (18), the specific mass response can be expressed as:

$$R_i^m = \frac{dR_i}{dC_i} = \frac{R_i}{C_i} = \frac{\int_{t_1}^{t_2} R_i \, dt}{\int_{t_1}^{t_2} C_i \, dt} = \frac{F \int_{t_1}^{t_2} R_i \, dt}{m_i} = \frac{FA_i}{km_i} \tag{19}$$

According to Eq. (9), the analyte **i** relative mass response is given by:

$$R_{i,r}^m = \frac{R_i^m}{R_r^m} \tag{20}$$

where R_r^m is the specific mass response for the internal reference compound **r**.

Considering Eq. (19), that is also valid for the reference compound **r**, the analyte **i** relative mass response is given by:

$$R_{i,r}^m = \frac{FA_i/km_i}{FA_r/km_r} = \frac{m_r A_i}{A_r m_i} \tag{21}$$

By definition the mass response factor can be expressed as:

$$f_i^m = \frac{1}{R_{i,r}^m} = \frac{A_r m_i}{m_r A_i} \tag{22}$$

*3.2.1.1. For a group of **n** components of a mixture*

According to Eq. (19) and assuming the group concept, the specific mass response for a group of **n** components of the separated mixture may be expressed as follows:

$$R_g^m = \frac{\sum_{i=1}^{n} R_i}{\sum_{i=1}^{n} C_i} = \frac{\int_{t_1}^{t_m} R_i \, dt}{\int_{t_1}^{t_m} C_i dt} = \frac{F}{k} \cdot \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} m_i} = \frac{FA_\Sigma}{km_\Sigma} \tag{23}$$

In particular, and considering the physical principle of FID the specific mass response ($R_i^m = A_i/m_i$) (also defined as the detector mass sensitivity to a substance), of a homologous series of compounds, that contain only carbon, hydrogen and oxygen

atoms in their structure, is assumed as a constant value [16,17]. Considering this assumption, the following mathematical relationship can be fulfilled:

$$\frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} m_i} = \frac{\sum_{i=1}^{n} \frac{A_i}{m_i}}{n} \tag{24}$$

Substituting Eq. (24) into Eq. (23), it may be obtained:

$$R_g^m = \frac{F}{k} \cdot \frac{\sum_{i=1}^{n} \frac{A_i}{m_i}}{n} = \frac{\sum_{i=1}^{n} \frac{FA_i}{km_i}}{n} \tag{25}$$

and taking into account Eq. (19), then:

$$R_g^m = \frac{\sum_{i=1}^{n} R_i^m}{n} = R_i^m \tag{26}$$

From the definition given by Eq. (19) and considering Eq. (23), the relative mass response of the group band may be expressed as follows:

$$R_{g,r}^m = \frac{R_g^m}{R_r^m} = \frac{(F/k) \cdot \sum_{i=1}^{n} A_i \Big/ \sum_{i=1}^{n} m_i}{(F/k) \cdot A_r/m_r} = \frac{m_r \cdot \sum_{i=1}^{n} A_i}{A_r \cdot \sum_{i=1}^{n} m_i}$$

$$= \frac{m_r \cdot A_\Sigma}{A_r \cdot m_\Sigma} \tag{27}$$

which can be rewritten as:

$$R_{g,r}^m = \frac{m_r}{A_r} \cdot \frac{\sum_{i=1}^{n} A_i/m_i}{n} = \frac{\sum_{i=1}^{n} m_r A_i/m_i A_r}{n} \tag{28}$$

considering Eq. (24), or as:

$$R_{g,r}^m = \frac{\sum_{i=1}^{n} R_{i,r}^m}{n} = R_{i,r}^m \tag{29}$$

if Eq. (21) is considered.

From Eqs. (11) and (27), the mass response factor of the group band (F_g^m) is:

$$F_g^m = \frac{1}{R_{g,r}^m} = \frac{A_r}{m_r} \cdot \frac{\sum_{i=1}^{n} m_i}{\sum_{i=1}^{n} A_i} \tag{30}$$

which can be rewritten as:

$$F_g^m = \frac{A_r}{m_r} \cdot \frac{\sum_{i=1}^{n} m_i/A_i}{n} = \frac{\sum_{i=1}^{n} A_r m_i/m_r A_i}{n} \tag{31}$$

if Eq. (24) is considered.

Finally, according to Eq. (11), the group-band mass response factor F_g^m for **n** homologous series components of a GC–FID analysed mixture may be written as:

$$F_g^m = \frac{\sum_{i=1}^{n} f_i^m}{n} = f_i^m \tag{32}$$

Eqs. (26), (29) and (32) apply only to the particular case we dealt with and they show that the group band quantitative parameters (R_g^m, $R_{g,r}^m$ and F_g^m) are equivalent to those of a single band. Hence, this deduction proves that the group-band integrating **n** separated single bands of **n** different analytes of a mixture, could be considered, from the quantitative point of view, as an individual analyte.

3.3. The relationship between the relative molar and mass response and the respective response factors

3.3.1. For a single mixture component

Multiplying by dC_i/dC_i Eq. (8), it holds that:

$$R_i^{\text{mol}} = \frac{dR_i}{dC_i} \cdot \frac{dC_i}{dN_i} \tag{33}$$

but since $dN_i = dC_i/M_i$ and Eq. (19) holds, then the specific molar response may be written as:

$$R_i^{\text{mol}} = R_i^m M_i \tag{34}$$

where M_i = the molecular mass of analyte **i**.

Similarly, for analyte **r** it holds that:

$$R_r^{\text{mol}} = R_r^m M_r \tag{35}$$

where M_r = the molecular mass of the internal reference compound **r**.

Then, replacing R_i^{mol} and R_r^{mol} in Eq. (9) with Eqs. (34) and (35) respectively and taking into account Eq. (20), the relationship between $R_{i,r}^{mol}$ and $R_{i,r}^m$ results:

$$R_{i,r}^{mol} = R_{i,r}^m \cdot \frac{M_i}{M_r} \tag{36}$$

Considering Eqs. (11) and (22), the relationship between the factors f_i^m and f_i^n may be obtained from Eq. (36), that is:

$$f_i^m = f_i^n \cdot \frac{M_i}{M_r} \tag{37}$$

*3.3.2. For a group of **n** components of a mixture*

Similarly, as for a particular analyte, for **n** hypothetically grouped components of an analysed mixture, the relationship between the group parameters R_g^{mol} and R_g^m may be obtained.

Therefore, dividing Eq. (12) by Eq. (23), leads to:

$$R_g^{mol} = R_g^m \cdot \frac{\sum m_i}{\sum n_i} \tag{38}$$

Replacing R_g^{mol} and R_r^{mol} in Eq. (15) with Eqs. (38) and (35), leads to:

$$R_{g,r}^{mol} = \frac{R_g^m}{R_r^m} \cdot \frac{\sum_{i=1}^{n} m_i \Big/ \sum_{i=1}^{n} n_i}{M_r} \tag{39}$$

Considering the $R_{g,r}^m$ definition given by Eq. (27), the relationship between the group-relative molar and the group-mass response ($R_{g,r}^{mol}$ and $R_{g,r}^m$) can be obtained:

$$R_{g,r}^{mol} = R_{g,r}^m \cdot \frac{\sum_{i=1}^{n} m_i \Big/ \sum_{i=1}^{n} n_i}{M_r} \tag{40}$$

Replacing appropriately in Eqs. (40), (15) and (30), it can be obtained the relationship between the group molar and the group mass response factors (F_g^n and F_g^m):

$$F_g^m = F_g^n \cdot \frac{\sum_{i=1}^{n} m_i \Big/ \sum_{i=1}^{n} n_i}{M_r} \tag{41}$$

4. The group-standard mixture (GSM)

The **GSM** is a mixture of a known composition made up of $\mathbf{n} \geq 1$ components that are unavailable as (a) pure compound(s) and it may contain some more analytes from which the standards are available.

The **GSM** contains those unavailable components that are present in the mixture matter of determination.

The **GSM** may be obtained by selective separation methods, chemical synthesis followed by effective purification procedures or by combining both related ways.

The identification of the **GSM** must be carried out by a suitable technique (GC, HPLC, GC–MS or HPLC–MS, etc.) and its purity may be determined by specific non-chromatographic methods or in combination with a chromatographic one.

4.1. The GSM requirements

(a) The **GSM** purity must be comparable to the standard compound

(b) All the components of the **GSM** must be capable to be separated and detected by GC

(c) The concentration of all the **GSM** components must be within the linear region of the detector response

(d) The **GSM** must be analysed in the same GC separation conditions as the mixture to be determined.

5. The group response factor

According to the group concept, it is possible to group $\mathbf{n} > 1$ GC separated analytes of a mixture no matter their elution order, and to consider the hypothetically formed group peak as a particular component.

By means of the GC analysis of the obtained **GSM** and taking into account the mentioned group concept, it is possible to determine the so called F_g^m for any group of components (to be available or not) that belong to the composition of the mixture to be quantified. If the **GSM** contains only a single unavailable component, it is feasible to obtain its response factor directly.

5.1. A *GSM* with a single unavailable component (a particular case)

The total mass of a mixture (M_T) can be expressed as the sum of the mass proportion of each mixture component (m_i).

Hence, for a **GSM**, it holds:

$$M_T = m_1 + m_2 + \cdots + m_n = \sum_{i=1}^{n} m_i = m_\Sigma \tag{42}$$

where $M_T(m_\Sigma)$ = total mass of the **n** mixture components. But, from Eq. (22):

$$m_i = \frac{m_r}{A_r} \cdot f_i^m A_i \tag{43}$$

Thus

$$\begin{aligned} M_T &= m_\Sigma \\ &= \frac{m_r}{A_r} \cdot f_1^m A_1 + \frac{m_r}{A_r} \cdot f_2^m A_2 + \cdots + \frac{m_r}{A_r} \cdot f_n^m A_n \\ &= \frac{m_r}{A_r} \sum_{i=1}^{n} f_i^m A_i \end{aligned} \tag{44}$$

where m_r/A_r = constant for a single analysis.

If the mixture contains a single unavailable analyte, then:

$$\begin{aligned} M_T &= m_1 + m_2 + \cdots + m_{n-1} + m_n = \sum_{i=1}^{n} m_i \\ &= m_\Sigma \end{aligned} \tag{45}$$

or

$$M_T = \sum_{i=1}^{n-1} m_i + m_n = \sum_{i=1}^{n} m_i = m_\Sigma \tag{46}$$

where

$$\sum_{i=1}^{n-1} m_i = m_{\Sigma d} \tag{47}$$

corresponds to the sum of all available components in the **GSM** and m_n = mass of a unavailable analyte.

Recalling Eq. (43), the term $m_{\Sigma d}$ can be rewritten as:

$$m_{\Sigma d} = \frac{m_r}{A_r} \cdot \sum_{i=1}^{n-1} f_i^m A_i \tag{48}$$

and therefore, by Eq. (47), the Eq. (46) identity could be rewritten as:

$$M_T = m_{\Sigma d} + m_n \tag{49}$$

Rearranging Eq. (49), it is possible to write, with respect to the identity [Eq. (48)]:

$$m_n = M_T - m_{\Sigma d} = M_T - \frac{m_r}{A_r} \sum_{i=1}^{n-1} f_i^m A_i \tag{50}$$

Finally, considering Eqs. (22) and (50), the corresponding f_n^m for a single unavailable analyte present in the **GSM** is given by:

$$\begin{aligned} f_n^m &= \frac{A_r(M_T - m_{\Sigma d})}{m_r A_n} \\ &= \frac{A_r}{m_r} \cdot \frac{M_T - \left(\frac{m_r}{A_r} \cdot \sum_{i=1}^{n-1} f_i^m A_i \right)}{A_n} \end{aligned} \tag{51}$$

where: A_n = area of the unavailable analyte obtained by GC analysis of the **GSM**.

In this particular case, the factor f_n^m is obtained taking into account the **GSM** defined properties and it can be employed for quantifying the component **n** by the conventional technique of GC quantitative analysis.

5.2. A *GSM* with **n** > 1 unavailable components

In general, for a **GSM** with **n** unavailable and **d** available components, the total mass (weight) of the mixture is given by:

$$\begin{aligned} M_T &= m_d + \cdots + m_{d+j} + m_n + \cdots + m_{n+k} \\ &= \sum_{i=d}^{n+k} m_i = m_\Sigma \end{aligned} \tag{52}$$

or

$$\begin{aligned} M_T &= \sum_{i=d}^{d+j} m_i + \sum_{i=n}^{n+k} m_i = \sum_{i=d}^{n+k} m_i = m_{\Sigma d} + m_{\Sigma n} \\ &= m_\Sigma \end{aligned} \tag{53}$$

where $m_{\Sigma n}$ = total mass of the unavailable components of the mixture.

Rearranging Eq. (53) yields:

$$m_{\Sigma n} = M_T - m_{\Sigma d} \tag{54}$$

Likewise, for the total peak areas of the **n**+**d** components of the GC separated **GSM**, it holds:

$$A_T = A_d + \cdots + A_{d+j} + A_n + \cdots + A_{n+k} = \sum_{i=d}^{n+k} A_i = A_\Sigma \quad (55)$$

which can be rewritten as:

$$A_T = \sum_{i=d}^{d+j} A_i + \sum_{i=n}^{n+k} A_i = \sum_{i=d}^{n+k} A_i = A_\Sigma \quad (56)$$

Let us denote the sum of the peak areas of the **d** available components and the **n** unavailable components of the **GSM** by:

$$\sum_{i=d}^{d+j} A_i = A_{\Sigma d} \quad (57)$$

and

$$\sum_{i=n}^{n+k} A_i = A_{\Sigma n} \quad (58)$$

respectively.

According to Eqs. (56)–(58):

$$A_{\Sigma n} = A_T - A_{\Sigma d} \quad (59)$$

Recalling Eqs. (48), (54) and (58) and considering the group-response Eq. (30), we can write:

$$F^m_{g,n} = \frac{A_r}{m_r} \cdot \frac{m_{\Sigma n}}{A_{\Sigma n}} = \frac{A_r}{m_r} \cdot \frac{(M_T - m_{\Sigma d})}{\sum_{i=n}^{n+k} A_i} = \frac{A_r}{m_r} \cdot \frac{M_T - \left(\frac{m_r}{A_r} \cdot \sum_{i=d}^{d+j} f^m_i A_i\right)}{\sum_{i=n}^{n+k} A_i} \quad (60)$$

where $F^m_{g,n}$ is the mass response factor of the group of the unavailable components present in the GC separated **GSM**.

If the **GSM** is composed by only **n** unavailable components, then the $F^m_{g,n}$ can be determined by Eq. (30).

6. Application procedures

6.1. The response factor determination for a unavailable component

This part of the paper also describes the group-correlation method as an application of the group-concept in order to reduce the uncertainty and improve the accuracy in the determination of the response factors of homologous series components.

By means of the present application, it can be possible to determine the response factor of any unavailable compound to be quantified. The obtained factor can be used for an analyte determination by the conventional internal standard method (ISM) of the GC quantitative analysis.

Procedure

1. Obtain a **GSM** that contains $\mathbf{d} \geq 1$ available components (already present or added to it) and the target analyte (**n**)
2. Prepare a **GSM** sample by dissolving a weighed amount of the latter with an appropriate solvent and add to it a defined amount of a chosen internal reference standard compound
3. Prepare a calibration sample containing approximately such proportion of those available components and an internal reference standard as the prepared **GSM** sample has
4. Submit the **GSM** and calibration samples to GC analysis using the same separation conditions
5. Determine the response factor f^m_i for the **GSM** available components by the expression: $f^m_i = m_i A_r / A_i m_r$ using the chromatogram data of the calibration sample where m_r is the mass of the internal reference compound **r** added to the calibration sample, A_r is the peak area of the **r** component, m_i is the mass (weight) of the available (standard) component and A_i is the peak area of the **i** component
6. Determine the response factor f^m_n for the unavailable component by the expression:

$$f^m_n = \frac{A'_r}{m'_r} \cdot \frac{M_T - \left(\frac{m'_r}{A'_r} \sum_{i=1}^{n-1} f^m_i A'_i\right)}{A_n}$$

using the **GSM** sample chromatogram data, where m'_r is the mass of the internal reference standard compound added to the **GSM** sample, A'_r is the peak area of the **r** component in the **GSM** chromatogram, M_T is the **GSM** weighed amount, A_n is the peak area of the unavailable component present in the **GSM**, f^m_i is the response factor of the available (standard) component and A'_i is the peak area of the available components in the **GSM** chromatogram.

The f_n^m found allows the quantitation of the mass proportion of the target unavailable analyte **n** present in a mixture to be determined by the conventional internal standard method.

6.2. Determination of the group-response factor

This application shows how to find, by means of the **GSM** GC analysis, the response factor of a group of unavailable components ($F_{g,n}^m$) that are present in a mixture to be determined.

The following procedure describes the steps for obtaining the $F_{g,n}^m$ by a **GSM** mixture that contains **n** unavailable components plus some of **d** available compounds, that might be present in the mixture to be determined or not.

Procedure

1. Prepare a **GSM** made up of a group of **n** unavailable components preferably with a similar quantitative proportion as it has in the mixture to be determined and some of **d** available compounds, which are already present in the obtained **GSM** or could be artificially added to it
2. Prepare a sample of the **GSM** by dissolving a weighed amount into an appropriate solvent and add a defined amount of a chosen internal reference standard compound to it
3. Prepare a calibration sample containing approximately such proportion of those available components and internal standard as the prepared **GSM** sample has
4. Submit the **GSM** and calibration samples to GC analyses using the same separation conditions
5. Determine the response factor f_i^m for the **GSM** available components by the known expression: $f_i^m = m_i A_r / A_i m_r$ using the chromatogram data of the calibration sample
6. Determine the group-response factor $F_{g,n}^m$ for the **n** unavailable component by the following relationship:

$$F_{g,n}^m = \frac{A'_r}{m'_r} \cdot \frac{M_T - \left(\frac{m'_r}{A'_r} \cdot \sum_{i=d}^{d+j} f_i^m A'_i \right)}{\sum_{i=n}^{n+k} A_i}$$

where m'_r is the mass of the internal reference standard compound added to the **GSM** sample, A'_r is the peak area of the **r** component in the **GSM** chromatogram, M_T is the **GSM** weighed amount, f_i^m is the response factor of the available (standard) component, A'_i is the peak area of the available components in the **GSM** chromatogram and $\Sigma_{i=n}^{n+k} A_i$ is the sum of the peak areas of the **n** unavailable components in the chromatogram of the separated **GSM**.

In the case that **GSM** contains only a group of **n**>1 unavailable components, the procedure steps are the same, but the $F_{g,n}^m$ is then calculated by the equation:

$$F_g^m = \frac{1}{R_{g,r}^m} = \frac{A_r}{m_r} \cdot \frac{\sum_{i=1}^{n} m_i}{\sum_{i=1}^{n} A_i}$$

6.3. Mixture composition determination

In general the mixture to be determined might be made up of **n**+**d** components, where **n** are unavailable and **d** are available**.** The mixture components could belong to an identical homologous series, or to different series.

According to the chosen quantitative strategy and taking into account the group method approach, it is possible to determine the quantitative proportion of a unavailable group of components of a mixture and to quantify its partial or total content.

General procedure

Let us obtain by the established procedure the $F_{g,n}^m$ for the group of **n** unavailable components of the mixture to be determined. Straight after, a weighed amount (M_P) of the problem mixture is dissolved into an appropriate solvent and mixed with a defined amount of a chosen internal standard compound and then the obtained sample is submitted to GC analysis.

Afterwards, the mass of the group of the **n** unavailable components can be calculated by means of the following expression:

$$m_n = \frac{m''_r}{A''_r} \cdot F_{g,n}^m \cdot \sum_{i=n}^{n+k} A''_i$$

where m''_r is the mass of the internal standard compound added to the mixture sample, A''_r is the peak area of the internal standard compound in the

chromatogram of the mixture to be determined and $\Sigma_{i=n}^{n+k} A_i''$ is the sum of the peak areas of the unavailable components in the chromatogram of the mixture to be determined.

The percentage mass proportion of the group of the **n** unavailable components in the mixture will be:

$$(\%) = \frac{m_n}{M_p} \cdot 100$$

Since: $M_p = m_n + m_d$ and $m_d = \frac{m_r''}{A_r''} \cdot \Sigma_{i=d}^{d+j} f_i^m A_i''$, then the experimental total mass of the mixture being determined (M_p') will be:

$$M_p' = \frac{m_r''}{A_r''} \cdot \left(\sum_{i=d}^{d+j} f_i^m A_i'' + F_{g,n}^m \cdot \sum_{i=n}^{n+k} A_i'' \right)$$

where m_d is the mass proportion of the **d** available components.

7. The group-correlation method (GCM)

The GCM can be used to improve the accuracy in the response factor determination by a correlation of the relative molar response ($R_{i,r}^{\mathrm{mol}}$) versus the molecular mass (M_i) of the homologous series compounds. For the homologous series of compounds, the relationship $R_{i,r}^{mol} = bM_i + a$ is well-obeyed [18,19].

At first sight, the above mentioned linear relationship could allow the determination of the $R_{i,r}^{\mathrm{mol}}$ parameter for any unavailable member of the series. However, in practice, the accuracy of the determination is very often low, mainly for the case when the $R_{i,r}^{\mathrm{mol}}$ is obtained by the extrapolation method, because the extent of the linear range is not known.

In similar fashion as for a single compound, for a group of components of a mixture it could be experimentally proved that $R_{g,r}^{\mathrm{mol}} = \beta \bar{M}_i + \alpha$, where: $\bar{M}_i$ is the mean value of molecular masses of the components that are considered into the group and $R_{g,r}^{\mathrm{mol}}$ the group-relative molar response.

The accuracy improvement in the unavailable component response factor determination by the linear regression approach can be fulfilled by including the group parameters ($R_{g,r}^{\mathrm{mol}}$, $\bar{M}_i$) into the available single component data ($R_{i,r}^{\mathrm{mol}}$, M_i).

In accordance with the group concept the GCM allows the determination of the response factor of one or more unavailable homologous series components of a mixture. In the first case, it is not necessary to prepare any **GSM**, but it is indispensable to suitably group some of the considered available components and to calculate their related group-relative response parameter.

The following procedure shows the steps for obtaining the response factor of **n**>1 unavailable homologous series components of a mixture.

Procedure

1. A **GSM** sample containing similar quantitative proportions of the target unavailable components as the mixture to be determined plus a minimum of three available compounds (already present in the **GSM** or artificially added to it), and a defined amount of a chosen internal standard compound is prepared in accordance with the **GSM** definition and requirements
2. A calibration standard mixture sample containing similar quantitative proportions of the considered available components as the obtained **GSM** and a defined amount of a chosen internal standard compound is prepared in accordance with the conventional ISM
3. The **GSM** and the standard calibration mixture samples are submitted to GC analysis using identical separation conditions
4. The response factor f_i^m of the standard calibration mixture components are calculated by the following known expression: $f_i^m = m_i A_r / A_i m_r$
5. The mass (m_i') of each available **GSM** component is computed by: $m_i' = f_i^m A_i' m_r' / A_r'$ where f_i^m is the response factor of the available (standard) component calculated by means of the calibration mixture prepared, A_i' is the peak area of the available component in the **GSM** chromatogram, m_r' is the mass of the internal reference standard compound added to the **GSM** sample and A_r' is peak area of the internal reference standard compound in the **GSM** chromatogram
6. The $R_{i,r}^{\mathrm{mol}}$ parameter of the available **GSM** components can be obtained using the calculated mass (m_i') of the latter and the **GSM** chromatogram data, that is:

$$R_{i,r}^{\mathrm{mol}} = \frac{m_r' A_i'}{A_r' m_i'} \cdot \frac{M_i}{M_r}$$

where M_i is the molecular mass of the available

component and M_r is the molecular mass of the internal standard component

7. The group parameter ($R^{\text{mol}}_{g,r}$) of the unavailable components of the **GSM** can be determined by the following empirical relationship:

$$R^{\text{mol}}_{g,r} \approx \frac{m'_r}{A'_r} \cdot \frac{\sum_{i=n}^{n+k} A_i}{M_T - \left(\frac{m'_r}{A'_r} \cdot \sum_{i=d}^{d+j} f^m_i A'_i\right)} \cdot \frac{\overline{M_n}}{M_r}$$

where $\overline{M_n}/M_r$ is numerically very close to the value of

$$\frac{\sum_{i=1}^{n} m_i \Big/ \sum_{i=1}^{n} n_i}{M_r}$$

referred to in Eq. (39), $\Sigma_{i=n}^{n+k} A_i$ is the peak area sum of the unavailable components in the **GSM**, $\Sigma_{i=d}^{d+j} f^m_i A'_i$ is the sum of the products ($f^m_i A'_i$) for the **GSM** available components, M_T is the **GSM** weighed amount and $\overline{M_n}$ is the mean molecular mass of the unavailable components of the **GSM**.

In the next step the $R^{\text{mol}}_{i,r}$, $R^{\text{mol}}_{g,r}$ and M_i, $\overline{M_n}$ values must be correlated by means of a least-squares procedure. Using the linear regression equation found, it is possible to obtain the value corresponding to the relative molar response ($R^{\text{mol}}_{n,r}$) of any unavailable component of the homologous series taken into account.

Afterwards, the $R^{\text{mol}}_{n,r}$ parameter found allows the determination of the molar response factor (f^n_n) or the mass response factor (f^m_n), by the following relationships:

$$f^n_n = \frac{1}{R^{\text{mol}}_{n,r}} \quad \text{and} \quad f^m_n = \frac{1}{R^{\text{mol}}_{n,r}} \cdot \frac{M_n}{M_r}$$

If the homologous series components taken into account in the response factor determination have an even and odd number of carbon atoms, it is expedient to group them separately. In such a way, the accuracy of the determination can be markedly improved.

The group-relative molar response ($R^{\text{mol}}_{g',r}$) for the available components with even or odd number of carbon atoms can be calculated using the following expression:

$$R^{\text{mol}}_{g',r} = R^m_{g',r} \cdot \frac{\sum_{i=d}^{d+j} m'_i \Big/ \sum_{i=d}^{d+j} n_i}{M_r} \approx R^m_{g',r} \cdot \frac{\overline{M_i}}{M_r}$$

where

$$R^m_{g',r} = \frac{m'_r}{A'_r} \cdot \frac{\sum_{i=d}^{d+j} A'_i}{\sum_{i=d}^{d+j} m'_i}$$

The procedure steps are the same as the ones related above. The pairs of values to be plotted are ($R^{\text{mol}}_{g',r}$, $R^{\text{mol}}_{g,r}$ and $\overline{M_i}$, $\overline{M_n}$).

As stated before, for the response factor determination of a single unavailable compound by the GCM, a **GSM** preparation is not necessary. In accordance with the group concept the considered available (standard) component can be appropriately grouped and the group parameters ($R^{\text{mol}}_{g',r}$) accordingly calculated by the above shown relationship.

Correlating three or more pairs of values as ($R^{\text{mol}}_{g',r}$ versus $\overline{M_i}$), a linear regression equation is obtained by which the f^n_n or f^m_n of a unavailable homologous series component can be calculated.

Employing the above-described approach, the accuracy of the conventional linear regression method for determining the response factor is improved.

8. Experimental corroboration

The main purpose of this part of the paper is to demonstrate the applicability of the group method procedures in the GC unavailable component determination of a mixture, by means of an analysis of acid model mixtures.

8.1. Reagents

The: 1-Nonadecanoic ($C_{19:0}$), 1-tetracosanoic ($C_{24:0}$), 1-hexacosanoic ($C_{26:0}$), 1-heptacosanoic ($C_{27:0}$), 1-octacosanoic ($C_{28:0}$), 1-nonacosanoic ($C_{29:0}$), 1-triacontanoic ($C_{30:0}$) and 1-hentriacontanoic ($C_{31:0}$) acids were obtained from Sigma (St. Louis, MO, USA), 99–100% GC.

Methanol, acetone and toluene (analytical-reagent grade) were from Merck (Darmstadt, Germany);

Table 1
Concentration of acids in the primary mixture[a]

Acid	$C_{19:0}$	$C_{26:0}$	$C_{27:0}$	$C_{28:0}$	$C_{29:0}$	$C_{30:0}$	$C_{31:0}$
C_i (mg/ml)	1.016	0.067	0.124	2.090	0.135	1.139	0.075

[a] ($C_{19:0}$) = internal standard.

further, aqueous HCl (37%)–methanol (5%, v/v) was used.

8.2. Instrumentation

A GC-14A gas chromatograph (Shimadzu, Kyoto, Japan), equipped with a FID system and coupled to a C-R4A computerised data processor (Shimadzu, Kyoto, Japan) was used. Also an analytical balance AG245 (precision: 0.01 mg) (Mettler, Toledo, Switzerland) and a Multi-Blok heater and sample concentrator (LabLine Instruments, IL, USA) were used.

8.3. Model mixture preparation

A primary mixture of standard acids was prepared dissolving a weighed amount of each chosen acid in 10 ml of toluene (see Table 1).

Four working samples were prepared taking 1.0 (A), 1.5 (B), 2.0 (X), 2.2 (C) and 2.5 (D) ml respectively of the primary mixture and pouring it into 3 ml vials.

Each working sample of acids was then gently evaporated under a nitrogen flow and methylated by adding 1 ml of 5% aqueous HCl–methanol and heating it tightly closed at 80°C for 1.5 h. Afterwards, the vials were opened and the samples were evaporated to dryness by a slow nitrogen flow. Then, a volume of 1 ml of toluene was added to each dry ester mixture and the vials were once again tightly closed and this way the samples were ready to GC analysis.

Samples A and C were taken as normal calibration mixtures, samples B and D as a model **GSM** and sample X as a problem mixture.

8.4. GC analysis

For GC separations two column types were used, a glass column (3.1 m × 3.0 mm) packed with Chromosorb W (HP) 80–100 mesh coated with 3% OV-101 and a BP5 Wide-bore fused-silica capillary column (25 m × 0.53 mm, 1.0 μm film thickness). The GC analysis conditions consisted of an injector temperature of 320°C, a detector temperature of 300°C, and an oven temperature program that went from 200 to 320°C (packed column) or 100 to 320°C (Wide-bore) at 10°C/min and then held for 20 min in both cases. The carrier gas flow-rates were for packed column (argon) 40 ml/min and for Wide-bore column (hydrogen) 13.4 ml/min. The hydrogen and air flow-rates for FID were 30 ml/min and 300 ml/min, respectively.

In Table 2 are reported the response factors calculated by the conventional ISM of each component of samples A (packed column) and B (Wide-bore column), respectively. The raw data from the GC separation of the samples B, D and X are presented in Tables 3–5, respectively.

9. Results and discussion

9.1. Response factor determination of a single unavailable component of a mixture

The response factor of an unavailable compound can be determined by the chromatographic analysis of a **GSM** containing such a component.

Table 2
Response factors (f_i^m) calculated by the GC separation of sample A (packed column) and C (Wide-bore column), respectively (n = 5)

Acid	$\overline{f_i^m} \pm$ SD	
	Packed column	Wide-bore column
$C_{26:0}$	1.164±0.022	0.981± 0.008
$C_{27:0}$	1.085±0.012	1.012±0.007
$C_{28:0}$	0.929±0.007	0.922±0.005
$C_{29:0}$	1.062±0.028	0.913±0.006
$C_{30:0}$	1.000±0.008	0.952±0.006
$C_{31:0}$	1.325±0.047	0.954±0.008

Table 3
Raw data from the GC separation of the sample B used as a model **GSM**. Weighed amount of the **GSM**, $M_T = 5.443$ mg. Internal standard ($C_{19:0}$) added mass, $m'_r = 1.524$ mg ($n = 5$)

Acid	Net response replicates (A')				
	1	2	3	4	5
$C_{19:0}$	72 923	74 613	79 145	77 648	77 527
$C_{26:0}$	4373	4205	4538	4292	4467
$C_{27:0}$	7848	8500	8379	8268	8923
$C_{28:0}$	161 518	164 697	173 978	170 645	170 605
$C_{29:0}$	9240	9592	10277	9426	9996
$C_{30:0}$	83 668	85 544	89 734	87 697	88 094
$C_{31:0}$	4384	4222	4179	4242	4298

Table 4
Raw data from the GC separation of the sample D used as a model **GSM**. Weighed amount of the **GSM**, $M_T = 7.977$ mg. Internal standard ($C_{19:0}$) added mass, $m'_r = 2.235$ mg ($n = 5$)

Acid	Net response replicates (A')				
	1	2	3	4	5
$C_{19:0}$	133 070	137 977	134 028	137 093	136 190
$C_{26:0}$	8465	8805	8483	8678	8442
$C_{27:0}$	15 866	16 494	15 969	16 314	16 022
$C_{28:0}$	297 281	309 603	300 350	307 571	304 510
$C_{29:0}$	18 777	19 355	19 035	19 317	18 958
$C_{30:0}$	157 183	162 952	158 567	162 348	160 435
$C_{31:0}$	9753	9989	10 077	9869	9638

Table 5
Raw data from the GC separation of the sample X used as a problem mixture. Weighed amount of the problem mixture, $M_P = 7.665$ mg. Internal standard ($C_{19:0}$) added mass, $m''_r = 2.032$ mg ($n = 5$)

Acid	Net response replicates (A'')				
	1	2	3	4	5
$C_{19:0}$	99 567	95 659	97 261	98 073	99 321
$C_{26:0}$	5563	5505	5342	5368	5649
$C_{27:0}$	11 344	11 145	11 461	11 256	11 373
$C_{28:0}$	218 495	212 394	213 515	214 487	218 653
$C_{29:0}$	12 731	12 373	12 220	12 883	12 737
$C_{30:0}$	113 844	110 673	112 378	112 168	112 536
$C_{31:0}$	5853	5817	5679	5872	5750

Table 6 shows the data of the group method (GM) application in the response factor determination of a single unavailable component (f_n^m, $n = C_{30:0}$) in a mixture (GC analysis of sample B taken as a model **GSM**).

The precision (relative standard deviation, RSD) and the accuracy (relative error, RE) of the f_n^m value compared with the value of the response factor (f_i^m) obtained from the same GC raw data (Table 3) by the conventional ISM were acceptable. Statistically, no differences were found between the f_n^m and f_i^m calculated.

*9.2. Group method determination of a mixture composed of **n** unavailable and **d** available components*

In order to demonstrate the possibility of quantifying a mixture composed of several unavailable components among others that can be available, an aliquot of a problem mixture (sample X) was analysed five times by GC (see Table 5).

Aimed at proving the influence of the acid type and its mass proportion in the mixture as well as its elution position (from $C_{19:0}$ up to $C_{31:0}$) in the GC fingerprint on the quantitative evaluation of the problem sample, the components of the latter were appropriately divided into two sets of available and unavailable compounds as follows: Case A (available acids, $\mathbf{d} = C_{26:0}$, $C_{28:0}$, $C_{30:0}$; unavailable acids, $\mathbf{n} = C_{27:0}$, $C_{29:0}$, $C_{31:0}$) and Case B (available acids, $\mathbf{d} = C_{27:0}$, $C_{29:0}$, $C_{31:0}$; unavailable, $\mathbf{n} = C_{26:0}$, $C_{28:0}$, $C_{30:0}$).

The mass proportions of those assumed as available components were obtained by means of the ISM using the f_i^m calculated by the GC packed column separation reported in Table 2. The quantitation of those grouped unavailable acids was done using the group-response factors ($\bar{F}_{g,n}^m = 1.0805$ for odd grouped acids and $\bar{F}_{g,n}^m = 0.9616$ for even grouped acids), calculated according to the previously described procedure from the **GSM** GC data reported in Table 3.

The precision and accuracy of the experimentally found acid sample mass (M'_P), independently of the even or odd carbon number of the acids and their concentration and the respective elution order, were quite good and very similar in both cases (see Table 7).

Table 6
Response factor of the analyte $C_{30:0}$ assumed as a unavailable component determined by the group method (GM) and by the conventional ISM (sample B). Total mass of sample introduced into GC column, $M_T = 5.443$ μg. ($n = 5$, $t_{tab.} = 2.306$, $\alpha = 0.05$). $t_{exp.} < t_{tab.}$ (NS: not significantly different)

(GM)$\bar{f}_n^m \pm$SD	RSD(%)	(ISM)$\bar{f}_i^m \pm$SD	RSD(%)	RE(%)[a]	$t_{exp.}$[b]
0.991±0.020	2.02	0.984±0.007	0.71	0.64	0.665(NS)

[a] $RE(\%) = \dfrac{|\bar{f}_i^m - f_n^m|}{\bar{f}_i^m} \cdot 100.$

[b] $t_{exp.} = \dfrac{|\bar{f}_n^m - \bar{f}_i^m|}{\sqrt{\dfrac{(SD)_n^2 + (SD)_i^2}{n}}}$

Table 7
Precision (RSD) and accuracy (RE) of the group method determination of a mixture (sample X) composed of **n** unavailable and **d** available components. Given sample mass ($M_P = 7.665$ mg). GC packed column separation ($n = 5$ analysis replicates)

Case A			Case B		
$\bar{M}_p' \pm$SD	RSD(%)	RE(%)	$\bar{M}_p' \pm$SD	RSD(%)	RE(%)
7.281±0.041	0.56	5.0	7.341±0.042	0.57	4.2

9.3. Response factor determination of homologous series unavailable components of a mixture by the group-correlation method approach

The GCM approach is based upon the experimental validity of the equations $R_{i,r}^{mol} = bM_i + a$ (for the individual components of a mixture) and $R_{g,r}^{mol} = \beta\bar{M}_i + \alpha$ (for the grouped components of a mixture). Following the steps described in the GM procedures and using the f_i^m (ISM) reported in Table 2 (sample C), the mass proportion of each assumed as an available component of the model GSM (acids $C_{26:0}$, $C_{27:0}$, $C_{28:0}$ and $C_{29:0}$ of sample D), was obtained and the corresponding $R_{i,r}^{mol}$ parameter was calculated.

Then, the group parameters $R_{g,r}^{mol}$ and $R_{g',r}^{mol}$ for unavailable grouped acids ($C_{30:0} + C_{31:0}$) and for the available pairs ($C_{26:0} + C_{28:0}$, $C_{27:0} + C_{29:0}$) respectively, were determined.

Using the previously calculated parameters and taking into account the molecular masses (M_i) of the acids: $C_{26:0}$ (410.7), $C_{27:0}$ (424.7), $C_{28:0}$ (438.8), $C_{29:0}$ (452.8), $C_{30:0}$ (466.8) and $C_{31:0}$ (480.9), there were obtained six different linear regression equations by which the individual f_n^m ($C_{30:0}$ and $C_{31:0}$) of each target acid was determined: Correlation of $R_{i,r}^{mol}$ versus M_i: C1 (i = $C_{26:0}$, $C_{27:0}$, $C_{28:0}$ $C_{29:0}$), and C2 (i = $C_{26:0}$, $\underline{C_{27:0}}$, $C_{28:0}$). Correlation of $R_{i,r}^{mol}$,$R_{g,r}^{mol}$ versus M_i, $\bar{M}_n$: C3 (i = $C_{26:0}$, $C_{27:0}$, $C_{28:0}$,$C_{29:0}$; g = $C_{30:0} + C_{31:0}$) and C4 (i = $C_{26:0}$, $C_{27:0}$, $C_{29:0}$; g = $C_{30:0}\underline{+C_{31:0}}$). Correlation of $R_{i,r}^{mol}$,$R_{g',r}^{mol}$, $R_{g,r}^{mol}$ versus M_i, $\bar{M}_i$, $\bar{M}_n$: C5 (i = $C_{26:0}$, g′ = $C_{27:0} + C_{29:0}$, $\underline{g}$ = $\underline{C_{30:0} + C_{31:0}}$). Correlation of $R_{g',r}^{mol}$, $R_{g,r}^{mol}$ versus $\bar{M}_i$, $\bar{M}_n$: C6 (g′ = $C_{26:0} + C_{28:0}$; g′ = $C_{27:0} + C_{29:0}$, g = $C_{30:0} + C_{31:0}$) (see Table 8).

Table 8
Confidence interval (CI), precision (RSD) and relative error (RE) of the response factors (f_n^m) of the acids $C_{30:0}$ and $C_{31:0}$ determined by means of the group-correlation method approach ($n = 5$, $t_{tab.} = 2.776$, $\alpha = 0.05$); C = Correlation[a]

Mean correlation coefficient ($\bar{r}$)	Acid	f_n^m (CI)	RSD(%)	RE(%)
C1 (0.9500)	$C_{30:0}$	0.888–0.891	0.15	6.59
	$C_{31:0}$	0.867–0.870	0.18	8.81
C2 (0.9454)	$C_{30:0}$	0.899–0.902	0.17	5.54
	$C_{31:0}$	0.888–0.882	0.22	7.56
C3 (0.8993)	$C_{30:0}$	0.939–0.947	0.41	1.77
	$C_{31:0}$	0.928–0.938	0.53	2.74
C4 (0.9279)	$C_{30:0}$	0.939–0.947	0.43	1.00
	$C_{31:0}$	0.921–0.931	0.56	2.04
C5 (0.9958)	$C_{30:0}$	0.952–0.962	0.53	0.50
	$C_{31:0}$	0.947–0.960	0.68	0.08
C6 (0.9981)	$C_{30:0}$	0.953–0.964	0.55	0.64
	$C_{31:0}$	0.953–0.968	0.75	0.83

[a] C1 and C2 are single component correlations.

As the results in Table 8 indicate, the conventional $R_{i,r}^{mol}$ versus M_i correlation carried out with individual component data (C1 and C2) showed the worse accuracy (expressed as relative error regarding the f_i^m

Table 9
Confidence interval (CI) of the mass response factors (f_n^m) of the regarded as unavailable acids from the model GSM (sample D), calculated by the numerical ISM. ($n=5$, $t_{tab.}=2.776$, $\alpha=0.05$)

Acid	$\bar{f}_n^m \pm SD$	$CI=\bar{f}_n^m \pm (SD)t_{tab.}/\sqrt{n}$
$C_{30:0}$	0.9524±0.0001	0.9523–0.9525
$C_{31:0}$	0.9524±0.0014	0.9510–0.9538

obtained by the numerical ISM), that are reported in Table 9. When it is used the way of grouping peaks (GM) the accuracy was considerably improved (C3 to C6).

It can be noted that the values of the f_n^m found by correlations 5 and 6 lie within the confidence interval of the response factors that were calculated by the numerical ISM (see Table 9).

10. Conclusions

The demonstrative experiences performed by means of model mixtures proved the applicability of the group method approach. The possibility of the response factor determination of a single unavailable component in a mixture as well as a precise and accurate quantitation of a mixture composed of several unavailable analytes among others that are available were demonstrated.

It was experimentally proved that the group-correlation method is a suitable approach for the practical improvement of the accuracy in the determination of a response factor of any unavailable component that belongs to a homologous series of compounds.

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