Chromametrics

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Chapter 3

Quantitative GC×GC analysis.*

Quantitative analysis using comprehensive two-dimensional gas chromatography is still rarely reported. This is largely due to a lack of suitable software. The objective of the present study is to generate quantitative results from a large GC×GC dataset, consisting of thirty-two chromatograms. In this dataset, six target components need to be quantified. We compare the results of conventional integration with those obtained using so-called "multiway analysis methods". With regard to accuracy and precision, integration performs slightly better than Parallel Factor (Parafac) analysis. In terms of speed and possibilities for automation, multiway methods in general are far superior to traditional integration.

3.1 Introduction

The demand for reliable, precise and accurate data in the analysis of complex mixtures is rapidly increasing. This is partly caused by an increased demand for comprehensive characterization of mixtures due to legislation, health concerns, controlled processing, *etc.*. Meeting this demand requires significant technological advances.

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One of the greatest and most significant advances for the characterization of complex mixtures of volatile compounds is comprehensive two-dimensional gas chromatography (GC×GC). This technique was pioneered and advocated by the late John Phillips [1–3]. In GC×GC, two GC columns are used. The fist-dimension column is (usually) a conventional capillary GC column, with a typical internal diameter of 250 or 320 μm. Most commonly, this column contains a non-polar stationary phase, so that it separates components largely based on their vapour pressures (boiling points). The second-dimension column is considerably smaller (smaller diameter, shorter length) than the first-dimension column, so that separations in the second dimension are much faster. The stationary phase is selected such that this column separates on properties other than volatility, such as molecular shape or polarity. Between the two columns, a modulator is placed. In the modulation process, small portions of the effluent from the first-dimension column are accumulated and injected into the second column. A large number of fractions are collected and the resulting gas chromatogram contains a large series of such fast chromatograms in series (and partly superimposed). When the second-dimension chromatograms are 'demodulated' [5], a two-dimensional representation of the separation is obtained and typically displayed as a colour or contour plot, a so-called chroma^{2}gram.

Many applications have shown the advantages of GC×GC over conventional GC, for instance in the petrochemical field [64, 77], essential oil [59, 60], fatty acids [69], pesticides [78], and polychlorinated biphenyls [50]. However, GC×GC is still largely a method for qualitative analysis. Quantitative analysis by GC×GC is much less commonly used. The first quantitative results obtained with GC×GC were reported by Beens *et al.* [79] in 1998. They applied an in-house integration package called "Tweedee" for the characterization of heavy gas oils. This program integrated 2D slices, followed by a summation along the first dimension. The program worked well on baseline-separated peaks, but it lacked sophisticated integration algorithms to cope with less-ideal situations. Several research groups working on GC×GC have developed their own software for quantification [80, 81].

Synovec *et al.* reported on the use of multiway methods using the so-called "second-order advantage" in order to retrieve quantitative data from GC×GC [15, 16, 76, 82, 83]. Multiway routines, such as the Generalized Rank-Annihilation Method (GRAM) were demonstrated to perform well in this

respect. For the flavour and fragrance industry, quantification of trace com-

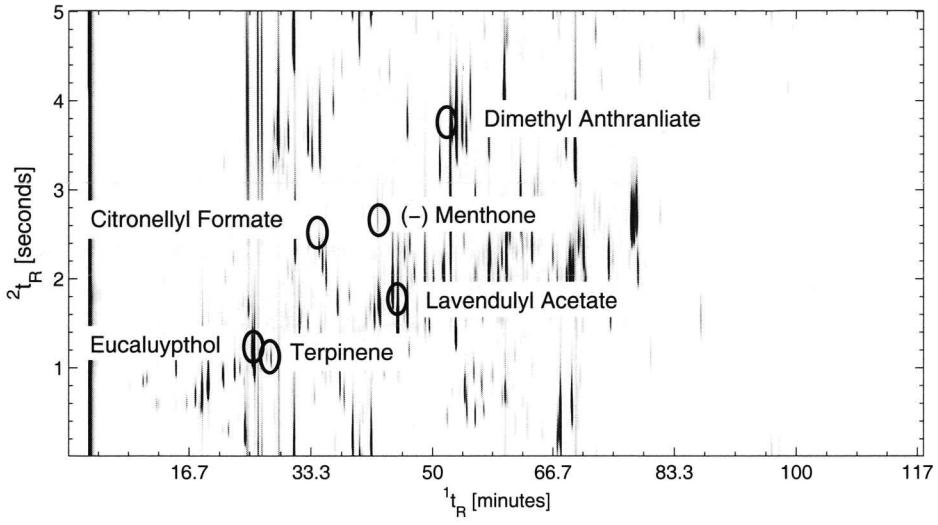

Figure 3.1: Chroma^{2}gram of a (synthetic) perfume sample.

pounds, such as essential-oil markers, is of high importance. The presence of essential oils has a big impact on both the olfactory quality and the price of a perfume. For quality control or competitor analysis, identification and quantification of essential oils is usually done through markers [56]. Cheap and chemically produced alternative ingredients often co-exist in the perfume composition. Markers are present at low levels in the essential oils and thus at trace levels in the entire formulation. GC×GC should yield accurate concentrations and low detection limits for these components.

This study describes the use of GC×GC to quantify essential-oil markers in full perfumes (*i.e.* complete formulations). Our goal has been to quantitate a limited number of target analytes in very complex GC×GC chromatograms by comparing integration with multiway-analysis methods.

3.2 Theory

3.2.1 Quantification

Integration of one-dimensional chromatograms to obtain quantitative data is well established. Typically, first-order and second-order derivatives are used to mathematically detect the peak "start", peak top, and peak "stop", as well as the presence of shoulders. Although far from trivial, integration is now generally regarded as reliable, reasonably fast, and accurate. However, for data obtained from a comprehensive two-dimensional separation, chromatographic integration yields only data that are integrated in the direction of the second-dimension chromatograms. A second step has to be performed to integrate the data along the direction of the first dimension. This can be done either automatically [84] or manually by drawing summation boxes, as is done in the present study.
Another approach can be to utilize the "second-order advantage", using the two-way nature of the measuring techniques. This can be achieved through so-called "multiway techniques", as described below. Synovec and Fraga described the application of the Generalized Rank-Annihilation Method (GRAM) to GC×GC data in order to retrieve both pure-component elution profiles and quantitative information [16, 85].

Nomenclature
In this article, standardized terminology is used, as proposed by Kiers [86] for multiway analysis and by Schoenmakers, Marriott and Beens [87] for comprehensive two-dimensional chromatography.

3.2.2 Multivariate analysis

Standard multivariate data analysis requires data to be arranged in a two-way structure, such as a table or a matrix. An example is a table in spectroscopy, where for different samples absorbances are measured at different wavelengths. The table can be indexed by sample-number and by wavelength and therefore is a two-way array. Two-way methods, such as principal-components analysis (PCA) can be used for the analysis of this type of data. When the relation between absorbances and, for instance, concentrations is wanted, techniques such as Partial Least Squares (PLS)

regression can be used. In many applications PCA and PLS are of prime importance. Near-infrared spectroscopy (NIR) essentially relies on these techniques [88].

In many other cases, a two-way arrangement of the data is not sufficient and a description in more directions is needed. One example is formed by the excitation/emission fluorescence spectra of a set of samples. Each data element can then be indexed by the sample number, emission wavelength, and excitation wavelength, which implies that we have a three-way matrix. When data can be arranged in matrices of order three or higher, it is referred to as "multiway" data. Multiway methods have been applied to a wide variety of problems [89]. Some examples are the decomposition of fluorescence-spectroscopy data of poly-aromatic hydrocarbons [90], the prediction of amino-acid concentrations in sugar with fluorescence spectroscopy [91], data exploration of food analysis with gas chromatography and sensory data [92], and the calibration of liquid-chromatographic systems [93, 94]. A dataset obtained from comprehensive two-dimensional gas chromatography (GC×GC) with flame-ionization detection can also be regarded as three-way. When all second-dimension chromatograms are stacked on top of each other, each data element can be indexed by first, - and second-dimension retention axes and by sample number and contains an FID response. When mass-spectrometry is used, data can be regarded as a four-way arrangement and indexed by first- and second-dimension retention axes, a mass axis and a sample number. Each element then contains an ion count.

Methods for multiway analysis are extensions of existing MVA routines. PCA can be generalized to higher order data in two different ways, Parallel Factor Analysis (Parafac) and Tucker models, while PLS can be expanded, for example, to multilinear PLS [95] or to multiway covariates regression [96].

Parafac

Parallel Factor (Parafac) analysis is a generalization of PCA toward higher orders. It is a true multiway technique, which decomposes a multiway dataset into one or more combinations of vectors ("triads"). The Parafac model was proposed in the 1970's, independently by Carrol and Chang under the name CANDECOMP (Canonical Decomposition) [97] and by Harshman under the name Parafac [98]. Essentially, Parafac models the

data as follows: In this schematic overview, the stacked chromatograms

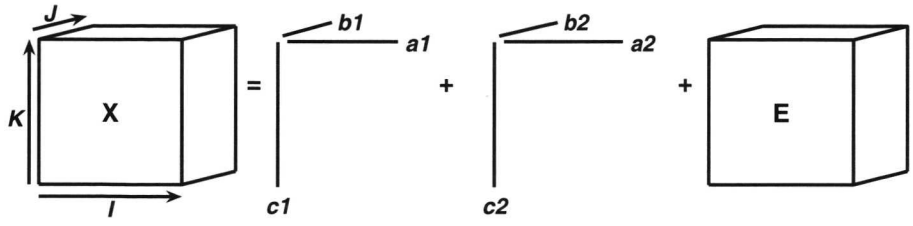

Figure 3.2: Schematic two factor Parafac model.

are represented by the matrix $\boldsymbol{X}$ with dimensions $(I \times J \times K)$. In our case I indicates the first-dimension fraction (retention time), J the second-dimension retention time, and K the specific sample or injection.
Tri-linear decomposition through Parafac into a two-component model yields two triads, $a1$, $b1$, $c1$ and $a2$, $b2$, $c2$ with the dimensions $a(I \times 1)$, $b(J \times 1)$ and $c(K \times 1)$. Matrix $\boldsymbol{E}$ contains the data not fitted in this two-component model. Each coordinate in the data cube $\boldsymbol{X}$ can be described by Parafac as the product of the first- and second-dimension points in both a and b, multiplied by the relative concentration in c:

$$x_{ijk} = \sum_{i=1}^{R} a_{ir} b_{jr} c_{kr} + e_{ijk} \tag{3.1}$$

Where:

x_{ijk}	FID response at ${}^1t_{R,i}$ and ${}^2t_{R,j}$ for the k^{th} sample
R	Number of factors (components)
a_{ir}	Value of ${}^1t_{R,i}$ (first-dimension elution time i) for component r
b_{jr}	Value for ${}^2t_{R,j}$ (second-dimension elution time j)for component r
c_{kr}	Relative concentration for sample k and component r
e_{ijk}	Residual for coordinate e_{ijk}

Described in a different (slab-wise) way the Parafac decomposition is given by:

$$\boldsymbol{X}_k = \boldsymbol{A}\boldsymbol{D}_k\boldsymbol{B}^T + \boldsymbol{E}_k \tag{3.2}$$

Where:

$\boldsymbol{X}_k$	chromatogram for k^{th} sample $(I \times J)$
$\boldsymbol{A}$	Matrix containing ${}^1t_R$ elution profile $(I \times R)$
$\boldsymbol{D}$	Diagonal containing weights (relative concentrations) of k^{th} sample of $\boldsymbol{X}$ $(R \times R)$ (From $\boldsymbol{C}$)
$\boldsymbol{B}$	Matrix containing ${}^2t_R$ elution profiles $(R \times J)$
$\boldsymbol{E}_k$	Residual for k^{th} sample in $\boldsymbol{X}$ $(I \times J)$

Constraints

In mathematical terms, empirical models are used to describe the data as well as possible. Negative values in the estimated loadings arise if these result in a better solution. However, negative values are often undesirable in chemical and physical applications. In our case, negative FID responses and concentrations are clearly unrealistic. By limiting the solution in the concentration direction to non-negative values, and peak profiles in both retention directions to be unimodal and non-negative, chemically meaningful results are obtained.

Uniqueness

For many bilinear methods there is a problem concerning rotational freedom. The loadings in spectral bilinear decomposition represent linear combinations of the rotated, pure spectra. Additional information is required to find the true (physical) pure-component spectra. Parafac, however, is capable of finding the true underlying pure-component spectra if the dataset is truly trilinear.

The Parafac and Parafac2 equations are solved through an alternating least-squares minimization of the residual matrix and yields direct estimates of the concentrations without bias.

Parafac2

Most multiway methods assume parallel proportional profiles (*e.g.* invariable absorbtion wavelengths or elution times). In some cases, such as batch-process analysis, the time required to process a batch may vary, resulting in unequal record lengths. In chromatography, peaks may shift due to minor deviations in conditions. Many multiway methods cannot deal with such shifted (time) axes. Parafac2 handles shifted profiles through the inner-product structure [99]. It uses this property to deal with stretched

time axes. The Parafac2 algorithm can be described schematically as follows:

$$\boldsymbol{X}_k = \boldsymbol{A}_k \boldsymbol{D}_k \boldsymbol{B}^T + \boldsymbol{E}_k \tag{3.3}$$

Where:

- $\boldsymbol{A}_k$ Matrix containing ${}^1t_R$ elution profile the for k^{th} sample$(I \times R)$
- $\boldsymbol{D}_k$ Diagonal containing weights (relative concentrations) of k^{th} sample of $\boldsymbol{X}$ $(R \times R)$
- $\boldsymbol{B}$ Matrix containing ${}^2t_R$ elution profiles $(R \times J)$.
- $\boldsymbol{E}_k$ Residual for k^{th} sample in $\boldsymbol{X}$ $(I \times J)$.

A useful property of $\boldsymbol{A}_k$ is that $\boldsymbol{A}_k^T \boldsymbol{A}_k = \boldsymbol{A}^T \boldsymbol{A}$ for $k = 1, .., K$. In other words, the cross-product of the $\boldsymbol{A}$ matrix is constant for all samples. In Table 3.1, a simulated GC×GC peak is given $(\boldsymbol{A})$, while $(\boldsymbol{B})$ and $(\boldsymbol{C})$ are the same distribution shifted by one and two positions, respectively. Figure 3.3 projects the data in the form of a two-dimensional peak The inner products ($\boldsymbol{A}^T\boldsymbol{A}$, $\boldsymbol{B}^T\boldsymbol{B}$ and $\boldsymbol{C}^T\boldsymbol{C}$) yield the square of each cell and on the diagonal the sum of squares appears. Note the three situations yield identical values.

In literature, Parafac2 has been used for the decomposition of LC-PDA

$\boldsymbol{A}$				$\boldsymbol{B}$				$\boldsymbol{C}$			
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	2	0	0	0	1	0	0	0	0	0
0	1	3	1	0	0	2	0	0	0	1	0
0	3	5	3	0	1	3	1	0	2	0	0
0	1	3	1	0	3	5	3	0	1	3	1
0	0	2	0	0	1	3	1	0	3	5	3
0	0	1	0	0	0	2	0	0	1	3	1
0	0	0	0	0	0	1	0	0	0	2	0
0	0	0	0	0	0	0	0	0	0	1	0
$\boldsymbol{A}^T\boldsymbol{A}$				$\boldsymbol{B}^T\boldsymbol{B}$				$\boldsymbol{C}^T\boldsymbol{C}$			
0	0	0	0	0	0	0	0	0	0	0	0
0	11	21	11	0	11	21	11	0	11	21	11
0	21	53	21	0	21	53	21	0	21	53	21
0	11	21	11	0	11	21	11	0	11	21	11

Table 3.1: Simulated GC×GC data for Parafac2.

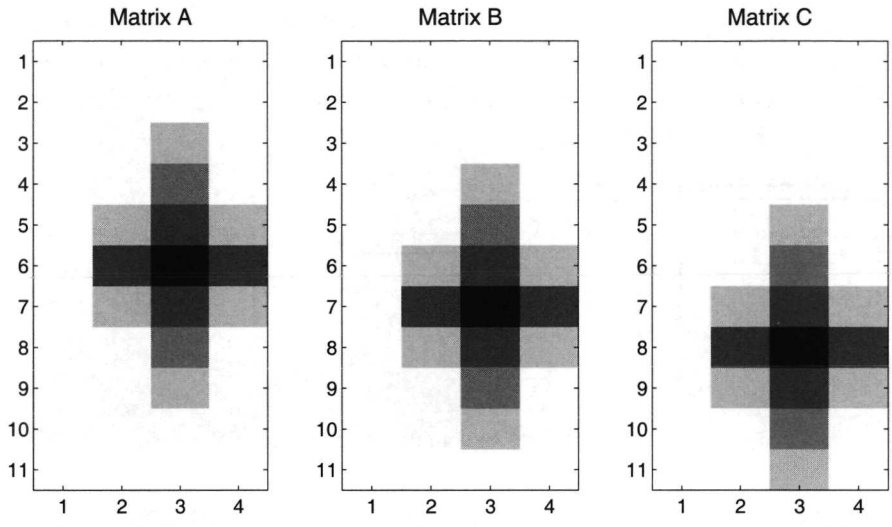

Figure 3.3: Effect of shift of peak position on inner-product.

(Liquid Chromatography - Photo-Diode Array) data [100] and for fault detection in batch-process monitoring [101].
Parafac2 only permits the inner-structure relationship in one direction. For LC-PDA this limitation is easy to justify, as retention-time shifts only occur in the LC direction. For GC×GC, however, shifts can (and will) occur in both retention directions, but they are not identical along the two retention axes. In the second dimension, a peak typically spans at least 15 points, while in the first dimension a maximum of 7 slices encompass a peak. Therefore, the flexibility of Parafac2 is applied along the first-dimension axis, to deal with differences in peak profiles between different injections.

Multilinear PLS

Partial-Least-Squares (PLS) regression is a method for building regression models between independent ($\boldsymbol{X}$) and dependent (y) variables. First, a regression model is calculated, based on calibration data. Decomposition is accomplished in such a way that the computed score vectors of $\boldsymbol{X}$ have maximum covariance with y. Applying the model to samples (unknowns) yields prediction of y.
One specific extension of PLS toward higher orders is called multi-linear

Partial-Least-Squares (NPLS) regression. In this method a multidimensional model is constructed to describe the variance in y. A schematic overview of NPLS is shown below: The NPLS method does not feature built-in con-

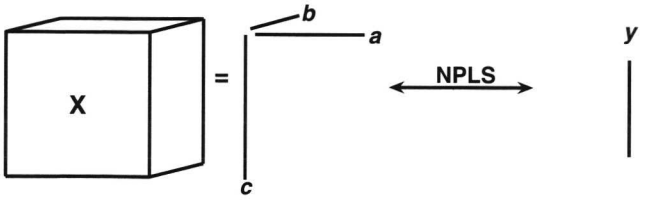

Figure 3.4: Schematic NPLS model.

straints, which may lead to erroneous predictions. Furthermore, in our case the NPLS model needs to be trained using a calibration dataset containing only standards. This may lead to the introduction of additional errors, since the samples contain many more components than the calibration mixtures. Bro has used the NPLS method for the determination of fly ash content in sugar by fluorescence spectroscopy [95] and for the quantification of isomers from tandem-MS experiments [102]. According to the nomenclature of Bro [95], the data presented in the present article can be described by a tri-PLS-1 model (three orders in $\boldsymbol{X}$ and one order in y).
The advantage of NPLS models is their ease of use. The construction of a model is straightforward and there is no external regression step involved. Application of the NPLS method directly yields concentrations for the samples.

3.3 Experimental

3.3.1 Instrumentation

The GC×GC system consists of an HP6890 series GC (Agilent Technologies, Wilmington, DE, USA), configured with a flame-ionization detector (FID) and a Gerstel Cis-4 PTV injector (Gerstel, Muhlheim an der Rühr, Germany) and retrofitted with a second-generation modulator (Zoex, Lincoln, NE, USA) as described by Phillips *et al.* [103]. This device contains a rotating "Sweeper" thermal modulator and a cassette system, which enables

independent heating of the second-dimension column.
The column-set consisted of a 10 m length × 0.25 mm i.d. × 0.25 mm film thickness DB-1 column (J&W Scientific, Folsom, CA, USA). The second-dimension column was 1.2 m × 0.1 mm × 0.1 mm DB-Wax (J&W). The modulation capillary was a 0.07 m × 0.1 mm × 3.5 mm SE-54 column (Quadrex, New Haven, CT, USA). Between the first-dimension column and the modulator, the modulator and the second-dimension column and the second-dimension column and the detector, diphenyltetramethyl-disilazane (DPTMDS) deactivated fused-silica tubing was used (0.1 m × 0.1 mm, TSP 100200-D10, BGB Analytik, Anwil, Switzerland). Columns were coupled with custom-made press-fits (Techrom, Purmerend, The Netherlands).
The carrier gas was helium set at a pressure of 200 kPa, resulting in a flow of approximately 0.8 ml/min at a temperature of 40°C, except for the second calibration mixture, which was analyzed at a carrier gas pressure of 175 kPa, with the intention of inducing retention-time shifts and variations in the first-dimension peak shapes.
The temperature of the first-dimension column oven was programmed from 35°C (5 min isothermal) to 225°C (5 min isothermal) at 2°C/min. The second-dimension column temperature was maintained at 30°C above that of the first-dimension column during the entire experiment.
The modulator was operated at 0.25 rev/s and a slit voltage of 70 V was used (resulting in approximately 100°C elevation of the slotted heater relative to the oven temperature). The modulation time (*i.e.* the time between successive modulations) was 5 seconds.

Instrument control and data processing
The detector signal was recorded with EZ-Chrom Elite software (version 2.61, SP1 SSI, Willemstad, The Netherlands) with an acquisition rate of 50.08 Hz in order to obtain a sufficient number of points across a peak. Data handling was performed with software written in MATLAB R13 (The Mathworks, Natick, MA, USA) running on a Compaq Evo 6000 equipped with two Xeon 2.2 GHz processors and 1 GB RAM. Data-handling routines were developed in-house. In addition, the NetCDF toolbox [104] and the N-way toolbox [105] version 2.10 of the KVL Food-Technology (Department of Dairy and Food Science, Copenhagen, Denmark) were used.

Samples

A set of seven different perfume mixtures for different purposes (detergents and personal care) was selected by Unilever's Perfume Competence Centre (PCC). The samples contained twelve target compounds, but this study is limited to the quantification of essential-oil markers which are γ-terpinene, citronellyl formate, dimethyl anthranliate, lavendulyl acetate, eucalypthol and (-) menthone. The other six components are not reported here for reasons of confidentiality.

The samples were diluted tenfold with 1-propanol (Lichrosolv grade; Merck, Darmstadt, Germany) containing accurately weighted concentrations of approximately 0.25% *n*-decane (Baker grade, min. 99%; Baker, Deventer, The Netherlands) as internal standard. Solutions were prepared in triplicate.

Calibration mixtures of all 12 components were prepared in the same internal-standard solution with concentrations at five levels ranging from 10 to 1500 mg/kg. All calibration solutions were measured in duplicate. To assess the accuracy of the quantification methods, a second calibration mixture was made, containing the same standards, but at concentrations of approximately 200 mg/kg. The calibration mixtures were measured in between the samples. The second calibration standard was measured using a slightly lower carrier gas pressure (175 kPa), forcing retention variations in both the first and second dimensions.

In Figure 3.1 a chroma^{2}gram of a typical synthetic perfume sample is shown. The broad peaks eluting around ${}^1t_R = 25$ min and ${}^2t_R = 3$ to 5 s result from dipropylene-glycol, which is used as an odourless solvent in the perfume industry. Due to the high polarity of the solvent severe wrap-around can be observed. Wrap-around occurs when the second-dimension retention time exceeds the modulation time. Components then elute in subsequent second-dimension chromatograms and show up as spurious, broad peaks.

3.3.2 Data handling and pre-processing

After acquisition and integration in EZ-Chrom, the data were exported to Common Data Format (CDF) format and imported into the MATLAB environment using the NetCDF toolbox [104].

Integration

In-house developed MATLAB routines were used for demodulation of both the detector output and the retention times of integrated areas. The chromatographic data is visualized through a colour plot in greyscales. Superpositioned onto the colour plot are the peak apices to visualize the quantitative information. Summated areas are calculated through a polygon summation box and processed further in Excel. Figure 3.5 gives the shows an apex plot. The dots in the chromatogram indicate identified and quantified peaks by the integration software.

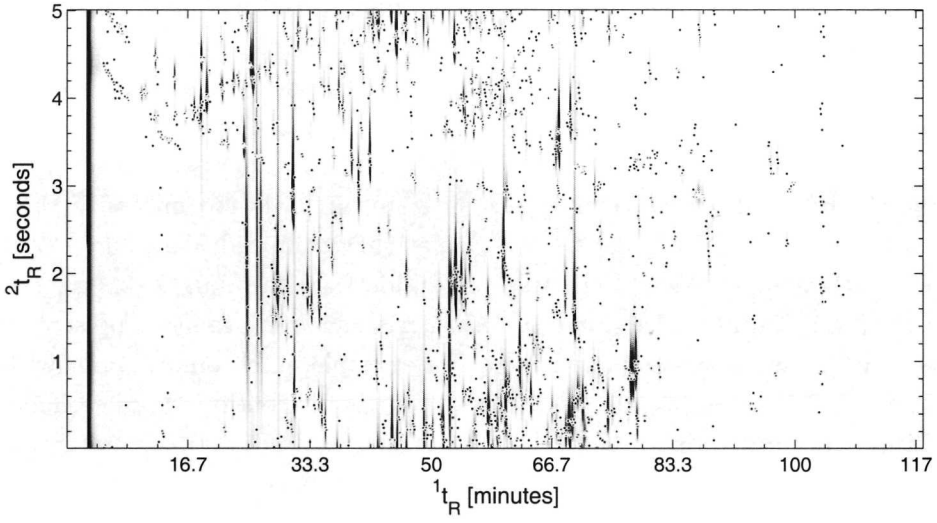

Figure 3.5: Apex plot of a typical perfume sample.

Peakfitting

Prior to the application of data analysis methods, data pre-processing is crucial. In this case the following steps were used:

Baseline removal: The offset, drift and wander of the baseline interfere with the quantitative information present in the chromatogram. Using a routine developed in-house, described in Section 4.2.2, page 59. The resulting baseline was subtracted from the original chromatogram. The baseline was calculated in such a way that no negative results in the baseline subtracted signal were produced.

Data stacking: Multiway methods require the data to actually be organized in a multiway orientation. Therefore, all GC×GC chromatograms are stacked on top of each other. The resulting matrix has the dimensions $(I \times J \times K)$ of $(1000 \times 250 \times 32)$.

Selection: Since in this study we are only interested in the concentration profiles of individual components, only the peaks of interest were selected. The typical selection window is 5 columns (first dimension) and 25 rows (second dimension) wide. The remaining (selected) matrix has typical dimensions of $(I \times J \times K)$ $(5 \times 25 \times 32)$. For each of the components of interest a separate sub-matrix was created.

Alignment: As in all chromatographic experiments, the actual retention times vary slightly from run to run due to small deviations in, for example, the temperature profile, the flow, the sample matrix and the (manual) injection. Shifted peaks are easily recognized by the human eye, because peak patterns remain identical. Thus, for user-supervised integration this is not a big issue. Data-analysis methods, however, are extremely sensitive towards shifts, and need a pre-processing step in order to minimize their effects. Bylund *et al.* [106] used Correlation Optimized Warping (COW) prior to Parafac analysis to eliminate retention-time shifts in LC-MS.

Elimination of shifts on a global scale, using all shift information present in the entire chromatogram, is preferred. For example, in chromatograms with a longer injection delay all peaks shift to higher retention times. Global shifting prevents individual peaks from being shifted to lower retention times. On a local scale the latter might occur, because no prior knowledge on shift profiles for individual peaks is present.

The observed shifts in this study are at most 4 points in the first dimension (20 seconds) and 20 points in the second dimension (0.4 seconds). The origin of these shifts is likely to be differences in the sample matrix, but also in operating conditions, which slightly differ from run-to-run. Synchronization (*i.e.* the simultaneous start of data acquisition and the GC run) is solved in the hardware.

Instead of solving all retention-time shifts (globally), we applied a correlation-optimized shifting based on the so-called inner product correlation [42] to the local selections. The inner-product correlation is defined as:

$$r_{(\boldsymbol{A},\boldsymbol{B})} = \frac{tr(\boldsymbol{A}^T\boldsymbol{B})}{\sqrt{tr(\boldsymbol{A}^T\boldsymbol{A}) \times tr(\boldsymbol{B}^T\boldsymbol{B})}} \tag{3.4}$$

Where:

$r_{(A,B)}$	Correlation coefficient between matrix $\boldsymbol{A}$ and matrix $\boldsymbol{B}$.
$\boldsymbol{A}$	Standard matrix.
$\boldsymbol{B}$	Sample matrix.
tr	Trace function (sum of all diagonal elements).

A standard was used as reference and all other selections were aligned with this standard. By shifting the selection window over a predefined grid and simultaneously calculating the correlation, a best-fit position was found and stored. Restricting the permissible number of steps in the shifting process prevents the selection of a neighbouring peak belonging to a different component.

The actual calculations with the Parafac, Parafac2 and NPLS routines are simple and fast. Decomposition of the selected sub matrix (with the dimensions 5×25×32) with Parafac takes about 1 second calculation time. Parafac2, and to a lesser extent NPLS, take considerably more time, but still not exceeding half a minute. The model inputs are the peak selection (after shifting), the number of expected components and constraints for the calculation. Normally, a one component Parafac model is sufficient. However, if the captured variance is too low (<80%), an additional component can be introduced. If the resulting calibration line does not yield a physically realistic description, the additional component does not contribute to a better model.

3.4 Results

Conventionally, chromatograms are integrated in order to obtain quantitative data. Thus, in the context of quantitative chromatography, integration can be regarded as a benchmark technique. The results obtained with other, multiway methods, such as Parafac, Parafac2, and NPLS, should not differ from those obtained by integration.

3.4.1 Alignment

The most critical step in the use of mathematical models to describe chromatographic data is alignment. Two chromatographic axes, as encountered in GC×GC, make this problem even more challenging. A global shifting routine experiences great difficulties in dealing with 'wrap-around'. Therefore, we selected a window around a peak in the GC×GC chromatogram of the standard ('reference') sample and used it as template. The same selection window was used for the next injection ('sample') and between the two matrices an inner-product correlation was calculated. The selection window for the sample was shifted across the chromatogram two columns to the left and to the right and up to ten points up or down. For each shift the inner-product correlation was calculated (105 shift positions). The shift with the highest correlation was assumed to be the best alignment. The same procedure was repeated for all injections, standards as well as samples. An inspection of the chromatograms revealed that the correlation-based shifting was a good and fast method to eliminate shifts on a local scale.

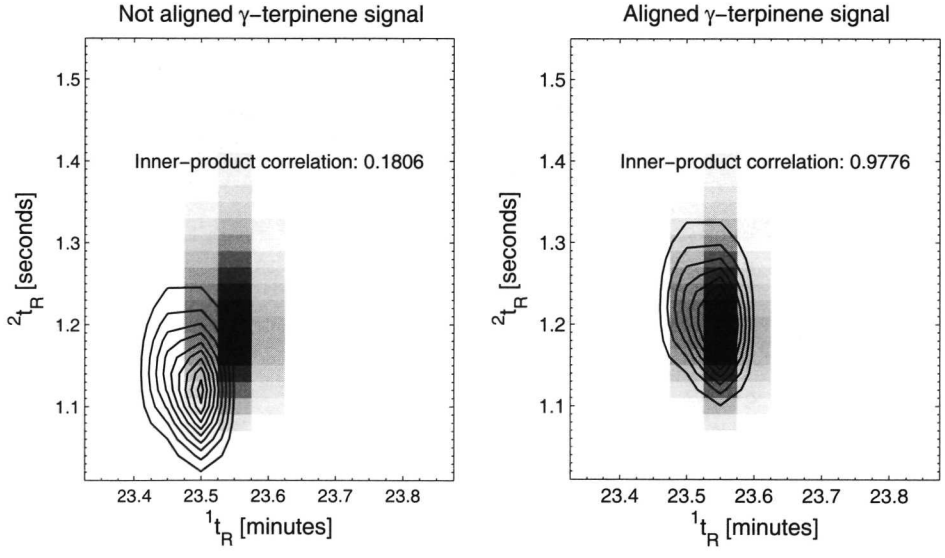

Figure 3.6: Effect of shifting (alignment) of a peak in a standard. Superpositioned on top of the chroma^{2}gram is a contour plot of a second chroma^{2}gram.

In this procedure no interpolation was involved and the automatic shifting

of 32 injections for a single component is completed in about 5-10 seconds. In Figure 3.6 the result of shifting was illustrated.

It should be emphasized that the improvement in correlation is not as dramatic in each sample as in the example of Figure 3.6. Samples containing low concentrations of the selected components yield lower correlation coefficients due to low signal-to-noise ratios (see Figure 3.7), but the highest value still corresponds to the best alignment. Even for samples containing other peaks in the immediate vicinity of the component of interest, shifting based on inner-product correlation appears to work properly.

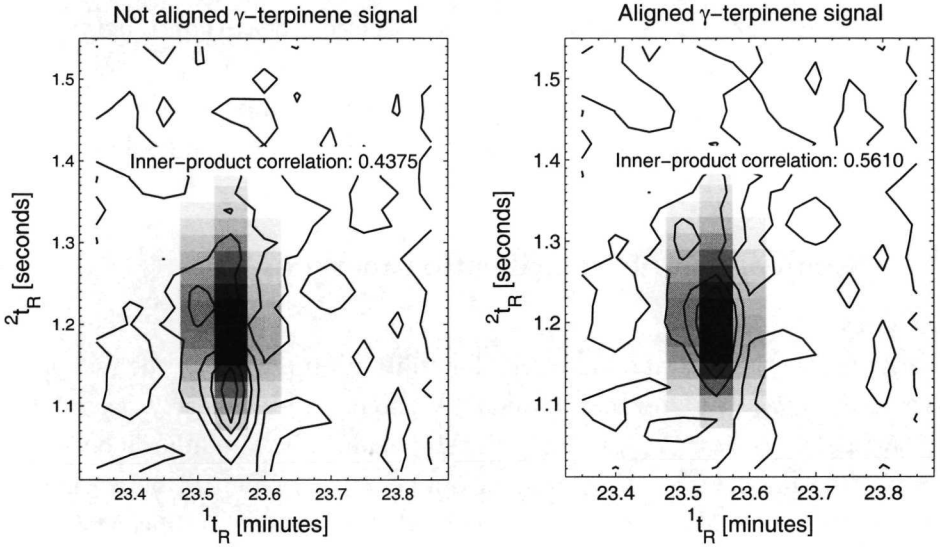

Figure 3.7: Result of shifting (aligning) performed on a peak in a sample.

After the alignment step the responses are calculated and corrected using the concentration and response of the internal-standard peak. In some samples, the selected local window contained more than one component. A theoretical advantage of the mathematical models described in the Theory section is the possibility of deconvolution, *i.e.* the reconstruction of pure-component elution profiles from overlapping peaks. The only condition is that the number of expected components is specified when applying the models. Overestimation of the number of components leads to an improved fit of the model, but the calculated factors (profiles) do not adequately describe the real factors.

Underestimation of the number of components also can lead to anomalies in the calculated peak profiles and responses. In the present samples and for the selected target analytes, a single component/factor model was sufficient to describe the variance in the local models. For samples containing two (or more) peaks in the selection window, additional factor(s) in the Parafac model can be considered. This should result in pure-component elution profiles for the target analyte and for the interfering component(s). However, if the additional peaks are found in only one or some of the samples, the introduction of additional factor(s) results in the modeling of the residuals of the first component. This is inherent to the least-squares criterion, which is used to minimize the residuals. The introduction of a second factor will always reduce the sum of squares, but it may lead to erroneous profiles and concentrations. The same aligned data are used as input for the different mathematical methods. Differences in calculated responses are solely originating from the methods.

3.4.2 Comparison of quantification methods

Linearity

In order to use the described methods for calibration purposes, the response (corrected using the internal standard) should vary linearly with the concentration. To test the linear relationship, calibration standards between 10 and 1500 mg/kg were measured in duplicate, interspersed between the samples. The correlation coefficient was used as a measure of linearity.

Correlation	Terpinene	Citronellyl	DMA	Lavandulyl	Eucalyptol	Mentone
Integration	0.9999	0.9997	0.9997	0.9996	0.9998	0.9997
Parafac	0.9979	0.9983	0.9988	0.9980	0.9973	0.9993
Parafac2	0.9987	0.9992	0.9989	0.9979	0.9976	0.9993
NPLS	0.9985	0.9986	0.9989	0.9972	0.9980	0.9993

Table 3.2: Correlation coefficients for all components with the various quantification methods.

Some differences in the correlation coefficients obtained using the three models are expected, since the ways in which the responses are calculated differ fundamentally due to constraints. In general, all methods revealed a good linearity (Table 3.2). It can be concluded that all methods result

in linear relationships between response and concentration. Integration performs best with respect to linearity.

Accuracy

A second calibration standard was measured as the last sample in this dataset under slightly different conditions (lower head pressure) to induce different peak shapes. This standard was treated as a sample and the concentrations were calculated for each component with integration, Parafac, Parafac2, and NPLS. Ideally, the calculated concentrations should be identical to of the true values. A deviation of 5% was thought to be acceptable.

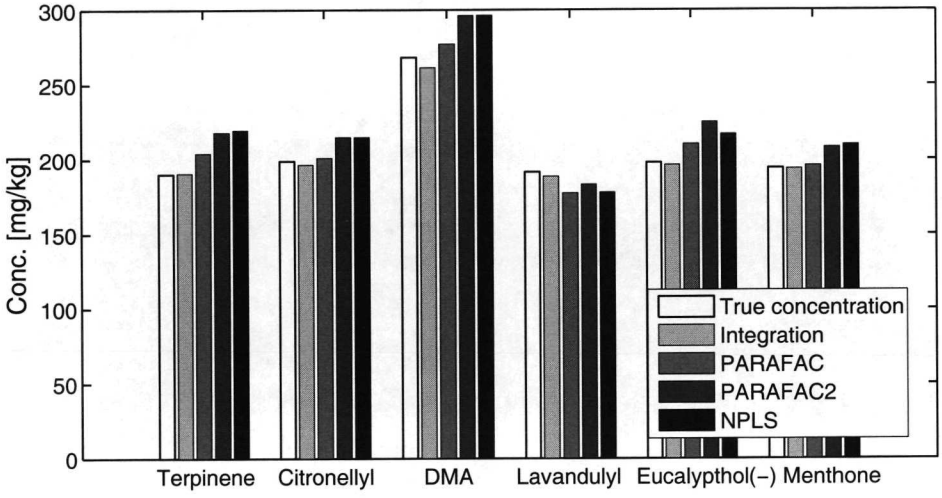

Figure 3.8: Accuracy of various methods based on the analysis of a reference mixture with known analyte concentrations.

As can be seen in Figure 3.8, integration performs best for (almost) all components. Parafac2 and NPLS tend to overestimate the concentrations. Parafac is the most accurate of the multiway methods in the present case. The influence of the peak shape seems to be more detrimental for Parafac2 than for Parafac. This result is surprising, since Parafac2 should theoretically be capable of dealing with shifted peaks.

Calculated concentrations

The results for the four samples, six target compounds and four quantification methods are given in Table 3.3.

Sample	Method	Terpinene	Citronellyl	DMA	Lavendulyl[a]	Eucalyptol	Mentone
M2	Integration	1830	405	16	58100	**800**	160
	Parafac	1880	405	40	55000	**310**	150
	Parafac2	1890	406	114	54200	**480**	157
	NPLS	1900	407	40	53300	**296**	150
M4	Integration	2.2	3.8	**100**	123000	16	36
	Parafac	4.3	6.8	**44**	115000	20	32
	Parafac2	6.2	11.8	**54**	118000	23	33
	NPLS	4.3	6.8	**44**	109000	21	32
M6	Integration	480	30	154	30300	**2790**	22
	Parafac	480	34	170	31000	**1330**	19
	Parafac2	498	36	254	29900	**1560**	22
	NPLS	491	34	172	29700	**1330**	19

[a] In real samples the peak of lavandulyl acetate is perfectly co-eluting with ortho-tertiary butyl cyclohexylacetate (OTBCA) present in concentrations up to 30% [*w*/*w*] in the sample. Both components have similar retention indices in both separation directions and completely overlap, even in GC×GC.

Table 3.3: Concentrations [mg/kg] in real samples obtained using integration and using the multiway methods. Bold numbers indicate large deviations.

In four cases there is a major difference between the methods (DMA/Sample4, Eucalypthol/Sample2 and Eucalypthol/Sample6, indicated in bold). These differences most likely originate from the shift routine, since the differences between the three multiway methods mutually are much smaller than those between the multiway methods and integration. Especially at low concentrations (<10 mg/kg), multiway methods systematically overestimate (assuming that integration provides the correct answer!). This might be due to the baseline removal, which does not allow negative baseline values. The result is a minor offset in the baseline, which can lead to overestimation at low concentrations. No experiments were performed to verify this (*e.g.* via standard addition). Surprisingly, the highest concentrations in almost all cases are found with Parafac2.

Limit of quantification(LOQ)

The limit of detection in GC×GC is primarily determined by the signal-to-

noise ratio of the peaks detected by the FID. The LOQ generally is defined as three times the S/N ratio and would obviously be identical in all four cases. Quantification, however, is also affected by the ability to differentiate between signal and noise. This is where integration and peak fitting approaches differ. In the case of integration, the minimum-area setting results in limits of quantification between 3 and 10 mg/kg, depending on the component of interest (purity, FID response factor). In the case of Parafac, Parafac2 and NPLS, the minimum detectable amount is less easy to determine, since it is also influenced by other samples in the dataset. If, for instance, the dataset is constructed solely from samples with low concentrations, then the minimum limit of quantification is expected to be lower then in case of a set of highly concentrated samples with only one dilute one. In this case, we estimate the limits of quantification for the multiway methods to be in the range of 6 to 20 mg/kg, somewhat higher than those obtained with integration.

Comparison of integration and multiway methods

The logarithmic scale forces the attention on the low concentration part of the comparison, where the largest deviations appear.

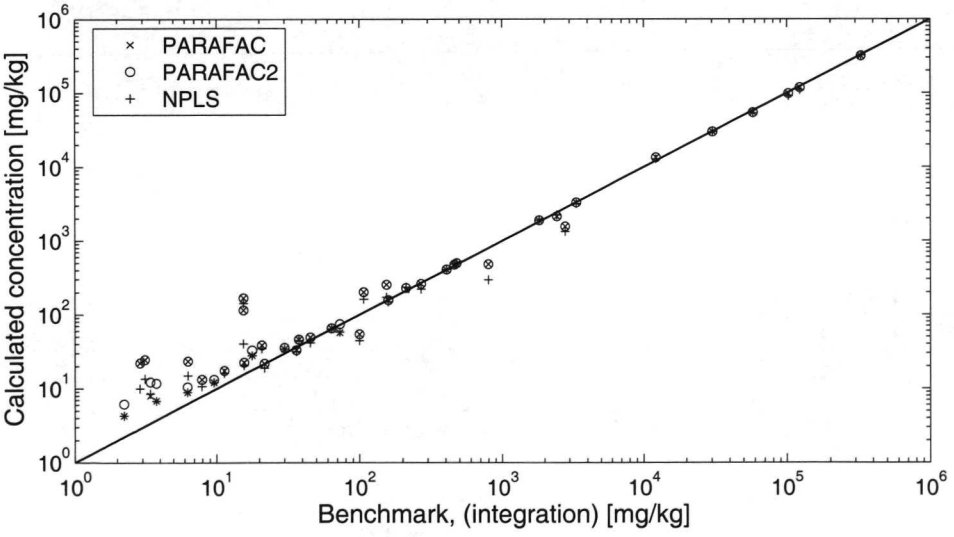

Figure 3.9: Comparison of quantification methods with to integration (regarded as benchmark technique).

On a logarithmic scale the results obtained with integration and with Parafac show a linear relationship without any real inconsistencies (Figure 3.9). The observed differences mainly appear in the low concentration region, near or below the LOQ.

Precision

One may expect that multiway methods yield a lower precision than conventional integration. This is probably true for simple (gas) chromatograms containing only a limited number of peaks, but in this particular case it turns out that precision is comparable, if relative standard deviations (r.s.d.) are used. In Figure 3.10, the r.s.d. for triplicates are shown as function of the calculated concentration. It appears that the three multiway methods do not show substantially higher r.s.d.'s then does integration. Differences appear in the low concentration region (<10 mg/kg), where the multiway methods are expected to perform worse. On average, multiway methods do not perform significantly worse than integration with respect to precision.

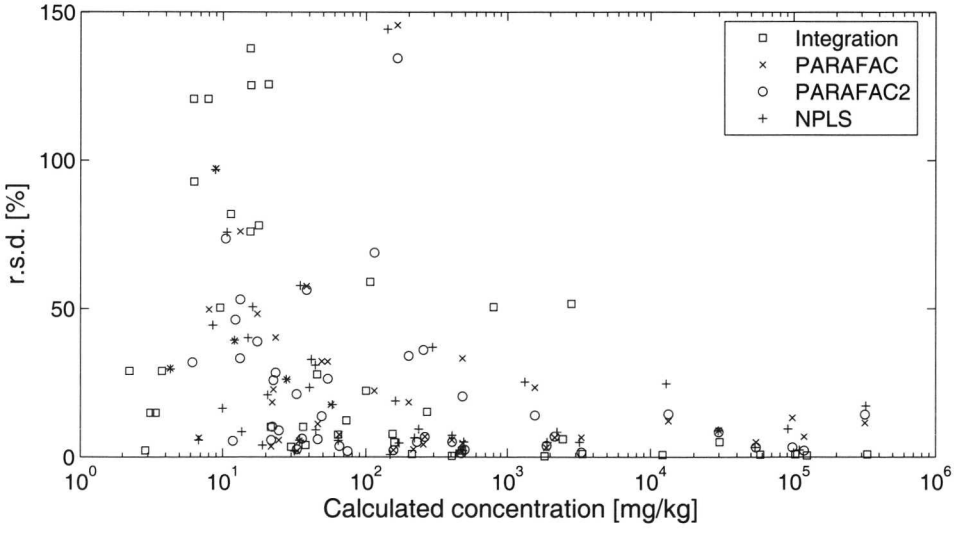

Figure 3.10: Errors (r.s.d.)obtained by various methods as function of concentration for seven target analytes.

Speed

The rigorous quantification of large GC×GC datasets with integration is a very time-consuming exercise. It requires about two minutes per component per chromatogram to integrate (GC×GC) slices, due to the manual combination of peaks. For the present dataset of 32 injections and 13 components, 13 hours of analyst effort were required to integrate all peaks. Further processing with Excel takes another three hours. This could be improved by the use of routines that combine the successive apices. However, this would lead to large result tables containing all the combined slices. From these, a selection has to be made of components of interest. The quantification by Parafac or NPLS takes only two minutes per component, regardless of the number of chromatograms. In the present study, 30 minutes proved sufficient to fully quantify all the target components in all the chromatograms. Further processing in Excel is easier (about 1.5 hours), since Parafac and NPLS yield an array of concentrations that can be directly imported. In total, integration takes about 16 hours, whereas Parafac and NPLS require about two hours for the total set.

3.5 Conclusions

Integration is the preferred method for accurately determining concentrations in GC×GC. This method is, however, very time-consuming and labour-intensive. Multiway methods, such as Parallel Factor (Parafac) analysis, its extension Parafac2, and multi-linear Partial Least Squares (NPLS), are all capable of estimating concentrations in the chromatograms. Especially constrained Parafac yields concentrations comparable to integration in terms of accuracy and precision. Due to different approaches in the multiway methods, a dramatic increase in productivity is found. Integration requires about 16 hours for the quantification of 13 components in 32 chromatograms, whereas Parafac and NPLS require only 2 hours. This aspect becomes increasingly important in the context of new GC×GC instruments equipped with jet-modulators and auto-injectors. The jet modulators permit higher data-acquisition rates (at least 100 Hz) and have the potential of increased numbers of peaks, while auto-injector units allow large numbers of analyses to give rise to large datasets. The shifting

routine developed for the multiway approach seems to work satisfactory on the dataset described in this Chapter. However, more experience is required to arrive at more definitive conclusions. It is also found in the present study that Parafac2 and, to a lesser extent, NPLS overestimate concentrations in comparison with integration. For NPLS this can be partly explained by the fact that the method calibrates using pure-component chromatograms, but predicts on multi-component samples. For Parafac2, however, this comes as a surprise, since the method was thought to be able to deal with retention-time shifts encountered in the first-dimension chromatograms, due to the inner-product structure. One of the reasons for this may be the fact that peaks in the first dimension are not shifted, but show a different peak profile, which is referred to in literature as "in-phase" and "out-of-phase" modulation [87]. This phenomenon leads to differences in the inner-structure property, but would only partially explain the systematic overestimation of the concentrations obtained by this method.

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