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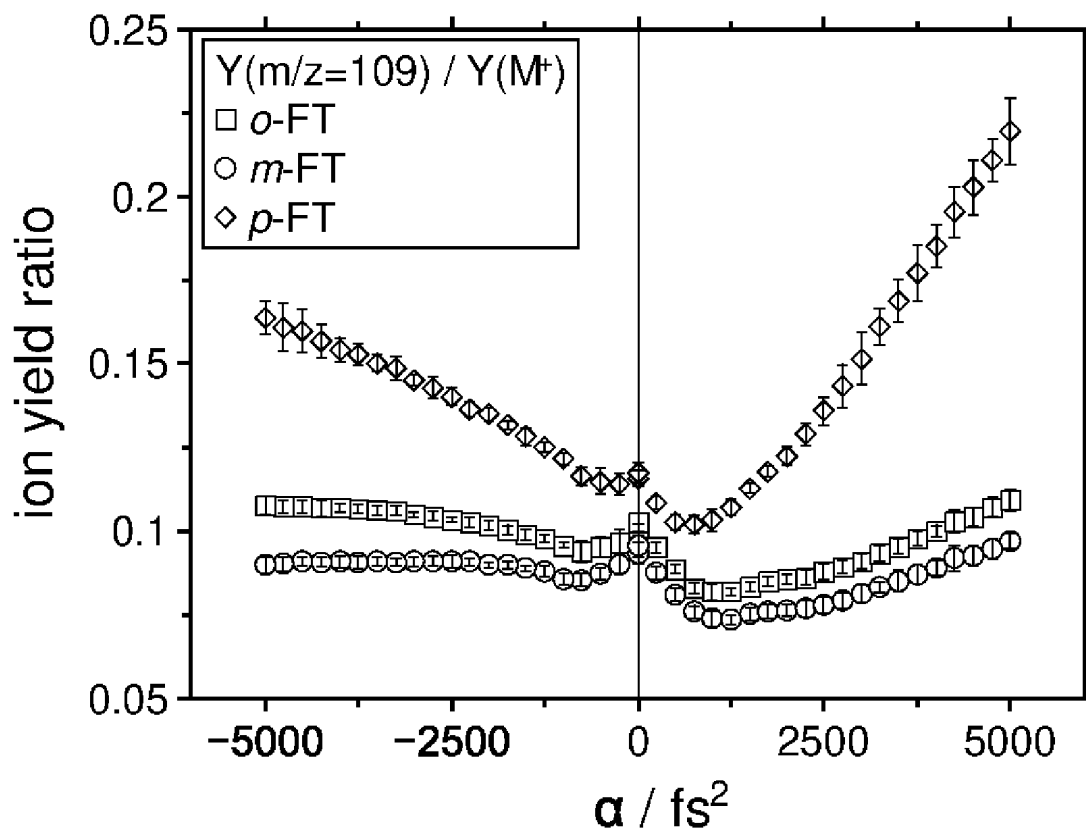
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(54) **METHOD AND SYSTEM FOR QUANTITATIVE ANALYSIS OF STRUCTURALLY ISOMERIC COMPOUNDS WITHIN MIXTURES OF COMPOUNDS**

(57) The computer implemented method provides the possibility of quantitatively determining the content of structural isomers within a mixture of compounds by mass spectroscopy without previously separating them.

Fig. 3A



Description

[0001] The invention relates to the qualitative and quantitative analysis of structurally isomeric compounds within mixtures of compounds by use of femtosecond laser ionization mass spectrometry (fs-LIMS).

Field of the invention

[0002] The field of the invention is the vast area of analyzing mixtures of chemical compounds, especially analyzing mixtures containing structural isomers. Quickly analyzing mixtures containing structural isomers is of great interest in numerous areas, e.g. environmental analysis, in-line process analysis etc.

Background of the technology

[0003] Femtosecond laser ionization mass spectrometry (fs-LIMS) is a powerful analytical method providing access to the qualitative distinction of structural isomers.

[0004] The quantitative analysis of mixtures of chemical compounds is a central task in classical analytical chemistry. Standard approaches involve a sequence of chemical separation steps followed by the actual identification. An example is coupling a gas chromatography with a (time of flight) mass spectrometer. There are situations, where separation is either too costly in terms of time or not accessible for other reasons, e.g. in quality control. There is great need within the state of the art for establishing methods which combine the chemical identification and the quantitative analysis in one experiment, for this would be saving time and money for analyzing mixtures containing structural isomers. This requires a multidimensional approach. Preferentially, at least one step consists of recording a mass spectrum. Multidimensionality usually can be introduced e.g. by combining several MS steps, as is already well known within the state of the art. Employing photons for ionization of target molecules opens a wide range of multidimensional experiments. Here, it appears most promising and in fact necessary to have an ionization concept which does not discriminate between components of the mixture. The same concept has also been the basis for success of the electron impact ionization, which in fact dominated organic mass spectrometry for decades. This goal of nondiscriminatory ionization automatically excludes all techniques employing high resolution in the spectral domain. Such techniques like resonance enhanced ionization have proven very powerful for the identification of components in a mixture but not for the quantification of compositions of mixtures. This directs the interest towards ultrashort laser pulses, which combine a very high peak power with a broad spectrum enabling non-resonant and thus nondiscriminatory ionization.

[0005] Due to the high peak power the mass spectra observed exhibit rich fragmentation patterns, however, with the molecular ion often prevailing. By systematical modulation of the spectral phase $\phi(\omega)$ of the fs-laser pulse a multitude of information contained in the signals of every mass spectrum is obtained. Here, the proper treatment of huge datasets poses a significant challenge. One established method is principal component analysis (PCA). In this statistical technique the dimensionality of a dataset is reduced without loss of information.

[0006] Another way to gain a multidimensional approach is coupling of a spectrally broad fs-laser system with a time-of-flight (ToF) mass spectrometer. The additional dimension is introduced by controlling the spectral phase of the fs-laser pulses. There are several concepts available for shaping fs-laser pulse, e.g. binary shaping of femtosecond laser pulses. Differences between structural isomers can also be enhanced in pump-probe experiments as demonstrated for 2-, 3- and 4-methylacetophenone. Changing the delay time led to characteristic variation of the ion yield of certain fragment ions.

[0007] It is already known within the state of the Art to qualitatively distinguish structural isomers of benzene derivatives by means of linear and quadratic chirping of fs-laser pulses. Linearly chirped fs-laser pulses enhance the differences between specific ion yield ratios of structural isomers, e.g. o- and p-xylene, and consequently allow for a qualitative distinction. In this context, a major influence of the pulse energy has been demonstrated. In additional work the qualitative distinction of all three structural isomers of difluorobenzene and benzenediamine has been achieved by linear and quadratic chirping of fs-laser pulses. A systematic variation of the linear chirp parameter helps in identifying common intermediate structures in the fragmentation pattern of difluorobenzenes.

[0008] In a chemical ionization mass spectrometry study within the state of the art small differences between the ion yield ratios of fluorotoluene isomers were found and interpreted as an indication that ring expansion to the FTr^+ ion may not be operative. Despite the difference between the ion yield ratios given in that report, they were discussed as being too small to allow distinction between the structural isomers. But variation of linear and quadratic chirp in fs-LIMS leads to an enhancement of small differences in the mass spectra of the isomers and enables the qualitative distinction of the isomers. In particular linear chirped laser pulses provide access to the mechanism of fragmentation pathways.

[0009] But despite greatest efforts and most intense scientific and technical research it was not possible up to date to quantitatively analyze mixtures containing three or more structural isomers by mass spectrometry alone, i. e. without combining the quantitative mass spectrometric analysis with a separation-step for the structural isomers in advance.

This is because within the known state of the art it is not capable to quantitatively discern between three or more structural isomers by mass spectrometry itself. Up to date it was necessary to chemically separate the at least three structural isomers, e.g. by HPLC, GC or other separation methods. Therefore the known methods for quantitatively analyzing mixtures containing three or more structural isomers are time-consuming and costly.

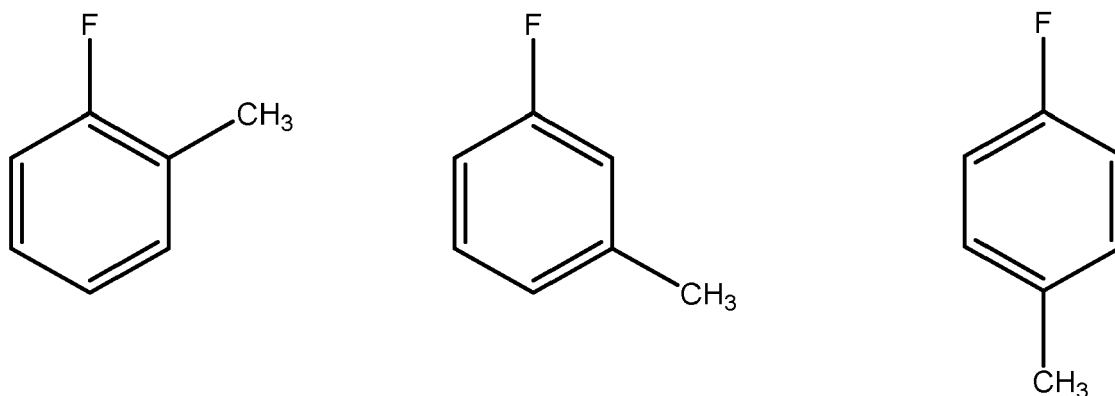
[0010] Therefore an objective of the present invention is to provide a method for a faster and more effective quantitative analysis of structural isomers within mixtures of compounds containing two, three or more structural isomers of at least one compound, i.e. a major objective of the present invention is to provide a method for quantitatively analyze mixtures of all isomers, i.e. to determine the fractional abundance of each isomer in a given mixture.

Content of the invention

[0011] In order to achieve the above objective, the present invention provides a method for a faster and more effective quantitative analysis of structural isomers by mass spectrometry using a fs-laser for ionization of the sample-components and a mass spectrometer for data acquisition, whereat the acquired data is analyzed mathematically by methods of statistical data-evaluation, e.g. principal component analysis (PCA).

[0012] It is well known to the person skilled in the art that the word "analysis" may have different meanings, depending on the scientific area one is talking about. There are several well-known mathematical meanings of the word "analysis" (e.g. "principal component analysis", "analysis" itself etc.). And there is also the well-known chemical meaning of the word "analysis", depicting either the determination of components of a mixture ("qualitative analysis") or the additional determination even of the amount of components of a mixture ("quantitative analysis"). Because the content of the invention described herein comprises both aspects, the word "analysis" is used herein in meanings regarding both areas of science. The meaning of the word "analysis" as it is used herein, may have the meaning according to mathematics or according to chemistry. The person skilled in the art well knows how to discern the correct meaning each time according to the current context. In cases where this might be somewhat difficult it may be clarified by adding "chemical" resp. "mathematical".

[0013] Herein we demonstrate how to distinguish between at least three structural isomers of a chemical compound, e.g. fluorotoluene (cf. scheme 1) by means of linear and quadratic chirp of fs-laser pulses. During fluorotoluene fragmentation a ring expansion and a rearrangement to the fluorotropylium (FTr^+) structure are conceivable and have been suggested in different reports. Another report discusses only partial rearrangement to the FTr^+ structure due to differences in the internal energies of the three structural isomers of, e.g., fluorotoluene.



Scheme 1: The three structural isomers of fluorotoluene (FT): ortho-FT (left), meta-FT (middle) and para-FT (right).

[0014] Typical mass spectra of the three fluorotoluene (FT) isomers recorded for transform-limited laser pulses are exemplarily shown in Fig. 1. The molecular ion peak (m/z 110) is the most intense signal under the experimental conditions exemplarily chosen. Throughout the text the molecular ions are marked as M^+ , while all fragments are characterized by the respective m/z value. Only the fragment ion at m/z 109 (regarding the exemplarily chosen isomeric fluorotoluenes) is termed $[M-H]^+$, indicating that it refers to a hydrogen loss. As an example the linear chirp dependence of the integrated ion yield (Y) for the *ortho* molecular ion (M^+) is shown in Figure 2A. It is well known to the person skilled in the art how to calculate and integrate the ion yield of a mass spectrum signal, therefore it is not necessary herein to explain it in more detail. The highest ion yield is observed for transform-limited laser pulses, with increasing linear chirp parameter

the ion yield decreases. As is known to the person skilled in the art, such characteristics are termed intensity driven chirp effect. The α -dependence of the molecular ion yield is isomer-specific. In the case of the fluorotoluenes investigated as an example the molecular ion yield for the *m*-FT and *p*-FT isomers are very similar to that of the *o*-FT. Consequently the linear chirp dependence of the molecular ion yield for *m*-FT and *p*-FT is shown in Figs. 2B and 2C respectively. As is known within the state of the art ion yield ratios (IYR) are more suitable quantities to emphasize characteristic differences between the isomers; the person skilled according to the state of the art well knows how to calculate/determine the ion yield ratio (IYR) of a mass spectrometric signal. The ion yields according to the invention, exemplarily shown in Figs. 2A, 2B and 2C are normalized to the maximum yield of, for example, singly charged molecular ions for *ortho*-, *meta*- and *para*-fluorotoluene as a function of the linear chirp parameter α . It lies well within the scope of the invention to normalize the ion yields of a measurement to another mass spectrometric signal of the measurement. The decision, which mass spectrometric signal to use for normalization is made by evaluating one or more calibration measurements of mixtures of isomers which are to be analyzed quantitatively. This evaluation of calibration measurements can be made manually and/or by use of computer implemented software and/or by use of computer implemented neuronal networks, i.e. artificial intelligence.

[0015] In addition to the α -dependency of the isomer-specific molecular ion yield, described above as one example, the isomer-specific molecular ion yield is also dependant from the chirp parameter β so that the scope of the invention also comprises the application of the dependancy of the normalized ion yield from the chirp parameter β additionally with or instead of the dependancy of the normalized ion yield from the chirp parameter α . The calculation and normalisation of the molecular ion yield in dependance of the β -parameter is done analogously to the way as is done for the α -parameter and is also well known by the person skilled in the art.

[0016] The herein described dependancy of the ion yield, resp. normalized ion yield, is exemplarily described by way of the molecular ion, because in this exemplary case the molecular ion shows the most intense mass spectrometric signal. It is well known to the person skilled in the art that these dependancies are also existing for the other mass spectrometric signals of the measurement also. Therefore the scope of the invention comprises the usage of any mass spectrometric signal of the measurement. Most preferred is the usage of the most intense signal of the measurement for normalisation.

[0017] For the qualitative distinction of structural isomers it is helpful to discuss ion yield ratios (IYR). These IYR characteristically depend on the spectral phase of the fs-laser pulses, most prominently on the linear and quadratic chirp. Conceptually these IYR may also be discussed in the context of thermodynamic or kinetic control. Thermodynamic control is dominant, if a reaction leads to the energetically favored product. This type of control is operative if the fragmentation channel with the largest number of e.g. mesomeric product structures dominates. Kinetic control on the other hand is operative, if the reaction channel with highest rate is dominant. This in general correlates with a variation of the effective activation energy. In the following, first the qualitative distinction of the isomeric fluorotoluenes with respect to the reaction pathway is described.

[0018] The hydrogen atom abstraction from the parent ions leading to ions of m/z 109 ($[M-H]^+$) is described here exemplarily. For this primary channel the ion yield ratio $Y(m/z=109)/Y(M^+)$ has been analyzed as a function of the linear and the quadratic chirp parameter (c.f. Figure 3A/B). Clearly, this IYR allows distinction of the three structural isomers of FT. The error bars are in many cases smaller than the data points shown and in that case not visible. The highest IYR is observed for the *p*-FT, while *m*-FT shows the lowest IYR corresponding to the lowest dissociation rate. For each isomer the IYR increases with increasing negative and positive linear and quadratic chirp parameter. Qualitatively, the possibility to distinguish the isomers is best for the largest values of $|\alpha|$ and $|\beta|$. For the transform-limited pulses a local maximum of the IYR is operative. H loss from fluorotoluene isomers is known to be the result of a complex competition between isomerization at the molecular ion level and the dissociation itself. There are literature reports that, for example, for all fluorotoluene isomers the H loss ultimately leads to a mixture of *p*-fluorobenzylum (*p*-FBz⁺) and fluorotropylium ions. The *p*-FBz⁺ ion is about 15 kJ/mol more stable than the *o*-FBz⁺ and 28 kJ/mol more stable than the *m*-FBz⁺. This order is based on a thermodynamic control and also reflected in the IYR shown in Figure 1 for the three isomers. This is analogously valid for other compounds.

[0019] For the linear chirp dependence exemplarily shown in Figure 3A a saturation of the IYR for *m*- and *o*-FT is found for high negative linear chirp parameters. Besides from the energetic aspects discussed above exemplarily dynamic aspects have to be considered as well. The relative position of the substituents in, e.g., fluorotoluene influences the internal rotation, the density of states and the life time of S_1 level just to mention a few effects exemplarily. One major aspect is, for example, the intramolecular vibrational energy redistribution (IVR), which can compete with a dissociation reaction. Such a competition could take place for high negative linear chirp parameter, leading to a stagnating in the IYR. Some scientists have found a higher IVR rate for *m*-FT than for *p*-FT, as a result of higher excited state level mixing in *m*-FT than in *p*-FT. The higher IVR rate in *m*-FT compared to *p*-FT is also in accordance with the results herein regarding qualitative discerning. Some scientists also discussed the state level mixing of *o*-FT, assuming an increase in ground state as well as excited state level mixing than in *m*-FT. Therefore an even faster IVR than in *m*-FT should occur. In that case, the ion yield ratio for the *ortho* trace in Fig. 3A would be expected to show a stagnation for smaller

negative linear chirp parameter than the *meta* trace. Experimentally the ion yield ratio for the *ortho* isomer lies between the *m*-FT and the *p*-FT data and higher negative chirp parameters are leading to a stagnation than for the *m*-FT suggesting that the IVR rate should also be in between the other two isomers.

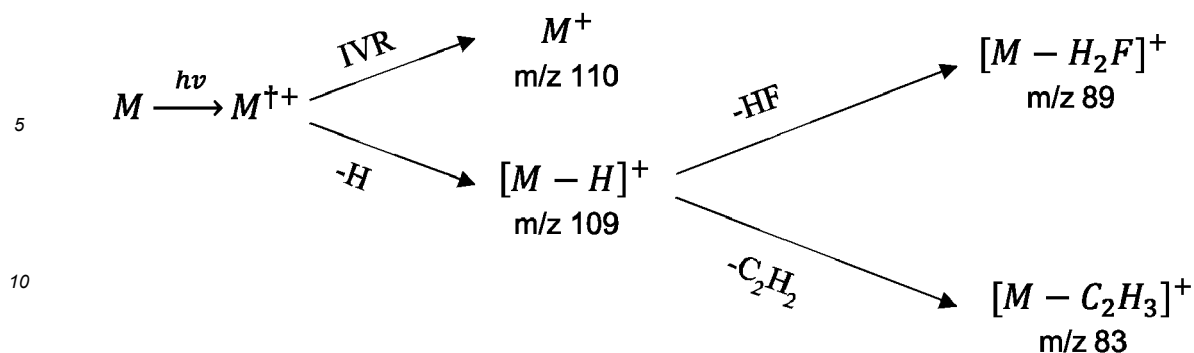
[0020] In the following the formation of fragment ions at m/z 83 and m/z 89 is described, which can be considered to originate from H-loss followed by hydrogen fluoride (HF) and C_2H_2 loss respectively, or possibly in reversed sequence. Three different hints for the sequence of H loss followed by HF and C_2H_2 loss are contained and discussed exemplarily below. The ion yield ratios are derived from the analysis of the mass spectra. The figures 5A and 5B exemplarily present the ion yield ratios $Y(m/z=89)/Y(M^+)$ and $Y(m/z=83)/Y(M^+)$ as a function of linear chirp parameter α . The order of ion yield ratios are different for the exemplarily presented fragmentation channel. This suggests that the molecular ion of the example is not the immediate precursor for signals at $m/z = 89$ and $m/z = 83$ but rather a sequential mechanism as depicted in Scheme 2 is operative.

[0021] The ion yield ratios explore the issue of sequence and point out that a sequential pathway with hydrogen atom elimination as a first step followed by C_2H_2 or HF elimination in a second step is more likely. The fragment ions at m/z 89 and m/z 83 are analyzed in relation to the molecular ion as well as in relation to the $[M-H]^+$ species. Different orders of the three isomers are observed for $Y(m/z=89)/Y(m/z=110)$ and $Y(m/z=83)/Y(m/z=110)$. Whereas the opposite is true for the fragment ion yields in relation to the $[M-H]^+$ species, what is shown in Figures 4A and 4B. Here, we observe the same order of the ion yield ratios for the three structural isomers of, for example, fluorotoluene. This could be an indication for the suggested sequence. The diagrams of $Y(m/z=89)/Y(m/z=110)$ and $Y(m/z=83)/Y(m/z=110)$ are shown in Figures 5A and 5B. The fragmentation channels of C_2H_2 and HF elimination show a similar linear chirp dependency and enable one to distinguish between the isomers, as it is shown in Figures 4A and 4B.

[0022] The corresponding ion signals in the mass spectra (c.f. Figure 1) also offer valuable clues to the issue of sequence. But a small signal in a mass spectrum could be due to a complete subsequent decay. The hydrogen fluoride loss from molecular ion is only a minor fragmentation channel because the ion signal at m/z 90 is very weak. This signal is even not observable in the fragmentation pattern of *para*-isomer. Hence, the hydrogen fluoride loss from molecular ion can be neglected. One can also consider C_2H_2 loss from molecular ion as a minor fragmentation channel, as the associated ion yield ratios occur a factor 10 less than with $[M-H]^+$ as precursor ion.

[0023] A further proof of the described sequence can be identified in the energy dependence of the IYR $Y([M-H]^+)/Y(M^+)$ (Figure 6). For small pulse energies the IYR of all three sample-isomers is rising with increasing pulse energy and passes through a maximum at about 15 μJ . The highest H elimination rate is located at this maximum in case of *o*- and *m*-FT isomers. Once the pulse energy is further increased, the IYR decreases and tends to an IYR of 0.1 for these two isomers. The IYR as a function of pulse energy for *o*- and *m*-FT isomers are very similar and almost indistinguishable. For *p*-FT the IYR is increasing again for pulse energies above 40 μJ , reaching the maximum value of about 0.125. The local maximum of IYR at a pulse energy of about 15 μJ and the decrease up to a pulse energy of 40 μJ is an indication for a further decomposition step following the H elimination.

[0024] In the exemplary case of fluorotoluene isomers, the rearrangement to fluorotropylium ($F-Tr^+$) structure and equilibration of isomers is known to be relevant as briefly discussed in the introduction. There are literature reports that up to 40% relative abundance of the tropylium compared to the benzylium structure under electron impact conditions can be formed at electron impact energies below 20 eV. That fraction decreases upon increasing the electron energy. A large fraction of ions transforming into the tropylium structure would lead to losing the memory of identity of the original isomer. For the ion yield ratio $Y(m/z=83)/Y(m/z=110)$ (c.f. Figure 5A) basically no distinction between the isomers is possible employing transform-limited laser pulses. Even there, laser pulses with imprinted linear chirp enhance the differences of the ion yield ratios of the three structural isomers. The elongation of pulse duration due to linear chirping constitutes more time for sequential molecular processes, e.g. for H loss and subsequent C_2H_2 loss. On the other hand, the ion yield ratios $Y(m/z=89)/Y(m/z=110)$ (c.f. Figure 5B) allow distinction of the three isomers for all spectral phases. This is incompatible with isomerization to a tropylium structure at the precursor level.



Scheme 2: Suggested fragmentation pathway for decomposition of the sample compound fluorotoluene.

[0025] In Scheme 2 a mechanism is presented which is based on a sequence of H loss followed by HF and C₂H₂ loss according to the experimental results presented herein. In addition to this sequence the IVR in the molecular molecular ion is added as a further process, which can compete with H loss.

[0026] Now, below, the quantitative analysis of mixtures of isomers according to the invention is presented exemplarily by way of an example of three isomers. It is obvious to the person skilled in the art that the aspects of the invention shown below exemplarily for a three-component-mixture do also apply for mixtures of only two components and for mixtures with more than three isomers accordingly, so that the scope of the invention also comprises these mixtures. Within the context of the invention "isomer"/"isomers" always denotes structural isomers, even if not explicitly mentioned. The example-mixtures of the three isomeric fluorotoluenes (FT) do not contain other components, e.g. solvents, but the person skilled in the art well knows that the features revealed herein do also accordingly apply to mixtures containing also other components. Therefor the scope of the invention comprises all types of analytes, i.e. mixtures of isomers with or without other compounds (solvents, bye-products, contaminations/impurities of all types etc.). Figure 7 shows exemplarily the ion yield ratio $Y(m/z=89)/Y(m/z=83)$ as function of the linear chirp paramter α for the sample isomers of fluoro toluene. This data set is exemplarily used in the exemplary quantitative analysis as specified below.

[0027] A mixture of, e.g., three isomers is characterized by three concentrations. In the present exemplary case, where no solvent but only the analytes are being discussed, the molar fraction of the three components, x_o , x_m and x_p , for the *ortho*-, the *meta*- and the *para*- isomer, are most suitable. Here, due to the fact that $x_o + x_m + x_p = 1$, the problem of quantitative analysis of chemical composition reduces to determining two molar fractions. (Regarding chemical compositions, resp. mixtures, containing b components, accordingly b-1 molar fractions have to be determined.) Conceptually one has to measure chosen ion yield ratios as a function of the chosen chirp parameters for a given mixture and compare this to the values for the pure isomers of which it is composed. In a first realization the most suitable ion signals and chirp parameters are chosen by hand based on criteria which identify differences between the isomers of interest. The person skilled in the art knows which parameters to apply, e.g. certain typical structure elements of the isomers, and which limit-values to use. At a later stage such choices can be generated by applying genetic algorithms to appropriate data basis. It is also according to the invention to use computer implemented neuronal networks for performing the choice of the most suitable ion signals. From a mathematical point of view given the amount of data (measurement values of the mass spectrometer) available and given the fact that only two parameters are aimed at, the numerical problem is over-determined. According to scientific findings, the measurable observables (the measurement values of the mass spectrometer) are sensitive to the molar fractions of the structural isomers due to the ionization with two or more dimensionally (at least linear and quadratic) chirped fs-laser pulses.

[0028] In order to investigate and demonstrate this sensitivity one has to set up a set of equations of the type given in equation 5.

$$IYR_{\varphi, \text{mixture}} = x_o \cdot IYR_{\varphi, o} + x_m \cdot IYR_{\varphi, m} + x_p \cdot IYR_{\varphi, p} \quad (5)$$

[0029] Herein, $IYR_{\varphi, \text{mixture}}$ is an ion yield ratio for a given spectral phase φ in the mixture. The person skilled in the art well knows how to calculate ion yield ratios from mass spectrometric data. $IYR_{\varphi, o}$, $IYR_{\varphi, m}$, and $IYR_{\varphi, p}$ are the corresponding ion yield ratios for the pure isomers. As ion yield ratio (IYR) according to the invention one has to consider, for example, the ratios $Y(m/z=109) / Y(m/z=110)$ and $Y(m/z=89) / Y(m/z=83)$. The latter one describes the ratio of the

two competing fragmentation channels shown in Figure 7. Herein, exemplarily a variable number of equations is chosen out of the large experimental parameter space (mass spectrometric data) until the molar fraction converge within a chosen limit of precision for the quantitative determination of the isomers. The equations chosen describe the relations between different signal-intensities of the acquired mass spectra at different combinations of chirp parameters. The choosing of the equations can be made either manually or by using computer implemented algorithms, either sequentially programmed by programming languages like, e.g. Fortran, C, Java, C++ etc. or being implemented by use of neuronal networks, AI (artificial intelligence) or the like.

[0030] Depending on the desired precision of the quantitative determination of the contents of the isomers within the mixture the scope of the invention comprises choosing a different number of equations, ranging from 2 to $n_i \times s \times c_p$, s being the number of mass spectrometric signals detected, c_p being the number of possible chirp-parameter combinations of all chirp-parameter values applied during the measurement, and n_i being the number of isomers to be quantitatively determined. It is preferred to use a number of equations within the range of 2 to 1000 equations; it is more preferred to use a number of equations within the range of 2 to 500 equations; it is even more preferred to use a number of equations within the range of 2 to 50 equations, and it is most preferred to use a number of equations within the range of 2 to 20 equations. Here and elsewhere within this revelation, whenever a range of numerical values is described by naming the limits of the range, all intermediate values are also included, even if they are not explicitly mentioned.

[0031] The selection of specific equations is therefore based on the optimization of the result. Besides, some further conditions are needed for the current example, e.g. $0 \leq x_i \leq 1$, where x_i is the molar fraction of isomer i and the sum over all x_i is one. In fact, for the two unknown parameters only two equations are required, however, more equations improve the result.

[0032] Respectively for three unknown parameters (unknown contents, expressed as, e.g., molar fractions, weight percentages etc. only three equations are needed, but more equations are improving the result. For any number of unknown parameters (unknown contents of isomers) the same number of equations is sufficient, but a higher number is improving the result.

[0033] A multitude of data points (mass spectrometric measurement values) is available due to the rich fragmentation pattern in the mass spectra shown in Figure 1 and the extent of applied chirp parameter.

[0034] Typically, seven equations have been used for the current examples and found to be sufficient for allowing a unique determination of the molar fractions according to the desired precision. Exemplarily, these equations are solved employing the software PTC Mathcad 15 using the *minerr* function. There are numerous more software tools (software programs) that can also be used without leaving the scope of the invention. The person skilled in the art knows that any type of software providing tools for analyzing data, e.g. PCA, least square fit method, and other statistical methods for data evaluation, can be used without leaving the scope of the invention. The MathCad algorithm, used exemplarily, requires starting values (estimated guess) for the parameters to be determined. The setting of these starting values is done either manually or automatically, e.g. by use of algorithms or neuronal networks (AI). Extensive checks ensure that the result does not depend on the starting value given to the algorithm. The equations actually used exemplarily in the current sample-analysis are presented below with the examples of detailed embodiments of the invention.

[0035] The real and the calculated compositions of the subsequently exemplarily measured ternary mixtures are listed in table 1 (Fig. 8). Evidently, the largest deviation between the experimentally and the real mixture is 5% for the amount of *m*-FT in mixture 1 and of *o*-FT in mixture 2. This is a relatively small deviation, which demonstrates the power of the method according to the invention.

[0036] Examples of ternary diagrams of the two exemplary ternary isomeric mixtures are shown in Figure 9A and Figure 9B. The open circles depict the result of the analysis, if only one of the seven equations is used. In this case the calculated compositions listed in table 1 have been taken as estimated start values. In case of only one equation used for calculation of the composition, the estimated start values influence the result. It is striking that the calculated value of the *para* isomer matches almost always its real portion (within 1% deviation). This exemplary set of equations seems to be very sensitive for this isomer. The fact that the quantitative analysis is possible by the method presented herein implies - regarding the current example - that the fragmentation of the molecular ions appears to be fast compared to the isomerization to tropylium-like structures, where the intermediate would lose memory of the precursor's identity. Similar conclusions seem to be possible in case of other isomeric mixtures.

[0037] Due to the massive over-determination of the numerical problem, the choice of the equations mentioned is neither unique nor critical. The huge number of mass spectrometric data sets for each single mass spectrometric signal in combination with the modification of the chirp parameters provides by far enough measurement values for creating numerous equations for performing the method according to the invention. Therefore the invention comprises the combination of any chirp-parameter-combination-specific mass spectrometric data set of any one mass spectrometric signal with any one else. Therefore it is obvious that, because manually choosing suitable equations is quite strenuous out of such a huge amount of measurement data, that it is a preferred method according to the invention to apply computer implemented algorithms and/or neuronal networks (artificial intelligence) for choosing the equations and performing all required calculations for performing the quantitative analysis of the isomeric mixtures.

[0038] At this point it suffices to state that in looking at the reference data, those regions will be preferred, where the observables, i.e. the ion yield ratios, differ characteristically between the different isomers. This step of the method is extraordinarily suitable for being performed via computer-implemented methods/AI-systems/neuronal networks. Since for a mixture of three isomers, the observable for one isomer inherently is located between the other two isomers, this leads to the subtlety, that there is an advantage of identifying points in parameter space (i.e. combination of chirp parameters), where two isomers have similar observable for one point but two other isomers for another point.

[0039] This leads to the question, what limits the accuracy of the quantitative analysis exemplarily presented herein. Clearly, the level of sophistication of defining the set of equations employed in the analysis can and will be improved in future work.

[0040] From another point of view the difference between the measured and the weight-in composition is correlated with the fact that the weighing-in occurs in the liquid phase, but the analysis occurs in the gas phase.

[0041] Since the vapor pressure of o-FT is about 10% higher than that of the other two isomers, the composition of the mixture in the gas phase is not automatically identical to that in the liquid phase. This is a well-known phenomenon discussed in every PhysChem textbook as the basis of distillation. Evidently, this is a physical limitation, which cannot be overcome by a better choice of equations. For the current experiment our data suggest that the difference between the measured and the weight-in composition is indeed mainly due to this systematic difference. But this physical limitation can be overcome by performing calibration experiments; therefore, it lies within the scope of the invention to perform calibration experiments for eliminating this effect as well as other effects also. It is also possible and completely comprised by the scope of the invention to employ a sample introduction not being subject to differences in the aggregate condition, e.g. a liquid spray technique.

[0042] The experiments described herein have exemplarily proven that distinction and quantitative determination of structural isomers is possible by means of chirped fs-LIMS. Linear and quadratic chirped pulses enhance the small differences in nearly all ion yield ratios of structural isomers. The systematic variation of linear chirp parameters provides information on the mechanism of fragmentation and evidence for sequential pathways and competing processes. The structural differences of the isomers are of prominent importance, because of stability and internal energy distribution in the individual isomers. The corresponding ion yield ratios, for example of the sequential reaction pathways, allows distinguishing between the isomers in a chirped fs-LIMS investigation. The quantitative in-situ analysis of ternary or even more complex isomeric mixtures has been proven. Exemplarily the molar fractions of isomers in mixtures containing structural isomers can be derived in a one-step experiment, i.e. without previously separating the structural isomers. This provides a method of isomer analysis making any chromatographic separation steps void. The deviation between the molar fraction (as one exemplary way of indicating quantitative composition of a mixture) of isomers derived from the fs-LIMS analysis and the one prepared in the liquid state is currently exemplarily on the order of 5%, but is easily reduced according to the inventive method to any desired value.

[0043] The key point of the method presented herein is a systematic variation of the spectral phase of the fs-laser pulses, which characteristically affects the fragmentation pattern observed in the mass spectra. Variation of the linear chirp parameter is also helpful for rationalizing the fragmentation mechanism. Exemplarily two ternary mixtures of three isomers are quantitatively analyzed in-situ with an accuracy of 5% for the molar fractions.

[0044] The scope of the invention is therefore summarized as follows:
The scope of the invention comprises a computer implemented method for determining at least partially the quantitative content of at least one structural isomer within a mixture of compounds containing at least two structural isomers comprising

- the controlling and timing of at least one femtosecond-laser for ionization of the components of the mixture of compounds and
- the controlling and timing of at least one mass-spectrometer analyzing the ionized components of the mixture of compounds,
- the acquiring of the intensity-values of at least three peaks of the mass spectrometer data

whereat

- the pulses of the at least one femtosecond-laser are formed by applying one and/or two dimensional chirping, whereat
 - the chirp-parameters of the femtosecond-laser are changed independently from each other in time and
 - the data acquisition of the at least one mass-spectrometer is timely linked with the change of the chirp parameters in time,
- characterized in that

- a) the intensity-values of at least three peaks of the mass spectrometer data are recorded at least at two

different combinations of chirp parameters, providing at least six chirp parameter-dependent intensity-values of the at least three peaks;

b) determining at least two chirp parameter-dependent ion yield ratios between at least three peaks of the mass spectrometer data ($IYR_{\alpha(n)/\beta(m)}$, $IYR_{\alpha(o)/\beta(p)}$) from the chirp parameter-dependent intensity-values according to step a) by dividing:

$$IYR_{\alpha(n)/\beta(m)} = \frac{\text{intensity-value } \alpha(n)/\beta(m) \text{ of a chosen peak}}{\text{intensity-value } \alpha(n)/\beta(m) \text{ of another chosen peak}}$$

respectively

$$IYR_{\alpha(o)/\beta(p)} = \frac{\text{intensity-value } \alpha(o)/\beta(p) \text{ of a chosen peak}}{\text{intensity-value } \alpha(o)/\beta(p) \text{ of another chosen peak}}$$

whereat

- the choice as to which peaks are used for the calculation of the ion yield ratios (IYR) is made manually or by use of a computer implemented algorithm or by use of a computer implemented neuronal network and
- the indexes n, m, o, p independently from each other have values belonging to the mathematical set of whole numbers between 1 and 50000, indicating the possible combinations of chirp parameter-values applicable, whereat
- the chirp parameters $\alpha(n)$, $\alpha(o)$ have values between -50000 fs² and +50000 fs² and/or
- the chirp parameters $\beta(m)$, $\beta(p)$ have values between -50000 fs³ and +50000 fs³;

c) setting up a set of at least two equations of the type

$$IYR_{\alpha(n)/\beta(m)} = \text{cal}_o \cdot x_o + \text{cal}_m \cdot x_m + \text{cal}_p \cdot x_p ,$$

$$IYR_{\alpha(o)/\beta(p)} = \text{cal}_o \cdot x_o + \text{cal}_m \cdot x_m + \text{cal}_p \cdot x_p ,$$

whereat cal_o , cal_m , cal_p , cal_o' , cal_m' , and cal_p' are calibration values of ion yield ratios (IYR) obtained by performing the inventive method on the pure structural isomers and x_o , x_m , x_p are units of quantitative content or concentration chosen from the list comprising mole fraction, percentage, promille, ppm, ppb, etc.

d) performing a numerical solution of the set of equations acquired in step c) manually or by use of a computer implemented method for numerically solving sets of equations.

[0045] Here and elsewhere within this revelation, whenever the scope of values or a range of values, e.g. the number of applied chirp parameters or the range of values of chirp parameters, is indicated by naming the upper-limit value and the lower-limit value, it is obvious to the person skilled in the art that any value in between the explicitly named limiting values is also included within the scope of the invention, even if not explicitly named.

[0046] The scope of the invention also comprises a method as described before, wherein for performing step d) between step c) and step d) an additional step is performed, comprising the definition of boundary conditions for performing the numerical solution of the set of equations, whereat the boundary conditions are:

- i) $0 \leq x_o \leq 1$, $0 \leq x_m \leq 1$, $0 \leq x_p \leq 1$, $1 = x_o + x_m + x_p$ if the unit of quantitative content is mole fraction;
- ii) $0 \leq x_o \leq 100$, $0 \leq x_m \leq 100$, $0 \leq x_p \leq 100$, $100 = x_o + x_m + x_p$ if the unit of quantitative content is per cent.

[0047] The scope of the invention also comprises methods as described before, characterized in that the chirp pa-

rameters are α and/or β .

[0048] The scope of the invention also comprises methods as described before, characterized in that the number of equations used is within the range of 2 to 1000 equations, preferred within the range of 2 to 500 equations, more preferred within the range of 2 to 50 equations, and most preferred within the range of 2 to 20 equations.

[0049] The scope of the invention also comprises methods as described before, characterized in that the choice of the at least three peaks of the mass spectrometer data which are to be recorded according to step a) of claim 1 is being performed by hand and/or via computer-implemented methods/AI-systems/neuronal networks. The person skilled in the art knows which parameters to apply, e.g. certain typical structure elements of the isomers, and which limit-values to use. At a later stage such choices can be generated by applying genetic algorithms to appropriate data basis. It is also according to the invention to use computer implemented neuronal networks for performing the choice of the most suitable ion signals.

[0050] The scope of the invention also comprises methods as described before, characterized in that the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies laser pulses with a duration between 1 fs and 10000 fs, more preferred between 5 fs and 1000 fs, even more preferred between 10 fs and 500 fs and most preferred between 20 fs and 200 fs.

[0051] The scope of the invention also comprises methods as described before, characterized in that the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies repetition rates of the laser pulses between 1 Hz and 100 kHz, more preferred between 10 Hz and 50 kHz, even more preferred between 100 Hz and 10 kHz and most preferred between 500 Hz and 5 kHz.

[0052] The scope of the invention also comprises methods as described before, characterized in that the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies a central wavelength of the laser pulses between 200 nm and 5000 nm, more preferred between 400 nm and 2000 nm, even more preferred between 500 nm and 1500 nm and most preferred between 600 nm and 1000 nm.

[0053] The scope of the invention also comprises methods as described before, characterized in that the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies a duration of the laser pulse of 45 fs and a repetition rate of the laser pulses of 1 kHz and a central wavelength of the laser pulses 805 nm.

[0054] The scope of the invention also comprises an apparatus determining at least partially the quantitative contents of at least two structural isomers within a mixture of compounds containing at least two structural isomers, having implemented a method as is previously described, comprising

- means for receiving mass spectroscopic data acquired from ionized molecules and/or parts of molecules emerging from the mixture of compounds, the ionized molecules and/or parts of molecules having been ionized by chirped fs-laser pulses,
- means for receiving the values of the chirp parameters used for creating the chirped fs-laser pulses, characterised in that the apparatus further comprises
- means for evaluating the mass spectroscopic data in temporal combination with the chirp parameters, acquiring ion yield ratios in dependence from chirp parameters,
- means for evaluating the chirp parameter dependent ion yield ratios in such a way that at least two equations are acquired representing the dependence of at least two chirp parameter dependent ion yield ratios from the quantitative content of at least two structural isomers of the mixture of compounds,
- means for solving the at least two equations, acquiring numerical values dependent from and representing the quantitative contents of the at least two isomers within the mixture of compounds and
- means for assigning the numerical values to values of concentration or values of content.

Detailed embodiments of the invention

[0055] Laser ionization experiments are exemplarily conducted by shining fs-laser light into the ion source of a linear time-of-flight mass spectrometer. The scope of the invention comprises the use of any other type of mass spectrometer available, e.g. sector mass spectrometer, quadrupole mass spectrometer, ion trap mass spectrometer, orbitrap mass spectrometer, fourier transform mass spectrometer, tandem mass spectrometer etc. This list is not confining the types of mass spectrometers that can be used according to the scope of the invention. It is well known to the person skilled in the art that any type of mass spectrometer may be used without leaving the scope of the invention. The laser system consists of a chirped pulse amplification system (e.g. Odin, Quantronix) seeded by a femtosecond oscillator (e.g. Synergy, Femtolasers). The system exemplarily generates laser pulses with a typical duration of 45 fs, 1 kHz repetition rate and a central wavelength of about 805 nm. Other repetition rates are usable as well according to the scope of the invention. Control of the spectral phase is exemplarily carried out in a folded 4f-pulse shaper including a liquid crystal display (e.g.

SLM-S640, Jenoptik) in the Fourier plane. The person skilled in the art well knows how to create chirped fs-laser pulses, e.g. as is described in the following paragraphs.

[0056] For further discussion, it is helpful to expand the spectral phase $\varphi(\omega)$ of the fs-laser pulse in a Taylor series (equation 1).

$$\begin{aligned} \varphi(\omega) = & \varphi_0(\omega_0) + (\omega - \omega_0) \cdot \frac{1}{1!} \frac{\partial \varphi}{\partial \omega} \Big|_{\omega=\omega_0} + (\omega - \omega_0)^2 \cdot \frac{1}{2!} \frac{\partial^2 \varphi}{\partial \omega^2} \Big|_{\omega=\omega_0} \\ & + (\omega - \omega_0)^3 \cdot \frac{1}{3!} \frac{\partial^3 \varphi}{\partial \omega^3} \Big|_{\omega=\omega_0} + \dots \end{aligned} \quad (1)$$

[0057] In the context of the current revelation the second and third order terms are pivotal, for example. The linear chirp parameter α (equation 2) is given by the second partial derivative of the spectral phase, which physically corresponds to a linear increase or decrease of the instantaneous frequency for positive or negative values of α , respectively.

$$\alpha = \frac{1}{2!} \cdot \frac{\partial^2 \varphi(\omega)}{\partial \omega^2} \quad (2)$$

[0058] An increasing linear chirp parameter α correlates with an increase of the pulse duration and hence a decrease of the peak intensity. The relationship between linear chirp parameter and pulse duration τ is given in equation 3.

$$\tau_\alpha^2 = \tau_0^2 + \left(\frac{8 \cdot \ln(2) \cdot \alpha}{\tau_0} \right)^2 \quad (3)$$

[0059] The actual pulse duration does not depend on the sign of the linear chirp parameter, i.e. the α function is symmetric with respect to $\alpha = 0$.

[0060] The third partial derivative in equation 1 defines the quadratic chirp parameter β (equation 4).

$$\beta = \frac{1}{3!} \cdot \frac{\partial^3 \varphi(\omega)}{\partial \omega^3} \quad (4)$$

[0061] Positive values of β lead to post-pulses after the main pulse, while negative values of β lead to pre-pulses before the main pulse. Combinations of values for α and β span a huge parameter space. For the examples revealed herein it is sufficient to explore only part of the complete parameter space. More specifically for experiments, in which α is changed, β remains unchanged ($\beta=0$). If β is changed, α is usually set to be zero. All pulse characteristics, e.g. the pulse duration, were analyzed by a Grenouille (8-50, Swamp Optics) during the course of the experiments. Other equipment for analyzing pulse characteristics can also be used without leaving the scope of the invention. Also, combined changing of α and β is also comprised by the scope of the invention. This will lead to even more precise quantitative determination of compositions containing isomers.

[0062] The fs-laser pulses are, e.g., focused into the ion source of the ToF mass spectrometer (or any other mass spectrometer) with a concave mirror ($f = 7.5$ cm). The ions are, e.g., accelerated towards the detector by an electric field in the ionization region. In a field free region they become separated according to their different mass to charge ratio (m/z). A more detailed description of the exemplary experimental setup is known within the state of the art. Typical (exemplary) experimental parameters have a pulse energy of 50 μ J and a constant pressure in the chamber of about $2 \cdot 10^{-6}$ mbar. All samples have been introduced through an effusive gas inlet either separately for the reference studies or premixed for the actual determination of chemical composition. Other forms of introducing the sample of interest to the ion source of the mass spectrometer are included as well within the scope of the invention, for example methods for introducing solid samples and/or methods for introducing liquid samples and/or methods for introducing gaseous samples. The person skilled in the art well knows methods for introducing solid, liquid or gaseous samples, which can be applied without leaving the scope of the invention.

[0063] First, the isomers to be determined, e.g. *o*-, *m*- and *p*-fluorotoluene, are separately brought into the chamber,

e.g., by effusive gas supply. This way calibration measurements are performed exemplarily. Time-of-flight spectra are measured while systematically varying the spectral phase. Conversion of the flight time in a mass to charge ratio provides the mass spectra of the compounds. After integration of the ion signals, specific ion yield ratios (IYR) are derived. These data exemplarily serve as a reference for subsequent analysis of unknown mixtures of the isomers.

[0064] The systematical varying of the spectral phase, i.e. the varying of α and β is done according to the invention either manually or by way of a computer implemented algorithm or by use of a computer implemented neuronal network or by any combination thereof. It is important that each data-set of the mass spectrometer is correlated to the values of α and β , i.e. for each mass spectrometric data-set it is known from which combination of values of α and β it originated.

[0065] Exemplary for the quantitative analysis of mixtures containing, e.g., *o*-, *m*- and *p*-fluorotoluene, premixed ternary mixtures of fluorotoluene isomers have been prepared. Due to new laser adjustments, the pure substances may be measured again (calibration measurement) just before the analysis of mixtures.

[0066] For the exemplary quantitative analysis of the exemplary isomer mixtures containing fluorotoluene isomers 7 points in the parameter space are defined by a specific ion yield ratio and a combination of chirp parameters α and β . Four points refer to the ion yield ratio $Y(m/z=109)/Y(M^+)$, i.e. the ion yield ratio between H-loss and molecular ion signals in the 1. step of Scheme 2. Three points refer to the ion yield ratio $Y(m/z=89)/Y(m/z=83)$, i.e. the branching ratio of the 2. step in Scheme 2. For book-keeping these data points are named as given in the second column of table 2 (Fig. 10). For these data points table 2 lists the measured ion yield ratios for both mixtures exemplarily investigated.

[0067] For the exemplary quantitative analysis a set of seven equations has been implemented as given below. The values in front of the molar fractions x_i are the IYR measured for the pure fluorotoluene isomers under conditions identical to the measurements of the mixtures (calibration measurement).

$$IYR(1.Step)\alpha_1 = 0.0813 \cdot x_o + 0.0782 \cdot x_m + 0.1299 \cdot x_p$$

$$IYR(1.Step)\alpha_2 = 0.0958 \cdot x_o + 0.0889 \cdot x_m + 0.1428 \cdot x_p$$

$$IYR(1.Step)\beta_1 = 0.0772 \cdot x_o + 0.0743 \cdot x_m + 0.1306 \cdot x_p$$

$$IYR(1.Step)\beta_2 = 0.0839 \cdot x_o + 0.0739 \cdot x_m + 0.1198 \cdot x_p$$

$$IYR(2.Step)\alpha_1 = 0.1948 \cdot x_o + 0.20597 \cdot x_m + 0.1336 \cdot x_p$$

$$IYR(2.Step)\alpha_2 = 0.1852 \cdot x_o + 0.1924 \cdot x_m + 0.1231 \cdot x_p$$

$$IYR(2.Step)\alpha_3 = 0.2011 \cdot x_o + 0.1968 \cdot x_m + 0.1361 \cdot x_p$$

[0068] Regarding the examples shown herein four further (boundary) conditions are relevant to solve this exemplary set of equations.

$$0 \leq x_o \leq 1$$

$$0 \leq x_m \leq 1$$

$$0 \leq x_p \leq 1$$

$$1 = x_o + x_m + x_p$$

[0069] Numerical solution of the exemplary set of equations then yields the molar fractions of the fluorotoluene isomers

in the respective exemplary mixture.

[0070] Table 3 (Fig. 11) and Table 4 (Fig. 12) exemplarily list the weight-in (line 2) as well as the experimentally determined (line 3) compositions of mixture 1 and 2 respectively. For the latter the seven equations listed above and the four boundary conditions were considered. To illustrate the stability of the set of equations the analysis was also repeated with only one of the seven equations shown above. The molar fractions obtain for that analysis are given in lines 4 to 10 of tables 3 and 4 respectively. As mentioned above the molar fraction of the ortho isomer appears to be matched best in all cases. We point out that the results for using one equation only are only given for illustration. This (using only one equation) is clearly not the recommended approach, nevertheless lies well within the scope of the invention. In fact, the result of the inventive method for quantitatively analyzing mixtures containing structural isomers depends on the choice of the start values given to the numerical algorithmic methods. The exemplary numbers shown apply to the values of line 2 (full analysis) used as the starting values. The choice of the start values for the application of the numerical algorithmic methods according to the invention may be made either manually or by use of computer implemented algorithms or computer implemented neuronal networks, i.e. artificial intelligence.

Description of the drawings

[0071]

Fig. 1: Exemplarily mass spectra of ortho fluorotoluene (o-FT, upper line as indicated by arrows), meta fluorotoluene (m-FT, center line as indicated by arrows) and para fluorotoluene (p-FT, bottom line as indicated by arrows) using a transform-limited laser pulse.

Fig. 2A: Normalized integrated molecular ion yield $Y(M^+)$ for o-fluorotoluene (exemplarily).

Fig. 2B: Normalized integrated ion yield of singly charged parent ion of m-FT as a function of linear chirp parameter α (exemplarily).

Fig. 2C: Normalized integrated ion yield of singly charged parent ion of p-FT as a function of linear chirp parameter α (exemplarily).

Fig. 3A: Linear chirp dependence for $Y(m/z=109) / Y(M^+)$ of fluorotoluene isomers (exemplarily).

Fig. 3B: Quadratic chirp dependence for $Y(m/z=109) / Y(M^+)$ of fluorotoluene isomers (exemplarily).

Fig. 4A: Linear chirp dependency of ion yield ratio belonging to C_2H_2 loss (exemplarily).

Fig. 4B: Linear chirp dependency of ion yield ratio belonging to HF loss (exemplarily).

Fig. 5A: Exemplary ion yield ratios $Y(m/z=89)/Y(M^+)$ of the fluorotoluene isomers as function of α .

Fig. 5B: Exemplary ion yield ratios $Y(m/z=83)/Y(M^+)$ of the fluorotoluene isomers as function of α .

Fig. 6: Exemplary presentation of $Y(m/z=109)/Y(M^+)$ of o-FT (black squares), m-FT (black dots) and p-FT (black diamonds) as a function of laser pulse energy.

Fig. 7: Exemplary presentation of ion yield ratio $Y(m/z=89)/Y(m/z=83)$ of the fluorotoluene isomers as function of α .

Fig. 8: Table 1, presenting exemplarily the real composition of the two exemplarily analyzed ternary isomeric mixtures as well as the experimentally determined amounts.

Fig. 9A: Exemplary ternary diagram of mixture 1 with the real composition (filled circle at the crosspoint of the three lines indicating the three molar fractions of the components), the calculated composition by use of all seven formed equations (open square) and the calculated compositions, if only one of the seven formed equations has been considered (open circles). For the latter ones the amount of ortho-, meta- and para-FT out of the calculation with all equations used has been taken into account as estimated start values.

Fig. 9B: Exemplary ternary diagram of mixture 2 with the real composition (filled circle at the crosspoint of the three lines indicating the three molar fractions of the components), the calculated composition by use of all seven

formed equations (open square) and the calculated compositions, if only one of the seven formed equations has been considered (open circles). For the latter ones the amount of ortho-, meta- and para-FT out of the calculation with all equations used has been taken into account as estimated start values.

5 Fig. 10: Table 2: Actual data points and measured ion yield ratios used in the exemplary quantitative analysis of mixtures 1 and 2.

Fig. 11: Table 3: Exemplary values for weight-in (line 2) and experimentally determined (line 3 to 10) molar fractions of the three fluorotoluene isomers in mixture 1. Line 3 gives the result of the full analysis, lines 4 to 10 give the result of the analysis employing one equation (as indicated) only.

Fig. 12: Table 4: Exemplary values for weight-in (line 2) and experimentally determined (line 3 to 10) molar fractions of the three fluorotoluene isomers in mixture 2. Line 3 gives the result of the full analysis, lines 4 to 10 give the result of the analysis employing one equation (as indicated) only.

Claims

1. A computer implemented method for determining at least partially the quantitative content of at least one structural isomer within a mixture of compounds containing at least two structural isomers comprising

- the controlling and timing of at least one femtosecond-laser for ionization of the components of the mixture of compounds and
- the controlling and timing of at least one mass-spectrometer analyzing the ionized components of the mixture of compounds,
- the acquiring of the intensity-values of at least three peaks of the mass spectrometer data whereat
- the pulses of the at least one femtosecond-laser are formed by applying one and/or two dimensional chirping, whereat

- the chirp-parameters of the femtosecond-laser are changed independently from each other in time and
- the data acquisition of the at least one mass-spectrometer is timely linked with the change of the chirp parameters in time,

characterized in that

- a) the intensity-values of at least three peaks of the mass spectrometer data are recorded at least at two different combinations of chirp parameters, providing at least six chirp parameter-dependent intensity-values of the at least three peaks;
- b) determining at least two chirp parameter-dependent ion yield ratios between at least three peaks of the mass spectrometer data ($IYR_{\alpha(n)/\beta(m)}$, $IYR_{\alpha(o)/\beta(p)}$) from the chirp parameter-dependent intensity-values according to step a) by dividing:

$$IYR_{\alpha(n)/\beta(m)} = \frac{\text{intensity-value}_{\alpha(n)/\beta(m)} \text{ of a chosen peak}}{\text{intensity-value}_{\alpha(n)/\beta(m)} \text{ of another chosen peak}}$$

respectively

$$IYR_{\alpha(o)/\beta(p)} = \frac{\text{intensity-value}_{\alpha(o)/\beta(p)} \text{ of a chosen peak}}{\text{intensity-value}_{\alpha(o)/\beta(p)} \text{ of another chosen peak}}$$

whereat

- the choice as to which peaks are used for the calculation of the ion yield ratios (IYR) is made manually or by use of a computer implemented algorithm or by use of a computer implemented neuronal network and

- the indexes n, m, o, p independently from each other have values belonging to the mathematical set of whole numbers between 1 and 50000, indicating the possible combinations of chirp parameter-values applicable, whereat

- the chirp parameters $\alpha(n)$, $\alpha(o)$ have values between -50000 fs^2 and $+50000 \text{ fs}^2$ and/or

- the chirp parameters $\beta(m)$, $\beta(p)$ have values between -50000 fs^3 and $+50000 \text{ fs}^3$;

c) setting up a set of at least two equations of the type

$$\text{IYR}_{\alpha(n)/\beta(m)} = \text{cal}_o \cdot x_o + \text{cal}_m \cdot x_m + \text{cal}_p \cdot x_p ,$$

$$\text{IYR}_{\alpha(o)/\beta(p)} = \text{cal}_o \cdot x_o + \text{cal}_m \cdot x_m + \text{cal}_p \cdot x_p ,$$

whereat cal_o , cal_m , cal_p , cal_o' , cal_m' and cal_p' are calibration values of ion yield ratios (IYR) obtained by performing the inventive method on the pure structural isomers and x_o , x_m , x_p are units of quantitative content or concentration chosen from the list comprising mole fraction, percentage, promille, ppm, ppb, etc.

d) performing a numerical solution of the set of equations acquired in step c) manually or by use of a computer implemented method for numerically solving sets of equations.

2. The method of claim 1, wherein for performing step d) of claim 1 between step c) and step d) an additional step is performed, comprising the definition of boundary conditions for performing the numerical solution of the set of equations, whereat the boundary conditions are:

i) $0 \leq x_o \leq 1$, $0 \leq x_m \leq 1$, $0 \leq x_p \leq 1$, $1 = x_o + x_m + x_p$ if the unit of quantitative content is mole fraction;

ii) $0 \leq x_o \leq 100$, $0 \leq x_m \leq 100$, $0 \leq x_p \leq 100$, $100 = x_o + x_m + x_p$ if the unit of quantitative content is per cent.

3. The method of one of the previous claims, **characterized in that** the chirp parameters are α and/or β .

4. The method of one of the previous claims, **characterized in that** the number of equations used is within the range of 2 to 1000 equations, preferred within the range of 2 to 500 equations, more preferred within the range of 2 to 50 equations, and most preferred within the range of 2 to 20 equations.

5. The method of one of the previous claims, **characterized in that** the choice of the at least three peaks of the mass spectrometer data which are to be recorded according to step a) of claim 1 is being performed by hand and/or via computer-implemented methods/AI-systems/neuronal networks. The person skilled in the art knows which parameters to apply, e.g. certain typical structure elements of the isomers, and which limit-values to use. At a later stage such choices can be generated by applying genetic algorithms to appropriate data basis. It is also according to the invention to use computer implemented neuronal networks for performing the choice of the most suitable ion signals.

6. The method of one of the previous claims, **characterized in that** the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies laser pulses with a duration between 1 fs and 10000 fs, more preferred between 5 fs and 1000 fs, even more preferred between 10 fs and 500 fs and most preferred between 20 fs and 200 fs.

7. The method of one of the previous claims, **characterized in that** the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies repetition rates of the laser pulses between 1 Hz and 100 kHz, more preferred between 10 Hz and 50 kHz, even more preferred between 100 Hz and 10 kHz and most preferred between 500 Hz and 5 kHz.

8. The method of one of the previous claims, **characterized in that** the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies a central wavelength of the laser pulses between 200 nm and 5000 nm, more preferred between 400 nm and 2000 nm, even more preferred between 500 nm and 1500 nm and most preferred between 600 nm and 1000 nm.

9. The method of one of the previous claims, **characterized in that** the controlling and timing of the at least one femtosecond-laser for ionization of the components of the mixture of compounds applies a duration of the laser pulse of 45 fs and a repetition rate of the laser pulses of 1 kHz and a central wavelength of the laser pulses 805 nm.

10. An apparatus determining at least partially the quantitative contents of at least two structural isomers within a mixture of compounds containing at least two structural isomers, having implemented a method according to one of the preceding claims, comprising

- means for receiving mass spectroscopic data acquired from ionized molecules and/or parts of molecules emerging from the mixture of compounds, the ionized molecules and/or parts of molecules having been ionized by chirped fs-laser pulses,
- means for receiving the values of the chirp parameters used for creating the chirped fs-laser pulses,

characterised in that the apparatus further comprises

- means for evaluating the mass spectroscopic data in temporal combination with the chirp parameters, acquiring ion yield ratios in dependence from chirp parameters,
- means for evaluating the chirp parameter dependent ion yield ratios in such a way that at least two equations are acquired representing the dependence of at least two chirp parameter dependent ion yield ratios from the quantitative content of at least two structural isomers of the mixture of compounds,
- means for solving the at least two equations, acquiring numerical values dependent from and representing the quantitative contents of the at least two isomers within the mixture of compounds and
- means for assigning the numerical values to values of concentration or values of content.

Fig. 1

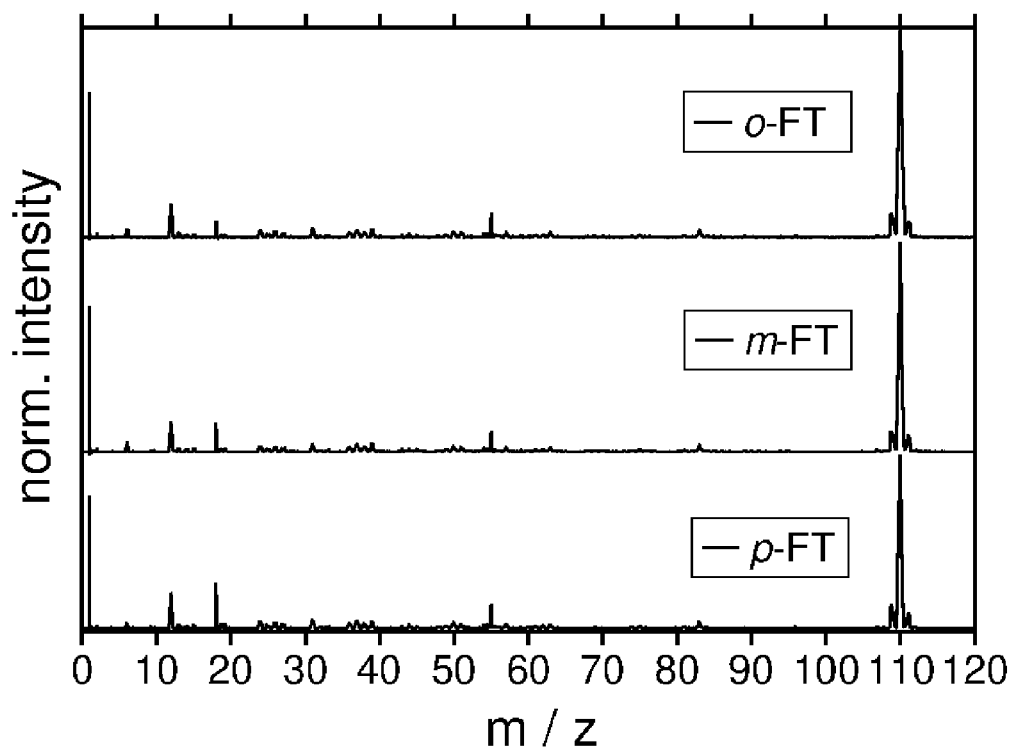


Fig. 2A

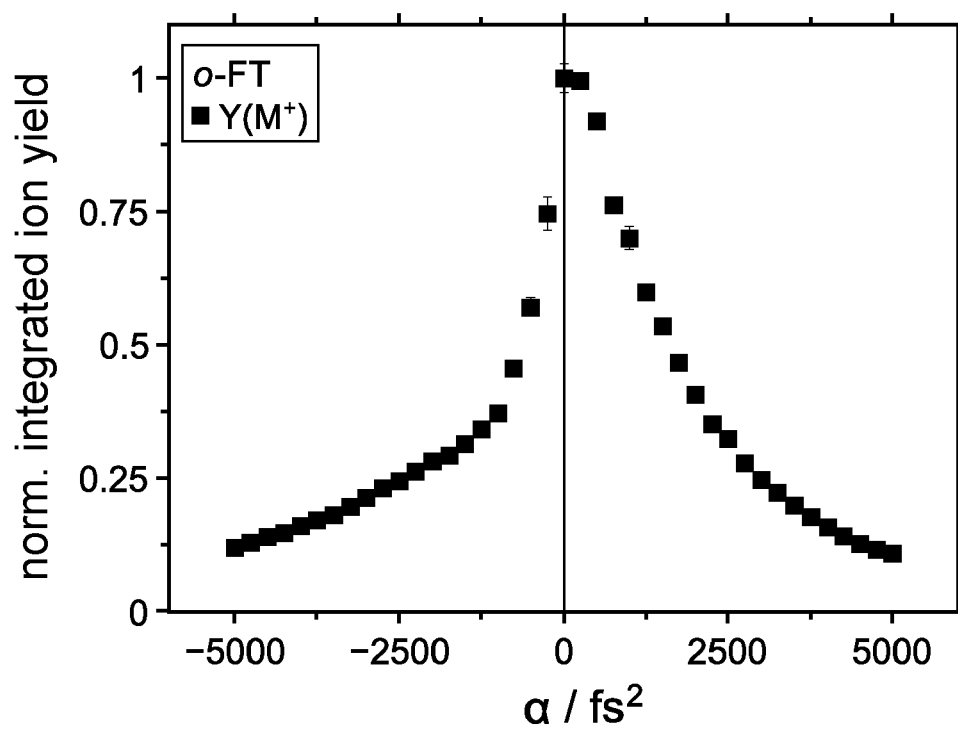


Fig. 2B

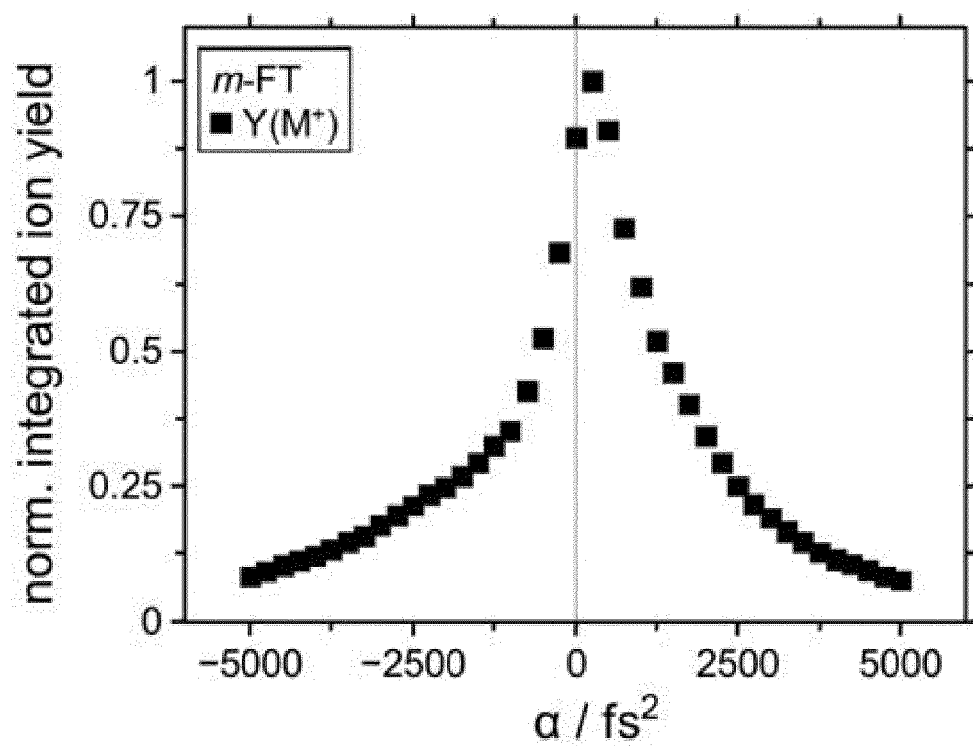


Fig. 2C

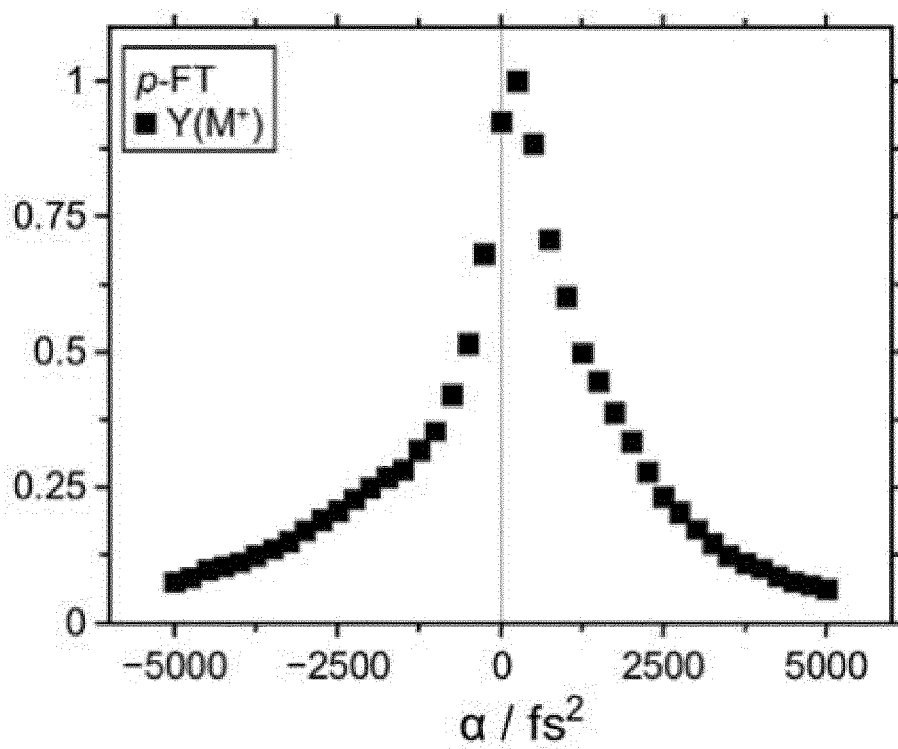


Fig. 3A

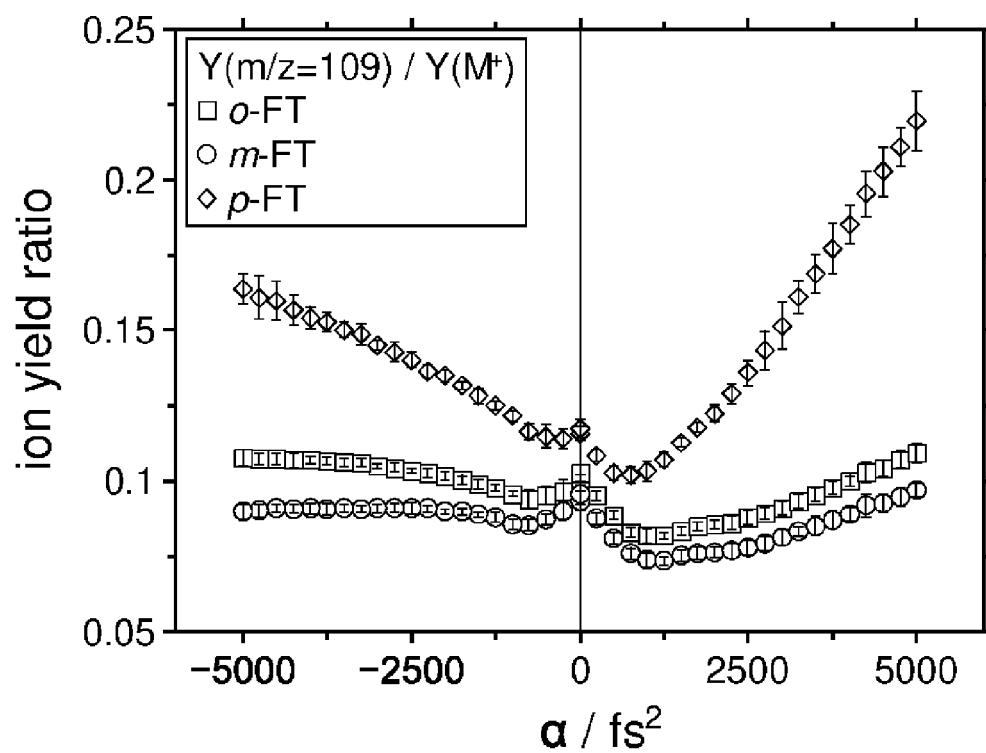


Fig. 3B

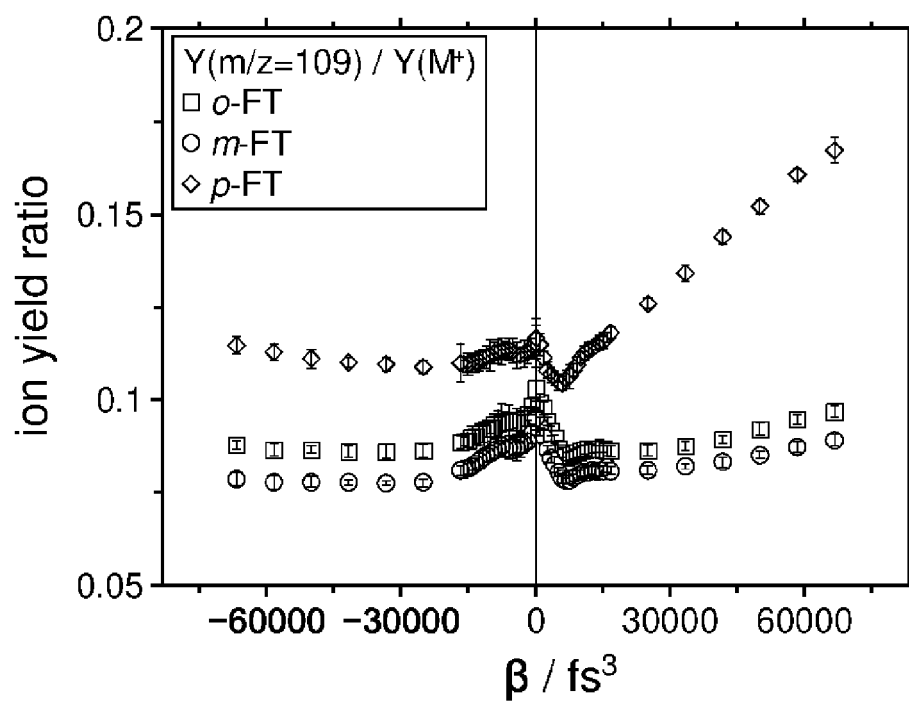


Fig. 4A

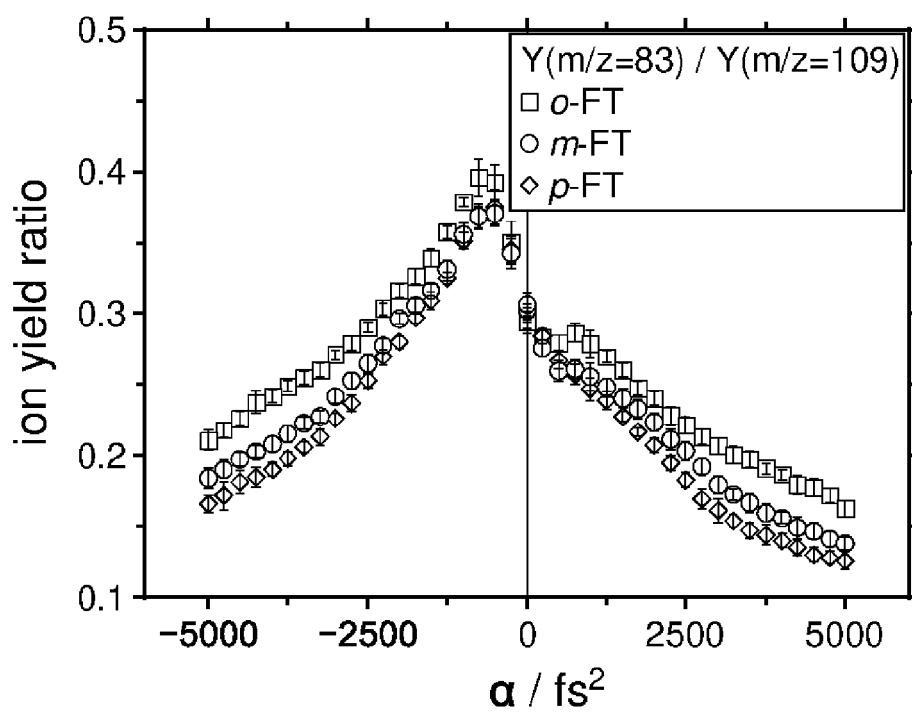


Fig. 4B

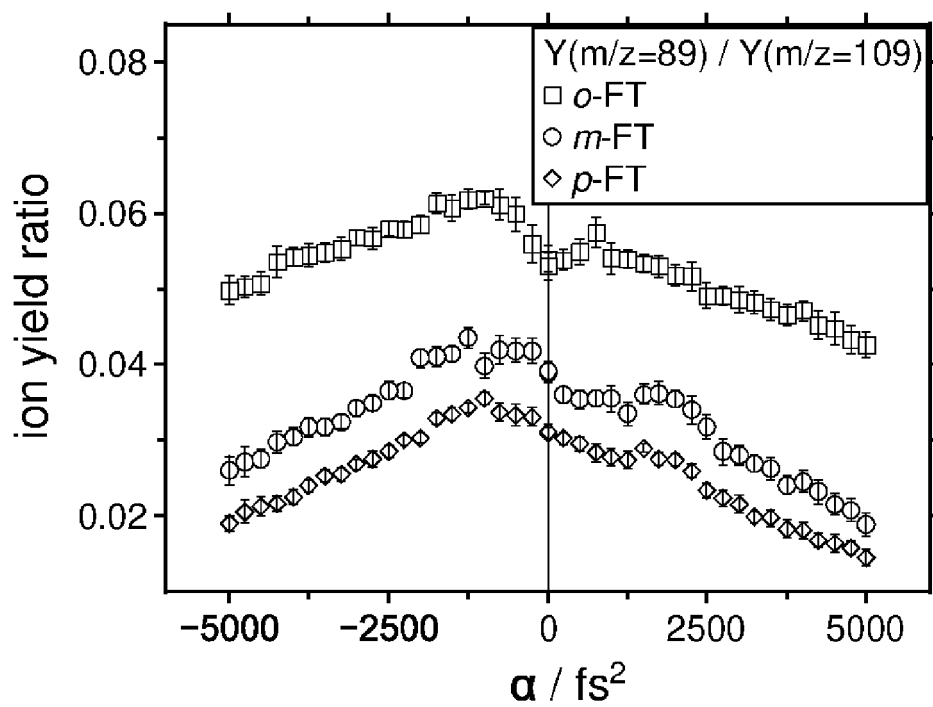


Fig. 5A

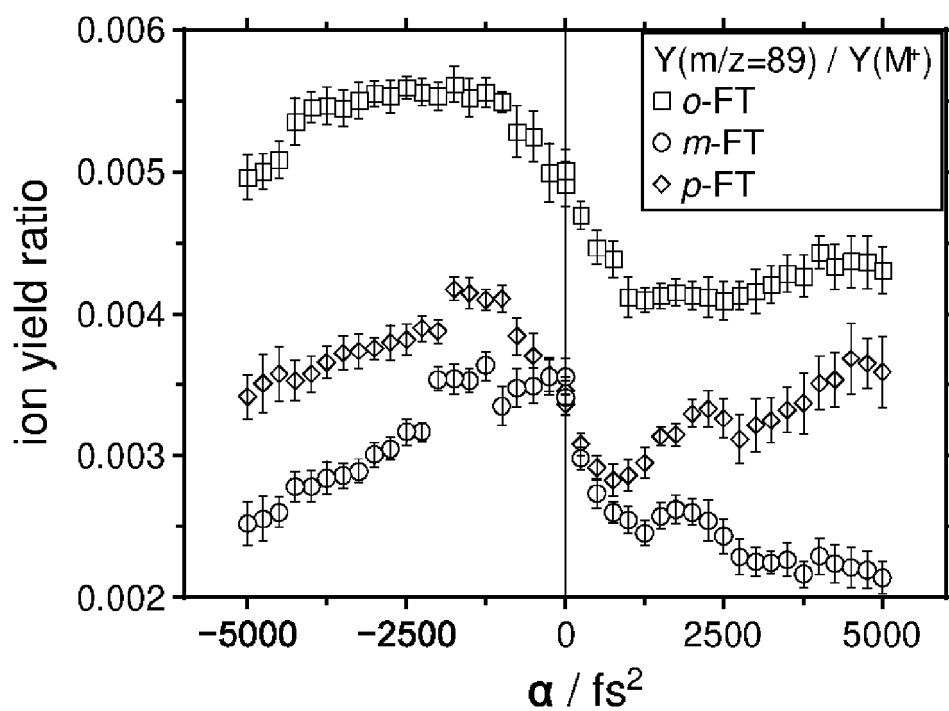


Fig. 5B

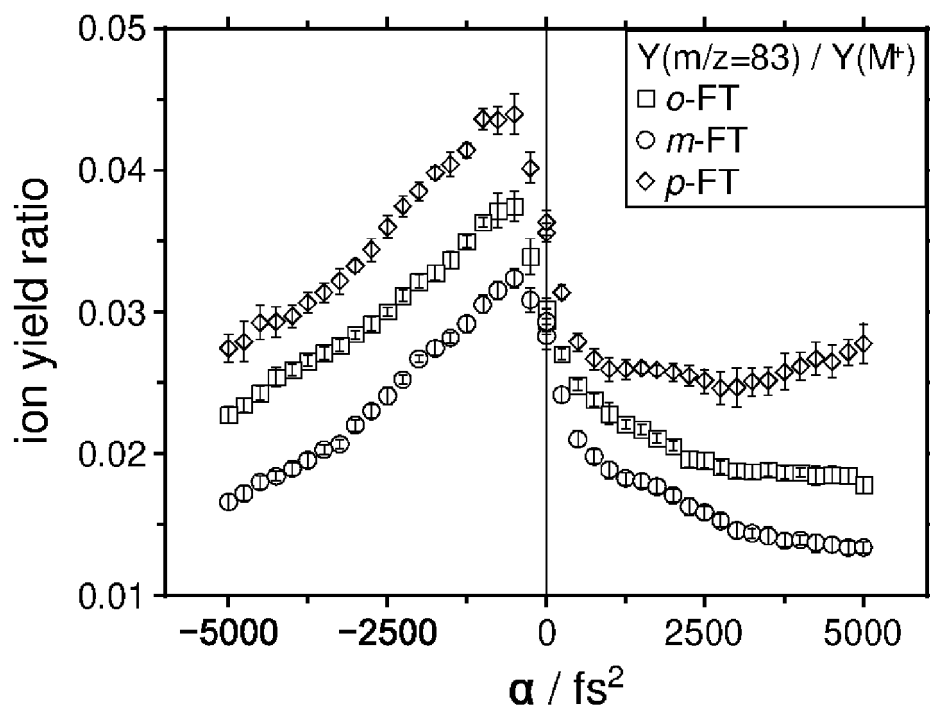


Fig. 6

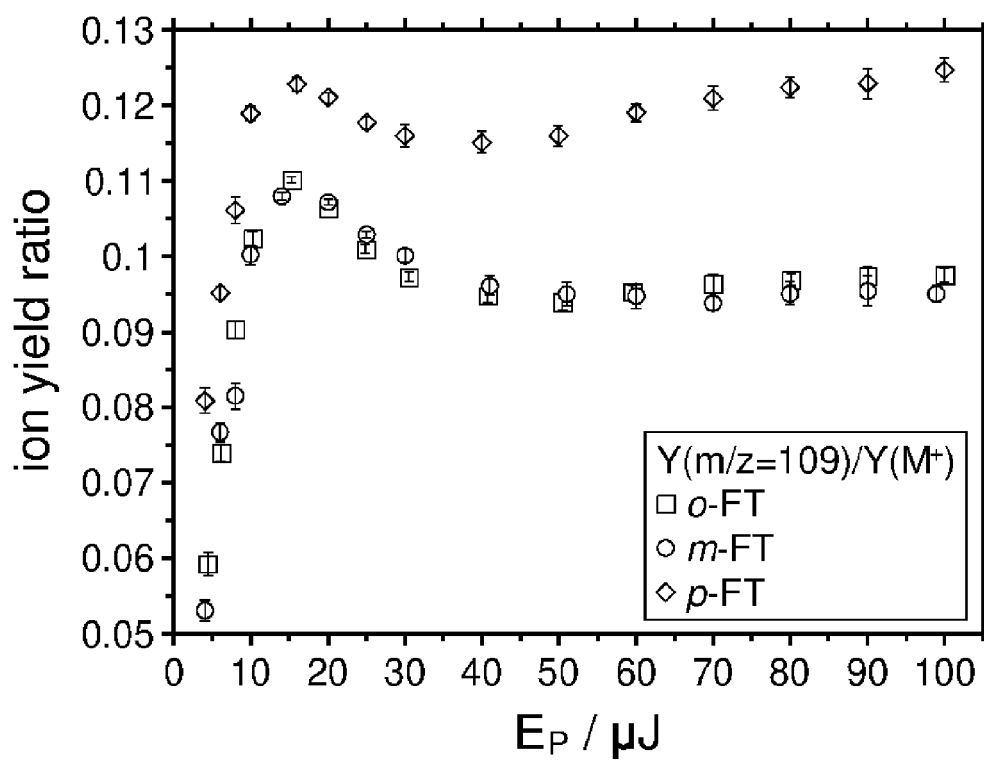


Fig. 7

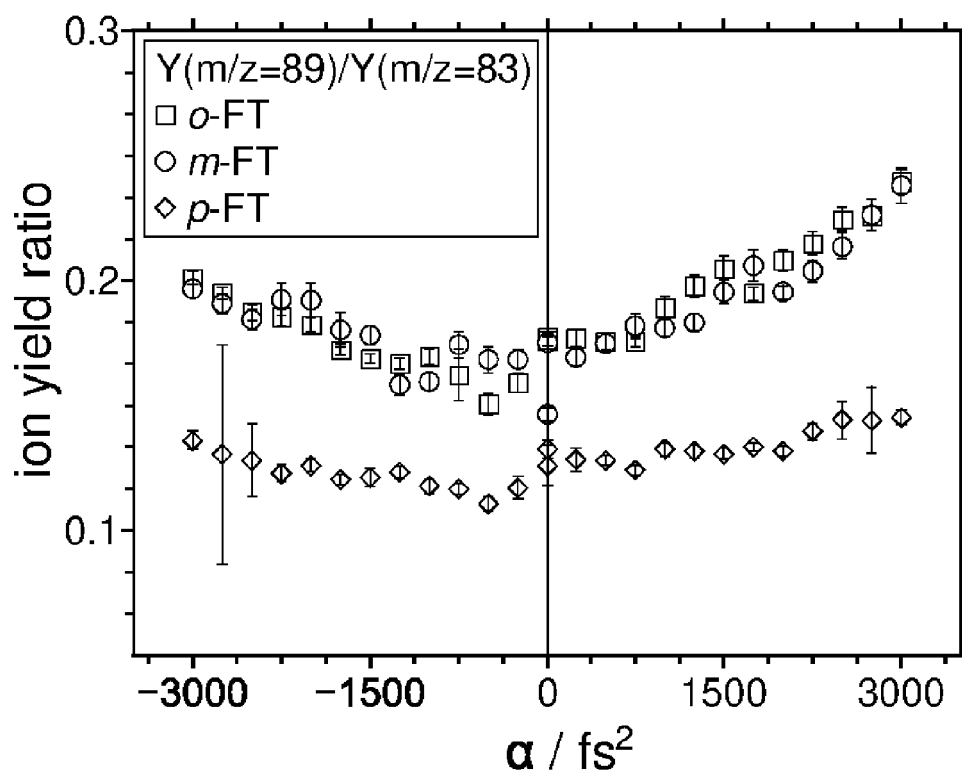
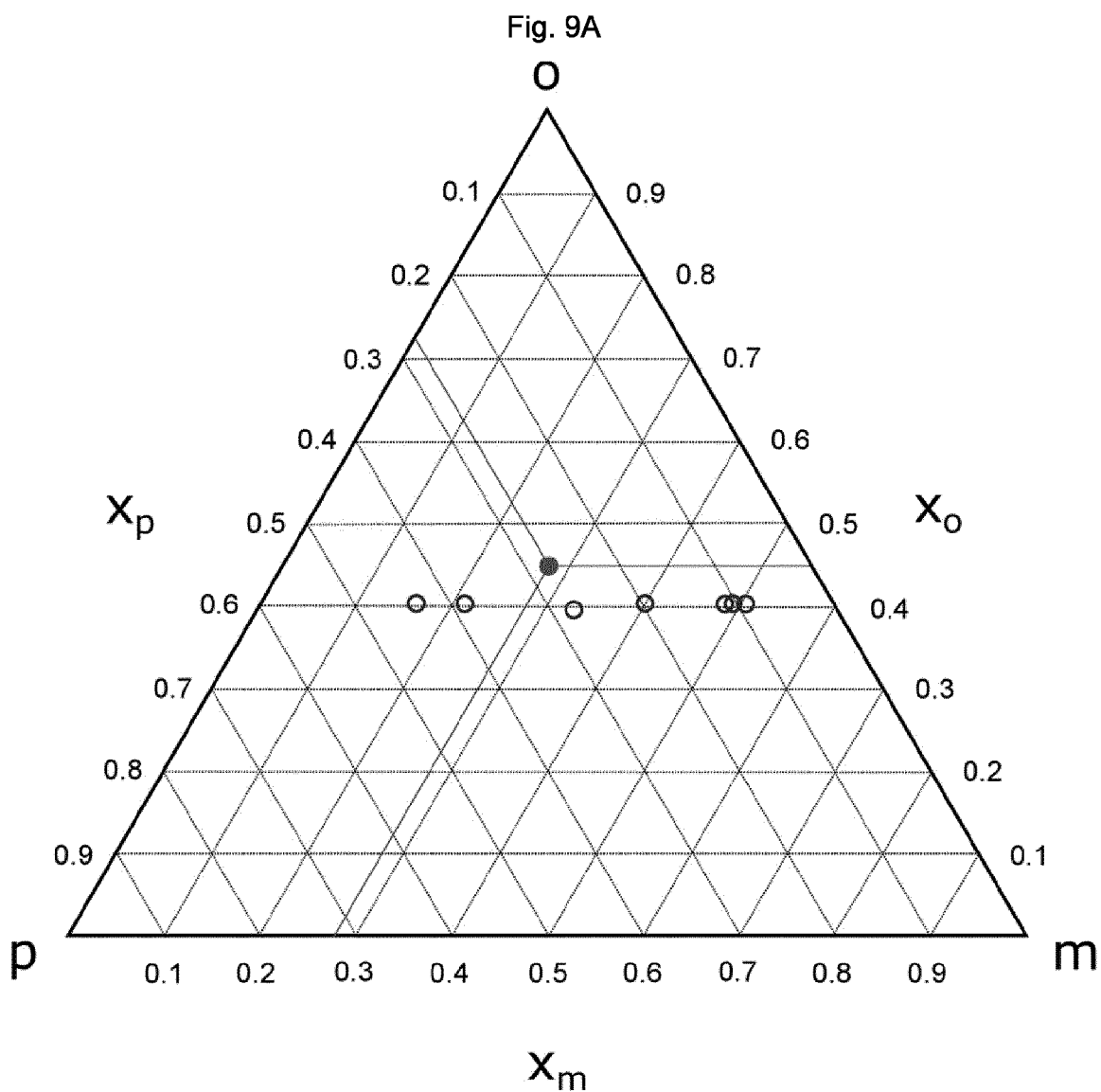


Fig. 8

Table 1:

	<i>o</i> -FT / %	<i>m</i> -FT / %	<i>p</i> -FT / %
Mixture 1: premixed composition	45	28	27
Mixture 1: experimental determination	41	33	26
Mixture 2: premixed composition	25	14	61
Mixture 2: experimental determination	20	17	63



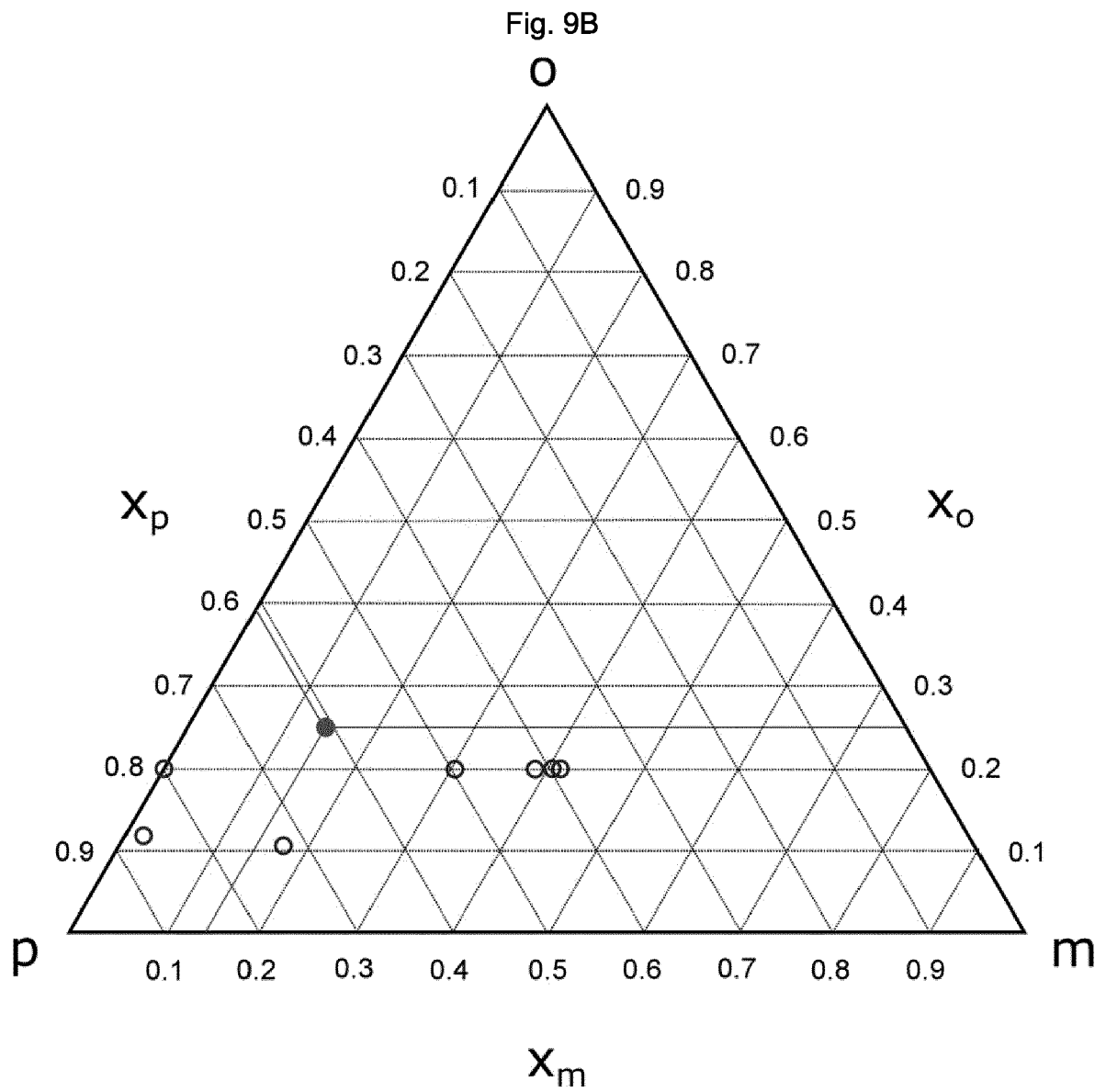


Fig. 10

Table 2

IYR	Name	α / fs ²	β / fs ³	Value mixture 1	Value mixture 2
Y(m/z=109)/Y(M ⁺)	IYR(1.Step) α_1	+1750	0	0.0895	0.09903
Y(m/z=109)/Y(M ⁺)	IYR(1.Step) α_2	-2250	0	0.098002	0.1165
Y(m/z=109)/Y(M ⁺)	IYR(1.Step) β_1	0	+25000	0.0815	0.0983
Y(m/z=109)/Y(M ⁺)	IYR(1.Step) β_2	0	-50000	0.0815	0.0932
Y(m/z=89)/Y(m/z=83)	IYR(2.Step) α_1	+1750	0	0.1744	0.1424
Y(m/z=89)/Y(m/z=83)	IYR(2.Step) α_2	-2250	0	0.1595	0.1353
Y(m/z=89)/Y(m/z=83)	IYR(2.Step) α_3	-3000	0	0.1813	0.1536

Fig. 11

Table 3

	x_o	x_m	x_p
Mixture 1: weight-in composition	0.45	0.28	0.27
Mixture 1: determined composition (full analysis)	0.41	0.33	0.26
IYR(1.Step) α_1	0.41	0.40	0.19
IYR(1.Step) α_2	0.41	0.47	0.12
IYR(1.Step) β_1	0.41	0.48	0.11
IYR(1.Step) β_2	0.41	0.51	0.08
IYR(2.Step) α_1	0.41	0.22	0.37
IYR(2.Step) α_2	0.41	0.16	0.43
IYR(2.Step) α_3	0.39	0.33	0.28

Fig. 12

Table 4

	x_o	x_m	x_p
Mixture 2: real composition	0.25	0.14	0.61
Mixture 2: determined composition	0.20	0.17	0.63
IYR(1.Step) α_1	0.20	0.41	0.39
IYR(1.Step) α_2	0.20	0.31	0.49
IYR(1.Step) β_1	0.20	0.38	0.42
IYR(1.Step) β_2	0.20	0.42	0.38
IYR(2.Step) α_1	0.12	0.02	0.86
IYR(2.Step) α_2	0.20	0.00	0.80
IYR(2.Step) α_3	0.11	0.17	0.72



EUROPEAN SEARCH REPORT

Application Number
EP 20 16 1225

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The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 31 July 2020	Examiner Peters, Volker
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

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The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of the search 31 July 2020	Examiner Peters, Volker
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
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