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Gas Chromatography

A New Dimension in Separation Science: Comprehensive Two-Dimensional Gas Chromatography

*Cornelia Meinert and Uwe J. Meierhenrich**

analytical methods · complex samples · gas chromatography · mass spectrometry

Dedicated to Professor Volker Schurig

The introduction and development of comprehensive two-dimensional gas chromatography offers greatly enhanced resolution and identification of organic analytes in complex mixtures compared to any one-dimensional separation technique. Initially promoted by the need to resolve highly complex petroleum samples, the technique's enormous separation power and enhanced ability to gather information has rapidly attracted the attention of analysts from all scientific fields. In this Minireview, we highlight the fundamental theory, recent advances, and future trends in the instrumentation and application of comprehensive two-dimensional column separation.

1. Introduction

Gas chromatography (GC) is a technique that has been used in separation science since 1951.[1] Several years later, the invention of capillary columns[2] (i.e., wall-coated open-tubular columns) allowed analytes to be highly resolved as sharp and often base-line-separated signals. Owing to this pioneering development—a quantum leap in separation science—approximately 400 000 GC instruments are currently in operation worldwide. The second quantum leap in chromatographic sciences occurred very recently, which was the introduction of multidimensional gas chromatography. By coupling two stationary phases with different selectivity in series, it becomes possible to resolve analytes that cannot be separated by conventional one-dimensional (1D) GC. Multidimensional GC separations are classified either as heart-cutting two-dimensional (2D) GC or as comprehensive two-dimensional gas chromatography (GC × GC).[3] Heart-cutting 2D GC selectively transfers a subset of analytes from a primary column (^{1}D, first dimension) to a secondary column (^{2}D, second dimension) using a valve or Deans switch device. An individual segment of the primary column effluent introduced to the secondary column is referred to as a heart-cut. Heart-cutting 2D GC is best suited for isolating and analyzing target compounds in complex mixtures and has contributed significantly to enhancing resolution in GC. In contrast, GC × GC—the most recent and most powerful two-dimensional gas chromatographic technique—passes the entire sample through both stationary phases. The key to GC × GC resides in the "modulation process": the manner in which sequential segments of the first dimension are continually transferred to the second dimension by a modulator.

Today, comprehensive two-dimensional gas chromatography is beginning to be successfully applied in advanced laboratories to detect and/or quantify trace-level constituents and contaminants in various types of samples. However, the real strength of GC × GC is in separating complex samples. The most prominent applications include the analyses of crude oils in petrochemistry,[4] soil, water, and air samples in environmental chemistry,[5] nutrient samples in food chemistry,[6] essential oils,[7] absolutes in the aroma and perfume industry,[8,9] and a wide variety of metabolites in biochemistry.[10] Coupling a GC × GC with a mass spectrometer or flame-ionization detector makes this analytical approach extremely powerful for the qualitative and quantitative determination of targeted and non-targeted substances.

This Minireview is not focused on the most recent applications of GC × GC techniques but rather highlights the state-of-the-art in comprehensive two-dimensional gas chromatography. Instrumental designs developed to realize continuous multidimensional gas chromatography are reviewed as well as recent advances and limitations of GC × GC.

[*] Dr. C. Meinert, Prof. Dr. U. J. Meierhenrich
Institut de Chimie de Nice ICN, UMR CNRS 7272, Université de Nice-Sophia Antipolis, Faculté des Sciences
Parc Valrose, 06108 Nice (France)
E-mail: uwe.meierhenrich@unice.fr
Homepage: http://www.unice.fr/meierhenrich/

2. The Fundamentals of Multidimensional Gas Chromatography

The invention of comprehensive multidimensional separation techniques is a milestone development in chromatography history. Since the first proposal of this technique by Giddings[11] in 1984, great advances have been made both in theory and practice. The first comprehensive two-dimensional gas chromatogram was recorded for an oil sample in 1991,[12] and immediately attracted attention. Complex real-life samples, which often contain several thousand components and isomers spread over a wide concentration and volatility range, are a routine challenge. The complete resolution and identification of each individual component has often been an unrealistic task, particularly using classical 1D GC. The revolutionary aspect of the new GC × GC methodology is that the entire sample is resolved on two distinct capillary columns of complementary selectivity, which results in enhanced peak resolution. The increased resolution in combination with a third selective detection dimension (e.g., mass spectrometry) currently makes the GC × GC methodology the most powerful analytical device for the analysis of volatile and semi-volatile organic compounds. Details regarding GC × GC and the applicability of GC × GC for various complex matrices have been described in depth in articles providing a background to the fundamentals of this technique.[13,14]

In ideal two-dimensional gas chromatography, the peak capacity, n_c, becomes the product of the peak capacity in each dimension as shown in Equation (1).[15]

$$n_{c,GC\times GC} = {}^1n_c\,{}^2n_c \quad (1)$$

The n_c is the maximum number of peaks a chromatographic system can resolve in an arbitrary time interval with a predetermined lowest acceptable resolution, R_S. An $R_S \geq 1.5$ is considered to be adequate for most applications, which corresponds to a standard deviation (σ) as a measure of peak width of 6σ.

2.1. Comprehensive Separation

The modulation process that connects two capillary columns containing stationary phases of different polarity is of crucial importance in GC × GC. Although this modulation process can be achieved through a variety of different approaches, the underlying principle remains basically the same: the modulator, placed between the two columns, ensures that (ideally) all of the effluent from the first column is periodically trapped and re-injected as very sharp bands onto the secondary column. Peaks eluting from the first dimension column are sliced into several segments, each of which is eluted through the second dimension column. In some systems, the second column is housed in a separate oven to allow more flexible and independent control of the temperature. Finally, enhanced resolution is achieved because compounds undergo two independent separations, which ensures that peak overlap arising from equivalent elution times on both columns is less likely.

The time required for one modulation cycle is defined as the modulation period (P_M) and is generally 2–8 s. Modulation has to be faster than the peak elution width at baseline on the ^{1}D column, so that multiple second dimension subpeaks (slices) are obtained and the peak resolution in ^{1}D is not degraded. Effective comprehensive GC × GC performance is achieved with at least three or four modulations per 1D peak.[13] Based on the modulation period, the time/response data stream is converted into a two-dimensional retention plane (contour plot) spanned by the two retention time axes (1D time × 2D time).[12] The effect of this continuous process is shown in Figure 1b.

Since GC × GC relies upon fast analysis of accumulated sub-peaks, it is usual to apply a short (1–2 m) ^{2}D column of narrow inner diameter (I.D.; normally 0.1 mm) with a relatively thin film thickness to achieve complete elution of pulsed sub-peaks in the second dimension, while the ^{1}D column is a conventional capillary column (length approximately 25 m) to give a normal GC elution. Ideally, analytes elute from the second column within the time frame of the modulation period, thereby preventing any potential overlap with peaks from a subsequent modulation event, which is a phenomenon called wrap-around. Wrap-around signals are usually avoided; however, can be accepted if compounds showing wrap-around do not co-elute or interfere with solutes from the next modulation.

As visualized in Figure 1, the effects from the modulator compress the analytes before they are released into the secondary column, that is, they have a much smaller width at base. The focusing effect combined with minimal band

Uwe J. Meierhenrich studied chemistry at the Philipps University of Marburg. After completing his Ph.D. at the University of Bremen with Prof. Wolfram H.-P. Thiemann, he identified amino acids in artificial comets at the Max Planck Institute for Solar System Research in Katlenburg-Lindau and at C.B.M. in Orléans in preparation for the Rosetta comet mission. In 2005, he became a full Professor at the University of Nice-Sophia Antipolis. He was awarded the Horst Pracejus Prize from the GDCh in 2011 for his work on chirality and enantioselective chromatography.

Cornelia Meinert studied chemistry at the Universities of Rostock and Leipzig. She received her Ph.D. on characterizing complex environmental mixtures using effect-directed analysis and preparative capillary GC at the Helmholtz Centre for Environmental Research in Leipzig. In 2009, she became a postdoctoral research fellow in the Meierhenrich group at the University of Nice. Her current research focuses on the origin of biomolecular asymmetry, especially enantiomer separation using GCxGC techniques.

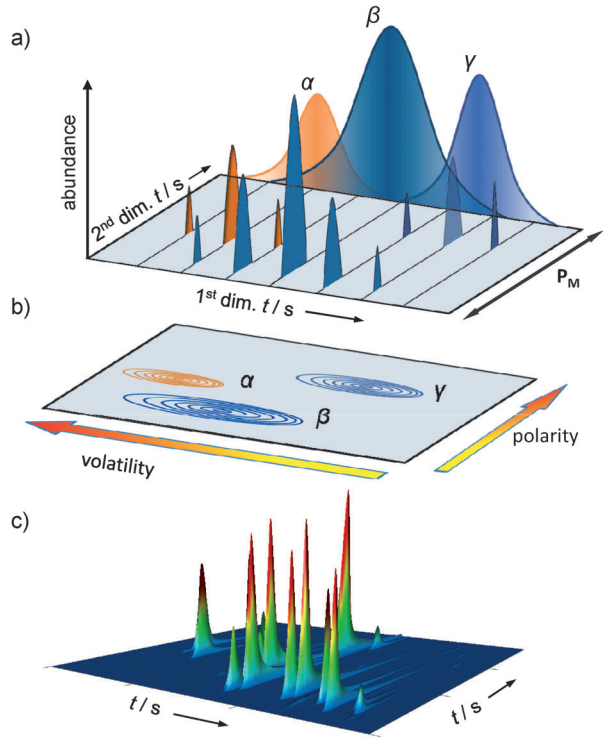

Figure 1. Illustration of a GC×GC chromatogram. a) Chromatographic peaks (α, β, and γ) eluted from a typical apolar ^{1}D column sequentially sliced into distinct fractions during a defined modulation period (P_M). Non-resolved analytes are often better resolved on a (generally polar) short micro-bore ^{2}D column. b) The data stream from the detector is then plotted based on the modulation in a 2D contour color plot format or c) directly showing signal intensities in 3D presentation as conical peaks.

broadening on the short secondary column results in narrow peaks with enhanced peak height and thus in improved detection limits.[16,17] Typical enhancements of the signal-to-noise ratio are on the order of 5–10-times depending on the detector acquisition rate, modulator system, secondary column characteristics (length, internal diameter, and stationary phase), gas velocity, and temperature program. Other major benefits in trace analysis of 2D GC over 1D GC are structured chromatograms, which reduce the possibility of interference through peak overlap and/or co-extracted matrix impurities. Additionally, the noise is minimal so a true surface area for peak integration is available.

2.2. Structured Separation Space in GC×GC Chromatograms

Apart from superior resolution of an analyte from chromatographic noise and co-eluting compounds, a comprehensive GC×GC analysis reveals the structural properties of analytes through the clustering of structurally related homologues, congeners, and isomers in the 2D plot.[18,19] Figure 2 highlights the important features of the 2D chromatogram and the following chemical trends can be determined to a further extent: well-resolved regions of saturated, cyclic-saturated, olefinic, heteroatomic, and aromatic compounds. Straight-chain and branched hydrocarbons are separated by retention time along the lower part of the second dimension, whereas the two- and three-ring aromatics (e.g., phenanthrene, biphenyl, dibenzothiophene, dibenzofuran, and the alkylated isomeric forms of each) can be observed at the top of the chromatogram as a result of their stronger interaction and higher relative retention on the secondary polar column. Within the group of geometrical isomers, patterns of elution of the different alkylated species can be determined. Generally, group-type separation can be helpful in the identification of unknowns and, in some cases, preferred over real 2D separation, in which the overall separation of adjacent peaks is the major goal.

In GC×GC, several parameters have a profound influence on the overall separation efficiency and have to be optimized before analytical performance. Dallüge et al. and Ong et al.[20] published optimization procedures and Beens et al.[21,22] developed programs that can predict the outcome of GC×GC separation based on the thermodynamics of the separation. Further studies have used computer modeling to predict and optimize the separation space in GC×GC, based on the enthalpy (ΔH_i) and entropy (ΔS_i) from experimentally determined retention times of target compounds.[23] However, the combined increase in experimental parameters—the relationship and interplay—causes the determination of optimal analytical settings for GC×GC to be difficult.[24] In addition to optimal modulation settings, the primary operational parameters to be considered are the chemistry of the stationary phases (type, film thickness), column dimensions (length, diameter), gas flow rate, outlet pressure conditions, temperature regime for both columns, and the detector settings. For example, suboptimal column selection can result in a loss of selectivity and overall efficiency of the separation process. Classically, the first dimension column has a non-polar stationary phase to separate a wide range of compounds in various matrices based on the partition coefficients between the mobile and stationary phases of analytes. The second column is usually polar and short to give fast separation in the second dimension based on different polarity interactions (i.e., dipole–dipole, hydrogen-bonding, and polarizability effects).

A separation is considered truly orthogonal if cross information (or synentropy) across both dimensions is zero[25] (i.e., the retention of molecules on each column must be independent). Minimizing synentropy is important in multi-dimensional separations because increased synentropy leads to an increased part of the separation space that is inaccessible. Sample constituents tend to cluster along a diagonal in the 2D retention time plane. By minimizing any correlation between the selectivities of the two stationary phases, the efficiency of GC×GC separations can be enhanced. Therefore, an increased percentage of separation-space usage is gained. The maximization of orthogonality of different stationary-phase combinations has been described using constants of a solvation parameter model, such as the Abraham model.[26] A separation-space evaluation method based on Denaulay's triangulation algorithms was proposed by Semard et al.,[27] and then further used to optimize the selection of column sets, geometric parameters of the secondary column,

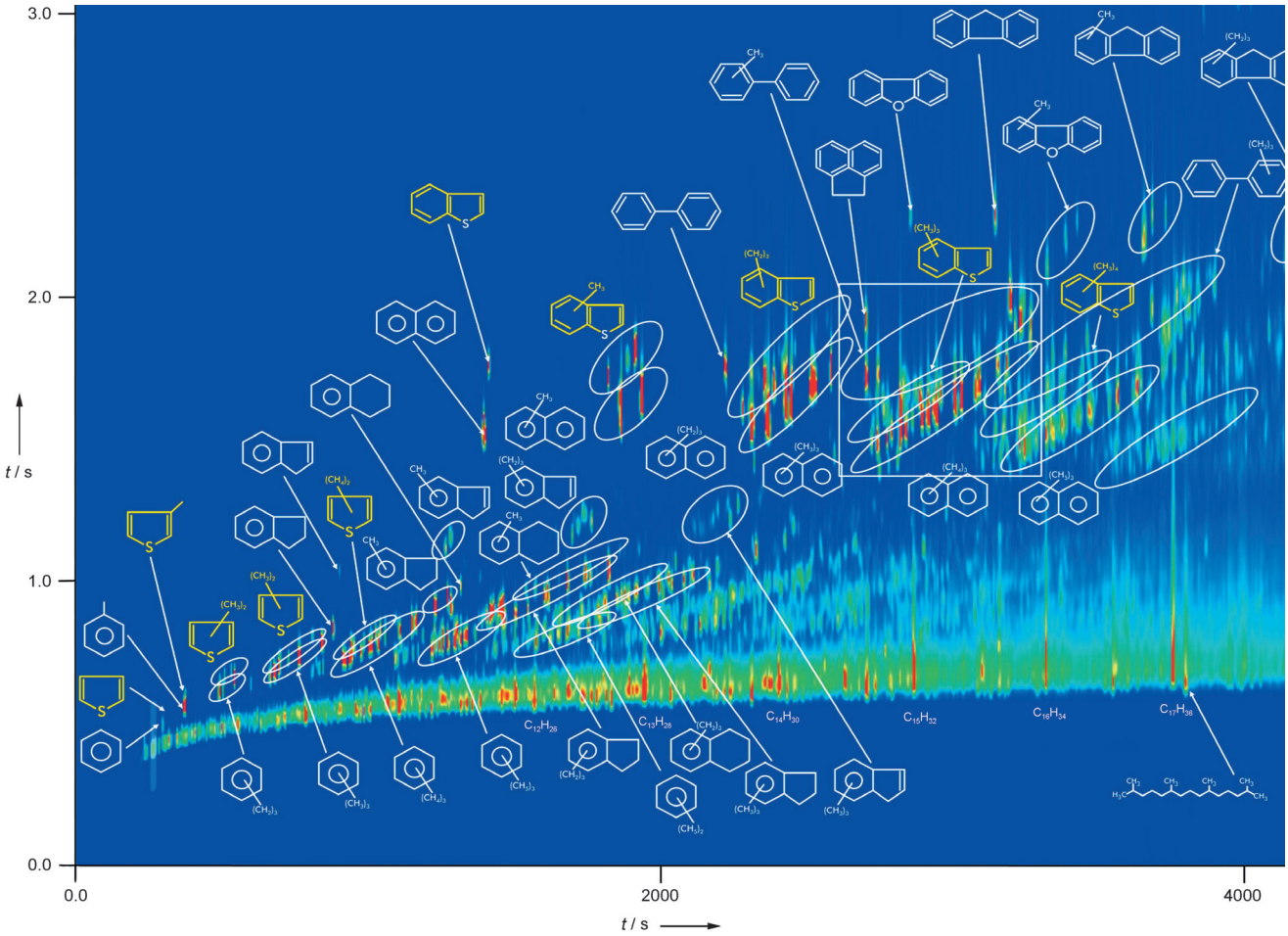

Figure 2. Section of a GC×GC-ToF-MS chromatogram of a diesel fuel using a non-polar×polar column set. Isomers line up as bands in the 2D chromatogram and congener groups or homologues appear as separate bands. Analytes at the lower separation space of the GC×GC chromatogram express less-polar interactions with the ^{2}D column. Image used with permission from LECO Corporation. Similar chromatograms can be obtained by the use of chromatographic GC×GC systems supplied by Agilent, Shimadzu or Thermo Scientific.

and other experimental conditions (e.g., gas flow and temperature).

Even though non-polar×polar column combinations are often stated as the preferred approach for GC×GC, non-classical conditions, that is, polar×moderately non-polar column combinations[28] as well as liquid crystalline,[29] ionic liquid,[30] and enantioselective stationary phases,[31–34] are gaining attention and can lead to high-resolution and group-type separations. Moreover, Omais and co-workers[35] recently suggested that the non-polar×polar column combination is not a necessary condition to achieve large peak distribution in the 2D plane, and non-classical conditions can occasionally provide a large occupation of 2D space. Thus, there are no fixed rules for the combination of column-phase types. The final decision regarding column selection will depend on the sample's composition and be based on fundamental considerations, such as "has an acceptable separation been achieved?"

3. Milestones in the GC×GC Techniques

3.1. Cutting the First Dimension Chromatogram into Fine Slices: Pneumatic versus Thermal Modulation

Various types of modulators have been designed and have demonstrated their suitability for GC×GC measurements. In principle, there are two approaches to achieve modulation: pneumatic[36] and thermal.[12,37–40] Generally thermal modulation provides a greater degree of sensitivity enhancement. Mondello et al.[41] recently listed all the GC×GC modulators developed and included the main characteristics, so our Minireview focuses only on the most significant devices and developments.

Pioneering work by Liu and Phillips[12] used an on-column two-stage thermal-desorption modulator, which was heated by a resistive film painted onto the capillary surface and cooled by ambient air. However, this modulator was difficult to operate and only had a short lifetime. The first reliable heated modulator, which was also the first to be commercialized, was developed in 1999 by Phillips et al.[37] The rotating

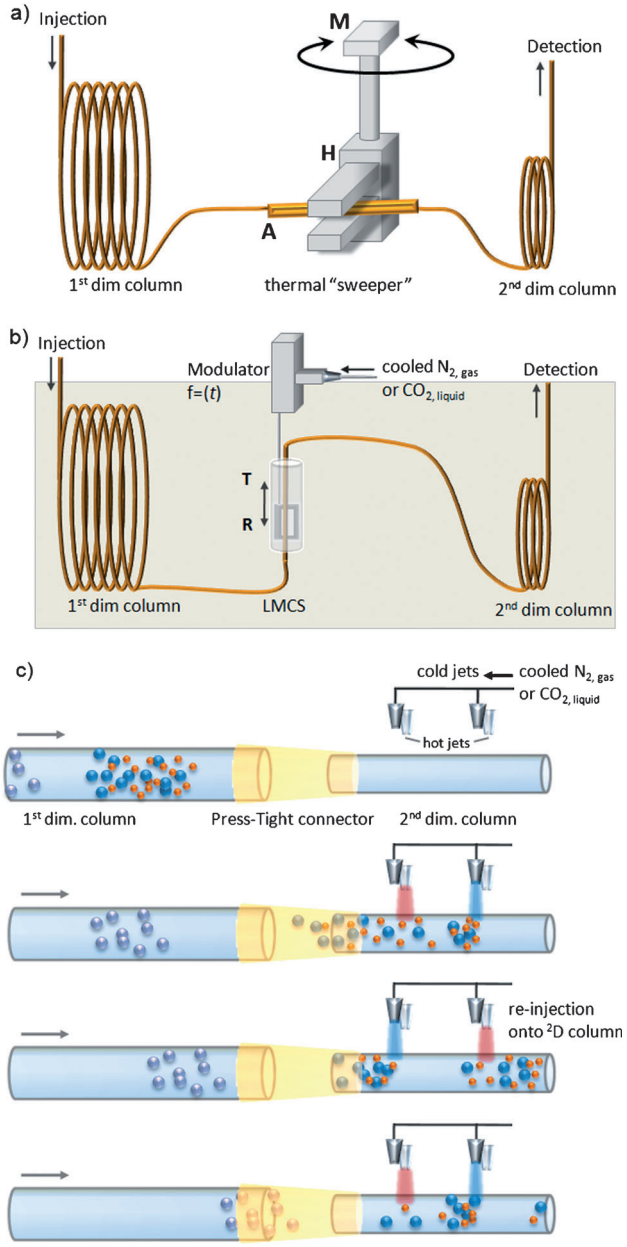

Figure 3. Illustration of the operational principles of various types of modulators. a) The thermal sweeper system (column A), placed between the first and second dimension, accumulates solutes from the ^{1}D until the slotted heater (H), rotated by the modulator (M), focuses and finally expels the solute onto the secondary column. b) Longitudinally modulated cryogenic system (LMCS). At T, LMCS is in the trapping position, and R is the release position when the LMCS is moved toward the injection direction to expose the trapped solute band to oven temperature. c) Cryogenic jet-based modulator. A cold-jet (depicted on the right-hand side) traps analytes eluting from ^{1}D. Subsequently a hot-jet switches on, the trapped solute band heats up rapidly and the analytes are released into ^{2}D while the left-hand side cold-jet is switched on to prevent sample breakthrough of ^{1}D; after the trap-release cycle the next modulation is started.

thermal modulator (thermal sweeper), shown in Figure 3a, was able to trap and re-inject a sample using a slotted heater connected perpendicular to a rotating shaft that periodically passed along a segment of a thick-film chromatographic column, which acted as a trap. The major drawback of the thermal sweeper is that the operation temperature of the oven must be approximately 100 °C lower than the maximum allowed temperature of the stationary phase in the modulation capillary. Today, the thermal sweeper and related modulation techniques are no longer used.

More efficient modulators using cryogenically cooled liquids, such as carbon dioxide, nitrogen, or air were introduced in the late 1990s. The first cryogenic modulator was described by Kinghorn and Marriott.[38] The longitudinally modulated cryogenic system (LMCS) uses expanding liquid carbon dioxide to cryogenically trap and focus the analytes in the first centimeters of the second column (Figure 3b). Re-injection is achieved by moving the modulator longitudinally away and heating the trapped fraction by means of the ambient oven air. Several types of contemporary cryogenic jet-based modulators[39] (Figure 3c) rely on carbon dioxide or liquid nitrogen for cooling conventional bench-scale GC × GC systems without any moving parts.

The principle parameter of the second class of modulators (valve-based modulators) relies on flow switching. A segment of the migrating peak is sampled, and upon switching the valve, pushed with high gas flow into ^{2}D in a differential flow arrangement. Valve-based modulators are very simple in design and were first introduced by Synovec and co-workers in 1998.[36a] However, early valve-based modulators send only a part of the effluent from the first column to the second column through. Therefore, use of the valve-based modulation technique was limited to relatively concentrated samples. The latest developments in valve/flow modulators, such as differential-flow modulation using a microfluidic Deans switch,[42] valve-switching modulation,[43] pulsed-flow modulation,[44] and capillary-flow technology, have allowed valve/flow modulators to become competitive with thermal modulators.[45] Thermal modulation mostly provides better resolution and is less restricted in column/flow combinations; however, a major drawback is the availability and relatively large consumption of liquid nitrogen or carbon dioxide. Novel differential flow modulation techniques are effective over the entire volatility range without requiring adjustments and cryogens. In addition, they allow portable and on-line process instrumentation to be possible.

3.2. Data Handling by Adequate Software

In the early history of GC × GC, advanced data processing methods were absent. The first automated data-handling-plus-interpretation results of one 2D-chromatogram required approximately 7 h.[46] The huge amount of information generated in comprehensive GC × GC applications causes the corresponding difficulties in data-handling to become immediately evident. The development of software algorithms that integrate all 2D peaks and subsequently identify and summarize the peak areas of each compound after modulation have contributed greatly to the increased use and availability of GC × GC systems. In a recent Review, Zeng et al.[47] detailed how state-of-the-art chemometric techniques aid analysts in data interpretation and analysis of GC × GC

investigations. This Review should be consulted for further details on multivariate statistics and software tools because they will not be presented herein.

A number of commercially available software packages that implement chemometric methods are currently available and continue to evolve: ChromaTOF, which was optimized for the Pegasus 4D system (LECO, USA), HyperChrom (Thermo Fisher Scientific, USA), Transform (ITT Visual Information Solutions, USA), GC Image (GC Image, Lincoln, USA), ChromaSquare (Shimadzu), and other software packages.[48] These systems can be employed for data acquisition, visualization, peak detection, discrimination, quantification, deconvolution, clustering and classification based on pattern recognition, calibration, mass-spectral similarity matching, result reporting, and other functions. For future advancements, novel and specialized in-house chemometric techniques currently being developed must be implemented in routine laboratory analyses.

3.3. Detecting GC × GC Signals with a Sufficient Acquisition Rate

As mentioned above, every modulation period produces fast secondary column chromatograms. This period can be as rapid as 50 ms for the peak width at baseline, so a fast scanning detector with an acquisition rate of at least 100 Hz is considered necessary for adequate quantification purposes. The flame-ionization detector (FID), which has negligible internal volume, can acquire data at frequencies of up to 300 Hz. Thus, FID is generally the high-quality workhorse for quantification and for most fingerprinting studies of complex samples. However, the considerable increase in the application of GC × GC in academic and industrial areas is attributed to the introduction of GC × GC setups featuring mass spectrometry (MS). GC × GCs coupled with MS systems benefit from having a third degree of separation in the unique m/z spectra of different compounds. Additionally, the fragmentation pattern in mass spectrometry acts as a molecule-specific fingerprint. The complementary nature of both principles is the reason for the strengths of GC × GC-MS.

Almost all GC × GC-MS applications have been performed using time-of-flight (ToF) or quadrupole mass analysis. The high data acquisition of ToF-MS instruments (up to 200 Hz) combined with improved mass spectral deconvolution software allows clean, unambiguous spectra to be produced, even for partially co-eluting compounds. Recently introduced rapid-scanning quadrupole mass spectrometers (qMS)[49] further increased the application of GC × GC. Even with limited mass ranges of 100–200 Da because of the limited acquisition rate (by the necessity to scan individual ions from each mass in the scan range) and the interscan duty cycle that restricts spectral quality and quantitative analysis, qMS systems are extremely popular and considerably less expensive than ToF-MS instruments. If trace amounts of compounds need to be adequately assigned, then ToF-MS provides better quality spectra than qMS, particularly for low-abundance first and last sub-peaks.[50] In both detection devices, the use of soft chemical ionization techniques (instead of conventional electron ionization) is mandatory if organohalogens have to be analyzed. In 2005, Korytár et al.[51] were the first to use a rapid-scanning qMS instrument in the electron-capture negative ion (ECNI) mode in a GC × GC experiment. The performance of the MS instrument was investigated and it was found to be capable of producing 23 spectra over a 300 Da mass range. Shortly after, the same research group introduced a ToF-MS system operating in the ECNI mode.[52] The ECNI-ToF-MS instrument used methane as the reagent gas, and data were recorded over a 50 to 700 Da range with a 40 Hz acquisition rate.

In addition, recent studies have used GC × GC in combination with combustion-isotope ratio mass spectrometry[53] and isotope-dilution ToF-MS.[54] A major benefit of the isotope-dilution detection method is the use of isotopically labeled internal standards for accurate quantification. Worthy of note is that a few high-resolution ToF applications have also been described in the GC × GC literature with mass resolution ($m/\Delta m$) up to 5000 full-width at half maximum (fwhm).[55] However, GC × GC-MS analysts normally look for unit-mass resolution to generate spectra suitable for MS library matching. Quadrupole MS and low-resolution ToF-MS analyzers are adequate for such a scope.

Other hybrid techniques, such as GC × GC in combination with fast, sensitive, and element-selective detectors can offer additional opportunities. Such techniques are usually limited to cases where the sensitivity and selectivity of mass spectrometers are inadequate. For instance, micro electron-capture detectors (μECD), typically with a 50 Hz sampling rate, have been reported as a useful alternative, specifically for the quantitative analysis and low-cost screening of polyhalogenated target compounds, such as polychlorinated paraffins, polychlorinated dibenzodioxins and dibenzofurans (PCDD/FS), and organochlorine pesticides.[18,29,56] Studies employing comprehensive GC × GC systems coupled to nitrogen phosphorus detection (GC × GC-NPD) demonstrated the potential and high sensitivity of NPD for the detection of nitrogen-containing substances in complex food matrices.[57] Furthermore, a comprehensive GC × GC dual-detection system coupled to ECD and NPD (GC × GC-ECD/NPD) for the simultaneous detection of halogenated and N-containing compounds has been developed for multiclass pesticide analysis.[58] Limited applications were reported for the combination of GC × GC with atomic emission detection,[59] sulfur[60] and nitrogen chemiluminescence detection,[61] and olfactory detection.[62] Olfactory (O) detection aims to identify odorous compounds that are the greatest contributors to the overall odor of an extract in the field of flavor and fragrances. The time between the reaction and the brain to perceive the reaction, makes characterization with GC × GC-O extremely difficult in terms of the short time frame provided by the fast and narrow peaks eluting after the GC × GC separation. Slower temperature programs could potentially overcome this problem; however, an increase in analytical run-time will be time-consuming and impracticable for the operators.

3.4. Enantioselective GC×GC using Chiral Stationary Phases

Another important instrumental design of GC×GC is enantioselective separation using dilute cyclodextrin derivatives[63] or Chirasil-Val[64] stationary phases as chiral selectors. Interest in enantiomeric separation by gas chromatography has continually increased with the aim of gaining better understandings of the differences in the biological properties (e.g., different odor structure–activity relationships), pharmacological effects, chiral recognition in biosynthetic pathways, and the determination of the authenticity, quality, and geographic origin of a sample. As the insight into chirality–activity relationships steadily improves and legislation of chiral compounds becomes more stringent, the development of reliable GC×GC methods for the quantification of the enantiomeric excess ($ee = 100\ (R-S)/(R+S)$) in complex biological and synthetic samples is desirable. Both enantioselective × moderately polar/polar[31] and non-polar × enantioselective[32] column combinations have been reported. Because the resolution of enantiomers by GC on enantioselective stationary phases is based on fast kinetics and is governed by thermodynamics,[65] the enantioselective × polar column combination is preferred for the efficient separation of enantiomers. If structured chromatograms are required, then the non-polar × enantioselective column combination generally gives better results. The challenge for achieving fast chiral analysis in the second dimension in GC×GC was addressed by Shellie and Marriott.[31]

The precise determination of *ee* values is also of paramount interest in the search for the origin of life's homochirality. Very recently, enantioselective GC×GC-ToF-MS analysis enabled the separation of chiral compounds, including amino acids, in simulated cosmic ices.[33] In a complementary experiment using the same analytical approach, statistically significant *ee* values were determined in the amino acid alanine (Figure 4).[34] The high chromatographic resolution of the analytes from interfering ^{1}D co-elution resulted a true surface area for peak integration that enabled the determination of *ee* values with high precision.

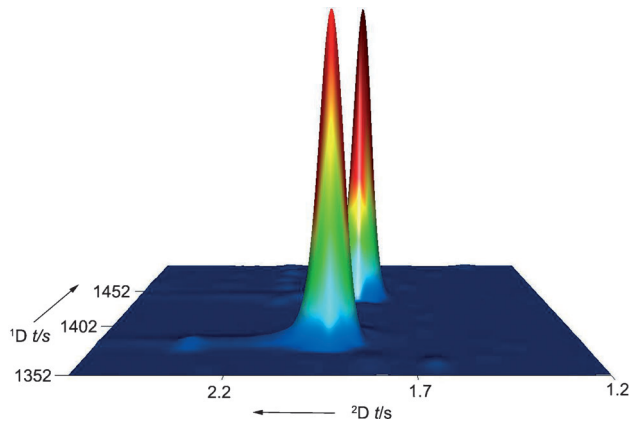

Figure 4. An enantioselective GC×GC-ToF-MS chromatogram depicting the resolution of a racemate of L-alanine (left signal) and D-alanine (right signal) as *N*-ethoxycarbonyl heptafluorobutanyl ester derivatives on 25 m Chirasil-D-Val coupled to a 1.4 m Carbowax phase. 2D separation results in a flat background around resolved enantiomers enabling the precise determination of enantiomeric ratios. Each point in the three-dimensional separation space has its individual mass spectrum. Mass spectra for both enantiomers are identical.

4. GC×GC in 2012: Where Are We Today and What is to Come?

Interestingly, the main potential of GC×GC, the multiplicity of chromatographic peak capacity, which is often claimed in the literature as "superior resolution", "unrivaled peak capacity", "exceptional separation power," and so forth, is not achieved in current GC×GC applications. Although a high number of entirely or partially resolved peaks in complex mixtures can be resolved, unresolved peaks are still present, as shown in Figure 2. Even though there is twice as much space available, the overall resolving power of GC×GC cannot be achieved in practice because component peaks (m) are not arranged in a regular fashion to minimize coincidental component overlap but randomly distributed in a chromatogram. By way of example, to resolve only 82% of random component peaks, n_c must exceed m by a factor of 10, requiring a number of N theoretical plates of 4×10^6.[15b] Therefore, even comprehensive GC×GC appears to fail to meet the enormous peak capacity requirements for such applications. Note, however, that optimized GC×GC analysis offers superior resolution compared to 1D GC even if there is still scope for improvements. Blumberg et al.[66] indicated that essentially every GC×GC report is on non-optimized systems. The substantial gap between actual and theoretically possible separation performance in comprehensive 2D GC is mainly caused by modulation pulses that are too wide, insufficient separation along the first dimension,[66] and suboptimal flow-rate regimes.[27,67] For these reasons, the theoretically $^1n_c{}^2n_c$ peak capacity value is overestimating the real value.

4.1. Qualitative and Quantitative Analysis using GC×GC

GC×GC produces better separation of particular peaks and groups of analytes in 2D plots; however, structural elucidation by GC×GC is challenging and not as straightforward as 1D GC. Owing to the lack of easy-to-use retention indices for both dimensions, insufficient spectral libraries, and the increased complexity of compound analysis through the generation of a greater number of possibilities, identification of all the resolved peaks with GC×GC remains difficult. The confirmation of structures using computer tools based on substructure identification combined with structure generation[68] can provide additional evidence for the identification of unknowns; however, computer-based tools have been rarely employed. Moreover, retention indices are important tools to minimize misidentification and play an important role in GC analysis. Although mathematical[69] and experimental[21,70] approaches have been reported that use retention information in 2D GC, user-friendly methods and databases of retention indices in both dimensions of GC×GC are still limited.

Some research focused on the correlation of retention indices with molecular structural features to estimate physico-chemical properties of GC × GC-resolved compounds, such as volatility, aqueous solubility, and the partitioning of octanol and water.[71] Recently, Seeley et al.[72] used a solvation-parameter model combined with physico-chemical compound descriptors and stationary-phase information[26] to predict the retention behavior of a diverse set of solutes.

While qualitative analysis has been of major interest in most GC × GC applications, there has been much less focus on quantitative analysis. Fast acquisition rates of 50 Hz or higher are needed to record at least 10 data points to properly and quantitatively describe the narrow, modulated GC × GC peaks.[21] Adequate quantitative analysis and calibration can be accomplished by selecting the major modulated peaks for solutes and internal standards.[73] One advantage of using the two or three most intense sub-peaks is the approximate independence from the exact timing of the modulation and, consequently, on the in-phase and out-of-phase modulation.

4.2. Miniaturization of GC × GC Devices

There is currently an increasing interest in developing faster methods of GC analyses by employing shorter, narrow-bore columns, faster temperature program rates, and higher carrier-gas velocities.[74] Optimizing method translation from "normal" to fast chromatographic conditions is always a compromise between speed, resolution, and peak capacity.[75] A challenge within the demand of higher sample throughput using fast GC × GC is to simultaneously allow the resolution of all the sampled peaks and preserve the system's peak capacity.

The first application of fast GC × GC was reported for suspected allergens (commonly present in perfume-type samples) and satisfactorily demonstrated full separation in approximately 5 min using a 5 m × 0.1 mm inner diameter ^{1}D column serially coupled to a ^{2}D column of 0.3 m × 0.05 mm I.D. providing improved lateral heat and mass transfer.[76] While conventional analysis of complex perfume-type samples requires approximately 75 min, fast separation with peak widths at half-height of approximately 25–50 ms resulted in the analysis time being reduced to a few minutes because of the introduction of shorter and smaller I.D. columns in both dimensions and increased temperature-program rates when cryogenic modulation was used.

Another interesting research direction is the miniaturization of GC × GC systems, which use micro-fabricated columns and miniaturized modulators. Recently, the use of 3 m long, 150 μm wide, and 240 μm deep columns that were etched onto a 3.2 cm × 3.2 cm silicon chip was reported, in which each column had 5000–6000 theoretical plates.[77] Two of the columns were coated with different stationary phases and were connected with a stop-flow valve, which further enhanced the resolution of adjacent peaks. In 2010, Reidy et al.[78] described the first microfabricated thermally modulated GC × GC system. A mixture of alkanes and alcohols was successfully resolved using 6 m and 0.25 m long microcolumns with first-dimension cross sections of 158 μm × 240 μm and second-dimension cross sections of 46 μm × 170 mm connected by a microfabricated two-stage thermal modulator[79] cycling from −35 °C to 250 °C. This miniaturized design is suitable for a wide spectrum of applications from the petrochemical industry to the study of the origin of life. The drastic decrease in physical size will allow a low-resource consuming, portable GC × GC system to be produced that is suitable for detecting in situ organic compounds that might provide insight on the origin of first cellular life[80] if the system is employed in future space missions to Mars, Titan, and/or comets.

4.3. Three-Dimensional Separation Space

Beside GC × GC, other comprehensive two-dimensional chromatographic techniques, such as LC × LC,[81] LC × CE,[82] CE × CE,[83] and LC × GC[84] (LC = liquid chromatography, CE = capillary electrophoresis) are becoming more and more common in modern analytical laboratories. Most recent advances in multidimensional separation are to invoke a third chromatographic dimension to provide additional chemical selectivity, such as offline LC-GC × GC,[85] LC × LC × CE,[86] and GC × GC × GC.[87] Recently, Synovec et al.[88] utilized two six-port diaphragm valves as the interface between three, serially coupled capillary columns housed in a conventional GC instrument attached to an FID. Increased chemical selectivity and peak capacity was reported for a diesel sample using a triflate ionic-liquid stationary phase (runtime: 4 s), which has a high selectivity for phosphorous compounds, between a non-polar primary column (DB-5, runtime: 20 min) and a complementary polar third-dimensional column (DB-Wax, runtime 200 ms). They noted that in principle, a thermally modulated instrument would provide higher sensitivity because a valve-based system generally transfers only a portion of the analyte from one dimension to the next. However, thermally modulated systems are presently not able to inject sufficiently narrow pulses in time to provide the narrow peak widths required for the third-dimensional column.

Welthagen et al.[89] have successfully applied GC × GC with soft single-photon ionization (SPI) ToF-MS. They used two selective chromatographic columns to separate molecules according to polarity and polarizability. The soft ionization mass separation step was again used to mimic a "volatility type separation". Venkatramani et al.[25] previously discussed that because of the lack of fragmentation and formation of single molecular ions, soft ionization mass spectrometry depicts similar separation characteristics to a non-polar gas chromatographic column. Consequently, the experiment resulted in a three-dimensional comprehensive separation space. A drawback of the laser-based SPI mass spectrometric method is the demanding requirement of a laser that can generate VUV-light.

In general, with each additional dimension, the selectivity of the system will increase, but the data set extends by one further separation dimension. Moreover, it is a challenge to keep the complexity of the instruments low and to ensure that the entirety of the effluent is led to the next separation step

without merging after each separation. Moreover, it is certainly challenging to fully utilize all of the tertiary retention space, this is because current GC phases lack completely different selectivities and because of the lack of polarity diversity within many samples.

5. Summary and Outlook

While GC × GC is still considered an emerging technology and is used primarily in research and development laboratories, it has undergone significant growth in the past few years. Today, the principles and instrumentations of GC × GC are well established. Robust and user-friendly modulators with various detectors (notably time-of-flight and/or fast scanning quadrupole mass spectrometers) are commercially available and have made this technique more suitable to routine analysis, screening of complex samples, and quantitative determination of components found within. As evidenced by recent literature, research and industrial applications for GC × GC are growing in a number of diverse areas including food safety, fragrances, forensics, petroleum, metabolomics, and environmental studies.

In general, there are several main advantages of comprehensive GC × GC coupled to mass spectrometry over conventional 1D GC-MS methods. First, the selectivity is increased using three separation dimensions of (commonly) volatility, polarity, and mass. Second, the gain in peak intensity as a result the modulator-driven solute band re-focusing effect causes this technique to be particularly suitable for trace-level component detection. Furthermore, GC × GC provides highly ordered chromatograms, in which structurally related compounds group together in patterns that facilitates the identification of unknown compounds. Generally, structure-based analyte identification might not necessarily require the resolution of all the constituents of mixtures. Additionally, GC × GC separation aids in minimizing both co-elution between analytes and chromatographic noise. These advantages minimize potential interference with selective detection strategies, leading to better quantitative results.

Nevertheless, GC × GC is not static and has not reached its full potential. One of the main areas in need of improvement is true peak identification. Further development of GC × GC analysis requires significant progress in user-friendly (semi) automated software that intelligently treats the huge number of peaks, which generally show up in a 2D plot. Moreover, the development of MS spectra libraries in combination with structure-generation tools, databases for retention indices in both dimension, and straightforward quantification techniques for targeted and non-targeted components are eagerly awaited. Progress is being made in comparing large sets of complex data, which require accurate alignment of chromatograms and background corrections.

Computational chemometric techniques in GC × GC have advanced steadily and have a tremendous impact on the increasing number of GC × GC applications; however, simultaneous 2D multivariate modeling approaches to correlate chromatographic properties and molecular structural descriptors will become necessary in future advancements to glean analytical information.

Another trend can be observed in expanding the distribution of analytes more efficiently in the 2D plane, that is, optimizing the peak capacity of GC × GC. Therefore, improved one-dimensional separation and faster modulation has to be realized. The speed of the second-dimensional separation may be required to increase, which will require faster scanning conditions and more powerful peak identification software. All those improvements will be challenging, however, the potential benefits will be great.

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[1] E. Cremer, R. Müller, *Z. Elektrochem.* **1951**, *55*, 217 – 220.
[2] M. J. E. Golay in *Gas Chromatography* (Ed.: V. J. Coates), Academic Press, New York, **1958**.
[3] L. Mondello, A. C. Lewis, K. D. Bartle, *Multidimensional Gas Chromatography*, Wiley, Chichester, **2002**.
[4] a) J. Blomberg, P. J. Schoenemakers, J. Beens, R. Tijssen, *J. High Resolut. Chromatogr.* **1997**, *20*, 539 – 544; b) G. S. Frysinger, R. B. Gaines, *J. High Resolut. Chromatogr.* **1999**, *22*, 251 – 255; c) S. J. Rowland, C. E. West, A. G. Scarlett, D. Jones, R. A. Frank, *Rapid Commun. Mass Spectrom.* **2011**, *25*, 1198 – 1204.
[5] a) A. C. Lewis, N. Carslaw, P. J. Marriott, R. M. Kinghorn, P. Morrison, A. L. Lee, K. D. Bartle, M. J. Pilling, *Nature* **2000**, *405*, 778 – 781; b) X. Xu, L. L. P. Stee, J. Williams, L. Beens, M. Adahchour, R. J. J. Vreuls, U. A. T. Brinkmann, *Atmos. Chem. Phys.* **2003**, *3*, 665 – 682; c) G. Semard, A. Bruchet, P. Cardinael, J. P. Bouillon, *Water Sci. Technol.* **2008**, *57*, 1983 – 1989; d) E. Jover, V. Matamoros, J. M. Bayona, *J. Chromatogr. A* **2009**, *1216*, 4013 – 4019.
[6] a) J. Dallüge, M. van Rijn, J. Beens, R. J. J. Vreuls, U. A. T. Brinkmann, *J. Chromatogr. A* **2002**, *965*, 207 – 217; b) P. Q. Tranchida, P. Dugo, G. Dugo, L. Mondello, *J. Chromatogr. A* **2004**, *1054*, 3 – 16; c) E. Humston, J. D. Knowles, A. McShea, R. E. Synovec, *J. Chromatogr. A* **2010**, *1217*, 1963 – 1970.
[7] a) R. Shellie, P. J. Marriott, C. Cornwell, *J. High Resolut. Chromatogr.* **2000**, *23*, 554 – 560; b) R. Shellie, J. P. Marriott, P. Morrison, *J. Chromatogr. Sci.* **2004**, *42*, 417 – 422; c) G. Eyres, P. J. Marriott, J. P. Dufour, *J. Chromatogr. A* **2007**, *1150*, 70 – 77; d) C. von Mühlen, C. A. Zini, E. B. Caramao, P. J. Marriott, *J. Chromatogr. A* **2008**, *1200*, 34 – 42.
[8] a) R. Shellie, P. J. Marriott, A. Chaintreau, *Flavour Fragrance J.* **2004**, *19*, 91 – 98; b) C. Kok Whye, T. Chin Ping, M. Hamed, C. Sung Tong, Y. B. Che Man, N. S. Abdul Hamid, O. Azizah, B. Mahiran, *Food Chem.* **2011**, *125*, 1481 – 1489.
[9] C. Debonneville, A. J. Chaintreau, *J. Chromatogr. A* **2004**, *1027*, 109 – 115.
[10] a) W. Welthagen, R. A. Shellie, J. Spranger, M. Ristow, R. Zimmermann, O. Fiehn, *Metabolomics* **2005**, *1*, 65 – 73; b) R. E. Mohler, B. P. Tu, K. M. Dombek, J. C. Hoggard, E. T. Young, R. E. Synovec, *J. Chromatogr. A* **2008**, *1186*, 401 – 411; c) M. Orešič, T. Hyötyläinen, S.-K. Herukka, M. Sysi-Aho, I. Mattila, T. Seppänan-Laakso, V. Julkunen, P. V. Gopalacharyulu, M. Hallikainen, J. Koikkalainen, M. Kivipelto, S. Helisalmi, J. Lötjönen, H. Soininen, *Transl. Psychiatry* **2011**, *1*, e57; d) M. F.

Almstetter, P. J. Oefner, K. Dettmer, *Anal. Bioanal. Chem.* **2012**, *402*, 1993 – 2013.

[11] a) J. C. Giddings, *Anal. Chem.* **1984**, *56*, 1258A – 1260A; b) J. C. Giddings, *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1987**, *10*, 319 – 323.

[12] Z. Liu, J. B. Phillips, *J. Chromatogr. Sci.* **1991**, *29*, 227 – 231.

[13] a) R. C. Y. Ong, P. J. Marriott, *J. Chromatogr. Sci.* **2002**, *40*, 276 – 291; b) T. Górecki, J. Harynuk, O. Panic, *J. Sep. Sci.* **2004**, *27*, 359 – 379; c) M. Adachour, J. Beens, U. A. T. Brinkmann, *J. Chromatogr. A* **2008**, *1186*, 67 – 108.

[14] L. Mondello, P. Q. Tranchida, P. Dugo, G. Dugo, *Mass Spectrom. Rev.* **2008**, *27*, 101 – 124.

[15] a) J. C. Giddings, *Unified Separation Science*, Wiley, New York, **1991**; b) J. C. Giddings, *J. Chromatogr. A* **1995**, *703*, 3 – 15.

[16] J. Beens, H. Boelens, R. Tijssen, J. Blomberg, *J. High Resolut. Chromatogr.* **1998**, *21*, 47 – 54.

[17] a) H.-J. de Geus, J. de Boer, J. B. Phillips, E. B. Ledford, Jr., U. A. T. Brinkman, *J. High Resolut. Chromatogr.* **1998**, *21*, 411 – 413; b) A. L. Lee, K. D. Bartle, A. C. Lewis, *Anal. Chem.* **2001**, *73*, 1330 – 1335; c) T. T. Truong, P. J. Marriott, N. A. Porter, *J. Anal. Chem.* **2001**, *84*, 323 – 335.

[18] a) P. Korytár, P. E. G. Leonards, J. de Boer, U. A. T. Brinkman, *J. Chromatogr. A* **2002**, *958*, 203 – 218.

[19] a) J. Dallüge, J. Beens, U. A. T. Brinkmann, *J. Chromatogr. A* **2003**, *1000*, 69 – 108; b) J. F. Focant, A. Sjodin, W. E. Turner, D. G. Patterson, Jr., *Anal. Chem.* **2004**, *76*, 6313 – 6320; c) P. Korytár, P. E. G. Leonards, J. de Boer, U. A. T. Brinkman, *J. Chromatogr. A* **2005**, *1086*, 29 – 44.

[20] a) J. Dallüge, R. J. J. Vreuls, J. Beens, U. A. T. Brinkman, *J. Sep. Sci.* **2002**, *25*, 201 – 214; b) R. Ong, P. J. Marriott, P. Morrison, P. Haglund, *J. Chromatogr. A* **2002**, *962*, 135 – 152.

[21] J. Beens, R. Tijssen, J. Blomberg, *J. Chromatogr. A* **1998**, *822*, 233 – 251.

[22] J. Beens, H.-G. Janssen, M. Adachour, U. A. T. Brinkmann, *J. Chromatogr. A* **2005**, *1086*, 141 – 150.

[23] F. L. Dorman, P. D. Schettler, L. A. Vogt, J. W. Cochran, *J. Chromatogr. A* **2008**, *1186*, 196 – 201.

[24] J. Harynuk, T. Gorecki, *Am. Lab.* **2007**, *39*, 36 – 39.

[25] C. J. Venkatramani, J. Xu, J. B. Phillips, *Anal. Chem.* **1996**, *68*, 1486 – 1492.

[26] S. K. Poole, C. F. Poole, *J. Sep. Sci.* **2008**, *31*, 1118 – 1123.

[27] G. Semard, V. Peulon-Agasse, A. Bruchet, J. P. Bouillon, P. Cardinal, *J. Chromatogr. A* **2010**, *1217*, 5449 – 5454.

[28] M. Adahchour, J. Beens, R. J. J. Vreuls, A. M. Batenburg, U. A. T. Brinkman, *J. Chromatogr. A* **2004**, *1054*, 47 – 55.

[29] P. Haglund, P. Korytár, C. Danielsson, J. Diaz, K. Wiberg, P. Leonards, U. A. T. Brinkman, J. de Boer, *Anal. Bioanal. Chem.* **2008**, *390*, 1815 – 1827.

[30] V. R. Reid, J. A. Clark, D. W. Armstrong, R. E. Synovec, *J. Sep. Sci.* **2008**, *31*, 3429 – 3436.

[31] R. A. Shellie, P. J. Marriott, *Anal. Chem.* **2002**, *74*, 5426 – 5454.

[32] M. Junge, H. Huegel, P. J. Marriott, *Chirality* **2007**, *19*, 228 – 234.

[33] C. Meinert, J.-J. Filippi, P. de Marcellus, L. d'Hendecourt, U. J. Meierhenrich, *ChemPlusChem* **2012**, *77*, 186 – 191.

[34] P. de Marcellus, C. Meinert, M. Nuevo, J.-J. Filippi, G. Danger, D. Deboffle, L. Nahon, L. d'Hendecourt, U. J. Meierhenrich, *Astrophys. J. Lett.* **2011**, *727*, L27.

[35] B. Omais, M. Courtiade, N. Charon, J. Ponthus, D. Thiébaut, *Anal. Chem.* **2011**, *83*, 7550 – 7554.

[36] a) C. A. Bruckner, B. J. Prazen, R. E. Synovec, *Anal. Chem.* **1998**, *70*, 2796 – 2804; b) J. V. Seeley, F. Kramp, C. J. Hicks, *Anal. Chem.* **2000**, *72*, 4346 – 4352; c) J. V. Seeley, *J. Chromatogr. A* **2012**, *1255*, 24 – 37.

[37] J. B. Phillips, R. B. Gaines, J. Blomberg, F. W. M. van der Wielen, J.-M. Dimandja, V. Green, J. Granger, D. Patterson, L. Racovalis, H.-J. de Geus, J. de Boer, P. Haglund, J. Lipsky, V. Sinha, E. B. Ledford, *J. High Resolut. Chromatogr.* **1999**, *22*, 3 – 10.

[38] a) P. J. Marriott, R. M. Kinghorn, *Anal. Chem.* **1997**, *69*, 2582 – 2588; b) R. S. Kinghorn, P. J. Marriott, *J. High Resolut. Chromatogr.* **1998**, *21*, 620 – 622; c) R. S. Kinghorn, P. J. Marriott, *J. High Resolut. Chromatogr.* **1999**, *22*, 235 – 238.

[39] a) J. Beens, M. Adahchour, R. J. J. Vreuls, K. Altena, U. A. T. Brinkman, *J. Chromatogr. A* **2001**, *919*, 127 – 132; b) J. Harynuk, T. Gorecki, *J. Chromatogr. A* **2003**, *1019*, 53 – 63; c) M. Adahchour, J. Beens, U. A. T. Brinkman, *Analyst* **2003**, *128*, 213 – 216.

[40] a) M. Libardoni, J. H. Waite, R. Sacks, *Anal. Chem.* **2005**, *77*, 2786 – 2794; b) M. Libardoni, E. Hasselbrink, J. H. Waite, R. Sacks, *J. Sep. Sci.* **2006**, *29*, 1001 – 1008.

[41] P. Q. Tranchida, G. Purcaro, P. Dugo, L. Mondello, *TrAC Trends Anal. Chem.* **2011**, *30*, 1437 – 1461.

[42] J. V. Seeley, N. J. Primeau, S. V. Bandurski, S. K. Seeley, J. D. McCurry, *Anal. Chem.* **2007**, *79*, 1840 – 1847.

[43] F. C. Y. Wang, *J. Chromatogr. A* **2008**, *1188*, 274 – 280; F. C. Y. Wang, US Patent 2007/0214866A1, **2007**.

[44] M. Poliak, M. Kochmann, A. Amirav, *J. Chromatogr. A* **2008**, *1186*, 189 – 195.

[45] H. J. Cortes, B. Winniford, J. Luong, M. Pursch, *J. Sep. Sci.* **2009**, *32*, 883 – 904.

[46] J. Dallüge, L. L. P. van Stee, X. Xu, J. Williams, J. Beens, R. J. J. Vreuls, U. A. T. Brinkman, *J. Chromatogr. A* **2002**, *974*, 169 – 184.

[47] Z.-D. Zeng, H. M. Hugel, P. J. Marriott, *Anal. Bioanal. Chem.* **2011**, *401*, 2373 – 2386.

[48] a) B. V. Hollingsworth, S. E. Reichenbach, Q. Tao, A. Visvanathan, *J. Chromatogr. A* **2006**, *1105*, 51 – 58; b) B. Wang, A. Q. Fang, J. Heim, B. Bogdanov, S. Pugh, M. Libardoni, X. A. Zhang, *Anal. Chem.* **2010**, *82*, 5069 – 5081; c) S. Dasgupta, K. Banerjee, S. H. Patil, M. Ghaste, K. N. Dhumal, P. G. Adsule, *J. Chromatogr. A* **2010**, *1217*, 3881 – 3889; d) P. Q. Tranchida, G. Purcaro, C. Fanali, P. Dugo, G. Dugo, L. Mondello, *J. Chromatogr. A* **2010**, *1217*, 4160 – 4166; e) S. Castillo, I. Mattila, J. Miettinen, M. Oresic, T. Hyotylainen, *Anal. Chem.* **2011**, *83*, 3058 – 3067; f) S. D. Johanningsmeier, R. F. McFeeters, *J. Food Sci.* **2011**, *76*, C168 – C177.

[49] a) R. A. Shellie, P. J. Marriott, *Analyst* **2003**, *128*, 879 – 883; b) R. A. Shellie, P. J. Marriott, C. W. Huie, *J. Sep. Sci.* **2003**, *26*, 1185 – 1192; c) M. Adahchour, M. Brandt, H.-U. Baier, R. J. J. Vreuls, A. M. Batenburg, U. A. T. Brinkman, *J. Chromatogr. A* **2005**, *1067*, 245 – 254; d) L. Mondello, A. Casilli, P. Q. Tranchida, M. Lo Presti, P. Dugo, G. Dugo, *Anal. Bioanal. Chem.* **2007**, *389*, 1755 – 1763; e) G. Purcaro, P. Q. Tranchida, C. Ragonese, L. Conte, P. Dugo, G. Dugo, L. Mondello, *Anal. Chem.* **2010**, *82*, 8583 – 8590.

[50] Z. L. Cardeal, M. D. R. Gomes da Silva, P. J. Marriott, *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2823 – 2836.

[51] P. Korytár, J. Parera, P. E. G. Leonards, J. de Boer, U. A. T. Brinkmann, *J. Chromatogr. A* **2005**, *1067*, 255 – 264.

[52] P. Korytár, J. Parera, P. E. G. Leonards, F. J. Santos, J. de Boer, U. A. T. Brinkmann, *J. Chromatogr. A* **2005**, *1086*, 71 – 82.

[53] a) H. J. Tobias, G. L. Sacks, Y. Zhang, J. T. Brenna, *Anal. Chem.* **2008**, *80*, 8613 – 8621; b) H. J. Tobias, Y. Zhang, R. J. Auchus, J. T. Brenna, *Anal. Chem.* **2011**, *83*, 7158 – 7165.

[54] J.-F. Focant, E. J. Reiner, K. MacPherson, T. Kolic, A. Sjödin, D. G. Patterson Jr., S. L. Reese, F. L. Dorman, J. Cochran, *Talanta* **2004**, *63*, 1231 – 1240.

[55] a) N. Ochiai, T. Ieda, K. Sasamoto, A. Fushimi, S. Hasegawa, K. Tanabe, S. Kobayashi, *J. Chromatogr. A* **2007**, *1150*, 13 – 20; b) H. Shunji, T. Yoshikatsu, F. Akihiro, I. Hiroyasu, T. Kiyoshi, S. Yasuyuki, U. Masa-aki, K. Akihiko, T. Kazuo, O. Hideyuki, A. Katsunori, *J. Chromatogr. A* **2008**, *1178*, 187 – 198.

[56] a) C. Danielsson, K. Wiberg, P. Korytár, S. Bergek, U. A. T. Brinkmann, *J. Chromatogr. A* **2005**, *1086*, 61 – 70; b) A. M. Muscalu, E. J. Reiner, S. N. Liss, T. Chen, G. Ladwig, D. Morse, *Anal. Bioanal. Chem.* **2011**, *401*, 2403 – 2413.

[57] a) D. Ryan, P. Watkins, J. Smith, M. Allen, P. Marriott, *J. Sep. Sci.* **2005**, *28*, 1075 – 1082; b) D. Ryan, P. Marriott, *J. Sep. Sci.* **2006**, *29*, 2375 – 2382.
[58] W. Khummueng, P. Morrison, P. J. Marriott, *J. Sep. Sci.* **2008**, *31*, 3404 – 3415.
[59] L. L. P. van Stee, J. Beens, R. J. J. Vreuls, U. A. T. Brinkman, *J. Chromatogr. A* **2003**, *1019*, 89 – 99.
[60] R. Hua, Y. Li, W. Liu, J. Zheng, H. Wei, J. Wang, X. Lu, H. Kong, G. Xu, *J. Chromatogr. A* **2003**, *1019*, 101 – 109.
[61] M. Z. Özel, J. F. Hamilton, A. C. Lewis, *Environ. Sci. Technol.* **2011**, *45*, 1497 – 1505.
[62] B. d'Acampora Zellner, A. Casilli, P. Dugo, G. Dugo, L. Mondello, *J. Chromatogr. A* **2007**, *1141*, 279 – 286.
[63] V. Schurig, H.-P. Nowotny, *Angew. Chem.* **1990**, *102*, 969 – 986; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 939 – 957; V. Schurig, M. Jung, S. Mayer, S. Negura, M. Fluck, H. Jakubetz, *Angew. Chem.* **1994**, *106*, 2265 – 2267; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2222 – 2223.
[64] H. Frank, G. J. Nicholson, E. Bayer, *Angew. Chem.* **1978**, *90*, 396 – 398; *Angew. Chem. Int. Ed. Engl.* **1978**, *17*, 363 – 365.
[65] U. Beitler, B. Feibush, *J. Chromatogr.* **1976**, *123*, 149 – 166.
[66] a) L. M. Blumberg, *J. Chromatogr. A* **2003**, *985*, 29 – 38; b) L. M. Blumberg, F. David, M. S. Klee, P. Sandra, *J. Chromatogr. A* **2008**, *1188*, 2 – 16.
[67] D. Ryan, P. Morrison, P. Marriott, *J. Chromatogr. A* **2005**, *1071*, 47 – 53.
[68] a) E. L. Schymanski, C. Meinert, M. Meringer, W. Brack, *Anal. Chim. Acta* **2008**, *615*, 136 – 147; b) E. L. Schymanski, M. Bataineh, K.-U. Goss, W. Brack, *TrAC Trends Anal. Chem.* **2009**, *28*, 550 – 561; c) HighChem. *Mass Frontier*, 5.0 trial version; HighChem. Ltd./Thermo Scientific, **2007**.
[69] J. V. Seeley, S. K. Seeley, *J. Chromatogr. A* **2007**, *1172*, 72 – 83.
[70] a) R. J. Western, P. J. Marriott, *J. Chromatogr. A* **2003**, *1019*, 3 – 14; b) S. Bieri, P. J. Marriott, *Anal. Chem.* **2008**, *80*, 760 – 768; Y. Zhao, J, Zhang, B. Wang, S. H. Kim, A. Fang, B. Bogdanov, Z. Zhou, C. McCain, X. Zhang, *J. Chromatogr. A* **2011**, *1218*, 2577 – 2583; c) C. von Mühlen, P. J. Marriott, *Anal. Bioanal. Chem.* **2011**, *401*, 2351 – 2360.
[71] J. S. Arey, R. K. Nelson, L. Xu, C. M. Reddy, *Anal. Chem.* **2005**, *77*, 7172 – 7182.
[72] J. V. Seeley, E. M. Libby, K. A. Hill Edwars, S. K. Seeley, *J. Chromatogr. A* **2009**, *1216*, 1650 – 1657.
[73] J. J. Harynuk, A. H. Kwong, P. J. Marriott, *J. Chromatogr. A* **2008**, *1200*, 17 – 27.
[74] a) C. A. Cramers, H.-G. Janssen, M. M. van Deursen, P. A. Leclercq, *J. Chromatogr. A* **1999**, *856*, 315 – 329; b) P. Korytár, H.-G. Janssen, E. Matisová, U. A. T. Brinkman, *TrAC Trends Anal. Chem.* **2002**, *21*, 558 – 572.
[75] a) L. M. Blumberg, M. S. Klee, *Anal. Chem.* **1998**, *70*, 3828 – 3839; b) M. S. Klee, L. M. Blumberg, *J. Chromatogr. A* **2002**, *40*, 234 – 247.
[76] M. Junge, S. Bieri, H. Huegel, P. J. Marriott, *Anal. Chem.* **2007**, *79*, 4448 – 4454.
[77] G. Lambertus, R. Sacks, *Anal. Chem.* **2005**, *77*, 2078 – 2084.
[78] S. M. Reidy, S.-J. Kim, K. Beach, B. P. Block, E. T. Zellers, K. Kurabayashi, K. D. Wise, *Solid-State Sensors, Actuators, and Microsystems Workshop*, Hilton Head Island, South Carolina, **2010**, pp. 78 – 81.
[79] S.-J. Kim, S. M. Reidy, B. P. Block, K. D. Wise, E. T. Zellers, K. Kurabayashi, *Lab Chip* **2010**, *10*, 1647 – 1654.
[80] U. J. Meierhenrich, J.-J. Filippi, C. Meinert, P. Vierling, J. P. Dworkin, *Angew. Chem.* **2010**, *122*, 3826 – 3839; *Angew. Chem. Int. Ed.* **2010**, *49*, 3738 – 3750.
[81] a) M. M. Bushey, J. W. Jorgenson, *Anal. Chem.* **1990**, *62*, 161 – 167; b) L. A. Holland, J. W. Jorgenson, *Anal. Chem.* **1995**, *67*, 3275 – 3283; c) D. R. Stoll, P. W. Carr, *J. Am. Chem. Soc.* **2005**, *127*, 5034 – 5035; d) D. R. Stoll, *Anal. Bioanal. Chem.* **2010**, *397*, 979 – 986; e) M. X. Gao, D. W. Qi, P. Zhang, C. H. Deng, X. M. Zhang, *Expert Rev. Proteomics* **2010**, *7*, 665 – 678.
[82] a) M. M. Bushey, J. W. Jorgenson, *Anal. Chem.* **1990**, *62*, 978 – 984; b) J. Zhang, H. Hu, M. Gao, P. Yang, X. Zhang, *Electrophoresis* **2004**, *25*, 2374 – 2383.
[83] a) D. A. Michels, S. Hu, K. A. Dambrowitz, M. J. Eggertson, K. Lauterbach, N. J. Dovichi, *Electrophoresis* **2004**, *25*, 3098 – 3105; b) H. Liu, C. Yang, Q. Yang, W. Zhang, Y. Zhang, *J. Chromatogr. B* **2005**, *817*, 119 – 126.
[84] a) W. C. Quigley, C. G. Fraga, R. E. Synovec, *J. Microcolumn Sep.* **2000**, *12*, 160 – 166; b) S. de Koning, H.-G. Janssen, M. vanDeursen, U. A. T. Brinkman, *J. Sep. Sci.* **2004**, *27*, 397 – 409.
[85] a) R. Edam, J. Blomberg, H.-G. Janssen, P. J. Schoenemakers, *J. Chromatogr. A* **2005**, *1086*, 12 – 20; b) M. Biedermann, K. Grob, *J. Sep. Sci.* **2009**, *32*, 3726 – 3737.
[86] A. W. Moore, J. W. Jorgenson, *Anal. Chem.* **1995**, *67*, 3456 – 3463.
[87] a) E. B. Ledford, C. A. Billesbach, Q. Y. Zhu, *J. High Resolut. Chromatogr.* **2000**, *23*, 205 – 207; b) N. E. Watson, W. C. Siegler, J. C. Hoggard, R. E. Synovec, *Anal. Chem.* **2007**, *79*, 8270 – 8280.
[88] W. C. Siegler, J. A. Crank, D. W. Armstrong, R. E. Synovec, *J. Chromatogr. A* **2010**, *1217*, 3144 – 3149.
[89] W. Welthagen, S. Mitschke, F. Mühlberger, R. Zimmermann, *J. Chromatogr. A* **2007**, *1150*, 54 – 61.