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30th Anniversary of comprehensive two-dimensional gas chromatography: Latest advances

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Abstract

In this review, we report on the latest (2020-Early 2021) instrumental advances and applications of comprehensive two-dimensional gas chromatography (GC×GC), including its hyphenation with novel upstream or downstream processes (sample preparation approaches or detection technologies). We also discuss software and analysis workflow developments necessary to elaborate the dense chemical information obtained. Thirty years after its inception, the use of GC×GC, as the main analytical tool or as a complementary platform, is undoubtedly shifting toward more applied challenges in a vast breadth of applications. Therefore, we consider the major fields (energy, fuel, food-stuff, plant, biological, and environmental) in which GC×GC has been successfully used, discussing some of the recent innovative research works.

KEYWORDS

aroma analysis, fingerprinting, GC×GC, mass spectrometry, metabolomics

1 | INTRODUCTION

The milestone paper from Liu and Phillips, in which they demonstrated for the first time the use of true comprehensive two-dimensional gas chromatography (GC×GC), was submitted back in April 1991.¹ The year 2021 marks the 30th anniversary of GC×GC, with the technique that has gone through considerable advancements since its first report. In the parallelism with human evolution, any novel analytical tool or technique can also be characterized by different evolutionary stages, from the birth to the maturity or death²: "Each successful new measurement tool passes through three stages: first, it is hyped by its

creators, and ridiculed by threatened competitors; second, it is accepted by a few for refinement and validation, and third, it accelerates to maturity or fades away". In view of this, it can be acknowledged that GC×GC is surely making its way through the maturity stage of its evolution, with its extended and growing breadth of applications and its presence in regulated methods.³

The separation power of GC×GC relies on the in-series combination of two capillary columns characterized by different stationary phases, ie, different analyte-stationary phase interactions, by means of a transfer device or interface, called modulator (Figure 1). Comprehensive GC×GC can be considered as the natural progression of the classical multidimensional gas chromatography (MDGC). However, unlike MDGC, in which selective transfers (or "cuts") from the first dimension (¹D) to the second dimension (²D) occur usually by means of a Deans' switch-based device, and consequently only a portion of the effluent is subjected to two different separation mechanisms, GC×GC enables the full transfer of the effluent into the second separation dimension, ensuring a true comprehensive 2D separation.⁴ The modulator is the key component of the system as it traps and transfers narrow pulses of

Abbreviations: ¹D, first dimension; ²D, second dimension; CDF, common data format; DMAs, dimethyl acetals; FAMEs, fatty acid methyl esters; FID, flame ionization detector; FIMS, field ionization mass spectrometry; FT-ICR MS, Fourier-transform ion cyclotron mass spectrometry; FWHM, full width at half maximum; GC, gas chromatography; GC×GC, comprehensive two-dimensional gas chromatography; GC×GC×GC, comprehensive three-dimensional gas chromatography; HR MS, high-resolution mass spectrometry; IL, ionic liquid; MDGC, multidimensional gas chromatography; MOAH, mineral oil aromatic hydrocarbons; MS, mass spectrometry; PCBs, polychlorobiphenyls; PDMS, polydimethylsiloxane; SBSE, stir-bar sorptive extraction; SPME, solid-phase microextraction; TIC, total ion chromatogram; VOC, volatile organic compound; VUV, vacuum ultraviolet

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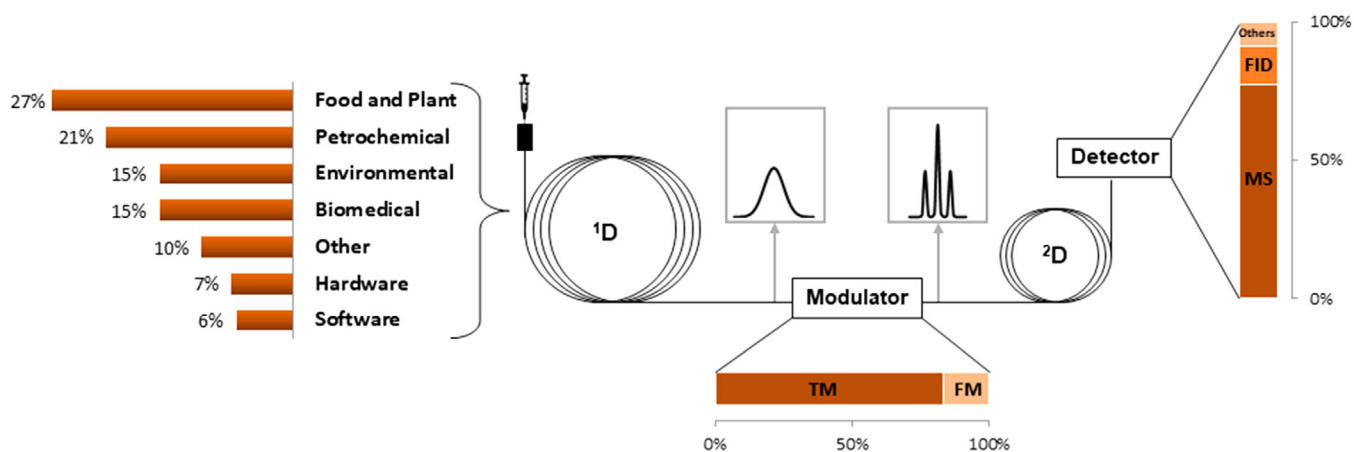


FIGURE 1 GCxGC overview of the latest advances: highlighted are the fields of applications (on the left), the modulator type (on the bottom), and the detector type usage (on the right). TM and FM are thermal and flow modulator, respectively

the entire effluent from the first to the second analytical column. The nature of this transfer principle identifies the two main families of modulators, that is, pneumatic- (or flow-) and thermal-based devices.

The primary column is usually a traditional gas chromatography (GC) column (30 - 60 m), while the secondary column is a shorter segment (commonly 1-5 m) to ensure a fast separation (1-6 s), hence maintaining the separation achieved in 1^{D} .

The resulting high peak capacity enables the chromatographic separation of several hundreds, or even thousands of compounds from a complex sample. The two retention mechanisms provide significantly more information about each component substance in the sample mixture, resulting in ordered 2D separations, based on the physicochemical properties of the analytes. The additional information provided by the second column may be used for a number of purposes, including providing a more reliable identification of an unknown compound, and informing about the differential polarity of the analytes when a proper combination of columns' stationary phases is used. In addition, the use of thermal modulators especially enables analytes enrichment through the focusing effect, particularly improving the detection limits.

Resuming, the increased sensitivity, the selectivity, the speed, the separation power, and the structured 2D separation, are considered the invaluable features of GCxGC.

Although thermal modulators are the elderly and most commonly used transfer device nowadays, we observed a trend toward an increasing use of flow modulators (Figure 1), with 16% of the studies in 2020 making use of flow modulators against 7% until 2017.⁵ Such trends could be explained, in part, by the continuous development and improvement of flow modulators, and more importantly by the increased interest of manufacturers, providing available commercial solutions. Although less sensitive, flow modulation indeed represents an attractive alternative to cryogenic modulation since no cryogenic fluids are necessary for its operation, significantly reducing the associated setup and running costs and the safety issues.

The power of GCxGC is further exploited when the detection is represented by mass spectrometry (MS), which provides unique identi-

fication capabilities. Indeed, MS detection remains, by far until now, the most popular choice, being used as a detector in 77% of the studies (Figure 1). This powerful analytical alliance (GCxGC-MS) allows the comprehensive characterization (fingerprinting and/or profiling), and/or the targeted analysis of highly complex samples, depending on the analyst research goals.

A book on the basic GCxGC principles and its application on food analysis, and a comprehensive review on the technical advances of GCxGC and general applications have been published in 2020.⁶⁻⁸ Likewise, in this last year review on specific applications have been released, like on fuel analysis,⁹ biomass analysis,¹⁰ pesticide residue analysis in biological matrices,¹¹ and exosome analysis.¹² Also dedicated topics have been reviewed recently on the use of GCxGC regarding the role sample preparation,¹³ the instrumental setup and the optimization of the experimental settings,^{14,15} the possible complex configurations for multidimensional separation,¹⁴ and the chemometric tools used for data processing.¹⁶

In this review, we report on the latest advances (2020-Early 2021) in hardware, software, and analysis workflows for GCxGC together with the various domains in which GCxGC has been successfully used. The review does not aim to give an exhaustive list of publications, but only a few selected, based on novelty/importance regarding various topics, are reported.

2 | INSTRUMENTATION DEVELOPMENT AND SEPARATION OPTIMIZATION

Although the use of GCxGC is penetrating more and more in different application areas, there is a small portion of research that aims at improving and advance the instrumental aspect of the technique or its hyphenation with innovative upstream or downstream processes (for example, sample preparation approaches or detection technologies).¹³ Considering the GCxGC's heart, there have been considerable developments, since its inception, especially in what concerns the modulation forms and designs, not surprisingly.

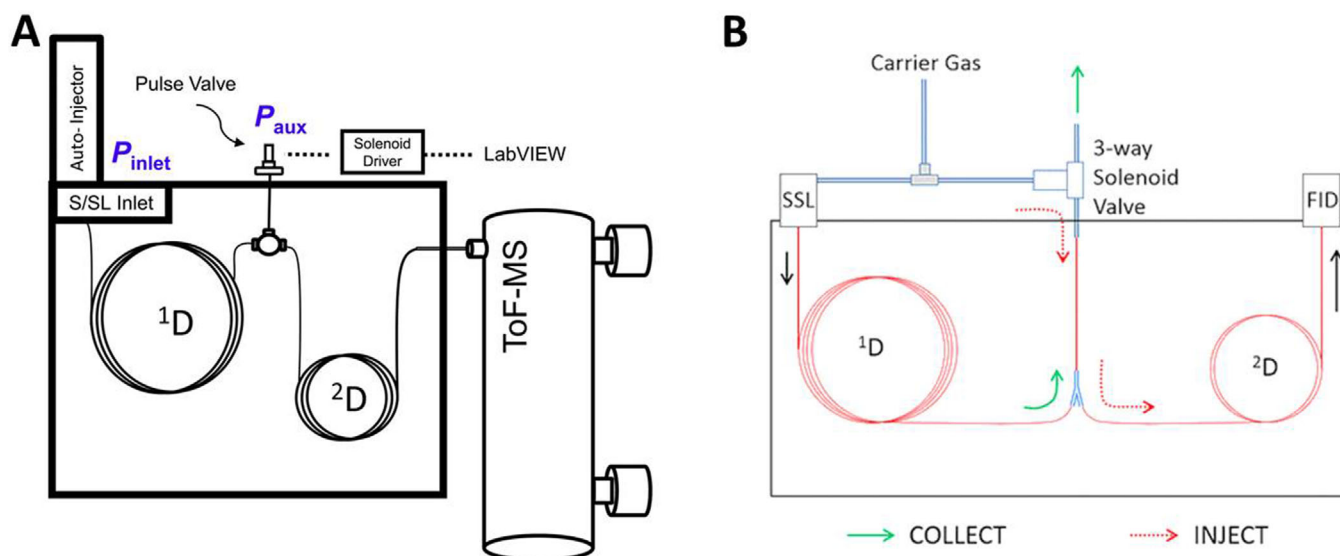


FIGURE 2 A and B, Schematic diagram of the two novel GCxGC flow modulation approaches: A, dynamic pressure gradient modulation, reproduced with permission from ¹⁷; B, quasi-stop-flow modulation, permission from ¹⁹

The latest developments regard the use of flow modulation, and specifically in a pursuit to increase its sensitivity and ease of use. Schöneich *et al.* developed a differential flow modulator characterized by unit duty-cycle and demonstrated its use with a mass spectrometer.^{17,18} It was called dynamic pressure gradient modulation, and consists of a single T-junction which bridges ¹D and ²D, and an auxiliary branch. The latter, through a pulse valve, generates the pressure pulse able to produce two-dimensional separations (Figure 2A).

Another recent design has been introduced, similar to and simpler than the original differential flow modulation from Seeley and Amirav, in which the auxiliary pressure controller is eliminated, and the same inlet pressure controller provides the gas flows to the first and second dimension and ensure the filling and flushing of the accumulation loop (Figure 2B).¹⁹

The last year has seen a few reports on the use and further development of three dimensional comprehensive GC, exploiting a thermal-flow modulation-hybrid GCxGCxGC-MS system.^{20,21}

As shown, the interest of developing novel forms of modulation that are simpler in hardware and hold limited running costs is confirmed also by the refinement or improvement of the separation capacity of existing forms of flow modulation. Aloisi *et al.* established an equivalent column set between flow and cryogenic modulation, obtaining approximately identical separation profiles and performance.²² The common and popular low-polar×mid-polar column set (a ¹D of 30 m × 0.25 mm ID × 0.25 mm d_f + a ²D of 1.5 m × 0.25 mm ID × 0.25 mm d_f) was used in the dual-loop cryogenic modulation system. In the flow modulated system, operating at MS-compatible flows,^{23,24} the best separation conditions were instead obtained using a ¹D (low-polarity) of 20 m × 0.18 mm ID × 0.18 mm d_f + a ²D (mid-polarity) of 5 m × 0.32 mm ID × 0.25 mm d_f .

Similarly, Stilo *et al.* demonstrated the possibility to translate GCxGC with dual detection (MS and flame ionization detection (FID))

methods from the more established cryogenic modulator to the flow modulator, maintaining similar separation power, coherent separation patterns, and avoiding chromatographic distortions.²⁵ Here, the best resulting column configuration for the reversed fill/flush flow modulation system consisted of a ¹D (low-polarity) of 20 m × 0.18 mm ID × 0.18 mm d_f and a ²D (mid-polarity) of 1.8 m × 0.18 mm ID × 0.18 mm d_f . It must be highlighted that the columns' dimensions and flows optimization are critical factors especially when using flow modulation, and that they are in addition strongly dependent to the system configuration (single/dual detection) and the flow modulator design (differential/diverting/low-flow).

A novelty in terms of detection coupling is represented by the first hyphenation of GCxGC with the orbitrap MS.²⁶ In this work, standards and a bio-oil sample were analyzed, exploiting only 15 000 full width at half maximum (FWHM) mass resolution and a 25 Hz frequency to obtain sufficient data points per modulated peak, suggesting that the power of the orbitrap MS has yet to be fully exploited with multidimensional GC.

3 | SOFTWARE AND ANALYSIS WORKFLOWS

The high-resolution nature of GCxGC intrinsically leads to highly complex and dense chemical information requiring long elaboration times and qualified analysts to interpret the results. In addition, the multiple instrumental configurations and detection technologies call for guided processing workflows together with accessible data elaboration tools to expand the use of such advanced multidimensional techniques.

A guided workflow, using commercially available software, was proposed for the processing of complementary data obtained from dual detection using MS and FID.²⁷ In an attempt to increase the transparency of data processing, different datasets have been published

as training tools, to enable the reproduction of the workflow applied, while discussing the implications of the chosen processing settings in different software packages.^{27,28} In another paper, an entire and detailed step-by-step protocol was proposed for pattern recognition in complex samples based on template matching algorithms.²⁹ The authors aimed at facilitating and standardizing chromatograms investigation and visualization, while dealing with analytes identification, analytes co-elutions, multiple calibration, group-type analysis, and parallel detection alignment, in both targeted and untargeted scenario. Such an approach enabled the cross-alignment of misaligned chromatograms, that is, from long-term studies or different instrumentations, and of features obtained from tandem ionization at 70 eV and 12 eV, while requiring limited computational time. Rosso *et al.* used the template-matching algorithm to fingerprint hazelnuts primary metabolome. The methodology enabled the cross-alignment of chromatograms obtained from two parallel detector signals with high and low electron ionization energies.³⁰ The authors pointed out that in addition to the complementary information obtained from the dual signal, the treatment of the signals separately enabled cross-validation of the fingerprinting results.

With the aim of getting greater confidence in analyte identification, a retention index (RI) system for GC×GC analysis, independent of the instrumental and analytical conditions, has been presented.³¹ This system is based on the combination of two well-established 1D GC RI systems; the non-isothermal Kovats index for the ¹D retention time, and the Lee index for the ²D retention time. Such approach provided good correlation with existing RI systems and predicted values ($r\bar{s} = 0.97$ in ¹D and $r\bar{s} = 0.99$ in ²D) and a coefficient of variance < 1% in ¹D and < 10% in ²D.

Retention modeling and prediction could greatly help in GC×GC method development. In this context, retention time prediction in both the first and second dimensions, using thermodynamic modeling, was introduced, in which the authors developed their model for both vacuum outlet (GC×GC-MS) and atmospheric outlet (GC×GC-FID).^{32,33}

Nolvachai *et al.* aimed at further enhancing analytes identification with the automated generation of peak centroid for each analyte in the 2D separation plane. The authors highlighted highly precise and reproducible retention times in both dimensions, with 0.003-0.066%RSD for the ¹D retention time and 0.305-0.551%RSD for the ²D retention time, from the analysis of peach aroma compounds using flow modulated GC×GC-MS. Practically, the peak centroid for each analyte was obtained after detection of the compounds using a conventional chromatographic integration followed by a curve-fitting approach identifying absolute retention times for all modulated peaks as can be seen in Figure 3. The total peak area was calculated from the sum of the area of the modulated peaks (Figure 3A). Finally, each compound was represented in the 2D space by its position at the intersecting coordinates with a height of the same magnitude as the total component summed area (Figure 3B), resulting in an evidently enhanced resolution.³⁴

An enhanced total ion chromatogram (TIC) algorithm was developed to improve peak detection and identification by identifying regions where the analytical signal is above a given threshold and zeroing the background noise.³⁵ The improved signal provided by the algo-

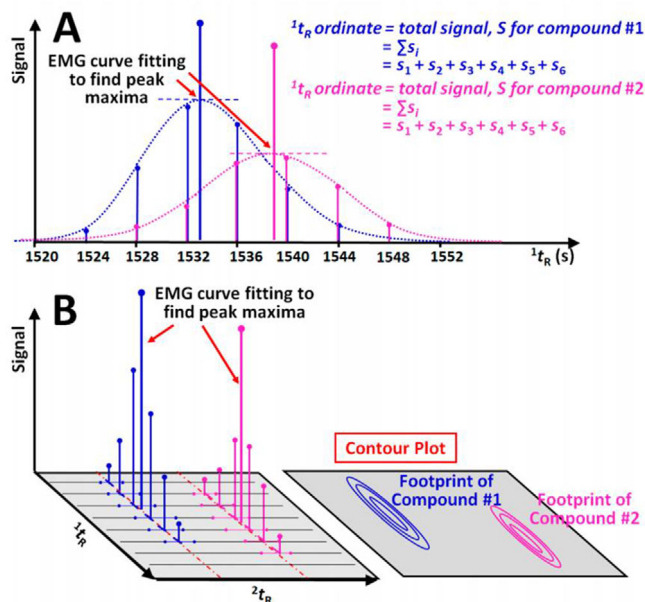


FIGURE 3 A and B, Automated peak centroid generation: A, approximation of first dimension retention time and total intensity of compounds from the modulated chromatogram, B, corresponding plot of the compound position (centroids) in a contour plot shown as the highest ordinates. Reproduced with permission from 34

rithm was demonstrated on standard test mixtures and on yeast cell metabolite extracts, in which the enhanced TIC found up to 64% more analytes than the classical TIC. Similar to analyte identification, analyte quantification in GC×GC can be challenging and needs additional delicate steps compared to 1D GC.

To improve the quantification process, Li *et al.* developed an automated processing based on image analysis to identify and get rid of streaks in the 2D chromatogram.³⁶ The algorithm enabled the detection of streaks in complex and low contrast background with a precision of 83.3% and a recall of 85.7%.

Although commercially available software present a high level of functionality, and are constantly being developed and improved, their accessibility is still limited. To increase accessibility for GC×GC data processing, several open-source platforms proposing an end-to-end procedure for GC×GC data processing and working with common data format (CDF) were developed.^{37,38} Moreno *et al.* made available an R package-based toolbox, which can be used for data preprocessing (baseline correction, signal smoothing, and alignment), multivariate data analysis, and with other R-based chemometric tools to perform further statistical analysis. The authors reported that less than 2 h were necessary to process 100 chromatograms and provided freely detailed and complete user manual and tutorial to help inexperienced users.³⁷ To maintain the user-friendly graphical interface of commercially available software, a web platform offering a one-window interface enabling basic GC×GC data visualization, spectral identification, environmental properties estimation (partition coefficients of octanol-water and air-water), and risk assessment of chemical mixture was created.³⁸

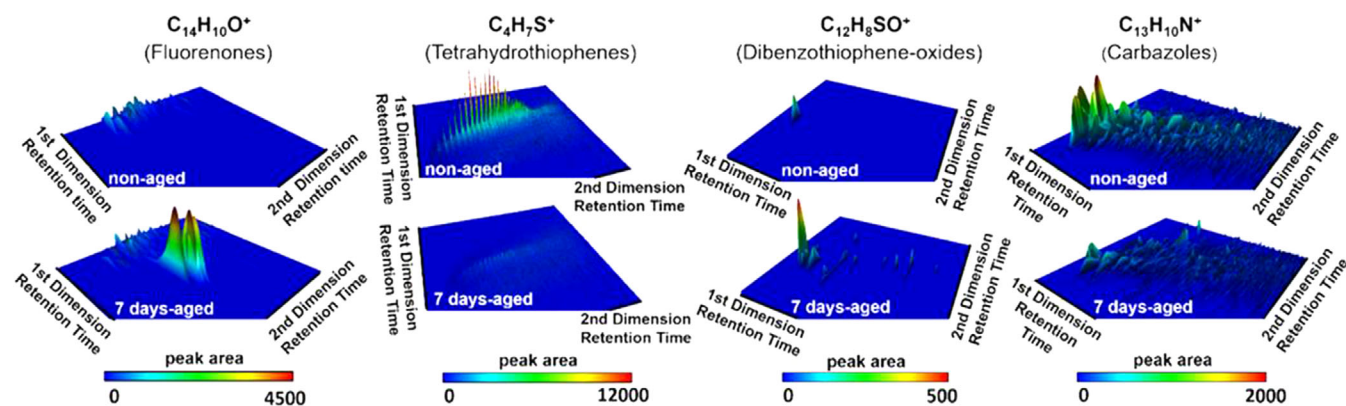


FIGURE 4 GCxGC-HR MS extracted ion chromatograms views of different fragments, illustrating the signal change after 7 days of aging. Reproduced with permission from 41

The recent implementation of GCxGCxGC analysis necessitated appropriate data processing strategies to get the full information potential of such high dimensionality data.^{17,39} The authors applied tile-based Fisher-ratio analysis, developed initially for GCxGC, to GCxGCxGC-FID. The approach was demonstrated on spiked diesel fuel samples. Practically, after using the F-ratio software enabling the location of the spiked analytes, the authors applied an in-house written S-ratio algorithm that provides relative quantification and peak purity for each hit compound. Nevertheless, the interpretation and quantification of peak overlapping in the 3D separation plane required the use of parallel factor analysis as decomposition tool.³⁹ In order to tackle this bottleneck, a more automatable approach was proposed and used to process 2D chromatographic data, consisting in the isolation of pure *m/z* combining the measure of lack of fit metric (for peak purity), F-ratio, and *P*-values; therefore avoiding the need for signal decomposition algorithm.⁴⁰

4 | LATEST APPLICATIONS

The use of GCxGC is more and more shifting toward application-oriented challenges. In the last year, more than 180 research papers have been published, in which GCxGC served as the main analytical tool, or as a complementary platform for a wide range of applications. In the following discussion, we gather selected applications. Instead, the complete list of the latest publications can be found in Table S1.

4.1 | Energy and fuels

Petrochemical samples have been exploited since the inception of the GCxGC technique to show its great potential in the rediscovery of the samples. Historically, and still contemporary as we have seen in Section 2,^{18,19} these kinds of samples are often the first choice to test the performance of novel pieces of hardware in GCxGC.

Corroborating its utility, GCxGC can be used as the reference technique to support and confirm structural estimations of Fourier-

transform ion cyclotron (FT-ICR) MS, within the upper GC elution limit compounds.⁴¹⁻⁴³ These two techniques have been recently used to investigate the aging process in bitumen.⁴¹ The supporting GCxGC high-resolution (HR) MS enabled the validation of FT-ICR MS data for the semi-volatile compounds and gave insights about aging processes which will help in improving the quality of bitumen binders and increase the durability of the pavement. Specifically, an increase of polar components (fluorenones, dibenzothiophene oxides), and a decrease of non-aromatic S-containing compounds (tetrahydrothiophenes), was observed (Figure 4).

In addition, the economical and societal impact on fossil fuels has been and is high, with ever-growing attention in fuels of biological origin.

Due to their chemical complexity, often a single technique is not capable of providing a complete separation or reliable identification and quantitation of petroleum and refined petroleum products. Wang reported an application of GCxGC on the complementarity of vacuum ultraviolet (VUV) and field-ionization MS (FIMS) detection on diesel qualitative and quantitative analysis.⁴⁴ VUV detection indeed may help in distinguishing coeluting components in a comprehensive two-dimensional gas chromatographic separation that are not resolved by molecular weight or ion fragmentation. In this application, the additional molecular structural group information from the VUV spectrum allowed the identification of isomers, which otherwise would be undistinguishable using the FIMS, since the isomers yield the same molecular ion. The author also observed and commented on the band broadening and the smaller linear dynamic range of VUV.

Bio-oils represent a valuable alternative to reduce fossil fuel consumption on one hand, and to reuse agricultural waste on the other. Thanks to GCxGC and MS, the detailed characterization of the waste products at various pretreatment stages is important to understand and refine their processing.⁴⁵⁻⁴⁸ For example, coconut shell represents an abundant and bulky waste, constituting about 80% of a coconut weight, and it can be used to produce bio-oil through a combination of pyrolysis and selective extractions.⁴⁵ The GCxGC-MS analysis of the fractions obtained from alkaline extraction and liquid-liquid biomass pretreatment showed the complementarity of the two approaches,

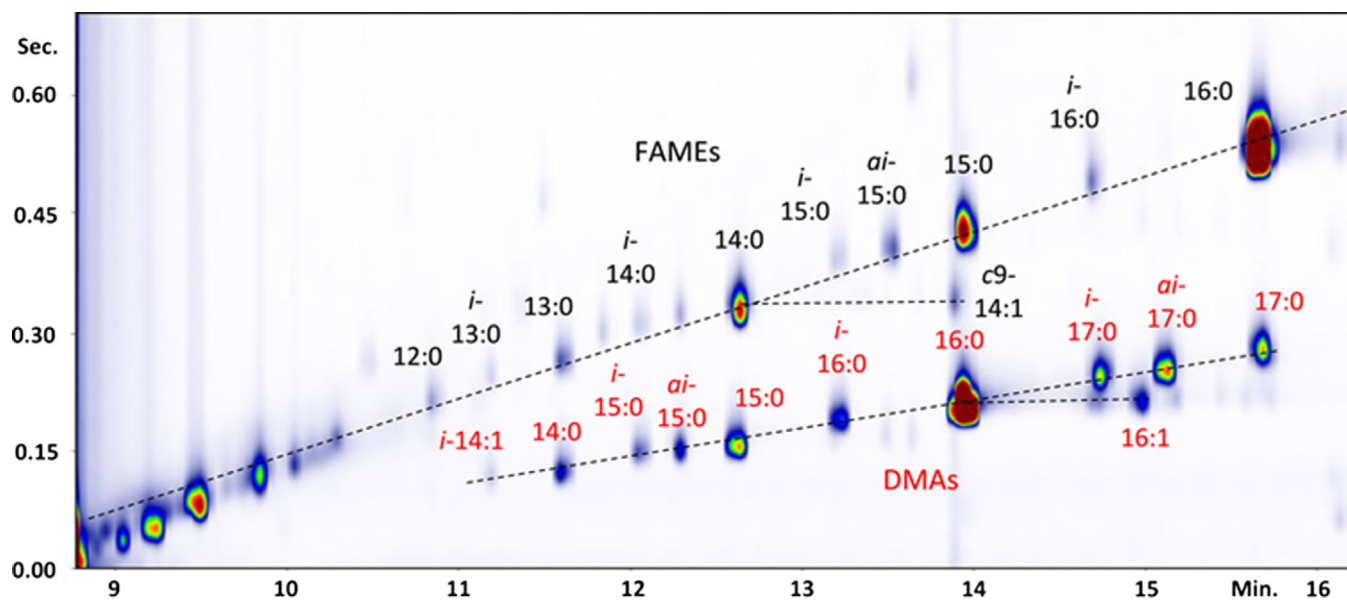


FIGURE 5 GCxGC (with online hydrogenation) separation of methanolyzed beef heart lipids from solvent front to FAME_{16:0}. Reproduced with permission from 56

giving higher recovery of oxygenate compounds, which increases the value of the final product.

4.2 | Foodstuff and plants

The majority of the GCxGC applications related to foodstuff and plant samples often aim at studying and monitoring the compounds responsible for sensorial properties or at improving the processing or quality.⁴⁹ Among them, the volatile fraction/aroma of foodstuff and beverages is very common.^{50–54} The volatile fraction reflects the composition of a sample, and often is used for classification and characterization. Aith *et al.* combined GCxGC and olfactometry analysis for the elucidation of wine aroma. Here, a complete and detailed characterization of wine volatiles and their olfactometric impact was obtained, and these results also provided support for the achievement of Geographical Indication denomination of origin labels for the wine investigated (Syrah wines of the São Francisco Valley) and to modify the processing to improve the quality of the final product.

The volatile fraction indeed holds rich information related to the heavier metabolites. Könen *et al.* developed a methodology for an analytical verification of grape variety classification in wine authenticity control, using *in vivo* tissue deuterium-labeling followed by GCxGC-MS.⁵²

Another common goal is the in-depth characterization and quality assessment of the samples or fraction of the samples. For instance, a GCxGC-HR MS method was used for the fingerprint of the unsaponifiable fraction of vegetable oils.⁵⁵ A laborious extraction and derivatization followed by GCxGC-HR MS allowed the detailed analysis of hydrocarbons, free sterols, linear alcohols, diterpene alcohols, and vitamins in virgin olive oils.

In another study, the elucidation of animal tissue lipids by an unconventional GCxGC setup was carried-out.⁵⁶ The authors exploited online hydrogenation during the GCxGC separation, for the reduction of lipids extracted from beef meat. The online hydrogenation added a degree of selectivity that generated uncommon group type separations on the 2D plane (Figure 5). In particular, fatty acids methyl esters (FAMES) and dimethyl acetals (DMAs) are typical acid-catalyzed methanolysis products from acyl lipids and enol ether lipids. As can be seen in Figure 5, the FAMES eluted on straight lines parallel to the ¹D time axis, each defined by the ²D retention time of a fully saturated FAME. Saturated DMAs eluted on another straight line bisecting the plane, characterized by a lower angular coefficient with the x-axis compared to the one of saturated FAMES. Similarly to FAMES, also DMAs differing for the number and/or position of double bonds were eluted on the same straight line parallel to the ¹D time axis, defined by the ²D retention time of the saturated form. FAMES and DMAs eluted as two independent sets of analytes with no co-elutions, and DMAs eluted below the separation space characteristic of FAMES.

A growing use of GCxGC is observed for some food safety applications, for example, the migration of packaging material into foodstuff. In this context, GCxGC is increasingly used as a complementary tool for a deeper understanding of the contamination source, which otherwise would be masked in conventional separations. For example, Biederman *et al.* compared two strategies to remove polyolefin interferences for mineral oil aromatic hydrocarbons (MOAH) determination in vegetable oils.⁵⁷ They increased chemically the polarity of olefins via epoxidation and used a polar×non-polar column configuration to enclose the interferences into specific elution zones (Figure 6).

The information acquired with the better understanding of plant metabolites can find practical application in the storage conditions,⁵⁸ cannabis plant variety discrimination,⁵⁹ or metabolism alteration.⁶⁰

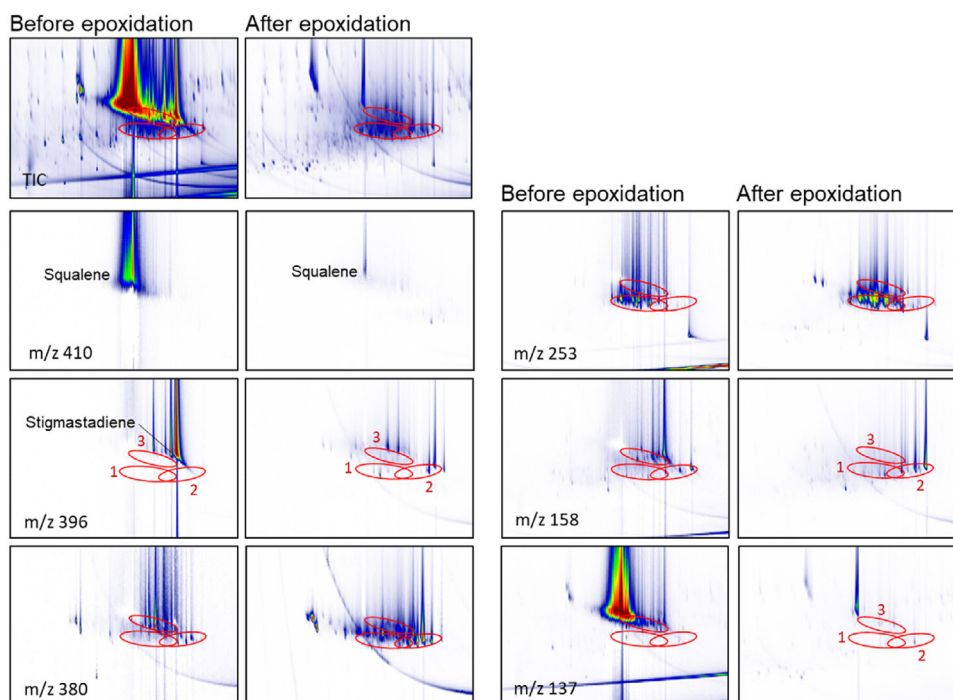


FIGURE 6 GCxGC-MS plots of the MOAH fraction from refined hazelnut oil before and after epoxidation: total ion chromatogram (TIC) and selected ions considered of interest. Framed in the ellipses, the three fields of interfering material in the MOAH fraction. Reproduced with permission from 57

Risticvic *et al* reported an *in vivo* solid-phase microextraction (SPME) sampling method for apples, in which the power of GCxGC resulted essentially for unraveling the complex diversity of metabolites.⁶⁰ They noticed the occurrence of metabolites known to be volatile end-products of lipid peroxidation in the *ex vivo* metabolic profiles, which are not present when sampling *in vivo*. This observation suggests that rapid degradation of metabolome integrity may be encountered in metabolomics studies that employ traditional methods of sample preparation.

4.3 | Biological applications

In the biomedical field, GCxGC is mainly used to identify novel biomarkers for disease diagnostic and prognostic, but also to fingerprint and characterize complex biological matrices (biological fluids, tissues, and biopsies), pathogenic microorganisms, and metabolic processes in cellular physiology associated to altered health status.

Over the past year, studies have been mainly oriented towards the profiling of biological fluids and microorganisms for biomarker discovery,^{61–65} and the fingerprinting of biochemical and physiological pathways resulting from altered conditions.^{66–69}

The volatilome of *C. vaccinii* was studied in monoculture and in co-culture to identify potential biogenic compounds inhibiting fungal growth, which could be beneficial for a wide range of applications. The characterization of *C. vaccinii* (wild-type MWU328 and mutant MWU328W) and of the fungus *Phoma sp.* using stir-bar sorptive extraction (SBSE) GCxGC-MS permitted the identification of 53 volatile

organic compounds (VOCs), among more than a thousand detected, characterized by a two-fold higher concentration than the media control. In their study, the authors highlighted that the volatile compounds emitted from the co-culture were not a linear combination of the bacterial and fungal VOCs. Indeed as can be seen in the principal component analysis of Figure 7, the co-cultures are not clustering between the fungal and bacterial monocultures, and thus cannot be predicted by the sum of these latter. In addition, three compounds (1-octanol, a carboxylic acid, and an unknown), were identified as specific inhibitory VOCs consistently produced by the wild-type *C. vaccinii*, detectable in the fungal co-culture, and absent in the fungal monoculture, which holds the potential to control >80% of fungal growth.⁶³

Mead *et al.* made use of GCxGC-MS in addition to RNA sequencing to identify active pathways in wild-type *Coccidioides posadasii* responsible for endospores formation and their release into the host causing respiratory infection. The comparison of the wild-type strain and the attenuated mutant strain used to produce the vaccine revealed 40 VOCs specifically produced (three-fold net production from the media blank) by the wild-type strain and 90 VOCs specifically consumed (five-fold net consumption) by the mutant strain. Among the produced VOCs in the wild-type strain, nitrogen-containing compounds were identified, which is in agreement with the up-regulation of transcripts for nutrient assimilation specific to nitrogen. Such up-regulation implicates that nitrogen co-factor is necessary to create and/or release endospores.⁶⁸ In another study, GCxGC enabled the identification of 97 compounds characteristic of the response of lung epithelial cells submitted to oxidative stress and biological inflammation, employing hydrogen peroxide and a pool of inflammatory sputum collected from

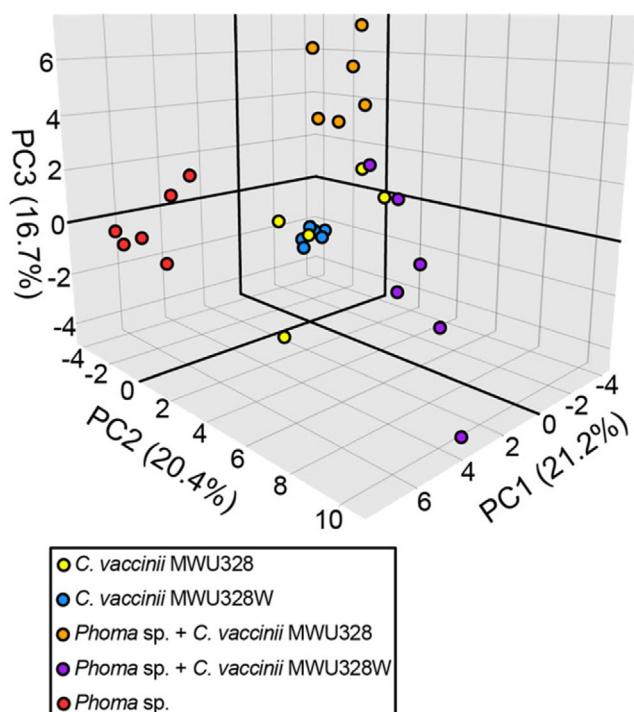


FIGURE 7 Principal component analysis score plot of monocultures of *Phoma* sp. (red), *C. vaccinii* (yellow), and *C. vaccinii* quorum sensing (QS) mutant (blue), and co-cultures of *Phoma* sp. and *C. vaccinii* (orange), and *Phoma* sp. and *C. vaccinii* QS mutant (purple) based upon 53 biogenic VOCs

asthmatic patients, respectively.⁶⁶ Thirty-five of these compounds, being mainly hydrocarbons and carbonyl compounds (aldehydes and ketones), were produced only after the induced biological inflammation. The exposition of lung epithelial cells to non-inflammatory sputum from healthy patients resulted in a similar volatile profile as the non-treated cells. In addition, the comparison of the chemical and biological inflammation highlighted only a 27% overlap of the altered compounds, featuring the complexity of the inflammatory mechanisms occurring in the lung of asthmatic patients.

Volatile compounds emitted from the skin surface present great potential for studying the skin microbiota, for diagnosis, and more largely in the metabolomics field. Wooding *et al.* developed bracelets and anklets made of a polydimethylsiloxane (PDMS) sorbent to sample VOCs from the skin of 20 human volunteers. Thermal desorption of the bracelet directly into the inlet of the GC×GC enabled the separation and detection of 69 compounds belonging to a wide range of chemical families.⁶⁵ Biological fluids were also used to monitor human exposure to chemical contaminants such as halogenated compounds. Tran *et al.* made use of untargeted GC×GC-MS analysis to characterize contaminants present in human breast milk.⁷⁰ In their untargeted analysis, they detected 172 potential contaminants in the lipid and/or water fractions of the breast milk, among which 40 were detected in all the samples. The mass spectra of 22 compounds out of the 40 revealed halogenated isotopic patterns characteristic of anthropogenic halogenated contaminants. Regulated persistent organic pol-

lutants such as p,p'-DDT, p,p'-DDE, PCB-153, BDE-47, HCB, and beta-HCH were detected together with 6 halogenated compounds, such as N-(4-Chlorophenyl)formamide, and 1-(4-chlorophenyl)pyrrole that are not regulated. This study demonstrated the potential of GC×GC-MS for biomonitoring human exposure to emerging and/or undiscovered contaminants.

4.4 | Environmental applications

Although nowadays many contaminants, such as pesticides, halogenated compounds, and polycyclic aromatic hydrocarbons, are recognized as priority pollutants regulated under environmental laws, contaminants of emerging concerns are not currently under any regulations but might be under investigation for future regulation. In such context, advanced separation methods such as GC×GC presenting enhanced peak capacity and sensitivity are preferable to monitor multi-class contaminants in diverse and complex environmental samples such as water,^{71,72} wastewater,^{73,74} aerosol,^{75–77} and oil.^{78,79}

Meza *et al.* used GC×GC to characterize biologically active micropollutants in hospital wastewater and to evaluate the efficiency of the wastewater treatment for such pollutants removal.⁷³ The analysis of the liquid-liquid extracts enabled the identification of 27 contaminants acting on bioreceptors such as the Androgen and Estrogen Receptor. The authors highlighted that the wastewater treatment using MnOx-coated coir fiber enabled to remove 19 micropollutants by >90%. In addition, the 2D chromatogram obtained from the analysis before and after treatment can directly inform on the efficiency of the wastewater treatment using pattern recognition. As can be seen in Figure 8, polyalkylene glycol monoalkyl ethers were not detected anymore in the total ion chromatogram obtained from the wastewater after treatment (bottom).⁷³

In another study, the coupling of SBSE with GC×GC was investigated for multiclass organic contaminants characterization in wastewater.⁷⁴ Compared to classical liquid-liquid extraction, similar extraction efficiencies were obtained for influent wastewater. However, greater sensitivities were obtained using SBSE-GC×GC in dilute samples, that is, effluent wastewater, thanks to the high concentration factor obtained with SBSE. This study pointed out the potential of SBSE-GC×GC to go toward greener and more automated qualitative analysis of contaminants.⁷⁴

Crucello *et al.* developed a fully automated method for the characterization of polychlorobiphenyls (PCBs) in complex matrices such as insulating oil, soil, and water. Flow modulated GC×GC, using a mid-polar×non-polar ionic liquid (IL) based column set, coupled to electron capture detection enabled the accurate quantification of PCBs (1–5%RSD) in oil, while being time- and cost-effective because no sample preparation was necessary.⁷⁸

The transport and accumulation of atmospheric aerosols were studied by Mazur *et al.* who analyzed Arctic snow by GC×GC-HR MS. Among the 100 compounds detected in the snow samples, only few priority pollutants (dimethyl-, diethyl-, and dibutylphthalate) were identified. However, the authors detected several fatty amides, with

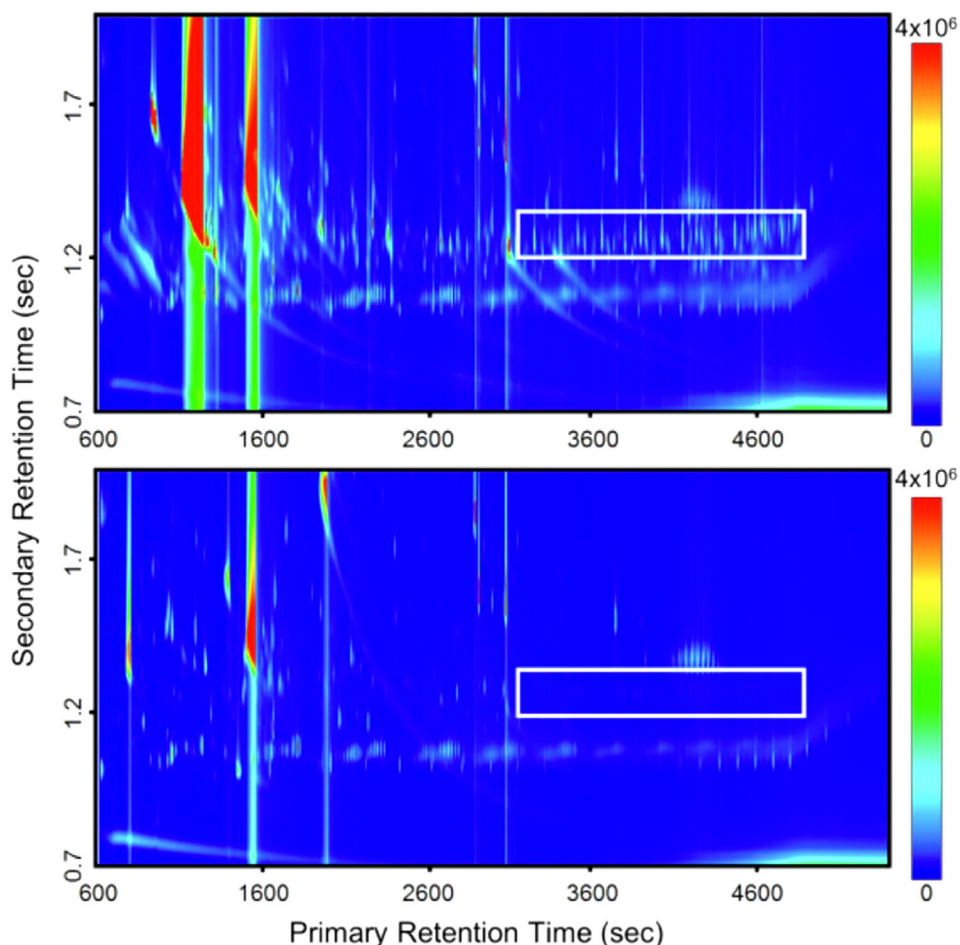


FIGURE 8 Total ion chromatogram (TIC) of hospital wastewater before treatment (top) and after 24 h reaction with MnOX-coated coir fiber (bottom). The rectangle in both chromatograms corresponds to polyalkylene glycol monoalkyl ethers region. Reproduced with permission from [73](#)

oleamide being the major component, resulting from biomass burning or plastic pollution. In addition, N,N-dimethylcyclohexylamine and N,N-dimethylbenzylamine were identified thanks to the accurate mass measurement of the molecular ion and the structurally important fragment ions by the high-resolution MS, informing on long-distance transport of pollutants depositing with the snow.⁷⁵

4.5 | Other applications

The increased separation power of GC×GC made it a useful technique to fingerprint and characterize volatile compounds from other application fields (forensic, microplastics, etc.) and complex matrices (for example, smoke, ignitable liquids, debris, etc.). Several hundreds of volatile compounds, comprising mainly hydrocarbons and oxygenated compounds, were identified in the mainstream of marijuana and tobacco smoke, revealing compounds associated with high health risks and toxicity which could be further targeted in health studies.^{80,81} Death-related VOCs were also studied using GC×GC and novel sampling phases (zwitterionic IL-based) for SPME with GC×GC enabled the reproducible (<18%RSD) extraction and determination of polar VOCs characteristics of body decomposition at the ng level.⁸²

The separation power of GC×GC-FID was demonstrated particularly valuable to profile fresh and weathered ignitable liquids⁸³ and, when coupled to MS, to identify traces of ignitable liquids in complex wildfire debris.⁸⁴ Increased identification confidence of arson was obtained thanks to the separation in the 2D space of natural interferences, such as pinene, camphene, and verbene, and characteristic ignitable liquids compounds (*n*-propylbenzene, 3-ethyltoluene, 4-ethyltoluene, 1,3,5-trimethylbenzene, and 2-ethyltoluene).

Finally, the technique also proved to be valuable as a novel method to identify illegal wildlife trafficking.⁸⁵ In their study, the authors showed that headspace SPME-GC×GC-MS enabled the accurate characterization of 85% of wildlife contrabands such as ivory, bones, and teeth; therefore highlighting the potential of GC×GC as nondestructive screening method.

5 | SUMMARY AND OUTLOOK

In the year of its 30th anniversary, GC×GC still experiences instrumental and software refinements and developments, and the latest advances appear to be in line with the evolution observed in the past decade, with the progression of comprehensive GC×GC into

multiple fields and more articulated research questions, determining a vast breadth of applications (Figure 1). In terms of instrumental advances, a few novel flow modulator designs were introduced this past year. This form of modulation still belongs to a small portion of the total GC×GC practice (16% of the reviewed papers), but thanks to the continuous developments and commercial availability, an increase in its deployment is to be expected in the future.

One of the biggest challenges still remains the data analysis and handling, and the extrapolation of useful information from the information-rich 2D chromatograms, especially in untargeted analysis.

The MS detection still represents and certainly will be an important ally for GC×GC to continue its journey through the maturation, expansion, and establishment of such a high-resolution multidimensional technique.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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