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REVIEW

Modulation in comprehensive two-dimensional gas chromatography: 20 years of innovation

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Abstract With almost 20 years having passed since John B. Phillips described the first comprehensive two-dimensional gas chromatography (GC×GC) separation, much has occurred in this ever-expanding field of separation science. GC×GC is currently one of the most effective techniques for the separation and analysis of complex mixtures, offering significantly greater peak capacities than conventional chromatographic methods. The technique is generally based upon separations performed on two chromatographic columns characterized by considerably different selectivities, joined together through a modulating interface. The modulator periodically traps or samples the primary column effluent, usually refocuses it into a narrow chromatographic band and injects the focused fraction into the secondary column. The modulator is often referred to as the 'heart' of the instrument, since a GC×GC separation is impossible without its use. This article reviews major innovations in GC×GC modulator development since its first use by Phillips in 1991. Emphasis has been placed on modulator design and function.

Keywords Comprehensive two-dimensional gas chromatography · Instrumentation · Modulation · Review

Abbreviations

DVM	diaphragm valve modulator
GC	gas chromatography
GC × GC	comprehensive two-dimensional gas chromatography
LMCS	longitudinally modulated cryogenic system
PDMS	polydimethylsiloxane
RTM	rotating thermal modulator
TDM	thermal desorption modulator
VOC	volatile organic compounds

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Introduction

The separation and quantification of compounds contained within increasingly complex mixtures is an ongoing challenge for the analytical chemist. The individual aspects of a typical analytical procedure, which generally includes sampling, sample preparation, separation and detection, are under continuous improvement. Special emphasis is often placed on the separation stage, as it is frequently the limiting factor in a complete analysis of complex chemical mixtures. Chromatography is currently the most valuable tool available for this task. Techniques such as gas chromatography (GC), high-performance liquid chromatography and capillary electrophoresis are routinely used in a wide variety of analyses. These separatory methods tend to rely upon one-dimensional separation, utilizing a uniform separation mechanism throughout. The ability of a chromatographic system to separate the individual constituents of a mixture is dependent on its peak capacity, which can be described as the number of individual components that can be placed, side by side, as single entities, within the separation space [1]. Ideally, the peak capacity of a column far exceeds the number of individual analytes in the mixture, but this is rarely ever the case. In reality, many samples are too complex for conventional separations and

peak capacity is often far exceeded. This results in peak overlap, which decreases the quality of the analysis [2]. Analytical challenges such as this are encountered often in the food, flavour and fragrance industries, in medical, pharmaceutical and forensic sciences, and in the petroleum and environmental sciences. Sensitive and selective trace analyses of target analytes within complex matrices, and full characterization of unknown compounds, are routinely performed within these fields [3].

The development of multidimensional chromatographic separations, the principles of which were described by Giddings [4] in his 1984 article entitled 'Two dimensional separations: concept and promise', was an important evolution for the separation sciences. The oldest, non-comprehensive multidimensional chromatographic separation techniques were termed 'heart cutting'. These techniques involved sampling a fraction of the effluent from one column and subsequently injecting it into another column with a differing selectivity. Heart cutting served to increase the selectivity and peak capacity over those of traditional GC by subjecting the sample effluent to a second separation dimension. This method proved to be effective only in target analysis, where information regarding one or several individual fractions, and not the entire sample, was required. The solution for those interested in separating an entire sample in two dimensions was to perform a comprehensive multidimensional separation. Giddings [4, 5] described a comprehensive multidimensional separation as one in which an entire sample is subjected to all separation dimensions, with further separation being achieved without the degradation of separations obtained in the previous dimension.

The first comprehensive two-dimensional GC (GC × GC) separation was performed by John Phillips [6] in the early 1990s. Noted as perhaps the most promising innovation in GC since the invention of the capillary column, GC × GC has evolved to become one of the most powerful separation methods available. Its use in many sectors of research and industry is a testament to the improved peak capacity, resolution, selectivity and sensitivity this technique provides [3]. The key to any quality GC × GC separation is the successful transfer of the effluent from the primary column to the secondary column while maintaining the separation achieved in the first dimension. For this task, a modulator or column interface is required. The modulator's role is to trap or sample the primary column effluent, usually refocus it into a narrow band and inject the focused fraction into the secondary column. The modulator is often referred to as the 'heart' of the system, since a GC × GC separation is impossible without its use. This article reviews GC × GC modulators development since their first use by Phillips [6] in 1991. All major contributions to modulator development are given consideration, with particular emphasis placed on modulator design and function.

The GC × GC interface

Principles of modulation

According to early interpretations of the Giddings definition, multidimensional GC separation qualified as comprehensive only if two requirements were met: (1) separation achieved in each dimension was preserved until the analysis was complete, and (2) complete transfer of the effluent from each dimension was accomplished [4, 5]. In the light of this interpretation, early work involving valve-based modulators did not qualify as comprehensive, as only small fractions of the primary column effluent were introduced into the secondary column. Following extensive discussions at the First International Symposium on Comprehensive Multidimensional Gas Chromatography held in 2003 in Volendam (The Netherlands), the GC × GC community agreed that those separations qualified as comprehensive as long as the resulting chromatogram was representative of the entire sample. The significance of modulation methods based on incomplete transfer of the primary column effluent to the secondary column has been decreasing steadily over the years; hence, in the following discussion focus will be mainly placed on modulators ensuring complete analyte transfer. However, the reader needs to keep in mind that in some cases the description does not apply to certain modulators, especially valve-based ones.

A GC × GC modulating interface is placed at the end of the primary dimension, at the beginning of the secondary dimension or between the primary and the secondary dimensions. Modulation can be defined as the periodic sampling, focusing and injection of primary column effluent into the secondary column. In the absence of a modulating device intermittently sampling the first dimension, chromatographic peaks of compounds that were previously separated on the primary column could overlap and the compounds could be coeluted at the detector. This would compromise the integrity of the first-dimension separation, thereby violating a key GC × GC requirement (Fig. 1). For modulation to be effective, it should occur frequently throughout the entire analysis. Murphy et al. [7] determined that each compound eluted from the primary column should be sampled between three and four times to preserve the first-dimension separation. The modulator must also be capable of preventing peak breakthrough, a condition whereby primary column effluent evades the trapping function of the modulator and is eluted unfocused into the secondary column. Breakthrough compromises the integrity of the second-dimension separation and should be avoided.

Peak wraparound occurs when analytes are retained strongly enough in the second dimension to be eluted with second-dimension retention times longer than one modulation period. This results in such analytes potentially being coeluted

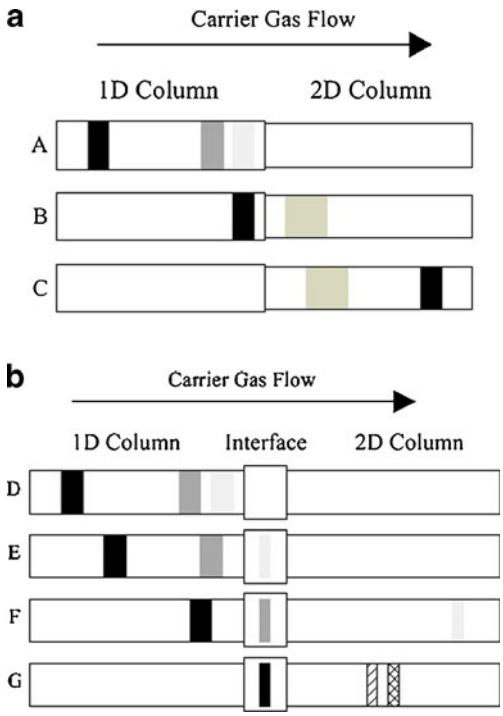

Fig. 1 **a** Without a modulator present between the primary and secondary columns, analytes separated in the primary column might be coeluted at the outlet of the secondary column (*A*–*C*). **b** A modulator between the primary and the secondary columns prevents coelutions of previously separated analytes (see the text)

with components of the subsequent fraction and appearing on the two-dimensional chromatogram in misleading locations.

Timing is a crucial factor in GC × GC separations, as the onset of operation of the interface must be coordinated with the GC to initiate the modulation sequence at precisely the same time during every analysis, and the modulation period must be strictly defined and reproducible throughout the run. Raw data collected from a GC × GC separation are in one-dimensional format prior to transformation. Computer software must be used to process this conventional chromatogram along with the modulation events to create a meaningful two-dimensional chromatogram (Fig. 2). Consistent reproducibility of chromatographic settings between runs allows the generation of reproducible chromatograms, with peaks appearing at the same locations in both dimensions. Designing the perfect modulator is clearly a challenging venture, as many have learned over the span of 20 years. This article traces the development of GC × GC modulators, separated into two distinct categories: thermal and valve-based models.

Thermal modulation

Heater-based interfaces

Thermal modulators can be further divided into heater-based interfaces and cryogenic-based interfaces. Heater-based interfaces trap primary column effluent at or above ambient temperatures, usually with the help of thick stationary phases. Cryogenic interfaces trap primary column effluent below ambient temperatures with the use of various cooling mechanisms.

Thermal desorption modulator

The two-stage thermal desorption modulator (TDM) was designed and implemented by John Phillips in 1991 and has the distinction of being the interface that allowed him [6] to perform the first truly comprehensive GC × GC separation that same year. First designed as a single-stage sample introduction device in multiplex and high-speed GC, this simple device was applied to GC × GC with very little alteration to its original design [8, 9]. The GC × GC system used by Phillips utilized a 250 μm inside diameter, 21 m long open tubular primary column with 0.25-μm Supelcowax-10 stationary phase, and a 100 μm inside diameter, 100 cm long open tubular secondary column with 0.5-μm 007 methyl silicone stationary phase from Quadrex. The two columns were joined together by shrinkable Teflon tubing along with a small splitter to adjust the flow rate through the secondary column. Electrically conductive gold paint was applied to a segment at the beginning of the secondary column, which was looped outside the oven and maintained at room temperature. The electrical resistance of the film was calculated after each coat of paint until a desired resistance was achieved. The total length of the modulating section was 15 cm. Electrical leads connected to the column provided current from a 40-V DC power supply to either end of the interface. The interface is shown in Fig. 3. As effluent from the primary column (a in Fig. 3) entered the secondary column (b in Fig. 3), it would become trapped and focused by the stationary phase coating. Electrical current was then applied to the first stage of the trap (S1 in Fig. 3) through leads L1 and L2 in Fig. 3. This caused rapid heating of the gold paint layer (c in Fig. 3), forcing analytes trapped within the capillary to partition into the carrier gas. Once in the mobile phase, the effluent was swept to the second stage (S2 in Fig. 3) of the capillary, where it became trapped once again. At this time the first stage of the modulator had cooled and would continue its trapping function, as the second stage was pulsed through leads L2 and L3 in Fig. 3, injecting the trapped components as a narrow band. The pulses were applied to each stage 100 ms apart for a duration of 20 ms. Modulation occurred every 2 s. The experimental results collected were acceptable, but left much to be desired. Wider than preferred injection pulses and component overlap were the main problems. These issues were recognized by Phillips and the dual-stage TDM was modified by increasing the length of the first trapping

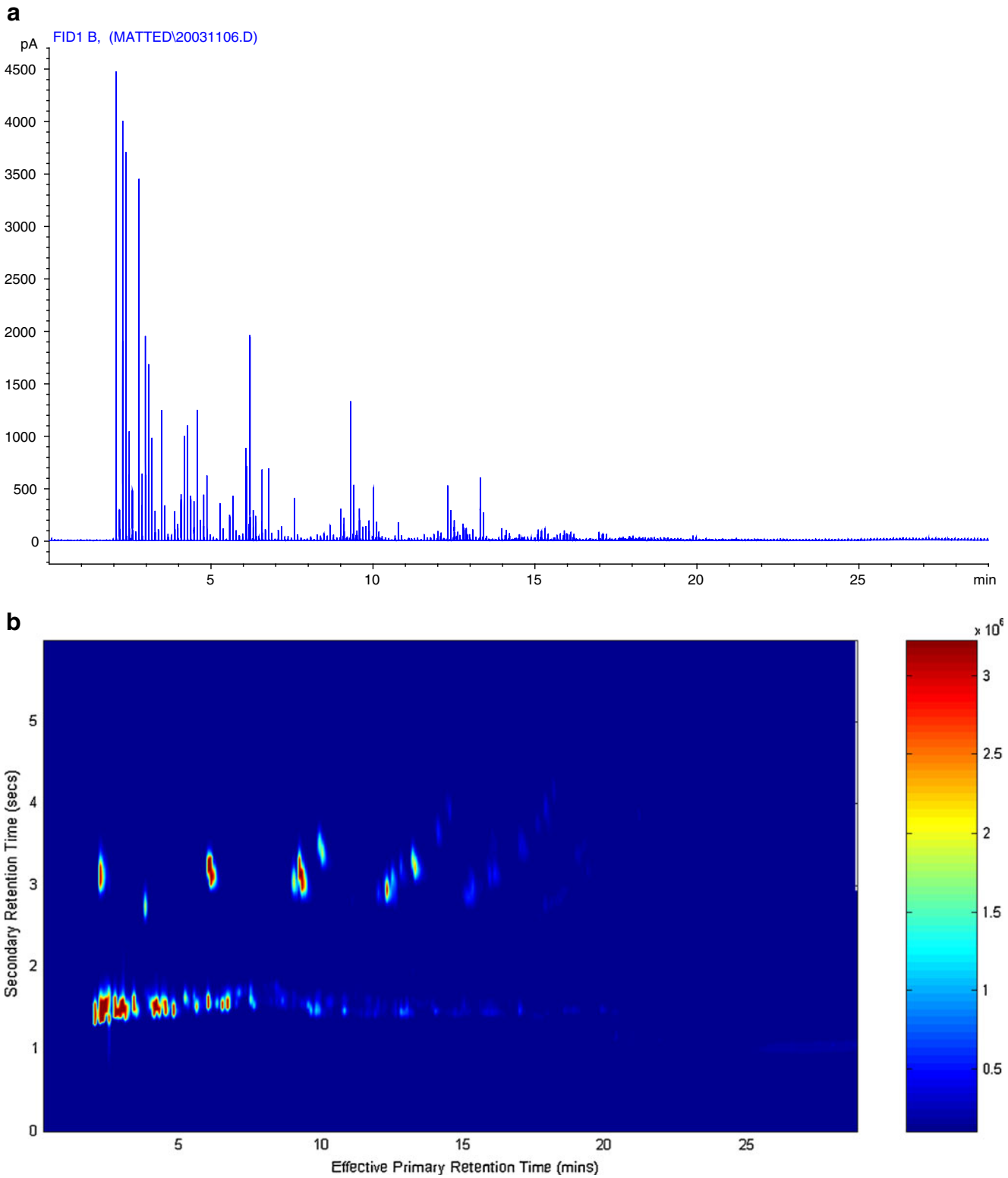

Fig. 2 Raw one-dimensional record of a two-dimensional separation of regular unleaded gasoline (*top*). The same separation transformed into a two-dimensional chromatogram (*bottom*)

stage, as well as by increasing the time between electrical pulses. It was shown that as a result of these modifications, sample breakthrough in the first stage was minimized, and the shorter second stage provided satisfactory focusing and injection of compounds into the secondary column [10–12]. Although the TDM was vital in achieving the first fully

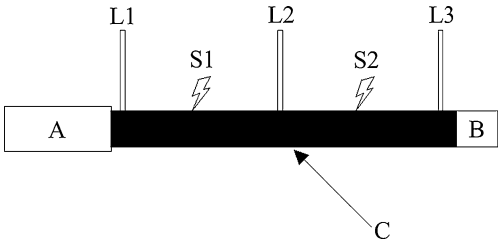

Fig. 3 The original thermal desorption modulator. The primary column (**a**) is connected to the secondary column (**b**). Gold paint (**c**) is applied to the beginning segment of the secondary column. Electrical leads (*L1*, *L2*, *L3*) allow current to flow alternately between stage one (*S1*) and stage two (*S2*) during modulation. (Based on [6])

comprehensive GC × GC separation, the gold-painted capillaries were not very robust and often required replacement [13].

An attempt was made at improving Phillips's design by de Geus et al. [14] in 1997 by evaluating a single-stage version of the TDM. The focus of this group was to increase the longevity and improve the modulation function of the TDM by experimenting with different electrically conductive materials applied to the outside of the capillary, as well as optimization of parameters such as voltage, pulse length and pulse interval. Some of the electrically conductive materials tested included an acryl-resin-based coating containing metallic silver, pure silver and pure aluminium that were applied through a metal evaporation technique, and copper wire that was tightly coiled onto the capillary surface. The authors found that pure-aluminium- and pure-silver-plated modulators outperformed their metallic painted counterparts, but were inferior to the copper coiled interface, which proved to be the most robust. Unfortunately, this design suffered from large power requirements because of its large thermal mass. The authors also showed that pulsed heating prevented the modulator from reaching undesirably high temperatures and was a good alternative to constant heating

Although the TDM was made more reliable, Phillips and his group had begun devising a new mechanical version of the resistively heated modulator referred to as the rotating thermal modulator (RTM). Development of resistively heated modulators did not stop here however, as the benefit of no moving parts and the simplicity of resistive heating were very attractive features that drew the attention of some researchers to this method of modulation. In 2000, Lee et al. [15] compared various modulating interface technologies and sought to use a new type of interface material, stainless steel capillary. These capillaries were coated internally with 3-μm polydimethylsiloxane (PDMS) and used in the same fashion as the previously described two-stage TDM. When compared with metal-paint-coated fused silica modulators, the stainless steel interface produced significantly broader peaks, although the superior mechanical properties of the material improved the robustness of the device.

An interface designed by Burger et al. [16] utilized a stainless steel capillary jacket in which a thick-film fused silica capillary was housed. Attached to the jacket through spot welds were a series of stainless steel connectors. Electrical charge could be supplied to each of these connectors individually through copper leads and an electrical sequencing system, thereby heating small sections of the capillary in sequence and consequently shuffling analytes down the column. The authors used this system successfully for 3 years without any modulator burnout and obtained pulsed widths at half height on the order of 145 ms, a result comparable with results for other TDM systems. Stainless steel capillary columns helped to improve the performance of resistively heated modulators, but also signalled the end of improvements that would be made to this style of interface for some time.

Rotating thermal modulator

The development of alternative methods of thermal modulation began shortly after the TDM had been proven as a functional GC × GC interface. Phillips and Ledford [17] described the theory behind the RTM (first introduced in 1996), also known as the 'sweeper', as well as its potential to improve field-portable GC instruments. It was not until early 1999 when its construction was described in detail along with 2 years of prototype performance results [18]. The RTM had four main functions: accumulate (a in Fig. 4), cut (b in Fig. 4), focus (c in Fig. 4) and launch (d in Fig. 4) [18]. As components exited the primary column, they would start to accumulate in the thick stationary phase of the modulator capillary (a in Fig. 4). The RTM cycle would be engaged via computer control, causing the slotted heater, which was kept at around 100°C above the GC oven temperature, to begin to rotate. The heater would move over the upstream portion of the capillary, rapidly heating a zone that moved along the tube at the speed of the heater's rotation, in the direction of the carrier gas. Analytes trapped in the capillary were instantly mobilized into the carrier gas by the moving heat zone, resulting in the creation of a thin 'slice' of accumulated components (b in Fig. 4). Flowing with the carrier gas, this fraction would not migrate far before becoming trapped and focused once again into a section of the capillary not yet exposed to the slotted heater (c in Fig. 4). As the heater continued its rotation, the moving heat zone propelled the focused analyte band, ultimately launching it out of the capillary, into a short section of uncoated capillary and onto the secondary column (d in Fig. 4). The trap cooling time was reduced by applying forced air cooling to the interface. The RTM prototype initially worked quite well in two of the five laboratories in which it was first tested [18]. Apart from its

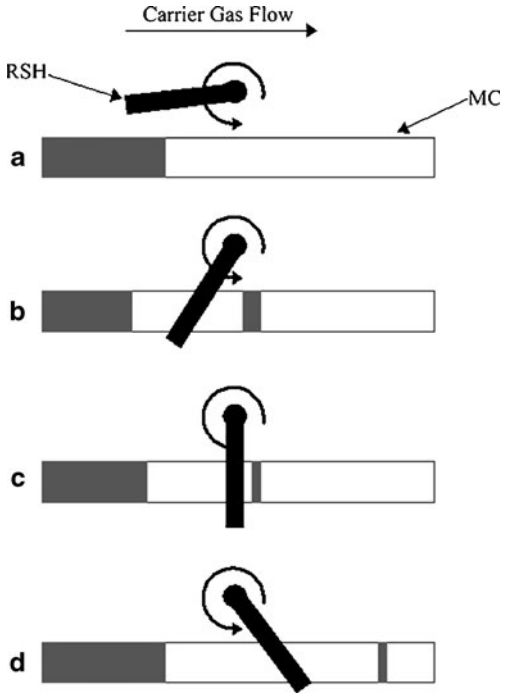

Fig. 4 The rotating thermal modulator . The rotating slotted heater (*RSH*) periodically passes over the modulating capillary (*MC*) as four main functions occur: accumulate (**a**), cut (**b**), focus (**c**) and launch (**d**). (Based on [18])

early success, the RTM experienced several technical issues related to keeping the capillary aligned between the slotted heater and preventing mounting hardware from experiencing thermal expansion at high oven temperatures. A temperature differential of about 100°C was also required for proper launching of the analyte bands, meaning that compounds with higher boiling points often had to be excluded from the analysis [13]. Even with some significant technical issues, the RTM became the first modulator to be commercially available and successfully functioned in many applications, such as analysis of chlorinated biphenyls and toxaphene [19], semivolatile aromatics [20] and other complex organic samples [21–26]. Dalluge et al. [27] reported that use of the RTM was reported in approximately 30% of all articles on GC × GC published before 2003. Its applicability to a wide range of samples, particularly those with very low or very high boiling points, was questioned by those experimenting and reviewing a new type of thermal modulator, the longitudinally modulated cryogenic system (LMCS) [27–31]. This was disputed by other researchers who were intimately involved with the RTM and had demonstrated its applicability in many analyses, albeit with a fair amount of customization [25, 26, 32–35]. The RTM is today considered inferior to other methods of modulation and is no longer commercially available.

Cryogenic interfaces

Longitudinally modulated cryogenic system As Phillips was developing thermal desorption methods of modulation, Marriott and coworkers were engineering a new kind of thermal modulator that utilized a cryogenic agent to trap analytes. Whereas both the TDM and the RTM trapped analytes at oven temperature and injected them at higher temperatures, cryogenic modulators trap analytes at temperatures significantly lower than that of the oven, and inject compounds into the second dimension at or close to the oven temperature. Marriott's first cryogenic modulator was referred to as the longitudinally modulated cryogenic system (LMCS) [36]. This design was based on the group's previous work on cryogenic trapping of solutes as a means of narrowing the chromatographic bands to provide sharper elution profiles in GC [37]. Their first cryogenic trap was constructed from two steel tubes of differing lengths and inside diameters that formed a cavity; a cryogen could be pumped into and out of this cavity. The analytical GC column was placed within this trap approximately 40 cm from the detector. Liquid CO_2 was pumped through the trap while compounds of interest were being eluted. The flow of the cryogen was then shut off, allowing the cooled capillary segment to return to oven temperature, thereby releasing any trapped analytes as focused bands into the detector. It was found that a GC analysis of *n*-alkanes (C_{13}–C_{16}) utilizing this trap produced greater sensitivity and lower detection limits than conventional GC. The main disadvantage was the time required for the capillary to reach temperatures high enough for effective desorption of trapped analytes. Their solution to this problem was to expose the portion of the capillary where components were trapped to the elevated temperatures of the GC oven, allowing desorption of analytes to occur while trapping continued upstream of the desorbed peak. In this way, the LMCS was born (Fig. 5) [36]. Modulation with this device was accomplished by moving the cryogenic trap longitudinally along the column towards the detector to trap components (a in Fig. 5) or away from the detector to release them (b in Fig. 5).

The LMCS was applied to GC × GC for the first time in 1998, and the separation of a commercially available kerosene sample was briefly described by Marriott and Kinghorn [38] in a short communication. Little modification from the original design was made and the authors demonstrated that the LMCS was a viable alternative to the RTM, if not a superior method of modulation. Some of the benefits highlighted included no need for a separate modulator capillary, the independence of oven temperature and modulator temperature (a major issue with the RTM) and the use of a single column connection. The LMCS proved to be applicable to other GC applications as well [39, 40]. After several years of use, the design of the LMCS

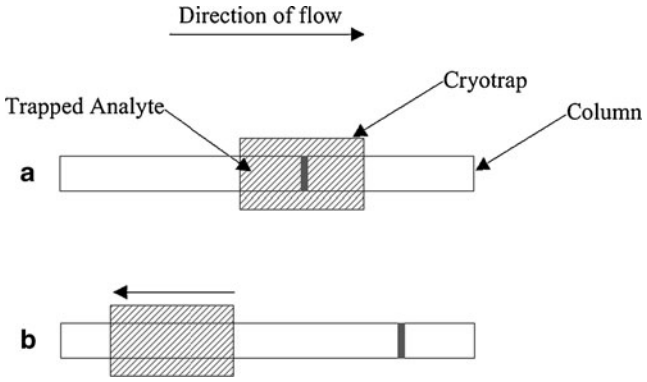

Fig. 5 The longitudinally modulated cryogenic system. Analytes travelling through the column become trapped in the segment of the column cooled by the cryotrap (**a**). Longitudinal movement of the cryotrap away from the detector releases the trapped segment as a narrow focused band (**b**). (Based on [36])

was improved to deal with several operational issues such as trap-column freezing, temperature control and inconsistent functionality during extended use [41]. A high-speed, electrically controlled, pneumatic ram that quickly moved the trap away from the cold zone of the column in less than 10 ms was installed. This exposed the cooled portion of the column much faster than in previous LMCSs and allowed for rapid heating of the column. The trap was modified to include a port for gaseous N_2 and was secured to control guides within the oven to ensure precise movement along the capillary column. The addition of gaseous N_2 helped prevent ice buildup within the trap. It was also reduced in size from 5 cm to 2.8 cm. The overall performance of the system was improved by utilizing a digital timer, controlled by computer software, to direct trap movement and control CO_2 valve operation. The authors studied the effect of this device on solute trapping and injection in great detail, evaluating the distribution of solute within the trap, the speed and time of remobilization of peaks, as well as its applicability to various GC × GC problems. The LMCS was arguably the biggest innovation in modulation technology since the RTM developed by Phillips. The LMCS was used extensively by the authors, as well as other scientists, to improve the quality of GC × GC separations, thereby bringing attention to this still new and relatively obscure method for separation of complex mixtures [42–44]. It was estimated that by 2004, cryogenic modulators had likely replaced almost all of the TDMs in use [45].

Post-LMCS advancements in cryogenic interfaces Although the LMCS was a significant improvement over thermal modulators, it was only able to cool the trap to a temperature of approximately −50°C, which was insufficient for trapping volatile organic compounds (VOCs). It also utilized a series of moving parts, an undesirable feature for long-term, maintenance-free use. Thus, the next stage in the evolution of cryogenic modulators was to utilize more effective cryogens and minimize the use of moving parts. One such device was developed by Ledford [46] in 2000. It utilized two liquid CO_2 cold jets and two hot gas jets. This design was based on earlier work of Ledford and Billesbach [47] using an RTM that was cooled between sweeps by a jet of liquid-N_2-cooled gas. As analytes exited the primary column, they were trapped in a cold spot created by the first (upstream) cryojet of the modulator. The first-stage hot jet and second-stage cold jet would then be engaged simultaneously, transferring trapped analytes to the second focusing stage of the modulator. To inject the focused chromatographic bands, the second hot jet was engaged and desorption of trapped analytes occurred. To prevent breakthrough while the second stage was hot, the first cryojet was engaged as injection occurred. Cryogenic modulation would evolve once again with Beens et al. [48] developing a system that utilized two liquid CO_2 cryojets for trapping, and ambient oven heat for analyte desorption and injection. This was a simplified version of Ledford's four-jet model and proved to be one of the most effective modulators at the time. This design would be modified further by the same group by using a single liquid CO_2 cryojet, single-stage modulator [49]. As component breakthrough had been recognized as the main issue with single-stage modulation systems, the authors evaluated this problem by studying the characteristics of the second-dimension peak as a function of the CO_2 valve switching times. Peaks of quality almost identical to those obtained with the dual-cryojet system were achieved, and the single-jet/single-stage system was deemed a success [49]. Another interface, developed by Harynuk and Górecki [50], utilized Silcosteel capillaries contained within a cryochamber, cooled with liquid N_2, to trap and freeze analytes being eluted from the primary column. Reinjection was achieved by resistively heating the Silcosteel capillaries, in single-stage or dual-stage modes. This modulator was effective in trapping VOCs and provided precise control over injection timing. The main drawback of this system was that liquid N_2 leaks often developed at the capillary entrance and exit locations in the cryochamber. This issue led to cold spots along the trap, and as a consequence band broadening occurred.

Ledford et al. [51] designed an alternative version of the single cryojet system that exploited a looped capillary to enable dual-stage modulation by cooling two segments of the same loop. This system was investigated in detail by Gaines and Frysinger [52] and was also made commercially available [53]. As analytes travelled out of the primary column, they would become trapped at the upstream cooled position on the capillary. The cryojet would be shut off and the hot jet engaged, allowing the fraction, along with some breakthrough, to travel through the loop. The cryojet would then be turned back on, allowing further trapping of components arriving from the primary column. The same

jet would also serve to focus the analytes travelling through the loop that had arrived at the second cooled section. Turning the cryojet off and the hot jet on would allow another fraction to be flushed into the loop, while the focused analytes from the previous modulation would be injected into the secondary column for further separation.

Further innovation in cryojet modulation technology came from Hyotylainen et al. in the form of rotating cryojet modulation. Their first design included two cryojets aligned on a single liquid CO_2 supply tube that would rotate alternately to spray the modulating capillary, effectively trapping analytes in two separate trapping zones [31]. The two zones were also fitted with a heating wire that could heat the area rapidly. The modulation cycle would proceed with the first cryojet trapping analytes arriving from the primary column. The CO_2 supply tube would rotate to expose the second cryojet to the modulator capillary as the first cryojet was moved away and the analytes trapped at the first cold stage were desorbed by the coiled wire heater. Analytes which were now trapped at the second cooled stage would be desorbed by the second coiled heater as the cryojet moved back to its original position, no longer exposing the capillary to liquid CO_2. Their second design, referred to as the semirotating cryogenic modulator, used one rotating CO_2 cryojet instead of two. It also dispensed with the wire heaters, as the authors believed that the oven temperature was sufficiently high to release the trapped analytes [54]. Both designs were successful and provided results similar in quality to those obtained with other cryogenic modulators utilizing liquid CO_2 cryojets. This design has recently been modified to include a new modulator control program unit, increasing retention time reproducibility. The authors successfully used the modified system to analyse a sample of forest aerosols [55].

Although some great advancements had been made in cryogenic modulation through the use of liquid CO_2 cryojets and intelligent designs, some difficulty was still being experienced with trapping VOCs. The next stage in cryogenic modulation saw the expanded use of liquid N_2 as a means of trapping analytes in the modulation capillary. Harynuk and Górecki [56] developed a cryogenic modulation system, based on the previous work of Ledford et al. [51], which utilized a single cryojet interface with a delay loop for effective dual-stage modulation (Fig. 6). A Dewar filled with liquid N_2 was used to cool in-house pressurized N_2 gas to just below its boiling point. This was done by passing the pressurized N_2 gas first through a heat exchanger and then through a length of coiled copper tubing that was immersed in the liquid N_2. Once cooled sufficiently, the now near boiling N_2 was directed to a phase separator which prevented gaseous N_2 from entering the cryojets. The liquid N_2 entered a custom-made low-volume T-fitting with the cryojet connected to the perpendicular port and an insulated N_2 recycling line connected to the opposite port. A set of solenoid valves worked in alternate fashion to direct the liquid N_2 either to the cryojet or back to the Dewar via the recycling line. Trapping (Fig. 6a) and launching (Fig. 6b) occurred within a deactivated fused silica capillary with alternating hot and cold jets functioning in the same fashion as the previously described cryojet system of Ledford et al. [51]. Effective trapping of a propane sample for 1 min was observed with no component breakthrough occurring [56]. A mixture of *n*-alkanes in CS_2 and a commercially available unleaded gasoline sample were analysed as well, with no tailing or breakthrough occurring [56]. Day-to-day reproducibility was also very good. This system has been used successfully for other analyses as well [57].

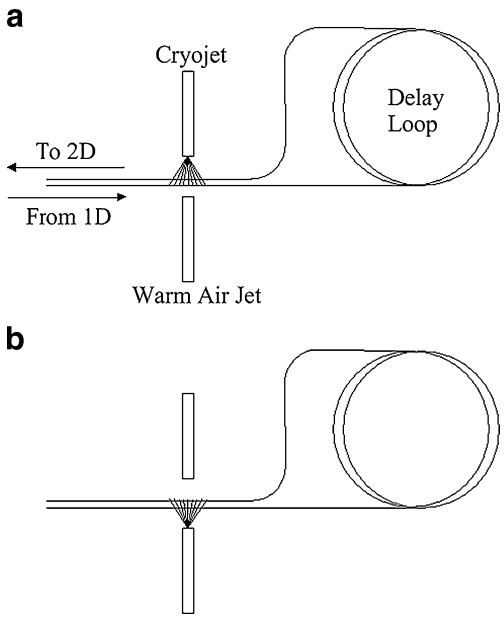

Fig. 6 The single jet delay loop modulator. Liquid N_2 from the cryojet traps analytes being eluted from the primary column and the delay loop in two separate spots on the loop capillary (**a**). Air from the warm jet liberates analytes periodically, allowing them to enter the loop (downstream) or the second-dimension column (upstream) (**b**). (Based on [52, 57])

Pursch et al. [58]designed a similar system that utilized liquid N_2 directly from the Dewar, a second cryojet, and the beginning segment of the secondary column as the modulator capillary. This system was also successful and provided quality separations of diesel and odour components in polymer coatings, as well as other samples [59]. Liquid N_2 interfaces proved to be the modulator of choice for the analysis of VOCs, as significantly lower trapping temperatures could be achieved with these devices. Although Harynuk and Górecki were able to minimize liquid N_2 consumption to about 30 L/day, the use of this cryogen proved to be expensive; thus, the search for a GC × GC

interface capable of trapping VOCs (C_3–C_6) without the use of expensive consumables is an important task. Several cryogenic modulators are commercially available through various instrument manufacturers.

Other thermal modulators Trapping in the modulator capillary assisted by compressed or cooled air has been explored in recent years. A single-stage modulator was developed by Libardoni et al. [60] which utilized a resistively heated stainless steel trapping capillary, with cooling assisted by a continuous flow of cold air. An aluminium block was fashioned to accommodate the trapping capillary, as well as entry and exit points for the cold air. A two-stage refrigeration unit served to cool a heat exchanger in which air was circulated at a rate of 35 L/min. This air would move from the heat exchanger, at a temperature of −45°C, into the aluminium block, and cool the modulator to a temperature between −32°C and −20°C, depending on the status of the chromatographic analysis. The device was used to successfully analyse a sample of gasoline and a 40-component text mixture spanning a range of volatilities [60]. However, component breakthrough was recognized for more volatile compounds. The authors attributed this to a relatively high trapping temperature compared with cryogenic devices, as well as a slow modulator cooling rate. This allowed components to evade trapping, and to escape into the secondary column in the period of time immediately after the electrical impulse had been delivered and before the trap had fully cooled. The device was later modified to use circulating ethylene glycol in place of cooled air and served as the basis for a consumable-free modulator introduced by LECO [61]. The design was altered once again in later years to become a dual-stage modulator with circulated air cooling [62].

Jover et al. [63] created an interface that employed two compressed air jets to trap analytes in a segment of the secondary column, and ambient oven heat for reinjection. Pizzuti et al. [64] developed a very similar system with a more robust construction. Both designs were successful in producing good separations of lipids and pesticide residues. The downside to these instruments was their incapacity to trap *n*-alkanes below C_{14}.

Forced air cooling has also been successfully used recently in a modulator designed by our group for the purpose of in situ analysis of the semivolatile fraction of organic aerosols. The device featured a uniquely flattened deactivated stainless steel capillary coated internally with PDMS at two individual trapping sections measuring 3 cm in length. The 15-cm capillary was looped outside the oven and exposed to a cooling blower or a vortex cooler, depending on the application. Two-stage thermal desorption of components trapped within the flattened capillary was accomplished using a custom-built capacitive discharge power supply. A more detailed description of the interface is provided in [65]. This device was successfully used in the thermal desorption aerosol comprehensive GC × GC–mass spectrometry system developed by Goldstein et al. [66]. Analysis of ambient aerosols was achieved and a considerable improvement in the system's capabilities was recognized. The device was also used to analyse a sample of gasoline and produced peak widths commonly seen only with cryogenic modulators [65]. The interface has been recently modified for single-stage modulation, and the use of a vortex cooler in place of a cooling blower has been evaluated in more detail. Analysis using the thermal desorption aerosol comprehensive GC × GC–mass spectrometry system was also achieved. These alterations and results will be described further elsewhere [67].

The most recent advancement in thermal modulation technology is the miniaturization of the device. Kim et al. [68] reported the microfabrication of a dual-stage, thermoelectrically cooled modulator for use in two-dimensional micro-GC. The authors utilized a boron doping process to create microchannels in an oxide layer that was thermally grown on a wafer. A more detailed description of the fabrication process can be found in [68]. These microchannels were coated with PDMS and measured 4.2 cm and 2.8 cm in length for the first and second stages, respectively. The inside dimensions were 250 μm×140 μm, with a wall thickness of 30 μm. Heating of each stage was achieved by providing a voltage to microheaters attached to the top portion of the microchannels. Rapid cooling was accomplished by a thermoelectric cooler secured to the bottom of the two stages. As analytes arrived at the first stage, they would become trapped in the first cooled microchannel. The microheater would be engaged briefly to allow transfer of the analytes to the second cold trapping stage, where further focusing would occur. Once the first stage had cooled sufficiently, the second stage would be heated and the analytes would be injected as a focused band into the secondary column. The thermoelectric cooler would remain operational throughout the whole analysis to prevent breakthrough while desorption of the analytes occurred. The authors could operate the modulator within the range from −30 to 250°C and effectively trap less volatile VOCs. To our knowledge, this is the first modulator to utilize thermoelectric cooling as a means of trapping analytes. This novel technique represents a significant contribution to the development of a GC × GC interface capable of trapping VOCs, as relatively low temperatures are achievable without the use of expensive consumables. We are currently evaluating the merit of a thermoelectrically cooled modulator for a benchtop GC × GC system.

Valve-based modulation

Unlike thermal modulators, which trap effluent from the primary column using temperature differentials, valve-based systems utilize pneumatic means to accomplish modulation of the primary column effluent. There is one exception which utilizes both cryogens and valves to modulate chromatographic peaks.

Diaphragm valve modulator

The diaphragm valve modulator (DVM) was the first valve-based interface to be successfully applied to GC × GC (Fig. 7). Bruckner et al. [69] first described their design in 1998 and suggested it may be used in place of thermal modulation, especially in the analysis of VOCs. The system utilized a wide-bore, PDMS-coated primary column and a narrow-bore, poly(ethylene glycol)-coated secondary column. A six-port diaphragm valve, with low-dead-volume fittings, was interfaced between the two columns within the oven. Actuation of the valve was achieved with nitrogen gas controlled by a three-port, normally closed Skinner solenoid valve. The nitrogen delivery system was modified to include a 1-L pressurized reservoir and large gas lines connected to the solenoid valve to ensure no drop in pressure would be experienced during actuation. Makeup helium was provided to the diaphragm valve, as was a vent line. Overall, four of the six ports were used. The flows of helium and the vented effluent were controlled using a low-flow controller and a fine metering valve, respectively. Gas flow was split between the secondary column and a 0.5-m length of fused silica capillary column to decrease the flow rate in the second dimension. The excess carrier gas was vented to the atmosphere. The diaphragm valve would be actuated twice per second for a very short period of time, allowing a small portion of the primary column effluent to enter the secondary column (Fig. 7a). As separation was occurring in the second dimension, all effluent from the first dimension would be vented to the atmosphere (Fig. 7b). Bruckner et al. [69] used the system to successfully analyse a sample of white gas (camping fuel) spiked with toluene, ethylbenzene, *m*-xylene, propylbenzene and *o*-xylene. Good repeatability between GC × GC runs was observed, but modulator performance was not studied in great detail. The same system was also used to analyse aromatic isomers in jet fuel [70], as well as aromatics and naphthalene in naptha [71]. Although rapid actuation allowed very narrow peaks of effluent to enter the secondary column, only approximately 10% of the primary column effluent was sampled and reinjected, leading to significant loss in mass transfer and the inapplicability of this system for trace analysis. Another issue was derived from the thermal limitations of the diaphragm valve. The valve could be operated at a maximum temperature of 175°C, restricting analysis to VOCs, thereby limiting this system's applicability. Flow disturbances during valve actuation were also seen as a potential detriment to the system. Seeley and coworkers recognized these imperfections and began engineering a new modulator using a six-port diaphragm valve that would greatly increase the capabilities of the valve-based interface.

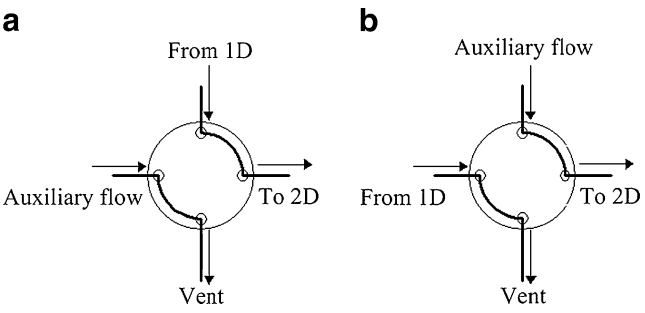

Fig. 7 The diaphragm valve modulator. **a** Effluent from the first-dimension column is directed to the second-dimension column. Auxiliary gas is vented to the atmosphere. **b** First-dimension effluent is vented to the atmosphere, while the auxiliary flow moves through the second dimension. (Based on [70])

Differential flow modulator

Seeley et al. [72] first introduced differential flow modulation in 2000. The design and function was based upon the DVM recently invented by Bruckner et al. [69]. Unsatisfied with poor sample transfer between the primary and secondary columns, Seeley et al. devised an interface capable of transferring significantly more of the primary column effluent into the secondary column. The design utilized a six-port diaphragm valve with a configuration similar to that of Bruckner et al.'s DVM, but featured the addition of a sample loop, thus making use of all six valve ports. The interface was kept outside the oven and its temperature was maintained at 125°C by block heaters. Effluent was transferred into and out of the interface by deactivated fused silica capillaries that were connected to the primary and secondary columns with fused silica unions. The modulator had two main stages: collection and injection. During the collection stage, effluent from the primary column entered the valve and was directed to a deactivated stainless steel sample loop measuring 10 cm long by 0.51-mm inside diameter. Effluent would collect in this loop and be vented to the atmosphere once the maximum volume had been reached. During the injection stage, the valve would be actuated, allowing a secondary flow of carrier gas to enter the sample loop and effectively sweep away the collected fraction into the secondary column. The flow rate in the secondary column was 20 times higher than in the primary column in order to produce

compressed injection bands and allow fast separation. For a 1-s modulation cycle, the collection stage would last 0.8 s, and the injection stage 0.2 s. This improved version of Bruckner et al.'s DVM increased the transfer efficiency of the effluent to the secondary column from approximately 10% to approximately 80%. The simple design and fast actuation speeds produced pulse widths similar to those described in cryogenic modulation studies. The system proved to be robust, but was still restricted by the maximum operating temperature of the diaphragm valve [73]. High flow rates in the secondary column also eliminated the possibility of using microbore columns, as very high head pressures would be required [74]. Because of the temperature limitations of the diaphragm valve design, steps were taken to increase its functionality at higher temperatures. Sinha et al. established that the valve temperature limitations were due to polymeric O-rings contained within the valve. Their solution was to mount the valve in such a way as to keep the O-rings outside of the oven, while the diaphragm and sample loop were kept inside [75, 76]. With the oven programmed to 250°C, the sample loop was found to reach 247°C, whereas the portion of the valve containing the O-rings maintained a temperature of approximately 80°C. With the diaphragm valve components exposed to effluent now capable of reaching higher temperatures, the range of compounds this interface could effectively modulate had increased to include semivolatiles [75].

Although differential flow modulators had been improved, valve-based interfaces were still inapplicable to the analysis of complex samples containing higher boiling point compounds (above 200°C). These limitations would not plague those in the field for much longer, as Bueno and Seeley were developing a novel system that would see the removal of the diaphragm valve from the flow path.

Flow-switching modulator

Recognizing that the diaphragm valve was the major detriment to the differential flow system, Bueno and Seeley [74] devised a flow-switching system that featured no valves within the GC × GC oven, and used materials capable of handling a much wider range of temperatures than the problematic valve (Fig. 8). Attached to the exit of the primary column was a T-union (T1 in Fig. 8) that allowed carrier gas to travel either left or right through deactivated fused silica tubing to two additional T-unions (T2 and T3 in Fig. 8). T2 and T3 were both connected to an auxiliary gas supply at their top port, and to a sample loop at their bottom port. All connections between unions were made with deactivated fused silica tubing. The auxiliary gas supply was controlled by a three-port solenoid valve (SV in Fig. 8) capable of directing gas flow to either T2 or T3. Both sample loops were connected at the bottom of the apparatus with another T-union (T4 in Fig. 8), whose bottom outlet was connected to the secondary column. Effluent from the primary column would be directed to either the fill or the flush sample loop, depending on the direction of the auxiliary flow. This would allow one sample loop to fill with the effluent, while the other was flushed with auxiliary gas. As the sample loop reached its effluent capacity, the solenoid valve would be activated, switching flow to the opposite side of the interface, allowing the auxiliary gas to inject the contents of the recently filled sample loop into the secondary column, while the now-flushed sample loop functioned as an effluent collector. The auxiliary gas flow valve would be switched at regular intervals to achieve consistent modulation throughout the chromatographic run. Differential flow was also featured in this design, with the ratio between the primary and secondary column flows fluctuating between

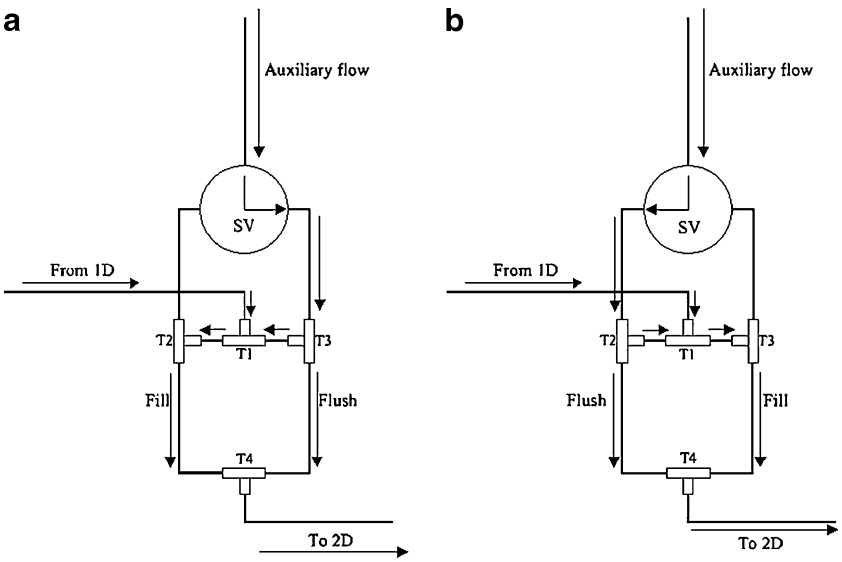

Fig. 8 The flow-switching modulator. **a** The capillary attached to T2 is filled with first-dimension eluate as the capillary attached to T3 is flushed to the second-dimension column. **b** Auxiliary flow is switched by the solenoid valve (*SV*) and the recently filled capillary is flushed into the second dimension, allowing the alternate capillary to fill with the eluate. Arrows indicate the direction of gas flow. (Based on [77])

25 and 30 depending on the analysis. The authors successfully used the flow-switching system to analyse a mixture of VOCs, diesel fuel and aromatics in gasoline [74, 77]. Narrow injection bands were observed throughout the chromatographic run. Placement of the switching valve outside the oven and all components in contact with the effluent within the oven allowed this interface to expand its range of analytes to include less volatile species. Another advantage of this interface was the removal of a primary column effluent vent. This ensured 100% mass transfer from the primary column to the secondary column, a significant improvement from the approximately 10% and approximately 80% mass transfer of the previously introduced valve-based modulators [69, 78]. The disadvantages of this system were mainly derived from its inflexibility [79]. Balancing gas flow rate with sample loop volume and modulation timing was a careful operation. If any single parameter was altered, the whole pneumatic system would have to be reevaluated and optimized, a rather tedious exercise. Modulation periods were also limited to around 2 s, thereby forcing separations in the second dimension to be very fast and perhaps not as effective as longer second-dimension separations. Seeley et al. [80] later introduced an alternative form of the flow-switching modulator called the simple fluidic modulator. This interface featured the primary and secondary columns connected with two T-unions, and a deactivated fused silica capillary in between. An auxiliary gas flow of 20 mL/min was introduced to the last port of the T-unions. Control of the auxiliary flow was accomplished by a three-port solenoid valve. A short segment of deactivated fused silica tubing was used to join the solenoid valve and the primary column, whereas the secondary column was joined with a long segment. The modulator operated in two stages, fill and flush. During the fill stage, effluent from the primary column would enter the sample loop for a desired period of time, while auxiliary gas flow moved through the secondary column T-union and into the second dimension. The second stage began when the solenoid valve was actuated, forcing auxiliary gas flow in the opposite direction through the primary column T-union. The significantly higher flow rate of the auxiliary gas temporarily stopped the flow of effluent from the primary column, and quickly flushed the sample loop into the second dimension. Each stage would alternate to effectively sample first-dimension peaks. Seeley et al. [80] used this modulator to analyse a sample of gasoline and obtained results of the same quality as those achieved by the previously described flow-switching interfaces. In later years, this method was investigated and modified further by Poliak et al. [81, 82] to become what is now known as the pulsed-flow modulator. The next innovation in flow modulation would see the improvement of both the first- and second-dimension separations through employment of an interface that combined valve-based techniques in combination with cryogens.

Stop-flow modulator

To preserve the first-dimension separation, multiple samples of the primary column effluent should be taken and injected into the secondary column. As previously mentioned, each fraction eluted from the primary column should be sampled at least three or four times [7]. With the need to sample the primary column so often, short modulation periods are often required; therefore, fast and sometimes less resolved separations in the second dimension can occur. The concept of stop-flow modulation was first introduced by Harynuk and Górecki [83] in an attempt to remedy this well-known compromise between first-dimension resolution and second-dimension separation time. The authors designed a system whereby flow in the first dimension could be stopped to allow a separation of primary column effluent in the second dimension to progress to completion. The design utilized an air-actuated, high-temperature, low-dead-volume six-port valve capable of withstanding up to 300°C. Three of the six ports were blocked. Connected to one port was the primary analytical column. Connected to a second port was a secondary column with the same pneumatic resistance as the primary column. This bypass column was connected to the primary gas supply using a Swagelok T-union at a location just before the injector. This was done to make certain that equivalent gas pressure was supplied to the inlet of both columns, and to prevent accidental transfer of sample components into the bypass column. Connected to the third port was a section of deactivated fused silica tubing which was connected to the secondary analytical column. The segment of deactivated fused silica tubing served as a trapping capillary when cooled with a previously described liquid N_2 cryojet interface [56]. After injection, the sample would make its way through the primary column, becoming separated. As the first components began to be eluted, the valve was in the sample position, and the eluate migrated through the valve to the deactivated fused silica capillary, where it was trapped and focused by the liquid N_2 cryojet. After a predetermined trapping time had passed, the valve was actuated and moved into the stop position, while the cryojet was halted and the warm jet was activated. With the valve in the stop position, no carrier gas flowed in the primary column. Carrier gas was now delivered instead through the bypass column, into the valve, and through the deactivated fused silica capillary, flushing the focused analytes into the secondary analytical column. Once separation was complete in the second dimension, the cryojet was engaged and the valve actuated. The primary column separation resumed once again, as did trapping of the effluent in the cryojet interface. Periodic stopping of the flow in the

primary analytical column allowed each second-dimension separation to proceed independently of the length of the trapping stage (which determines the modulation period in standard modulators), permitting the adjustment of individual parameters, such as column length and stationary phase, to create optimal separation conditions. This interface was compared with conventional GC × GC systems and proved to be superior by providing better resolution for peaks corresponding to compounds that were eluted early. It also allowed the use of long, efficient second-dimension columns (up to 6 m × 0.15-mm inside diameter), and consequently an increase of the separation power of the system (albeit at the expense of longer separation time). Wraparound peaks were also eliminated, as enough time was permitted for a full separation on the secondary column to occur [84]. The design was altered in later years with the removal of the six-port valve from the effluent path, and the inclusion of a pneumatic system to stop the flow in the primary column [85]. A diesel fuel sample was successfully analysed using this method and the authors also saw better overall performance. The main disadvantages of stop-flow GC × GC are its somewhat complicated setup and long analysis times.

Deans switch modulator

Seeley et al. [86] once again introduced an alternative approach to modulation featuring a microfluidic Deans switch. Their design resembled previously described differential flow modulators with a few modifications. The switch was designed to include a monolithically constructed manifold with five ports and a three-way solenoid valve. Two ports of the manifold were connected to the three-way solenoid valve, which directed the flow of auxiliary gas to one of the two ports. One of these two ports was connected with a T-union to the secondary column and the primary column, whereas the other port was connected to a flow-restricting column and the primary column. Primary column flow was split with a T-section that was connected to both of the previously described unions. The manifold portion of the switch remained in the oven, whereas the solenoid was kept outside. The Deans switch operated in two stages, bypass and injection [86]. The bypass stage involved the solenoid directing an auxiliary flow of gas in such a way that effluent from the primary column was directed away from the secondary column and towards the flow-restricting column. No separation occurred on this column, as it was made of deactivated fused silica and functioned only to maintain gas pressure within the system and to vent non-sampled effluent. During the injection stage, the direction of auxiliary gas flow was reversed, and primary column effluent was directed towards the secondary analytical column. The switch remained in the injection state for only 5-10% of the modulation period, thereby producing sharp injection bands into the secondary column. The Deans switch permitted a greater range of oven temperatures to be used, extending its use to semivolatile compounds. The greatest disadvantage of the Deans switch modulator, similarly to that of the first diaphragm valve interface, was that only a small portion of the primary column effluent was directed to the secondary analytical column, greatly reducing sensitivity. Consequently, this interface is not suitable for trace analysis.

Wang's differential flow modulator

The most recent development in valve-based modulation was introduced by Wang [87]. It utilizes two four-port, two-position switching valves that are actuated electronically (Fig. 9). The interface operates in two stages, referred to as X and Y by the author. In stage X, effluent from the primary column enters the first valve (V1 in Fig. 9) and travels down a transfer line to the second valve (V2 in Fig. 9). Effluent is vented to the atmosphere if the transfer line volume is exceeded. During this stage, auxiliary gas flow enters the second valve (V2) and is swept through the opposite transfer line, flushing it into the secondary column. The Y-stage begins when the valves are actuated into their second position. First-dimension eluate that had filled the transfer line is now flushed into the secondary column by the auxiliary gas flow. Eluate leaving the primary column now fills the opposite transfer line, with

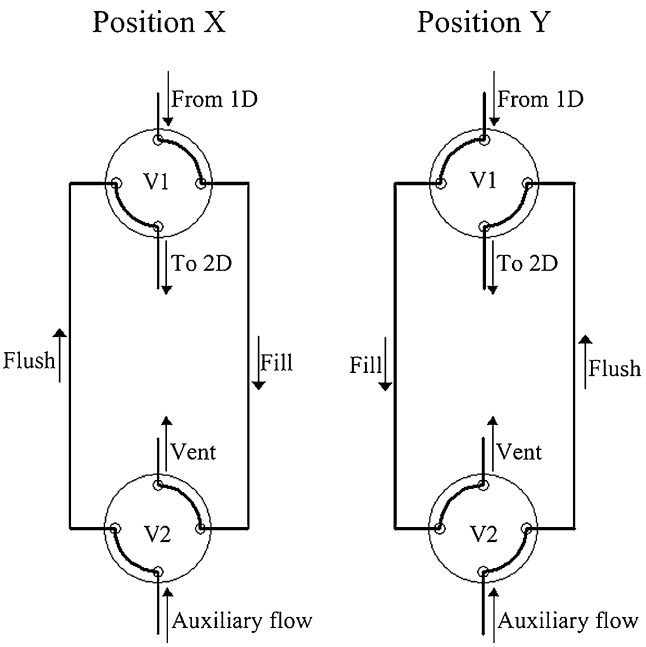

Fig. 9 Wang's differential flow modulator. Valves V1 and V2 are actuated at the same time to either fill or flush the transfer columns. (Based on [87])

excess being vented once the maximum volume is exceeded. The valves (V1, V2) are actuated between these two stages to achieve modulation. Wang tested this device by analysing samples of naptha, diesel, fatty acid methyl esters and PCBs [87]. Performance equivalent to that of thermal modulators was observed, and 100% transfer of the effluent from the primary column to the secondary column was achieved. The valves used were capable of withstanding temperatures of up to 350 °C, allowing a wider range of analyses to be performed with this device. Wang utilized high flow rates and a wide-bore column for second-dimension separation, an attractive feature as a greater variety of stationary phase coatings are available for use in this type of column [88]. Swapping the two four-port, two-position valves used in the study for one 12-port, two-position valve or one eight-port, two-position valve was described as a simple alternative. Wang's differential flow modulator helped expand the range of analyses performed by valve-based modulation.

Concluding remarks

In the past decade, GC × GC has become generally accepted as the most powerful technique for the analysis of complex samples, as evidenced by the ever-increasing number of articles on the technique being published every year. Initially, they were mainly devoted to the principles and the basic theory of the technique. In the first year after the introduction of the technique, only four articles devoted to GC × GC were published. By the end of 2002, the total number of publications had reached nearly 100 [27]. Three years later, a further 150 articles had been published [89]. In the period from 2006 to the end of 2010, close to 360 new articles were published, indicating nearly exponential growth of the technique. The focus of the articles gradually shifted over time from fundamental developments to applications. Nearly 83% of the over 110 articles published in 2010 were devoted to applications of GC × GC. Regarding today's trends in GC × GC modulation, cryogenic methods remain very popular because of their ability to produce very small peak widths at half height and minimize breakthrough. The commercial availability of modern cryogenic devices from several vendors, for which the designs of Ledford et al. [46, 51] and Beens et al. [48] served as the foundation, has also contributed to the popularity of this modulation technique. Valve-based interfaces are increasing in popularity owing to their expanded volatility range and increasingly simple setup. They are also less expensive to operate and easier to maintain than their cryogenic counterparts, but are incapable of producing peaks of the same quality. A valve-based modulator derived from work of Seeley et al. [80] and Poliak et al. [81, 82] is commercially available as well. In addition, coupling of differential flow modulators with mass-spectrometric detectors is very problematic owing to the large carrier gas flows in the second dimension. Widespread use of GC × GC systems depends on the development of versatile, inexpensive, low-maintenance and easy-to-operate modulators. Valve-based systems might fill this role with further improvements in some (limited) applications. Thermal modulation with the use of thermoelectric cooling also looks like a promising alternative if temperatures can be lowered enough to trap VOCs. The next 20 years will surely see more exciting innovations in GC × GC modulation.

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