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Review

Multidimensional chromatography in food analysis

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ABSTRACT

In this work, the main developments and applications of multidimensional chromatographic techniques in food analysis are reviewed. Different aspects related to the existing couplings involving chromatographic techniques are examined. These couplings include multidimensional GC, multidimensional LC, multidimensional SFC as well as all their possible combinations. Main advantages and drawbacks of each coupling are critically discussed and their key applications in food analysis described.

Contents

1. Introduction

Single column (one-dimensional) chromatography analysis has been used for many years as a standard separation tool for analyzing compounds in a broad variety of fields including food analysis [1–6]. At present, attention is being paid to avoid laborious sample pre-treatments that can in fact be an important source of errors mainly for complex matrices as e.g., food or food-related matrices. One-dimensional chromatography does not always provide the resolution and separation power required to obtain the best results in terms of identification of analytes in food samples. This problem frequently comes out even considering the large number of detectors developed in the past years, including MS detectors.

Multidimensional chromatography has emerged as an interesting alternative to analyze complex samples in a situation in which technological improvements, such as new column technologies, seem to be close to their maximum level. Thus, peak capacity enhancement achievable by multidimensional chromatography is by far higher than the obtained after improving by any mean one-dimensional separations. Multidimensional chromatography allows combination of two or more independent or nearly independent separation steps, increasing significantly the separation power of the corresponding one-dimensional techniques and, therefore, the physical separation of compounds in complex samples.

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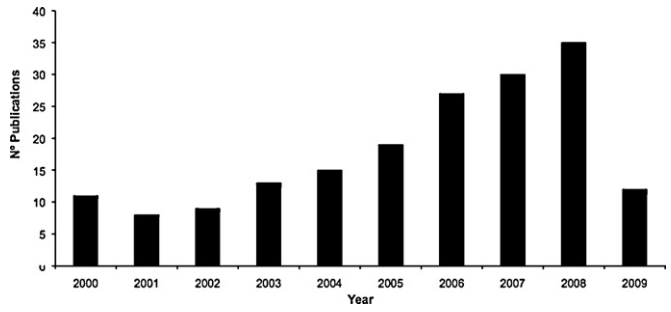

Fig. 1. Evolution of the published works in the last decade in food analysis using multidimensional chromatography (data up to July 2009). The sources of information were the databases: Isi-Web of Knowledge, Scirus, Scopus and Science Direct. The search has been done using as keywords. [(Multidimensional Liquid Chromatography) or (Multidimensional Gas Chromatography) or (Two-dimensional Liquid Chromatography) or (Two-dimensional Gas Chromatography) or (Comprehensive Liquid Chromatography) or (Comprehensive Gas Chromatography) or (Supercritical Fluid Chromatography) or (LC × LC) or (GC × GC) or (LC–LC) or (GC–GC) or (LC–GC) or (MDGC) or (MDLC) or (SFC × SFC) or (SFC × LC)] and (Food or Beverages).]

The main goal of this review is to describe the present state-of-the-art of multidimensional chromatography in the field of food analysis. For this purpose, the different existing couplings are described and their most important advantages and drawbacks are commented. Besides, the most remarkable applications in food analysis are critically discussed. Different couplings involving gas, liquid and supercritical fluid chromatography, for the analysis of a great variety of compounds in different food matrices (honey, wine, milk, cheese, oils, etc.) are considered.

To illustrate the increasing importance of the use of multidimensional chromatographic techniques in food analysis, Fig. 1 shows the evolution of the number of published papers on this topic (i.e., multidimensional chromatography in food analysis) in the last decade.

Several interesting works have already been published on general aspects (fundamentals, design and applications) of the different chromatographic multidimensional techniques [7–14]. Therefore, only a brief description of each multidimensional approach will be given in the following sections. Readers interested on more specific instrumental or fundamental details can take resort of the aforementioned review works.

2. Multidimensional gas chromatography (MDGC)

Single-column gas chromatography actually offers quite high peak capacities along with a diverse number of available configurations and detectors. However, when highly complex samples (like food matrices) have to be studied, some peak overlapping can occur leading to ambiguous or problematic peak identification. One option to improve the separation power is to couple, through an interface, two or more independent columns giving rise to the so-called multidimensional gas chromatography (MDGC). MDGC was developed several decades ago [15] and it has been used to analyze a large variety of samples [9]. Few years ago, Bertsch published two reviews dealing with the principles of MDGC [9,11]; it was stated that two-dimensional gas chromatography (and higher dimensional systems) consists of an arrangement of two or more columns where distinctive segments of effluent from the first column are fed into one or more columns. Different designs of MDGC systems are shown in Fig. 2 including the use of heartcut valves to transfer one or few fractions eluting from the first column to the second dimension (the typical MDGC configurations) and the more sophisticated use of modulators to transfer the whole eluted fraction from the first dimension to the second one (i.e., comprehensive GC × GC).

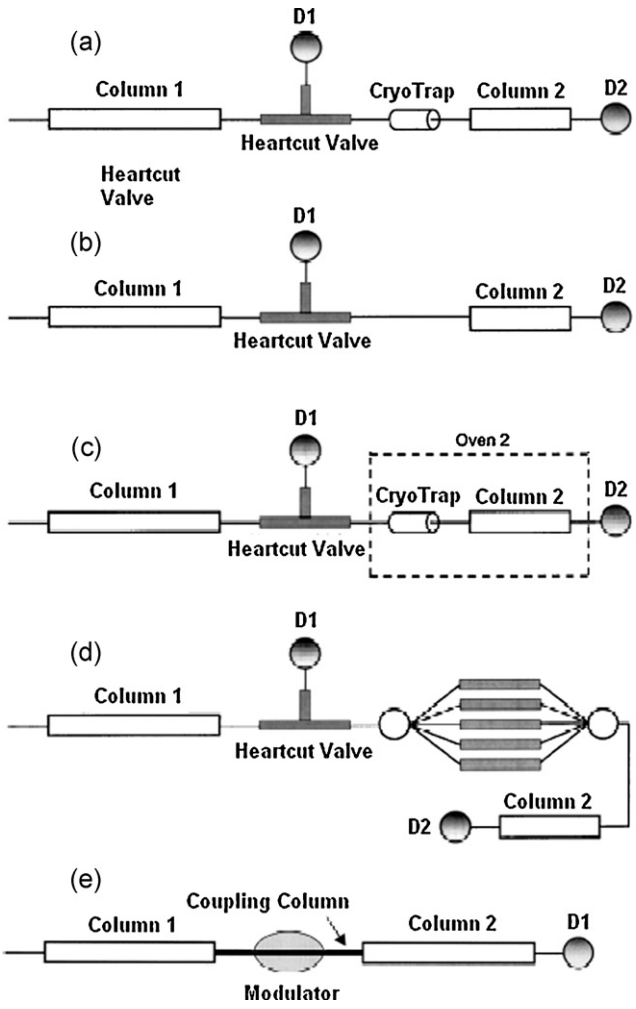

Fig. 2. Designs of different multidimensional gas chromatography systems incorporating (a–d) heartcut valves, multiple detectors (D1, D2), multiple columns, dual ovens and cryogenic traps (conventional MDGC) and (e) first dimension column, modulator device with coupling column, second dimension column and detector (D1) in a typical comprehensive chromatography system. Reprinted from [8]. Copyright (2000) with permission from Elsevier.

In this review, the applications of both, MDGC and GC × GC in food analysis will be divided in two sections according to the type of multidimensional chromatography employed. Although the use of these techniques in food analysis has been really broad, it is also remarkable their use in other fields, like petrochemical, environmental and biological studies [16–18].

2.1. Conventional and advanced heart cutting MDGC

MDGC is a well-known technique that has reached its maturity through the development of new configurations and designs. The most critical issue of MDGC is the control and sequential transfer of compounds from the first to the second column; this transfer is usually done using an on-line heartcut, that allows the transportation of only some key analytes from the first to the second column. [19]. There are some other options like off-line techniques, but they require more time, specific and sophisticated hardware [20,21]. The heart cut could be done using either a valve or a pneumatic switcher [22–26]. By using a valve, control of pressure and flow is not needed, when two columns with similar physical characteristics are used [22]; however, some problems have been reported regarding memory effects due to analyte stability and adsorption [23]. Other problems related to thermal inertia can be avoided by

Table 1
MDGC applications in food analysis.

Matrix	Compounds of interest	Sample treatment	First dimension	Second dimension	Transfer	Detection	Ref.
Essential oils	Monoterpenes	Hydrodistillation	HP-Innowax (60 m × 0.25 mm i.d., 0.25 μm film thickness)	Lipodex (25 m × 0.25 mm i.d.)	Heart cut	FID, MS	[31]
Rosemary essential oil	Chiral components	Dilution	SE 52 (10 m × 0.1 mm i.d., 0.1 μm)	DEtTBuSililBETA-086 (10 m × 0.1 mm i.d., 0.1 μm)	Heart cut	FID	[32]
Essential oils (Bergamot, *Pistacia lentiscus*, *Cymbopogon winterianus* Jowitt, etc.)	Monoterpene hydrocarbons and alcohols, enantiomeric chiral compounds, etc.	Hydrodistillation	SLB 5MS, (30 m × 0.25 mm i.d., 0.25 μm)	PS 086 (25 m × 0.25 mm i.d., 0.25 μm)	Heart cut	FID, MS	[33–36]
Gin essential oil	Aroma compounds	None	HP5-MS (30 m × 0.25 mm i.d., 0.25 μm)	Supelcowax-10 (30 m × 0.25 mm i.d., 0.25 μm)	Heart cut cryotrapping	FID, MS	[37]
Mint flavored food products	Pulegone	Simultaneous distillation–extraction	DB-5 column (30 m × 0.25 mm i.d., 0.25 μm)	DiActButylsilyl-γ-CDX (25 m × 0.25 mm i.d.)	Heart cut	FID	[38]
Hazelnuts	Filbertone	Dynamic headspace extraction	Glass capillary column coated with poly(5% diphenyl 95% dimethylsiloxane) (30 m × 0.25 mm i.d., 0.1 μm)	Glass capillary column coated with Chirasil-β-Dex (permethyl-β-cyclodextrin) (25 m × 0.25 mm i.d., 0.25 μm)	Pneumatically controlled six-port valve	FID, MS	[39]
Kiwi	Volatile components	Liquid extraction and steam distillation	5% cross-linked phenyl-methylsiloxane (30 m × 0.25 mm i.d., 0.25 μm)	5% cross-linked phenyl-methylsiloxane (30 m × 0.25 mm i.d. with 0.25 μm)	CTS1 cryotrapping device	Olfactory detector	[41]
Guava	Volatile components	Liquid extraction	HP-5 (30 m × 0.25 mm with 0.25 μm)	HP-5 (30 m × 0.25 mm with 0.25 μm)	CTS1 cryotrapping device	Olfactory detector	[42]
Coriander leaf and hop essential oils	Odorants	Molecular distillation under high vacuum	HP5 (30 m × 0.32 mm i.d., 0.25 μm)	Solgel Wax (30 m × 0.32 mm i.d., 0.5 μm)	Heart cut (Deans switch)	Olfatory detector	[43,44]
Orange oil	Valencene	None	SolGel-Wax (30 m × 0.53 mm i.d., 0.5 μm)	DB-5 (30 m × 0.53 mm i.d., 0.5 μm)	Heart cut (Deans switch)	Olfactory detector and FID	[45]
Malt whisky	Green note compounds	Simultaneous distillation–extraction	DB 1701 (30 m × 0.32 mm i.d., 1.0 μm)	DB-Wax column (25 m × 0.25 mm, i.d., 0.25 μm)	Heart cut (Deans switch)	Olfactory detector	[46]
Red wines	Volatile compounds	Dilution and liquid extraction	SPB1 (30 m × 0.25 mm i.d., 0.25 μm)	BP20 (50 m × 0.25 mm i.d., 0.25 μm)	Heart cut	Olfactory detector and MS	[47]
Wines, brandy and whisky	Aroma compounds	SPE	DBWAX (30 m × 0.32 mm i.d., 0.50 μm)	FactorFour-5ms (30 m × 0.32 mm i.d., 0.1 μm)	Heart cut cryotrapping	Olfactory detector FID and MS	[48–51]
Red wine	Trans-resveratrol	SPME with on-fiber silylation derivatization	SGE BP5 (12 m × 0.53 mm i.d., 1 μm)	SGE BP50 (30 m × 0.53 mm i.d., 1 μm)	Heart cut Deans switch cryotrapping	MS	[52]
Wine	Ethyl lactate	SPE and liquid extraction	SE 52 (30 m × 0.25 mm i.d.)	BGB 176 (DiMeβ-CD) (30 m × 0.25 mm i.d.)	Heart cut (ITD transfer with an open split interface)	MS	[53]
Human breast milk, milk and cheese	Polybrominated and polychlorinated compounds	Matrix solid phase dispersion (MSPD) and liquid extraction	DB-5 and an HT-8 (30 m × 0.25 mm i.d., 0.25 μm)	DB 17 and HT 8 (30 m × 0.25 mm i.d., 0.25 μm) Chirasil-Dex (25 m × 0.25 mm i.d., 0.25 μm).	Heart cut (Deans switch)	ECD	[54,55]
Raspberries products	(*E*)-α-Ionone and (*E*)-β-ionone	Simultaneous distillation and extraction (SDE)	SE 52 (30 m × 0.25 mm i.d., 0.23 μm) Rtx-1701 (30 m × 0.25 mm i.d., 1 μm)	DIME-β-CD (30 m × 0.25 mm i.d., 0.23 μm) ZB 5 (30 m × 0.25 mm i.d., 0.50 μm)	Multi Column Switching System MCS 2	Isotope ratio mass spectrometry (IRMS)	[56]

using an additional heating valve. On the other hand, when narrow columns are used, wider peaks are obtained due to high dead volume of the valve. The approach based on a pneumatic switcher, developed by Deans in 1968, is based on pressure equilibrium [24]. Even being a more sophisticated system, it has also some problems associated mainly to peak width for which different solutions have been proposed [25,26].

Another configuration, called longitudinally modulated cryogenic system (LMCS) [27,28], allows controlling the peak transfer between the two columns by blocking and releasing different sections of the chromatogram sequentially. With this technique, peak broadening can be overcome but some limitations are still encountered dealing with the fast analysis required in the second column that restricts the use of some types of columns and makes necessary to employ very fast detectors after the second dimension. Different approaches have been lately developed to overcome some limitations of the LMCS system, one of them was the use of micro switching and cryogenic modulators [29] which allowed the use of a double cool strand interface making possible to hyphenate the LMCS based system to slower detectors [30].

The use of conventional MDGC provides better peak capacity and take less time than two separate GC runs, allows increasing the signal-to-noise (S/N) ratio as a result of the focusing effect at the modulator and can be used together with most of the detectors usually employed in one dimension GC (FID, ECD, ODO and MS). On the other hand, MDGC requires more equipment (supplementary gases, pumps, detectors, etc.), usually takes longer run times and more attention than single column analyses, it is not possible to do unlimited cuts prior to second dimension and several runs have to be done to cover the whole separation. So, as it could be seen the use of MDGC presents advantages and disadvantages that should be taken into account prior to select this technique Table 1, summarizes the most relevant applications of MDGC in food analysis. One of the most widespread applications of MDGC is the characterization of compounds responsible for the aroma of several food matrices, especially essential oils analysis. The enantiomeric distribution of the monoterpenes α-pinene and camphor in essential oils from *Tanacetum argenteum* subsp. *flabellifolium* was studied [31]. Fast enantiomeric analysis of rosemary essential oil was carried out by MDGC [32], thanks to the use of a rapid-multi sequential heart-cutting method. Other essential oils that were also studied by the same group, using similar MDGC systems were Bergamot [33], *Cymbopogon winterianus* Jowitt oil [34], *Pistacia lentiscus* L. [35] as well as essential peel oils of two Brazilian mandarin cultivars [36]. Also, the analysis of gin essential oil by one-dimensional GC/MS and two-dimensional GC/MS has been reported [37]. The enantiomeric composition of mint flavored food products [38] and hazelnuts [39] have also been studied. In the first work, a MDGC system with two GCs and two FIDs was used; a non-chiral column was placed in the first GC while a chiral column was employed in the second one. The two GCs were connected via a heated transfer line thermostated at 170 °C. In this work, pulegone (a natural monoterpene) enantiomers were determined in mint essential oils and mint products (syrups, dried leaves, toothpaste,

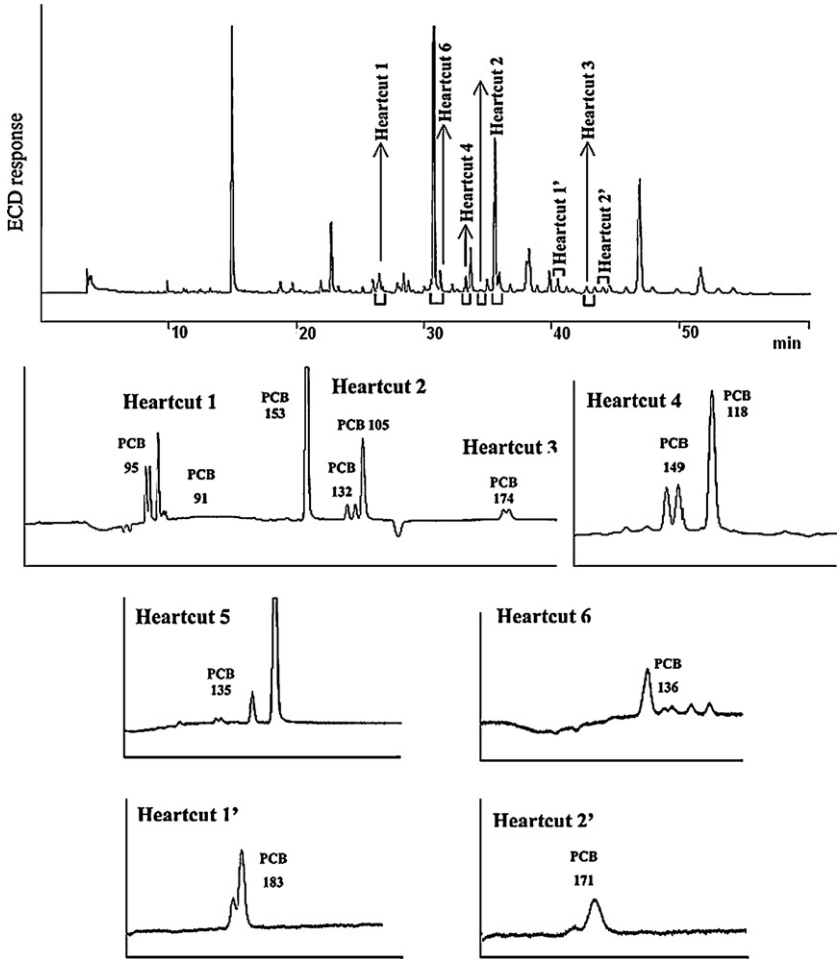

Fig. 3. MDGC-ECD chromatogram of a goat milk sample and subsequent heart-cuts to Chirasil-Dex for the determination of PCBs 91, 95, 132, 135, 136, 149, and 174 (heart-cut 1 to heart-cut 6), and to BGB-172 for the determination of PCBs 171 and 183 (heart-cut 1′ and 2′). Taken from [55]. 2005, Copyright Wiley–VCH Verlag GmbH & Co. Reproduced with permission.

lozenges, candy and chewing-gum). On the other hand, a MDGC system with two separate ovens, one on-column injector, and two FIDs was employed to determine the enantiomeric composition of filbertone in hazelnuts [39]. Column switching was achieved with a pneumatically controlled six-port valve using both, a chiral and a non-chiral column. Using this set-up, a matrix effect produced by hazelnuts was observed that allowed the racemization of this important odor compound.

Combination of MDGC and olfactometry (MDGC–O) has already shown important capabilities in different applications in food flavor analysis [40], including the identification of aroma components such as alcohols, esters, and aldehydes in kiwi [41], guava [42] fruit purees and essences and the presence of odorants in coriander leaf and hop essential oils [43,44]. Some applications about the use of multidimensional GC–O/GC–MS to identify aroma active compounds have also been published, for instance for commercial orange essential oil [45] or malt whisky [46] analysis. One of the most extended applications of MDGC–O is wine analysis. It was possible to identify the volatile compounds responsible for prune aroma in prematurely aged red wines [47]. In this work, a GC–O was first employed to distinguish the characteristic odoriferous zones in several wines, and later on, a MDGC–MS method was employed to identify the compounds responsible for the unwanted aroma; γ-nonalactone, β-damascenone, and 3-methyl-2,4-nonanedione were identified as possessing strong odor of prunes. This latter compound was also found in prunes for the first time whereas its perception threshold was determined to be 16 ng/ml in hydroalcoholic solution. A home made-MDGC system was developed by Campo et al. and used to analyze red wines, Sherry white wine, Madeira wines, brandy and whisky [48–51]. Other key compounds have also been studied in wine samples, like trans-resveratrol in red wine samples [52] or ethyl lactate [53].

Regarding food safety, MDGC has proven to be a useful analytical technique for the separation of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in human breast milk [54] and milk or cheese samples [55]. Fig. 3 shows the chromatograms obtained for the analysis of goat milk sample by this MDGC methodology. Using this methodology, 15 chiral PCBs were, at least, partly separated. MDGC significantly enhanced the separation compared to conventional GC, allowing the determination of the enantiomeric fractions of the chiral PCBs free of interferences [55].

Another further development allowed the hyphenation of constant flow multidimensional gas chromatography to combustion/pyrolysis–isotope ratio mass spectrometry (MDGC–C/P–IRMS), in order to authenticate (*E*)-α (β)-ionone from different raspberries products [56]. The proposed method was helpful to differentiate among natural and synthetic raspberry aroma compounds presented in different commercial products.

From the above commented contributions, it can be easily deduced the importance of MDGC in food analysis as well as its potential to enhance traditional GC-based separations, both in terms of separation power and information provided. As an evolution to these techniques, comprehensive two-dimensional GC has emerged, providing some excellent advantages over the less evolved heart-cutting systems.

2.2. Comprehensive two-dimensional gas chromatography (GC × GC)

Although the principles and the first system for comprehensive two-dimensional gas chromatography (GC × GC) were developed in the late 80s [57,58], in the last few years its expansion has been outstanding, as it can be inferred from the numerous research works and review papers published so far [11,14,59–63]. The enhancement achieved by GC × GC compared to one-dimensional and multidimensional approaches is very significant. In this regard, the huge amount of data that can be produced after a comprehensive GC separation has increased the need for specific software as well as for the use of chemometric tools.

In GC × GC, the whole first dimension effluent is transferred onto a second column, which must be operated at high speed. Thus, a rapid sampling of the first column effluent should be achieved without affecting the second dimension analysis. In this technique, the interface between the two columns (dimensions) is a modulator whose main functions are to increase the amplitude of the signal and facilitate its transfer to the second dimension. The modulator plays an important role in any GC × GC system, since it entraps and releases smaller and more manageable portions of the effluent from the first dimension [60]. There are two main classes of modulators: flow switching modulators, which operate as high-frequency division valves, and thermal modulators that sample the first dimension eluate more completely. These last ones are divided in three groups, namely, heat [64–66], cryogenic [67–69] and jet pulsed modulators [70,71]. The valve modulators-based GC × GC system [72,73] are more like a heart-cut MDGC set-up, being cheaper and easier to maintain than the thermal modulators. In contrast, are less efficient.

Another important factor to be considered is the columns combination. In GC × GC, two columns of different composition are used. The composition of the stationary phase of the first dimension is generally less polar than the one used in the second dimension, so that the separation is ruled by boiling point properties in the first dimension and polarity in the second [74]. It should be noticed that set-ups with low orthogonality have also been successfully employed [75]. However, their use is less frequent than the more orthogonal combinations. Due to the high-speed needed for the second dimension separation, detectors with fast response times are required. In a first approach, FID and other element selective detectors as electron capture (ECD), nitrogen chemiluminescence (NCD) and nitrogen-phosphorus (NPD), have been employed [63]. Quadrupole and magnetic sector high-resolution mass spectrometers have also been used coupled to GC × GC, being their main limitation the slow nature of their operation [76,77]. Time of flight mass spectrometers (TOF-MS) were introduced as an alternative to the mentioned MS detectors and quickly became the best option for a comprehensive detector; main features of TOF analyzers are the high resolution and mass accuracy and good scanning speed that can simultaneously be achieved compared to the mentioned MS analyzers. Besides, as many others MS instruments, TOF-MS use deconvolution software to resolve compounds that coelute after the separation. Although new rapid scanning quadrupoles have been introduced [63], a TOF-MS is preferred when the target analytes cover a wide mass range, or when dealing with unknown compounds. The relevance of the combination between GC × GC and MS detectors has been summarized in a recent review [62], where the principles, practical and theoretical aspects were discussed along with some applications.

GC × GC offers faster run times, increased peak capacity, improved resolution and enhanced mass sensitivity. In addition, they generate structured two-dimensional (2D) chromatograms helpful for classification and identification of analytes. Sample preparation procedures can often be minimized, or even eliminated in some cases, due to the high separation power offered by this technique. However, GC × GC also shows some limitations as the high cost of equipment and maintenance, difficult method optimization, and although the GC × GC peaks are sharp because the second dimension is short, it should be noted that the short-second-dimension column also reduces the sample capacity of GC × GC, so the improvement in sensitivity sometimes is not so high. As it has been previously commented, the high speed and generated amount of data of GC × GC make necessary the use of fast detectors and

Table 2
GC × GC applications in food analysis.

Matrix	Compounds of interest	Sample treatment	First dimension	Second dimension	Modulator	Detection	Ref.
Fish oils	Pesticides (PCBs and PCDDs)	Gel permeation and SPE	Restek Rtx-Dioxin 2 (60 m × 0.25 mm i.d., 0.25 μm film thickness)	Rtx-PCB (3 m × 0.18 mm i.d., 0.18 μm film thickness)	Non-moving quad-jet dual stage	TOF-MS	[78]
Fish oil, milk, cheese and salmon	Toxaphene enantiomers, PCBs and PBDs	Column extraction	BGB-172 (30 m × 0.25 mm, i.d., 0.18 μm)	BPX-50 (2 m × 0.10 mm i.d., 0.10 μm)	Cryogenic loop KT2003	μECD	[79,83]
Fish oil, salmon, milk, vegetable oils, eel extracts.	Pesticides (PCBs, PCDDs and dioxins),	Column extraction, soxhlet, liquid extraction	DB-XLB (30 m × 0.25 mm i.d., 0.25 μm)	LC 50 (1.4 m × 0.15 mm i.d., 0.1 μm)	LMCS and loop-type carbon dioxide jet KT2002	FID, μECD, ECD	[80,81]
Fish	Brominated flame retardants	Soxhlet and automated gel permeation chromatography	DB-XLB (30 m × 0.25 mm i.d., 0.10 μm)	BPX-50 (1.25 m × 0.1 mm i.d., 0.1 μm)	Dual stage jet	TOF-MS	[82]
Milk	PCBs	SPE	DB-1 (15 m × 0.25 mm i.d., 0.25 μm)	HT-8 (1.2 m × 0.10 mm i.d., 0.10 μm)	Non-moving quad-jet dual stage thermal	Isotope dilution time-of-flight mass spectrometry (ID-TOF-MS)	[84]
Mussels	Aromatic hydrocarbons	Alkaline saponification	DB-5 (30 m × 320 μm i.d., 0.25 μm)	DB-17 (1.9 m × 100 μm i.d., 0.1 μm)	Non-moving quad-jet dual stage thermal	TOF-MS	[85,86]
Olive and sunflower oils	Polycyclic Aromatic hydrocarbons	SPME	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BPX50 (1 m × 0.1 mm i.d., 0.1 μm)	LMCS	TOF-MS	[87]
Carrots and celeriacs	Pesticides	Liquid extraction	CP-SIL 5CB (15 m × 0.25 mm i.d., 0.25 μm)	BPX50 (0.8 m × 0.1 mm i.d., 0.1 μm)	LMCS based	TOF-MS	[88]
Baby food	Pesticides	Gel permeation chromatography	DB-XLB (30 m × 0.25 mm i.d., 0.25 μm)	DB-17 (1 m × 0.1 mm i.d., 0.1 μm)	Dual stage jet	TOF-MS	[89]
Tea	Pesticides	Head-space solid-phase microextraction (HS-SPME)	BPX-5 (40 m × 0.18 mm i.d., 0.18 μm)	Supelcowax (2.5 m × 0.1 mm i.d., 0.1 μm)	Dual stage jet	TOF-MS	[90]
Berry grape	Pesticides	Solvent extraction	RTX-5MS (10 m × 0.18 mm i.d., 0.2 μm	TR-50MS (1 m × 0.1 mm i.d., 0.1 μm)	Non-moving quad-jet dual stage thermal	TOF-MS	[91]
Red grapefruit extracts	Pesticides	ISTISAN 23/97 guidelines	SLB-5MS (30 m × 0.25 mm i.d., 0.25 μm)	Omegawax (1 m × 0.10 mm i.d., 0.10 μm)	KT-2006 loop system	qMS	[92]
Brussels sprouts	Fungicides	Liquid extraction	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BPX50 (1 m × 0.15 mm i.d., 0.15 μm)	LMCS	NPD	[93]
Lemon essential oil	Sesquiterpene and oxygenated monoterpene components	Dilution	Supelcowax-10 (30 m × 0.25 mm i.d., 0.25 μm)	SPB-5 (1 m × 0.10 mm i.d., 0.10 μm)	LMCS	FID	[94]
Lemon thyme essential oil	Volatile compounds	Dilution	RTX-1 (30 m × 0.25 mm i.d., 0.25 μm)	RTX-Wax (2 m × 0.1 mm i.d., 0.1 μm)	Single-stage, liquid-cooled thermal	FID	[95]
Hop essential oil	1-alkenes, methylketones and acids	Dilution	DB-5 (10 m × 0.18 mm i.d., 0.18 μm)	DB-17 (1.9 m × 0.1 mm i.d., 0.1 μm)	Cryogenic trapping	TOF-MS	[96]
Eucaliptus dunii essential oil	Volatile compounds	Steam distillation	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BP20 (1.5 m × 0.1 mm i.d., 0.1 μm)	LMCS	qMS, TOF-MS	[97]
Peppermint essential oil	Polar components	Dilution	OV1 or CW20M (30 m × 0.25 mm i.d., 0.25 μm)	OV1701 or CW20M (0.8 m × 0.1 mm i.d., 0.1 μm)	Dual-stage CO_2 jet	Dual stage quadrupole (MS)	[98]
Wine	Methoxypyra -zines	HS-SPME	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BP20 (1 m × 0.1 mm i.d., 0.1 μm)	LMCS	NPD, TOF-MS	[99]
Gingseng roots	Terpenoids	Steam distillation	DB-5ms (30 m × 0.25 mm i.d., 0.25 μm)	DB-1701 (1.6 m × 0.1 mm i.d., 0.1 μm)	Cold jet KT2001	TOF-MS	[100]
Roasted coffee beans, hazelnut	Volatile compounds	HS-SPME and SPME	Supelcowax-10 or BPX-5 (30 m × 0.25 mm i.d., 0.25 μm)	BPX-5 or SPB-5 (1 m × 0.1 mm i.d., 0.1 μm)	LMCS, two-stage thermal device	FID, qMS, TOF-MS	[101–105]

Table 2 (*Continued*)

Matrix	Compounds of interest	Sample treatment	First dimension	Second dimension	Modulator	Detection	Ref.
Vitis vinifera L. cv. 'Fernâo-Pires' white grape	Monoterpenoids	HS-SPME	Equity-5 (60 m × 0.25 mm i.d., 1 μm)	Supelcowax-10 (2.5 m × 0.1 mm i.d., 0.1 μm)	Dual stage jet cryogenic	TOF-MS	[106]
Cheddar chesse	Volatile compounds	Direct thermal desorption (DTD)	DB5 (10 m × 0.18 mm i.d., 0.18 μm)	DB17 (1.6 m × 0.18 mm i.d., 0.18 μm)	Jet-type	TOF-MS	[107]
Olive oil	Volatile compounds	DTD	VF-5ms (30 m × 0.25 mm i.d., 0.25 μm)	BPX50 (2 m × 0.1 mm i.d., 0.10 μm)	Quad-jet cryogenic	TOF-MS	[108]
Olive oil and vanilla extracts	Flavour compounds	Dilution and liquid extraction	CP-Wax 58 (25 m × 0.32 mm i.d., 0.30 μm) BP21 (30 m × 0.25 mm i.d., 0.25 μm)	BPX-35 (1 m × 0.10 mm i.d., 0.10 μm)	Home-made CO_2-cooled dual-jet	FID, TOF-MS	[109]
Honey	Volatile compounds	SPME	DB-5ms (30 m × 0.25 mm i.d., 0.25 μm)	BPX-50 or Supelcowax 10 (1.25 m × 0.1 mm i.d., 0.1 μm)	Cryogenic	TOF-MS	[110]
Ginger	Volatile compounds	SPME	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BP20 (0.8 m × 0.1 mm i.d., 0.1 μm)	Cryogenic	FID	[111]
Strawberry	Volatiles	SPME	EtTBS-β-CD (20 m × 0.25 mm i.d., 0.25 μm) CycloSil B coated column (26 m × 0.25 mm i.d., 0.25 μm)	BPX50 (1 m × 0.1 mm i.d., 0.10 μm)	LMCS	FID	[112]
Cachaça, vodka, gin, whiskey		SPME	BPX5 (30 m × 0.25 mm i.d., 0.25 μm)	BPX20 (1.5 m × 0.1 mm i.d., 0.1 μm)	LMCS	TOF-MS	[113–114]
Lemon flavored beverages	Flavour components	Liquid extraction and column chromatography	SPB-1 (15 m × 0.25 mm i.d., 1 μm)	Supelcowax-10 (0.7 m × 0.1 mm i.d., 0.1 μm)	Hot jet	FID	[115]
Sour cream	Flavour compounds	Solvent-assisted flavour evaporation (SAFE)	CP-Sil 5 CB (15 m × 0.25 mm i.d., 0.25 μm	BPX50 (0.8 m × 0.1 mm i.d., 0.1 μm)	LMCS	TOF-MS	[116]
Butter	Flavour compounds	SPE and SPME	BP21 (30 m × 0.25 mm i.d., 0.25 μm)	BPX35 (1 m × 0.10 mm i.d., 0.10 μm)	Home-made CO_2-cooled dual-jet	FID, TOF-MS	[117]
Beer, honey and wine	Amino acids	Dilution or liquid extraction and derivatization	BPX5 (30 m × 0.25 mm i.d., 0.25 μm) Chirasil-L-Val (25 m × 0.25 mm i.d., 0.16 μm)	BPX50 (2 m or 1 m × 0.1 mm i.d., 0.1 μm)	LMCS	FID, TOF-MS	[118,119]
Brown rice seeds	Metabolites	Liquid extraction and derivatization	Rtx-5 Sil MS (30 m × 0.25 mm i.d., 0.25 μm)	Rtx-50 (1 m × 0.18 mm i.d., 0.20 μm)	Thermal	TOF-MS	[122]
Coatings in contact with food	Food contaminants	Liquid extraction	PSS-255 (30 m × 0.25 mm i.d., 0.15 μm) DB-5 (10 m × 0.18 mm i.d., 0.18 μm)	SOP-50 (1.5 m × 0.1 mm i.d., 0.1 μm) BPX-50 (2 m × 0.10 mm, i.d., 0.10 μm)	Dual cryojet and thermal devices	FID, TOF-MS	[123–125]
Vegetal, animal and fish oils	Fatty acid methyl esters (FAMEs)	Transesterification and dilution	BPX5 or Supelcowax-10 (30 m × 0.25 mm i.d., 0.25 μm)	Supelcowax-10 or SPB-5 (1 m × 0.1 mm i.d., 0.1 μm)	LMCS	FID	[126]
Nutritional supplements	Anabolic steroids	Liquid extraction	DB5-MS (30 m × 0.25 mm i.d.,. 0.25 μm)	BPX50 (2 m × 0.1 mm i.d., 0.1 μm)	Cold jet	TOF-MS	[127]

sophisticated and powerful software in order to obtain, evaluate and show the data.

Up to now, a good number of GC × GC applications have been developed for food analysis. A summary of the most remarkable among these applications is presented in Table 2. One of the main uses of GC × GC in food analysis is for safety purposes including the determination of pesticides, contaminants and harmful compounds in different food matrices, such as fish, oil, fruit and milk [78–81]. As polychlorinated and polybrominated compounds are widely known for their toxic properties including carcinogenicity, immunotoxicity and induction of adverse effects in reproductive and endocrine systems, their presence in foods should be strictly controlled. Hoh et al. [78] developed a screening analysis of 17 polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/Fs) and 4 non-*ortho* polychlorinated biphenyls (PCBs) in fish oil. By using a TOF-MS as detector, low detection limits could be attained. In a different work, several toxaphenes contaminants were determined in the same matrix [79]. An enantioselective β-cyclodextrin-based column was employed in the first dimension together with a non-enantioselective column in the second dimension. The enantiomeric ratios and concentration levels of five chiral toxaphenes were determined in different commercial fish oil samples with adequate reproducibility, repeatability and low LODs [79]. Fig. 4 shows the GC × GC-μECD analysis of a fish oil sample. The different mechanisms of retention employed in each dimension permitted the correct separation of highly correlated dioxins. The possibilities of using GC × GC-ECD for determining dioxins and dioxin like PCBs in different food matrices (salmon, milk, oil, etc.) have been also recently described [81]. Numerous columns combinations were studied in order to select the pair of columns which maximized the separation obtained. Finally, a non-polar column was selected in the first dimension whereas a LC-50 (50% liquid crystal) column was employed in the second dimension. These columns were interfaced by a loop-type jet modulator.

Aromatic hydrocarbons, which also possess toxicity and harmful properties, have been studied in mussels using GC × GC–TOF-MS [85,86]. Polycyclic aromatic hydrocarbons (PAHs) have been determined in several edible oils like olive or sunflower [87]. In this case, GC × GC was directly combined with SPME for sample preparation. Later on, the extracted PAHs were analyzed by GC × GC using a TOF-MS detector. Besides, the fast modulation times (3 s) provided by a longitudinally cryogenic modulator enabled the multiple modulation of the components present on the samples. This SMPE-GC × GC–TOF-MS procedure allowed the elution of each compound free of interferences enhancing their identification with LOQs as low as 0.4 ppb [87].

Regarding to the analysis of pesticides and fungicides in foods, GC × GC–TOF-MS offers a great separation power in multiresidue analysis. Thus, Dallüge et al analyzed 58 pesticides in extracts of carrots and celeriac in the first reported application of GC × GC–TOF-MS in multiresidue analysis of food samples [88]. One year later, 20 pesticides were analyzed at trace level in baby food [89]. In a recently reported work, 36 pesticides acting as tea contaminants have been determined [90]. In this latter work, the LODs of the pesticides could be effectively lowered by selectively extracting the pesticides from the matrix, thanks to the use of HS-SPME coupled with comprehensive GC. Under these conditions, LOQs from 1 to 28 μg/kg were obtained for the studied pesticides, being 34 out of 36 detected below the permitted MRLs. Considering the more traditional labour-intensive solvent extraction approach, it was concluded that their development could be a useful tool in routine control of large sample batches [90]. In summary, regarding multiresidue analysis, comprehensive GC is able to provide with more separation power and significant improvements in sensitivity when compared to conventional GC [91].

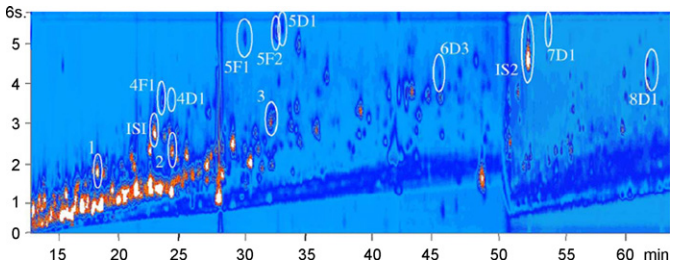

Fig. 4. GC × GC-μECD contour plot of a fish oil analysis (PCDD/F and non-*ortho*CBfraction) with DB-XLB × LC-50 column combination. Reprinted from [80]. Copyright (2005) with permission from Elsevier.

Besides food safety and analysis of contaminants, an important field of application of comprehensive GC is essential oils analysis. Lemon essential oil, which is considered one of the most complexes profiles among all citrus oils, has been analyzed using comprehensive GC with an FID detector and a LMCS modulating system [94] and compared to conventional GC–MS; results showed the superiority of GC × GC in terms of resolution and information achieved compared to conventional GC and MDGC. Lemon thyme essential oil has been analyzed using a transportable GC × GC instrument [95], consisting on an electrically heated and liquid-cooled single-stage thermal modulator with a conventional FID detector.

Comprehensive GC has emerged also as an important tool for the food industry. It is well known, that the particular composition of hops is a major determinant in beer flavor. Hops are composed of dozens of important volatile compounds which separation is greatly limited using conventional GC. For this reason, a new GC × GC–TOF-MS method was developed in order to evaluate the volatile profile of these important products [96]. Thanks to the MS detector employed, 119 compounds could be identified, 45 of them for the first time, demonstrating the great abilities of this technique to successfully separate and characterize extremely complex samples in a single chromatographic run. The same can be applied to the winemaking industry. For instance, comprehensive GC has been applied to the determination of potent odorant which are found at very low levels in wines such as methoxypyrazines. A combination of HS-SPME with GC × GC using a TOF-MS detector allowed the detection of this kind of compounds at concentrations ca. 1 ng/l avoiding the time-consuming traditional extraction procedures as well as any matrix effect in the determination [99]. The combination of SPME with GC × GC–TOFMS has been also employed in the analysis of the monoterpenoid profile of *Vitis vinifera* L. cv. 'Fernão-Pires' white grapes [106]. Very promising results were obtained in terms of compound classification based on the organized structure of the peaks of structurally related compounds in the GC × GC contour plot, as can be seen in Fig. 5. Moreover, GC × GC has been also proved useful in the food industry to determine and identify possible contaminants migrated from can coatings used for food storage [123,124] or ion exchange resins used in contact with foods [125].

Other valuable compounds, such as volatiles of roasted coffee beans [101–105] have been analyzed with three different detectors (FID, TOFMS and qMS), and again it was found that TOFMS was the best available option for identifying and quantifying peaks in comprehensive GC. Nevertheless, the qMS detector allowed the correct fingerprinting of several real samples, so that they could be grouped according to the processing carried out by selecting the appropriate markers in the 2D plane [102]. This particular sample, coffee and roasted coffee, exemplify perfectly the true potential of comprehensive GC, since it is widely considered as one of the most complex volatile profile [103]. Cheese volatiles are also regarded as important for their influence in its organoleptic properties. Direct thermal desorption was used to enrich the Cheddar cheese volatiles prior

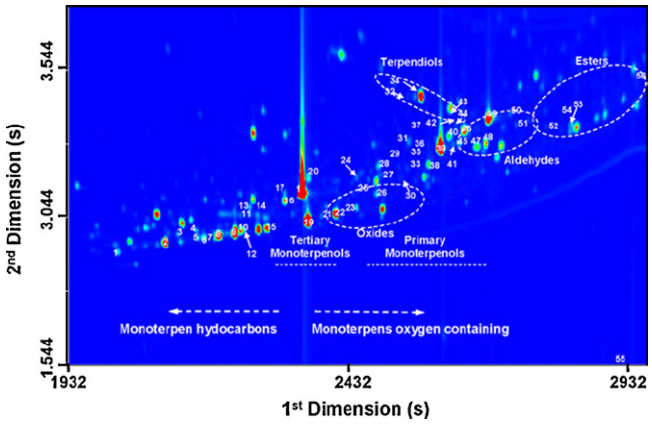

Fig. 5. GC × GC–TOF–MS extracted ion chromatogram contour plot of *m*/*z* 93, 121 and 136. Bands or clusters formed by structurally related compounds are indicated. Reprinted from [106]. Copyright (2007) with permission from Elsevier.

to their analysis [107]. DTD has also been employed to characterize volatile compounds in olive oil [108].

Honey is another complex matrix whose volatile composition has also been widely studied. The use of GC × GC for its analysis is no exception. The volatile profile can be used as an alternative to the analysis of pollen for the authentication of the honey botanical origin. Since it is a very complex matrix, solid phase microextraction (SPME) was used as sample treatment. [110]. Among the different kinds of fibers tested a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm fiber provided the best sorption capacity and, therefore, the highest amount of volatiles extracted from the headspace of a mixed honey sample. Using this fiber together with a relatively short GC × GC method (19 min run), 164 volatile compounds were identified, which could be used, later on, to establish authenticity markers.

Volatiles from spirits have also been analyzed by this comprehensive technique, like cachaça (a Brazilian sugar cane spirit), vodka, gin, whiskey and other flavored spirits [113,114]. By using a 5% phenyl-dimethyl polysilphenylene-siloxane primary column interfaced to a polyethyleneglycol short column in the second dimension by a LMCS modulator, the developed method allow the attainment of different profiles, or fingerprints, that could be useful to follow the distillation process and to identify its several phases, to observe the effect of wood storage and ageing period as well as for product quality analysis or even fraud detection [114].

Another kind of compounds that could act as quality markers and that have been studied by GC × GC are amino acids [118,119]. The enantioselective separation of chiral AAs previously derivatized with ethyl chloroformate has been achieved by using a GC × GC-FID equipped with a LMCS modulator [119], combining an enatioselective column (1D) with two non-enantioselective columns (2D) for the analysis of AAs in beer samples. In another approach, alkylchloroformate derivatised AAs have been separated with a GC × GC–TOF-MS/FID (LMCS modulator) system in three different food matrices (honey, wine and beer) [118].

A different application of comprehensive GC is related to Metabolomics, which focuses onto the analysis of low molecular weight, endogenous metabolites in tissues and biofluids. A large variety of analytical techniques as GC–MS, LC–MS, CE-MS or NMR have been used to reach this goal. Several papers have been recently published dealing with this topic [120–122] while only one is related to metabolic profiling of a food commodity (brown rice seeds) [122]; in that work, a GC × GC–TOF-MS (thermal modulation) method was developed and applied for the non-targeted metabolic profiling of brown rice seeds from the world rice core collection.

3. Multidimensional liquid chromatography (MDLC)

Different approaches have been used to couple several liquid chromatography-based separations, including two-dimensional or multidimensional LC performed in either off-line or on-line modes. From a practical point of view, multidimensional LC (MDLC) can be divided in three groups of techniques: off line MDLC, on line MDLC and comprehensive LC (LC × LC).

In off line MDLC, the fractions of interest eluting from the first LC separation are collected manually, evaporated and injected in a second LC separation. Thus, the results of these analyses will be a series of second dimension separations equal to the number of fractions collected from the first dimension.

In on-line MDLC, special interfaces are used to allow the coupling between the two separation dimensions. Thus, these systems automatically transfer selected fractions from the first to the second dimension. Like in off-line MDLC, only some fractions are reinjected in the second dimension.

A third mode of MDLC is comprehensive LC (or LC × LC) in which the whole sample is submitted to two independent separation processes. In this configuration, an interface is usually employed to couple the two dimensions in order to automatically and continuously collect the eluate from the first dimension and inject it into the second dimension separation.

First multidimensional LC works were based in the off-line coupling approach. However, an increase in the on-line and comprehensive applications is being observed in the last 10 years. In this section, representative applications of these three modes are described separately. Besides, the advantages and shortcomings of each MDLC mode are critically discussed.

3.1. Off-line MDLC

Off-line MDLC was the first mode of MDLC developed and thus, a lot of applications can be found in several different fields [128–131]. The instrumental set-up necessary to carry out off-line MDLC is quite simple; basically, a first separation is performed, and as a result one or more interesting fractions eluting from this separation are collected. Later on, these fractions are concentrated or evaporated (if needed) and re-injected in a second dimension, which will be ideally based on a different separation mechanism. Although it is relatively simple to enhance the separation power of one-dimensional analysis by using this type of analytical tool, off-line MDLC has some important drawbacks. Thus, apart from the laborious and time consuming methods, there is a great risk of degradation or losses of analytes when transferring the fractions from the first to the second dimension. Besides, other kind of artifacts can be formed. This problem can be even more important in the food analysis field, since the interesting compounds are usually quite unstable or labile and can be affected by different factors such as light or oxygen. Moreover, like almost every manual (non automatic) technique, the reproducibility is quite poor. In spite of these problems, a relatively high number of applications have been developed using off-line MDLC in the food analysis field.

One of the most important applications of this technique is related to the analysis of contaminants in food. Off-line MDLC has been extensively used to determine PAH in foods, mainly in fats and oils [132]. Typically, a silica column is employed in the first dimension to isolate the PAH fraction of the fat sample after injecting a quite high amount of sample (0.5 ml). After evaporating the solvent, a reversed phase separation is carried out to separate every single PAH [133,134]. This procedure has demonstrated to be reproducible and sensitive to detect PAH in olive oil and other edible oils [133], as well as in smoked fish samples [134]. Other contaminants have been also studied using this technique. In fact, a combination of off-line MDLC, ICP-MS and ESI-MS has been employed to

characterize the arsenic species found in *Laminaria* edible algae [135]. After the extraction of the arsenic species from the sample, three different chromatographic steps were carried out. Firstly an anion exchange step to purify the arsenic compounds, and then two different reversed phase separations followed by the parallel ICP-MS and ESI-MS detection. Using this methodology, it was possible to detect a good number of arsenic species avoiding the ambiguities related to peak purity observed when using only LC–ICP-MS [135].

Among the food related applications, the analysis of dairy products to determine or identify interesting proteins or peptides with functional properties is also extended. In most of these applications, the first dimension is used to isolate fractions from the water soluble extracts that are subsequently analyzed by LC–MS in order to precisely determine the sequence of the peptides contained in the active fractions [136–142]. One of the preferred strategies was applied by Quiros et al. [143], consisting on a first fractionation of the protein hydrolysates by using semipreparative HPLC followed by an HPLC–MS/MS analysis of the most active fractions in terms of antihypertensive activity. Following this workflow, several new antihypertensive active peptides were identified in fermented milks [143]. Similar strategy was also applied to the detection of active peptides from cheeses [144].

Related to the analysis of proteins, off-line MDLC has been also used to identify markers of meat adulteration using soybean proteins. Due to their low cost and technological properties, soybean proteins are often added to meat products to enhance their properties or to act like emulsifiers. However, the use of these proteins is limited by the regulation. A novel approach was developed [145] to determine soybean proteins in heat-processed meat products. A first protein fractionation step by perfusion chromatography was used, and then, the fraction of interest, containing the soybean proteins, was collected, evaporated, digested with trypsin and analyzed by nano-LC coupled to MS [145]. These steps can be observed in Fig. 6. As a result, different peptides corresponding to soybean proteins could be identified demonstrating the ability of the off-line MDLC methodology to carry out this kind of food quality control.

Triacylglycerides (TAG) have also been extensively analyzed by Laakso et al. [146–148]; who developed a series of applications for the elucidation of butterfat TAGs. In the mentioned works, two different dimensions were employed; a first silver ion HPLC analysis was carried out with the aim to fractionate the TAG contained in the butterfat according to the type and degree of unsaturation. Later on, the acyl carbon number distribution of the TAG in each fraction was elucidated by RP-HPLC–MS. The same type of LC separations have been recently used to elucidate the TAG profile of donkey milk [149]; in this application, a RP-HPLC separation was used in the first dimension, and the eluate fractionated, the interesting fractions were afterwards injected into a silver ion column to confirm peak identifications. As a result, 55 different TAGs were successfully identified in donkey milk, based on the different retention mechanisms in the two different dimensions and the APCI-MS detection used. The same strategy allowed the determination of beef tallow in lard [150], demonstrating how even 5% additions of tallow to lard can modify the positional isomer distribution of some TAG.

Other food related applications have been developed using off-line approaches, for instance, to quantitatively determine vitamin D3 and 25-hydroxyvitamin D3 in different meats [151,152]. This technique has been also used to study the speciation of selenium and manganese in garlic [153] and pine nuts [154], respectively. In this latter work, different orthogonal separations were carried out based on size exclusion chromatography and strong ion exchange chromatography together with ICP-MS detection.

3.2. On-line MDLC

On-line MDLC can be seen as a further development of the previously described off-line approach. In on-line MDLC, at least two different dimensions are coupled by using a special interface that is able to automatically transfer selected fractions from

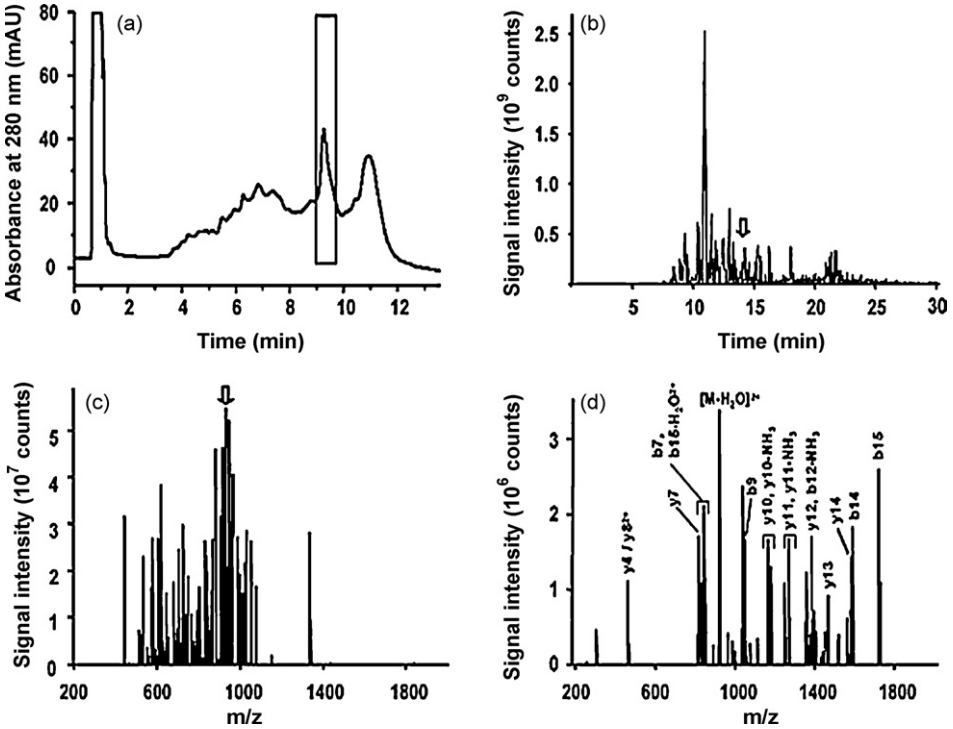

Fig. 6. Identification of a peptide from the soybean protein glycinin in heat-processed turkey meat sample. (A) Peak collected using perfusion chromatography. (B) MS/MS total ion chromatogram from the tryptic digest of the former fraction. (C and D) different MS and MS/MS spectra, respectively, corresponding to the identified soybean peptide. Reprinted with permission from [145]. Copyright (2006) American Chemical Society.

the first dimension eluate to the second dimension column. The most used interfaces are electronically controlled switching valves. This automation solves some of the problems associated to the off-line MDLC, such as the lack of reproducibility or the possibilities of degradation. However, the coupling of different separation mechanisms is not so easy to achieve, given that solvent incompatibilities and immiscibility problems can arise. Besides, the system is generally more difficult to operate. Nevertheless, this technique is regarded as a powerful one, and is commonly known as heart-cutting, illustrating the concept that only selected interesting fractions from the first dimension will be re-injected into the second dimension. This technique has been successfully employed in different fields [155,156], and food analysis is not an exception.

The determination of food constituents usually implies a high degree of difficulty due to the complex matrices under study, and heart-cutting LC can provide a step forward to solve problems in food analysis. A two-dimensional method was optimized to analyze several flavonoids in berry wines [157]. A first column was employed to perform an on-line sample clean-up before the transfer to the secondary column. Myricetin, quercitrin, kaempferol, rutin and isoquercitrin could be determined in these wines [157]. A more recent method has been developed to characterize and quantify other flavonoids such as naringin, hesperidin, neohesperidin, narirutin and eriocitrin in citrus juices as diasteromers [158]. The optimized method allowed the separation of every single flavanone glycoside in a C_{18} column and their transfer into a carboxymethylated β-cyclodextrin-based column to be further separated into their respective stereoisomers. This way an interesting comparison could be established between freshly squeezed and commercial juices. It could be observed that for some of the separated compounds, the diastereomeric ratios found in the freshly squeezed citrus juices were significantly different than those from the commercial counterparts.

Heart-cutting MDLC has been recently employed for safety purposes. THI (2-acetyl-4-(1,2,3,4-tetrahydroxybutyl)imidazole) is a immunotoxic compound that it is formed during the Class III caramel color manufacture, a dye frequently used in the food industry. Two different methods using either a C_{18} column or a strong cation exchange column in the first dimension together with a porous graphitic carbon column in the second dimension [159] have been reported for THI analysis. Both heart-cutting methods provided acceptable quantitative results, according to the European Commission requirements. Other contaminants such as pesticides [160] or antibiotics [161] have been also determined by on-line MDLC. For pesticides analysis, two different C_{18} columns have been connected by means of a switching valve. The elution conditions varied so that a desalting step could be performed in the first dimension to enhance the separation in the second dimension using MS as detection. Under these conditions, several phenylurea herbicides could be detected in water at levels below 0.01 μg/l in about 25 min as total analysis time [160].

3.3. Comprehensive two-dimensional LC

Comprehensive liquid chromatography (LC × LC) is based on the analysis of a whole sample, typically, in two different consecutive separation procedures. Using LC × LC configuration the whole sample is analyzed in the two dimensions. This is a significant difference compared to heart cutting or off-line MDLC and has allowed to improve dramatically the separation power to values that otherwise could not be obtained (e.g., by technological modifications such as column technology improvements or high temperature separations). A comprehensive separation is attained by coupling two different separation processes by means of an interface (also called modulator) able to continuously collect the eluate from the first dimension separation and re-inject it in the second dimension. Usually, switching valves are used as interfaces and theoretically different combinations of the diverse LC separation modes (SEC, RP, NP, IEX or AC) can be coupled. As a result, the peak capacity can be increased using LC × LC [162–166]. Even considering that the product of the single peak capacities obtained in the two dimensions overestimates in great extent the total effective peak capacity [163], it is clear that the enhancement achieved by LC × LC compared to one-dimensional approaches is very significant. It is also important to remark that the huge amount of data that can be produced after a comprehensive LC separation will increase the need for specific software as well as for the use of chemometric tools [167].

Despite the great advantages associated to LC × LC, some problems have to be still addressed in order to be able to take full advantage of the potential of this technique. Namely, solvent immiscibility (since there is not an evaporation step between the two separations (like in off-line MDLC)), solvent incompatibility (between the first and the second dimension); analysis time in the second dimension (since separation has to finish before the next fraction is transferred) and transfer velocity (since the transfer has to be made fast enough to avoid eluate losses from the first dimension). A basic requirement is that two components separated in the first dimension have to remain separated in the second dimension. For this reason, the sampling of fractions from the first dimension to the second dimension is a critical parameter to consider when optimizing any LC × LC method [164].

Today, LC × LC can be considered as a mature technique. Since the first published attempt to put in practice a comprehensive LC system in 1978 [168], LC × LC has found a large number of applications in different analytical fields [166,169–175]. Table 3 shows a summary of the most representative LC × LC food-related applications.

Although to maximize the resulting peak capacity different separation mechanisms with non correlated selectivity (i.e., orthogonal) in the two dimensions are preferred, some degree of orthogonality can be also attained by using two different stationary phases, even though the same separation mechanism is used. This effect has been widely studied by Jandera et al. for the separation of phenolic antioxidants from beer and wine samples [176,178,180,186,190,192,193]. Different stationary phases and columns have been tested; generally, it can be confirmed that a combination of a polyethylene glycol-silica column in the first dimension and a C_{18} stationary phase in the second dimension produced the highest degree of orthogonality [192]. This approach could be useful to separate and identify phenolics and flavonoids from beer and wines samples without any pretreatment other than filtration of the sample prior injection. Besides, different interfaces were compared. The most common strategy in LC × LC is the employment of a switching valve with two identical injection loops installed, so that the first dimension eluate can be simultaneously collected and injected into the second dimension. However, it has been already shown that the use of two trapping columns instead of the injection loops in the interface can be also a valid approach for the separation of phenolic compounds [186]. The same group also explored the use of two parallel columns in the second dimensions to separate these compounds [180]; the use of two alternative columns allowed longer re-equilibration periods of the second dimension columns, although it was difficult to find two columns identical. Besides, the use of high temperatures in the second dimension separation was explored using zirconia stationary phases; results demonstrated that the use of temperatures up to 120 °C improved the resolution and speeded up the second dimension separations [180].

Wine is a challenging food complex matrix containing a wide variety of polyphenolics. New approaches, different from the ones mentioned above, have been tested by different authors. For exam-

Table 3
LC × LC applications in food analysis (GE, gradient elution; IE, isocratic elution).

Matrix	Compounds of interest	Sample treatment	First dimension	Second dimension	Interface	Fraction Collection	Modulation time	Detection	Observations	Ref.
Standards, beer, hop extract	Phenolic antioxidants	Dilution and filtration	PEG (50 × 2.1 mm, 3 μm) 10 mM CH_3COONH_4 (pH = 3)/ACN (99:1), F: 0.4 ml/min	C_{18} (125 × 2.0 mm, 5 μm) MP: 10 mM CH_3COONH_4 (pH = 3)/ACN; F: 0.4 ml/min	Six port switching valve	–		UV-Vis	RP × RP Stop flow mode	[176]
Red Orange essential oil	Carotenoids	Filtration	CN (250 × 1.0 mm, 5 μm) MP (GE): hexane/butyl acetate/acetone 80:15:5, and hexane. F: 10 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN/water 80:20. F: 5 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	2 min	DAD APCI-MS	NP × RP	[177]
	Phenolic acids and flavonoids		PEG (150 × 2.1 mm, 5 μm). MP (GE): 5 mM ammonium acetate (pH = 3) and ACN. F: 0.05 ml/min	C_{18} monolithic (50 × 4.6 mm). MP (GE): 5 mM ammonium acetate (pH = 3) and ACN. F: 3.5 ml/min	Ten port-2 position switching valve	2× 100 μl injection loops	2 min	DAD	RP × RP	[178]
Stevia rebaudiana	Glycosides		C_{18} (150 × 2.1 mm, 3 μm). MP (GE): water and ACN. F: 0.1 ml/min	NH_2 (50 × 2 mm, 3 μm). MP (IE): ACN/water 1:1. F: 1.8 ml/min	Ten port-2 position switching valve	2× 100 μl injection loops	1 min	TOF-MS	RP × RP MS based quantification	[179]
Standards, beer and wines	Phenolics	Filtration	C_{18} (150 × 2.1 mm, 5 μm). MP (GE): 10 mM ammonium acetate (pH = 3) and methanol	ZR-Carbon (20 × 2.1, 5 μm). MP (IE): methanol/0.06 M H_3PO_4 + 0.01 M CH_3COONH_4 40:60. F: 1 ml/min	Ten port-2 position switching valve	Two parallel second dimension columns	3 min	DAD	RP × RP 2nd D at 120 °C	[180]
Lemon oil	Coumarins and psoralens		SI (300 × 1 mm, 5 μm). MP (IE): hexane/ACN 75:25. F: 20 μl/min	C_{18} monolithic (25 × 4.6 mm). MP (GE): water and ACN. F: 4 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	1 min	DAD	NP × RP	[181]
Wines and juices	Phenolics	Filtration and dilution	C_{18} (150 × 2.1 mm, 3 μm). MP (GE): Water (0.5% acetic acid) and ACN. F: 0.1 ml/min	C_{18} (50 × 3 mm, 2.5 μm). MP (IE): 15 mM tetrapenty-lammonium bromide in ACN/water (0.05% acetic acid) 21:79. F: 1.35 ml/min	Ten port-2 position switching valve	2× 200 μl injection loops	1.5 min	DAD	RP × IPC	[182]
Lemon oil	Coumarins and psoralens		Diol (250 × 1 mm, 5 μm). MP (GE): hexane/ethyl acetate 90:10 and ethyl acetate. F: 30 μl/min	C_{18} (50 × 4.6 mm, 3.5 μm). MP (GE): water and ACN. F: 4 ml/min	2 ten port-2 position switching valves and 2 secondary columns	2× 30 μl injection loops	1.9 min	UV (254 nm)	NP × RP 2 parallel detectors after each secondary column	[183]
Lamiaceae	Phenolic acids	Dynamic sonication assisted extraction	C_{18} (150 × 2.1, 3 μm). MP (GE): water (0.5%acetic acid)/ACN 85:15 and ACN. F: 0.1 ml/min	CN (75 × 4.6 mm, 3 μm). MP (IE): water (0.5% acetic acid)/ACN 65/35. F: 1.9 ml/min	Ten port-2 position switching valve	2× 130 μl injection loops	35 s	TOF-MS	RP × RP MS based quantification. LOD: 18-90 ng/ml	[184]

Table 3 (*Continued*)

Matrix	Compounds of interest	Sample treatment	First dimension	Second dimension	Interface	Fraction Collection	Modulation time	Detection	Observations	Ref.
Plant oils	Triacyl glicerols		Ag+ (150 × 1 mm, 5 μm). MP (GE): hexane with 0.7% ACN and hexane with 0.9% ACN. F: 11 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN. F: 4 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	2 min	APCI-MS	Ag+xRP	[185]
Beer and wines	Phenolics and flavonoids		PEG (150 × 4.6 mm, 5 μm). MP (GE) water and ACN. F: 0.3 ml/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): water and ACN. F: 2 ml/min	Ten port-2 position switching valve	Two C_{18} (30 × 4.6 mm, 2.5 μm) trapping columns	8 min	DAD	RP × RP	[186]
Corn oil	Triacyl glicerols		Ag+ (250 × 2.1 mm, 5 μm). MP (GE): methanol and 6%MeCN in methanol. F: 20 μl/min	C_{18} (30 × 4.6 mm, 1.8 μm). MP (IE): methanol/MTBE 70/30. F: 3.0 ml/min	Ten port-2 position switching valve	2× 40 μl injection loops	1 min	UV (210 nm) ELSD APCI-MS	Ag+xRP	[187]
Mandarin essential oil	Carotenoids	Filtration	SI (300 × 1.0 mm, 5 μm). MP (GE): hexane and etanol. F: 10 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN. F: 4.7 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	2 min	DAD APCI-MS	NP × RP	[188]
Citrus oil	Psoralens and flavones		Diol (250 × 1 mm, 5 μm). MP (IE): hexane/ethyl acetate 90/10. F: 30 μl/min	C_{18} (50 × 4.6 mm, 3.5 μm). MP (GE): water and ACN. F: 5 ml/min	Ten port-2 position switching valve	2× 30 μl injection loops	1 min	UV (315 nm)	NP × RP	[189]
Beer	Phenolic antioxidants	Filtration	PEG (150 × 4.6 mm, 5 μm). MP(IE): 0.010 M ammonium acetate (0.1% ACN, pH = 3.0). F: 0.3 ml/min	C_{18} monolithic (50 × 4.6 mm). MP (GE): water and ACN. F: 2 ml/min	Ten port-2 position switching valve	Two C_{18} (30 × 4.6 mm, 2.5 μm) trapping columns	9 min	DAD	RP × RP 2nd D separation at 40 °C	[190]
Orange	Carotenoids	Carotenoid extraction and saponification	SI (300 × 1.0 mm, 5 μm). MP (GE) hexane and ethyl acetate. F: 10 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN/water 80:20. F: 4.7 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	2 min	DAD	NP × RP	[191]
Beer	Phenolic antioxidants	Filtration	PEG (150 × 2.1 mm, 5 μm). MP (GE): ACN and 0.01 M ammonium acetate. F: 25 μl/min	C_{18} (30 × 3.0 mm, 2.7 μm). MP (GE): ACN and 0.01 M ammonium acetate. F: 1 ml/min	Ten port-2 position switching valve	2× 100 μl injection loops	4 min	DAD ESI-MS	RP × RP	[192]
Wines	Phenolic antioxidants	Filtration	PEG (150 × 2.1 mm, 5 μm). MP (GE): ACN and 0.01 M ammonium acetate. F: 25 μl/min	C_{18} (30 × 3.0 mm, 2.7 μm). MP (GE): ACN and 0.01 M ammonium acetate. F: 4.8 ml/min	Ten port-2 position switching valve	2× 100 μl injection loops	35 s	DAD	RP × RP 2nd dimension operated at 60 °C	[193]
Citrus essential oils	Aurapten		SI (300 × 1.0 mm, 5 μm). MP (IE): hexane/ethyl acetate 80:20. F: 18 μl/min	C_{18} monolithic (25 × 4.6 mm). MP (GE): ACN and water. F: 4 ml/min	Ten port-2 position switching valve	2× 20 μl injection loops	1 min	DAD	NP × RP Quantification based on 2D plots	[194]

Donkey milk	Triacyl glicerols	TAGs extraction	Ag^+ (150 × 1 mm, 5 μm). MP (GE): hexane with 0.7% ACN and hexane with 0.9% ACN. F: 11 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN. F: 4 ml/min	Ten port-2 position switching valve	2 × 22 μl injection loops	2 min	APCI-MS	Ag+xRP	[195]
Rice oil	Triacyl glicerols		Ag^+ (150 × 1 mm, 5 μm). MP (IE): hexane with 0.7% ACN. F: 13 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN. F: 4 ml/min	Ten port-2 position switching valve	2 × 20 μl injection loops	1.5 min	APCI-MS	Ag+xRP	[196]
Wines	Phenolic antioxidants	Filtration	Phenyl (250 × 1.0 mm, 5 μm). MP (GE): water and ACN. F: 10 μl/min	C_{18} (30 × 4.6, 2.7 μm). MP (GE): water and ACN. F: 4 ml/min	Ten port-2 position switching valve	2 × 20 μl injection loops	2 min	DAD ESI-IT-TOF-MS	RP × RP	[197]
Orange juices	Epoxy carotenoids	Carotenoids extraction	CN (250 × 1.0 mm, 5 μm) MP (GE): hexane/butyl acetate/acetone 80:15:5, and hexane. F: 10 μl/min	C_{18} monolithic (100 × 4.6 mm). MP (GE): 2-propanol and ACN/water 80:20. F: 5 ml/min	Ten port-2 position switching valve	2 × 20 μl injection loops	2 min	DAD APCI-MS	NP × RP	[198]

ple, Dugo et al. [197] developed an RPLC × RPLC comprehensive method using a microbore phenyl column in the first dimension operated at low flow rates (10 μl/min) and a short C_{18} column with partially porous particles in the second dimension at high flow rates (4 ml/min). Thanks to the use of a DAD together with an IT-TOF-MS detector, 18 different polyphenolic compounds from different families could be adequately separated and identified [197]. Other TOF-MS approach used to study the phenolic compounds from different wines and juices, employed a RPLC × IPLC (ion pairing liquid chromatography) development [182] in which the separation is based on hydrophobicity (in the first C_{18} dimension) while the separation, in the second dimension is based on the ionic properties of the compounds (the IPC retention mechanism is not yet completely understood) [182].

TOF-MS detection has also been employed to identify and quantify different phenolics from plants. In these studies, different RPLC × RPLC methods were optimized using a C_{18} column in the first dimension and either an amino based column [179] or a cyano column in the second dimension [184]. Moreover, in these works, reduced modulation times were employed in order to limit the undersampling of interesting compounds eluting from the first dimension. Besides, the MS extracted ion based quantification allowed the attainment of good LODs [184].

Although a good number of applications are being developed, in practice there are only few in which the quantification of the separated compounds is also considered. This can be due to the fact that some parameters in LC × LC do not have the same interpretation than in conventional LC [199] and also to the fact that most analysts use their own in-lab developed software to obtain the useful 2D plots [200]. Mondello et al. recently studied this topic in detail [194] and some applications have been already developed in which direct contour plots based quantifications are shown [201].

Although, some relatively orthogonal separations have been obtained coupling two RPLC separations using different stationary phases, other couplings are by far more adequate to obtain orthogonal two-dimensional separations. Among them, NPLC × RPLC has been explored in several food applications. NPLC × RPLC presents a series of practical problems, mainly related to the different mobile phases incompatibility, which could generate broadened and distorted peaks in the second dimension [189]. To overcome this problem, Dugo et al. [181] proposed a methodology that included the use of a long microbore column in the first dimension and a monolithic column in the second dimension. This combination allowed maintaining really low flow rates in the first dimension, as low as 10 μl/min, whereas the monolithic column, due to its high permeability, allowed the use of high flow rates (more than 4 ml/min) being possible to perform fast second dimension separations. In this way, the first dimension eluate volume injected in the second dimension could be set to 20 μl, avoiding any incompatibility. Using this approach, it was possible to study the coumarins and psolarens composition of several citrus oils [181,183,189], as well as the orange essential oil, orange juice and mandarin carotenoids composition [177,188,191,198]. In fact, this technique was used not only to elucidate the orange free carotenoids pattern [191], but also to show the complex orange carotenoids native pattern [188]. Analysis of intact carotenoids in real samples, such as orange, is a challenge considering that these compounds can be found in nature either in their free form or as fatty acids esters. Due to this complexity, a saponification step is generally performed prior to carotenoid analysis, although the study of the carotenoid pattern of a saponified sample could not give a complete true picture of the carotenoids naturally contained in a sample. Thanks to the high resolving power of LC × LC and to the combined use of a DAD and APCI-MS detector, it was possible for the first time to separate and identify the native carotenoids found originally in orange without any pre-treatment [188]. Fig. 7 shows the separation of

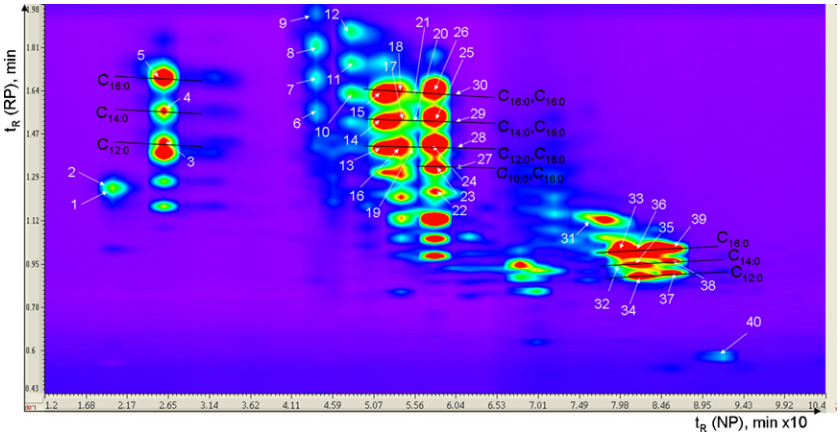

Fig. 7. Two-dimensional plot of the orange native carotenoids (free carotenoids and carotenoid esters) by NPLC × RPLC. Reprinted with permission from [177]. Copyright (2008) American Chemical Society.

orange native carotenoids under the optimum NPLC × RPLC separation conditions.

TAG analysis from food samples has been continuously regarded as challenging. Taking advantage of the combination of two dimensions composed by a silver ion separation (elution according to the increasing number of double bonds) and a reversed phase separation (elution according to the partition number) together with the use of a MS detector, some interesting LC × LC applications in this area were developed. Mondello et al. optimized a method using a microbore silver ion column and a short monolithic column [196]. The flow rates in the first dimension were kept low, and two identical injection loops were employed in the modulator to transfer the eluate to the second dimension. In the secondary column the flow rate was set at 4 ml/min, and each individual separation could be finished in less than 1.5 min, which was the modulation time. Using this approach, TAG pattern from rice [196] and other plant oils [185] as well as donkey milk [195] were obtained. Thanks to the APCI-MS detection, it was even possible to differentiate between TAG isomers. Following this methodology, the complex TAG profile of corn oil was lately studied [187], as can be observed in Fig. 8.

4. Multidimensional LC–GC

The idea of a coupling between liquid chromatography and gas chromatography was born some decades ago, the first work describing this coupling and some applications was published in 1980 [7]. However, the first automated system was not constructed till 1987 [202].

One of the main problems of LC–GC is the transfer of the eluent from LC to GC since the liquid fraction from LC must be vaporized before entering into GC. Thus, removal of the eluent is a challenge in LC–GC, moreover, considering the different liquid phases used depending on the separation mechanism employed. Normal-phase (NP) solvents are usually compatible with GC because they are organic solvents, and thus easily evaporated. But when reversed-phase LC (RPLC) is used, the transfer to GC becomes more difficult due to the aqueous nature of the mobile phase, thus requiring special techniques. A last problem is to match the volumes commonly used for LC and GC, since LC fractions are normally hundreds of microliters while GC uses few microliters; therefore, the need of specially designed interfaces is mandatory [203].

There are several works devoted to the design of interfaces for coupling LC–GC [204–206]. Grob classified them according to the LC mode used [10]. One of the most used transfer technique in NPLC–GC is based on wire interfaces, which was previously introduced for fully or partially concurrent eluent evaporation [207] and could be used with or without co-solvent trapping. For more volatile components, the retention gap technique with different modifications is preferred [208,209]. The programmed-temperature vaporizer (PTV) is used as an interface when large volumes of injection are needed [210]. In RPLC–GC analysis, the mobile phase usually contains water so the transfer will require special conditions and devices: one possibility is to transfer via phase switching, that behaves like an on-line liquid-liquid extraction of the mobile phase [211] while another option is to employ a vaporization with hot injectors of the RPLC eluent [10,212].

There are two modes of working with LC–GC, on-line and off-line. In the on-line approach, a first separation occurs in the LC and then the whole eluent with the isolated analytes is transferred to

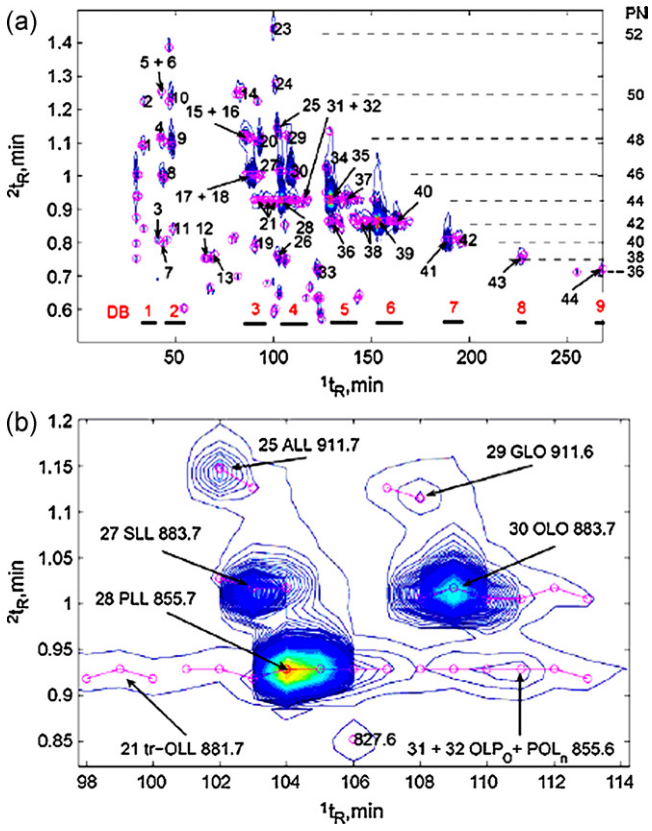

Fig. 8. Contour plot of corn oil constructed on the basis of the TIC chromatogram (top) and expansion (bottom). Separation on the basis of the number of double bonds in the first dimension and according to their partition number (PN) in the second dimension. Reprinted from [187]. Copyright (2008) with permission from Elsevier.

Table 4
LC–GC applications in food analysis.

Matrix	Compounds of interest	Sample treatment	LC Column	GC column	Transfer	Detection	Observations	Ref.
Nuts, coffee, cocoa chocolate	Hydrocarbons	Decantation	SpherisorbS-5-W (l0 cm × 2 mm i.d.)	PS-255 (25 m × 0.32 mm i.d.)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[213–216]
Powdered baby food	Hydrocarbons	Decantation	Spherisorb Si 5 (10 cm × 2 mm i.d.)	DB1 (30 m × 0.32 mm i.d.)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[217]
Infant food (potato, broccoli, meat)	Epoxidized soybean oil	Transesterification	Grom-Sil 100 Cyano-2 PR, (25 cm × 2 mm i.d., 5 μm)	PS-255 (30 m × 0.25 mm i.d., 0.2 μm)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[218]
Fatty foods	Polyaromatic hydrocarbons	On-line solvent extraction	Spherisorb Si (25 cm × 4.6 mm i.d., 5 μm) Spherisorb NH_2 (10 cm × 4.6 mm i.d., 5 μm).	PS-255 (15 m × 0.25 mm i.d., 0.3 μm)	Concurrent eluent evaporation (loop interface)	UV, FID	NPLC–LC–GC	[219]
Chicken, potatoes, sea foods, shrimps	Hydrocarbons	Solvent extraction, decantantion	SpherisorbS-5-W (l0 cm × 4.6rnm i.d.) Lichrospher. (25 cm × 2 mm i.d.)	PS-255 (35 m × 0.25 mm i.d.) 0.3 μm)	Concurrent eluent evaporation	UV, FID	NPLC–LC–GC	[220]
Vegetables	Fungicide (fenarimol)	Decantation	LiChrosorb Si 50 (12.5 cm × 4.6 mm i.d., 5 μm)	CP-Si15 CB (22 m × 0.25 mm i.d., 0.4 μm)	Concurrent eluent evaporation (loop interface)	FID, ECD	RPLC–GC	[221]
Olive oil	Pesticides	Filtration	Kromasil C_4 (5 cm × 4.6 mm i.d.)	Phenyl methyl silicone (30 m × 0.32 mm i.d., 0.25 μm)	TOTAD	FID	RPLC–GC	[223–224]
Lemon peel essential oils	Methyl epijasmonate	Solid-liquid extraction	C_6 (15 cm × 4.6 mm i.d., 10 μm)	5% methyl polysiloxane. (30 m × 0.25 mm i.d.) 0.25 μm)	TOTAD	FID	RPLC–GC	[225]
Rice	γ-Oryzanol	Solid-liquid extraction	Eurospher, 100. (25 cm × 2 mm i.d., 5 μm)	Trifluoropropylmethyl polysiloxane (27 m × 0.25 mm i.d., 0.1 μm)	Concurrent eluent evaporation (loop interface)	LC-UV GC-FID	NPLC–GC	[226–227]
Olive oil	Furan fatty acids	Transmethylation	Lichrospher 60. (25 cm × 2 mm i.d., 5 μm)	PS-255 (30 m × 0.32 mm i.d., 0.2 μm)	Concurrent eluent evaporation (loop interface)	LC-μUVis GC-PID-FID GC–MS	NPLC–GC	[228]
Olive oil and butter	Fatty acids methyl esters and tri-(-acyl)glycerides	Solvent extraction, decantantion	Chromspher Lipids 5 μm (25 cm × 2.0 mm i.d.)	VF-25MS (25 m × 0.25 mm i.d., 0.25 μm)	At-line coupling with intermediate fraction collection	TOF-MS FID	NPLC × GC	[229]
Meat derived products	Nervonic acid	Solvent extraction, decantantion	LiChrosorb Si 50, 5 μm. (12.5 cm × 4.6 mm i.d.)	DB5 (60 m × 0.32 mm i.d., 0.25 μm)	Six port valve and uncoret precolumn connected by a special stainless steel T	QMD (MS) FID	NPLC–GC	[230]
Cocoa butter	Steryl esters	Solvent extraction	Zorbax-SB 80. (15 cm × 2.1 mm i.d.5 μm)	DB-5ht (15 m × 0.25 mm i.d.)) 0.1 μm)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[231]
Coffee bean	16-O-Methylcafestol and sterols	Solvent extraction	Hypersil 60 Å, (2.1 cm × 10 mm i.d., 5 μm)	CP-Sil8CB (25 m × 0.25 mm i.d., 0.4 μm)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[232]
Olive oil	Sterols, fatty alcohols, esters	Solvent extraction	Spherisorb S-5-W (10 cm × 2 mm i.d.)	PS-255 (15 m × 0.32 mm i.d.)	Concurrent eluent evaporation (loop interface)	FID	NPLC–GC	[233]
Olive oil	*n*-Alkanes	Filtration and dilution	Spherisorb (100 cm × 4.6 mm i.d)	PS-255 (12 m × 0.25 mm i.d., 0.3 μm)	Concurrent eluent evaporation (loop interface	FID	NPLC–GC	[234,235]
Fruits	Chiral lactones	Steam distillation solvent extraction	Vydac 214 TPB 10 (5 cm × 4.6 mm i.d.)	Chirasil-β-Dex (25 m × 0.25 mm i.d., 0.25 μm)	PTV	FID	RPLC × GC	[236]
Essential oils	β-Pinene and limonene	Dilution	C_6 (10 cm × 4.6 mm i.d., 10 μm)	Heptakis (6-O-t-butylsilyl-2,3-di-O-ethyl)-β-CD doped into 14%cyanopropylphenyl/86% dimethylpolysiloxane (30 m × 0.32 mm i.d., 0.25 μm)	PTV	FID	RPLC–GC	[237]

the capillary column of the GC via the selected interface. In the off-line mode not all the LC eluent is transferred onto the GC, and LC is employed as a pre-fractionation method before the GC analysis. It is useful when it is not possible to separate the compounds of interest in a single GC run. Nevertheless, on-line LC–GC is more employed than off-line LC–GC in food analysis, since on-line LC–GC requires less amount of sample and less sample manipulation. In Table 4, several applications of LC–GC in food analysis are summarized.

An interesting example of this group of applications is devoted to the analysis of food contaminants; Grob et al. developed the analysis of mineral oil polyaromatic hydrocarbons in fatty foods using an automated on-line LC-solvent evaporation system (LC-SE–LC-UV–GC-FID), being the first application of the solvent evaporator in an on-line system [219]. Fig. 9 shows the LC-SE–LC-UV and GC-FID chromatograms of a mineral oil material in a non-refined linseed oil. The approach of LC–LC–GC-FID has been also used for the determination of food irradiation in complex samples (soup mixes, spices, fish and shrimps) [220].

As it has been commented, on-line LC–GC techniques are highly sensitive and selective, making possible the determination of pesticide residues in complex matrices as foods. The fungicide fenarimol was determined in fruiting vegetables such as cucumbers, tomatoes and sweet peppers by an on-line LC–GC method [221] using a loop-type interface with concurrent eluent evaporation; two different detectors were used, ECD for the qualitative identification of fenarimol and FID for the quantitative determination. On the other hand, Hyötyläinen et al. used another interesting interface for determining pesticides in red wine samples by RPLC–GC-FID [212]. A vaporizer/pre-column solvent split/gas discharge interface was used for the coupling between LC and GC; this interface allowed the transfer of the aqueous eluent from the LC to the GC.

The use of a PTV injector, packed with a suitable trapping material has been described; this interface, called through-oven-transfer-adsorption/desorption (TOTAD) has been used for on-line RPLC–GC coupling [222] and applied to the analysis of pesticides in olive oil [223,224] and to the isolation of (+)-methyl epijasmonate, one of the four different stereoisomers of methyl jasmonate, an endogenous plant growth regulator, from lemon [225].

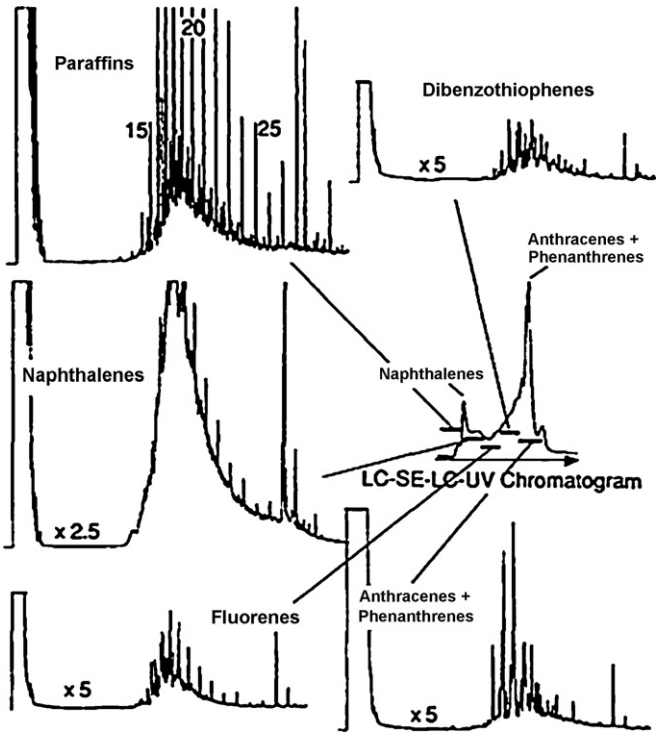

Fig. 9. LC-SE–LC-UV and GC-FID for the analysis of mineral oil material in a non-refined linseed oil. Reprinted from [219]. Copyright (1996) with permission from Elsevier.

Fatty acids, esters, sterols, lipids and several other organic compounds have been analyzed in different food matrices by different LC–GC methodologies. Off line comprehensive NPLC × GC has been employed to determine fatty acids methyl esters (FAMEs) and tri-(acyl) glycerides (TAGs) in olive oil and butter [229]. LC × GC as a comprehensive technique has more resolution power than the LC–GC and allows the characterization of the whole sample; if LC × GC is hyphenated with TOFMS, a third dimension analysis is included, increasing the application range of the technique. In fact, in this work, the use of a silver ion LC column in the first dimension allowed the separation of the TAGs according to their number of double bonds, whereas in the second GC dimension, the compounds were further separated according to their number of carbon atoms. The fractions were collected and transferred to the second dimension every minute [229]. This NPLC–GC–MS approach has been also use in other applications for fatty acids analysis, even via a concurrent eluent evaporation interface [231].

Another interesting application of a LC–GC coupling was oriented to the development of a method capable of distinguishing natural and non-natural commercial essential oils [237]. To do that, the enantiomeric analysis of β-pinene and limonene in several essential oils was carried out. A reversed phase separation was employed in the first dimension. The eluate transfer was performed through the chromatograph PTV injector filled with PDMS. The determination of s-enantiomers proved the predominace of synthetic aromas employed to enhance the quality of the natural commercial essential oils [237].

5. Other multidimensional chromatography couplings

The increase on the use of hyphenated and coupled techniques has favored the emergence of new multidimensional couplings other than multidimensional GC and LC based approaches, for instance using supercritical fluid chromatography (SFC). Some of them have been applied in food analysis. Hirata et al. developed a comprehensive SFC × SFC system to separate TAG from different edible oils and fats [238]. The system included a first dimension using an ODS packed column operating under constant pressure and stop-flow mode, using carbon dioxide as mobile phase; afterwards, a capillary trap mounted in a switching valve was employed to retain the compounds eluting from this first dimension that were then transferred into the second dimension. The capillary trap was packed with stationary phase material, which enhanced the trapping efficiency compared to a bare capillary. The secondary column was packed with the same ODS material than the first dimension column and was operated under constant flow conditions; an FID was used as detector.

In a recent work the construction of an interface to carry out comprehensive separations combining SFC and LC is described [239]. A modified interface was employed based on the use of a ten-port switching valve equipped with two packed C_{18} loops to trap the solutes eluting from the first dimension. Besides, a water make-up flow was added to the SFC effluent in order to enhance the focusing of the analytes in the trapping stationary phase material and to reduce interferences from the carbon dioxide in the second dimension. SFC-LC could be an alternative to NPLC × RPLC systems due to its high orthogonality and the lack of miscibility problems due to the complete removal of the CO_2 employed as solvent in the first dimension. The suitability of the set-up was demonstrated analyzing a lemon oil sample; a high amount of compounds could be separated and capacity values higher than 600 were obtained [239]. Separation of phenacyl esters of fatty acids from a fish oil

extract was also achieved through a comprehensive analysis using silver-ion (SI) supercritical fluid chromatography (SFC) followed by RPLC [240].

6. Conclusions and future trends

In this work, we have tried to present an overview on the vast field of multidimensional chromatographic techniques and their main applications in food analysis. These techniques provide with extraordinary gains in separation power and resolution that make them ideal for the analysis of complex matrices such as foods. Although the coupling between different chromatographic separations is not new, the technological development has led, above all, to the increase of comprehensive applications in which the whole sample is analyzed in two different, independent dimensions, reducing the sample preparation steps. The number of applications regarding the use of such comprehensive techniques increases every year also in the food analysis domain.

From a technical point of view, it is already possible to have a wide range of modulators commercially available, thus enabling the comprehensive operation. Nevertheless, some problems inherent to the connection of the two systems still persist, for instance related to the relatively costly operation conditions in GC × GC or the loss in sensitivity in LC × LC. In the coming years, new solutions should appear in order to facilitate these couplings as well as to further increase the orthogonality of the systems and, consequently, their separation power and applications. Keeping the cost of analysis as low as possible should be also a priority when designing efficient and new comprehensive GC and SFC modulators. Besides, the development of on-line sample preparation steps in multidimensional systems can be also expected: Moreover, the extended use of powerful MS detectors would enhance even more the applications and identification power of these techniques in food analysis.

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