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Supercritical fluid chromatography in food analysis: a survey of the last three-year period

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Supercritical fluid chromatography (SFC) has evolved into a versatile, high-performance analytical technique with compelling advantages in food analysis in terms of speed, resolution, and sustainability. This minireview surveys the substantial advancements in SFC technology and applications from 2023 to 2025, focusing on nutritional and bioactive compound profiling, underpinning food authenticity and fraud prevention, and emphasizing chiral and regiochemical separations. Furthermore, applications in quality control and food safety demonstrate the capability of SFC for rapid, multi-residue contaminant screening with minimal environmental impact. The integration of green analytical chemistry principles through solvent minimization and on-line extraction consolidates SFC as a sustainable platform for comprehensive food analysis. Challenges and future perspectives including standardization, method automation, artificial intelligence integration, and portable systems are discussed.

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1. Introduction

In the ever-evolving landscape of food analysis, robust, rapid, and sustainable methodologies to comprehensively characterize complex food samples have become fundamental. The increasingly globalized food supply chains, along with stringent regulatory frameworks and increased consumer awareness, demand precise monitoring of food quality, authenticity, and safety. Supercritical fluid chromatography (SFC) has increasingly established itself as a robust and environmentally sustainable technique, well-suited to addressing contemporary analytical demands.[1] SFC uniquely exploits supercritical carbon dioxide ($scCO_2$) as the bulk mobile phase, which owing to its intermediate properties between gases and liquids enables fast mass transfer. Additional features of the SFC mobile phase consist of low viscosity and tunable solvent strength, through the addition of organic co-solvents (modifiers) such as methanol (MeOH) and ethanol (EtOH).[2] These properties can offer several advantages over traditional liquid chromatography (LC) and gas chromatography (GC) in many applications, including higher throughput, efficient separations, and reduced organic solvent consumption, although the performance depends on the specific analytical conditions and application.[3,4] These factors closely align with the modern green analytical chemistry paradigm, emphasizing reduced environmental impact and improved operator safety.[5,6] Technological advances in SFC instrumentation were remarkable in recent years, for example, ultrahigh-performance SFC (UHPSFC) setups that incorporate columns packed with sub-2 μm particles[7] and hybrid (organic–inorganic particles) stationary phases. Such technology provides reduced silanol activity, improved chemical and thermal stability, as well as a significant increase in high-pH stability compared to conventional silica-based stationary phases.[8] Spectrophotometric ultraviolet (UV) or diode-array detectors (DADs) remain widely used in routine SFC applications, particularly when target compounds are present at high concentrations and possess characteristic UV spectra, typically arising from the presence of chromophores such as conjugated double bonds, aromatic rings, or carbonyl groups. However, in recent years, SFC has been increasingly coupled with high-sensitivity mass spectrometers (MS), ranging from triple quadrupole (QqQMS) to high-resolution (HR) detectors such as Orbitrap and hybrid Quadrupole-Time of Flight MS (QToF). These detectors provide enhanced selectivity and sensitivity, especially at trace levels, while enabling structural elucidation, differentiation of stereoisomers and regioisomers, and accurate quantification of contaminants as well as nutritious and bioactive compounds in authenticity studies.[5,9,10] In particular, the use of $scCO_2$ as the bulk mobile phase makes this technique suitable for coupling with MS detectors, facilitating efficient desolvation and reducing solvent load into the ion source. Nevertheless, when low proportions of an organic co-solvent are employed, the addition of a make-up solvent may be required to

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improve ionization efficiency and ensure MS response. Supporting these instrumental improvements, method development has become highly efficient through the use of software optimization tools and design of experiments, facilitating tailored method performance to suit the high heterogeneity of food matrices (such as oils, cereals, fruits, dairy, and processed foods).[11,12] Moreover, emerging automated and miniaturized sample preparation approaches, including supercritical fluid extraction (SFE), coupled with SFC, have substantially decreased preparative steps, solvent consumption, and sample degradation, thereby enhancing the throughput.[13,14] Such hyphenation can be implemented in off-line and on-line configurations, with the latter additionally providing full automation, improved reproducibility, along with reduced operator exposure.

Recent literature evidences the wide application of SFC in food science, including detailed profiling of micro- and macronutrients, lipidomics for quality assessment, analysis of contaminants, and chiral separations. This minireview synthesizes insights of peer-reviewed articles published across the last three-year period, reflecting the state of the art in SFC technology and its role in cutting-edge food analysis. Through the Scopus engine, a literature analysis across the 2023–2025 period was performed. Using "supercritical fluid chromatography" as the keyword, close to 900 papers were found. The inclusion of "food" as an additional keyword was intentionally avoided, as it was deemed too generic, threatening to narrow the results to approximately 150 articles and potentially exclude pertinent contributions. Therefore, a manual screening was performed to select those studies specifically addressing food-related applications, with particular attention to the most innovative and methodologically advanced contributions according to the authors' opinion. For a simplified overview, the following classification was made herein: food constituents, food contaminants, and multidimensional approaches. The research papers selected for discussion are detailed in Table 1.

2. Food constituents

The characterization of food chemical composition provides detailed information about the type and amount of nutrients, bioactive compounds, and other minor constituents, helping to meet the growing demand for transparency to consumers and scientifically grounded information.

Feng and co-workers developed an SFC-UV method for the separation and detection of 42 representative molecules spanning a broad range of polarities and molecular weight, including vitamins, fatty acids, saponins, flavonoids, phenylpropanoids, phenols, terpenoids, steroids, anthraquinones, alkaloids, and peptides.[15] A modified ordered mesoporous core–shell silica (OMCS) microspheres stationary phase was synthesized *via* a micelle-templating method. Following the preparation of nonporous silica particles, mesoporous growth was induced using cetyltrimethylammonium bromide as a template. The obtained OMCS particles showed excellent monodispersity, well-defined particles (of *circa* 1.2 μm) with uniform pore sizes (4.0 nm), and high surface area (261 $m^2 g^{-1}$). By these means, rapid mass transfer and ultra-high separation speed and efficiency (up to 320 000 theoretical plates per meter) were pursued. All the analytes exhibited stronger retention than acetone (used as an unretained marker), confirming the absence of size-exclusion effects within the 4-nm pore structure. The authors further validated the practicality and stability of the developed stationary phase by the analysis of samples such as notoginseng, ginsenoside, and stevia extracts, demonstrating how tailored stationary phase architecture can push SFC applicability for the rapid and selective analysis of natural products.

Zhang and colleagues employed an SFC-QqQMS system for the separation of 42 amino acids (AAs), including 20 chiral pairs and 2 achiral AAs, important components in tea, contributing to its flavour and potential bioactivity.[16] To compensate for their high polarity, AAs were derivatized to overcome their low solubility in non-polar $scCO_2$ and to reduce their retention. A teicoplanin-bonded stationary phase (100 × 4.6 mm, 2.7 μm d_p) was used for the separation. Among the tested co-solvents, MeOH provided better resolution compared to acetonitrile (ACN), although peak distortion was observed for highly polar AAs. The addition of water (10%, v/v) significantly improved the peak shape and enantiomeric resolution by increasing mobile phase polarity and enhancing hydrogen-bonding interactions. The incorporation of ammonium formate (200 mM) further enhanced the chromatographic performance by improving peak symmetry and resolution. Column temperature, back-pressure, and flow rate were initially evaluated during method development as parameters which affect resolution and peak symmetry. Since full factorial design demonstrated a negligible effect of column temperature, only pressure and flow rate were further optimized through a central composite design, yielding optimal values of 132 bar and 1 mL min^{-1}, respectively. The obtained SFC method outperformed LC separation performance when applied to tea samples. As shown in Fig. 1, although LC afforded baseline separation of standard mixtures, D-leucine coeluted with previously eluted L-isoleucine in the tea sample, highlighting the challenges posed by the large enantiomeric abundance disparities in chiral analysis. In contrast, SFC provided a clear separation of D-leucine and L-isoleucine ($R_s \geq 2.0$), enabling their reliable quantification.

Another recent study exploited the performance of SFC-QToF MS to investigate the distribution of nine cannabinoids in hemp-based tea infusions, including six neutral cannabinoids, namely cannabidiol (CBD), cannabigerol, cannabinol, cannabichromene, Δ^9-tetrahydrocannabinol, and tetrahydrocannabivarin, and three acidic ones, namely cannabidiolic acid (CBDA), cannabigerolic acid (CBGA), and tetrahydrocannabinolic acid (THCA).[17] The 2-ethylpyridine stationary phase provided the best resolution of the cannabinoids within a total run time of 17 min. Chromatographic conditions were optimized by evaluating different organic modifiers, with MeOH containing 0.1% formic acid selected as the co-solvent to improve peak symmetry and enhance the separation of closely related analytes. Under the optimized gradient conditions, acidic compounds were strongly retained, thus enabling the use of the negative ionization mode, which significantly improved the detection sensitivity for CBDA,

Table 1 List of the research papers investigated in the present review (from 2023 to 2025)[a]

Instrument	Stationary phase	Mobile phase	Flow rate	Back pressure (bar)	Make up	Oven	Inj. Vol. (μL)	Analytes	Samples	Ref
SFC-UV/vis	Homemade ordered mesoporous core–shell silica microspheres (50 × 4.6 mm, 1.2 μm d_p)	CO_2 (A); MeOH (B) 0 min, 2% B 0–10 min, 2–50% B	2.0 mL min^{-1}	145	NR	40 °C	1	42 representative compounds (vitamins, fatty acids, saponins, flavonoids, phenylpropanoids, phenols, terpenoids, steroids, anthraquinones, alkaloids, peptides)	Notoginseng, ginsenoside, and stevia extracts	15
SFC-QqQMS	InfinityLab Poroshell 120 chiral T (100 × 4.6 mm, 2.7 μm d_p)	CO_2 (A) MeOH/H_2O, 90 : 10, v/v, with 200 mM AF and 0.1% FA (B) 0–3 min, 35% B 3–4 min, 35–55% B 4–22 min, 55% B 22–24 min, 55–85% B 24–40 min, 85% B	1.0 mL min^{-1}	132	NR	40 °C	4	42 derivatized aminoacids	Dark, black, oolong, yellow, white, and green teas	16
SFC-QToF MS	Reprospher 100-2EP column (150 × 3 mm, 5.0 μm d_p)	CO_2 (A) MeOH with 0.1% FA (B) 0–8.2 min, 2% B 8.2–16.2 min, 2–29% B	2.0 mL min^{-1}	400	MeOH 0.1 mL min^{-1}	35 °C	2	6 neutral and 3 acidic cannabinoids	Hemp tea infusions	17
SFC-QqQMS	Ascentis Express dimethylpenta -fluorophenylpropyl (150 × 2.1 mm, 2.7 μm d_p)	CO_2 (A); MeOH (B) 0 min, 2% B 0–10 min, 2–10% B	1.0 mL min^{-1}	120	MeOH 1 mL min^{-1}	40 °C	2	28 oxygen heterocyclic compounds	Citrus juices, jams, infusions, alcoholic and soft drinks, and bakery products	18
SFE//SFC-MS	HSS C18 SB (150 × 2.1 mm, 1.8 μm d_p)	CO_2 (A) MeOH/ACN, 80 : 20, v/v (B) 0 min, 1% B 0–5 min, 1–10% B 6–10 min, 10–20% B	1.8 mL min^{-1}	138	NR	40 °C	5	14 free fatty acids and theobromine	Cocoa beans husk	19
SFC-QToF MS	Acquity UPC^2 BEH (100 × 3 mm, 1.7 μm d_p)	CO_2 (A) MeOH/H_2O, 33 : 1, v/v, with 20 mM AmA (B) 0 min, 5% B 0–5.5 min, 5–48% B 5.5–10 min, 48% B	1.1 mL min^{-1}	103	NR	55 °C	2	195 polar lipids	Human breast milk	23

Table 1 (*Contd.*)

Instrument	Stationary phase	Mobile phase	Flow rate	Back pressure (bar)	Make up	Oven	Inj. Vol. (μL)	Analytes	Samples	Ref
SFC-MS	ACQUITY UPC^2 HSS C18 SB (100 × 3.0 mm, 1.8 μm d_p)	CO_2 (A); MeOH/IPA 80 : 20, v/v, (B) 0–6 min, 5–15% B 6–6.1 min, 15–25% B 6.1–7 min, 25% B; 7–9 min, 25–5% B	1.0 mL min^{-1}	172	MeOH (0.1% ammonia) 0.2 mL min^{-1}	40 °C	3	13 free fatty acids	Corn, soybean, coconut, peanut, olive oils	24
SFE-SFC-QqQMS	Unips 5–100 (250 × 4.6 mm, 5 μm d_p)	CO_2 (A) MeOH (B) 0–30 min, 5–40% B	3 mL min^{-1}	100	NR	35 °C	NR	3 free fatty acids	Wild mushrooms	25
SFC-MS	4 Kinetex C18 (150 × 4.6 mm, 2.6 μm d_p) + 1 Accucore C18 (150 × 4.6 mm, 2.6 μm d_p)	88% CO_2, 12% ACN/MeOH 90 : 10, v/v	1.6 mL min^{-1}	100	None	17 °C	1	94 triacylglycerols	Almond, apricot kernel, argan, avocado, camelina, corn, cotton, hazelnut, linseed, macadamia, olive, palm, peanut, pistachio, rapeseed, safflower, sesame, sunflower, walnut, wheat, tamanu, castor oils, shea butter	26
SFC-MS	Ascentis Express C18 (100 × 30 mm, 2.0 μm d_p)	CO_2 (A); ACN (B) 0–5 min, 5% B 5–15 min, 5–20% B	1.0 mL min^{-1}	150	None	20 °C	2	121 triacylglycerols	Borage, corn, refined hazelnut, extravirgin olive, palm, peanut, and soybean oils	27
SFC-QToF MS	BEH-2EP (100 × 3 mm, 1.7 μm d_p)	CO_2 (A) MeOH/ACN, 50 : 50, v/v with 0.1% FA (B) 0 min, 0.8% B 1 min, 1% B 8 min, 1% B 8–9 min, 1–15% B	1.2 mL min^{-1}	170	MeOH (0.1% AF) 0.1 mL min^{-1}	50 °C	1	59 triacylglycerols	Walnut, corn, soybean, sunflower, and wheat germ oils	28

Table 1 (*Contd.*)

Instrument	Stationary phase	Mobile phase	Flow rate	Back pressure (bar)	Make up	Oven	Inj. Vol. (μL)	Analytes	Samples	Ref
SFC-QToF MS	Acquity UPC^2 BEH-2EP (100 × 3.0 mm, 1.7 μm d_p)	CO_2 (A) MeOH/ACN, 50 : 50, v/v with 0.2% FA (B) 0 min, 0.1% B 2 min, 0.1% B 2–6 min, 0.1–1% B 6–9 min, 1–2% B 9–15 min, 2% B 15–20 min, 2–7% B	1.0 mL min^{-1}	117	NR	50 °C	1	113 triacylglycerols	Holstein cattle, goat, yak, buffalo, horse, and camel milks	29
SFC-APPI-MS	Viridis HSS C18 SB (100 × 3 mm, 1.8 μm d_p)	CO_2 (A); MeOH (B) 0 min, 5% B 0–5.25 min, 5–25% B 5.25–6 min, 25–100% B	1.0 mL min^{-1}	100	MeOH 0.2 mL min^{-1}	40 °C	NR	57 acylglycerols	Linseed oil	30
SFC-QqQMS	Synergi Hydro-RP (250 × 4.6 mm, 4 μm d_p)	CO_2 (A) ACN, with 0.1% FA (B) 0–5 min, 5–50% B 5–6 min, 50–100% B 6–11 min, 100% B	1.5 mL min^{-1}	NR	MeOH 0.3 mL min^{-1}	40 °C	NR	Malondialdehyde and 7 α,β-unsaturated aldehydes	Edible oils and oil-containing foods	31
SFC-UV	Homemade column, packed with octadecylamine-modified poly(GMA-DVB) microspheres (for BADGEs retention) + Ultimate XB-C18 (250 × 4.6 mm, 5 μm d_p)	CO_2/MeOH 96 : 4, v/v	2.0 mL min^{-1}	100	MeOH 0.3 mL min^{-1}	40 °C	NR	Bisphenol A diglycidyl ether and 6 derivatives	Canned beverages	32
SFC-QqQMS	ZORBAX RX-SIL 1 (150 × 4.6 mm, 5 μm d_p)	CO_2/MeOH 90 : 10, v/v	2.0 mL min^{-1}	130	MeOH 0.2 mL min^{-1}	40 °C	5	9 pesticides	Rice, wheat, and maize	33
SFC-QqQMS	CHIRALPAK AD-3 (150 × 3.0 mm, 3 μm d_p)	CO_2/MeOH 90 : 10, v/v	3.0 mL min^{-1}	100	MeOH (5 mM AF and 0.1% FA) 0.1 mL min^{-1}	40 °C	1	Metconazole	Grape, peach, pear, jujube	34
SFC-QqQMS	CHIRALPAK AD-3 (150 × 3.0 mm, 3 μm d_p)	92% CO_2, 2% MeOH/IPA 3 : 1, v/v	2.5 mL min^{-1}	100	MeOH (5 mM AF and 0.1% FA) 0.1 mL min^{-1}	30 °C	NR	Fenpropathrin	Cabbage, lettuce, pak choi, celery, cucumber	35
SFC-QqQMS	CHIRALCEL OZ-3 (100 × 3.0 mm, 3 μm d_p)	CO_2/MeOH 97 : 3, v/v	3.0 mL min^{-1}	100	MeOH (5 mM AF and 0.1% FA) 0.1 mL min^{-1}	35 °C	NR	Bitertanol	Cabbage, lettuce, pak choi, celery	36

Table 1 (*Contd.*)

Instrument	Stationary phase	Mobile phase	Flow rate	Back pressure (bar)	Make up	Oven	Inj. Vol. (μL)	Analytes	Samples	Ref
SFC-QqQMS	CHIRALCEL OZ-3: penflufen CHIRALPAK AD-3: fluindapyr, benzovindiflupyr, sedaxane CHIRALCEL OD-3: Penthiopyrad, furametpyr, pydiflumetofen, isopyrazam (150 × 3.0 mm, 3 μm d_p)	CO_2/MeOH 93 : 7, v/v: Penflufen CO_2/MeOH 88 : 12, v/v: Fluindapyr, pydiflumetofen CO_2/MeOH 80 : 20, v/v: Benzovindiflupyr CO_2/EtOH 88 : 12, v/v: sedaxane CO_2/EtOH 90 : 10, v/v: Penthiopyrad, furametpyr CO_2/ACN 93 : 7, v/v: isopyrazam	2.5 mL min^{-1} (3.0 mL min^{-1} for penflufen)	100	MeOH (5 mM AF and 0.1% FA) 0.1 mL min^{-1}	40 °C	NR	8 pesticides	Celery, tomato, citrus, peach, apple, grape, chicken, pork, beef, egg white, egg yolk, chicken liver, chicken intestine	37
SFC-IM-QToF MS	ZORBAX RX-SIL (150 × 4.6 mm, 5 μm d_p)	CO_2 (A) MeOH, with 0.2% FA (B) 0–5 min, 2–10% B 5–10 min, 10–20% B	2.5 mL min^{-1}	140	MeOH (0.1% FA) 0.1 mL min^{-1}	40 °C	1	9 pesticides	Ginger	38
SFC-IM-QToF MS	ZORBAX RX-SIL (150 × 4.6 mm, 5 μm d_p)	CO_2 (A); MeOH, with 0.1% FA (B) 0 min, 2% B; 0–6 min, 0–10% B 6–10 min, 10–20% B	2.5 mL min^{-1}	140	MeOH (0.1% FA) 0.5 mL min^{-1}	40 °C	3	20 pesticides	Potato, yam	39
SFE-SFC-QqQMS	Chiralcel OD-H (250 × 4.6 mm, 5 μm d_p)	CO_2/EtOH 79 : 21, v/v	2.0 mL min^{-1}	160	MeOH (10 mM AF and 1% FA) 0.25 mL min^{-1}	30 °C	2	Pyraclostrobin, mefentrifluconazole	Mango and mango juice	40
SFC-SFC-DAD-MS	^{1}D: ACQUITY UPC^2 Torus DEA (100 × 3.0 mm, 1.7 μm d_p) ^{2}D: CHIRALPAK IB-3 (150 × 4.6 mm, 3.0 μm d_p)	CO_2 (A) MeOH, with 0.1% MSA (B) ^{1}D: 0.0 min, 20% B 0.5–8 min, 20–100% B 8–10 min, 100% B ^{2}D: 0.0 min, 15% B 0.5–17 min, 15–50% B 17–19 min, 50% B	^{1}D: 1.7–0.6 mL min^{-1} ^{2}D: 2 mL min^{-1}	^{1}D: 150–110 ^{2}D: 150	MeOH (20 mM NH_4OH and 2% H_2O) 0.4 mL min^{-1}	30 °C	5	Naringin, neohesperidin	Bitter orange peel	44 and 45

Table 1 (*Contd.*)

Instrument	Stationary phase	Mobile phase	Flow rate	Back pressure (bar)	Make up	Oven	Inj. Vol. (μL)	Analytes	Samples	Ref
SFE-SFC-SFC-DAD-MS	^{1}D: ACQUITY UPC^2 Torus DEA (100 × 3.0 mm, 1.7 μm d_p)	CO_2 (A)	^{1}D: 1.7–0.6 mL min^{-1}	^{1}D: 150–110	MeOH 0.4 mL min^{-1}	30 °C	5	Naringin, neohesperidin	Bitter orange flowers, peels, and leaves	46
	^{2}D: CHIRALPAK IB-3 (150 × 4.6 mm, 3.0 μm d_p)	MeOH, with 0.1% MSA (B) ^{1}D: 0 min, 20% B 0.5–8 min, 20–100% B 8–10 min, 100% B ^{2}D: 0–0.5 min, 15% B 0.5–8 min, 15–50% B 8–10 min, 50% B	^{2}D: 2 mL min^{-1}	^{2}D: 150						
SFC-SFC-DAD-MS	^{1}D: ACQUITY UPC^2 Torus DEA (100 × 3.0 mm, 1.7 μm d_p)	CO_2 (A)	^{1}D: 1.7–0.6 mL min^{-1}	^{1}D: 150–110	MeOH 0.4 mL min^{-1}	30 °C	5	7 flavonoids	Honey	47
	^{2}D: CHIRALPAK IG-3 (150 × 4.6 mm, 3.0 μm d_p)	MeOH, with 0.1% MSA (B) ^{1}D: 0.0 min, 2% B 1 min, 2% B 1–7 min, 2–30% B; 7–10 min, 30–90% B 10–11 min, 90% B ^{2}D: 0–0.5 min, 5% B 0.5–12 min, 5–50% B 12–13 min, 50% B	^{2}D: 2 mL min^{-1}	^{2}D: 150						

[a] MeOH: methanol; H_2O: water; AF: ammonium formate; FA: formic acid; IPA: isopropanol; AmA: ammonium acetate; ACN: acetonitrile; EtOH: ethanol; MSA: methanesulfonic acid; NR: not reported.

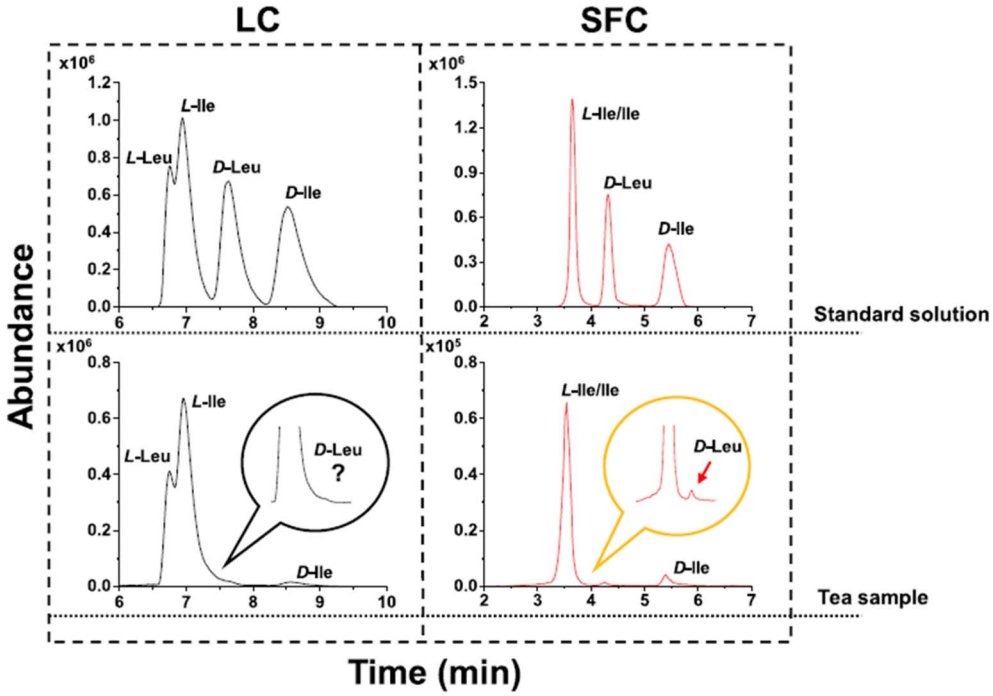

Fig. 1 Impact of enhanced resolution and peak shape on large abundance disparities issue in chiral analysis using SFC compared to LC. Reproduced with permission from *Food Chemistry*, 2025, **493**, 146028 (ref. 16). Copyright 2026, Elsevier.

CBGA, and THCA. Therefore, considering the relatively long polarity-switching time of the QToF instrument, chromatographic separation of acidic and neutral cannabinoids proved beneficial for their accurate detection. When applied to commercial hemp tea infusions, the method highlighted variable cannabinoid profiles among the investigated samples, with CBDA, THCA, and CBD being the predominant compounds.

In 2023, Cafeo *et al.* investigated 28 oxygen heterocyclic compounds by SFC-QqQMS, namely coumarins, furocoumarins, and polymethoxyflavones in different food samples, including citrus juices, jams, infusions, alcoholic and soft drinks, and bakery products.[18] Numerous beneficial effects are associated with the daily consumption of polymethoxyflavones, including antioxidant, anti-inflammatory, and antibacterial properties. However, alongside these beneficial effects, negative health effects have been linked to coumarin, for which a tolerable daily intake was established by the European Food Safety Authority. The chromatographic separation was achieved on a pentafluorophenyl column in 8 min and by using less than 10% of MeOH as the co-solvent, highlighting SFC as a greener alternative to standard HPLC techniques.

A SFE/SFC-MS off-line coupling was employed for the valorisation of cocoa bean husk, the major by-product of cocoa processing.[19] The optimization of SFE under different temperature and pressure conditions maximized lipid recovery, yielding an extraction efficiency of 31.5%. The SFC-MS analysis of the obtained cocoa butter revealed a predominance of unsaturated FAs, namely oleic and linoleic acid, in addition to saturated stearic and palmitic acids. Additionally, theobromine was also quantified showing its preferential partitioning into the defatted solid fraction. This approach ensured the recovery of value-added ingredients, strengthening the valorisation of waste by-products as strategic resources for bioeconomy.

2.1 Lipid analysis

Lipids, including free fatty acids (FAs), polar lipids, and triacylglycerols (TAGs), constitute a heterogeneous class of compounds which represent a key target of modern food analysis. Owing to their generally low polarity and hydrophobic character, these molecules are particularly suitable for SFC analysis.[20] A significant advantage of SFC lies in its ability to modulate the polarity and eluotropic strength of the pressurized CO_2-based mobile phase through the addition of organic solvents and additives at different concentrations. Moreover, SFC is compatible with a wide range of stationary phases with different polarities and selectivities, thus enabling separation of a diverse range of compounds, from non-polar to polar. Under SFC conditions, adsorption phenomena involving the CO_2-based mobile phase and the stationary phase play a central role in separation mechanism. The mechanism of this adsorbed phase is strongly influenced by the presence of co-solvents and additives, which can alter the effective polarity of the stationary phase and lead to unpredictable changes in retention behaviour.[21,22]

Jiang *et al.* employed SFC-QToF MS to comprehensively investigate polar lipids in human milk.[23] A panel of 126 samples, including colostrum (1–7 days postpartum), transitional milk (8–14 days postpartum), and mature milk (>14 days postpartum), were collected from 42 breastfeeding mothers from northern, central, and southern China. Employing

a MeOH/H_2O (33 : 1, v/v) gradient elution up to 48%, a total of 195 molecular species within 11 lipid classes were identified. The study demonstrated how lipid composition systematically varies with lactational stage and also geographical region, reinforcing the importance of detailed lipid profiling in food analysis to support nutritional quality assessment. However, to better assess the variations in lipid composition, additional mature milk samples (longer than 1 or 6 months postpartum) would be required, particularly to facilitate the design of infant formulas that more closely resemble human milk.

Within the context of lipid analysis, several research groups have exploited the capabilities of SFC for direct and rapid profiling of FAs, offering a greener and more time-efficient alternative to conventional GC-based methods that require derivatization.

A sample preparation-free protocol, based on an isopropanol (IPA) dilution, coupled with SFC-QMS was proposed for the characterization of FAs in edible vegetable oils.[24] Following optimization of the flow rate, solvent composition, temperature, and backpressure, optimal chromatographic performance was achieved at 40 °C and 172 bar, using MeOH/IPA (80 : 20, v/v) as the modifier at a total flow rate of 1.0 mL min^{-1}. To gain deeper insight into FA composition and distribution patterns, the authors applied multivariate statistical analysis. Principal component analysis (PCA) revealed a clear separation of coconut oil from other plant oils, due to the distinctive presence of myristic acid and the high content of saturated fatty acids. In contrast, soybean and olive oils showed a partial overlap, reflecting their similar FA compositions. To improve discrimination, orthogonal partial least squares discriminant analysis (OPLS-DA) was employed as a supervised model, exhibiting a classification accuracy of 95.2%. Subsequently, a set of 45 high-priced oils (coconut and olive oils) mixed at different adulteration proportions (10%, 20%, 40%, and 50%) with low-priced ones (corn, soybean, and peanut oils) were analysed. Nevertheless, both chemometric approaches showed limited capability in accurately identifying adulteration at low levels (*e.g.*, 10%), due to the minimal compositional differences between genuine and adulterated samples. To overcome these limitations, supervised machine learning models were developed using FA profiles as input variables. All models yielded high classification accuracy; notably, random forest achieved 100% accuracy in both classification and adulteration detection. These results demonstrate the robustness of data-driven methodologies for lipid profiling and authentication.

An SFE-SFC-QqQMS coupling was investigated for the efficient extraction and fractionation of fatty acid-related compounds from edible wild mushrooms.[25] SFE with pure $scCO_2$ was employed as a solvent-free approach to selectively recover lipophilic compounds. The resulting extract was then subjected to an initial lipid fractionation by preparative-scale SFC, using a polystyrene/divinylbenzene (PS-DVB) stationary phase and a CO_2/MeOH elution gradient. The ten collected fractions were re-analysed on the same stationary phase using both SFC and reversed-phase LC (RPLC), with a water/ACN gradient applied in the RPLC mode. Such analyses revealed significantly different chromatographic profiles compared to SFC, with several fractions showing improved resolution and the presence of multiple minor components not evident in the SFC chromatograms. This highlighted that retention under SFC conditions on PS-DVB is mainly governed by van der Waals and π–π interactions, rather than by hydrophobic interaction in conventional aqueous RPLC, thereby emphasizing the different selectivity. This complementarity justified the implementation of a second-step purification by RPLC, enabling further resolution of complex fractions and isolation of linoleic acid and derived compounds, namely methyl linoleate and ethyl linoleate, with purities exceeding 95%.

In the context of lipid analysis, the detailed characterization of TAGs provides critical insights for verifying product authenticity and revealing adulterations, consisting in blending and mislabeling. The TAG composition and abundance in different natural products, mainly vegetable oils, were examined using SFC hyphenated to a single-quadrupole MS.[26] The authors employed five tandem octadecylsilica (C18) columns to improve chromatographic resolution. Considering the entire set of samples, 94 different TAGs were identified and quantified, comprising low-abundance ones, regio- and geometrical isomers, within a total run time of 80 min.

Similarly, in their SFC-QMS approach, Salerno and co-workers identified a total of 121 TAGs, again considering the entire set of analysed edible vegetable oils (Fig. 2).[27] Employing a single C18 column (100 × 3.0 mm ID) packed with 2.0 μm partially porous particles and an ACN gradient elution up to 20%, the method enabled high-efficiency separations by minimizing band broadening and enhancing mass transfer under SFC conditions. This approach allowed the achievement of a compromise between reduced elution times and resolution of the most abundant TAGs belonging to the same partition number, while prioritizing analysis time (8 min, with the exception of borage oil) and instrumental simplicity. This approach provided efficient TAG qualitative fingerprinting, suitable for high-throughput routine screening and quality control of edible oils. However, as stated by the authors themselves, the proposed method was not assessed for its quantitative capabilities.

The same C18 stationary phase with similar dimensions (100 × 3.0 mm ID, 1.7 μm d_p) was employed by Zhang and co-workers for the quantification of 59 TAG compounds in walnut, corn, soybean, sunflower, and wheat germ oils, in comparable analysis time (10 min).[28] The accurate and information-rich QToF MS spectra provided reliable identification of TAGs, minimizing misidentification in complex lipid mixtures while offering high sensitivity across a wide mass range. To further explore the differences in TAG composition, both unsupervised (PCA) and supervised (OPLS-DA) statistical approaches were conducted. Although TAGs were widely distributed and most of them overlapped, the high-dimensional multivariate analysis accurately distinguished walnut oil adulterated with low quality oils down to 5%. In particular, the linolenic-containing TAGs significantly contributed to the classification of adulterated samples.

Similarly, a chemometrics-assisted SFC-QToF MS fingerprinting approach for TAGs in livestock milks was developed by

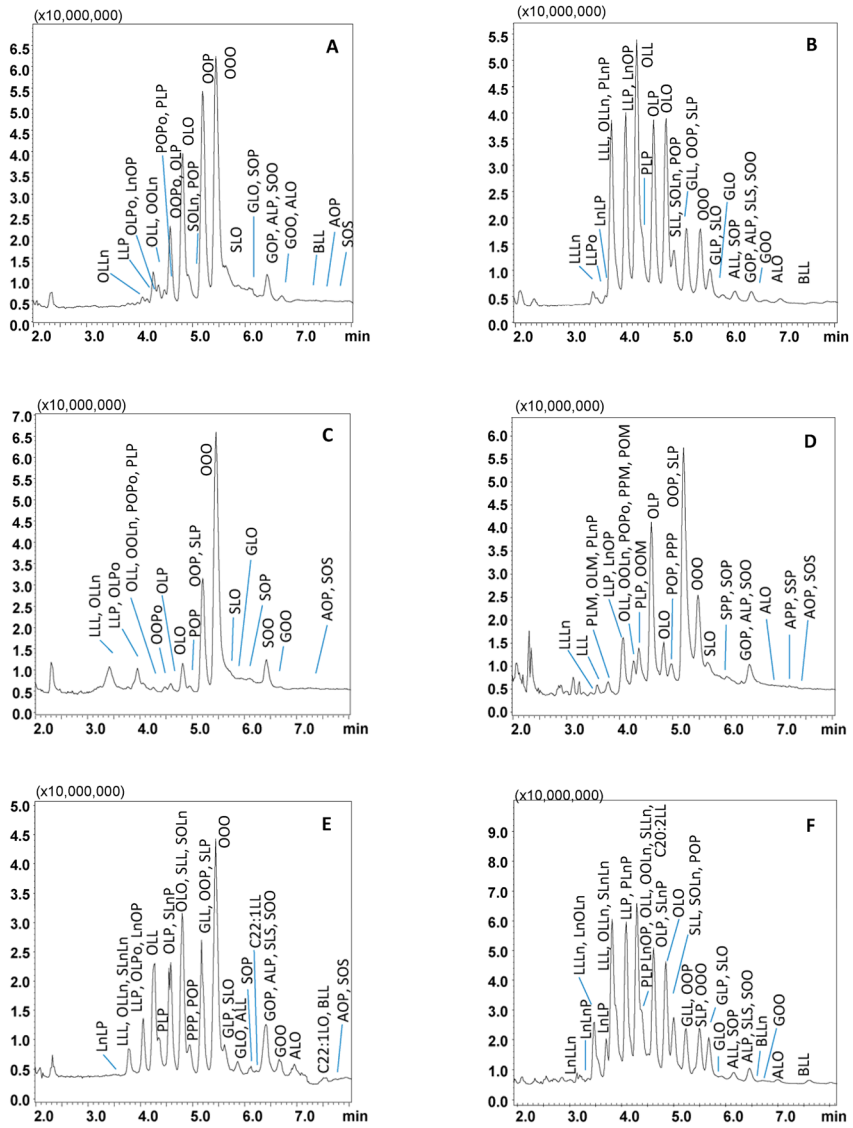

Fig. 2 SFC-QMS TAG profiles of extra virgin olive oil (A), corn oil (B), hazelnut oil (C), palm oil (D), peanut oil (E), and soybean oil (F). Reproduced with permission from *Separations*, 2023, **10**, 56 (ref. 27). Copyright 2026, MDPI.

Wang *et al.*[29] Using a 100 × 3.0 mm ID, 1.7 μm d_p 2-ethylpyridine column, the authors achieved the first systematic separation and characterization of TAG compositions from Holstein cattles, goats, yaks, buffaloes, horses, and camels (Fig. 3).

The combination of an optimized SFC method with QToF MS enabled the detection of both abundant and low-abundant TAGs, resulting in comprehensive lipid fingerprints suitable for chemometric analysis and for food authenticity studies. A multivariate analysis of the total 113 identified TAGs revealed clear species-dependent clustering consistent with livestock taxonomy. Although the method proved to be robust for single-species milk authentication, the authors recognized limitations in discriminating blended milk samples due to composition similarity. As a conclusion, the need to improve the bioinformatic models within complex adulteration scenarios was foreseen.

Mueller and Hopfgartner combined SFC with chlorobenzene-assisted atmospheric-pressure photoionization MS (APPI-MS) for TAG analysis.[30] Such a coupling exploited the high efficiency of SFC for the separation of neutral lipids together with the superior ionization capability of APPI for weakly polar and non-polar compounds, poorly ionized by electrospray (ESI). This approach enabled the generation of radical cations ($M^{\bullet+}$) and $[M–H]^+$ ions, referred to as electron-deficient precursor (EDP) ions, which undergo characteristic collision-induced dissociation (CID), allowing a statistically supported rule-based *de novo* annotation of double bond positions, FA attachments, and *syn*-positions. As a result, highly informative EI-like fragmentation spectra were obtained, suitable for commercial library search. Distinct intensity ratios for positional isomers and multiple fragmentation series related to double-bond position were observed, thereby allowing direct

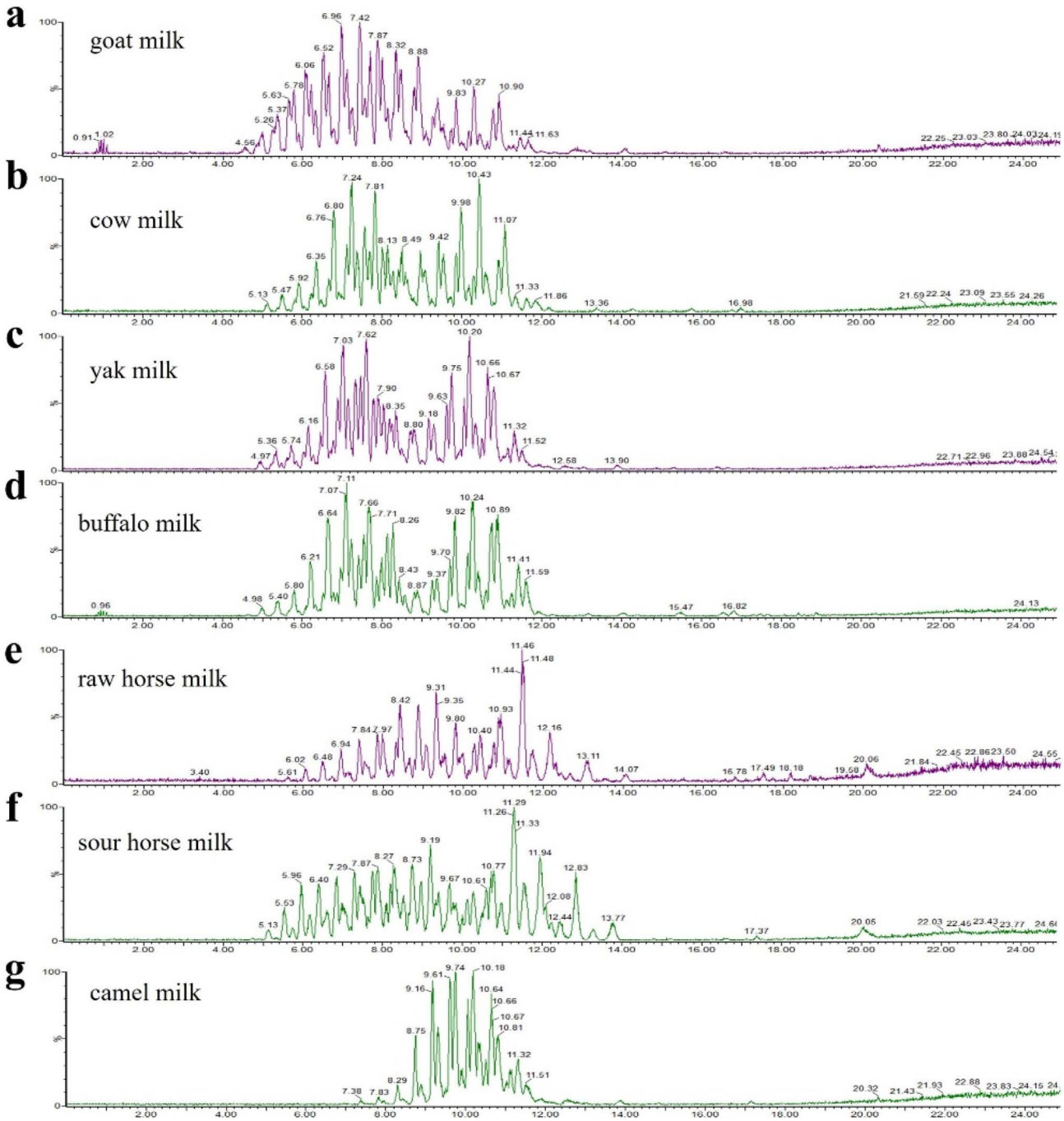

Fig. 3 TAG chromatograms of goat (a), cow (b), yak (c), buffalo (d), raw horse (e), sour horse (f), and camel (g) milks. Reproduced with permission from *Food Chemistry*, 2025, **480**, 143940 (ref. 29). Copyright 2026, Elsevier.

assignment of FA chains and their positional distribution on the glycerol backbone. Following optimization with standard materials, a sample of linseed oil (consisting approximately 90% of unsaturated FAs), was analysed to benchmark the EDP-CID capability for TAG analysis. Similarly to the previously reported studies, a C18 (100 × 3 mm, 1.8 μm d_p) column was used along with MeOH as the modifier, allowing the detection of 57 acylglycerols, including 44 TAGs, 12 diglycerides, and 1 monoglyceride. The FA distribution derived from *de novo* annotation was consistent with preliminary GC-EI analysis performed after acylglycerol saponification and derivatization.

3. Food contaminants

The determination of food contaminants represents one of the main efforts of the scientific community and analytical research due to their potential impact on human health and environmental safety.

An SFC-QqQMS method was recently proposed for the simultaneous determination of malondialdehyde (MDA) and seven α,β-unsaturated aldehydes in edible oils and oil-containing foods.[31] Such toxic compounds are secondary lipid oxidation products obtained through heat-induced chemical transformations of polyunsaturated FA-rich oils. The SFC method was compared to a conventional LC-QqQMS approach, performed on an SFC/LC switching system, using the same Synergi Hydro-RP column. For reversed phase LC-QqQMS analysis, a 32-min gradient with 0.1% (v/v) formic acid in ACN and 0.1% (v/v) formic acid in water was used at a flow rate of 1 mL min^{-1}. For SFC-MS/MS analysis, ACN (0.1%, v/v formic acid) was used as the modifier, the flow rate was increased to 1.5 mL min^{-1}, and MeOH (0.3 mL min^{-1}) was added as the make-up solvent. The SFC method was characterized by significantly shorter elution times (<4 min) and higher sensitivity (5–23 times), as $scCO_2$ readily vaporized within the ESI source, leaving only the organic modifier, thereby facilitating ionization. In contrast, the high boiling points of aqueous LC mobile phases hinder droplet vaporization and analyte ionization in LC-QqQMS. The application of the method to edible oils (flaxseed, rapeseed, soybean, peanut, corn, sunflower, palm, and lard oils) and oil-containing baked and fried foods, revealed substantial differences in aldehyde formation, strongly correlated with FA composition. The linolenic acid content (ω-3 PUFA) drove the formation of MDA, 4-hydroxy-2-hexenal, acrolein, and related aldehydes, while the linoleic acid content (ω-6 PUFAs) was linked to 4-hydroxy-2-nonenal, *trans*-2-heptenal, and *trans*,*trans*-2,4-decadienal. Flaxseed oil and fried foods showed the highest levels, whereas high-oleic and saturated-fat oils remained relatively stable, except for *trans*,*trans*-2,4-decadienal. These results underscored the need for effective antioxidant strategies and processing controls, especially for deep-fried foods, given the absence of clear regulatory limits and the health risks associated with dietary intake.

A novel online column-switching SFC-UV method was proposed for the rapid and automated determination of cytotoxic and mutagenic emerging pollutants, namely bisphenol A diglycidyl ether (BADGE) and its six derivatives in canned beverages.[32] The authors designed an integrated system for BADGE enrichment and matrix interference elimination. A homemade column, packed with octadecylamine-modified poly(GMA-DVB) microspheres, was used to selectively retain BADGEs prior to online transfer to a C18 separation column. The unretained matrix impurities were directly eliminated by an external pump. After optimizing the stationary phase, switching time, modifier composition, temperature, and backpressure, the method afforded extraction yields higher than 85%, low quantification limits (in the 8.0–11.6 ng mL^{-1} range), and baseline separation of the seven BADGE-related compounds in 12 min, thus, marking the method as fully automated and faster than conventional LC or GC approaches. The elimination of matrix interferences allowed the use of a simple UV detector (set at 230 nm), with the possibility to integrate MS to expand the method applicability to more complicated matrices, such as lipid-rich food products. Despite the flexibility of the packing material, with particle size, functional groups, porosity, and surface chemistry customized to the specific physicochemical properties of BADGE derivatives, the column cost-effectiveness was substantially reduced.

Although SFC has been applied to different classes of contaminants, the majority of the reported studies primarily focused on pesticide residue determination. Over the years, a wide range of sample preparation procedures have been developed to ensure accurate and selective extraction of these compounds. Among them, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure, based on an ACN-mediated extraction followed by dispersive solid phase extraction (d-SPE) with different adsorbents to remove interfering compounds, is widely recognized as the method of choice for pesticide analysis in complex food matrices. Xie *et al.* developed and validated an SFC-QqQMS method for the determination of carbendazim, isoprocarb, paclobutrazol, isoprothiolane, flusilazole, quinalphos, piperonylbutoxide, propargite, and bioresmethrin in rice, wheat, and maize, following QuEChERS extraction and matrix-matched calibration.[33] The nine pesticides were separated on a silicone-coated ZORBAX RX-SIL 1 column (150 × 4.6 mm, 5 μm d_p) using isocratic elution with CO_2/MeOH (90 : 10, v/v) at 40 °C and 2 mL min^{-1} flow rate. Method validation showed excellent linearity, low LoQs (0.4–40 μg kg^{-1}), recoveries between 70 and 107%, acceptable precision, and matrix effect values carefully assessed and corrected.

Within the context of pesticide analysis, the in-depth characterization and separation of chiral pesticides is receiving increasing attention from researchers, given the impact of enantiomeric differences on the biological activities and degradation rate.

In 2024, Diao *et al.* proposed the chiral separation of metconazole stereoisomers using SFC-QqQMS.[34] A modified QuEChERS method was employed for the efficient recovery of metconazole from fruit samples. Specifically, magnetic iron nanoparticles were employed as the d-SPE adsorbent to selectively bind the matrix interfering substances. Similarly, using the QuEChERS protocol and SFC-QqQMS, the stereoselectivity and diastereoselectivity of fenpropathrin and bitertanol stereoisomers in different types of vegetables were also investigated.[35,36] An efficient enantioseparation of the investigated molecules was obtained using an amylose-based and a cellulose-based polysaccharide stationary phase, respectively, in less than 5 and 2.5 min, using a low amount of pure MeOH or a mixture of MeOH/IPA as the organic modifier. Di and coworkers investigated the SFC-QqQMS enantioseparation of eight chiral succinate dehydrogenase inhibitor fungicides, including fluindapyr, benzovindiflupyr, penflufen, penthiopyrad, furametpyr, pydiflumetofen, isopyrazam, and sedaxane.[37] Rather than developing a simultaneous method, analysis of each fungicide was individually optimized in terms of the stationary phase, mobile phase composition, and gradient. Again, an amylose-based or cellulose-based polysaccharide stationary phase was employed depending on the compound, with modifiers including CO_2/MeOH, CO_2/EtOH, or CO_2/ACN at various ratios. Pesticide residues were then quantified in representative foods of plant (celery, tomato, citrus, peach, apple, and grape) and animal origin (chicken, pork, beef, egg white, egg yolk, chicken liver, and chicken intestine).

Comprehensively, chiral pesticides exhibited varying degrees of stereoselectivity and diastereoselectivity, leading to distinct dissipation patterns in different matrices, probably related to plant enzymatic systems and/or their associated microbial communities. These enantio-SFC-MS approaches highlight the evolving need to improve pesticide efficiency in agricultural applications, reducing unnecessary pollution, and allowing harmonization of the risk assessment evaluation.

In 2024, Shi *et al.* first coupled SFC with ion mobility QToF MS (IM-QToF MS) for the analysis of carbamate residues in ginger, namely methomyl, isoprocarb, carbaryl, promecarb, aldicarb, mesurol, thiodicarb, benfuracarb, and indoxacarb, using a carbon black-assisted miniaturized SPE.[38] The SFC optimization involved the selection of ZORBAX RX-SIL as the optimal stationary phase and MeOH with 0.2% formic acid as the modifier to ensure effective separation and response values (at 140 bar and 60 °C). The SFC-IM-QToF MS method developed provided multidimensional information, namely retention time, drift time, and collision cross-sectional (CCS) values. The same authors further broadened the potential application of SFC-IM-QToF MS to the analysis of different pesticide classes, such as phenylurea, triazine, organophosphorus, and pyrethroid, in potato and yam tubers by performing a simple ACN extraction without purification steps.[39] The IM-QToF MS analysis revealed that pesticides within the same structural class underwent a characteristic cleavage pattern, such as amide-bond breakage in phenylureas, alkyl-group loss and ring cleavage in triazines, and C–S bond cleavage in organophosphates. Single-field and multi-field CCS values were measured as highly specific parameters to support compound identification (to four decimal places) and further improve accuracy. The combined use of m/z and CCS values enabled a clear discrimination among pesticide classes, with compounds of the same class showing linear CCS trends due to their similar structures. All these analytical methodologies highlight the feasibility and robustness of SFC-MS for multi-pesticide residue analysis, proposing it as a valuable alternative to the solvent- and time-consuming HPLC and/or GC reference methods. These approaches exhibit comparable quantitative performance, meet regulatory requirements, and minimize environmental impact.

An online SFE-SFC-QqQMS approach for the analysis of pyraclostrobin and chiral mefentrifluconazole (MFZ) residues in mango and mango juice was proposed by Yang and co-workers.[40] In parallel with conventional SFE optimization in terms of organic co-solvent amount, static and dynamic extraction times, extraction temperature, and dynamic extraction flow rate, a computational study was also performed to evaluate several materials with purification purposes, including primary secondary amine (PSA), octadecysilane, graphitized carbon black (GCB), and multi-walled carbon nanotubes (MWCNTs). The simulations showed that all the target pesticides, particularly pyraclostrobin, selectively accumulated around MWCNTs, leading to unsatisfactory recovery. Consequently, a combination of GCB and PSA was selected in order to obtain a good purification effect, maintaining the recovery values higher than 90%. Similarly, dynamic simulations were used to investigate molecular docking and to gain deeper insights into enantiomer separation behaviour. Six chiral stationary phases were tested, including three cellulose-based and three amylose-based ones, with measured resolution values in the 1.61–4.51 range. Molecular docking was also employed to elucidate the enantioseparation mechanism of MFZ on the cellulose tris(3,5-dimethylphenylcarbamate) selected column. The calculated binding energies were slightly more favourable for *S*-MFZ (-4.6 kcal mol^{-1}) than for *R*-MFZ (-4.5 kcal mol^{-1}). This could be related to a different hydrogen bond distance between the hydrogen of CSP and the nitrogen of MFZ, equal to 2.6 Å and 2.7 Å for *S*-MFZ and *R*-MFZ, respectively. Overall, integrating computational studies with experimental data represented a valuable strategy to enhance chiral discrimination and to guide further enantioseparation optimization.

The accurate prediction of chromatographic retention times remains challenging in SFC, as the retention behaviour is highly dependent on multiple experimental parameters, including pressure and temperature, along with modifier composition and stationary-phase chemistry. In this context, quantitative structure–property relationship (QSPR) modelling offers a promising *in silico* strategy to support method development and compound identification, as recently applied by Consonni and co-workers for pesticide retention time prediction.[41] A set of 438 pesticides, previously analysed by another research group using SFC-HRMS,[42] were used to select molecular descriptors, including charge, molecular properties, functional groups, and to calibrate the QSPR model. The trained model was successfully applied to retention time prediction of 213 pesticides previously analysed in a different SFC-LRMS system,[43] demonstrating consistent performance within the defined reliability domain and effectively compensating for instrumental variability. Although not focused on food matrices, the study addressed the poor retention time transferability typical of SFC, thereby supporting faster method development, improved reproducibility, and more reliable compound identification.

4. Multidimensional approaches

Technological and methodological advances have become a pivotal challenge, essential for improving analytical performance and supporting progress in both scientific research and applied fields.

Two-dimensional (2D) separation techniques have emerged as powerful analytical strategies to address the complexity of food samples, thereby significantly enhancing peak capacity, resolving power, and selectivity compared to one-dimensional approaches.

In such a context, a loop-based 2D-SFC system, operating in the multiple heart-cutting mode (mSFC-SFC), coupled with DAD and MS detection, was recently proposed by Réset *et al.* for the characterization of flavonoids in *Citrus aurantium* extracts.[44] Three external 6-port 2-position valves were online connected between the first (^{1}D) and the second dimension (^{2}D). Predefined fractions, eluted from the ^{1}D, were temporarily stored in 50 or 100 μL loops and subsequently re-injected into the ^{2}D for a further separation with a reproducible and efficient transfer rate

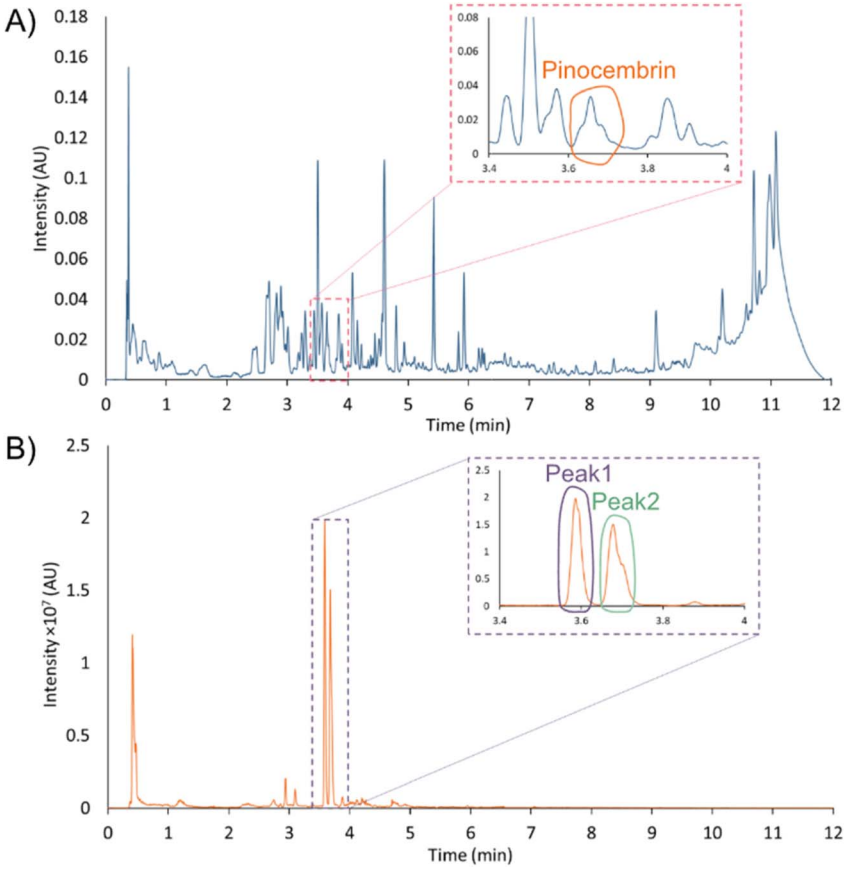

Fig. 4 [1]D SFC chromatograms of a honey sample. (A) SFC-UV analysis (280 nm). (B) SFC-MS analysis (*m*/*z* 257). Reproduced with permission from *Chirality*, 2025, **37**, e70058 (ref. 47). Copyright 2026, Wiley & Sons.

(exceeding 80%). This configuration allowed multiple heart cuts within a single run, enabling targeted improvement of resolution for critical regions of the chromatogram, rather than applying comprehensive 2D chromatography. Specifically, the diastereomers of two chiral flavonoids, namely neohesperidin and naringin, were separated on a stereoselective polysaccharide ^{2}D stationary phase, and the stereoisomeric excesses were determined. Despite the injection into the ^{2}D of large volumes containing a high proportion of co-solvent, excellent peak shapes were observed, thus, probably linked to the lower elution strength of the ^{2}D mobile phase, promoting compounds focusing at the column inlet. Starting from this proof-of-concept study, the same authors subsequently provided a more in-depth and systematic investigation of fraction-transfer conditions, evaluating the influence of loop volume, pressure, and mobile-phase composition mismatches,[45] thus, defining the system robustness and limitations for reliable routine analyses. Storage loops of 50–100 μL volumes provided an effective compromise between transfer efficiency and peak shape, while neither reducing loop size nor splitting fractions into multiple transfers significantly improved the resolution. Additionally, an appropriate pressure control also acted as a compressing factor of the injected fractions. To demonstrate the feasibility of the proposed configuration, complex food-related matrices were subsequently analysed. First, the developed system was employed for the determination of the diastereomeric excesses of chiral flavonoids in *Citrus aurantium* organs, including peel, flowers, leaves, and fruits at different stages of maturity.[46] Glycosylated flavanones were optimally recovered by SFE through a Box–Behnken experimental design, and the diastereomeric excess of naringin and neohesperidin was identified as a critical response parameter. Similarly, the stereoisomeric separation of pinocembrin, a bioactive flavanone aglycone of honey, was investigated.[47] A multi-criteria optimization strategy based on Derringer desirability functions was employed to investigate the separation capability of five different chiral columns. Four criteria, namely retention time, co-solvent proportion at the elution time of the second enantiomers, resolution, and peak asymmetry, were considered. The analytical responses of each function were normalized into a single desirability value, enabling direct identification of a single broadly applicable column or columns tailored to specific classes of chiral flavonoids. Despite the SPE clean-up, the honey extract remained too complex for single-dimension enantiomeric analysis (Fig. 4A, acquired at 280 nm). A selected ion monitoring (SIM) chromatogram in the positive ionization mode (Fig. 4B, *m*/*z* 257) revealed two peaks near the expected retention time (identified by standard injection).

5. Conclusions, challenges, and future perspectives

SFC has consolidated its position as a powerful, versatile, and environmentally friendly analytical platform for food analysis. In particular, speed and low solvent consumption position SFC as a competitive alternative to LC and GC in many applications, particularly in routine quality control laboratories and high-throughput screening environments. The surveyed three-year period literature demonstrates how continuous improvements in instrumentation, stationary phase design, and multidimensional configurations have expanded SFC applicability to complex food matrices, ranging from lipidomic studies and bioactive profiling to multi-residue contaminant analysis and chiral separations. In such a context, most of the SFC applications in food analysis are currently based on the coupling with advanced MS detectors, which, despite their high cost and complexity, remain crucial for achieving proper structural information and selectivity, along with sensitivity, especially for contaminant trace analysis.

Despite these advances, several challenges still limit the broader routine implementation of SFC. Some of the main bottlenecks remain restricted inter-laboratory validation studies, standardization, and regulatory acceptance. Although SFC is inherently aligned with green analytical chemistry due to its reduced organic solvent consumption, further efforts toward sustainable instrumentation design should still be encouraged. In this context, the implementation of closed-loop CO_2 recycling systems in analytical SFC requires a careful evaluation of the overall environmental and energy balance, which could be further improved through the integration of renewable energy sources. At the same time, bio-based organic solvents of suitable purity from renewable sources are becoming available, to be employed as mobile phase modifiers or make-up fluid to support MS ionization.[48]

Another promising, although poorly explored, direction concerns the broader implementation of compact, energy-efficient, and portable SFC systems. That remains challenging due to the need for reliable high-pressure fluid delivery, back-pressure regulation, and integrated CO_2 management.

As already stated, the incorporation of artificial intelligence, machine learning, and predictive modeling could represent a transformative perspective for the field, further highlighting the method potential for high-throughput, information-rich, and sustainability-oriented workflows. In particular, data-driven tools may support automated method development, retention time prediction, intelligent peak annotation, and the handling of multidimensional datasets. Such approaches could also improve the classification of complex food samples, strengthen authenticity and adulteration studies, and facilitate the transfer of methods across laboratories and instrument configurations. More broadly, the integration of these computational strategies with green analytical chemistry principles may help reduce experimental trial-and-error, shorten development time, and maximize the analytical value extracted from each run.

Looking ahead, such continued efforts could finally lead to the SFC transitioning from a promising alternative to a recognised reference method.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this mini-review.

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