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Review

Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food

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Abstract

An overview is presented of chromatographic methods currently in use to determine sugars, carboxylic acids and amino acids in foods: high-performance liquid chromatography, gas chromatography and capillary electrophoresis. As a basis of selection the following approaches can be distinguished: quantitation of constituents of several food matrices, without derivatization and in the form of different derivatives, in the presence of the matrix, or subsequently to various work-up procedures.

Keywords: Reviews; Food analysis; Sugars; Carboxylic acids; Amino acids

Contents

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1. Introduction

The quality, safety, attractive performance and taste are the most important characteristics that determine the value of food. To be able to guarantee and prove the optimum level of all of these requirements, suitable also for comparison, exact, reliable and reproducible testing procedures are needed. Of the analytical chemical procedures, without doubt, chromatographic ones are the methods of choice: since, by means of any, quantitative chromatographic separation, constituents to be determined, are emerging on the chromatogram one by one, providing unambiguous, selective proof of their presence and quantity.

The need for the determination of sugars and carboxylic and amino acids in foods is very well known and can be attributed to the fact that both numerous crude foods (fruit, vegetable, drug and industrial plants, animal and vegetable oils, meat, mushrooms, honey, milk, etc.) and the overwhelming majority of processed food products (marmalades, fruit juices, ciders, soft drinks, wines, beers, alcoholic beverages, milk products, etc.) consist of a considerable amounts of these three types of organic compounds.

The increasing interest in chromatography in the field of food analysis can be best evaluated by the increasing amount of research papers presented at international forums. On the basis of proceedings available of the International Symposia on Chromatography (ISC) and High-Performance Liquid Chromatography (HPLC) meetings of the last 10 years, the following conclusions can be drawn: (i) the interest in all chromatographic methods, including the topic of foods, has been increased in the last 10 years. (ii) Food related papers are presented mainly in the poster sections. (iii) HPLC methods are less involved in comparison to the GC methods revealing in 1999 a slight increasing tendency. (iv) The most promising sign was the appearance of oral presentations on the food topic at the last two ISC conferences: the particularly high number of research papers in Germany can be associated with the world famous work of food scientists at the University of Hamburg.

The aim of this review was to present the state of the art in the development and application of chromatographic methodologies devoted to the analysis of sugars and carboxylic and amino acids in foods. In order to save space and furnish a clearly arranged overview, suitable also to reproduce the proposals in

question, the details of practical conditions are presented in Tables 1–14 in a uniform format.

This selective review is based on the literature of chromatographic analyses published mainly in the last decade in Journal of Chromatography, *Chromatographia*, *Journal of Chromatographic Science*, *Journal of Liquid Chromatography and Related Technologies*, *Analytical Chemistry*, *Analytical Biochemistry*, *Analytica Chimica Acta*, *Analyst*, *Rapid Communications in Mass Spectrometry*, *European Journal of Mass Spectrometry*, *Journal of Agricultural and Food Chemistry*, *Food Chemistry*, *Journal of Dairy Science*, etc.

The main principle of the grouping was to give a separate discussion of the methods dealing with the free and with the derivatized sugars and carboxylic and amino acids. The chromatographic methodology serves as classifier of subdivisions, such as GC, HPLC and capillary electrophoresis (CE). Recent compilations [1,2] furnish a short overview on the analytical chemistry of food analysis, including the chromatography of sugars and carboxylic and amino acids.

2. Chromatography of sugars

Most of the organic matter on earth consists of free carbohydrates, i.e., mono-, di-, trisaccharides, oligosaccharides and polysaccharides and/or are integrated in glycoproteins and glycolipids. Carbohydrates are responsible for several sensory properties and texture of foods, as well being involved in many life processes. Monosaccharides such as glucose, fructose, sucrose taste sweet, oligo- and polysaccharides define the texture and consistency of fruits, vegetables, etc. Their role is associated with the metabolism and transformation of carbon dioxide. The primary importance of saccharides can be explained by the fact that they can serve as source and fuel of numerous biological procedures, playing also a key role in the cell–cell recognition phenomena [3].

The intrinsic difficulty in the analysis of carbohydrates can be attributed, on one hand, to the number of their possible isomeric forms: a single monosaccharide, such as glucose can exist in six forms (the simple non glycoside form, its hydrate version, the α- and β-glycopyranoses, as well as the α- and β-glycofuranoses). On the other hand several monosaccharides differ from each other very slightly: in the direction of their hydroxyl group(s), only. Thus, in the case of a highly selective, sophisticated chromatographic separation, theoretically, we have account for six glucose isomers, rendering the evaluation of all others, possibly, also six isomers eluting monosaccharides (mannose, galactose, etc.) more difficult.

In this particular case, in the analyses of food saccharides, when, with few exceptions, the distribution of isomers is of secondary importance, we can state that overinformation can result in misinformation. Consequently, quantitation of the most common mono- and disaccharides (glucose, fructose, sucrose, maltose), routinely, is performed in the most simple way: without any isolation and/or derivatization process, applying HPLC. Accepting, however, that the HPLC of their aqueous solutions allows the separation even of these most frequent, three sugars, often, with limited resolution, sensitivity and selectivity.

2.1. High-performance liquid chromatography

Considerable progress was achieved on the topic of HPLC of underivatized sugars due to efforts at the basic research level [4–6]. The advantage of the graphitized carbon column coupled with pulsed amperometric detection (PAD) [4] has been shown in the separation of several mono-, disaccharides and cyclomaltaoses. The usefulness of this procedure has been proved also in food analyses [5–7]: in the identification and determination of saccharides in plants [5], in fermentation media [6] and in onions and garlics [7]. Indirect detection of saccharides in reversed-phase (RP) HPLC ensured the quantitation of sugars at trace levels, including also the non reducing ones, applying gradient elution [8]. The power of HPLC with electrospray ionization mass spectrometry (ESI-MS) was demonstrated by characterization of the size, sugar sequences and branch structures of closely related oligosaccharides without derivatizations [9].

2.1.1. Without derivatization

The special applications of HPLC in the separation

Table 1
HPLC of sugars without derivatization[a]

Matrix; No./min*	Sample preparation	Column	Eluent	Detection	Detection limit	Ref.
HPLC–IR						
Beer; 7 (2,3-butanediol, gly, glu, suc, mal, malto-tri, maltotetr)/12	2 ml fermentation liquor+0.65 ml ACN (for protein+starch: precip); syr filtr (0.45 μm); I: –	250+15(guard)×4.6 mm (Dynamax 5 μm, amino)	Isocr., ACN–water (60:40, v/v); 1.0 ml/min	RI	62.5 n*M*	[10]
Infusion, soft drinks; 4 (xyl, glu, malt, lact)/12	Soft drinks were diluted 20-fold with eluent; I: 50 μl	150×6 mm (laboratory-modified silica gel, 5 μm)	Isocr., 10 m*M* borate buffer (pH 6.5)–ACN (10:90, v/v), 1.0 ml/min; 30°C	RI	*R*: 1.5%	[11]
Fruits, ice cream, etc.; 4 (suc, glu, fruc, sorbitol)/30	10 g fruits homog with 2×30 ml water, centrif. Extracts compl to 100 ml. Filt (0.45 μm); I: 20 μl	300×65 mm (Polyspher CHCA, Merck)	Deionized water; 0.3 ml/min; 90°C	RI		[12,13]
HPLC–ELSD						
Beet juice; 4 (fruc, glu, suc, raf)/6	Juice diluted with water 1:32 ratio, purif. on Hamilton resin catridge, dil. by MeOH, 1:3 ratio; I: –	250(125)×4.0 mm (LiChrospher 100 Diol 5 μm)	Isocr., CH_2Cl_2–MeOH (83:17, v/v); 1.0 ml/min; 40°C	ELSD	*R*: 0.999 1 ppm	[14]
Plant tissues; 7 (rham, xyl, fruc, glu, suc, mal, malto-triose)/30	Details are not available; I: –	250×4.0 mm, 100NH_2, 5 μm (RP 60 mm guard, Merck)	Isocr., ACN–water (80:20, v/v); 1.0 ml/min; 25°C	ELSD+RI, connected in series	– 10 n*M*	[15]
Beer; 8 (fruc, glu, malto-oligosac up to DP8)/30	0.5 ml beer injected into a tube of 500 mg C_{18} SPE, flushed with 10 ml water; I: 10 μl	250×4.0 mm, Polymeric NH_2 (Adv. Sep. Technol.)	Grad., ACN–water (70:20, v/v), 5 min, (50:50) in 20+5 min; 1.0 ml/min; 40°C	ELSD	<11.1 5–10 mg/l	[16]
HPLC analysis by electrochemical detection (PAD and others)						
Plants; 10 (inositol, ribitol, fuc, 2-deoxyglu, arab, gal, rham, glu, xyl, man)/30	1 g air dried plant material was refluxed with 3 *M* H_2SO_4 for 5 h at 75°C, followed by strong anion+strong cation-exchange; I: –	250×4.0 mm+25×3 mm guard (CarboPac PA1)	Grad., 15 m*M* NaOH with 1.5 m*M* NaAc for 15 min, ramped to 50 m*M* NaOH at 25 min; 0.8 ml/min; amb. temp.	PAD	0.05–0.5 mg/l	[5]
Beer, drinks; 9 (fuc, methylglu, arab, glu, fruc, lac, suc, cellob, mal)/30	10–100-fold dilution by deionized water of beer, cola, sprite, grape soda, etc.; I: –	250×4.0 mm (Dionex CarboP anion-exchange)	Isocr., 0.1 *M* NaOH; 1.0 ml/min	Cu–Ag/AgCl (0.55 V) cons. potential	3.1–20 n*M*	[17]
Food, beverages; 4 (mal, mal-totetrose, cellob, glu)/100	Solutions containing 0.5 g/l of each sugar; I: –	Two size-exclusion columns in line (Superdex Peptide HR 10/30, Pharmacia Biotech)	50 m*M* phosphate buffer (pH 6)	Laboratory-made amperometric	25 μ*M*–3 m*M*	[18]
Broth; 4 (glu, xyl, gal, ethanol)/20	On-line microdialysis probe; I: –	Ligand-exchange (Aminex HPX-67-P, Hercules, USA)	Water; 06 ml/min; 85°C	Laboratory-made[#]	0.1–0.4 g/l	[19]
HPLC–MS						
Broth; 5 (glu, xyl, gal, arab, man)/25 (RI);/12 (PAD); /25 (TSP-MS)	Hydrolyzate (purified by SPE); I: –	300×7.8 mm (Aminex HPX/87P Pb); 250×4.0 mm (CarboPac PA1)	RI: Milli-Q purif. water, 0.6 ml/min; PAD: 10 m*M* NaOH, 1.0 ml/min;	RI+PAD; TSP-MS	RI, PAD, MS: 0.2 m*M*, 2 μ*M*, ~5 n*M*	[6]
Onion, shallot, garlic; 13 (glu, fru, +fru.ol. up to DP=12)/60	1 g, peeled, chopped freese dried sample extr. 2× (40 ml water, 1 h reflux), centrif., filt. (0.45 μm), stored (−20°C). I: 50 μl extr. +50 μl 0.01 *M* KCl	250×4.0 mm+50×4 mm guard (CarboPac PA1)	Terner grad., A: 100 m*M* NaOH; B: 100 m*M* NaOH+400 m*M* NaAc; C: 300 m*M* NaOH, 0.7 ml/min	MALDI-MS; PAD	PAD: 5 μg MALDI-MS: 2 μg	[7]

[a] Abbreviations: No./min*=number (name) of compounds determined/min; I=injection, μl; –=no data available; *R*=correlation coefficient; arab=arabinose; xyl=xylose; rham=rhamnose; fuc=fucose; fru=fructose; glu=glucose; gal=galactose; man=mannose; suc=sucrose; lac=lactose; mal=maltose; cellob=cellobiose; raf=raffinose; fru.ol.=fructooligosaccharides; other abbreviations as in Nomenclature section. [#]=Bio+carbohydr+alcohol sensors.

Table 2
HPLC of sugars as various derivatives[a]

Matrix; No./min*	Sample preparation; derivatization; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
HPLC of pre-column derivatized sugars measured by UV detection (nm)						
Tree sap glycoprotein sugars; 23 (pentoses, hexoses methylamino sugars)/80	P: 1 mg sap trees+2 ml 2 *M* TFAA (6 h, 100°C), reduced by $NaBH_4$ (2 h, 40°C), acidif. to pH 3, evap. by MeOH, dry (2 h, 50°C), evap.+vac. dist. (60°C), extr. $CHCl_3$, evap. followed by#; De: benzoic anh.+4-dimethylaminopyrine; I: –	150×4.6 mm (Develosil 60-3, 3 μm)	Grad., C_6H_6–dioxane–CH_2Cl_2 (110:10:5) to (20:10:5), 1.25 ml/min	UV, 275	– –	[27]
Food; 7 (monosaccharides in 14 anomeric peaks)/25	De: 1 ml DMF cont. 1 mg sugar+PHI 55 μl DMF, [sugar/PIC=1/92 (mol/mol)], 95 min, 55°C. Exc. PIC destr.: 0.5 ml MeOH, 5 min +6 ml DMF. Stor 4°C. I: 10×dil., 10 μl	220×4.6 mm (Brownlee ODS 224 RP18, 5 μm)	Isocr, ACN–water (60:40, v/v); 2 ml/min	UV, 240	– 0.2–1 ng	[28]
Milk (cow, human goat), infant formulas; myo-inositol**	P: 2 g milk powder or (15 g milk)+25 ml water titr. to pH 4.5, compl. by water to 100 ml, filtr. De: 100 μl filtr. (lyoph)+70 μl Pyr+20 μl PIC, 70 min, 55°C. Exc. PIC destr.: 20 μl MeOH (10 min, 55°C), dil. ad. 20 μl by Pyr.; I: –	Resolve C_{18} (5 μm), length –	Grad., ACN–water (45:55) to (10:90, v/v); 2–2.5 ml/min	UV, 240 and FAB	1.4–3.1 –	[29]
HPLC of pre-column derivatized sugars measured by fluorescence (FL) detection, $\lambda_{ex}/\lambda_{em}$ (nm)						
Beverages; 16 (pentoses, hexoses, disaccharides)/24	P: Samples deprot., dilut., filtered; De: 100 μl filtrate+ 10 μl TCA (1%, w/v)+ 50 μl Dnshydr (5%, w/v, in ACN) 65°C, 20 min, sealed tube). I: 10–300 μl within 2 h	250×6 mm (Nucleosil, ODS, 5 μm);	Isocr, 80 m*M* CH_3COOH–ACN (79:21, v/v); 1.0 ml/min	FL, 360/470	<0.4 0.5 n*M*	[30]
Corn syrup; 7 (maltose-maltoheptaose)/30	P/De: 100 μl aqueous sugar samples+100 μl 3%TCA +200 μl freshly prepared Dnshydr. dried (vacuum centrif.), dissolv. 10–200 μl ACN–water (1:1), cont. 0.05% DAB. I: –	250×4.6 mm (LiChrosorb Si-60)	Grad.: A, ACN–water (90:10) (0.05% DAB), B, water (0.05% DAB); 1 ml/min	FL, 336/487	– 0.25–280 n*M*	[31]
HPLC of post-column derivatized sugars measured by UV [32] and FL [33]						
Wein sugars; 9 (treh, rham, arab, glu, rut, fru, suc, genti)/30	P: Diluted wine (1:100), 2 μl injected directly; De: post-column, in 10 m×0.3 mm I.D. PTFE coil (2% thymol in 96% sulfuric acid); 90°C	Anion-exchange (HPIC-AS 6, Dionex)	Isocr, 0.15 *M* NaOH; 0.5 ml/min; 36°C	UV, 500 nm	– ng range	[32]
Natural+comm. fruits/juices, cola beverages, plant extracts; 3 (suc, glu, fru)/30	P: dilut. 50×, filtr. (0.22 μm filter). De: post-column, effluent's non reducing sugars hydr. to reducing ones (75×3.8 mm column, Dowex 50W-X4, etc., mixed with 30 m*M* benzamidine +1 *M* KOH (1 ml/min, at 100°C, in a 530-μl coil)	300×6.5 mm, microparticulate resin, Ca form	Isocr, water; 0.4 ml/min; 70°C	FL, 60/470 nm	<4.8 30–60 p*M*	[33]

[a] Abbreviations as in Table 1, as well as/or: P=preparation; De=derivatization; #=gel permeation chromatography (TSK gel, G2000HG, 60×2.2 cm×2, $CHCl_3$); DMF=dimethylformamide; PIC=phenylisocyanate; **=together with four sugars (glu, gal, lac, suc)+sorbitol/50 min; FAB=fast atom bombardment; Pyr=pyridine; TCA=trichloroacetic acid; Dnshydr=5-dimethylamino-naphthalene-1-sulfonylhydrazine; DAB=1,4-diaminobutane; genti=gentiobiose.

Table 3
CE of sugars: UV detection with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) [34] as derivatization reagent and underivatized sugars applying BGE [35–38][a]

Matrix; No./min*	Sample preparation; derivatization; injection	Capillary	Voltage	Electrolyte	Detection UV (nm)	RSD (%) Detection limit	Ref.
Model; 24 (maltooligosaccharides, DP=1–24)/6	De: 1 μ*M* of stand saccharide of each+200 μl, 0.2 *M* ANTS [in acetic acid–water (3:17, v/v)]+ 200 μl, 1 *M* $NaCNBH_3$ (in dimethylsulfoxide); vortexing, incubating in water bath (15 h, 40°C), 20–40-fold dil., filtr. (0.22 μm); I: at the cathodic end	270 mm×50 μm,	V: 230/cm	30 m*M* phosphate buffer, pH 2.5, 50°C	214	– 31 ng	[34]
Fruit juices; 3 (fru, glu, suc)/8, or 8/22	P: fruit juices 50–100-fold dil., orange juice filtr. (0.22 μm, Waters); I: 4 nl	420–900 mm×50 μm,	230/s	6 m*M* potassium sorbate (pH 12.2) as BGE	256	<2.4	[35,36]
Citrus, apple, beet pectins; 1/90	P: powdered pectins were dissolved by heating with deionized water, 2.5 mg/ml (60°C, 30 min); I: 190 nl	570 mm×100 μm	15 kV	50 m*M* phosphate buffer (pH 7.0) as BGE; 30°C	192	– 0.5 mg/ml	[37]
Saccharides; 21 (neutral+acidic+amino sugars/alcohols of glycoprot.)/30	P: 500 μg fetuine dissolved in 100 μl, 2 *M* HCl (95°C, 2–6 h). Evaporated, dried, redissolved in 100 μl hot water, centrif., filtered+100 μl 4 *M* HCl; I: 6 nl	805 mm×50 μm,	25 kV	20 mm 2,6-pyridinecarboxylic acid+0.5 CTAB (pH 12.1) as BGE; 20°C	350	<2.7 23–71 μ*M*	[38]

[a] Abbreviations, as in Tables 1 and 2, as well as/or: BGE=background electrolyte; CTAB=cetyltrimethylammonium bromide.

Table 4
GC of sugars as their trimethylsilylsilyl (TMS) (oxime) derivatives[a]

Matrix; No./min*	Sample preparation; extraction; derivatization; injection	Column	Gradient	Inj (°C)	Det (°C)	RSD (%)	Ref.
Derivatized in the presence of the matrix with HMDS+TFAA [39–54,62] or with HMDS+trimethylchlorosilane (TMCS) [52,54]							
Corn syr; 4 (glu, mal, maltotri +maltotetr)/50	De: 60–70 mg corn syrup (80%, i.e., max. 40 mg water), dissolve in 1 ml Pyr+ 0.9 ml HMDS+0.1 ml TFAA, shake 30 s, let stand 15 min; I: 5 μl	2/6 ft.×0.25 in. O.D., filled with Chromosorb W cont. 2–3% SE52 or SE30;	140–275°C, 15°C/min	300	FID, 300	≤3.6	[62]
Fruits, honey, etc.; 19 (mono-, di- and trisaccharides)/50	De: ~0.02 g sugar/equiv. matrix, vacuum evap. 1. Step: 500 μl oxim reag.[#] (70°C, 30 min), 2. Step: 900 μl HMDS+ 100 μl TFAA (100°C, 60 min), dil. from 10× to 100×; I: 1 μl	30 m×0.248 mm, 0.25 μm film (DB-5 J&W)	60–320°C, dif. progr.	60–320 progr.	GC–MS	≤0.5[&] ≤10[&&]	[39–51]
Vinegar; 7 (2,3-butanediol, 1,3-propanediol, erytritol, arabitol, mannitol, sorbitol, inositol/30	De: 500 μl vinegar evap. to dryness by N_2, then dissolved in 500 μl of Pyr–HMDS–TMCS (10:5:3) (80°C, 1 h on water bath), I: 1 μl	25 m×0.32 mm, glass capillary, 0.4 μm film (SE52, Alltech)	80–300°C, 10°C/min	300	FID 300	≤5.0	[52]
Fruit leaves, soy-shot; 6 (suc, glu, fru, myoinositol, sorbitol)/–	De: 100 mg freeze–dried leave tissue, 1. step: 125 μl Pyr+ 125 μl Stox* reagt., vortex (70°C, 40 min), 2. step: 200 μl HMDS+20 μl TFAA (room temp.); I: 1–2 μl	Packed column of 3% OV-17 on Chromosorb WHP	–	–	–	–	[53]
Caramel; 13 (1,6-anh.glu, fru, dimer fructoses, genti, isomalt/30	P: 100 μl caramel solution (16 mg/ml) evap. to dryn. (60°C, 1 h, oven); De: 1. step: 1 ml Pyr (cont. 20 mg/ml hydroxylamine) (60°C, 45 min), 2. step: 200 μl HMDS+100 μl TMCS (60°C, 30 min); I: 1 μl	30 m×0.25 mm, with 0.25 μm film (HP5 MS, Hewlett-Packard),	180–310°C, 5°C/min	300	GC–MS	≤5.0	[54]
Derivatized in various extracts							
Soybean; 15 (raf family of oligosaccharides, DP=1–5)/20	E: 50% MeOH (refl., 30 min, sample–MeOH, 1:20); De: 0.02 g sugar/equiv. matrix of extract (vacuum evap.), further on as in Refs. [39–50]; I: 10 μl, without dilution	50 cm×3 mm, filled with: 3% SP-2250 on Supelcoport 80–100 mesh (Supelco)	80–380°C, 16°C/min	410	FID 430	≤11.1	[55,56]
Fruits; 7 (rham, ara, xyl, man, gal, glu, uronic acid)/–	E: 500 g fruits refl. with EtOH (water in total 80 %). Extr repeated 2× with EtOH, evap. to 400 ml, extracted: ($CHCl_3$, 3×400 ml). Water solution was concentrated, cation- and anion-exchanged; De: [60]	25/12.5 m×0.2 mm (CP-Sil-5/CP-Sil-88, WCOT) (Chrompack)	–	–	FID	–	[63]
Pea flour; 4 (suc, raf, stachiose, verbascose)/30	E: 80 % MeOH (homogenized 2 min, 2 g pea flour/30 ml MeOH); De: 0.5–5.0 mg sugar (equiv. extract)+Tri-Sil Z reagent (Brockville, Canada) 70°C, 30 min; I: –	10 m×0.32 mm, 25 μm (DB5-60; J&W)	188–316°C, 8°C/min	250	FID (300)	≤4.7	[64]

[a] Abbreviations as in Tables 1–3, and/or: TFAA=trifluoroacetic acid; Stox*=Pierce Chemical, oximation reagent (25 mg/ml hydroxyl-amine·HCl in Pyr); oxim reag.[#]=2.5 g hydroxylamine·HCl 100 ml Pyr; [&]=in the case of main constituents (≥1 μg); [&&]=in the case of minor constituents (≤1 μg); *r*=linear regression coefficient (Pearson's correlation). 1 ft=30.48 cm; 1 in.=2.54 cm.

Table 5
HPLC [67–72] and ion chromatography (IC) [70,72,73] of aliphatic carboxylic acids without derivatization[a]

Matrix; No./min*	Sample preparation; extraction; injection; detection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
HPLC analysis in extracts						
Dairy products; 9 (formic, pyruvic, orotic, lactic, acetic, uric, citric, propionic, butyric acid)/30	E: 7 g (yogurt, cheeses)+50 ml eluent, extr. (1 h). Centrif., filtered (1. paper, 2. membr., 0.45 μm); milk diluted with eluent (1:5); I: 10 μl	250+15×4.6 mm (Beckman C_8, 5 μm)	Isocr., 0.5% (w/w) $(NH_4)_2HPO_4$–0.4% (v/v) ACN (pH 2.24), 1.2 ml/min	UV (210)	>85.3[#] –	[67]
Wheat, pea; 8 (oxalic, maleic, citric, aconitic, malic, tartaric, formic, acetic acid)/30	E: 0.1 g, lyophilized, pulverized (1–2 μm) 2× extr. with 5 ml water; water bath: 50°C, 60 min; filtered (0.45 μm); I: 20 μl	300×7.8 mm (HPX-87, Richmond, USA)	Water, or 2 m*M* H_2SO_4, or 0.1–10 m*M* ''bspt'' acids; 0.5 ml/min; 40°C	UV (250–300)	<3.8 <70 μ*M*	[68]
HPLC and IC analysis in the presence of the matrix						
Wine; 4 L- and D-, 8 (tartaric, malic, lactic, ascorbic acid)/10	P: 1 ml vine eluted through 400 mg C_{18} SPE cartr; I: 20 μl	250×4.6 mm (C_{18}, 5 μm, Alltech)	Isocr., 0.5 *M* NaH_2PO_4 (pH 2.5 adj. with H_3PO_4); 1.0 ml/min; 23±1°C	UV (210) and CD	<6.5 1 m*M*	[69]
Wine; 9+2 (acetic, lactic, succinic, malic, maleic, citric, tartaic, fumaric acid)/15 (RP-HPLC); /30 (IEC, IC)	P: dil. 5× [deionized water, filt. (0.45 μm)], I: 25 μl (for RP-HPLC, IC), 50 μl for IEC	HPLC, 250×4.6 mm (Zorbax ODS)	HPLC, 0.75 m*M* H_2SO_4	UV (210) and CD	– <10–4000 ppm	[70]
Royal jelly and products; 1 (*trans*-10-hydroxy-2-decenoic acid)/15	P: 50 μl weighed in a 100-ml volumetric flask, sonicated for 30 min, made up to the mark with eluent. Filtered (1 μm paper; I: –)	300×39 mm (Waters μBondapak C_{18})	ACN–THF–water (50:22: 28, v/v) (pH 2.5 by 0.1% H_3PO_4); 1 ml/min; 35°C	UV (215)	<7.8 –	[71]
Raw juice, molass; 9 (citric, malic, lactic, formic, acetic, propionic, butyric, valeric, pyroglutamic acid)/18	P: Solutions were diluted to 1% sugar content	HPLC: HPX-87 H sulf; IC: HPIEC-AS1 sulf	RP-HPLC: 5 m*M* H_2SO_4, 0.6 ml/min, 50°C; IC: 2 m*M* HCl; 0.8 ml/min	UV (210) and Cond.	– 100 ppm	[72]

[a] Abbreviations, as in Tables 1–4, and/or: fru.ol.=fructooligosaccharides; [#]=recovery %; ''bspt'' acids=benzo-, or salicylic-, or phthalic-, or trimesic acids; CD=circular dicroism; Cond.=conductivity detection for IC.

Table 6
HPLC [73–81] of aliphatic and aromatic carboxylic acids simultaneously, without derivatization[a]

Matrix; No./min*	Extraction/sample preparation; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
HPLC analysis in extracts						
Apple extract, cider; 7 (quinic, malic, shikimic, lactic, citric, succinic)/15	E: 1 kg apple, 1 quarter of each exc+blend (80% EtOH, cont. 1% HCl), refl., 2 h, filt. (1. gooch cruc., 2. Sep-Pak C_{18} cartr. 3. membr (0.45 μm); resid washed, evap., filled up to 500 ml; I: 5–60 μl	250×4.6 mm/100×2.1 mm or (Spherisorb/Hypersil ODS-2)	Isocr., 0.01 *M* $(NH_4)_2HPO_4$–H_3PO_4 (pH 2.25–2.7; 0.1–0.5/2 μl ml/min, 25°C)	UV (206/210)	>3.0–5.0 –	[73–75]
Fruit juices, nectars; 5 (oxalic, citric, malic, quinic, succinic acid)/25	E: 5 g/ml juice, or 2 g conc.+40 ml MeOH mixed, heated (20 min), centr.+50 ml MeOH, centr. MeOH evap., resid. dissolv. in 50 ml water. Filtr: Sep-Pak C_{18}, membr. (0.45 μm); I: 20 μl	300×6.5 mm (Ion 300 Acero Inox)	0.00425 *M* H_2SO_4; 0.8 ml/min, 42°C	UV (214)	– –	[76]
Honey; 17 (galacturonic, gluconic, tartaric, pyruvic, quinic, malic, isocitric, lactic, acetic, α-OH-butyric, citric, succinic, dimethylglyceric, fumaric, propionic, 2-oxopentanoic, glutaric a)/50	E: Cart washed: 50 ml water (3 ml/min)+5 ml CH_3COOH, pH 2.05, 0.5 ml/min+50 ml, pH 4.5, 3 ml/min) dried. 0.5–1 g honey in 10 ml water. Transf. to (cartr., 0.3 ml/min), washed (30–40 ml water), 3 ml/min; I: –	250×4.6 mm (Spherisorb ODS-1 S5, 5 μm)	H_2SO_4 (pH 2.45); 0.7 ml/min	UV (210)	<3.2 <3 ppm	[77]
HPLC and IC analysis in the presence of the matrix						
Vine, fruit juices 8 (tartaric, malic, shikimic, lactic, actic, citric, fumaric, succinic a)/9	P: filtered by membr. (0.45 μm); no more data available	250×4.6 mm (Spherisorb ODS-2, 5 μm) precol.: 30×3.9	Isocr., H_2SO_4 (pH 2.50), ionic str.: 0.1 *M* Na_2SO_4; 0.5 ml/min, 35°C	UV (210)	<6.1 1 m*M*	[78]
Must; 10 (glucuronic, tartaric, malic, shikimic, ascorbic, acetic, fumaric, citric/citramalic, succinic acid)/40	P: 25-fold dilution with mobile phase; filtered (0.45 μm); I: 2.5–15 μl	250×4.6 mm, Beckmn ODS Ultrasphere C_{18}, 5 μm	Isocr., 0.01% HCOOH; 0.3 ml/min, room temp.	UV (190)	<4.8 1 m*M*	[79]
Orange juice; 12 (oxalic, quinic, tartaric, malic, isocitric, lactic, ascorbic, citric, fumaric, succinic, propionic, *cis*-aconitic acid)/40	P: 8 ml juice+1 ml HPO_3+1 ml 2 % propionic ac. centr. (5 min), 1 ml filtr. (SCX pretr. cartr.: 1 ml MeOH+10 ml water). Centr: tube washed: 2 ml water+4 ml El, filtr. (0.45 μm); I: 20 μl	250×4.6 mm (YMC-Pack ODS-AQ, 5 μm)	20 m*M* KH_2PO_4 (pH 2.8); 0.7 ml/min, 25°C	UV/DAD (210)	<2.8 –	[80]
Fruit juices; 12 (acetic, lactic, quinic, shikimic, Cl^-, galacturonic, NO_3^-, malic, tartaric, SO_{4+} ascorbic, PO_4, citric, isocitric a)/55	P: fruit juices, 10–25-fold dilutions filtered (0.45 μm); I: 20 μl	250×4 mm (OmniPac Pax-500)	Grad., 0.2–0.6 m*M* NaOH in water–EtOH–MeOH (66:20:14), or in water–EtOH (65:35, v/v); 1.0 ml/min	Cond.	<2.9 –	[81]

[a] Abbreviations as in Tables 2–5.

Table 7
HPLC of aromatic (phenolic) carboxylic acids and catechins without derivatization, in extracts[a] [82–91]

Matrix; No./min*	Extraction/sample preparation; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
Apple pulp, wine, 7 (gallic, protocatechuic, rezorcylic, gentisic, *p*-OH-benzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, chlorogenic+4 aldeh+2 catechin)/55	E: 25 ml apple pulp (15 g crushed MeOH–HCl, 1000:1), or 25 ml wine was extracted with 4×15 ml EtAc or DieEt (1 min) dried (Na_2SO_4), evap., dissolved in 1 ml MeOH–water (1:1); I: –	250×4.6 mm (Bondapak Ultrasphre C_{18})	Grad., A: CH_3COOH–water (2:98); 1.0–1.2 ml/min	UV (280/340)	<10.1 (for DieEt) <17 (for EtAc)	[82]
Wine; 8 (gallic, 3,4-dihydroxybenzoic, vanillic, syringic, caffeic, *p*-coumaric, ferulic acid)/67	E: 1 ml (red wine), or 2 ml (white wine) evaporated; residue dissolved in 1 (red wine), or 2 ml (white wine) phosphate buffer; both extracted by 3×1.5 ml diethyl ether, dried (Na_2SO_4, evap., dissolved in 50 μl MeOH); I: 1 μl	250/500×1.1 mm (slurry, laboratory-made, Spherisorb ODS2, 5 μm)	Grad., A: MeOH–pho buf (pH 2.4) (95:5), B: pho buf (pH 2.4)–water (5:95); 40 μl/min	UV (280)	<11.5 –	[83]
Fruit juices; 4 (gallic, chlorogenic, caffeic, ferulic acid)/50; +1 (ellagic)/30	15 residues dissolved in 10 ml EtOH. Extraction validated by standards (0.05–10.00 mg) added to juice samples; I: 20 μl	120+4×4 mm (LiChrospher 100 RP18, 5 μm)	Isocr., water–EtAc–CH_3COOH (95.6:4.1:0.3, v/v); 0.5 ml/min	UV (280/320)	<4.7 0.1 mg/l	[84]
Apples, pears; 11 (arbutin, gallic, (+)-catechin, chlorogenic, caffeic acid, (−)-epicatechin, coumarin, rutin, phlorizdin, quercetin, kaempferol)/35	E: 5–10 g apple and pear pulp extracted by 2×10 ml+1×5 ml MeOH (ultras. bath, in dark, with 1% 2,6-di-*tert*.-butyl-4-methyl phenol, 90 min) extracts comb. to 25 ml, filtr. (0.5 μm); I: 20 μl	Co: 250×4.6 mm (Nucleosil 120 C_{18}, 5 μm)	Grad., A: 0.01 *M* H_3PO_4, B: ACN; 2 ml/min	DAD (210/350)	<1.0 –	[85]
Green cofee [86]; 7 (variously substituted cinnamic acids)/47; quince jam [87]; 12 (3,4,5-caffeo-ilquinic+benzoic+8 glycosides)/56	E: ([86]) 5 g fine powdered coffee+60 ml 40% MeOH (24 h). Filt, evap. to 5 ml. Hydr: 5 ml 2 *M* NaOH (240 min). Acidif. to pH 7, liquid–liquid extr. EtAc 3×20 ml. Comb. extr. evap. to 7 ml; I: 20 μl; E: ([87]) 40 g jam	Co: 250×4.6 mm (Spherisorb ODS-2, 5 μm)	Grad., A: water–HCOOH (19:1), B: MeOH; 0.9 ml/min	UV (320/280) [86,87]	<7.6 [86] 0.5–400 μg/ml <2.8 [87]	[86,87]
Tea leaves; 20 (theogallin, gallic a, theobromine, 3 chlorogenic a, caffeine, 2 epicatechine+theoflavine gallates, 5 quercetin/kaemferol glycoside)/50	P: 4 g black tea leaves added to 100 ml boiling distilled water and further heated for 10 min at 80°C. The hot solution filtered through cotton wool; I: 20 μl	250×4.6 mm (Hypersil ODS, 5 μm)	Grad., A: 2% CH_3COOH, B: ACN; 1.5 ml/min	DAD (280–600)	– –	[88]
Coffee; 4 (5-hydroxymethylfurfurol, furfurol, chlorogenic a, caffeine)/28	E: 1 g finely powdered coffee bean+150 ml water boiled for 2 min, filled up for 200 ml, thereafter filtered; I: 20 μl; D	250×4.6 mm (Spherisorb ODS-2, 5 μm)	Grad., A: water+0.2 % CH_3COOH, B: MeOH; 1 ml/min	UV (280)	<7.8 0.1–200 μg/ml	[89]
Honeys; 12 (gallic, *p*-hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic, *p*-coumaric, ferulic, *m*-coumaric, *o*-coumaric, ellagic, cinnamic acid)/45	P: 50 g honey+250 ml HCl (pH 2), filtr. Ion ex. (25×2 cm, Amb XAD-2 Fluka). Column washed: 100 ml HCl (pH 2)+300 ml water (sugars+polar comp. moved). Phenolics elut: 300 ml MeOH Sephadex LH20 (15×1 cm). Evap. (40°C), dis. 5 ml MeOH; I:20 μl	125×3 mm, 5 μm (Merck LiChrospher 100 RP-18)	El: grad., A: water–HCOOH (19:1), B: MeOH; 0.4 ml/min	DAD (280, 320)	– –	[90]
Wine; 12 (protocatechuic, *p*-hydoxybenzoic, vanillic, ferulic, *p*-coumaric, caffeic, gallic a+5 aldeh)/70	P: direct I: 5 μl wine;	250×4.6 mm (3 μm Spherisorb ODS-2)	Grad., A: HCOOH, 4. %, B: A eluent–ACN (90:10); 0.7 ml/min	UV (280) APCI-ESI	– –	[91]

[a] Abbreviations as in Tables 1–6.

Table 8
HPLC of FAs [92–98] derivatized with 1-naphthyldiazomethane (NDM), or 1-naphthyldiazoethane (NDE), with 9-bromomethylacridine (BEA), with 9-(chloromethyl)antracene (CMA), with 2-nitrophenylhydrazine hydrochloride (2-NPH·HCl), with phenacylbromide (PB), with 4-hydroxymethyl-7-methoxycoumarin (HMC) and with coumarin (CM), in extracts[a]

Matrix; No./min*	Sample preparation/extraction; derivatization; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
Butter, margarine, oil; 9 (NDE de: $C_{10:0}$, $C_{12:0}$, $C_{18:3}$, $C_{14.0}$, $C_{18:2}$, $C_{16:0}$, $C_{18:1}$, $C_{17:0}$, $C_{18:0}$)/90; 8 (NDM de: $C_{14:0}$+$C_{18:3}$)/75	De: 1–3 mg dis in 0.5 ml NDM–NDE cont C_6H_6–DieEt (N_2). Red reagent colour (1 h); I: 2–5 μl; D	300×(I.D.?) mm (C_{18} μBondapak)	Isocr., NDE: ACN–water (85:15); NDM: MeOH–water (85:15), 1.5 ml/min	UV (254/260) –	–	[92]
Butter, margarine, oil; 15 (caprylic, capric, lauric, eicosapentaenoic, linolenic, docosahexaenoic, arachiic, palmitoleic, myristic, linoleic, eicosatrienoic, oleic, palmitic, heptadecanoic, stearic acid)/62	De: 20–40 mg+5 ml $CHCl_3$, remove 10 μl+1.5 ml 25 m*M* tetraethylamm. carbonate in MeOH (70°C, 5 min capped tube, 70°C, 30 min open tube). Evap., resid. dissolv.: 1 ml DMF. Remove 100 μl+200 μl 5 m*M* BEA in DMF (10 min, room temp.); I: 10 μl	150×4.6 mm (TSK-gel ODS 120A, Toyo Soda)	Grad., MeOH–water from (90:10) to (97:3) in 32 min, +30 min; 0.8 ml/min	UV (252) FL (365/425)	– –	[93]
Beer; 9 (lauric, linolenic, myristic, palmitoleic, linoleic, palmitic, oleic, *n*-heptadecanoic, stearic acid)/85	E: 240 ml heat 60°C, cool rem 200 ml+10 ml 3 *M* H_2SO_4+200 ml DieEt–C_5H_5 (50:50, v/v) +60 g NaCl (shake: 30 min, centr.: 5 min). Rem 150 ml org layer, wash: 150 ml 5% $NaHCO_3$, evap. dry; De: 1 ml 6 m*M* tetra-met amm. hydr.+1 ml 7.5 m*M* CMA, in DMF (75°C, 20 min); I: 50 μl	250×4.6 mm (Kaseisorb LC ODS-60-5S C_{18}, Tokyo)	Grad., ACN–water from (90:10) to (100:0); 1.1 ml/min; 40°C	–	– 0.4 ng/ml	[94]
Milk, milk products; 14 LCFA** ($C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$ $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1c}$, $C_{18:2c,c}$, $C_{18:3}$, $C_{20:2}$, $C_{20:3}$, $C_{20:4}$)/28; 10 SCFA*** (lactic, C_2–C_6 *i*- and *n*-acid, 2-ethylbutyric, I.S.)/24	De: FFA, 100 μl milk/20 mg butter/cheese, 50 mg yogurt/condensed milk/ice cream+100 μl water+200 μl EtOH+0.02 *M* 2-NPH·HCl+ 200 μl 0.25 *M* 1-EDC* (80°C, 5 min). Then 200 μl 10% (w/v) KOH, in MeOH–water (1:1, v/v) heated (80°C, 5 min). Neutr.: 4 ml 1/30 *M* pho buf (pH 6.4)–0.5 *M* HCl (7:1, v/v). LCFA** hydraz extr.: 5 ml *n*-C_6H_6; SCFA*** hydraz extr. (water layer: 2×4 ml DieEt. Extracts evap.: N_2; Dissolv.: 200 μl MeOH; I: 10–20 μl	250×6 mm (YMC-FAC-8, 5 μm)+guard	Isocr., ACN–MeOH–water (30:20:50, v/v); 1.2 ml/min; 35°C	UV (400)	<4.2 0.5–2 p*M*	[95]
Wine, must; 5 (heptanoic, octanoic, nonanoic, decanoic, dodecanoic acid)/30	P: must/wine centr. (5000 *g*, 10 min); FAs sep from carboxylic acids: Sep-Pak C_{18}, i.e., FAs elut: 2 ml MeOH; De: into 2 ml screw-capped Pyrex tube 180 μl sample+400 μl pho buf (pH 6.8)+400 μl 0.2 *M* PB+400 μl 0.02 *M* 18-crown-6 (both in acetone)+200 μl acetone.(90°C, 90 min); I: 2 μl	250×6 mm, 5 μm (Spherisorb ODS2)+guard	Grad., A: water; B: MeOH; 1 ml/min; 30°C	UV (254)	<9.4 ppm	[96]
Milk, dairy products; 16 ($C_{4:0}$, $C_{6:0}$,$C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:1}$, $C_{13:0}$, $C_{18:3}$, $C_{14:0}$, $C_{16:1}$, $C_{18:2}$, $C_{15:0}$, $C_{16:0}$, $C_{18:1}$, $C_{17:0}$, $C_{18:0}$)/50	E: milk, yogurt 5 g, butter 0.5 g, cheese 0.3 g+5 ml DieEt+5 ml water (for butter/cheese)+ 5 g NaCl+5 ml 2.4 *M* HCl+5 ml C_6H_6 (shake 1 min, centr. 10 min, separ. layers). Comb. C_6H_6–DieEt layers dehyr.: anh. Na_2SO_4, prep: 1 ml extr.; De: 1 ml extr.+1 ml HMC reagent; I: 10 μl	250×4.6 mm (L column ODS-)	Grad., A: water, B: EtOH–ACN (1:1, v/v); 1 ml/min	FL (330/395)	<10.2 –	[97]
Vegetable oil; 11 ($C_{18:3c}$, $C_{18:3t}$, $C_{14:0}$, $C_{18:2c}$, $C_{18;2t}$, $C_{16:0}$, $C_{18:1c}$, $C_{18:1t}$, $C_{18:0}$, $C_{20:0}$, $C_{22:0}$)/40	E: 0.4 g oil+25 ml THF+5 ml 1 *M* NaOH (sap.: 45°C, 1 h). Acidify 10 ml 1 *M* HCl, extr., 3×25 ml $CHCl_3$. Comb. extr.+anh. Na_2SO_4, prep. 1 ml extr.; De: 1 ml extr.+equim. Na_2SO_4–$KHCO_3$, dibenzo-18- crown-6+slight excess CM (50°C, dark). Evap. dry (N_2), dissolv.: acetone; I: 20 μl	250×4.6 mm (Zorbax Rx-C_{18}, 5 μm)	Grad., ACN–water–MeOH from (82:15:3) to (0:0:100); 1 ml/min; 55°C	FL (320/375)	– 1 ppm	[98]

[a] Abbreviations as in Tables 1–7, as well as/or: FA=fatty acid; FFA=free fatty acid; 1-EDC·HCl=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl; LCFA**=long-chain FAs; SCFA***=short-chain FAs.

Table 9
HPLC analysis of aliphatic- and aromatic acids derivatized with phenacylbromide (PB), with *O*-(4-nitrobenzyl)-*N*,*N*′-diisopropylisourea (NBDI) and by micellar electrokinetic capillary chromatography (MECC) as ion pairs[a]

Matrix; No./min*	Sample preparation; derivatization	Column	Eluent	Detection (nm)	RSD (%)	Ref.
Wine; 33 (galacturonic, glycolic, glyoxylic, pyruvic, lactic, acetic, propionic, mandelic, tartaric, ascorbic, salicylic, *p*-OH-benz., vanillic, butyric, malic, α-ketoglutaric, citramalic, succinic, phenylacetic, cinnamic, benzoic, glutaric, valeric, sorbic, fumaric, anisic, gallic, isocitric, citric, benzilic, protocatechuic, enanthic, caprylic acids)/3×20	De: 1 ml wine (pH adjusted to 7–8 by $KHCO_3$)+ 1 ml 170 m*M* PB (in acetone)+1 ml 17 m*M* 18-crown-6 in acetone+1 ml acetone (water bath 65 min); I: 10 μl	250×4 mm, 7 μm, RP8, RP18 (Merck); guard: Bondapak, C_{18} (Corasil)	Grad., MeOH–water from (35:65) to (85:15); 2 ml/min	UV (254)	<5.0	[99]
Instant coffee, wine, fruit juices; 9 (quinic, glycolic, pyro-glutamic, lactic, formic, acetic, malic, phosphoric, citric acids)/24	P: 0.5 g coffee+15 ml 5 m*M* benzylmalonic acid (ultras. bath, 10 min), compl. to 25 ml. 4–5 ml filtr.+0.5 g Dowex 50W-X8. De: 50 μl. Wine/juice filt.+dil.; De: 50 μl+10 mg NBDI in 500 μl dixane (80°C, 60 min); I: 5 μl	250×4 mm (Nucleosil-5), or 100×2.1 mm (Hypersil ODS)	Grad., A: water–HCOOH (19:1), B: MeOH; 0.4–1 ml/min; 25°C	DAD (265)	<6.7	[100]
Beverages, jam; 13 additives (aspartame, propyl gal, methyl paraben, sorbic acid, ethyl paraben, *tert.*-butylhydroquinone, benzoic acid, saccharin, acesulfame-K, octyl gal, butylated OH-anisole, butylated OH-toluene, dodecyl gal)/20	P: beverages filtered, 1 g jam+10 ml water compl. to 15 ml then filtr. (0.45 μm); hydrodynamic, 3 s	60 cm (52 cm effective length) ×75 μm fused-silica capillary	Running buffer, 25–75 m*M* SDS–SC–SDC in 20 m*M* sodium tetraborate (pH 9.5)	UV (214)	–	[101]

[a] Abbreviations, as in Tables 1–8, and/or: 1-EDC·HCl=1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; SDS=sodium dodecyl sulfate; SC=sodium cholate; SDC=sodium deoxycholate; gal=gallate.

Table 10
GC of carboxylic-, mainly FAs without derivatization[a]

Matrix; No./min*	Extraction/preparation; injection	Column	Gradient	Inj (°C)	FID (°C)	RSD (%) detection limit	Ref.
Wine; 3 (caproic, caprylic, capric)/60	E: 1 ml wine neutr., evap.+5 ml 0.5 *M* H_2SO_4. Extr: EtAc–pentane (1:1, v/v) shake 10 min; I: 5 μl (solv layer)	1 m×3 mm, Chromosorb WAW, 60–80 mesh, 5% DEGS+1% H_3PO_4	Isocr., 110°C: caproic acid, 130°C: caprylic/capric acids	290	290	≤5.0 –	[103]
Royal jelly; 1 (10-hydroxydecenoic acid)/14	E: 2–5 ml sample+5 ml 3-OH-myristic acid (I.S., 0.1 % in 0.01 *M* KOH), to pH 8–9, washed: 3×10 ml light petroleum. Adj to pH 2–6 (1 *M* HCl), shake: 3×20 ml DieEt. Dehydr Na_2SO_4, evap. to 2 ml; I: 2 μl	1 m×2.6 mm, Tenax GC, 60–80 mesh, 5% OV-17	Isocr., 240°C	240	240	≤3.4 10–500 ng	[104]
Wine, must, milk, cheese; 21 (C_2–C_{20}, incl. iso-C_{4-6} and crotonic acid)/72	250 ml extr continuous: 150 ml Freon 11 (24 h). 30 ml ext. concentr. to 0.3 ml (32°C). 250 ml cowmilk (precip. 10% CCl_3COOH), filtr.+extr.: 150 ml Freon 11; 10 g blue cheese refl.: 2 ml *n*-pentane, concentr: 1 ml; I: 5 μl	7.5 m×0.6 mm Pyrex tube, Volaspher A-2, 120–140 mesh (Merck), 4% FFAP	90°C (5 min) then 5°C/min to 275°C, hold 10 min	350	350	≤4.4 –	[105]
Dairy products; 10 (C_2–C_7 FAs, incl. iso-C_{4-6} and crotonic acid)/10	10 g cheese {steam dist. [107], neutr. (0.1 *M* NaOH, phenolpht.)+5 ml sodium crotonate (250 mg/l). Salts dried (40°C) and mixed with 10 ml H_3PO_4 (20 mg/l in CH_2Cl_2), passed through anh. Na_2SO_4; I: 1 μl	15 m×0.53 mm, 0.5 μm (Nukol, fused-silica, Supelco)	100–180°C (10°C/min); on	Cold (on col)	200	– –	[106,107]
Canola seed; 6 (palmitic, stearic, oleic, linoleic, linolenic, C_{17} acid, I.S.)/8	E: 10 ml seed ground, dried (80°C, 8 h). 200–400 mg sample+ml heptane (contg. C_{17} I.S. 1 g/l)+4 ml solvent (isopropanol–H_2SO_4, 40:1, v/v), shake +2 ml heptane+ 4 ml water shake again; I: from top layer	30 m×0.53 mm, 1 μm (J&W, J1253232, DBFAP3)	220°C, then 1°C/min to 225°C, +3.5 min, then to 230°C, 5°C/min+1.5 min	270	330	≤1.1 –	[108]
Dairy products; 16 [$C_{5,\ 7,\ 13,\ 17}$ (I.S.)+ $C_{2:0-18:0}$+$C_{18:1}$+lactic+benzoic acid]/30	10 ml milk/yogurt+1 ml EtOH+1 ml 2.5 *M* H_2SO_4+15 ml (DieEt–C_7H_7, 1:1, v/v), centr. (2 min). Top layer+1 g anh. Na_2SO_4. Extr. 2× again. 1 g cheese+3 g anh. Na_2SO_4+0.3 ml 2.5 *M* H_2SO_4. Extr. 3×3 ml. Comb extracts: 1 g SPE. Elut: neutr lipids: 10 ml $CHCl_3$– 2-propanol (2:1); FVFAs: 10 ml DieEt contg. 20 ml/l HCOOH; I: 0.5 μl	15 m×0.53 mm, 1 μm (J&W, FFAP)	65–240°C (10°C/min)	–	–	≤3.0 –	[109,110]

[a] Abbreviations as in Tables 1–9, as well as/or: FVFAs=free volatile FAs; I.S.=internal standard; DEGS=diethylenglycolsuccinate.

Table 11
GC of carboxylic acids as trimethylsilyl (TMS) [111–117], methyl [118,119], butyl [120] and isopropyl [121] esters[a]

Matrix; No./min*	Extraction/preparation; derivatization; injection	Column	Gradient	Inj (°C)	Det (°C)	RSD (%) detection limit	Ref.
Breast milk; 16 [lactic, caprylic, urea, succinic, fumaric, 3,4-hydroxybutyric, capric, malic, 2,3-dideoxypentonic, 2-hydroxymethyl-3-deoxy-2,4-dihydroxybutyric, 3-deoxypentonic, lauric, acid, 2-hydroxymethyl-3-deoxy-2,5-dihydroxypentono-lacton, citric, myristic, $C_{16:0-1}$, $C_{17:0}$, (I.S.) $C_{18:0-2}$ acid]/30	P: 2 ml milk (centr. 30 000 rpm, 30 min)+1 ml water acidif.: pH 1 (2 *M* HCl +1 g NaCl+100 μg *n*-heptadec ac (I.S.). E: 3× with DieEt+EtAc, evap.; De: silylate: BSTFA (details not given); I: –	2 m×3 mm, glass, Gas Chrom Q, 3% OV-17	80°C for 3 min increasing to 280°C (6°C/min)	–	GC–MS	– –	[111]
Food oils; 7 ($C_{14:0}$, $C_{16:0}$, $C_{18:0-3}$, $C_{20:0}$, $C_{22:0}$) acid/26	P+De+Inj: 200 μl oil+10 μl tetradecane+10 μl 2-heptenoic acid (I.S.)+2 μl BSTFA+1 μl oil sample (order of listing); I: –	30 m×0.322 mm, DB-170l (J&W Scientific)	50–300°C (5°C/min)+5 min	220 pyro int f	GC–MS	≤13.3 40 μg/ml	[112]
Grape must, yeast; 31 (caprylic, decanoic, lauric, $C_{7:0}$–$C_{21:0}$, $C_{23:0}$, linoleic, linolenic acid ethyl esters+10 sterols)/77	E: sep funnel 50 ml must 3×25 ml $CHCl_3$. (centr. after each extr.). Evap.,to 1 ml+anh. Na_2SO_4. Washed: 1 ml $CHCl_3$. De: 200 μl+100 μl Pyr +100 μl BSTFA+10 μl TMCS (80°C, 1 h); I: 1 μl	30 m×0.25 mm, 0.25 μm film, DB-1 (J&W, Scientific)	40–200°C (6°C/min+15 min), increasing to 290°C (2°C/min)	280	FID (320)	≤15 –	[113]
Fruits, vegetables, mushroom, honey, etc., 37 (fatty-, aliph hydoxy, mono-, di- and polycarboxylic acid; substituted benzoic-, substituted aralkyl, satur+unsatur, fenolic acid)/54	P: 25, 50, 100 μl (20 mg/ml, in total), equiv. natural matrix rotary evap., 50–60°C); De: res+500 μl Pyr (contg 2.5 g $NH_2OH·HCl$/100 ml) (70°C, 30 min), then 900 μl HMDS+100 μl TFAA (100°C, 60 min). Diluted (25–200×); I: 1 μl	30 m×0.248 mm, 0.25 μm film (DB-5 J&W, Scientific)	60–320°C: applying different programs	60–320 progr	GC–MS	≤6.7 20 ng	[114–116]
Vegetable oils; 3 (pentacyclic hydroxy triterpene acids: oleanolic, maslinic and ursolic acid)/34	E: SPE (3 ml prewashed cartr., aminopropyl, Supelco). 1 ml sample (contg 0.2 g oil/6 ml C_6H_6). Elut acids: 2× +4 ml DieEt–CH_3COOH (98:2). Evap.+200 μl reag: (HMDS–TMCS–Pyr, 3:1:9); I: 1 μl	2 m×0.25 mm, 0.25 μm (SGL5, Suge-lab, Spain)	260°C for 4 min increasing to 320°C (2°C/min)	320	FID (320)	≤4.7 –	[117]
Vegetable oils; 10 ($C_{16:0}$, $C_{18:0-3}$, $C_{20:0-1}$, $C_{22:0}$, $C_{22:1}$, $C_{24:0}$ acid)/25	P+De+Inj: 0.25 g, from 10 g finely ground seed+ 3 ml C_6H_6+1 ml 0.4 *M* $NaOCH_3$ (in MeOH) vortex-mixed, stand for 10 min+10 ml water; I: 1 μl from C_6H_6 layer	2 m×2 mm glass, Chrom WAW, 100–120 mesh, 3% GP-2310/2% SP 2300	185°C (8 min), increasing to 220°C (4°C/min), hold 8 min	–	–	≤5.0 –	[118]
Cheese fat; 10 ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{9:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0-1}$ acid)/48	E: 10 g cheese+5 ml water acidif pH 2 (H_2SO_4+15 ml ice-cold DieEt shake 3 min, centr. (3000 rpm, 5 min, 0°C). Upper layer+ 1 g anh. Na_2SO_4 (5 min). 3 ml DieEt layer+0.2 ml 20% $(CH_3)_3HOH$ in MeOH shake for 1–2 min; I: –	2 m×3.2 mm, Chrom G, AW-DMCS, 80–100 mesh, 4% DEGA	70°C for 3 min increasing to 190°C (4°C/min) hold 40 min	275	FID	≤2.2 –	[119]
Organic acids in crude sugars; 14 (propionic, butyric, *i*-valeric, lactic, glycolic, levulinic, oxalic, malonic, fumaric, succinic, malic, aconitic, citric acid)/40	De: 2 mg acids/equiv. sugar neutr (1 *M* NaOH), evap. (40°C)+ 5 ml *n*-butanol+0.7 g Amberlite CG 120 (H form, 200–300 mesh)+2 g anh. Na_2SO_4 (refl. 2 h). After esterif 3–5 ml C_6H_6, (paper filtr), subjected to GC; I: –	–	–	–	–	– 0.4–6 μg	[120]
Vegetable oil, cheese; 4 ($C_{18:3\ c,c,t}$, $C_{18:3c,c,c}$, $C_{18:3t,c,t}$, $C_{18:3t,c,c}$ acid)/32	E: 10 ml (80°C, 8 h). 200–400 mg sample+1 ml C_7H_7+4 ml solvent (isopropanol–H_2SO_4, 40:1, v/v), shake; I: from top layer	30 m×0.53 mm, 1 μm, DBFAP (J&W Scientific)	220–230°C (1°C/min)+5 min	270	FID (330)	≤1.1 –	[121]

[a] Abbreviations as in Tables 1–10, as well as/or: DEGA=diethyleneglycol adipate; pyro int f=pyrolysis interface.

Table 12
HPLC of amino acids as their phenylthiocarbamyl (PTC) [132–143], butylthiocarbamyl (BTC) [138] and benzylthiocarbamyl (BzTC) [139] derivatives[a]

Matrix; No. of compounds (name)/min	Hydrolysis; preparation; derivatization; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
Wine; 22 (Asp, Glu, OH-Pro, Ser, Asn, Gly, Gln, Cit, Thr, Ala, Arg, Pro, AABA, Tyr, Val, Met, Ile, Leu, Phe, Trp, Orn, Lys)/60	P: (1) Sep-Pak, Waters; activated C_{18} Sep-Pak cartridge+0.5 ml wine washed by water–ACN (60:40) (recovery>90%). (2) Micropartition method (Centrifree MPS-1, Amicon); sample reservoir+300 μl wine+YTM membr. (cut-off M_r 10 000, 4000 rpm/60 min), De: [130]; I: 5 μl	250×4.6 mm, 5 μm (Spherisorb ODS), guard: 30×3.9 mm (Bondapak C_{18})	Grad., A: 0.05 *M* NaAc in 0.25% ACN–water, B: 0.1 *M* NaAc– ACN (50:50), pH 6.8; C: ACN–water (70:30); 1 ml/min; 52°C	UV (254)	<7.8 –	[132]
Wine; 24 [Asp, Glu, OH-Pro, Ser, Asn, Gly, Gln, His, GABA, Thr, Ala, Arg, Pro, a-ABA, Tyr, Histam, Val, Met, $(Cys)_2$, Ile, Leu, Phe, Trp, Orn, Lys]/40	P: (1) 24 amino acids incl. histamine, micropartition method [129]. (2) Histamine: SPE of derivatized sample: Bond Elut, SAX 100 mg bed, sample dissolved in 200 μl A El+100 μl A El, mixed; I: 5 μl	As in Ref. [129]	Grad., (1) 24 amino acids [129], (2) hist: A, 0.05 *M* NaAc (0.25/0.05% ACN–TEA), 1 ppm EDTA (pH 6.8), B, ACN; 1 ml/min; 52°C	UV (254)	<5.0 0.1 ppm	[134,135]
Fresh muscle, cured ham; 21 (Asp, Glu, Asn, Ser, Gln, Gly, His, Tau, Arg, Thr, Ala, Pro, Tyr, Val, Met, Ile, Leu, Phe, Trp, Orn, Lys, AABA)/51	P: 8 g muscle/4 g dry cured ham homog 0.1 *M* HCl (5°C, 8 min), centr. (10 000 *g*, 20 min), filtr. (glass wool). Deprot: 2 ml supernat+4 ml ACN centr. (10 000 *g*, 15 min), evap (38°C); De: [130]; I: 20 μl	250×4.6 mm, 5 μm (Supelcosil Lc-18 DB), guard: 20×2.1 mm	Grad., A: 0.14 *M* NaAc contg. 0.5 ml/l TEA (pH 6.4); B: ACN–water (60:40); 0.8 ml/min; 40°C	UV/ AD (254)	– –	[136]
Must, wine; 23 (CYA, Asp, OH-Pro, Glu, Ser, Asn, Gly, Gln, Thr, Ala, His, Pro, Arg, GABA, ETA, Tyr, Val, Met, Ile, Leu, Phe, Trp, Orn, Lys)/65	De: 40 μl must/100 μl wine+20 μl I.S. (Nle)+ 20 μl solution contg. TEA–water–EtOH (1:2:2) evap. to dry (35×4 mm tube). De: [130], kept in freezer, before injection dissolv. ACN–water (95:5), filtered (0.45-μm cellulose acetate); I: –	250×4.6 mm, 5 μm (Spherisorb ODS), guard: same filling	Grad., A: 0.1 *M* NaAc (TEA–Na sulf, 0.68/0.2 ml/l, pH 5.3), B: water, C: ACN; 1 ml/min; 50/35°C	UV DAD (254)	<7.0 0.025 n*M*	[137]
Soybean, egg; 22 [BTC/BzTC Asp, Glu, OH-Pro, Asn+Ser, Gly, Gln, His, Thr, Ala, Arg, Pro, Tyr, Val, Met, Ile, Leu, $(Cys)_2$, I.S., Phe, Trp, Lys, Cys]/50	H: 0.2 g soy meal+0.5 ml 6 *M* HCl contg 0.1% phenol (4 h, 145°C), filt., evap. (50°C). Resid. dissolv. in 0.01 *M* HCl filled to 50 ml. 5 ml aliq: (Dowex 5-X8, 100×13 mm I.D.), elut: 4 *M* NH_3, evap. (50°C), dissolv.: in 50 μl: ACN–MeOH–TEA (10:50:2)+3 μl PITC (20 min), 3 μl BITC (30 min, 40°C), 3 μl BzTC (30 min, 50°C). Reag moved: pierced needle; I: 10 μl	300×3.9 mm, 4 μm (Novapak C_{18}, Waters), ml/min	Grad., A: 0.05 *M* AmAc (pH 6.7), B: 0.02 *M* NaH_2PO_4 (5% MeOH in THF–ACN, 50:50); C: ACN–water (70:30); 1 ml/min	UV (246/240) BzTC/BTC	<7.2 BzTC 3.9 p*M*	[138,139]
Green bean; 17 (Asp, Glu, OH-Pro, Ser, Gly, His, Arg, Thr, Ala, Pro, Tyr, Val, Met, Ile, Leu, Phe, Trp, Lys)/37	H: 0.1 g homog+lyphyl green bean+15 ml 6 *M* HCl (screw-cap Pyrex tube, 125×16 mm, N_2, capped, 110°C, 24 h). Filtr adj 25 ml De: 5–50 μl sol. dried (65°C)+ 30 μl MeOH–water–TEA (2:2:1), r: vac. Next MeOH–water–TEA–PITC (7:1:1:1) (20 min) excess rem: N_2 (sealed, stored 4°C). Dissolv. in 150 μl 5 m*M* Na phosph. contg. 5 % ACN; I: 20 μl	250×4.6 mm, 5 μm (Spherisorb ODS)	Grad., A: 0.14 *M* NaAc, contg 0.5 ml/l TEA (pH 6.2); B: ACN–water (6:4, v/v); 30°C	UV (254)	<4.0 >260 p*M*	[140]
Wine, must; 17 [Asp, Glu, Ser, Gly, Gln, His, GABA (I.S.), Thr, Ala, Arg, Pro, Tyr, Val, Met, Ile, Leu, Phe, Lys]/28	P: wine/must centr. (30 min/6000 rpm) 1 ml supernatant ultafiltr.+centr. (40 min/6000 rpm); De: 200 μl filtr+50 μl 2.5 m*M* AABA (I.S.) dried 10 ml tube, further on: [129]; I: –	Co: 250(+30 guard)× 4.6 mm (Vydac C_{18}, 5 μm)	Grad., A: 50 m*M* AmAc (pH 6.5); B: 50 m*M* AmAc (pH 6.5), in ACN–water (1:1); 2 ml/min; 50°C	UV (254)	<3.6 –	[141,142]
Apple; 27 [Asp, Glu, OH-Pro, Ser, Gly, Asn β-Ala, Gln, Hser, GABA, His, Thr, Ala, ACPCA, Arg, Pro, H-Arg, Tyr, Val, Met, Cys+$(Cys)_2$, Ile, Leu, Phe, Trp, Orn, Lys]/40	P: 5 g apple pulp filtr. Whatman GF/F glass microfibre filter (particle retention from liquid 0.7 μm) Cat. exch. clean-up [141]; De: [142]; I: 1 μl: 10–20 μl	150(+20)×4 mm (Hypersil ODS, Shandon, 5 μm)	Grad., A: 0.05 *M* NaAc (pH 7.2); B: 0.1 *M* NaAc–ACN–MeOH (46:44:102, v/v); 2.1 ml/min; 50°C	UV/ DAD (254)	<4.0 15 p*M*	[143]

[a] Abbreviations as in Tables 1–11, and/or: AABA=α-aminobutyric acid: GABA=γ-aminobutyric acid; TEA=triethylamine; ETA=ethanol amine; H=hydrolysis.

Table 13
HPLC of amino acids as their *o*-phthaldialdehyde–3-mercaptoethanol (OPA–ME) [148–150], OPA–*N*-isobutyryl-L(D)-cysteines (OPA–iBC) [151–154], OPA–3-mercaptopropionic acid–9-fluorenylmethyl chloroformate (OPA–MPA–FMOC) [155] and OPA–MPA or OPA–*N*-acetyl-L-cysteine (NAC) [156] derivatives[a]

Matrix; No./min*	Preparation/extraction; derivatization; injection	Column	Eluent	Detection (nm)	RSD (%) detection limit	Ref.
Fruit juices/drinks; 16 (Asp, Glu, Asn, Ser, Gln, His, Gly, Arg, Ala, Trp, Met, Val, Phe, Ile, Leu, Lys)/16	P: 0.1 ml juice+0.1 ml MeOH+0.1 ml ACN, shaken, centr. (500 rpm, 3 min). 10 μl supernat dil. to 1 ml; De: 5 μl supern+5 μl water+15 μl OPA reagent (50 mg OPA+40 μl ME dissolv. in 0.2 *M* $Na_2B_4O_7$ contg. water–MeOH (9:1) (60 s); I: 2.5 μl	75×4.6 mm, 3 μm (Ultrasphere ODS)	Grad., A: 0.01 *M* NaAc+0.01 *M* CH_3COOH (water–MeOH–ACN (8:1:1); B: water–MeOH–ACN (1:2:2); 1 ml/min	FL (356/450)	<9.3 –	[148]
Apple; 18 (Asp, Glu, Asn, Ser, Gln, His, Hse, Gly, Thr, Arg, β-Ala, Ala, GABA, Tyr, Val, Phe, Ile, Leu)/45	P: [73]; De: 100 μl extract filtr. (0.45 μm, Millex HV membr., Millipore)+100 μl OPA reag. (100 mg OPA+9 ml MeOH+100 μl ME+1 ml 0.4 *M* borate buf., pH 10, adj: NaOH, mixed 24 h before use) compl to 1 ml with water (1 min); I: 2 μl	150×4.6 mm, 5 μm (Spherisorb Tracer, Spain ODS-2)	Grad., A: 0.01 *M* NaH_2PO_4, contg. 1% (v/v) THF+$NaNO_3$ for ionic strength to 0.08 *M*); B: MeOH; 1.3 ml/min; 30°C	FL (340/425)	<9.0 –	[149,150]
Different foods[§]; 36 (17 pairs, L+D-Asp, Glu, Asn, Ser, Gln, His, Arg, Thr, Ala, Tyr, Val, Met, Ile, Leu, Phe, Trp, Lys, Gly, L-homo-Arg I.S.)/90	P: 200 g grape+70% EtOH. Squeezed. Juice filtr.+centr. 1 ml supernat+3 μl I.S. (1.6 m*M* L-homoarg in 0.1 *M* HCl) adj. to pH 2. (Dowex 50W-X8, 5 cm×1 cm). Eluted: 30 ml 4 *M* NH_4OH. Evap., dissolved in 1 ml 0.1 *M* HCl; De: OPA/iBC reag; I: 2 μl	250×4.6 mm, 5 μm, guard 20×2.1 mm (Hypersil ODS)	Grad., A: 0.023 *M* NaAc (3.13 g NaAc·H_2O/1 l (pH 5.95 adj. 10% AcAc); B: 474 g MeOH+39 g ACN; 1 ml/min; 25°C	FL (230/445)	<1.2 5–10 p*M*	[151–154]
Potato tuber; 21 (Asp, Glu, Asn, Ser, Gln, His, Gly, Thr, Ala, Arg, Tyr, Val, Met, Norval, Trp, Phe, Ile, Leu, Lys, OH-Pro, Pro)/13.5	E: 100 g peeled, cut, boiled (100 ml water, 2 h), filtr. (0.45 μm Millex FH membr., Millipore). 100 μl extract +900 μl water contg. 100 ng Nval I.S.); De: OPA/MPA and OPA/MPA/FMOC (autosampler); I: 1 μl	100×4 mm, 3 μm (Hypersil cartr type, ODS)	Grad., A: 0.018 *M* NaAc (0.02/0.3%, v/v, TEA/THF, pH 7.2; B: ACN–MeOH–0.1 *M* NaAc (2:2:1, v/v); 1.4 ml/min; 40°C	FL, (264/313a) (340/450b)	>0.984 20 p*M*	[155]
Apple; 25 (29[£]) (Asp, Glu, Asn, Ser, Gln, His, Gly1, Hser, Thr, β-Ala1, Arg, Ala, GABA1, Harg, Tyr, Gly2, Val, Met, Cys, β-Ala2, GABA2, Trp, Phe, Ile, Leu, Orn2, Orn3, Lys2, Lys3)/40	P: 2–5 g apple pulp (pealed, homog. sieved weighed into a funnel (5 cm I.D.), covered with GF/F glass microfib paper. Filtered residue washed (4×1 ml water), justif. to pH 9.3–10.3, filled to 10.0 ml, stored −20°C/used immed). De: 100 μl stock sol+100 μl reag: [OPA]/[MPA]/{[NAC]}/$[AA]^T$=20/60/1; I: 10–20 μl	Co: 200(+20 guard) ×4.0 mm (Hypersil C_{18}, 5 μm)	Grad., A: 0.05 *M* NaAc (1%, v/v, THF, pH 7.2); B: 0.1 *M* NaAc–ACN–MeOH (46:44:10, v/v) (pH 7.2); 1.3 ml/min(0–11 min, 2.3 ml/min 11–40 min; 50°C	FL (337/454) UV/DAD (334)	<7.1 (model) <9.0 (apple)	[156]

[a] Abbreviations as in Tables 1–12, as well as/or: [§]=fruit juice, wine, vinegar, beer, fermented cabbage juice, coumiss, dietetic whey drink, honey, etc.; [£]=including the more than one derivative furnishing amino acids; a, b=excitation/emission wavelengths of the OPA/MPA (a) and OPA/MPA/FMOC (b) derivatives.

and determination of non derivatized sugars in food samples [5–7,10–23] are detailed in Table 1.

The most simple procedure carried out on a silica-based aminopropyl stationary phase with refractive index (RI) detection proved to be satisfactory for monitoring the relevant sugars in malted barley, worth, fermenting and finished beers: baseline separation has been presented within 12 min for seven compounds [10]. The modified column packing material (3-morpholinopropylsilyl-silica gel) was found to be chemically stable under continuous operation for over 500 h [11]. Determinations of sucrose, fructose, glucose and sorbitol in fresh fruits (with water eluent, at 90°C), is an inexpensive but time-consuming process [12,13].

Evaporative light-scattering detection (ELSD) being an universal detection mode suffers from low selectivity: although, compared to RI detection, ELSD provides better sensitivity, more stability of the chromatographic baseline and no incidence of the temperature [14–16]. IR detection and ELSD systems, connected in series, allowed the superimposition, i.e., the comparison of the two detector features on a single chromatogram, obtained from the same injection [14].

Electrochemical detection [5–7,17–22], in particular the common PAD [5,17–22] proved to be superior to RI detection and ELSD, in respect of selectivity, sensitivity and in the possibility of gradient elution. However, because of the continual pulsing of the platinum and gold electrodes to extreme oxidizing and reducing potentials (needed for the stable response of sugars), a successive passivation of the electrode can be expected (due to the adsorption of the sugar oxidation products). To avoid this drawback, alternative solutions have been investigated [17–19]: electrochemical detection by using the constant potential providing Cu electrode was found to be well suited, also in the undergraduate laboratory, for the determination of the sugar content of common food products [17]. Simultaneous amperometric determination of some mono-, di- and oligosaccharides has been developed by a dual electrode system prepared by cross-linking cellobiose dehydrogenase or oligosaccharide dehydrogenase with an osmium-containing polymer to form a redox hydrogel on the surface of solid graphite electrodes [18]. (This electrode system was also used for detection of sugars after their separation by size-exclusion chromatography). The laboratory-constructed amperometric biosensors made possible the simultaneous, selective identification and quantitation of sugars (pyranose oxidase+horsradish peroxidase) and alcohols (alcohol oxidase) [19]. High-performance anion-exchange chromatography (HPAEC) with PAD [20–22] was successfully applied to the determination of sorbitol and the major sugars in fruit juices and concentrates [20], to the quantitation of alditols applied as artificial sweeteners [21] and to the separation and quantitation of sugars and disaccharide alditols in dietetic foods, simultaneously [22].

The necessity of mass spectrometric identification would be obligatory [6,7]: in particular in the analysis of underivatized compounds in the presence of the natural matrix. Because of the high cost of the on-line HPLC–MS systems, today, only a few of us can enjoy the reliability, safety and the feeling of a 100% security of our results obtained by a single HPLC detection. Thus, in the analysis of natural matrices, possibly at least two different types of detection (RI+ELSD) [14], or dual enzyme electrodes [18,19] can increase the reliability of our data: in the most fortunate case completed also by TSP-MS [6], by RI+PAD+TSP-MS, or by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and PAD+MALDI-MS [7].

The structure-elucidation of unknown oligosaccharides obtained enzymatically from pear and apple fruits was reported by means of ESI-MS [23].

An interlaboratory study [24] with 20 participant laboratories gives a compilation on sources of influencing reproducibility criteria: concerning the determination of mono- and disaccharides in 12 food products, using HPLC with RI detection.

2.1.2. With derivatization

Several approaches [25–33] can be found in the literature aiming on one hand to enhance selectivity and sensitivity and on the other hand to make RP-HPLC possible also for sugars. However only a few of them have been applied in the everyday practice of food analysis (Table 2 [27–33]).

On-line HPLC–ion-spray (ISP) MS was used in the identification of oligosaccharides labeled with 2-aminopyridine [25]. The pyridylamino (PA)-oligo-

Table 14
GC analysis of amino acids as their *N*-(*O*,*S*) trifluoroacetyl (TFA) [165], pentafluropropionyl (PFP) [166–168], propyl- [165–168], *N*-(*O*,*S*) heptafluoroburyl (HFB) isopropyl [169], *N*-(*O*,*S*) HFB isobutyl esters [170,171] and *N*-(*O*,*S*) isobutyloxycarbonyl (isoBoc) *tert*.-butyldimethylsilyl (TBDMS) TBDMS esters [172][a]

Matrix; No./min*	Extraction/preparation; hydrolysis; derivatization; injection	Column	Gradient	Inj (°C)	Det (°C)	RSD (%)	Ref.
Meat; 1 (OH-proline)/14	P: Muscle's acetone powder: [162]; H: 0.5 mg amino acids (oven, 6 *M* HCl, 110°C, 16 h), filtr. (W 541 paper); De: Esterific: 0.5 mg amino acids (5–16.5 μg+10 μg I.S., OH-Pro), dry (N_2, sand bath, 100×14 mm tube, 70°C). Res+CH_2Cl_2, evap. +0.3 ml *n*-propanol– 3.5 *M* HCl, refl. (oil bath, 110°C, 25 min). Evap. dry (sand bath 40°C); acylation: 0.4 ml CH_2Cl_2+0.2 ml TFA (sealed, oil bath, 150°C, refl. 5–6 min). Evap., dry (30°C N_2). Res. dissolv.: 0.5 ml EtAc; I: –	25/12 m×0.2 mm (BP-1, BP-10, SGE, HP-1, Hewlett-Packard)	100°C for 2 min increasing to 250°C (15°C/min)	250	GC–MS	–	[165]
Fermented foods, juices; 18 (D/L amino acid separately: Ala, Thr, Val, Leu, iLeu, Pro, Asp, Glu, Orn, Gly, GABA)/40	P+E+ion-exchange: [148–151]; De: Esterific: 3 mg isol. sample+ 200 μl 1-/2-propanol–acetyl chloride (8:2)+1.5 mg antioxidant BHT (2,6-di-*tert*.-butyl-*p*-cresol, reacti vial Wheaton, 100°C, 1 h), Solv. moved: N_2; acylation: 200 μl CH_2Cl_2+50 μl PFP (100°C, 20 min). Solv moved: N_2. Res. dissolv.: 50–100 μl CH_2Cl_2; I: 0.6 μl	25 m×0.25, Chirasil-L-Val (Macherey–Nagel)	80°C for 4 min, increasing to 190°C (4.5°C/min+10 min)	250	FID (250)	–	[166,168]
Potato; 21 (Ala, Gly, ABA, Val, Thr, Ser, iLeu, Asn, GABA, Pro, Asp, Gln Met, Glu, Phe, Orn, Lys, Tyr, Arg, His, Trp)/15	E: 5 g peeled potato+250 ml 70% EtOH (8 h, shake), centr. (10 min), filtr. (0.45 μm), evap. Res. dissolv. in 10–20 ml 0.1 *M* HCl (kept frozen); cat. exch.: 100 mg Rexyn 101H (Fischer Scientific). amino acids eluted: 1 ml 2 *M* NH_4OH (1 drop/min), collect. Pierce vial evap. De: Esterif.: 200 μl acetyl chloride– isopropanol (1:5) (80°C, 120 min) evap. (room temp); acylation: 100 μl EtAc+200 μl HFBA (110°C, 10 min); I: –	3.5 m×2 mm, Gas-Chrom Q, 80–100 mesh, 3% SE30 (Appl. Sci. Lab.)	70°C for 1 min, increasing to 225°C (20°C/min+quant. elution)	225	FID (280) GC–MS	–	[169]
Cheese; 18 (Ala, ABA, Gly, Val, Thr, iLeu, Leu, Ser, Pro, GABA, OH-Pro, Met, Asp+ Asn, Phe, Glu+Gln, Tyr, Orn, Lys)/25	P+E+ion exch.: 1 g cheese+50 ml (95% EtOH–1 *M* HCl (70:25), warmed to 75°C+2 h, room temp.), centr. (5 min). 2.5 ml upern evap. (40°C); Res dissolv.: 0.95 ml water+7 drops 2% LiOH (pH 2); into 1 g resin (1 drop/5 s). Washed: 5 ml water (1 drop/s). amino acids eluted: 20 ml 7% NH_4OH (1 drop/s). Dried, dissolv.: 10 drops 1 *M* HCl, dried: N_2, 50°C; De: Esterif: 200 μl isobutanol–3 *M* HCl (110°C, 30 min), dried; acylation: 120 μl CH_2Cl_2+35 μl HFBA (110°C, 10 min), dried. dissolv.: 30 μl CH_2Cl_2; I: 0.5 μl	25 m×0.32 mm, OV 1701 (film 0.2 μm)	80–280°C (8°C/min)	280	FID (280)	≤3.40	[170]
Honey; 17 (Ala, Gly, Val, Thr, Ser, iLeu, Leu, Pro, Met, Asp, Phe, Glu, Lys, Tyr, Arg, Try, Cys)/50	P: 25 g honey+500 μg I.S. (norleucine) adjust. to 50 ml (pH~2.3), 0.1 *M* HCl. Ion exch.: 1 ml into column (10 mm, Dowex WX, 100–120 mesh, 1 g). Sugars wash: 6 ml water (pH 2.3). amino acids elut: 30 ml 2 *M* NH_4OH, 1 drop/s). Evap. (N_2, 35°C). Res transf.: NH_4OH (screw cap vial) evap. De: Esterific: 600 μl isobutanol–3 *M* HCl (110°C, 1 h) dried; acylation: 150 μl CH_2Cl_2+120 μl HFBA (125°C, 20 min), dried, dissolv.: 30 μl CH_2Cl_2; I: –	30 m×0.32 mm, SPB/5, Supelco (film 0.2 μm)	80°C for 2 min raised to 280°C (5°C/min) hold 10 min	290	FID (290)	≤4.5	[171]
Almond, sunflower walnut, seeds; 53 (protein and non protein amino acids)/65	E: 500 mg ground seed vortex: 4×1 ml water centr. (5 min). 1 ml supern.+200 μl picric acid, shaken, centr. (2 min). Supern. 10% H_2SO_4 (few drops) washed: 3×2 ml EtAc+2×2 ml DieEt. Aqueous layer basif.: pH 11; De: acylation: 100 μl basif sol.+0.5 ml isoBoc, vortex-mixed. Excess isoBoc mov: (3×2 ml DieEt); SPE: isoBoc amino acids elut with DieEt. 100 μl dried: N_2 (50°C)+20 μl THF+20 μl MTBSTFA (60°C, 20 min); I: 1 μl	30 m×0.25 mm, DB-5/DB-17, J&W (film 0.241 μm)	150°C, for 2 min, then 3°C/min to 280°C	260	GC–MS (300) interface	≤5.0	[172]

[a] Abbreviations as in Tables 1–13.

saccharides have been separated on a RP column. Complex mixtures of oligosaccharides with molecular masses between 1400 and 2000 could be identified also in the cases of incomplete separations. The fragmentation patterns of 30, different sugar derivatives were reported [26], obtained by HPLC–TSP-MS. On the basis of an exhaustive study of chromatographic and detection conditions it has been proved that the correct setting of the capillary tip, temperature and the repeller voltage is fundamental for the ionization of carbohydrates. Optimum results have been obtained by using an acetonitrile (or methanol)–0.1 *M* ammonium acetate (1:1, v/v) eluent, resulting in the TSP spectra of most carbohydrates in the very informative ions of high masses $[M+NH_4]^+$.

Detailed conditions of practical tasks are presented in Table 2. Twenty-three sugar components of tree sap glycoproteins have been measured (after reduction with sodium borohydride, followed by acylation with benzoic anhydride), as their alditol benzoate derivatives [27]. Phenylisocianate (PIC) derivatives of food [28] and milk [29] sugars furnished sensitive (detection limit: 0.2–1 ng [28]) and reproducible (relative standard deviation, RSD<1.8% [29]) determinations. The pre-column derivatization with dansyl chloride [30,31] similarly to the post-column reactions with thymol-sulfuric acid [32] and benzamidine in alkaline media [33] resulted in highly sensitive fluorescent derivatives.

2.2. Capillary electrophoresis

Although CE (Table 3) has been used successfully in various fields of application, such as biochemistry, biotechnology and pharmaceutical and clinical chemistry, its impact on food science and food quality control, up to now, has had limited acceptance [34–38]. The reason for that can be attributed to detection difficulties. In four proposals [35–38], out of five, indirect UV detection was used, while in the fifth one, derivatization of maltosaccharides has been achieved via reductive amination with 8-aminonaphthalene-1,3,6-trisulfonic acid, in the presence of $NaCNBH_3$ [34].

2.3. Gas chromatography

Sugars, being extremely polar compounds can be volatilized as derivatives prepared in non aqueous media. Consequently, the two main questions are: out of several derivatization reactions, which of them should be preferred? The common, simple answer, i.e., it depends on the task to be solved, in the case of sugars, it is in particular true. Namely: acylated derivatives are the optimum ones if slight differences, such as the direction of a hydroxyl group(s) is the basis in distinction of species, such as in cases of pentitols (arabitol, xilytol, ribitol, etc.), or hexitols (mannitol, galactitol, sorbitol, etc.), or aldohexoses (mannose, galactose, glucose, etc). However, in the case of acetylated saccharides with glycosidic linkages, due to their relatively high polarity, and high molecular mass, they cannot be eluted under practical conditions. In latter tasks the best choice proved to be the separation of the trimethylsilyl (TMS) or *tert*.-butyldimethylsilyl (TBDMS), in the case of reducing saccharides the TMS or TBDMS (oxime/methoxime) derivatives.

The second main question, associated with the first step of preparation, prior to derivatization, relates to isolation/extraction phenomena of sugars: how to isolate them from other constituents, present in food matrices (if any isolation procedure is needed). In the author's understanding the extraction of sugars, in the case of high saccharide-containing matrices – such as different fruits and vegetables – is resulting in considerable loss of sugars (investigated in apricots [39] applying several isolation procedures). Consequently, derivatization should be carried out in the presence of the matrix [39–54], with the exception of high protein-containing foods, such as soy samples [55], when extraction of sugars proved to be inevitable necessary: in these cases best results can be obtained by using 80% MeOH (H_2O: MeOH= 20:80, v/v) in the ratios of 5 g sample in H_2O:MeOH (20:80) [56].

2.3.1. As acetates, trifluoroacetates, etc., and/or as the corresponding oximes

First of all it needs to be underlined that in the analysis of food carbohydrates the GC separation of the acylated derivatives (i) played a limited role, and (ii) everything that could be found in the literature has been published in the 1980s. The monosaccharide contents of wine and vinegar, subsequent to their reduction by $NaBH_4$, have been determined as alditol acetate derivatives [57]. The trifluoroacetyla-

tion of the glucose, sorbitol, maltose, maltitol, maltotriose and maltotriitol content of the synthetic sweetener maltitol syrups [58] resulted in two or three peaks due to the incomplete derivatization of compounds. The neutral sugar compositions of cell walls from 17 fruits were determined as aldonitril acetates [59] based on a long derivatization process [60].

2.3.2. As trimethylsilyl and tert.-butyldimethylsilyl and/or the corresponding oxime derivatives

On the basis of pioneering work [61] several improvements have been introduced into the topic of sugar's analysis as silyl derivatives: all of them, somehow, proved to be in connection with the isolation and derivatization of carbohydrates. Sugars and polyalcohols are ready to provide fully silylated products even under soft conditions (70–80°C, 20–30 min) applying also the less basic, i.e., the less strong silylation agent, such as hexamethyldisilazane (HMDS). The major shortcoming arises in the cases of reducing saccharides, similarly also to all of their derivatization processes: due to the fact that all of their anomers elute in separate silylated products, such as the α- and β-anomers of the furanose and pyranose acetal, ring products, which are in equilibrium with the open chain-, anhydrous and/or hydrate forms. Consequently, theoretically we have to take into account six silylated derivatives, practically only four, since the amount of the open chain species are of secondary importance.

To overcome this phenomenon, reducing sugars are to be transformed into their oximes prior to their silylation process resulting in the anti- and syn-oximes of the silylated derivatives. Thus, also in the case of reducing saccharides we have to reckon with two reaction products only, while the non reducing sugars remain intact under the oximation process providing the single silylated product.

2.3.2.1. As their trimethylsilyl (oxime) derivatives prepared in the presence of the matrix without preliminary isolation

The first proposal [62], of the direct silylation of sugars, in the presence of the matrix (corn syrup) with HMDS+trifluoroacetic acid (TFAA), in a single step, was a milestone in the analysis of food carbohydrates as their TMS derivatives. In addition, in this work it has also been proved, at first, that the presence of water, up to 40 mg (detailed reactant ratios in Table 4) does not affect the quantitative silylation of the glucose, maltose, maltotriose and maltotetrose content of corn syrup.

To exhaust the advantage of the direct oximation/silylation process several advancements [39–52], have been introduced by proving that (i) in a wide concentration range of sugars (2–5 ng/injection) the ratios of their syn- and anti-oximes are stable, independent of their amounts analyzed [48,49]. (ii) Applying the above detailed principle the quantitative evaluation of several mono-, di- and trisaccharides present in fruits, vegetables, and/or mushrooms, as well as in honeys has been solved from a single injection: partly based on their single peaks, partly either on both, or on one of their completely resolved oximes, or performing calculations in cases of coeluting silyl (oxime) derivatives [48,49]. (iv) In addition to the above-mentioned points the advantage of the direct derivatization by avoiding the hydrolysis of sucrose to fructose+glucose, under the common ethanol extraction process [53] has been also accurately documented. In order of listing, subsequent to extractions, significant hydrolysis of sucrose, has been measured. Consequently, without extraction considerably higher amounts of sucrose and lower amounts of fructose+glucose were determined as follows: for sucrose (s), fructose (f) and glucose (g) contents (derivatized directly/in extract, w/w, %), in leaves of apple (s: 14.6/8.3, f: 11.1/20.0, g: 0.9/4.4), grape (s: 40.6/11.2, f: 2.8/24.7, g: 2.0/23.3), corn (s: 67.3/49.2, f: 10.0/15.2, g: 4.3/8.9) and tomato (s: 17.6/15.9, f: 6.8/11.0, g: 8.9/13.5).

2.3.2.2. As their trimethylsilyl (oxime) derivatives prepared in extracts

For an overview, see Table 4. As mentioned above the saccharide components of the high protein-containing foods should be derivatized in extracts: due to the fact that to dry the matrix by means of rotary evaporation, prior to derivatization, in the presence of strongly foaming proteins, is quite impossible [55,56].

To avoid losses of sugars their quantitative recovery under their isolation process, especially in various matrices, should be determined cautiously. In

the case of soy saccharides [56], the 30 min reflux with 50–80% methanol provided the optimum amounts of sugars in accordance with the extraction of pea flours [63]. The complicated and time-consuming procedure of sugar-extraction performed with several fruits is to be mentioned as an example, which should not be, followed [64].

3. Chromatography of acids

Foods consist of members of various homologous series of acids: consequently, those methods that provide the separation of various types of acids, simultaneously, are to be regarded as the methods of choice. For all that, numerous papers are restricted to the identification and quantitation of selected types of acids, only: exhausting their special physical and chemical properties. For this reason, in addition to the usual groupings (HPLC, GC, underivatized/derivatized acids), in this paper, the homologous series of acids form further subdivisions.

3.1. High-performance liquid chromatography

3.1.1. Without derivatization

3.1.1.1. Underivatized fatty acids

Two special fields are worthy of mention [65,66]. The analysis of the volatile fatty acid (FA) contents of landfill leachates (formic, acetic, propionic, iso- and *n*-butyric acids) [65], subsequent to their steam distillation, have been performed in a gradient HPLC–UV process (methanol–water, 3:97, v/v, adjusted to pH 4.0 with 0.05 *M* sulfuric acid). Elutions have been carried out at a concentration of ~500 μg/ml of each, in 25 min, with a reproducibility of <3.4% RSD. The composition of the triacylglycerol (TAG) contents of various oils [66] (soybean, corn, safflower, sunflower) has been determined by HPLC combined with FID. TAG molecular species were identified on the basis of the theoretical carbon numbers. Samples of crude oils were hexane extracted, filtered (Celite and activated carbon), as well as isolated by solid-phase extraction (SPE) prior to their HPLC–FID analysis. FA composition was calculated by multiplying the concentration of each TAG by the percent of each FA species in the TAG molecule. Results calculated from HPLC–FID analysis of TAG(s) were compared with their composition determined by GC. Data revealed excellent agreement between the two methods (HPLC–FID, GC–FID), proving that (i) TAG molecular species could be identified on the basis of the theoretical carbon number conception, and (ii) the FA composition of oils can be obtained from their HPLC–FID elutions, without the need for special response factors for their quantitation.

3.1.1.2. Underivatized aliphatic carboxylic acids

For an overview, see Table 5. The overwhelming part of carboxylic acids in fruit juices and wine belong to the aliphatic series, although, as minor constituents also aromatic (phenolic) acids are present. Due to the different p*K* values and various UV maxima of the aliphatic and aromatic carboxylic acids, in order to apply optimum conditions for the HPLC, or ion chromatography (IC) of aliphatic ones, the possibility to determine also the minor aromatic acids has been neglected [67–72]. Aliphatic carboxylic acids in dairy products [67] have been analyzed in aqueous extracts with 85.3% recovery. The indirect photometric detection [68] of the aliphatic carboxylic acids of wheat and peas, in the concentration range of 0.1–10 m*M* was performed with 1 m*M* phthalic acid as eluent. The combination of flow cell containing circular dichroism (CD) spectrophotometry with UV detection resulted in sensitive and accurate determination of the chirality of tartaric, malic and ascorbic acid contents of wines [69]. Three chromatographic systems, ion-exchange chromatography (IEC), ion-exclusion chromatography and RP-HPLC were compared in the simultaneous quantitation of aliphatic carboxylic acids of wine [70]: the shortages and advantages of all three systems have been evaluated from the point of view of sensitivity, linear concentration range of analysis, as well as the possibility of the quantitation of the two important inorganic anions (Cl^-, SO_4^{2-}). A rapid technique, with high recovery was proposed for the quantitation of the *trans*-10-hydroxy-2-decanoic acid content of royal jelly proving its authenticity in the concentration range of 0.75–2.54% [71]. IC, RP-HPLC and enzymatic analysis have been compared in the fermentation process of a sugar beet factory [72].

3.1.1.3. Underivatized aliphatic and aromatic carboxylic acids

For an overview, see Table 6. Analytical chemists of high expertise have not abandoned the possibility of the simultaneous quantitation of the aliphatic and aromatic carboxylic acids characteristic to apple juice and cider [73–75], or to other different fruit juices [76–81], including also honey [77], wine [78] and must [79]. Determinations have been performed both after isolation processes (by alcoholic extracts [73–76] or after a very complicated cleaning by cartridge [77]) and in the presence of the matrix [77–80]. In all the reported cases diluted and filtrated (0.45 μm membrane) samples have been injected. Exhaustive optimization studies [73–75] performed as a function of the size of column and silica particle size revealed that fast/best resolution can be expected by using a short column (Spherisorb ODS-2, 3 μm [75]).

Direct proposals [77–80], due to the reliability and reproducibility of all of these methods, proved without exception that the extraction step can be omitted, unless, it has been performed in order to concentrate the analyte.

3.1.1.4. Underivatized aromatic (phenolic) carboxylic acids (Table 7)

Investigations of phenolic acids in fruits, beverages and vegetables became of increasing interest, especially in the last decade [82–91]: due to the proven contribution of selected phenolics to enzymatic processes, acting as inhibitors, as well as to their role in the organoleptic characteristics of matrices, such as color, bitterness, flavor, browning, etc. The main task to be solved is the difficulty of isolation of these phenolics, being complex compounds, present in very low concentrations. Thus, it is understandable that their quantitation needs preconcentration [82–90], unless, the advantages of the highly selective and sensitive, on-line HPLC–atmospheric pressure ionization (API) ESI-MS can be enjoyed [91] (Table 7). A comparative extraction study was carried out with ethyl acetate and diethyl ether. Test matrices were apple pulp and wine [82]. Later on for the non volatile, phenolic acids from wine: diethyl ether [83] and from fruit juices: ethyl acetate were preferred [84,85]. Methanol was selected for the isolation of hydroxycinnamic acid content of green coffee [86], and for the isolation of sodium benzoate of jams [87]. Aromatic acids of tea [88] and coffee [89] have been extracted into water. The phenolic acids from various honey samples have been measured from cation- followed by anion-exchanged concentrates [90].

The rate of extraction of wine and apple pulp phenolics, into two different solvents [82] (diethyl ether, ethyl acetate) proved that ethyl acetate has a better extraction rate for acids and aldehydes of low molecular mass, while, reproducibility of extractions is greater in the case of diethyl ether. Analysis of wine phenolics in the diethyl ether extracts, provided excellent separation, on a microbore column, for eight constituents, within 67 min [83]. Methods proposed to determine five pharmacological active phenolic acids, constituents of cherry and grape juices seem to be time-consuming (50 min elution for gallic, chlorogenic, caffeic and ferulic acids+ extra 30 min elution for ellagic acid) [84].

The very recently developed quantitation of apple and pear phenolics provided an attractive fast separation (35 min) for 11 compounds [85]. The simple isolation by methanol of various cinnamic acids from coffee [86] and quince jam [87] furnished good and fast separation for seven [86] and 12 [87] cinnamic acids, together with the corresponding aldehydes and flavonol glycosidic compounds: including also convincing validation data of all compounds in question. The quantitation of black tea [88] and coffee [89] phenolics, in water extracts, revealed that also the simplest isolation can offer excellent results. Twenty black tea constituents have been separated and identified by using photodiode array detection (DAD) spectra of phenolic compounds believed to influence tea quality [88]. The very time consuming and tedious cation+anion-exchange ''isolation/preconcentration'' technique of honey phenolics [90] resulted in the identification of 12 compounds: unfortunately recovery studies were not presented. A basic research study was reported to define the most suitable ion source conditions to determine low-molecular-mass phenolics in wine by API-ESI-MS coupled on-line to HPLC from a 5-ml sample, without any pretreatment [91]. Detailed investigations of MS conditions revealed that the flavan-3-ol molecules can be determined with better sensitivity using the positive ion mode; however, because the

natural matrices, like wine samples, are complex and usually composed of neutral and acidic constituents, the negative ion mode is to be selected: since acidic compounds are not detected in the positive ion mode, at all.

3.1.2. With derivatization

3.1.2.1. Fatty acids

For an overview, see Table 8. FAs as various derivatives [92–98] have been prepared in different extracts, with the exception of milk products [92,93,95]. In general in can be stated that derivatization processes are time consuming, thus, if isolation is not necessary it should be omitted, since the analysis in the form of derivative can be regarded as a method that enhances selectivity. The quantitation of FAs as their 9-bromoacridine derivatives [93] seems to be more advantageous than that based on the use of the very hazardous diazoalkanes [92]: although reproducibility data are not reported in either case [92,93]. The reproducibility and the low detection limit of the analysis of the short-chain and long-chain FAs [95], obtained separately, as their hydrazides require tedious preliminary reaction and extraction steps: analysis data seem to be convincing and reproducible, with a low detection limit.

3.1.2.2. Aliphatic and aromatic carboxylic acids

The main constituents of fruit juices, beverages consist of short-chain fatty, aliphatic hydroxy and selected phenolic acids [73–91,99–101].

In comparison with the above group of acids without derivatization (Tables 6 and 7: [73–91]) and as derivatives (Table 9: [99–101]) it turns out that by means of IC fast analysis can be achieved [101], applying various surface active agents. The unambiguous advantage of analysis as derivatives lies in the possibility of detection of improved selectivity: higher region of the UV scale and DAD could be utilized. Underivatized aliphatic and aromatic acids are detected in the range of 190–214 nm [73–79,101], phenacyl derivatives at 254 nm, and *O*-nitrobenzyl-*N*,*N*′-diisopropylurea derivatives at 265 nm.

On the basis of data shown in Tables 6, 7 and 9, according to the author the following lessons can be drawn: (i) extraction should be omitted, (ii) analysis should be performed without derivatization, (iii) and in the higher region of the UV scale, possibly with DAD.

3.2. Gas chromatography

The only group of carboxylic acids suitable to be separated and quantitated by GC, without derivatization, was the group of FAs: including also, in selected cases, short-chain hydroxy carboxylic acids, such as lactic or malic acid. Having a look at proposals to determine ‘‘fatty acids’’ in the actual Supelco Catalogue [102], the current trend of developments/efforts is unambiguous: out of eight proposals seven suggest GC and only one HPLC method. It means, in order to have optimum conditions for the determination of the homologous series of FAs, GC is to be preferred: in terms of selectivity, sensitivity, optimum peak shape, shortest analysis time and for the separation of maximum number of constituents. The special polyethylene glycol bonded phase (trade name Nukol) offers spectacular separations. For 21 FAs (C_2–C_{22}, including iso-C_4–C_6, two species of C_{16} and four species of C_{18}, on Nukol, 15 m×0.53 mm, 0.5 μm film) within 24 min, or, for the C_2–C_7 series (including the iso-C_4–C_6 members), i.e., in total for nine acids (on Nukol, 30 m×0.25 mm, 0.25 μm film) within 9 min. Similarly, complete resolution can be obtained in the cases of positional and geometrical isomers of 34 FA methyl esters (within 40 min), or the C_3–C_{10} dicarboxylic, as well as the three phthalic acid dimethyl esters (within 11 min) by using the SP-2380 (poly-90% biscyanopropyl–10% cyanopropylphenyl siloxane) stabilized phase developed for high resolution and efficiency. The analysis of methyl esters furnishes about 200-times higher sensitivity in comparison to the analysis of the underivatized FAs. Concerning the optimum conditions for the identification and quantitation of members of any other homologous series of acids, without doubt, their analysis in the form of their silyl derivatives is the method of choice [51]. This topic, in context with the simultaneous analysis of members of various homologous series of acids, together with sugars, polyalcohols and selected amino acids, in particular relevant for food analysis, has been reviewed recently [51]: therefore, in this paper will not be repeated

in detail. Although, the author would like to call the reader's attention to the fact that sugars and acids, in considerable part of foods are inseparable. Unfortunately, in spite of this, their separation, identification and quantitation used to be performed by separate isolation and chromatographic techniques: with the exception of those determined as their TMS derivatives by GC [39–51].

3.2.1. Without derivatization

For an overview, see Table 10 [103–110]. Volatile FAs in wines indicate possible adulteration, microbial activity furnishing a characteristic odor. Early proposals have been performed on filled columns [103,105]. A shorter column (1 m) [103] allowed the separation of three acids needing a relatively long time (60 min), while on a longer column (7.5 m) [105] 21 FAs and crotonic acid have been separated and determined with good reproducibility, within an acceptable time (72 min). The importance of quantitation of 10-hydroxydecenoic acid in royal jelly resulted in special method development both by HPLC [71] and by GC [104]. Comparing these two chromatographic methods GC proved to be more accurate (RSD$<$3.4% for GC: Table 10 and $<$7.8% for HPLC: Table 5) and also more sensitive (for HPLC detection limit was not given). The knowledge of free volatile fatty acid (FVFA) content of dairy products [105–107,109,110] is an important area of FA analysis by GC. FVFAs are formed under ripening processes of cheeses, yogurts, or any fermented dairy products from fermentation of lactose and from degradation of amino acids. Thus, the qualitative and quantitative evaluation of FVFAs can characterize the quality, the level of ripening, as well as the source of dairy products they are originating from. An important step in the quantitation of FVFAs proved to be their isolation from lactic acid which is present in overwhelming excess compared to FVFAs disturbing their unambiguous, selective resolution by GC. The isolation of FVFAs was carried out by continuous extraction [105], by steam distillation [106,107], by simple extraction [108], or by solvent extraction of three steps [109] followed by SPE [109,110]: all are time-consuming and tedious isolation procedures.

3.2.2. With derivatization

For an overview, see Table 11 [111–121]. The knowledge of the changing composition of free FAs in breast milk [111], during milk secretion, is of primary importance: metabolic profiling data of organic acids in the colostrum and mature milk by GC–MS enhance the optimum feeding of babies in preparing baby-formulas. On-line derivatizations [112,119] of FAs, which means co-injection of sample and reagent is resulting in gas phase reaction, both the silyl- [112] and methyl [119] esterifications are worthy of mention due to their fast limited sample preparation requirement. The unique efficiency/advantage of the GC–MS analysis of TMS derivatives was shown in the quantitation of 37 acids, members of various homologous series (together with sugars, sugar alcohols and selected amino acids), from a single solution, by one injection [114–116]. Outstanding efficiency has been obtained, as silyl [112,118] and isopropyl [121] derivatives equally, in the analysis of the variously polyunsaturated C_{18} acids ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ [112,118]; $C_{18:3c,c,t}$, $C_{18:3c,c,c}$, $C_{18:3t,c,t}$, $C_{18:3t,c,c}$ [121],): due to the higher selectivity in the analysis of derivatives in comparison to the underivatized compounds. It is of interest to note the only exception cited [108], i.e., the separation of stearic, oleic, linoleic and linolenic acids of canola seed, without derivatization (Table 10) (the author assumes that the separation of canola seed's FAs could be performed as their isopropyl esters based on their extraction with isopropanol containing H_2SO_4 [108]).

4. Chromatography of amino acids

The first approach to the automatic liquid chromatography (LC) of amino acids, known today as IEC, was published in the late 1950s [122]. Now, under special conditions takes less than 5 min, both by GC [123–125] and by HPLC [126] to separate and quantitate the essential protein amino acids instead of 2 days [122]. Early separations were carried out by IEC applying post-column derivatizations with ninhydrin. Later on, first GC, thereafter HPLC, achieved wide acceptance instead of IEC, which requires dedicated apparatus suitable for the analysis of amino acids, exclusively.

In the last 20 years LC, offered quite unlimited possibilities both at the preparative and at the

analytical scale. The wide choice and sophisticated improvement of columns, detectors, derivatization procedures, development of modern instrumentation and data handling systems are time consuming, in work requirement and cost basically versatile, if needed automated and by good laboratory practice (GLP) also controlled conditions. The most popular LC method for the analysis both of free amino acids (present in several natural matrices, including numerous fresh and prepared foods) and of those constituents of protein hydrolyzates, is now RP-HPLC after pre-column derivatization of amino acids.

Current trends in amino acid analysis are associated with the development of HPLC–MS and identifying the best conditions for enantiomer separations: in particular relevant endeavors aim to prove the adulteration of food products by the presence of the unnatural enantiomers, D-amino acids.

4.1. High-performance liquid chromatography

4.1.1. Without derivatization

The main, ''believed'' advantage of HPLC, i.e., to separate a selected group of organic compounds without derivatization, in the case of amino acids has remained of secondary importance. However, the characteristics of the free amino acids are considerably different from each other their various structural properties do not permit their easy resolution. Consequently, the HPLC of underivatized amino acids proved to be fruitful for special tasks only [127–131]. Tryptophan was measured directly [127–129], within 8 min, in neutralized alkaline hydrolyzates of feed and foodstuffs, using an RP column, 5% methanol containing acetate buffer (pH~4.0) and UV detection (280 nm). A selective and sensitive method was developed in order to study tryptophan and other indols (indole-3-carboxylic, indol-3-acetic and indole-3-propionic acids, indol-3-methanol, indol, ethyl-indol-3-acetate, skatole and indole-2-carboxylic acid ethylester) in wine and must responsible for their ''untypical aging off-flavor'' [130]. These 10 compounds were determined after SPE cleaning, separated on a 250×4.6 mm (Purospher RP-18, 5 μm, Merck) column with fluorescence (FL) detection, within 35 min. The aspartame content (aspartylphenylalanine, i.e., Nutrasweet) of diet soft drinks (Coke, Pepsi, 7-Up) [131] was separated with a mobile phase of methanol–1% triethylammonium acetate (pH 4.5), on a β-cyclodextrin bonded silica gel column (250×4.6 mm), monitored at 214 nm (UV).

4.1.2. With derivatization

The use of the two most popular techniques, i.e., derivatization with phenylisothiocyanate (PITC) and *o*-phthaldialdehyde (OPA), in the presence of different SH group containing additives, are represented also in the determination of amino acids in food samples.

4.1.2.1. Phenylthio-, butylthio- and benzylthiocarbamyl derivatives

For an overview, see Table 12. The popularity of the HPLC of phenylthiocarbamyl (PTC) amino acids [132–145] lies in the fact that, in undissolved form, they can be stored for unlimited time in the freezer. Numerous practical tasks have been solved by the analysis of PTC-amino acids, including various food samples, such as the free amino acids in wine and musts [132–135,137,141,142], in various apple pulps [143], as well as the total, i.e., the free and protein amino acids of pork meats [136], soybean [138,139] and green bean [140] samples. On the basis of our earlier experiences [144,145] (in accordance with literature data [146] relating to the optimization of the buffer and pH dependence on the retention of the PTC-amino acids [144] and that of the recovery of amino acids under cation-exchange clean-up [145]) recently [143], we aimed to find the possibility for the simultaneous analysis of 27 amino acids, including the possible apple constituents. We quantitated (i) the main component asparagine (being present in overwhelming excess), as well as the minor constituents glutamine, β-alanine, γ-aminobutyric acid (GABA), homoserine, homoarginine and 1-amino-1-cyclopropanecarboxylic acid (ACPCA), (ii) together with all others, within a reasonable retention time, utilizing the advantages of the detailed spectra furnished by UV/DAD. In the course of this exhaustive study [143] seven columns of different length and fillings have been tested, applying flow-rates from 1.3 to 2.3 ml/min (also in programmed version), at different temperatures (15–50°C).

The recently introduced butylisothiocyanate (BITC) [138] and benzylisothiocyanate (BzITC) [139] furnish the only advantage over the classical PITC that cysteine and cystine can be separately

eluted; However, derivatization needs to be performed for 30 min, at an elevated temperature {40°C for the butylthiocarbamyl (BTC) [138], 50°C for benzylthiocarbamyl (BzTC) derivatives [139]}.

4.1.2.2. o-Phthaldialdehyde derivatization in the presence of various SH containing additives (Table 13)

In this section, in order of listing, the quantitation of food amino acids are listed as OPA–3-mercaptoethanol (ME) [148–150], as OPA–*N*-alkyl-L/D-cysteines (achiral and chiral separations) [151–154], as OPA–3-mercaptopropionic acid–9-fluorenylmethyl chloroformate (MPA–FMOC) [155] and as OPA–MPA or OPA–*N*-acetyl-L-cysteine (NAC) derivatives [156]. Based on the pioneer work appearing three decades ago [147], the reaction of amino acids with OPA, in the presence of various SH-group containing agents, has gained wide acceptance in the chromatographic analysis of amino acids. Because of the instability of the isoindoles obtained from amino acids with the primarily introduced OPA–ME [148–150] reagent, also in the analysis of food amino acids, as SH-group containing alternatives, *N*-alkyl-L/D-cysteines [151–154] and MPA [155,156] were proposed. The common advantages of the OPA–MPA and OPA–NAC reagents, can be characterized by the fact that they provide more stable isoindoles, compared to those formed with the OPA–ME one, while in the optical resolution of enantiomeric amino acids the advantages of the OPA–NAC and/or OPA–*N*-alkyl-L-cysteine reagents have been extensively utilized [151–154].

Conditions for the separation of OPA derivatives in various food products are summarized in Table 13. Evaluating the preparation step in the quantitation of amino acids as OPA derivatives it is unambiguous that this step is the rate limiting one: due to the fast derivatization reaction in aqueous media. The simple and fast isolation procedure is to be preferred instead of the time consuming and unsatisfactory recoveries [145] furnishing ion-exchange procedures [151–154]. The simple filtration on glass fiber paper [156] resulted in good reproducibility in the case of three apple samples combined by the fast OPA derivatization technique resulted in substantially reduced preparation time and the complete recovery of amino acids. The main drawback of the OPA derivatization, i.e., OPA does not react with secondary amino groups was solved by the consecutive reactions with OPA–MPA–FMOC [155].

4.1.2.3. Derivatives obtained with fluorenylmethyl chloroformate (FMOC), with dimethylaminonaphthalene-1-sulfochloride (DnsCl) and with 4-dimethylaminoazobenzene-4′-sulfonyl chloride (DbsCl)

Although, from the basic research studies it is well known that derivatizations of amino acids with FMOC alone, or both with dimethylaminonaphthalene-1-sulfonyl (dansyl) chloride (Dns-Cl) and 4-dimethylaminoazobenzene-4′-sulfonyl chloride (Dbs-Cl) result in inconveniences, due to the interfering effects of side products originating from these reagents, for all that, in the analysis of food amino acids these processes have been proposed also recently [157–161].

FMOC was used in the analysis of the ornithine, OH-proline, β-alanine and 3-methyl-histidine content of green coffee beans [157] and in the quantitation of the enantiomeric ratios of the proline, leucine and phenylalanine concentrations in honeys [158] responsible for their authenticity. Twenty-two amino acids in wheat flour, unfermented and fermented bread dough and wheat bread samples have been determined as their dansyl derivatives [159]: amino acids were isolated by a cation-exchange process and separated on a short column [83+36 (guard)×4.6 mm, 3 μm], within 42 min, at 30°C (flow-rate 1.5 ml/min, detection 250 nm, UV). The lysine, histidine and tyrosine content of food proteins (milk casein, chicken egg white, lysozyme) were quantitated in their hydrolyzates as didansyl derivatives [160]. Derivatization was carried out at 40°C for 60 min, separation of these three amino acids, including two reagent components (Dns-NH_2, Dns-$MeNH_2$), was completed in 16 min. Selected members of proteinogenic and physiological amino acids together with biogenic amines, simultaneously, more than 40 compounds have been separated, within 90 min, as their dabsyl derivatives in parmesan cheese [161]. The repeatability of the procedure ranged between 1.3 and 3.1%, with a low detection limit (0.12–0.52 p*M*). The amounts of D-amino acids [162] labeled with a very complex fluorescent chiral reagents [*R*(−)- and *S*(+)-4-(3-isothiocyanopyrrolidin-1-yl)-

7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazoles] have been determined in several food samples, such as milk, cream, fermented dairy products (yogurt, yacult), tomato products (juice, puree, catchup), beer and red wine. Derivatization was performed in basic medium by using triethylamine (TEA), at 55°C for 20 min. Isocratic separation (water–acetonitrile–methanol) on a short column (150×4.6 mm, 3 μm, Wacosil-II $3C_{18}$ RS), for 75 min at 40°C resulted in the separation of eight enantiomer pairs in addition to glycine and β-alanine. Fluorescence detection ($\lambda_{ex}/\lambda_{em}$=460/550 nm) ensured the detection limit in the range of 0.16–0.75 p*M*.

4.2. Capillary electrophoresis

The aspartame content of diet soft drinks has been determined by CE using electrochemical detection with a copper electrode [163]. The free amino acid contents of orange juice and beer samples have been measured in a very simple manner, without any pretreatment, applying CE with UV detection (185 nm) [164]. Separations of nine amino acids (arginine, alanine, serine, asparagine, tryptophan, glutamic acid, phenylalanine, tyrosine, proline) have been carried out under acidic conditions, in 35 min (running buffer pH 2.36, 10 m*M* NaH_2PO_4+30 m*M* octanesulfonic acid, containing 0–20% acetonitrile, injection time 15 s, applied voltage 30 kV).

4.3. Gas chromatography

According to the classical methods, in order to obtain volatilazable amino acids, suitable for GC analysis, both of their functional groups need to be derivatized. Commonly, the first step relates to the esterification of the carboxyl group using different short-chain, aliphatic alcohols while in the second step the *N*-(*O*,*S*) function will be acylated with various acid anhydrides. In the late 1980s a one-step derivatization of amino acids, applying various alkyl chloroformates, was introduced: resulting in the corresponding *N*-(*O*,*S*)-alkoxycarbonyl alkyl esters. Unfortunately, this procedure suffers from several shortcomings: the guanidino-, indolyl-, imidazolyl and the hydroxyl groups do not react and the molecular response of amino acids varies between 0.3 and 1.07 [124]. Thus, it is understandable that this one-step process was not favored for the analysis of food amino acids.

4.3.1. Derivatives: variously acylated different esters

In this section, the quantitation of food amino acids is discussed measured as *N*-(*O*,*S*) trifluoroacetyl (TFA) [165], pentafluoropropionyl (PFP) [166–168], *n*-propyl [165–168], *N*-(*O*,*S*) heptafluorobutyryl (HFB) isopropyl [169] and *N*-(*O*,*S*) HFB isobutyl ester [170,171] derivatives (Table 14).

The identification and quantitation of OH-proline in meat by GC–MS [165] was of importance because of legislative and nutritive point of view. To get unambiguous proof of the presence of unadulterated meat products, which do not contain added, connective tissues, a selective method was required. Performing chiral phase capillary chromatography [166–168] an exhaustive study was performed in order to determine the D-amino acid content of several fermented foods (kefir, cheese, beer, instant coffee, fermented sausage [166]), or sour milk, Emmentaler cheese, carrot juice, fermented black beans [167]. The advantages/disadvantages in respect of the choice of chiral derivatization [151–154], or chiral stationary phase, associated with the difference of the methods (GC [168], or HPLC [151–154]) have been evaluated in detail [168]. The free amino acid content of potato has been studied by GC–MS using the *N*-heptafluorobutyryl isopropyl ester derivatives [169]. As a result of this selective, fast GC method (21 amino acids/15 min) revealed that γ-aminobutyric acid is abundant in potato (27% of the total of free amino acids). Eighteen free amino acids in cheese [170] and 17 amino acids in honey [171] have been determined in 25 min [170] and in 50 min [171], respectively: proving the fact that a well established elution procedure can be the rate-limiting step of a chromatographic separation.

4.3.2. Derivatives: N-(O,S)-tert.-butyldimethylsilyl, N-(O,S)-isobutyloxycarbonyl, N-(O,S)-trimethyl silyl tert.-butyldimethylsilyl and trimethyl silyl esters

The identification and determination of 51 protein and non protein amino acids as their *N*-(*O*,*S*)-isobutyloxycarbonyl (isoBoc) TBDMS esters in almond, walnut and sunflower seed, within 65 min

elution time is an outstanding study [172]: the optimization both of derivatization (three pH values, pH 11, 12, 13) and that of chromatographic conditions (ULTRA-1, DB-5, DB-17 capillaries, paired in dual systems), have been investigated in detail. The advantages of proposal can be characterized by the facts that (i) SPE was proposed to be carried out with the *N*-(*O*,*S*)-isoBoc derivatives allowing efficient, rapid and highly selective extraction of free amino acids, prior to their esterification with *N*-methyl-*N*-(*tert*.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA), (ii) optimized conditions ensured the satisfactory reproducibility of measurements, and (iii) the use of dual capillaries of different polarity provided the complete separation of selected amino acids. It would be great that only the OH group containing serine, threonine and OH-lysine would have been furnishing double derivatives as shown [172]: since on the basis of our recent study, in accordance also with the experience of others [173] it has been repeatedly proven that the reaction of the overwhelming part of amino acids with HMDS [39,115,116,174], with bistrifluoroacetamide (BSTFA) [174] and with MTBSTFA [173,174], equally, under different conditions [174], result in multiple derivatives [39,173,174].

An excellent compilation of the classical basis of the GC separation and quantitation of amino acids was published by one group of pioneer scientists of this topic [175].

5. Conclusion

Chosen examples are summarized where chromatographic methods were applied both at the basic research level aiming to identify/determine important food constituents and in the routine analyses suitable for use in everyday practice, in food quality control.

It was encouraging that also in control laboratories – instead of the earlier applied, ''so called total'' of sugars/acids/amino acids providing procedures – chromatographic techniques have become preferred. In order to apply the best fitting process to the given task the analytical chemist should be aware of the most important characteristics of the chromatographic method selected, i.e., selectivity, sensitivity, reproducibility and the most convenient type of chromatography (GC, HPLC, CE, etc.).

This review has been prepared on the basis of the most relevant, and in the overwhelming part but not exclusively, on the most recently published works, including various food matrices, requiring different work up steps before the chromatographic analysis: more than 2000 papers have been overviewed thoroughly. The main aim was (i) to choose the most relevant ones, (ii) to select those, most important parameters of methods that mirror their suitability to the reader: which of them should/could be applied to the task to be solved, and (iii) compiling those conditions in a detailed manner into tables ensuring that only a few or none of the original texts need to be read.

6. Nomenclature

ACN	Acetonitrile
AABA	α-Aminobutyric acid
ACPCA	1-Amino-1-cyclopropanecarboxylic acid
APCI	Atmospheric pressure chemical ionization
API	Atmospheric pressure ionization
BGE	Background electrolyte
BzTC	Benzylthiocarbamyl
BSA	Bovine serum albumin
BSTFA	bis-(Trimethylsilyl)trifluoroacetamide
BTC	Butylthiocarbamyl
CD	Circular dichroism
CE	Capillary electrophoresis
CEC	Capillary electrochromatography
CSP	Chiral stationary phase
dbs/dabsyl	4-Dimethylamino-azobenzene-4-sulfonyl
dns/dansyl	5-Dimethylaminonaphthalene-1-sulfonyl
DieEt	Diethyl ether
DMF	Dimethylformamide
DP	Degree of polymerization
ESI	Electrospray ionization
EtOH	Ethanol
EtAc	Ethyl acetate

ELSD	Evaporative light-scattering detection
FID	Flame ionization detection
FMOC	9-Fluorenyl methylchloroformate
FL	Fluorescence
FA	Fatty acid
FVFA	Free volatile fatty acid
GABA	γ-Aminobutyric acid
GC	Gas chromatography
GLP	Good laboratory practice
HPLC	High-performance liquid chromatography
HMDS	Hexamethyldisilazane
HFB	Heptafluorobutyryl
IEC	Ion-exchange chromatography
isoBoc	Isobutyloxycarbonyl
ISP-MS	Ionspray mass spectrometry
LC	Liquid chromatography
NAC	*N*-Acetyl-L-cysteine
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
MeOH	Methanol
MS	Mass spectrometry
ME	3-Mercaptoethanol
MPA	3-Mercaptopropionic acid
MEKC	Micellar electrokinetic chromatography
MTBSTFA	*N*-Methyl-*N*-(*tert*.-butyldimethyl)trifluoroacetamide
OPA	*ortho*-Phthaldialdehyde
PFP	Pentafluoropropionyl
PIC	Phenylisocianate
PITC	Phenylisothiocyanate
DAD	Photodiode array detection
Pr	Propyl
PAD	Pulsed amperometric detection
RMR	Relative molecular response
RI	Refractive index
RP	Reversed-phase
Pyr	Pyridine
SDS	Sodium dodecyl sulfate
SPE	Solid-phase extraction
TAG	Triacylglycerol
TBDMS	*tert*.-Butyldimethylsilyl
TEA	Triethylamine
TSP-MS	Thermospray mass spectrometry
TFA	Trifluoroacetyl
TFAA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIC	Total ion current
TMS	Trimethylsilyl
WCOT	Wall-coated open tubular

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