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Review

Simultaneous quantitation of acids and sugars by chromatography: gas or high-performance liquid chromatography?

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Abstract

As is well known, the knowledge of the qualitative and quantitative distribution of sugars and acids, present in various biological (urine fermentation liquor) and several natural matrices (fruits, vegetables, drug- and industrial plants, mushrooms, honeys) proved to be of primary importance from several points of view.

In accordance with the chronological order of the development of the chromatographic methods, first, the possibilities of gas chromatography, thereafter, those of high-performance liquid chromatography have been shown. The advantages/disadvantages of these two main chromatographic methods, relating to this special topic will be presented in details.

Keywords: Reviews; Sugars; Organic acids

Contents

1. Introduction

The demand for the simultaneous analysis of acids and sugars is contemporaneous with the recognition that a number of natural matrices (fruits, vegetables, drug- and industrial plants, mushrooms, honeys, ferment liquors, etc.) can consist of an overwhelming part (~90%) of these two groups of organics. The knowledge of the quality and quantity of sugars and

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Table 1
Characteristics, advantages/disadvantages in the analysis of sugars and acids by HPLC and by GC

Sugars/(sugar alcohols)/organic acids			
HPLC		GC	
Characteristics			
Advantage	Disadvantage	Advantage	Disadvantage
Derivatization			
not necessary			necessary
Column			
one/two		one	
Detector			
one/two		one	
Selectivity			
low		excellent	
Resolution			
low		excellent	

acids, as well as the ratios of the single saccharides to each other and the ratios of the sugars to the acids proved to be of particular importance: providing information on the general quality, freshness, maturity, storability and/or on the optimization of selected technological processes. Thus, in all of those laboratories, which are specialized in the analysis of the above detailed matrices, at least some of the total acid-, or total sugar determinations are performed.

The development of a wide variety of chromatographic methods, including, with reference to, the group of acids and sugars, furnish up-to-date possibilities to solve this analytical task by means of chromatography.

In accordance with the chronological order of the development of chromatographic methods, first, the possibilities of GC [1–27,44] and, thereafter, those of HPLC [28–43] were described.

The aim of this paper is to compile all those efforts that have been performed in order to determine the two, in particular, important groups of organics from the same matrix, on the basis of literature data [1–4,8–16,28–44] and on those of personal experiences [5–7,17–27].

The possibilities in general of the main two techniques are schemed in Table 1.

The advantage/disadvantage phenomena which reflect the view of an analytical chemist, [having access to both, relatively new systems (GC–MS–flame ionization detection, Varian Saturn, 1995, HPLC–photodiode array–fluorescence detection, Waters, 1997)], needs more detailed explanation.

1. The advantage/disadvantage phenomena reveal the possibilities, which should be investigated as a function of the task to be solved (matrix, number of components expected, reproducibility required, etc.) and the facilities available, one by one.
   1.1 No doubt about it, if a given task, such as the separation of 10–20 acids, (members of various homologous series), +1–5 sugar alcohols, (belonging to the C_3–C_6 series), +5–15 sugars (of various degree of polymerization), can be solved by a single procedure, using one apparatus, one detector and one column, saving time, cost and work by using a single elution procedure of high selectivity, then this is a highly desirable procedure. These are the characteristic criteria of the advantage of GC, in spite of the fact that it needs the derivatization of the analyte.
   1.2 In order to achieve the same results detailed above you could need more than one column,

Table 2
Analysis of sugars and acids from the same matrix by separate methods[a]

[Ref] date	Extraction	Derivatization (De), Detection (D), Column (C), Elution (El)	RSD (%)	Compounds/min[b]
GC analysis				
[1] 1974	50 g apricot/150 ml 95% Et, 60 min, centr., residue+2×25 mlEt=250 ml. Acids precip. by lead acetate, extr., evapor.	De: Sugars, Et extr+Try Sil, 70°C, 20 min; Acids, lead precip.+Tri Sil, 50°C, 30 min; D: GC–FID, C: stainless steel, 1.5 m×2.28 mm (5% SE 52, Aeropak 30, 60–80 mesh)	≤12	3 sugars+1 sugar-alcohol/40 14 acids/20
[2] 1981	Sugars: 50 g cheese/100 ml water, mixing at 42°C, 7 min, centr.; 5 ml filtrate+20 ml Met.; Acids: 50 g/150 ml 0.43 *M* HCl, filtrate adjusted to pH 10	De: Sugars, HMDS+TMCS, 70–80°C, 30 min; D: GC–FID, C: 3.84 m 4 mm (3% OV-1, Supelc 80–100 mesh); De: Acids, Est. 10% cc. H_2SO_4+90% abs. Met., 25 h, 50 ml water, extr. $CHCl_2$, C: 1.83 m×2 mm (10% DEGA+20% H_3PO_4, Gas Chrom A)	–	3 sugars/32 3 acids/32
HPLC analysis				
[28] 1981	50 g aloquat/100 ml water, blending, slurry filtr. residue 2×25 ml water wash,+50 ml blend, filtr, unify; Sugars: C_{18} Sep Pak; Acids: cat.+ an. exch.	Sugars: D: RI, Column 30 cm (Waters μBondapak carbohydrate + 4 cm precol), El: ACN–water (85:15, v/v); Acids: D: UV, C: 30 cm (Waters μBondapak C_{18}+4 cm precol, E: 2% $NH_4H_2PO_4$ (pH=2.4 with H_3PO_4)	– –	3 sugars/- 6 acids/23
[29] 1986	20 g tomato juice/60ml 8% Et., 1 h, 80°C, refl. filtr. residue+150 ml 80% Et.	Sugars: D: RI, C: 30 cm (Waters μBondapak carbohydrate), El: ACN–water (80:20 v/v); Acids: D: RI, C: Bio-Rad HPX-87, E: 0.005 *M* H_2SO_4, 60°C		2 sugars/15 5 acids/-
[30,31] 1986	water melon/water=1/9 (homogenized)	Sugars: D: RI, C: Bio-Rad HPX-87C (300×7.8 mm); Acids: D: UV, C: Bio-Rad HPX-87H, El: 0.0004 *M* H_2SO_4, 75°C	–	3 sugars/- 2 acids/-
[32] 1988	20 lbs apple crushed by hammer, pressed, juice clarified by pectic enzyme treatment, filtered	Sugars: D: RI, C: Bio-Rad HPX-87C (300×7.8 mm+ 3.6×40 mm cation Microguard), El: water; Acids: D, UV, C; MCH-10 Micro-pak (10 μm, 300×4 mm), El: 2% KH_2PO_4+2% NaCl in water, or, 0.1 *M* KH_2PO_4+Met	≤9.5	3 sugars+1 sugar-alcohol/24 5 acids/25
[33] 1991	extraction study: 1 g dry mass/50 ml solution, 0–80% Et	Sugars: D: RI, C: 300×6.5 mm (Sugar-Pak I, Ca^{2+}), El: water cont. 15 mg/L CaEDTA, 85°C; Acids: D: UV, C: Aminex HPX-87H^+, El: 0.00445 *M* H_2SO_4, 65°C	≤9.1	3 sugars/12 8 acids/18

[a] Indications: –=no data available; Met=methanol; Et=ethanol; Tri Sil=silylation reagent (Pierce Chemical Co.); Est=esterification; ACN=acetonitrile; 1 lb=454 g.
[b] Number of compounds/elution time, min (column equilibration not included).

Table 3
Simultaneous analysis of sugars and acids from one solution with a single injection by GC–FID[a]

[Ref] date	Extraction (E), Derivatization (De)	Chromatography: Column (C), Gradient (G), Injector (Inj), Detector (Det)	RSD (%)	Compounds/min[a]
[3] 1982		C: 183×2 mm (3% OV-17 on ABS-100 110 mesh); G: 140–250°C, 15°C/min; Inj and Det=280°C	≤3.44	3 sugars+ 2 acids/10
[4] 1987	E: 3 g ground coffee+25 ml DMSO, water bath, 90°C, 60 min, (filt.: 0.5 μm); De: DMSO filtr./Tri-Sil reagent=1/1, 50°C, 30 min (not homog. mixture: vigourously shaken in every 5 min);	C: 25 m×0.25 mm I.D. (CP-SIL5-CB, Chrompack); G:, 100–240°C (8°C/min), 240–300°C (15°C/min), Inj and Det=310°C	≤4.73	1 sugar (sucrose) +30 acids/32
[5] 1990	E: No; De: ≤20 mg sugars+acids, or equivalent apple juice (in total dried) 1st step sample+ 500 μl oxim reag[b] (70°C, 30 min), 2nd step 900 μl HMDS+100 μl TFAA	C: 3 m×4 mm (15% Dexsil GC 300 on Chromosorb W AW DMCS, 80–100 mesh); G: 60–360°C (12°C/min), Inj=380°C, Det=400°C	≤10.7[c] ≤4.0[d]	3 sugars+ 19 acids/30
[6] 1991	E: Extraction study: 2-, 5-, 10- and 20 g fruits/100 ml 80% Et, or *80% Met, at 0°C (overnight), 25°C (5, 10 min), refl. (15 min); De: [5]	C:,10 m×0.25, I.D. (CP-SIL-5CB, df 0.12, Chrompack), G: 120–280°C (10°C/min, hold 3 min at 160°C and 6 min at 280°C), Inj and Det=300°C	≤5.0	7 sugars+1sugar alcohol+ 9 acids/30
[7,8] 1992	E: No; De: according to [5,6]	C: as in [6], G:, 60°C (1 min), 60–84°C(12°C/min), 84–168°C (14°C/min, hold 4 min), 168–270°C (10°C/min, hold 12 min)	≤10.7[c] ≤3.7[d]	12 sugars+ 2 sugar alcohols+ 19 acids/35
[9] 1993	E: No; De: oxymation+silylation in one step by *N*-methoxy-*N*,*O*-bistrimethylsilyl carbamate (BSMOC),i.e., ≤20 mg sugars+acids, or equivalent fruit (in tota, dried)+1 ml pyridine+ 400 μl BSMOC+ 100 μl TFAA	C and G as in [6–8]	≤6.5	11 sugars+3 sugar alcohols+ 15 acids/35
[10,11] 1996-97	E: 5 g/10 ml 50% Et (blended, centr., the supernatant diluted to 50 ml by 50% Et); De: 1 ml extract (10 mg fruit)+400 μl pyridine+ 100 μl TMCS+ 400 μl HMDS, 60°C, 2 h	C: 25 m×0.25 mm I.D. (CP-Sil-5CB, DF 0.12, Chrompack, k, G:, 120°C (1 min), 120–152°C (8°C/min), 152–176°C (12°C/min), 176–198°C (16°C/min), 198–238°C (20°C/min), 238–300°C (24°C/min, hold 5 min), Inj=280°C, Det=320°C	r 0.919–0.999	7 sugars+3 sugar-alcohols+ 6 acids/18

[a] Indications as in Table 1, as well as; Stox[a]=Pierce, oximation reagent (25 mg/ml hydroxylamine HCl in pyridine); oxim reag[b]=2.5 g hydroxylamine. HCl dissolved in 100 ml pyridine; Inj=Injector; Det=Detector; [c]=in the cases of main constituents (≥1 μg); [d]=in the case of minor constituents (≤1 μg); r=linear regression coefficient (Pearson's correlation). DMSO=Dimethyl sulfoxide.

Table 4
Simultaneous analysis of sugars and acids from one solution with a single injection by GC–MS

[Ref] date	Extraction (E), Derivatization (De)	Chromatography: Column (C), Gradient (G), Injector (Inj), Detector (Det)	RSD (%)	Compounds/min[a]	
				TIC SFI[c,d]	
[12] 1984	E: propolis/96% Et=1/5, (filtered, evaporated), De: 200 mg extract+ *N*,*N*-bis(trimethylsilyl)trifluoroacetamide	C: 2 m×2 mm (3% OV-17 on Gas Chrom 100–120 mesh); G: 50–260°C (8°C/min); Inj and Det no data	–	6 sugars+1sugar alcohol+ 8 acids/25	1 acid/25[c]
[13] 1989	E: 80% Et; De: TMS derivatives (no more details available)	C: 25 m×0.2 mm, I.D. (CBP 1,Chrompack); G: 100–250°C (5°C/min), Inj=280°C, Ion source 250°C, Ionizing voltage (IV)=70 eV	–	3 sugars+1 sugar alcohol+ 3 acids	1 acid/30[c]
[14,15] 1989	E: 5 g sweet potato (peach) diced+finely ground (mortar)/5 ml 75% Et+Et to 25 ml (filt. after 10 min; De: 0.5 ml extr. 1st step+ 500 μl oxim reag[b] (75°C, 30 min), 2nd step 500 μl BSTFA+1% TMCS (Pierce) (20 min)	C: 15 m×0.25 mm (DB-1, 0.25 μm); G:, 150°C (4 min), 150–192°C (4°C/min+0.5 min), 192–240°C (10°C/min+7 min), Inj=–, Ion source=–, IV=–;	–	6 sugars+2 sugar alcohols+ 4 acids/25	(6 sugars+2 sugar alcohols+ 4 acids/25)[c]
[16] 1991	E: No; De: 0.5–1.0 ml, urease treated urine+MSTFA (25–100% of urine's volume) (70°C, 1 h)	C: 30 m×0.32 mm I.D. (OV-5, 0.05 μm, Ohio Valley Specialty Chemical); G: 80°C (1 min), 80–130°C (2°C/min), 130–200°C (3°C/min), 200–280°C (6°C/min+10 min),	–	9 sugars+7 sugar acids (alcohols)+24 acids+ 46 amino acids+3 amines	(9 sugars+7 sugar acids (alcohols)+ 24 acids+46 amino ac +3 amines/67+9)[c] /67+9
[17–19] 1994-96	E: No; De: according to [5–8]	C: 30 m×0.248 mm I.D., (DB-5, J&W); G: 60–120°C (16°C/min), 120–155°C (4°C/min, +12 min), 155–210°C (4°C/min), 210–320°C (16°C/min+12 min), Inj=60°C (2 min), 60–320°C (180°C/min+10 min); IV=70 eV	≤10.6 [17]	16 sugars+9 sugar alcohols/acids+ 16 acids+2 aldehydes/61	(16 sugars+9 sugar alcohols/acids 16 acids+2 aldehydes)[c] 2 acids[d]
[20–27] 1997-98	E: No; De: according to [5–8,17–19]	C: as in [17–19], G: 60°C (2 min), 60–155°C (13°C/min+10 min),155–250°C (14°C/min+12 min), 250–320°C (20°C/min+10 min); Inj, IV as in [17–19]	≤ 12 (SFI) ≤ 5 (TIC)	22 sugars+3 sugar alcohols/acids +39 acids+proline+ HMF/52	(22 sugars)[c]+(3 sugar alcohols/acid+ 39 acids+proline+ HMF/52)[d]

[a,b,c,d] Indications as in Tables 2, 3 as well as: SFI[c,d]=identified ([c]) or identified and determined ([d]) on the basis of selective fragment ions (SFI); HMF=hydroxymethylfurfural

Table 5
Simultaneous analysis of sugars and acids from one solution, by single or separate detections with HPLC

[Ref] date	Extraction (E)/Sample preparation (P), Detection (D)	Chromatography, Column (C), Elution (El)	RSD (%)	Compounds/min[a]
HPLC analysis by a single detection				
[34]	P: must diluted 1/2 (dist. water, wine without dilution,	C: 300×7.8 mm, I.D. (Aminex HPX87H$^+$, Biorad);	r	1sugar+
1987	1st membrane-filtered (0.22 μm), 2nd removal phenolics (Sep-Pak cartridge, elution by 0.065 *M* H_2SO_4); D: UV (210 nm)	El: isocratic, 0.0013 *M* H_2SO_4, 0.8 ml/min, 65°C	0.977–0.999	6 acids/20
[35]	E: 1 kg apple, one quarter of each, covered by 80% Et, blended	C: 300×0.65 mm, I.D. (Sugar Pak I, Water Associates);		3 sugars+2 sugar alcohol+
1988	(2 min, high speed), slurry refluxed (2 h), compl. to 500 ml, filtered (Sep-Pak C_{18}), filtered (0.45 μm); D: RI	El: isocratic, water containing 50 ppm $Ca(Na)_2$ EDTA, 0.5 ml/min, 80°C	<6	1 acid+ Et/20
[36,37]	P: All grape must/wine samples filtered (0.45 μm); Removal of	C: 300×7.8 mm I.D. (+GC-801 ion guard column, ION-300,		2 sugars+glycerol+
1992	phenolics, (1 ml red wine/grape must C_{18} Sep-Pak, elut. by 1.5 ml 0.005 *M* H_2SO_4); Sep. of neutral- from acidic compounds by LC-Sax, strong anion-exchange chromatography; D: RI	Interaction); El: 0.013 *M* H_2SO_4, isocratic, 0.6 ml/min, 71°C	<5.62	6 acids+ Et/30
HPLC analysis by separate detections				
[38]	P: 1 ml fermentation liquor filtered (0.45 μm, Millipore disposable);	C: 300×7.8 mm I.D. (Aminex HPX87H$^+$, Biorad); El:	<7.6(RI)	5 sugars+10 acids +Et/40
1987	D: RI+UV (21 nm)	0.026 *M* H_2SO_4, isocratic, 0.6 ml/min, 40°C	<9.6(UV)	
[39]	P: 3 ml fresh or fermented cucumber juice filtered	C: 300×7.8 mm I.D. (Aminex HPX87H$^+$, Biorad); El: isocratic,	r	2 sugars+
1989	(0.45 μm, Millipore disposable); (D: UV (210 nm)+RI	0.013 *M* H_2SO_4, 0.6 ml/min, 60°C	0.997–0.999	4 acids/20
[40]	P: Standards, containing sugars and acids between 12 and	C: 300×7.8 mm I.D. (+GC-801 ion guard column, ION-300,	r	3 sugars+8 acids+ Et+Met/50
1991	2614 ppm; D: UV (210 nm)+RI	polymer resin, Interaction); El: 0.004 *M* H_2SO_4, isocratic, 0.4 ml/min, 25°C	0.997–0.999	
[41]	E: 5 g cheddar cheese+ 25 ml 0.0045 *M* H_2SO_4 (mixing,	C: 300×7.8 mm I.D. (Aminex HPX87H$^+$, Bio-rad); El: isocratic,	–	3 sugars+ 10acids/30
1991	magn. stirrer, 1 h), centr. 10 min, filt. (Whatman No. 1 paper+ 0.20 μm membr. (Bio-Rad); De UV (220, 280 nm)+RI	0.0045 *M* H_2SO_4, 0.7 ml/min, 65°C		
[42]	P: Centrifugation (Eppendorf microcentrifuge, 15 000 *g*);	C: Phenomenex ROA organic acid column, (No. OOH-0138-KO);	r	2 sugars+2 sugar alc.+
1993	D: Conductivity (Dionex CDM)+PDA (Pulsed Amperometric Detector (Dionex PAD, gold electrode)	El: isocratic, 1.6 m*M* heptafluorobutyric acid, 0.7 ml/min, 65°C	0.993–0.999	1 acid+Et+n-Prop/20
[43]	P: Filtration (0.45 μm, Millipore); D: RI+UV (214 nm)	C: 300×7.8 mm I.D., (Aminex HPX87H$^+$, Bio-Rad); El:	<9.16	2 sugars+glycerol+
1996		0.65 m*M* H_2SO_4, 0.7 ml/min, 75°C		6 acids+Et/40

[a] Indications as in Tables 2–4.

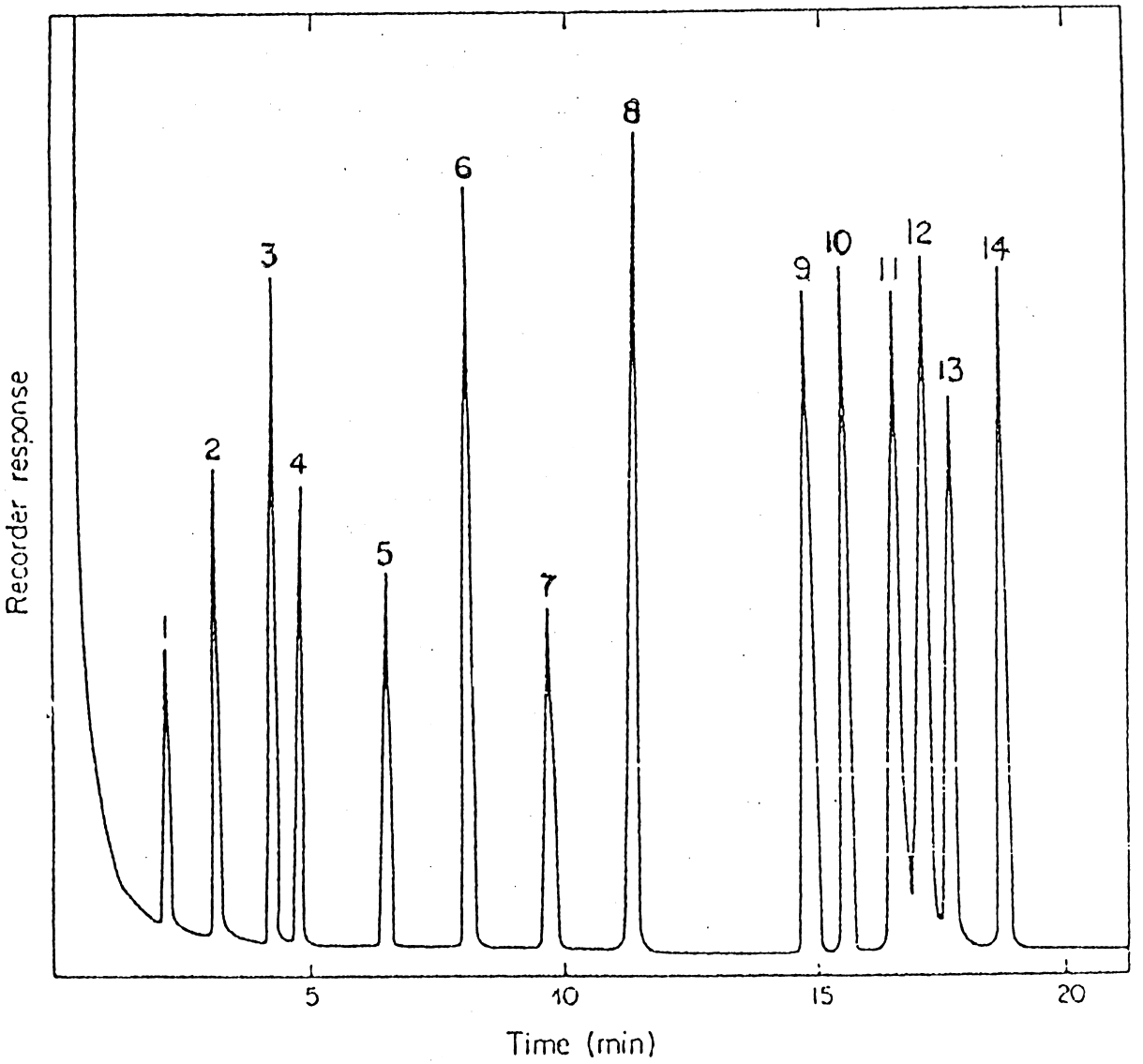


Fig. 1. Separate GC–FID determination of the TMS derivatives, prepared from lead salts of acid standards on SE-52 column: (1) oxalic; (2) malonic; (3) phosphoric; (4) succinic; (5) glutaric; (6) malic; (7) oxalacetic; (8) tartaric; (9) citric; (10) quinic; (11) p-coumaric; (12) ascorbic; (13) α-glucuronic+α-galacturonic; (14) β-glucuronic+β-galacturonic acids. From Ref. [1] with permission, ©ACS.

more than one eluent system, more than one detector, more than one apparatus (or applying the methods one after the other, on the same apparatus), and all these can be regarded as the disadvantageous characteristics of HPLC quantitation of the underivatized samples.

2. What should it mean in the practice? When can we exhaust the advantages of the HPLC?
3. For example, to determine the various sugar/acid constituents of a syrup obtained from the hydrogenolysis of a natural matrix, such as corn cobs, the most effective results can be expected from

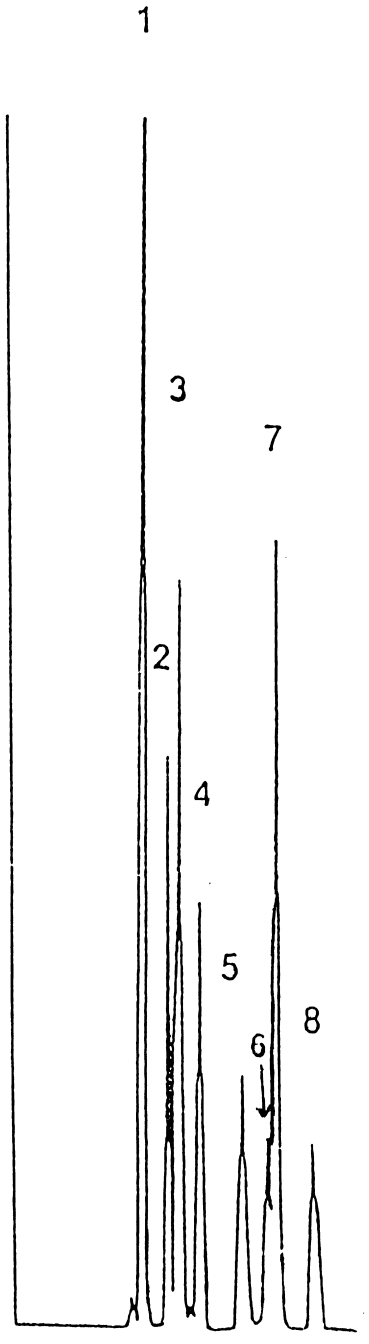


Fig. 2. Separate HPLC of a standard organic acid solution using water as the eluent and UV detection: (1) oxalic; (2) citric; (3) tartaric; (4) malic; (5) succinic; (6) lactic; (7) fumaric; (8) acetic acids. From Ref. [33] with permission, ©ACS.

GC analysis of the trimethylsilyl (TMS) derivatives, from one solution, by a single injection.

4. But, in simple cases, such as to determine the free malic acid, glucose, fructose and sucrose content of a solution in order to follow the stability of its constituents, or, to quantitate the increasing fumaric acid content of the analytical grade malic acid in the presence of limited number of saccharides (1–3) during storage conditions, HPLC determination of the underivatized samples could be the method of choice.

Compilation of derivatization, chromatographic conditions and reproducibility data will be given in detail (Tables 2–5, Figs. 1–9).

2. Analysis of acids and sugars from the same matrix by separate methods (Table 2, Fig. 1)

These separate determinations of sugars and acids, nowadays, have been substituted by really simultaneous chromatographic processes, which means separation from the same solution, by a single injection [3–27,34–43]: however, these separate methods [1,2,28–33] are still used in practice and, consequently, are worthy to be dealt with. The main peculiarities of these procedures are their high cost and time consumption. Elutions have been performed with the extracts of samples, except for the diluted matrix of watermelon [30,31]. The methods of extractions were particularily complicated (Table 2, second vertical column) also in comparison to the recent proposals. The advantage of GC–flame ionization detection (FID), even in one of the earliest proposal, is obvious: excellent separation has been obtained for 14 acids within 20 min ([1], Fig. 1), while performing the task by HPLC, an additional seventeen years later, furnished only tentative separation for 8 acids, within 18 min ([33], Fig. 2). In spite of the 30 cm long HPLC columns, due to the isocratic elutions, they result in the separation of a few sugars, or a few acids only (Table 2, last vertical column). Concerning separate detections in HPLC, commonly, for the sugars a refractive index (RI) detector while for the acids a UV detector was used [29–33]. The only exception [29] was the analysis of the sugars and acids in tomato juice: according to

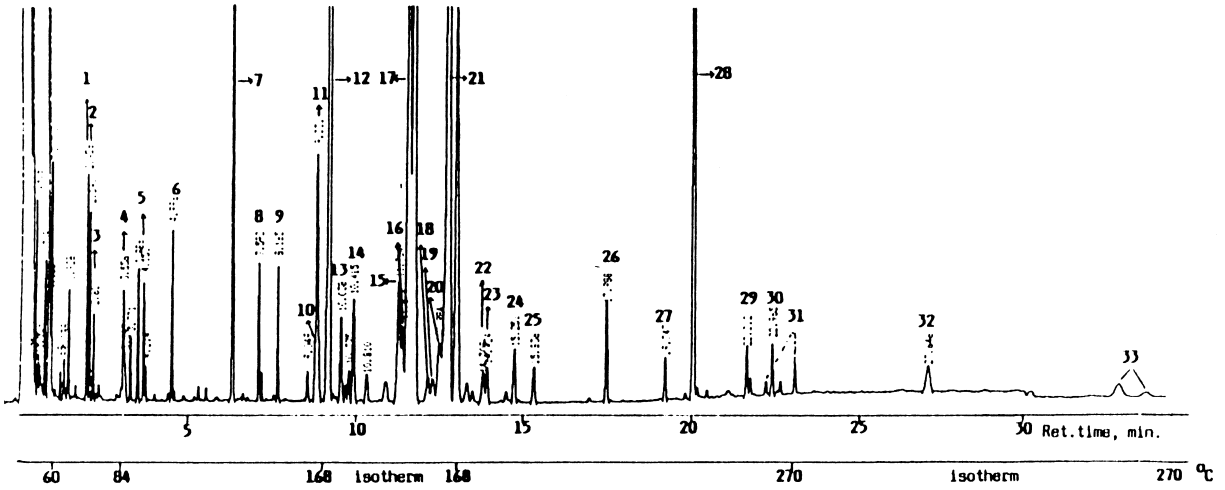


Fig. 3. Simultaneous GC–FID quantitation of the TMS/TMS-oxime derivatives of acids and sugars obtained from a model solution: (1) glycolic; (2) lactic, (3) oxalic; (4) sorbic; (5) benzoic; (6) succinic; (7) malic; (8) pimelic; (9) tartaric acids; (10) arabinose; (11) xylose; (12) citric+isocitric acids; (13) rhamnose; (14) quinic acid; (15) mannitol; (16) sorbitol; (17) fructose; (18) ascorbic acid; (19) galactose; (20) mannose; (21) glucose; (22) palmitic; (23) caffeic; (24) linoleic; (25) stearic; (26) arachidic; (27) behenic acids; (28) sucrose; (29) maltose; (30) chlorogenic acid; (31) isomaltose; (32) raffinose; (33) maltotriose. From Ref. [7] with permission, ©Vieweg.

this proposal both groups have been determined, separately, by RI detection.

3. Simultaneous analysis of acids and sugars from one solution by a single injection

3.1. Gas chromatographic methods

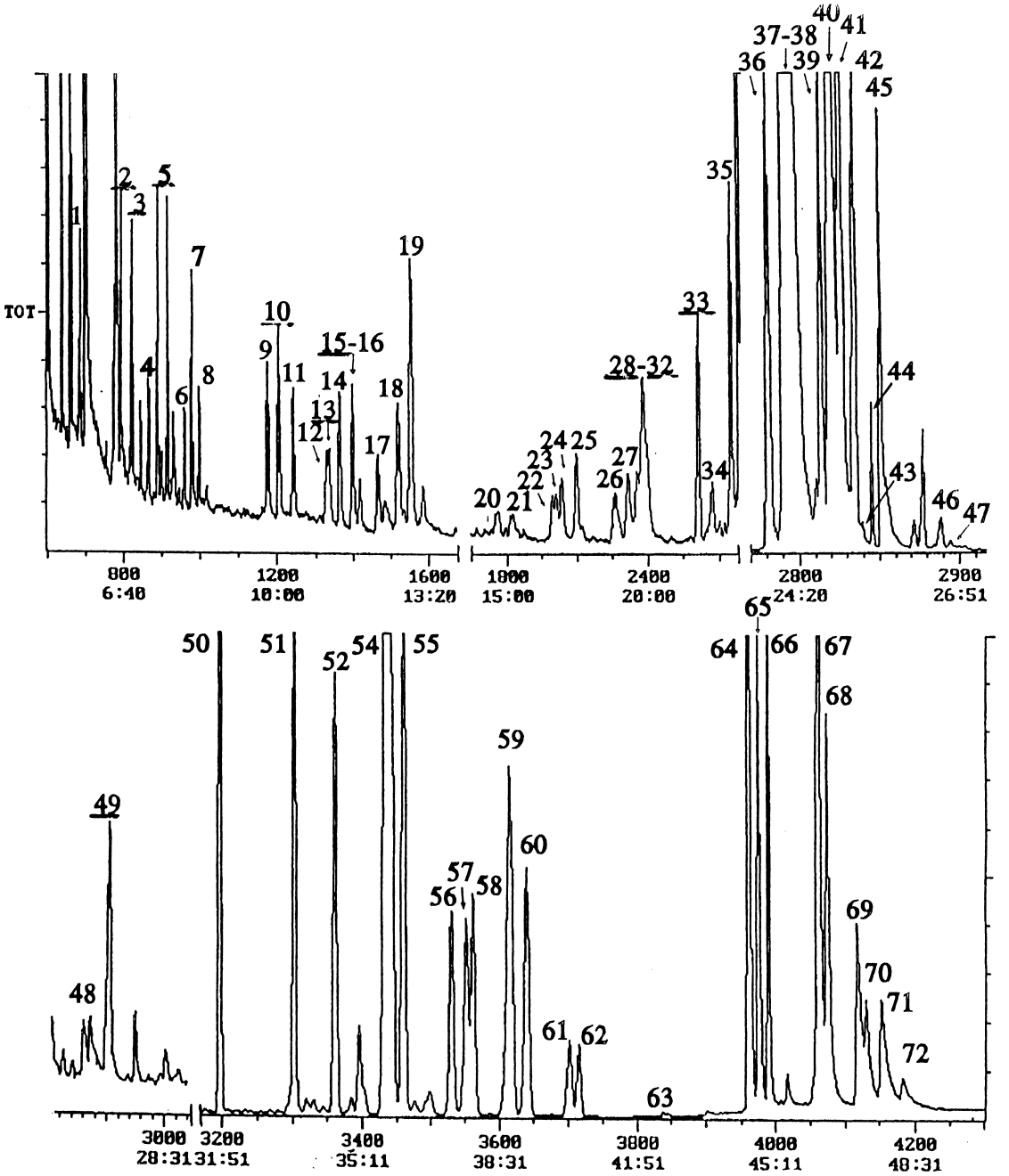
3.1.1. Simultaneous GC analysis with flame ionization detection (Table 3, Fig. 3)

The first simultaneous determination of three sugars and two acids, within 10 min and with a spectacular separation on a short packed column [3] (Table 3) was developed, on the basis on Brobst's proposal [44] (who advised direct silylation of sugars, in the presence of the matrix of corn syrup, with hexamethyldisilazan+trifluoroacetic acid), in a single step. The optimization of chromatographic conditions [5] using packed columns, resulted in the separation of 3 sugars and nineteen acids, simultaneously. The use of capillaries [4,6–11] extended the number of sugars and acids that have been determined in a single run, as well as, allowing the identification of trace amounts of aromatic carboxylic acids in the presence of an enormous excess of sugars [7] (Fig. 3). Based on the discovery that silylated solutions of acids and sugars can be evaporated, without irreversible changes, allowing the quantification of compounds in the concentration range of 0.001–60% (calculated on the dry matter content of the fruit matrices) [8].

3.1.2. Simultaneous GC analysis with MS detection (Table 4, Figs. 4 and 5)

In the first applications of the mass selective detector its advantages had not been fully utilized [12–15] since the amounts of constituents have been calculated on their total ion current (TIC) values, exclusively. However, identification on the basis of fragment ions, were reported for cinnamic acid in propolis [12], for citric acid in the skin of apple fruit [13] and for sugars in sweet potato extracts [14].

Quantitation of selective ion monitoring (SIM) was reported for the identification and quantitation of the TMS derivatives obtained from urine samples: sugars, acids, amino acids and amines [16] have been measured on the basis of one or two of their main fragment ions, not necessarily on the ion of highest intensity. Reproducibility studies on the linear responses of these fragment ions was not presented [16].



Basic studies with citrus fruits, [18] revealed that the selective fragment ion(s) (SFIs) for citric/isocitric acids appeared with $m/z=273$ ([M–TMSCOO–TMSOH]$^+$), with $m/z=347$ ([M–TMSO–COO]$^+$) and with $m/z=375$ ([M–TMSOH–CH_3]$^+$), respectively. In the case of honeys citric- and isocitric acids are present in commensurable concentrations [25].

Recently [17–27], exhaustive GC–MS studies [22–27] were performed with various minor com-

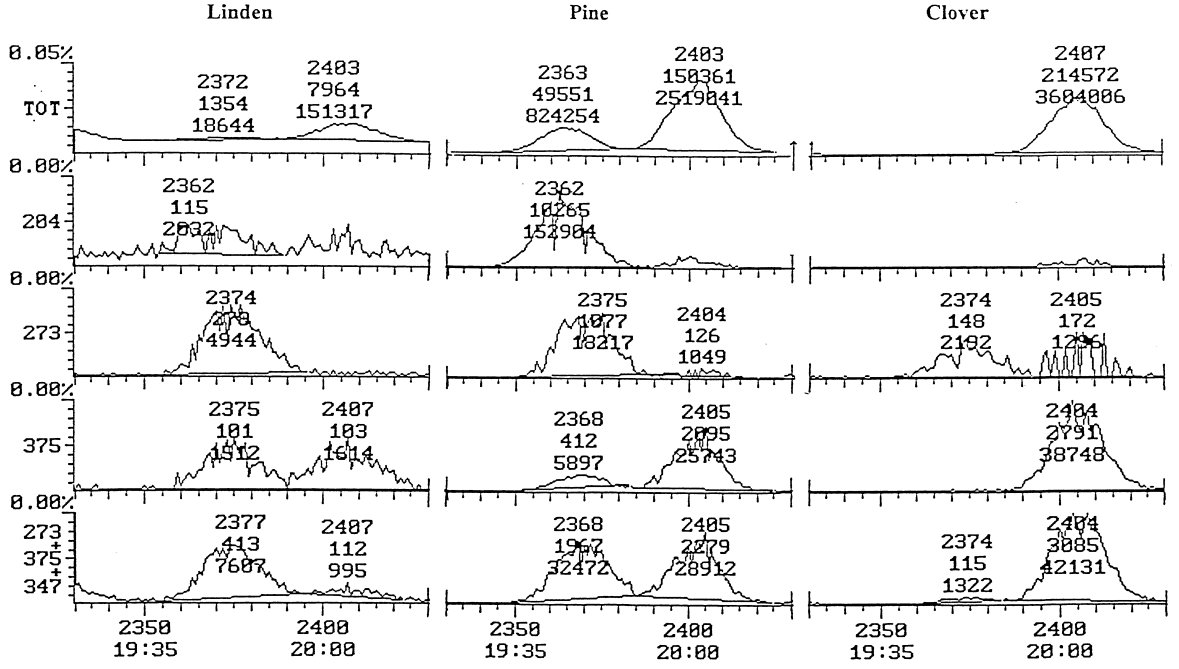


Fig. 5. GC–MS quantitation of the shikimic, citric and isocitric acid contents of honeys determined on the basis of their SFI values [25]: selected parts of chromatograms (2330–2430 scans) obtained from SFI values characteristic for shikimic (SFI, $m/z=204$), citric (SFI, $m/z=273$), (isocitric SFI, $m/z=375$), and for the total of citric/isocitric acids (SFIs, $m/z=273+347+375$). Samples: linden, pine and clover honeys. From Ref. [25] with permission, ©Vieweg.

pounds, possible constituents of fruits [17–21,27], mushrooms [22], honeys [21,25] etc., including a number of acids, members of various homologous series. Fragmentation patterns and quantitation studies of thirty-four aromatic- [23,24], fourteen aliphatic acids, *ortho*-phosphoric acid, HMF (hydroxymethylfurfural) and proline [24], in the presence of an enormous excess of saccharides of different degrees of polymerization (DP), also provided practicable, utilizable results (Figs. 4 and 5). On the basis of these experiences minor acids, together with the very important HMF and proline, have been determined quantitatively. Also, in those cases where they could not be resolved completely from their neighbours, (Fig. 4, compounds: 12 from 13, 15 from 16, 22–24 and 28–32 from each other, etc.), they could be evaluated by means of their SFI values, at the low ng concentration level, in the presence of each other (Fig. 5) [24,25].

The determination of three, not resolved, minor compounds, such as shikimic-, citric- and isocitric acids (Fig. 4, peaks 28 and 32, i.e.,), were performed

Fig. 4. Simultaneous GC–MS of acids, sugars, HMF and proline as their TMS/TMS-oxime derivatives, obtained from a model solution: (1) pyruvic; (2) benzoic; (3) *ortho*-phosphoric; (4) phenylacetic; (5) succinic; (6+7) levulinic; (8) β-phenylbutyric; (9) mandelic; (10) malic; (11) salicylic; (12) cinnamic acids; (13) 5-hydroxymethylfurfurol; (14) 3-hydroxybenzoic acid; (15) proline; (16) β-phenyllactic; (17) 3-hydroxyphenylacetic; (18) 4-hydroxybenzoic; (19) 4-hydroxyphenylacetic; (20) 3,5-dimethoxybenzoic; (21) veratric; (22) 2,6-dihydroxybenzoic; (23) 3-(4-hydroxyphenyl)propionic; (24) vanillic; (25) 2,5-dihydroxybenzoic; (26) o-coumaric; (27) 2,4-dihydroxybenzoic; (28) shikimic; (29) protocatechuic; (30) 4-methoxycinnamic; (31) 3,5-dihydroxybenzoic; (32) citric+isocitric; (33) quinic; (34) azaronic; (35) syringic acids; (36) mannitol; (37) 4-hydroxycinnamic acid; (38) fructose; (39) galactose; (40) galactose+glucose; (41) glucose; (42) galacturonic; (43) palmitic; (44) glucuronic acids; (45) inozitol+ferulic; (46) caffeic; (47) margaric; (48) oleic; (49) stearic acids; (50) sucrose; (51) trehalose; (52,53) cellobiose; (54) turanose+maltose; (55) maltose; (56) palatinose; (57) gentiobiose; (58) palatinose; (59) gentiobiose+melibiose; (60) isomaltose; (61) melibiose; (62) isomaltose; (63) chlorogenic acid; (64) raffinose; (65) erlose; (66) melezitose; (67,68) maltotriose; (69) panose; (70) isomaltotriose; (71) panose; (72) isomaltotriose. From Ref. [25] with permission, ©Vieweg.

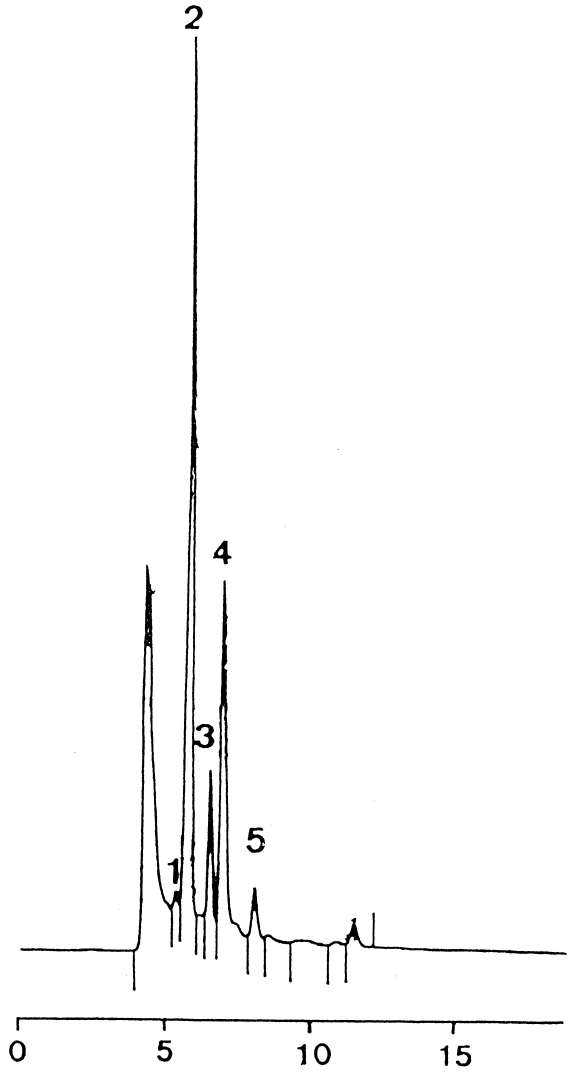


Fig. 6. Simultaneous HPLC separation of the constituents of a grape must using 0.013 *M* H_2SO_4 as the mobile phase and UV detection [34]: (1) citric; (2) tartaric; (3) malic acids; (4) fructose; (5) succinic or shikimic acid. From Ref. [34] with permission, ©ACS.

on the basis of their SFI values for linden-, pine- and clover honeys (Fig. 5) [18,23–25]. The tiny amounts of shikimic/citric/isocitric acids, covered by unknown impurities, indicated by the total of ions (TOT) does not reflect the presence of these three acids (Fig. 5, TOT, first display line). But, SFIs for shikimic acid (Fig. 5, second display line, at 2362 scans, by $m/z=204$ $[(TMSO–CH=CH–OTMS)]^+$), for citric acid (Fig. 5, third display line, at 2372–2377 scans, by $m/z=273$), for isocitric acid (Fig. 5, forth display line, at 2403–2407 scans, by $m/z=375$), and, for the total of citric/isocitric acids (Fig. 5, fifth display line, by mz=273+347+375, all three present in the citric/isocitric acid spectra), made possible their separate, quantitative evaluation [25].

3.2. Simultaneous analysis by HPLC

3.2.1. Simultaneous HPLC analysis with a single detection method (Table 5, Figs. 6, 7)

In the HPLC determination of acids and sugars simultaneously, both UV [34,35] and RI [36,37] detection were used. In spite of the consecutive steps requiring sample-preparation, the frequent change of the guard columns proved to be also obligatory: resulting in high cost of the method. The separation of compounds is poor (Figs. 6 and 7). Comparing RI and UV detection, in this particular case, out of these two possibilities, UV seems to be the preferred detection method.

3.2.2. Simultaneous HPLC analysis with separate detection methods (Table 5, Figs. 8, 9)

The comparison of methods described in papers [38–41,43] seems to be very simple because, with one exception [40], the same coupled detection methods (UV and RI), the same column (Aminex $HPX86H^+$, 300×7.8 mm), the same eluent H_2SO_4 (0.65–26 m*M*), while, various elution temperatures were selected. Evaluating the resolution of compounds it can be stated that the lower the temperature of elution the better the separation. However, none of the separations proved to be suitable for the quantitation of neighbouring compounds being present in not comensurable and/or in extremely different concentrations. Comparing conductivity (Cond) and pulsed amperometric (PAD) detection ([42], Fig. 8) with the RI/UV couple ([43], Fig. 9) the advantage of the Cond/PAD pair is obvious: in terms of selectivity (excellent resolution on both panels of Fig. 8) and sensitivity, equally.

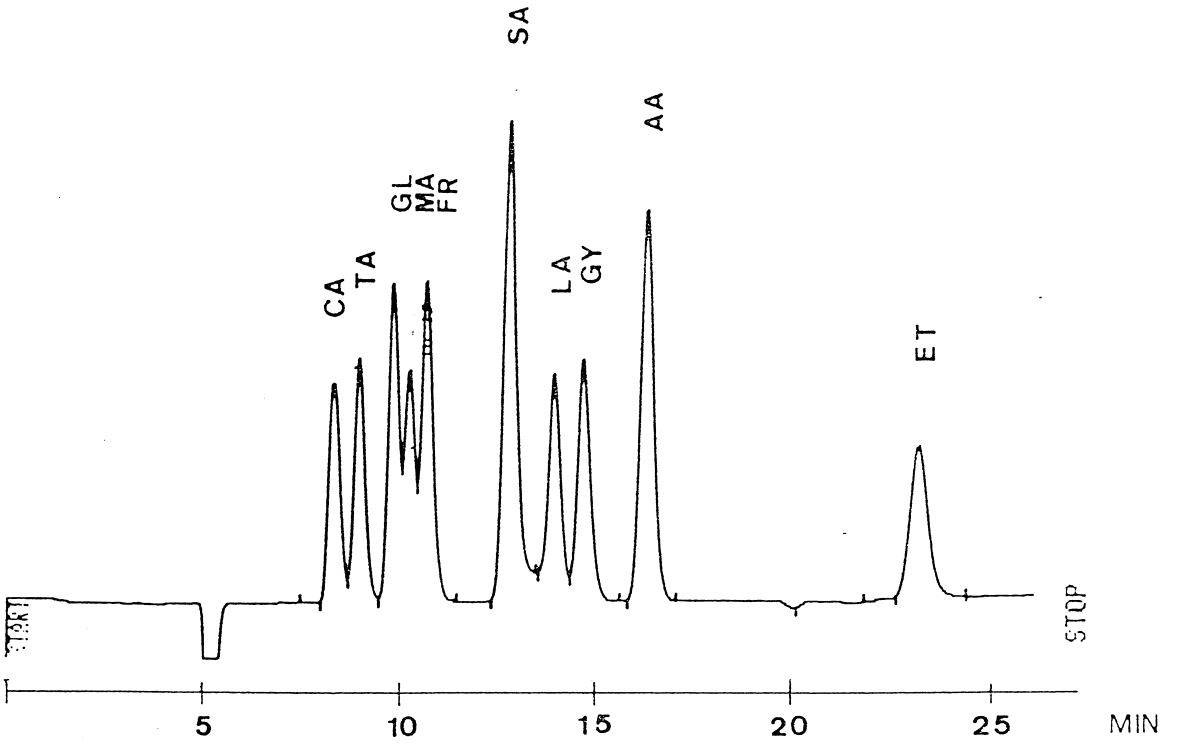


Fig. 7. Simultaneous HPLC separation of the sugar, acid and ethanol constituents of a standard solution using 0.01 *M* H_2SO_4 as the mobile phase and RI detection [37]: CA, citric; TA, tartaric acids; GL, glucose; MA, malic acid; FR, fructose; SA, succinic; LA, lactic acids; GY, glycerol; AA, acetic acid; ET, ethanol. From Ref. [36] with permission, ©Elsevier.

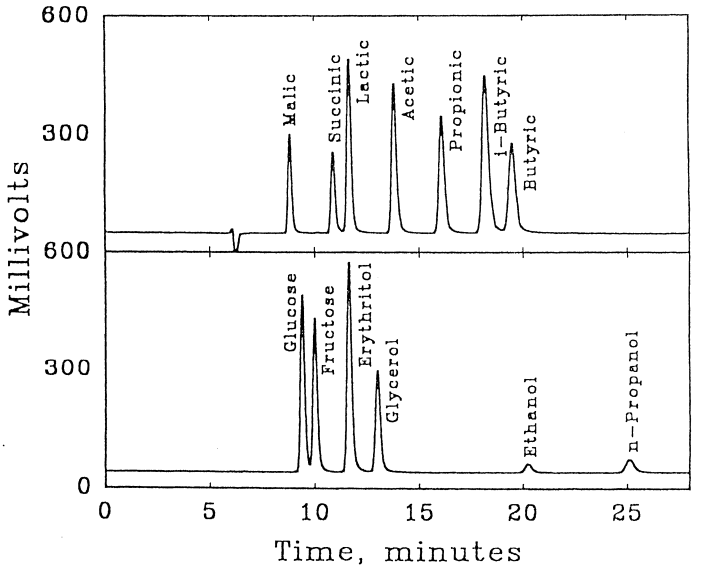


Fig. 8. Simultaneous HPLC separation of a standard solution of acids and sugars using as mobile phase 1.6 m*M* heptafluorobutyric acid and conductivity (upper panel) and pulsed amperometric (lower panel) detections [42]. From Ref. [42] with permission, ©ACS.

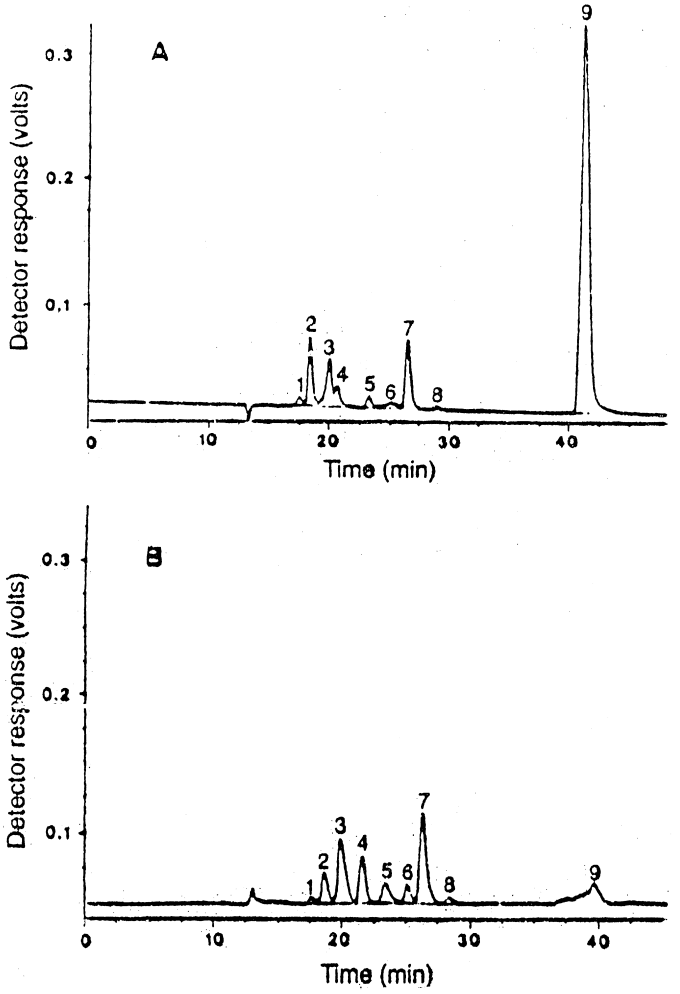


Fig. 9. Simultaneous HPLC separation of a standard solution of acids and sugars using as mobile phase 0.65 m*M* H_2SO_4 and RI (A panel) and UV (B panel) detections [43]: (1) citric; (2) tartaric; (3) malic acids; (4) fructose; (5) lactic; (6) succinic acids; (7) glycerol; (8) acetic acid; (9) ethanol. From Ref. [43] with permission, ©Preston.

4. Conclusion

Comparing, in general, GC and HPLC on the basis of their efficiencies, time and cost requirement this paper was intended to give a compilation. The widespread believed advantage of the HPLC methods i.e., the immediate separation of acids and sugars in their underivatized, free forms proved to be its largest obstacle: resulting in the low selectivity of the separations.

In summary it can be stated that in this particular case, i.e., in the simultaneous analysis of (1) acids, members of different homologous series, (2) sugars of various degree of polymerization and (3) sugars/sugar alcohols of different chain length, from one solution by a single injection GC provides: (i) better selectivity, (ii) higher sensitivity, (iii) the separation and quantitation of considerably higher number of constituents, being present in extremely various amounts (10^{-4}–$\geq$ 50%), on the same column, with the same detector, (iv) for a lower cost.

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