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Rapid Separation of Soft Drinks Ingredients using High Performance Liquid Chromatography

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ABSTRACT

A method is described which uses high performance liquid chromatography in the reverse phase mode to separate amaranth, quinoline yellow, quinine sulphate, sunset yellow, caffeine, aspartame, saccharin, vanillin, sorbic acid, benzoic acid and green S in 4 min in samples of soft drinks. The final separation was achieved using 17·5% acetonitrile, 12·5% methanol, 70% buffer (0·85% v/v sulphuric acid and 17·5% mM potassium dihydrogen orthophosphate in water with the pH adjusted to 1·8), with a flow rate of 1·35 ml/min through a 100 mm × 4·6 mm column containing 3 μm Spher-isorb octylsilane. The octylsilane material is fully capped with monolayer coverage and a carbon loading of 6% w/w, pore diameter of 8 nm and a surface area of 220 m^2/g. This material was used in preference to the more widely used octadecylsilane as it provides shorter analysis time and allows for the use of more polar eluting solvents. Detection was at 220 nm, 0·1 AUFSD with an injection of 5 μl samples using a rotary injection valve. The standard solution concentration was 100 mg $litre^{-1}$ for aspartame; 20 mg $litre^{-1}$ for quinine sulphate, caffeine, vanillin, sorbic acid and benzoic acid and 5 mg $litre^{-1}$ for amaranth, quinoline yellow, sunset yellow, green S, tartrazine, indigo carmine and carmoisine.

INTRODUCTION

The cited literature gives examples of the separation of sorbic acid and sodium benzoate in fruit juices (Bennet & Petrus, 1977; Archer, 1980),

saccharin, sorbic acid and benzoic acid (Leuenberger *et al.*, 1979), saccharin, benzoic acid and caffeine (Woodward *et al.*, 1979) and, finally, the determination of quinine in soft drinks (Jeuring *et al.*, 1979). The analysis times ranged from 5·5 min to 13 min.

An HPLC method to analyse soft drinks for quinine sulphate, caffeine, saccharin and benzoic acid in 8 min had been available for some time in the Britvic laboratories. It then became necessary to determine two additional artificial sweeteners, as well as vanillin and sorbic acid, in one chromatographic separation.

This proved to be a difficult separation to achieve in a reasonable time. The shortest analysis took 18 min, which was not acceptable for routine use and was therefore only carried out on specified samples. Work was undertaken to reduce this analysis time, in order that the method could be used routinely to quantify the quinine sulphate, caffeine, aspartame, saccharin, vanillin, sorbic acid and benzoic acid levels in soft drink samples.

The method was then used in the Central Quality Control laboratories to monitor the manufacture of soft drink bases, syrups and finished products.

MATERIALS AND METHODS

Chemicals

HPLC grade acetonitrile and methanol were purchased, together with Puriss grade quinine sulphate, from Koch Light (Haverhill, Suffolk). Benzoic acid, caffeine, potassium dihydrogen orthophosphate, sodium saccharin, sulphuric acid, vanillin and HiPerSolv water were from B.D.H. Chemicals (Poole, Dorset). Aspartame was from G. D. Searle and sorbic acid was purchased from Sigma Chemical Company (Poole, Dorset). The synthetic food dyes (amaranth, quinoline yellow, sunset yellow, green S, tartrazine, indigo carmine and carmoisine) were from Pointing Limited (Prudhoe, Northumberland).

Preparation of standards

Stock solutions of 1000 mg litre^{-1} saccharin, caffeine, sorbic acid, benzoic acid, amaranth, quinoline yellow, sunset yellow, green S, tartrazine, indigo carmine, carmoisine and 5000 mg litre^{-1} aspartame were prepared by dissolving 1000 mg of each (except aspartame where 5000 mg was taken) with 1000 ml of distilled water. 1000 mg litre^{-1} of quinine sulphate was prepared by dissolving 1000 mg into approximately 500 ml of 0·5% v/v

sulphuric acid; the volume was then made up to 1000 ml with distilled water. 1000 mg litre^{-1} vanillin was prepared by dissolving 1000 mg into approximately 500 ml of 10% v/v methanol in distilled water; the volume was then made up to 1000 ml with distilled water. The stock solutions were then filtered through a Whatman GF/C glassfibre filter to remove any particulate matter.

Preparation of eluting solvents

All eluting solvents were prepared using HPLC grade acetonitrile and methanol, which were added to a previously prepared buffer solution comprising 17·5 mM potassium dihydrogen orthophosphate in 0·85% v/v sulphuric acid in HiPerSolv water. The eluting solvents were passed through Whatman GF/C filters into screw-top reagent bottles and thoroughly de-gassed in a sonic bath.

Preparation of samples

The carbonated soft drinks were de-gassed in the sonic bath and diluted 5:1 with 25% v/v methanol using an autodiluter into 1·5 ml plastic centrifuge tubes. The tubes were then spun for 4 min at 12000 rpm (9980 *G*) in a mini centrifuge in order to precipitate any fruit cell or other particulate matter.

Instrumentation

Two HPLC systems were used. The first, on which the majority of the optimisation was carried out, consisted of an Altex 110A pump with pulse dampener and solvent de-bubbler fitted, a Rheodyne 7125 rotary injection valve with a 5 μl sample loop. The columns used were 250 mm × 4·6 mm stainless steel packed with 5 μm Spherisorb octylsilane and a 100 mm × 4·6 mm stainless steel packed with 3 μm Spherisorb octylsilane supplied by HPLC Technology (Macclesfield, Cheshire). Detection was made using a Pye Unicam LC–UV variable wavelength detector with a standard 8 μl flow cell. The output of the detector was connected to a Pye Unicam twin pen PM8252A chart recorder.

The second system, on which the fine tuning of the optimisation was carried out, consisted of an LDC Constametric III double reciprocating pump with a solvent de-bubbler and a three-way purge valve, a Rheodyne 7126 rotary injection valve with a 5 μl injection loop and an LDC Model 713 autosampler. The column used was a 100 mm × 4·6 mm stainless steel packed with 3 μm Spherisorb octylsilane material. Detection was by an

LDC Spectromonitor III variable wavelength detector with a standard 12 μl flow cell. Output from the detector was to an LDC 301 computing integrator and a Houston printer plotter. Connected in parallel to this was a Pye Unicam PM8251 single pen chart recorder.

Instrumental conditions and procedure

The first HPLC system was operated with the detector wavelength set at 220 nm with AUFSD at 0·16 and with the time constant at 0·5 s.

The pump was set at 1·50 ml/min eluting solvent flowing through a 250 mm × 4·6 mm 5 μm octylsilane column PP/16346 from HPLC Technology. A set of standard solutions (prepared from the stock solutions) containing 100 mg litre^{-1} quinine sulphate, 100 mg litre^{-1} caffeine, 500 mg litre^{-1} aspartame, 100 mg litre^{-1} saccharin, 100 mg litre^{-1} vanillin, 100 mg litre^{-1} sorbic acid and 100 mg litre^{-1} benzoic acid in eluting solvent were passsed through the system with a series of different eluting solvents (Table 1).

TABLE 1
Eluting Solvent Composition

Per cent v.v composition	*Solvent* 1	2	3	4	5
Acetonitrile	25	20	15	10	5
Methanol	5	10	15	20	25
Buffer	70	70	70	70	70
Decreasing strength i.e.	increasing solvent polarity →				

The eluting solvents were changed in the order of strongest to weakest, in order to avoid a strongly retained solute in a weak solvent interfering in a subsequent run using a stronger eluting solvent. The retention time t_R and retention of a non-sorbed substance, t_0, were recorded for three runs of each solute with the five eluting solvents.

The average value of each parameter was then taken and the capacity ratio K' was calculated for each solute in each eluting solvent (Table 2).

The log of the capacity ratio was then calculated for each case (Table 3) and a graph of log K' versus methanol % v/v in the eluting solvent was plotted (Fig. 1) using linear regression to obtain the best straight line fit between the points.

TABLE 2
Retention Times and Capacity Ratio—Variations with Solvent Strength

Eluting solvent	*1*			*2*			*3*			*4*			*5*		
	t_R	t_0	K'	t_R	t_0	K'	t_R	t_0	K'	t_R	t_0	K'	t_R	t_0	K'
Quinine sulphate	2·03	1·3	0·56	2·24	1·3	0·72	2·50	1·3	0·92	2·84	1·3	1·18	3·32	1·3	1·55
Caffeine	2·44	1·3	0·88	2·67	1·3	1·05	2·99	1·3	1·30	3·40	1·3	1·62	4·04	1·3	2·11
Aspartame	2·63	1·3	1·02	3·06	1·3	1·35	3·59	1·3	1·76	4·10	1·3	2·15	4·66	1·3	2·59
Saccharin	3·33	1·3	1·56	3·49	1·3	1·63	3·74	1·3	1·88	3·95	1·3	2·04	4·25	1·3	2·27
Vanillin	3·48	1·3	1·68	3·75	1·3	1·88	4·14	1·3	2·18	4·55	1·3	2·50	5·14	1·3	2·95
Sorbic acid	4·52	1·3	2·45	5·14	1·3	2·95	5·98	1·3	3·60	6·87	1·3	4·28	8·12	1·3	5·25
Benzoic acid	4·72	1·3	2·63	5·39	1·3	3·15	6·30	1·3	3·85	7·27	1·3	4·59	8·64	1·3	5·65

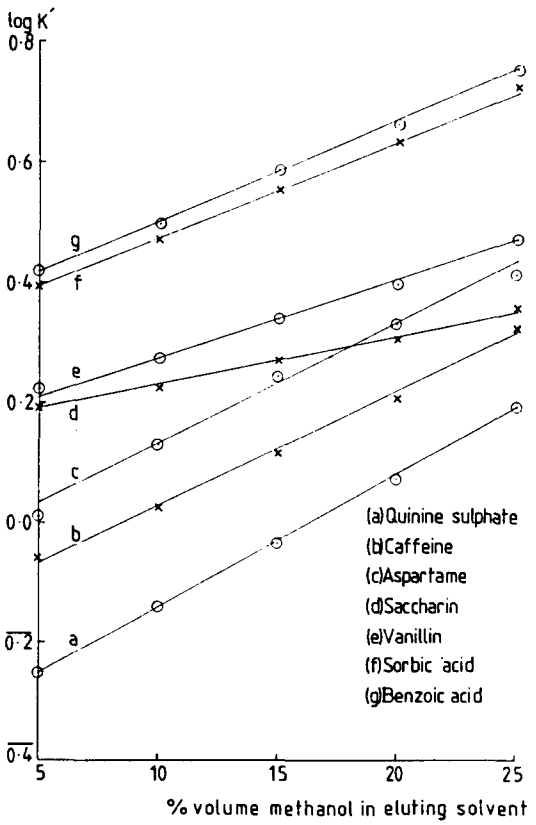

Fig. 1.

TABLE 3
Log Capacity Ratio Variation with Respect to Solvent Strength

Eluting solvent	*1*	*2*	*3*	*4*	*5*
Quinine sulphate	0·251	0·140	0·035	0·073	0·191
Caffeine	0·057	0·023	0·114	0·208	0·323
Aspartame	0·009	0·131	0·245	0·333	0·412
Saccharin	0·193	0·226	0·273	0·309	0·356
Vanillin	0·224	0·275	0·339	0·398	0·470
Sorbic acid	0·394	0·470	0·556	0·632	0·719
Benzoic acid	0·419	0·497	0·585	0·662	0·751

Where: t_R is the retention time of a solute (measured from the injection point)
t_0 is the retention time of a non-sorbed substance (measured from the injection point)
K' is the capacity ratio of a solute

$$K' = \frac{t_R - t_0}{t_0}$$

RESULTS AND DISCUSSION

The graph of log K' versus solvent composition highlighted the area in which there would be no resolution between aspartame and saccharin (i.e. at approximately 19% methanol, 11% acetonitrile, 70% buffer) and the area in which the optimum resolution between all the components lay (i.e. between 10% and 15% methanol in the eluting solvent) (Fig. 1). A sixth eluting solvent was then prepared containing 17·5% acetonitrile, 12·5% methanol and 70% buffer. Once the system had reached equilibrium with the new eluting solvent the test mixture was chromatographed, producing the expected resolution between pairs of solutes. This methodology was then transferred directly onto the second HPLC system, where a 100 mm × 4·6 mm column containing 3 μm Spherisorb octylsilane material was connected. This column exhibited approximately the same number of theoretical plates per column as the 5 μm column that was previously used. It therefore followed that an optimised system using the 3 μm column would be capable of providing sufficient resolving power in a reduced analysis time.

The system was optimised for the use of short 3 μm columns by reducing the connecting tube lengths between the injector and column and the column and detector to 2·5 cm and 2·0 cm, respectively (including ferrules

and fittings). The bore of the tubing was also reduced to the finest available which was 0·006 in ID stainless steel. The test mixture concentration was also reduced fivefold in order to reduce the possibility of overloading the column with solute and consequently reducing the observed chromatographic performance. With the eluting solvent flow rate set at 1·50 ml/min, baseline resolution between aspartame and saccharin, and saccharin and vanillin, was not achieved. The flow rate of the mobile phase was progressively reduced until baseline resolution was achieved. This was found to be at a flow rate of 1·35 ml/min using 17·5% acetonitrile, 12·5% methanol and 70% buffer. The injection volume was 5 μl onto the 100 mm × 4·6 mm column packed with 3 μm Spherisorb octylsilane. Detection was at 220 nm with AUFSD set at 0·1 (Fig. 2). The parameters of the separation are given in Table 4 showing capacity ratio, selectivity and efficiency.

Samples of soft drinks were prepared and injected into the system using the optimised conditions and examples of the application are shown in Figs 3 and 4.

Since the dyes absorb light in the ultraviolet and visible portions of the electromagnetic spectrum it was important to ensure that there was no

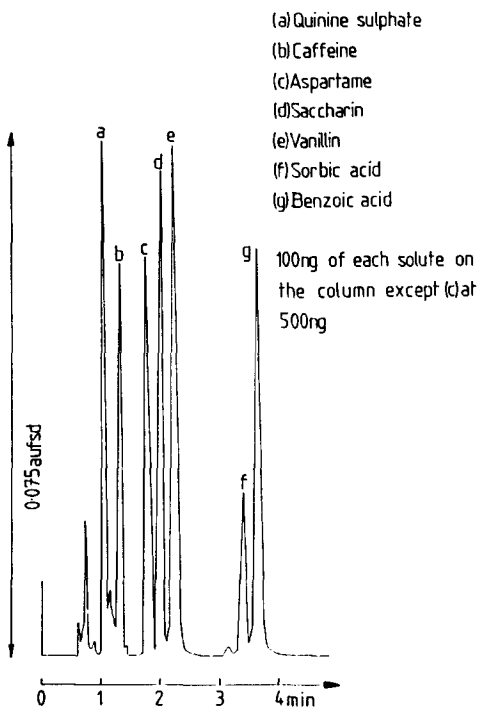

Fig. 2. Test mixture on 100 × 4·6 mm, 3 μm column.

TABLE 4
Capacity Ratio, Selectivity and Efficiency of the Optimised Separation

	t_R	t_0	t'_R	K'	*log K'*	*Plates per metre N*	*Selectivity* α
Quinine sulphate	1·03	0·625	0·40	0·64	$\overline{0·194}$	1 800	1·69
Caffeine	1·30	0·625	0·68	1·08	0·034	10 090	1·70
Aspartame	1·78	0·625	1·15	1·84	0·265	14 950	1·22
Saccharin	2·03	0·625	1·40	2·24	0·350	25 700	1·15
Vanillin	2·24	0·625	1·61	2·58	0·412	29 380	1·75
Sorbic acid	3·45	0·625	2·83	4·52	0·655	61 190	1·08
Benzoic acid	3·68	0·625	3·05	4·88	0·688	54 210	

Where $\alpha = \frac{K'_2}{K'_1}$

and K'_1 = Capacity ratio of the first member of the pair.

K'_2 = Capacity ratio of the second member of the pair.

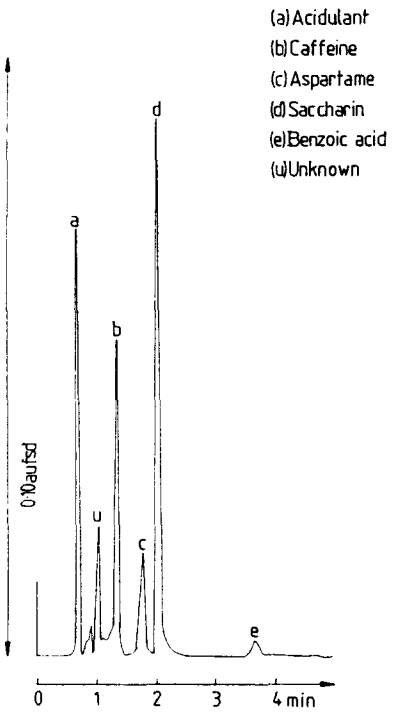

Fig. 3. Low calorie kola drink type 1 on 100 × 4·6 mm, 3 μm column.

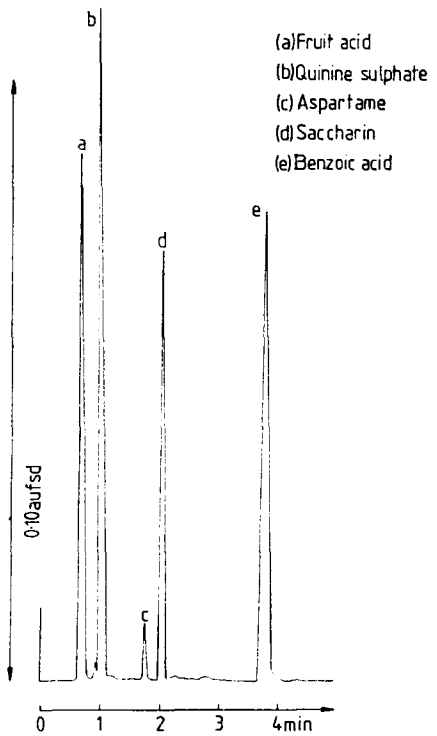

Fig. 4. Low calorie indian tonic water on 100 × 4·6 mm, 3 μm column.

TABLE 5
Capacity Ratio, Selectivity and Efficiency of Food Dyes Chromatographed in the System

	t_R	t_0	t'_R	K'	*Selectivity* α
Amaranth	0·75	0·625	0·125	0·20	2·20
Quinoline yellow	0·90	0·625	0·275	0·44	2·00
Sunset yellow	1·18	0·625	0·550	0·88	5·95
Green S	3·90	0·625	3·275	5·24	1·06
Carmoisine	4·10	0·625	3·475	5·56	1·00
Indigo carmine	4·10	0·625	3·475	5·56	1·00
Tartrazine	4·10	0·625	3·475	5·56	

interference between the dyes and the other solutes. Their behaviour was then investigated under the same chromatographic conditions. Solutions of 5 mg litre^{-1} were prepared from the synthetic dye stock solutions and chromatographed. Once the retention times of the individual dyes had been established, a seven component dye mixture was prepared containing 5 mg litre^{-1} of each of the synthetic food dyes. This test mixture was then chromatographed and the parameters recorded in Table 5. A comparison

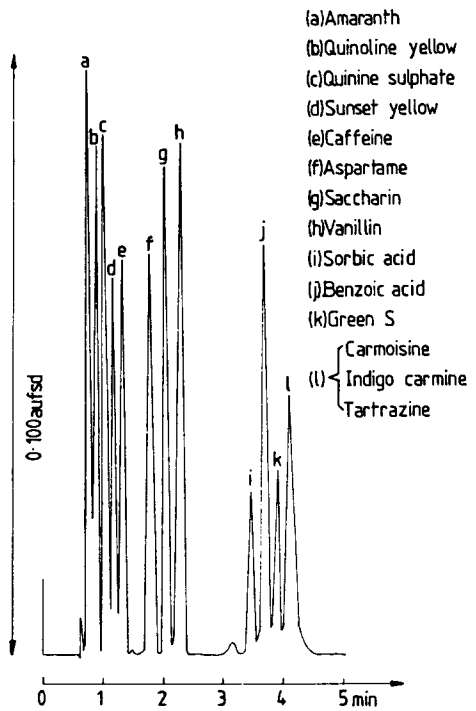

Fig. 5. Final test mixture on 100 × 4·6 mm, 3 μm column.