CHROMSYMP. 1200

Note

Gradient liquid chromatographic method for the simultaneous determination of sweeteners, preservatives and colours in soft drinks

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Quality control of soft drinks is commonly achieved by isocratic chromatography with detection at 254 nm, yet this limits precision and sensitivity for aspartame, saccharin and benzoic acid which are better detected at 214 nm[1,2].

Because the presence of colours (particularly from tropical fruit) or quinine tends to interfere with the quantitation of saccharin by such methods[1,3] there is a need to use gradient chromatography to obtain good separation of colours from the other constituents. The separation of synthetic colours has been accomplished by isocratic ion-exchange chromatography[4] or ion-pair chromatography[5]. Puttemans *et al.*[6] have reported the simultaneous separation of colours, sweeteners and preservatives by gradient reversed-phase chromatography, but this requires a lengthy extractive pretreatment of the sample with limited recovery of sweeteners and preservatives. We therefore developed a gradient reversed-phase separation with detection at 214 nm, which requires minimal sample pretreatment and would allow the quantitation of all the components of interest.

EXPERIMENTAL

High-performance liquid chromatography

The liquid chromatograph used was a Philips Analytical PU4100 system, Incorporating the PU4110 variable-wavelength detector and the PU4700 autojector (Philips Analytical, Cambridge, U.K.). The detector was connected to a PU4810 computing integrator and to a PM8252 dual-pen recorder (Philips Analytical). Peak purity was assessed using a PU4021 diode-array detector in conjunction with a PU4850 Data station (Philips Analytical). A 250 × 4.6 mm I.D. stainless-steel Spherisorb-5 ODS column (Philips Analytical) was used. The binary mobile phase was methanol with 50 mM phosphate buffer at pH 3.6 After a 3-min equilibration with 10% methanol, an increase to 60% methanol in 10 min provided the gradient, and a 2-min isocratic elution with 60% methanol cleaned the column in preparation for the next injection.

Reagents and samples

AnalaR-grade saccharin (dihydrate), benzoic acid, phosphoric acid and sodium dihydrogenphosphate were purchased from BDH (Poole, U.K.), HPLC-grade methanol and water were obtained from Rathburns (Peebleshire, U.K.). The pH of

the buffer was measured using a Philips PW9420 pH meter. A Sonicor (New York, NY, U.S.A.) sonic bath was used to degas the soft drinks and Whatman 1.2 μm glass-fibre filters were used to filter all soft drinks and soft drinks matrices, many of which were provided by Cadbury Schweppes (Dollis Hill, London, U.K.).

Solutions of the reference compounds (1 mg/ml) were prepared in HPLC grade water and stored at 4°C.

Efficient quality control requires a minimum of sample preparation. The soft drinks were degassed by sonication and filtered before direct injection of 10 μl into the chromatograph. Dilution of high sugar content soft drinks was necessary to prevent jamming of the syringe.

RESULTS AND DISCUSSION

Perchloric acid is often used for ion suppression in soft drink analysis, but because this acid is a strong oxidant and particulary corrosive, it was replaced by phosphate buffer in the present work.

Detection at 254 nm is commonly employed despite the low sensitivity to certain components. Fig. 1a shows the chromatogram obtained from a mixture of stan-
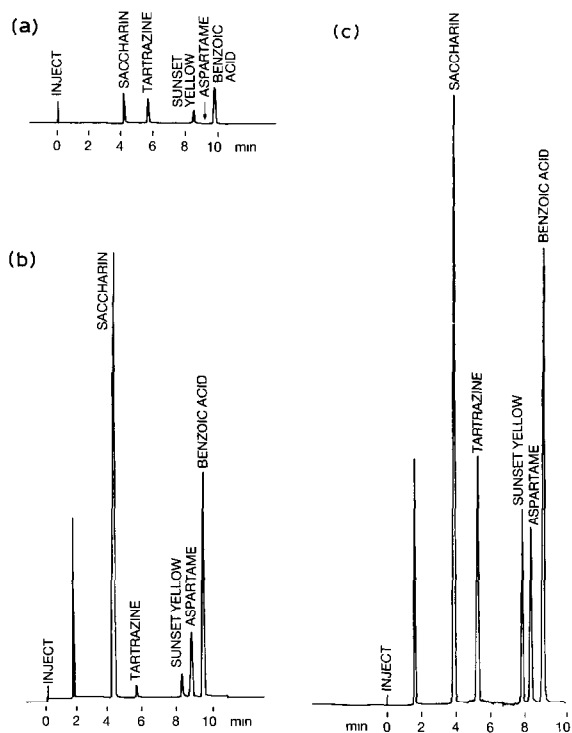

Fig. 1. Comparison of detection wavelengths. (a) 254 nm, attenuation 1.0 a.u.f.s.; (b) 214 nm, attenuation 1.0 a.u.f.s.; (c) programmed wavelength and attenuation: 4.8 min, 207 nm, 1.0 a.u.f.s.; 2.2 min, 423 nm, 0.1 a.u.f.s.; 1.5 min, 485 nm, 0.1 a.u.f.s.; 0.7 min, 210 nm, 0.5 a.u.f.s.; 1.8 min, 225 nm, 1.0 a.u.f.s.
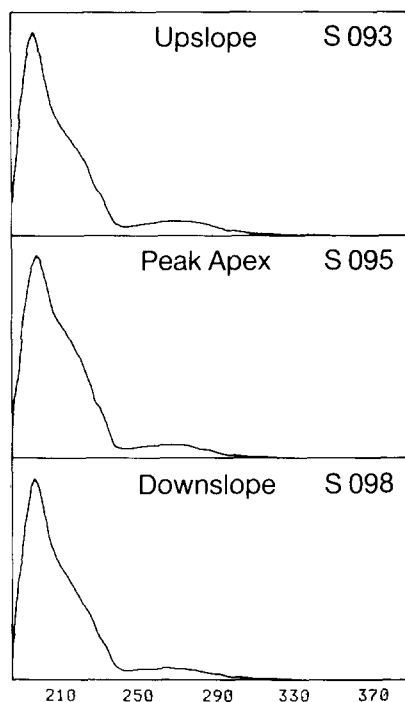
Fig. 2. Peak purity of saccharin derived from fruit drink. UV spectra were taken at three points in the peak, *i.e.* at retention times of 93 s (S 093), 95 s (S 095) and 98 s (S 098).

dards monitored at 254 nm. Aspartame demonstrates an almost negligible response. Fig. 1b shows the improved sensitivity obtained by using a detection wavelength of 214 nm. Fig. 1c shows further improvement in sensitivity when programmed wavelength and absorbance are used. However, monitoring at 214 nm provides adequate sensitivity and simplicity.

Peak purity is an important consideration in quantitation. It can be assessed

TABLE I

COMPONENT RECOVERY AND REPEATABILITY FOR THE ANALYSIS OF SOFT DRINK ADDITIVES

C.V. = Coefficient of variation; $n = 5$.

Analyte	Fruit drink			Tonic		
	Claim level (mg/l)	Recovery (%)	C.V.	Claim level (mg/l)	Recovery (%)	C.V.
Saccharin	74	101.2 ± 0.9	0.67	120	100.8 ± 0.7	0.60
Tartrazine	15.35	77.3 ± 2.7	1.3			
Sunset yellow	10.96	81.5 ± 1.0	0.76			
Aspartame				84	99.4 ± 1.6	0.54
Benzoic acid	150	103.4 ± 0.9	0.15	150	102.3 ± 2.8	1.32

TABLE II

LINEARITY OF RESPONSE OF SOFT DRINK ADDITIVES IN BLANK MATRIX OF WATER

y = (area of peak) $\cdot$ 10^5. x = percentage claim. n = 3.

Analyte	Sample	Linearity of response	
		Matrix	Water
Saccharin	Fruit drink	$y = 0.4099x + 1.0800$ ($r = 0.999$)	$y = 0.3965x + 0.5003$ ($r = 0.999$)
	Slimline tonic	$y = 0.5584x + 0.5884$ ($r = 0.999$)	$y = 0.5629x + 0.1823$ ($r = 0.999$)
Benzoic acid	Fruit drink	$y = 0.4500x + 0.2942$ ($r = 0.999$)	$y = 0.4534x + 0.2155$ ($r = 0.999$)
	Slimline tonic	$y = 0.3120x + 0.7035$ ($r = 0.999$)	$y = 0.3086x + 0.6182$ ($r = 0.999$)
Aspartame	Slimline tonic	$y = 0.1137x + 0.0507$ ($r = 0.999$)	$y = 0.1151x + 0.0654$ ($r = 0.999$)
Tartrazine	Fruit drink	$y = 0.0212x - 0.2861$ ($r = 0.996$)	$y = 0.0252x + 0.0308$ ($r = 0.997$)
Sunset yellow	Fruit drink	$y = 0.0333x - 0.2441$ ($r = 0.993$)	$y = 0.0403x + 0.0755$ ($r = 0.999$)

by obtaining spectra across an eluted peak for comparison. Fig. 2 shows spectra taken across a saccharin peak derived from orange and passion fruit drink. No changes in the profile of the spectra were seen, indicating high peak purity. Similar
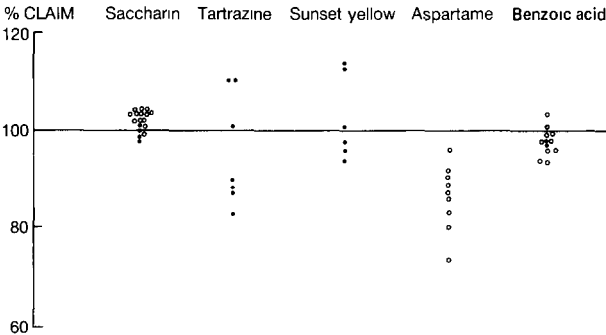

Fig. 3. Batch-to-batch variation of soft drink additives. Each point represents the mean of a triplicate determination on a unique batch of soft drink.

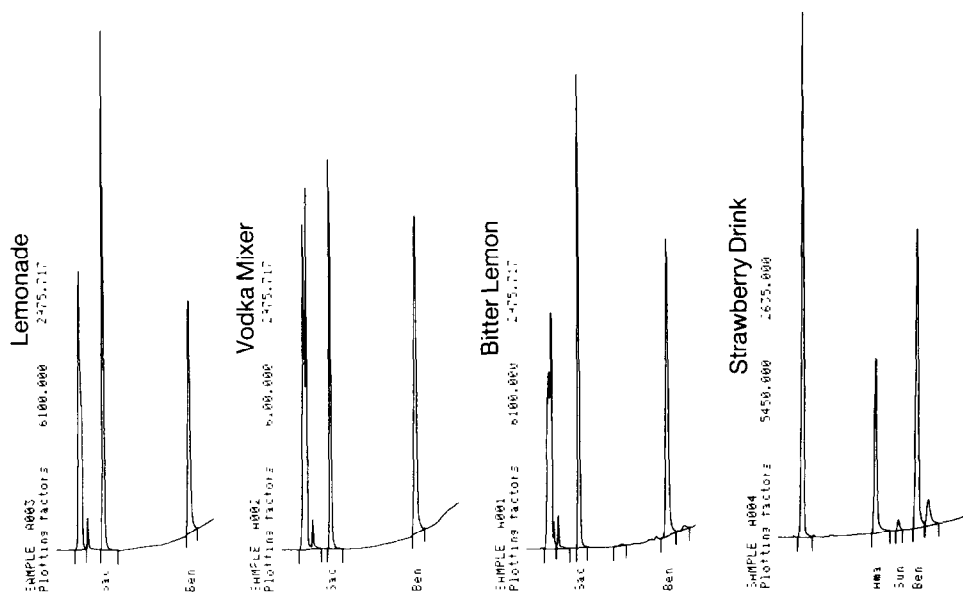| Analyte	Precision of assay (C.V.)		Variation in composition (C.V.)
	Tonic (○)	Orange and passion fruit (●)	
Saccharin	0.60	0.67	1.95
Tartrazine	—	1.30	11.39
Sunset yellow	—	0.76	7.90
Aspartame	0.54	—	6.96
Benzoic acid	1.32	0.15	3.35

Fig. 4. Chromatograms derived from a range of soft drinks. Sac = saccharin; Ben = benzoic acid; Sun = sunset yellow.

procedures were applied to all components under analysis, and peak purity confirmed.

Repeatability and component recovery was determined from quintuplicate injections of a mixture of standards at the claim level in water and spiked in an additive free soft drink matrix. The results are shown in Table I.

The linearity of response of standards in water or soft drink matrix was investigated using six levels ranging from 22 to 175% of claim. All values used to generate the linear regression equations (Table II) were the mean of triplicate determinations. The response was found to be linear for saccharin, aspartame and benzoic acid across the range of supplementation, whether water or matrix were used as the injection medium. The matrices had no effect on recovery of saccharin, aspartame or benzoic acid and consequently, quantitation is possible by calibration with a single aqueous standard. The response from tartrazine and sunset yellow was again linear over the range of supplementation in both water and matrix. However, the presence of matrix reduced the recovery considerably (77.3 ± 2.7% for tartrazine and 81.5 ± 1.0% for sunset yellow, see Table I). This reduced recovery appeared to be due to adsorption on the glass-fibre filter itself or on suspended particles retained by the filter. Quantitation is therefore only possible by multilevel calibration with standards in matrix. Binding of dyes by food matrices is well known and may be overcome by enzymic[7] or extractive[8] pretreatment of the sample, but for quality control the speed of direct injection outweighs any gain in precision from such pretreatment.

A number of bottles of the diet tonic and orange and passion fruit drink from different batches were assayed (mean of triplicate determination). The batch-to-batch variation was found to be much greater than the precision calculated from quintuplicate injections of standards in matrix (Fig. 3).

All values for aspartame were found to be below claim level, and a weak correlation ($r^2 = 0.54$) was found between expiry date and assay value, due perhaps, to the widely recognised labile nature of aspartame.

Chromatograms of several soft drinks are presented (Fig. 4) to illustrate the wide applicability of the method. The early eluted components appear to derive from the fruit ingredients and are well resolved from the analytes of interest.

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