DETERMINATION OF BENZOIC ACID IN CHILLI SAUCE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

J.S. CHIA* and Z. BADRIAH*

RINGKASAN

Satu kaedah untuk penentuan asid benzoik dalam sos cili dengan kromatografi cecair tekanan tinggi (HPLC) menggunakan turus Hewlett Packard RP–8 dan 5% metanol dan metanol (50:50) sebagai fasa bergerak diterangkan. Ia dikesan pada 254 nm dengan menggunakan pengesan jarak gelombang pemboleh-ubah. Pemulihan 81.5% ± 2.11% telah didapati apabila 5 µg asid benzoik standard digunakan dalam kajian ini. Persamaan yang baik didapati antara hasil daripada penggunaan HPLC dan kaedah penyulingan wap, kecuali sedikit gangguan yang mungkin disebabkan oleh asid volatil dalam hasil keputusan yang lebih tinggi yang diperolehi dengan kaedah penyulingan wap.

INTRODUCTION

Advancements in methodology and instrumentation have made high performance liquid chromatography (HPLC) a very powerful tool for the analysis of food components and additives. High performance liquid chromatography methods for the determination of benzoic acid and sorbic acid in fruit juices and other food products have been reported (NELSON, 1973; SMYLY, WOODWARD and CONRAD, 1976; EISENBEISS, WEBER and EHLERDING, 1977; CARNEVALE, 1980). Advantages of this technique are associated with the speed, the specificity of the analysis and most important of all, minimal sample preparation was required. Titrimetric and spectrophotometric methods are the official methods recommended (PEARSON, 1970; A.O.A.C., 1975). However, these methods are tedious and at times results are not reproducible.

Chilli sauce is a product prepared from chilli, sugar, vinegar, salt and spices with pH values around 3.5. Sodium benzoate, which is a preservative with a pH range of 2.5–4.0 for optimum microbial inhibition (FURIA, 1968), is a common preservative used in this product. The present paper described a simple and rapid estimation by reverse phase HPLC of benzoic acid in chilli sauce, and the percentage recovery as compared with titrimetric analysis (PEARSON, 1970).

MATERIALS AND METHODS

Instrument

Analyses were performed with a Hewlett Packard 1084B Liquid Chromatography equipped with a variable wavelength detector (190–600 nm), a variable volume injector injection system, a built-in printer/plotter and a digital integrator. A reversed phase RP–8, 10 µm Hewlett Packard Column (200 x 46 mm i.d.) was used with 5% methanol: methanol (Merck, HPLC grade) as the mobile phase (A:B). The solvent was filtered through a 0.8 µ Millipore filter with the aid of gentle suction. The flow rate of the mobile phase was set at 1.5 ml/minute. The variable wavelength detector and the reference wavelength were set at 254 nm and 400 nm respectively. The oven and solvent temperatures were set at 35° Centigrade.

Sample Preparation

Seven bottles of chilli sauce were randomly collected from the market. The pH of 4 g of homogenized sauce sample was adjusted to 2 with dilute sulphuric acid and extracted twice with 10 ml of diethyl ether (BDH, Anala grade). The ether solution was extracted twice with 10 ml of 0.1 N

*Food Technology Division, MARDI, Serdang, Selangor, West Malaysia.

sodium hydroxide (BDH, Anala grade) and the ether portion was discarded. The aqueous phase was acidified to pH 2 and re-extracted first with 20 ml and then with 10 ml of ether. The ether was evaporated off and the residues were dis-solved in 10 ml of methanol (Merck, HPLC grade).

The extracted sample was filtered through a 0.5 μ Millipore filter with the aid of Luer Lock syringe into a sample vial and 10 μl of this extract was injected into the column.

Preparation of Standard Benzoic Acid (500 μg/ml)

Twenty-five milligrammes of benzoic acid (BDH, Anala grade) was accurately weighed in a weighing boat and dissolved in methanol in a 50-ml volumetric flask. The standard solution was filtered through a 0.5 μ Millipore filter with the aid of Luer Lock syringe into a sample vial. Ten microlitres of this standard benzoic acid was used in the external standard calibration.

RESULTS AND DISCUSSION

External standard calibration method was adopted in this study. The RP−8 column was equilibrated with the mobile phase solvent at a maximum flow rate of 4 ml/minute. Very stable baselines were obtained at slope sensitivity (ss) set at 0.5 and zero. Trial runs at various flow rates of 1, 1.5 and 2.0 ml/min as well as at different solvent compositions were carried out. *Table 1* shows the retention times (R.T.) of the standard benzoic acid at different flow rates and solvent compositions. A flow rate of 1.5 ml/min and a mobile phase of 50% composition B was chosen. A typical chromatogram of standard benzoic acid is shown in *Figure 1*.

Solvent temperature was found effecting the R.T. of the peak. This was observed when the solvent temperature was set at

Table 1. Retention time of standard benzoic acid at different flow rates and % methanol

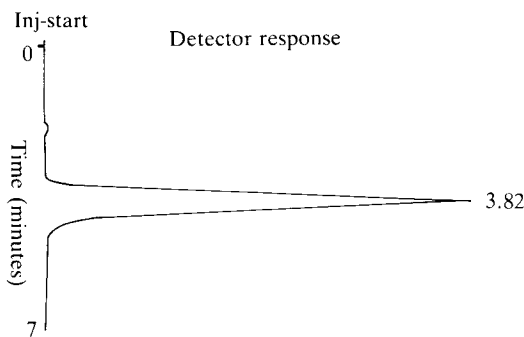| Flow rate (ml/min)	% B (methanol)	Retention time (min)
1	70	4.05
1.5	50	3.75
1.5	80	2.47
2.0	30	3.94
2.0	40	3.20
2.0	50	2.86
2.0	75	2.10

Figure 1. Chromatogram of 10 μl of standard benzoic acid (500 μg/ml).

ambient temperature (25°C) where fluctuations in the R.T. occurred. When the solvent temperature was set at 35°C or at a slightly higher temperature than the room temperature, reproducibility of the retention time was within the 5% retention time window.

In the solvent container A, a 5% methanol solution was used instead of the pure distilled water. This was to avoid excessive heat produced when methanol (50%) was mixed with H_2O (50%) in the mixing chamber prior to flowing into the chromatography column.

Recovery tests of the standard benzoic acid (5 μg) were carried out in order to test the efficiency of the method of extraction. *Table 2* shows the percentage recovery and the reproducibility of the peak. An average of 81.5% ± 2.11% of the standard were recovered if standard benzoic acid was extracted using the method described for

191

Table 2. Retention times and percentage recovery of the extracted benzoic acid (amount injected = 5 μg)

Retention time (min)		Standard benzoic acid recovered	
Standard benzoic acid	Extracted benzoic acid	Amount (μg)	%
3.85	3.80	4.13	82.8
3.85	3.81	4.18	83.6
3.85	3.81	4.12	82.4
3.83	3.83	4.12	82.4
3.60	3.60	3.93	78.6
3.60	3.60	3.95	79.0
		Ave: 81.5% ± 2.11%	

sample preparation. Ten microlitres (5 ±g) of non-extracted standard benzoic acid was used as the external standard in the calibration run. *Figure 2* shows the chromatogram of the extracted standard benzoic acid.
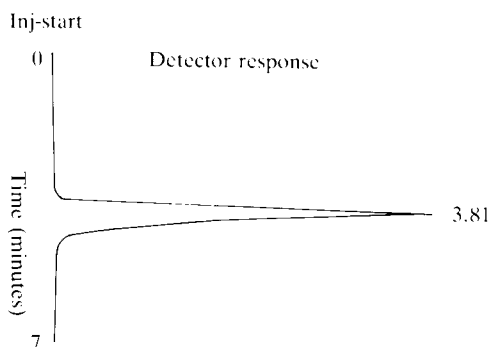
Inj-start

Figure 2. Chromatogram of standard benzoic acid extracted in methanol.

Table 3 shows the results of a comparative study of the amounts of benzoic acid in some chilli sauce samples obtained by the HPLC method described and the steam distillation method (A.O.A.C., 1975). In samples CS3 and CS3−A agreeable results were obtained by both methods. In samples CS−1, CS−2 and CS−4 benzoic acid was not detected by the HPLC method but 12 to 21 ppm were obtained by the steam distillation method. The higher results obtained by the latter method might be due to the interference of other volatile acids present in the products.

The extract of the benzoic acid from the sample stayed rather stable in methanol. This was shown by results obtained in *Table 3*. In samples CS3 and CS3−A, on the first day of extraction, the amounts obtained

Table 3. Comparison of results for the estimation of benzoic acid by HPLC and steam distillation methods

Sample code	Estimated benzoic acid (μg/g)	
	HPLC method	Steam distillation method
CS3	1 318 ± 20 (n=4)	1 200 ± 70 (n=2)
	*1 332 ± 27 (n=3)	
CS3−A	1 144 ± 30 (n=2)	1 300 ± 60 (n=2)
	*1 206 ± 41 (n=2)	
CS−1	N.D. (n=2)	21 ± 8 (n=2)
CS−2	N.D. (n=2)	12 (n=1)
CS−4	N.D. (n=2)	17 ± 4 (n=2)
SC−3	525 ± 7 (n=3)	N.A.
SC−3A	541 ± 13 (n=3)	N.A.

n = No. of determinations carried out.
N.D. = Not detectable.
N.A. = Not analysed.
* = The extract was kept for two days before it was analysed.

192

were 1 318 $\pm$ 20 ppm and 1 144 $\pm$ 30 ppm respectively. When the extracts were kept in sample vials for two days, the amounts obtained were 1 332 $\pm$ 27 ppm and 1 206 $\pm$ 41 ppm, thus non-significant changes were observed.

Under the chromatographic conditions used in this study, benzoic acid was not totally resolved from sorbic acid, a preservative which is not permitted to be used in sauce. However, this problem could be overcome by using a U—Bandapak CN reverse phase column with 2% acetic acid: methanol (CARNEVALE, 1980) as the mobile phase, if quantitation is required for these two preservatives.

ACKNOWLEDGEMENTS

ABSTRACT

A method is described for the determination of benzoic acid in chilli sauce by high performance liquid chromatography (HPLC) using a RP–8 Hewlett Packard column, and 5% methanol and methanol (50:50) as the mobile phase. It was detected at 254 nm by using the variable wavelength detector. Recoveries of 81.5% $\pm$ 2.11% was obtained when 5 μg of standard benzoic acid was used in the recovery studies. Good agreement was obtained between results from HPLC and steam distillation methods, except some interference, possibly due to the presence of volatile acids was observed in the higher results obtained by the steam distillation method.

REFERENCES

A.O.A.C. (1975). Official Methods of Analysis of the Association of Official Analytical Chemists (ed. HORWTIZ, W., SENZEL, A., REYNOLDS, H. and PARK, D.L.) 12th ed., pp. 19—27. Washington D.C.

CARNEVALE, J. (1980). Determination of ascorbic, sorbic and benzoic acids in citrus juices by high-performance liquid chromatography. *Food Technol. Aust.* **32**, 302—5.

EISENBEISS, F., WEBER, M. and EHLERDING, S. (1977). Determination of sorbic acid in the presence of benzoic acid by high-performance liquid chromatography. *Chromatographia* **10**, 262—4.

FURIA, T.E. (1968). *Handbook of Food Additive* pp.

142. Ohio: The Chemical Rubber Co., Cleveland.

NELSON, J. (1973). Quantitation of sodium saccharin, sodium benzoate and other food additives by high-speed liquid chromatography. *J. Chromatog. Sc.* **11**, 28—35.

PEARSON, D. (1970). *The Chemical Analysis of Foods* 6th ed., pp. 34—6. London: J & A Churchill.

SMYLY, D.S., WOODWARD, B. and CONRAD, E.C. (1976). Determination of saccharin, sodium benzoate and caffeine in beverages by reverse phase high pressure liquid chromatography. *J. Assoc. Off. Anal. Chem.* **59**, 14—9.

193