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# STUDIES ON THE ANALYSIS OF FOOD ADDITIVES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

## V*. SIMULTANEOUS DETERMINATION OF PRESERVATIVES AND SACCHARIN IN FOODS BY ION-PAIR CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic method for the simultaneous determination of sorbic acid, benzoic acid, *p*-hydroxybenzoic acid and its methyl, ethyl, isopropyl, *n*-propyl, isobutyl, *n*-butyl esters and saccharin in foodstuffs is described. For good separations of these compounds, acetonitrile–water–0.2 *M* phosphate buffer pH 3.6 (7:12:1) containing 2 m*M* cetyltrimethylammonium bromide as an ion-pair reagent and a Nucleosil 5C18 column are required. A steam distillation method and a Sep-Pak $C_{18}$ cartridge method for the sample preparation are compared. The recoveries from a coffee drink were generally better than 93.8% and the relative standard deviations were 0.85–2.15% for the Sep-Pak $C_{18}$ cartridge method.

## INTRODUCTION

Sorbic acid (SOA), benzoic acid (BA), the esters of *p*-hydroxybenzoic acid (ethyl-, isopropyl-, *n*-propyl-, isobutyl- and *n*-butyl-PHBA) and saccharin (SA) are commonly used food additives in Japan. Many methods for their analysis have been reported, including UV spectrophotometry[1], thin-layer chromatography (TLC)[1,2], gas chromatography (GC)[1] and high-performance liquid chromatography (HPLC)[3–17]. However, TLC procedures are difficult to quantify accurately, and UV spectrophotometric and GC procedures require tedious pre-treatments and do not allow the simultaneous determination of these food additives. Although HPLC has great advantages over other conventional methods, simultaneous separations of these food additives is difficult because the polarities of SOA, BA and SA are extremely different from those of the esters of PHBA. Several reports have been made on simultaneous separations of these compounds using gradient elution[9–14] or ion depression[17]. Recently, Aitzetmüller and Arzberger[15] reported an isocratic method for the

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simultaneous determination of preservatives in foods using normal phase HPLC

The use of ion-pair chromatography enables the highly polar compounds to be separated as weakly polar compounds. We previously reported[16] an HPLC method for the determination of SOA, BA and SA this technique. However, the method could not to be applied to the esters of PHBA. The purpose of the present study was to investigate the chromatographic behaviour of the nine preservatives SOA, BA, PHBA, methyl-, ethyl-, isopropyl-, *n*-propyl-, isobutyl- and *n*-butyl-PHBA and to establish a method for the simultaneous determination of these compounds and SA in food products.

## EXPERIMENTAL

### *Apparatus and reagents*

The HPLC system consisted of a Twincle pump (Jasco, Tokyo, Japan), a VL614 variable loop injector with a 100-$\mu$l sample loop, a Uvidec 100 II UV detector operating at 235 nm and an U125M recorder (Nippon Denshi Kogaku, Kyoto, Japan). Separations were carried out by using stainless-steel columns (15 cm × 4.3 mm I.D.; Umetani, Osaka, Japan) packed by the balanced slury technique with LiChrosorb RP-18 (5 $\mu$m) (E. Merck, Darmstadt, F.R.G.), Develosil ODS-5 (Nomura Kagaku, Seto, Japan) and Nucleosil 5C18 (Macherey-Nagel, Düren, F.R.G.), respectively. The columns were water-jacketed for temperature control (27°C). A Sep-Pak $C_{18}$ caretridge was obtained from Waters Assoc. (Milford, MA, U.S.A.).

PHBA, esters of PHBA, *n*-decyltrimethylammonium bromide (DTA), *n*-dodecyltrimethylammonium bromide (DDTA), *n*-tetradecyltrimethylammonium bromide (TTA) and cetyltrimethylammonium bromide (CTA) were purchased from Tokyo Kasei (Tokyo, Japan), SOA and BA from Katayama (Osaka, Japan) and SA from Wako (Osaka, Japan).

The phosphate buffer was prepared from 0.2 *M* potassium dihydrogen phosphate by titration to the required pH with 0.2 *M* phosphoric acid or 0.2 *M* dipotassium hydrogen phosphate. All the water used was purified by the Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.).

### *Chromatographic procedure*

Mobile phases were prepared by mixing the calculated amounts of acetonitrile, water, 0.2 *M* phosphate buffer and ion-pair reagents and degassed in an ultrasonic bath immediately before use. The operating conditions are given in the figure captions. The capacity factors were calculated from the equation

$$k' = (t_R - t_0)/t_0$$

where $t_R$ is the retention time of the sample peak and $t_0$ is the retention time for a non-retained peak.

### *Sample preparations*

*Steam distillation method.* To an accurately weighed quantity (*ca.* 10 g) of ground sample in a 500-ml kjeldahl flask, 60 g of sodium chloride, 150 ml of water and 10 ml of 15% tartaric acid were added. The mixture was then steam distilled at a rate of 10 ml/min until *ca.* 480 ml of distillate had been collected in a 500-ml

volumetric flask, the volume was adjusted with water. To aliquots (10 ml) of this solution in a 20-ml volumetric flask, 5 ml of acetonitrile were added and the volume was adjusted with water; 100 μl of this solution were subjected to HPLC.

Quantitation was carried out using calibration graphs obtained from a standard solution (preservatives and SA in the concentration range 0.25–1.25 μg in 1.0 ml of 25% aqueous acetonitrile).

*Sep-Pak $C_{18}$ cartridge method.* To an accurately weighed quantity (*ca.* 2 g) of liquid sample in a 50-ml volumetric flask, 5 ml of 0.2 *M* phosphate buffer pH 3.0 and 5 ml of 5 m*M* CTA were added and the volume adjusted with water. After mixing the solution, 5 ml of the mixture were poured into a Sep-Pak $C_{18}$ cartridge at a rate of 2 ml/min and the cartridge was washed with 10 ml of water. The cartridge was attached to a 20-ml glass syringe and pre-conditioned with 20 ml of methanol, 20 ml of water and 2 ml of 5 m*M* CTA prior to use. The preservatives and SA were eluted from the cartridge with 10 ml of acetonitrile–water (1:1) into a 20 ml volumetric flask and the volume was adjusted with water. Aliquots (100 μl) of this solution were subjected to HPLC.

Quantitation was carried out using calibration graphs obtained from a standard solution which was treated with a Sep-Pak $C_{18}$ cartridge according to the above analytical procedure.

## RESULTS AND DISCUSSION

The influence of the support on the retention and the selectivity of the chromatographic system was studied using LiChrosorb RP-18, Develosil ODS-5 and Nucleosil 5C18 as supports. Although similar chromatographic behaviours were ob-

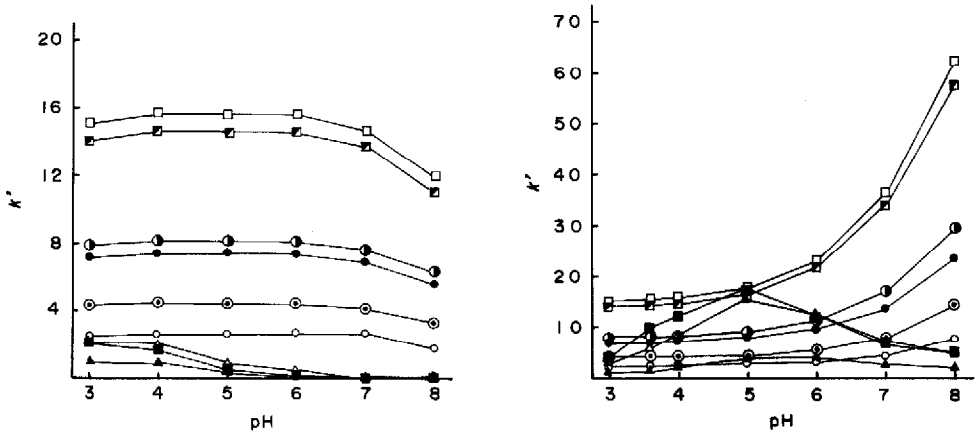


Fig. 1. Effect of the pH of the phosphate buffer added to the mobile phase containing no ion-pair reagent on the *k'* of the preservatives: ▲—▲, PHBA; △—△, SOA; ■—■, BA; ○—○, methyl-PHBA; ⊙—⊙, ethyl-PHBA; ●—●, isopropyl-PHBA; ◑—◑, *n*-propyl-PHBA; ◩—◩, isobutyl-PHBA; □—□, *n*-butyl-PHBA. Operating conditions: column; Nucleosil 5C18 (15 cm × 4.3 mm I.D.); mobile phase, acetonitrile–water–0.2 *M* phosphate buffer (7:12:1); column temperature, 27°C; flow-rate, 1.0 ml/min; detector, UV 235 nm.

Fig. 2. Effect of the pH of the phosphate buffer added to the mobile phase containing 2 m*M* CTA as an ion-pair reagent on the *k'* of the preservatives. Other details as in Fig. 1.

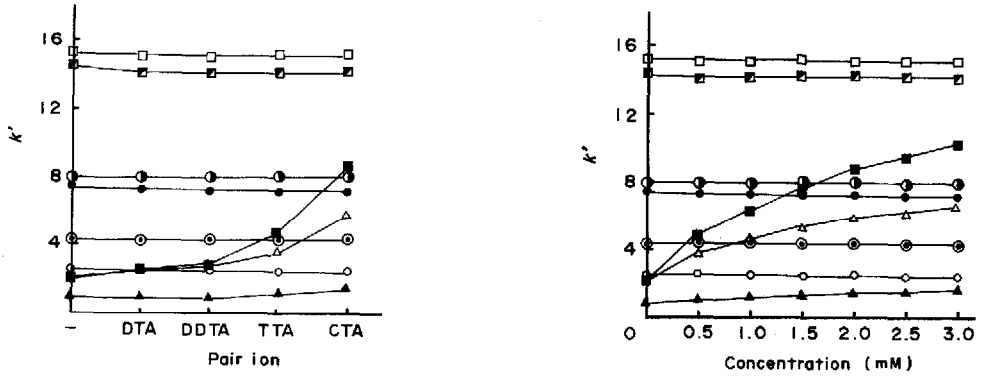


Fig. 3. Relationship between the alkyl chain length of the ion-pair reagent (2 m*M*) and $k'$ of the preservatives. Mobile phase: acetonitrile–water–0.2 *M* phosphate buffer (pH 3.6) (7:12:1) containing 2 m*M* ion-pair reagent. Key to symbols as in Fig. 1.

Fig. 4. Effect of the concentration of CTA on the $k'$ of the preservatives. Mobile phase: acetonitrile–water–0.2 *M* phosphate buffer (pH 3.6) (7:12:1) containing 0–3.0 m*M* CTA. Key to symbols as in Fig. 1.

served, the LiChrosorb RP-18 and Develosil ODS-5 columns did not give a good separation of isobutyl-PHBA and *n*-butyl-PHBA. The Nucleosil 5C18 column, however, gave good separations of all compounds investigated. Therefore, it was adopted for further investigations.

Fig. 1 shows the effect of the pH of the phosphate buffer added to the mobile phase containing no ion-pair reagent on the capacity factors ($k'$) of the preservatives. In the range pH 3–6, the $k'$ values of each ester of PHBA were constant, but at pH $> 7$ they were slightly decreased. The $k'$ values of the highly polar PHBA, SOA and BA also decreased with increasing pH. Fig. 2 shows the effect of the pH of the

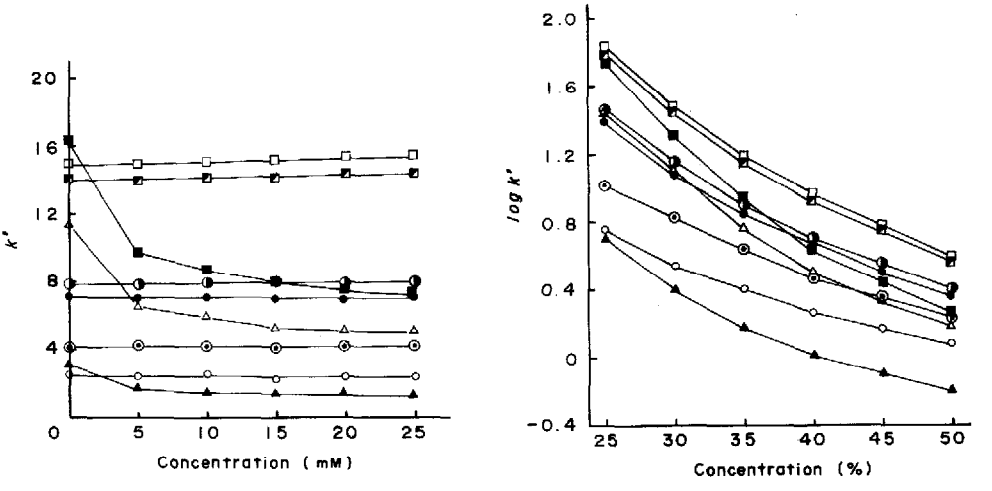


Fig. 5. Effect of the concentration of phosphate buffer pH 3.6 on the $k'$ of the preservatives. Mobile phase: acetonitrile (35%, v/v)–water containing 0–25 m*M* phosphate buffer pH 3.6 and 2 m*M* CTA.

Fig. 6. Effect of the concentration of acetonitrile on the log $k'$ of the preservatives. Mobile phase: acetonitrile (25–50%, v/v)–water containing 10 m*M* phosphate buffer pH 3.6 and 2 m*M* CTA.

phosphate buffer added to the mobile phase containing 2 m*M* CTA as an ion-pair reagent on the *k'* of the preservatives. The *k'* values for the esters of PHBA increased with increasing pH, in contrast to the behaviour in the mobile phase containing no ion-pair reagent. On the other hand, the *k'* of PHBA, SOA and BA had a maximum at pH 5.

Fig. 3 shows the relationship between the alkyl chain length of the ion-pair reagent and the *k'* of the preservatives. The *k'* values for the esters of PHBA were constant, regardless of the ion-pair reagents, but the values for PHBA, SOA and BA increased with increasing alkyl chain length of the ion-pair reagent.

Figs. 4–6 show the effect of the concentration of CTA, phosphate buffer pH 3.6 and acetonitrile, respectively, on the *k'* of the preservatives. The values for the esters of PHBA were independent of the concentration of CTA and slightly increased with increasing concentration of phosphate buffer, the values for PHBA, SOA and BA increased with increasing concentration of CTA and decreased with increasing concentration of phosphate buffer and the values for all the compounds decreased with increasing concentration of acetonitrile, especially in the case of the acids.

On the basis of these results, acetonitrile–water–0.2 *M* phosphate buffer pH 3.6 (7:12:1) containing 2 m*M* CTA was used as a mobile phase. Fig. 7 shows a typical chromatogram for a standard mixture. SA could be separated simultaneously under the same chromatographic conditions.

For the sample pre-treatment, a steam distillation method, commonly used to analyze preservatives such as SOA, BA and esters of PHBA[1], and a Sep-Pak $C_{18}$ cartridge method were employed. In previous studies, we established a method of preparation for SOA, BA and SA using a Sep-Pak $C_{18}$ cartridge[16]. The packing material of the Sep-Pak $C_{18}$ cartridge is similar to that present in the analytical

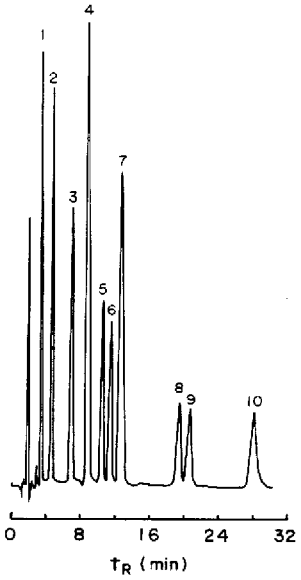


Fig. 7. Liquid Chromatogram obtained from a standard mixture (containing 0.1 μg/ml of each compound): 1 = PHBA; 2 = methyl-PHBA; 3 = ethyl-PHBA; 4 = SOA; 5 = isopropyl-PHBA; 6 = *n*-propyl-PHBA; 7 = BA; 8 = isobutyl-PHBA; 9 = *n*-butyl-PHBA; 10 = SA.

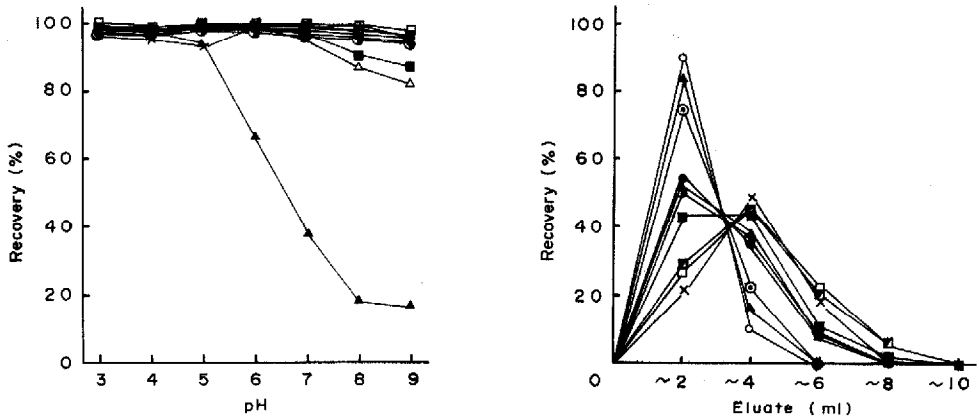


Fig. 8. Effect of the pH of the sample solution on the recoveries of the preservatives and SA (×) from a Sep-Pak $C_{18}$ cartridge. See Fig. 1 for key to symbols.

Fig. 9. Elution pattern of the preservatives and SA (×) from a Sep-Pak $C_{18}$ cartridge. See Fig. 1 for key to symbols.

column. Accordingly, not only the esters of PHBA but also the highly polar SOA, BA and SA can be adsorbed in the cartridge from aqueous solutions containing an ion-pair reagent such as CTA. Therefore, the method was modified for the preparation of PHBA and its esters.

Fig. 8 shows the effects of the pH of the sample solution on the recoveries of the preservatives and SA from a Sep-Pak $C_{18}$ cartridge. In the range pH 3–5, the recoveries were over 95%, but at pH > 6 for PHBA and 8 for SOA and BA the recoveries decreased. Consequently, 0.2 *M* phosphate buffer pH 3.0 was added to the sample solution in order to maintain in a pH < 5.

Fig. 9 shows the elution pattern of the preservatives and SA from a Sep-Pak $C_{18}$ cartridge. All of the compounds were eluted completely with 10 ml of acetonitrile–water (1:1).

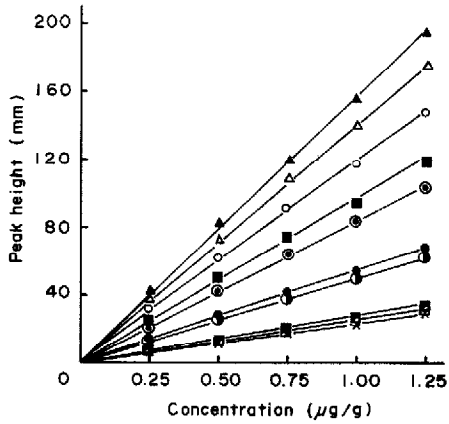


Fig. 10. Calibration curves of the preservatives and SA (×) obtained by use of the Sep-Pak $C_{18}$ cartridge method. See Fig. 1 for key to symbols.

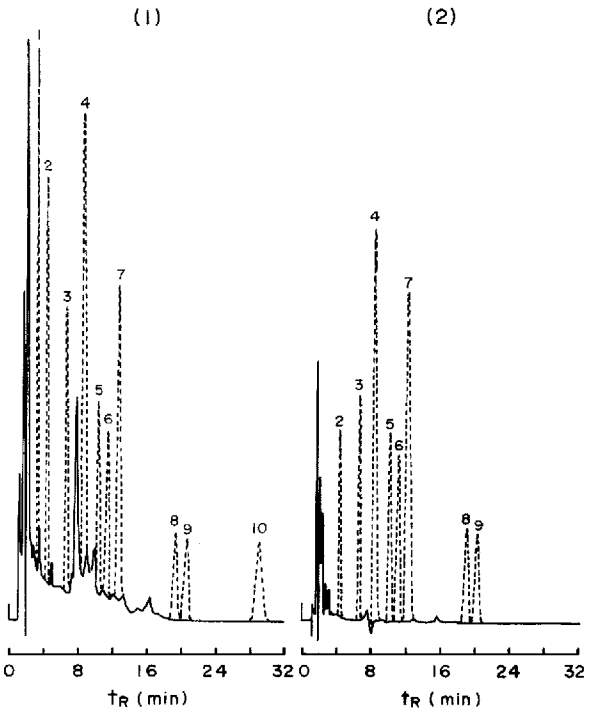


Fig. 11. Chromatograms obtained from a coffee drink prepared by use of the Sep-Pak $C_{18}$ cartridge method (1) and the steam distillation method (2). Broken line: sample spiked with 100 $\mu$g/g of the standard mixture. Peaks as in Fig. 7.

TABLE I

RECOVERIES OF THE PRESERVATIVES AND SA FROM A COFFEE DRINK

Methods: A = steam distillation method; B = Sep-Pak $C_{18}$ cartridge method. C.V. = Coefficient of variation.

| *Compound* | *Method A* | | *Method B* | |
|---|---|---|---|---|
| | *Recovery (%)* | *C.V. (%)* | *Recovery (%)* | *C.V. (%)* |
| PHBA | – | – | 100.3 | 1.01 |
| SOA | 76.1 | 5.97 | 101.6 | 0.90 |
| BA | 97.2 | 1.34 | 99.9 | 1.22 |
| SA | – | – | 102.4 | 1.18 |
| Methyl-PHBA | 32.6 | 8.05 | 99.0 | 1.14 |
| Ethyl-PHBA | 77.1 | 4.29 | 96.7 | 0.85 |
| Isopropyl-PHBA | 95.5 | 1.13 | 93.8 | 1.20 |
| *n*-Propyl-PHBA | 91.3 | 2.56 | 96.1 | 1.53 |
| Isobutyl-PHBA | 95.8 | 0.92 | 100.9 | 2.15 |
| *n*-Butyl-PHBA | 95.9 | 1.58 | 100.3 | 1.80 |

Fig. 10 shows the calibration curves of the preservatives and SA obtained by use of the Sep-Pak $C_{18}$ cartridge method. Linear plots of relative response passing through the origin were obtained for all of the compounds studied in the concentration range 0.25–1.25 μg in 1.0 ml of the solution subjected to HPLC.

Fig. 11 shows the chromatograms obtained from a coffee drink spiked with the preservatives and SA after treatment by the Sep-Pak $C_{18}$ cartridge method (1) and the steam distillation method (2).

In a recovery test, the steam distillation method and the Sep-Pak $C_{18}$ cartridge method were applied to a coffee drink spiked with the preservatives and SA each at a level of 100 μg/g. The reproducibility was determined by carrying out five identical analyses, and the results are summarized in Table I. The steam distillation method had a few advantages, namely it provided a cleaner sample solution that contained few interfering compounds, and it was applicable to samples of various types. Nevertheless, it was time-consuming and provided no recovery of PHBA and SA and low recoveries of SOA, methyl- and ethyl-PHBA. The Sep-Pak $C_{18}$ cartridge method generally provided better results.

The detection limits of the present method were 5 μg/g for PHBA, SOA, BA, methyl- and ethyl-PHBA and 10 μg/g for isopropyl-, *n*-propyl-, isobutyl-, *n*-butyl-PHBA and SA. They, however, could be enhanced for a specific analysis by using the appropriate absorption maximum (BA, 225 nm; SA, 205 nm; SOA, PHBA and the esters of PHBA, 260 nm), because the detector wavelength used, 235 nm, was a compromise made in order to detect all of the compounds concerned simultaneously.

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