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DETERMINATION OF FOOD PRESERVATIVES AND SACCHARIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The quantitative analysis of benzoic and sorbic acid, methyl, ethyl and propyl esters of *p*-hydroxybenzoic acid and saccharin in foodstuffs is described. These compounds are quantitatively extracted with disposable clean-up columns packed with Extrelut® and simultaneously determined by high-performance liquid chromatography on reversed-phase columns. Complicated matrices such as cheese, cake, ketchup and chocolate were tested and recoveries were generally better than 95% in the concentration ranges normally used in the food industry.

INTRODUCTION

The existing approaches for determining quantitatively compounds in a complicated matrix generally consist of two steps: extraction and quantification. The isolation of acidic compounds such as benzoic and sorbic acid, the esters of *p*-hydroxybenzoic (PHB) acid and saccharin may be time consuming. The common method of isolating these compounds from substances that might interfere in the quantitative determination is extraction either with a Soxhlet apparatus or manually with a separating funnel[1,2]. As such extractions are common in the routine work of almost every analytical laboratory, an alternative, easier method of extraction has been devised, namely the use of disposable Extrelut® clean-up columns (Merck, Darmstadt, G.F.R.), which simplifies considerably the isolation of organic compounds, especially when working with complex matrices that tend to form emulsions[3,4]. This approach is used extensively in forensic and clinical chemistry and has recently been applied in food chemistry to extract the mycotoxin patulin from apple and grape juice[5].

The second step, the quantitative determination of the extracted compounds, can be performed either by chromatography [gas–liquid chromatography (GLC)[6], thin-layer chromatography (TLC)[7] or high-performance liquid chromatography (HPLC)[8–12]] or by measuring the UV absorbance of the total extract[13]. Whereas the GLC determination requires derivatization prior to injection, commercially available silica-coated thin-layer plates lack the separation power for sorbic and benzoic acid. These problems can be eliminated by using reversed-phase systems and HPLC. This

chromatographic approach has great advantages over those involving the use of silica gel in the ease of equilibration and in cleaning the column from accumulated contaminants by simply flushing with methanol. HPLC is not only rapid, but shows also a greater linear calibration range than TLC. One gradient and two isocratic systems are proposed for the simultaneous identification of the above-mentioned compounds.

EXPERIMENTAL

Materials and chemicals

Extrelut pre-packed columns (Art. 11737) and an Extrelut refill pack (Art. 11738) were obtained from Merck.

The chemicals used were diethyl ether, acetone, chloroform and ethanol (all distilled in glass), pure methanol (Art. 6008, Merck), isopropanol (pro analysi) (Art. 9634, Merck), 0.1 *N* sodium hydroxide solution, 0.5 *N* sulphuric acid, 5 *N* sulphuric acid (140 ml of concentrated acid diluted to 1000 ml with distilled water), aqueous solution of concentrated ammonia, aqueous saturated solution of sodium chloride and ethyl acetate (distilled).

Phosphate buffer solution was prepared by dissolving 2.5 g of $K_2HPO_4 \cdot 3H_2O$ (Art. 5099, Merck) and 2.5 g of KH_2PO_4 (Art. 4873) in doubly distilled water and passing the solution through a 0.45-μm filter (for instance, a "solvent clarification kit", Art. 85122, Waters Assoc., Milford, Mass., U.S.A.).

Standard solutions

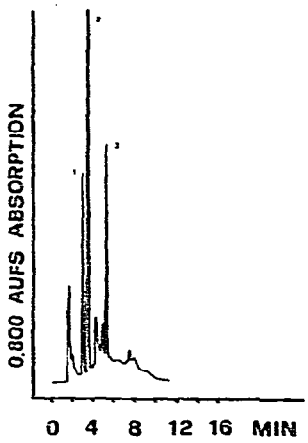
A 25-mg amount of each of the following compounds was dissolved together in 25 ml methanol and diluted to 50 ml in a volumetric flask: saccharin (Art. 38, Fisher Scientific, Zürich, Switzerland), benzoic acid (p.a.) (Art. 136, Merck), sorbic acid (Art. 662, Merck), methyl *p*-hydroxybenzoate (Art. 6757, Merck), ethyl *p*-hydroxybenzoate (Art. 887, Merck) and propyl *p*-hydroxybenzoate (Art. 7424, Merck).

This stock solution was diluted 1:10 for the HPLC method for PHB esters alone and the two acids plus saccharin (see *Chromatography*) resulting in a concentration of 0.05 μg/μl per compound. For the determination of the five preservatives (see *Chromatography*) the undiluted parent solution had to be applied.

Experimental procedures

Direct analysis. Fruit juices such as orange juice, wines and other aqueous media generally need no clean-up. In any case, a filtration (0.45-μm pore diameter, for instance waters sample clarification kit, Art. 26870) is recommended for eliminating any particulate matter. The detection limit for benzoic acid, which has the smallest absorption coefficient of the compounds analysed, is far lower than the concentrations normally used in the food chemistry. Fig. 1 shows such a chromatogram of commercial orange juice containing 300 ppm each of benzoic and sorbic acid and methyl PHB. The baseline shift is due to the gradient used. Fig. 2 shows a similar analysis of white wine spiked with 170 ppm of sorbic acid.

Samples containing mainly triglycerides and similar matrices such as butter and margarine. Extremely fatty samples such as margarine have to be extracted by the



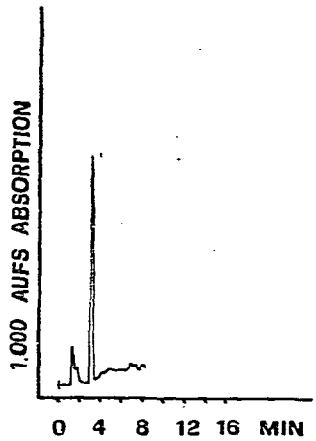


Fig. 1. Orange juice spiked with 300 ppm of benzoic acid (1), sorbic acid (2) and methyl *p*-hydroxybenzoate (3).

Fig. 2. White wine spiked with 170 ppm of sorbic acid (1).

following procedure. A 5-g sample is dissolved in 50 ml of diethyl ether and extracted twice with 10 ml of 0.1 *N* sodium hydroxide solution in a separating funnel. The basic aqueous extracts are acidified with 1 ml of 5 *N* sulphuric acid in a 50-ml volumetric flask and diluted to volume with methanol.

This solution is filtered as in described under *Direct analysis* and analysed.

Extrelut clean-up. This method is suitable for most other foodstuffs such as cheese, cakes, yoghurts and other samples that tend to form emulsions during the extraction. The pre-packed or refilled Extrelut column in a plastic tube consists of a wide-pore Kieselguhr of grainy structure and is characterized by a high pore volume. The tube is capped at both ends with screens containing a filter disc. The elution rate is controlled by the diameter of a cannula stuck on to the effluent cone. A 5–20-g sample is homogenized for 3 min in 50 ml of 0.5 *N* sulphuric acid using a beaker and a high-speed blender. The homogenate is transfered quantitatively into a 100-ml volumetric flask and diluted to volume with water. When analysing material that sediments quickly, it is homogenized again in order to obtain a representative aliquot. A 20-ml volume of this pre-treated sample is pipetted on to the Extrelut column and allowed to filter in for at least 15 min. It is important to replace the original filter disc by some glass-wool when working with samples of thicker consistency. Subsequently it may also be necessary to cover the retained particulate matter again with some glass-wool.

The absorbed preservatives and saccharin can now be eluted with 350 ml of chloroform–isopropanol (9:1) using a closed 500-ml separating funnel as a solvent reservoir above the column (Mariott flask). The eluate is collected in a 500-ml round-bottomed flask and evaporated carefully nearly to dryness. The last few millilitres of isopropanol are removed with a gentle flow of nitrogen in order to prevent substantial losses of benzoic and sorbic acid, which have relatively high vapour pressures. The residue is transferred with methanol into a 10-ml volumetric flask and diluted to volume with methanol. To speed up the dissolution, the use of an ultrasonic bath is recommended. The filtered extract is now ready for analysis.

Instrumental

An Ultra Turrax high-speed mixer (type TP 18/2, IKA Werk, Stauffen im Breisgau, G.F.R.) was used.

For HPLC, a rheodyne Model 7105 injector (Perkin-Elmer, Norwalk, Conn., U.S.A.), two Altex Model 100 pumps (Altex Scientific, Berkeley, Calif., U.S.A.), a Model 420 microprocessor programmer (Altex), a μBondapak C_{18} column (Waters), a Uvikon UV detector (Kontron, Zürich, Switzerland) with a variable-wavelength detector, Model LCD 725 (wavelength used, 235 nm) and an SP 4000 integration system (Spectra-Physics, Santa Clara, Calif., U.S.A.) were employed.

Chromatography

Determination of the five preservatives. In order to analyse the PHB esters in the same run as the two acids, it is necessary to use a gradient. Pump A delivers phosphate buffer solution as described under *Materials and chemicals*, whereas pump B delivers methanol. The initial chromatographic conditions were 20% B, a linear gradient from 20% to 80% B within 1 min, and flow-rate 2 ml/min. Fig. 3 shows a calibration chromatogram with an injection of a 10-μl standard (5 μg of each compound). The same chromatographic conditions were used in obtaining Figs. 1 and 2.

PHB esters alone. Isocratic elution was carried out with 60% methanol in phosphate buffer (for preparation see *Materials and chemicals*), flow-rate 1.2 ml/min. A 10-μl volume of the standard (0.05 μg/μl, see *Materials and chemicals*) was used for calibration.

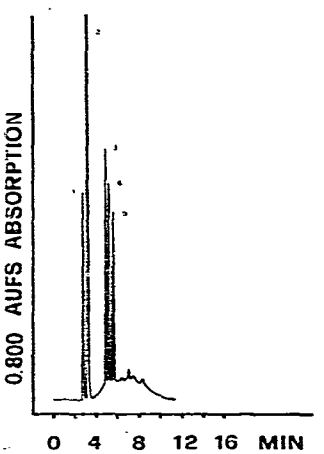


Fig. 3. Standard chromatogram of benzoic acid (1), sorbic acid (2), methyl (3), ethyl (4) and propyl *p*-hydroxybenzoate (5) (5 μg of each).

The two acids plus saccharin. The isocratic use of pure buffer solution (for preparation see *Materials and chemicals*) and a rapid flow-rate of 2.8 ml/min led to the separations shown in Figs. 4 and 5.

RESULTS AND DISCUSSION

As shown above, the isolation and extraction of preservatives and saccharin by using the disposable clean-up column for routine analysis offers considerable

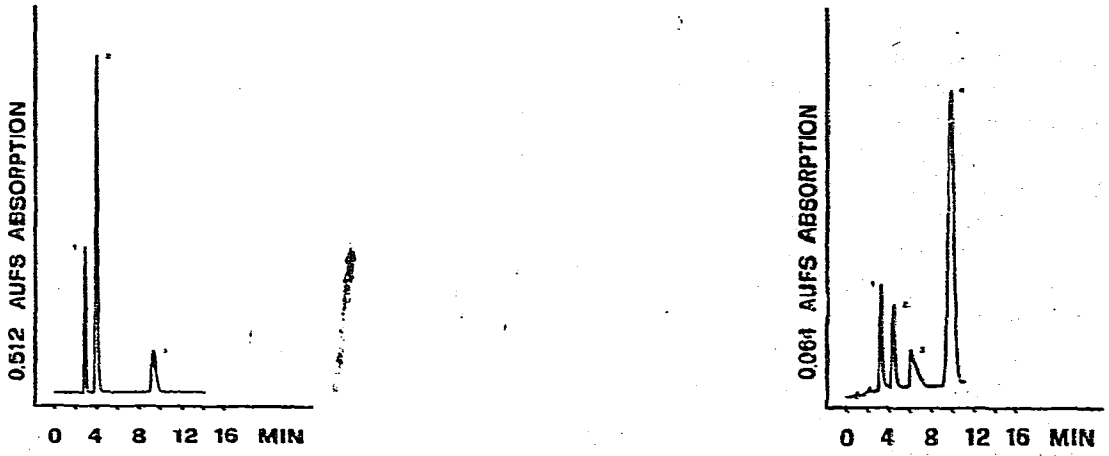


Fig. 4. Standard chromatogram of benzoic acid (1), sorbic acid (2) and saccharin (3) (4 μg of each).

Fig. 5. Standard chromatogram of benzoic acid (1; 0.4 μg), sorbic acid (2; 0.4 μg), cyclamate (3; 91 μg) and saccharin (4; 1 μg). Detection at 207 nm.

savings in time and materials, especially when dealing with samples that tend to form emulsions. The advantages are great enough to predict further applications in routine extractions, especially in trace analysis.

Typical recoveries found with this rapid extraction mode are summarized in Table I. The detection limits of all six compounds are at least ten times below those commonly used in applications of preservatives and saccharin.

The extracts from this clean-up column are generally so clean and free from interfering compounds that not even matrix peaks can be observed in the chromatogram, although some of the neutral fraction is eluted together with the acidic compounds.

TABLE I

RECOVERIES FROM FOOD SAMPLES FOLLOGING EXTRELUT CLEAN-UP

Sample	*Recovery (%)*				
	Benzoic acid	*Sorbic acid*	*PHB esters*		*Saccharin*
			Methyl	*Propyl*	
Peppermint candies	—	—	—	—	98.0
Milk chocolate	95.2	87.6	—	—	97.0
Chestnut paste	98.3	90.7	93.6	92.9	96.0
Toothpaste	—	—	98.0	100.0	91.0
Marmalade	96.7	92.7	97.4	99.9	98.5
Ketchup	99.8	86.2	99.5	97.7	98.7
Fruit cocktail	99.8	80.0	93.0	93.3	100.4
Canned Russian salad	96.7	98.3	95.9	99.2	95.8
Canned mixed vegetables	87.4	87.7	100.9	101.1	98.5
Mixed pickles	92.0	—	100.5	100.8	96.3
Yoghurt (plain)	98.5	97.9	100.5	100.5	101.4
Cake	—	—	—	—	99.7
Cheese	96.3	95.3	99.3	96.6	—

Preliminary approaches to load the Extrelut column with alkaline samples in order to elute first the basic and neutral fractions were found to be unnecessary, and later esters were partially saponified. Although it is easy to change the column to a basic pH, problems arise on acidification as both organic and inorganic acids obviously are adsorbed on the column. Gaseous HCl is also strongly adsorbed, changes the filling material and is not suitable for routine work.

The detector wavelength used of 235 nm is a compromise made in order to detect all of the compounds concerned simultaneously (absorption maxima: benzoic acid 227 nm, saccharin 207 nm, sorbic acid and PHBs 250–260 nm). The detection limit can be greatly enhanced for a specific analysis by using the appropriate absorption maximum.

The simultaneous determination of benzoic acid, sorbic acid and saccharin as shown in Fig. 4 or the quantification of all five preservatives (Fig. 3) within one run makes this analysis faster than with previously described methods earlier.

REFERENCES

1 *Schweiz. Lebensmittelbuch*, EDMZ, Berne, 1973, Ch. 44.
2 R. Battaglia, *Mitt. Geb. Lebensmittelunters. Hyg.*, 68 (1977) 28.
3 J. Breiter, R. Helger and H. Lang, *Forensic Sci.*, 7 (1976) 131.
4 J. Breiter, A. Sachs and B. Küpper, *GIT Fachz. Lab.*, November (1976) 20.
5 U. Leuenberger, R. Gauch and E. Baumgartner, *J. Chromatogr.*, 161 (1978) 303.
6 E. Fogden, M. Fryer and S. Urry, *J. Ass. Publ. Anal.*, 12 (1974) 93.
7 R. Duden, A. Fricker, R. Calverley, K. H. Park and V. M. Rios, *Z. Lebensm.-Unters.-Forsch.*, 151 (1973) 23.
8 J. J. Nelson, *J. Chromatogr. Sci.*, 11 (1973) 28.
9 W. A. Wildanger, *Chromatographia*, 6 (1973) 381.
10 M. McCalla, F. G. Mark and W. H. Kipp, *J. Ass. Offic. Anal. Chem.*, 60 (1977) 71.
11 D. S. Smyly, B. B. Woodward and E. C. Conrad, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 14.
12 M. C. Bennett and D. R. Petrus, *J. Food Sci.*, 42 (1977) 1220.
13 M. Hussein, H. Jacin and F. Rodriguez, *J. Agr. Food Chem.*, 24 (1976) 36.