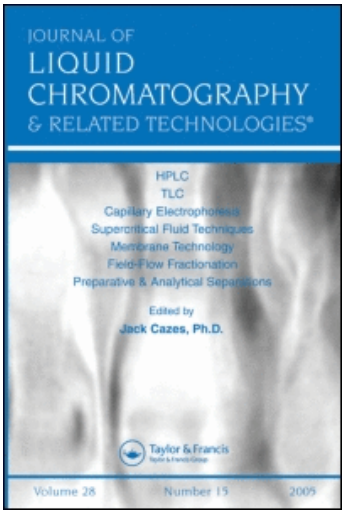

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of Extraction Methods and Column Types for the Determination of Additives by Liquid Chromatography

B. H. Chen[a]; S. C. Fu[a]
[a] Department of Nutrition and Food Sciences, Fu Jen Catholic University, Taipei, Taiwan, R.O.C.

To cite this Article Chen, B. H. and Fu, S. C.(1996) 'Comparison of Extraction Methods and Column Types for the Determination of Additives by Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 19: 4, 625 — 643
To link to this Article: DOI: 10.1080/10826079608005525
URL: http://dx.doi.org/10.1080/10826079608005525

J. LIQ. CHROM. & REL. TECHNOL., 19(4), 625–643 (1996)

COMPARISON OF EXTRACTION METHODS AND COLUMN TYPES FOR THE DETERMINATION OF ADDITIVES BY LIQUID CHROMATOGRAPHY

B. H. Chen,* S. C. Fu

Department of Nutrition and Food Sciences
Fu Jen Catholic University
Taipei, Taiwan 24205, R.O.C.

ABSTRACT

Several extraction methods and column types were compared, with respect to extraction and separation efficiency of 14 additives from soy sauce, sugared fruit and dried roast beef, by paired-ion liquid chromatography. Results showed that the application of a Sep-Pak C_{18} cartridge was the best method for extraction, because it resulted in higher recovery than those given by acetone extraction and steam distillation. The purification of acetone extract by a Sep-Pak silica gel cartridge can result in recovery loss. Monomeric column was superior to polymeric column for simultaneous separation of preservatives, antioxidants and sweeteners. The capacity factor (k') of each additive was also determined.

INTRODUCTION

Food additives, such as preservatives (sorbic acid, sodium dehydroacetate, benzoic acid and p-hydroxybenzoic acid esters), sweeteners (dulcin, saccharin -

Na and acesulfame-K) and antioxidants (BHA and TBHQ) are frequently used in Taiwan to enhance product quality and shelf life. However, it has been reported that the consumption of these additives in excess may exhibit toxic effects to the human body.[1,2,3] Therefore, a method for rapid extraction and simultaneous determination of these additives is necessary.

The extraction of additives from foods has been previously achieved by direct injection,[4,5] steam distillation[6,7] and solvent extraction.[8,9] Direct injection was used for extraction of additives from liquid food samples such as carbonated drinks. This method is simple and easy to use. However, some impurities can also be extracted which interfere with the subsequent separation of additives from foods. Steam distillation can be used for solid and liquid types of foods. However, this method is time-consuming and the recoveries for some additives are low. Organic solvents such as methanol, acetonitrile, ethyl acetate and ethanol can also be used for extraction of additives. However, it is difficult for one solvent to extract all the additives because of polarity differences among these additives. Thus, it is necessary to find a better solvent for the possibility of simultaneous extraction all of these additives. More recently, a Sep-Pak C_{18} cartridge was used for extraction of preservatives and sweeteners in foods.[7] Compared to the other extraction methods, this method is fast and accurate. Also, the recovery is high (>93.8%). Nevertheless, this method has to be modified because some more additives were included in this study. In addition, the possibility of using a Sep Pak™ silica gel cartridge to purify additives has to be investigated.

The simultaneous separation of preservatives, sweeteners and antioxidants by HPLC has been difficult because of polarity differences among these additives. In recent years, paired-ion liquid chromatography was developed for separation of these additives. Terada and Sakabe[7] used a mobile phase of acetonitrile-water-0.2M phosphate buffer solution (7:12:1, v/v/v) containing 2 mM cetyltetrabutylammonium chloride (CTA) to separate nine preservatives and one sweetener, within 32 min, with flow rate at 1.0 mL/min and detection at 233 nm. In a later study, Ikai et al.[9] developed a solvent system of methanol-acetonitrile-0.05M acetonic acid solution (pH = 4.5; 1.5 : 1 : 3.1, v/v/v) containing cetyltrimethylammonium chloride (2.5mM) to separate eight preservatives and one sweetener, within 20 min, with flow rate at 1.0 mL/min and detection at 233 nm. Recently, Chen and Fu[10] employed a mobile phase of acetonitrile-50 mM aqueous α-hydroxyisobutyric acid solution (pH = 4.5; 2.2 : 3.4, v/v) to separate twelve preservatives and three sweeteners, within 40 min, with flow rate at 1.0 mL/min and detection at 233 nm. As these authors used a

monomeric C_{18} phase column to separate additives, it is necessary to compare the separation efficiency between monomeric and polymeric phases of C_{18} columns.

It has been reported that polymeric C_{18} phase exhibits better selectivity for structurally similar compounds than monomeric C_{18} phase.[11] The purposes of this study were (1) to compare the extraction efficiency of four extraction methods, i.e., steam distillation, acetone extraction, Sep-Pak C_{18} cartridge and Sep-Pak silica gel cartridge, and (2) to compare the separation efficiency of additives by monomeric and polymeric phases of C_{18} columns.

MATERIALS AND METHODS

Instrumentation

The HPLC system consisted of a Jasco PU-980 pump, a Jasco UV-970/975 detector (Jasco Co., Tokyo, Japan) and a SIC chromatocorder 12 integrator (System Instruments, Tokyo, Japan). Separations were performed on a Shoko stainless-steel monomeric C_{18} column (25 cm X 4.6 mm I.D., 5μm) (Kyoto, Japan) and a stainless-steel polymeric C_{18} column (25 cm X 4.6 mm I.D., 5μm) (J.T. Baker Co., Frankfurt, Germany). A Sep-Pak C_{18} cartridge, containing 360 mg packing material, and a Sep-Pak silica gel cartridge, containing 690 mg packing material, were all from Waters Co. (Milford, MA, USA)

Reagents

p-Hydroxybenzoic acid esters (methyl-, ethyl-, propyl-, and n-butyl-PHBA), salicyclic acid, 3-t-butyl-4-hydroxyanisole (BHA), and hexadecyltrimethylammonium bromide (HTA) were purchased from Nacalai Co., (Kyoto, Japan). Isopropyl-PHBA, isobutyl-PHBA, and t-butyl hydroxyquinone (TBHQ) were from Tokyo Chemical Co. (Tokyo, Japan). Dulcin, saccharin-Na and α-hydroxyisobutyric acid were from Sigma Co (St.Louis, Mo, USA). Acesulfame-K was from Hoechst Co. (Frankfurt, Germany). Succinic acid and sodium dehydroacetate (DHA-Na) were from Kwok Wah Co. (Taipei, Taiwan). Sodium hydroxide was from J. T. Baker Co. (NJ, USA).

Solvents used for extraction, including methanol, acetonitrile, hexane, ethyl acetate and acetone, were analytical grade and were from Merck (Darmstadt, Germany). HPLC-grade solvents such as methanol and acetonitrile were filtered through a 0.2 μm membrane filter and degassed under vacuum prior to HPLC analysis. All the water used was purified by the Milli-Q water purification system (Millipore, Bedford, MA, USA).

Extraction of Additives by Steam Distillation

A method similar to that used by Terada and Sakabe,[7] for extraction of additives from solid type of foods, was used in our work. A ten gram sample each of soy sauce, roast beef and sugared fruit, was placed in a 500 mL flask. Internal standard TBHQ (250 mg), sodium chloride (60 g), deionized water (150 mL) and 15% tartaric acid solution (10 mL) were added to the flask. The mixture was then steam distilled at a rate of 10 mL/min until approximately 300 mL of distillate was collected in a 500 mL flask; the volume was adjusted to 500 mL with acetonitrile. The solution was filtered through a 0.2 μm membrane filter and subjected to HPLC analysis.

Extraction of Additives by a Sep-Pak C_{18} Cartridge

A modified method used for extraction of additives from both solid and liquid types of foods by a Sep-Pak C_{18} cartridge, as described by Chen and Fu,[10] was used.

Extraction of Additives by Acetone

A two gram sample of soy sauce, and 1 gm sample of sugared fruit and roast beef, each, was placed in a 50 mL volumetric flask. Ten gm sodium chloride was added to the flask, and the volume adjusted with acetone. After swirling vigorously, the mixture was allowed to stand for 30 min, then vacuum-filtered through a Buchner funnel. The filtrate was collected in a 50 mL volumetric flask, and the residue washed with 10 mL acetone. The filtrate was also added to the flask, and the volume adjusted with acetone. The solution was filtered through a 0.2 μm membrane filter and subjected to HPLC analysis.

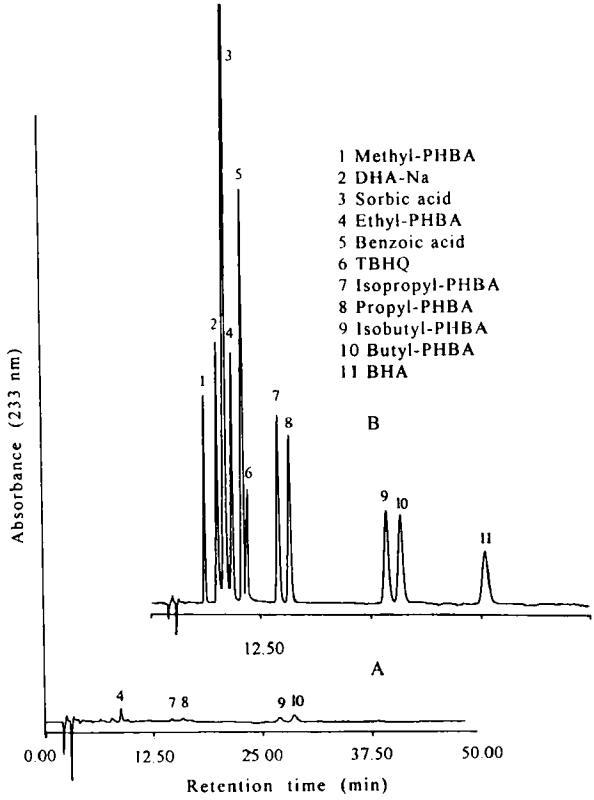

Figure 1. Chromatograms of food additives in soy sauce, extracted by steam distillation. A solvent system consisting of acetonitrile/50 mM aqueous αhydroxyisobutyric acid (pH4.5; 2.2:3.4, v/v) containing 2.5 mM HTA was used. A: soy sauce only; B: soy sauce spiked with food additive standard at a concentration of 25 μg/g each.

Purification of Acetone Extract by a Sep-Pak Silica Gel Cartridge

Five mL acetone extract obtained from 1 gm sample of roast beef, as described above, was poured into a silica-gel cartridge, which was previously activated with 10 mL acetone at a rate of 2.0 mL/min, and filtrate A was collected. Preservatives adsorbing to the packing were then eluted with 10mL ethyl acetate, acetone or methanol; filtrates B, C and D were collected.

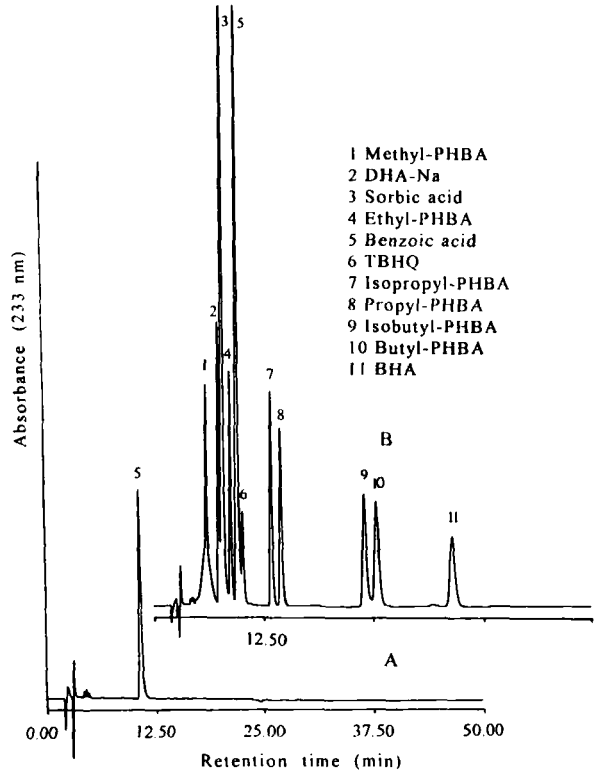

Figure 2. Chromatograms of food additive in sugared fruit, extracted by steam distillation. A solvent system of acetonitrile/50 mM aqueous α-hydroxy isobutyric acid (pH4.5; 2.2/3.4, v/v), containing 2.5 mM HTA was used.
A: sugared fruit only; B: sugared fruit spiked with food additive standard at a concentration of 25 μg/g each.

Each filtrate was diluted to volume (25 mL) with acetone, and filtered through a 0.2 μm membrane filter and subjected to HPLC analysis.

HPLC Analysis of Additives

A mobile phase of acetonitrile-50mM α-hydroxyisobutyric acid solution (pH=4.5; 2.2:3.4, v/v), containing 2.5 mM HTA, was used to separate 14 food

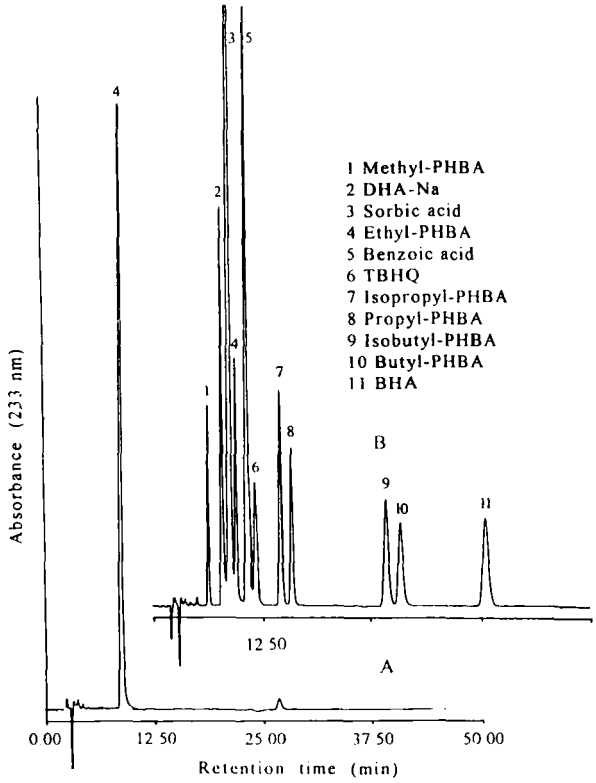

Figure 3. Chromatograms of food additive in dried roast beef extracted by steam distillation. A solvent system of acetonitrile/50 mM aqueous α-hydroxy-isobutyric acid (pH4.5; 2.2/3.4, v/v) containing 2.5 mM HTA was used. A: dried roast beef only; B: dried roast beef spiked with food additive standard at a concentration of 25 μg/g each.

additives, including dulcin, methyl-PHBA, DHA-Na, sorbic acid, ethyl-PHBA, benzoic acid, TBHQ, isopropyl-PHBA, propyl-PHBA, saccharin-Na, acesulfame-K, isobutyl-PHBA, butyl-PHBA and BHA, with detection at 233 nm and flow rate at 1.0 mL/min.[10] Sensitivity was 0.08 AUFS and injection volume was 20 μL. Recovery was determined by adding 25 or 2.5 mg standard to each sample, and steam distillation was performed for the former and the other three extraction methods for the latter. Recovery data were then calculated by dividing the amount of each additive standard added to the sample by the amount of standard obtained following extraction and

Table 1

Recoveries of Food Additives from Various Foods Extracted by Steam Distillation[1,2]

Compound	Recovery[3] (%) Soy Sauce		Sugared Fruit		Dried Roast Beef	
Dulcin	[4]		[4]		[4]	
Methyl-PHBA	86.80[a]	(1.10)	87.39[a]	(0.48)	48.32[b]	(1.35)
DHA-Na	71.63[a]	(2.59)	66.19[b]	(1 20)	65.27[b]	(2.67)
Sorbic Acid	94.77[a]	(3.35)	104.21[b]	(1.22)	52.18[c]	(1.99)
Ethyl-PHBA	80.02[a]	(1.02)	81.46[a]	(2.59)	34.31[b]	(1.81)
Benzoic acid	94.50[a]	(0.56)	109.28[b]	(4.58)	77.00[c]	(2.25)
TBHQ	69.29[a]	(1.84)	83.03[b]	(2.30)	71.35[c]	(1.76)
Isopropyl-PHBA	94.57[a]	(1.65)	97.59[b]	(1.68)	49.64[c]	(1.84)
Propyl-PHBA	90.73[a]	(1.86)	85.95[b]	(3.90)	34.71[c]	(3.44)
Saccharin	[4]		[4]		[4]	
Acesulfame-K	[4]		[4]		[4]	
Isobutyl-PHBA	84.88[a]	(1.12)	83.33[a]	(2.77)	40.38[b]	(1.72)
Butyl-PHBA	85.88[a]	(2.41)	93.05[b]	(2.33)	41.71[c]	(3.44)
BHA	78.42[a]	(5.66)	90.17[b]	(3.51)	65.24[c]	(2.25)

1. a-c symbols bearing different letters in the same row are significantly different ($p<0.05$).
2. Values in parentheses represent coefficient of variation (%).
3. Mean of duplicate determinations.
4. Not extracted by steam distillation.

quantification. Quantitation was carried out using calibration graphs obtained from a standard solution containing six concentrations of additives (5, 10, 25, 50, 75 and 100 ppm) and 25 ppm internal standard (TBHQ).

All the data were subjected to analysis of variance (PROC ANOVA) and Duncan's multiple range test procedures for statistical analysis.[12]

Table 2

Recoveries of Food Additives from Various Foods Extracted by Acetone[1,2]

Compound	Recovery[3] (%) Soy Sauce		Sugared Fruit		Dried Roast Beef	
Dulcin	83.88[a]	(3.13)	78.77[a]	(1.53)	76.42[a]	(1.72)
Methyl-PHBA	87.56[a]	(1.92)	85.00[a]	(1.39)	81.59[a]	(0.97)
DHA-Na	75.04[a]	(2.02)	74.25[a]	(2.39)	69.54[b]	(3.33)
Sorbic Acid	88.39[a]	(1.30)	84.41[a]	(3.66)	89.05[a]	(4.66)
Ethyl-PHBA	82.96[a]	(1.97)	86.04[a]	(2.22)	80.00[a]	(2.49)
Benzoic acid	89.19[a]	(2.27)	89.35[a]	(2.29)	81.54[b]	(1.63)
TBHQ	90.42[a]	(1.87)	88.45[a]	(1.23)	83.51[b]	(1.16)
Isopropyl-PHBA	93.68[a]	(2.78)	91.55[a]	(4.29)	86.74[b]	(1.70)
Propyl-PHBA	92.94[a]	(1.79)	91.76[a]	(1.96)	86.41[a]	(3.88)
Saccharin	89.88[ab]	(2.74)	95.68[a]	(2.25)	84.19[b]	(4.24)
Acesulfame-K	92.76[a]	(1.31)	92.11[a]	(1.89)	85.02[b]	(5.05)
Isobutyl-PHBA	85.44[b]	(4.10)	81.30[b]	(2.21)	85.00[b]	(3.25)
Butyl-PHBA	96.19[a]	(3.25)	92.28[a]	(2.40)	92.76[a]	(1.49)
BHA	88.13[a]	(5.64)	82.88[b]	(3.04)	83.13[ab]	(3.28)

1. a-b symbols bearing different letters in the same row are significantly different ($p<0.05$).
2. Values in parentheses represent coefficient of variation (%).
3. Mean of duplicate determinations.

RESULTS AND DISCUSSION

Extraction by Steam Distillation

Figures 1, 2 and 3 show the HPLC chromatograms of additives in soy sauce, sugared fruit and dried roast beef, extracted by steam distillation, respectively. Five additives, ethyl-, isopropyl-, propyl, isobutyl- and butyl-PHBA were found in the soy sauce, while benzoic acid and sorbic acid were found in both sugared fruit and dried roast beef. Table 1 shows the recoveries

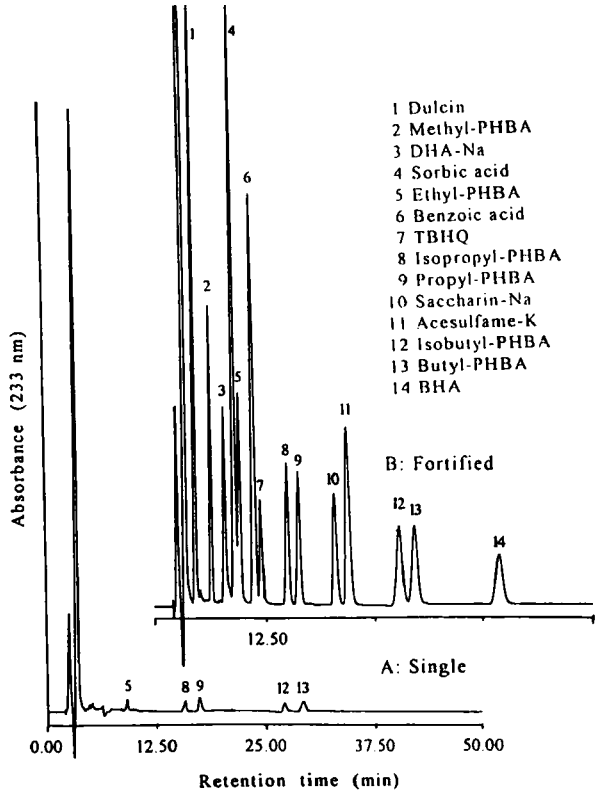

Figure 4. Chromatograms of food additive in soy sauce extracted by acetone. A solvent system of acetonitrile/50 mM aqueous α-hydroxyisobutyric acid (pH4.5; 2.2/3.4, v/v) containing 2.5 mM HTA was used. A: soy sauce only; B: soy sauce spiked with food additive standard at a concentration of 2.5 μg/g each.

of food additives from soy sauces, sugared fruit and dried roast beef by steam distillation. One drawback for steam distillation is that it failed to extract dulcin, saccharin-Na and acesulfame-K from foods. This is probably because sweeteners can be decomposed in the presence of tartaric acid during sample preparation. Compared to the other additives, DHA-Na has lower recovery for soy sauce and sugared fruit, probably because of its high boiling point, which results in partial extraction from food samples. Dried roast beef has lower recovery for all the additives than soy sauce or sugared fruit, mainly because

Table 3

Influence of Various Elution Solvents on Recoveries of Food Additives of Dried Roast Beef Acetone Extract using a Sep-Pak Silica Gel Cartridge

Recoveries[1] (%)

Compound	Acetone Extact	Elution solvent[2] None[3]	Ethyl Acetate[4]	Methanol[4]	Acetone[4]
Dulcin	76.42	61.38	76.99	75.31	75.94
Methyl-PHBA	81.59	81.49	81.52	80.46	82.86
DHA-Na	69.54	44.14	41.91	63.69	51.27
Sorbic Acid	89.05	64.99	75.69	77.46	80.73
Ethyl-PHBA	80.00	80.25	80.46	78.89	81.81
Benzoic acid	81.54	71.54	60.96	62.29	61.63
TBHQ	83.51	83.41	75.28	77.33	76.63
Isopropyl-PHBA	86.74	86.53	79.35	77.81	80.69
Propyl-PHBA	86.41	84.21	80.19	78.46	81.08
Saccharin-Na	84.19	36.92	64.40	71.87	70.22
Acesulfame-K	85.02	69.01	71.72	81.35	71.63
Isobutyl-PHBA	95.00	83.24	85.36	83.19	85.22
Butyl-PHBA	92.76	87.15	90.08	92.56	88.51
BHA	83.13	74.97	78.08	76.46	73.44

1. Mean of duplicate analyses.
2. For purification of acetone extract using a Sep-Pak silica gel cartridge
3. Acetone extract passed through Sep-Pak silica gel cartridge without elution solvent.
4. Acetone extract passed through Sep-Pak silica gel cartridge followed by elution solvent.

the former contains more unwanted substances, i.e., fat and protein, which can interfere with the subsequent separation of additives.

To remedy this problem, the removal of fat and protein is necessary prior to extraction of additives from high fat- or protein-containing foods. Solvents, such as ether, are often employed to extract the fat, followed by potassium

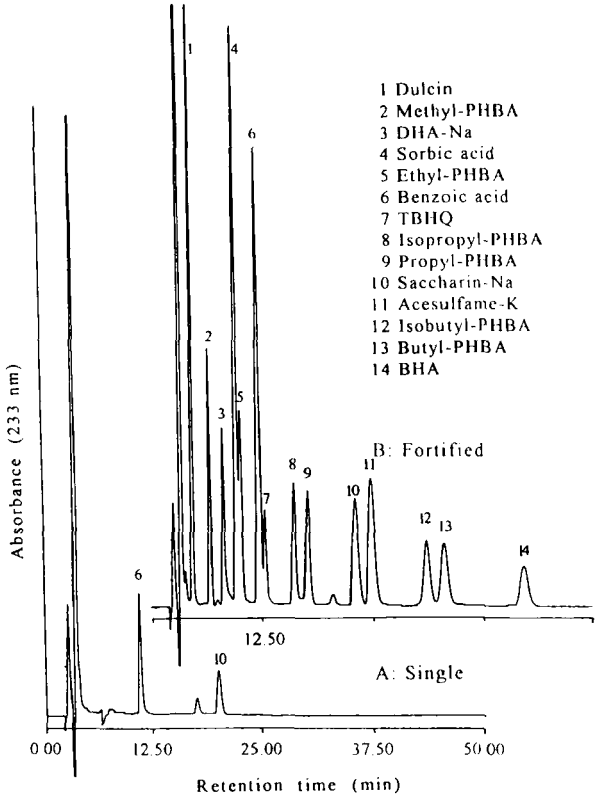

Figure 5. Chromatograms of food additive in sugared fruit extracted by acetone. A solvent system of acetonitrile/50 mM aqueous α-hydroxyisobutyric acid (pH4.5; 2.2/3.4, v/v) containing 2.5 mM HTA was used.
A: sugared fruit only; B: sugared fruit spiked with food additive standard at a concentration of 2.5 μg/g each.

hydroxide, to saponify triglycerides.[13] Likewise, ethanol is often used to precipitate protein.[5]

Nevertheless, the coefficient of variation for all the additives was between 0.48-5.66%, indicating that steam distillation can be applicable to low fat- and protein-containing foods such as soy sauce and sugared fruit.

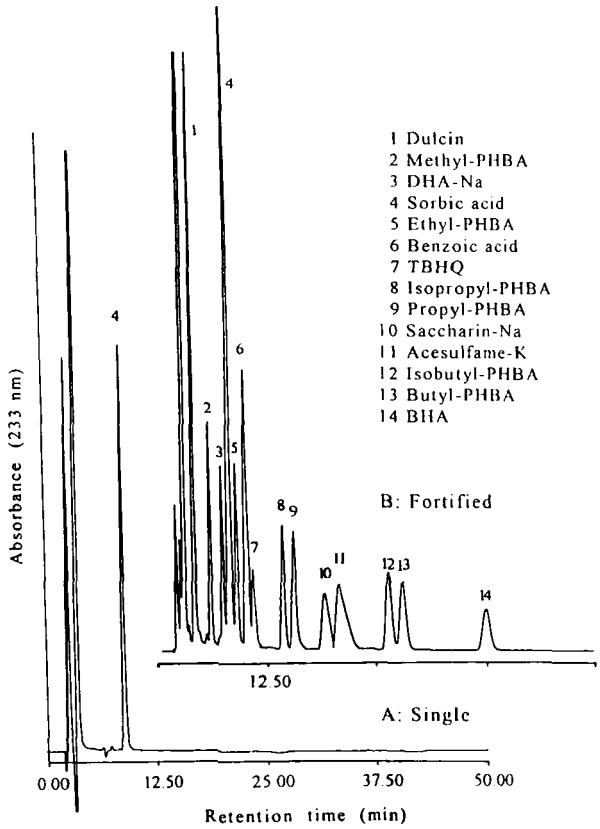

Figure 6. Chromatograms of food additive in dried roast beef extracted by acetone. A solvent system of acetonitrile/50 mM aqueous acetonic acid (pH4.5; 2.2/3.4, v/v) containing 2.5 mM HTA was used.
A: dried roast beef only; B: dried roast beef spiked with food additive standard at a concentration of 2.5 μg/g each.

Extraction by a Sep-Pak C_{18} Cartridge

The recovery data for additives extracted by a Sep-Pak C_{18} cartridge was described in a previous report.[10] Compared to the other additives, saccharin-Na has lowest recovery in dried roast beef, probably because of its partial decomposition under acidic conditions. Unlike steam distillation, the short

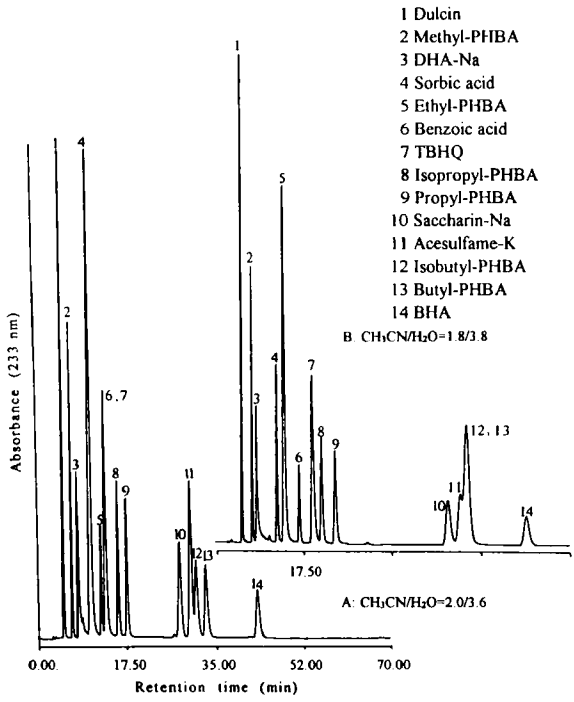

Figure 7. Chromatograms of food additive using a polymeric column and two mobile phases. A: $CH_3CN:H_2O$ = 2.0:3 6; B: $CH_3CN:H_2O$ = 1.8:3.8, containing 50 mM aqueous α-hydroxyisobutyric acid (pH 4.5) and 2.5 mM HTA with detection at 233 nm.

exposure time of saccharin-Na to acid only results in partial decomposition. Also, this method is superior to steam distillation because the latter failed to extract sweeteners from food samples. Nevertheless, one drawback of using a Sep-Pak C_{18} cartridge is that some impurities were also coeluted. Terada and Sakabe[7] used a Sep-Pak C_{18} cartridge to extract additives from coffee drinks and good recovery was obtained.

Our study showed that Sep-Pak C_{18} cartridge can also be applicable to solid type of foods as long as appropriate steps were taken before extraction, and the recoveries of most additives between soild and liquid types of foods were not significant ($p>0.05$).[10]

Extraction by Acetone

It has been reported that organic solvents such as alcohol and ethyl acetate can be used to extract sweeteners and preservatives from foods, and the former (alcohol) results in higher recovery than the latter (ethyl acetate).[14] Figures 4-6 show the HPLC chromatograms of additives in soy sauce, sugared fruit and dried roast beef extracted by acetone, respectively. Soy sauce was found to contain ethyl-PHBA, isopropyl-PHBA, propyl-PHBA, isobutyl-PHBA and butyl-PHBA, while benzoic acid and saccharin-Na were found in sugared fruit and sorbic acid in dried roast beef. Table 2 shows recoveries of additives extracted by acetone. Compared to the other additives, dulcin and DHA-Na have lowest recovery, probably because of their low solubility in acetone. Dried roast beef was also found to have lower recovery for most additives than soy sauce or sugared fruit, mainly because the former contains more protein and fat. Nevertheless, the coefficient of variation was between 0.97 and 5.64%, indicating that using acetone to extract additives can result in high reproducibility.

Purification of Acetone Extract by Sep-Pak Silica Gel Cartridge

From Figures 4-6 it can be seen that dried roast beef contained more impurities than soy sauce and sugared fruit. Thus, it is necessary to investigate the possibility of purifying the acetone extract by employing a Sep-Pak silica gel cartridge, so that column lifetime can be enhanced.

Table 3 shows the effect of various elution solvents on recoveries of additives of dried roast beef using Sep-Pak silica gel cartridge. Without elution solvent, the recovery loss of PHBA esters was low, mainly because of their low polarity, which results in weak interaction with silica gel. In contrast, saccharin-Na has highest recovery loss because of its strong interaction with silica gel. After elution with various solvents, the recoveries of most additives increased. However, saccharin-Na and DHA-Na were only partially eluted, indicating that the solvent strength of elution solvent was too low.

To remedy this problem, the selection of an eluant with high solvent strength, such as water, is necessary. Nevertheless, some impurities can also be coeluted. Thus, using a Sep-Pak silica gel cartridge to purify additives is not an appropriate method.

Table 4

Effect of Monomeric and Polymeric Columns on Capacity Factors (k') of Food Additives[a]

Compound	k' Monomeric C_{18} column	k' Polymeric C_{18} column
Dulcin	0.75	0.65
Methyl-PHBA	1.51	1.18
DHA-Na	2.07	1.61
Sorbic Acid	2.32	2.13
Ethyl-PHBA	2.78	2.13
Benzoic acid	3.15	2.83
TBHQ	3.50	2.83
Isopropyl-PHBA	4.89	3.75
Propyl-PHBA	5.43	4.18
Saccharin-Na	7.24	6.25
Acesulfame-K	7.95	7.00
Isobutyl-PHBA	10.03	7.72
Butyl-PHBA	10.71	8.22
BHA	14.76	11.29

a. A mobile phase of acetonitrile -50 mM aqueous α-hydroxy- isobutyric acid (pH 4.5; 2.2:3.4, v/v), containing 2.5 mM HTA with flow rate at 1.0 mL/min and detection at 233 nm was used.

Comparison of Four Extraction Methods

One drawback of steam distillation is that it failed to extract sweeteners from foods. Despite the drawback, steam distillation can be applicable to extraction of preservatives and antioxidants from liquid, solid or viscous types of foods. Also, the extracts contained less impurities in comparison with the other methods. Sep-Pak C_{18} cartridge is also a good method to choose, because it has higher recovery than the other three methods. The extraction of food additives by acetone is rapid and convenient. However, the acetone extract contained more impurities than the other methods. Also, this method resulted in low recovery for dulcin and DHA-Na in sugared fruit and dried roast beef.

The selection of an appropriate elution solvent is difficult for Sep-Pak silica gel cartridge because of wide polarity difference of additives. Hence, the application of Sep-Pak silica gel cartridge for purification of acetone extract should be disregarded.

Comparison of Monomeric and Polymeric Column

Reverse phase materials can be divided into monomeric and polymeric phases, depending on the difference of modification method on the silica gel surface. The former results from the reaction of monofunctional silane reagent, e.g., chlorodimethyloctylsilane, with silanol sites at the silica surface. Trifunctional silane reagents, e.g., octadecyltrichlorosilane, may also be used to produce monomeric phases. However, silane hydrolysis and polymerization can possibly form a polymeric bonded phase in the presence of water.[11,15] Because of steric hindrance, the selectivity of residual silanol is low and thus solute stability is greatly enhanced.[11]

Table 4 shows the effect of monomeric and polymeric columns on k' (capacity factor) of food additives. Monomeric column is superior to polymeric column in terms of separation efficiency. It has been reported that a polymeric column provided better selectivity for structurally similar compounds.[11] In this study this effect cannot be accounted for, because of wide structural differences among sweeteners, antioxidants and preservatives. Nevertheless, the separation time of using a monomeric column was longer, as shown by k' values. Although the k' values of 14 additives could be reduced to 11.29 by employing a polymeric column, some peaks (sorbic acid and ethyl-PHBA; benzoic acid and TBHQ) were overlapped. This result implied that the solvent strength of the mobile phase was too high. By changing mobile phase as acetonitrile-aqueous α-hydroxyisobutyric acid (2.0:3.6 or 1.8:3.8, v/v) and thus decreasing solvent strength, it was found that the retention times of 14 additives increased substantially (Figures 7A and 7B). Although the separation efficiency was improved, additives benzoic acid and TBHQ coeluted for the former system, and isobutyl-PHBA and butyl-PHBA coeluted for the latter system. From the above discussion, it can be concluded that a monomeric column provided better resolution of 14 additives than a polymeric column as long as a mobile phase of acetonitrile-aqueous α-hydroxyisobutyric acid was employed.

In conclusion, the application of a Sep-Pak C_{18} cartridge is the method of choice because it can extract all the additives from foods and high recoveries

were observed. Monomeric column was found to be superior to polymeric column for simultaneous separation of preservatives. sweeteners and antioxidants.

ACKNOWLEDGMENT

This study was supported by a grant (NSC84-409-B-030-002) from the National Science Council, Taiwan, R.O.C.

REFERENCES

1. J. Y. Hsu, **The Proper Use of Additive**, Hua-Siang-Yuan Publishing Co., Taipei, 1985, pp.227-333.

2. J. J. Chen, G. R. Shen, **Food Additive**, Wen-Yuan Publishing Co., Taipei, 1986, pp.173-201.

3. H. W. Chiu, Food Industry, **18**, 36-38 (1986).

4. M. Veerabhadrarao, M. S. Narayan, O. Kapur, J. Assoc. Off. Anal. Chem., **70**, 578-582 (1987).

5. J. F. Lawrence, C. F. Charbonneau, J. Assoc. Off Anal. Chem., **71**, 934-937 (1988).

6. AOAC, Assoc. Offic. Anal. Chem., 14th ed. Washington, D.C., 1984.

7. H. Terada, Y. Sakabe, J. Chromatogr., **346**, 333-340 (1985).

8. L. V. Bui., C. Cooper, J. Assoc. Off Anal. Chem., **70**, 892-896 (1987).

9. Y. Ikai, H. Oka, N. Kawamura, M. Yamada, J. Chromatogr., **457**, 333-343 (1988).

10. B. H. Chen, S. C. Fu, Chromatographia, In Press, 1995.

11. L. C. Sander, S. A. Wise, Anal. Chem., **56**, 504-510 (1984).

12. **SAS/STAT Guide for Personal Computer**, Version 6 ed, SAS Instruments, Cary, NC, 1985.

13. U. Leuenberger, R. Gauch, E. Baumgartner, J. Chromatogr., **173**, 343-348 (1979).

14. K. H. Wu, H. F. Lee, M. H. Shen, Annual Report of Food and Drug Bureau of Taiwan, **1**, 246-249 (1989).

15. M. Verzele, P. Mussche, J Chromatogr., **254**, 117-122 (1983).

Received August 13, 1995
Accepted September 5, 1995
Manuscript 3949