Journal of Food and Drug Analysis
1999. 7(4) : 291-304

藥物食品分析
第七卷　第四期

A Simple Method for the Simultaneous Determination of Various Preservatives in Liquid Foods

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ABSTRACT

A direct injection gas chromatography (GC) instrument equipped with an intermediate polar column (CP-SIL 8CB, 30 m × 0.53 mm) was used to determine nine preservatives (including sorbic acid, dehydroacetic acid, benzoic acid, methyl paraben, ethyl paraben, isopropyl paraben, propyl paraben, isobutyl paraben and butyl paraben) in vinegar, soy sauce, pickle condiment liquid and fish sauce. A water soluble compound 1,4-dihydroxybenzene (DHB) was used as an internal standard. The detection limits of the above preservatives were found to be lower than 0.5 ppm. A recovery study was performed by spiking 200 μg target compounds into 1 ml of vinegar or soy sauce and then analyzed by GC. Results showed the recoveries of the above nine preservatives were in the range of 95-106% with coefficients of variation less than 7.2%. These results indicate that the direct injection GC method is an accurate, simple and rapid method to simultaneously screen and quantify sorbic acid, dehydroacetic acid, benzoic acid and six parabens in liquid foods without further sample preparation. Thirty-seven liquid food samples of vinegar, soy sauce, pickle condiment liquid and fish sauce were analyzed using the developed method. The contents of sorbic acid, benzoic acid, dehydroacetic acid, and 6 parabens in vinegar samples were found to be 0-407, 0-519, 0 and 0-102 μg/mL; in soy sauce samples were 0-311, 0-266, 0 and 0-243 μg/mL; in pickle condiment liquid samples were 0-462, 0-3, 0 and 0-209 μg/mL; and in fish sauce samples were 0-1044, 0-266, 0 and 0-163 μg/mL, respectively. Results also showed most of the test samples were fortified with two or more preservatives. Thirteen out of 14 samples labeled "preservative-free" were detected to be preservative containing products. The total preservative contents in some test samples were over the regulation levels of 100 μg/mL in vinegar and 500 μg/mL in soy sauce or fish sauce.

Key words: **preservative, liquid food, direct injection, gas chromatography, determination.**

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Accepted for Publication: Sep. 27, 1999

INTRODUCTION

Some food preservation methods such as drying, salting, sugaring, heating and adding preservatives have been developed to reduce food deterioration. Preservatives have long been used to prolong the shelf-life of foods. They are widely used in soy sauce, candy, preserved fruit, meat products, cheese, butter, margarine, juice, and baked goods. Seventeen preservatives have been approved in the ROC. Among them, sorbic acid, benzoic acid and its potassium and sodium salts, and parabens are used most often. Their usage levels and applicable products are also regulated. Food which is over-fortified with additives such as preservatives, antioxidants, and sweeteners, or the inadequate use of food additives could adversely affect human health as they are consumed. Therefore, the identification and quantification of food additives are important in terms of food additive inspection[1-2]. With respect to the usage of preservatives, except parabens, the use of more than one preservative in the same product is not allowed[3].

Sorbic acid, benzoic acid and its potassium and sodium salts and parabens are capable of preventing vinegar from turbidity caused by the decrease of acidity due to the *Acetobacter* species growth. The above preservatives are also able to inhibit the growth of mold in high salt products such as soy sauce, soy paste, and condiment liquids.

Traditional methods for determination of benzoic acid are based on its chemical property. Benzoic acid could react with iron chloride to form an iron benzoate precipitate, which appears salmon red in color[3]. Benzoic acid could also be quantified using a modified Mohler method where an alkaline titration is conducted[4]. Sorbic acid was usually quantified using a spectrophotometric method where the absorbance at 530 nm was measured[4-6]. This method detects a red complex formed by the reaction of thiobarbituric acid with malonaldehyde, which is produced when sorbic acid is oxidized by $K_2Cr_2O_7$. The above methods, however, are complex in operation and subject to interference by many factors. The analytical techniques have been much improved by using spectrophotometry with UV detection[7-8], thin layer chromatography (TLC)[9], gas solid chromatography (GSC)[10-11], gas liquid chromatography (GLC)[12] and high performance liquid chromatography (HPLC)[13-26]. These methods require a complicated sample preparation procedure, which involves steam distillation or direction extraction under acid conditions, alkalization of distillates or extracts, removal of neutral and alkaline materials with an organic solvent such as diethyl ether, petroleum ether or chloroform, acidification of aqueous phase, and finally preservatives extraction with solvent. This sample preparation is time-consuming and therefore not suitable for routine analysis.

Gas chromatography (GC), which can provide an analysis with high resolution as well as excellent sensitivity, is one of the most important analytical techniques. In our experience, the insertion of glass wool into the glass liner of the injection port or adaptation of a guard column (about 1~2 m) in front of the analytical column is capable of preventing the analytical column from being contaminated by non-volatiles, inhibiting the interference from contaminants, and reducing the tailing effect of peaks so as to improve the resolution in GC chromatogram, as well as prolong the column life[27-30]. In our previous study[31], we established a simple method using non-polar organic solvents to extract benzoic acid and sorbic acid, which were then analyzed by GC. Analysis of other preservatives such as dehydroacetic acid and parabens, has not yet been set up in our laboratory. In this study, a direct injection GC method was researched to simultaneously analyze multiple preservatives in liquid foods including vinegar, soy sauce, pickle condiment liquid and fish sauce. This method is expected to be a routine analytical method for determination of multiple preservatives in liquid foods.

METERALS AND METHODS

I. *Materials*

(I) Thirty-seven test samples including 10 samples of vinegar, 10 samples of pickle condiment, 13 samples of soy sauce and 4 samples of fish sauce were purchased from the local supermarkets of Pintung and Tainan.

(II) The standards of 1,3-butanediol, 1,4-dihydroxybenzene, 1,5-pentanediol, 1,6-hexanediol, sorbic acid, dehydroacetic acid, benzoic acid, methyl paraben, ethyl paraben, isopropyl paraben, propyl paraben, isobutyl paraben and butyl paraben (of purity > 98%) were obtained from Tokyo Chemical Inc. (TCI, Tokyo). Methanol and propanol were LC grade and purchased from ALPS (Taiwan).

II. *Methods*

(I) *Preparation of Standard and Internal Standard Solutions*

Two hundred mg of sorbic acid (SA), dehydroacetic acid (DHA), benzoic acid (BA), methyl paraben (Me-P), ethyl paraben (Et-P), isopropyl paraben (IPr-P), propyl paraben (Pr-P), isobutyl paraben (IBu-P), butyl paraben (Bu-P) and 1,4-dihydroxybenzene (DHB, as an internal standard) were separately weighed and placed in a 100-mL volumetric bottle. Twenty-mL of methanol was then added to bottle to dissolve above chemicals. Standard and internal standard solutions were thus prepared by adding distilled water to the volume.

(II) *Test for the Relative Response Factor (RRF) of Preservatives to 1,4-Dihydroxybenzene*

The mixtures of various ratios of 0.2% (w/v) preservatives (sorbic acid, dehydroacetic acid, benzoic acid, and 6 parabens) to 0.2% (w/v) 1,4-dihydroxybenzene (2:1, 1:1 and 1:2) were injected to GC. The RRF of preservatives to 1,4-dihydroxybenzene was calculated as follows:

$$RRF = \frac{A_{preservatives}}{W_{preservatives}} \div \frac{A_{IS}}{W_{IS}} \quad \text{.....(1)}$$

The contents of preservatives were determined according to the following equation.

$$\text{Preservatives}(\mu g/mL) = \left(\frac{A_{preservatives}}{A_{IS}} \times \frac{W_{IS}}{RRF} \right) \times \frac{1}{V}$$

.....(2)

Where $A_{preservatives}$ is the peak area of preservatives; A_{IS} is the peak area of internal standard; $W_{preservatives}$ is the weight (μg) of preservatives; W_{IS} is the weight (μg) of internal standard;

RRF is the relative response factor of various preservatives to internal standard; V is the volume (mL) of test samples.

(III) *Determination of Preservatives*

1. *Direct Injection Method*

The sample of vinegar, pickle condiment liquid, soy sauce and fish sauce (1 mL) was transferred into a 7-mL vial where a 0.5 mL of 0.2% (w/v) internal standard solution (1,4-dihydroxybenzene, DHB, dissolved in 20% methanol) was added. The mixture was then acidified with one drop of 5% (w/v) hydrochloride solution, vortex-mixed, and 0.1 μL of which was injected to GC for analysis.

2. *AOAC Method*

Test sample was neutralized with 10% sodium hydroxide or 10% hydrochloride in a 50-mL beaker. This neutral solution was then transferred into a 500-mL round-bottomed flask containing 15 mL of 15% tartaric acid, 60g sodium chloride and one drop of silicon resin. The solution was then diluted with water to the volume of 200 mL and steam-distilled at a rate of 10 mL/min. Fifty mL of distillate was transferred to a separation funnel, acidified with 10% sulfuric acid, saturated with sodium chloride, and extracted with 100 mL (2 X) of diethyl ether. The combined diethyl ether layer was washed with 30 mL of saturated sodium chloride solution and partitioned twice with 50 mL of 1% $NaHCO_3$ solution. The combined aqueous phase containing benzoic acid, sorbic acid, and dehydroacetic acid was designated to solution A; while diethyl ether layer, which contained parabens was designated to solution B. Solution A

was acidified with 10% sulfuric acid, saturated with sodium chloride, and extracted with 50 mL (3 X) of diethyl ether. The combined diethyl ether layer was dehydrated with anhydrous sodium sulfate, spiked with 1 mL of nonane, and evaporated at 40˚C under vacuum. The residue was then added with acetone to a volume of 5 mL. The test solution of benzoic acid, sorbic acid, and dehydroacetic acid was thus prepared. The test solution of parabens was obtained when solution B was dehydrated with anhydrous sodium sulfate followed by evaporation under vacuum, and added with acetone to a volume of 5 mL. One µL of above solutions were then injected into GC for analysis.

(IV) *Test for the Detection Limit of Preservatives*

Standard solutions of 0.1% (w/v) SA, DHA, BA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P were individually diluted to serious concentrations of 5.0, 1.0, 0.5, and 0.1 ppm. One mL of each dilution was spiked with 0.1 mL of 0.2% (w/v) internal standard prior to GC analysis. Each analysis was carried out in triplicate.

(V) *Fortification Recovery Test*

Standards (200 µg) of SA, DHA, BA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P were separately added into a 7-mL vial containing 1 mL of soy sauce or vinegar. Each vial was then spiked with 0.5 mL of internal standard and acidified with one drop of 5% (w/v) hydrochloride solution. After mixing, 0.1 µL of final solution was then injected to GC for analysis. The analysis of each fortification was carried out in triplicate and the sample blank without spiking standards was also performed.

(VI) *GC Conditions*

A Hitachi G-3000 GC equipped with FID (hydrogen and air flow at 30 and 300 mL/min, respectively) was used in this study. The temperatures of detector and injection port were 290˚, and 260˚C, respectively. Separation column was Chrom Pack CP-SIL 8CB fused silica column (30 m x 0.53 mm i.d., 1.5 µm film thickness, Netherlands). Helium as carrier gas was delivered at a flow rate of 4 mL/min. The oven temperature was set at 100˚C for 3 min, raised to 230˚C at 6˚C/min, and then rapidly increased to 300˚C at 50˚C/min. Direct injection mode with 0.1 µL injection volume was used in this study.

RESULTS AND DISCUSSION

I. *Study on GC Conditions*

The purpose of this study was to develop a simple and rapid method using a direct injection GC technique to simultaneously quantify multiple preservatives (including benzoic acid, sorbic acid, dehydroacetic acid, and parabens) in liquid foods (including vinegar, soy sauce, pickle condiment liquid, and fish sauce). This method skipped a sample preparation procedure. The only two fac-

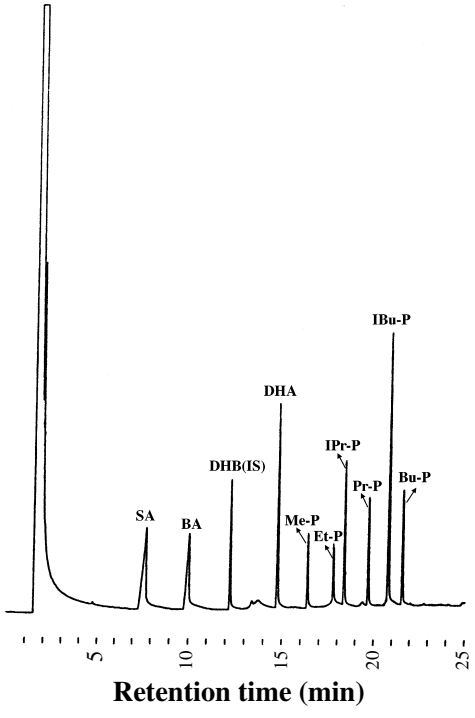

Figure 1. Gas chromatogram of authentic standard. SA=sorbic acid, BA=benzoic Acid, DHA=dehydroacetic acid, DHB=1,4-dihydroxybenzene, Me-P=methyl paraben, Et-P=Ethyl paraben, IPr-P=isopropyl paraben, Pr-P=propyl paraben, IBu-P=isobutyl paraben and Bu-P=butyl paraben.

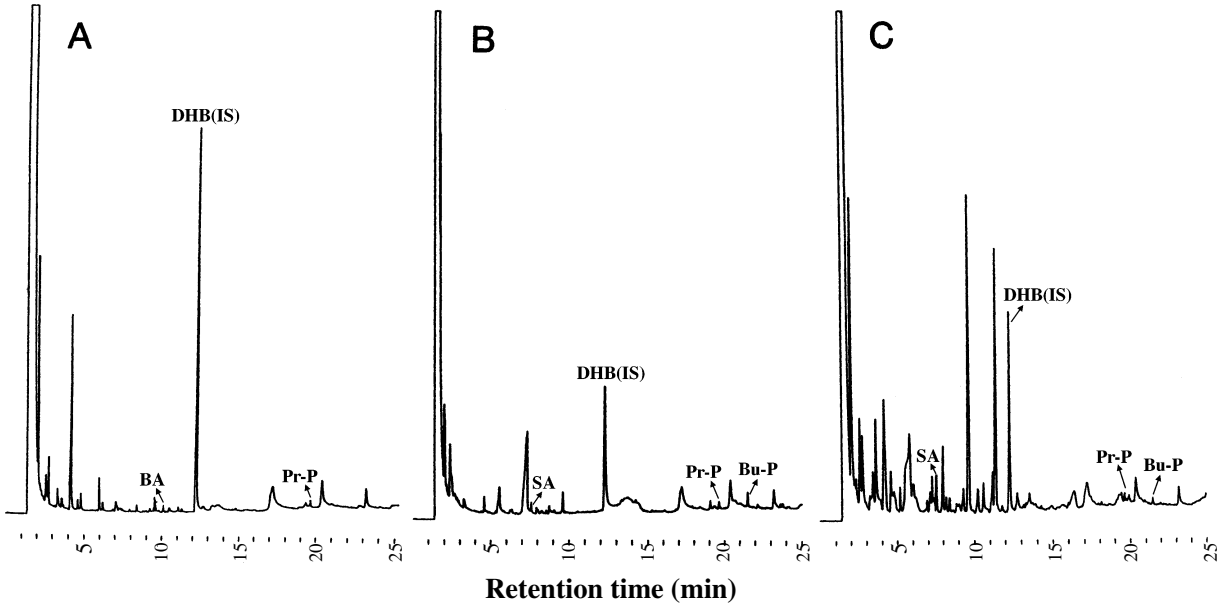

Figure 2. Gas chromatograms of various preservatives in (A) vinegar, (B) soy sauce, and (C) fish sauce by direct injection method. SA= sorbic acid, BA=benzoic acid, DHB=1,4-dihydroxybenzene, Pr-P=propyl paraben and Bu-P=butyl paraben.

tors that needed to be attention were GC column and GC conditions selections.

Using a medium polar CP SIL 8 CB column and GC conditions as described in Method (VI), the standards of SA, BA, DHA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P were found to appear at 7.55, 10.01, 14.78, 16.39, 17.79, 18.40, 19.72, 20.83 and 21.60 min, respectively, as shown in Figure 1. GC chromatograms of test samples of vinegar, soy sauce and fish sauce are shown in Figure 2. This method allows one sample to be analyzed in 25 min.

With respect to the internal standard selection, 4 compounds including 1,3-butanediol, 1,5-pentanediol, 1,6-hexanediol and 1,4-dihydroxybenzene (DHB) were injected to GC and their retention times were shown to be 5.68, 8.34, 10.12 and 12.27 min, respectively (Table 1). Compared to the GC chromatograms of test samples (vinegar, soy sauce and fish sauce), the DHB peak had much better resolution. In addition, its structure containing a benzene ring was similar to those of preservatives. Therefore, DHB was thus selected as an internal standard in this study.

II. *The RRF of Preservatives to Internal Standard*

To accurately quantify the preservatives in liquid food, determination of the RRF of preservatives including SA, BA, DHA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P to internal standard, DHB, is required. Based on the equation (2), the RRFs of above 9 tested preservatives were calculated to be 0.84, 0.85, 0.78, 1.20, 1.24, 1.33, 1.26, 1.75, and 1.51, respectively (Table 2). These data were obtained by plotting a standard curve of peak area ratios of preservative to internal standard versus concentrations. The regression coefficients of above 9 standard curves were higher than 0.98.

Table 1. Gas chromatographic retention times of internal standard candidates

Compounds	Retention time (min)[a]
1,3-Butanediol	5.68
1,5-Pentanediol	8.34
1,6-Hexanediol	10.12
1,4-Dihydroxybenzene	12.27

[a] CP-Sil 8CB(0.53mm × 30m, 1.5 μm) was used. Oven condition = 100˚C(3min)←6˚C/min ← 230˚C/min ←50˚C/min ←300˚C.

Table 2. Relative response factors of various preservatives to 1,4-dihydroxybenzene internal standard and their retention times

Compound	Relative response factor (REF)[b]	Retention time (min)[c]
Sorbic acid (SA)	0.84	7.55
Benzoic acid (BA)	0.85	10.01
1,4-Dihydroacetic acid (DHB)[a]	1.00	12.27
Dehydroacetic acid (DHA)	0.78	14.78
Methyl paraben (Me-P)	1.20	16.39
Ethyl paraben (Et-P)	1.24	17.79
Isopropyl paraben (IPr-P)	1.33	18.40
Propyl paraben (Pr-P)	1.26	19.72
Isobutyl paraben (IBu-P)	1.75	20.83
Butyl paraben (Bu-P)	1.51	21.60

[a] Used as internal standard.

[b] See "materials and method" for the determination of relative response factor.

[c] CP-Sil 8CB (0.53 mm × 30 m, 1.0 μm) was used. Oven condition = 100˚C(3min)←6˚C/min ←230˚C/min ←50˚C/min ←300˚C.

III. *Detection Limits of Preservatives*

The detection limits of 9 tested preservatives were in the range of 0.1~0.5 ppm as listed in Table 3.

IV. *Fortification Recovery Test*

The recoveries of 9 tested preservatives from vinegar and soy sauce are listed in Table 4 and 5, respectively. The results show that the recoveries of above 9 preservatives with a 200 μg fortification level were in the range of 94~107% with the coefficient of variation (CV%) less than 7.2%. This indicates the developed method is not only simple and rapid (one sample run for only 25 min) but precise enough to quantify preservatives in liquid food.

Analytical methods for preservatives are well documented. The GC method is the most popular for preservative analysis and therefore recognized as an official method by many countries[3]. However, these methods require a complicated sample preparation procedure, which involves steam distillation or direct extraction under acid condition, alkalization of distillates or extracts, removal of neutral and alkaline materials with organic solvent such as diethyl ether, petroleum ether or chloroform, acidification of the aqueous phase, and finally preservatives extraction with solvent. The preservative extracts usually need to be derivatized prior to GC analysis[3-4, 10-12]. A precise quantification of preservatives was difficult to achieve due to the loss of analytes during such a complicated sample preparation process. Solid phase extraction (SPE) is an alternative method to rapidly extract preservatives from a food matrix followed by GC or GC-MS analysis [32-34]. However, the improvement of reproducibility and accuracy in quantification is required. The direct injection GC method developed in this study requires no sample preparation procedure, which saves the cost of solvents and the time for steam distillation and solvent extraction. The developed method is capable of quantifying 9 preservatives simultaneously and allowing one sample to be analyzed in 25 min. This method is economical, simple and precise, and therefore highly recommended for routine analysis.

V. *Comparison of the Direct Injection GC and AOAC Methods*

The results of using direct injection GC and AOAC methods for the analysis of preservatives in vinegar, soy sauce and fish sauce samples were compared as shown in Table 6. Sorbic acid was

Table 3. The detection limit of nine preservatives by gas chromatography with FID detector

Compounds	Concentration (μg/mL)	Detectable[a]	Recovery[b] (%)	CV[c] (%)
Sorbic acid (SA)	5.0	yes	102.4	6.7
	1.0	yes	106.4	5.6
	0.5	yes	111.3	7.7
	0.1	no	—	—
Benzoic acid (BA)	5.0	yes	98.4	4.8
	1.0	yes	99.7	5.6
	0.5	yes	114.2	8.1
	0.1	no	—	—
Dehydroacetic acid (DHA)	5.0	yes	102.4	4.9
	1.0	yes	96.7	6.2
	0.5	yes	115.1	8.2
	0.1	yes	—	—
Methyl paraben (Me-P)	5.0	yes	102.5	3.7
	1.0	yes	95.9	5.5
	0.5	yes	114.4	7.3
	0.1	yes	120.3	12.7
Ethyl paraben (Et-P)	5.0	yes	104.7	2.9
	1.0	yes	99.2	4.8
	0.5	yes	111.5	8.5
	0.1	yes	123.4	11.7
Isopropyl paraben (IPr-P)	5.0	yes	96.8	1.8
	1.0	yes	98.5	6.4
	0.5	yes	108.9	6.5
	0.1	yes	128.5	15.1
Propyl paraben (Pr-P)	5.0	yes	101.7	2.8
	1.0	yes	97.1	4.6
	0.5	yes	104.1	8.1
	0.1	yes	134.5	20.4
Isobutyl paraben (IBu-P)	5.0	yes	104.6	4.6
	1.0	yes	95.7	3.9
	0.5	yes	105.9	6.5
	0.1	yes	127.3	18.7
Butyl paraben (Bu-P)	5.0	yes	97.4	5.1
	1.0	yes	104.7	6.9
	0.5	yes	121.5	9.4
	0.1	yes	138.9	12.7

[a] FID range = 1, Attenuation = 2.

[b] Average of triplicate analyses.

[c] Coefficient of variation (cv%).

the only preservative found in vinegar samples and its content was 412.4 μg/mL detected by the direct injection GC method or 372.9 μg/mL detected by the AOAC method. In soy sauce, sorbic acid and butyl paraben were found to be 140.9 and 35.8 μg/mL, respectively, detected by the

Table 4. Recoveries of the nine preservatives spiked into vinegar

Preservatives	Blank[a] (μg)(A)	Added amount (μg)(B)	Found amount[b] (μg)(C)	Recovery (%)[c]	CV (%)[d]
SA	412.7	200.0	598.8	97.7	4.9
BA	ND[e]	200.0	189.7	94.9	6.7
DHA	ND	200.0	213.4	106.7	5.4
Me-P	ND	200.0	210.1	105.1	6.1
Et-P	ND	200.0	191.7	95.9	4.8
IPr-P	ND	200.0	208.4	104.2	5.3
Pr-P	ND	200.0	212.1	106.1	7.2
IBu-P	ND	200.0	187.9	94.0	6.5
Bu-P	ND	200.0	207.3	103.7	5.9

[a] SA, BA, DHA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P in 1.0 mL vinegar.

[b] Average of triplicate analyses.

[c] Recovery (%) = (C - A)/B × 100%.

[d] Coefficient of variation (cv%).

[e] ND = not detected.

Table 5. Recoveries of the nine preservatives spiked into soy sauce

Preservatives	Blank[a] (μg)(A)	Added amount (μg)(B)	Found amount[b] (μg)(C)	Recovery (%)[c]	CV (%)[d]
SA	ND[e]	200.0	198.9	99.4	3.8
BA	ND	200.0	209.7	104.9	5.9
DHA	ND	200.0	203.6	101.8	4.4
Me-P	ND	200.0	207.8	103.9	5.3
Et-P	ND	200.0	209.7	104.9	7.2
IPr-P	ND	200.0	196.1	98.1	3.6
Pr-P	ND	200.0	199.2	99.6	5.7
IBu-P	ND	200.0	211.4	105.7	6.4
Bu-P	140.9	200.0	350.2	102.7	5.8

[a] SA, BA, DHA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P in 1.0 mL soy sauce.

[b] Average of three analyses.

[c] Recovery (%) = (C - A)/B × 100%.

[d] Coefficient of variation (cv%).

[e]ND = not detected.

direct injection GC method, or 126.3 and 30.7 μg/mL, respectively, detected by the AOAC method. Fish sauce samples were found to contain sorbic acid, benzoic acid and butyl paraben, with levels at 997.6, 247.9 and 170.3 μg/mL, respectively, using the direct injection GC method, or 874.3, 232.8 and 156.5 μg/mL, respectively, using the AOAC method. The above results reveal that levels of preservatives detected by direct injection GC method were higher than those detected by the AOAC method. This could be due to the procedures of steam distillation and solvent extraction by using the AOAC method which reduced the analyte recoveries. These results indicate that the direct injection GC method is not only reliable and simple, but also fast. It allows one sample to be analyzed in 25 min; while the AOAC method requires 3 hours to complete one sample run.

Table 6. Preservative content in vinegar, soy sauce and fish sauce analyzed by using direct injection GC and AOAC methods

Method	Preservative content (μg/mL)[b]								
	SA	BA	DHA	Me-P	Et-P	IPr-P	Pr-P	IBu-P	Bu-P
					in vineger				
Direct injection[a]	412.4	ND[c]	ND	ND	ND	ND	ND	ND	ND
AOAC	372.9	ND	ND	ND	ND	ND	ND	ND	ND
					in soy sauce				
Direct injection	140.9	ND	ND	ND	ND	ND	ND	ND	35.8
AOAC	126.3	ND	ND	ND	ND	ND	ND	ND	30.7
					in fish sauce				
Direct injection	997.6	247.9	ND	ND	ND	ND	170.3	ND	ND
AOAC	874.3	232.8	ND	ND	ND	ND	156.5	ND	ND

[a] Direct injection method = proposed method in this study.
AOAC method = solvent extraction and then determined by GC[(4)].

[b] SA = sorbic acid, BA = benzoic acid, DHA = dehydroacetic acid, Me-P = methyl paraben, Et-P = ethyl paraben, IPr-P = isopropyl paraben, Pr-P = propyl paraben, IBu-P= isobutyl paraben, Bu-P = butyl paraben.

[c] ND = not detected.

Table 7. Preservative contents in some commercial vinegars

Sample	Preservative contents (μg/mL)[b]								
	SA	BA	DHA	Me-P	Et-P	IPr-P	Pr-P	IBu-P	Bu-P
V-1	407.1	ND	ND	ND	ND	ND	ND	ND	ND
V-2[a]	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND
V-3[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
V-4	102.3	96.7	ND	ND	ND	ND	0.0	ND	5.8
V-5	ND	ND	ND	ND	ND	ND	182.7	ND	ND
V-6	ND	40.7	ND	ND	ND	ND	ND	ND	ND
V-7	ND	125.9	ND	ND	ND	ND	95.2	ND	6.8
V-8	ND	519.1	ND	ND	ND	ND	ND	ND	40.7
V-9[a]	ND	ND	ND	ND	ND	ND	2.7	ND	ND
V-10	ND	ND	ND	ND	ND	ND	ND	ND	20.8

[a] Labeled with “preservative-free”.

[b] SA = sorbic acid, BA = benzoic acid, DHA = dehydroacetic acid, Me-P = methyl paraben, Et-P = ethyl paraben, IPr-P = isopropyl paraben, Pr-P = propyl paraben, IBu-P= isobutyl paraben, Bu-P = butyl paraben.

[c] ND = not detected.

VI. *Investigation of the Varieties and Contents of Preservatives in Vinegar*

The purpose of adding preservatives to vinegar is to inhibit the growth of *Acetobacter* species, which could reduce the vinegar acidity so as to increase the turbidity of the product. Parabens including Et-P, IPr-P, Pr-P, IBu-P and Bu-P are preservatives commonly applied in vinegar products, and the allowable level to be used is limited to less than 0.1g/kg. The direct injection GC method developed in this study was used to determine the above preservatives in the vinegar samples obtained from Tainan and Pintung supermar-

Table 8. Preservative contents in some commercial soy sauce, pickled condiment liquid and fish sauce

Sample	Preservative contents (μg/mL)[c]								
	SA	BA	DHA	Me-P	Et-P	IPr-P	III. Pr-P	IBu-P	Bu-P
S-1[b]	ND[d]	ND	ND	ND	ND	ND	ND	ND	142.4
S-2[b]	ND	ND	ND	ND	ND	ND	ND	ND	65.7
S-3[a]	134.2	ND	ND	ND	86.4	ND	158.2	ND	ND
S-4[a]	ND	ND	ND	ND	ND	ND	ND	ND	137.5
S-5[b]	129.1	ND	ND	ND	ND	ND	122.1	121.3	ND
S-6[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
S-7[b]	108.2	ND	ND	ND	ND	ND	99.5	ND	ND
S-8[b]	117.1	ND	ND	ND	ND	54.2	103.7	ND	92.1
S-9[b]	311.2	ND	ND	ND	ND	ND	123.1	ND	101.1
S-10[b]	142.3	ND	ND	ND	ND	ND	ND	ND	33.4
LC-1[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
LC-2[a]	56.2	ND	ND	ND	ND	ND	ND	ND	ND
LC-3[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle1[a]	ND	ND	ND	ND	ND	ND	148.7	ND	ND
Pickle2[a]	ND	ND	ND	ND	ND	ND	208.9	ND	ND
Pickle3[a]	461.9	ND	ND	ND	ND	ND	ND	74.1	ND
Pickle4[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle5[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle6[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle7[a]	ND	ND	ND	ND	ND	ND	ND	22.7	ND
Pickle8[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle9[a]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pickle10[a]	ND	3.2	ND	ND	ND	ND	ND	ND	ND
FS1[a]	151.2	ND	ND	ND	ND	ND	109.4	ND	ND
FS2[b]	1044.1	265.5	ND	ND	ND	ND	162.9	ND	ND
FS-3[b]	ND	85.5	ND	ND	97.1	ND	ND	ND	ND
FS-4[b]	ND	ND	ND	ND	ND	ND	ND	ND	32.4

[a] Labeled with "preservative-free". S = A grate soy sauce; LS= Low salt soy sauce.

[b] Labeled with paraben-containing. FS = Fish sauce.

[c] SA = sorbic acid, BA = benzoic acid, DHA = dehydroacetic acid, Me-P = methyl paraben, Et-P = ethyl paraben, IPr-P = isopropyl paraben, Pr-P = propyl paraben, IBu-P= isobutyl paraben, Bu-P = butyl paraben.

[d] ND = not detected.

kets. Table 7 lists the preservative contents detected in 10 commercial vinegar products. As can be seen, two out of three samples labeled "preservative-free" were found to contain Bu-P (11.9 μg/mL) and Pr-P (2.7 μg/mL). Nine out of ten test samples were detected to include the following preservatives: SA (0-407 μg/mL), BA (0-519 μg/mL), Pr-P (0-183 μg/mL) and Bu-P (0-41 μg/mL). Five samples were found to contain preservatives higher than the regulation level of 100 μg/mL.

VII. *Investigation of the Varieties and Contents of Preservatives in Soy Sauce, Pickle Condiment Liquid and Fish Sauce*

Twenty-seven samples of liquid foods includ-

ing 13 samples of soy sauce, 10 samples of pickle condiment liquid, and 4 samples of fish sauce were analyzed using the developed method. The results are shown in Table 8. Seven out of ten samples of soy sauce labeled with "preservative-free" and 4 of them were detected to be products containing preservatives. Ten samples of soy sauce were found to contain preservatives as follows: SA (0-311 μg/mL), BA (0-266 μg/mL), Et-P (0-97 μg/mL), IPr-P (0-54 μg/mL), Pr-P (0-158 μg/mL), IBu-P (0-121 μg/mL) and Bu-P (0-142 μg/mL). These results indicate the producers are likely to fortify multi-preservatives in one product. It is worthy to note that one sample was detected to include 535 μg/mL total preservatives, which was higher than the regulation level (500 μg/mL). All pickle condiment liquid samples were labeled as "preservative-free". However, 5 samples were detected to contain the following preservatives: SA (0-462 μg/mL), BA (0-3.2 μg/mL), Pr-P (0-209 μg/mL) and IBu-P (0-74 μg/mL). All fish sauce samples were found to contain the following preservatives: SA (0-1044 μg/mL), BA (0-266 μg/mL), Et-P (0-97 μg/mL), Pr-P (0-163 μg/mL) and Bu-P (0-32 μg/mL), although one was labeled as "preservative-free". The total preservative content in one test sample even reached 1469 μg/mL, which was 3 fold of the regulation level (500 μg/mL).

CONCLUSIONS

The samples of vinegar, soy sauce, pickle condiment liquid and fish sauce were spiked with an internal standard (1, 4-dihydroxybenzene, DHB) and 0.1 μL of which was directly injected into GC for preservatives analysis. The direct injection GC method developed in this study is simple, rapid and precise. It allows one sample to be done in 25 min., and 9 preservatives (including SA, DHA, BA, Me-P, Et-P, IPr-P, Pr-P, IBu-P and Bu-P) to be analyzed simultaneously. Investigation of the preservatives in 37 commercial products of vinegar, soy sauce, pickle condiment liquids and fish sauce was also conducted by using the developed method. Most of the test samples were found to contain two or more than two preservatives. Thirteen out of fourteen samples labeled "preservative-free" were detected to contain preservatives, indicating the labeling was inconsistency with the product content. The contents of preservative in five vinegar samples were detected to exceed the regulation level of 100 μg/mL. One each of soy sauce and fish sauce samples contained preservatives higher than the regulation level of 500 μg/mL.

ACKNOWLEDGEMENT

We would like to thank Dr. C.-W. Chen for his translation work.

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Journal of Food and Drug Analysis. 1999. 7(4)

液體食品中多種防腐劑之同步簡易定量分析

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摘　要

本研究建立了液體食品(包括：食醋、醬油、醬菜、醬瓜汁及魚露等)中九種防腐劑(己二烯酸、去水醋酸、苯甲酸及六種對羥苯甲酸酯類)之同步簡易快速之氣相層析檢測定量方法。以中間極性之CP-SIL 8CB管柱(30m x 0.53mm)，採用直接注入(direct injection)之方式分析定量上述液體食品中之九種防腐劑。選擇水溶性之1,4-二羥基苯(1,4-dihydroxybenzene, DHB)為內標準。己二烯酸、去水醋酸、苯甲酸及六種對羥苯甲酸酯類等九種防腐劑之最低檢出量均在0.5ppm以下。添加上述九種防腐劑200 μg於1mL檢體中，直接注入GC分析，其回收率為95-106%，變異係數均在7.2%以下，顯示直接注入法之精確性高，且檢體不需經前處理即可直接注入GC分析，簡單又快速。以本研究發展之方法，檢驗市售不同廠牌之食醋、醬油、醬菜、醬瓜汁及魚露等共37件液體食品之防腐劑。結果顯示，己二烯酸、苯甲酸、去水醋酸及對羥苯甲酸酯類之含量(μg/mL)分別為：食醋，0-407、0-519、0及0-102；醬油，0-311、0-266、0及0-243；醬菜醬瓜汁，0-462、0-3、0及0-209；及魚露，0-1044、0-266、0及0-163。且上述食品中多含兩種或兩種以上之防腐劑。37件商品中，14件標示"不含防腐劑"，而檢出含防腐劑者達13件；防腐劑超過法規之用量標準者，10件食醋中有5件；10件醬油中有1件；4件魚露中亦有1件。

關鍵詞：防腐劑，液體食品，直接注入法，氣相層析，定性定量分析。