

# Determination of Parabens by Injection-Port Derivatization Coupled With Gas-Chromatography-Mass Spectrometry and Matrix Solid Phase Dispersion

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**Abstract.** A rapid determination of four parabens preservatives (methyl paraben, ethyl paraben, propyl paraben, and butyl paraben) in marketed seafood is presented. Analytes were extracted and purified using matrix solid-phase dispersion (MSPD) method, followed by Injection port acylation gas chromatography–mass spectrometry (GC-MS) with acetic anhydride reagent. In this method, acylation of parabens was performed by acetic anhydride at GC injection-port generating reduction of the time-consuming sample-processing steps, and the amount of toxic reagents and solvents. The parameters affecting this method such as injection port temperature, purge-off time and acylation (acetic anhydride) volume were studied. In addition, the MSPD influence factors (including the amount of dispersant and clean-up co-sorbent, as well as the volume of elution solvent) were also investigated. After MSPD method and Injection port acylation applied, good linearity of analytes was achieved. The limits of quantitation (LOQs) were 0.2 to 1.0 ng/g (dry weight). Compared with offline derivatization commonly performed, injection port acylation employs a rapid, simple, low-cost and environmental-friendly derivatization process. The optimized method has been successfully applied for the analysis of parabens in four kind of marketed seafood. Preliminary results showed that the total concentrations of four selected parabens ranged from 16.7 to 44.7 ng/g (dry weight).

## 1. Introduction

Parabens (esters of 4-hydroxybenzoates) are employed as antimicrobial preservatives in food, industrial, pharmaceutical and personal care products due to their broad spectrum of action against numerous microorganisms, biodegradability, efficiency in wider pH range, non-volatility, and no color [1-3]. Parabens were considered quite low toxicity compounds. Nevertheless, some studies have suggested that parabens acted as endocrine disruptors which affect the functions of the male reproductive system, potentially impacted on female reproductive and enhanced the risk of breast cancer [4,5]. Earlier researchers have also exposed the significant relationship between urinary paraben concentration and sperm DNA damage [6].



Parabens were potentially polute aquatic system through recreational activities pathways such as showering, laundering, washing, swimming and bathing in rivers or lake. They entered in aquatic food chain and bioaccumulate in aquatic biota because of their high lipophilic nature and degradation resistance. Previous studies found parabens in aquatic systems, including environmental water, sediment and sludge [1,7]. The finding parabens in human urine, blood, breast milk and serum proved that human has been exposed parabens [1,8].

Parabens concentration in aquatic biota assumed are low to pose, lead some difficulties regarding the limitation of methods and instrumentation. As the result, the pretreatment process was needed to enrichment analytes before determined by instrumentation. Matrix Solid Phase Dispersion (MSPD) is the most common technique for pretreatment biota samples since it has many advantages, such as extraction and clean-up is integrated into a single step, thus making the procedure simple, low-cost, and convenient [9].

Gas Chromatography-Mass Spectrometry (GC-MS) is well suited for the quantity identification organic volatile compound due to its high chromatographic resolution capacity and reproducible ionization efficiency. Otherwise, derivatization process before GC analysis is needed to establish compound more amenable to standard GC-MS analysis, by improving volatility, thermal stability, and increasing chromatographic performance [10]. Derivatization reactions are frequently performed off-line, otherwise off-line derivatization need multi-step reactions, the procedure was laborious, tedious, time-consuming, and use toxic and harmful reagents [11]. Injection-port derivatization (IPD) was developed method to derivatize analytes and enhance the analytical efficiency of organic compounds in short time reaction and need small amount of organic solvent [12].

This paper demonstrate a injection-port derivatization using GC-MS instrument and matrix solid phase dispersion as a pretreatment method for determination of parabens in seafood. Acetic anhydride was a derivatization reagent used in this study. The derivatization factors affecting the derivatization process efficiency, such as, the injection port temperature, purge-off time, and derivatization reagent volume, were examined.

## 2. Experimental

### 2.1 Chemicals and reagent.

All chemicals and solvents are analytical grade and used without further purification. Standard of Methylparaben (MP), Ethylparaben (EP), Propylparaben (PP), and Butylparaben (BP) (purify 99%) are supplied by Alfa Aesar. P-Terphenyl- $d_{14}$  (purify 98%, used as internal standard), was supplied by Sigma-Aldrich. Acetonitrile (purity 99.9%) are supplied by Sigma-Aldrich. Sodium sulfate anhydrous (purity > 99%) was supplied by Fluka. Activated magnesium silicate (Florisil, <200 mesh) was supplied by Sigma- Aldrich. Supelclean ENVI-18 ( $C_{18}$ ) was supplied by Supelco. Silica gel (70-230 mesh) was supplied by Merck. Acetic Anhydride was supplied by Sigma-Aldrich. Methyl alcohol (purity 99.9 %) supplied by Merck. Deionized water was further purified using Millipore Elix 10 RO system and a Millipore Synergy UV system (Millipore SAS, Molsheim, France).

### 2.2 Sample Preparation using MSPD

Two freshly killed fishes (perch) were purchased from a local fish market in Chung-Li city, Taiwan. The fish samples were stored in a thermo-insulator box and then brought to the laboratory. The fishes were washed several times with Milli-Q deionized water. The fish skin were removed and the muscle tissue was cut into small pieces, and then homogenized in a blender. The homogenate was then freeze-dried for 3 days, and ground into powder. The lyophilized sample was stored in sealed container at -20°C.

A portion of powdered fish sample (0.5 gram) was mixed with 0.5 gram of anhydrous sodium sulfate and 1.0 gram of Florisil as a dispersant and placed in polypropylene centrifuge tube, then shaken in tube shaker to homogenize this mixture. This blend was transferred to a polypropylene SPE

cartridge containing mixture 1.5 gram of silica + C18 (w/w, 9:1), which was packed at the bottom as the clean-up co-sorbent. The analytes were eluted with 10 mL of acetonitrile by gravity flow. The extract was evaporated to dryness by a gentle stream of nitrogen at room temperature, then re-dissolved with 50  $\mu$ L of methanol. A small fraction of extract (10  $\mu$ L) was placed in micro-vial, added 1  $\mu$ L internal standard, 1  $\mu$ L derivatization reagent and subjected to online acylation GC-MS analysis. This procedure is appropriate with previous MSPD studies [9,13,14].

### 2.3 GC-MS analysis and derivatization

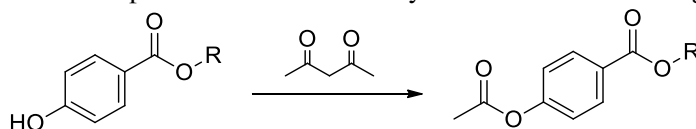
The GC-MS analysis was performed using a Varian 450 GC directly connected to a Varian 220 ion-trap mass spectrometer (Walnut Creek, CA, USA) operating in the SIS (Selected Ion Storage) mode. A ChromatoProbe (Varian) and a temperature-programmed injector (liner: 3.4 mm i.d.) were used to introduce large-volume samples for injection-port acylation. ChromatoProbe device with a disposable micro-vial was used in these experiments. The micro vial containing 10  $\mu$ L sample extract, 1  $\mu$ L internal standard and 1  $\mu$ L acetic anhydride was placed into a ChromatoProbe vial holder, then positioned in the GC injection port.

The temperature of the injection- port acylation was maintained at 90  $^{\circ}$ C for 1.5 min then rapidly increased to 300  $^{\circ}$ C (the temperature ramping rate: 120  $^{\circ}$ C/min). Separations were carried out in a DB-5MS capillary column (30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film thickness; Agilent, Santa Clara, CA, USA). The GC oven was programmed as follows: 2.5 min at 60  $^{\circ}$ C, elevated 30 $^{\circ}$ C/min to 200 $^{\circ}$ C hold for 2 min and at 40  $^{\circ}$ C min $^{-1}$  to 280  $^{\circ}$ C. The GC-MS interface and the ion trap temperatures were set at 270 $^{\circ}$ C and 220 $^{\circ}$ C, respectively. The carrier gas used was Helium (99.999%) at a constant flow of 1 mL/min. The temperature of the transfer line was set at 280  $^{\circ}$ C. Full-scan EI spectra were acquired under the following conditions: scan time 1 s, mass range 100–500 m/z, ion trap temperature 200  $^{\circ}$ C.

## 3. Result and Discussion

### 3.1 Parabens Derivatization

Parabens derivatization is needed to improve parabens volatility thus it could be amenable for GC-MS analysis. In this study, injection-port derivatization with acylation reagent was applied. Acetic anhydride is one of popular acylation reagent which commonly used for injection-port derivatization. The derivatization reaction between parabens and acetic anhydride is described in Figure 1.



**Figure 1** The paraben derivatization reaction by acetic anhydride

The paraben derivatization reaction by acetic anhydride involved the replacement of active hydrogen in hydroxyl group with the acyl group from acetic anhydride and converted into ester group. The ester group attached in derivative of parabens could improve the volatility of analytes. The ester group in parabens derivative has no hydrogen atom attached directly to an oxygen atom. Therefore, it is incapable of engaging in intermolecular hydrogen bonding thus has considerably lower boiling points than originally parabens.

An overview of the retention times, and the ions used to quantitate the SIS signal was provided in Table 1. The success of injection-port acylation was confirmed by appearance molecular ion peak at m/z 195,208, 222, and 236 for derivatives of MP, EP, PP, and BP, respectively, which represented the molecular weight of the parabens derivatives. The fragment at m/z 121 for fourth parabens derivatives was observed, which is confirmed by the loss of  $-\text{COCH}_3$  (acetyl group) and  $-\text{O}(\text{CH}_2)_n$  ( $n = 1$  to 4, for derivatives MP, EP, PP and BP, respectively). Moreover, the fragment at m/z 151 for derivative-methyl paraben was attributed to the loss of  $-\text{COCH}_3$ ; the fragment at m/z 137 for other three parabens

was attributed to the loss of  $-\text{COCH}_3$  (acetyl group) and  $-(\text{CH}_2)_n$  ( $n = 2$  to  $4$ , for EP, PP and BP, respectively).

**Table 1** The logarithm of the octanol/water coefficient, retention time, molecular ion and fragment ion of parabens observed in mass spectra

Analytes	Log $K_{ow}$	$t_R$ (min)	Molecular ion	Fragment ion
Methylparaben	1.91	7.645	195	121+ 151
Ethylparaben	2.34	8.040	208	121 + 137
Propylparaben	2.94	8.713	222	121 + 137
Butylparaben	3.50	9.517	236	151 + 137

### 3.2 Evaluation of Injection-Port Acylation

Injection-port acylation was evaluated in order to study the influence of various parameters on the acylation derivatization process, three parameter (injection-port temperature, purge-off time, and the volume of acylation reagent) was examined. The RRF (Relative Response Factors) was a parameter used to compare derivatization efficiency to evaluate the best compromise condition of each variable. RRF is the ratio of analyte response factor and internal standard response factor.

#### 3.2.1 Selection of injection port temperature

Injection-port temperature was an important parameter in the derivatization efficiency due to thermally catalyse the derivatization reaction process [11]. The different injection-port temperature ranging from  $80$  to  $110^\circ\text{C}$  (at  $10^\circ\text{C}$  increments) were evaluated. As shown in Figure 2, the RRF value of parabens derivatives increased gradually from  $80$  to  $90^\circ\text{C}$  indicating that parabens was derivatized well as the higher temperature could overcome the energy barrier of the reaction and steric interference, thus made increasing of the derivatization reaction efficiency [12]. Therefore, in this study,  $90^\circ\text{C}$  was chosen as the optimised injection-port temperature. The RRF value was significantly decreased gradually from  $90$  to  $110^\circ\text{C}$ . It was corresponding with some previous study which reported that too high temperature of injection-port may cause the derivative decomposition [11,12,13].

#### 3.2.2 Selection of Purge-off Time

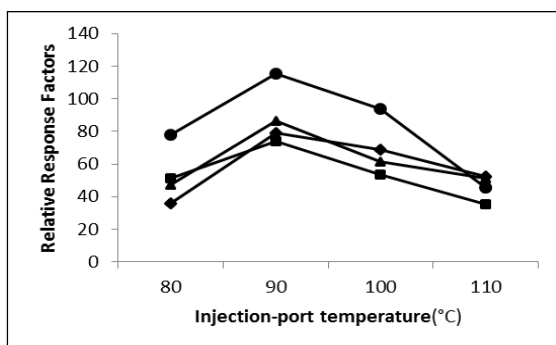
The other parameter affect the injection port acylation process is the purge-off time. The purge-off time had a correlation with detection sensitivity. A long purge-off time time was related with long introduction of majority of analytes into the column which guarantee that all the derivatives was derivatized well. Otherwise, solvent tailing was observed when the purge-off time was too long which followed the decreasing detection sensitivity [12].

In this study, purge-off time was evaluated for  $0.5$ ;  $1.0$ ;  $1.5$ ;  $2.0$  and  $3.0$  min (shown in Figure 3). It was clearly observed that the RRF value increased with increasing purge-off time from  $0.5$  min  $1.5$  min followed the lowering RRF value in  $2.0$  min. Thus,  $1.5$  min was chosen as the purge-off time.

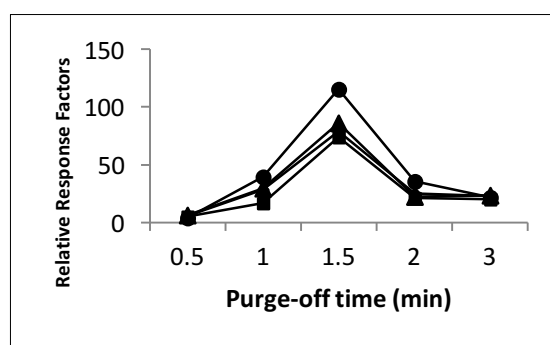
#### 3.2.3 Selection of acylation reagent volume

Acylation reagent volume had also effect to the derivatization efficiency. The excessive of acylation reagent could disturb separation of analytes once the excess of derivatization reagents was detected [15]. In the other side, insufficient of acylation reagent cause incomplete derivatization, poor separation and unreliable quantification [16]. The volume of *acylation reagent* was optimized by

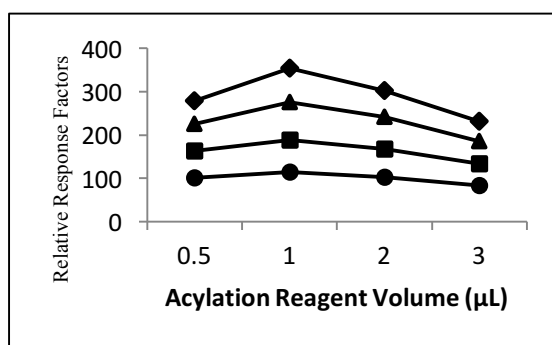
evaluating various *acylation reagent* volume: 0.5; 1.0; 2.0 to 3.0  $\mu\text{L}$ . As shown as Figure, the poor RRF value was observed when 0.5; 2.0 to 3.0  $\mu\text{L}$  were used. Therefore, 1.0  $\mu\text{L}$  was selected as the best volume of acylation reagent.



**Figure 2.** Effect of injection-port temperature. MP (●), EP (■), PP (▲) and BP (◆).

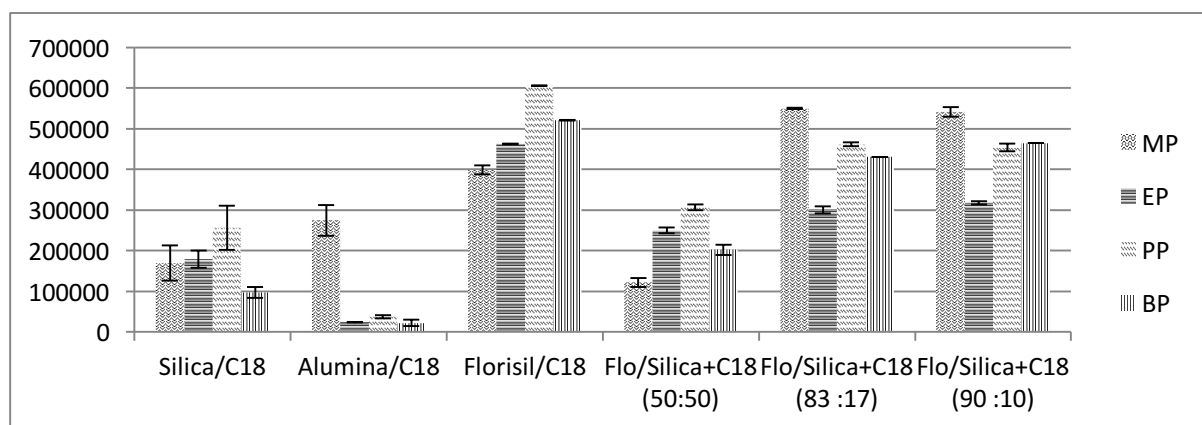


**Figure 3.** Effect of injection-port temperature. MP (●), EP (■), PP (▲) and BP (◆).



**Figure 4.** Effect of acylation reagent volume. MP (●), EP (■), PP (The paraben derivatization reaction by acetic anhydride (▲) and BP (◆).

### 3.3 Optimization of MSPD



**Figure 5.** Effects of Florisil (as a dispersant) combined with various clean-up co-sorbents on the recoveries of analytes extracted by MSPD technique.

One of the most attractive advantage of MSPD procedure is analytes can be extracted from the sample and separated from lipid and other interfering species in a single step. In order to achieve this aim, experimental conditions like type of dispersants, co-sorbents and extraction solvent were optimized. All the optimization were determined based on relative peak area of analytes to the internal standard, from the average of three replicate measurements.

The preliminary experiments were carried out using several combinations of C<sub>18</sub>, silica, Florisil, alumina, activated carbon (AC) as dispersants and/or co-sorbents. For this purpose, 0.5-gram of spiked fish sample was dispersed was mixed with 0.5 g of anhydrous sodium sulfate and dispersed with 1.0 gram of Florisil using a vortex for 2 min. Acetonitrile was applied as elution solvent in this first study corresponding with previous study which use acetonitrile to elute polar compound in fish sample [9]. Samples were prepared and extracted by MSPD method as described in session 2.2. The experimental results of dispersants and/or co-sorbents types were presented in Figure 5. The used of Florisil/C18 as dispersant/co-sorbent gave good recoveries. Otherwise, a light yellow coloration was present on the drying extract, perhaps as a result of colored polar interfering species co-elution. The intense peaks also appeared in the GC-MS chromatograms, and interfered with peaks quantification of the target analytes that cause lower recoveries were observed. Therefore, the silica gel was added to co-sorbent to improve the chromatograms and eliminate the colored polar interferences. Figure 5 shows that better recoveries and precisions were achieved when Florisil as dispersant and the mixing ratio of silica gel + C18 (9:1,w/w) as co-sorbent were applied. This may be due to the high ratio silica gel capable to receive and retain polar species resulting cleaner extracts and better chromatograms for peaks quantitation. These results are in agreement with previous findings reported by Gar'cia de Llasera and Reyes-Reyes [17].

The second experiment, type of elution solvent was tested. Elution solvent could travel down the analytes through the cartridge. Selecting type of elution solvent was performed using four solvent with enhancing polarities: dichloromethane, ethyl acetate, methanol, and acetonitrile. 1 gram Florisil, silica gel + C18 (9:1,w/w) and 10 mL of each elution solvent were used.

### 3.4 Method Performance

In the interest to determine the feasibility and efficiency of the evaluated injection-port derivatization and MSPD method, the analytical characteristic, such as, linearity, repeatability, reproducibility, LODs, and LOQs were investigated (summarized in Table 2). As shown in table 2, good linearity (ranging from 0.4 to 500 ng/g) was confirmed by the  $r^2$  which higher than 0.998. Instrumental quantification detection limit (LOQ) was defined for signal-to-noise (S/N) ratio of 10, ranged from 0.2 ng/g to 1.0 ng/g. The limit of detection (LOD) was confirmed at the S/N of 3, ranged from 0.06 ng/g to 0.30 ng/g.

Precision of the whole method was evaluated with relative standard deviation (RSD) value of intra-day and inter-day analysis. Under repeatability condition (intra-day, 5 extractions performed in a day), RSD values remained below 8% for all analytes. Reproducibility (inter-day, 20 extractions performed 4 days with 5 extraction in a day) RSD value range from 3% to 8%. The method accuracy was determined by average recovery of all the analyte, range from 98% to 118%. These results denoted that the optimized injection-port derivatization give an excellent linearity, reproducibility, repeatability, accuracy and precision for determination of parabens.



**Table 2** Performance of the proposed method

Analytes	Linear Range (ng/g)	r <sup>2</sup>	LOQ (ng/g)	LOD (ng/g)	Intra-day (n=5)	Inter-day (n=20)	Recovery (%)
MP	0.4-500	0,9999	0.2	0.06	107 <sup>a</sup> (6) <sup>b</sup>	111 <sup>a</sup> (5) <sup>b</sup>	98 (6) <sup>a</sup>
EP	0.4-500	0,9996	0.4	0.12	93 (6)	102 (8)	98 (2)
PP	0.4-500	0,9996	0.4	0.12	112 (3)	106 (6)	84 (1)
BP	0.4-500	0,9994	1.0	0.30	101 (7)	102 (3)	118 (1)

<sup>a</sup> Average spiked recovery (final spiked concentration of 10 ng/g).<sup>b</sup>Relative standard deviation (% RSD) of spiked recovery.

### 3.5 Application in real samples

The optimized injection port derivatization and MSPD method was applied to assess the seafoods in order to evaluate method feasibility and applicability. Four kinds of seafoods commonly consumed and commercially sold were pretreated as described in section 2.2. Table 3 listed the recovery of unspiked sample under 10 ng/g of final concentrations. Almost four kinds of parabens detected in four seafood samples. However, propylparaben didn't find in tilapia fish sample. The finding parabens in fish samples represent that the optimize MSPD method is sensitive enough to allow the quantification of target analytes.

**Table 3** Concentration (ng/g, dry weight) of parabens detected in seafood samples.

Samples	Analytes			
	MP	EP	PP	BP
<b><i>Shrimp</i></b>				
Unspike conc. (ng/g) <sup>a</sup>	10.75 (2) <sup>b</sup>	8.01 (1)	5.45 (4)	7.44 (1)
<b><i>Cod</i></b>				
Unspike conc. (ng/g)	11.47 (0.7)	5.63 (7)	6.81 (0.1)	5.60 (9)
<b><i>Tilapia</i></b>				
Unspike conc. (ng/g)	6.23 (4)	5.47 (2)	n.d.*	5.02 (5)
<b><i>Perch</i></b>				
Unspike conc. (ng/g)	18.53 (7)	15.12 (3)	4.89 (9)	6.24 (3)

\*n.d., not detected at LOQ

<sup>a</sup>Original concentration (ng/g) of analytes found in fish samples (n = 3)<sup>b</sup>Relative standard deviation (%RSD) are given in parentheses (n = 3)

## 4 Conclusion

A rapid, effective and simple MSPD method coupled with on-line derivatization GC-MS was demonstrated and optimized to determine trace level parabens in seafood samples. The optimized MSPD method does not involve high-priced instrumentation and take moderate volume of organic solvent, amount of dispersant and clean-up co-sorbent. Furthermore, MSPD employs a single clean-up step thus make this method simple and rapid in extraction. Compared with off-line derivatization, on-line acylation which coupled with MSPD method provides time-saving and environment-friendly that can be reacted in hot injection port without requiring further treatment process. On the whole, the proposed method appears to be a good technique for determination of hydroxylated semi-volatile trace pollutant in biota sample as it provided acceptable recoveries and good sensitivity of real samples

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