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Optimization of Silylation for Parabens Determination by Gas Chromatography-Mass Spectrometry

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ABSTRACT

A low cost, environmental friendly and convenient method for parabens derivatization using Gas Chromatography-Mass Spectrometry (GC-MS) analysis is investigated. Derivatization is needed to enhance the thermal stability, detectability, and volatility of parabens to make them amenable for gas chromatographic analysis. This method involved on-line derivatization by silylating reagent: N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA), N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), and N,O-bis(trimethylsilyl)acetamide (BSA). The variables affected the derivatization process, such as, types and volumes of silylating reagents, injection port temperature, and purge-off time, were evaluated to obtain the optimal condition for determination of parabens. The Relative Response Factors (RRF) was used as a parameter of parabens derivatization efficiency to obtain the best compromise condition of each variable. On a comprehensive level, a comparison of the optimal condition of each silylating reagent was evaluated. Moreover, 1 µL of MSTFA at 260°C of injection port temperature and 2.5 min purge-off time (in splitless mode) obtained the most effective of derivatization process.

Keywords: Parabens, on-line silylation, Gas Chromatography-Mass Spectrometry

1. Introduction

Parabens or esters of p-hydroxybenzoic acid that include methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) are commonly used as antimicrobial preservatives in cosmetics, food, industrial and pharmaceuticals products due to their low toxicity, low cost, inert nature and worldwide regulatory acceptance (Han, Xia, Chen, Shen, Miao, & Shen, 2016). The widespread use of parabens causes their ubiquitous existence in environment. Parabens have been found in indoor dust (Tran, Minh, Kumosani, & Kannan, 2016), surface water (Rocio-Bautista et al., 2015), soil, sediment/sludge (Ferreira, Moder, & Laespada, 2011), cosmetics and personal care products (Rodas, Portugal, Avivar, Estela, & Cerda, 2015), seafood (Han et al., 2016), and other foodstuff (Liao, Chen, & Kannan, 2013). The finding parabens in human urine (Moos et al., 2015), blood, breast milk (Azzouza, Rascon, & Ballesteros, 2016) and serum (Hines, Mendola, van Ehrenstein, Ye, Calafat, & Fenton, 2015) proved that human have been exposed parabens. Although parabens have been considered low acute toxicity compounds, but controversy about side effect of parabens arose due to their effects on the endocrine system. Previous studies have

reported that parabens have endocrine disruptive effect which cause human male reproductive disorders and play a role in enhance the risk of breast cancer (Shanmugam, Ramaswamy, Radhakrishnan, & Tao, 2010). Because of parabens have potential health effects of endocrine disrupting factors, developing method for parabens analysis got great interest.

Gas chromatography–mass spectrometry (GC–MS) is commonly used for identification and separation parabens. To improve GC chromatographic separation, derivatization is typically used to increase the volatility of parabens and to improve sensitivity (Bowden, Colosi, Mora-Montero, Garret, & Yost 2009). However, derivatization reactions commonly are performed off-line, but it needed multi-step reactions, long time-consuming, and used toxic reagents (Wang, Ma, Yin, & Xu, 2013). On-line derivatization reduces solvent waste, simplifies sample preparation, and avoids the need for hazardous reagents (Wu, Hu, Yue, Yang, & Zhang, 2009).

On-line silylation was a derivatization using silylation reagent which developed and applied for the analysis of parabens with GC–MS. Silylation is the most commonly used in derivatization procedures for GC-MS analysis since it could improve the GC sensitivity, accuracy and resolution by enhancing thermostability, detectability and decreasing peak tailing (Wang et al., 2013). The active hydrogen on hydroxyl groups of parabens is replaced with silyl groups of the silylation reagent during derivatization process. There are many kinds of silylation reagents, such as N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide (MTBSTFA), N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA), and N,O-bis(trimethylsilyl)acetamide (BSA) which play important role in the derivatization efficiency. The derivatization factors affected derivatization efficiency, such as the types and volumes of silylating reagent, injection port temperature, and purge-off time were also investigated.

2. Materials and methods

2.1. Materials

All chemicals are analytical grade and used without further purification: methylparaben (Alfa Aesar), ethylparaben (Alfa Aesar), propylparaben (Alfa Aesar), butylparaben (Alfa Aesar), *p*-Terphenyl-d_{14} as an internal standard (Sigma-Eldrich), dichloromethane (Macron), methyl alcohol (Merck), acetone (Macron), acetic anhydride (Sigma-Aldrich), and deionized water, Milli-Q water produced by Millipore Elix 10 RO system and a Millipore Synergy UV system. The silylation derivatization reagents used: N-Methyl-N-tertbutyldimethylsilyltrifluoroacetamide (MTBSTFA), N,O-bis(trimethylsilyl)- trifluoroacetamide (BSTFA), N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA), and N,O-bis-(trimethylsilyl)-acetamide were purchased from Sigma-Aldrich.

2.2. Preparation of stock solutions

All solution used in this experiments was prepared using microsyringes to measure accurately which conditioned before by rinsing the syringes with dichloromethane, acetone, and methanol. The methylparaben stock solution was made by dissolving 10 mg of methylparaben solid standard with 10 mL methanol to make 1000 ppm of methylparaben (MP). The same procedure was applied to prepare ethylparaben (EP), propylparaben (PP), and butylparaben (PP) stock solutions. The internal standard stock solution was prepared by dissolving 10 mg of internal standard with 10 mL dichloromethanes. These stock solutions were stored at 4°C in the dark to prevent degradation by light. A stock solution of internal standard was prepared by dissolving mg of 10 mg *p*-Terphenyl-d_{14} in 10 mL dichloromethane to make 1000 ppm.

2.3. Preparation of working solutions

The working solution was prepared by diluting stock solution. To prepare MP, EP, PP, BP, and internal standard working solution, the stock solutions of parabens standard was diluted with methanol and internal standard stock solution was diluted with dichloromethane until reach concentration 1.0 ppm of MP, EP, PP, and BP.

2.4. GC-MS analysis

Analysis was performed by gas chromatography-mass spectrometry (GC-MS) system: Varian 450 GC directly connected to a Varian 220 ion-trap mass spectrometer (Walnut Creek, CA, USA) operated in electron ionization (EI) which set to full scan at mass range 100-500 m/z (at 70 eV electron energy). Gas

chromatography column used was DB-5MS capillary column (30x0.25 mm i.d., 0.25 µm film thickness). A ChromatoProbe (Varian) was used to introduce large-volume samples for on-line silylation. High purity helium (99.999%) at a flow rate of 1 mL/min was used as carrier gas.

The transfer line and ion source temperatures were set to 250 and 180°C, respectively. Oven temperature was programmed to begin at 100°C for 4.0 min, increased by 25°C/min to 300°C and maintained for 3 min. The injection-port temperature was held at 260°C for 2.5 min in order to complete silylation reaction and solvent vaporisation, then the temperature was accelerated rapidly to 300°C for silylated derivatives introduction into the analytical column.

2.5. On-line silylation procedure

Each sample solution contained standard mixed of 4 types of parabens (methylparaben, ethylparaben, propylparaben and butylparaben). In a typical run, 10 µL volume of standard parabens mixed containing internal standard solution was added various silylating reagent in different volume. The mixing solution then introduced into a micro-vial, the vial was took into a ChromatoProbe vial holder, and then placed in the GC injection-port to obtain on-line silylation. Important silylation parameters, such as types and volumes of silylating reagent, injection port temperature and purge-off time were investigated to obtain the optimal condition of silylation process.

3. Result and discussion

3.1. GC-MS analysis of on-line silylation

Parabens are polar and thermally fragile compounds thus need derivatization to convert into more volatile compound for GC-MS analysis. Derivatization turned them into less polar, thermally stable and more volatile compounds for GC-MS analysis. Silylation is a derivatization procedure which substitute active hydrogen of the hydroxylated molecule with a silyl group of silylating reagent. Silylation reagents will convert hydroxyl alcohol to form tertiobutyldimethylsylil- (TBDMS) ethers (for derivatizations with MTBSTFA) and trimethylsilyl (TMS) ether (for derivatizations with BSTFA) via SN_2 substitution reaction, resulting a derivative for each compound. The silylated derivatives formed are volatile and for the most part, are easily separated (Scott, 2003). Fig. 1 displays the reactions of parabens with various silylating reagents (MTBSTFA, MSTFA, BSTFA, and BSA). The success of on-line silylation was evaluated with the appeared of molecular ion of silylated derivatives. Table 1 summarises the molecular ion and the fragment ions for each silylated derivatives observed in mass spectra.

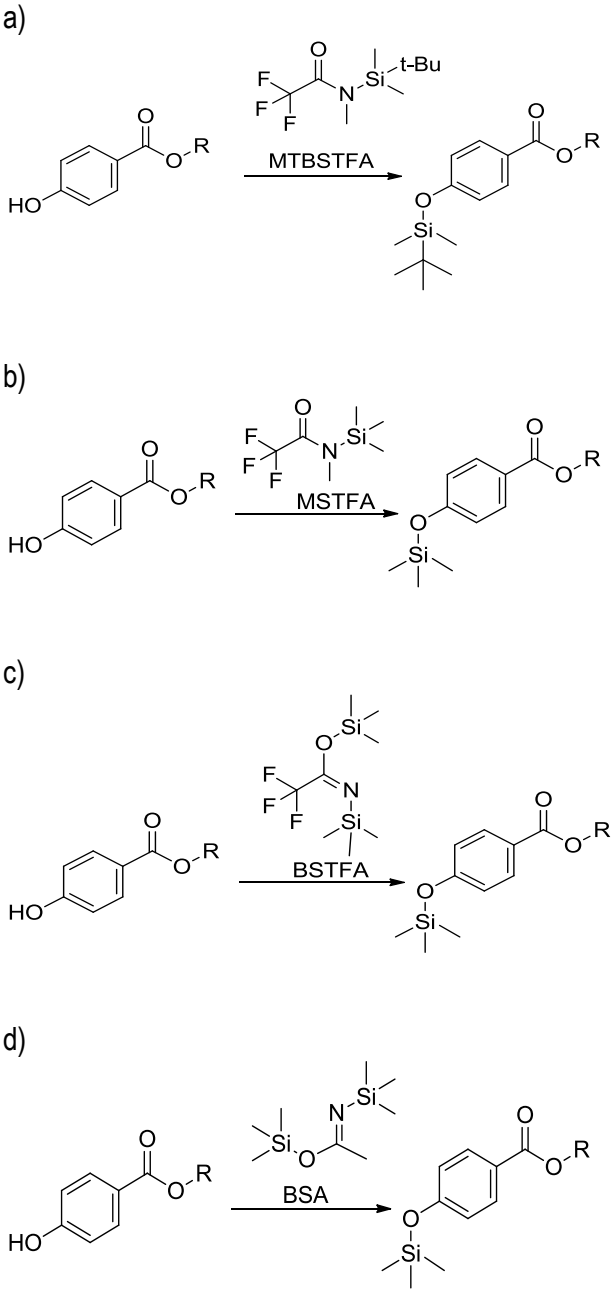

Fig. 1. The reactions of parabens with various silylating reagents: a) MTBSTFA, b) MSTFA, c) BSTFA, d) BSA.

Molecular ions ($[M]^+$) showed in Table 1 is the molecular ion of silylated derivatives of each analyte. For MTBSTFA derivatization, [M-57] is corresponding to the loss of tert-butyl group of

MTBSTFA-derivatives. Whereas for MSTFA, BSTFA, and BSA derivatization, [M-15] is corresponding to the loss of methyl group. The loss of tert-butyl and methyl group resulting silylated derivatives possessed good thermal and hydrolytic stability (Wang et al., 2013). Ion at 195 m/z ($[(CH_3)_3SiO\text{-}Ar\text{-}CO + 2H]^+$) observed in all of molecule was confirmed by loss of tert-butyl group (for MTBSTFA derivate) or $–CH_3$ group (for other silylated derivatives) of the $Si(CH_3)_3$ and by loss of $–(CH_3)n$ (n=1 to 4 for MP, EP, PP, and BP).The intense signals detected in MS spectra were ions of m/z 151, 163 and 177 which attributed to the loss of CO_2 (carbondioxide), O_2 (oxygen) and H_2O (water), respectively.

Table 2.
The optimal condition of each silylating reagent

Silylating Reagent	Temperature of Injection-port (˚C)	Derivatization Time (min)	Silylating Reagent Volume (µL)
MSTFA	260	2.5	1.0
BSA	300	3.0	2.0
BSTFA	280	2.5	2.0
MTBSTFA	300	2.0	1.0

Table 1.
The molecular and the fragment ions for each silylated derivatives observed in mass spectra.

Silyl Reagents	Analytes	$[M]^+$	[M-57]/ [M-15]	Fragment ions
MTBSTFA	Methylparaben	266	209	195, 177, 151
	Ethylparaben	280	223	195, 177, 163, 151
	Ethylparaben	294	237	195, 163, 151
	Butylparaben	308	251	210, 195, 151
MSTFA	Methylparaben	224	209	195, 177, 151
	Ethylparaben	238	223	195, 177, 163, 151
	Ethylparaben	252	237	195, 163, 151
	Butylparaben	266	251	210, 195, 151
BSTFA	Methylparaben	224	209	195, 177, 151
	Ethylparaben	238	223	195, 177, 163, 151
	Ethylparaben	252	237	195, 163, 151
	Butylparaben	266	251	210, 195, 151
BSA	Methylparaben	224	209	195, 177, 151
	Ethylparaben	238	223	195, 177, 163, 151
	Ethylparaben	252	237	195, 163, 151
	Butylparaben	266	251	210, 195, 151

Table 3.
The RRF of the optimal condition for each silylation reagent

Silylating Reagent	MP	EP	PP	BP
MSTFA	45.06±0.03	40.45±0.07	81.02±0.05	93.64±0.06
BSA	66.38±0.02	37.60±0.09	27.91±0.06	21.86±0.05
BSTFA	53.74±0.05	50.53±0.03	80.07±0.14	84.44±0.09
MTBSTFA	6.32±0.11	11.73±0.01	29.41±0.03	19.06±0.03

3.2. Optimisation of the silylation

Optimisation of the silylation was evaluated using standard mixed of parabens consisted of examining injection-port temperature, purge-off time, and volume of silylating reagent with various silylating reagents: MTBSTFA, MSTFA, BSTFA, and BSA. The optimal condition of injection-port temperature, purge-off time, and volumes of silylating reagent of each reagent was used for comparison, as shown in Table 2. The RRF was used as a derivatization efficiency parameter to evaluate the best compromise condition of each variable. Table 3 shows RRF of the optimal condition for each silylating reagent.

According to Table 2 and Table 3, MSTFA and BSTFA as silylating reagents have good RRF due to both are appropriate reagent choice for derivatization of hydroxylated compounds (Bowden et al., 2009). Otherwise, MSTFA offers better compromise condition than BSTFA since it had higher RRF values than others and only required 1.0 µL of MSTFA reagent for 2.5 min for silylation reaction and offers acceptable reproducibility. This result was corresponding with previous study mentioned that MSTFA is more potent and selective trimethylsilyl donor for reactions, mainly with OH groups, thus it could give good RRF for parabens derivatization (Wu, & Lee, 2006).

3.3 Injection-port temperature

Injection-port temperature had effect to the derivatization efficiency as it thermally catalyse the derivatization reaction process. The various injection-port temperatures ranging from 220 to 320°C (at 20°C increments) were examined at a 2.5 min purge-off time. As shown in Figure 3, the RRF values of TMS-derivatives enhanced gradually when the injection-port temperature was increased from 220-260°C, but thereafter it decreased significantly. Therefore, in this study, 260°C was chosen as the optimised injection-port temperature. It was corresponding with some previous study which reported that the injection-port was set between 250 to 300°C to obtain fast and completely reaction (Wang et al., 2013). The high temperature could overcome the energy barrier of the reaction and steric interference, thus made increasing of the reaction efficiency (Wu & Lee, 2006). Otherwise, a too high temperature could reduce analytical signals due to derivatives decomposition (Wang et al., 2013). In the other side, a low injection-port temperature resulted the low of GC signal because the analytes did not derivatized completely.

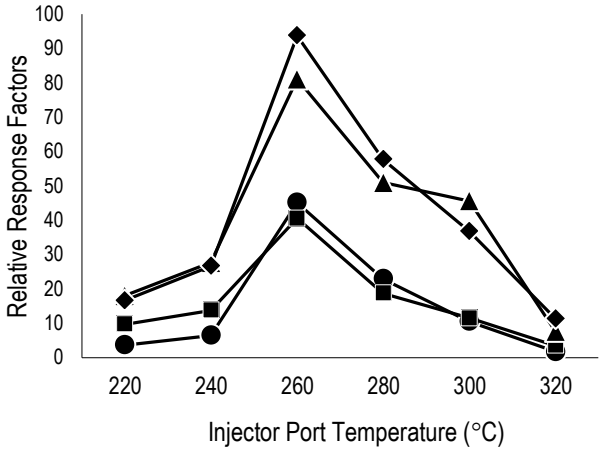

Fig. 3. Effect of injection-port temperature. MP (●), EP (■), PP (▲) and BP (♦).

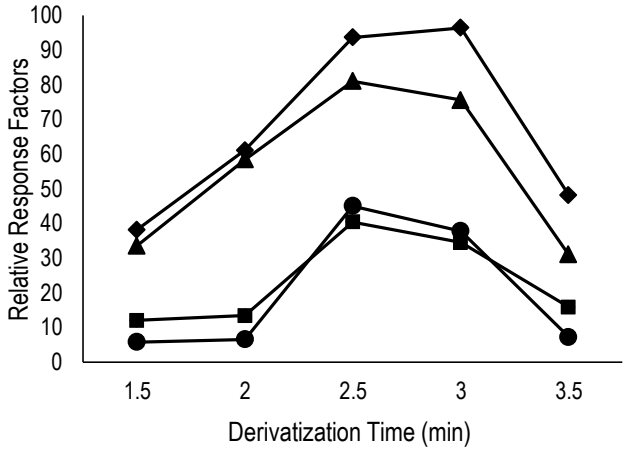

Fig. 4. Effect of the time of silylating. MP (●), EP (■), PP (▲), and BP (♦).

3.4. Purge-off time

Other parameter influenced the on-line silylation process is the purge-off time, which evaluated for 1.5, 2.0, 2.5, 3.0, and 3.5 min in splitless mode with injection-port temperature at 260°C. It was observed that RRF values increased with increasing purge-off time from 1.5 to 2.5 min, then decreased with the purge-off time increasing to 3.5 min (shown in Figure 4). A possible reason of the decreasing RRF during the longer time is that the produced solvent "cloud" in the injector after a longer purge-off time prevented the derivatives which would be brought out by the carrier gas (Elie, Baron, & Birkett, 2012). Thus, 2.5 min was selected

as the optimised purge-off time that analytes got highest RRF at this value. This result agreed with Ho's study which reported 2.5 min gave the highest yield for on-line silylation process (Ho & Ding, 2012).

3.5. Volumes of derivatization reagent

The volume of derivatization reagent had also a significant effect on the derivatization efficiency. The excessive of the volume of silylating reagent could disturb the analytes separation, thus led the reduction of derivatization efficiency (Toledano, Cortes, Andini, Vazquez, & Villen, 2012). As well as the insufficient of silylation obtain poor separation due to incompletely derivatization (Basheer, Parthiban, Jayaraman, Lee, & Valiyaveettil, 2005). In this present work, the volume of silylation reagent was varied, 0.5; 1.0; 2.0 to 3.0 µL. When 0.5; 2.0 and 3.0 µL were used, the poor peak resolution was observed, as shown in Figure 5. Therefore, 1.0 µL was selected as the best volume of silylating reagent.

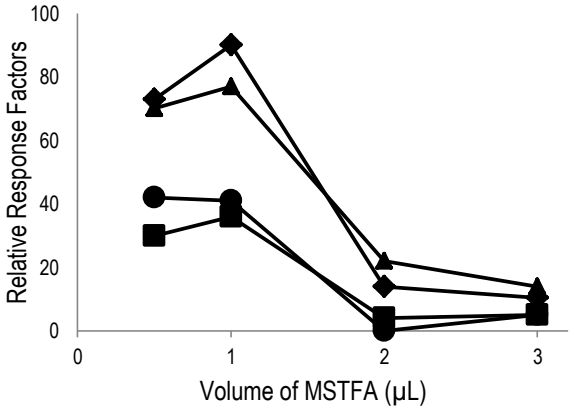

Fig. 5. Effect of the volumes of silylating. MP (●), EP (■), PP (▲), and BP (♦).

4. Conclusion

The study was focused on the optimization of on-line silylation for parabens analysis using gas chromatography-mass spectrometry. On-line silylation appears to be a promising method for parabens derivatization due to it is low cost, environmental friendly and convenient method. MSTFA (N-methyl-N-trimethylsilyl-trifluoroacetamide) was selected as the most effective silylating reagent for derivatization of parabens. Many factors influenced the derivatization process, such as, injection-port temperature, purge-off time and the volume of silylating reagent were optimised, resulting the good derivatization efficiency which conditioned by 260°C of injection-port temperature for 2.5 min purge-off time (in splitless mode) and 1 µL of MSTFA.

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