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# Determination of phthalate in PVC food wrap by gas chromatography mass spectrometry

**Nguyen Thi Ha Binh*, Dang Thu Hien, Tran Cao Son**

*National Institute for Food Control, Hanoi, Vietnam*

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## Abstract

Toxic phthalates that can be released from food packaging into food are currently a matter of concern, affecting consumers' health. However, in Vietnam, no studies have been published on this group of substances in food packaging and films. In this research, a method for the simultaneous determination of 7 typical phthalates, including benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), di-(2-ethylhexyl) adipate (DEHA) in food wrap samples by gas chromatography mass spectrometry (GC-MS) using internal standards has been developed and validated. The limit of detection and limit of quantification for all substances was 0.03 mg/kg and 0.10 mg/kg, respectively. The recoveries of the method were in the range of 82 - 105%. Relative standard deviation (RSD) ranged from 5.6 to 7.5%. The method has been applied effectively to analyze the content of phthalates in 35 food wrap samples that were collected from markets in Hanoi. Analytical results showed that ten samples were detected with phthalates, of which three samples contained DEHA exceeding the specific migration limit (SML). This study contributes to consumer health protection and advises the regulatory agency on the addition of safety regulations for PVC food wrap in particular and other food packaging materials in general.

***Keywords:*** *GC-MS, internal standard, phthalate, DBP, BBP, DEHP, DEHA, DNOP, DIDP, DINP.*

## 1. INTRODUCTION

Food wraps used to wrap food for storage, maintain food freshness and prevent external contamination are extensively used. They are manufactured mainly from thermoplastic Polyvinyl chloride (PVC) and are softened by the addition of plasticizers to increase the toughness of food wrap. In the production of flexible films, the most common ingredients are 1,2-benzene dicarboxylic acid (phthalic acid) esters, including BBP, DBP, DEHP, and DEHA [1]. However, the utilization of phthalates can be a potential risk of contaminants in food, adversely affecting human health.

* *Corresponding author: Tel: +84 988479022* *Email: habinhsp86@gmail.com*

Phthalates are a group of carcinogens, mutagenic and endocrine disruptors, and sex hormone disruptors. DEHP and DEHA are typical plastics that have been banned for use because they can affect hormones, disrupt the endocrine, cause estrogen to increase, and male hormones to decrease, causing early puberty in women and male infertility while creating a significant influence on the genital development of infants and young children [1].

Some countries have imposed restrictions on phthalates used in food contact materials. The European Food Safety Authority (EFSA) published reassessments of phthalates in 2005, highlighting concerns about the use of phthalates in food contact with PVC materials. Currently, five phthalates have been concerned by the European Union (EU) and are restricted for use in food packaging, including BBP, DBP, DEHP, DIDP, and DINP [1, 4]. Korea also has regulations on the SML in PVC for DBP, BBP, DHEP, DEHA, DNOP, DIDP, and DINP [2] (Table 1). In Vietnam, according to Decision No. 2204/QD-BYT, the SML of DEHP in food has been set at 1.5 mg/kg. Vietnam has not yet regulated the SML for these substances in the packaging material in contact with food.

***Table 1.*** *SML regulation of phthalates in food contact material*

| ***Analyte*** | ***SML in Korea, mg/L [2]*** | ***SML in EU, mg/L [3]*** |
|---|---|---|
| DBP | 0.3 | 0.3 |
| BBP | 30 | 30 |
| DEHP | 1.5 | 1.5 |
| DNOP | 5 | - |
| DIDP | 9 | 9 |
| DINP | 9 | 9 |
| DEHA | 18 | - |

Several studies conducted in different countries have shown that DEHP is still used as a plasticizer for PVC plastics [1, 4-5]. A study in Brazil found that food packaging materials containing plasticizers di-2-ethyl-hexyl (DEHA), di-2-ethyl-hexyl phthalate (DEHP), and di-iso-decyl phthalate (DIDP) in films with concentrations ranging from 12 to 19%, 15 to 44% and 10 to 11%, respectively. This percentage has exceeded the Brazilian regulation because the required concentration of DEHP and DIDP in plastic materials in Brazil should not be greater than 3% [1]. In Canada, Cao Zhao et al. (2014) investigated the concentration of decontaminated DEHP in 40 cheese samples covered with PVC film and found that seven cheese contaminants of DEHP levels ranged from 0.29 to 15 mg/kg [4], where the SML is 1.5 mg/kg. In Japan, Tsumura et al. (2001) studied the amount of DEHP contaminating food from PVC gloves containing 30% DEHP in contact with food and found that the level of DEHP migration in chicken meat was 16.9 mg/kg, exceeding the SML regulations [5]. In Vietnam, TCVN 6238-6:2015 has been issued guiding the determination of phthalate group

content in children's toys. In addition, there are several domestic studies aimed at identifying phthalates in air and soft drinks [6]. However, no studies have identified phthalates in food contact packaging. Recognizing the risk of phthalate migration from PVC food wrap, we have developed a method for simultaneous determination of phthalate immigrant from food wrap and applied this method for the determination of phthalates in some PVC wrap samples.

## 2. MATERIALS AND METHOD

### 2.1. Chemicals and reagents

Standards of DBP, BBP, DEHP, DEHA, DNOP, DIDP, and DINP (Sigma Aldrich Company) have purity > 95%. Analytical chemicals: n-heptane, ethanol, acetic acid, and acetone (Merck company) are analytical-grade chemicals.

### 2.2. Sampling

The random sampling method is implemented by Circular 14/2011/TT-BYT of the Ministry of Health [7]. 19 PVC imported samples from Japan, Korea, Malaysia, and Thailand, and 16 PVC domestic samples were collected, then coded before analysis.

### 2.3. Instrumental analysis

The main equipment used is the gas chromatography-mass spectrometry (GC-MS) system ISQ 7000 of Thermo Scientific, analytical balance with an accuracy of 0.0001 g (MS-205DU, Mettler), Shellab incubator (Germany). Common laboratory equipment includes a 100 mL beaker, 50 mL measuring tube, 10 mL volumetric flask, and 1.8 mL sealed sample vial. Instruments used for analysis should be washed, dried, and shaken with n-heptane before use. Particularly, glassware can be heated at 450°C for 30 minutes to remove phthalate impurities that can be adsorbed on the surface. Plastic tools (pipette tips, centrifuge tubes) are only used once. The usage of Teflon tools was prioritized.

## 3. RESULTS AND DISCUSSION

### 3.1. Optimizing of procedure

*3.1.1. Optimizing of chromatography conditions GC-MS*

In this study, the Korean Standard for the determination of phthalates in direct food contact packaging was referenced for the development of the analytical methods [1]. The main equipment used was GC-MS with electronic ionization EI and SIM mode. The sample injection chamber temperature was 240°C, in the split mode of 10:1, with an ionization energy of 70 eV. Helium carrier gas was at a flow rate of 1 mL/min. Column DB-5MS (30 m × 0.25 mm; 0.25 μm) was used. The gradient program was conducted as follows. With an initial temperature of 100°C, it increased at a rate of 20°C/min to 280°C, continued to increase by a rate of 10°C/min to 310°C, and kept for 3 minutes. The results of

chromatographic analysis of a mixture of 7 phthalates at a concentration of 1.5 mg/L are shown in Figure 1.

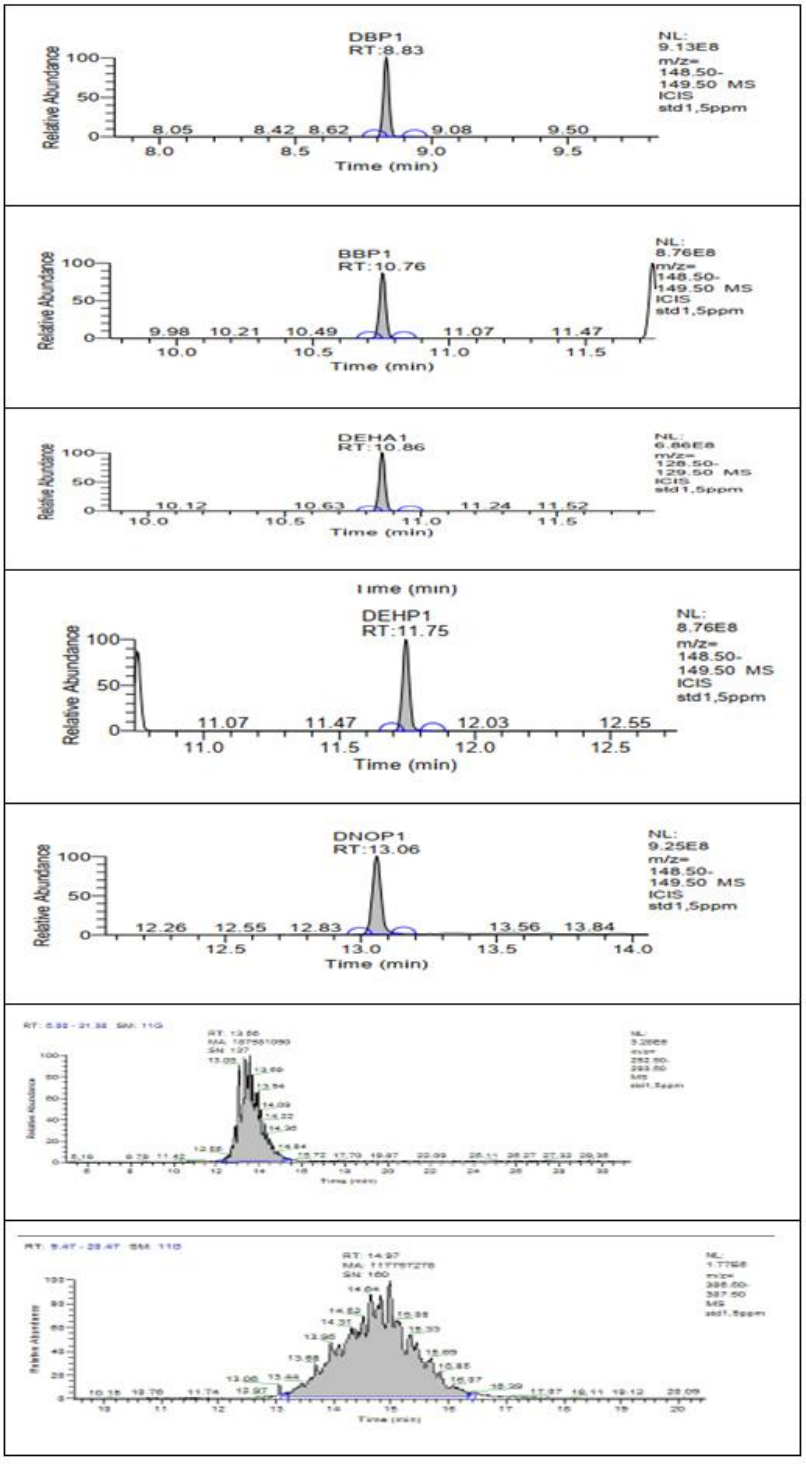


***Figure 1.*** *Chromatogram of standards at a concentration of 1.5 mg/L*

From the chromatograms in Figure 1, it can be seen that the peaks of DBP, BBP, DEHP, DEHA, and DNOP have a retention time of about 8.8 to 13 minutes, with sharp

peaks, and well-symmetric peak shapes. Particularly, the two peaks, DIDP and DINP, are quite obtuse because these are the peak of the collection of esters of phthalic acid and isomer decyl alcohol or different isononyl esters of phthalic acid.

The Korean standard uses an external standard method for the analysis of the phthalate group; however, not using an internal standard may cause fluctuations in the results of the analysis. Our method had a new change when using an internal standard of benzyl benzoate (refer to ISO 8124-6:2014) to correct the analyte change during mass analysis. The use of internal standards is effective in analysis, making analytical results more stable and accurate than external standard analytical methods.

*3.1.2. Preparation of test solution*

Through reference to documents [2-3], the standard decontamination procedures of Korea and the EU have certain similarities. The main parameters include the test solution, the immigration time, and the temperature of the immigrant. Three immigrant solutions are modeled with the food in the packaging: watery, greasy, and acidic.

In this study, the immigrant conditions were referenced according to the Korean Standard, as shown in Table 2.

***Table 2.*** *Conditions for immigrants of packaging samples*

| ***Environmental migration (Immersion solution)*** | ***Migration Time*** | ***Migration Temperature*** | ***Treatment of extracted solution*** |
|---|---|---|---|
| n-heptan | 1 hour | 25°C | Transfer the immersion solution to the sample injection vial and analyze it on GC-MS. |
| Ethanol 50% | 30 min | 70°C | Take 25 mL of soaking solution, add 50 mL of 1:1 n-hexane, shake for 5 minutes, and let it rest. Take the n-hexane layer. Extract three times with n-hexane. Evaporate, and dissolve the residue with 25 mL of acetone. Transfer to a sample syringe and analyze by GC-MS. |
| Acid acetic 4% | 30 min | 70°C | |
| Water | 30 min | 70°C | |

The PVC samples were cut to size 3 × 3 cm and immersed in 36 mL of leaching solution as described in Table 2, ensuring a soaking ratio of 2 mL/cm$^2$. Blank samples (or solvent blank) and quality control (QC) samples were performed with each analysis batch. The QC sample was subjected to standard addition at a concentration of 0.3 mg/L by adding

108 µL of the 100 mg/L standard to the packaging blank, allowing it to dry for about 10 minutes before proceeding with further analysis. Samples with a high phthalate content that exceeds the calibration curve need to be diluted to the appropriate concentration to calculate the content of the substance.

**3.2. Method validation**

The analytical method was validated for the following parameters: specificity, linear range, the limit of detection/limit of quantification, recovery, repeatability, reproducibility, and precision, measurement uncertainty, according to AOAC International [8]. Solvent blanks were analyzed with each batch to eliminate sources of phthalate contamination (if any).

*3.2.1. Specificity*

The results of the specificity evaluation showed that the PVC wrap blank sample did not give a signal at the retention time of the analyte. The calibrator and the spiking sample give the analyte signal at the same retention time with a deviation value < 1%, suitable for the AOAC International requirement (Figure 2).

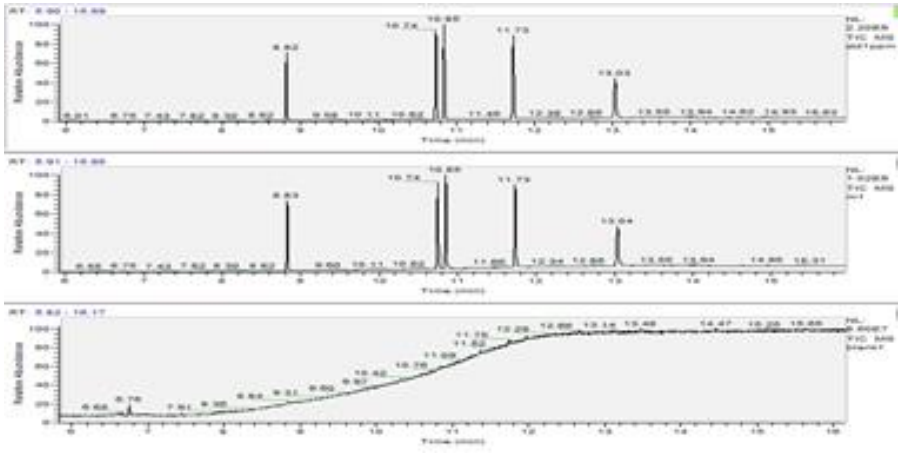

***Figure 2.*** *Results of method specificity assessment*

*3.2.2 Limit of detection and limit of quantitation*

The spiked sample at a concentration level close to the estimated MQL value was analyzed, and the ratio of signal to noise of peak height was calculated. The standard spiked level with an S/N ratio of about 3 is defined as the MDL of the method. Experimental results show that the MDL is 0.03 mg/L. The quantitative limit of the method was 0.10 mg/L.

*3.2.3. Linearity*

The standard solutions with concentrations from 0.1-20.0 mg/L were analyzed on the GC-MS instrument to determine the correlation between peak area and substance's concentration with a required $R^2$ factor greater than 0.995, degree bias at the benchmarks < 15%. Experimental results show that the acceptable linear range was from 0.1 to 10.0 mg/L.

*3.2.4. Repeatability and recovery*

Spiked samples at three concentrations of 0.1 mg/L; 1.0 mg/L; 5.0 mg/L in 4 solutions of n-heptane, 50% ethanol, 4% acetic acid, and water (Table 2) were analyzed. The

recoveries of the method were in the range of 82 - 105%, and the RSD% repeatabilities were in the range of 5.6 - 7.5%, suitable for the AOAC requirements.

*3.2.5. Internal reproducibility*

The samples in 6 duplicates at 1 mg/L standard were analyzed by two individual staff and determined the repeatability. The reproducibility was determined based on the analysis results of this dataset. Reproducibility results were in the range of 7.5 - 8.2%, suitable for AOAC's requirements.

*3.2.6. Measurement uncertainty*

Method uncertainty (U) is determined based on the results of the analysis recovery, internal reproducibility, and standard uncertainty (Bottom-Up method) [9]. The measurement uncertainty of the method is considered to be 30%.

The evaluation results show that the analytical method meets the requirements in terms of reliability and sensitivity for analyzing phthalates in packaging samples.

**3.3. Application to real PVC food wrap samples**

In this study, 35 samples (including 19 samples of wrapping film imported from Japan, Korea, Malaysia, and Thailand and 16 samples of wrapping film produced domestically at stores and supermarkets) were collected and analyzed 7 phthalates by this method. Samples were analyzed according to the optimized procedure in the above section (section 3.1).

Blank and quality control samples were performed for each batch of samples analyzed. In the results of the analysis of 35 food wrap samples, 10 samples were detected with phthalates, accounting for 29% of the total number of samples (Figure 3).

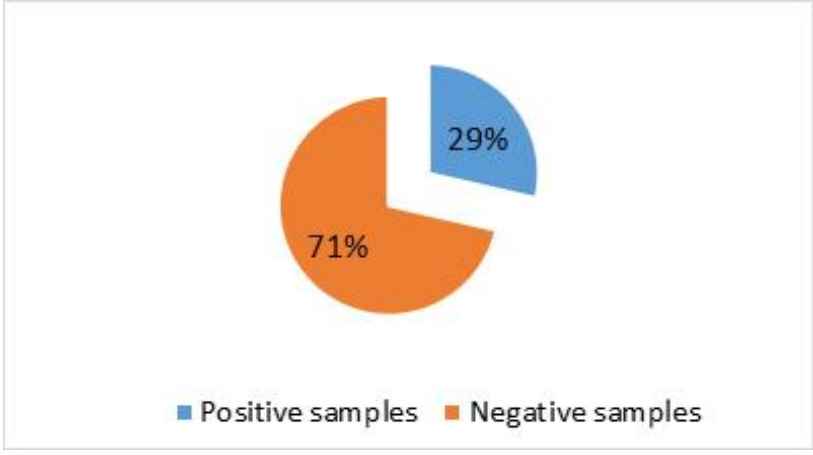


***Figure 3.*** *The ratio of negative and positive samples*

The real PVC sample analysis results revealed that the phthalates detected in the samples were DEHA and DEHP. The concentrations of DEHP and DEHA ranged from 0.3 to 0.7 mg/L and 0.5 to 134.0 mg/L, respectively. The number of DEHA-positive samples that exceeded the regulation was 3/35 samples (accounting for 8.5% of the total samples), and the concentration exceeded 1.4 to 7.4 times the SML level. DEHA is a plastic that has been banned for use because of its toxicity, which can affect hormones and cause endocrine

disruption. A study at Chen Kung University (Taiwan) in 2009, surveying 30 girls with precocious puberty compared with 33 normal girls, showed that the urine of girls with precocious puberty contained much higher levels of phthalates compared to 33 normal girls and concluded that phthalates might be a cause of precocious puberty in Taiwanese girls. Therefore, Taiwan has recalled products containing phthalate derivatives such as DEHP and DEHA detected in products in their domestic market and issued a global food hygiene warning.

In terms of origin, the proportion of samples containing phthalates in domestic products is higher than that of imported products; the positive rate in imported samples was 16%, while that of domestic products was 44% (Figure 4).

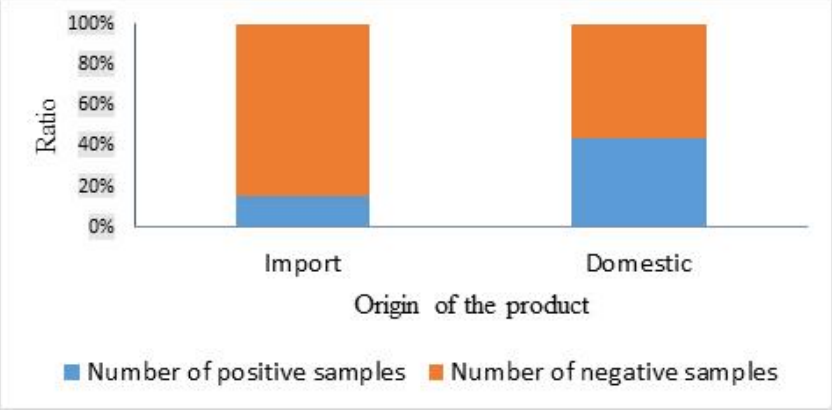


***Figure 4.*** *Sample rate chart exceeding SML regulation*

The results show that the research has detected DEHA and DEHP in some samples of PVC food wraps. This result is also quite consistent with previous studies in the world, which can be mentioned by the study of Xi-Liang Cao et al. 2014, DEHA was detected in most of the PVC wrap samples but not in PE wrap samples. DEHA was detected in 30 cheese samples packaged in PVC film containing DEHA plasticizer at levels ranging from 0.71 to 879 µg/g, with an average level of 203 µg/g. Levels of DEHA found in most cheese samples from this study were above the European migration limit of 18 mg/kg [4]. The results of this study show that toxic substances of the phthalate group in food wrap products need to be more closely controlled in terms of usage, especially for domestic products.

## 4. CONCLUSION

Analytical methods have been developed and validated to analyze 7 toxic chemicals of the phthalate group including DEHP, DEHA, DBP, BBP, DNOP, DIDP, and DINP. The procedure was effectively applied to analyze 35 samples of PVC food wrap produced domestically and imported and detected 3 samples with DEHA exceeding the Korean Standards's SML. Therefore, the issue of phthalate contamination from PVC food wrap is a matter of concern today to ensure the benefits and health of consumers better.

## ACKNOWLEDGMENT

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# Nghiên cứu xác định hóa chất độc hại nhóm phthalate trong màng bọc thực phẩm PVC bằng phương pháp GC-MS

**Nguyễn Thị Hà Bình, Đặng Thu Hiền, Nguyễn Ngọc Sơn, Trần Cao Sơn**

*Viện Kiểm nghiệm an toàn vệ sinh thực phẩm quốc gia, Hà Nội, Việt Nam*

***Tóm tắt***

Chất độc hại phthalate có thể thôi nhiễm từ bao bì chứa đựng thực phẩm vào thức ăn đang là vấn đề được quan tâm hiện nay, gây ảnh hưởng đến sức khỏe người tiêu dùng. Tuy nhiên tại Việt Nam chưa có nghiên cứu xác định nhóm chất này trong các bao bì, màng bọc thực phẩm. Nhóm nghiên cứu đã xây dựng phương pháp xác định đồng thời 7 phthalate điển hình gồm benzyl butyl phthalate (BBP), dibutyl phthalate (DBP), di(2-ehtylhexyl) phthalate (DEHP), di-n-octyl phthalate (DNOP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), di-(2-ethylhexyl) adipate (DEHA) trong mẫu màng bọc thực phẩm bằng phương pháp sắc ký khí (GC-MS) khối phổ sử dụng nội chuẩn. Giới hạn phát hiện và giới hạn định lượng của phương pháp cho các chất lần lượt là 0,03; 0,10 mg/kg. Độ thu hồi của phương pháp trong khoảng 82 - 105%. Độ lệch chuẩn tương đối (RSD) trong khoảng 5,6 - 7,5%. Phương pháp đã được áp dụng hiệu quả để phân tích hàm lượng các phthalate trong 35 mẫu màng bọc thực phẩm thu thập trên thị trường Hà Nội. Kết quả phân tích cho thấy đã phát hiện 10 mẫu có chứa phthalate, trong đó có 3 mẫu chứa DEHA vượt quy định cho phép. Nghiên cứu này góp phần bảo vệ sức khỏe người tiêu dùng và tham mưu cho cơ quan quản lý về việc bổ sung các quy định an toàn cho màng bọc thực phẩm PVC nói riêng và bao bì tiếp xúc, chứa đựng thực phẩm nói chung.

***Từ khóa:*** *GC-MS, nội chuẩn, phthalate, DBP, BBP, DEHP, DEHA, DNOP, DIDP, DINP.*