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Permanently Positively Charged Stable Isotope Labeling Agents and Its Application in the Accurate Quantitation of Alkylphenols Migrated from Plastics to Edible Oils

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ABSTRACT: A new permanently positively charged stable isotope labeling (SIL) agent pair, 4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)-*N*,*N*,*N*-trimethylbenzenaminium iodide(DPTBA) and its deuterated counterpart d_3-DPTBA, was designed and synthesized. The SIL agents were applied to the liquid chromatography−tandem mass spectrometry analysis of alkylphenols. Light labeled standards and heavy labeled samples were mixed and analyzed simultaneously. Matrix effect which mainly occurred during the ionization process was minimized because of the identical ionization processes between samples and standards. Meanwhile, derivatization made alkylphenols be positively charged, and thus the sensitivity was enhanced. The limits of detection were in the range of 1.5−1.8 ng/L, and the limits of quantitation were in the range of 4.8−6.1 ng/L. The developed method was applied to analyze alkylphenols migrated from plastics to edible oils. The recoveries for all analytes were in the range of 88.6−95.3%, while the matrix effects for all analytes were in the range of 96.2−99.6%.

KEYWORDS: *stable isotope labeling, alkylphenols, matrix effect, HPLC−MS/MS*

1. INTRODUCTION

Plastics have been widely used in food packaging because of their stable, flexible, and low density properties.[1,2] The production of plastics needs many compounds such as monomers, stabilizers, plasticizers, ultraviolet light absorbers, lubricants, and so on.[1,3] Besides, impurities of raw materials might also be introduced into plastics.[2] Alkylphenols might be used as raw materials of plastics, and some of them exist in the form of contamination or impurities.[3] They showed high incidence of migrating into oil samples because of their good solubility. Alkylphenols can bring about adverse effect on our hormonal system and are regarded as endocrine disrupting compounds.[3−5] Some of them can cause immune system damage and induce tumor or cancer at low concentrations.[6,7] A large proportion of oil samples are packaged in plastic materials in the Chinese market, which renders a big health threat to local people. It is urgent to study the level of alkylphenols migrating from plastics to oils.

Various methods have been developed for the analysis of alkylphenols.[3,8,9] Liquid chromatography and gas chromatography distinguished themselves from these methods because of their high selectivity and sensitivity.[10] However, misidenfication often occurs because of the coelution of the interferences with the analytes. Gas chromatography−mass spectrometry (GC-MS) and liquid chromatography−tandem mass spectrometry (LC−MS/MS) methods can provide not only retention time data but also molecular weight and fragment ion information.[11] They greatly decreased the misidentification ratio and were often used in the analysis of samples with complex matrices.[12] However, there are still great challenges in accurate GC−MS and LC−MS/MS quantification. The matrix effect is one of the most important drawbacks, and a large number of studies indicate that the method accuracy is greatly influenced by the matrix effect.[13−15] The matrix effect can be reduced by a dilution of the sample,[14] but this strategy might not work well because the sensitivity of different instruments differs greatly. Another often used method is the application of an internal standard (IS) which coelutes with the analytes and thus compensates for the alteration in the LC−MS/MS signal,[16−19] but it is expensive to use IS for all analytes, and IS is not always available. The matrix-matched calibration curve has also been used to enhance method accuracy, but it is difficult to find identical matrices and more contaminants might be introduced in to the MS instrument during this process.[20] The stable isotope labeling (SIL) technique provides an alternative strategy to overcome the matrix effect.[21]

In the SIL method, there is a pair of stable isotope labeling agents which have identical functional groups and differ only in isotopic composition.[22,23] Light and heavy SIL agents react with the standard and analytes, respectively, and then they are mixed and analyzed in the same run.[24] Analytes and standards elute simultaneously, and thus the matrix effect which mainly occurs during the ionization process is overcome. It is desirable that the sensitivity of MS be enhanced simultaneously during this process. In such conditions, samples can be injected in small volume, and fewer matrices are introduced into the

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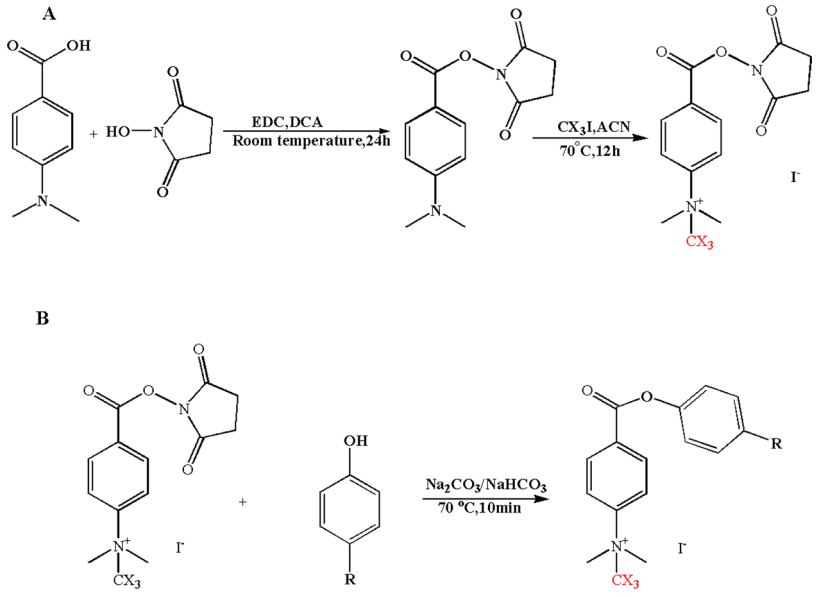

Figure 1. Synthesis route for DPTBA (A) and the derivatization scheme for alkylphenols(B). X = H or D.

instrument.[25] Phenolic compounds were usually analyzed in negative mode, the sensitivity of which was regarded to be lower than that in positive mode.[26] It is desirable that charge reverse be achieved during the derivatization process.

In this study, a pair of permanently positively charged SIL agents were designed and synthesized to enhance the MS sensitivity, and to overcome the matrix effect too. This SIL strategy showed the following merits: (1) charge reverse derivatization which enable the analysis of alkylphenols in more sensitive positive mode; (2) overcome of matrix effect because of the coelution of the analytes and standards; and (3) multiple reaction monitoring (MRM) identification of unknown analytes because of the identical MRM conditions for all analytes. This strategy can be well applied in the accurate analysis of alkylphenols in complex matrices.

2. MATERIALS AND METHODS

2.1. Chemicals. 4-Propylphenol (C3), 4-butylphenol (C4), 4-pentylphenol (C5), 4-*n*-hexylpheno (C6), 4-*n*-heptylphenol (C7), 4-*t*-octylphenol (C8), and 4-*n*-nonylphenol (C9) were purchased from national standard material research center of China. Methanol, 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC), dichloromethane (DCA), *N*-hydroxysuccinimide (NHS), 4-dimethylaminobenzoic acid, *n*-hexane, and acetonitrile were of high performance liquid chromatography (HPLC) grade and purchased from Sigma-Aldrich (U.S.A.). Dichloromethane (DCM) and dansyl chloride (DNS-Cl) were purchased from Aladdin Company (Shanghai, China). Pure water was purchased from Watsons (Guangzhou, China).

2.2. Synthesis of 4-(((2,5-Dioxopyrrolidin-1-yl)oxy)-carbonyl)-*N*,*N*,*N*-trimethylbenzenaminium iodide(DPTBA) and D_3-DPTBA. *2.2.1. Synthesis of 2,5-Dioxopyrrolidin-1-yl 4-(dimethylamino)benzoate.* The synthesis of 2,5-dioxopyrrolidin-1-yl 4-(dimethylamino)benzoate was based on the reaction of carboxylic acid with NHS.[27] NHS (1 g) and 4-dimethylaminobenzoicacid (1 g) were dissolved in 40 mL of DCM and then cooled to 0 °C in an ice bath. Then 20 mL of DCM solution of EDC (100 g/L) was then added slowly into the mixture through a constant pressure dropping funnel. The mixture was stirred at room temperature for 24 h. After the completion of the reaction, the solvent was removed under reduced pressure to obtain a crude product. The crude product was purified by silica gel column chromatography to obtain the target product, and the yield was 75%. The $[M]^+$ of the product was m/z 263.1, in good accordance with the theoretical molecular weight (Figure S1).

2.2.2. Synthesis of DPTBA and Its Deuterated Counterpart d_3-DPTBA. The final products were synthesized based on the reaction of tertiary amine with iodomethane.[28] Then 0.1 g of 2,5-dioxopyrrolidin-1-yl 4-(dimethylamino)benzoate was dissolved in 20 mL of acetonitrile, meanwhile iodomethane (0.5 g, 15 mmol) or iodomethane-d_3 (0.5 g, 15 mmol) was dissolved in 10 mL of acetonitrile. The former solution was added dropwise into the latter solution under stirring. Then, the mixed solution was further reacted at 60 °C for 12 h. After removing excess solvent under reduced pressure, a crude product was obtained. Finally, it was recrystallized from ethanol to obtain a pure product, yield 0.11 g (70%). The m/z value for DPTBA was 277.1 (Figure S2), in good accordance with the $[M]^+$ of the DPTBA, indicating the positive charge of DPTBA. The m/z value for d_3-DPTBA was 280.1, and the synthesis route was depicted in Figure 1A. DPTBA ethanol solution could react with $AgNO_3$ to afford a yellow precipitate, which further confirmed the ionic property of DPTBA (Figure S3).

2.3. Sample Extraction. *2.3.1. Migration Test.* A migration test was performed using edible peanut oil according to the dietary habit of China. Plastics made of polyethylene terephthalate (PET), polycarbonate (PC), polyvinyl chlorid (PVC), polystyrene (PS), high density polyethylene (HDPE), polyethylene (PE), and polypropylene (PP) were chosen as examples because they were frequently used in the packaging of oils. Plastics (0.6 dm^2) were purchased from supermarket and cut to squared rectangles. They were immersed in 100 mL of oil samples and incubated at 70 °C for 2 h. After cooling to room temperature, 30 mL of the liquid oil sample was transferred to 50 mL centrifuge tubes.

2.3.2. Liquid–Liquid Extraction (LLE) of the Analytes from Oil Migrant. $NaHCO_3$ buffer, aquatic NaOH solution, and NaOH methanol solutions with concentrations of 0.1 mol/L were used to extract acidic alkylphenols from oil samples. They were added into oil samples containing 100 ng/L of alkylphenols, respectively. The mixture was vortexed vigorously for 3 min to achieve the LLE process. After centrifugation, the oil phase was discharged, and the methanol phase was adjusted to neutral with 6 mol/L HCl aqueous solution. The extract was evaporated to dryness for later derivatization. Each sample was analyzed in three parallels.

2.4. Derivatizaiton of Alkylphenols. Alkylphenols were derivatized under basic condition, and the derivatization scheme was shown is Figure 1B. Briefly, dried sample extract, 100 μL of acetonitrile, 100 μL of 500 mg/L d_3-DPTBA acetonitrile solution, and 100 μL of 0.1 mol/L pH 9 Na_2CO_3 buffer were mixed and react in a

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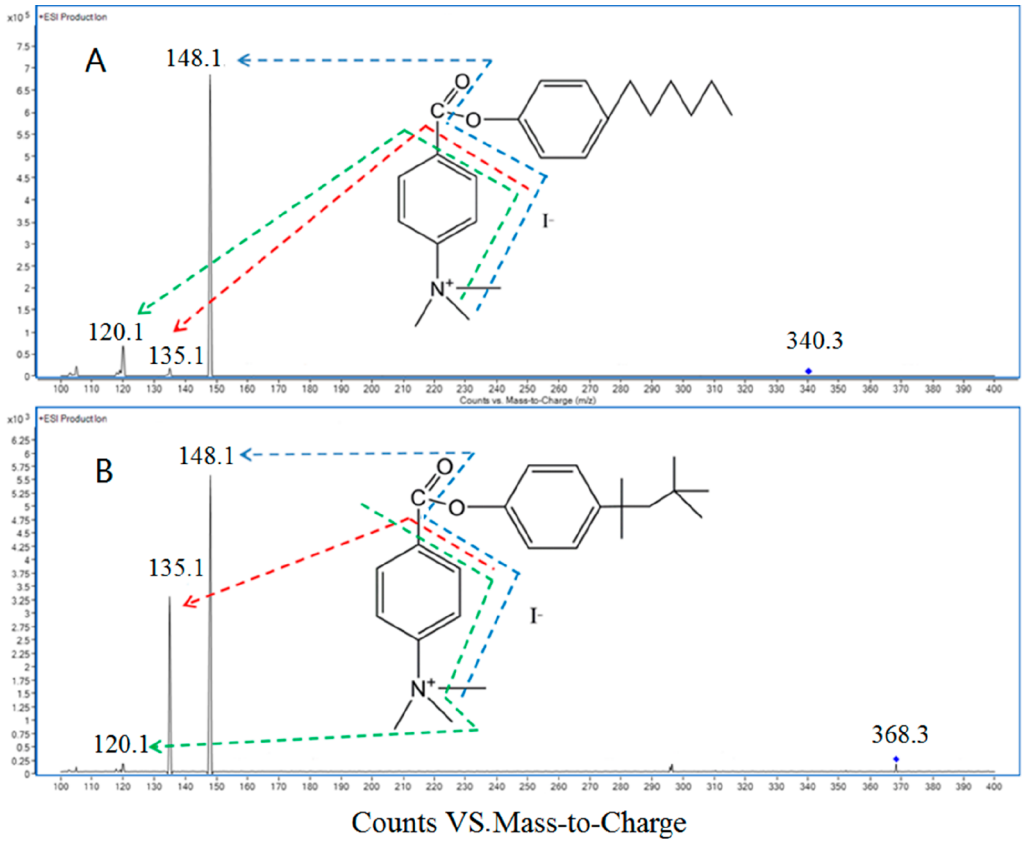

Figure 2. Illustration of the generation path of the production ions for DPTBA-C6 (A) and DPTBA-C8 (B).

water bath at 70 °C for 10 min. Standard samples were derivatized under the same conditions with DPTBA as labeling reagent. After the reaction was completed, the mixture was cooled to room temperature. Then heavy labeled samples and light labeled standards were mixed and analyzed by LC−MS/MS in three replicates.

To achieve the sufficient labeling of the analytes, derivatization conditions including DPTBA concentration, reaction temperature, reaction time, and pH of buffer solutions were optimized in detail.

2.5. HPLC−MS/MS Analysis. An Agilent 6460 Triple Quadrupole LC/MS System (Agilent, Santa Clara, U.S.A.) coupled with an Agilent 1290 series HPLC system was used for the HPLC−MS/MS analysis. An Agilent Jet Stream electrospray ionization source (ESI source) was used as ionization source. HPLC separation was achieved using an Eclipse Plus C18 column (2.1 × 50 mm, 1.8 μm i.d., Agilent). Mobile phase A was distilled water containing 5% acetonitrile, and 100% acetonitrile was used as the mobile phase B. The column flow rate was set at 0.15 mL/min, and the column temperature was kept at 30 °C. The elution conditions were as follows: 40%−95% B from 0 to 5 min and then hold for 2 min. The first 1.5 min was operated in the "to waste" mode to make the excess labeling reagent be discharged. The injection volume was 2 μL. The derivatives were permanently positively charged and was analyzed in a positive ion mode for the monitoring of $[M]^+$. The ESI source conditions were capillary voltage, +4.0 kV; nebulizer pressure, 40 psi; dry gas flow rate, 11.0 L/min; dry gas temperature, 300 °C; sheath gas temperature, 280 °C; sheath gas flow rate, 10 L/min. The MRM conditions were fragmentor, 140 V; collision energy, 40 V. The precursor ions were 298.2 (C3), 312.2 (C4), 326.2 (C5), 340.3 (C6), 354.2 (C7), 368.3 (C8), and 382.4 (C9). The production ions for lighted labeled alkylphenols were m/z 135.1, 120.1, and 148.1, and the production ions for heavy labeled ones were m/z 138.1, 123.1, and 151.1.

2.6. Method Validation. Limits of detections (LODs) which were defined as signal-to-noise (S/N) ratio of 3 were evaluated first, and then limits of quantitation (LOQs) based on S/N = 10 were studied. Experimental peak abundance ratio between light and heavy labeled standard, was compared with the theoretical concentration ratio to obtain the linearity information. The six ratio levels were 12:1, 9:1, 6:1, 3:1, 1:1, and 1:3, respectively. Recoveries obtained by the comparison between determined value and spiked value were used to evaluate the accuracy of the method. They were carried out by spiking blank oil samples with standard solutions, and the concentrations of the analytes in oil samples were 10, 30, and 100 ng/L, respectively. Intraday precision was performed with six replicates, and interday precision was calculated with three replicates on three different days. They were carried out at the same three levels with those of recoveries. The relative standard deviations of the parallel results were used to evaluate the precision. The matrix effect was evaluated by comparing the MS responses of alkylphenols in blank oil migrants to those of an equivalent amount in a neat standard solution. They were calculated by the formula of (determined value of standard in migrant/determined value of neat standard) × 100. All spiked samples were heavy labeled and standard samples were light labeled. Samples and standard were mixed in equal volume before HPLC−MS/MS analysis. If the peak abundance ratios obtained were beyond the linear range, the volume ratio of the sample and standard were adjusted, and the new mixture was analyzed again to obtain accurate results.

3. RESULT AND DISCUSSION

3.1. Confirmation of the Derivatives. DPTBA is vulnerable to fragmentation, and thus it can produce common production ions for different derivatives. As shown in Figure 2, the DPTBA-C6 (m/z 340.3) and DPTBA-C8 (m/z 368.3) derivatives had identical production ions, i.e., m/z 148.1, 135.1, and 120.1. The production ion of m/z 148.1 comes from the breaking of the C−O bond and a methyl group of DPTBA, while the ion with m/z of 120.1 comes from the further breaking of the chemical bond between the benzene ring and the C═O bond. The production ion of m/z 135.1 comes from

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Table 1. Linearity, Detection Limits, Precision, and Matrix Effect

analyte	linearity	R^2	LOD (ng/L)	LOQ (ng/L)	spiked level (ng/L)	intraday precision (RSD%)	interday precision (RSD%)	recovery (%)	matrix effect (%)	matrix effect of control group (%)
C3	$Y = 1.1176X^a - 0.0536$	0.9980	1.7	5.5	10	3.5	5.2	95.2 ± 2.5[b]	96.8 ± 2.9	86.4 ± 2.6
					30	3.3	4.8	95.1 ± 2.6	96.2 ± 2.5	86.2 ± 2.8
					100	3.5	4.6	95.3 ± 2.8	96.4 ± 2.7	86.9 ± 2.4
C4	$Y = 1.0494X - 0.0292$	0.9995	1.6	5.0	10	3.4	5.0	93.1 ± 3.0	97.3 ± 2.6	90.7 ± 3.2
					30	3.1	4.7	93.6 ± 2.6	97.2 ± 2.8	90.5 ± 2.9
					100	2.9	4.5	93.4 ± 2.5	97.5 ± 2.9	91.1 ± 3.4
C5	$Y = 1.0193X - 0.0290$	0.9990	1.5	4.8	10	3.4	4.5	92.6 ± 3.1	99.3 ± 2.8	92.5 ± 3.3
					30	3.1	4.6	92.4 ± 3.2	98.8 ± 3.0	92.7 ± 3.2
					100	3.2	4.2	92.3 ± 3.1	98.7 ± 2.6	92.1 ± 2.8
C6	$Y = 1.0032X - 0.0253$	0.9993	1.8	6.1	10	3.1	4.3	91.2 ± 2.6	99.0 ± 2.8	96.3 ± 2.7
					30	2.8	4.6	91.5 ± 2.8	99.6 ± 2.5	96.1 ± 2.9
					100	3.2	4.2	91.6 ± 2.9	99.2 ± 2.4	96.7 ± 2.6
C7	$Y = 1.0587X - 0.0244$	0.9996	1.7	5.6	10	3.4	4.7	90.3 ± 3.0	98.7 ± 3.1	95.2 ± 3.4
					30	3.1	4.2	90.6 ± 2.9	98.4 ± 2.9	95.8 ± 3.6
					100	3.3	4.1	90.2 ± 2.7	98.5 ± 2.7	95.1 ± 2.6
C8	$Y = 1.0249X - 0.0537$	0.9991	1.7	5.6	10	3.6	4.5	89.5 ± 3.1	99.4 ± 2.5	98.8 ± 3.2
					30	3.4	4.3	89.8 ± 2.8	99.5 ± 2.8	98.2 ± 3.5
					100	3.5	4.4	89.6 ± 3.2	99.6 ± 2.7	99.2 ± 3.6
C9	$Y = 1.0727X - 0.055$	0.9993	1.8	6.0	10	3.3	4.8	88.6 ± 3.0	98.6 ± 2.8	92.4 ± 3.2
					30	3.1	4.6	88.8 ± 2.9	98.5 ± 2.6	92.9 ± 2.8
					100	3.4	4.5	88.9 ± 2.6	98.7 ± 2.5	93.4 ± 3.1

[a]X, theoretic concentration ratio of light/heavy labeled alkylphenols; Y, experimental mass spectrometric peak intensity ratio of light/heavy labeled alkylphenols. [b]data are expressed as mean value.

the breaking of the chemical bond between benzene ring and the carboxylate ester. These common production ions from different derivatives confirmed the successful derivatization of the analytes.

The peak abundance ratio of the production ions showed obvious correlations with the alkyl substituent of alkylphenols. For compounds with linear alkyl substituent, m/z 120.1 ion was more abundant. When the abundance of m/z 148.1 was set as 100, the abundance of m/z 120.1 was in the range of 9.06−9.31, while the abundance of m/z 135.1 was lower than 3. For C8 with branched alkyl substituent, the m/z 135.1 ion was more abundant. The peak abundance ratios of all DPTBA derivatives were listed in Table S1, while the production ion chromatograms were shown in Figures S4 and S5.

3.2. Optimization of Derivatization Condition. *3.2.1. Effect of DPTBA Concentration on Derivatization.* According to our previous experience in derivatization,[29] DPTBA was studied in the concentration level of 200 to 800 mg/L with acetonitrile as solvent. The results indicated that the peak abundance reached the maximum when DPTBA concentration was 500 mg/L. Further increasing the concentration of DPTBA beyond this level had no obvious influence on peak abundance. As a result, the labeling reagent was prepared at a concentration of 500 mg/L.

3.2.2. Effect of pH on Derivatization. The derivatization of DPTBA was carried out in basic acetonitrile condition. $Na_2CO_3/NaHCO_3$ buffer was studied in the pH range of 8−11. As shown in Figure S6A, the highest peak abundance was obtained when the pH was 9, further increasing pH beyond this level, the peak abundance decreased. Therefore, the subsequent derivatization was carried out in 0.1 mol/L $Na_2CO_3/NaHCO_3$ buffer with pH of 9.

3.2.3. Effect of Temperature on Derivatization. Derivatization can be accelerated at high reaction temperature, but decomposition of the derivatives might also occur. The influence of temperature on derivatization was studied in the temperature range of 50−90°C with the reaction time fixed at 30 min. The results showed that the derivatization could be quickly finished at 70 °C (Figure S6B). Further increasing the temperature to 80 °C, the signal abundance decreased obviously. The reaction time was further studied when temperature was fixed at 70 °C, and the results indicated that stable peak abundance was obtained at 10 min. As a result, the derivatization was carried out at 70 °C for 10 min.

3.3. Optimization of the Extraction Method. Most of the migration test was performed using food simulates. Although food simulate is easier to analyze, the migration behavior in food simulate might be different from that in real food samples. Therefore, it is ideal to use real food samples to study the migration behavior.[30] However, food matrices are usually much more complex than those of food simulate, and thus efficient sample preparation and analysis skills are necessary. In this study, the migration test was carried out using peanut oil, a kind of preferred edible oil in China. The oil matrices were removed by a simple but effective LLE method.

The extraction was based on the weak acidic property of alkylphenols. $NaHCO_3$ buffer which had been used to extract bromophenols from biological matrices was tried first,[31] but the recoveries were lower than 40% for most of the analytes. Then aquatic NaOH solution with stronger basic property was applied, but the recoveries were still not satisfying. The poor recoveries were due to the low solubility of alkylphenols in water. In consideration of the immiscibility of methanol with oil samples, 0.1 M NaOH methanol solution was applied to extract the analytes from the oil migrants, and good recoveries were obtained. As shown in Table 1, the obtained recoveries were in the range of 88.6−95.3%.

3.4. Evaluation of Matrix Effect. Matrix effect was reported to be mainly caused by the ionization competition form matrices,[16,32] and a severe matrix effect (40%−65%) of

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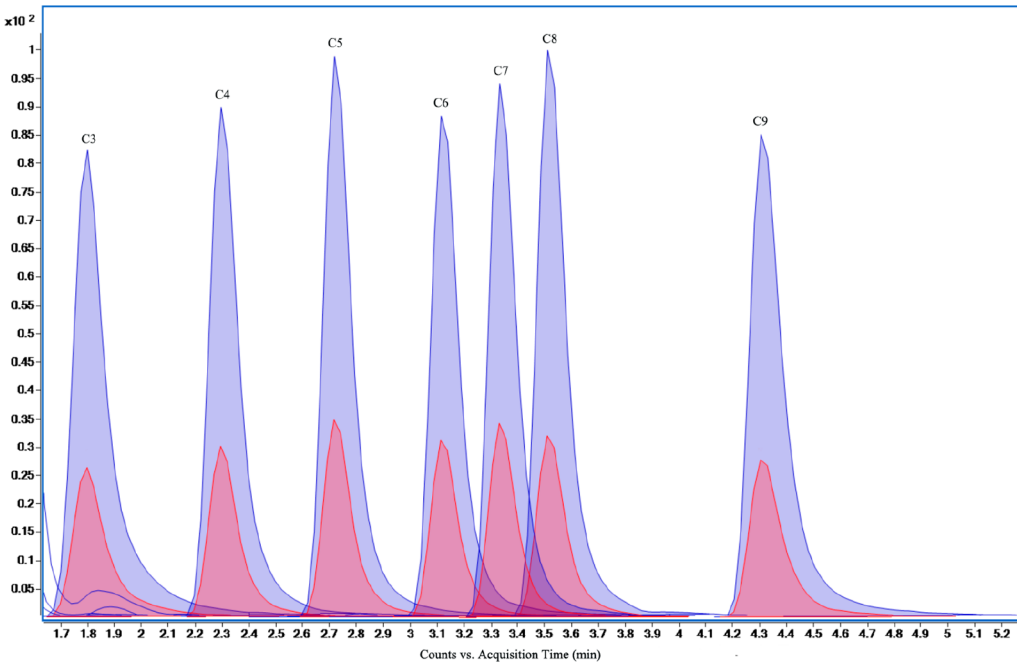

Figure 3. Extracted ion chromatograms of light (blue chromatogram) and heavy (red chromatogram) labeled alkylphenols.

oil samples have been reported.[33] In this study, two strategies were applied to overcome matrix effect. The first one is the permanent positive charge of the SIL agents. The derivatives were permanently positively charged, and thus they did not suffer from the ionization competition from matrices. Moreover, all samples were analyzed using neutral mobile phase, but not the traditional acidic mobile phase. This change in mobile phase greatly restrained the ionization of neutral matrices and thus lowered the matrix effect. The second strategy is the SIL method during which light labeled standard and heavy labeled samples eluted simultaneously (Figure 3). Sample and standard experienced the same evaporation and ionization process, which further eliminated the matrix effect. In this study, standard samples were added in oil extract, but the matrix effect could be neglected. The obtained values of spiked standards were about 96.2−99.6% of that in neat standard solutions (Table 1). The performance of the developed method was also compared with previously reported methods. As shown in Table 2, the recoveries and matrix effect was obviously improved in comparison with normal MS method.[34−37]

3.5. MS Enhancement Effect. It is commonly regarded that the MS sensitivity is higher in the positive mode than that in the negative mode, especially for phenolic compounds which do not dissociate completely in aqueous solution. To illustrate the mass enhancement effect of the positively charged DPTBA, a comparison was made between DPTBA and dansyl chloride (DNS-Cl), a commercially available labeling reagent. OP and NP were selected as examples, and the derivatization and MS conditions for DNS-Cl derivatives were shown in section S1 and S2. The DPTBA and DNS-Cl derivatives were mixed and analyzed in a single run. As shown in Figure S7, the sensitivity obtained using DPTBA as the derivative was much higher than that of DNS-Cl derivatives. Meanwhile the background noise was lowered. The detection limit (signal-to-noise ratio of 3) was lowered by 11 and 16 times for OP and NP, respectively, further validating the MS enhancement effect of the positively charged DPTBA.

Table 2. Comparison of the Accuracy of This Method with Previously Reported Methods

NO	method	matrix	recovery	matrix effect	ref
1	GC−MS	water	73−107%	73−121%	34
2	GC−MS	water	83.6−118.6%		35
3	LC−MS	water	57−136%	43−138%	36
4	LC−MS	soil	67−114%	80.8−82.5%	37
5	LC−MS	oil	88.6−95.3%	96.2−99.6	this article

3.6. Method Validation. The proposed method was validated based on the method described in experimental section. As shown in Table 1, the correlation coefficients (R^2) for all analytes were higher than 0.998, indicating the good linearity of the SIL method. LODs were in the range of 1.5−1.8 ng/L, while LOQs were in the range of 4.8−6.1 ng/L. Recoveries were in the range of 88.6−95.3%, and the matrix effects were in the range of 96.2−99.6%. Intraday precision was in the range of 2.8−3.6%, while interday precision ranged from 4.1% to 5.2%.

3.7. Sample Analysis. The proposed method was applied to the analysis of alkylphenols in the oil migrants from different plastic materials. All standard samples were light labeled and all migrants were heavy labeled, and they were mixed in equal volume before MS analysis. As shown in Table 3, C9 was detected in all samples with a concentration higher than 10 μg/L, while the other alkylphenols were detected in some samples. The determined concentrations were in the range of 0.17−33.2 μg/L, and a representative MRM chromatogram was shown in Figure 4. These concentrations were lower than some reported median inhibitory concentrations (IC 50) of alkylphenols (199 and 44 μg/L for OP and NP, respectively).[38] However, there are various intake routes of alkylphenols, and their total concentration might exceed these levels and pose an adverse effect on our body. Although alkylphenols can cause obvious adverse effect at low levels, there is still a great lack of regulations on them.[6] It is good to see that some regulations

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Table 3. Concentrations of Alkylphenols Determined in Peanut Oil Migrants

plastic type	plastic character	C3 (μg/L)	C4 (μg/L)	C5 (μg/L)	C6 (μg/L)	C7 (μg/L)	C8 (μg/L)	C9 (μg/L)
HDPE	white, rigid, and semitransparent	*a*	*a*	*a*	17.7	*a*	1.63	11.5
PET/PE	opaque and flexible	*a*	*a*	*a*	5.72	*a*	2.81	14.8
PET	rigid and transparent	*a*	*a*	*a*	2.43	*a*	*a*	13.4
PP	rigid and transparent	*a*	*a*	*a*	4.92	*a*	*a*	11.3
PC	rigid and transparent	1.52	0.19	0.25	*a*	0.18	0.20	33.2
PVC	rigid and white	0.49	*a*	0.18	*a*	0.17	*a*	16.1
PS	rigid and white	1.36	0.18	0.31	*a*	0.19	*a*	14.5

[a]Not detected.

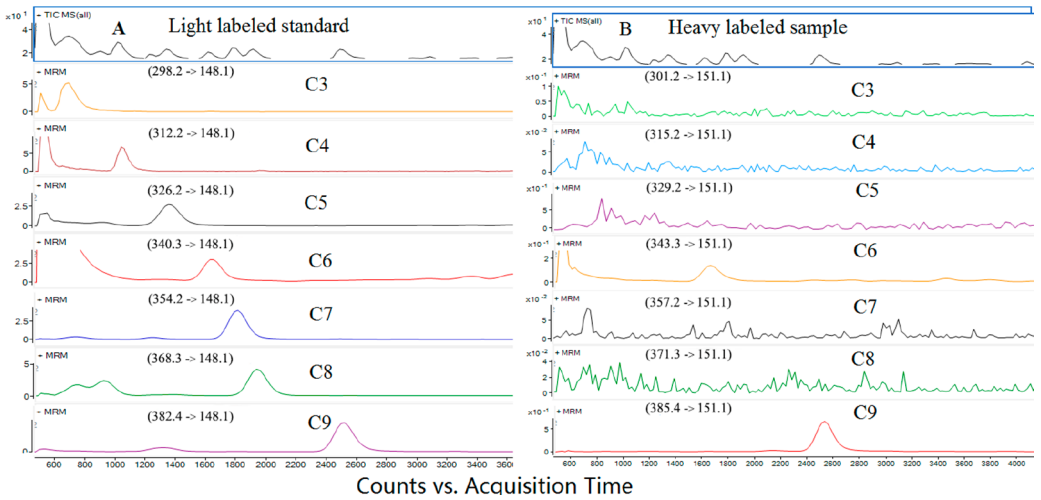

Figure 4. MRM chromatogram of light labeled standard (A) and heavy labeled sample (B).

on them are being made now. For example, there is a local standard for C9 in food contacting materials in China, and the national standard is in the consultation process.[39] European Union will forbid the use of nonylphenol ethoxylates after 3 Feburary 2021.[40]

3.8. Application in the MRM Identification of New Compounds. DPTBA is more vulnerable to fragmentation than alkylphenols, and thus all of the analytes showed common production ions. Since these ions come from the breaking of the same chemical bond, thus the collision energy needed for these ions was also equal. This means that all of the alkylphenols derivatives can be analyzed in the same MRM condition. Even if there is no standard sample available, as long as the molecular information is available, the compounds can still be analyzed in MRM mode, which is much more sensitive than the scan mode. In this study, we found 4-dodecylphenol in the absence of corresponding standard using the common MRM conditions (Figure S8A). The compound eluted after C9, and its precursor ion was m/z 427.3, in good accordance with the character of 4-dodecylphenol. To verify our predication, the standard sample of 4-dodecylphenol was purchased and analyzed. As shown in Figure S8B, the retention time and MRM information on the standard and sample were identical, validating the MRM identification result.

In conclusion, permanently positively charged SIL agents, DPTBA and d_3-DPTBA, were designed and synthesized. The derivatives were positively charged, and thus the MS sensitivity was enhanced. Samples and standards were mixed before HPLC−MS/MS analysis, and thus they experienced the same electrospray process, which minimized the matrix effect. The developed method was applied to the analysis of alkylphenols migrated from plastics to edible oil, and little matrix effect was observed. It also showed great potential in the MRM identification of unknown phenolic compounds.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c03413.

Derivatization of OP and NP with DNS-Cl, the MRM conditions for DNS-Cl derivatives, the mass spectrum of 2,5-dioxopyrrolidin-1-yl 4-(dimethylamino)benzoate, the picture for DPTBA, $AgNO_3$ and a mixture of DPTBA and $AgNO_3$, the mass spectrum of DPTBA (A) and D3-DPTBA (B), the production ion chromatograms for all alkylphenol derivatives, the influence of pH (A) and temperature (B) on derivatization, the MRM chromatogram of DPTBA and DNS-Cl derivatized alkylphenols, MRM chromatogram of 4-dodecylphenol detected by the established MRM method (A) and standard MRM chromatogram of 4-dodecylphenol (B), peak abundance ratio of the DPTMA derivatives (PDF)

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Notes

The authors declare no competing financial interest.

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