



Development of microwave-assisted extraction and dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry for the determination of organic additives in biodegradable mulch films

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ABSTRACT

Organic additives are extensively used as ingredients in biodegradable mulch films. They are swiftly released into the environment, which may have an ecotoxicological impact on plant growth and development, as well as on soil microbial community abundance and function. Herein, a method based on the application of microwave-assisted extraction (MAE) and dispersive liquid–liquid microextraction (DLLME) was developed to analyze eighty organic additives by gas chromatography–mass spectrometry in poly(butylene adipate terephthalate) (PBAT) biodegradable mulch films. This was a comprehensive study, including the identification of organic additives, optimization of MAE and DLLME methods, analysis of isocyanate conversion, and evaluation of the matrix effect (ME). Under the optimized experimental conditions, this method exhibited excellent detection capabilities for organic additives, except for 5 kinds of isocyanates and their reaction products, with coefficients of determination $R^2 > 0.999$ and lack of fit $P > 0.05$ in linear regression parameters. A negligible ME was observed. The relative recoveries were 93.0–109.8%, and the repeatability and reproducibility varied within the ranges of 2.06–8.76% and 2.38–10.23%, respectively. The limits of detection and limits of quantitation were 0.0008–0.0586 $\mu\text{g g}^{-1}$ and 0.003–0.195 $\mu\text{g g}^{-1}$, respectively. The developed method was further successfully applied to the analysis of organic additives in PBAT biodegradable mulch films from four different manufacturers. Interestingly, the Venn diagram and principal component analysis showed that different manufacturing origins display obvious characteristic differences in the organic additive types and concentrations.

1. Introduction

Each year, vast amounts of traditional plastics (e.g., PE, PP, PET) are produced and used worldwide. Although these plastics offer many benefits to society, there are numerous drawbacks [1]. Due to their stability and high durability, plastics have long degradation times and tend to extensively accumulate in different environments, affecting wildlife and human health [2]. In contrast to the majority of traditional plastics, biodegradable plastics such as poly(lactic acid) (PLA), poly(butylene succinate) (PBS), and poly(butylene adipate terephthalate) (PBAT) decompose into benign water, carbon dioxide in a short period

once exposed to the environment and thus, exhibit minimal environmental impact [3]. PBAT, which is obtained by the polycondensation of butanediol (BDO), adipic acid (AA), and terephthalic acid (PTA), is a completely biodegradable aliphatic-aromatic copolyester. It is a flexible material, that has a high elongation at break, as well as good hydrophilic and processing properties [4]. Thus, PBAT has been widely used in the production of blown films and their associated membrane products [5].

In order to improve the physical and chemical characteristics of biodegradable plastics, equal in performance to the traditional plastics, organic additives of different properties have been widely added. The intentionally added substances (IAS) such as antioxidants, UV

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stabilizers, fillers, rubbers, lubricants, flame retardants, have been reported [6]. Moreover, non-intentionally added substances (NIAS) such as impurities, and reaction, degradation products can form during the manufacture [7]. Given their extensive use, the abovementioned organic additives are released into the environment, which may pose a risk to the environment and human health. The effect of additives needs to be considered in a life cycle assessment of biodegradable alternatives [3]. Hence, it is necessary to evaluate the type and concentration of organic additives in PBAT biodegradable mulch films.

Recently, reliable analytical techniques were reported for target or non-target analysis of organic additives in traditional or biodegradable plastics. These techniques mainly include gas chromatography (GC) or liquid chromatography (LC) coupled to mass spectrometry (MS) or tandem mass spectrometry (MS/MS) [8–10]. However, Tinuvin® is poorly ionized and a high matrix effect is often found in benzophenone and its derivatives (BPs) in the electrospray source of LC-MS systems [11,12]. Compared to other techniques, the favorable features of GC-MS include its powerful separation, high reproducibility and lower sensitivity toward matrix effects, which makes it the most suitable separation technique for the analysis of complex organic additives [13]. Regarding the extraction method of the organic additives, they are most often extracted with an organic solvent using soxhlet extraction (SE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) [14–18]. Compared to other methods, MAE is advantageous in that it allows for a high extraction recovery whilst using less solvent and shorter extraction times [19]. Regarding the large amounts of co-extracts for MAE such as low molecular weight polymers, these may affect subsequent analysis. It is also difficult to analyze low levels of organic additives by direct injection in GC-MS. Thus, the sample enrichment and purification are important steps for accurate and sensitive analysis of the organic additives. Traditional enrichment and purification methods, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE), are laborious and consume large amounts of organic solvent [20]. Dispersive liquid-liquid microextraction (DLLME) was first introduced by Rezaee et al. for the extraction of organic compounds from aqueous samples [21]. Subsequently, a series of modified DLLME methods were developed and applied in different matrices [22–25]. These environmentally friendly extraction techniques offer several advantages, including rapidity, easier manipulation, less organic solvent consumption, less time, lower cost, higher recovery, and easier linkage to separation techniques [26]. Therefore, the use of MAE combined with DLLME technology can effectively improve the extraction efficiency, reduce the extraction time, interference of non-target compounds and then improve the sensitivity. To the best of our knowledge, this pretreatment method has not been used to analyze the organic additives in PBAT biodegradable mulch films.

In the present study, we developed a method based on MAE and DLLME with GC-MS for the determination of organic additives in PBAT biodegradable mulch films. These organic additives were extracted by MAE, followed by enrichment and purification by DLLME. The MAE conditions were optimized using the Doehlert design (DD) for maximum extraction efficiency. The organic additives were tentatively identified and quantified using GC-MS. The proposed method was employed in the analysis of twelve PBAT biodegradable mulch film samples from four different manufacturers. The type and concentration of organic additives could be used to discriminate between the different manufacturers. Therefore, the proposed method may be used to trace the manufacturing origins.

2. Experimental

2.1. Standards and reagents

Organic additive standards and an internal standard of phenethyl acetate (with purities of >98%) were purchased from Sigma-Aldrich (St

Louis, MO, USA). *n*-paraffins C6–C40 (purity > 95.5%) for the determination of the linear retention indices (LRI), were also obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical purity and were procured from Sinopharm Chemical Reagent Co., Ltd. (Beijing, PRC). Ultra-pure water was obtained by purifying demineralized water in a Milli-Q® system (Millipore, Bedford, MA, USA).

2.2. Preparation of standard solutions and samples

The standard stock solutions of 2,6-diisopropylphenyl isocyanate (5.16 mg mL⁻¹), 2,6-diisopropylaniline (5.12 mg mL⁻¹), butylated hydroxytoluene (1.16 mg mL⁻¹), 1,6-dioxacyclododecane-7,12-dione (1.28 mg mL⁻¹), 2,2,4-trimethyl-1,3-pentanediol diisobutyrate (0.6 mg mL⁻¹), cedrol (0.56 mg mL⁻¹), heptadecane (0.68 mg mL⁻¹), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (1.52 mg mL⁻¹), phenanthrene (0.96 mg mL⁻¹), hexadecanenitrile (1.32 mg mL⁻¹), dibutylphthalate (1.04 mg mL⁻¹), octyl 4-methoxycinnamate (1.24 mg mL⁻¹), bis(2-ethylhexyl)adipate (5.72 mg mL⁻¹), eicosanamide (1.08 mg mL⁻¹), octabenzene (0.84 mg mL⁻¹), squalene (1.32 mg mL⁻¹) and internal standard of phenethyl acetate (1.852 mg mL⁻¹) were prepared in acetone and stored in brown bottles at 4 °C. Standard working solutions were prepared fresh immediately prior to use by diluting the standard stock solution with acetone. All solutions were brought to ambient temperature prior to use.

To investigate the differences of organic additives in the PBAT degradable mulch films from different manufacturers, four common samples of PBAT A, PBAT B, PBAT C, and PBAT D, which are widely used in agricultural production, were selected. Six repetitive samples were taken and then cut into small square-shaped pieces of ~5 mm. These samples were stored in glass bottles for further use.

2.3. Experimental procedure

The MAE was performed using the microwave extraction system (MARS™ 6, CEM Corp., USA). The sample (0.1 g) and the internal standard solution (5 µL) were placed in an extraction vessel. An aliquot of methanol (1.8 mL) was added and the mixture was mixed using a vortex mixer for 1 min. The vessel was transferred into a microwave chamber. The extraction was performed for 20 min at 80 °C with microwave power of 800 W. After extraction, the vessel was cooled to room temperature. The extraction mixture was remixed for 1 min and passed through a 0.22 µm nylon membrane into a 2 mL centrifuge tube for further use. The DLLME was conducted as follows: ultra-pure water (5 mL) was transferred into a 10 mL conical-bottomed glass centrifuge tube and spiked with the extraction solution (0.8 mL). A 5:2 v/v acetone/carbon tetrachloride mixture (280 µL) and NaCl (0.08 g mL⁻¹) were added in sequence. The mixture was extracted by vortex oscillation at 2000 rpm for 1–2 min. After centrifugation at 3000 rpm for 5 min, the settled phase was collected using a 100 µL Hamilton syringe and transferred into a 100 µL autosampler vial insert with polymer feet. A 1.0 µL aliquot was injected for GC-MS analysis.

2.4. Instrument conditions

An Agilent 7890A-GC system equipped with a 5975C-MS (Agilent Technologies, Palo, CA, USA) and an HP-5 ms capillary column (60 m × 0.25 mm, 0.25 µm) was used. An injector temperature of 280 °C, split injection, and a split ratio of 5:1 were used. The oven temperature was initially held at 60 °C for 2 min, raised at 15 °C min⁻¹ to 230 °C for 5 min, second rate 5 °C min⁻¹ to 280 °C for 20 min and then the post run was conducted for raised at 3 °C min⁻¹ to 290 °C for 5 min. The constant flow rate of helium carrier gas (99.999%) was maintained at 1 mL min⁻¹. The MS transfer line temperature was 280 °C. The ionization chamber and quadrupole temperatures were 230 °C and 150 °C, respectively. Electron impact mass spectrometric data in the range *m/z* 50–500 *amu* were collected, using a scan rate of 3.99 s⁻¹ with an

ionization voltage of 70 eV, and the solvent delay was 7 min. The quantitative and qualitative characteristics are shown in Table 1.

Organic additives were identified by comparison of the mass spectra and LRI with the authentic standards and published data, as well as by comparison with the Wiley08 and Nist14 MS libraries. The LRI of each additive compound was calculated using the retention time associated with the *n*-paraffin standard (C6–C40) using AMDIS software. The sixteen standard compounds were quantified by the internal standard method, and the other sixty-four identified compounds were semi-quantified by the internal standard method, using phenethyl acetate as a reference without considering the calibration factors, i.e. $F = 1.00$.

2.5. Statistical analysis

The response surface methodology of the DD was used to optimize the MAE extraction efficiency. The analysis of variance (ANOVA) of DD was conducted using Design-Expert version 8.0.6 software (Stat-Ease Inc., Minneapolis, MN, USA). The ANOVA for the response surface quadratic model of the relative response factors (RRF) was used to justify the adequacy of the models. The RRF values were calculated by dividing the peak area of the analyte by the peak area of the internal standard. The internal standard (5 μL with a concentration of 1.852 mg mL^{-1}) was added to the extracted aqueous solution (5 mL). To measure how well the proposed model fits the experimental data, parameters such as the model *p*-value, lack of fit, coefficient of variation, and R^2 were used. The model *p*-value and coefficient of variation (CV) were less than 0.05 and 10, respectively, and the lack of fit and R^2 were greater than 0.05 and 0.90, respectively, which represent a reliable model. DLLME extraction efficiencies can be indirectly calculated by $R \times V_{\text{org}}$, where *R* is the peak area and V_{org} is the volume of the sedimentary organic phase. Significant differences were assessed with one-way ANOVA for method optimization. Fisher's least square difference test (*p*-value < 0.05) was used to compare mean values using the SPSS® 16.0 software package (SPSS, Chicago, IL, USA). SIMCA-P software version 13.0 (Umeå, Sweden) and online tools (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) were used for principal component analysis (PCA) and Venn diagram analysis to discriminate the manufacturing origin based on the organic additive composition. Other diagrams were drawn using Origin 8.0 Software (Origin Lab Corp., USA).

2.6. Calculation of analytical parameters

To evaluate the matrix effects (ME), the slopes ratios of the calibration curves of the solvent and matrix solutions were compared using Eq. (1) below. Positive or negative values of the ME (%) were considered as matrix enhancement or suppression. The limits of detection (LOD) and limits of quantitation (LOQ) ($\mu\text{g g}^{-1}$) were calculated as 3 and 10 times the signal-to-noise ratio of the lowest solvent matched-calibration standard solution (taking DLLME into account), respectively, based on the sample weight, dilution ratio of the sample, and recoveries of the target analytes.

$$\text{ME}(\%) = \left(\frac{\text{Slope of matrix matched curve}}{\text{Slope of solvent matched curve}} - 1 \right) \times 100 \quad (1)$$

The accuracy was evaluated by the relative recovery (RR) that was determined by spiking analytes with approximately half of the initial concentration and the initial concentration. The RR (%) was calculated using equation: $\text{RR}\% = (C_{\text{spiked sample}} - C_{\text{sample}}) \times 100 / C_{\text{spiked}}$. $C_{\text{spiked sample}}$ and C_{sample} represent the amounts of target analytes in the spiked and unspiked samples, respectively. C_{spiked} is the amount of spiked standard analytes. The DLLME absolute recoveries (ARs) were calculated from the peak area ratios of the DLLME and standard solutions with the same volume. The enrichment factors (EFs) were calculated by $\text{AR} \times V_{\text{aq}} / V_{\text{org}}$, where V_{aq} and V_{org} are the aqueous and sedimentary organic volumes, respectively. The stability of organic additives were

tested by relative differences (RDs) between the analyte concentrations at the start ($t = 0$) and the end of the storage period ($t = 48$ h).

3. Results and discussion

3.1. Organic additive identification and analysis

Due to the high enrichment factor of DLLME and the high separation capacity of GC–MS, this approach tentatively identified or confirmed up to eighty compounds. This included 23 alkanes, 15 esters, 9 amides, 5 acids, 4 ketones or aldehydes, 4 UV absorbers, 4 nitriles, 4 phenols, 3 isocyanates, 3 olefins, 3 unknown compounds, 1 alcohol, 1 amine, and 1 polycyclic aromatic hydrocarbon (PAH). A typical GC–MS chromatogram is shown in Fig. 1. The three unknown compounds displayed similar fragmentation behaviors and the difference in molecular weight between each compound was $m/z = 28$ (ethyl group). It was speculated that the three unknown compounds were homologous structures.

Table 1 gives a detailed description of the identified additives in PBAT biodegradable mulch films. These additives are often added during the processes of injection molding, extrusion, blow molding, vacuum molding, etc. [27]. They impart several important functionalities to the PBAT polymer material, acting as lubricants, plasticizers, stabilizers, UV absorbers, antioxidants. For example, amides such as erucamide, octadecanamide, and hexadecanamide, etc. were detected. Erucamide and hexadecanamide, which are fatty acid derivatives, are the most common slip agents used in polyethylene and other polymers [28], while octadecanamide is used as a release agent. Octadecanamide is considered highly toxic (class III) according to the Cramer rules [6]. Phthalates are widely used to increase the flexibility, solubility, or stability of packaging materials, adhesives, cosmetics, pharmaceuticals, and toys, among others. These are believed to exhibit reproductive toxicity and other harmful effects on the human body [29]. In contrast to traditional plastic, only three types of phthalates were detected in the PBAT films, namely dibutyl phthalate, diisobutylphthalate, and bis(2-ethylhexyl) phthalate. Antioxidants are often used to reduce oxidative degradation from exposure to the air. Butylated hydroxytoluene is widely used as it is a powerful antioxidant [30]. The compound 2,4-di-*tert*-butylphenol (possibly a degradation product of Irgafos 168 and Irganox 1010) that could be a NIAS was also detected [31]. Bisphenol A is often used as plasticizer in plastic packaging and presents high endocrine-disrupting activities [32,33]. Some UV absorbers such as octabenzene, bumetizole, triphenyl phosphate, and *p*-cresyl diphenyl phosphate were also detected. There are generally used to slow down the oxidation process due to exposure to UV light. It is preferentially degraded and helps to stabilize the plastic [34].

3.2. Isocyanate conversion and analysis

Isocyanate and amine derivatives displayed poor calibration curves in the range 0.1–50 $\mu\text{g mL}^{-1}$ with coefficients of determination (R^2) less than 0.99. The recoveries were 64.5–121.3%, indicating relatively poor accuracy. This may be attributed to the high reactivity of isocyanates due to the cumulative double bonds and the strongly electronegative oxygen and nitrogen atoms on both sides of the carbon atom. In PBAT degradable mulch film samples, three isocyanates including 2,6-diisopropylphenyl isocyanate, *p*-methyl-2,6-diisopropylphenyl isocyanate, and hexamethylene diisocyanate were identified. Employing 2,6-diisopropylphenyl isocyanate in acetone as a standard solution, we studied its conversion by using MAE combined with DLLME experimental procedures, according to the following six protocols: (1) Direct GC–MS analysis for standard solutions; (2) Methanol-free DLLME for standard solutions and then analysis; (3) DLLME for standard solutions and then analysis; (4) MAE and DLLME for standard solutions and then analysis; (5) Extraction of the PBAT biodegradable mulch films with the aprotic solvent methyl *tert*-butyl ether and then analysis; (6) MAE with DLLME for the PBAT biodegradable mulch films and then analysis. As shown in

Table 1

The detailed description of the identified organic additives in PBAT biodegradable mulch films.

No	LRI/RLRI ^a	Organic additives	Function	ID ^b	RT ^c /min	Characteristic ions, m/z ^d	Match factor ^e
<i>Alkanes</i>							
1	1100/1100	Henecane	Lubricant	B	9.713	57, <u>71</u> , 85	90%
5	1400/1400	Tetradecane	Lubricant	B	13.120	57, <u>71</u> , 85	85%
10	1500/1500	Pentadecane	Lubricant	B	14.005	57, <u>71</u> , <u>85</u>	85%
18	1700/1700	Heptadecane	Lubricant	A	15.825	57, <u>71</u> , <u>85</u>	90%
19	1710/—	2,6,10,14-tetramethyl-pentadecane	Lubricant	B	15.901	57, <u>71</u> , 85	92%
21	1771/1771	3-methylheptadecane	Lubricant	B	16.589	57, <u>71</u> , 85	90%
24	1800/1800	Octadecane	Lubricant	B	16.901	57, <u>71</u> , <u>254</u>	90%
26	1810/1774	2,6,10,14-tetramethyl-hexadecane	Plasticizer	B	17.025	57, <u>71</u> , 43	91%
36	2000/2000	Eicosane	Lubricant	B	19.785	57, <u>71</u> , <u>282</u>	92%
41	2100/2100	Heneicosane	Lubricant	B	21.447	57, <u>71</u> , <u>85</u>	99%
45	2200/2200	Docosane	Lubricant	B	23.12	57, <u>71</u> , <u>310</u>	98%
47	2300/2300	Tricosane	Lubricant	B	24.816	57, <u>71</u> , <u>324</u>	95%
49	2362/—	2-methyl-tricosane	Lubricant	B	25.945	43, <u>57</u> , 71	98%
52	2400/2400	Tetracosane	Lubricant	B	26.601	57, <u>71</u> , 85	95%
56	2462/2462	2-methyltetracosane	Lubricant	B	27.666	57, <u>71</u> , 85	99%
57	2500/2500	Pentacosane	Lubricant	B	28.275	57, <u>71</u> , <u>352</u>	99%
61	2562/2562	2-methylpentacosane	Lubricant	B	29.353	57, <u>71</u> , <u>85</u>	97%
66	2600/2600	Hexacosane	Lubricant	B	30.012	57, <u>71</u> , <u>366</u>	97%
68	2661/2673	3-methylhexacosane	Lubricant	B	31.201	57, <u>71</u> , 85	91%
69	2703/2700	Heptacosane	Lubricant	B	31.931	57, <u>71</u> , <u>380</u>	98%
71	2800/—	Octacosane	Lubricant	B	34.217	57, <u>71</u> , <u>394</u>	94%
76	2900/2900	Nonacosane	Lubricant	B	36.861	57, <u>71</u> , <u>408</u>	96%
77	3000/3000	Triacotane	Lubricant	B	39.853	57, <u>71</u> , 85	97%
<i>Olefins</i>							
14	1590/1587	1-hexadecene	Lubricant	B	14.821	57, <u>71</u> , 85	90%
23	1793/1793	1-octadecene	Lubricant	B	16.829	57, <u>71</u> , <u>85</u>	92%
75	2835/2835	Squalene	Lubricant	A	35.148	69, 81, <u>410</u>	98%
<i>Nitriles</i>							
30	1906/—	Hexadecanenitrile	Lubricant	A	18.389	97, <u>110</u> , 69	95%
40	2088/—	Oleanitrile	Lubricant	B	21.325	69, <u>122</u> , 136	97%
42	2106/—	Octadecanenitrile	Lubricant	B	21.687	97, <u>110</u> , <u>124</u>	95%
58	2508/—	Docosenenitrile	Lubricant	B	28.392	69, <u>122</u> , <u>319</u>	95%
<i>Alcohols</i>							
17	1665/—	Cedrol	Lubricant	A	15.488	95, 150, <u>222</u>	93%
<i>Acids</i>							
20	1765/1768	Myristic acid	Adhesion promoter	B	16.434	73, 129, <u>228</u>	90%
33	1953/1929	Palmitic acid	Adhesion promoter	B	19.156	129, 213, <u>256</u>	98%
37	2013/—	3,5-di- <i>tert</i> -butyl-4-hydroxyphenylpropionic acid	Adhesion promoter	B	19.947	263, <u>278</u> , 55	90%
43	2156/—	Octadecanoic acid	Adhesion promoter	B	22.552	129, <u>284</u> , 241	90%
65	2590/—	<i>Cis</i> -11-eicosenoic acid	Adhesion promoter	B	29.863	59, 72, <u>309</u>	95%
<i>Amines</i>							
8	1457/—	2, 6-diisopropylaniline	—	A	13.606	162, <u>177</u> , 120	96%
<i>Amides</i>							
22	1776/—	Undecanamide	Slip agent	B	16.637	59, 55, <u>185</u>	90%
44	2182/2182	Hexadecanamide	Slip agent	B	23.007	59, 72, <u>255</u>	96%
50	2397/2397	Oleamide	Slip agent	B	26.202	59, 72, <u>281</u>	95%
51	2398/2398	Octadecanamide	Release agent	B	26.573	59, 72, <u>283</u>	95%
63	2587/—	<i>Cis</i> -11-eicosenamide	Slip agent	B	29.752	59, 72, <u>309</u>	95%
67	2605/—	Eicosanamide	Slip agent	A	30.123	59, 72, <u>311</u>	95%
72	2809/—	Erucamide	Slip agent	B	34.284	59, 72, <u>337</u>	95%
73	2816/—	Docosanamide	Slip agent	B	34.619	59, 72, <u>339</u>	95%
78	3001/—	<i>Cis</i> -15-tetracosenamide	Slip agent	B	40.138	59, 72, <u>365</u>	95%
<i>Keto and aldehydes</i>							
2	1108/1108	1-Nonanal	—	B	10.245	57, <u>98</u> , 114	91%
9	1480/1470	1,4-diacetylbenzene	—	B	13.825	147, <u>162</u> , 119	91%
25	1803/—	3,5-di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	—	A	16.963	219, <u>191</u> , <u>234</u>	95%
38	2018/—	Octadecanal	—	B	20.218	82, <u>96</u> , 109	91%
<i>Phenols</i>							
11	1525/1525	2,4-di- <i>tert</i> -butylphenol	Antioxidant	B	14.209	191, 57, <u>206</u>	97%
12	1533/1533	Butylated hydroxytoluene	Antioxidant	A	14.292	205, <u>220</u> , 177	94%
46	2238/—	Bisphenol A	Plasticizer	B	23.526	213, <u>228</u> , 119	96%
55	2460/—	2,2'-methylenebis(6- <i>tert</i> -butyl-4-methylphenol)	Antioxidant	B	27.390	177, 161, <u>340</u>	98%
<i>PAHs</i>							
28	1841/—	Phenanthrene	—	A	17.567	178, <u>176</u> , 89	87%
<i>Esters</i>							
3	1180/—	3,6-dimethyl-1,4-dioxane-2,5-dione	—	B	10.953	56, 100, <u>144</u>	80%
13	1585/—	1,6-dioxacyclododecane-7,12-dione	—	A	14.793	84, <u>100</u> , 129	90%
15	1601/1591	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	Plasticizer	A	14.924	71, 111, <u>243</u>	85%

(continued on next page)

Table 1 (continued)

No	LRI/RLRI ^a	Organic additives	Function	ID ^b	RT ^c /min	Characteristic ions, <i>m/z</i> ^d	Match factor ^e
16	1649/—	2,6-diisopropylphenylcarbamic acid methyl ester	Plasticizer	B	15.33	176, <u>235</u> , 160	91%
27	1823/1823	Isopropyl myristate	Mold release agent	B	17.223	102, <u>228</u> , <u>270</u>	95%
29	1888/—	Phthalic acid, isobutyl octyl ester	Plasticizer	B	18.061	149, <u>223</u> , 167	90%
31	1925/—	Methyl palmitate	Mold release agent	B	18.644	74, 87, <u>270</u>	99%
32	1929/—	7,9-di- <i>tert</i> -butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	—	B	18.988	205, <u>217</u> , 276	99%
34	1962/1943	Methyl 3-(3,5-di- <i>tert</i> -butyl-4-hydroxyphenyl) propionate	Plasticizer	B	19.221	277, <u>292</u> , 147	94%
35	1967/1967	Dibutyl phthalate	Plasticizer	A	19.491	149, <u>223</u> , 278	90%
39	2086/—	Adipic acid, butyl 2-ethylhexyl ester	Plasticizer	B	21.258	129, <u>185</u> , 111	97%
48	2340/—	Octyl 4-methoxycinnamate	Plasticizer	A	25.624	178, 161, <u>290</u>	98%
53	2407/2398	Bis(2-ethylhexyl) adipate	Plasticizer	A	26.722	129, 57, <u>112</u>	90%
59	2519/2519	2-monopalmitin	Mold release agent	B	28.601	98, <u>239</u> , 134	95%
62	2564/2550	Bis(2-ethylhexyl) phthalate	Plasticizer	B	29.382	149, <u>167</u> , 279	81%
<i>Isocyanates</i>							
4	1379/—	Hexamethylene diisocyanate	Chain extender	B	12.911	56, <u>85</u> , 99	92%
6	1430/—	2,6-diisopropylphenyl isocyanate	Chain extender	A	13.344	188, <u>233</u> , 146	99%
7	1433/—	<i>P</i> -methyl- 2,6-diisopropylphenyl isocyanate	Chain extender	B	13.448	202, <u>188</u> , <u>217</u>	90%
<i>UV absorbers</i>							
54	2436/—	Triphenyl phosphate	UV absorber	B	27.241	326, <u>77</u> , 170	90%
60	2521/—	<i>P</i> -cresyl diphenyl phosphate	UV absorber	B	28.65	340, <u>77</u> , 263	99%
64	2588/—	Bumetrizole	UV absorber	B	29.767	<u>300</u> , 315, 272	98%
70	2796/—	Octabenzene	UV absorber	A	34.052	213, <u>326</u> , 137	98%
<i>Unknown compounds</i>							
74	2819/—	Unknown 1	—	C	34.795	204, 177, 162, 344, <u>387</u>	—
79	3038/—	Unknown 2	—	C	41.147	204, 177, 162, 372, <u>415</u>	—
80	3324/—	Unknown 3	—	C	50.361	204, 162, 400, <u>443</u>	—

^a LRI: linear retention index on HP-5 ms; RLRI: reference linear retention index according to NIST database on HP-5 or HP-5 ms (<http://webbook.nist.gov/chemistry/>).

^b Method of identification, where A: authentic compounds; B: tentative identification from RI and Wiley08 and Nist14 MS libraries; C: Unidentified compounds.

^c Retention time.

^d Quantitative ion for the first ion, qualitative ion for underlined ion.

^e Comparison with the Wiley08 and Nist14 MS libraries.

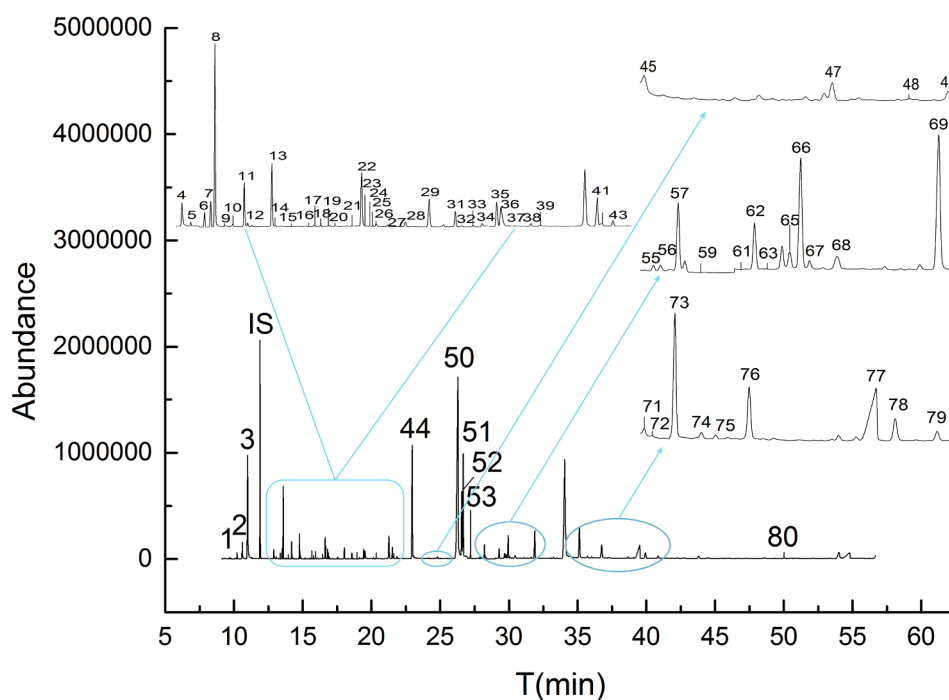


Fig. 1. GC-MS selected ion scan chromatogram of PBAT biodegradable mulch films (PBAT-A). The compound numbers and characteristic ions are shown in Table 1.

Fig. 2A, protocols (2) and (3) resulted in the new peaks 1 (amine) and 2 (amine and methyl carbamate), respectively. This was attributed to the presence of protic solvent of water and methanol which can react with isocyanates. Protocol (4) resulted in higher peak areas for the methyl carbamate, indicating that the MAEs conducted at high temperature and pressure speed up reaction with methanol. Since methyl *tert*-butyl ether

does not react with isocyanate, 2,6-diisopropylphenyl isocyanates were the main compounds when using protocol (5), followed by 2,6-diisopropylaniline. This was attributed to moisture in the air which would react with 2,6-diisopropylphenyl isocyanate to produce 2,6-diisopropylaniline. Since PBAT biodegradable mulch films inevitably come into contact with the protic solvent water during use, it can be rationalized that

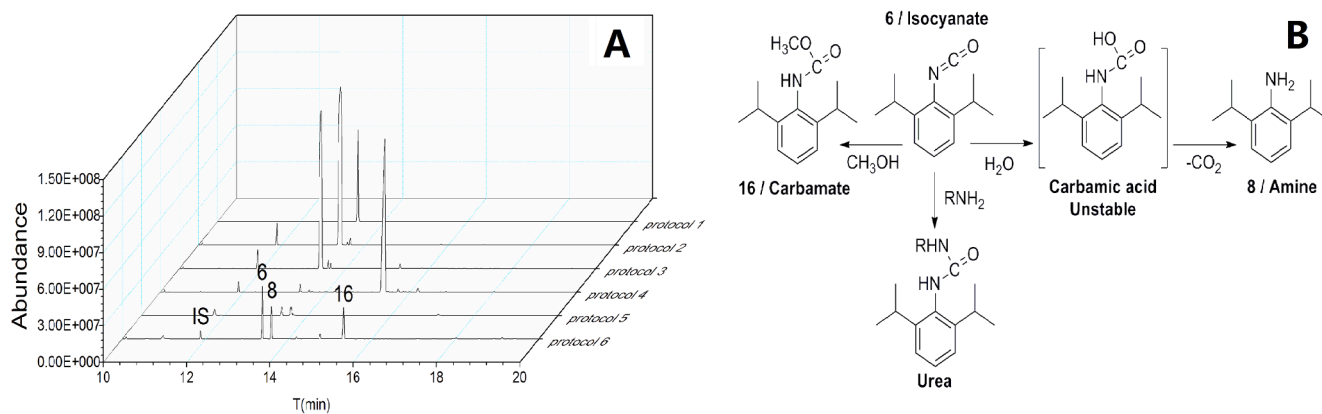


Fig. 2. (A) GC–MS chromatogram of 2, 6-diisopropylphenyl isocyanate using MAE combined with DLLME and then GC–MS analysis experimental processes in 6 protocols: (1) Standard solutions; (2) Methanol-free DLLME for standard solutions; (3) DLLME for standard solutions; (4) MAE and DLLME for standard solutions; (5) Extraction of the PBAT biodegradable mulch films with the aprotic solvent methyl *tert*-butyl ether; (6) MAE with DLLME for the PBAT biodegradable mulch film; (B) Possible conversion process of 2, 6-diisopropylphenyl isocyanate reaction with water, methanol, and amine.

2,6-diisopropylaniline is the most important reaction product. Protocol (6) gave three peaks, attributed to isocyanate, amine, and methyl carbamate, further proving that methanol and isocyanate can react by MAE. A scheme of 2,6-diisopropylphenyl isocyanate reaction with water, methanol, and amine compounds is given in Fig. 2B. Urea was not detected in this experiment, because the amine concentration was relatively very low compared to methanol or water. Based on the above experimental results, it was deduced that isocyanates may be IAS, while amines may be NIAS. Moreover, MAE combined with DLLME was deemed unsuitable for analyzing isocyanates and amines. Considering that isocyanates were mainly converted to amine and methyl carbamate, the total concentration of the three types is evaluated in this study.

3.3. Optimization of the MAE

3.3.1. Optimization of extraction solvent

It was deemed necessary that the extraction solvent should favor the subsequent DLLME procedure. Therefore, five water-soluble organic

solvents including methanol, ethanol, acetonitrile, acetone, and isopropanol were selected. The results are shown in Fig. S1. Methanol provided the highest extraction efficiency, followed by ethanol, and isopropanol. Acetonitrile and acetone extracted large quantities of low molecular weight polymers, which could be enriched with carbon tetrachloride. These polymers have high boiling points and stabilities, which can contaminate the GC–MS inlet severely, resulting in the appearance of ghost peaks and erroneous quantification. Although alcohol will cause side reactions of isocyanate, very little polymers were extracted. Considering the comprehensive effect, methanol was selected in the subsequent studies.

3.3.2. Optimization with response surface methodology

Previous studies reported that three variables including the liquid–material ratio (A), extraction time (B), and temperature (C) can affect the MAE extraction efficiency [35]. Based on the preliminary studies and experiments, the values and levels were selected based on the DD protocol. When applied to the three variables, seventeen experiments were

Table 2

DD with coded variables, real variables, RRF, and model coefficient for ANOVA.

No	Coded variables			Real variables			RRF ^a
	A	B	C	A/mL g ⁻¹	B/min	C/°C	
1	0.000	0.000	0.000	18.50	20.00	65.00	16.7472
2	0.000	1.000	0.000	18.50	30.00	65.00	13.2986
3	0.866	0.500	0.000	24.13	25.00	65.00	14.6098
4	0.289	0.500	0.817	20.38	25.00	85.43	16.9195
5	0.000	-1.000	0.000	18.50	10.00	65.00	15.5565
6	-0.866	-0.500	0.000	12.87	15.00	65.00	16.5092
7	-0.289	-0.500	-0.817	16.62	15.00	44.57	14.7761
8	-0.866	0.500	0.000	12.87	25.00	65.00	14.7984
9	-0.289	0.500	-0.817	16.62	25.00	44.57	12.5879
10	0.866	-0.500	0.000	24.13	15.00	65.00	15.1919
11	0.577	0.000	-0.817	22.25	20.00	44.57	13.0729
12	0.289	-0.500	0.817	20.38	15.00	85.43	15.9596
13	-0.577	0.000	0.817	14.75	20.00	85.43	16.8748
14	0.000	0.000	0.000	18.50	20.00	65.00	17.1068
15	0.000	0.000	0.000	18.50	20.00	65.00	16.4172
16	0.000	0.000	0.000	18.50	20.00	65.00	17.0234
17	0.000	0.000	0.000	18.50	30.00	65.00	16.9943
ANOVA	A <i>p</i> -value		0.0109	Model <i>p</i> -value			<0.0001
	B <i>p</i> -value		0.0002	Lack of fit			0.4955
	C <i>p</i> -value		< 0.0001	Coefficient of variation			1.78
	BC <i>p</i> -value		0.0021	R ²			0.9849
	A ² <i>p</i> -value		0.0008	Adj. R ²			0.9655
	B ² <i>p</i> -value		< 0.0001	Pred. R ²			0.8857
	C ² <i>p</i> -value		< 0.0001	Adeq. precision			21.149

^a Relative response factors.

performed in random order. The experimental design expressed in terms of the coded variables, real variables and levels, and the RRF is displayed in Table 2. To evaluate how well the proposed model fits the experimental results, the DD was evaluated at a 5% level of significance and validated using ANOVA. Table 2 shows that the model was highly significant, with a p -value of 0.0001; there was only a 0.01% chance that this value could occur due to noise. A lack of fit p -value of 0.4955 indicated that the lack of fit was not significantly associated with the pure error. Furthermore, R^2 and adjusted- R^2 were greater than 0.90 with values of 0.9849 and 0.9655, respectively. A higher R^2 value indicated that the data satisfactorily fit the model. Since the CV is the standard deviation as a percentage of the mean, smaller values give better reproducibility; thus, each CV less than 10 indicated that the model was reproducible. All of these statistical parameters demonstrated the reliability of this model.

To predict the optimum value of RRF, the variables were set according to our specific requirements. The goals for the variables A, B, C were set “in investigated range”, i.e. the three variables varied from the -1 to $+1$ level. The extraction efficiency was set to “maximize”. Then, the solutions were obtained using the Design-Expert version 8.0.6 software. The result showed that the highest RRF value was 17.37 and the optimum conditions in terms of the liquid–material ratio was 17.87 mL g^{-1} , the extraction time was 19.52 min, and the extraction temperature was 77.24 °C. For simplicity of operation, the values were rounded off to 18 mL g^{-1} , 20.0 min, and 80.0 °C. Using these optimal conditions, three experiments were performed to validate this result. The experimental value of RRF was 17.05 ± 0.54 , which was very similar to the theoretical value. This result showed that the model was adequate for reflecting the expected optimization.

3.4. Optimization of the DLLME

3.4.1. Selection of disperser solvent and extraction solvent

Five solvents including methanol, ethanol, acetonitrile, acetone, and isopropanol were selected as possible disperser solvents and the results are shown in Fig. 3. Acetone had higher extraction efficiencies than other solvents. The extraction solvent of chloroform, dichloromethane, carbon tetrachloride, tetrachloroethylene, tetrachloroethane, 1,2-dichloroethylene, and chlorobenzene were investigated. Since PBAT biodegradable mulch films were highly soluble in chloroform, dichloromethane, tetrachloroethane, and 1,2-dichloroethylene, a large amount of low molecular weight polymers were extracted in the sedimentary organic phase during the DLLME and then only carbon tetrachloride, tetrachloroethylene, and chlorobenzene were selected for further optimization. According to Fig. 3, carbon tetrachloride displayed the highest extraction efficiency, which is primarily due to the non-polarity of the organic additives.

3.4.2. Effect of the disperser solvent and extraction solvent volumes

To investigate the effect of the disperser solvent volume, various volumes of acetone (100–500 μ L) containing carbon tetrachloride were tested. According to Fig. 3, the extraction efficiency increased with increasing acetone volume in the range of 100–200 μ L and decreased with increasing acetone volume in the range of 200–500 μ L. Therefore, 200 μ L of acetone was deemed suitable for subsequent studies. At low volumes of acetone, a cloudy state could not be satisfactorily formed, so the extraction efficiency of the analytes was low. At high volumes of acetone, the solubility of carbon tetrachloride in aqueous solution increased, leading to a lower extraction efficiency. Various extraction solvent volumes of carbon tetrachloride (40–120 μ L) were also studied. According to Fig. 3, the extraction efficiency increased markedly in the range of 40–80 μ L carbon tetrachloride. When the extraction volume were over 80 μ L, no distinct changes were observed in the extraction

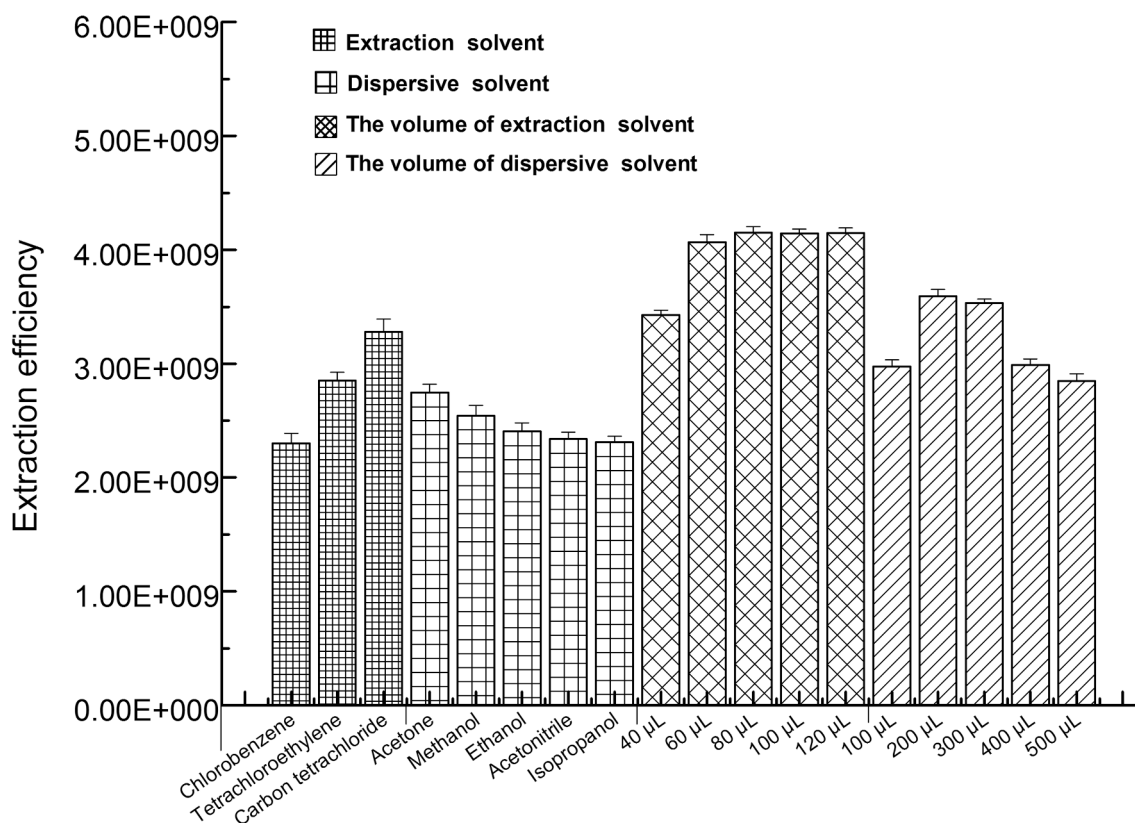


Fig. 3. Optimization of the type and volume of extraction and dispersive solvent in DLLME with one-factor-at-a-time. The error bars indicate standard deviations of three repeated determinations.

efficiency. Therefore, 80 μL carbon tetrachloride was chosen as the optimum volume.

3.4.3. Effect of salt addition

Increasing the ionic strength is known to reduce the solubility of organic compounds in water and has been extensively utilized to increase the extraction efficiencies. On the other hand, it can increase the volume of the sedimentary phase by decreasing the solubility of the organic solvent in the aqueous phase, leading to a decrease in the enrichment factors [36]. To this end, the effect of the ionic strength was studied by adding different amounts of NaCl (0–0.2 g mL^{-1}). Fig. S2 indicated that the extraction efficiency increased with NaCl in the range 0–0.08 g mL^{-1} and slightly decreased in the range 0.08–0.2 g mL^{-1} , and the maximum extraction efficiency was obtained at 0.08 g mL^{-1} NaCl.

3.5. Method validation

The proposed method was evaluated in terms of linearity, matrix effect, sensitivity (LODs and LOQs), accuracy (RR), precision (repeatability and reproducibility) and stability (RDs) according to method performance validation guideline [37]. A total of sixteen standard compounds including each organic additives classes were used for validation. The mixed standard solutions were prepared in duplicate at six concentrations using the solvent (methanol) and blank matrix extract solution. The blank matrix of PBAT films was performed by re-precipitation in methanol as described previously [38]. The solvent and matrix matched-calibration curves were constructed using weighted (1/X) least-squares linear regression models. Linearity was evaluated

using regression analysis and was expressed by R^2 and lack of fit test. Table 3 reveals that satisfactory linearity was obtained, with R^2 and statistical p values greater than 0.9990 and 0.05, respectively. ME (%) of the analytes ranged from –4.39 to 7.09% showing slight matrix enhancement or suppression. These results indicated that the solvent matched-calibration curve could be used to quantify real samples with errors in their quantification below 10%. Table 4 showed that LODs and LOQs ranged from 0.0008 to 0.0586 $\mu\text{g g}^{-1}$ and 0.003–0.195 $\mu\text{g g}^{-1}$, respectively. The RR was in the range of 93.0–109.8 %, which indicated that the proposed method exhibited high extraction efficiency. The repeatability and reproducibility were assessed by conducting six independent analysis between one and six days. The repeatability and reproducibility varied from 2.06 to 8.76% and 2.38–10.23%. The AR and EFs were 82.3–93.5% and 105–120, respectively. The stability of extracts was very good over the 48 h period, with RDs less than 4.35%. The results indicated that the proposed method was accurate, precise, and stable enough to permit highly sensitive analysis of organic additives.

3.6. Real samples analysis

The organic additives have different functional effects, therefore, the PBAT biodegradable mulch films with different manufactures may display specific characteristic profiling. We investigated the type and concentration change of organic additives in PBAT biodegradable mulch films from four manufacturing origins using the proposed method. The type and concentration change of organic additives were analyzed using the Venn diagram and biplot graph in Fig. 4. The Venn diagram showed

Table 3
The linearity of solvent-matched and matrix-matched standard curve and ME evaluation.

Compounds	LR ^a / $\mu\text{g mL}^{-1}$	Solvent-matched standard curve			Matrix-matched standard curve			ME ^b / %
		Regression equation ^c	R^2 ^d	P values of lack-of-fit	Regression equation	R^2	P values of lack-of-fit	
2,6-diisopropylphenyl isocyanate	0.1–50	$y = 0.2754x - 0.0149$	0.9872	0.0231	$y = 0.2443x - 0.0080$	0.9794	0.0311	—
2,6-diisopropylaniline	0.1–50	$y = 0.7738x + 0.0720$	0.9795	0.0122	$y = 0.8169x + 0.0429$	0.9654	0.0236	—
Butylated hydroxytoluene	0.025–10	$y = 1.0375x - 0.0041$	0.9994	0.312	$y = 1.0241x - 0.0036$	0.9996	0.526	–1.29
1,6-dioxacyclododecane-7,12-dione	0.05–20	$y = 0.4041x - 0.0042$	0.9990	0.089	$y = 0.4039x - 0.0052$	0.9991	0.215	–0.05
2,2,4-trimethyl-1,3-pentanediol diisobutyrate	0.01–5	$y = 1.322x + 0.0017$	0.9991	0.366	$y = 1.3475x + 0.0002$	0.9996	0.435	1.92
Cedrol	0.01–5	$y = 0.2965x + 0.0004$	0.9994	0.587	$y = 0.3114x - 0.0009$	0.9999	0.511	5.03
Heptadecane	0.02–10	$y = 0.726x - 0.0011$	0.9997	0.075	$y = 0.7775x + 0.0008$	0.9990	0.092	7.09
3,5-di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	0.02–10	$y = 0.6704x + 0.0030$	0.9990	0.317	$y = 0.6512x - 0.0038$	0.9993	0.623	–2.86
Phenanthrene	0.02–10	$y = 1.138x + 0.0015$	0.9994	0.253	$y = 1.133x - 0.0061$	0.9992	0.361	–4.39
Hexadecanenitrile	0.1–40	$y = 0.1881x + 0.0002$	0.9992	0.526	$y = 0.1813x - 0.0019$	0.9991	0.405	–3.62
Dibutyl phthalate	0.02–10	$y = 1.638x + 0.0131$	0.9991	0.343	$y = 1.6297x + 0.0025$	0.9996	0.351	–0.51
Octyl 4-methoxycinnamate	0.04–20	$y = 0.8658x - 0.0049$	0.9994	0.086	$y = 0.8659x - 0.0102$	0.9995	0.344	0.12
Bis(2-ethylhexyl) adipate	0.1–50	$y = 0.5717x - 0.0045$	0.9993	0.329	$y = 0.5735x - 0.0228$	0.9991	0.310	3.15
Eicosanamide	0.1–50	$y = 0.3047x - 0.0043$	0.9993	0.446	$y = 0.3033x - 0.0058$	0.9993	0.366	–0.46
Octabenzene	0.02–10	$y = 0.3687x - 0.0030$	0.9991	0.310	$y = 0.3675x - 0.003$	0.9996	0.272	–0.33
Squalene	0.02–10	$y = 0.7252x - 0.0109$	0.9991	0.354	$y = 0.7272x - 0.0017$	0.9994	0.361	0.27

^a Linear range.

^b Matrix effect.

^c y : peak area ratio of the standard compound to internal standard; x : mass ratio of the standard compound to internal standard (9.26 μg).

^d Coefficient of determination.

Table 4
Recovery, precision, ARs, EFs, LODs, LOQs, and stability studies.

Compounds	Concentration ($\mu\text{g g}^{-1}$)			RRs ^a %/n = 6	Precision		ARs ^b %/n = 6	EFs ^c	LODs ^d / $\mu\text{g g}^{-1}$	LOQs ^e / $\mu\text{g g}^{-1}$	RDs ^f / %
	Content	Added	Found		Repeatability %/n = 6	Reproducibility %/n = 6					
2,6-diisopropylphenyl isocyanate	1398.70	700	1862.1	66.2	11.15	17.89	75.2	83	0.0202	0.067	6.69
		1400	2301.7	64.5	10.01	15.56					
2,6-diisopropylaniline	1047.86	500	1640.4	118.5	13.09	19.76	98.4	136	0.0018	0.006	7.37
		1000	2260.9	121.3	11.97	17.31					
Butylated hydroxytoluene	3.45	1.50	5.07	108.0	7.39	8.69	84.2	108	0.0008	0.003	2.51
		3.00	6.56	103.7	6.33	7.24					
1,6-dioxacyclododecane-7,12-dione	97.33	48.00	147.92	105.4	3.50	4.85	86.1	110	0.0058	0.019	2.21
		96.00	193.91	100.6	2.87	3.62					
2,2,4-trimethyl-1,3-pentanediol diisobutyrate	0.42	0.20	0.63	105.0	6.37	8.62	86.6	111	0.0032	0.011	3.17
		0.40	0.82	100.0	5.20	7.31					
Cedrol	0.97	0.50	1.47	100.0	7.09	8.39	83.4	107	0.0124	0.041	4.04
		1.00	1.90	93.0	6.36	7.16					
Heptadecane	1.5	0.70	2.26	108.6	5.79	6.69	82.7	106	0.0586	0.195	2.08
		1.40	2.88	98.6	4.37	5.45					
3,5-di- <i>tert</i> -butyl-4-hydroxybenzaldehyde	1.38	0.60	2.00	103.3	3.60	3.91	93.5	120	0.0039	0.013	2.75
		1.20	2.57	99.2	2.11	2.38					
Phenanthrene	1.32	0.60	1.95	105.0	8.02	8.54	92.1	118	0.0018	0.006	1.18
		1.20	2.55	102.5	8.76	10.23					
Hexadecanenitrile	3.52	1.80	5.44	106.7	7.69	7.31	91.3	117	0.0015	0.005	2.04
		3.60	6.96	95.6	6.89	6.52					
Dibutyl phthalate	1.82	0.90	2.78	106.7	7.03	8.37	82.3	105	0.0177	0.126	4.35
		1.80	3.58	97.8	6.11	8.66					
Octyl 4-methoxycinnamate	0	0.60	0.63	105.0	4.06	4.17	85.8	110	0.0011	0.004	3.02
		1.20	1.21	100.8	2.78	3.12					
Bis(2-ethylhexyl) adipate	2.95	1.50	4.56	107.3	2.89	4.17	86.4	111	0.0079	0.026	1.31
		3.00	6.03	102.7	2.09	3.91					
Eicosanamide	142.49	70.00	216.62	105.9	3.69	5.09	85.2	109	0.0393	0.131	2.25
		140.00	277.97	96.8	2.16	3.63					
Octabenzene	2.48	1.20	3.75	105.8	3.01	3.69	88.7	114	0.0193	0.064	2.64
		2.40	5.01	105.4	2.06	2.75					
Squalene	11.22	5.00	16.71	109.8	6.29	8.47	87.4	112	0.0149	0.050	1.66
		10.00	21.45	102.3	5.63	6.34					

^a Relative recoveries.

^b Absolute recoveries.

^c Enrichment factors.

^d Limits of detection ($S/N = 3$).

^e Limits of quantitation ($S/N = 10$).

^f Relative differences.

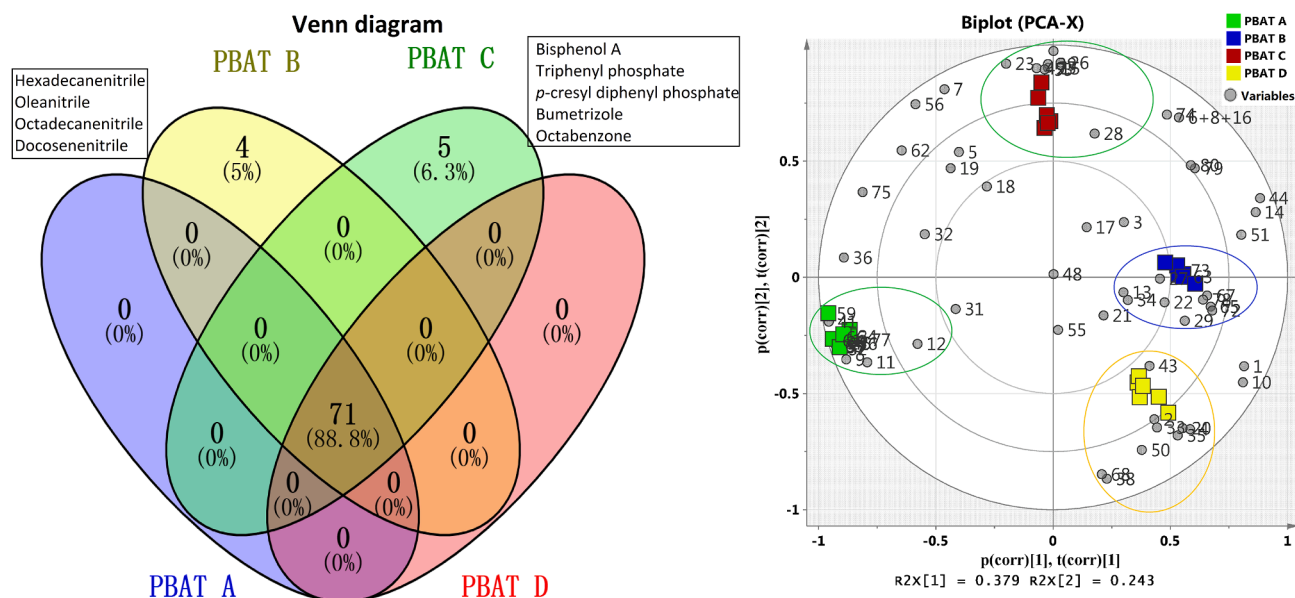


Fig. 4. Venn diagram and PCA biplot analysis of the type and concentration change of organic additives in PBAT biodegradable mulch films from four manufacturing origins. Venn diagram showed that PBAT B and C had four and five kinds of unique organic additives with displaying compound name. The PCA biplot shows PC1 against PC2, and the locations of the common organic additive variables (grey circle) and the four manufacturing origins (color square) (Print in color).

that PBAT B and C had four and five kinds of unique organic additives, respectively. The PBAT B had unique nitrile additives, mainly including hexadecanenitrile, olefinitrile, octadecanenitrile, and docosenitrile with concentrations of 1.68, 3.15, 5.26, and 13.3 $\mu\text{g g}^{-1}$, respectively. The PBAT C had unique UV absorbers and an plasticizer, mainly including triphenyl phosphate, *p*-cresyl diphenyl phosphate, bumetrizole, octabenzene, and bisphenol A with concentrations of 451.9, 2.18, 1506.9, 8833.3, and 39.8 $\mu\text{g g}^{-1}$, respectively. Unique organic additives were not found in PBAT A and D. Among the four PBAT biodegradable mulch films, seventy-one kinds of common organic additives were found.

PCA was used to cluster and display the relationships/correlations of common organic additives with manufacturing origins. The PCA biplot is a two-dimensional plot that shows PC1 against PC2, and the locations of the common organic additive variables and the four manufacturing origins. The first two principal components (PCs) can explain 62.2% of the total variance, and the PC1 and PC2 represented 37.9% and 24.3% of the variance, respectively. Fig. 4 shows that four manufacturing origins could be differentiated by seventy-one kinds of common organic additives. PBAT A, C, and B + D were well separated and located on the negative, middle, and positive sides of PC1, respectively, while PBAT B and D were separated and located on the middle and negative sides of PC2. The PBAT A and C samples displayed a strong, positive association with organic additives (NO. 9, 11, 12, 24, 41, 45, 47, 52, 57, 59, 61, 66, 69, 71, 76, 77) and (NO. 15, 23, 25, 26, 28, 39, 49, 53), which mainly belonged to alkanes and ester classes, respectively. In particular, a very high concentration (18635.1 $\mu\text{g g}^{-1}$) of the plasticizer bis(2-ethylhexyl) adipate was found in PBAT C. The PBAT B and D samples displayed strong, positive associations with organic additives (NO. 13, 22, 27, 29, 34, 63, 65, 67, 72, 73, 78) and (NO. 2, 4, 20, 33, 35, 43, 50), which mainly belonged to the amide and acid classes, respectively. These results demonstrated that PBAT biodegradable mulch films from different manufacturing origins have obvious characteristic differences in organic additive types and concentrations. Previous studies report that the additives released in biodegradable materials may play a vital role in ecotoxicology for plant growth and development, and soil microbial community abundance and function, thus, toxicity evaluation must be considered the effects of additives [3,39]. Although the proposed method can simultaneously analyze eighty organic additives, future work will involve further improvement and optimization of this method for the identification of more organic additives.

3.7. Comparison of the validated method with others

The proposed method was compared with other methods in terms of features such as extraction, enrichment, LODs, LR, ME, etc in Table S1 [6,16,40,41]. The experimental procedures of this method is simple, fast and less solvent used. The repeatability and reproducibility is good, and RSDs% are lower than or comparable with those of the mentioned methods. The proposed method provides wider LRs and lower LODs except high sensitive detection system of MS/MS or time of flight mass spectrometry (TOF-MS). Moreover, lower matrix effect is obtained with less interference of co-extracts. All these results reveal that the proposed method is a sensitive, rapid, and stable technique that can be used for analysis of organic additives.

4. Conclusions

In this study, a combined method based on MAE and DLLME with GC-MS has been reported for extraction, enrichment, purification and determination of eighty organic additives from PBAT biodegradable mulch films. Through careful optimization of MAE and DLLME conditions, the results reveal that the developed method exhibit high selectivity, lower LODs, broad linear ranges, adequate accuracy, high precision, and no obvious matrix effects, except for isocyanates and their reaction products. The newly developed method facilitate the

identification and quantitation of a broad range of known and unknown organic additives in PBAT biodegradable mulch films, which may contribute to better monitoring of their environmental impact.

CRedit authorship contribution statement

Huan Cui: Conceptualization, Methodology, Data curation, Writing - original draft. **Weichang Gao:** Data curation, Resources. **Yechun Lin:** Data curation, Resources. **Jie Zhang:** Writing - review & editing. **Runsheng Yin:** Writing - review & editing. **Zhangmin Xiang:** Visualization, Investigation. **Song Zhang:** Visualization, Investigation. **Shuping Zhou:** Software, Validation. **Wensheng Chen:** Writing - original draft, Supervision, Conceptualization, Project administration. **Kai Cai:** Writing - original draft, Supervision, Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2020.105722>.

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