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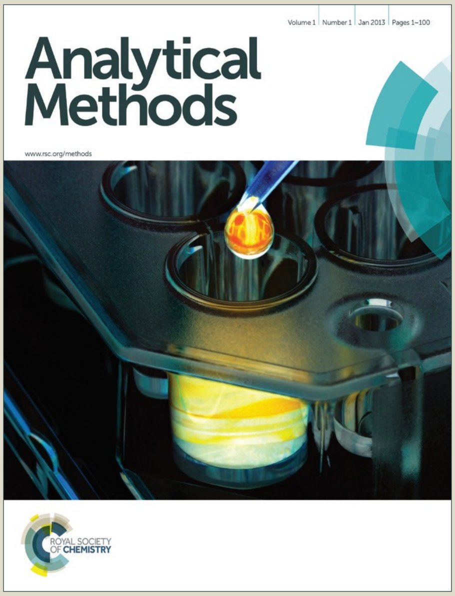

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Development of a Rapid Detection Method for eight Organophosphate Esters in Plastic samples as automobile Interiors using Ultra-Performance Liquid Chromatography tandem mass spectrometry with Microwave Assisted Extraction

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Abbreviations: OPEs, organophosphate eaters; **TEP**, Triethyl phosphate; **TCEP**, Tris(2-chloroethyl) phosphate; **TPrP**, Tripropyl phosphate; **TCPP**, Tris(2-chloroisopropyl) phosphate; **TPhP**, Trisphenyl phosphate; **TBEP**, Tris(2-butoxyethyl) phosphate; **TCP**, Tricresyl phosphate; **DPOP**, Diphenylisooctyl phosphate; **RSM**, Response Surface Methodology;

Keyword: automobile interiors, Microwave Assisted Extraction, organophosphate eaters (OPEs), Ultra-Performance Liquid Chromatography

Abstract

A rapid, effective and eco-friendly method for determination of eight organophosphate esters in plastic samples as car interiors by microwave assisted extraction followed ultra-high performance liquid chromatography combined with tandem mass spectrometry was established. Acetone/ethyl acetate (3/1, v/v) was used as extractant. Response surface methodology was applied as the experimental design strategy to screen and optimize the extracting variables, which affected the extraction efficiency, such as the volume of the extraction solvent, time of extraction, and temperature of extraction. Baseline separation of eight OPEs was achieved in 4.5 minutes. The method showed excellent linearity 1-250 μg/L for TBEP, TCP, and DPOP; 2-500 μg/L for TPhP; 5-1250 μg/L for TEP; 10-2500 μg/L for TPrP and TCPP; 15-3750 μg/L for TCEP (R>0.9907). For eight OPEs, the LODs ranged between 0.25μg/L and 5 μg/L and LOQs ranged between 0.5μg/L and 15 μg/L.

Introduction

Organophosphate esters (OPEs) used as flame retardants and plasticizers were applied in many industrial products, such as electronics, building materials, automotive trim, furniture, and textiles to delay ignition and slow the spread of fire [1]. These OPEs are potential replacements for the Penta-BDE commercial formulations that have been taken off the market [1]. In 2005, the global amount of OPEs used as flame retardants was 270000 tons. In Europe, OPEs used as flame retardants were about 84,000 metric tons in 2005, and an increase was about 7% from 2005 to 2006. Additionally, in the United States Production output for TDCPP, TPP, and TCPP increased from 500−5,000 metric tons in 1990 to 5,000−25,000 metric tons in 2006 for each of these Chemicals. OPEs could easily release to the environment because they were not chemically bonded to the matrix. And their bioaccumulation, toxicity, and long-range atmospheric transport arouse more and more concerns [2]. It was reported that Trichloropropyl phosphate (TCPP) exhibits potential carcinogenicity. Trichloroethyl phosphate (TCEP) is carcinogenic. Triphenyl phosphate (TPhP) is neurotoxins and inhibits hormone levels. In addition, chlorinated phosphate esters such as TCEP and TCPP are hard to be degraded naturally [3-6].

As living standard improved, more and more people own their private cars, so more plastic materials containing OPEs were used as automotive trim. Concerning the safety of health, it is urgent to develop a fast green method for the detection of OPEs to control the qualities of the plastic materials. Recently, there were many articles reported about the concerns of OPEs. For instance, Zheng et al developed a method to detect 8 OPEs in sediments [7]. Gao et al established a method to quantify 14 OPEs in water samples [8]. Fan et al built a simultaneous determination method to detect 13 OPEs in indoor house dust [9]. MahibaShoeib et al determined the concentrations in air of OPEs in Toronto, Canada [10]. To our knowledge, there were few articles reported about the determination of OPEs in plastic samples.

In this study, microwave assisted extraction (MAE) technique was applied to extract the target molecules from the plastic materials because MAE has characteristics of saving solvent amount and energy consumption. In order to increase the recoveries of the OPEs, response surface methodology (RSM) as an effective method was used to design experiments, determine the interaction effects of different parameters and at last obtain the optimum conditions of MAE.

A straightforward method based on MAE and UHPLC-MS/MS was developed for the quantification of 8 OPEs in plastic materials. 8 OPEs were separated and detected within 4.5 minutes (Fig 1C). In addition, MAE extraction efficiencies have been optimized by response surface method. The method was rapid and sensitive. The whole method also has been applied to real sample analysis. Furthermore, this method could be potentially extended to investigate the pollution by OPEs in other fields.

Experimental

Reagents and chemicals

All OPEs standards were purchased from Dr. Ehrenstorfer GmbH (Germany). Formic acid was bought from Dikma (Lake Forest, US). Acetonitrile (ACN, Optima grade) and methanol (MeOH, Optima grade) were purchased from Fisher Scientific. Ultra-pure water (18.3 MΩ) produced with a Milli-Q Gradient system (Millipore, Bedford, USA) was used throughout. Microwave

Accelerated Reaction System (CEM, North Carolina, USA) was used for extraction. Acetone, ethyl acetate and chloroform were purchased from Beijing Chemical Plant (Beijing, China).

Individual OPE stock solutions (10 mg/mL) were prepared by dissolving the OPEs in ACN. All of the stock solutions were kept in a 4 ℃ refrigerator.

Sample preparation

The simulated polypropylene (PP) sample was prepared by mixing each 8 OPEs including TCP, TCEP, DPOP, TBEP, TPhP, TPrP, TCPP, and TEP 1.00 g with 200 g blank PP power for 30 min, next processing with a plastic extruding machine at 190 ℃, then, pulverizing the PP sample to particles as a size of 2 mm with a universal high-speed smashing machine.

The real samples was cleaned with alcohol and water, dried and putted into the universal high-speed machine, and the obtained particles with the size of 2 mm.

Microwave Assisted Extraction

A single factor experimental method was used to study the effect of each factor on the OPEs yield from PP sheets. In this part, the range of the level for the different factors was as follows: the extraction temperature was from 75 ℃ to 95 ℃ step with 10 ℃, under 30 min extraction time and 15 mL extraction solvent. The extraction time was selected at the range from 20 min to 40 min (interval 10 min), under 85 ℃ extraction temperature and 15 mL extraction solvent. The extraction solvent volume was set at the level of 10-20 mL (interval 5 mL) under 85 ℃ extraction temperature and 30 min extraction time. According to the results from single factor experiments, a RSM design method with three variables (extraction time, extraction temperature and extraction solvent volume) was used to determine the interaction effects and optimal process of variables and last obtain the optimized parameters.

UHPLC–MS/MS analysis

Separation of the 8 OPEs was accomplished by using an ultra-performance liquid chromatography system (ACQUITY UHPLC, Waters, USA) coupled with a Waters BEH C18 column (2.1 mm × 50 mm, 1.7 μm). The column temperature was 30 ℃. For the gradient elution, a binary mobile phase of an aqueous solution of 0.2% formic acid (A) and acetonitrile (B) at a flow rate of 0.3 mL/min was utilized. The gradient was set as follows: 0 min (45% B), 2 min (75% B), 4 min (99% B), 4.2 min (45% B), and 4.5 min (45% B). The injection volume was 2 μL

A Quattro Premier Micromass® mass spectrometer (Waters/Micromass, Milford, USA) was interfaced to the UHPLC for the determination of the 8 OPEs. The electro spray ionization (ESI) was run in the positive ion mode. Source conditions were typically as follows: capillary 3.0 kV, source temperature 120 ℃ and desolvation temperature 350 ℃. The desolvation and cone gas flow rate were 650 and 50 L/h respectively. Argon was used as collision gas and the flow rate was 0.14 mL/min. Multipliers were set to 650 V. The parameters of MRM mode were summarized in Table 1. All aspects of data acquisition were controlled using MassLynx 4.1 software with QuanLynx^{TM} program (Waters).

Results and discussion

Optimization of the UHPLC–MS/MS conditions

In the previous studies, methanol was selected as the mobile phase in LC-ESI$^+$-MS/MS, because methanol can offer protons and improve ionization efficiency. In this work, the acetonitrile was chosen as mobile phase because acetonitrile can provide a much cleaner background [11, 12]. Under the optimized condition, a baseline separation for 8 considered OPEs was accomplished in 4.5 min (Fig 1C). Comparing to earlier studies for OPEs, this method shortened the separation time (e.g. 11 min for 12 OPEs [9], 24 min for 9 OPEs [11]) and improved the separation resolutions of OPEs [11, 12].

Optimization of MAE conditions

Optimization of extraction solvent

The solvents selected for the extraction were Acetone, chloroform, ethyl acetate and acetone/ethyl acetate due to their good microwave absorbing property and high selectivity towards the analytes of interest excluding unwanted matrix. The recoveries of 8 OPEs were shown in Fig 2 (A). The result showed that acetone/ethyl acetate (3/1, v/v) has greater extraction efficiency for most OPEs. However, the recoveries of TEP and TPrP were low because they have greater volatility and the losses were bigger than the others during the whole process.

Optimization of extraction solvent volume

From Fig 2 (B), the recovery of OPEs increased with the extraction solvent volume increased, and reached its maximum at 15 mL, while it decreased at 20 mL. This may be due to the solution capacity of the solvent [13]. When 15 ml extraction solvent was enough to extract the

OPEs in the sample, increase of the volume of extractant resulted in the more loss of OPEs during concentration with nitrogen flow controller.

Optimization of extraction temperature

As shown in Fig 2 (C), the OPEs recovery increased with the extraction temperature rose, and achieved the highest OPEs recovery at 85 ℃, and then, dropped. It may be that the increase of the extraction temperature caused the solvent viscosity decreasing and improved the solvent and solute diffusivity within extraction system, which enhanced the solubility of OPEs in solution. However, too high temperature may cause the degradation of the additives. So the temperature of 85 ℃ was suitable.

Optimization of extraction time

Fig 2 (D) showed that the OPEs recovery first increased with extraction time extended and then decreased, and reached its maximum value at 30 min. It is inferred that the thermal accumulation within extraction solution due to the absorption of microwave energy promoted dissolution process of OPEs into solution until 30 min. However, the excessive time exposure in the microwave field may cause the degradation of OPEs.

Optimization of factors of MAE by RSM

The response surface methodology was used to study the interactive effects of different independent parameters of MAE to get the best extract conditions. The results were shown in Fig 3. The operation conditions and results were shown in Table 2.

The results were analyzed by multiple regression analysis. ANOVA was used to estimate the effects of main factors and their potential interaction on the recovery of 8 OPEs. Table 3 below showed the statistical results of variance analysis (ANOVA) for the model. The F-value and related probability (Prob > F) are important outputs of the model. The value of the F-test on the model was 94.31 and the value of Prob > F was less than 0.05, which indicated that the developed model was significant at the 95% confidence interval. 0.97 as the “Lack of Fit F-value” which was relative to the pure error means showed that the lack of fit was not significant. ANOVA results in table 3 showed that model terms A (temperature), B (time), C (volume), A^2, B^2, C^2 and AB^2 had significant effects on the recovery of 8 OPEs. Consequently, the selected model could be considered to represent the actual process of recovery of 8 OPEs. As shown in Fig 3(A), when the volume was kept at 15 mL, the recovery increased with extraction time and extraction temperature up to 35 min and 85 ℃, respectively. A normal interaction of both extraction time and extraction temperature was found.

Keeping the extraction time at 30 min, the higher recovery would be obtained with extraction temperature and extraction solvent volume at 87.5 ℃ and 19 mL, respectively. (See Fig 3(B)). The interactive effect of the extraction time and extraction solvent volume on recovery in condition of 85 ℃ extraction temperature was shown in Fig 3(C). It was found that the recovery achieved the highest level at the extraction time of 40 min, and the extraction volume of 20 mL.

Based on Fig 3, the condition of maximum recovery came up in the range of variables, as the highest for all of the three dimensional response surface plots in each plot was kept on the center point. The optimized conditions were obtained as: extraction temperature of 87.05 ℃, extraction time of 35.77 min, extraction solvent volume of 19.08 mL. In consideration of the practical operate; the final solution was adjusted as extraction temperature of 88.5 ℃, extraction time of 36 min, extraction solvent volume of 19 mL. The real sample preparation was operated under this condition.

Method evaluation

The LODs and LOQs were determined at the concentration at which S/N was 3 and 10 respectively. The blank PP powder was spiked with different concentrations of OPEs to test the linear range of the method. As expound in Table 4, the correlation coefficients (R) ranged between 0.9907 and 0.9996 with wide linear ranges. Such wide linear ranges make the methodology suitable for analysis of both high concentration and low concentration samples.

Intra-day repeatability of the UHPLC-ESI-MS/MS method was evaluated by performing 6 repetitive analyses of 10 and 100 ppb of each 8 OPEs, which gave the RSDs between 0.57% and 3.82%, showing an excellent precision. (Table 5)

The matrix effect study was investigate to confirm the feasibility of this method, according to the study of M. Careri et al [17], the mathematically description as follow: $x_f = a_f + b_f x_c$, which x_f means found concentrations and x_c means original calibration concentrations. The value of slope b_f was between 0.9290 and 1.0165, which is closed to the ideal case $b_f = 1$ indicated that the matrix effect was not significant. So the developed UHPLC-ESI-MS/MS method could be used for real plastic samples.

Application to Real Samples

Finally, the established method was utilized to determine OPEs in different real samples. The real samples were from different brand cars as auto-interiors. The result was shown in Table 6. Sample 1, 3, 4 were obtained from dashboard and sample 2 was obtained from automotive outlet. In general, the 8 concerned OPEs were used more in dashboard than in outlet. TEP, TCPP, TPhP, DPOP, TCP, TBEP were detected in the all samples. TCEP and TPrP were under the LOQ in sample 1 while TCEP was under the LOQ in sample 2.

Conclusions

In this study, a simple, sensitive and fast method utilizing MAE coupled UHPLC-MS/MS for the detection of 8 OPEs in plastic automobile trim samples was developed and validated. The optimized extraction conditions and separation method enabled accurate detection of eight OPEs within 4.5 min. This study provided an effective method to control the qualities of plastic materials as auto-interiors in the future.

Acknowledgements

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Table 1.The parameters of 8 studied OPEs with UPLC-ESI^{+}-MS/MS detection

Compound name	Abbreviation	Retention time (min)	transitions	Cone voltage (V)	Collision energy(eV)
Triethyl phosphate	TEP	0.71	182.80→154.78 a)	28	10
			182.80→126.72		15
Tris(2-chloroethyl) phosphate	TCEP	1.01	286.68→124.80 a)	25	20
			286.68→98.70		20
tripropyl phosphate	TPrP	1.45	225.06→183.06 a)	30	10
			225.06→140.92		10
Tris(2-chloroisopropyl) phosphate	TCPP	1.65	326.85→251.07 a)	25	15
			326.85→174.77		15
Triphenyl phosphate	TPhP	2.46	326.91→215.08 a)	45	30
			326.91→153.05		30
Tris(2-butoxyethyl) phosphate	TBEP	2.79	399.06→299.17 a)	25	15
			399.06→199.02		15
tricresyl phosphate	TCP	3.33	368.88→243.13 a)	33	30
			368.88→166.07		30
diphenyl isooctyl phosphate	DPOP	3.70	362.89→251.07 a)	18	15
			362.89→153.02		30

a) Quantification of MRM transitions.

Table 2.The operations and results of RSM

number	Temperature/℃	Time/min	Volume/mL	Recovery[a)]/%
1	75	20	15	43.26
2	95	20	15	59.27
3	75	40	15	51.49
4	95	40	15	63.66
5	75	30	10	40.44
6	95	30	10	47.54
7	75	30	20	59.17
8	95	30	20	64.64
9	85	20	10	45.19
10	85	40	10	54.29
11	85	20	20	58.75
12	85	40	20	68.38
13	85	30	15	66.12
14	85	30	15	64.68
15	85	30	15	64.73
16	85	30	15	65.99
17	85	30	15	63.07

[a)] average recovery of 8 OPEs

Table 3.ANOVA results for the regression model equation and coefficients of model terms for the recovery of 8 OPEs.

source	Sum of squares	DF	Mean square	F value	Prob > F
model	1275.89	9	141.77	94.31	$<0.0001^a$
A	39.50	1	39.50	26.28	0.0014^a
B	87.70	1	87.70	58.35	0.0001^a
C	191.13	1	191.13	127.15	$<0.0001^a$
A^2	212.34	1	212.34	141.27	$<0.0001^a$
B^2	48.57	1	48.57	32.31	0.0007^a
C^2	99.82	1	99.82	66.41	$<0.0001^a$
A^2B	4.67	1	4.67	3.10	0.1215^b
A^2C	8.36	1	8.36	5.56	0.0504^b
AB^2	30.46	1	30.46	20.26	0.0028^a
Residual	10.52	7	1.50		

$R^2 = 0.9918$; adjusted $R^2 = 0.9813$; predicted $R^2 = 0.9376$.

[a] Significant at 95% confident interval.

[b] Not significant at 95% confident interval.

Table 4.Equations of linear regression, instrument linear range, correlation coefficients, and instrument LODs and instrument LOQs of 8 OPEs

Analytes	Linear range	Correlation coefficient	LOD(S/N=3) μg/L	LOQ(S/N=10) μg/L
TEP	5-1250	0.9969	2	5
TCEP	15--3750	0.9924	5	15
TPrP	10-2500	0.9981	3.30	10
TCPP	10-2500	0.9938	3.30	10
TPhP	2-500	0.9980	0.70	2
TBEP	1-250	0.9996	0.25	0.5
TCP	1-250	0.9983	0.25	1
DPOP	1-250	0.9907	0.30	1

Table 5.UHPLC-ESI-MS/MS intra-day repeatability of 8 OPEs (n=6)

Analyte	Concentration level (ng/mL)	RSD (%)	Analyte	Concentration level (ng/mL)	RSD (%)
TEP	10	1.41	TPhP	10	0.79
	100	1.20		100	0.57
TCEP	10	1.17	TBEP	10	3.60
	100	1.95		100	1.38
TPrP	10	2.52	TCP	10	2.79
	100	2.60		100	2.58
TCPP	10	2.70	DPOP	10	3.82
	100	2.35		100	2.26

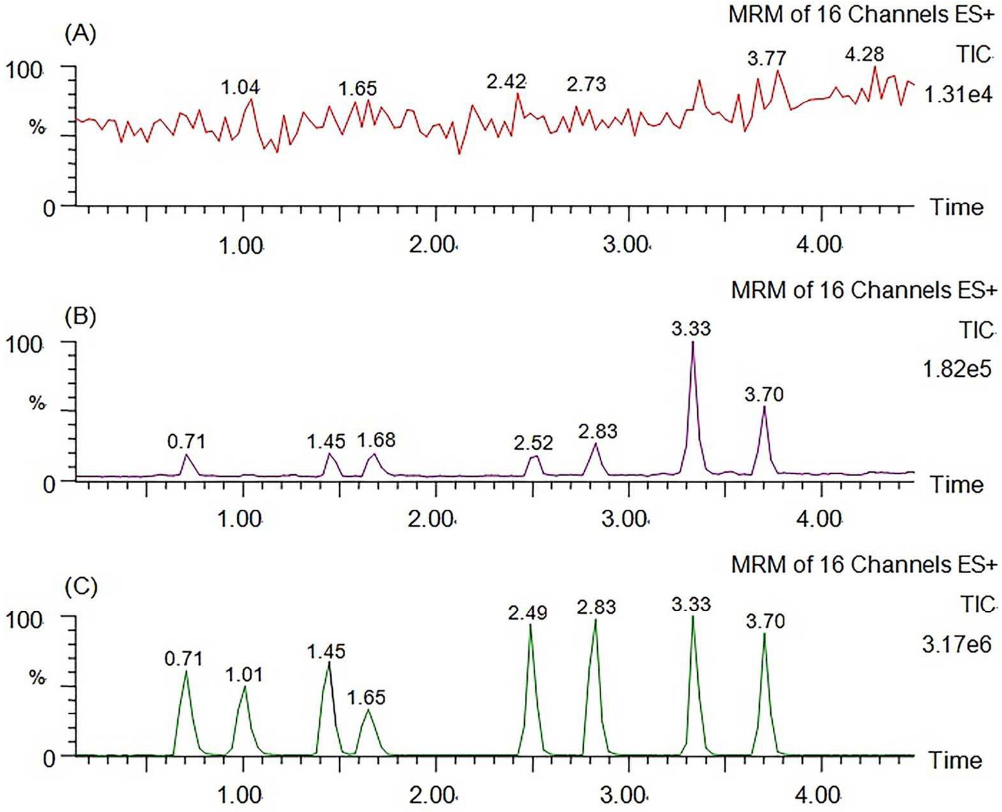
Table 6.The concentrations of eight studied OPEs detected in automotive trim Samples

Analytes	Sample 1(n=4)	Sample 2(n=4)	Sample 3(n=4)	Sample 4(n=4)
TEP	37.10±8.20	21.22±3.02	48.85±2.33	33.00±4.38
TPrP	/	41.68±1.51	28.20±1.13	15.90±0.85
TCEP	/	/	872.15±30.90	507.50±33.66
TCPP	29.55±6.32	78.77±7.07	337.05±13.36	229.40±16.40
TPhP	11.45±0.78	7.38±1.82	31.35±1.63	23.75±.0.35
DPOP	15.40±0.85	10.05±1.40	34.75±0.78	25.20±0.85
TCP	11.95±0.78	19.78±4.26	37.75±4.17	30.50±1.13
TBEP	9.10±0.57	4.38±0.28	11.85±0.21	10.85±0.78

Fig 1.The chromatogram of a blank sample (A), real sample (B), simulation sample (C)

Fig 2.Optimization of extraction solvent (A), extraction solvent volume (B), extraction temperature (C), extraction time (D)

Fig 3.Response surface plots of RSM. (A) Extraction Time to extraction Temperature; (B) extraction solvent volume to extraction Temperature; (C) extraction solvent volume to extraction time.



The chromatogram of a blank sample (A), real sample (B), simulation sample (C)
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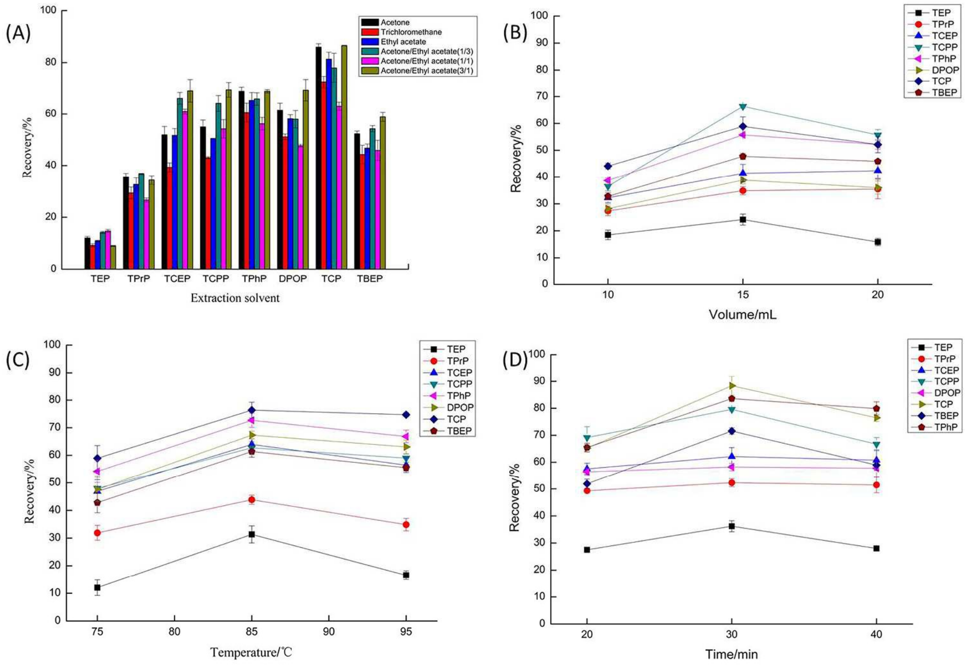

Optimization of extraction solvent (A), extraction solvent volume (B), extraction temperature (C), extraction time (D)
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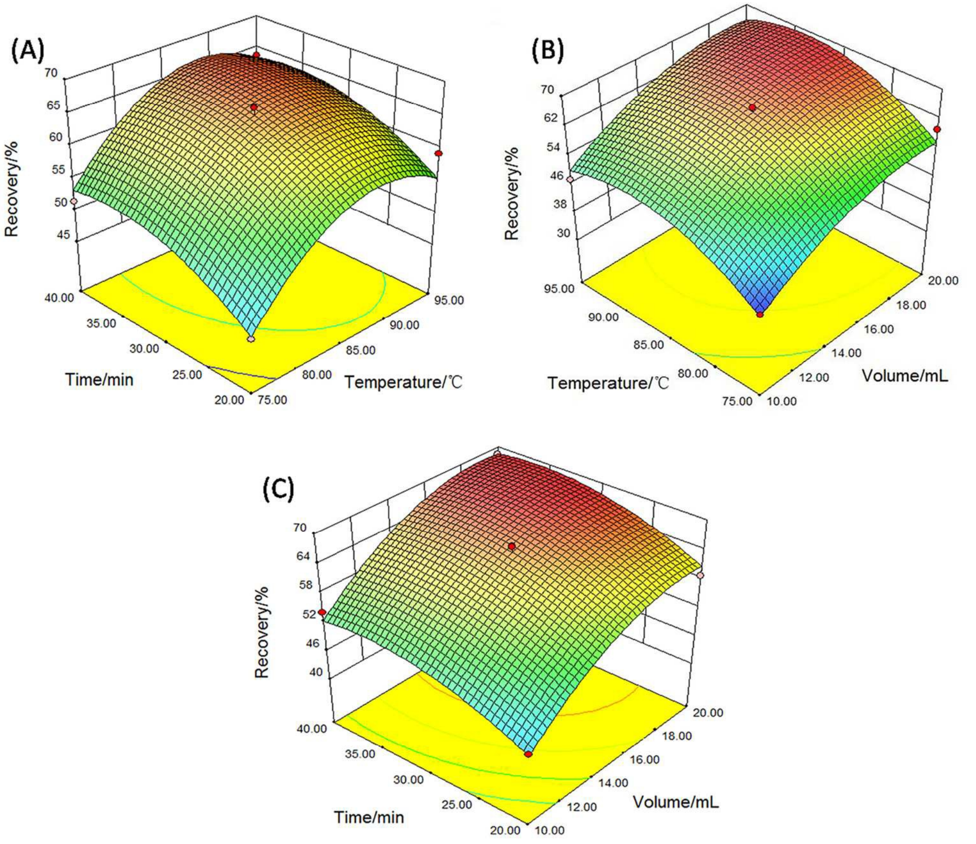

Response surface plots of RSM. (A) Extraction Time to extraction Temperature; (B) extraction solvent volume to extraction Temperature; (C) extraction solvent volume to extraction time.
68x58mm (600 x 600 DPI)

A UPLC-MS/MS method combined with MAE for detection of OPEs in automobile Interiors. The method is rapid, effective and eco-friendly.
39x25mm (600 x 600 DPI)