RESPONSE SURFACE METHODOLOGY USED FOR PARAMETERS OPIMULIZATION IN DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS RESIDUES BY GAS CHROMATOGRAPHIC ION TRAP MASS/MASS SPECTROMETRY

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Abstract

The response surface methodology (RSM) was used to determine the parameters of gas chromatographic ion trap mass/mass spectrometry (GC/IT-MS/MS) for analyzing polybrominated diphenyl ethers (PBDEs) in this study. Emission current and trap offset were selected to examine the methodology. The results showed that the two variables were quadratic to the PBDEs response ($P<0.05$) as standard solution applied to GC/IT MS/MS analysis. The fitted second degree equations could explain experimental results ($P > 0.05$) and the response of BDE-47 from the analysis of variance and go through a maximum at emission current 516 µA and trap offset 11 V. Meanwhile, the predictive results from equations for each congener fell between the experimental responses of 95% confidence levels. Emission current has influence on PBDEs sensitivity by using GC/IT-MS/MS analysis, however, the higher emission current does not promise higher sensitivity unless the trap offset is considered together.

Introduction

Polybrominated diphenyl ethers (PBDEs) are brominated compounds which were used as flame-retardant additives in polymers such as textiles, furniture and electronic equipment. The concentration of PBDEs was found to increase gradually by years in human milk [1]. Because of the persistence properties of PBDEs, it could be accumulated into human body through the route of food chain or environmental exposure. Many researches are concerning the problem and making efforts on the distribution of PBDEs residues. Recently, more and more studies are focusing on the PBDEs residues in foods which amounts are trace mostly [2,3]. A highly specific and sensitive analytical instrument is helpful doing the work. The most reliable analytical instrument for PBDEs analysis is high resolution gas chromatography/high resolution mass (HRGC/MS) which were used in many field, such as USEPA draft method 1614. Nevertheless, GC/IT-MS/MS could be an alternative analytical method since its high sensitivity, specificity and especial economy. However, the parameters setting in GC/IT-MS/MS are complicated for new users to operate. In general, optimization of parameters should be completely done before real sample analysis. Many researchers had reported studies on the parameters (such as the resonant excitation energy, emission current, excitation time, excitation voltage etc.) optimization [4,5,6]. For all of studies, they used single variable analysis in each parameter. In this study, we aimed to offer a valid solution for setting up suitable condition in MS/MS analysis through 3-level-2-factor RSM design and proposed fitted equations for the parameters decision.

Materials and Methods

Native PBDE standards, including 2,4,4'-TriBDE(BDE-28), 2,2',4,4'-TeBDE (BDE-47), 2,2',4,4',5-PeBDE (BDE-99), 2,2',4,4',6-PeBDE (BDE-100), 2,2',4,4',5,5'-HxBDE (BDE-153), 2,2',4,4',5',6-HxBDE (BDE-154), 2,2',3,4,4',5',6-HpBDE (BDE-183) and DeBDE (BDE-209) were purchased from AccuStandard (New Haven, CT, USA). *n*-Nonane purchased from Sigma-Aldrich (St. Louis, MO, USA) were used for preparing the standard solution and standard mixture solution with BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 at 400 pg/µL, and BDE-209 at 4000 pg/µL. The GC/IT-MS/MS analysis of PBDEs was performed using a Thermo Finnigan Trace GC Ultra/Polaris Q ion trap mass spectrometer (Thermo, Austin, TX, USA). One DB-5HT column (15 m × 0.25 mm i.d., 0.1 µm film) was used to separate tri- through deca-BDE. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The injector was set at 170℃. Two µL of the standard mixtures were injected into the GC system operated in spiltless injection by following temperature program: the temperature were maintained at 170℃ for 1.5 min, then ramped at 10℃/min to 250 ℃, then ramped at 20℃/min to 300℃ and maintained for 25 min. The optimized temperature of the MS ion source and transfer line temperature were set at 265℃ (data not shown here) and 240℃, respectively. The mass spectrometer was operated with electron impact of 70 eV. The most abundant ions (*m/z* 406, 486, 564, 644, 721, and 959) from the full-scan mass spectrum of each PBDE congener were selected as the precursor ion followed by collision-induced dissociation (CID) for MS/MS spectrum. The resonant excitation voltage for each congener and product ions for quantitative analysis were shown in table 1. The other instrumental parameters were set as follows: 0.3 for q value, 50 for AGC target value, 15 ms for excitation time, and 12 ms for isolation time.

Experiments were done according to the central composite design with five repetitions of the centre point as shown in table 2 and table 4. The design makes it possible to calculate response surface equations of the type shown in Eq. $\hat{Y} = a_0 + a_1X_1 + a_2X_2 + a_3X_1X_2 + a_4X_1^2 + a_5X_2^2$, where X_1 and X_2 are variables and a_0, a_1, a_2, a_3, a_4, and a_5 are the coefficients. In this study we selected two parameters, emission current and trap offset, for the parameter optimization test. The independent variables were emission current and trap offset. The PBDEs congeners were injected to GC/ion trap MS/MS using the condition mentioned above. The response of each congener was collected for RSM analysis. The designs and data analyses were done by the StatSoft statistical software, version 6.0.

Results and Discussion

The BDE-47 responses in GC/IT-MS/MS analysis in table 2 were selected here to show the first-order design and experimental datas. The analysis of variance (ANOVA) test of the BDE-47 responses was shown in table 3, in which the *P* value (< 0.05) indicates the emission current and trap offset had a quadratic relationship rather than linear. Therefore, we used the central composite rotatable second-order design to evaluate the parameters responses as shown in table 4. The variability between the 5 centre points for the emission current and trap offset was considerably smaller showing the experimental error was small. The response surface coefficients and *P* values were given in table 5. The quadratic terms of two parameters were significant ($P < 0.05$), indicating that the response of BDE-47 goes through a maximum around at emission current 516 µA and trap offset 11 V as shown in figure 1. Besides, lack of fit ($P > 0.05$) in ANOVA analysis indicated that the fitted equation ($\hat{Y} = 441166 + 7390\ X_1 + 151131\ X_2 - 112233\ X_1^2 - 109385\ X_2^2 + 1111\ X_1X_2$) could be used to predict the response values before the instrumental analysis. The other PBDEs including BDE-28, 99, 100, 153, 154, 183 and 209 were also evaluated by the RSM and the predicted values obtained from the fitted equations were quiet the same as the experimental values as shown in table 6. The results all fell between the 95% confidence levels showing that the equations were reliable and the RSM were useful for determining parameters in GC/IT-MS/MS method. The GC/IT-MS/MS chromatogram (see figure 2) of PBDEs standard solution with BDE-28, BDE47, BDE-99, BDE-100, BDE-153, BDE-154, and BDE-183 at 2 pg and BDE-209 at 20 pg applied to GC/IT-MS/MS analysis with parameters set up above showed excellent sensitivity.

The emission current will influence the intensity of electron beam and be used to increase sensitivity. The higher emission current seems to have higher MS/MS response. Because the instrument we used is an outer ionization mode, the trap offset is needed for attracting ions into trap. In other words, trap offset will play an important role for ions capture. In this study, we have done the relationship between emission current and trap offset through RSM design. The higher emission current does not promise higher sensitivity unless the trap offset is considered together.

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Table 1. The precursor ion and product ions of PBDE congeners analyzed by GC/IT-MS/MS

PBDE congener #	Precursor ion (*m/z*)	Product ions (*m/z*)	REV (V)	RT (min)
28	406	246, 248	4.75	3.75
47	486	324, 326, 328	4.75	4.86
100, 99	564	404, 406	4.6.	5.65, 5.87
154, 153	644	482, 484, 486	4.7	6.47, 6.76
183	721	562, 564	3.2	7.76
209	959	797, 799, 801	3	13.32

REV: resonant excitation voltage, RT: retention time.

Table 2. First-order design and experimental data of BDE-47 in GC/IT-MS/MS method

Natural variable		Coded variable		Response
X_1	X_2	x_1	x_2	y
300	5	-1	-1	7358
300	15	-1	1	227152
700	5	1	-1	3456
700	15	1	1	229533
500	10	0	0	452709
500	10	0	0	497054
500	10	0	0	507975
500	10	0	0	520974
500	10	0	0	444431

X_1: emission current (μA), X_2: trap offset (V).

Table 3. Analysis of variance of first order design on BDE-47 responses in GC/IT-MS/MS method

Model		DF	SS	MS	*F*	*p*
Regression	Linear	2	3.374E+10	1.687E+10	0.296954	0.753399
Residual		6	3.409E+11	5.682E+10		
	Crossproduct	1	8.844E+07	8.844E+07	0.0760	0.796452
	Quadratic	1	3.362E+11	3.362E+11	288.8533	0.000070
	Total error	4	4.655E+09	1.164E+09		
Total		8	3.747E+11			

SS: sum of squares, MS: mean square, DF: degree of freedom.

Table 4. Central composite rotatable second-order design and experimental datas of BDE-47 in GC/IT-MS/MS method for 3-level-2-factor response surface analysis

Natural variable		Coded variable		Response
X_1	X_2	x_1	x_2	y
300	5	-1	-1	7358
300	15	-1	1	227152
700	5	1	-1	3456
700	15	1	1	229533
500	10	0	0	452709
500	10	0	0	497054
500	10	0	0	507975
500	10	0	0	520974
500	10	0	0	444431
782.8	10	1.414	0	320435
217.2	10	-1.414	0	274951
500	17.07	0	1.414	484960
500	2.93	0	-1.414	522

X_1: emission current (μA), X_2: trap offset (V).

Table 5. Analysis of variance of central composite rotatable second-order design of BDE-47 in GC/IT-MS/MS method for 3-level-2-factor response surface analysis

Factor	DF	SS	MS	*F*	*P*
X_1 (L)	1	3.728E+08	3.728E+08	0.3204	0.601631
X_1 (Q)	1	8.776E+10	8.776E+10	75.4094	0.000968
X_2 (L)	1	2.602E+11	2.602E+11	223.6581	0.000116
X_2 (Q)	1	2.0194E+11	2.019E+11	173.5204	0.000192
$X_1 \times X_2$	1	1.695E+10	9.869E+06	0.0085	0.931056
Lack of fit	3	1.696E+10	5.653E+09	4.8572	0.080458
Pure error	4	4.655E+09	1.164E+09		
Total	12	4.834E+10			

L: linear, Q: quadratic, X_1: emission current (μA), X_2: trap offset (V)

Table 6. Predicted and experimental values of PBDE congeners

PBDE congener #	Predicted	Confidence level		Experimental
		-95%	+95%	
28	361328	313653	409002	387921
47	493299	454432	532165	513290
100	635391	611569	659212	656706
99	472703	449150	496255	460671
154	449415	424170	474659	483508
153	365575	342172	388977	354733
183	315115	279499	350731	285981
209	165116	80597	249635	89450

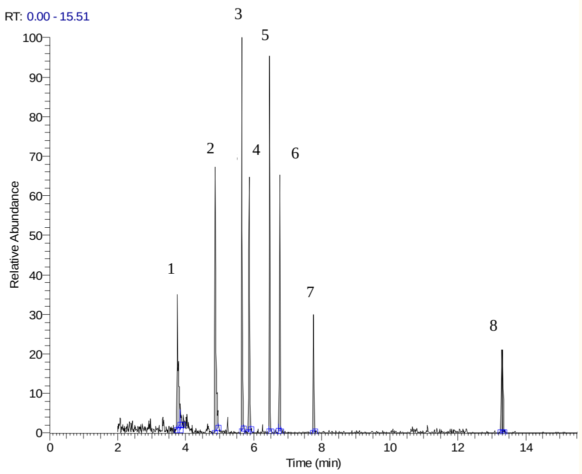

Figure 2. The GC/IT-MS/MS chromatogram of PBDE standards solution for BDE-28 – BDE-183 (2 pg injected) and BDE-209 (20 pg injected).1, BDE-28; 2, BDE-47; 3, BDE-100; 4, BDE-99; 5, BDE-154; 6, BDE-153; 7, BDE-183; 8, BDE-209.

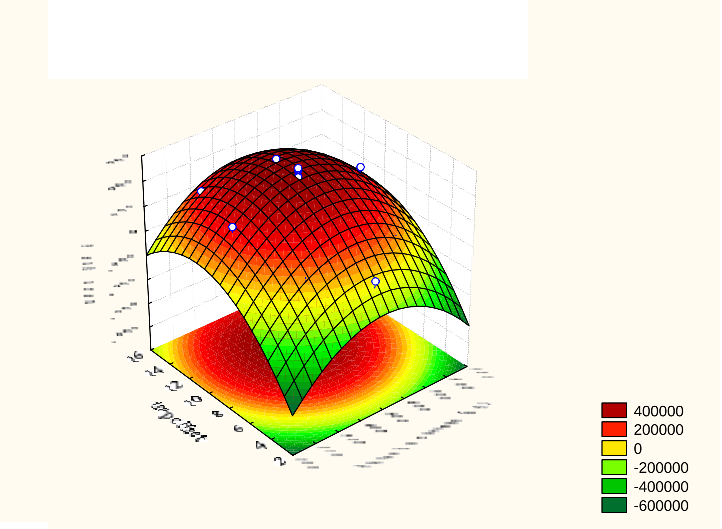

Figure 1. The response surface plot of emission current and trap offset for BDE-47.