Supporting Information

Fully-automated dynamic in-syringe liquid-phase microextraction and on-column derivatization of carbamate pesticides with gas chromatography-mass spectrometric analysis

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ABSTRACT

A new fully-automated dynamic in-syringe liquid-phase microextraction (LPME) and on-column derivatization approach, with gas chromatography-mass spectrometric (GC-MS) analysis, was developed to determine carbamate pesticides from water samples. By using a CTC CombiPal autosampler and its associated Cycle Composer software, a sample preparation-GC-MS method was enabled that allowed consecutive multiple experiments of sample extraction, extract injection and analyte derivatization followed by GC-MS analysis, to be carried out completely automatically. Optimization of the extraction parameters was performed by using orthogonal array design (OAD). In the OAD approach, considerable amount of time was saved and key variables and interactions amongst extraction parameters could be identified easily (which would not be possible via the univariate approach that is normally employed in many microextraction studies). With the use of OAD, the number of sampling cycles, sampling volume and their interaction were identified to be the more significant parameters amongst the rest of the five variables and eight interactions considered. All the 16 experiments (each comprising LPME, derivatization and GC-MS analysis) necessary for the OAD optimization were consecutively conducted completely automatically, without any human intervention. This automated dynamic in-syringe LPME approach demonstrated the feasibility of a complete analytical system comprising sample preparation and GC-MS that might be operated onsite, fully automatically without human intervention.

GC-MS-Selective Ion Monitoring (SIM) chromatogram

Figure S-1 shows a total ion chromatogram under SIM mode showing sharp and symmetrical peaks of the carbamate standards (at 1 mg/L), employing on-column derivatization. This shows that the derivatization step as part of the automated procedure was efficient and unproblematical.

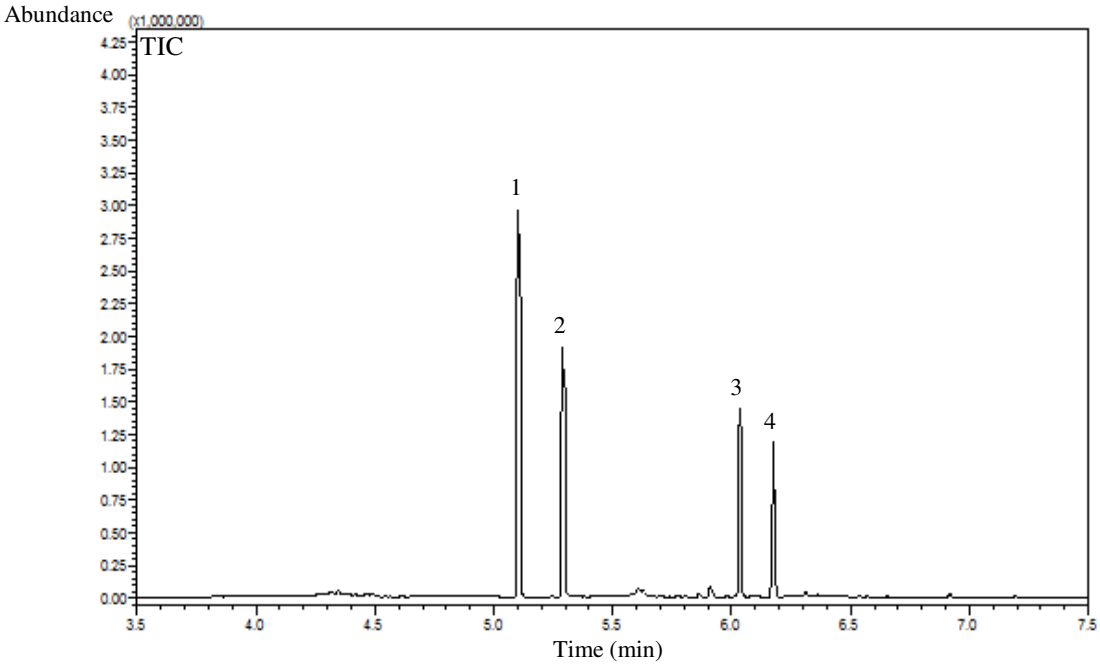

Figure S-1. GC-MS-SIM chromatogram of on-column derivatized standards of the carbamate pesticides at 1 mg/L. Peaks: 1) Promecarb, 2) Aminocarb, 3) Methiocarb, 4) Carbaryl.

Orthogonal array design (OAD) optimization[2,3]

Table S-1. Assignment of factors and level settings for initial experimental runs in the OA_{16} (2^{15}) matrix

Level	Column no.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	A	**B**	**(A × B)**	**(B × C)**	**#**	**C**	**(A × C)**	**#**	**(C × E)**	**D**	**(A × D)**	**(C × D)**	**(B × E)**	**(A × E)**	**E**
1	0.1	8 s				8 µL				15					0%
2	0.8	2 s				3 µL				3					30%

A = rate of plunger movement; B = dwell time; C = sampling volume; D = sampling cycles; E = salt concentration (w/v); # = dummy factors

A × B = interaction between rate of plunger movement & dwell time; B × C = interaction between dwell time & sampling volume; A × C = interaction between rate of plunger movement & sampling volume; C × E = interaction between sampling volume & salt concentration; A × D = interaction between rate of plunger movement & sampling cycles; C × D = interaction between sampling volume & sampling cycles; B × E = interaction between dwell time & salt concentration; A × E = interaction between rate of plunger movement & salt concentration

By choosing a two-level OAD, many variables together with their interactions can be simultaneously examined by the least number (i.e. 16) of experiments. The effects of five variables

(rate of plunger movement, dwell time, sampling volume, number of sampling cycles and salt concentration) and eight possible interactions were evaluated (Table S-1).

The responses (enrichment factors, EFs) from the array were calculated, and are tabulated in Table S-2. The mean responses for each factor at the two different levels (r_1 and r_2) are also presented in Table S-2. Direct observation analysis, also known as range analysis, was used and the mean value difference (d) was subsequently found by determining the range between the maximum and minimum mean responses for each variable and interaction.[1] The results showed that the number of sampling cycles (D) was the most significant variable, followed by the sampling volume (C). Interaction between sampling volume and sampling cycles (C × D) was the most significant amongst the eight interactions considered. On the contrary, the effects from the rate of plunger movement (A), dwell time (B) and salt concentration (E) were negligible. The other interactions were also comparatively of less importance. Moreover, the percentage contribution owing to error (unknown or uncontrolled factors), calculated from the dummy factors, was low (4.6 %).

Table S-2. OA_{16} (2^{15}) matrix with experimental results

Trial	Factors & interactions															Response (EF)				
no.	**A**	**B**	**(A × B)**	**(B × C)**	**#**	**C**	**(A × C)**	**#**	**(C × E)**	**D**	**(A × D)**	**(C × D)**	**(B × E)**	**(A × E)**	**E**	PC	AC	MC	CB	Sum
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	120	67	54	72	313
2	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	63	60	52	56	230
3	1	1	1	2	2	2	2	1	1	1	1	2	2	2	2	88	81	54	71	293
4	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	58	56	51	54	219
5	1	2	2	1	1	2	2	1	1	2	2	1	1	2	2	57	56	52	53	218
6	1	2	2	1	1	2	2	2	2	1	1	2	2	1	1	80	64	52	62	258
7	1	2	2	2	2	1	1	1	1	2	2	2	2	1	1	65	59	53	57	234
8	1	2	2	2	2	1	1	2	2	1	1	1	1	2	2	96	81	54	71	302
9	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	104	81	53	75	313
10	2	1	2	1	2	1	2	2	1	2	1	2	1	2	1	65	58	52	57	231
11	2	1	2	2	1	2	1	1	2	1	2	2	1	2	1	66	59	51	56	232
12	2	1	2	2	1	2	1	2	1	2	1	1	2	1	2	56	55	52	53	217
13	2	2	1	1	2	2	1	1	2	2	1	1	2	2	1	57	56	52	54	219
14	2	2	1	1	2	2	1	2	1	1	2	2	1	1	2	72	66	54	60	252
15	2	2	1	2	1	1	2	1	2	2	1	2	1	1	2	65	62	54	57	238
16	2	2	1	2	1	1	2	2	1	1	2	1	2	2	1	111	71	54	72	309
r_1	258	256	259	254	252	271	250	258	258	284	259	264	251	256	252					
r_2	251	254	251	255	258	238	260	252	251	226	251	246	259	254	258					
D	**7**	**2**	**8**	**1**	**6**	**33**	**10**	**5**	**7**	**58**	**8**	**18**	**8**	**1**	**6**					

PC = promecarb; AC = aminocarb; MC = methiocarb; CB = carbaryl

This demonstrated that there was no important factors or interactions excluded in the initial array design, and therefore all other interactions between factors could be neglected in this present study.

According to the initial results, a four-level OA_{16} (4^5) array (Table S-3) was chosen to effectively study the effects of sampling volume, number of sampling cycles, and their interaction for a more comprehensive optimization of the fully-automated dynamic in-syringe LPME procedure. The extractions were carried out with the rest of the less significant variables fixed at the level that gave the higher mean response (r) from the OA_{16} (2^{15}) array. Therefore, the rate of plunger movement, dwell time and salt concentration were fixed at 0.1 µL/s, 8 s and 30 % (w/V) respectively.

Table S-3. Assignment of factors and level settings for optimization of dynamic in-syringe LPME experiments by OA_{16} (4^5) matrix

Level	Column no.				
	1	2	3	4	5
	A	**B**	**$(A \times B)_1$**	**$(A \times B)_2$**	**$(A \times B)_3$**
1	9 µL	9			
2	8 µL	12			
3	7 µL	15			
4	6 µL	18			

A = sampling volume; B = sampling cycles; A × B = interaction between sampling volume & sampling cycles

Experimental results of the four-level array are presented in Table S-4. Data analysis was carried out according to the equations and methods given for data analysis strategy employing ANOVA (analysis of variance) and percentage contribution techniques.[2] ANOVA results are tabulated in Table S-5. The effects of the variables on the response function can be evaluated by both their significance (F ratio) and their percentage contribution (PC %). The F-test results shown in Table S-5 established that the two variables and their interaction were all statistically significant at $P < 0.001$, this was in good agreement with the results obtained from the first array.

The results were further confirmed by the analysis of PC %, which indicates how important each variable or interaction is amongst the rest of the considered factors.[3] It can be seen that the most important variable is B (sampling cycles, 61.9 %), and variable A (sampling volume)

contributed 13.6 %. Results from Table S-5 indicate that at the superior levels, the interaction between sampling volume and sampling cycles (A × B) was of higher importance. A significantly low contribution from error (1.2 %) was attained as well.

Table S-4. OA_{16} (4^5) matrix with experimental results

Trial no.	Factors & interactions					Response (EF)		
	A	**B**	**$(A \times B)_1$**	**$(A \times B)_2$**	**$(A \times B)_3$**	Sum 1	Sum 2	Sum 3
1	1	1	1	1	1	291	289	292
2	1	2	2	2	2	353	340	340
3	1	3	3	3	3	439	435	461
4	1	4	4	4	4	400	407	403
5	2	1	2	3	4	279	272	277
6	2	2	1	4	3	264	273	270
7	2	3	4	1	2	385	378	392
8	2	4	3	2	1	432	424	431
9	3	1	3	4	2	284	282	283
10	3	2	4	3	1	270	276	274
11	3	3	1	2	4	371	352	366
12	3	4	2	1	3	398	396	404
13	4	1	4	2	3	291	288	290
14	4	2	3	1	4	324	313	333
15	4	3	2	4	1	301	307	307
16	4	4	1	3	2	332	337	323
r_1	1112	855	940	1049	974			
r_2	1019	908	993	1070	1007			
r_3	989	1123	1110	993	1052			
r_4	937	1171	1013	945	1024			

Table S-5. ANOVA table for experimental responses in the OA_{16} (4^5) matrix

Source	SS	d.f.	MS	F	SS'	PC (%)
Sampling volume (A)	21566	3	7189	173 **	21442	13.6
Sampling cycles (B)	97925	3	32642	785 **	97800	61.9
(A × B)	37226	9	4136	100 *	36852	23.3
Error	1330	32	42		1953	1.2
Total	158047	47			158047	100.0

SS = sum of squares; d.f. = degrees of freedom; MS = mean squares; F = critical values; SS' = purified sum of squares; PC = percentage contribution.
** $F_{(3,32)} = 6.94$ at $P < 0.001$; * $F_{(9,32)} = 4.30$ at $P < 0.001$

Based on the OAD experiments, the optimized extraction conditions were: 9 µL sampling volume, 18 sampling cycles, 8 s dwell time, addition of 30 % (w/v) salt concentration to the sample and 0.1 µL/s for the rate of the plunger movement.

REFERENCES

(1) Wu, J.; Lee, H.K. *J. Chromatogr. A* **2005**, *1092*, 182-190.

(2) Lan, W.G.; Wong, M.K.; Chen, N. *Analyst* **1994**, *119*, 1669-1675.

(3) Lan, W.G.; Wong, M.K.; Chen, N. *Analyst* **1994**, *119*, 1659-1667.