Supporting Information

Bubbles in Solvent Micro-Extraction: The Influence of Intentionally Introduced Bubbles on Extraction Efficiency

D. Bradley G. Williams*[1], Mosotho J. George[1], Riaan Meyer[2] and Ljiljana Marjanovic[1]

[1]Department of Chemistry and [2]Department of Biochemistry, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa

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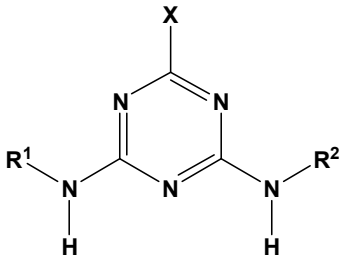
Experimental

1. Molecular structures of the ten triazines used in the study

The molecular structures of the various triazine pesticides investigated as part of this study are given below in Supplementary Table S1.

Supplementary Table S1. Information on triazine structures for the components used in this study.



	X	R^1	R^2	CAS number
Prometron	OCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$	1610-18-0
Atraton	OCH_3	CH_2CH_3	$CH(CH_3)_2$	1610-17-9
Propazine	Cl	$CH(CH_3)_2$	$CH(CH_3)_2$	139-40-2
Atrazine	Cl	CH_2CH_3	$CH(CH_3)_2$	1912-24-9
Simazine	Cl	CH_2CH_3	CH_2CH_3	122-34-9
Terbutylazine	Cl	CH_2CH_3	$C(CH_3)_3$	5915-41-3
Prometryn	SCH_3	$CH(CH_3)_2$	$CH(CH_3)_2$	7287-19-6
Ametryn	SCH_3	CH_2CH_3	$CH(CH_3)_2$	834-12-8
Simetryn	SCH_3	CH_2CH_3	CH_2CH_3	1014-70-6
Terbutryn	SCH_3	CH_2CH_3	$C(CH_3)_3$	886-50-0

2. GC programs used for FID and MS detection of triazines

Supplementary Table S2. GC program for the use of the ZB1701 column for FID detection

GC-FID set-up				
Injector temperature		280 ºC		
Split ratio		1:10		
Detector temperature		360 ºC		
Column flow		0.50 mL/min		
Oven Programme				
Temperature (ºC)		Ramp (ºC/min)	Time lapse (min)	
Initial	Final		Comment	Time
100	100	-	Hold	3
100	200	50	Ramp	1
200	230	30	Ramp	1
230	235	2.5	Ramp	2
235	260	25	Ramp	1
260	260	-	Hold	5

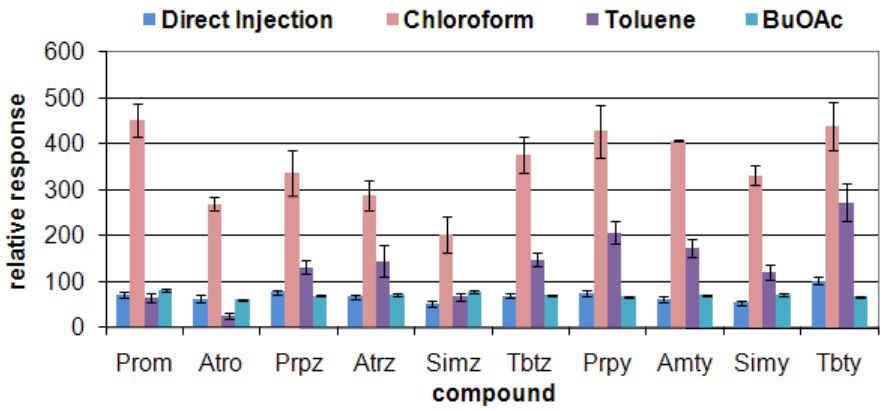
Supplementary Table S3. GC method for the use of the ZB35MS column for MS detection

Injector set-up				
Injector Temperature		280 ºC		
Split ratio		1:10		
MS settings				
Ion source temperature		250 ºC		
Transfer line		250 ºC		
Analysis window		8-14 minutes		
Acquisition mode		Scan at 0.2 second intervals		
Scanning mass range		150–250 at 208 scans/s		
Detector voltage		1.75 V		
Oven Programme				
Temperature (ºC)		Ramp (ºC/min)	Time lapse (minutes)	
Initial	Final		Comment	Time
100	100	-	Hold	4
100	200	50	Ramp	2
200	240	5	Ramp	8
240	260	20	Ramp	1
260	260	-	Hold	3
Column flow		1.0 mL/min		

Results

3. Selection of solvent for optimum extraction of triazines

Of the many solvents tested, three stood out above the rest for their ability to extract triazines from aqueous solutions, namely toluene, chloroform and butyl acetate, the latter of which has been cited as a solvent of choice for such processes.[1] In our hands, chloroform dramatically outperformed the extraction ability of the other two solvents (Supplementary Fig. S1) and was selected for the remaining investigations.

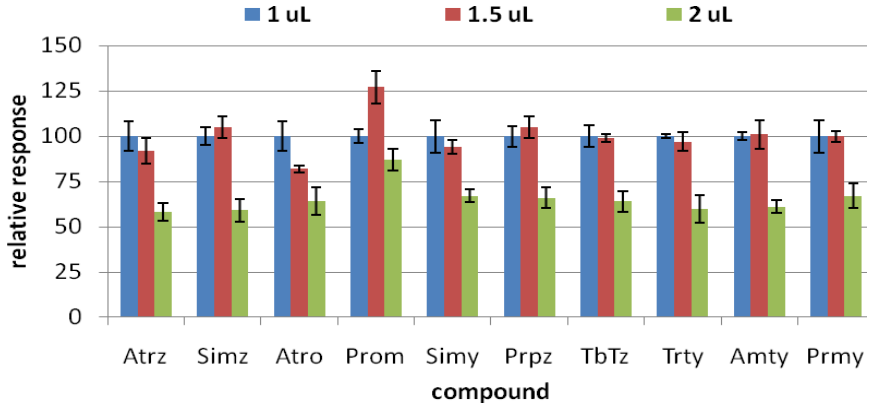


Supplementary Figure S1. SDME extraction results of triazines (10 µg/L) in HPLC grade water with various solvents.

4. Bubble size and droplet volume influence on extraction of triazines

The effect of bubble size housed within a 1 µL droplet (bubble-in-drop, BID) was investigated. Clear improvements in the extraction ability of the BID system were demonstrated for increasing bubble volumes up to and including 0.5 µL (see Fig. 2, main paper for optimum bubble influence effect). Thereafter, no further gains were secured. Instead, the arrangement became unstable leading to erratic results (see Fig. 1, main paper, for oversize bubble destabilizing effect).

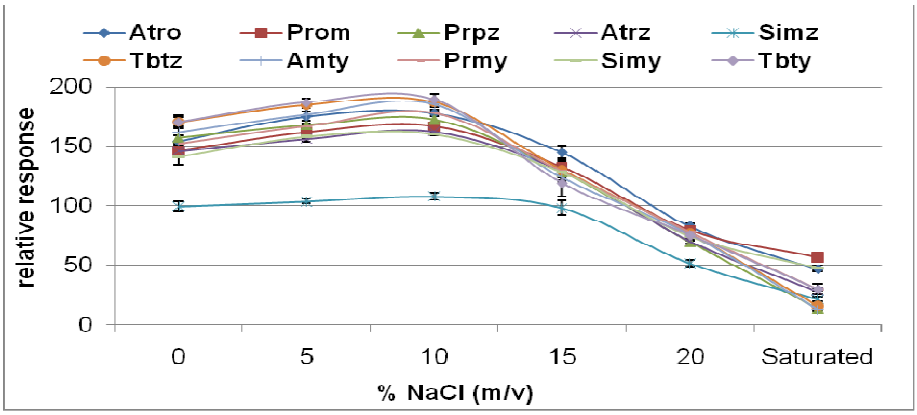
The BID-SMDE method was compared to droplets containing larger volumes of solvent (no bubble). Droplet volumes of 1 µL, 1.5 µL and 2 µL showed generally poorer extraction efficiency along this series (Supplementary Fig. S2). For the sake of consistency, 1 µL samples were injected into the GC instrument to assess concentration effects.



Supplementary Figure S2. The effect of droplet volume using SDME (no bubble) on the extent of extraction of triazines (10 µg/L) from HPLC grade water containing 10% (m/v) NaCl. Data are normalised to 100 for each triazine using the 1 µL droplet case.

5. Effect of NaCl on BID-SDME extraction efficiency

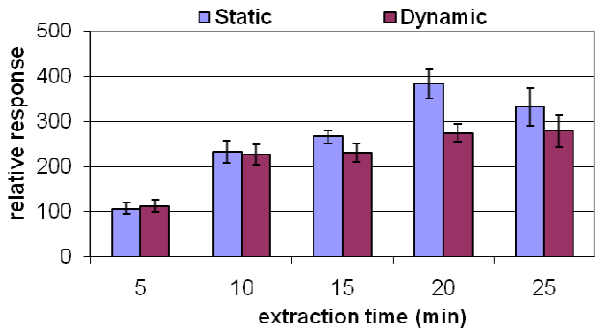
The added NaCl was mainly to assist in stabilising the bubble-in-drop (BID) set-up. We found that the BID was overall less stable in salt-free solutions. In contrast, NaCl-containing solutions stabilised the BID and led to improved extraction efficiencies for the triazine analytes. 10% (m/v) NaCl added to the triazine solutions was found to be optimal for a stable BID system with high extraction efficiencies (Supplementary Fig. S3).



Supplementary Figure S3. The effect of NaCl concentration on the extractability of triazines (10 µg/L) making use of HPLC grade water (with added NaCl) and BID-SDME.

6. Influence of stirring and time allocation on the extraction efficiency of triazines

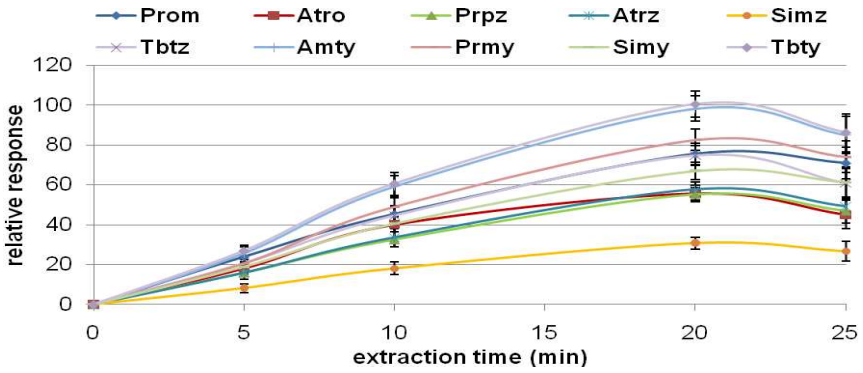
Despite dynamic extraction being understood to generally enhance mass transfer with a net increase in the overall extraction efficiency,[2] our new method is compromised by medium or strong stirring due to the increased mechanical instability of the droplet/bubble set-up. This is shown by poorer levels of extraction of the analytes compared to those obtained without stirring (Supplementary Fig. S4), for various time allocations for the extractions.



Supplementary Figure S4. The effect of stirring and time of extraction on the enrichment of triazines into the micro-droplet from aqueous triazine solutions (10 µg/L) containing 10% (m/v) NaCl using BID-SDME. Averaged data for the ten component mixture are provided.

7. Influence of time on the extraction of triazines

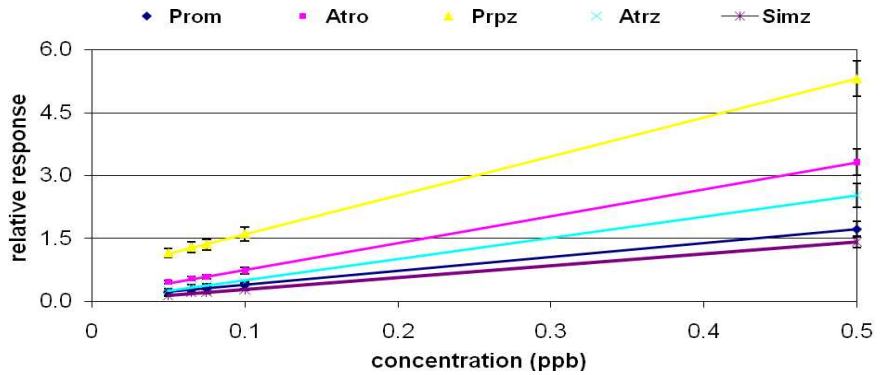
The length of time allocated to the extractions was investigated and found to be an optimum at 20 minutes (Supplementary Fig. S5). The graph provides the detail not shown in the averaged data presented in Supplementary Figure S4.



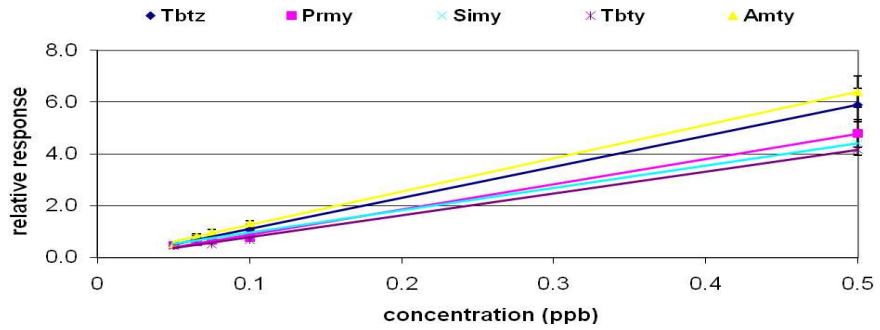
Supplementary Figure S5. The effect of extraction time on efficiency for BID-SDME pre-concentration of triazines (10 µg/L) from HPLC grade water containing 10% (m/v) NaCl.

8. Linearity of the BID-SDME method for extracting trace levels of triazines

The method demonstrates good linearity, as shown in Supplementary Figs. S6 and S7, the analytical data of which are captured in Supplementary Table S4, in the range 0.05-0.5 μg/L concentrations. The 0.5 μg/L concentration on the calibration curve is to reflect the concentration of one of the CRM standards.



Supplementary Figure S6. Calibration curves for prometron, atraton, propazine, atrazine and simazine.



Supplementary Figure S7. Calibration curves for terbutylazine, prometryn, simetryn, terbutryn and ametryn.

Supplementary Table S4. Analytical data from the calibration curves (0.05–0.5 µg/L spiking).

	Prom	**Atro**	**Prpz**	**Atrz**	**Simz**	**Tbtz**	**Prmy**	**Amty**	**Simy**	**Tbty**
SIM (*m/z*)	210	211	200	214	201	214	241	227	213	226
Retention time (min)	9.99	10.04	10.26	10.15	10.36	10.39	11.88	12.01	12.12	12.25
R^2	0.9999	0.9997	1.0000	0.9992	0.9993	0.9996	0.999779	0.9992	0.9992	0.9999
Intercept	0.0556	0.1165	0.6788	0.0007	0.0017	-0.0942	-0.03381	-0.0085	0.0913	-0.1562
Slope	3.3418	6.4088	9.2769	5.0462	2.8414	11.9636	9.640063	12.8483	8.6428	8.6356
SD(Intercept)	0.0034	0.0139	0.0068	0.0194	0.0104	0.0328	0.025934	0.0484	0.0336	0.0300
F_{calc}[b]	51574	11532	101611	3697	4083	7267	9063	3835	3607	7347

[a]LOQ calculated as 10 × SD(intercept)/slope.

[b]F_{crit} = 7.709 for a significant regression.

Accuracy validation

The method was validated for accuracy using a certified reference material (5.0 and 0.5 ppb ± 5% each of atraton in water. The student t-test was used to compare the t-value obtained from the following equation with the t-critical from statistical tables (n = 6).

$$t = \pm \frac{\bar{x} - \mu}{s/\sqrt{N}}$$

Where:

$\bar{x}$ = Experimental mean
μ = Known value
s= Standard deviation
N = Number of measurements

9. Reproducibility studies

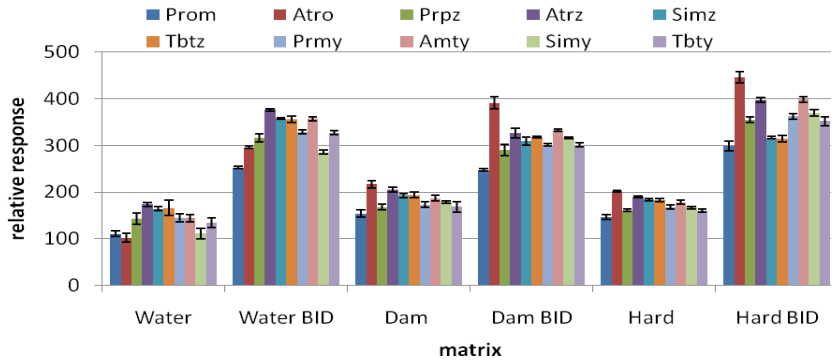
Solutions of the triazines (5 µg/L) in HPLC grade water containing 10% m/v NaCl were made up on three different days and analysed using GC-MS analysis under optimised BID-SDME conditions. The results are shown in Supplementary Table S5, demonstrating the reproducibility of the method.

Supplementary Table S5. Reproducibility data for BID-SDME extraction of 10 µg/L samples of the ten component triazine mixture from HPLC grade water containing 10% m/v NaCl. Data are provided as relative responses.

	Prom	**Atro**	**Prpz**	**Atrz**	**Simz**	**Tbtz**	**Prmy**	**Amty**	**Simy**	**Tbty**
Day 1	6.51	12.74	7.50	8.77	16.69	11.54	16.00	9.27	16.28	17.31
Day 2	5.90	13.22	7.57	9.02	16.98	11.43	16.21	9.29	16.48	17.53
Day 3	6.48	13.17	7.74	9.23	16.45	12.08	16.42	9.33	16.84	17.52
Day 4	7.10	13.06	7.66	9.19	15.98	11.91	15.67	8.95	15.69	16.52
Average	**6.50**	**13.05**	**7.62**	**9.05**	**16.53**	**11.74**	**16.08**	**9.21**	**16.31**	**17.22**
SD	0.49	0.22	0.11	0.21	0.42	0.31.	0.32.	0.18	0.48	0.48
% RSD	7.5	1.7	1.4	2.3	2.6	2.6	2.0	1.9	2.9	2.8

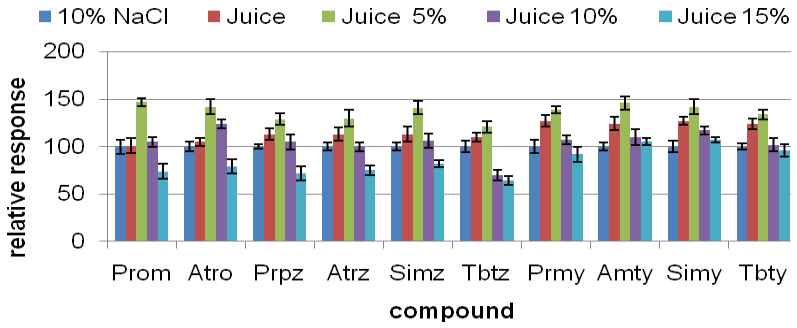
10. Application of the BID-SDME method to various matrices

Supplementary Fig. S8 compares SDME and BID-SDME under the optimised conditions, making use of various water matrices. Here, HPLC grade water, dam water sourced from an urban dam nearby the campus of our institution and synthetic hard water, made up to contain 10% m/v NaCl, were spiked with the ten component triazine mixture (to achieve 10 µg/L levels of triazines) and the mixtures extracted.



Supplementary Figure S8. Comparison of extraction efficiency for various matrices with and without a bubble (BID-SDME vs SDME) for 10 µg/L triazines. Water sources were: water = HPLC grade water; dam = dam water (brown silty water) sourced from an urban dam nearby our institute; hard = synthetic hard water prepared as in *reference 3*.

When the BID-SDME method was applied to triazine-spiked orange juice, 5% NaCl (m/v) afforded optimum stability of the BID-SDME set-up (Supplementary Fig. S9). These data reflect a rare overall positive matrix effect.



Supplementary Figure S9. BID-SDME extraction of triazines (10 µg/L) from orange juice (5% m/v NaCl compared to efficiencies of extraction of spiked HPLC grade water containing 10% NaCl (m/v) as the reference solution and normalised to 100.

11. References

1. Bagheri, H.; Khalilian, F. Immersed solvent microextraction and gas chromatography–mass spectrometric detection of *s*-triazine herbicides in aquatic media. *Anal. Chim. Acta* **537**, 81-87 (2005).

2. Zhao, E.L., Huan, L., Jiang, S., Wang, Q.; Zhou, Z. Application of a single-drop microextraction for the analysis of organophosphorus pesticides in juice. *J. Chromatogr. A* **1114**, 269-273 (2006).

3. Weber, C.I. *Environmental Monitoring Systems Laboratory*, 1993, Method 600/4-90/027F US EPA.