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Inter-laboratory validation of solid-phase microextraction for the determination of triazine herbicides and their degradation products at ng/l level in water samples

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Abstract

The accuracy and precision of solid-phase microextraction (SPME) were validated in an inter-laboratory study including ten laboratories for the analysis of triazine herbicides and their metabolites at ng/l level in aqueous samples. The SPME conditions were optimised in order to obtain maximum sensitivity. Especially, salt addition and choice of the SPME fibre coated with Carbowax–divinylbenzene increased the sensitivity. The average detection limits were in the range from 4 to 24 ng/l for the triazine herbicides, and 20 and 40 ng/l for desisopropylatrazine and desethylatrazine, respectively. The average r^2 values of the calibration curves were above 0.99 for all of the analytes. The statistical data treatment was performed in accordance with the International Standardisation Organisation (ISO) standard 5725. Relative repeatability standard deviations between 6 and 14% and relative reproducibility standard deviations between 10 and 17% were found. The determined concentrations of the reference sample compared well to the ''true'' values, thus proving the good accuracy of the method. It is concluded that SPME is a reliable technique for the quantitative analysis of water samples containing triazine herbicides in concentrations around the European limit of 100 ng/l for individual pesticides in drinking water.

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1. Introduction

Solid-phase microextraction (SPME) [1–4] combined with gas chromatography (GC) has been optimised for the analysis of a large number of organochlorine pesticides [5,6], organophosphate pesticides [7–9] and organonitrogen pesticides [7–12] in aqueous samples. These pesticides were all extracted well by the SPME fibre coated with polydimethylsiloxane or polyacrylate after appropriate optimisation, and generally detection limits in the ng/l range were obtainable. Also an inter-laboratory study was organised with the participation of eleven laboratories who analysed a reference water sample containing twelve pesticides in the range from 2 to 25 μg/l in order to examine the precision and accuracy of SPME [13]. Another inter-laboratory validation of SPME was carried out for volatile organic compounds at low μg/l levels in water [14] for the purpose of testing the only standard method using SPME [15]. Satisfactory performance in such validation studies is required in order for SPME to be accepted as a reliable alternative to the traditional methods. In both inter-laboratory studies SPME proved to be a valid technique as regards precision and accuracy. We report here the results of an inter-laboratory study concerning the analysis of triazine herbicides in which two new tasks were addressed, namely (i) the analysis at concentrations near the limit of 0.1 μg/l for individual pesticides in European drinking water [16] and (ii) the analysis of the polar metabolites of the triazine herbicides. The detection limits reported previously for triazine herbicides were in the range from 0.3 ng/l for propazine [10] to far above the 0.1 μg/l limit [11]. Typical values were in the range from 10 to 80 ng/l with mass spectrometry (MS) or nitrogen–phosphorus detection [7–9]. However, in order for the quantitation to be reliable around the 0.1 μg/l limit for drinking water, preferably, the detection limit should be 5 to 10-times below this limit for all of the analytes [17]. Therefore, firstly the sensitivity of SPME was optimised considering the effect of stirring, extraction time, salt addition, pH adjustment and choice of fibre coating material. Subsequently, a reference sample was analysed in accordance with the standardised optimum SPME conditions by ten laboratories and the repeatability, reproducibility and accuracy of the method was estimated according to the International Standardisation Organisation (ISO) standard 5725 concerning inter-laboratory statistics [18].

2. Experimental

2.1. Analytes and sample preparation

Solutions of the pure triazine herbicides (Dr. Ehrenstorfer) were prepared in methanol for spiking of mineral water from glass bottles in order to obtain samples of the desired concentrations. The reference water sample containing the herbicides at concentrations between 50 and 120 ng/l unknown to the participants was prepared in one batch by Unità Operativa Chimica, PMIP, Parabiago, Italy, and subsequently divided into 50 ml amber glass bottles for distribution to the participants. Besides this sample, 5 ml of a standard solution containing 500 μg/l of each herbicide in methanol was also distributed in amber glass vials for preparation of the calibration samples. The extractions were performed from 5 ml water samples in glass vials equipped with PTFE-lined septa. The methanol content of the water samples was minimised and never exceeded 0.5%, because it has been reported that the extraction efficiency of SPME for triazines decreases with increasing methanol content [7].

2.2. SPME conditions

Firstly, the effects of stirring, extraction time and salt addition were examined using the SPME fibre coated with 85 μm of polyacrylate (Supelco) and varying the values around those reported in earlier studies of triazine herbicides with the same type of fibre [7,8,10]. Subsequently, two new SPME fibres also coated with 65 μm of polydimethylsiloxane–divinylbenzene or Carbowax–divinylbenzene (Supelco) were tested. The fibre coated with Car-

bowax–divinylbenzene was chosen for the inter-laboratory study and a final optimisation of extraction time, salt addition and pH was carried out with this fibre. The resulting optimum conditions used in the inter-laboratory study were: extraction for 30 min at ambient temperature and neutral pH from a rapidly stirred sample containing 0.3 g/ml NaCl. The SPME was performed using the device for manual operation (Supelco).

2.3. Analytical conditions and quantitation

The analyses were carried out as soon as possible after the receipt of the reference sample. Nonetheless, in most cases the recommended maximum storage time of 7 days at 4°C [19] was exceeded. However, at neutral pH and low temperature the hydrolysis of triazine herbicides is avoided [20], and practically no biological degradation should occur in the mineral water used for the sample preparation, so the reference sample was expected to be reliable. The calibration curves for quantitation of the reference sample were based on the analysis of spiked mineral water samples containing 50, 100, 200 and 500 ng/l of each analyte. The curves were forced through origin, i.e., the intercept b in the linear expression $y=ax+b$ was set to zero, because the blank values were zero. The entire procedure was repeated on three consecutive days and the replicate results for the reference sample were reported without rounding. The desorption of the analytes from the SPME fibre was performed directly in the injection port of a gas chromatograph for 5 min at 240°C. With a split–splitless injector the split was closed immediately before the injection and kept closed during the desorption. Separation was performed using a capillary column with a 5% phenylmethylsilicone or similar stationary phase. The approximate temperature program was 50°C for 5 min followed by a rapid increase of the temperature to 150°C and then 5°C/min to 250°C. The exact conditions varied with the choice of column. Separation to baseline was achieved in all cases. For the detection a nitrogen–phosphorus detector or an ion-trap or quadrupole mass spectrometer was used. With the mass spectrometer single ion monitoring was performed and the quantitation was based on one or both of the characteristic ions listed in Table 1, i.e., target-oriented analysis was performed.

2.4. Statistical data treatment

The statistical data treatment was performed in accordance with the ISO standard 5725 [18]. A complete explanation of the statistics used for calculation of the results has been given previously [13,14,21]. The statistics are based on three assumptions, namely (i) the replicate results within a given laboratory follows the normal distribution law, (ii) the laboratory mean distribution follows the normal distribution law and (iii) the intra-laboratory variances are equal. Initially, two statistical tests are carried out for the purpose of detecting and eliminating data that do not conform with the assumptions. When the disagreement is significant at 99% confidence level the data cell, i.e., the replicate results for a given analyte in a given laboratory, is called an outlier and excluded from the further data process-

Table 1
Characteristic ions, linearity expressed as average r^2 values and detection limits

Compound	Characteristic ions	Linearity	Average detection limit (ng/l)	Lowest detection limit (ng/l)
Desisopropylatrazine	158 and 173	0.994	20	20
Desethylatrazine	172 and 187	0.993	40	20
Simazine	186 and 201	0.996	20	3.3
Atrazine	200 and 215	0.995	7.4	1.8
Propazine	214 and 229	0.995	6.8	1.5
Terbuthylazine	214 and 229	0.993	4.1	0.1
Ametryn	212 and 227	0.989	10	2.2
Prometryn	184 and 241	0.990	10	1.9
Cyanazine	225 and 240	0.992	24	7.3

ing. When the disagreement is significant at 95% confidence level only the data cell is called a straggler and left in the data set. Cochran's test addresses the assumption of equal variances. Grubb's test concerns normal distribution of the data.

3. Results and discussion

3.1. Optimisation of the SPME conditions

Most attention was dedicated to the polar metabolites and cyanazine in the optimisation of the SPME conditions, because these compounds were more difficult to extract. The best extraction efficiency was observed with the SPME fibre coated with Carbowax–divinylbenzene. Also the equilibration was faster with this fibre than with the fibre coated with polyacrylate. As reported previously [8], rapid stirring of the sample proved essential to obtain acceptable equilibration times. An alternative way would have been to perform the extraction at an elevated temperature. However, this approach would have influenced the sensitivity negatively [22], and consequently was not considered in the present study. In spite of the rapid stirring, an extraction time of 30 min was not sufficient to reach the equilibrium completely. The reason for the choice of this extraction time in the inter-laboratory study was that, preferably, as many analyses as possible should be carried out in one day. Thus, the limiting factor should be the optimised time of the chromatographic analysis rather than the total time of desorption and extraction. Furthermore, the response after 30 min extraction was around 70% of the response at equilibrium, so the sensitivity of the method would be improved only slightly by increasing the extraction time. Salt addition was of major importance for the extraction efficiency. Previously, optimum values between 50 and 100% saturation of the samples with NaCl have been reported for different triazine herbicides [7]. In the present study, the responses obtained with 0.3 g/ml NaCl (~83% saturated solution) were significantly higher for all of the analytes than with 0.2 g/ml NaCl. Upon variation of the pH from neutral to base (pH 9) the response for cyanazine decreased drastically, while no effect on the extraction of the other analytes was observed. The latter observation is in accordance with earlier results [10].

3.2. Stragglers and outliers

Two of the ten laboratories who participated in the inter-laboratory study encountered severe problems regarding linearity and repeatability in the calibration. Consequently, they were not able to perform a reliable quantitation. In the first case, the problems were caused by detection difficulties. This was also the reason why some of the remaining laboratories were not able to report results for all of the analytes. It is seen from Table 1 that the analysis of the metabolites and cyanazine was especially complicated. In the second case, apparently the problems were caused by depositing of salt on the liner of the GC injector. However, in the other laboratories such problems were not encountered or had no impact on the results. A subsequent study showed that the problem can be avoided easily by washing the fibre with clean water before inserting it into the GC injector for desorption. Theoretically, this approach might lead to some loss of analytes. However, no loss of practical importance was observed for the triazine herbicides. Cochran's statistical test showed one outlier and no stragglers, while one outlier and one straggler were found in Grubb's statistical test. In order for an inter-laboratory study to be considered reliable from a statistical point of view, preferably it should include no less than eight laboratories ($p \geq 8$), and no more than two out of nine cells should be outliers [21]. The present inter-laboratory study does not fulfil the first condition for all of the analytes (see Table 2). However, it is considered valid for all of the analytes, but desisopropylatrazine, because practically no data were eliminated, i.e., only the outliers for desethylatrazine and simazine and the straggler for cyanazine were excluded from the further data processing. Thus, the number of laboratories considered in the validation of the precision and accuracy was satisfactory for all of the analytes except desisopropylatrazine. In the case of desethylatrazine and cyanazine the number of laboratories was on the limit. The motivation for eliminating also the straggler for cyanazine, although the ISO method prescribes to leave stragglers in the data set [21], is that when basing the calculations on

Table 2
Number of participating laboratories (p) and results of the statistical data treatment expressed in ng/l

Compound	p	s_r	s_L	s_R	r	R	$\bar{x}$	CI	''True'' value
Desisopropylatrazine	1	7.4	–	–	21	–	102	[81:123]	120
Desethylatrazine	5	7.8	15	17	22	47	98	[76:120]	100
Simazine	6	7.0	1.7	7.2	20	20	54	[34:73]	50
Atrazine	6	6.3	4.5	7.7	18	22	78	[61:96]	70
Propazine	6	8.7	4.8	10	24	28	63	[38:87]	60
Terbuthylazine	7	9.5	0.6	9.5	26	27	96	[69:122]	90
Ametryn	7	10	11	15	29	42	123	[93:152]	110
Prometryn	8	12	15	19	33	55	134	[100:167]	120
Cyanazine	5	5.5	14	15	15	43	87	[71:102]	80

Repeatability standard deviation (s_r), inter-laboratory standard deviation (s_L), reproducibility standard deviation (s_R), repeatability limit (r), reproducibility limit (R), gross average ($\bar{x}$), confidence interval (CI) and ''true'' value.

only five cells ($p=5$), instead of minimum eight as prescribed, it is very difficult to obtain significance at 99% confidence level. The straggler was more than twice the average of the other results which is considerably more than the typical repeatability standard deviations.

3.3. Linearity and detection limits

The results are shown in Table 1. Only the results from the laboratories who could perform a reliable calibration in the range from 50 to 500 ng/l were considered. The detection limits were calculated on a signal-to-noise basis ($S/N=3$) from the results for the 50 ng/l sample. The linearity of SPME was good as in the previous inter-laboratory studies [13,14]. The detection limits were below the typical values reported earlier [7–9] and below the method detection limits of the Environmental Protection Agency (EPA) standard methods for triazine herbicides [9,19]. The lowest detection limits were obtained with nitrogen–phosphorus detection.

3.4. Precision

The results of the statistical data treatment are listed in Table 2. The relative repeatability standard deviations were between 6 and 14% and the relative reproducibility standard deviations were between 10 and 17%. These results are slightly better than the results of the previous inter-laboratory study for simazine and propazine [13]. The fact that each replicate analysis included a new calibration was a minor deviation from the standard definition of repeatability [15]. This implies that repeatability standard deviations might be even lower than reported.

3.5. Accuracy

The determined concentrations of the reference sample compared well to the ''true'' values which were included in the confidence intervals in all cases (see Table 2). Thus, the accuracy of the analysis of triazine herbicides by SPME has proven to be good also at ng/l level, as previously observed at μg/l level [13]. In one laboratory the reference sample was analysed by traditional solid-phase extraction as well for comparison of the techniques. The results were comparable to those obtained by SPME for all of the analytes. Previously, also SPME and liquid–liquid extraction have been compared and similar results were obtained for the analysis of triazine herbicides in groundwater samples [12]. The fact that all of the results were close to the ''true'' values confirms that the sample storage conditions were satisfactory.

4. Conclusions

Reliable quantitation could be performed at concentrations below the European limit for individual pesticides in drinking water, the precision was satisfactory for most routine analysis, and the accuracy was good in all cases. Thus, SPME is a valid alternative to the traditional methods for analysis of triazine herbicides in drinking water. Furthermore,

also the analysis of surface and ground water samples may be feasible considering that humic acids at concentrations typical for such waters have been reported to interfere little with the SPME process [8,23].

References

[1] D. Louch, S. Motlagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[2] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[3] J. Pawliszyn, Trends Anal. Chem. 14 (1995) 113.
[4] R. Eisert, K. Levsen, J. Chromatogr. A 733 (1996) 143.
[5] S. Magdic, J. Pawliszyn, J. Chromatogr. A 723 (1996) 111.
[6] B.D. Page, G. Lacroix, J. Chromatogr. A 757 (1997) 173.
[7] R. Eisert, K. Levsen, Fresenius J. Anal. Chem. 351 (1995) 555.
[8] R. Eisert, K. Levsen, J. Am. Soc. Mass Spectrom. 6 (1995) 1119.
[9] T.K. Choudhury, K.O. Gerhardt, T.P. Mawhinney, Environ. Sci. Technol. 30 (1996) 3259.
[10] A.A. Boyd-Boland, J. Pawliszyn, J. Chromatogr. A 704 (1995) 163.
[11] I.J. Barnabas, J.R. Dean, I.A. Fowlis, S.P. Owen, J. Chromatogr. A 705 (1995) 305.
[12] W. Bechmann, P. Volkmer, GIT Fachz. Lab. 39 (1995) 1129.
[13] T. Górecki, R. Mindrup, J. Pawliszyn, Analyst 121 (1996) 1381.
[14] T. Nilsson, R. Ferrari, S. Facchetti, Anal. Chim. Acta, in press.
[15] UNICHIM 1210-1997, Qualità dell'acqua, Determinazione di Idrocarburi e Idrocarburi Alogenati Volatili, Metodo per Microestazione su Fase Solida (SPME) e Gascromatografia Capillare, Unichim, Milan, 1997.
[16] M. Fielding (Ed.), Water Pollution Research Report 27, Pesticides in Ground and Drinking Water, Commission of the European Communities, Brussels, 1991.
[17] J. Lindstedt, Metal Finishing 91 (1993) 64.
[18] ISO 5725-1994, Accuracy (Trueness and Precision) of Measurement Methods and Results, International Standardisation Organisation, Geneva, 1994.
[19] C.J. Koester, R.E. Clement, Crit. Rev. Anal. Chem. 24 (1993) 263.
[20] C.R. Worthing (Ed.), The Pesticide Manual, A World Compendium, British Crop Protection Council, Thornton Heath, 8th ed., 1987.
[21] M. Feinberg, Trends Anal. Chem. 14 (1995) 450.
[22] T. Nilsson, F. Pelusio, L. Montanarella, B. Larsen, S. Facchetti, J.Ø. Madsen, J. High Resolut. Chromatogr. 18 (1995) 617.
[23] T. Nilsson, L. Montanarella, D. Baglio, R. Tilio, G. Bidoglio, S. Facchetti, Int. J. Environ. Anal. Chem., (1997) in press.