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Short communication

Determination of atrazine and simazine in drinking and surface waters by solid-phase extraction and high performance thin layer chromatography

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Abstract

A simple high-performance thin layer chromatographic (HPTLC) method has been developed for the determination of atrazine and simazine herbicides in drinking and surface waters. The method involves solid-phase extraction on C_{18} Bakerbond cartridges followed by development of the concentrated extracts on HPTLC silica plates with a nitromethane–tetrachloromethane (1:1, v/v) mobile phase and quantitation by UV scanning densitometry. Using the proposed mobile phase composition, pronounced background suppression on the chromatograms of the real water samples was accomplished. The detection limits of the method were 30 and 60 ng/l for atrazine and simazine, respectively, at the 80–400 ng/l fortification level in the surface waters. The method was successfully applied to the analyses of tap and surface waters with overall recoveries between 58 and 93% and a relative standard deviation below 12%. The results show, that the HPTLC method is sufficiently selective and sensitive to be employed in screening of contaminated waters containing the triazines below the maximum residues limits of the European Community.

1. Introduction

Triazine herbicides such as atrazine and simazine have been extensively applied in agriculture over the last three decades. Triazines are usually used for pre- and post-emergent control of broadleaf and grassy weeds in corn, soybeans and other field crops. As a result of the extensive application, the herbicides may contaminate crops and also drinking, surface and ground waters. In European countries, the maximum residue limit (MRL) of individual pesticide in drinking water is 100 ng/l. Routine analysis of large series of samples is therefore needed to monitor environmental pollution and satisfy the regulatory requirements.

A number of sample processing and quantitation schedules has been used for the determination of triazines including thin-layer chromatography (TLC) [1–5]. Up to now, application of TLC in the field of pesticide residue analysis has been limited, particularly because of its lack of its selectivity [6]. Since the 1960s, various approaches have been proposed in order to over-

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come this problem, including post-chromatographic labeling of the pesticide spots [2–4], the use of HPTLC layers precoated with modified hydrophilic surfaces [5] or automated multiple wavelength detection [6,7]. In this paper, a simple HPTLC method is described for the determination of atrazine and simazine residues in tap and surface waters. The method is based on solid-phase extraction (SPE) with C_{18} cartridges followed by elution of the extracts on the fluorescently labeled silica HPTLC plates with a mobile phase allowing sensitive and sufficiently selective detection of the pesticide spots by UV scanning densitometry.

2. Material and methods

2.1. Chemicals

Methanol, tetrachloromethane, chloroform, acetone, ethyl acetate and anhydrous sodium sulfate were purchased from Lachema (Brno, Czech Republic); dichloromethane, toluene, hexane and isooctane from Fluka (Buchs, Switzerland); nitromethane from P.P.H. Polskie Odczynniki Chemiczne (Gliwice, Poland); and [$^2H_{10}$]anthracene from Aldrich (Milwaukee, WI, USA). Atrazine and simazine were obtained from Supelco (Gland, Switzerland). All the chemicals and solvents used were of analytical grade. Methanol, toluene and water were redistilled in glass prior to use.

2.2. Standard solutions

Working calibration standards of each triazine were prepared by serial dilution from the individual stock solutions (1 mg/ml) in methanol and used for spiking of water samples and formation of calibration plots.

2.3. Samples

Surface water samples were obtained from Římov and Orlík water reservoirs located in south and central Bohemia (Czech Republic), respectively. Drinking water samples originating from Římov water reservoir were collected from the municipal water supply in the authors' laboratories. The samples were taken in February–June 1992 and stored in 2-l glass bottles rinsed with Nanopure, deionized water prior to sampling.

2.4. SPE and HPTLC equipments

Bakerbond spe octadecyl 6-ml cartridges (1000 mg) and a vacuum manifold Baker SPE 10 column processor system from J.T. Baker (Gross-Gerau, Germany) were used for extraction of the pesticides from water samples. HPTLC precoated silica gel 60 F_{254} plates, 10 × 10 cm, article No. 5628, series 19760707 (Merck, Darmstadt, Germany) and HPTLC Nano-Plates SIL-20 UV 254, 10 × 10 cm, article No. 811 022, charge 11.88 from Macherey–Nagel (Düren, Germany) were used for chromatography. The samples were applied on the plates with a Linomat IV applicator from Camag (Muttenz, Switzerland). A Camag horizontal developing chamber and a Camag TLC Scanner II densitometer were used for the elution and detection of the pesticides. The plates were scanned in the reflectance mode at 220 nm. Data were processed with a SP 4270 integrator (Spectra-Physics, Darmstadt, Germany).

2.5. SPE

Atrazine and simazine were removed from water samples by using Bakerbond C_{18} SPE cartridges according to a procedure similar to that described elsewhere [8]. Shortly, each sample was split in six 250-ml aliquots; four of these were fortified with two known concentrations of each triazine in the range of 80–400 ng/l. The cartridges were conditioned with 2 × 6 ml of methanol followed by 2 × 6 ml of redistilled water. Each sample aliquot was then aspirated through the preconditioned cartridge at a flow-rate of 8.3 ml/min. The cartridges were further washed with 3 × 6 ml of redistilled water, 3 × 6 ml of 5% aqueous methanol and dried under vacuum for 15 min. Triazines were desorbed from the cartridges with 4 × 500 μl of methanol into 4-ml screw vials. The eluate was evaporated to dryness under a stream of nitrogen on a water

bath at 45°C, redissolved in 200 μl of methanol and used for HPTLC and/or GC–MS analysis.

2.6. HPTLC analysis

A 40-μl volume of each SPE methanolic extract was applied by means of the Linomat IV applicator in 3-mm strips at a rate of 8 s per μl on the HPTLC plates. The samples and the triazine standards were applied alternatively at 2-mm intervals to both halves of the plates, 18 strips on each side, 8 mm from the edge. The calibration was based on the peak heights obtained from densitometric responses of the standards applied on each plate. The plates were developed using an appropriate eluent on the 45-mm developing path at ambient temperature (25°C). The spots were dried under a stream of air and scanned immediately. The conditions for the quantitative evaluation were: deuterium lamp wavelength, 220 nm; monochromator bandwidth, 30 nm; slit width, 0.4 mm; slit length, 2 mm; scanning speed 1 cm/min.

2.7. GC–MS analysis

Two aliquots of each water sample processed by the SPE procedure, a spiked and an untreated one, were subjected to the GC–MS analysis. A 80-μl volume of each methanolic SPE extract was evaporated to dryness under a mild stream of nitrogen and redissolved in 100 μl of toluene, containing [$^2H_{10}$]anthracene as an internal standard (2 ng/μl). Analyte identity in the water samples was verified by GC–MS on a 30 m DB-5 column using a Finnigan MAT ion trap detector as described by Pereira et al. [9]. The detection limit of the GC–MS method ($S/N = 5$) was in our hands about 10 ng/l for both triazines.

3. Results and discussion

In order to meet criteria of the pesticide MRLs, conventional SPE in combination with chromatographic techniques, such as HPLC [10–14], was utilized prior to the HPTLC analysis. Optimization of the HPTLC step was primarily focused on two factors which showed to be critical in preliminary experiments; the mobile phase composition and performance of the HPTLC plates. A series of solvent mixture eluents was evaluated in terms of selectivity. R_F values of atrazine and simazine calculated for the examined mobile phase compositions are summarized in Table 1. The triazines can easily be separated on the HPTLC silica gel plates with most tested eluents, which is consistent with the results reported by other authors [2]. Moreover, using UV scanning densitometry triazine spots were easily detected at the low nanogram level, corresponding to 100 ng/l of each herbicide in a water sample. The results were less satisfactory when spiked real water samples were subjected to the analysis; this was evidently due to the background effects. Fortunately, as only small amounts of mobile phase were needed (typically 4.8 ml for up to 18 analysis runs available on a HPTLC plate), rather uncommon solvents could be used as eluents for the selectivity adjustment. We found nitromethane–tetrachloromethane (50:50, v/v) the best elution system leaving impurities mostly on the start and front positions of the eluent, which resulted S/N values approximately 5 times higher than those achieved with other tested eluents. For calculations of sensitivity (S = peak height/ng of each standard on the HPTLC plate) and detectability [16] ($D = 2 \times$ noise/S), six spiked surface water samples, obtained from the Římov water reservoir and preliminary checked to be free of triazines by GC–MS, was analysed by the developed method. The results, summarized in Table 2, show detection limits of the method ($S/N = 2$) to be 30 and 60 ng/l for atrazine and simazine, respectively.

Performance of the HPTLC plates was found to be another critical factor considerably influencing the HPTLC analysis at the MRL concentration level. With a batch of Merck HPTLC silica gel plates sensitivity and selectivity obtained from densitometric detection was acceptable for trace analysis of both triazines in water samples. However, when a similar batch of the Macherey–Nagel HPTLC silica gel plates was examined, the results were less satisfactory. This

Table 1
R_F values of atrazine and simazine obtained with the 12 tested mobile phases on the Merck silica gel 60 F_{254} plate by UV scanning densitometry

Mobile phase	R_F		Ref.[a]
	Atrazine	Simazine	
$CHCl_3$–MeOH (80:20, v/v)	0	0	[15]
$CHCl_3$–acetone (90:10, v/v)	0	0	[2]
Hexane–MeOH (90:10, v/v)	0.09	0.06	[3]
Toluene–acetone (85:15, v/v)	0	0	[4]
$CHCl_3$–MeOH–water (97:2:1, v/v)	0.53	0.43	
$CHCl_3$–MeOH–water (97:2.5:0.5, v/v)	0.80	0.69	
$CHCl_3$–MeOH–water (96.5:2.5:1, v/v)	0.57	0.51	
$CHCl_3$–MeOH–EtAc[b]–water (97:2:0.5:0.5, v/v)	0.73	0.63	
CH_2Cl_2–MeOH–water (97:2.5:0.5, v/v)	0.51	0.44	
CH_2Cl_2–MeOH–water (97:2:1, v/v)	0.43	0.36	
Nitromethane–$CHCl_3$ (50:50, v/v)	0.58	0.45	[2]
Nitromethane–CCl_4 (50:50, v/v)	0.61	0.47	[2]

[a] References, where the same solvents were employed for the elution of the triazines on TLC silica gel plates.
[b] EtAc = Ethyl acetate.

is illustrated in Fig. 1, where densitograms of 15 ng of atrazine and simazine applied on these two HPTLC silica gel plates are depicted. As the silica gel layer on the Merck HPTLC plates proved also to be more compact and mechanically resistant to manipulation, only these plates were further evaluated for quantitative analysis.

3.1. HPTLC calibration curves

Calibration curves exhibited good linearity on the Merck HPTLC precoated silica gel plates in the range of 2–45 ng for both triazines. Typical linear regression equations calculated from the densitometric peak height measurements were $y = 3.504 \times + 3.604$ ($r^2 = 0.999$) for atrazine and $y = 1.803 \times + 0.576$ ($r^2 = 0.999$) for simazine.

The calibration curves were found to be non-linear in the range 50–250 ng.

3.2. Quantitation of atrazine and simazine in real water samples

The proposed HPTLC method was further evaluated by determining atrazine and simazine levels in tap and surface waters collected from various water reservoirs in central and south Bohemia. The overall recoveries of atrazine and simazine from real water samples and their relative standard deviations (R.S.D.) are sum-

Table 2
Sensitivity, detectability, detection limits (DLs) and precision of the HPTLC method calculated from the determination of atrazine and simazine in six surface water samples fortified at the 100 ng/l level

Analyte	Sensitivity (mm/ng)	Detectability (ng/spot)	DL (ng/l)	R.S.D.[a] (%)
Atrazine	3.8	2	30	3
Simazine	1.9	3	60	4

Each sample obtained from the Římov water reservoir in March 1992 was analysed in triplicate.
[a] Relative standard deviation of the densitometric peak height measurement.

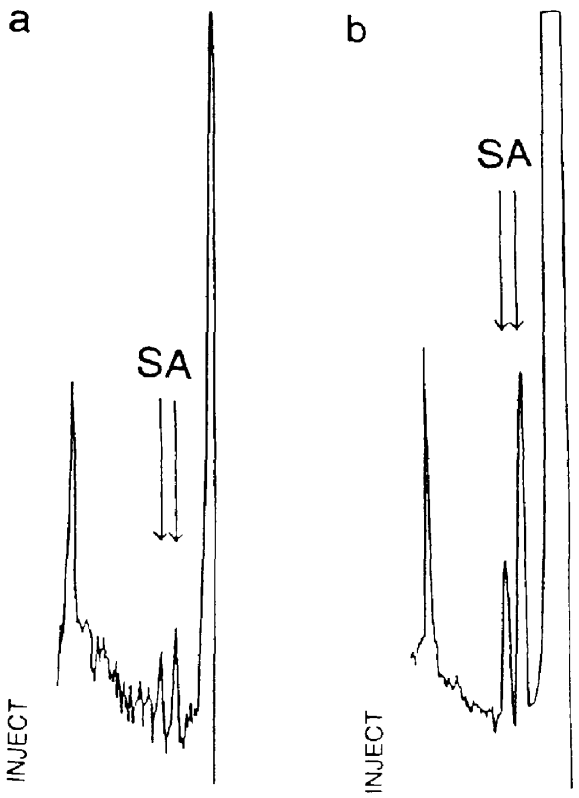

Fig. 1. Densitograms of 15 ng of atrazine (peak A) and simazine (peak S) obtained by UV scanning densitometry on the Macherey–Nagel (a) and Merck (b) HPTLC silica gel plates. Mobile phase: nitromethane–tetrachloromethane (1:1, v/v); 220 nm.

marized in Table 3. The results are satisfactory showing recoveries in the range of 58–93% and R.S.D. values not exceeding 12%. Rather varying recoveries in some cases may be explained by occurrence of the herbicides below the detection limit of the method that could influence recovery calculations on the 100 ng/l concentration level.

Typical densitograms obtained from a HPTLC analysis of a surface water sample from the Římov water reservoir and the same sample spiked with each triazine at 400 ng/l level (sample 8 in Table 3) are shown in Fig. 2a and b, respectively. Although the water samples were collected in Spring and early Summer period, when pollution of water reservoirs in central Europe from run-off waters is most serious, the amounts of atrazine and simazine in all tap and natural waters were found below the detection limit of the HPTLC method as well as below the legal tolerance levels. This knowledge was further checked by the GC–MS method, used as a confirmatory technique. The use of another independent method such as GC–MS is essential in cases when spots corresponding to atrazine and simazine are present on the densitograms in order to avoid false positive results. Using GC–MS only traces of atrazine (at 15 ng/l) were found in samples collected from the Orlík water reservoir in April 1992.

Sample throughput of the HPTLC method is high because 36 analysis runs can be performed simultaneously on each HPTLC plate. The time of analysis is limited by the SPE procedure; particularly by the capacity of the vacuum manifold column processor and sample evaporation

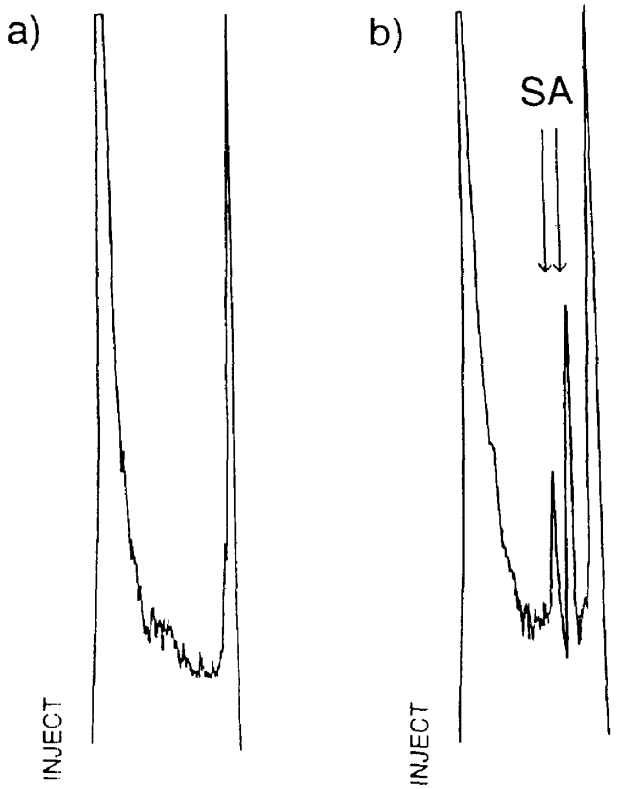

Fig. 2. Densitograms of (a) the SPE extract obtained from the Římov water reservoir collected 13 April 1992 and (b) the SPE extract of the same sample aliquot spiked with each triazine at the 400 ng/l level.

Table 3
Mean recovery and R.S.D. of atrazine and simazine in real tap and surface water samples

Water sample	No.	Date[a]	Atrazine			Simazine		
			Spike[b] (ng/l)	Recovery (%)	R.S.D. (%)	Spike[b] (ng/l)	Recovery (%)	R.S.D. (%)
Tap water	1	19 February 1992	80	93	4	80	79	4
	2	19 February 1992	200	85	3	200	70	3
Římov reservoir	3	19 February 1992	120	77	8	120	67	10
	4	19 February 1992	240	81	4	240	58	4
	5	20 March 1992	120	79	8	120	84	9
	6	20 March 1992	240	64	6	240	62	7
	7	13 April 1992	200	72	8	200	70	8
	8	13 April 1992	400	62	5	400	65	6
	9	15 June 1992	200	93	5	200	74	5
	10	15 June 1992	400	88	6	400	79	7
Orlík reservoir	11	23 April 1992	200	62	8	200	65	12
	12	23 April 1992	400	73	7	400	72	7
	13	9 June 1992	200	84	9	200	88	9
	14	9 June 1992	400	62	10	400	66	10

The triazines were detected only in the spiked samples. Each value is the average from the analysis of four replicates ($n = 4$).
[a] Date of the water sample collection.
[b] Spiking sample level.

after desorption of analytes from the SPE column. Considering the HPTLC step only, which involves application of six standards for calibration, application of a sample and the corresponding spike, development of the spots, densitometric scanning, and integrator data processing, 15 water sample extracts can be analysed on each HPTLC plate in 2 h; i.e. complete quantitation of 60 water samples can be achieved by the proposed HPTLC method within 8 working hours.

4. Conclusions

The proposed HPTLC method is sufficiently selective and sensitive for determination of atrazine and simazine in drinking and surface waters below the MRLs established in the European Community. Due to its performance, particularly high sample throughput, the method seems to be suitable for routine screening of contaminated waters, reducing the use of more powerful and expensive methods such as GC–MS for confirmatory purposes.

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References

[1] V.N. Kaveckii, L.I. Bublik and G.V. Fuzik, *Zh. Anal. Khim.*, 42 (1987) 1302–1304.
[2] D.C. Abbott, J.A. Bunting and J. Thomson, *Analyst*, 90 (1965) 356–361.
[3] J. Sherma, *J. Liq. Chromatogr.*, 11 (1986) 2121–2130.
[4] G. Szökely, P. Weick and B. Abt, in P. Henschel and P.G. Lauberean (Editors), *Proceedings of a Workshop organized within the framework of Working Party 1 of the Concerted Action "Organic Micropollutants in the Aquatic Environment" COST 641, Berlin (West), 16–17 March 1989*, Guyot, Bruxelles, 1989, pp. 102–110.

[5] H.-E. Hauck, M. Mack, S. Reuke and H. Herbert, *J. Planar Chromatogr.*, 2 (1989) 268–275.
[6] K. Burger, *Fresenius' Z. Anal. Chem.*, 318 (1984) 228.
[7] U. de la Vigne and D.E. Jänchen, *J. Chromatogr.*, 553 (1991) 489–496.
[8] *Bakerbond Application Note EN-022*, J.T. Baker, Gross-Gerau, 1991.
[9] W.E. Pereira, C.E. Rostad and T.J. Leiker, *Anal. Chim. Acta*, 228 (1990) 69–75.
[10] M. Battista, A. Di Corcia and M. Marchetti, *Anal. Chem.*, 61 (1989) 935–939.
[11] A. Di Corcia, M. Marchetti and R. Samperi, *J. Chromatogr.*, 405 (1987) 345.
[12] R.J. Bushway, L.B. Perkins, L. Fukal, R.O. Harrison and B.S. Fergusson, *Arch. Environ. Contam. Toxicol.*, 21 (1990) 365–370.
[13] M. Stahl, M. Lührmann, H.-G. Kicinski and A. Kettrup, *Z. Wasser-Abwasser-Forsch.*, 22 (1989) 124–127.
[14] C. Schlett, *Fresenius' J. Anal. Chem.*, 339 (1991) 344–347.
[15] R.W. Frei, N.S. Nomura and M.M. Frodyma, *Microchim. Acta*, 6 (1967) 1099–1104.
[16] C.F. Poole, M.E. Coddens, H.T. Butler, S.A. Schuette, S.S.J. Ho, S. Khatib, L. Piet and K.K. Brown, *J. Liq. Chromatogr.*, 8 (1985) 2875–2926.