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Review

High-performance separations in the determination of triazine herbicides and their residues

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Abstract

The present state of analysis for triazine herbicides and their residues is critically surveyed. After a brief introduction, summarizing the properties of triazines and the history of the determination of the parent compounds and their residues, attention is primarily paid to preconcentration techniques for water and soil samples, such as liquid–liquid, supercritical-fluid and solid-phase extraction, and modern separation methods employed for the actual determination, e.g., gas and liquid chromatography, especially in combination with selective detection, capillary electrophoresis and, to a lesser extent, thin-layer chromatography. Important immunoanalytical procedures are also briefly discussed.

Keywords: Reviews; Environmental analysis; Extraction methods; Triazines; Pesticides

Contents

1. Introduction

Triazine herbicides form a wide group of substances that belong among the most common agrochemicals applied to pre- and post-emergence weed control (about 30% of all agricultural herbicides are triazines). The history of their use is quite long, starting in 1952 when J.R. Geigy S.A. synthesized and screened the first triazine derivatives [1]. Since that time, the spectrum of commercially available

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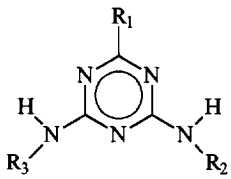
triazines has widened greatly. Simultaneously, the approach to pesticides, including triazines, has considerably changed over the years — whereas Ref. [1], published in 1970, primarily discusses the properties of triazines and their residues from the point of view of their herbicidal efficiency and the economic aspects of agriculture, at present we are mainly aware of the ecological and health hazards of their use. This is reflected in the fact that the use of some triazine pesticides has been banned in certain countries (e.g., atrazine in Germany in 1991, further prohibiting the use of terbutylazine in water-protection areas) and that their permitted levels in potable waters are of the order of 0.1 μg/l [2]. All this places great demands on analytical chemistry, as systematic monitoring of pesticides and their residues produced by a combination of hydrolytic, photochemical and microbial degradation processes, is required in complex matrices of soil, water and biological samples, at analyte levels in the subnanogram range (see e.g. Refs. [3–6]).

A great majority of triazine herbicides are derived from *s*-triazine, a six-membered heterocycle with symmetrically located nitrogen atoms,



that is substituted in positions 2, 4 and 6. Unsubstituted *s*-triazine is somewhat similar to benzene, however, its stability is lower because the perfectly delocalized π-bond system of benzene is disturbed by the introduction of nitrogen atoms to positions 1, 3 and 5, with a subsequent increase in the electron density in these positions and a corresponding decrease in the electron density in positions 2, 4 and 6; nucleophilic substitution in the latter positions is thus facilitated. Nevertheless, the stereochemical stability of *s*-triazines is still large and the persistence of triazine pesticides and their degradation products in soils, waters and plant and animal materials is considerable, from several months for the parent compounds to many years for their degradation products, which are often even more toxic.

The physico-chemical properties of *s*-triazine derivatives are primarily determined by the substituent in position 2; this is most often -Cl (the commercial names ending with -azine), -SCH_3 (-tryn) and -OCH_3 (-ton). The properties of chloroderivatives considerably differ from those of the other two types, whereas the thiomethyl- and methoxy-derivatives behave very similarly. Positions 4 and 6 are usually occupied by substituted amino groups and exert substantially smaller effects on the derivative properties.

A list of common *s*-triazine derivatives and some of their properties are given in Table 1 [7–10] and a few toxicity levels are listed in Table 2 [11].

As can be seen, *s*-triazines are weakly basic, poorly soluble compounds of a low polarity, stable both in the solid phase and in solution. The basicity increases with the order of substituents in position 2 of Cl<SCH_3<OCH_3; the substituents in positions 4 and 6 affect the basicity to a lesser degree, but the basicity increases with an increasing number of hydrogen atoms in the substituted amino group and with increasing length and branching of the alkyl chain. The solubility in water increases with increasing acidity, due to protonation. Hydrolysis occurs in strongly acidic and basic solutions, especially at elevated temperatures; hydroxy derivatives are formed but the *s*-triazine nucleus is preserved. A great volume of useful data on the behaviour of *s*-triazine derivatives can be found in Ref. [1], especially those on interactions with the soil components, persistence and methods of degradation; of course, the contents are dated in many places. More recent information, especially on degradation, can be found in Ref. [12].

It should be pointed out that some triazine herbicides (e.g., metribuzine and metamitron) are based on non-symmetrical 1,2,4-triazine. Basically, their properties are similar to those of *s*-triazines, but their molecules are somewhat more polar, as reflected, e.g. in a lower value of log P_{oct} (partition coefficient between octanol and water) (1.65), compared with atrazine (2.7) [13].

Analytical methods for the determination of triazine herbicides and their degradation products must then be chosen on one hand on the basis of the requirements, i.e., trace or ultratrace determinations of multiple analytes in complex matrices, and, on the other hand, on the physico-(bio)chemical properties available for measurement. The latter involve the

Table 1
Selected characteristics of some *s*-triazine herbicides and their derivatives [7–10]

Compound	Substituents			pK_a	Absorption maxima and the corresponding absorption coefficients				Partition coefficient between octanol and water and water
	R_1	R_2	R_3		λ_1 nm	ϵ_1 1 mol^{-1} cm^{-1}	λ_2 nm	ϵ_2 1 mol^{-1} cm^{-1}	log $P_{oct/w}$
Simazine	Cl	NHC_2H_5	NHC_2H_5	1.65	222	36 000	263	3100	2.3
Atrazine	Cl	NHC_2H_5	$NHCH(CH_3)_2$	1.68	222	41 000	263	3900	2.7
Propazine	Cl	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	1.85	221	32 000	268	3100	2.91
Terbutylazine	Cl	NHC_2H_5	$NHC(CH_3)_3$	1.94	223	19 500	263	1800	3.06
Trietazine	Cl	NHC_2H_5	$N(C_2H_5)_2$	1.88	227	44 300	267	4300	3.07
Ipazine	Cl	$N(C_2H_5)_2$	$NHCH(CH_3)_2$	1.85	228	43 100	266	4300	
Deethylatrazine	Cl	NH_2	$NHCH(CH_3)_2$	1.3					1.6
Deisopropylatrazine	Cl	NHC_2H_5	NH_2	1.3					1.2
Deethyldeisopropylatrazine	Cl	NH_2	NH_2	1.5					0
Hydroxysimazine	OH	NHC_2H_5	NHC_2H_5		218				
Hydroxyatrazine	OH	NHC_2H_5	$NHCH(CH_3)_2$	5.15	218				1.4
Hydroxypropazine	OH	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	5.2	217				
Hydroxydeethylatrazine	OH	NH_2	$NHCH(CH_3)_2$	4.75	212				0.2
Hydroxydeisopropylatrazine	OH	NHC_2H_5	NH_2	4.65	213				−0.1
Simeton	OCH_3	NHC_2H_5	NHC_2H_5	4.15	222	39 300			
Atraton	OCH_3	NHC_2H_5	$NHCH(CH_3)_2$	4.2	217				2.69
Desmetryn	SCH_3	$NHCH_3$	$NHCH(CH_3)_2$	3.93	221	33 700			
Simetryn	SCH_3	NHC_2H_5	NHC_2H_5	4.0	222	44 400			2.8
Ametryn	SCH_3	NHC_2H_5	$NHCH(CH_3)_2$	4.0	222	40 000			3.07
Prometryn	SCH_3	$NHCH(CH_3)_2$	$NHCH(CH_3)_2$	4.05	223	42 000			3.34
Terbutryn	SCH_3	NHC_2H_5	$NHC(CH_3)_3$	4.4	223	21 200			3.74

absorbance in the UV range and the electrical current produced by the reduction or oxidation of the substances and, of course, much more selective parameters, such as mass spectra, immunological interactions, or selective interactions of the nitrogen heteroatoms in a N–P detector.

Most triazines exhibit absorption maxima in aqueous solutions around 220 to 225 and/or 255 nm, the magnitude of which is affected by the acidity of the solution, while their hydroxy derivatives absorb at lower wavelengths (around 215 nm) (Table 1) (Ref. [1], p. 373; [14,15]). In methanol, in which triazines are much more soluble, single maxima appear of methoxy- and thiomethyl-derivatives at 219–230 nm (ϵ of ca. 10^3), while chloroderivatives also exhibit another maximum at 263–270 nm (ϵ ca. 10^3) [15]. Triazines are polarographically reducible at potentials below −1.0 V (Ag/AgCl), the reduction involving two electrons in acidic solutions and being kinetically controlled by protonation at pH values above 2 [16]; they can also be oxidized, e.g. at potentials somewhat below +2.0 V (Ag/AgCl) in acetonitrile [14]. Hydroxy derivatives, commonly produced by degradation processes, are readily oxidized in aqueous solutions, at potentials close to +1.0 V (Ag/AgCl) [7].

Photolysis of triazine herbicides has been studied (e.g. Refs. [7,17,18]). It has been found that the

Table 2
Toxicity of selected triazines [11]

Triazine	LD_{50} for rats (mg/kg)
Atrazine	1869–3080
Metamitron	3343
Metribuzin	2200–2345
Propazine	>7700
Simazine	>5000
Terbutylazine	2000
Terbutryn	2000

presence of humic material influences the photodegradation pathway [17,19] and accelerates the photodegradation rate. In aqueous solutions without humic acids, only hydroxy derivatives are formed [19], while in humic media the dealkylation reactions also occur [17]. The photolysis rate strongly depends on the substituent in position 2 and decreases in the order methoxy<chloro<thiomethyl. A comparison of the photolysis of desmetryn, ametryn and prometryn, i.e., triazines differing only in the substituent in position 4, indicates that the photolysis rate is independent of the alkyl bond. The photolysis rate decreases with decreasing pH for atrazine and ametryn and is independent of the pH for atraton [7].

The nature of the samples necessitates the use of preconcentration, clean-up and separation techniques; the systems employed must take into account that the test substances involve groups of substances of low polarity (parent triazines) and more polar compounds (their degradation products), whose behaviour depends on the acid–base conditions. Therefore, various extraction methods are suited for matrix separation and analyte preconcentration, followed by clean-up and GC, HPLC and CE procedures with various detection techniques and their combinations for the actual determination. Furthermore, immunochemical techniques are rapidly gaining in importance.

These current methods are still sometimes accompanied, especially in less-equipped laboratories, by methods common in the earlier history of triazine herbicides, namely, thin-layer chromatographic procedures that must be considered only semi-quantitative and by spectrophotometric and electrochemical procedures, whose sensitivity and selectivity are rarely up to the present requirements. Even the very popular gas chromatography, that has been used for a much longer time than have HPLC and CE, has serious shortcomings, with difficulties primarily with polar analytes, i.e., with triazine degradation products.

Nevertheless, even simple spectrophotometric measurements may still be useful in certain cases, especially when using modern measuring and chemometric approaches for the analysis of pesticides of different chemical classes. For example, atrazine and diuron were simultaneously determined at a ppb level in pesticide formulations, soils and waters employing derivative spectrophotometry [20], or three herbicides including atrazine were simultaneously quantitated by spectrophotometry with multivariate calibration optimized with the use of principal component and partial least squares procedures [21]. Specialized selective determinations can also be carried out electrochemically when using, e.g., adsorptive stripping procedures [22,23]; electrochemistry is also useful in following the interactions of triazines and their degradation products with water or soil matrices [24]. For a recent survey of the possibilities of electrochemical stripping methods see, e.g., Ref. [25].

Below, combinations of efficient preconcentration and separation determinations of triazine herbicides and their degradation products are discussed. Immunochemical methods are also treated, in view of their importance and the role often played by separations in these procedures, but the treatment is brief, as the principles are outside the scope of this review. The literature on triazines is very voluminous; therefore, we refer only to a selection of important recent publications. Further references can be found in the works cited.

2. Preconcentration of triazines

Analyses of triazines and their residues in water, soil and biological materials are usually only a part of more complex multiresidual analyses; therefore, the sample matrix is very complex, not only in major components, both organic and inorganic, but also in trace and ultratrace components. Isolation and preconcentration procedures are thus almost always necessary. The selection of the procedure depends on the sample character, the presence of further trace compounds and the concentration of the analytes. The most common techniques are liquid–liquid, supercritical-fluid and solid-phase extraction, variously combined with subsequent separation methods, either off- or on-line.

The selectivity of these clean-up techniques varies widely — some simple extraction and adsorption procedures are very poorly selective and their main effect lies in the analyte preconcentration; on the other hand, certain modified adsorbents and chemi-

cally bonded stationary phases exhibit considerable selectivity that can be tuned appropriately by judicious choice of experimental conditions. In general, inorganic sample components that might strongly interfere, e.g. in spectroscopic (especially in mass spectroscopic) detection and also in some reversed-phase HPLC separations, are usually removed during the clean-up procedures. Very large organic molecules are often at least partially removed. The remaining mixture is still complex and must be adequately handled by the actual analytical separation procedure.

2.1. Liquid–liquid extraction

Liquid–liquid extraction (LLE) has a very long history and does not require special instrumentation and materials; these are the main reasons for which it is employed in most methods for the determination of pesticides, including official ones (e.g. Refs. [26–31]), rather than much more recent methods of supercritical-fluid extraction (SFE) and solid-phase extraction (SPE). LLE has an advantage in a great variety of extraction systems available which have been thoroughly studied theoretically, optimized and widely tested in practice and offers many possibilities for fine-tuning of the extraction efficiency and selectivity by variation of the experimental conditions. However, it also has several serious drawbacks, for which it is gradually being replaced by SFE and SPE, namely [32]:

1. The extraction yield is often insufficient and another preconcentration step must be included prior to actual analysis;
2. Phase separation is often complicated by the formation of emulsions;
3. Large volumes of solvents are required;
4. The extraction procedure tends to be tedious, slow and difficult to automate;
5. The extraction efficiencies for individual triazine derivatives differ, which greatly complicates quantitation.

Dichloromethane was commonly used as the extraction agent (e.g. Ref. [29]), but its replacement by a mixture of ethyl acetate and cyclohexane (1:1) [33] has recently been recommended for toxicological and ecological purposes. The recovery for simazine, atrazine and terbutylazine from plant material has been determined with this method and ranges from 88 to 105%.

The extraction is usually followed by a clean-up step. Silica gel and alumina columns have been used for this purpose, separating the solutes according to their polarities, or gel-permeation columns have been employed, distributing the solutes according to their size (e.g. Refs. [31,34]).

A combination of extraction into light petroleum–ethyl acetate (1:1, v/v) and a clean-up procedure has been applied to the determination of *s*-triazines and other xenobiotics in fish [34]. Triazine degradation products are usually extracted from soils and sediments in acidic media, using water–methanol solutions [35]. Extraction of atrazine and its degradation products from soil, followed by concentration of the aqueous extract through lyophilization, has been used prior to HPLC analysis [36]. The non-symmetrical triazine, metribuzin, has been extracted from soil into methanol–0.01 *M* $CaCl_2$ (4:1, v/v), followed by a SPE preconcentration step [37].

Although most extractions are carried out off-line, an on-line LLE of *s*-triazines from oil with membrane barriers followed by flow injection analysis (FIA) or HPLC has also been described [38].

2.2. Supercritical-fluid extraction

SFE is a very efficient extraction method that is primarily applicable to solid samples. Liquid samples can also be extracted with supercritical fluids, but must first be immobilized on solid sorbents. Membranes have also been used to immobilize liquid samples in flow systems, but so far this method has been applied only to substances other than triazines.

The advantages of SFE involve the high extraction efficiencies attained and good selectivity, short extraction times, simple preconcentration steps and a great reduction in the volumes of toxic and environmentally hazardous solvents used [39–41]. However, some advantages claimed have yet to be proved, namely, a reduction in the cost of analyses and the feasibility of on-line coupling with chromatographic techniques for routine use.

There are also serious drawbacks:

1. The choice of extraction system is very limited; in fact, carbon dioxide with certain modifiers is virtually the only system that is routinely used;
2. The selection and optimization of the experimental conditions are difficult and still largely empirical;
3. The maintenance of constant experimental conditions during the procedure is demanding and the measurement may suffer from high blank and noise levels;
4. The procedures involving intermediate washing and drying steps may be rather lengthy.

The extraction efficiency for triazines directly depends on their solubility in the supercritical fluid and the latter in turn depends on the fluid density, i.e., on the experimental pressure and temperature, the triazine vapour pressure and on the kind and content of modifier(s). A modifier can be mixed with the supercritical fluid beforehand, to increase the triazine solubility, or be added to the extraction cell to improve the sample wettability and thus facilitate displacement of the analytes from the sample matrix.

SFE of triazines from solid samples has been described in several papers [39,41–45] and its conditions have been optimized for soils using multiple linear regression [41], arriving at the following conclusions: The recovery increases with increasing pressure and when methanol is added as the modifier; an increase in the temperature somewhat decreases the recovery. The modifier must be added directly to the extraction cell and its effect increases with increasing polarity of the solutes. Small effects are exerted by the soil type and the extraction time. The maximum recovery has been obtained at the maximum attainable pressure (50 MPa), when adding 100 μl of methanol per ml of cell content, with the minimum possible temperature of 50°C.

The recoveries have been compared [42] for SFE and Soxhlet extraction, using soil samples spiked with terbutylazine, atrazine and their metabolites (see Table 3). The yields of the parent triazines are high and comparable for both the methods, whereas those of the hydroxy metabolites are higher when using SFE.

2.3. Solid-phase extraction

SPE is a very important alternative to LLE in preconcentration of triazines from liquid samples. Its advantages include the ability to preconcentrate traces of analytes from very dilute solutions, provided that sufficiently large sample volumes are available, and easy automation of the procedure. Its drawback lies in its high cost, as the adsorbent cartridge is disposed of after each analysis. SPE is becoming very popular and has been introduced into some standard methods [46].

The use of SPE in analyses of water for pesticides has been reviewed [32]. SPE is based either on analyte adsorption on active carbon, graphitized carbon black, an organic polymer (e.g., Tenax, styrene–divinylbenzene copolymers, Porapak Q, polyurethanes) (e.g., Refs. [47–52]), or on analyte partition between the sample solution and chemically modified HPLC phases, such as C_8 or C_{18} [32,52–57]; this is followed by a suitable desorption step. The solid phases used in SPE are packed in tubes or

Table 3
A comparison of the recoveries of SFE and Soxhlet extraction from spiked soil [42]

Spike (ppm,w/w)	Recovery (%)											
	SFE						Soxhlet extraction					
	T	A	OHT	OHA	OHDEA	OHDIA	T	A	OHT	OHA	OHDEA	OHDIA
0.5	91±7	93±9	83±5	81±11	80±9	79±17	95±9	93±10	53±11	50±7	57±9	40±9
2.0	95±3	90±5	78±7	78±4	70±6	76±2	94±4	92±7	50±6	48±10	50±8	37±13
5.0	88±5	87±4	73±7	66±3	81±8	70±8	90±5	94±3	48±8	39±7	43±9	48±7
Mean	91.7	90.0	78.0	75.0	77.0	75.0	93.0	93.0	50.3	45.6	50.3	41.7
Range	82–101	84–104	64–88	63–90	75–83	61–96	84–103	83–102	41–64	32–56	33–66	24–55

n=3, SFE: 15 min, 1 ml/min, 10% methanol, 50 MPa; Soxhlet extraction: 2 h, 150 ml methanol, spiked soil: sandy humic soil stored at 4°C for two days after spiking. T=terbutylazine, A=atrazine, OHT=hydroxyterbutylazine, OHA=hydroxyatrazine, OHDEA=hydroxydeethylatrazine, OHDIA=hydroxydeisopropylatrazine.

immobilized on membrane disks (e.g., Refs. [52,58,59]). The use of the latter is increasing, as the membrane disks permit higher sample flow-rates and the danger of extraction of impurities from polymer tubes and frits is eliminated. The desorption step consists of leaching with solvents, or thermal desorption is employed, provided that the analytes are sufficiently thermally stable, or supercritical carbon dioxide is used, which is the best alternative. Off-line SPE techniques are often preferred for their simplicity, but both precolumns and disks have also been used on-line.

The SPE performance is affected by several factors, namely [32]:

1. Sample type (water or a solid sample that must be converted into a solution, the kind and complexity of the matrix, the presence and concentration of organics, such as humic acids or detergents, the ionic strength, etc.);
2. Sample volume (the breakthrough volumes for reversed-phases are proportional to the capacity ratio, k; volumes of around 200 ml of water samples must usually be analyzed to ensure analyte detectability);
3. The sample pH, which affects both the solute form and its interaction with the solid phase;
4. Solid phase pretreatment, such as adsorbent activation or wetting of organic polymers with water–organic solvent mixtures;
5. Washing of the solid phase with adsorbed or dissolved triazine analytes in order to remove interferents.

If the trapped triazines are desorbed by organic solvents, there is a danger of contaminating the sample by plasticizers leached from plastic cartridges [55], which is especially detrimental when gas chromatography with electron-capture detection is used for determination; glass microcolumns should then be used.

Various solvents and their mixtures have been tested for desorption of triazines from the C_{18} phase, e.g., ethyl acetate, hexane and light petroleum [55], mixtures of light petroleum with toluene at ratios of 2:1 [47] and 1:1 [49], dichloromethane with methanol (6:4) [48] or acetonitrile (6:4) [50], acetonitrile [60] and methanol [61]. The highest recovery (close to 100%) has been obtained for ethyl acetate [53,55,62,63].

An example of the use of SPE in automated trace determination of triazine herbicides in water [64] is based on preconcentration in Sep-Pak C_{18} cartridges followed by GC–MS SIM analysis. The automation shortens the analysis time by 70%, increases the sample throughput by 200% and permits a precision of 5% to be obtained. The method is robust, reliable and permits determination of triazines at a sub-ppb level in water samples as small as 100 ml. An SP microextraction has been described [65,66] with on-line thermal desorption in the injection system of a capillary gas chromatograph; fused-silica fibres coated with polydimethylsiloxane, 100 μm I.D., have been found optimal for the extraction [66]. To improve the sensitivity, the fibre was repeatedly immersed in the sample solution, the triazines then desorbed and the combined desorbed portions injected into the analytical column, thus determining triazine herbicides at a 0.1-ppb level [66].

As the sorbents used in SPE are highly selective, it is impossible to preconcentrate both parent triazines and their degradation products in a single cartridge. Reversed-phase C_{18} materials are commonly used for parent triazines but are unsuitable for their hydroxy derivatives because the recoveries are very poor [65]. Mixed-mode cartridges have been proposed [67] to improve the recovery, containing, e.g., a C_{18} phase and a strong cation exchanger (SCX) [35,63], graphitized carbon black with SCX [50,68], reversed-phase material PRP-1 with Porapak G [60], etc. Another possibility is the use of a double trap [20], containing a LiChrospher RP-18 material in the first column, which retains all lipophilic compounds present in the sample and exhibits a very low retention for triazines and a specifically developed reversed-phase material, Supelclean Envi-18, in the second compartment, where the triazines are preconcentrated and eluted onto the separation column.

3. Determination of triazines

3.1. Thin-layer chromatography

Although GC and HPLC are by far the most common in analyses of triazine residues and the application of electrophoretic and immunochemical

methods is spreading rapidly, TLC is still accepted as an inexpensive and simple screening method (see, e.g., reviews [70,71]), especially in less developed countries, as it does not require sophisticated instrumentation. The modern version of TLC, RP-HPTLC, was employed to determine atrazine in polymeric microcapsules with densitometric detection [72], as an alternative to elemental analysis. Fluorescence quenching [73] or Hill's inhibition reaction (based on the ability of *s*-triazines to inhibit the enzyme system of an isolated chloroplast [74]) have also been used for detection and quantitation of *s*-triazines. Metribuzin and its metabolites were determined in soil and water [75] by using SPE preconcentration on a C_{18} material followed by RPTLC with densitometric detection. TLC has further been applied to determining the lipophilicity (partition coefficients) of *s*-triazines [76].

3.2. Gas chromatography

Together with HPLC, gas chromatography is at present the most important method for the determination of triazines. A great variety of stationary phases have been used for the purpose, including OV-1, OV-101, SE-30, DB-1, DB-5, polyethylene glycol-based phases and SPWax 10 [77]. The retention indices have been measured for triazines on various stationary phases [14,78]. Packed columns have recently been replaced by capillary ones. The problem encountered in GC of triazines lies in the fact that the degradation products of parent triazines, especially 2-hydroxy-*s*-triazines, are polar and are not amenable to direct analysis. Therefore, derivatization is required; e.g., silylation, alkylation, acylation and methylation have been used (e.g. Ref. [65]).

s-Triazines can be detected with flame ionisation detection (FID), but a more sensitive and selective response is obtained when using the nitrogen–phosphorus detector (NPD), because of the presence of nitrogen atoms in the analyte molecules (Fig. 1, [35]). The detection limits obtained for GC–NPD have been reported to be around 5–10 μg per kg of soil [79], which is much less than the values obtained by HPLC with diode-array detection (see Table 4) [35]. Gas chromatography [80] and GC–

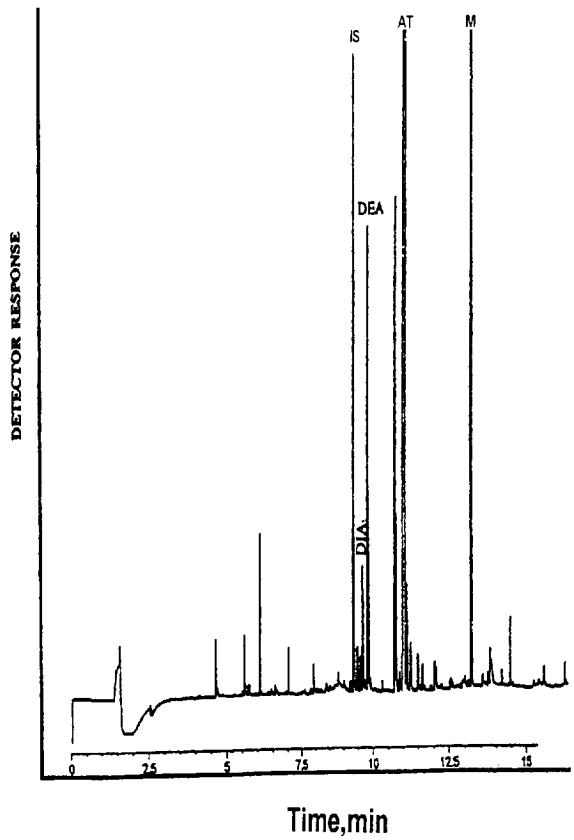


Fig. 1. GC chromatogram of an extract of a sediment sample (According to Ref. [35], with permission). IS (internal standard, diphenylamine), 5 μg/l; DIA (deisopropylatrazine), 27 μg/kg; DEA (deethylatrazine), 47.2 μg/kg; AT (atrazine), 629.5 μg/kg; M (metolachlor), 824.3 μg/kg. Conditions: extraction with C_{18} cartridges, capillary column DB-5 (30 m×0.25 mm I.D., d_f=0.25 μm), 2 ml of He/min, NP detector, temperature program from 50 to 160°C at 20°C/min, then 5°C/min to 185°C and then 20°C/min to 240°C. Injection, splitless, 1 μl.

Table 4
A comparison of the detection limits of GC–NPD and HPLC–DAD in water and sediments (in ppb) [35]

Compound	Water		Sediment	
	GC	HPLC	GC	HPLC
Atrazine	0.05		0.5	
Deethylatrazine	0.05		0.5	
Deisopropylatrazine	0.05		0.5	
Metolachlor	0.15		1.5	
Deethyldeisopropylatrazine		0.15		1.5
Hydroxyatrazine		0.15		1.5

MS [81] have also been applied to the determination of 1,2,4-triazines.

GC–LC on-line coupling has been described [82,83] for preconcentration and determination of *s*-triazine herbicides. Aqueous samples are preconcentrated on a polymer-packed precolumn in the LC system, the adsorbed analytes are extracted with ethyl acetate, dried to remove residual water and fed into a GC–FID system. Concentrations of 27 ppt to 2 ppb can be determined in 10 ml samples (Fig. 2) [82].

GC–MS systems have the obvious advantage in the possibility of identifying the triazines and their degradation products [34,51,52,84–86] and determining them together with other pesticides [52]. In addition to the electron-impact (EI) mode, chemical ionization (CI) (positive- or negative-ion) is used, thus improving the identification power of the system. The stability of analytes under electron impact increases in the series, chloro<methoxy<methylthio derivatives. CI mass spectra, using methane as the reagent gas, have been tabulated [78]. The ease of fragmentation of the protonated molecular ions of chlorotriazines is related to the low proton affinity of the leaving neutral HCl group. In the isobutane CI mass spectra, all the triazines exhibit the protonated ion as the base peak, with only minor fragmentation. Negative chemical ionization yields a higher sensitivity [52]. The detection limits are comparable to those obtained in the GC–NPD system (low μg/l levels). The Rhine river and drinking water have been analyzed by GC–MS and concentrations of tens of ppt were determined [87].

The GC–MS system is definitely best for confirmation of the structure of triazine residues, but is not available in all laboratories. Other possibilities to confirm the structures of analytes involve the sequential or parallel use of two columns of different polarities in GC analysis [55], a combination of GC with HPLC and chemical derivatization [77]. The use of the latter approach has been reviewed [88]; the most common methods replace the chlorine atom in position 2 by the methoxy group using sodium methoxide, methylate the secondary amino substituents in positions 4 and 6 with methyl iodide–sodium hydride in dimethylsulphoxide or apply trifluoroacetylation to deethyl- and desisopropyl atrazine. *s*-Chlorotriazine structures have recently been

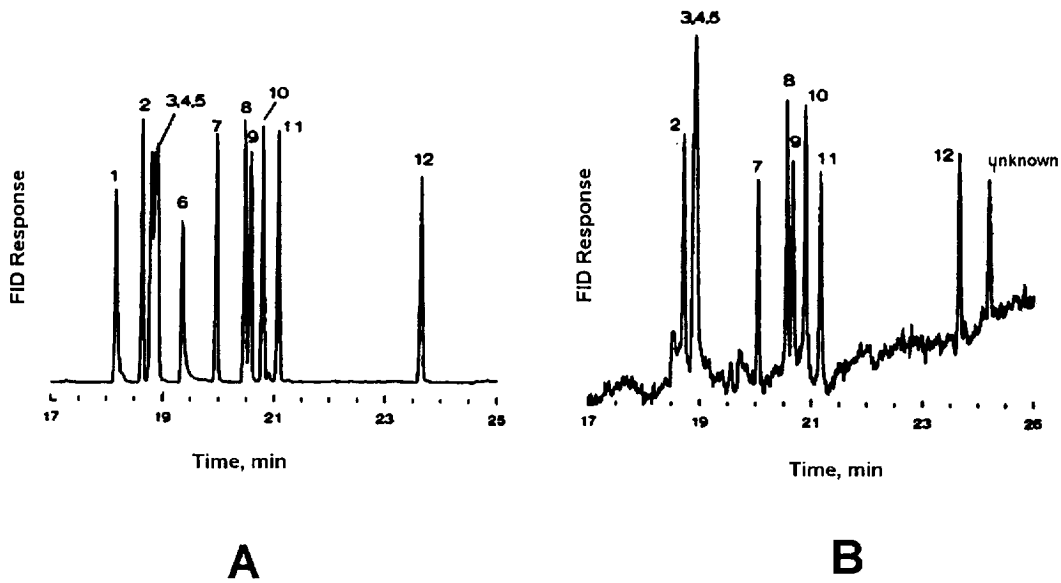


Fig. 2. On-line LC–GC–FID analysis of 10 ml of an aqueous solution containing twelve triazine standards at a concentration level of (A) 0.2 ng/ml and (B) 27 pg/ml. (According to Ref. [82], with permission). 1, atraton; 2, trietazine; 3, simazine; 4, atrazine; 5, propazine; 6, secbumeton; 7, sebutylazine; 8, prometryn; 9, simetryn; 10, terbutryn; 11, dipropetryn; 12, cyanazine. Preconcentration on SDB copolymer, elution by ethyl acetate, column DG 1701 (15 m×0.25 mm I.D., d_f=0.25 μm).

confirmed by derivatization with pyrrolidine in dimethylsulphoxide [77]; the retention times of the derivatives were markedly increased compared with the parent compounds. The reaction takes 30 min at 60°C and is virtually quantitative.

3.3. Liquid chromatography

Gas chromatography fails with compounds that decompose under the experimental conditions, e.g. cyanatryn, or with compounds that are non-volatile and highly polar, such as hydroxy-*s*-triazines. Therefore, when using GC for herbicide screening, these substances escape attention. HPLC provides direct determination without derivatization of both nonpolar *s*-triazines and their polar degradation products. Various stationary phases have been tested for the separation of *s*-triazines, e.g., silica gel and cyanopropyl-, nitrile- and octadecyl-modified phases [89–91]. Reversed-phases are most often employed for the separation of *s*-triazine degradation products (e.g. Refs. [7,35,42]), but ion-pair and ion-exchange separations have also been reported [69].

As discussed in Section 1, the ability of triazines to strongly absorb UV light is dependent on the character of the substituents and the pH and composition of the solution. Therefore, UV photometric detection is quite sensitive (Fig. 3) [51]; e.g., the detection limits for *s*-triazines at 220 nm amount to 0.8–3.0 μg per kg of soil [67]. Amperometric detection has also been tested [7]. Only hydroxy derivatives can be electrochemically oxidized at +1.1 V (Ag/AgCl), but the detection limits (ca. $2 \cdot 10^{-5}$ mol/l) are high compared with UV photometric detection. However, the selectivity of amperometric detection can be advantageous in the analyses of hydroxy derivatives in complex matrices. All triazines should be detectable polarographically, but the detection has not been tested and it can be expected that it would suffer from insufficient sensitivity.

Recently, HPLC has been often coupled with MS detection (e.g., Refs. [42,92–97]), in order to elucidate the structures of triazine residues. Atrazine metabolites have been characterized using positive-ion low-collision-energy MS. The structure and origin of the main daughter ions was investigated using desorption CI with NH_3 or D_3 as the reagent gas. Parent-ion spectra, based on $[XC{=}N\text{-}C(=NH)NH_2]^+$ ions, where X is Cl or OH, are highly diagnostic for chloro- and hydroxy triazines [92].

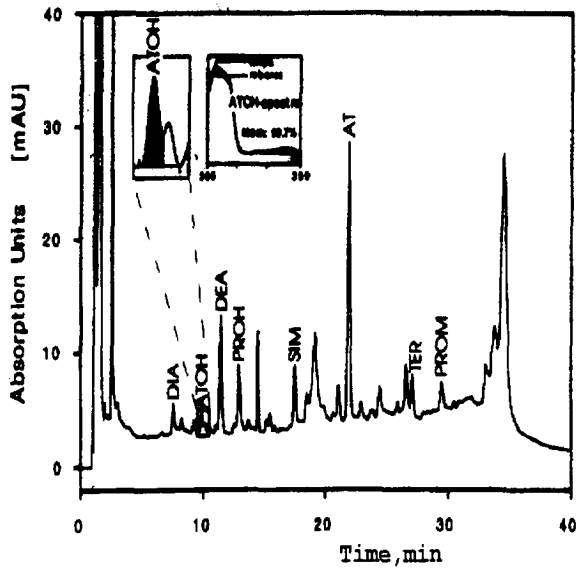


Fig. 3. HPLC chromatogram of a concentrated eluate of unspiked ground water (according to Ref. [51], with permission). The UV spectra are the reference spectrum of hydroxyatrazine (ATOH) and the ATOH spectrum obtained in the sample. Concentrations in μg/l: DIA (deisopropylatrazine), 0.05; ATOH (hydroxyatrazine), 0.031; DEA (deethylatrazine), 0.193; PROH (hydroxypropazine, internal standard), 0.1; SIM (simazine), AT (atrazine), 0.330; TER (terbutylazine), 0.056; Pryn (prometryn), 0.034. Conditions: SPE on graphitized carbon black, stationary phase ODS(30) Ultracarb (5.15×4.6 mm); mobile phase, gradient elution, 1 min isocratically in 15% acetontrile + 1 μmol/l phosphate buffer, pH 7.0, then gradient of acetonitrile to 75% in 32 min, then isocratically for 4 min. Diode-array detection.

Using the LC–MS system, triazines can be determined in several modes [42]:

1. Direct liquid introduction (DLI);
2. Thermospray (TSP) in "filament on" and "filament off" modes;
3. Particle beam (PB) mode;
4. With atmospheric pressure ionization interface.

In less complex samples, the MS–MS tandem can be used for structure elucidation. The limits of detection for hydroxy atrazine and hydroxy terbutylazine are compared in Table 5 [42].

Table 5
A comparison of methods for the analysis of hydroxyterbutylazine and hydroxyatrazine [42]

Methods	Detection limit (in ng)	Structural information
LC–DAD	0.2	UV spectra
LC–TSP–MS (SCAN)	1–2	Molecular ion
DLI–MS–MS	20	SID mass spectra
LC–PB–MS	50	Three selected ions
LC–PB–MS (SCAN)	400	EI mass spectra

LC–MS and MS–MS methods have permitted the elucidation of the structure and trace analysis for triazines and their hydroxy metabolites in humic soils, down to a concentration of 0.5 mg per kg of soil [42].

3.4. Capillary electrophoresis

Capillary electrophoresis (CE) has recently become extremely popular in analytical practice, primarily because the modern instrumentation that is now commonly available permits the attainment of highly efficient separations in relatively short times, is reasonably sensitive and reliable, while requiring extremely small samples for analysis and is generally cheaper and simpler than HPLC. Of course, HPLC still retains its importance, mainly in analyses of extremely low concentrations of analytes, as large volumes of sample (hundreds of μl) can be injected into the HPLC system compared to CE (a few nl). The number of papers describing CE analyses of triazines is not very high [98–103], but it can be expected that it will increase rapidly.

In separations of triazines and their degradation products, their acid–base properties can be utilized in capillary zone electrophoresis (CZE) [99,100] or isotachophoresis (ITP) [101,102], whereas the differences in the polarity of their molecules are exploited in micellar electrokinetic chromatography (MEKC) [99,103]. MEKC is actually a hybrid of electrophoresis and HPLC. A surfactant is added to the running buffer at a concentration slightly exceeding the critical micelle concentration (CMC). The micelles formed represent a pseudostationary phase with which the analytes interact to an extent that is dependent on their hydrophobicity. The whole system is driven by the electroosmotic flow. Therefore, even uncharged substances can be separated in a manner similar to that of HPLC, whereas the local velocity distribution in the separating capillary remains flat, leading to high efficiency compared to HPLC with a parabolic velocity profile. ITP is at present the least important of these methods; an example of its use is a determination of some herbicides in milk extracts [101], without any preconcentration step.

CZE separations have mainly been applied to the separation of triazines [101] and their degradation products [101] (Fig. 4), e.g., hydroxy- and alkoxy-*s*-triazines produced by solvolysis of chloro-*s*-triazines [99], atrazine photodegradation products [100] and their determination in soil [102]. The differences in the migration velocities of the charged protonated species are used in these determinations. A micellar system containing sodium dodecylsulphate was used to determine atrazine and simazine in river water samples [98].

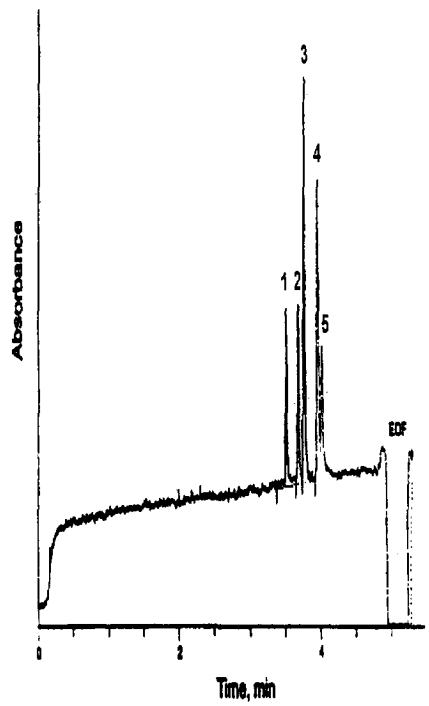


Fig. 4. CE separation of hydroxytriazines in 50 m*M* acetate buffer, pH 4.65 (according to Ref. [100], with permission). 1, hydroxyterbutylazine; 2, desisopropylatrazine; 3, hydroxyatrazine; 4, hydroxydesethylterbutylazine; 5, hydroxydiaminotriazine (ametryn).

3.5. Immunochemical methods

Immunochemical methods, especially enzyme immunoassay (EIA) in its heterogeneous form, enzyme-linked immunosorbent assay (ELISA), immunosensors, immunolabelling and immunochromatography, are important approaches to the analysis of pesticides, complementary to the above separation methods [104–118]. The main advantage of these methods is their high selectivity, sensitivity, simplicity and a relatively low operational cost. A disadvantage of ELISA lies in considerable sample-to-sample variations of the calibration curves. The sources of error have been examined [114] and ways of reducing the cost suggested. Immunomagnetic beads have been used as an alternative to conventional screening, based on tagging surface receptors of hybridoma cells with binding properties of the secreted antibodies by magnetic beads coated with hapten conjugates [104].

The EIA analysis of triazines is based on an immunoreaction caused in an organism by the addition of the antigen, an *s*-triazine derivative, bound to a protein (usually hemoglobin) [111]. The antibody is then obtained from the organism (usually from the blood) and immobilized on a microtitre plate covered with agar gel. The triazine sample is then added, together with an enzyme tracer, which is a covalent complex of the triazine with a suitable enzyme, usually horseradish peroxidase. The triazine from the sample competes for the active sites on the antibody with the enzyme-labelled triazine. Finally, the gel is washed with water and the tracer still bound to the antibody is determined by a suitable colour reaction (with horseradish peroxidase, hydrogen peroxide and tetramethylbenzidine are added, leading to the formation of a blue colour). Of course, many other approaches to labelling can be taken.

Antibody production is a key step of ELISA methods [114], as the specificity and sensitivity of the method depends on the antibody. Recombinant technology should allow the production of antibodies with new binding properties [104]. EIA kits for environmental samples, including triazines, have been developed [117]. Comparative trials on commercially available microtitre plate tests have been done [114], together with validation by GC–NP and HPLC–diode array detection. The sensitivity, reproducibility and cross-reactivity have been tested. A detection limit lower than 0.1 μg/l and inter- and intra-assay precision below 20 and 5%, respectively, have been obtained. An immunoassay for the simultaneous analysis of atrazine, simazine, cyanazine and prometon in a low sub-ppb region has been published [117].

Table 6
Peak concentration of atrazine after determination by EIA and HPLC [105]

Sample of soil number	Soil concentration by EIA (μg/kg)		Soil concentration by HPLC (μg/kg)
	Dilution 1:150	Dilution 1:500	
238	606± 61	706± 95	699
271	490± 79	595± 35	488
343	738±138	757± 61	725
428	368± 48	477±101	474
509	413± 28	531± 60	594
513	340± 38	385± 10	387
582	504± 78	573± 98	501
620	632± 51	655± 20	703
624	1008± 64	1118± 33	1240
638	1345± 86	1545± 98	1422

EIA has been shown to be a useful screening method as long as the positive samples can be validated by other methods such as HPLC or GC. The EIA results have been found to correlate well with HPLC [105] (Table 6) (correlation coefficient, r=0.96) and GC–MS [112].

Immunoaffinity chromatography [113,118], in which a non-specific antibody is bound to a suitable sorbent and packed in a column, can be used to advantage to preconcentrate triazines prior to HPLC or GC–MS analysis.

4. Conclusions

Analysis for triazines and their degradation products is a demanding task of trace or ultratrace analysis of complex samples for substances of differing polarity. In addition to simple spectrophotometric and electrochemical methods that are suitable only in special cases, high performance separation methods combined with efficient isolation and pre-

concentration techniques are best suited for the purpose, together with selected immunochemical methods.

Future development will probably stress capillary electrophoresis and powerful combinations of methods, such as GC–MS, LC–MS, CE–MS, MS–MS, SFE–GC or HPLC, SPE–GC or HPLC, HPLC–GC–MS, CE–HPLC, etc., with a great emphasis being placed on automation of the procedures. Furthermore, the general use of simple immunochemical screening tests can be expected, in the form of kits that can be operated by personnel of low qualification. However, it should be borne in mind that meaningful results can only be obtained from these screeining tests if they are properly validated and regularly checked by well-defined, robust, efficient methods, such as GC–MS, LC–MS and other hyphenated techniques.

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