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Review

Determination of triazine herbicides in environmental samples

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Abstract

The extraction and subsequent chromatographic analysis of the triazine herbicides from environmental samples is reviewed. Particular emphasis is focused on the extraction of the herbicides from aqueous samples (e.g. natural waters). For completeness, however, an indication of the methods used for extraction from solids (e.g. soils) is also included. The range of chromatographic separation techniques involved in the separation and subsequent detection are described. Advantages and drawbacks of both the extraction and chromatographic analysis are discussed. The characteristics of the extraction methodology and chromatographic techniques involved for the determination of the triazine herbicides are summarised and future developments outlined.

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

Contents

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1. Introduction

Herbicides are used in agriculture to remove weeds that would otherwise compete with the crop. In order to be successful as a herbicide, the weedkiller must have an intrinsic selectivity between the weed species and crop plants [1]. In 1952, J.R. Geigy, of Basel, Switzerland investigated the use of triazine derivatives as possible herbicides. The first triazine herbicide, chlorazine, was introduced in 1954, and this was followed by simazine in 1955 [2]. The development of new and improved triazines for crop protection is a continuing process such that, in 1993, the Advisory Committee on Pesticides Annual Report [3] included provisional approval for products based on two additional triazines, anilazine and cyromazine. Anilazine has been developed for use in the UK as a cereal fungicide while cyromazine has been proposed for use as an insect growth regulator to control housefly larvae on manure in and around intensive calf, pig and poultry units.

In order to protect the quality of potable and surface water in Europe a priority list of pesticides has been compiled, also called 'red' and/or 'black lists' [4]. The EEC Directive on the Quality of Water Intended for Human Consumption sets a maximum admissible concentration (MAC) of 0.1 μg/l per individual pesticide [5]. The triazines listed in the 76/464/EEC Directive ('black list') on pollution are atrazine and simazine. In addition, for pesticides for which over 50 000 kg per annum are used, a further list has recently been published [5]. The pesticides included in this additional list have the potential for probable or transient leaching. Within the list, the herbicides cited in addition to atrazine and simazine are cyanazine, prometryn, terbutylazine and terbutryne. The Drinking Water Inspectorate for England and Wales reported that the total number of individual pesticide determinations carried out by Water Supply and Water Service Companies in 1993 was 1 006 458 [6]. Of these determinations, 8.5% were attributable to the following triazine herbicides: atrazine, prometryn, propazine, simazine, terbutryne and trietazine. The most popular triazine monitored was atrazine (with 37 647 determinations) closely followed by simazine (with 36 130 determinations). The number of determinations contravening the MAC was 18.8 and 13.0% for atrazine and simazine, respectively. In the report [6], the maximum detected concentration of atrazine was 5.57 μg/l; but only a single sample was reported containing this concentration.

In the USA, a joint project of the Environmental Protection Agencies (EPA's) Office of Drinking Water (ODW) and the Office of Pesticide Programs carried out a national pesticide survey [7,8]. The results were released between 1990 (Phase I) [7] and 1992 (Phase II) [8]. The result is one of the most comprehensive lists used to conduct a monitoring programme on pesticides. The list arranges the pesticides according to their method of analysis. The triazines included in Method 1 [EPA method 507: dichloromethane extraction followed by gas chromatography (GC) with nitrogen–phosphorus detection (NPD)] are ametryn, atrazine, prometone, prometryn, propazine, simazine, simetryn and terbutryne. Unlike in Europe, the ODW of the EPA has established drinking water regulations and health advisory levels for individual pesticides. Examples of the health advisory levels for the atrazine, cyanazine and simazine are 3, 9 and 4 μg/l, respectively [8,9].

1.1. General properties and uses of the triazine herbicides

The triazine herbicides are solids, with a low vapour pressure at room temperature, and water solubilities in the range 5–750 ppm. The water solubility of each triazine compound is dependent on the substituent in the 2-position [10], e.g.

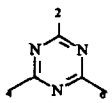
prometone ($-OCH_3$)	750 ppm,
prometryne ($-SCH_3$)	48 ppm,
propazine	8.6 ppm.

It should also be noted that the ending of the herbicides common name is indicative of the substituent in the 2-position, e.g.

-azine	chlorine atom,
-etryne	methylthio group,
-tone	methoxy group.

The most common triazine herbicides are shown in Table 1 and their uses in Table 2.

Table 1
Common triazine herbicide structures



Triazine herbicide	Position		
	2	4	6
Ametryne	$-SCH_3$	$-NHC_2H_5$	$-NHC_3H_7$ (iso)
Anilazine	-Cl	-Cl	$-NHC_6H_4Cl$ (aromatic)
Atrazine	-Cl	$-NHC_2H_5$	$-NHC_3H_7$ (iso)
Chlorazine	-Cl	$-N(C_2H_5)_2$	$-N(C_2H_5)_2$
Cyanazine	-Cl	$-NHC(CN)(CH_3)_2$	$-NHC_2H_5$
Cyromazine	$-NHC_3H_5$ (cyclo)	$-NH_2$	$-NH_2$
Prometone	$-OCH_3$	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)
Prometryne	$-SCH_3$	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)
Propazine	-Cl	$-NHC_3H_7$ (iso)	$-NHC_3H_7$ (iso)
Simazine	-Cl	$-NHC_2H_5$	$-NHC_2H_5$
Simetryn	$-SCH_3$	$-NHC_2H_5$	$-NHC_2H_5$
Terbuthylazine	-Cl	$-NHC_2H_5$	$-NHC(CH_3)_3$
Terbutryne	$-SCH_3$	$-NHC_2H_5$	$-NHC(CH_3)_3$
Trietazine	-Cl	$-N(C_2H_5)_2$	$-NHC_2H_5$

The two most common triazines analysed in natural waters are atrazine and simazine. Both are used as pre- and post-emergence herbicides for the control of annual and perennial grass and annual broad-leaved weeds [3]. Their main uses have been for total weed control on non-crop land such as railways, roadsides and industrial areas. In addition, atrazine has a significant use in maize production for which there are no alternative herbicides. Simazine is frequently used on crops such as winter beans, rhubarb and asparagus for which there are no alternative herbicides [3].

1.2. Mode of action

The triazine herbicides are mainly soil-acting against seedling weeds. The herbicides can either be applied during the relatively narrow time interval between sowing the crop and its emergence, prior to crop planting or on established crops. Triazine herbicides can persist for many months in some soils and seasonal carry-over can sometimes cause difficulties. The effectiveness of triazines, when applied to soils, is dependent on several variables, which include the soil structure, organic matter content, moisture content and particle size distribution. The way the herbicide is applied to soil can affect its persistence, wettable powders and dust formulations are less persistent then granule applications. For a similar herbicide effect, much less is normally required in a sandy soil type (low adsorption properties allow rapid leaching) and when rainfall is high than in organic soils and when rainfall is low, during the first few weeks after application [13]. This was illustrated by Rahman et al. [14], who investigated the phytotoxicity of atrazine on soil organic matter; the equitoxic dose of atrazine increased three-fold as the content of organic matter increased from 8 to 19.3%.

The triazine herbicides are one of the most common classes of chemical pollutants monitored by water authorities. The direct application of the triazines, as wettable powders, to the ground makes their determination in soil, plant and water samples of importance. This importance is manifested in the wide use of the triazine herbicides for various agricultural crops. The herbicides are readily absorbed by the plant roots [2] and spread rapidly, to the tops of the weed seedlings i.e. tips and margins of leaves.

Table 2
Properties and uses of some triazine herbicides [1,2,11,12]

Common name	Trade name	Chemical name	Melting point (°C)	Solubility in water (20–25°C) (ppm)	Vapour pressure (20°C) ($\times 10^{-6}$ mmHg)	pK_a	LD_{50} administered orally (mg kg^{-1})	Uses
Ametryne	Evik; Ametrex; Gesapax	2-Methylthio-4-ethylamino-6-isopropylamino-*s*-triazine	88–89	185	0.84	3.93	965 (in mice); 1100 (in rats)	Pre- and post-emergence selective herbicide for the control of broad-leaved and grassy weeds in pineapple and sugar cane; in banana, citrus, stone and pome fruits, corn and coffee
Anilazine	Dyrene	2,4-Dichloro-6-(*o*-chloroanilino)-*s*-triazine	159–160	insoluble			>5000 (in rats)	Fungicide effective against pathogens including *Botrytis*, *Septoria* and *Colletotrichum* spp. Ineffective as a seed protectant
Atrazine	AAtrex; Atranex; Gesaprim; Primatol A	2-Chloro-4-ethylamino-6-isopropylamine-*s*-triazine	171–174	70	0.3	1.68	1750 (in mice); 3080 (in rats)	Pre- and post-emergence herbicide on many crops including maize, pineapples, sorghum, sugar cane, raspberries, roses and young forest trees
Chlorazine		2-Chloro-4,6-bis(diethylamino)-*s*-triazine		10				

Cyanazine	Bladex; Fortrol	2-Chloro-4-(1-cyano-1-methyl-ethylamino)-6-ethylamino-*s*-triazine	167.5–169	171	0.0016	1.1	182 (in rats); 380 (in mice)	Pre- and post-emergence herbicide of short persistence. It is valuable for general weed control applied pre-emergence for maize, peas and broad beans. Applied post-emergence in combination with hormone weedkillers (e.g. MCPA or mecoprop) for the control of hard-to-kill broad-leaved weeds in wheat and barley
Cyromazine	Vetrazine	2-Cyclopropyl-amino-4,6-diamino-*s*-triazine	219–222					Insecticide
Prometone	Pramitol; Primatol	2-Methoxy-4,6-bis(isopropyl-amino)-*s*-triazine	91–92	750	2.3	4.28	2980 (in rats)	Non-selective herbicide for the control of most annual and perennial broad-leaved and grass weeds
Prometryne	Caparol; Gesagard	2-Methylthio-4,6-bis(isopropyl)-amino-*s*-triazine	118–120	48	1.0	4.05	3750 (in rats)	Pre- and post-emergence herbicide used for selective weed control in cotton, peas, carrots, celery, potatoes, sunflowers and onions. At high application rates prometryne persists in soil for 1–3 months

(*Continued on p. 300*)

Table 2 (*continued*)

Common name	Trade name	Chemical name	Melting point (°C)	Solubility in water (20–25°C) (ppm)	Vapour pressure (20°C) ($\times 10^{-6}$ mmHg)	pK_a	LD_{50} administered orally (mg kg^{-1})	Uses
Propazine	Milogard; Gesamil; Prozinex	2-Chloro-4,6-bis(isopropyl)-amino-*s*-triazine	212–214	8.6	0.029	1.85	>5000	Pre-emergence herbicide for the control of broad-leaved and grass weeds in millet and umbelliferous crops
Simazine	Princep; Gesatop; Primatol	2-Chloro-4,6-bis(ethylamino)-*s*-triazine	226–227	5	0.0061	1.65	5000 (in rats)	Total weed control on paths. Pre-emergence herbicide for the control of broad-leaved and grassy weeds in deep-rooted crops such as citrus, deciduous fruits, olives, asparagus, broad beans, grape vines, forest nurseries, coffee, tea and cocoa. A major use is on maize
Simetryn	Gy-bon	2-Methylthio-4,6-bis(ethylamino)-*s*-triazine	82–83	450			1830 (in rats)	Used in a mixture with *S*-4-chlorobenzyl diethyldithiocarbamate to control wood leafed weeds in rice

Terbuthylazine	Gardoprim	2-Chloro-4-ethylamino-6-*tert.*-butyl-*s*-triazine	177–179	8.5	1.12		2160 (in rats)	Pre-emergence herbicide in sorghum; also for selective weed control in maize, vineyards, citrus and pome fruits. Mixed with terbumeton it will control perennial weeds in established stands of apples, citrus and grapes
Terbutryne	Igran; Prebane	2-Methylthio-4-ethylamino-6-*tert.*-butylamino-*s*-triazine	104–105	58	0.96	4.07	2400 (in rats); 5000 (in mice)	Pre- and post-emergence herbidicide. Pre-emergence use on winter cereals for the control of blackgrass and annual meadow grass; control of autumn germinating broad-leaved weeds such as chickweed, mayweeds, speedwell and poppies. Other pre-emergence uses are on sunflower, peas and potatoes; post-emergence directed on maize
Trietazine	Gesafloc	2-Chloro-4-diethylamino-6-ethylamino-*s*-triazine	100–102	20			2830 (in rats)	Inhibits the Hill reaction and is taken up by roots and foliage. As a herbicide it is used for weed control, with linuron, in potatoes and, with simazine, in peas

They then interfere with the enzyme system responsible for the photolysis of water [10], thereby halting the process of photosynthesis. The foliage of the seedling then dies (chlorosis), and some herbicides (e.g. atrazine) can also reduce transpiration in plants as well as inhibit photosynthesis.

1.3. Triazine loss after application to soil

After application of a herbicide to soil [15], only a fraction will be available for uptake by the plant roots, with probably less than 1% of the herbicide applied reaching the site of action within the plants. This is due to losses occurring via several processes in the top few cm of soil. These processes include: volatilisation; adsorption by clay soils or soils of high organic content; leaching by heavy or prolonged rainfall (some rainfall is required to allow the herbicide to enter the soil solution for weed control); photochemical degradation by sunlight (replacement of Cl atom by hydroxyl group in simazine and atrazine); microbiological degradation by soil microorganisms; chemical degradation by soil constituents; and thermal degradation [16–19].

Monitoring of residue samples is maintained in order to determine the fate of the triazine herbicides and their toxic degradation products in plants, soils, water and the general human environment. In the former case, it is the application of the herbicides to agricultural crops that allows the triazines to be transported to surface waters by various mechanisms, such as non-point source run-off, groundwater discharge or atmospheric deposition [20]. One of the most common herbicides used in the USA and Europe over the past thirty years is atrazine [21]. Typical application rates for atrazine on corn have been 2.2–4.4 kg/ha active ingredient, depending on the soil properties, the nature of the crop, atmospheric conditions and/or type of irrigation programme [22].

This paper reviews the analysis of the triazine herbicides in environmental samples, with a particular emphasis on water samples. Although other sample matrices, e.g. soils and plant materials, have been included for convenience. Previously, other reviews have highlighted chromatographic methods for the determination of pesticides, including triazine herbicides, in foods [23] and crops, food and environmental samples [24]. Triazine herbicides are commonly determined by gas chromatography with flame ionisation, nitrogen–phosphorus and mass spectrometric detection because they do not require any preliminary derivatisation. However, because they are one of the most common water pollutants (especially atrazine), they have been determined by a whole range of other techniques including chromatography [high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), thin-layer chromatography (TLC)], immunoassay [25–31] and electroanalysis [32,33].

1.4. Preservation and stability of triazines

In order to preserve triazine herbicides (ametryn, atrazine, prometon, prometryn, propazine, simazine and simetryn) in water after collection in glass containers the following procedure is recommended [34]. To each sample container is added mercuric chloride to give a concentration in solution of 10 mg/l. This should be done at the collection site before transportation to the laboratory. Then, the water samples should be kept at −4°C and away from light until ready to be extracted and analysed. Under these conditions the triazines should be stable in solution for at least 14 days.

The stability in water of atrazine and simazine has also been reported elsewhere [35]. It was noted that atrazine and simazine were stable for at least one month (25°C). This result also agrees with previously reported data [36] that indicated that only about 2% losses were estimated for atrazine and simazine under real environmental conditions for 20 days.

It has recently been reported [37], during routine stability monitoring, that atrazine and simazine are unstable (at room temperature) at a concentration of 500 μg/ml in methanol, the solvent in which the analytes were formulated. In each case, nucleophilic displacement of the chlorine atom (position 2 on the ring) on atrazine and

simazine occurs and it is replaced by a methoxy group to form atraton and simeton, respectively. Using accelerated stability studies, both atrazine and simazine have been shown to be stable in acetone for at least 16 months at 25°C.

2. Sample preparation

The quality of the analytical data is probably more dependent upon the variability introduced by the method of sample preparation used than any other single variable. This is in contrast to the high technology of manufacture involved in the production of chromatographic equipment and all its components including the analytical separation column. The analytical precision obtained by modern analytical instruments is testimony to the total quality management operated by the manufacturers. This is in complete difference to the often low technology base of sample preparation involved in preparing liquid and solid samples for analysis. The sample preparation methods used are frequently based on labour intensive and slow methods of extraction. Traditionally, the methods of extraction used involve liquid–liquid (solvent) extraction and liquid–solid (Soxhlet or Soxtec) extraction. More modern accomplices for these established methods are solid-phase extraction (SPE), supercritical fluid extraction (SFE), microwave extraction and solid-phase microextraction (SPME). In this part of the review we will examine the different approaches employed for the extraction of the triazine herbicides from environmental matrices.

2.1. Liquid–liquid extraction

Liquid–liquid extraction is the method of choice for the extraction of the triazines from environmental aqueous samples [34]. In this case, the sample (up to 1 l and pH adjusted to 7) is shaken with an immiscible organic solvent, e.g. methylene chloride, isolated, dried and concentrated to a volume of 5 ml during a solvent exchange to methyl *tert.*-butyl ether. The extract is then separated and detected using GC–NPD. In order to achieve the required sensitivity for many of the target pesticides it is essential to preconcentrate. However, conventional liquid–liquid extraction employs samples of <1 l. For many target pesticides the preconcentration is insufficient. For larger-volume samples (up to 120 l) the Goulden large-sample extractor can be used [38]. Pesticide concentrations in river water samples at the ng/l level have been reported using this technique [38]. A summary of common liquid–liquid extraction methods is shown in Table 3.

2.2. Liquid–solid extraction

The most common method employed for extraction of triazines from soil and plant samples is liquid–solid extraction, typified by Soxhlet extraction. Prior to extraction the samples may require some pretreatment, for example, soil samples may require air drying [19,42,43,52] and/or grinding [44]; crop and food samples may require chopping and milling [45,46]. Soxhlet extraction involves placing a representative sample into a thimble and extracting by distillation of a suitable solvent (methanol [18,19,47–49] and chloroform [50]) for a predetermined length of time, e.g. 3–12 h. Alternatively, extraction involves shaking the sample (soil, sediment, food or crop) with an organic solvent (e.g. methanol [36,43,45,52], dichloromethane [43,53], diethyl ether [43,44], chloroform [43,52,54,55], and acetone [56,57]), a mixed solvent (water–acetonitrile [42,54,59,60], methanol–water [36,51,52] or acetone–hexane [61]) or hot water [62], followed by filtration, drying and evaporation of the solvent. Additional cleanup may be required, and this can be done using either liquid–liquid solvent extraction [36,44,45,60] or column cleanup using alumina [42,45,55,59], Florisil [18,36,47,63,64], or silica gel [53,56,58] columns prior to analysis.

2.3. Solid-phase extraction

Solid-phase extraction (SPE) provides a useful medium for trace enrichment of triazines from aqueous samples. The application of SPE, in either disk or cartridge form, allows a high degree of versatility and flexibility to be included

Table 3
Liquid–liquid extraction of triazines from water samples

Analyte	Matrix	Sample preparation	Method of detection	Recovery data	Ref.
Ametryne, atrazine and terbutryne	Natural water (pond and canal water)	Water samples spiked with triazines (0.1, 0.01, 0.001 and 0.005 ppm); shaken for 1 min with successive portions (10 and 50 ml) of DCM in a separating funnel. Combined extracts filtered through glass wool and 10 g anhydrous sodium sulphate prior to evaporation to 0.5 ml	GC–alkali flame detector	87–107% recovery ($n = 46$) with a R.S.D. of 5.3%	[32]
Atrazine	Water	Spiked samples (1 l) spiked and then shaken for 2 min with methylene chloride (200 ml); extraction repeated three times. Extracts combined, dried with anhydrous sodium sulfate and evaporated to 1 ml prior to determination	GC–MS	Average recovery of 95.2 ± 5.5% ($n = 3$) at the 1 ng/ml spike level using isotope dilution GC–MS	[61]

Atrazine	Natural water (river water)	Spiked sample (1 l) acidified to pH < 1 with H_2SO_4; 50 ml DCM added and stirred for 30 min prior to transfer to a separating funnel. Extraction repeated with two successive portions of DCM (50 ml). Combined extracts shaken for 1 min with 100 ml 2% $KHCO_3$ solution, then filtered through 80 g anhydrous sodium sulfate. Then, 50 ml DCM added and shaken for 1 min; extract filtered again through anhydrous sodium sulfate and washed with 50 ml DCM. To combined DCM extracts add 3 ml isooctane, evaporate to 10 ml under reduced pressure and then add 50 ml hexane or petroleum ether and evaporate to 3–5 ml. Concentrated extract then cleaned up using a Florisil column containing 20 g 10% deactivated Florisil and 1 cm of anhydrous sodium sulfate. Column eluted with 1% acetone in benzene; extract evaporated to 3 ml as before. Concentrated extract then applied to a mini-column (23 cm × 5 mm I.D.) containing 4 cm of Florisil and 0.5 cm of anhydrous sodium sulfate and eluted with 1.5% acetone in benzene; 10 ml eluate analysed	GC–NPD	At a spike level of 2.0 ng/ml the recovery was 95.2 ± 2.5% ($n = 9$) while at a spike level of 0.2 ng/ml the recovery was 89.7 ± 2.8% ($n = 9$)	[39]
Ametryne, atratone, atrazine, cyanazine, cyprazine, prometone, prometryne, propazine, simazine, simetone and simetryne	Natural water	Extraction with methylene chloride followed by a Florisil column clean-up	GC–NPD	Detection limit 25 pg/ml. Recoveries ranged from 87–108% ($n = 6$) irrespective of spike level (10, 1 and 0.1 ng/ml)	[40]

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Table 3 (*continued*)

Analyte	Matrix	Sample preparation	Method of detection	Recovery data	Ref.
Atrazine, cyanazine, prometon, prometryn, propazine, simazine and terbuthylazine	Surface water	Sample volumes ranging from 10 to 120 l were preconcentrated using the Goulden large-sample extractor using 200 ml of DCM on site. In the laboratory, 1-l samples were extracted with DCM; eluate filtered through anhydrous sodium sulphate and evaporated to 0.5 ml prior to analysis. Comparison with conventional 1-l liquid–liquid extraction using DCM	GC–MSD	Mean % recoveries ranged from 34 to 89% (9–56% R.S.D.) (n = 3–18). Typical concentrations in river water ranged from 7 ng/l for simazine to 37 ng/l for cyanazine	[38]
Atrazine and simazine	Estuarine water samples	Spiked samples (1–4 l) were adjusted to pH 1 with H_2SO_4 and extracted with DCM (50–100 ml); extract evaporated to dryness and residue dissolved in methanol	HPLC–UV	At the 2.5 ng/ml level the recovery was 93–100% (9% R.S.D.) (n = 9)	[41]
Atrazine and simazine	Natural water (drinking water)	Spiked (0.02–7 ng/ml) water samples (150 l), with 0.6% glycine added as a stabilizer, were freeze-dried after thorough mixing. Freeze-dried water samples (2.5 g) were reconstituted by addition of 1 l of HPLC-grade water and bubbling CO_2. Each sample was then extracted with hexane (1 × 20 ml) and DCM (2 × 40 ml). Combined extracts were then evaporated to dryness. For GC–NPD analysis further clean up was required; samples were cleaned up using Florisil cartridges (6 ml) and eluted with a 1 + 1 mixture of n-hexane and diethyl ether	GC–NPD; HPLC–UV	95–110% recovery	[35]

in the sample preconcentration step of any analysis. SPE media are used in two procedures: the first allows off-line preconcentration, while the second procedure allows for on-line trace enrichment. Both procedures have recently been compared and their limitations and advantages noted for the determination of organic pollutants in water [65].

As can be seen in Table 4, the use of SPE is both popular and robust, providing quantitative recovery of triazine herbicides from aqueous samples. However, for deethylatrazine and desisopropylatrazine the recovery is often less than expected when using C_{18} SPE media [71,73,78,82,83,86]. Cassada et al. [73] investigated the influence of sample volume on the recovery of atrazine, deethylatrazine and deisopropylatrazine from three different volumes of water (200, 400 and 800 ml). As the sample volume increased, it was noted that the recovery of atrazine remained within experimental error ($95.1 \pm 8.3\%$) whereas the recovery for deethylatrazine decreased from $89.0 \pm 8.1\%$ to $63.1 \pm 4.4\%$ to $32.1 \pm 2.0\%$ and for deisopropylatrazine from $41.4 \pm 2.2\%$ to $24.0 \pm 2.0\%$ to $12.8 \pm 0.8\%$. These results were in agreement with those reported by Thurman et al. [86], who reported recoveries from a 100-ml sample of $99 \pm 5\%$, $90 \pm 5\%$ and $55 \pm 5\%$ for atrazine, deethylatrazine and deisopropylatrazine, respectively. Also, Chiron et al. [78], who investigated the breakthrough volume and recovery of four triazines (atrazine, deethylatrazine, deisopropylatrazine and simazine) from two different spiked (0.3 ng/ml) water samples (50 and 150 ml). Both breakthrough volume and recovery were investigated for two different on-line SPE procedures, the first involving ten Empore disks, and the latter, a precolumn containing PRP-1. The results indicate that the breakthrough volume for ten Empore disks and the PRP-1 precolumn was: 8 and 14 ml for deisopropylatrazine; 70 and 64 ml for deethylatrazine; >150 and >160 ml for simazine; and >150 and >320 ml for atrazine, respectively. These breakthrough volumes also concurred with recovery data obtained using the ten Empore disks and PRP-1 precolumn. For a 50-ml sample the recoveries were as follows: 60 and 65% for deisopropylatrazine; 90 and 90% for deethylatrazine; 95 and 94% for simazine; and, 97 and 100% for atrazine, respectively. However, for the 150-ml sample the chlorotriazine transformation products provided even lower recoveries: 17 and 22% for deisopropylatrazine; 55 and 51% for deethylatrazine, respectively, as compared to simazine and atrazine, which maintained high recoveries (87 and 92% for the Empore disks, respectively, and 90 and 92% for the PRP-1 precolumn, respectively). In this paper [78], the lower recoveries were attributable to the presence of fulvic and humic substances present in the river water sample. Similarly, using an automated SPE system (Millilab 1A workstation) [71] 12 triazine herbicides were preconcentrated from a 100-ml spiked water sample. Only deethylatrazine and deisopropylatrazine were detected in the breakthrough determinations (100-ml sample, spiked at the 1 ng/ml level). As the flow-rate was increased (20 to 60 ml/min), the breakthrough of deethylatrazine increased from 5 to 10% and for deisopropylatrazine from 35 to 40%. It was suggested that deisopropylatrazine has less sorption capacity than deethylatrazine for the C_{18} medium because it has one less carbon group in the alkyl sidechain and can therefore undergo less hydrophobic interaction.

Automated trace enrichment of triazines from natural waters is advantageous for several reasons, including: cost savings in terms of personnel, less risk of sampling and handling errors, low contamination risk, increased sample throughput, and increased safety. If the preconcentration process can also be directly coupled to a chromatographic setup for separation and detection, then we have a fully automatable system. Two groups have applied this technology to the analysis of triazines in environmental samples [71,76]. In the first case [76], a dedicated SPE system (Prospekt) for trace enrichment was used. In this system, disposable SPE cartridges are used; the system is both automated and programmable for sample preparation allowing direct coupling to an HPLC column. The group [76] investigated the quantitative aspects of trace enrichment from environmental samples of a range of pollutants including triazine herbicides.

Table 4
Solid-phase extraction of triazine herbicides from aqueous matrices

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Ametryn, atrazine, desmetryne, prometryne, propazine, simazine and terbutryne	Aqueous	Preconcentration of sample solution (pH 3.8–11) by sorption on a macroporous polymer sorbent (1 + 2, Separon SE50/50 mixed with ground silica); column dried by passage of a nitrogen stream for 20 min at a temperature of 110°C. The column was eluted with either acetone or methanol	GC–NPD and HPLC–UV	Recoveries ranged from 94.9 to 105.5% at spiking levels between 0.2 μg and 200 μg (typical R.S.D.s were 1.4–3.0%) ($n = 5$)	[66]
Atrazine	Water (tap water)	Sample (10 ml) preconcentrated on a Spherisorb ODS column (100 × 2 mm I.D.); desorption with methanol–water (60:40) + 5% 1-propanol. Transfer of eluent to GC for separation and detection using concurrent eluent evaporation	GC–alkali flame ionisation detector	No recovery data available. Detection limit 3–5 ppt	[67]
Ametryn, atrazine, promazine, prometon, prometryn, simazine, simetryn and terbutryn	Natural (tap) water	A two-step tandem system used; one column containing Carbopack B and the other column a sulphonic acid type silica-based cation exchanger (SCX). Desorption from SCX with 0.7 ml of aqueous methanol containing 70 mmol/l KCl	HPLC with UV detection	At the 15 pg/ml level average recoveries ranged from 95.0 ± 3.65% for prometryn to 99.4 ± 3.46% for atrazine ($n = 7$)	[68]

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Atrazine and simazine	Water	Spiked samples (200 ml) preconcentrated on C_{18} SPE cartridge; elution with 2 ml benzene. Eluate concentrated to 0.5 ml under a stream of air	Isotope dilution GC–MS	Detection limit was 0.05 ng/ml. Recoveries ranged from 90.5 to 105.0% (at the spike levels of 25, 5 and 1 ng/ml)	[51]
Ametryne, atrazine, cyanazine, deethylatrazine, deisopropylatrazine, didealkylatrazine, hydroxyatrazine, metribuzin, propazine, prometryn, prometon, simazine, terbuthylazine and terbutryn	Natural water (surface and ground water)	Spiked samples (100 ml) preconcentrated on C_{18} cartridge using a Millipore workstation; elution with 2.0 ml of ethyl acetate. Eluate dried over sodium sulfate, evaporated under N_2 to 100 μl prior to determination	GC–MS	The recoveries for deisopropylatrazine and didealkylatrazine were 55 and 10%, respectively. For all other triazines, the average recoveries ranged from 90 to 113%	[86]
Atrazine, propazine, simazine and terbutylazine	Natural water (drinking and river water)	On-line preconcentration using two precolumns, one packed with C_{18} or C_8 and the other with a styrene–divinyl benzene copolymer (PRP-1). Effect of *n*-alkyl bonded silica investigated. A 500-ml sample was spiked with 1 ng/ml of each triazine	HPLC–UV detection	Recoveries on the PRP-1 precolumn when coupled to a first precolumn packed with different *n*-alkyl bonded silicas ranged from 12% for propazine using ODS-2 and 15% to 98% for simazine using RP-8 ($n = 3$)	[69]
Prometryn, propazine and simazine	Natural water (surface and ground water)	A 1-l sample was preconcentrated using 500 mg of 50–100 μm C_{18} bonded porous silica. Elution with 5 ml of ethyl acetate, evaporated to 200 μl prior to determination	GC–alkali flame ionisation detection	For spiked water samples at pH 7, the mean recoveries ($n = 5$) were 94.8 ± 8.2%, 79.6 ± 9.7% and 75.6 ± 9.7% for prometryn, propazine and simazine, respectively. Similar recoveries were obtained for spike natural waters (tap, lake and sea water), the exception being simazine from sea water were the recovery was 59.9%	[70]

(*Continued on p. 310*)

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Atrazine, deethylatrazine and simazine	Sea water	Sample (5 l) was pre-filtered (down to 0.45 μm), then passed through an Empore SPE disk (500 mg C_{18} bonded silica); the disk was washed with 2 × 10 ml MeOH under vacuum. The disk was eluted with MeOH (20 ml), the extract evaporated to dryness and re-dissolved in ethyl acetate prior to determination	GC–MS; GC–NPD	Screening method for triazines at levels down to 1 pg/ml	[20]
Atrazine, deethylatrazine, deisopropylatrazine and terbuthylazine	Surface and ground water	Samples (250–500 ml), pH adjusted to 7.0–7.5, preconcentrated using SPE cartridges (cyclohexyl-modified silica sorbent); cartridges centrifuged prior to elution. Elution by air-displacement of 2.0 ml of acetonitrile	HPLC with UV detection	Over the concentration range 1–20 ng/ml, the mean recoveries for atrazine, deethylatrazine and terbuthylazine were within 115–90%, with an R.S.D. of <25%. Recovery of deisopropylatrazine was <40%	[62]
Ametryn, atrazine, cyanazine, deethylatrazine, deisopropylatrazine, prometon, prometryn, propazine, simazine, terbuthylazine and terbutryn	Surface and ground water	Automated SPE method (Millilab 1A workstation) used. Spiked samples (100 ml) were preconcentrated onto preconditioned SPE extraction cartridge; determinand eluted with 2.5 ml of ethyl acetate and evaporated to 100 μl prior to determination	GC–MS	SPE cartridge has a limited capacity for deethylatrazine and deisopropylatrazine. Precision of method ±10% ($n >$ 200). Accurate results reported on inter-laboratory comparison studies and duplicate samples	[71]

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Atrazine and simazine	Drinking water	A 1-l spiked sample (pH adjusted to 2) was preconcentrated onto a 500 mg C_{18} SPE column; retained triazines eluted with 1 ml of MeOH, evaporated to dryness and the residue dissolved in 0.25 ml MeOH and then in an equal volume of 0.1 *M* acetic acid–sodium acetate buffer (pH 3.8) to a final volume of 0.5 ml	HPLC–UV	For atrazine the % recoveries ranged from 82.7 ± 5.2 to 97.5 ± 2.9 when determined at 5 × and 20 × the limit of detection (n = 5). For simazine the % recoveries ranged from 77.3 ± 7.9 to 98.3 ± 4.2 when determined at 5 × and 20 × the limit of detection (n = 5)	[72]
Atrazine, deethylatrazine and deisopropylatrazine	Ground and surface water	Spiked samples (800 ml) were preconcentrated using C_{18} SPE cartridges; eluted with 2 ml ethyl acetate and concentrated to 50 μl	Isotope dilution GC–MS	The effect of sample volume on recovery was noted. As sample volume increased (200, 400 and 800 ml) the recoveries for atrazine remained constant (95.1 ± 5.3%), whereas the recovery for deethylatrazine decreased from 89.0 ± 8.1% to 63.1 ± 4.4% to 32.1 ± 2.0% and that for deisopropyl-atrazine from 41.4 ± 4.4% to 24.0 ± 2.0% to 12.8 ± 0.8%, respectively	[73]
Atrazine, metamitron, metribuzin, propazine, simazine, terbuthylazine and terbutryn	Natural water	A sample (250 ml) was filtered (0.45 μm) and then passed through a SPE cartridge (RP-18), after washing with water and dilute ammonia solution, the column was eluted with 2 × 1 ml of acetonitrile. The extract was evaporated to dryness and the residue dissolved in 0.5 ml of acetonitrile	HPLC–UV	At a spike level of 0.16 ng/ml the recovery for simazine was 90.6 ± 2.0% (n = 5); while at a spike level of 3.0 ng/ml the recovery for metamitron was 89.7 ± 10.0% (n = 5)	[74]

(*Continued on p. 312*)

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Ametryn, anilazine, atraton, atrazine, desethylatrazine, desisopropylatrazine, cyanazine, hexazinone, desmetryn, prometryn, propazine, secbumeton, sebutylazine, simazine, terbutyrn and terbuthylazine	Natural water	Samples (1 l) extracted using 2 g C_{18} SPE cartridges; elution with 4×1 ml MeOH. Extract reduced to 1 ml under a stream of N_2	Thermospray LC–MS	R.S.D.s for different sample amounts (15, 30 and 300 ng) ranged from 1.2 to 11.8%	[75]
Atrazine, cyanazine, deethylatrazine, deisopropylatrazine, hydroxyatrazine, hexazinone, prometon, propazine, sebutylazine, simazine, simetryne and terbuthylazine	Drinking and surface water	Automated on-line trace enrichment using Prospekt system. Preconcentration of samples using a 10 mm × 2 mm I.D. cartrdige prepacked with styrene–divinylbenzene copolymer (15–25 μm, PLRP-S); triazines eluted using acetonitrile gradient with phosphate buffer (pH 7)	HPLC–UV detection	Determination below the 1 ng/ml level can be achieved with an average R.S.D. of 15%	[76]
Ametryn, atrazine, cyanazine, metribuzin, propazine, sebuthylazine, simazine and terbuthylazine	River water	Sample (1 l) filtered through glass wool and then preconcentrated using 2 g C_{18} cartridge; elution with 5×1 ml MeOH followed by solvent reduction to 1 ml under a stream of N_2	GC–AED	Limits of detection ranged from 30–50 pg	[77]

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Atrazine, deethylatrazine, deisopropylatrazine, simazine	Natural water	Spiked water samples (100 ml) were preconcentrated on SPE discs (using ten Empore disks or a column packed with PRP-1 evaluated); gradient elution using acetonitrile	LC–thermospray MS	At the 0.4 ng/ml level typical R.S.D.s ranged from 6 to 9% (n = 5). At a spike level of 0.3 ng/ml average recoveries ranged from 17% for deisopropylatrazine to 97% for atrazine (using ten Empore disks), while for the PRP-1 column the average recoveries ranged from 22% for deisopropylatrazine to 100% for atrazine	[78]
Hydroxyatrazine, deethylhydroxyatrazine and deisopropylhydroxyatrazine	Water	Sample (250 ml) adjusted to pH 2.5 with 1 *M* KH_2PO_4 and preconcentrated on a 500 mg SPE column (SCX bonded phase); elution with 2 ml acetonitrile–0.5 *M* KH_2PO_4 (1:3) at pH 7.5. Eluate filtered prior to analysis	HPLC–UV detection	Recoveries from spiked samples (1 and 5 ng/ml) ranged between 87.1 and 91.5%	[79]
Ametryn, atrazine, cyanazine, prometryne, propazine, simazine, terbutryne and terbuthylazine	Water (tap and river water)	Spiked sample (100 ml) preconcentrated onto SPE column; various column packings materials evaluated. The triazines were eluted with acetonitrile–buffer (70:30, v/v), the buffer was 1.0 m*M* phosphate (pH 4.5) containing 30 m*M* NaCl	Cation exchange chromatography–UV detection	The highest recoveries were obtained using the Supelclean Envi-18 column; the recoveries ranged from 93 ± 5 to 104 ± 5% (n = 3). The system was also investigated with a double SPE trap and applied to a natural water sample	[80]

(*Continued on p. 314*)

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Ametryn, atrazine, promazine, prometryn, prometon, simazine and terbutryn	Natural water	Spiked sample (250 ml), pH adjusted to 1.5, preconcentrated onto 100 mg aromatic sulfonic acid sorbent SPE cartridge; elution with 3×1 ml of 70 m*M* KCl in MeOH–water (90:10). Eluate concentrated to 250 μl prior to analysis	HPLC with UV detection	Recoveries ranged from 74% for propazine to 103% for ametryn ($n = 5$) at a spike level of 1.5 ng/ml. Minimum detectable concentrations ranged from 14 pg/ml for simazine to 56 pg/ml for terbutryn	[109]
Atrazine and simazine	Drinking and surface water	A spiked sample (250 ml) preconcentrated onto C_{18} SPE cartridge; elution with 4×500 μl methanol followed by evaporation to dryness with N_2. Residue reconstituted with 200 μl of methanol prior to analysis	HPTLC and/or GC–MS	Recoveries for atrazine ranged from 62 to 93% determined at spike levels of 400 and 800 pg/ml, respectively; while for simazine the recoveries ranged from 62 to 88% determined at spike levels of 240 and 200 pg/ml, respectively. Average R.S.D.s ranged from 3 to 12% ($n = 4$)	[81]
Atraton, atrazine, desethylatrazine, prometon, prometryn, propazine, simazine, terbuthylazine and terbutryn	Lake and rain water	Sample preconcentrated on C_{18} cartridge; elution with 5 ml of methylene chloride onto silica minicolumns containing anhydrous sodium sulphate. Elution with 5 ml of ethyl acetate and evaporated to 200 μl	GC–MS	Recoveries >80% at the 4–43 ng/l level, except desethylatrazine which had only a 20% recovery at the 50 ng/l level	[82]

Table 4 (*continued*)

Analyte	Matrix	Sample preparation	Method of determination	Comments on procedure and/or recovery data	Ref.
Ammelide, ammeline, atrazine, cyanuric acid, deethylatrazine, deisopropylatrazine, hydroxyatrazine, hydroxydeethylatrazine and hydroxydeisopropylatrazine	Water	Sample (250 ml) preconcentrated using C_{18} SPE cartridges followed by elution with 2 × 2 ml MeOH for atrazine and hydroxyatrazine or a cation exchange support followed by elution with 2 × 5 ml fractions of ammonium acetate solution in water–acetonitrile 75:25 (v/v) with pH adjusted to 8.6 for the metabolites	HPLC–MS or SFC–MS	Recoveries ranged from 20 to 100% using C_{18} cartridge while for the cation exchange support recoveries ranged from 65 to 91% ($n = 5$)	[83]
Atrazine and simazine	River water	Spiked sample (500 ml) preconcentrated onto either C_{18} or styrene–divinylbenzene copolymer Empore discs; elution with 2 × 15 ml of ethyl acetate. Eluate evaporated to 100 μl prior to analysis	GC–EI-MS	Recoveries ranged from 82 to 98% (3.6–6.5% R.S.D.) ($n = 3$)	[84]
Atrazine and simazine	Ground and drinking water	A spiked sample (1 l) preconcentrated onto either a XAD-2 column or a C_{18} SPE cartridge. With the XAD-2 column elution with 100 ml of acetone followed by evaporation to 50–100 μl. For the C_{18} cartridge elution with 5 ml DCM onto a sodium sulphate minicolumn. After washing, elution with DCM and evaporation to dryness. Residue reconstituted with 100–200 μl hexane prior to GC–NPD analysis	GC–NPD or GC–MSD	Recoveries ranged from 74 to 85% at a spike level of 0.1–5 ng/ml (3–15% R.S.D.) (n = 3–5)	[85]

Several triazines were investigated: atrazine and simazine and important breakdown products such as deisopropylatrazine, deethylatrazine, hydroxyatrazine, hexazinone, cyanazine, simetryne, prometon, sebutylazine, propazine and terbutylazine. The trace enrichment procedure involved the following steps: (1) washing the cartridges with 10 ml of acetonitrile; (2) conditioning cartridges with 10 ml of methanol and then 10 ml of HPLC-grade water; (3) percolation of samples; and (4) desorption from the cartridge to the analytical column by an acetonitrile gradient with phosphate buffer (pH 7). The preconcentration parameters investigated were: choice of sorbent, breakthrough volumes, reproducibility and flow-rate. The results showed that determinations were possible below the 1 ng/ml level with average R.S.D.s of 15%. However, identification of unknown compounds is difficult if relying on retention time and a UV spectrum. The interfacing of LC with mass spectrometry would obviously eliminate this problem.

In the second paper [71], automated isolation and preconcentration was done using a Millilab 1A workstation. In this paper [71] samples of surface and ground water samples were spiked (0.05–5 ng/ml) with ametryn, atrazine, cyanazine, deethylatrazine, deisopropylatrazine, metribuzin, prometon, prometryn, propazine, simazine, terbutryn and terbuthylazine. Prior to extraction the SPE cartridge was preconditioned by passing 2 ml of methanol, 6 ml of ethyl acetate, 2 ml of methanol and 2 ml of distilled water. Each sample was then preconcentrated from solution using an SPE cartridge incorporated into the workstation. Elution from the cartridge was with 2.5 ml of ethyl acetate; eluates were then reduced to 100 μl ready for analysis by GC–MS. As noted above, deethylatrazine and deisopropylatrazine were detected in the breakthrough determinations. It was recommended for quantitative work that the precise control of flow-rate is necessary when analysing compounds with limited sorption capacity (e.g. deisopropylatrazine). The precision achievable using this automated workstation was reported to be ±10% R.S.D. (based on hundreds of determinations), for each triazine analysed. Accuracy was demonstrated by the analysis of interlaboratory comparisons and the analysis of duplicate samples. Additional advantages of using this automated SPE system were noted: reduction in the number of man-hours required for extraction by >70%; greater precision (5%); increased sample throughput (200% when using two Millilab workstations); and a significant reduction in technician exposure to solvent fumes.

2.4. Supercritical fluid extraction

The use of supercritical fluids continues to develop as a potential technique for the extraction of analytes from solid matrices. This development has been most notable in the area of environmental sample preparation. It is perhaps not surprising therefore to discover that supercritical fluid extraction (SFE) has been applied to the extraction of the triazine herbicides. The primary advantages of SFE relate to its use of CO_2 and its inherent benefits of low cost, availability in high purity, non-toxicity, mild critical conditions and compatibility with detectors. These benefits are further enhanced when considering the potential for highly selective extraction of analytes from matrices achieved by the low surface tension, low viscosity and variable solvent power of a supercritical fluid. However, with every new technique caution is also required when reality does not approach that of experimental data. All to often, however, the recoveries of analyte spikes from matrices as obtained with SFE can be lower than expected. These non-standard recoveries may be compensable by the addition of a modifier, typically methanol, or the use of an alternative supercritical fluid to CO_2. The latter is normally not the preferred option as it may require instrumental alterations.

Table 5 summarises the SFE of triazines from environmental matrices. The complex nature of the extraction process is often further complicated by the variability introduced by the extraction technique e.g. SFE. The many operating variables that can be considered for SFE can increase the number of experiments required dramatically. An effective tool in these cases is to

Table 5
Supercritical fluid extraction of triazine herbicides from environmental matrices

Analyte	Matrix	Sample preparation	SF conditions	Method of detection	Recovery data	Ref.
Atrazine, simazine, propazine, terbutylazine and cyanazine	River sediment	0.5 g of dried lyophilised sample was slurry spiked using a methanolic solution of triazines, left overnight and extracted. Sample placed into a 0.57-ml volume cell prior to extraction	230 bar; 48°C (density of 0.80 g/ml) for 30 min of CO_2. 20 μl of methanol also added as modifier	GC–FID and HPLC–UV	CO_2 only: 42.5% recovery for simazine (spiked at the 28.0 ppm level); 82.4–96.4% recovery for other triazines (spiked at the 40.2–81.2 ppm level). CO_2 + MeOH: 92.0–103.4% recovery for simazine (spiked at the 28 ppm to 28 ppb level) and 90.2–103.5% recovery for the other triazines (spiked at the 81.2 ppm to 60.2 ppb level)	[87]
Atrazine	Sandy loam soil	2 g samples of soil (1.1% organic matter) were air dried and spiked at the 100 μg/g level. Sample placed in 7-ml volume cell prior to extraction	Variable pressures used at 50 and 80°C; flow-rate kept constant at 300 ml/min (measured at atmospheric pressure and room temperature)	On-column GC–FID	Solubilities predicted using the Peng–Robinson equation of state compared with measured values	[88]
Atrazine, deethylatrazine, deisopropylatrazine and terbthylazine	Soil	10 g samples of soil (4.48% total organic carbon) amended with 10% water and spiked at the 5 ppm level. Sample placed in 7-ml volume cell prior to extraction	First extraction. Density: 0.3 g/ml at 121 bar; temperature: 80°C; flow-rate: 1 ml/min. Static extraction: 2 min; dynamic extraction: 5 min. Second extraction. Density: 0.9 g/ml at 350 bar; temperature: 50°C; flow-rate: 4 ml/min; extraction time: 20 min	HPLC with UV detection	Additional co-extracted material noted in HPLC trace when compared to extraction with hot water	[62]

(Continued on p. 318)

Table 5 (*continued*)

Analyte	Matrix	Sample preparation	SF conditions	Method of detection	Recovery data	Ref.
Atrazine, cyanazine, desethylatrazine and desisopropylatrazine	Soils (Kenyon loam, Ida/ Monona silt loam, synthetic soil matrix and sand)	10.0 g samples of soil were spiked with the triazines (in acetonitrile); stirred for several hours and evaporated to dryness (48 h)	Pressures varied over the range 10.5 to 34.5 Pa at temperatures of 40–120°C. Extraction conditions: CO_2 only; static addition of 20% water; and dynamic addition of 20% methanol in water evaluated. A combination of both static and dynamic addition of methanol and water was also studied	HPLC–UV	Recoveries of each analyte from fortified soils (0.1–2.0 mg/kg) ranged from 25 to 120%, depending upon the soil matrix and analyte extracted	[89]
Atrazine, simazine, terbuthylazine, desisopropylatrazine and desethylatrazine	Soil (sand, peat, clay)	6-g or 14-g samples were extracted. All soils were dried at 40°C, passed through a 2.8-mm sieve and homogenised in a ball mill. Spike levels ranged from 30 to 170 μg/kg for each triazine	Pressure: 20, 25, 30 and 35 MPa. Temperature: 50, 70, 80, 80, 100°C. CO_2 only and modified-CO_2 (methanol or acetone) used. Extraction times varied from 30 to 70 min	GC–NPD	Experimental design approach; recoveries ranged from 'not detected' to 113%. A repeatability study gave average recoveries of between 9 and 66% ($n = 7$) with %R.S.D. of 11–165, whereas a linearity study gave average recoveries of 92–99% ($n = 7$) with %R.S.D. of 5–11	[90]

Table 5 (*continued*)

Analyte	Matrix	Sample preparation	SF conditions	Method of detection	Recovery data	Ref.
Atrazine, deisopropylatrazine and deethylatrazine	Sediment	100 g sediment was sampled from a sediment core and frozen until required. 20 g subsamples were obtained and ^{13}C-labelled triazines added at the 5 ng/g level. The subsample was then mixed with 10 g of silica sand and 10 g anhydrous sodium sulfate and transferred to an extraction vessel that contained 10 g of PTFE boiling chips. 2 ml of methanol was also added to the extraction cell prior to commencement	Pressure: 10 MPa; temperature, 43°C. Methanol-modified (4% v/v) CO_2 used	GC–MSD	For atrazine the precision was determined to be ±16% R.S.D. ($n = 13$) at a concentration level of 1.91 ng/g; for deethylatrazine ±32% R.S.D. ($n = 13$) at a concentration level of 0.25 ng/g; while, for deisopropylatrazine ±9.3% R.S.D. ($n = 13$) at a spiked concentration of 5 ng/g	[73]
Atrazine, 2-hydroxyatrazine and desisopropyl-desethyl-2-hydroxyatrazine	Soil	samples were pounded then dried for 24 h at 120°C. The samples were then slurry spiked with triazines (20 ppm). 5 g subsamples were extracted using 4.1-ml volume cells	Pressure: 300 bar; temperature: 65°C. Extraction time: 60 min. Methanol-modified (10% v/v) CO_2 used	HPLC–UV detection	Recoveries ranged from 5% (±2%) for desisopropyl-desethyl-2-hydroxyatrazine to 96% (±4%) for hydroxyatrazine. The recoveries were improved by the use of a non-polar wash	[91]

(*Continued on p. 320*)

Table 5 (*continued*)

Analyte	Matrix	Sample preparation	SF conditions	Method of detection	Recovery data	Ref.
Atrazine and simazine	Freeze-dried water samples	150 l of drinking water were spiked (0.02–7 ng/ml), stirred and freeze-dried. 2–3 g sub-samples of freeze-dried residue were introduced into extraction cell (5 ml)	Pressure: 20 MPa; temperature 50°C. CO_2 only used (30 ml)	GC–NPD detection	Recoveries ranged from 10% to 98% depending on extraction conditions	[35]
Simazine, propazine and trietazine	Water	200-ml water sample spiked (10 μg level); preconcentration of triazines on solid-phase extraction disk (Empore). Disk placed in extraction cell (10 ml)	Pressure: 250 kg/cm^2; temperature: 50°C. Methanol-modified CO_2 (10% v/v) used	HPLC–UV detection	Recoveries ranged from 86.6 to 100.4% with 7.4 to 12.9% R.S.D. ($n = 5$)	[92]
Atrazine and simazine	Natural water (tap, ground and river water)	Water sample (1000 ml) spiked; preconcentration on SPE Empore disk. Disk placed in extraction cell (3.0 ml). Void volume in cell filled with glass wool; 1.5 ml acetone added to top of sample disk and glass wool	Pressure: 400 atm; temperature: 50°C. Acetone-modified CO_2 (25 ml) used	GC–ion trap MS	Average recoveries of 106 to 107% with 12 to 15% R.S.D. ($n = 7$). Method detection limit was 0.11 ng/ml for atrazine and 0.24 ng/ml for simazine	[93]

employ a chemometric approach for both experimental design and/or data manipulation. The use of a chemometric approach has been shown to be useful for the SFE of triazines from soils [89,90].

In the first case [89], atrazine, cyanazine, desethylatrazine and desisopropylatrazine were extracted from four different soils types (Kenyon loam, Ida/Monona silt loam, synthetic soil matrix and sand). The soils were chosen to reflect the agricultural characteristics of Midwest, USA soils onto which herbicides are applied each year. Each soil was characterised according to standard methods to determine the organic carbon content, cation exchange capacity, pH, %sand, %silt and %clay content. Then each spiked soil was extracted under conditions in which the fluid density or pressure, flow-rate, extraction cell

temperature, trap temperature and cosolvent (modifier) were varied. Furthermore, using dynamic modifier addition the following variables were held constant: extraction time, rinse solvent, vessel equilibration time, number of trap rinses per cycle and trap eluant volume. A principal component analysis design was used to analyse the influence of each extraction variable upon all of the observed variables. Initial results indicated that extraction time, CO_2 flow-rate, trap elution volume and equilibration time exhibited little influence on extraction recovery. In addition, and perhaps most strikingly, was the trend between triazine recovery and carbon content of the soil. It was shown that the greater the polarity of the triazine derivative, the greater the effect of soil carbon content upon extraction efficiency with the most polar analyte, deisopropylatrazine, having the lowest recovery from the Kenyon loam soil. The importance of soil organic content was further highlighted with the reported recoveries of 93% for all herbicides studied when extracted from sand (organic content <0.05%). Temperature was also reported to have an influence on extraction recovery; higher temperatures (120°C) yielding lower recoveries. Similar behaviour was also reported for pressure; higher pressures yielding higher recoveries, the exception being cyanazine — a triazine with a cyano functional group which is capable of undergoing hydration, hydrolysis or methanolysis reactions. As the primary role of a polar modifier is to overcome the chemisorption bonding between the herbicide and a specific molecular environment within the soil particle, it is perhaps not surprising to find that the addition of cosolvent (modifier) was significant in the recovery of the triazines. The authors concluded that for atrazine, desethylatrazine and desisopropylatrazine, the optimal combination consists of high extraction pressure (up to 34.5 MPa) and low extraction temperature (40°C), using a maximum of 4–5% water. For cyanazine, lower extraction pressure is recommended.

Secondly, an experimental design approach has been applied to the extraction of atrazine, simazine, terbuthylazine, desisopropylatrazine and desethylatrazine from three types of soil [90]. The three soils investigated had organic contents of 0.3, 3.3 and 6.8% for sand, peat and clay, respectively. Within the experimental design the SFE operating parameters investigated were as follows: pressure: 20, 25, 30 and 50 MPa; temperature: 50, 70, 80, 90 and 100°C; extraction time: 30, 35, 40, 60 and 70 min; type of modifier: methanol, mixed CO_2–methanol and mixed CO_2–acetone; amount of modifier: 100, 200, 300 and 1000 μl; cell volume: 3.5 and 10 ml; and amount of triazines used: 50 and 100 μl. After extraction all samples were analysed by GC–NPD and the results, more than 200 data points, analysed using multilinear regression. It was concluded that the two important operating parameters for the extraction of triazines from soil samples were the pressure and the amount of modifier with respect to cell volume. The effect of temperature was not noted to be significant, however, the use of a particular pressure–temperature combination, i.e. density of the supercritical fluid, should prove to be beneficial. The effect of modifier was particularly important when added to the extraction cell and for triazines of higher polarity. Only a small effect was noted from soil type. The optimised operating conditions were: pressure 50 MPa; methanol modifier (100 μl/ml cell volume); temperature 50°C; and a short extraction time. The use of an experimental design approach allows fewer experiments to be performed, allows evaluation of recovery data, and to identify possible interactions between operating parameters.

One of the features of SFE is its controllable solvent power that allows the potential for selective extraction. Barnabas et al. [92] report the selective extraction of organochlorine pesticides (OCPs) from two classes of herbicide (urons and triazines) in aqueous samples. The aqueous samples were preconcentrated on solid-phase extraction media (Empore disks) prior to SFE. It was found that with CO_2 only, the OCPs could be quantitatively extracted (OCP, mean % recovery and %R.S.D.: heptachlor, 91.7%, 2.3%; isodrin, 101.6%, 8.1%; dieldrin, 84.8%, 2.8%, respectively) with no significant loss of the herbicides. When methanol (10% v/v)-modified CO_2 was used to extract the herbicides from the same

Empore disk, quantitative recovery was obtained (herbicide, mean % recovery, %R.S.D.: simazine, 100.4%, 12.9%; propazine, 88.5%, 7.4%; trietazine, 86.6%, 9.0%; chlorotoluron, 86.1%, 9.3%; isoproturon, 90.2%, 5.2%; and diuron, 87.8%, 7.7%, respectively). This selectivity of extraction was further enhanced by the method of separation–detection used. The OCPs were analysed using GC–mass selective detection while HPLC with UV detection was used for both classes of herbicides.

2.5. Microwave extraction

The use of microwave-assisted extraction for the extraction of environmental pollutants from solid matrices provides an alternative strategy for extraction. At present, the scientific development of this technique is in its infancy. However, the utilisation of microwave ovens is not new to the user because of their domestic use. Therefore, provided microwave-assisted extraction can provide some benefits to the analyst its niche will be guaranteed. At the present time, few applications have been reported in the literature [94,95]. However, the simplicity of operation, the reduction in solvent usage, speed of extraction (compared to Soxhlet extraction) and the good recoveries reported for environmental pollutants indicate that microwave-assisted extraction should be more widely reported in the next few years.

2.6. Solid-phase microextraction

The application of SPME to the determination of triazine herbicides in aqueous samples has been recently demonstrated [96]. In this situation the triazines (atrazine, simazine, propazine and trietazine) are adsorbed onto silica fibres coated with poly(dimethylsiloxane) and mounted in a modified GC syringe holder. After a suitable adsorption time, the syringe is inserted into the injection port of the GC and the fibre protruded from its protective holder. Desorption rapidly occurs and the triazines are then separated and detected using a gas chromatograph fitted with a nitrogen–phosphorus flame thermionic detector. In the work reported [96], the system was optimised with respect to adsorption time, desorption time, desorption temperature and column focusing temperature. The approach was found to be linear over three orders of magnitude (0.001–1 ppm). However, the required level of determination for drinking water is 0.1 ppb (ten times lower than that detectable above). In order for SPME to be useful as a screening technique for triazines at the 0.1 ppb level an alternative strategy was required. This was achieved by carrying out multiple adsorptions and desorptions of a 0.1-ppb triazine solution. But instead of analysing each successive desorption as previously, the triazines were stacked at the front of the column which was maintained at the focusing temperature. After ten successive adsorptions (10 min) followed by 5-min desorptions the chromatograph was run. The whole process was repeated three times and the precision obtained ranged from 6% R.S.D. for propazine to 20% R.S.D. for atrazine.

3. Chromatographic analysis

The sample preparation methods, discussed previously, are the first stage in the analysis of herbicide residues and their degradation products. Once the sample has been extracted from its matrix, usually into a suitable solvent, it is then ready to be analysed by the appropriate technique. Chromatographic methods and in particular GC and HPLC are the modern methods of choice. These fast, precise and user friendly analysis tools have almost completely replaced UV–Vis spectrophotometry and thin-layer chromatography (TLC), which were used extensively around the early 1970s. GC is often the first choice of analysis for triazines because of their good response with a nitrogen–phosphorus detector (NPD), which offers sensitivity and selectivity to organic compounds containing nitrogen or phosphorus atoms in their structure. The standard EPA protocol for the determination of triazines (and other nitrogen-containing pesticides) states the use of GC with NPD detection [34]. However, triazines also chromato-

graph well by HPLC, and this is often used in conjunction with UV detection when other neutral herbicides, which cannot be gas chromatographed, are to be analysed in the same run. HPLC with diode-array detection (DAD) also offers the possibility of avoiding matrix interferences, by choosing different wavelengths. In this second half of the review we consider the development of modern chromatographic techniques for the analysis of triazine herbicides.

3.1. Gas chromatography

The triazine herbicides have previously been analysed both spectrophotometrically in the visible and UV regions and by paper and thin-layer chromatography. Spectral methods were proven to be sensitive but lacked any separation potential, whereas both paper and thin-layer chromatography allowed some separation but could only be used semi-quantitatively [33]. The disadvantages of these methods are overcome by GC, which, in the early stages, was performed using packed-column systems, the short glass or stainless-steel columns being packed with a small percentage of stationary phase held on an inert support. A comparison between UV spectrophotometry and GC with an alkali flame detector (AFD) revealed that 0.001 ppm of atrazine, ametryne and terbutryne could be determined from two different water sources by using GC [32]. The packed glass column used was 1 m in length and 4 mm I.D. and was packed with 2% neopentylglycol succinate on 80–100 mesh Chromosorb W. However, UV analysis was only adequate at the 0.01 ppm level, being unreliable at lower concentrations.

The alkali flame detector has been used extensively for triazine analysis because of its sensitivity and selectivity towards nitrogen-containing compounds, and is actually another name for the modern-day NPD. The detector was conventionally made by modifying a standard FID and inserting a salt tip of rubidium bromide or sulphate [32,50]. Precise control of both hydrogen and nitrogen gas to the detector is essential and was achieved with a flow controller. In the latter publication the AFD was used in conjunction with a 1.9 m × 3 mm I.D. column packed with 10% w/w Reoplex 400 on 80–100 mesh Gas Chrom Z for the analysis of atrazine and simazine. Minimum detectable limits for the triazines were better than 0.5 ng.

The selectivity of triazines towards different types of GC detector was further demonstrated by comparing four detectors for the analysis of twelve triazines with differing functional groups [45]. Two of the detectors chosen offered specificity towards general triazines (alkali flame and electrolytic conductivity detectors) and these were used together with a microcoulometric detector (specific for chlorotriazines) and a flame photometric detector (specific for methylthiotriazines), each detector having its own column conditions and set of operating parameters. The results indicate the feasibility of each detector in the analysis of specific herbicides from a variety of food crops and show that in all cases the AFD can be used as a replacement for the electrolytic conductivity detector.

The triazine herbicides have also been determined in packed systems using flame ionisation as a detector [55]. Pacakova and Kozakova [42] successfully used FID to analyse atrazine in soil using a stainless-steel column (144 cm × 3 mm I.D.) packed with a mixture of 2% Reoplex 400 and 5% SE-30 supported on Chromaton. The GC method was evaluated against a standard spectrophotometric technique and the GC method was used to compare the amount of triazines in a technical product (Semparol). In this case a FID was used because the atrazine present in soil samples was at a relatively high level (of the order of a few ppm) and the amounts of other substances in the extract was low. Had other interfering compounds also been present, or the herbicide been present at low concentrations, a more specific detector (AFD) would have been beneficial.

Detectors other than those already mentioned have been successfully used to determine triazines in multi-residue mixtures. Lee and Chau [39] used a ^{63}Ni electron capture detector in conjunction with columns (1.8 m × 2 mm I.D.) packed with four different stationary phases. The GC system was used to analyse chlorinated

herbicides including atrazine since the detector is particularly sensitive to halogenated species. The best resolution and response was obtained from a 3% OV-1 column.

Although the use of packed-column GC has solved many of the problems associated with UV spectrophotometric and TLC herbicide analysis, some problems still exist. The main disadvantage with packed systems is the lack of resolution between eluting compounds and hence the inability of the system to analyse complex multi-component mixtures. Many *s*-triazine peaks overlap on non-polar stationary phases, with separations on polar phases (e.g. Carbowax 20M) better but still inadequate. The advent of high-resolution capillary columns has allowed a great increase in the ability to completely resolve complex mixtures. These columns have been used to separate very complex mixtures of triazine herbicides which have proved impossible to resolve using packed GC instrumentation. Matisova and Krupcik [97] demonstrated the use of capillary columns for the separation of 18 *s*-triazines in a mixture. The columns, made from soda-lime glass, were made from tubes approximately 0.7–1.5 m long, with 2–3 mm I.D., from which capillaries up to 130 m long with 0.2–0.3 mm I.D. were drawn. The columns were etched with gaseous hydrogen chloride for several hours to roughen the surface and allow stationary phase to be coated onto the column. A GC fitted with stream splitters and FID detection was used for the analysis. It was found that glass capillary columns with non-polar stationary phase were not suited for the analysis of the triazines as peak tailing was often observed. However, symmetrical peaks and the separation of 17 of the 18 analysed *s*-triazines was obtained on capillary columns coated with Carbowax 20M.

The same authors also compared the separation of chloro-, methoxy- and methylthio-*s*-triazines on a glass capillary column coated with Carbowax 20M to that obtained from a packed system filled with 3% Carbowax 20M on a Chromosorb W AW support [98]. A GC fitted with a stream splitter and FID detection was once again used for the analysis. The optimal temperature for the analysis of the triazines (isothermal) was determined from the dependence of log(retention time) on $1/T$.

Further work was carried out by Roseboon and Herbold [53] on the advantages of capillary over packed columns for the analysis of a complex mixture of 13 triazines. Both an electrolytic conductivity detector and a thermionic detector were used in the analysis, with the latter detector found to be approximately ten times more sensitive than the former. As expected, the capillary column was shown to offer about five times greater sensitivity than the packed system and was also capable of giving far greater separation. The best separation was obtained using a capillary column (40 m × 0.3 mm I.D.) coated with OV-225, as this had the advantage of shorter retention times for selected triazines. However, the column was found to have the disadvantage of producing very high backgrounds when using the thermionic detector, so a 12 m × 0.3 mm I.D. capillary column coated with Carbowax 20M was subsequently used. The GC system was then shown to be effective in analysing triazines in various crops. A similar comparison was undertaken by Lee and Stokker [40], who evaluated the use of both Ultrabond 20M and 3% OV-1 columns compared to a 30-m DB-1 capillary column. Eleven triazines were used for the evaluation and were extracted from natural waters prior to GC analysis with an NPD. Results using the Ultrabond 20M column (1.8 m × 2 mm I.D.) showed that it was capable of baseline separating ten of the eleven components at a column temperature of 170°C. However, it was not possible to separate cyprazine or simetryne, with the retention time of the former being too long (>70 min). A packed column with identical dimensions but packed with OV-1 was therefore used and the eleven triazines chromatographed at 160°C isothermal. Under these conditions many of the peaks merged (the overall retention time was reduced to <15 min) but it was possible to baseline separate both cyprazine and simetryne. Therefore a combination of the two columns could be used to achieve complete resolution. These results were then compared with those obtained from a DB-1 fused-silica capillary column, which was chosen over other

capillaries because of its high-temperature and long lasting stability. Although incomplete resolution was observed using this column, the eleven peaks were readily identifiable and quantitation was possible for each triazine. The detection limit for all eleven compounds was found to be 0.025 μg/l.

The use of capillary columns has now been established as the obvious choice for analysing complex herbicide mixtures. Other publications focused on the use of the capillary column with both FID and AFD detectors; comparing packed performance against that of capillary and FID only [99]. A glass capillary column coated with Carbowax 20M successfully separated twenty-nine *s*-triazines and their N-dealkylated degradation products [100].

Capillary GC with NPD (or AFD) is now the chosen technique for much of the triazine analysis. In recent years commercial GC instrumentation and capillary columns have become so widespread that their use has become of a secondary nature within environmental analytical research. Publications now concentrate on achieving a similar status for sample preparation protocols which have already been discussed. Two recent papers demonstrate GC's popularity as the chosen analysis technique. Capillary GC with AFD was used to analyse triazines extracted from natural water samples using SPE [70], while GC with NPD detection was used as the mode of analysis for the herbicides extracted from soil samples using SFE [90].

3.2. Gas chromatography linked mass spectrometry

The discussion so far has concentrated solely on GC analysis using a variety of specific and non-specific detectors, non of which are capable of positively identifying the eluting compounds. Conformation of positive results has been previously determined by chromatographing the unknown sample on columns of differing polarity or by using extensive clean-up procedures to selectively separate different classes of pesticides and herbicides. By far the most sophisticated and reliable approach for the identification of unknowns is that of mass spectrometry, which allows the unambiguous determination of unknowns by using the mass spectra 'fingerprint' which they produce. Early work in the field used magnetic sector instruments to obtain spectra but had the disadvantage of being complex to operate, requiring specialist knowledge. Twenty-two different triazines were chromatographed using a packed-column GC fitted with an FID followed by independent mass spectrometry [101]. A magnetic sector instrument was used for positive ion electron impact mass spectrometry, which yields excellent fragmentation patterns, with further conformation achieved using chemical ionisation of the sample with a quadrupole instrument. Chemical ionisation has the advantage of producing far less fragmentation of the compound (since it is a much softer ionisation method) and so allows a greater chance of the molecular ion being present, which can aid interpretation.

The main disadvantage with early mass spectrometry was the degree of specialist training required to operate the complex instrumentation and the amount of maintenance needed. However, it was soon recognised as the ultimate GC detector for the confirmation of positive results obtained from the very sensitive detectors already discussed. The advent of the modern commercially available mass selective detector (MSD) which is linked to a capillary GC system has meant that not only a detector can be as sensitive as most of the others available but can also be capable of positively confirming the identity of the eluants in a single determination. By far the most popular instrument used is the quadrupole MSD, which has the advantage of being very compact as well as offering all of the above benefits [56]. The MSD can be operated in two modes, both of which are useful in environmental analysis: total ion scanning or selected ion monitoring (SIM). In total ion scanning the MSD acquires a series of mass spectra over the whole time the GC separation is taking place. The addition of all the ions from the ionisation results in the total ion current (TIC) trace, which resembles the chromatogram obtained from a conventional detector, the difference being that each point on the TIC represents a full mass

spectrum at that time and can be retrieved at any time for inspection or comparison to library spectra contained on computer. In SIM the sensitivity of the MSD is increased, but at the expense of the TIC information. Instead of continually scanning the entire spectral range, only a few ions are inspected which are representative of the compound of interest. The sensitivity is increased because of the longer specific sampling times for each ion selected. SIM therefore requires prior knowledge of the mass spectrum of the compounds of interest and their retention time under the GC conditions chosen, which means that TIC and SIM are often used in conjunction with each other.

There are many examples of GC–MS being used as a conformation tool after analysis of triazines by a specific detector such as a NPD. Electron impact GC–MS was used in SIM mode for the conformation of atrazine, deethylatrazine and simazine after analysis by GC–NPD following SPE [20]. GC–MS in SIM mode has also been used as a stand-alone detector, once again after SPE, for the determination of triazines and their metabolites from SPE cartridges [71,82,85]. Selected ion monitoring in conjunction with SPE may hold an advantage over conventional specific detectors in that impurities which may be present at the surface of the disk or cartridge can be eluted with the solvent and can interfere with the final analysis when using FID or NPD. The nature of GC–MS in SIM mode means that it does not suffer from these possible problems. The GC–MS–SIM technique was compared with GC–NPD by Psathaki et al. [85] for a range of pesticides including atrazine and simazine in ground and drinking water. In this work [85], the MS was found to be more suitable for identification and determination of a range of pesticides. It was advised that a second capillary GC column was required for identification purposes if NPD was the only available detection method. Other examples include the use of GC–MS to verify the presence of atrazine, simazine and terbuthylazine in sea water after initial GC with AFD [102] and its use after GC–NPD to avoid false positive identification of triazines in freeze-dried water samples extracted by SFE [35]. Chlorotriazine herbicides and their degradation products have also been subsequently analysed by GC–MS after initial screening using GC–NPD [48]. Fragmentation was achieved conventionally by electron impact ionisation, which is the common mode of operation with further positive- and negative-ion chemical ionisation recommended for unequivocal identification. The main advantages observed in using chemical ionisation (CI) are the reduction in the limit of detection when using negative-ion CI (by a factor of 50) and the enhancement of the signal to noise ratio.

Although GC–MS is conventionally used as a conformation tool after GC–NPD analysis for triazines, the technique has also been compared with enzyme-linked immunosorbent assay (ELISA) [86]. ELISA has been extensively used in clinical chemistry but has only recently been introduced into environmental analytical chemistry. Commercially available ELISA kits were used for the immunosorbent analysis of water samples (surface and groundwater) which use polyclonal antibodies, coated to the walls of a polystyrene tube, and an enzyme conjugate that was prepared, in this case, by covalently binding atrazine to horseradish peroxidase. The ELISA technique proved linear between 0.2 to 2.0 μg/l and allowed for the rapid screening of water samples both in the field and laboratory. Other advantages with the technique include the lack of false negatives observed, which is typical of an ELISA method, and the ability of the method to work with only small sample volumes (ca. 160 μl) which require no sample preparation. ELISA proved useful as an initial screening method and showed good correlation with GC–MS analysis of both spiked and field samples.

The use of mass spectrometry in environmental analysis has one other advantage in that it can be used it conjunction with isotope dilution analysis (IDA) for the quantitation of environmental pollutants. IDA is an analytical procedure in which a known quantity of a stable isotope is added to the sample prior to the final analysis in order to quantitate a particular compound. The ratio of the naturally abundant and the labelled isotope can be used to measure the concentration of the naturally occurring compound. Although

the availability of stable isotopes still remains a problem, there are many benefits to their use in environmental monitoring. The obvious similarity between the stable isotope compound and the naturally abundant one can be used in two ways. Firstly, by adding the isotope immediately after sample collection, then the extent to which the naturally abundant compound has degraded can be assessed. Secondly, addition prior to sample extraction allows a continuous quality control check throughout the analysis. A multi-residue method was developed for the simultaneous determination of low ppb concentrations of various herbicides including atrazine and simazine [51]. Isotopically labelled forms of each herbicide were added, prior to extraction of the sample containing matrix, to monitor the extraction and subsequent analysis of each compound. A conventional quadrupole GC–MS was used for the analysis, in SIM mode, using a 25 m × 0.25 mm OV-17 capillary column for separation. Using this technique, the limit of detection was found to be 0.05 ppb for water and 0.5 ppb for soil. IDA has also been used to analyse pesticides and herbicides (including atrazine) in soil where atrazine-d_5 was spiked in the sample prior to extraction [61,82]. The analysis was performed using quadrupole GC–MS operated in SIM mode. Accuracy of better than 86% with a precision of 8% was reported. The technique has also been used to compensate for differences in the physical recovery of atrazine and its metabolites when extracted using solid-phase extraction (from water samples) or SFE (from soil) [73]. Over 400 water and 800 sediment samples were analysed by IDA with, average accuracy (bias) for atrazine-fortified water samples of +6.4% ($n = 200$) and precision from duplicate analyses as ±6.0%. For atrazine-fortified soil samples, the precision for the SFE method was ±11% at the 2 ng/g level with an accuracy of −3.2% ($n = 8$) at the 5 ng/g level [73].

The discussion of GC–MS in triazine analysis has so far been restricted to magnetic sector or quadrupole instruments. However, one other technique which has also been used for triazine quantitation is that of GC–ion-trap MS [93]. The components of an ion-trap system are much less complicated than the instrumentation previously mentioned and rely on electronic control to obtain mass spectra. In this example, GC–ion-trap MS was used to identify and analyse 43 semi-volatile pollutants, including triazines, from water samples. As with other systems, the capillary column outlet from the GC is placed directly into the ion-trap MS, which was scanned from 45 to 450 m/z using selected ions for quantitation.

3.3. Gas chromatography with atomic emission detection

The use of GC linked to atomic emission detection (AED) offers an alternative to GC–MS for the unambiguous determination of unknowns. GC–AED was demonstrated by Eisert et al. [77] as a potentially useful new screening tool for triazines in water samples. The coupling technique was shown to be a very selective tool with element-characteristic chromatograms acquired using different element emission lines, therefore enhancing the selectivity of the method for environmental monitoring. A helium purification kit was used to obtain helium of sufficient quality for both the GC carrier and AED plasma gas. The following elements can be monitored: nitrogen, phosphorus, sulphur, carbon, chlorine, hydrogen, bromine and oxygen. Typical limits of detection for all investigated compounds were reported to be between 0.5 and 5 μg/l using the carbon and sulphur emission lines. This AED sensitivity is poorer than classical GC detection methods, however, AED is nevertheless selective, allowing compounds to be characterised by their elemental emission lines.

3.4. Liquid chromatography

Although gas chromatography is still the preferred choice of analysis for triazine herbicides (since it normally offers much lower detection limits), they can also be well separated by liquid chromatography (LC). In addition, as triazines strongly absorb in the UV region they make excellent compounds for UV detection, the commonly used method of detection in liquid chromatography. LC, or more commonly 'high-per-

formance' liquid chromatography (HPLC), also has other advantages over GC since it can be used to analyse compounds which would normally require chemical derivatisation for GC analysis. Urons, another class of neutral herbicides, fall into this category, and as they are often found in water samples together with triazines, separate analysis would be required if the triazines were to be detected by GC. Also, if SPE is used as the sample preparation technique, it is normal to elute analytes in solvents directly amenable to HPLC (i.e. methanol). This would normally necessitate solvent switching by evaporation if the sample was to be analysed by GC.

Early publications focused on comparing HPLC with standard methods for triazine analysis. Reversed-phase HPLC using a C_{18} column and UV detection at 254 nm was compared with a colorimetric method for determining atrazine residues in soil [59]. The HPLC method used a mobile phase for atrazine of either 100% $CHCl_3$ or methanol–water (65:35) and gave over double the recovery efficiencies of the colorimetric technique. HPLC has also been evaluated for triazine analysis in water samples and compared with GC [66]. Here HPLC using a 15 cm long column (3.5 mm I.D.) packed with Separon SIC_{18} sorbent, was used to separate seven different triazines and gave a limit of detection around the ppb level. Packed GC–NPD was found to allow tenths of ppb quantities to be detected, although the HPLC gave more complete resolution of the seven compounds. Janda et al. [87] used both HPLC with diode-array detection (DAD) and GC–FID for the analysis of triazines in soil samples which had previously been extracted by SFE. The non-specific nature of the GC detector meant that *n*-alkanes, present in the soil, caused interference and GC could not therefore be used to detect low triazine levels. HPLC–DAD did not suffer from this problem since the interfering compounds do not absorb at the wavelength chosen for the triazine quantitation (225 nm). Diode-array detection also has the advantage over conventional UV detection in that it can be used to scan the entire range of the UV–Vis spectrum, allowing interfering or overlapping peaks to be identified. HPLC–DAD has been used to confirm the presence of triazines in extracts by means of their UV spectrum [41]. Atrazine (λ_{max} = 220 nm) was shown to exhibit a characteristic spectrum when eluting from a reversed-phase HPLC column, although compounds with maxima at lower wavelengths could not be identified because of interference from the mobile phase. This method was also compared against GC–NPD with GC–MS conformation and was found to show slightly less spectral resolution between some compounds and poorer limits of detection, depending on the compounds UV maxima. However, HPLC–DAD permitted the direct determination of bentaone (herbicide) without the need for derivatisation.

A more recent paper describes the use of HPLC with DAD for the analysis of atrazine and its principal degradation products (deethylatrazine and deisopropylatrazine) in soil, surface and ground waters [62]. Instrumental chromatographic parameters involved complex ternary system pumping (methanol, acetonitrile and water) and flow programming using a LiChrospher 100 RP-18 column (5 μm, 124 × 4 mm I.D.). Photodiode-array detection was performed at 220, 235 and 254 nm with a 4-nm bandwidth. The chromatograms obtained show excellent separation of the three compounds as well as terbuthylazine, which serves as a surrogate standard which mimics the chemical behaviour of atrazine. The method also showed excellent response linearity between 1 and 100 ppm; the extended range was necessary for concentrations normally found in the samples of interest. DAD was again successfully used to analyse terbuthylazine and its degradation products in soil using a variety of different columns [43]. Reversed-phase C_8 and C_{18} together with normal-phase NH_2 columns were employed with a water–acetonitrile mobile phase at various ratios and flow-rates. The reversed-phase columns were reported to give good separation of the four compounds studied with a 50:50 mix of mobile phase, with the metabolites being retained more efficiently by the C_8 column. With the normal-phase column, using acetonitrile with 1% water as an eluant, the peak elution order was reversed, with a moderate overall reduction in retention time. The work indicates that the

NH_2 column could be valuable as a confirmatory tool.

Photodiode-array detection is probably the preferred method for LC analysis of triazines, although less sophisticated detection systems have been employed. HPLC separation, using reversed-phase C_{18} columns (150 mm × 3.5 mm I.D.) with both UV and amperometric detection, was used to analyse 18 *s*-triazine derivatives [103]. Capacity factors for the triazines were obtained at various methanol concentrations, pH and ionic strengths of a mobile phase consisting of aqueous sodium dihydrogenphosphate and methanol. The optimum methanol content was found to be 70% (except for the degradation products of atrazine, for which it was found to be 40%) and was held at this value when determining the optimum pH and ionic strength. The optimum conditions were determined to be: a pH of 6.8 and an ionic strength of 0.01 *M* aqueous sodium dihydrogenphosphate. Overall, UV detection was found to give a good overall response for all of the triazines studied. Amperometric detection (with a carbon-fibre array electrode) proved useful for selective detection of the *s*-triazine hydroxy derivatives.

There are many examples of HPLC being used as the preferred technique for triazine analysis after preconcentration on a solid adsorbent. Grob and Li [67] employed a multi-dimensional chromatographic protocol, coupling reversed-phase LC to capillary GC for the determination of atrazine in water. An amount of 10 ml of water containing atrazine was passed through a small C_{18} chromatographic column (100 × 2 mm I.D.) and eluted with methanol–water (60:40). Using nitrogen–phosphorus detection (for the GC) allowed 15 ppt atrazine to be detected.

The use of small precolumns, connected in series, prior to HPLC analysis has also been shown to be effective in determining triazine residues in soil [104], vegetables [68] and water [105,69]. In the first example, eight triazines were extracted from soil, which was contained in the first column, by allowing 3 ml of acetone to be passed through and into a second column which was packed with a strong acid exchanger (sulphonic acid-type silica-based cation exchanger, SCX). The triazines were then selectively re-adsorbed via salt formation on the SCX surface. After disconnection of the two columns, the triazines were eluted from the cation exchange column with potassium chloride saturated methanol (this elution procedure was also adopted on the first column). Methanol was then removed from the combined eluates and the triazines were fractionated and analysed by reversed-phase HPLC using a 25 cm × 4.6 mm I.D. analytical column packed with LC-18-DB, in conjunction with a guard column. The mobile phase used was acetonitrile–phosphate buffer (10 mmol/l; pH 6.7) (38:62) and the triazines were monitored on a UV detector set at 220 nm. The mean limit of detection for the procedure was reported to be about 1 ng/g in soil. Similar protocols have also been reported [68,105]. In these cases, the first column was filled with a non-specific adsorbent material (graphitised carbon) connected in series to a second column packed with SCX. Vegetable extracts are analysed for the same eight triazines after suitable extraction, first with an acetonitrile–water mixture and then by passage through the graphitised carbon filled precolumn [68]. Identical HPLC conditions are used as previously, giving limits of sensitivity for the method of 10 ng/g. River water samples have also been determined using the same precolumn setup [105]. Here simazine, atrazine and propazine together with phenylurea herbicides have been shown to be extracted and analysed from 1 l of river water. In addition, a two column setup, one packed with alkylsilica (C_{18} or C_8) and the other with a styrene–divinylbenzene copolymer (PRP-1), has been used to extract triazines and other selected herbicides from water samples before analysis by HPLC [69]. To achieve acceptable detection limits the PRP-1 column was used in series with a specific ion exchanger column (10 × 2 mm I.D. packed with 15–20 μm BC-X8 with 3 ml 0.1 *M* perchloric acid modified with 25% acetonitrile) which effectively removes both inorganic and organic interferents, therefore lowering the limit of detection to 0.1 μg/l.

HPLC has also been used as a stand-alone analysis technique for the monitoring of triazine samples after solid-phase extraction (SPE). Both

C_{18} and cyano columns were used to separate a mixture of acidic herbicides and triazines, with the retention times of all triazines being shorter on the latter column [72]. Vitali et al. [74] used HPLC to separate ten compounds (seven triazines and three dinitroanilines) after extraction onto a C_{18} cartridge. A 250 × 4 mm I.D. C_{18} analytical column was used to perform the separation, using gradient elution (mobile phase acetonitrile–water; 4 min at 40% acetonitrile, from 40% to 75% in 26 min, convex gradient curve, then 5 min at isocratic conditions). A DAD was used at 222 nm with a bandwidth of 3 nm for the triazine and dinitroaniline analytes. Identification and quantitation was achieved by comparing retention times and spectral data with those from known standards. Hydroxylated atrazine degradation products have been quantified using reversed-phase C_8 (25 cm × 4.6 mm I.D.) HPLC after adsorption from water onto an SCX column [79]. Two different mobile phases were used to separate hydroxyatrazine (water–40% methanol) and deethylhydroxyatrazine and deisopropylhydroxyatrazine (water–15% methanol). The limit of detection and quantitation were reported as 0.12 and 0.4 ppb, respectively, with a linear response quoted between 0.2 and 8 ppb. In the final example, HPLC has been used in the development of an on-line SPE procedure for the analysis of different classes of herbicides including triazines [76]. On-line analysis has several advantages, including no sample manipulation between preconcentration and analysis and therefore no loss or contamination risk. In river water samples, the low levels of detection required that the method of standard additions was employed for quantitation because of the high matrix interference due to low detector attenuation of the DAD. Other quantitation methods specific to the HPLC–DAD instrumentation were also used, which are based upon spectrophotometric identification. Detection limits of the order of 0.1 μg/l were obtained using 150 ml of river water without the need for sample clean-up.

In addition to SPE analysis, HPLC is often the method of choice after extraction of triazines using SFE. The widespread use of non-polar carbon dioxide as a supercritical extraction solvent has also lead to the equally extensive use of polar modifiers, such as methanol. This has meant that HPLC (for similar reasons to SPE) has become a favoured technique for polar compound analysis. Examples include its use in analysing triazines: atrazine, 2-hydroxyatrazine and deisopropyl-deethyl-2-hydroxyatrazine [91]; and, atrazine, cyanazine, desethylatrazine and desisopropylatrazine [89] from soil samples; and the analysis of triazines (simazine, propazine, trietazine) and urea herbicides, selectively extracted from water samples [92].

3.5. Liquid chromatography linked mass spectrometry

One of the major disadvantages with LC is the lack of conformation ability of the UV detector. Photodiode-array detection is capable of some identification, but often interferences from the matrix or the mobile phase affect spectral interpretation. In the case of triazines, conformation can be carried out using GC–MS, but often other herbicides are present, which can prove problematic to GC without derivatisation due to their thermal instability at the column temperatures typically used. One potential approach to overcoming these problems, which has been reported, is the use of liquid chromatography–mass spectrometry (LC–MS) in conjunction with thermospray coupling using either a high-resolution or a quadrupole mass spectrometer [47,75,78,83,106]. Other interface techniques have been used (i.e. moving belt, direct liquid introduction, fast atom bombardment and particle beam), but thermospray is the one most widely used today.

Thermospray LC–MS using a double-focusing mass spectrometer was used for the determination of selected herbicides, including triazines, in water samples [106]. The heated thermospray source was used in the discharge ionisation mode, with a source block temperature of 250°C, tip temperature of 155°C and vapour temperature of 190°C. The spectrometer was scanned repetitively from m/z 110 to 650 using a 2.5-s cycle time. Gradient elution was used for evaluation of a river water sample using a mobile phase

consisting of methanol containing 0.1 *M* ammonium acetate (40% methanol to 60% methanol in 30 min then 90% methanol in 10 min). A reversed-phase C_{18} column was used for the separation. Application of this method to a river water extract showed the absence of problematic interferences and allowed the detection of herbicides at levels of a few nanograms.

One of the main disadvantages with the thermospray interface is the lack of fragmentation and therefore structural information observed. Normally positive-ion mode is used and the only ions usually produced are the protonated molecular ion and/or the adduct ion $[M + NH_4$, etc.] (depending on mobile phase composition). Barcelo et al. [47] explored the possibility of obtaining structural information in filament-on thermospray LC–MS for the identification of pesticides in environmental samples. Both positive-ion (PI) and negative-ion (NI) modes were used, although NI gave additional structural information, with the ions showing a great resemblance to the ions observed under negative chemical ionisation MS. Thermospray LC–MS, combined with post-column techniques was used for the unambiguous determination of 128 pesticides including 16 triazines [75]. An LC separation in conjunction with post-column addition of a volatile salt solution was developed which allowed the determination of 95 of the pesticides within a single run using reversed-phase gradient elution (methanol–water mixtures). Salt is added after separation to enhance selectivity and sensitivity since these are dependent on the dielectric constant of the final carrier stream. By using narrow-bore columns and post-column salt addition, the detection limits for triazines were found to be 0.5–5 ng in PI mode (full scan, m/z 130–450). Both positive-ion and negative-ion LC–MS were also studied for the determination of pesticides in environmental waters [78]. Two multi-residue analyses were carried out depending on the method of extraction. The first involved a 250 × 4.6 mm I.D. C_{18} analytical column (used after preconcentration onto a small precolumn packed with PRP-1 copolymer) and used gradient elution to accomplish separation within 35 min. The eluant used contained water, ammonium formate (0.05 *M*) and acetonitrile mixtures of various composition. The second analysis (after Empore C_{18} SPE) used a cartridge column with identical dimensions and used a complex gradient elution programme. All detection was performed using MS with two main ions being produced (usually $[M + H]^+$ and $[M + NH_4]^+$ or $[M + CH_3CN]^+$) in positive-ion mode while the $[M + H]^-$ and $[M + HCOO]^-$ ions were used in negative-ion mode. Good linearity is reported for levels varying from 0.025 to 1.2 μg/l, and a limit of detection of 0.01 to 0.4 μg/l was achieved depending on the particular compound and the operational mode.

3.6. Ion chromatography

Initial studies in using ion chromatography for triazine analysis compared conventional LC techniques with ion interaction procedures [107]. For LC studies, sodium phosphate buffer (15.0 m*M* H_3PO_4 + NaOH) was used with acetonitrile (55:45) as the mobile phase. In comparison, for ion interaction chromatography (IIC) sodium dodecyl sulphate (SDS) at 1.0 m*M* and sodium phosphate buffer (15.0 m*M* H_3PO_4, pH adjusted to 7.0 with NaOH) was used in conjunction with acetonitrile (50:50). An RP-C_{18} analytical column with UV detection was used throughout, with the detector set at a compromise wavelength of 220 nm. In the LC work, the influence of organic modifier concentration, pH, ionic strength and modification of the stationary phase polarity on triazine retention time was investigated. The greatest effect on retention time was observed with changes in modifier concentration and ionic strength, with little change noted with pH and stationary phase modification. The addition of lithium perchlorate, to increase ionic strength, was shown to improve the resolution and chromatographic performance with a significant effect on retention times. IIC was achieved by working at a suitable pH (depending on the triazine pK_a) so that the analytes can be treated as cations. Optimisation experiments were carried out to assess the effectiveness of SDS as a counter ion. SDS is used because it modifies the stationary phase, which assumes a negative

charge, introducing dynamic ion-exchange sites on the phase which can interact with the cationic triazines. The optimised IIC conditions (described above) were shown to give better resolution for the triazines when compared to the optimised LC method and allow for a more flexible technique than LC in the analysis of herbicides. The same group also developed a full ion chromatographic method for the determination of triazines in environmental samples [80]. The polar nature of the triazines was used to effect a separation on a cation exchange column (OmniPac PCX 500, 250 × 4 mm I.D.) with the organic modifier concentration and ionic strength optimised. The eluant composition after optimisation was found to be: acetonitrile–buffer (70:30), the buffer being 1.0 m*M* phosphate (pH 4.5) containing 30 m*M* NaCl, with a flow-rate of 0.7 ml/min. UV detection was usually performed at 220 nm. Detection limits between 10 and 80 μg/l with a linear range between 0.5 and 10 mg/l were reported, depending on the triazine.

3.7. High-performance thin-layer chromatography

Conventional thin-layer chromatography (TLC) was used extensively in the late 1960s and early 1970s for the determination of triazine herbicides since it offered a simple and fast screening method. However, it was often found to lack selectivity and sensitivity compared to GC and LC. More recently, a high-performance thin-layer chromatography (HPTLC) method has been evaluated for the analysis of atrazine and simazine in drinking and surface waters [81]. The procedure used HPTLC precoated silica gel 60 F_{254} plates (10 × 10 cm) and SIL-20 UV 254 (10 × 10 cm) for the chromatography. The samples (40 μl), which had previously been extracted using C_{18} solid-phase extraction cartridges, were applied to the plate using a commercially available applicator in 3-mm strips at the rate of 8 s per μl. The samples and the standards were applied alternatively at 2 mm intervals to both halves of the plate with the calibration based on peak heights obtained from densiometric responses using a UV scanning densitometer. The plates were then developed using a mobile phase of nitromethane–tetrachloromethane (1:1) at 25°C, which was found to be the best composition, leaving impurities from the samples mostly on the start and front positions of the eluent. The system showed good linearity and gave detection limits of 30 and 60 ng/l for atrazine and simazine, respectively, at the 80–400 ng/l fortification level in surface waters. The method was compared against GC–MS and was found to be advantageous due to its high sample throughput at reduced cost.

3.8. Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) has gained popularity for the determination of thermally labile compounds since it does not require the high temperatures of GC but will allow the use of typically GC-based detection systems not possible with LC. SFC also offers other advantages over LC such as faster analysis and higher resolution per unit time. Analytes are eluted using a supercritical fluid (usually carbon dioxide) which can have its solvent strength altered by changing the pressure and hence the density of the fluid. The temperature normally remains constant (isothermal) during a chromatographic run.

Few publications exist on the use of SFC in triazine analysis [83,108]. However, Shah et al. [108] have demonstrated its applicability. In this work, an LC pump, modified for supercritical fluids, was used to deliver CO_2 to the system. Since several of the triazines are markedly polar, the system would not elute the mixture with 100% CO_2, and therefore the mobile phase was modified with methanol to increase the mobile-phase polarity. A second pump was used to add methanol which was mixed in a T-mixing chamber prior to passage through the analytical column (cross-linked cyanopropyl bonded silica column, 250 × 4.6 mm I.D.). A variable-wavelength UV detector was used throughout the analysis. During the initial study, it was found impossible to obtain adequate separation using isocratic CO_2–methanol conditions, even with a variety of stationary phases and column tempera-

tures. The complete separation of all the eight triazines was, however, achieved with gradient elution. The percentage methanol was varied from 0% to around 33% at the end of the run, which was performed at 60°C, at a CO_2 flow-rate of 2 ml/min and 4000 psi (2.76×10^7 Pa). Under these conditions separation was achieved in under 6 min. Both CO_2 pressure and flow-rate were also investigated for their effect on resolution, and it was found that little separation was lost when the flow-rate was doubled, giving an even greater time saving. The authors concluded that packed SFC was a viable alternative to conventional LC analysis, providing faster analysis times without loss of resolution.

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