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Solid-phase extraction with styrene–divinylbenzene sorbent for high-performance liquid or gas chromatographic determination of urinary chloro- and methylthiotriazines☆

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Abstract

A solid-phase extraction (SPE) procedure on a styrene–divinylbenzene (SDB-1 cartridge) for extraction and cleaning of the triazine herbicides atrazine, simazine, ametryn, and prometryn and atrazine monodealkylated metabolites from urine samples was developed and optimised for final high-performance liquid chromatographic (HPLC–UV diode array detection) and gas chromatographic (GC–electron-capture detection and GC–thermionic-sensitive detection) analyses. Interfering polar matrices were eliminated by rinsing SDB-1 with 1% acetonitrile in water or with pure water. Extraction recoveries were from 78 to 101% with an RSD of about 10% for all studied compounds. The extraction recovery for the didealkylated atrazine metabolite was significantly lower and this compound cannot be determined with these procedures. Sorbent matrix generated interferences, although not detected by the chromatographic system, lowered the response of nitrogen–phosphorus and electron-capture GC detectors for monodealkylated chlorotriazines when compared to standards prepared in *n*-hexane. HPLC and GC analysis with SPE (SDB-1) preconcentration showed excellent linearity over the concentration range tested, with detection limits in urine of 10 ng ml^{-1} for the parent herbicides (HPLC and GC analysis) and 20 ng ml^{-1} for monodealkylated chlorotriazines (HPLC analysis).

Keywords: Chlorotriazines; Methylthiotriazines; Triazines; Pesticides

1. Introduction

Occupational exposure to atrazine and other triazine herbicides results in urinary excretion of traces of both parent compounds and of their metabolites [1–5]. The spectrum of urinary metabolites of atrazine, the most common triazine herbicide studied, comprises unmodified atrazine [2–4], its mono- and didealkylated amino metabolites [1,3–5], and atrazine mercapturic acid conjugate [5]. Deethyldeisopropylatrazine and deisopropylatrazine, at total concentrations ranging from 30 to 110 μmol l^{-1}, were the only metabolites identified in urine samples of railway workers engaged in the weeding of railway lines and exposed to liquid atrazine formulation [1]. A different finding resulted from biological monitoring of workers exposed to atrazine

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during its industrial production [2,3]. The atrazine excretion was followed through measurement of unmodified herbicide and its mono- and didealkylated metabolites in 8-h urine samples of manufacturing workers. Approximately 80% of the excreted metabolites was didealkylated atrazine (on average 1.614 μmol per 24-h sample), 10% deisopropylated (on average 0.206 μmol per 24-h sample), 8% deethylated (on average 0.112 μmol per 24-h sample) and 1–2% was unmodified atrazine (on average 0.021 μmol per 24-h sample) [3]. The levels of urinary metabolites found in 8-h urine samples of workers manufacturing atrazine [3] were comparable to those determined in post-exposure spot urine samples collected from workers in an atrazine and simazine production plant [4]. Atrazine and simazine and/or their mono- and didealkylated metabolites were detected in all spot urine samples at concentrations of 20–98 ng ml^{-1} for deethylatrazine, 25–35 ng ml^{-1} for deisopropylatrazine, 30–667 ng ml^{-1} for deethyldeisopropylatrazine, 5–29 ng ml^{-1} for unmodified atrazine and 10–20 ng ml^{-1} for simazine. A series of urine samples from workers applying atrazine confirmed a mercapturic acid conjugate as a major urinary metabolite [5]. The mercapturate was found in concentrations at least 10 times that of any of the N-dealkylated metabolites or the parent compound. Urinary atrazine mercapturate levels, determined by an enzyme-linked immunosorbent assay, ranged from <1 to 1756 ng ml^{-1}.

Due to the low concentrations of urinary metabolites in occupationally exposed persons, very sensitive analytical methods are required for a direct exposure assessment or to study the metabolic fate of triazine compounds in humans. High-performance liquid chromatography (HPLC) [5,6] and gas chromatography (GC) with selective detectors [1–4,7,8] are techniques directly applicable both to unmodified triazines and their metabolites. However, to achieve sufficiently low detection limits, a sample pretreatment is required providing enriched sample extracts and eliminating matrix interferences as far as possible. We recently summarised papers dealing with the development of analytical methods for determination of triazine compounds in human urine and compared critically extraction methods for atrazine, simazine, prometryn, ametryn and three chlorodealkylated atrazine metabolites from human urine using diethyl ether and C_{18} solid-phase extraction (SPE) [4]. The non-polar unmodified herbicides can be efficiently extracted by a solvent or trapped on hydrophobic sorbents like C_2 and C_{18} [4,5,9,10]. An efficient and very sensitive solid-phase microextraction procedure with a polydimethylsiloxane-coated fibre was also reported for determination of chloro-, methylthio- and methoxy-*s*-triazines, as well as for non-symmetric triazinones in human body fluids [11]. The compounds were quantified by capillary GC with nitrogen–phosphorus detection with detection limits in urine of 0.4–2.0 ng ml^{-1}. However, quantitative liquid–liquid or SPE of traces of more polar triazine N-dealkylated metabolites is more difficult. In our experience [4], and contrary to some literature data [3,7], liquid–liquid extraction of urine with diethyl ether did not prove uniformly efficient for trace enrichment of atrazine and its mono- and didealkylated metabolites and the additional extraction of urine with ethyl acetate did not improve the recoveries of dealkylated chlorotriazines. On the other hand, SPE of urinary atrazine metabolites with octadecyl-bonded silica packed in a Sep-Pak C_{18} cartridge resulted in variations of metabolite recoveries with sample concentrations, indicating a breakthrough of compounds during the sample percolation [4].

Two recent comprehensive reviews of SPE procedures for multiresidue analysis of organic contaminants [12] and priority pesticides, including triazines and degradation products [13], pointed out the higher potential of polystyrene–divinylbenzene resins over C_{18} silicas for trace enrichment of polar compounds from water. Styrene–divinylbenzene copolymer (PRP1, PLRP-S) was shown to be highly efficient for the on-line preconcentration of chloro- and methylthiotriazine herbicides using reversed-phase liquid chromatography with UV [14–17] and electrochemical detection [15]. Using the same on-line systems the atrazine monodealkylated metabolites were recovered less efficiently due to their significantly lower breakthrough volumes compared to the parent herbicides [15,17]. The recoveries of deethyl- and deisopropylatrazine at the 5 μg l^{-1} level in Milli-Q water at sample volumes of 25–50 ml ranged from 75 to 83% and dropped to about 50% on percolation of 100 ml of water [17]. However, commercially available styrene–divinylbenzene res-

ins vary in their cross-linking degree, porosity and specific area, properties significantly influencing the extraction efficiency of polar compounds from aqueous media [12,13]. The highly cross-linked styrene–divinylbenzene adsorbent LiChrolut EN was successfully applied for extraction of atrazine, terbutylazine and their polar N-monodealkylated degradation products and for hydrophilic hydroxyatrazine degradation products from tap and river water [18]. All chlorotriazines were recovered almost quantitatively from 200 ml of tap water spiked with 0.1 μg l^{-1} of each compound.

The aim of this study was to investigate the ability of styrene–divinylbenzene sorbent to retain chloro- and methylthiotriazine herbicides and mono- and didelkylated chlorotriazine metabolites as a method for their enrichment from human urine and simultaneous elimination of polar matrix interferences. This sorbent seemed to be promising for an efficient accumulation not only of the parent herbicides but also of more polar dealkylated metabolites from relatively small urine volumes not exceeding the breakthrough volume. The final analysis was performed by reversed-phase HPLC with UV diode array detection (DAD) or capillary GC with nitrogen-selective or electron-capture detection.

2. Experimental

2.1. Chemicals

Atrazine (6-chloro-*N*-ethyl-*N′*-isopropyl[1,3,5]-triazine-2,4-diamine) 99.0%, simazine (6-chloro-*N,N′*-diethyl[1,3,5]triazine-2,4-diamine) 92.2%, prometryn (6-methylthio-*N,N′*-diisopropyl[1,3,5]-triazine-2,4-diamine) 99.7%, ametryn (6-methylthio - *N* - ethyl - *N′* - isopropyl[1,3,5]triazine - 2,4-diamine) 98.0%, deethylatrazine (6-chloro-*N*-isopropyl[1,3,5]triazine-2,4-diamine) 99.0%, deisopropylatrazine (6-chloro-*N*-ethyl[1,3,5]triazine-2,4-diamine) 95.0%, and deethyldeisopropylatrazine (6-chloro[1,3,5]triazine-2,4-diamine) 96.7%, were purchased as standards of Pestanal quality from Riedel-de Haën, Seelze, Germany.

HPLC-grade acetonitrile and acetone were products of Rathburn, Walkerburn, UK. LC-grade water was obtained by purifying distilled water with a Milli-Q water purification system (Millipore, Bedford, MA, USA). *n*-Hexane for organic trace analysis, Suprasolv, was purchased from Merck (Darmstadt, Germany). All other chemicals used were products of analytical-reagent purity of Kemika (Zagreb, Croatia). Acetone for GC analysis was redistilled before use.

SPE was carried out using 3 ml Bakerbond spe extraction SDB-1 columns (J.T. Baker, Deventer, Netherlands) packed with 200 mg of styrene–divinylbenzene copolymer (SDB) of 43–123 μm particle size and specific surface area of 965 m^2 g^{-1}.

2.2. Equipment

2.2.1. HPLC conditions

Triazine compounds were analysed on an HP Series 1100 LC system with a diode-array detector (Hewlett-Packard, Palo Alto, CA, USA). The chromatographic column was 250×4 mm I.D. of 5 μm particle size Hypersil ODS from Hewlett-Packard.

The mobile phase consisted of acetonitrile and 20 m*M* phosphate buffer (pH 7). The gradient elution was carried out according to the following programme: 3 min at 0% acetonitrile, from 0 to 100% in 22 min. The flow-rate was 1 ml min^{-1}. The amount injected was 100 μl. UV spectra of all peaks were recorded from 200 to 400 nm. The working wavelengths for quantitative analysis were 225, 240 and 260 nm.

2.2.2. GC conditions

Triazine compounds were analysed on a Varian STAR 3400 Cx gas chromatograph (Varian, Walnut Creek, CA, USA) with a septum-equipped programmable injector (SPI) and a thermionic-sensitive detection (TSD) system. Chlorotriazines were also analysed on a Varian STAR 3380 gas chromatograph equipped with a split/splitless injector and ^{63}Ni electron-capture detection (ECD) system. Separation was performed in both instruments on a fused-silica capillary column of SPB-5 [poly(5%diphenyl–95%dimethylsiloxane)], 60 m×0.25 mm I.D., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA).

Operating conditions of GC analysis were: column temperature programmed from 60°C (with 1 min hold) up to 160°C at 40°C min^{-1} with a hold of 1 min, then from 160°C to 240°C at 2°C min^{-1} with a

final hold of 1 min. SPI and split/splitless injector temperatures were 260 and 270°C, respectively. Both detectors were at 300°C. The carrier gas in GC–TSD was hydrogen produced with an HG 200 hydrogen generator (Claind, Lenno, Italy) and in GC–ECD helium (99.9999% pure) supplied by Messer (Gumpoldskirchen, Austria). Column head pressure in both systems was 25 bar and the injection volume 2 μl (splitless injection 45 s).

2.3. Standard solution and sample preparation

Stock solutions of 1 mg ml^{-1} of atrazine, simazine, prometryn, ametryn, deethylatrazine, and deisopropylatrazine and of 0.5 mg ml^{-1} deethyldeisopropylatrazine were prepared in acetone and further diluted with acetone, *n*-hexane or deionised water for preparation of spiked urine samples, standard solutions for GC analysis and standard solutions for HPLC analysis, respectively. The concentrations of single herbicides in the final aqueous HPLC and *n*-hexane GC standards ranged from 23 to 1403 ng ml^{-1} and those of mono- and didealkylated chlorotriazine metabolites from 42 to 2580 ng ml^{-1}. For recovery experiments, blank urine samples were spiked with acetonic solutions of triazine compounds at the ng ml^{-1} level.

2.3.1. SPE of urinary triazine residues with styrene–divinylbenzene copolymer

The SDB extraction column was conditioned by the passage of 2 ml of acetone and 5 ml of deionised water. A vacuum was applied to draw acetone and water through the cartridge, preventing the cartridge from drying completely. A 5-ml urine sample was passed through the cartridge under an adjusted vacuum at a flow-rate of 3–5 ml min^{-1}. The cartridge was rinsed with 20 ml of 1% acetonitrile in water, when the final eluate was analysed by HPLC, or with 20 ml of water, when triazines were determined by GC analysis. Triazine compounds were eluted with 2 ml of acetone and the eluate was evaporated under a gentle stream of nitrogen to an aqueous residue. For HPLC analysis the residue was diluted with 2 ml of water and for GC analysis extracted with 2 ml of *n*-hexane by vortexing the sample for 1 min.

3. Results and discussion

3.1. HPLC analyses

Fig. 1a shows the HPLC–DAD chromatogram, recorded at 225 nm, of a standard solution of chloro- and methylthiotriazine compounds prepared in water. Similar chromatograms were recorded at 240 and 260 nm but the detection wavelength of 225 nm was selected as the best because of its optimum sensitivity of determination. Gradient elution with the acetonitrile–phosphate buffer (pH 7) system enabled an efficient separation and well-shaped peaks of all triazine compounds analysed.

The ability of the SDB cartridge to retain chloro- and methylthiotriazines was preliminarily tested by passing through 5 ml Milli-Q water samples spiked

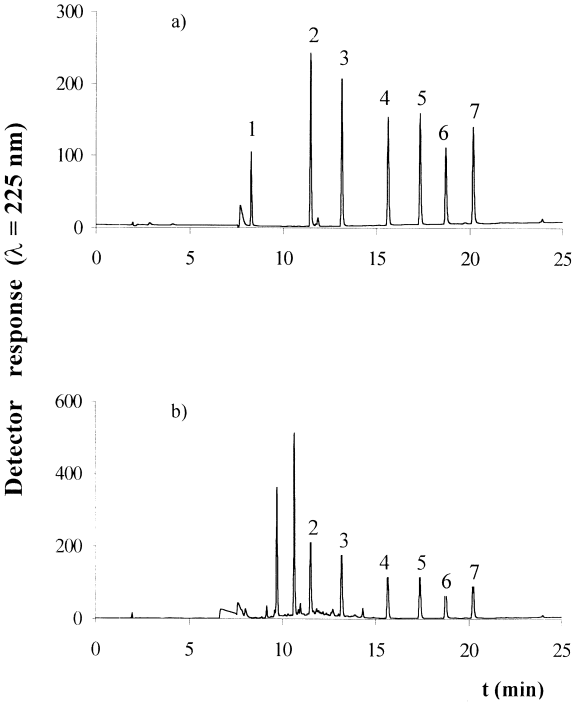

Fig. 1. HPLC–DAD chromatograms of chloro- and methylthiotriazine compounds: (a) in aqueous standard solution; (b) accumulated from 5 ml spiked urine sample by SDB solid-phase extraction. Column: Hypersil ODS. Peaks: 1, deethyldeisopropylatrazine; 2, deisopropylatrazine; 3, deethylatrazine; 4, simazine; 5, atrazine; 6, ametryn; 7, prometryn. Compound concentrations in standard: 577–1219 ng ml^{-1}. Compound concentrations in urine: 231–518 ng ml^{-1}. Volume injected: 100 μl.

with 462–561 ng ml^{-1} of the parent herbicides and 834–1035 ng ml^{-1} of dealkylated chlorotriazines. After sample percolation the cartridge was washed with 20 ml of water and then eluted with acetone. The recoveries of all compounds, including the most polar didelkylated atrazine, were higher than 90%, confirming the efficiency of the SDB sorbent for enrichment of triazines from aqueous samples. However, the percolation of spiked urine samples through the SDB sorbent resulted not only in trace enrichment of target triazine compounds but also of polar urinary components. Very complex HPLC–DAD chromatograms were obtained if the sorbent was washed only with pure Milli-Q water before the elution of triazines with acetone. A number of interfering peaks overlapped with the peaks of chloro- and methylthiotriazine compounds, especially in the range of atrazine metabolite retention times. Washing the cartridge after urine percolation with either 20 ml of a 0.5% or 10 ml of a 1% solution of acetonitrile in water eliminated the polar urinary interferences only partially. To achieve an interference-free chromatogram, as shown in Fig. 1b, it was necessary to precede the elution of triazines with acetone by washing the cartridge with 20 ml of the 1% solution of acetonitrile in water. This procedure resulted in well resolved peaks of two monodealkylated atrazine metabolites and four triazine herbicides, but the peak of the didealkylated atrazine was missing. Despite its efficient retention on the SDB sorbent, the most polar of the three atrazine metabolites analysed was completely lost during wash out of the cartridge with aqueous acetonitrile solution.

Table 1 shows the SDB solid-phase extraction recoveries of chloro- and methylthiotriazine compounds from spiked urine samples at concentrations ranging from about 10 to 500 ng ml^{-1} for the parent herbicides and from 20 to 1000 ng ml^{-1} for the monodelkylated chlorotriazines. The recoveries of all compounds, calculated by quantification at three different wavelengths, were nearly quantitative with relative standard deviations ranging from 4 to 10% for parent compounds and from 7 to 18% for monodealkylated atrazine metabolites. The lowest recovery was calculated for deisopropylatrazine at a detection wavelength of 225 nm but was not significantly different from the recoveries of other compounds ($P<0.05$). In the concentration ranges listed in Table 1 the relationships between the compound peak areas and their urinary concentrations were linear with correlation coefficients (r) ranging from 0.9934 to 0.9992. The slopes of the calibration lines constructed by detection of the compounds at 225 nm ranged from 1.2 for deisopropylatrazine to 2.7 for atrazine and simazine. The detection limits determined at a detection wavelength of 225 nm and based on a signal-to-noise ratio of 3 were twice as high for monodealkylated metabolites as for unmodified compounds. The sensitivity of the method was thus comparable with the sensitivity of the GC determination of atrazine, simazine,

Table 1
Mean recoveries, standard deviations and detection limits of triazine compounds in human urine using SDB solid-phase extraction and HPLC–DAD analysis

Compound	Concentration (ng ml^{-1})	Extraction recovery, %±SD (n)			Detection limit (ng ml^{-1}) 225 nm
		260 nm	240 nm	225 nm	
Deisopropylatrazine	21–1035			78±12 (12)	20
	52–1035	83±11 (9)	87±16 (10)		
Deethylatrazine	21–971			96±9 (12)	20
	49–975	88±12 (8)	101±7 (8)		
Simazine	10–462			99±4 (12)	10
	23–462	95±6 (10)	93±8 (10)		
Atrazine	10–522			94±6 (12)	10
	26–522	93±6 (11)	92±6 (12)		
Ametryn	11–540	92±9 (12)	95±4 (12)	95±5 (12)	10
Prometryn	11–561	93±6 (12)	96±6 (12)	93±6 (12)	10

n=Number of samples analysed.

prometryn and ametryn accumulated from urine by C_{18} SPE [4]. Compared to the same C_{18} method, the SDB solid-phase extraction technique offered more reliable determination of monodealkylated atrazine metabolites due to their significantly higher recoveries, not depending on the initial compound concentration in urine.

3.2. GC analysis

For GC determination of urinary triazine compounds the SDB solid-phase extraction procedure was slightly modified as follows: (a) after urine sample percolation the cartridge was washed with a volume of 20 ml of pure water (instead of with aqueous acetonitrile solution); and (b) the triazine compounds concentrated in the aqueous residue after evaporation of the acetonic eluate were reextracted into *n*-hexane, a non-polar solvent suitable for capillary GC. By omitting washing of the SDB cartridge with acetonitrile solution we expected to improve the recovery of the urinary didealkylated atrazine metabolite, which was completely lost during the sorbent washing step included in the HPLC procedure.

Due to the high selectivity of the nitrogen-selective detector, the GC–TSD chromatograms of blank and spiked urine samples were clear of any visible intereferences. Fig. 2 compares GC–TSD chromatograms of five chlorotriazine and two methylthiotriazine compounds in a standard *n*-hexane solution and in the extract of a spiked urine sample. The applied chromatographic conditions enabled a good separation of all analytes. However, in the chromatogram of the urine extract, the peak heights of two monodealkylated chlorotriazines and especially of the didealkylated chlorotriazine were surprisingly low, indicating much lower recoveries than expected. Indeed, the recoveries of monodealkylated chlorotriazines calculated against the calibration standards prepared in *n*-hexane (Table 2) were not comparable with those determined by the HPLC method (Table 1). We presumed that some interferences generated by the sorbent or sample matrix, although not visible in the chromatogram, lowered the detector response compared to its response for *n*-hexane calibration standards. This presumption was tested by comparison of recoveries from spiked urine samples with

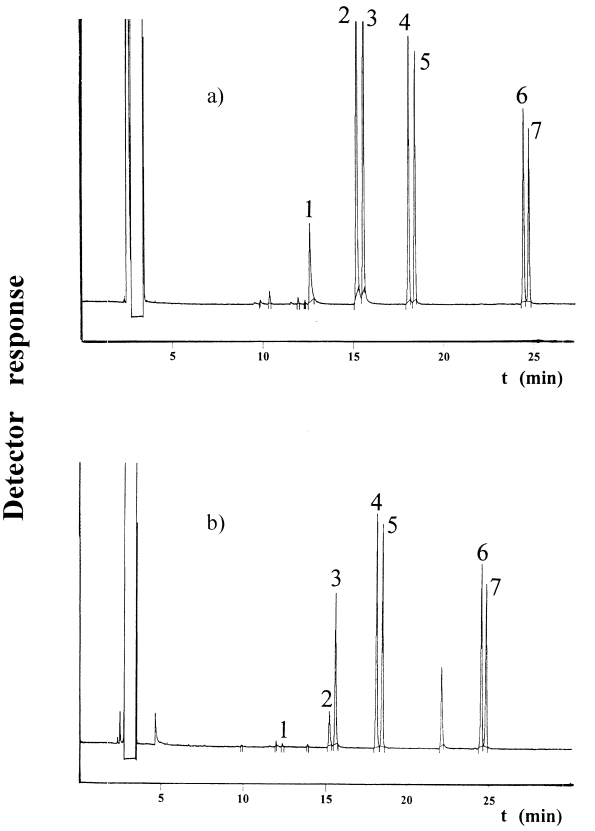

Fig. 2. GC–TSD chromatograms of chloro- and methylthiotriazine compounds: (a) in *n*-hexane standard solution; (b) accumulated from 5 ml spiked urine sample by SDB solid-phase extraction; final extract volume 2.0 ml. GC column: SPB-5. Peaks: 1, deethyldeisopropylatrazine; 2, deisopropylatrazine; 3, deethylatrazine; 4, simazine; 5, atrazine; 6, ametryn; 7, prometryn. Compound concentrations in standard: 577–1219 ng ml^{-1}. Compound concentrations in urine: 231–518 ng ml^{-1}. Volume injected: 2 μl.

recoveries from the spiked acetonic eluate of an activated SDB cartridge through which no urine or a blank urine sample was percolated. As shown in Table 2 the recoveries from both spiked urines and spiked acetonic eluates of the activated SDB cartridge were low and nearly equal, indicating the sorbent as the origin of interference. Consequently, also in the GC method the determination of didealkylated chlorotriazines continues to be a problem, despite the fact that it is expected to be retained by the SDB sorbent. The recoveries of monodealkylated chlorotriazines from urine are compar-

Table 2
Mean recoveries of mono- and didealkylated atrazine metabolites accumulated from spiked human urine by SDB solid-phase extraction and from the spiked acetonic eluate of an activated SDB cartridge determined by GC–TSD analysis of final *n*-hexane extracts against the calibration standards prepared in *n*-hexane

Compound	Recovery from urine		Recovery from acetonic eluate		
	Concentration (ng ml^{-1})	%±SD (*n*)	Concentration (ng ml^{-1})	%	(*n*)
Didealkylated atrazine	233–933	2±0.8 (6)	1165	<2	(4)
Deisopropylatrazine	21–1053	13±8 (11)	1286	5	(4)
Deethylatrazine	21–975	32±7 (13)	1234	32	(4)

n=Number of samples analysed.

able with those in the HPLC method only if calculated with calibration standards prepared in the acetonic eluate of an activated SDB cartridge instead of directly in *n*-hexane. However, due to the lowered detector response, the sensitivity of GC determination of these compounds becomes critical. A similar interfering effect, resulting in poor and non-reproducible recoveries, was noticed in GC–ECD analysis of urinary dealkylated chlorotriazines (Fig. 3), previously proved to be sensitive for analysis of chlorotriazine compounds [4]. The recoveries of didealkylated atrazine, deisopropylatrazine and deethylatrazine calculated against standards prepared in *n*-hexane were (%±SD) 18±19, 29±9 and 56±15, respectively.

In contrast to the poor results obtained for urinary dealkylated chlorotriazines, the GC method was highly efficient and sensitive for determination of unmodified chloro- and methylthiotriazines (Table 3). The recoveries were as high as those in the HPLC method, with relative standard deviations ranging from 9 to 17%. Atrazine and simazine were detected equally sensitively by both detectors. The ECD response for methylthiotriazines was low and non-reproducible and prevented reliable quantitation. Calibration lines for GC–TSD and GC–ECD determination of urinary chloro- and methylthiotriazine herbicides were constructed by analysis of spiked urine samples which were prepared in triplicate at six different concentrations. In the concentration ranges listed in Table 3 the relationship between the compound peak areas and their concentrations in urine were linear with correlation coefficients (*r*) ranging from 0.9905 to 0.9982 for GC–TSD analysis of chloro- and methylthiotriazines, and from 0.9809 to 0.9856 for GC–ECD analysis of chlorotriazines. The slope of the calibration lines for GC–TSD analysis ranged from 206 for prometryn to 389 for simazine,

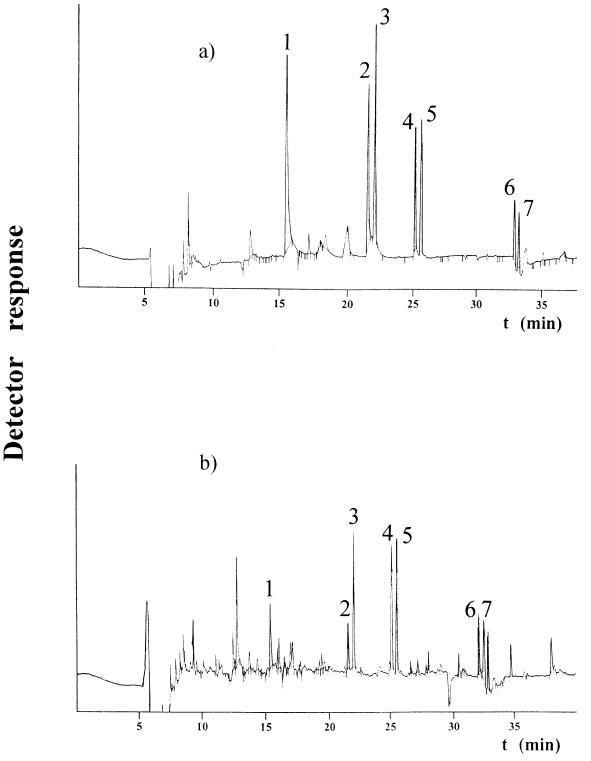

Fig. 3. GC–ECD chromatograms of chlorotriazine compounds: (a) in *n*-hexane standard solution; (b) accumulated from 5 ml spiked urine sample by SDB solid-phase extraction; final extract volume 2.0 ml. GC column: SPB-5. Peaks: 1, deethyldeisopropylatrazine; 2, deisopropylatrazine; 3, deethylatrazine; 4, simazine; 5, atrazine; 6, ametryn; 7, prometryn. Compound concentrations in standard: 577–1219 ng ml^{-1}. Compound concentrations in urine: 231–518 ng ml^{-1}. Volume injected: 2 μl.

Table 3
Mean recoveries, standard deviations and detection limits of parent triazine herbicides in human urine using SDB solid-phase extraction and GC–TSD or GC–ECD analysis

Compound	Concentration (ng ml^{-1})	Extraction recovery %±SD (*n*)		Detection limit (ng ml^{-1})	
		TSD	ECD	TSD	ECD
Simazine	10–462	91±10 (15)	95±9 (15)	10	10
Atrazine	10–522	97±12 (15)	101±17 (13)	10	10
Ametryn	11–540	100±16 (15)		10	
Prometryn	11–561	97±10 (11)		10	

n=Number of samples analysed.

and for GC–ECD analysis was 67 for atrazine and 77 for simazine.

4. Conclusion

The SDB cartridge can quantitatively retain chloro- and methylthiotriazine herbicides as well as monodealkylated chlorotriazines from urine samples. Polar urinary interferences can be eliminated by washing the cartridge with 1% acetonitrile in water for HPLC analysis or with pure water for GC analysis. Despite its efficient retention on SDB sorbent from aqueous solution neither the HPLC nor GC method allows the determination of urinary didealkylated chlorotriazine: in the HPLC procedure it is lost during the wash out of the cartridge with an aqueous solution of acetonitrile and in the GC–ECD procedure its recovery is low and non-reproducible. Both HPLC and GC methods enable determination of the parent herbicides in the same concentration range and with the same detection limits. Nevertheless HPLC was found to be more reliable for determination of monodealkylated chlorotriazines because of sorbent generated interferences which decrease the response of nitrogen–phosphorus and electron-capture detectors for these metabolites. The HPLC method is also less time consuming because before GC analysis reextraction of atrazine compounds into *n*-hexane is needed. Considering the levels of urinary triazine compounds detected in workers manufacturing or applying triazine herbicides, the methods are sufficiently sensitive for biological monitoring of occupationally exposed persons [1–4]. The sensitivity of determination can be further increased by reduction of the final sample volume to less than 1 ml.

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