Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 794 (2003) 389–395
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Triazinic herbicide determination by gas chromatography–mass spectrometry in breast milk

L. Balduini[a], M. Matoga[a], E. Cavalli[a], E. Seilles[b], D. Riethmuller[c], M. Thomassin[a], Y.C. Guillaume[a,*]

[a] *Equipe Sciences Séparatives et Biopharmaceutiques (2SB)/Laboratoire de Chimie Analytique, UFR des Sciences Médicales et Pharmaceutiques, 2 Place Saint Jacques, 25030 Besançon Cedex, France*
[b] *Equipe Interaction Hôte-Greffon et Ingénierie Cellulaire et Génique en Transplantation/Laboratoire de Thérapeutique Immunomoléculaire, Centre Hospitalier Universitaire, 1 Boulevard Alexandre Fleming, 25030 Besançon Cedex, France*
[c] *Service gynécologie obstétrique, Centre Hospitalier Universitaire, 2 Place Saint Jacques, 25030 Besançon Cedex, France*

Received 24 February 2003; received in revised form 21 May 2003; accepted 23 May 2003

Abstract

A solid-phase extraction procedure using a graphitized carbon black cartridge for extraction and cleaning of a series of five triazines (atrazine, deethylatrazine, deisopropylatrazine, ametryne and prometryne) from breast milk samples was developed. Using a chemometric methodology, the optimisation of both the analysis time and the triazinic herbicide separation by gas chromatography–mass spectrometry (GC–MS) was then carried out with only 18 experiments. Detection and quantification limits for 1 ml breast milk sample were, respectively, 0.3 and 1 ppb for each studied compound. The variation coefficients were less than 5% over the concentration range from 1 to 100 ppb. The accuracy was between 98.63 and 104.62% for each triazinic herbicide. The recovery was between 58.64 and 63.22% for the concentration range from 1 to 100 ppb for each triazinic herbicide. The assay was successfully applied to the analysis of several breast milk samples.

Keywords: Triazines

1. Introduction

Since their introduction more than 40 years ago, triazines have been extensively used in agriculture as herbicides. Atrazine, ametryne and prometryne are applied to corn, soybean, sorghum, vine, legumes and several fruit crops for broadleaf weed control.

Today, use of triazines is the subject of significant concerns because these herbicides (and two desalkyl metabolites of atrazine: deethylatrazine and deisopropylatrazine) are found not only in plant and soil directly treated, but also in surface and ground waters in the following years and in the cultures grown [1,2] as well as in agricultural products as e.g. forage and milk [3–7].

Studying breast milk contamination is important from a health standpoint because lactation can be an important process for eliminating triazine compounds (which are gradually ingested with the diet and accumulate in adipose tissue) from the human organ-

*Corresponding author. Tel.: +33-3-8166-5544; fax: +33-3-8166-5655.
E-mail address: yves.guillaume@univ-fcomte.fr (Y.C. Guillaume).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00455-0

ism. Moreover, breast milk is the only food taken in the first months of a baby's life.

The expected concentration in this material is rather low and triazinic herbicides cannot be detected directly in the raw material. Thus, an extraction and preconcentration step has to be included [8]. The triazine proper determination is then predominantly performed using gas chromatography–mass spectrometry (GC–MS) [3,4,9–18].

The aim of this study was to develop and validate an efficient solid-phase extraction (SPE) procedure for the quantitative determination of these triazinic herbicides at trace levels in breast milk using a GC–MS technique.

2. Experimental

2.1. Materials

The chromatographic system consisted of a Shimadzu QP5050 mass spectrometer with a Shimadzu GC-17A gas chromatograph fitted with the Class 5000 Vs 2.2 software (Shimadzu, Sarreguemines, France). The capillary column used was a BPX-5 SGE (25 m×0.22 mm I.D., film thickness 0.25 μm) 5% phenyl-polysilphenylene siloxane (Touzard & Matignon, Courtaboeuf, France). The gas used was helium. The injector was used in splitless mode and its temperature was 250 °C. The GC oven temperature program applied was: an initial temperature fixed to 100 °C followed by a first temperature gradient up to 160 °C followed by a second temperature gradient up to 180 °C followed by a third temperature gradient.

For SPE, an extraction system consisted of a VisiPrep Vacuum Unit (Supelco, Saint-Quentin, France) with graphitized carbon black (GCB) cartridges, Carbograph Extract Clean (150 mg, 4 ml, Alltech, Templemars, France).

2.2. Chemicals

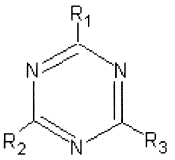
Ten breast milk samples were collected in the Besançon maternity hospital (France) between the 1st June and 1st July 2002. The studied breast milk volume was only 1 ml. They were stored at −18 °C until analysis.

Methanol, dichloromethane, hydrochloric acid (37%) were used for SPE (Carlo Erba, Val-de-Reuil, France). Water was obtained from an Elgastat water purification system (Odil, Talan, France) fitted with a reverse osmosis cartridge. Ethanol (99%) was used for triazinic herbicide mixture (Carlo Erba, Val-de-Reuil, France).

Atrazine, deethylatrazine and deisopropylatrazine were obtained from Dr Ehrenstörfer Laboratory (Augsburg, Germany). Ametryne and prometryne were obtained from Supelco (Saint-Quentin, France). Naphthalene was used as internal standard (Merck, Nogent-sur-Marne, France). The molecular structures of triazines are given in Table 1. All the studied molecular mass spectra are given in Fig. 1.

Table 1
Molecular structure of triazinic herbicides

General structure



Compound	Substituent in position R_1	R_2	R_3
Atrazine	Cl	$NH–C_2H_5$	$NH–CH(CH_3)_2$
Deethylatrazine	Cl	NH_2	$NH–CH(CH_3)_2$
Deisopropylatrazine	Cl	$NH–C_2H_5$	NH_2
Ametryne	$S–CH_3$	$NH–C_2H_5$	$NH–CH(CH_3)_2$
Prometryne	$S–CH_3$	$NH–CH(CH_3)_2$	$NH–CH(CH_3)_2$

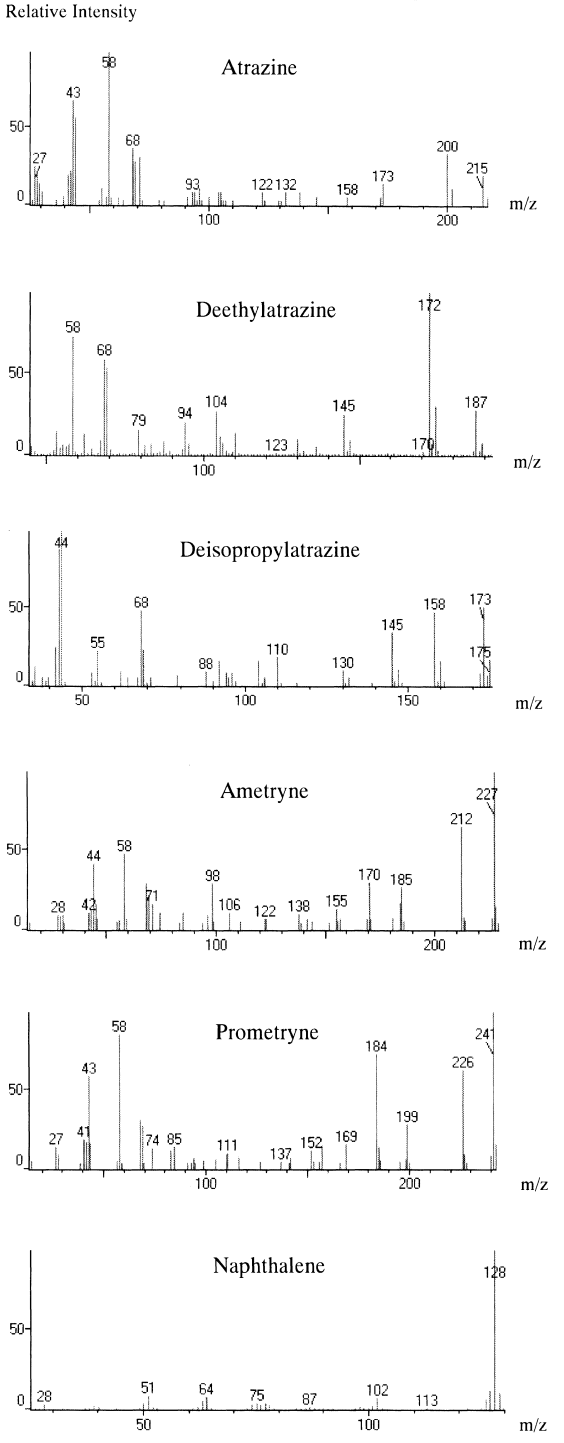


Fig. 1. Mass spectra of naphthalene (I.S.) and five triazinic herbicides. The axes represent relative intensity (%) versus m/z ions.

2.3. Detection conditions

The mass spectrometer detector was used in selected ion monitoring (SIM) mode for the triazinic herbicide quantitative determination in breast milk. The target m/z ions of each triazinic herbicide are given in Table 2 and used as qualifiers for the quantification.

2.4. Standard solutions

Stock standard solutions of each triazinic herbicide and naphthalene (I.S.) were prepared at concentrations of 100 mg/l in ethanol. The I.S. stock solution was diluted in ethanol to yield a 1 mg/l working solution.

From the five triazinic herbicide stock solutions, five working solutions (1 mg/l) were made up and the calibration standards were prepared freshly for each assay. Calibration standards were added into herbicide-free milk to yield concentrations of 1, 25, 50, 75 and 100 μg/l of each studied triazinic herbicide. In the same manner, milk quality controls spiked with the same concentrations were prepared to measure the accuracy and the precision of the method.

2.5. Milk sample preparation: extraction process

In comparison with instrumental clean-up techniques (liquid–liquid extraction, size-exclusion chromatography, sweep co-distillation), solid-phase extraction (SPE) is a simple, rapid and inexpensive method, which does not require the preparation or maintenance of costly apparatus.

Graphitized carbon black (GCB) is a non-specific sorbent of generally hydrophobic nature [19,20]. It has been shown that a GCB cartridge is much more

Table 2
Triazinic herbicides and naphthalene m/z ions

Compound	m/z ions		
Atrazine	200	215	173
Deethylatrazine	172	187	145
Deisopropylatrazine	173	158	145
Ametryne	227	212	170
Prometryne	241	184	226
Naphthalene	128	102	75

efficient than a C_{18} cartridge [21] and is extensively used in the analysis of pesticide residues [22].

To 1 ml of calibration or breast milk were added 10 μl of I.S. (1 mg/l). Firstly, the GCB cartridge was conditioned by passing 5 ml of a dichloromethane–methanol (80:20) mixture, 2 ml of methanol and 5 ml of acidified and distilled water. Next, milk sample was percolated through the cartridge at about 5 ml/min using the extraction system. Then, 5 ml of distilled water were poured onto the cartridge, eluted and discarded, to clean up the extract. Subsequently, the cartridge was dried for 3 min with air. The extract was eluted with 6 ml of a dichloromethane–methanol (80:20) mixture and evaporated to dryness. The eluted triazines were reconstituted in 2×250 μl of ethanol and analyzed by GC–MS.

2.6. Selectivity, linearity, recovery, precision and accuracy

Blank samples (herbicide-free and naphthalene-free milk) were performed to verify the selectivity. In order to determine the assay linearity, five samples (one for each triazinic herbicide) at each concentration (1, 25, 50, 75 and 100 μg/l) were extracted from breast milk and analyzed to construct five independent calibration curves, performing six measurements at each concentration level.

Extraction recovery from breast milk was determined by comparison of GC–MS responses from extracted samples, containing known amounts (two levels of the calibration range: 1 and 100 μg/l) of each triazinic herbicide, to those from ethanol standard solutions spiked with the same amounts.

The intra-day precision of the method was assessed by calculating coefficient of variation (%C.V.) for six replicates ($n = 6$) of milk quality control samples prepared as described above.

Accuracy, expressed as % bias was calculated as the percentage ratio of the difference between each triazinic herbicide observed concentration and each triazinic herbicide theoretical concentration to each triazinic herbicide theoretical concentration.

3. Results and discussion

The elution order of the five triazinic herbicides on the chromatogram was the same whatever the experimental conditions. The internal standard was eluted first and never interfered with the five triazinic herbicides.

It is of interest to obtain an efficient GC–MS separation of atrazine, deethylatrazine, deisopropylatrazine, ametryne and prometryne in a minimum analysis time. To reduce the analysis time, a chromatographic response function ξ has been developed and defined by Guillaume's team [23] as:

$$\xi = \text{Min}\,(R_{ij}) \quad \text{if Min}\,(R_{ij}) \leq R_L$$
$$\xi = R_L + 1/t_a \quad \text{if not}$$

where Min (R_{ij}) is the resolution for the worst separated pair of peaks (ij) on the chromatogram. R_L is the limit resolution accepted. In our application, R_L was 0.8. The analysis time t_a was given by the retention time of the last peak on the chromatogram (i.e. prometryne).

With an experimental design developed by Guillaume's team [24,25], a second order polynomial model, which links the ξ-values with the three temperature gradients and the gas flow-rate, was determined. Using a simplex methodology, the chromatographic conditions, which allow to maximize the ξ-value, were calculated. These ones were 39.80 °C/min; 1.00 °C/min; 0.10 °C/min and 0.60 ml/min, which necessitate carrying out only 18 experiments. In summary, the following GC oven temperature program was applied: 100 to 160 °C at 39.8 °C/min, then 160 to 180 °C at 1.00 °C/min and 180 °C held for 5 min. The chromatogram with these conditions is given in Fig. 2. The analysis time was below 25 min.

Herbicide-free milk and internal standard-free milk analyses showed no interference with the triazinic herbicide and the internal standard peaks, indicating a good selectivity for the method.

The detection limit and the quantitation limit for 1 ml breast milk sample were, respectively, 0.3 μg/l and 1 μg/l for each studied compound.

As shown in Table 3, the curve peak-area ratio of the triazinic herbicides to internal standard (y) versus the concentration of the triazinic herbicide (x) were linear over the evenly distributed calibration range. The r values of the calibration curves for each triazinic herbicide in milk were between 0.9964 and 0.9997, showing a good linearity for the assay.

Table 4 gives the detailed intra-day precision and

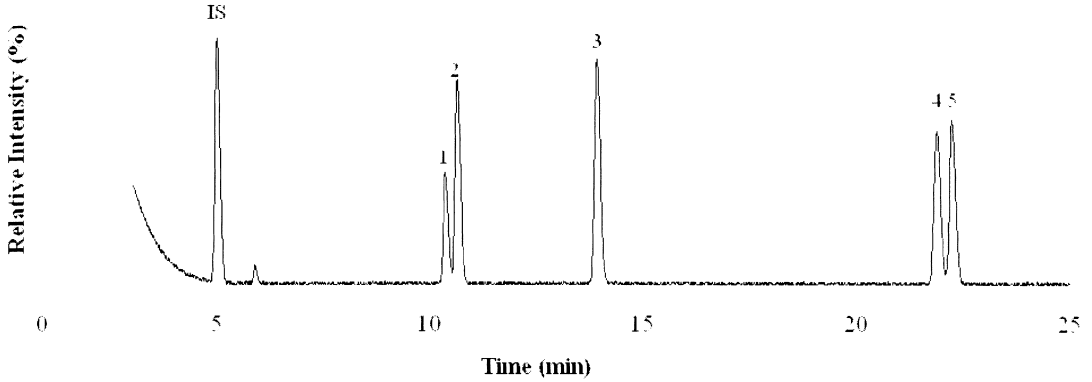


Fig. 2. Representative optimal chromatogram of naphthalene (I.S.) and a five-triazinic herbicide mixture controlled by four chromatographic parameters: $x_1 = 39.80\,^{\circ}C/min$, $x_2 = 1.00\,^{\circ}C/min$, $x_3 = 0.10\,^{\circ}C/min$ and $x_4 = 0.60\,ml/min$. Concentration at 1 mg/l for each triazinic herbicide and I.S. Peaks: I.S., naphthalene; 1, deisopropylatrazine; 2, deethylatrazine; 3, atrazine; 4, ametryne; 5, prometryne.

Table 3
Linear regression of the peak-area ratio of the triazinic herbicides to internal standard versus the concentration of the triazinic herbicide

Compound	Slope	Intercept	r^a
Atrazine	2.00×10^{-4}	0.04×10^{-3}	0.9964
Deethylatrazine	4.00×10^{-4}	-1.20×10^{-3}	0.9976
Deisopropylatrazine	5.00×10^{-4}	-3.50×10^{-3}	0.9977
Ametryne	5.00×10^{-4}	1.40×10^{-3}	0.9997
Prometryne	4.00×10^{-4}	4.60×10^{-3}	0.9984

[a] r is the correlation coefficient.

the accuracy. High precision and good accuracy for the assay were obtained: C.V.% lower than 2% at the highest concentration and lower than 5% at the lowest one, whereas the accuracy was between 98.63% and 104.62%.

The recovery procedure gave values between 58.64% and 63.22%. Following the ISO 10695: 2000 standard ''Water quality—determination of selected organic nitrogen and phosphorus compounds—gas chromatographic methods'', these results were considered as good extraction recovery.

Ten breast milk samples were collected in the Besançon maternity hospital (France): five samples were obtained for the first lactation day and five others for the fourth day. After SPE/GC–MS analyses, three of them contained atrazine: one at 1.3 μg/l (Fig. 3) and two below the quantification limit. The other triazine concentrations were below the detection limit for all the breast milk samples.

Table 4
Precision, accuracy and recovery of triazinic herbicides in spiked breast milk using GCB solid-phase extraction and GC–MS

Compound	Concentration μg l^{-1}	Precision %	Accuracy %	Recovery %
Atrazine	1	4.57	103.37	59.32
	100	1.94	102.47	63.22
Deethylatrazine	1	1.98	99.12	58.89
	100	1.01	98.63	61.74
Deisopropylatrazine	1	2.21	99.45	61.98
	100	1.23	99.04	62.17
Ametryne	1	3.32	104.62	58.85
	100	1.82	104.32	60.76
Prometryne	1	4.75	101.13	58.64
	100	1.78	98.88	60.63

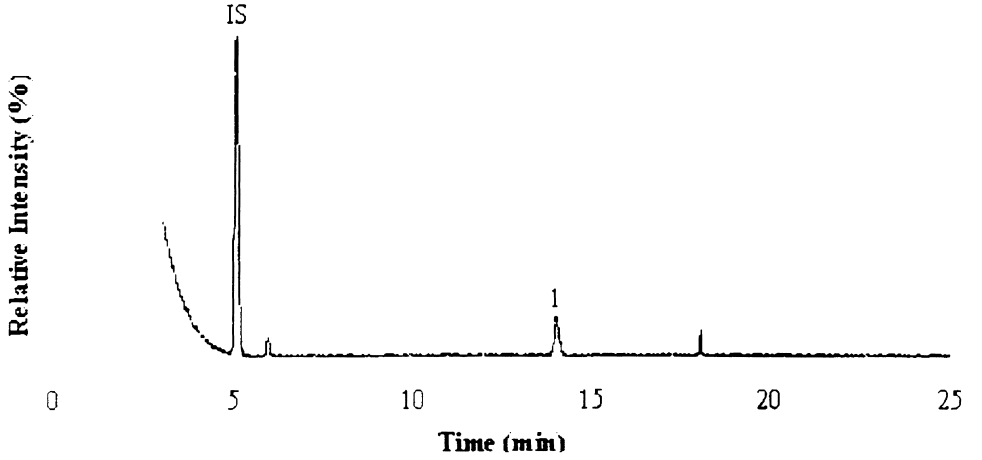


Fig. 3. Chromatogram of the breast milk sample containing 1.3 μg/l of atrazine. Peaks: I.S., naphthalene; 1, atrazine.

Because triazinic herbicides were persistent and widespread, the levels presented in breast milk were independent of restrictive measures taken in France. This study confirmed the triazinic herbicide presence in the environment. Considering the atrazine tolerance level set at 20 μg/l in the Environmental Protection Agency (EPA) Tolerance Index [26] and the level of triazinic herbicide compounds detected in milk studies (0.018 mg/kg atrazine and 0.004 mg/kg prometryne) [3–7], the proposed method was sufficiently sensitive for biological monitoring.

4. Conclusion

In this paper, an efficient SPE/GC–MS technique associated with a chemometric methodology was developed and validated for the simultaneous determination of five triazinic herbicides at trace levels in a very low volume of breast milk (1 ml).

The intra-day precision was less than 2% at 100 ppb and less than 5% at 1 ppb for all the five triazinic herbicides. The method showed an accuracy with a bias ranging from 98.63 and 104.62% and a recovery ranging from 58.64 and 63.22%.

This preliminary study indicated a low dietary exposure rate for babies in Franche-Comté (France). In order to confirm these results, it would be of interest to extend this study in more breast milk samples and in other French areas.

References

[1] J.R. Pardue, R. Bong, Am. Chem. Soc. 12 (1998) 123.
[2] C.M. Torres, Y. Pico, J. Manes, J. Chromatogr. A 754 (1993) 301.
[3] J. Tekel, P. Farkas, K. Schultzova, J. Kovacicova, A. Szokolay, Z. Lebensm. Unters. Forsch. 186 (1998) 319.
[4] I. Viden, Z. Rathouska, J. Davidek, J. Hajslova, Z. Lebensm. Unters. Forsch. 185 (1987) 98.
[5] L. Krivankova, P. Bocek, J. Tekel, J. Kovacicova, Electrophoresis 10 (1989) 731.
[6] J. Tekel, J. Kovacicova, A. Szokolay, Agrochemia 27 (1987) 277.
[7] Z. Rathouska, J. Hajslova, L. Babicka, J. Davidek, Potr. Vedy 5 (1987) 109.
[8] L. Krivankova, P. Bocek, J. Tekel, J. Kovacicova, Electrophoresis 10 (1989) 731.
[9] L.Q. Huang, Anal. Chem. 72 (1989) 349.
[10] R. Hu, J.M. Berthion, I. Bodereau, Chromatographia 43 (1996) 181.
[11] R. Hüskes, K. Levsen, Chemosphere 35 (1997) 3013.
[12] C.G. Zambonin, F. Catucci, F. Palmisano, Analyst 123 (1998) 2825.
[13] F. Hernández, C. Hidalgo, J.V. Sancho, F. López, Anal. Chem. 70 (1998) 3322.
[14] R. Loos, R. Niessner, J. Chromatogr. A 835 (1999) 217.
[15] C.G. Zambonin, F. Palmisano, J. Chromatogr. A 874 (2000) 247.
[16] T. Kumazawa, O. Suzuki, J. Chromatogr. B 747 (2000) 241.
[17] S. Schachterle, C. Feigel, J. Chromatogr. A 754 (1996) 411.
[18] J. Tekel, S. Hatrik, J. Chromatogr. A 754 (1996) 397.
[19] M. Battista, A. Di Corcia, M. Marchetti, Anal. Chem. 61 (1989) 935.
[20] A. Lagana, A. Marino, G. Fago, B.P. Martinez, Analysis 22 (1994) 63.
[21] A. Di Corcia, R. Samperi, A. Marcomini, R. Samperi, Anal. Chem. 65 (1993) 907.

[22] J. Tekel, S. Hatrik, J. Chromatogr. A 754 (1996) 397.
[23] Y.C. Guillaume, E.J. Cavalli, E. Peyrin, C. Guinchard, J. Liq. Chromatogr. Relat. Technol. 20 (1997) 1741.
[24] Y.C. Guillaume, E. Peyrin, Talanta 50 (1999) 533.
[25] Y.C. Guillaume, C. Guinchard, J. Chromatogr. Sci. 35 (1997) 14.
[26] EPA Toleranca Indes For Pesticides: http://www.epa.gov/opprd001/tolerance/tisinfo.