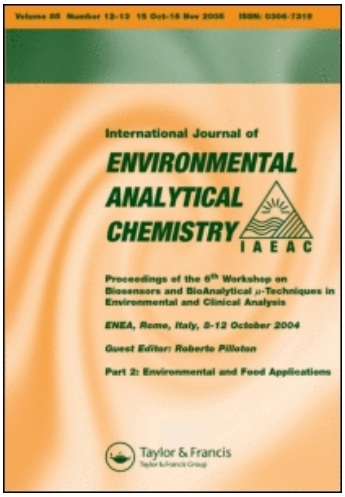

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A COMPARISON OF CHROMATOGRAPHIC METHODS FOR DETERMINATION OF s-TRIAZINES IN MILK*

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Both the selectivity and the sensitivity of several chromatographic methods used for a determination of s-triazine residues in milk have been evaluated. Employing GLC-NPD the detection limit for all analytes was as low as 0.2 μg/kg. Acceptable separation of s-triazines was achieved on wide-bore HP-20 M capillary column. Due to the high selectivity of the mass fragmentography technique even less polar stationary phase could be employed for analysis of milk extracts. The detection limit of GLC-MS was comparable to that achieved by GLC-NPD. Additional clean-up step had to be included prior to HPLC-UV determination. Good resolution of s-triazines was accomplished on reverse phase octadecyl-bonded column, nevertheless, the sensitivity was lower one order of magnitude in this case.

KEY WORDS: S-triazines, atrazine, cyanazine, desmetryn, metoprotryn, prometryn, simazine, terbutryn, terbuthylazine, milk

INTRODUCTION

A variety of substituted s-triazines has been widely employed as selective pre- and postemergence herbicides for a weed control in many agricultural crops. Residues of s-triazines as well as products of their either chemical or microbial degradation are wide-spread in the environment. Occurrence of these contaminants in food chain has been consequently recorded.

Nowadays, chromatographic methods have been exclusively used for the determination of trace levels of s-triazines. Several detailed studies dealing with the analyses of their multicomponent mixtures by GLC were carried out.[1–4] Efficient resolution of analytes was achieved[3] on capillary column coated with Carbowax 20 M stationary phase. In recent years, attention has been paid to the separation

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of s-triazines by HPLC, especially with a respect to the possibility of simultaneous determination[5] of both their polar (and often thermolabile) metabolites such as 2-hydroxytriazines that are not amenable to GLC without previous derivatization.[6,7] Conditions under which complex mixtures of s-triazines can be separated on octadecyl-[8] and octyl-[9] reversed phases as well as on amino-[10] and cyano-[11] chemically bonded phases have been studied.

Besides of common selective detectors (i.e. above all NPD in GLC and UV in HPLC), mass detector can be used. Mass spectral studies have been published aiming to describe fragmentation pathways both by electron impact[12] and chemical ionization[9,13] in order to select suitable ions for MS detection following chromatographic separation.

For the monitoring purpose, TLC can be employed. Highly selective and sensitive detection of s-triazines based on the inhibition of Hill's reaction in isolated chloroplasts contained in spray reagent together with an acceptor of electrons (2-chlorophenol indophenol) has been developed.[14,15]

In a comprehensive study by Jork and Rothe[16] there have been compared several chromatographic methods from point of view of their analytical application, nevertheless, the adverse effects of co-extracted compounds arising during analysis of environmental samples have not been considered. Multiresidue methods for a determination of s-triazines in water,[17] soil[18] and/or plant matrix,[19] respectively, have been developed. Less attention, however, has been paid to s-triazines in materials of animal origin.

Based on our previous results,[20] the residue levels of s-triazines in milk correspond to their content in cows' feed. Besides of health hazard, it should be taken into account that even trace levels of these contaminants in milk intended for the manufacture of fermented products can influence the growth of microbial population.

Considering the obvious need for selective and sensitive determination of s-triazine residues, our study aimed at a comparison of several analytical methods suitable for a routine milk quality control.

EXPERIMENTAL

Materials

Analytical standards of s-triazines were purchased from Supelco Inc. Standard stock solutions in methanol (0.1 g/l) were appropriately diluted either in acetone (1 μg/ml for GLC) or in methanol–water, 1: 1 (10 μg/ml for HPLC).

All solvents (analytical grade) were redistilled prior to the use. Silica cart™ cartridges were provided by Tessek Ltd. (Czechoslovakia)

Methods

Isolation of s-triazines from milk

1. precipitation of proteins: To the mixture of 200 ml milk and 200 ml of methanol

20 ml of acetate buffer (2 M acetic acid, 2 M sodium hydroxide, 1:1) were added. Filtration of cooled (30 min, 4 °C) suspension followed. The filter cake was then gently squeezed out and transferred back into the beaker by means of water. 200 ml of methanol and 10 ml of 1 M sodium hydroxide were added and the precipitation was repeated using 10 ml of 2 M acetic acid. The mixture was again cooled and then filtered.

2. extraction: Residues were extracted from combined filtrates with 1 × 100 and 2 × 60 ml of chloroform. Extract was dried over anhydrous sodium sulphate and then evaporated to the dryness. Remainder was dissolved either in acetone (for GLC) or in 10 ml of hexane (for further clean-up).

3. *clean-up (prior to HPLC)*: Silica-cart SGX cartridge (Tessek Ltd.) was gradually prewashed with 2 ml of 0.1% pyridine in diethyl ether, 5 ml of diethyl ether and 5 ml of hexane. 9 ml aliquot of milk extract in hexane was applied on this cartridge and the most of co-extracts were removed with 5 ml of hexane followed by 2 ml of hexane- diethyl ether (3:1). s-Triazines were eluted with 10 ml of diethyl ether. After evaporation of the solvent sample was dissolved in 1 ml of methanol and then 1 ml of water was added. Cooled sample was filtered by pulling it through the small disposable filter unit into the syringe and then injected into the HPLC apparatus.

Analyses of extracts

GLC

apparatus: Hewlett Packard 5890 A gas chromatograph equipped with NPD and split-splitless capillary injection port (temperature held at 230 °C); carrier gas nitrogen.

column A: 10 m × 0.53 mm capillary HP-20 M; oven temperature 230 °C; flow rate of nitrogen 6.5 ml/min; splitless injection (40 sec).

column B: 60 m × 0.32 mm capillary SPB-1; temperature programmed from 90 to 170 °C at 40 °C/min then to 265 °C at 3 °C/min; flow rate of nitrogen 1.0 ml/min; splitless injection (40 sec).

GLC-MS

apparatus: Shimadzu QP 1000 mass detector was used. Analyses were carried out under the same conditions as described above (see GLC) for column B, the sole exception being the final oven temperature 280 °C and the use of helium as a carrier gas (1 ml/min).

Ion source temperature was 280 °C, accelerating voltage 2.5 kV, ionization energy 70 eV. For mass fragmentography version 9.1 of the original Shimadzu "MF" software was used selecting 4 channels in two time periods.

Table 1 Determination of s-triazines in milk (5 replicates, recoveries in %, standard deviations)

Compound	*GLC*[a]	*S.D.*	*GLC*[b]	*S.D.*	*HPLC*[b]	*S.D.*
Cyanazine	76.1	0.9	98.2	0.4	99.0	1.0
Simazine	81.5	0.8	91.4	0.2	91.2	0.4
Atrazine	89.5	0.6	94.2	0.2	90.8	0.5
Desmetryn	90.7	0.7	92.3	0.5	88.8	0.6
Metoprotryn	88.1	0.9	96.6	0.5	81.6	0.7
Terbuthylazin	81.3	0.8	88.3	0.3	78.4	0.5
Prometryn	86.8	0.9	90.0	0.4	78.6	0.8
Terbutryn	90.5	1.0	96.2	0.4	81.3	0.9

[a]Spiking level 0.001 mg/kg.
[b]Spiking level 0.05 mg/kg.

HPLC

apparatus: Liquid chromatography system Spectra Physics SP 8700 equipped with variable wavelength UV detector SP 8400 (set at 226 nm) and injection valve Rheodyne 7125 (10 μl or 50 μl sample loop was used).
column A: 250 × 4 mm stainless column with Separon SGX C 18, 7 μm particles (Tessek Ltd.), mobile phase was methanol – 0.005 M KH_2PO_4 (70:30), flow rate 0.8 ml/min.
column B: 150 × 3 mm glass cartridge with Si 100-Polyol octyl, 5 μm particles (Serva), mobile phase was methanol-water (65:35), flow rate 0.5 ml/min.

RESULTS AND DISCUSSION

Residues of 8 s-triazines were reliably recovered, see Table 1, from spiked milk samples using the method described formerly by Knusli[21] for the spectrophotometric determination of simazine. Milk extracts obtained by this procedure were suitable for GLC-NPD analyses. As mentioned earlier, Carbowax 20 M proved to be the most convenient stationary phase for separation of s-triazines mixture. Resolution of all analytes was achieved on wide-bore fused silica capillary HP-20 M within 20 min of isothermal analysis with the exception of metoprotryne (RT = 26.3 min). An example of the separation of a mixture of s-triazines in the presence of milk matrix is given in Figure 1. Detection limits of all investigated compounds were as low as 0.2 μg/kg.

Since low bleeding thermally stable columns are generally suited for GC-MS analyses, the separation of s-triazines on non polar stationary phase (OV-1) was carried out, too. Figure 2 shows that the use of the high resolution SPB-1 fused silica capillary enables an acceptable separation and low detection limits of all analytes.

The sensitivity of mass fragmentography was better in comparison with mass chromatography method described in our previous paper.[12] Selective ions of s-triazines were chosen, see Table 2, mainly on the basis of their high abundance in the respective EI mass spectra. For all compounds their base peaks were

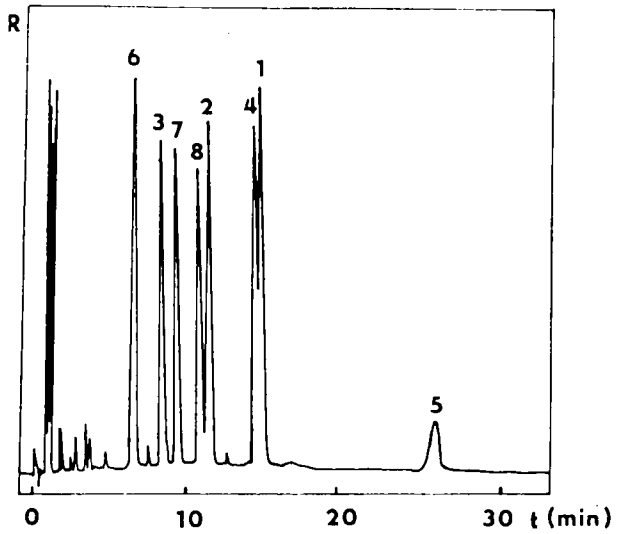

Figure 1 Chromatogram of milk extract, column *A*, s-triazines at spiking level 0.05 mg/kg. Compounds corresponding to individual peaks: 1-cyanazine, 2-simazine, 3-atrazine, 4-desmetryn, 5-metoprotryn, 6-terbuthylazine, 7-prometryn, 8-terbutryn.

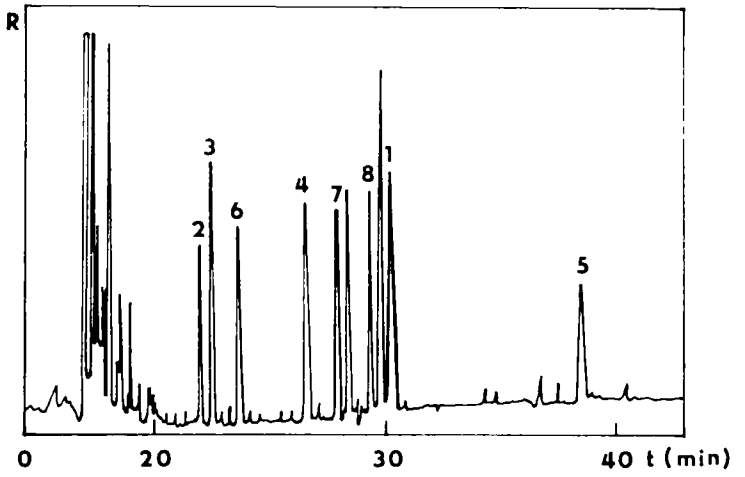

Figure 2 Chromatogram of milk extract, column *B*, s-triazines at spiking level 0.002 mg/kg. Compounds corresponding to individual peaks: see Figure 1.

selected with the exception of m/z 215 (atrazine) whose abundance was 62% of the base peak. Mass fragmentograms are given in Figure 3, that is divided into two parts (10–15 min and 15–24 min) according to the retention times of analytes for easy data processing. Table 2 shows, furthermore, the relative mass responses of the selective ions compared to cyanazine, the selective ion m/z 212 of which was the least sensitive. On the other hand the best sensitivity was found for m/z 214 of terbutylazine. All responses of the injected amounts of s-triazines were found to be

Table 2 Selective ions of s-triazines and relative mass responses (related to cyanazine)

Compound	*Selective ions (m/z)*			*Relative mass responses*	
				MS-SIM	*NPD*
Cyanazine	240	225	212[a]	1.0	1.0
Simazine	201[a]	186	173	1.5	1.0
Atrazine	215[a]	200	173	2.0	1.0
Desmetryn	213[a]	198	171	3.0	1.0
Metoprotryn	271	256[a]	240	1.5	0.5
Terbuthylazine	229	214[a]	173	5.0	1.2
Prometryn	241[a]	226	184	3.0	0.9
Terbutryn	241	226	185[a]	3.0	0.8

[a]Ions used within our work

linear in the range between 10 pg and 1 ng of each compound. Detection limits in the majority of s-triazines (at S/N 10:1) by mass fragmentography were comparable to those achieved by GLC-NPD. Detection limits of mass chromatography under the same instrumental conditions were found to be only 1–5 ng of s-triazines.

Considering the alternative ways of analysis of milk extracts, HPLC was also employed for this purpose. Using a reversed phase analytical column Separon SGX C-18 and methanol-0.005 M KH_2PO_4 (65:35) as a mobile phase, satisfactory resolution of analytes was feasible. Nevertheless, on account of both the generally lower selectivity of UV detector and the lower efficiency of analytical HPLC columns (in comparison with the parameters of the used GC columns), the additional clean-up step was inevitable. This step described in Experimental was performed on commercial disposable Silicagel cartridge. In order to eliminate acidic active sites of this sorbent that caused irreproducible sorption of s-triazines esp. 2-methylthio-derivatives, prewashing with 0.1% pyridine in diethyl ether before the use of such cartridge was recommended. This further clean-up procedure resulted, however, in a slight decrease of recoveries of 2-methylthio triazines (metoprotryn, prometryn and terbutryn), see the comparison of GLC and HPLC given in Table 1. Due to the limited capacity of Silicagel cartridge only samples containing less than 12% of milk fat could be handled in the described way. Although the sensitivity of HPLC-UV was approximately ten times lower when compared to GLC-NPD and mass fragmentography, it was suficient for a routine residues monitoring. From practical point of view it is convenient to perform HPLC analyses under isocratic conditions, nevertheless k′ values of prometryn and terbutryn are rather high. In order to achieve higher performance of separation and also to diminish peak broadening of these compounds, octyl-bonded reversed phase column was used. Owing to the different selectivity and lower retention of analytes, the time of analysis was significantly reduced (12 min vs. 25 min). Unfortunately, poor resolution of atrazine and desmetryn was observed and moreover interfering substances originating from milk did not permit reliable quantitation of cyanazine at spiking level below 50 μg/kg. The separation of s-triazines isolated from spiked milk on octadecyl- and octyl-bonded stationary phases is presented in Figures 4 and 5.

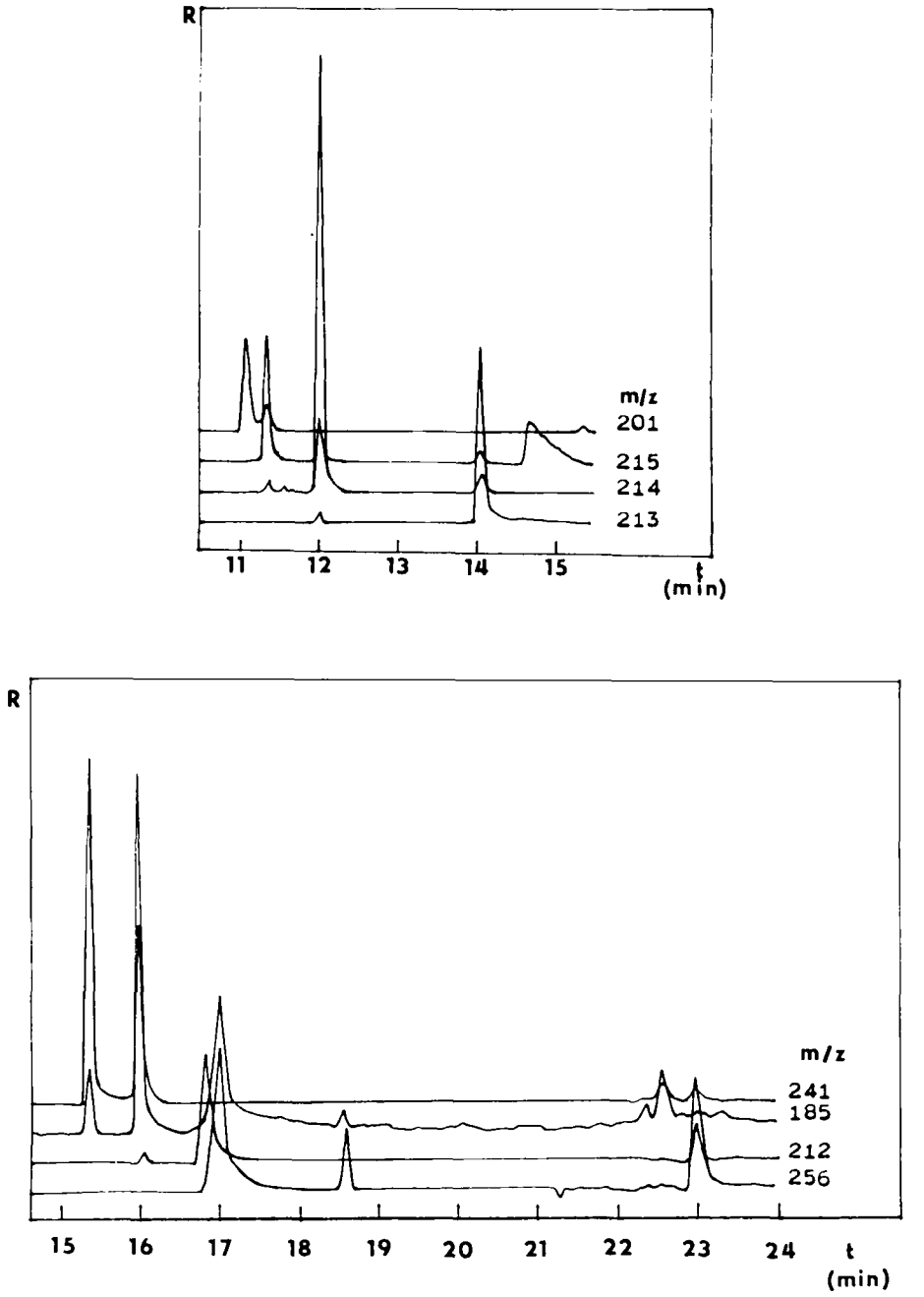

Figure 3 Mass fragmentogram of 8 selective ions (4 ions in each channel) of s-triazines, analytes in milk at level 0.005 mg/kg. Compounds corresponding to individual ions: see Table 1.

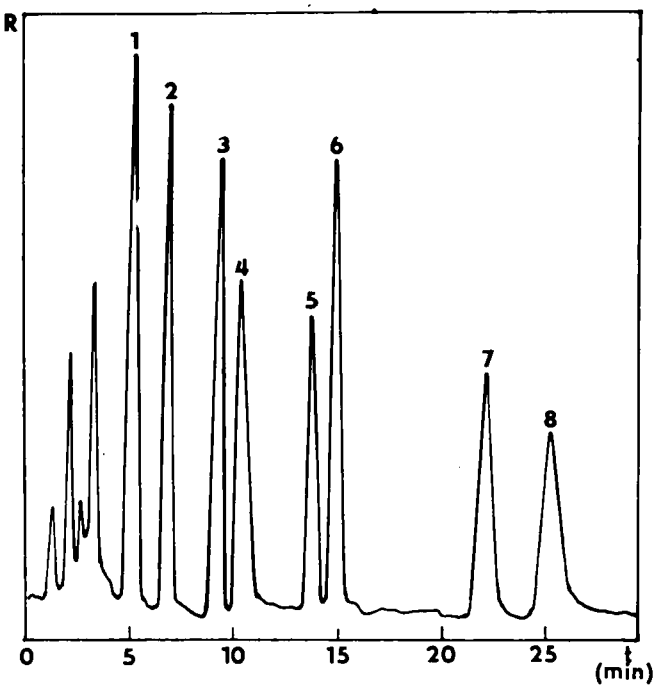

Figure 4 Chromatogram of milk extract, HPLC – column *A*, s-triazines at spiking level 0.06 mg/kg. For compounds corresponding to individual peaks see Figure 1.

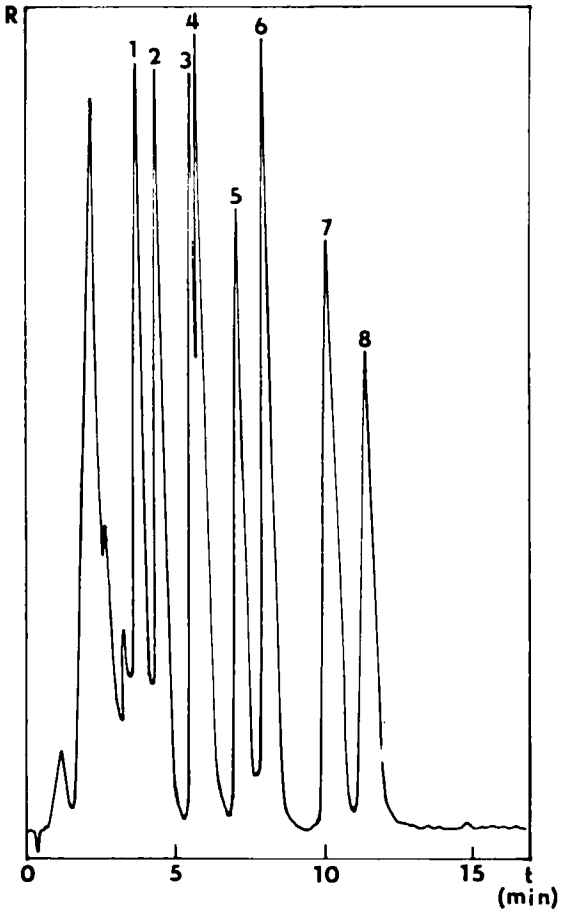

Figure 5 Chromatogram of milk extract, HPLC – column *B*, s-triazines at spiking level 0.06 mg/kg. For compounds corresponding to individual peaks see Figure 1.

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