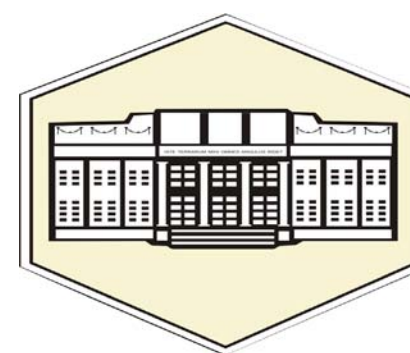


# LIQUID CHROMATOGRAPHY ANALYSIS OF SULFONAMIDE RESIDUES IN HONEY

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## INTRODUCTION

In the EC regulation there are no residue limits for antibacterial substances (antibiotics and sulfonamides) in honey. Hence the finding of any amount of above-mentioned substances may provide the basis to disqualify this product even though the amounts of the detected contaminants are varied and the results are directly affected by the analytical methods used. **The aim** of our study was to optimise the procedure for sulphonamide residues determination in honey by HPLC with fluorescence detection and their control in commercial honey.

## MATERIAL AND METHOD

The hydrolysis parameters of sugar-sulfonamide bonded derivatives (temperature, mixing conditions) for better repeatability and recovery were developed based on method elaborated by Diserens et al. (2002). The HPLC procedure was adapted from Diserens et al. (2002) and Posyniak et al. (2002, 2003).

Separation, identification and quantity analysis of sulphonamides in honey were done using SHIMADZU HPLC and fluorescence detection with excitation wavelength  $\lambda=405$  nm and emission wavelength  $\lambda=495$  nm, after synthesis of sulfonamide derivatives with fluorescamine. Chromatographic conditions were performed on Phenomenex Synergi 4 $\mu$  Fusion - RP 80Å column (250 x 4.60 mm) at 55°C, with mobile phase of: eluent A – 0.02 M/l H<sub>3</sub>PO<sub>4</sub>, eluent B – methanol:acetonitrile (1:1). Flow rate of 0.6 ml/min of mobile phase and gradient mode were used for separation of analytical standards of 9 sulphonamides and p-aminobenzoic acid (PABA), naturally present in some kinds of honey.

The external standard method was used for calculation of sulphonamides in honey samples. Validation of the procedure was done by calculation of: detection and quantification limits, repeatability, reproducibility and recovery for each of analysed sulphonamide in honey. Recovery for sulfonamide residues in honey were calculated on the basis of results found in spiked samples at the level of 50 and 100  $\mu$ g/kg. Repeatability and reproducibility were established by serial analysis of secondary reference material (honey with established content of sulfonamide residues).

The prepared procedure were used for control of commercial honey samples sent to Bee Products Quality Testing Laboratory, Apiculture Division, Research Institute of Pomology and Floriculture, Puławy, Poland. During 2007-2008 241 commercial honey samples were analysed. All samples were stored in dark and dry place at room temperature prior to analysis.

## RESULTS

**Table 1.** Recovery of sulphonamides in honey (%).

Sulfonamide	50 $\mu$ g/kg		100 $\mu$ g/kg	
	mean	SD	mean	SD
sulfanilamide	92.5	6.9	86.2	7.2
sulfatiazole	81.4	7.8	75.7	6.9
sulfacetamide	37.2	8.1	41.2	7.9
sulfamethazine	85.2	4.8	77.8	4.9
sulfamerazine	82.8	6.1	79.1	5.3
sulfamethoxy pyridazine	78.4	6.9	71.8	6.0
sulfachloropyridazine	70.0	4.6	65.6	5.3
sulfamethoxazole	83.4	5.3	74.1	5.7
sulfadimethoxine	75.0	6.2	64.5	5.9

**Table 2.** Repeatability and reproducibility for sulphonamides determination in honey – coefficient of variation (%).

Sulfonamide	Repeatability (n=5)		Reproducibility (n=10)	
	from - to	mean	from - to	mean
sulfanilamide	1.7 – 2.1	1.9	4.9 – 5.6	5.1
sulfatiazole	2.2 – 3.0	2.7	6.9 – 8.2	7.7
sulfacetamide	1.2 – 1.8	1.4	10.8 – 15.1	14.4
sulfamethazine	1.9 – 2.6	2.2	12.3 – 14.2	13.4
sulfamerazine	3.2 – 3.8	3.6	11.6 – 13.8	12.8
sulfamethoxy pyridazine	4.1 – 4.9	4.4	14.8 – 17.9	16.8
sulfachloropyridazine	5.0 – 5.7	5.3	10.6 – 12.8	11.6
sulfamethoxazole	4.1 – 4.8	4.3	12.8 – 14.2	13.9
sulfadimethoxine	7.4 – 8.5	8.1	14.6 – 15.9	15.5

**Table 3.** Sulphonamides contamination of honey samples sent to Laboratory over the 2007-2008.

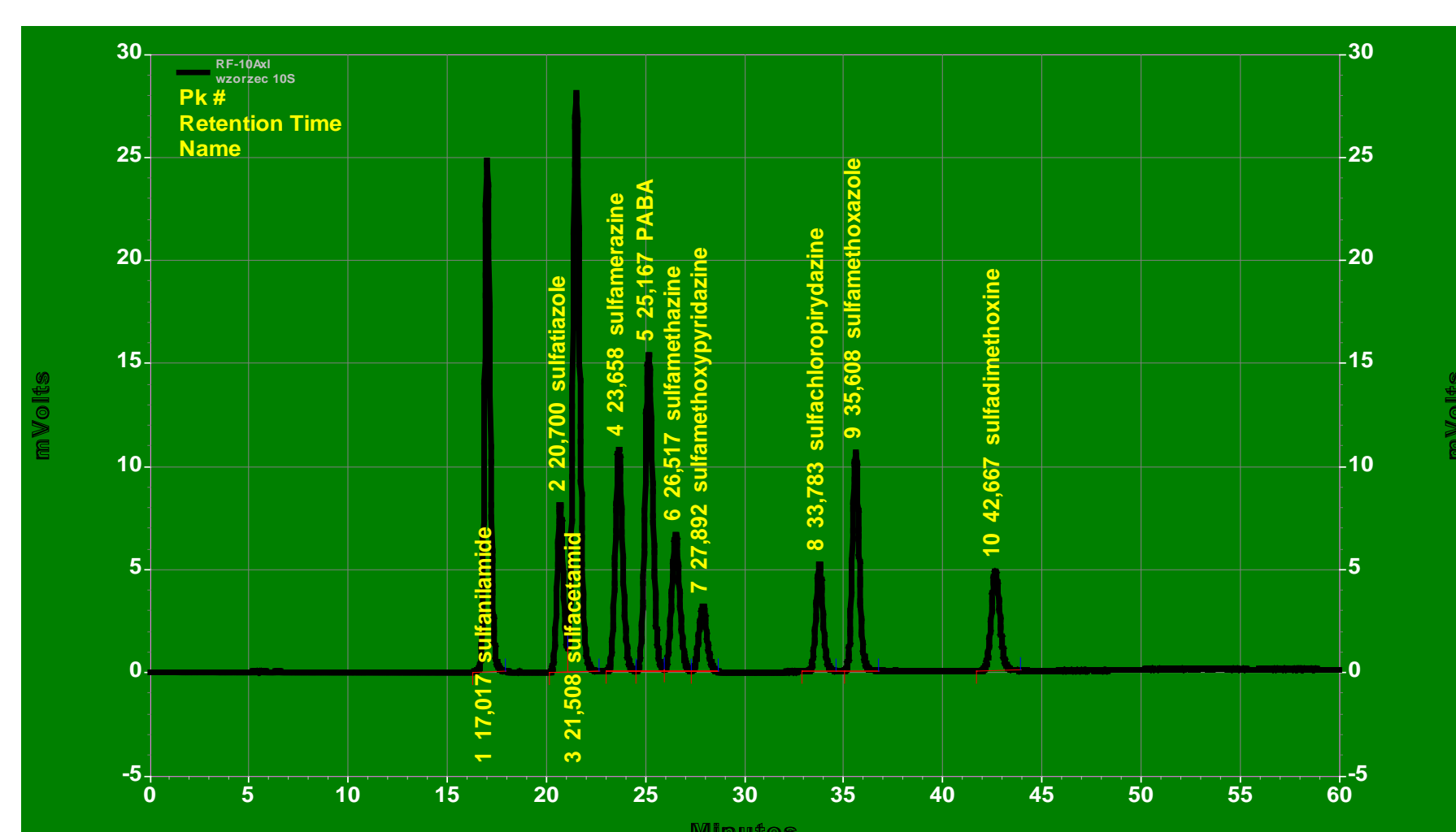
Year	Number of samples	Number of samples >50 $\mu$ g/kg*	Percentages of samples >50 $\mu$ g/kg*
2007	160	29	18%
2008	81	12	15%
2007 - 2008	241	41	17%

\*quantification limit according Polish National Monitoring Program for 2008

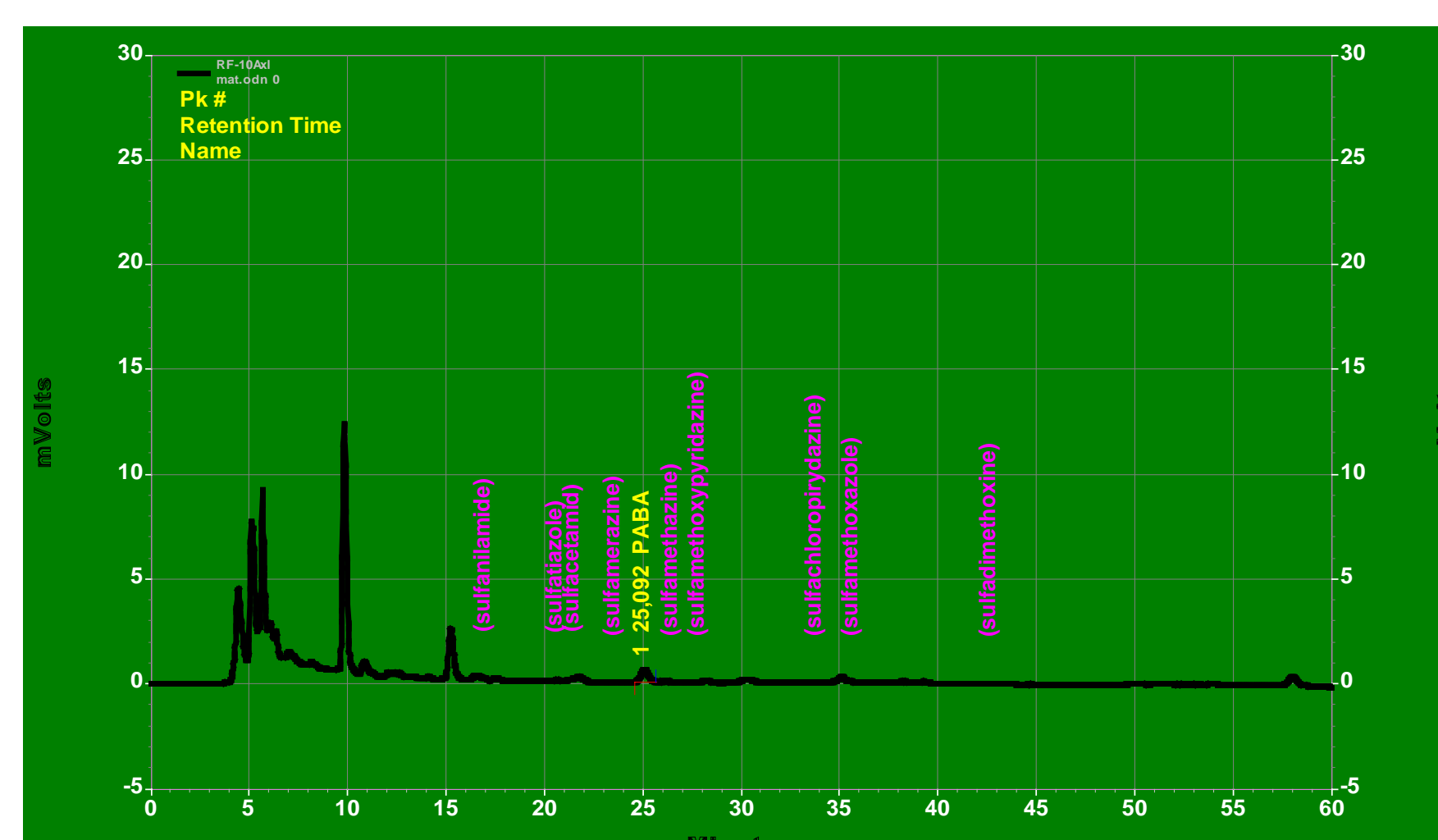
**Table 4.** Ranges of sulphonamides concentration detected in honey samples ( $\mu$ g/kg).

Sulphonamide	Concentration from - to ( $\mu$ g/kg)
Sulfanilamide	9 - 927
Sulfatiazole	5 – 1090
Sulfacetamide	8 - 207
Sulfamethazine	6 – 1270
Sulfamerazine	5 - 1308
Sum of sulphonamides*	5 - 2891

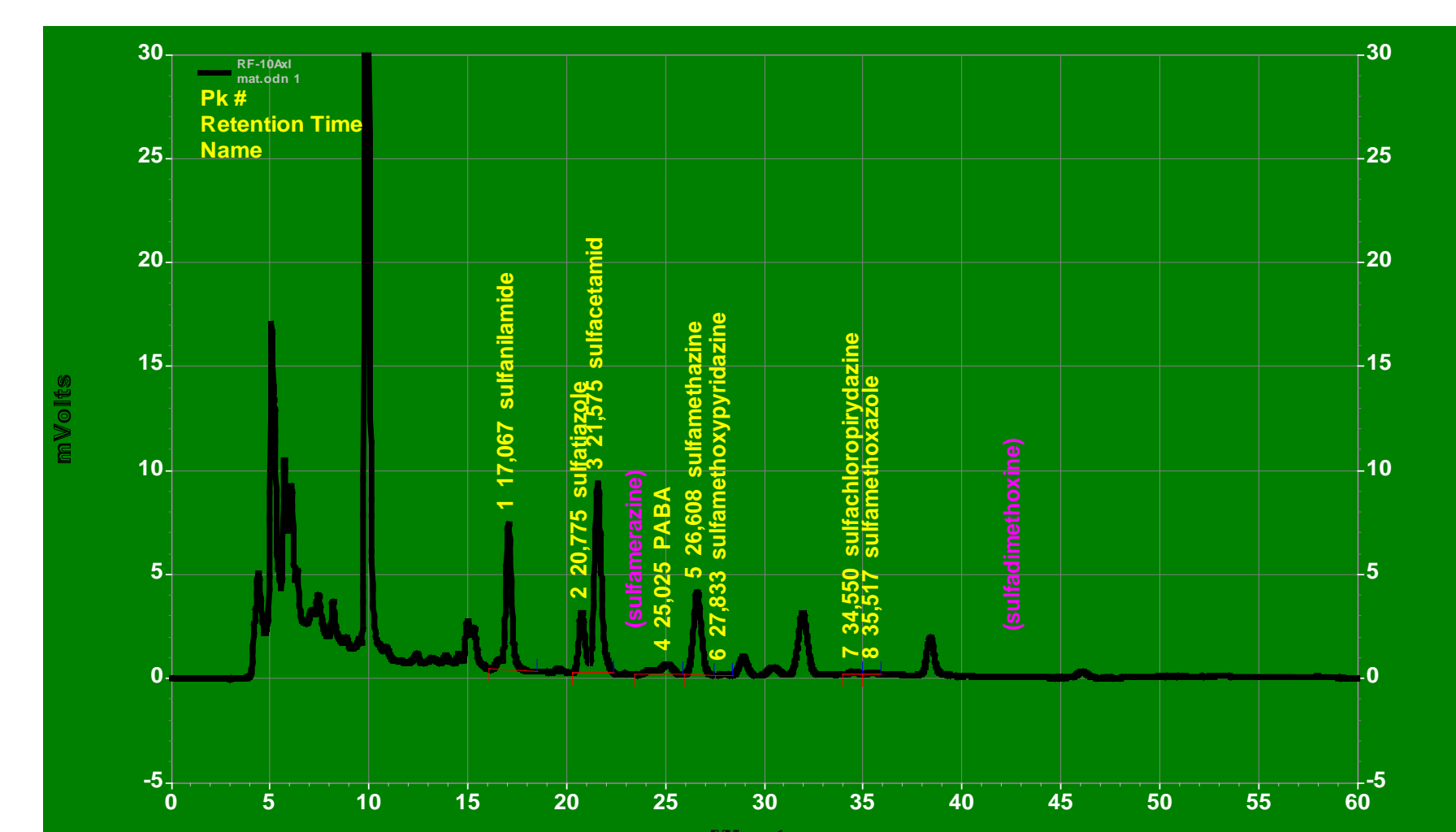
\*- sum of all sulphonamides detected over 5  $\mu$ g/kg



**Fig. 3.** HPLC chromatogram of standard solution of sulphonamides.



**Fig. 2.** HPLC chromatogram of blank honey sample.



**Fig. 3.** HPLC chromatogram of contaminated honey sample.

## SUMMARY AND CONCLUSIONS

- The following parameters for hydrolysis of sugar-sulphonamide derivatives in honey were established: 2M/l hydrochloric acid and shaking for 60 min at room temperature on mechanical shaker.
- Phenomenex Synergi 4 $\mu$  Fusion HPLC column and chromatographic conditions applied in this study gave good separation of 4-aminobenzoic acid (PABA) (naturally occurring in some honeys) from sulfamethazine and simultaneous analysis of the following sulfonamides: sulfanilamide, sulfacetamide, sulfatiazole, sulfamethazine, sulfamerazine, sulfamethoxy pyridazine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine.
- Elaborated procedure will contribute to improvement of quality of honey by extension of monitoring of sulphonamide residues in this product available on the market.
- The results of honey tests indicate that the beekeepers have not ceased to administer sulphonamides to control American and European foulbrood, in spite of the prohibition of their use.

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