Sulfonamide Residues In Honey. Control and Development of Analytical Procedure

Andrzej Posyniak, Jan Zmudzki, Jolanta Niedzielska, Tomasz Sniegocki, Agnieszka Grzebalska

National Veterinary Research Institute, Department of Pharmacology and Toxicology, *Al. Partyzantow 57,* 24-100 Pulawy, Poland, *phone: +48-81-8863051ext287,fax: +48-81-8862595, e-mail: aposyn@piwet.pulawy.pl*

Abstract

The suitability of analytical protocol for determination of sulfonamides in honey was investigated. Sulfonamides were extracted from honey with acetate buffer, and cleaned up by solid phase extraction (SPE) procedure. The liquid chromatography (LC) separation was carried out on RP C_{18} column and sulfonamides were monitored with fluorescence detector, after pre-column derivatisation. Recoveries from spiked honey were above 80%, and detection limits were 0.1 μg/kg for sulfacetamide and 0.2 μg/kg for sulfathiazole and sulfamethazine. The prepared procedure was used in the regular control of sulfonamide residues in honey.

Key words: *honey, sulfonamide, residue, method, control.*

Introduction

Sulfonamides are relatively stable chemotherapeutics known to control of American or European foulbroods but they are not permitted to use because of the potential of sulfonamide residues to contaminate honey.

Regulatory agencies are responsible for assuring that potentially harmful residues of these drugs are not present in honey or honey products. However so far, maximum residue limits have not been established for sulfonamide compounds in honey, within the European Union [4] and Poland as well.

A variety of chromatographic methods have been used to measure sulfonamide residues in honey and other biological materials [1-3, 5-12].

The objective of the present studies was to optimise the conditions for sulfonamide isolation from honey samples and control of the sulfonamide levels in honey. The whole procedure was validated for sulfacetamide (SCA), sulfomethazine (SMT) and sulfathiazole (STZ), active compounds of Polisulfamid® (figure 1), that is used in veterinary practice in Poland.

Material and Methods

Material. Sulfacetamide, sulfamethazine, sulfathiazole and fluorescamine were obtained from Sigma Chemical Co. (St Luis, MO, USA). Acetic acid was from Merck (Darmstadt, Germany). Acetonitrile and Bakerbond SPE octadecyl (3 ml) columns and SPE manifold were from J. T. Baker (Philipsburg, NJ, USA).

Stock solution and standards. Stock solutions of 1 mg ml^{-1} were prepared in acetonitrile. The working solutions for LC and sample spiking were prepared by dilution of 1 ml of each stock – solution to serial 10-fold dilutions in mobile phases or in water to concentration of 100, 10, 1 and 0.1 µg ml^{-1}. All solutions were stored in the dark at 4˚C.

Liquid chromatography. A Shimadzu VP Series liquid chromatograph (Duisburg, Germany) equipped with a fluorescence detector FR-10AXL with excitation wavelength λ = 405 nm and emission wavelength λ = 495 nm was used to analyse the tested solutions. LC control, data acquisition and peak integration were performed by system controller SCL-10A utilizing RS-232C interface for communication with CLASS-VP chromatography workstation.

The chromatographic analyses were performed on a Phenomenex Luna column (250x4,6 mm, 5 µm) with mobile phase 2% (v/v) acetic acid (AA) – acetonitrile (ACN). Flow 0.9 ml/min was used for the separation of analytes in gradient mode at the following program: 0-2 min, AA+ACN (70:30); 2-5 min, AA+ACN (80:20); 5-12 min, AA+ACN (60:40); 12-19 min, AA+ACN (70:30);. Aliquots of 20 µl were injected into the column.

Sample preparation. The honey sample (2.5g) was diluted with 12.5 ml of acetic buffer at pH from 3 to 7 and than was immersed in ultrasonic water bath, homogenised or shaked. The whole solution was applied on to an octadecyl phase. After percolation the column was washed with 3 ml of acetic buffer (pH 5.0) and dried. The sulfonamides were eluted with 5 ml of acetonitrile.

Extract was evaporated. The dry residue was dissolved in 900 µl of acetic buffer (pH 3.5) and 100 µl of 0.2% fluoroescamine in acetone was added. The sample was analysed after 20 min.

Validation studies. For validation purposes of the procedure that is illustrated in figure 2 have been taken. Samples of honey were spiked with sulfonamides at levels of 5 and 10 µg/kg. The recovery and precision of the assay was measured using the same samples.

Results

The choice of pH for the acetate buffer is the result of a compromise that takes into account the differences in a tested concentration of SCA, STZ and SMZ (table I). Ultrasonic treatment of the sample rather than homogenization or shaking was more efficient.

As shown in figure 3, the sulfonamides were well separated when the chromatographic separation was obtained at 55˚C and a gradient mode was used. The detection limits were set at the level of 0.1 µg/kg for SCA, and 0.2 µg/kg for SMZ and STZ, respectively.

The day–to day reproducibility for several series is shown in table II. The variation coefficients are satisfactory, and mean extraction recoveries are above 80%. The study intraserial reproducibility is shown in table III. The observed variation coefficients are satisfactory.

Discussion

Traditionally, sulfonamides are extracted by treating the sample with an organic solvent, and clean–up in solid–phase by passage through disposable cartridges [5, 8, 10].

As it was found in our studies, optimum extraction efficiency for isolation of sulfonamides from honey matrix was when acetate buffer at pH 5.0 was used. The results of the present study show that proposed LC technique is an efficient and reliable method for detection of sulfonamide residues in honey. The use of acetate buffer and SPE on octadecylsilane cartridges makes the isolation of sulfonamides from honey matrix easer and cheaper than commonly used procedures.

The prepared procedure was used for control of sulfonamides in honey. For control of sulfonamide residues in honey, the samples were routinely collected as part of the Polish National Monitoring Program for Chemical Residues in Food of Animal Origin. Additionally commercial honey samples were analysed (the samples were taken during the internal control of beekeepers). As it was shown in table IV, the samples from monitoring program were free from the residue of sulfonamides at concentration above detection limits of used procedure. However in some commercial honey samples the presence of SCA, SMZ and STZ was found at levels from 1.0 to 5.6 μg/kg.

References

[1] Agarwal V. K., High-performance liquid chromatographic methods for the determinations of sulfonamides in tissue, milk and eggs. J. Chromatogr. 624 (1992), 411-423

[2] Balizs G., Benesch-Girke L., Hewitt S. A. Comparison of the determiantion of four sulphonamides and their N^4-acetyl metabolites in swine muscle tissue using liquid chromatography with ultraviolet and mass spectral detection. J. Chromatogr. B. 661 (1994), 75-84

[3] Barry C.P. and Mc Eachern G., Reverse Phase Liquid Chromatographic Determination of Sulfathiazole Residues in Honey. J. Assoc. Off. Anal. Chem. 66 (1983), 4

[4] Commision Regulation (EEC) No. 2377/1990. Off. J. Eur. Commun. L224 (1990), 1

[5] Diserens J.-M., Renaud-Bezot C. and Savoy-Perround M.-C., Simplified determination of Sulfonamides Residues in Milk, Meat and Eggs. D. Lebensmitt.-Rund. 87 (1991), 205

[6] Gehring A.T., Rushing G. L. and Thompson H. C., Jr., Determination of Sulfonamides in Edible Salmon Tissue by Liquid Chromatography with Postcolumn Derivatization and Fluorescence Detection. J. AOAC Int. 80 (1997), 751

[7] Horie M., Saito K., Hoshino Y. and Nose N., Simultaneous determination of residual synthetic antibacterials in fish by high-performance liquid chromatography. J. Chromatogr. 538 (1991), 484

[8] Ikai Y., Oka H., Kawamura N. And Yamada M., Improvement of chemical analysis of antibiotics. Application of an amino cartridge to the determination of residual sulphonamide antibacterials in meat, fish and egg. J. Chromatogr. 541 (1991), 393

[9] Takeda N. and Akiyama Y., Pre-column derivatization of sulfa drugs with fluorescamine and high-performance liquid chromatographic determination at their residual levels in meat and meat products. J. Chromatogr. 558 (1991), 175

[10] Takeda N. and Akiyama Y., Rapid determination of sulphonamides in milk using liquid chromatographic separation and fluorescamine derivatization. J. Chromatogr. 607 (1992), 31

[11] Tarbin J. A. Shearer C. G., Screening of sulphoamides in egg using gas chromatography-mass-selective detection and liquid chromatography-mass spectrometry. J. Chromatogr. B. 729 (1999), 127-138

[12] Walker L.V., Walsh J. R. and Webber J.J., High-performance liquid chromatography of sulphonamides extracted from bovine and porcine muscle by solid-phase dispersion. J. Chromatogr. 595 (1992), 17

Legend of tables and figures:

table I - Recovery of sulfonamides after different sample preparation

table II - Recovery and day-to-day reproducibility of SCA, SMZ and STZ determination in honey

table III - Within-day reproducibility for SCA, SMZ and STZ determination in honey

table IV - Control of sulfonamides in honey

figure 1 - Chemical structure of analysed sulfonamides

figure 2 - Flow diagram of analytical procedure

figure 3 - Typical chromatograms: a – control honey sample, b – mixture of sulfonamide standards, c – honey sample spiked with sulfonamide standards

table I

Recovery of sulfonamides after different sample preparation

Combinations	Isolation	Extractant	SCA	SMZ	STZ
1	Homogenisation	Acetate buffer, pH 3	74,8	68,4	66,7
2		Acetate buffer, pH 5	78,2	69,2	65,7
3		Acetate buffer, pH 7	65,7	65,4	67,8
4	Ultasolic bath	Acetate buffer, pH 3	76,2	74,8	71,4
5		Acetate buffer, pH 5	82.6	83.4	81.7
6		Acetate buffer, pH 7	76,8	78,5	77,8
7	Shaking	Acetate buffer, pH 3	63,2	63,8	62,4
8		Acetate buffer, pH 5	71.6	74.4	72.7
9		Acetate buffer, pH 7	75,8	76,5	66,8

SCA – sulfacetamide, SMT – sulfamethazine, STZ – sulfathiazole

table II

Recovery and day-to-day reproducibility of SCA, SMZ and STZ determination in honey

	SCA		SMZ		STZ	
Amount added, μg/kg	5.0	10.0	5.0	5.0	10.0	5.0
Amount found, μg/kg	4.3	8.2	4.1	4.3	8.2	4.1
Replicates (n)	6	6	6	6	6	6
Coefficient of variation (%)	6.2	7.8	5.8	6.2	7.8	5.8
Mean recovery (%)	82.6	83.4	81.7	82.6	83.4	81.7

SCA – sulfacetamide, SMT – sulfamethazine, STZ – sulfathiazole

table III

Within-day reproducibility for SCA, SMZ and STZ determination in honey

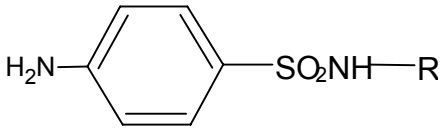
	SCA		SMZ		STZ	
Amount added, μg/kg	5.0	10.0	5.0	10.0	5.0	10.0
Amount found, μg/kg	4.4	8.6	4.2	8.4	4.3	8.2
Replicates (n)	6	6	6	6	6	6
Coefficient of variation (%)	5.7	4.6	4.3	4.8	3.7	5.4

SCA – sulfacetamide, SMT – sulfamethazine, STZ – sulfathiazole

table IV

Control of sulfonamides in honey

Year	Control	No. of samples	Positives	% of positives
2000	National monitoring program	20	0	0
	Commercial samples	30	3	10
2001	National monitoring program	20	0	0
	Commercial samples	42	5	11,6



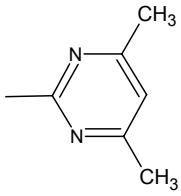
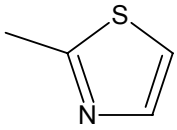
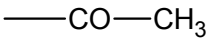
Compounds	R
Sulfamethazine	
Sulfathiazole	
Sulfacetamide	

figure 1. Chemical structure of analysed sulfonamides

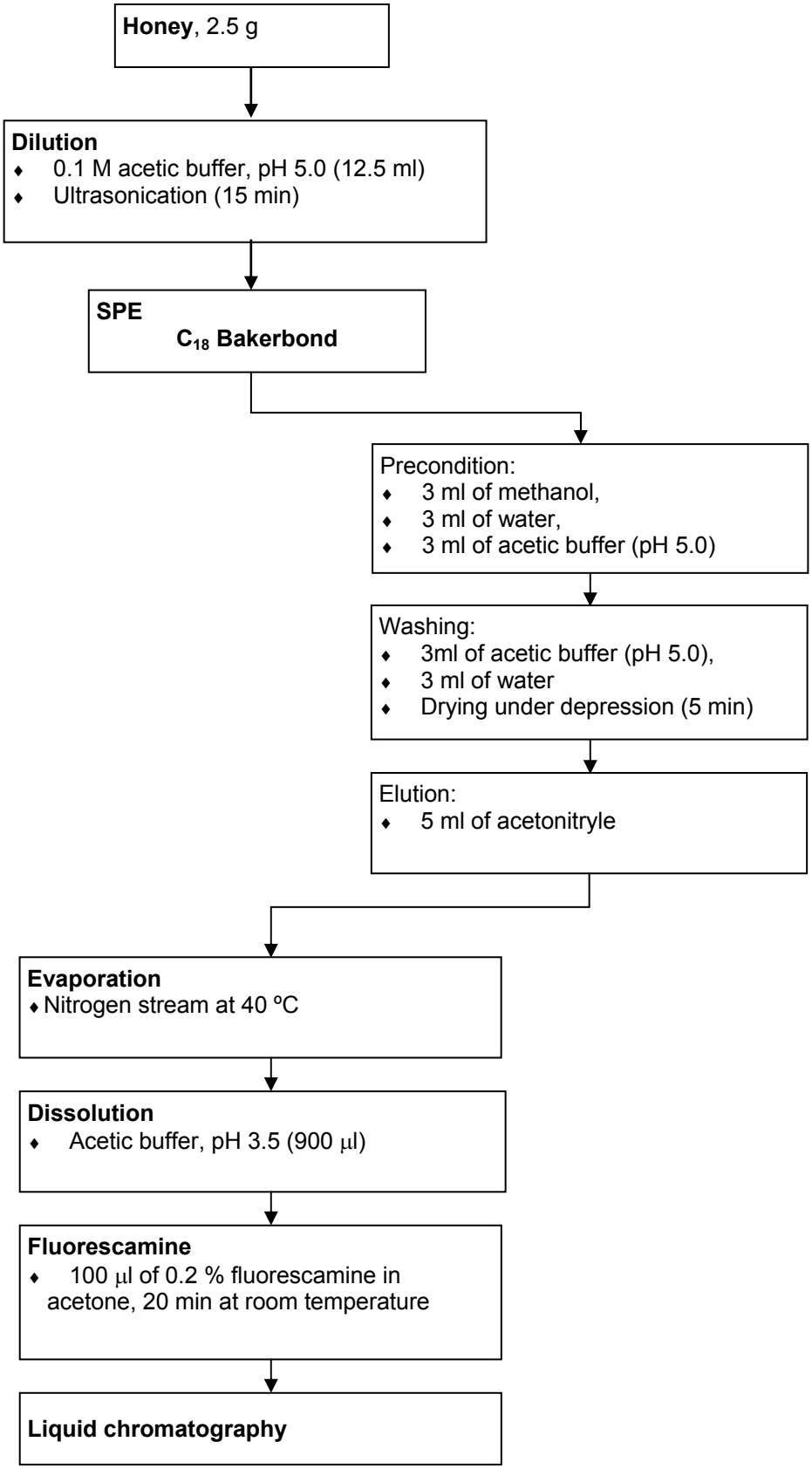


figure 2. Flow diagram of analytical procedure

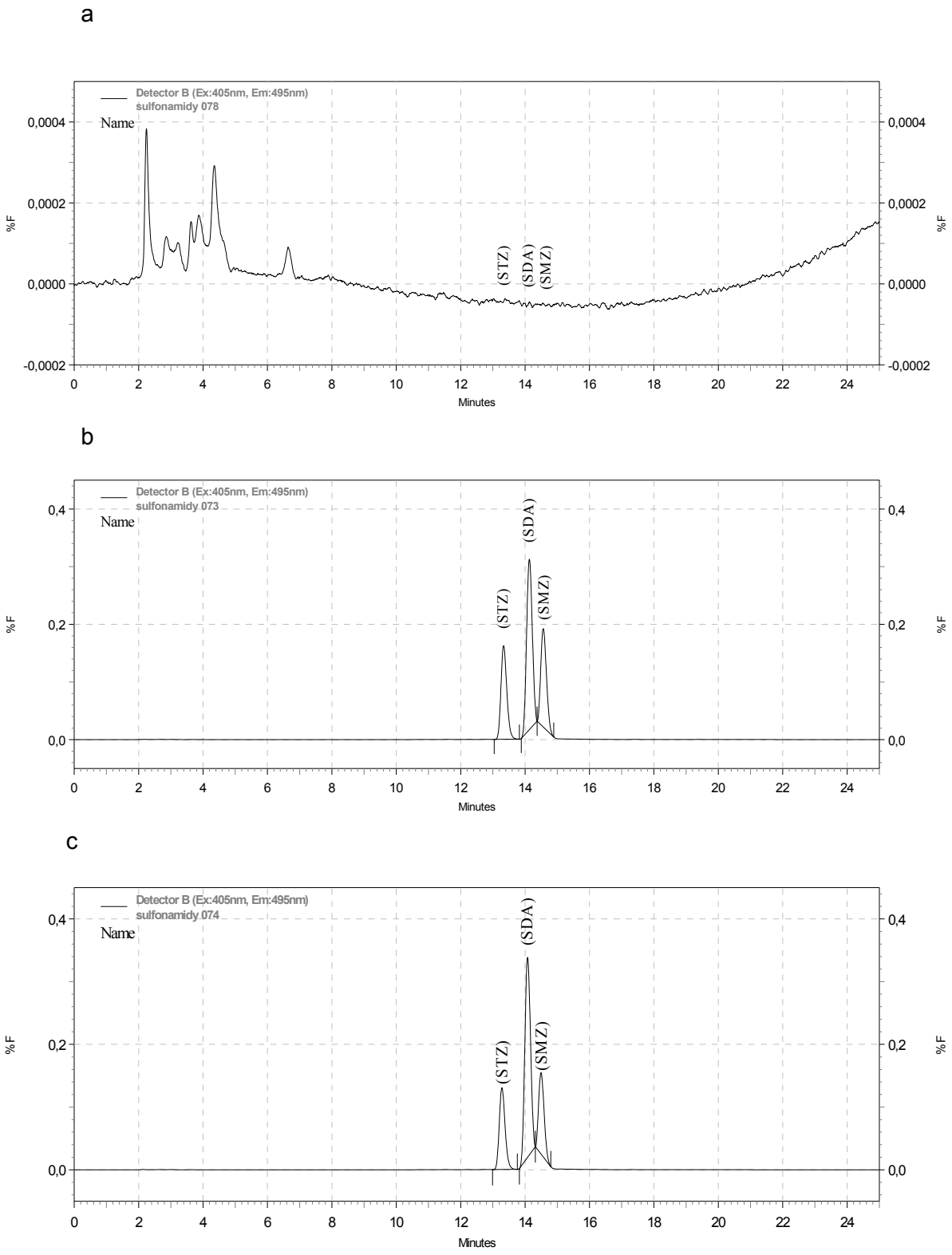


figure 3. Typical chromatograms: a – control honey sample,
b – mixture of sulfonamide standards, c – honey sample spiked with sulfonamide standards