Determination of sulfonamides in honey: model study for method optimization

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Abstract

The study was focused on optimization of methods for the determination of chemotherapeutics from the group of sulfonamides (sulfadiazine, sulfathiazole, sulfamethoxazole, sulfadoxin) in honey by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Three working processes were selected for the optimization: water, acid hydrolysis 1, acid hydrolysis 2. The aim of the study was to determine a suitable methodology in which extraction of selected sulfonamides would be acceptable in terms of their physico-chemical properties. The model study was performed on a set of twelve parallel determinations at concentrations of 0.5, 1 and 5 µg/L for each procedure. The gained results indicated following analytical recoveries: from 79 % to 121 % (water), from 88 % to 127 % (acid hydrolysis 1) and from 61 % to 95 % (acid hydrolysis 2). The repeatability, expressed as relative standard deviation (RSD) was from 7.73 % to 29.60 % (water), from 4.37 % to 14.74 % (acid hydrolysis 1) and from 13.96 % to 31.58 % (acid hydrolysis 2). Regarding the physico-chemical properties of sulfonamides, acid hydrolysis 2 was chosen as a suitable process.

Key words: drug residues, acid hydrolysis, liquid chromatography, mass spectrometry

Introduction

Sulfonamides (SAs) are bacteriostatically acting broad-spectrum chemotherapeutic agents used to treat and prevent bacterial diseases. It is a large group of substances with antimicrobial properties. Sulfonamides have found rapid application in veterinary medicine to treat pet and food animals. Especially among farm animals, there are certain specifics for the application of veterinary medicines. The main requirements are compliance with withdrawal periods and compliance with the criteria for residues of veterinary medicines, the so-called maximum residual limits (MRLs). Honey as an animal origin foodstuff, produced by honeybee species (*Apis mellifera*), is quite different in this concept. It is not allowed to treat honeybee colonies with antibiotics in the European Union (EU). Therefore, MRLs requirements are not legally set. This prohibition does not apply to non-EU countries. Beekeepers in these countries often choose to use antibiotics in the presence of honeybee larvae diseases - American and European foulbrood diseases. The etiological agents of these diseases are *Paenibacillus larvae* and *Melisococcus plutonius* along with other accompanying pathogens. In the EU, radical destruction of honeybee colonies by incineration is required in the occurrence of these dangerous infections (Jin, Zhang, Zhao, Zhang, Wang, Zhou & Li, 2017; Reybroeck, Daeseleire, De Brabander & Herman, 2012; Su, Li, Liu, Wang & Yang, 2016; Orso, Floriano, Ribeiro, Bandeira, Prestes & Zanella, 2016; Juan-Borras, Periche, Domenech & Escriche, 2015; Dmitrienko, Kochuk, Apyari & Tomacheva, 2014; Act No. 166/1999 Coll., as amended; Regulation EC No. 37/2010; Regulation EC No. 470/2009).

Due to honey tradability there is an increased risk of the presence of honey containing residues of veterinary medicines. As part of compliance with European legislation, monitoring of extraneous substances is carried out in the EU countries (Council Directive 96/23/EC).

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Honey is a unique foodstuff with a valuable nutritional profile, though its production is challengeable. From the chemical analysis point of view, honey is a very problematic food matrix. This is due to the composition of the honey and the ingredients that may cause problems in the determination of residues. Sulfonamides are by their physico-chemical properties very specific analytes. They bind firmly to their constituents (saccharides/ sugars or proteins) in food matrices. For their proper isolation it is necessary to choose the procedure of preanalytical preparation for the most effective release from the matrix and their appropriate detection (Guillen, Guardiola, Almela & Nunez-Delicado, 2017; Galarini, Saluti, Giusepponi, Rossi & Moretti, 2015; Dubreil-Cheneau, Pirotais, Verdon & Hurtaud-Pessel, 2014; Economou, Petraki, Tsipi & Botitsi, 2012; Thompson & Noot, 2005; Maudens, Zhang & Lambert, 2004; Verzegnassi, Savoy-Perroud & Stadler, 2002; Dmitrienko, Kochuk, Apyari & Tomacheva, 2014; Hu, Zhao, Xi, Mao, Zhou, Chen & Yan, 2017; Commission Decision 2002/657/EC).

In the study, three methods were applied to determine four sulfonamides (sulfadiazine, sulfathiazole, sulfamethoxazole, sulfadoxin) in honey by liquid chromatography with tandem mass spectrometry.

Material and methods

Chemicals and instrumentation

The chemicals were of analytical grade. Standards SAs sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethoxazole (SMZ), sulfadoxin (SDX; Sigma-Aldrich, Germany), trichloroacetic acid p.a. (Penta, Czech Republic), acetonitrile for HPLC and LC-MS, dichloromethane for HPLC (Merck, Germany), formic acid 98-100% (Sigma-Aldrich, Germany), sodium hydroxide p.a. (Penta, Czech Republic), UPLC BEH C18 2.1 x 100 mm chromatography column, 1.7 µm (Waters, Ireland), argon (Linde Gas, Germany). Deionized water was prepared using Aqua Osmotic 03 device (Tisnov, Czech Republic). Other instrumentations were: analytical laboratory scales Kern ALJ 160-4NM (Balingen, Germany), ultrasonic bath (Kreintek, Slovakia), water bath GFL 1002 (GmbH, Germany), vacuum rotary evaporator with water bath (Büchi Labortechnik AG Flawil, Switzerland), orbital shaker GFL 3005 (Merci, Czech Republic), Hermle centrifuge type 326 K (Labortechnik GmbH, Germany), pH meter 211 Microprocessor (Hanna Instruments, Romania). Mobile phases were filtered through a membrane filter (0.2 µm) and degassed in an ultrasonic water bath for 15 minutes. Flower honeys from the market network (Billa, Lidl, Czech Republic) were used as model honeys.

Preparation of solutions

Stock solutions were prepared by weighing SAs standards and diluting to methanol to give a final concentration of 0.4 g/L. Calibration solutions were prepared at a concentration of 0.1–100 µg/L. SAs infusion solutions were diluted at a concentration of 2 mg/L for analytes mass detection optimization and for the detection of precursor and product ions.

For the model study, working solutions of SAs were prepared in concentrations of 0.5, 1 and 5 µg/L using the selected solvent according to the working procedure (water, acid hydrolysis 1 and acid hydrolysis 2). Using the water procedure, honey samples were dissolved in deionized water, filtered into vials and analyzed. Acid hydrolysis 1, included a 5% aqueous solution of trichloroacetic acid. The mixture was filtered and subjected to chromatographic analysis. The acid hydrolysis 2, included the use of a 10% trichloroacetic acid, an ultrasonic water bath (15 minutes), a water bath temperature (64 °C/1 hour), pH adjustment with sodium hydroxide and two extractions into acetonitrile and dichloromethane. The extracting agents were then evaporated, and the analytes were reconstituted with 1 ml of mobile phase A (0.05% aqueous formic acid). The sample was filtered into a vial for chromatographic determination. This workflow was a modification of the method described by Verzegnassi, Savoy-Perroud & Stadler (2002).

Conditions of chromatographic analysis and mass detection

The Acquity H Class UPLC liquid chromatograph in conjunction with a triple quadrupole mass detector (TQD) was used to determine SAs. Acquity UPLC BEH C18 2.1 x 100 mm, 1.7 µm column was used for the separation. The mobile phases were: 0.05% aqueous formic acid solution (A) and 0.05% formic acid in acetonitrile (B) at a flow rate of 0.3 ml/min and a gradient elution of 0-3 min: 10% B; 3 - 3.6 min: 90% B; 3.6-5 min: 10% B. The column temperature was 35 °C and run time was 5 minutes.

In optimizing the analytes for mass detection, precursor ions 251 (SDZ), 256 (STZ), 254 (SMZ), 311 (SDX) were followed, same as produced fragments 156, 108 and 92 m/z. Two product ions were selected for each sulfonamide - quantification and confirmatory (Table 1). The evaluation was performed using the external calibration curve method in the software MassLynx 4.1 (Waters, USA).

Table 1. Mass detection parameters for the determination of sulfonamides in honey

Analyte	Cone [V]	Precursor ion $[M+H]^+$	Product ions *(m/z)*	Collision [V]	Q *(m/z)*	C *(m/z)*
			>156	17		
SDZ	34	251	>92	25	92	156
			>65	39		
			>156	15		
STZ	33	256	>108	24	156	92
			>92	30		
			>156	14		
SMZ	37	254	>108	23	92	156
			>92	27		
			>156	17		
SDX	38	311	>108	30	92	108
			>92	31		

SDZ, sulfadiazine; STZ, sulfathiazole; SMZ, sulfamethoxazole; SDX, sulfadoxin; Q, quantification fragment; C, confirmatory fragment

Results and discussion

From the model experiment, the average values of recoveries (from 12 parallel determinations) were following: for water (79 – 121 %), acid hydrolysis 1 (88 – 127%) and acid hydrolysis 2 (61 – 95 %). The obtained results were close to the recovery values reported by Thompson & Noot (2005): 84 – 112%, Economou, Petraki, Tsipi & Botitsi (2012): 70 – 106%, Dubreil-Cheneau, Pirotais, Verdon & Hurtaud-Pessel (2014): 85.8 - 110.2%, Juan-Borras, Periche, Domenech & Escriche (2015): 89 – 114%, Orso, Floriano, Ribeiro, Bandeira, Prestes & Zanella (2016): 70 – 120% and Guillen, Guardiola, Almela & Nunez-Delicado (2017): 89 – 118%. Hammel, Mohamed, Gremaud, Le Breton & Guy (2008) found very low recoveries in the range of 26% to 61% despite the complex honey preparation prior to analytical determination. Maudens, Zhang & Lambert (2004) achieved only 37.4% to 66.8% recoveries using acid hydrolysis and solid phase extraction (SPE). Verzegnassi, Savoy-Perroud & Stadler (2002) used acid hydrolysis 2 for the method modification. The authors achieved a recovery of only 44% to 73%.

The repeatability of the methods (n = 12) expressed as relative standard deviation (RSD) was 7.73% to 29.60% (water), 4.37% to 14.74% (acid hydrolysis 1) and 13.96% to 31.58% (acidic hydrolysis 2). Declining repeatability is related to the difficulty of the workflow. Experimental papers report following values: 10% (Maudens, Zhang & Lambert, 2004; Hu, Zhao, Xi, Mao, Zhou, Chen & Yan, 2017), 20% (Hammel, Mohamed, Gremaud, Le Breton & Guy, 2008; Economou, Petraki, Tsipi & Botitsi, 2012; Dubreil-Cheneau, Pirotais, Verdon & Hurtaud-Pessel, 2014; Juan-Borras, Periche, Domenech & Escriche, 2015; Orso, Floriano, Ribeiro, Bandeira, Prestes & Zanella, 2016; Guillen, Guardiola, Almela & Nunez-Delicado, 2017), and 30% (Thompson & Noot, 2005).

Physico-chemical properties of honey samples have been considered in the optimization of methods for the determination of SAs. The results of the water workflow were very satisfactory, though authors favor the inclusion of an acid hydrolysis step. The aim was to extract the SAs from the food matrix components, to convert them to the free form and to separate them correctly. The acid hydrolysis 1 workflow did not quantify one of the four

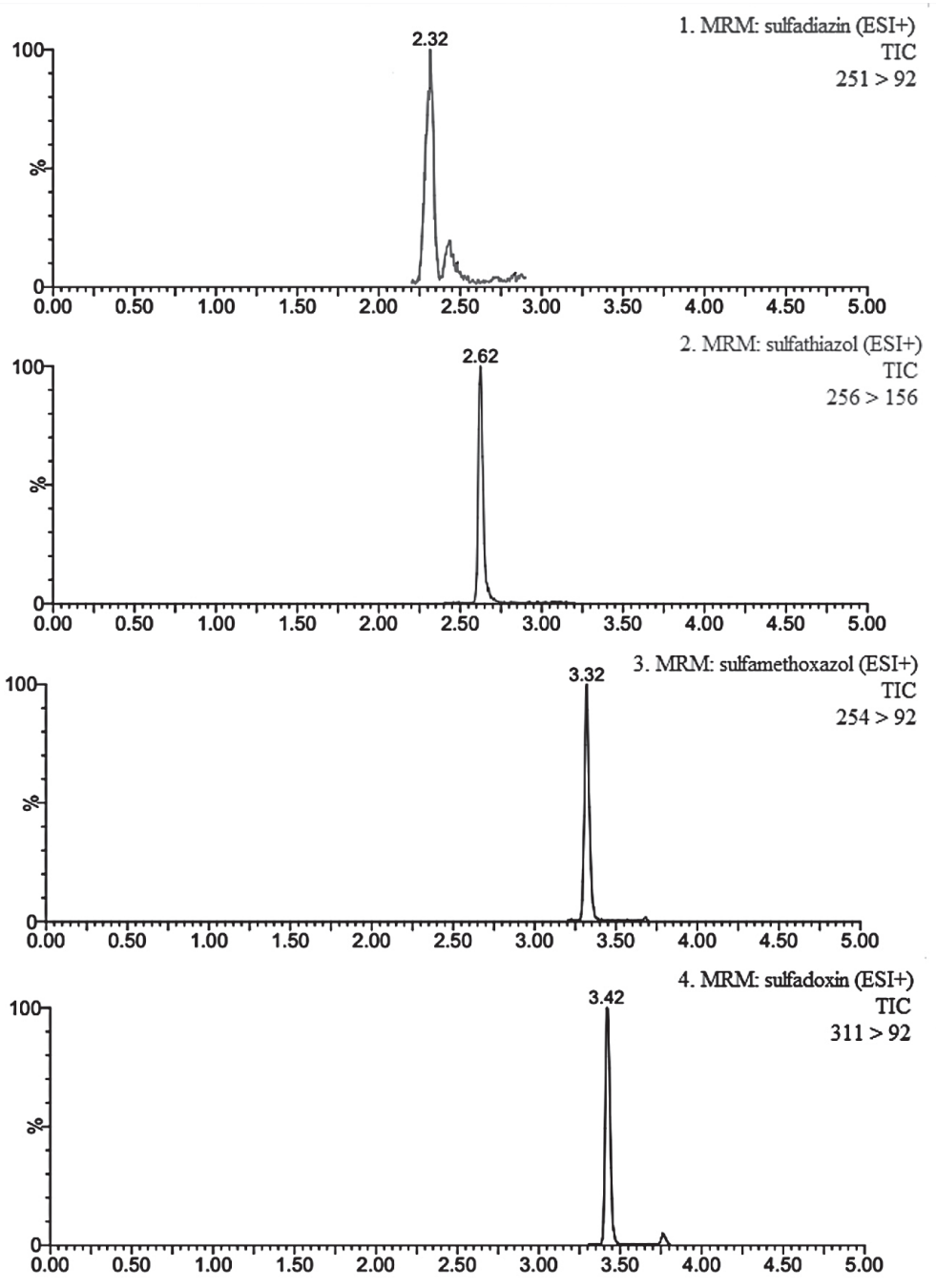

Fig. 1. Acid hydrolysis 2: Chromatograms of method optimization for the determination of sulfonamides in honey (concentration 5 µg/L).

selected analytes, sulfadiazine. The authors emphasized the importance of the synergistic effects of the use of acid hydrolysis, ultrasonic bath and tempering (Hammel, Mohamed, Gremaud, Le Breton & Guy, 2008; Pang, Cao, Fan, Zhang, Li, Li & Jia, 2003). Therefore, the acid hydrolysis procedure 2 was chosen as suitable for the determination of selected analytes in honey (Fig. 1, 2). This fact was used in another experimental work Dluhošová, Borkovcová, Kaniová & Vorlová (2018).

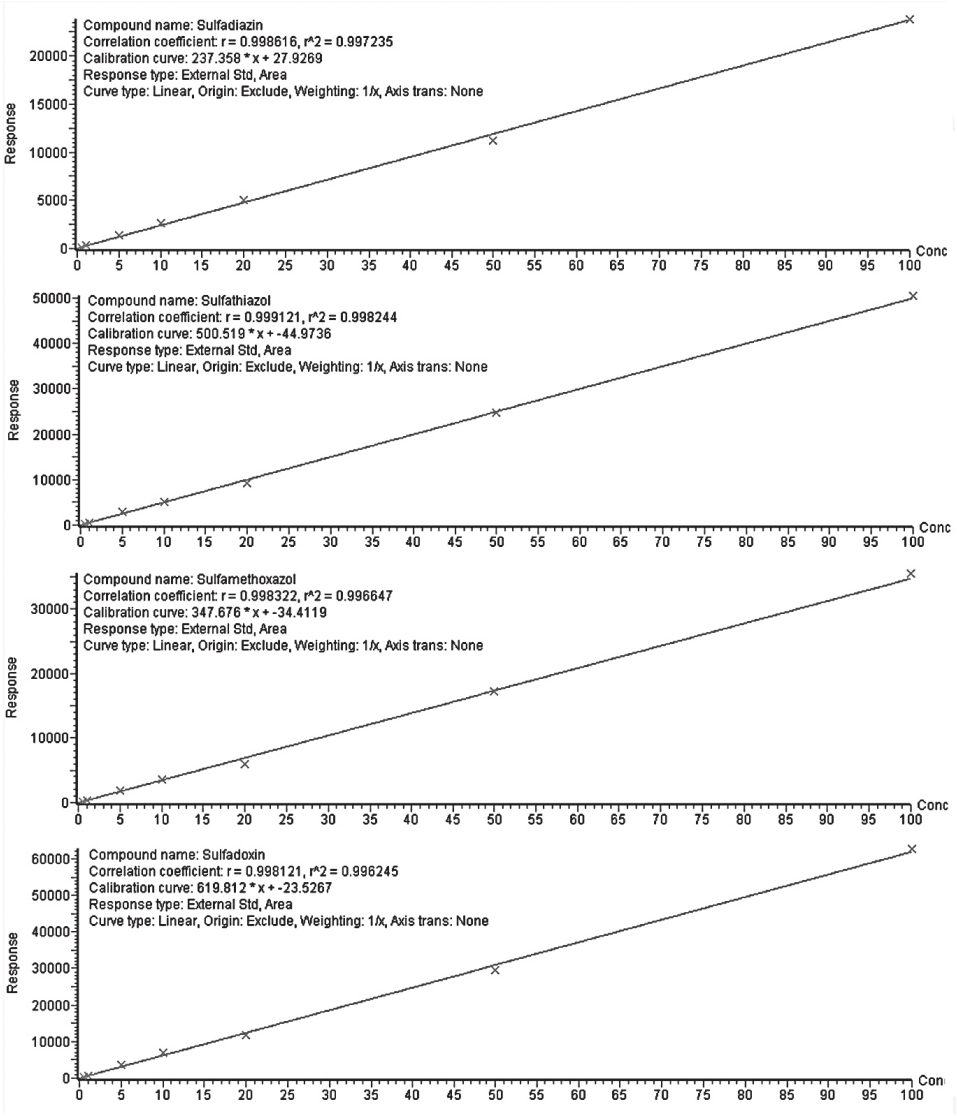

Fig. 2: Acid hydrolysis 2: Matrix calibration curve parameters (linearity 0.1 µg/L–100 µg/L)

Conclusion

The research was focused on the optimization of methodology for the determination of sulfonamides in honey. Three procedures were selected – without extraction using deionized water (water), with extraction using 5% trichloroacetic acid (acid hydrolysis 1) and 10% trichloroacetic acid, acetonitrile and dichloromethane (acid hydrolysis 2). The recoveries of the analytes were 79 % to 121 % (water), 88 % to 127 % (acid hydrolysis 1) and 61 % to 95 % (acid hydrolysis 2). Acid hydrolysis 2 was chosen as a suitable procedure for extracting all four analyzed analytes regarding physico-chemical properties and behavior of sulfonamides in food matrices.

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