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# Application of liquid chromatography–electrospray ionization tandem mass spectrometry to the detection of 10 sulfonamides in honey

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**Abstract**

Liquid chromatography (LC) in combination with tandem mass spectrometry (MS–MS) has been applied to the separation and detection of 10 different sulfonamides in honey. The methodology encompasses a simple hydrolysis of the honey sample to liberate sugar-bound sulfonamides followed by liquid–liquid extraction of the 10 analytes, filtration, and analysis by LC–MS–MS. Conditions for reversed-phase LC and electrospray ionization (ESI) MS–MS in the positive ion mode were optimized for the 10 compounds under study, monitoring two characteristic mass transitions simultaneously for each analyte. The procedure is a qualitative confirmatory method for 10 sulfonamides at the low μg/kg level in honey. Typical recoveries of the analytes in honey ranged from 44 to 73% at a fortification level of 50 μg/kg. This study also addresses the issue of matrix-induced suppression of ionization, an effect often encountered in trace residue analysis of food matrices using LC–ESI-MS–MS based methods.


*Keywords:* Honey; Liquid–liquid extraction; Food analysis; Sulfonamides; Antibiotics

## 1. Introduction

Sulfonamides are antimicrobial agents widely used in food producing animals as growth promoters as well as for therapeutic and prophylactic purposes [1–3]. Such antibiotics are, for example, employed to treat bees infected with bacterial diseases such as the American or European foulbrood [4,5]. However, if bees are treated during the harvesting season, residues of these compounds may be found in honey. Currently, no maximum residue level (MRL) exists for this antibiotics/commodity combination in Europe [6], and legislation in Switzerland has set an MRL of 50 μg/kg for ''total'' sulfonamides in honey [7]. Furthermore, residues of antibiotics in the food chain are of increasing concern due to their overall contribution to the increase of antibiotic resistance of pathogenic bacteria, as well as the potential allergenic reactions they may illicit in certain individuals [8].

Sulfonamides in food may be determined by a number of different analytical methods, based for example on enzyme immunoassay [9], thin-layer

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chromatography [10], gas chromatography (GC) [11], and reversed-phase high-performance liquid chromatography (HPLC) [12–15]. GC coupled to mass spectrometry (GC–MS) is both sensitive and specific for the detection of sulfonamides, but requires derivatization of the polar analytes. The major technical advancements made in the past decade on interfacing LC and capillary electrophoresis (CE) systems to mass spectrometers have also resulted in a number of applications of CE–MS- [16] and LC–MS-based methods for the detection of sulfonamides in food, employing various ionization techniques such as thermospray (TSP) [17], electrospray (ESI) [18–24] and atmospheric pressure chemical ionization (APCI) [25,26].

LC coupled to tandem mass spectrometry has been employed for the determination and confirmation of several sulfonamides at low μg/kg levels in different matrices [17,22,24,27]. However, to our knowledge, no ''selective'' multiresidue method for the determination of 10 sulfonamides in different honeys has been reported to date.

We describe a qualitative confirmatory method for the simultaneous detection of 10 different sulfonamides (Fig. 1) by LC–ESI-MS–MS. The study addresses in particular the impact of the food matrix on ion suppression and, consequently, the challenges encountered in the trace-level quantification of the analytes in honey. The experiments conducted here compare standard and matrix-matched calibration curves in different honey samples, determining linearity ranges and recoveries of the individual analytes. Finally, the application of the analytical method using honeys of various origins and with incurred residues is demonstrated.

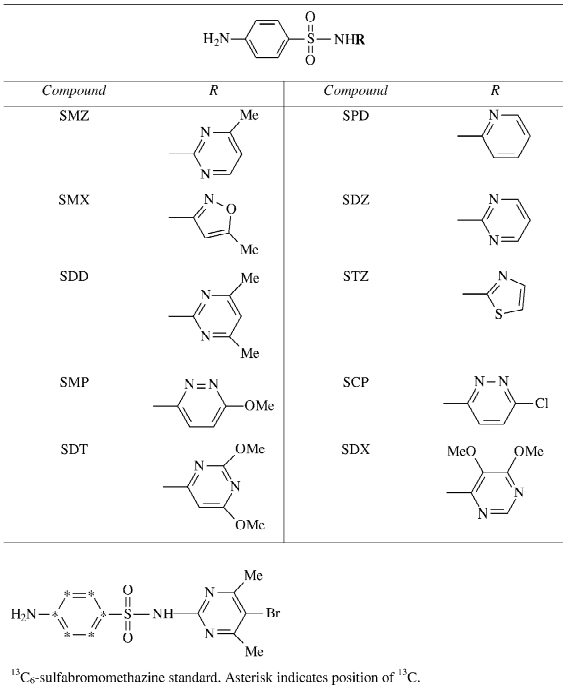


Fig. 1. Chemical structures of the compounds investigated.

## 2. Experimental

### 2.1. Materials and reagents

Sulfadimidine (SDD), sulfamethoxypyridazine (SMP), sulfachloropyridazine (SCP), sulfamerazine (SMZ), sulfadiazine (SDZ), sulfadimethoxine (SDT), sulfamethoxazole (SMX), sulfathiazole (STZ), sulfadoxine (SDX) and sulfapyridine (SPD) standards were purchased from Riedel-de-Haen (Seelze, Germany). Stock solutions of all standards were prepared by dissolving each compound in methanol at a concentration of 1 mg/ml and were stored at −16 °C. Working standards were prepared fresh daily by diluting the stock solution with distilled water, and intermediate storage at +4 °C. The labeled standard $^{13}C_6$-sulfabromomethazine (chemical purity >98%, isotopic purity >90%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). A stock solution of labeled standard was diluted accordingly to afford a working solution of 2.2 μg/ml, which was stored at −16 °C. Formic acid, water (LiChrosolv), and disodium hydrogen phosphate dihydrate were obtained from Merck (Darmstadt, Germany). Acetonitrile (Ultra Gradient HPLC Grade) was purchased from J.T. Baker (Deventer, The Netherlands). Trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) were from Merck.

### 2.2. Honey samples

Honey samples of different geographical origin,

i.e. Mexico, France, Switzerland, New Zealand, and Vietnam, were used in this study. All honeys were multiflower honeys either provided by various honey suppliers or purchased from retail outlets ''off-the-shelf'' in Switzerland. All samples were stored in dark and dry places at room temperature.

### 2.3. Sample extraction

The extraction procedure follows that of Schwaiger and Schuch [28], which has been developed for STZ and other sulfonamide residues in honey. Typically, a honey sample (5 g) was weighed in a centrifuge tube and dissolved with 5 ml trichloroacetic acid (10%, v/v, in water). The sample was agitated on a mechanical shaker for 10 min and then heated to 63±2 °C for 60 min in a water bath. Thereafter, the sample was allowed to cool to room temperature in an ice bath and the pH was adjusted to 6.5 using a saturated solution of $Na_2HPO_4$ (1 *M* at pH 12, adjusted with NaOH). Solvents were added (10 ml of acetonitrile and 2.5 ml of dichloromethane) and the mixture agitated in the centrifuge tube for 10 min. The mixture was then centrifuged for 10 min at 4690 *g* (Beckmann Coulter Avanti J-25 I centrifuge, Ja-17 rotor) at 10 °C. The upper organic layer was carefully transferred to a 25-ml marked round bottom flask. The sample was again extracted with the same solvent preparation and the organic phases combined. The tube was shaken for a further 10 min. The mixture was centrifuged as described above and the upper organic layer transferred to the 25-ml flask and made up to the mark with dichloromethane. An aliquot of 10 ml was transferred to an amber glass tube and evaporated to dryness in a heater block at 40 °C under a constant stream of nitrogen. A sulfonamide standard ($^{13}C_6$-sulfabromomethazine) was added (115 μl, corresponding to 125 μg/kg) after sample preparation. The residue was taken up in 1 ml of water–acetonitrile (90:10, v/v) and passed through a syringe filter (Spartan 13/0,2 RC, Schleicher & Schuell). An aliquot of 10 μl was injected onto the LC–MS system.

### 2.4. LC–ESI-MS–MS

All measurements were carried out with an ''Alliance'' 2690 HPLC system (Waters, Rupperswil, CH) coupled to a Quattro LC tandem mass spectrometer (Micromass, Manchester, UK). The separation of the different sulfonamides was achieved on a Nucleosil $C_{18}$ HD column (50×2 mm I.D., particle size 3 μm) and running a linear gradient from 100% solvent A (0.3% formic acid and 5% acetonitrile in water, v/v/v) at 0 min to 70% solvent A and 30% solvent B (0.3% formic acid in pure acetonitrile, v/v) at 12 min. At 12.1 min, solvent A was increased to 100% until 23 min at a flow-rate of 0.2 ml/min. The LC column and autosampler temperatures were set at 35 and 5 °C, respectively.

The analytes were detected using electrospray in the positive ionization mode. Typical MS settings were: needle voltage 3.08 kV; RF lens 0.2 V; source block and desolvation temperatures 120 and 350 °C, respectively. The nebulizer gas flow ($N_2$) was set to 80 l/h and the desolvation gas flow ($N_2$) to 600–650 l/h. The collision gas (argon) was used at an indicated pressure of 2.3 mTorr. The ion energies were set to 1.0 V for both quadrupoles. Two different characteristic fragmentation reactions were monitored for each analyte in the selected reaction monitoring (SRM) mode using a dwell time of 0.1 s. Specific settings for cone voltages and collision energies as listed in Table 1.

### 2.5. Calibration

For quantitation of the 10 sulfonamides in the incurred honey samples, both solvent and matrix-matched calibration curves using different blank honeys were constructed. A multi-component standard stock solution was prepared from which aliquots were taken, mixed with a fixed amount of labeled standard solution (final standard concentration in the calibration solutions and in the extracts was 125 μg/kg, unless otherwise stated). These solutions were prepared to cover a calibration range up to 100 μg/kg, i.e. blank, 10, 25, 50 and 100 μg/kg. Aliquots of these standard solutions were mixed with concentrated extracts to obtain matrix-matched calibration solutions. All samples were injected in triplicate. Area ratios of the SRM transitions showing the most intense signal and standard were plotted against their respective amount ratios.

Table 1
LC–MS parameters used in this study for the determination of 10 sulfonamides and the standard

| Compound | Precursor $[M+H]^+$ ion $m/z$ | Transitions observed | Collision voltage (V) | Cone voltage (V) | Typical retention time (min) |
|---|---|---|---|---|---|
| SPD | 250 | 250>156 | 17 | 30 | 3.5 |
| | | 250>108 | 20 | 30 | |
| SDZ | 251 | 251>156 | 17 | 27 | 2.7 |
| | | 251>108 | 20 | 27 | |
| STZ | 256 | 256>156 | 15 | 20 | 3.7 |
| | | 256>108 | 20 | 20 | |
| SMZ | 265 | 265>156 | 17 | 25 | 3.9 |
| | | 265>190 | 13 | 25 | |
| SMX | 254 | 254>156 | 15 | 24 | 9.0 |
| | | 254>108 | 20 | 24 | |
| SDD | 279 | 279>156 | 20 | 20 | 5.6 |
| | | 279>204 | 20 | 20 | |
| SMP | 281 | 281>156 | 17 | 20 | 7.2 |
| | | 281>108 | 25 | 20 | |
| SCP | 285 | 285>156 | 15 | 25 | 8.3 |
| | | 285>108 | 23 | 25 | |
| SDX | 311 | 311>156 | 20 | 25 | 9.4 |
| | | 311>108 | 25 | 25 | |
| SDT | 311 | 311>156 | 20 | 25 | 11.4 |
| | | 311>108 | 25 | 25 | |
| Standard* | 363 | 363>114 | 33 | 30 | 14.5 |
| | | 363>162 | 33 | 20 | |

*$^{13}C_6$-Sulfabromomethazine.

## 3. Results and discussion

### 3.1. Analyte extraction and LC separation

A common approach to extract sulfonamides in honey entails the use of a simple extraction step with organic solvents [10–12,14,22,29]. However, levels of sulfonamides in honey are known to decrease over time when the honeys are stored at room temperature [28,30]. This apparent reduction is attributed to the formation of glucose adducts ($N^4$-glucopyranosyl derivatives) [28]. Therefore, an initial acid hydrolysis step is required to ensure complete release of bound residues, enabling a better estimation of the incurred amount of residual compounds in the food sample.

In the method described here, the acid hydrolysis is followed by a common liquid–liquid extraction step. The target analytes are extracted into the organic phase of the solvent mixture, their extraction efficacy being dependent on their individual p$K_a$ values, which range from 5.1 to 8.6 [29]. The pH of the hydrolyzed solution is therefore carefully raised to 6.5, a value which was found most suitable for the simultaneous extraction of all 10 compounds within an acceptable recovery (69–84% at a fortification level of 50 μg/kg) (J.-M. Diserens, personal communication).

The LC procedure was adapted from Kaufmann et al. [31] and the main goal was to achieve a short overall run time for the 10 sulfonamides and the standard. Due to the low content of apolar constituents such as lipids in the honey matrix (<0.5%) [32], the organic composition of the LC solvent was only raised up to 30% during the gradient. However, we observed that a wash step with 100% acetonitrile was necessary after a series of sample injections (30–40), to avoid augmentation of background signal noise probably due to build-up of poorly eluting matrix constituents at relatively low concentrations of organic solvent.

All sulfonamides included in this study showed easy to integrate chromatographic peaks (Fig. 2),

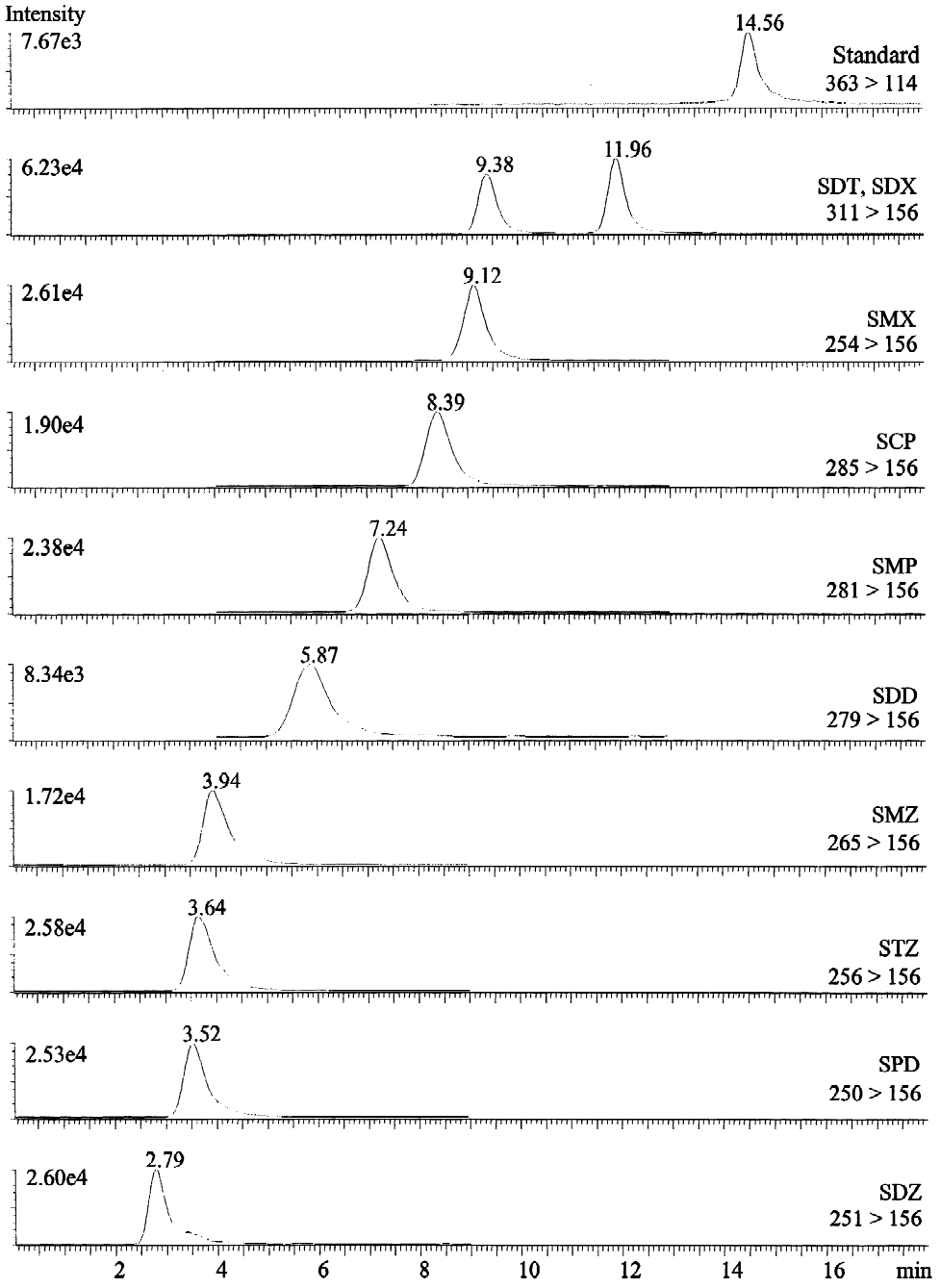


Fig. 2. SRM traces ($m/z$) recorded for the 10 sulfonamides (100 μg/kg) and the $^{13}C_6$-standard (125 μg/kg) in solvent.

eluting at typical retention times from 2.7 to 14.5 min. The overall run time per sample is thus <25 min, enabling the LC–MS analysis of up to 30 samples in a 12-h overnight sequence.

### 3.2. Mass spectrometry

Sulfonamides show relatively simple ESI mass spectra. The only molecular-ion species formed in the acidic mobile phase are protonated molecules. Under the given conditions, no sodium or potassium adducts were observed. However, high background signals and potential interferences warranted additional characteristic fragmentation of the analytes. For example, the molecular ion of SDD corresponds to that of the very common elasticizer di-butyl phthalate [22]. Additional mass transitions are thus required to confirm the presence of sulfonamide residues. For this reason, we chose to acquire two characteristic SRM transitions for each compound. Since the compounds of interest differ only in the heterocyclic base attached to the sulfonamide moiety, ESI-MS–MS spectra of the analytes portray a common pattern (Fig. 3), i.e. all are characterized by $m/z$ 92 ($[H_2NPh]^+$), $m/z$ 108 ($[H_2NPhO]^+$), $m/z$ 156 ($[H_2NPhSO_2]^+$) and $[M+H-155]^+$ ions. In addition, ions corresponding to $[M+H-93]^+$ and $[M+H-66]^+$ are also observed in mass spectra of SDD, SPD, SMX, SMZ and SDX, and most probably correspond to $[O_2SNHR]^+$ and $[M+H-H_2SO_2]^+$ [17]. Quantification was done on the most intense ion transition. However, the base peak observed at $m/z$ 204 in the product-ion spectrum of SDD, most probably due to the $[M+H-93+H_2O]^+$ ion, does not give the most intense fragment ion in the matrix, and thus the $[M+H]^+>156$ transition was chosen.

### 3.3. Calibration and method performance characteristics

All of the 10 sulfonamides showed good response linearity in solvent and honey matrix up to 100 μg/kg, although calibration equations differed for most of the sulfonamides. Matrix-matched calibration curves displayed slopes which not only differed significantly from those observed in the solvent, but which also varied amongst the honey extracts of different origin (Table 2). Variations between these slopes exceeded the repeatability of our samples (RSD<10%). Food matrices can vary in terms of complexity/content in natural products, and it is well established that co-eluting matrix constituents may interfere with the ionization process of the analytes [33–35]. Indeed, this is most probably the cause of variation observed in the different matrix-matched calibration slopes. To circumvent this problem, every matrix-matched calibration curve was always acquired together with its analog in solvent, and the ratio of the two slopes taken into account for a comparison of different honey matrices (Table 2). Examples considered as ''extreme'' are STZ and SMZ, which showed responses from 62 to 89% and 62 to 92%, respectively, versus those obtained in solvent. On the other hand, slopes less dependent on the honey matrix were observed for the compound SDZ.

Signal suppression as observed in our samples present difficulties that need to be addressed especially in quantitative analysis. The effect of solution composition (incorporation of low concentrations of additives or buffers) has been described, and although very efficient in the improvement of the sensitivity in qualitative analysis, it does not help to reduce the matrix impact [36–38]. Addition of a selective clean-up step can occasionally assist in decreasing the matrix effect, although this depends on the matrix as well as on the compounds to be analyzed [27,34]. The employment of isotopically labeled internal standards is, on the other hand, advantageous [39] as this approach compensates for signal irreproducibility associated with the general signal instability mentioned above. However, especially for a multiresidue method that encompasses 10 chemically different compounds, a single labeled standard would not be able to compensate for extraction losses of compounds baring so different chemical properties.

In this study, we chose to spike the final extracts with a $^{13}C_6$-labeled sulfonamide which elutes toward the end of the chromatogram (Fig. 2). It is clear that this standard can be regarded only as an indicator of the variability of the overall analytical system (e.g., column performance, MS response) during a long series of runs. In most cases, we have observed that using the ratio with the labeled standard the slope difference between matrix-matched and solvent cali-

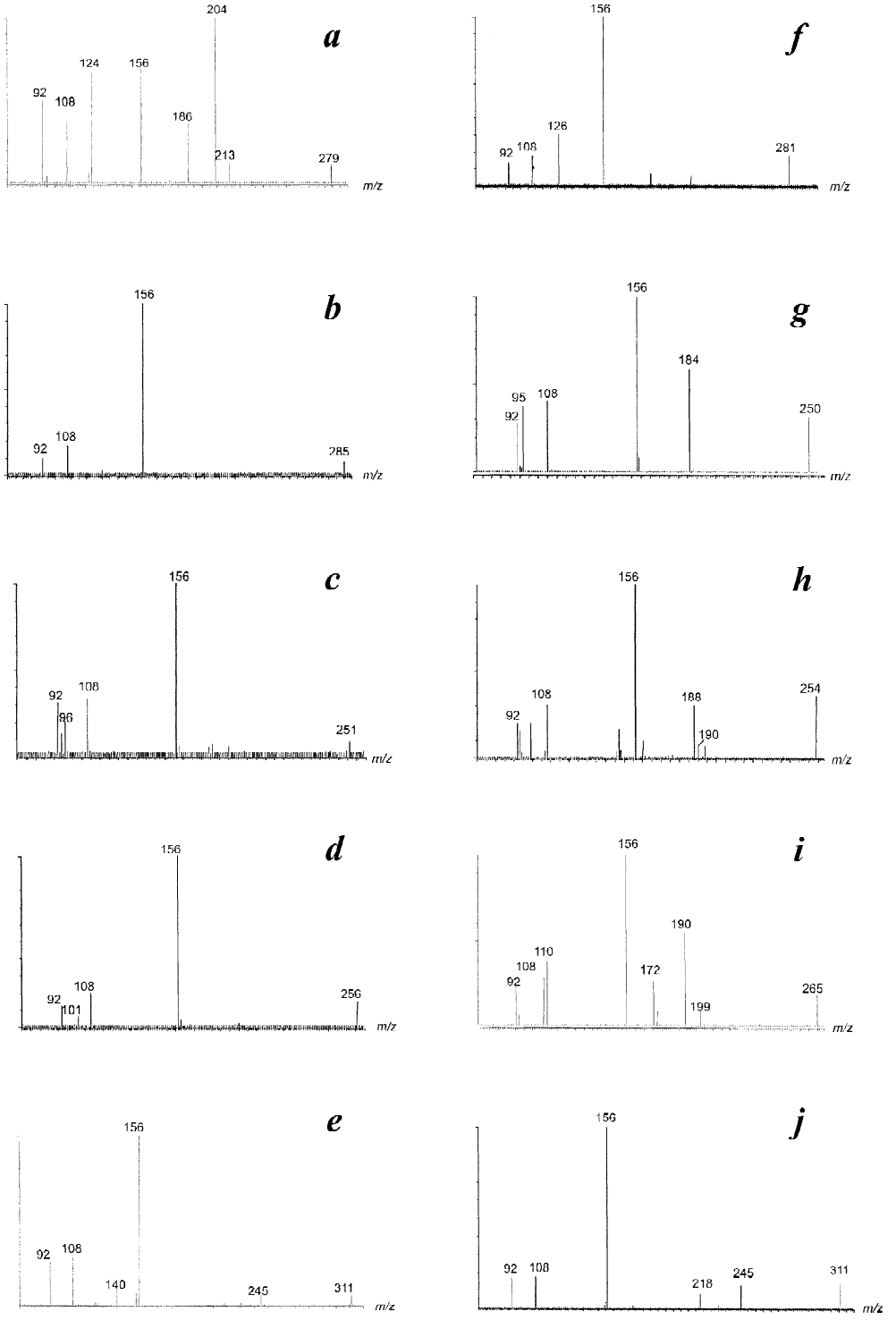


Fig. 3. ESI-MS–MS product ion spectra of the 10 sulfonamides: (a) SDD, (b) SCP, (c) SDZ, (d) STZ, (e) SDX, (f) SMP, (g) SPD, (h) SMX, (i) SMZ, (j) SDT.

Table 2
Matrix-matched calibration curves ($r^2 > 0.995$) established in five different honey extracts (fortified with all 10 analytes after extraction) compared as a ratio to solvent calibration curves: the most intense SRM transition for each compound is considered

| Compound | Honey sample* | Slope (matrix) | Matrix/solvent slope ratio (%) |
|---|---|---|---|
| SPD | FR | 3.115 | 72.5 |
| | MX | 3.928 | 86.2 |
| | NZ | 4.455 | 77.0 |
| | VT | 3.504 | 81.5 |
| | CH | 3.503 | 60.5 |
| SDZ | FR | 3.043 | 107.7 |
| | MX | 3.456 | 95.6 |
| | NZ | 4.037 | 95.3 |
| | VT | 2.707 | 95.8 |
| | CH | 3.757 | 88.7 |
| STZ | FR | 3.502 | 71.5 |
| | MX | 4.217 | 88.5 |
| | NZ | 4.793 | 77.5 |
| | VT | 4.161 | 84.5 |
| | CH | 3.811 | 61.6 |
| SMZ | FR | 2.758 | 76.6 |
| | MX | 3.141 | 92.0 |
| | NZ | 3.455 | 78.4 |
| | VT | 3.067 | 85.2 |
| | CH | 2.714 | 61.6 |
| SMX | FR | 3.665 | 76.6 |
| | MX | 4.331 | 94.0 |
| | NZ | 4.821 | 89.9 |
| | VT | 4.023 | 84.0 |
| | CH | 4.366 | 81.4 |
| SDD | FR | 1.425 | 77.2 |
| | MX | 2.089 | 93.6 |
| | NZ | 2.464 | 90.0 |
| | VT | 1.618 | 87.6 |
| | CH | 2.344 | 85.7 |
| SMP | FR | 4.703 | 76.2 |
| | MX | 4.765 | 92.8 |
| | NZ | 6.087 | 78.6 |
| | VT | 5.476 | 88.7 |
| | CH | 5.814 | 75.0 |
| SCP | FR | 2.818 | 82.6 |
| | MX | 3.439 | 94.5 |
| | NZ | 3.372 | 90.3 |
| | VT | 2.919 | 95.6 |
| | CH | 3.090 | 82.8 |
| SDT | FR | 9.403 | 87.6 |
| | MX | 8.817 | 95.4 |
| | NZ | 10.04 | 87.5 |
| | VT | 9.885 | 91.7 |
| | CH | 9.041 | 78.8 |
| SDX | FR | 8.052 | 82.5 |
| | MX | 8.598 | 99.6 |
| | NZ | 9.808 | 86.1 |
| | VT | 8.970 | 91.9 |
| | CH | 8.783 | 77.1 |

*Honey origin: FR. France; MX, Mexico; NZ, New Zealand; VT, Vietnam; CH, Switzerland.

Table 3
Recoveries (in %) of the 10 sulfonamides spiked in different honey samples

| Compound | Honey origin | | | Average ±SD |
|---|---|---|---|---|
| | Mexico | New Zealand | France | |
| SPD | 54 | 49 | 44 | 49±5 |
| SDT | 54 | 51 | 53 | 53±1 |
| SMX | 55 | 53 | 54 | 54±1 |
| SDZ | 62 | 55 | 57 | 58±4 |
| SCP | 60 | 52 | 58 | 57±4 |
| SDX | 56 | 59 | 56 | 57±2 |
| STZ | 69 | 64 | 65 | 66±3 |
| SMP | 66 | 63 | 63 | 64±2 |
| SMZ | 72 | 66 | 67 | 68±3 |
| SDD | 73 | 67 | 56 | 66±9 |

Recoveries of blank samples spiked at 50 μg/kg before extraction are based on the corresponding matrix-matched calibration curves. Each entry represents the average of three consecutive injections. Only the most intense SRM transition of each analyte was considered for quantitation.

bration curves decreases, but remains consistent (Table 2).

In an attempt to better understand the observed differences in matrix-induced signal suppression, we conducted a number of chemical analyses on the multiflower honeys. In these tests, we focussed on the main constituents of the honey, i.e. sugars (65–75% of the total solids), also including the fructose degradation product hydroxymethylfurfural (HMF). HMF is an indicator of honey aging and is legally permitted in honeys up to a level of 80 mg/kg [40]. The five honeys investigated had very different concentrations of HMF, but no evident correlation could be established between the observed suppression of ionization in extracts and the sugar/HMF profiles.

Recoveries of the analytes at a fortification level of 50 μg/kg were determined by comparing the peak areas obtained from three different honey samples spiked with standards before and after sample preparation. A summary of the data (Table 3) shows an average recovery for all analytes in the individual honey matrices ranging from 44 to 73%. Furthermore, a SD $\leq$5 was determined for nine of the residues, showing similar consistency in recoveries (extractability) of the analytes in the different honeys. Only SDD gave a SD of 9.0, indicating that the matrix can have a significant impact on the extraction procedure. Typical signal-to-noise ($S/N$) ratios of the analytes in different honeys at a fortification level of 10 μg/kg ranged from $S/N=6$ (STZ) to $S/N=100$ (SDX and SDT).

### 3.4. Analysis of incurred honey samples

A number of honey samples of various geographical origins were analyzed by the LC–MS–MS method, and a typical MS chromatogram of a contaminated sample is shown in Fig. 4. The antibiotics of concern are frequently employed as formulations composed of more than one active compound. Estimations of the incurred amounts of residues were conducted based on solvent calibration curves. The sample shown, containing SMX and STZ at estimated levels of 10.5 and 114 μg/kg, respectively, is clearly in violation of current Swiss MRLs [7].

## 4. Conclusion

A qualitative confirmatory LC–MS–MS method for the determination of 10 sulfonamides in honey at trace levels has been developed, entailing acid hydrolysis to liberate the residues, and followed by a liquid–liquid extraction step. Sulfonamides are determined using two mass spectrometric fragmentation reactions and their LC retention times. We have shown that matrix-matched calibration curves cannot be used for quantitation of the residues in honey extracts due to variation in signal suppression. The matrix suppression effect in ESI-MS has no apparent correlation with the chemical composition of the honey sample, and could eventually be remedied by an additional clean-up procedure [34]. However, this will remain a significant challenge in multiresidue methods based on LC–MS techniques.

To our knowledge, this is the first confirmatory multiresidue method by LC–MS for the determination of 10 sulfonamides in honey so far reported. Although current EU legislation forbids the detectable presence of sulfonamide antibiotics in honey, analysis of commercially obtained samples of different origin show that in violative cases sulfonamide levels may reach up to more than 100 μg/kg. This emphasizes the requirement of systematic quality

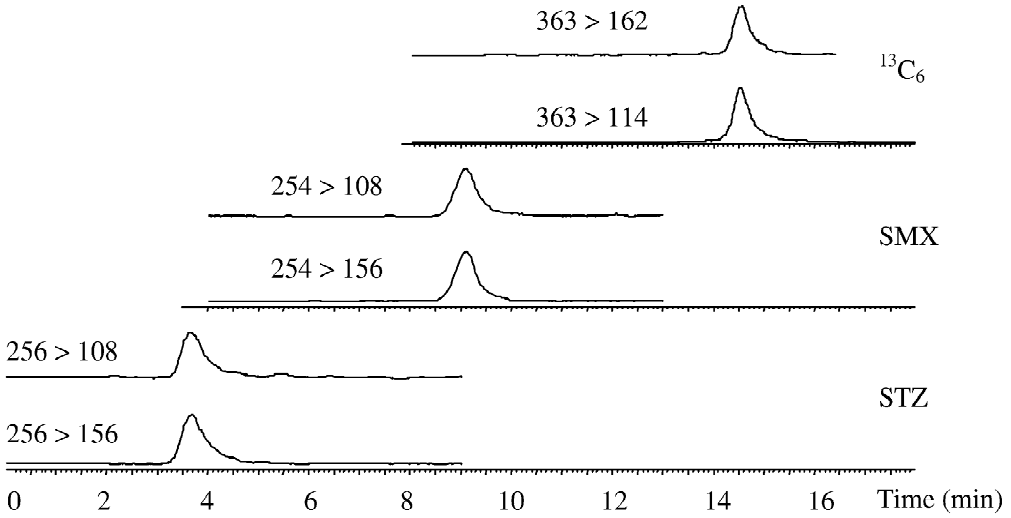


Fig. 4. Analysis of an incurred honey sample, showing residues of STZ and SMX. The concentration of the $^{13}C_6$ standard, added after extraction, is 125 μg/kg. Two SRM transitions are monitored for each compound.

control to avoid that residues enter the food chain at unacceptable levels.

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## References

[1] Feed Additives with Synergic Effect for Growth Promotion. Jpn. Kokai Tokkyo Koho. Patent no. JP 60227641, 1985.
[2] N.T. Crosby, in: Determination of Veterinary Residues in Food, Ellis Horwood, Chichester, 1991, p. 97.
[3] N.A. Botsoglou, D.J. Fletouris, in: Drug Residues in Foods, Marcel Dekker, New York, 2001, p. 85.
[4] T.A. Gochnauer, Bee World 35 (1954) 187.
[5] P.S. Milne, Nature 155 (1945) 335.
[6] Commission of the European Communities, Council Regulation EEC 2377/90. Off. J. L 224 (1990) 1.
[7] Schweizer Honig teilweise verunreinigt, Bundesamt für Gesundheit Bulletin 24/00 (2000) 461.
[8] D.G. Harle, B.A. Baldo, J.V. Wells, Mol. Immunol. 25 (1988) 1347.
[9] A.B. Sheth, P. Sporns, J. Assoc. Off. Anal. Chem. 73 (1990) 871.
[10] M.H. Thomas, K.E. Soroka, S.H. Thomas, J. Assoc. Off. Anal. Chem. 66 (1983) 881.
[11] K. Takatsuki, T. Kikuchi, J. Assoc. Off. Anal. Chem. 73 (1990) 886.
[12] J.O.K. Boison, L.J.-Y. Keng, J. Assoc. Off. Anal. Chem. Int. 77 (1994) 558.
[13] A.R. Long, L.C. Hsieh, M.S. Malbrough, R.C. Short, S.A. Barker, J. Assoc. Off. Anal. Chem. 73 (1990) 868.
[14] A.R. Long, L.C. Hsieh, M.S. Malbrough, C.R. Short, S.A. Barker, J. Agric. Food Chem. 38 (1990) 423.
[15] M.D. Smedley, J.D. Weber, J. Assoc. Off. Anal. Chem. 73 (1990) 875.
[16] K.P. Bateman, S.J. Locke, D.A. Volmer, J. Mass Spectrom. 32 (1997) 297.
[17] J. Abian, M.I. Churchwell, W.A. Korfmacher, J. Chromatogr. 629 (1993) 267.
[18] C. Hartig, T. Storm, M. Jekel, J. Chromatogr. A 854 (1999) 163.
[19] R. Hirsch, T.A. Ternes, K. Haberer, A. Mehlich, F. Ballawanz, K.-L. Kratz, J. Chromatogr. A 815 (1998) 213.
[20] S. Porter, Analyst 119 (1994) 2753.
[21] N. Van Eeckhout, J. Castro Perez, C. Van Peteghem, Rapid Commun. Mass Spectrom. 4 (2000) 2331.
[22] B. Casetta, R. Cozzani, A.L. Cinquina, S. di Marzio, Rapid Commun. Mass Spectrom. 10 (1996) 1497.
[23] S. Pleasance, P. Blay, M.A. Quilliam, J. Chromatogr. 558 (1991) 155.
[24] D.A. Volmer, Rapid Commun. Mass Spectrom. 10 (1996) 1615.
[25] M.T. Combs, M. Ashraf-Khorassani, L.T. Taylor, J. Pharm. Biomed. Anal. 25 (1999) 301.
[26] K. Dost, D.C. Jones, G. Davidson, Analyst 125 (2000) 1243.
[27] T. Pfeifer, J. Tuerk, K. Bester, M. Spiteller, Rapid Commun. Mass Spectrom. 16 (2002) 663.

[28] I. Schwaiger, R. Schuch, Dtsch Lebensm.-Rundsch. 3 (2000) 93.
[29] P. Edder, A. Cominoli, C. Corvi, Trav. Chim. Aliment. Hyg. 88 (1997) 554.
[30] O.W. Parks, J. Assoc. Off. Anal. Chem. 67 (1984) 566.
[31] A. Kaufmann, S. Roth, B. Ryser, M. Widmer, D. Guggisberg, J. Assoc. Off. Anal. Chem. 85 (2002) 853.
[32] European Commission, Directorate C-Scientific Opinions: Opinion of the Scientific Committee on Veterinary Measures relating to Public Health on Honey and Microbiological Hazards (adopted on 19–20 June 2002).
[33] K. Bester, G. Bordin, A. Rodriguez, H. Schimmel, J. Pauwels, G. Van Vyncht, Fresenius J. Anal. Chem. 371 (2001) 550.
[34] B.K. Choi, D.M. Hercules, A.I. Gusev, Fresenius J. Anal. Chem. 369 (2001) 370.
[35] J.L. Sterner, M.V. Johnston, G.R. Nicol, D.P.J. Ridge, Mass Spectrom. 35 (2000) 385.
[36] A.M. Kamel, P.R. Brown, B. Munson, Anal. Chem. 71 (1999) 5481.
[37] F.E. Kuhlmann, A. Apffel, S.M. Fischer, G. Goldberg, P.C. Goodley, J. Am. Soc. Mass Spectrom. 6 (1995) 1221.
[38] S. Zhou, M. Hamburger, Rapid Commun. Mass Spectrom. 9 (1995) 1516.
[39] R.K. Boyd, Rapid Commun. Mass Spectrom. 7 (1993) 257.
[40] Codex Standard for Honey, CX-STAN 12-1981, Rev. 1 (1987).