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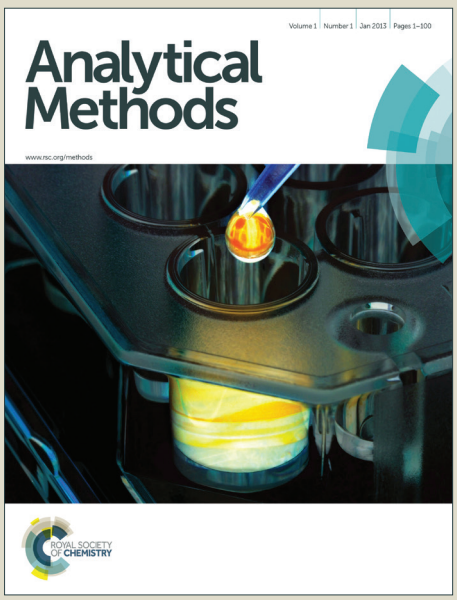

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A multi-residue analysis of sulphonamides in edible animal tissues using QuEChERS extraction and HPLC – MS/MS

Hiba Abdallah,[ab] Carine Arnaudguilhem,[b] Ryszard Lobinski[bc] & Farouk Jaber [*ad]

[a] *CNRSL, Lebanese Atomic Energy Commission (LAEC), Laboratory for Analysis of Organic Compound (LAOC), Beirut, Lebanon*

[b] *CNRS/UPPA, Laboratory of Bio-Inorganic Analytical and Environmental Chemistry (LCABIE), UMR5254, Hélioparc, 2, Av. President Angot, 64053 Pau, France*

[c] *Department of Chemistry, Warsaw University of Technology, ul. Noakowskiego 3, 00-664 Warsaw, Poland*

[d] *Laboratory of Analysis of Organic Compounds (509), Faculty of Sciences I, Lebanese University, Hadath, Beirut, Lebanon*

Abstract

A HPLC- double reaction monitoring MS/MS method was developed for the determination of a wide range (>20) sulphonamide residues in several edible animal (sheep, pork, beef, chicken and dromedary) tissues. Sample preparation was based on the simultaneous extraction into acetonitrile solution followed by a clean-up using primary secondary amine beads. Quantification was carried out using matrix-matched calibration curves. The limit of detection (LODs) and limit of quantification (LOQs) ranged from 0.5 to 14.5 $\mu g.kg^{-1}$ and from 1.8 to 48.4 $\mu g.kg^{-1}$, respectively. Decision limit (CCα) and decision capability (CCβ) obtained were below 100 $\mu g.kg^{-1}$ for sulphonamides and below 5 $\mu g.kg^{-1}$ for dapsone. The method was validated in terms of recoveries and inter and intra-day precision by reference analyses of meat samples using LC-Orbitrap MS and by the analysis of a reference material. The method was applied to the analysis of several animal tissue samples collected in Lebanon. The highest values were observed for sulfamethazine and sulfadimethoxine at 70.2 and 62.5 $\mu g.kg^{-1}$ in sheep tissues.

[*] *Corresponding author: Farouk Jaber*

Address : Laboratory for Analysis of Organic Compound Lebanese Atomic Energy Commission, B. P. 11- 8281, Riad El Solh 1107 2260 Beirut, Lebanon

Tel : 00 96 11 45 08 11 Fax : 00 96 11 45 08 10

E-mail address: fjaber@cnrs.edu.lb

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1. Introduction

Sulphonamides (SAs) are the most common (after tetracyclines) veterinary antibiotics used in the EU. They are relatively cheap and efficient to combat many common bacterial infections.[1,2] SAs are *N*-substituted derivatives of the *p*-aminobenzenesulfonic acid with amphoteric properties. They can be metabolized in the animal body to produce N1 (oxidation) and N4 (acetylation) derivatives. Glucuronide conjugation and aromatic hydroxylation can also take place leading to sulfinamide, AZO-SAs or nitro-SAs (Fig. 1).[3,4] As a consequence of the extensive usage of SAs, their residues (parent compounds or metabolites) can persist in edible tissues of farm animals.[4-6] The exposure of consumers to SAs can lead to allergies and hematological, gastrointestinal and neurological diseases.[7,8] The use of SAs in animals is regulated; according to the EU regulation 37/2010, SAs are authorized substances whereas dapsone is a prohibited one. The maximum residue limit (MRL) for the total amount of SAs in edible tissues, such as muscle, liver, kidney and milk, is 100 $\mu g.kg^{-1}$ [9] which requires the development of relevant monitoring analytical methods.

High performance liquid chromatography (HPLC) coupled to triple quadrupole mass spectrometry (MS),[10-13] operated in "Multiple Reaction Monitoring" (MRM) or "Selected Reaction Monitoring" (SRM),[10,12,14-16] mode, is a common technique of choice for a wide range of chemical residues. The analytes are usually detected by monitoring the ions corresponding to at least two mass transitions which, in combination with their chromatographic retention time, offer sufficient analytical selectivity. The high throughput of HPLC- MS/MS analysis is dependent on the simultaneous multispecies efficient extraction method. These criteria are fulfilled by leaching with aqueous acetonitrile solution followed by the extract cleanup. This principle, referred to as QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) was first developed for the extraction of pesticides [17] but has been increasingly used for the recovery of veterinary drugs from various types of matrices, offering an increased sample throughput and reducing the cost of analysis.

The literature concerning the simultaneous HPLC - triple quad MS/MS analysis of SAs residues in edible animal tissues is relatively scarce and limited to few tissue varieties

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(poultry and fish [16,18-20]) and to a limited number of compounds (6 [16], 7 [19] and 16 compounds [18]). The objective of this work was to develop a wide-scope method in terms of the number of compounds determined (23 – the most complete list reported recently by non-targeted high-resolution MS[21]) and in terms of the variety of matrices analysed (sheep, chicken, beef, pork, and dromedary kidney, liver and muscle).

2. Materials and methods

2.1. Reagents and samples

The structures of the studied SAs are summarized in ***Fig. 1***. SAs were obtained from Ehrenstorfer (Augsburg, Germany) (Sulfaguanidine (SGN), Sulfadiazine (SD), Sulfathiazole (STZ), Sulfamerazine (SM), Sulfamethoxypyridazine (SMP), Sulfamonomethoxine (SMM), Sulfadoxine (SDO), Sulfaphenazole (SNZ), Sulfadimethoxine (SDM) and Sulfaquinoxaline (SQX)) and Sigma Aldrich (China) (Sulfacetamide (SAA), Sulfisomidine (SIM), Sulfapyridine (SP), Sulfameter (SME), Sulfamethizole (SMT), Sulfamethazine (SMZ), Sulfachloropyridazine (SCP), Sulfamethoxazole (SMX), Sulfisoxazole (SIX), Sulfabenzamide (SB), Sulfanitrane (SNT), Sulfaclozine (SCL) and Dapsone (Da)). The internal standard SMX-D_4 was obtained from C/D/N Isotopes (Pointe-Claire, QC, Canada). All the standards were of high purity grade (>95 %).

LC-MS grade methanol (MeOH), acetonitrile (MeCN), acetic acid (AA) and formic acid 98% (FA) were purchased from Honeywell (Germany), Fluka (Germany), Sharlu (Spain) and BDH AnulaR (England), respectively. Water was purified using Easypure™ II (Thermo Scientific, USA). For the "QuEChERS" extraction sodium citrate, sodium hydrogencitrate sesquihydrate, magnesium sulfate and primary secondary amine were purchased from Sigma Aldrich. Sodium chloride was purchased from Riedel de Haen. Purified extracts were filtered through a 0.2 µm Ultrafree-CL Centrifugal filter with a low-binding Durapore PVDF membrane (Millipore, France).

Edible beef, sheep, chicken, pig and dromedary tissues (liver, kidney, muscle) were collected from slaughterhouses and farms in Lebanon**.** A reference material (FAPAS pig kidney N°02227) was obtained from the Food and Environment Research Agency (United Kingdom).

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2.2. Standards solutions

Individual standard stock solutions (ca 1000 $mg.l^{-1}$) were prepared by dissolving an appropriate amount of each compound in MeCN and MeOH depending on their solubility. A mixed standard working solution (10 $mg.l^{-1}$) used for the spiking of the control samples was prepared by appropriate dilutions with MeCN. Another mixed standard working solution (1 $mg.l^{-1}$) was prepared by dilution of the 10 $mg.l^{-1}$ mixed standard working solution with the initial mobile phase (water/MeOH 0.01% formic acid (95:5, *v/v*)). A working internal standard solution (10 $mg.l^{-1}$) of SMX-D_4 was prepared by dilution of the stock solution (ca. 550 $mg.l^{-1}$) in MeCN. All stock and working solutions were stored in dark at -20 ^{0}C.

2.3. Extraction

Extraction and cleanup were based on QuEChERS extraction as described elsewhere.[21] Extraction efficiency was evaluated using samples spiked with appropriate amounts of solutions of SAs and SMX-D_4 (IS) at 100 $\mu g.kg^{-1}$. A 5-g finely ground sample of meat was weighed. Then, 5 mL of water and 10 mL 1% acetic acid in MeCN (v/v) were added to the sample. After agitation for 1 min, 0.5 g of sodium hydrogencitrate sesquihydrate, 1.0 g sodium citrate, 4.0 g of anhydrous magnesium sulfate and 1g of sodium chloride were added. The mixture was vigorously shaken, vortexed for 1 min and centrifuged at 3500 rpm for 5 min. 6 mL of the supernatant was purified with 150 mg of primary secondary amine and 900 mg of anhydrous magnesium sulfate followed by shaking and centrifugation in the conditions as above. 4 mL of the supernatant was evaporated to dryness with N_2 (35 ^{0}C), reconstituted with 500 µl 0.01% (v/v) formic acid in 95% (v/v) MeOH and then filtered through a 0.2µm PVDF, low-binding Durapore (Millipore) filter.

2.4. Liquid chromatography–mass spectrometry ($LC–MS^2$)

Chromatographic analysis was performed using an Agilent 1200 HPLC system (Agilent, USA). Separations were achieved with a Zorbax Eclipse XDB-C_8 column (3.5µm, 2.1×100 mm, Agilent). The column was kept at 30 ^{0}C. The flow-rate and injection volume were 0.2 ml/min and 5µL, respectively. The mobile phases used were: (A) 0.01% formic acid and (B) 0.01% formic acid in MeOH. The gradient elution program was: 0-10 min (5% -10%) B, 10-12 min (10% - 50%) B, 12-15 min (50% - 100%) B, 15-17 min (100%) B. Then, the elution gradient was linearly ramped down to 5 % B for 2 min and maintained for 11 min to allow the conditioning of the column prior to next injection.

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Mass spectrometry analysis was carried out using an Agilent 6410 electrospray triple quadrupole mass spectrometer operated in positive mode. All the SAs were measured in the same chromatographic run by tandem MS carried out in the MRM acquisition mode. Two precursor-to-product ion transitions were monitored of each analyte ***(Table 1)***. The most intense transition was used for quantification ("quantification transition") and the second transition for confirmation of the presence of the analyte ("confirmation transition").

The optimization of MS parameters (precursor ions, skimmer voltage, collision energy, and quantification and confirmation transitions) was performed by flow injection analysis for each compound dissolved in the mobile phase. Data acquisition was carried out using MassHunter software (Agilent).

2.5. Validation

Linearity, accuracy, intra-day and inter-day precision, limits of detection (LOD) and quantification (LOQ), decision limit (CC_α), detection capability (CC_β) and stability were studied to validate the whole procedure according to the European Commission 2002/657/EEC recommendations [22]. SAs quantification was performed using matrix-matched calibration. Linearity was verified by spiking meat samples with the target compounds at 5 levels (blank, 50, 100, 150, 200 $\mu g.kg^{-1}$) for SAs and (blank, 1.25, 2.5, 3, 5 $\mu g.kg^{-1}$) for dapsone and a fixed concentration of SMX-D4 (100 $\mu g.kg^{-1}$). Calibration curves were obtained by least-squares linear regression analysis of the peak area versus concentration corrected with a deuterated internal standard SMX-D_4.

Accuracy of the method was assessed by determining the concentration of 3 uncontaminated meat samples spiked with 100 $\mu g.kg^{-1}$ and 5 $\mu g.kg^{-1}$ of SAs and dapsone respectively, using matrix-matched calibration, and comparing the calculated concentration with the theoretical concentration. Precision (intra- and inter-day) was investigated at the same concentration level. The values of CC_α and CC_β were calculated for all analytes using a matrix-matched calibration curve. CC_α was calculated at the statistical certainty of $1-\alpha$ (α = 0.05 for authorized compounds and 0.01 for unauthorized compounds) and CC_β for $1-\beta$ (β= 0.05 for both authorized and unauthorized compounds) to detect the concentration at the spiked levels 100 and 5 $\mu g.kg^{-1}$ for SAs and dapsone, respectively.[23,24] LOD and LOQ were determined as the lowest amount of analyte which could be detected and quantified, respectively. The LOD and LOQ were estimated at 3 and 10 times the standard deviation of the response obtained for 10 samples spiked at 25 and 5 $\mu g.kg^{-1}$ for SAs and dapsone

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respectively, divided by the slope of the calibration curve. All the experiments were repeated for each concentration level on 3 different days. The stability was assessed by spiking beef samples at different concentration (50 (1.25), 100 (2.5), 150 (3) and 200 (5) $\mu g.kg^{-1}$ for SAs (and dapsone) compounds, respectively, stored at -18 ^{0}C for 12 weeks.

3. *Results and Discussion*

The choice of the analytes was made to match the most complete list reported so far. [21]

3.1. Extraction procedure

The extraction procedure was developed for beef muscle in a previous study.[21] In order to evaluate the efficiency of this procedure, samples of liver, muscle and kidney derived from beef and pork were spiked at 100 $\mu g.kg^{-1}$ of SAs and 5 $\mu g.kg^{-1}$ of dapsone.

For pork matrices (***Fig.2a)*** recoveries of 70-120% for 19 SAs and of 4 SAs 50-70% were achieved for muscle and kidney samples. Most SAs were extracted from liver with recoveries higher than 50%. For beef matrices ***(Fig.2b)***, QuEChERS allowed the extraction recoveries of 70-120% for 21 SAs from kidney and for 16 SAs in muscle tissues. In liver, most of the tested SAs yielded recoveries of 50-70%. Similar results were obtained for sheep ***(Fig.2c)*** (70-100% from muscle and kidney and 54-80% from liver samples) and dromedary ***(Fig.2d)*** (70-120% from muscle and kidney and 60-90% from liver samples), and chicken (70-90% and 55-70%, respectively) ***(Fig.2e)***. In general, the recoveries decreased in the order kidneys > muscle > liver for beef, pork, sheep, and in the order: muscle > kidneys > liver for dromedary.

3.2. LC-MS/MS determination

ESI source and positive ionization mode were selected due to the presence of primary or secondary amino groups in the SAs. MRM mode was applied; two transitions per analyte were selected. The more sensitive one was used for quantitation whereas the other one for the identity confirmation. A typical Total Ion Count (TIC) chromatogram for a beef muscle spiked with 23 SAs at the fixed levels (100 and 5 $\mu g.kg^{-1}$ for SAs and dapsone respectively) is shown in ***Fig. 3***.

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Typical fragment ions were observed for most SAs at *m/z* 156 (cleavage of the S-N bond $[M\text{-}RNH_2]^+$), *m/z* 108 (elimination of the RNH_2SO group) and at *m/z* 92 (cleavage of the $M\text{-}RNH_2\text{-}SO_2$ group]$^+$. Another number of specific transitions were detected for some compounds due to the variable amine substituent, such as, e.g., ions of *m/z* 124 and 186 for SIM and SMZ, *m/z* 215 for SME and SMM, *m/z* 184, 126, 113 and 130 for SP, SMP, SIX and SCL, respectively. For the di-substituted SAs, SNT was detected at *m/z* 134 and 156. The values of the parameters optimized and the MRM transitions selected are given in ***Table 1***.

3.3. Figures of merit

The monitoring of SAs in the animal tissues was performed by Multiple Reaction Monitoring (MRM). The identity confirmation was accomplished by comparing the retention time and the ion ratio of the 2 transitions within 2% and 20%, respectively. The quantification was performed with the most intense transition by matrix-matched calibration. The method was validated following the criteria defined in the Decision 2002/657/EC for quantitative confirmatory methods.[22] Method detection limit (LOD), quantitation limit (LOQ), precision (intra-day and inter-day), accuracy, decision limit (CC_α), detection capability (CC_β) and stability were evaluated for all compounds using spiked beef tissue. No SAs compound was detected in any of the blank beef tissue samples.

Linearity was that of a matrix-matched calibration curve obtained by spiking a beef tissue with the selected antibiotics in the range from 50 to 200 and 1 to 5 $\mu g.kg^{-1}$ for SAs and dapsone, respectively. A correlation coefficient (R^2) higher than 0.990 was obtained for all the compounds, except for SNT (***Table 2*****).** Accuracy (expressed as *A (%) = mean measured concentration * 100 / theoretical concentration),* intra-day and inter-day precision (expressed as *Relative Standard Deviation, RSD*) of the analytical method were assessed by the analysis of 3 different samples spiked at 100 and 5 $\mu g.kg^{-1}$ levels for SAs and dapsone, respectively. The analysis was performed by the same operator on three separate days (3 experiments per day) (***Table 2***). The A% value varied from 71% to 117%. The inter-day precision (*RSD R*) values were below 23% except for SGN and SNT and the intra-day precision (*RSD r*) below 15% for all SAs except for SNT. These results obtained for *A%*, *RSD R* and *RSD r* are consistent for all the analytes with the requirements of the 2002/657/EC decision.[22]

The decision limit (CC_α) was defined as "the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant", and the detection capability (CC_β) as "the smallest content of the substance that may be detected, identified

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and/or quantified in a sample with an error probability of β". In the case of SAs, α and β errors were set at 5% (authorized antibiotics) and 1% in the case of dapsone (unauthorized antibiotic). The decision limit (CC_α) and the detection capability (CC_β) were calculated from the matrix matched calibration curve using the ISO 11843 method by using the following equations: [24]

$$\text{Eq1}: \quad CC_\alpha = C_{MRL} + t_{v,\alpha}\frac{\hat{\sigma}}{a}\sqrt{1+\frac{1}{IJ}+\frac{(X_{MRL}-\bar{X})^2}{\sum(X_{ij}-\bar{x})^2}}$$

$$\text{Eq 2}: \quad CC_\beta = C_{MRL} + \delta_{v,\alpha\beta}\frac{\hat{\sigma}}{a}\sqrt{1+\frac{1}{IJ}+\frac{(X_{MRL}-\bar{X})^2}{\sum(X_{ij}-\bar{x})^2}}$$

where a is the slope of the regression line which equals the recovery of the analyte, C_{MRL} is the MRL value of the analyte, $t_{v,\alpha}$ the associated t-value, σ is an estimation of the residual standard deviation of the regression function, I the number of replicates per concentration, J the number of concentrations of the spiked samples, x_{MRL} is the referenced MRL value of the analyte, x is the mean of the x_{ij} values (Eq.1) and $\delta_{v,\alpha,\beta}$ is a statistical function that can be fairly approximated by $2t_{v,\alpha}$ (Eq.2).

The results reported for CC_α and CC_β values in ***Table 2*** ranged from 101 to 118 $\mu g.kg^{-1}$ which is similar to the SAs MRL level. For dapsone, CC_α and CC_β values were 0.5 and 0.6 $\mu g.kg^{-1}$ respectively, which is less than the lowest spiked concentration. We can thus conclude that the developed method is applicable for the detection of SAs and dapsone with a statistical certainty of 95 and 99%, respectively. In comparison with the values reported in literature for SAs,[10,15,18] the calculated CC_α and CC_β values from this study are equal (in most cases) indicating a high sensitivity of the reported methodology.

The limit of detection (LOD) is the smallest value of the concentration of an analyte which can be detected and the limit of quantification (LOQ) is the smallest value of the concentration of an analyte which can be quantified. These limits were calculated as the standard deviation (SD) of the intensity obtained for tissues spiked at levels close to the LOD and LOQ divided by the slope (a) of the calibration curve according to the formulae: LOD = 3.3 (SD/a) and LOQ = 10 (SD/a). In each case, LOD was found to be lower than the MRL and ranged from 1.7 to 15 $\mu g.kg^{-1}$; LOQ ranged from 5.8 to 49.7 $\mu g.kg^{-1}$for SGN and SQX,

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respectively. For dapsone, LOD and LOQ values of 0.5 and 1.8 µg.kg^{-1}, respectively, were found (***Table 2***).

In order to evaluate the stability of 23 SAs in meat samples, different beef muscles were spiked with the analytes at 4 concentration levels (50 (1.25), 100 (2.5), 150 (3) and 200 (5) µg.kg^{-1} for SAs (and dapsone) respectively, stored at -18 ^{0}C for 1, 2, 6 and 12 weeks and extracted as described in section 2.3. All SAs were found to be stable for at least 12 weeks at -18 ^{0}C (***Appendix A, Fig. A1***).

3.4. Analysis of marketed samples

The developed method was tested on different matrices (kidney, muscle, liver) collected from beef, pig, sheep, chicken and dromedary. Forty samples were analyzed: 12 beef (6 muscle, 3 liver and 3 kidney), 12 sheep (4 muscle, 4 liver and 4 kidney), 8 pig (4 muscle, 2 liver and 2 kidney), 4 chicken (2 muscle and 2 liver) and 4 dromedary (2 muscle, 1 liver and 1 kidney). The concentrations of the detected compounds are summarized in ***Table 4***. LC/MS/MS chromatograms obtained for samples S5 and S20 are shown in ***Appendix B, Fig. B1***.

Seventeen samples showed the presence of SDM, SMZ, SD and SQX with some detected at MRL/2<C<MRL. SDM was detected at 62.5, 59.1 and 50.5 µg.kg^{-1} levels in beef and pork muscle, respectively. SMZ was detected at 70.2 µg.kg^{-1} in sheep liver and at 25.6 and 23.2 µg.kg^{-1} in sheep muscle and kidney, respectively. Traces of SMZ and SD (LOD<C<LOQ) were observed in some muscle, liver and kidney of beef, sheep, pork and chicken as shown as in ***Table 4***. The confirmation according to the Commission Decision 2002/657/EC[22]: the correct ratio of the intensities of the two transitions and the correct retention time was successful in all cases.

3.5. Quality assurance

The samples with the detectable concentrations of SAs were analysed using HPLC-Orbitrap MS according to the procedure reported elsewhere.[21] Note that in terms of precision, linearity, accuracy, CC_{α} and CC_{β}, HR Orbitrap-MS matches the LC-MS/MS performance for the most compounds but shows lower sensitivity. The results presented in ***Fig. C1*** show good ($R^2 > 0.992$) correlation between LC-Orbitrap MS and LC - MS/MS.

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A FAPAS (Food Analysis Performance Assessment Scheme) test material 02227 of pig kidney containing SP, SMZ and SDO at concentrations of 120, 68.7 and 63.4 $\mu g.kg^{-1}$ was analysed. The concentrations found for SP, SMZ and SDO were 106, 44 and 62 $\mu g.kg^{-1}$ with Z scores -0.52, -1.6 and -0.53, respectively. The fitness for purpose of the presented method was thus demonstrated with all $-2 \leq Z \text{ scores} \leq 2$ (***Table 3***).

4. Conclusion

HPLC – double reaction monitoring MS/MS preceded by HPLC allows a rapid, sensitive, precise and accurate determination of 23 sulfonamides in different edible animal tissues required to be monitored by the European Commission 2002/657/EEC decision. The method offers lower detection limits (1-3 times) in comparison with the use of Orbitrap mass analyser making triple Quad MS better for quantitative analysis. However, unlike MS/MS, the Orbitrap-MS methods using both "full scan" and "MS^n" mode may offer the possibility to explore sets of data retrospectively.

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Caption to the tables

Table 1: SAs retention time (RT) and MS/MS parameters

Table 2: Extraction recovery, AR (%) and validation parameters of analysis of SAs in beef muscle using QuEChERS-LC-MSMS

Table 3: Results for analysis of CRM (pig kidney) using QuEChERS extraction method, LC-MSMS and Orbitrap-MS

Table 4: Concentration of SAs in positively analysed samples

Caption to the figures

Fig.1: Structures of SAs antibiotics

Fig.2: Recovery values (%) obtained from a) pork samples, b) beef, c) sheep, d) dromedary and e) chicken with selected buffered QuEChERS method

Fig.3: Total Ion Counts (TIC) of a beef muscle spiked with 23 sulphonamides at the fixed levels: 100 $\mu g.kg^{-1}$ and 5 $\mu g.kg^{-1}$ for SAs and dapsone, respectively. a) SGN, b) SAA, c) SD,

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d) SIM, e) SMZ, f) STZ, g) SP, h) SM, i) SME, j) SMP, k) SMM, l) SMT, m) Da, n) SCP, o) SCL, p) SMX, q) SDO, r) SDM, s) SIX, t) SB, u) SNZ, v) SQX, w) SNT and x) SMX-D_4

Captions to supplementary data

Appendix A

Fig.A1: Stability of SAs in spiked beef muscle at different concentrations a) 50 (1.25), b) 100 (2.5), c) 150 (3) and d) 200 (5) $\mu g.kg^{-1}$ for SAs and dapsone, respectively .

Appendix B

Fig.B1: LC-MS/MS chromatograms of some analysed samples (a) S5 containing SDM (59.1 $\mu g.kg^{-1}$) and (b) S20 containing SMZ (70.2 $\mu g.kg^{-1}$)

Appendix C

Fig.C1: Correlation graph of concentrations ($\mu g.kg^{-1}$) determined of studied compounds with triple quadrupole mass spectrometry (QqQ) and Orbitrap analyzers in meat samples

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Table 1:SAs retention time (RT) and MS/MS parameters.

Analyte	RT(min)	Quantification transition - m/z (collision energy - V)	Cone voltage (V)	Confirmation transition - m/z (collision energy - V)	Ion ratio* (%)
SGN	1.9	214.9 > 156 (10)	60	214.9 > 108 (20)	55.1
SAA	4.94	215 > 156 (5)	60	214.9 > 107.9 (15)	58.9
SD	8.8	251.2 > 92 (25)	60	251.2 > 108 (20)	92.5
SIM	9.8	279.1 > 124 (20)	60	279.1 > 186 (15)	48.0
STZ	12.9	256 > 156 (10)	60	256 > 108 (20)	85.0
SP	13.8	250.2 > 156 (10)	80	250.2 > 184.1 (15)	55.0
SM	15.3	265 > 156 (10)	60	265 > 172 (10)	84.4
SME	17.8	281 > 155.9 (15)	60	281 > 215.1 (15)	28.8
SMZ	18	279 > 186 (15)	60	279 > 124 (15)	79.9
SMT	18	271 > 155.9 (10)	60	271 > 107.9 (25)	62.5
Da	18.1	249 > 108 (20)	120	249 > 156 (10)	80.7
SMP	18.3	281.6 > 108 (25)	60	281.6 > 126.5 (20)	65.2
SCP	18.5	285 > 156 (10)	100	285 > 107.9 (25)	71.2
SMX	18.7	254.1 > 108 (25)	100	254.1 > 156 (10)	79.5
SMM	18.8	281 > 155.9 (15)	60	281 > 215 (15)	65.4
SDO	18.8	311.2 > 156 (15)	60	311.2 > 107.9 (25)	73.4
SIX	18.9	268.2 > 156 (10)	60	268.2 > 112.9 (10)	94.6
SB	19.17	277.2 > 156 (5)	60	277.2 > 108 (20)	65.0
SCL	19.4	285 > 155.7 (10)	60	285 > 130 (25)	24.2
SNZ	19.3	315.5 > 92 (35)	60	315.5 > 156 (30)	77.4
SDM	19.4	311.2 > 156 (20)	60	311.2 > 107.9 (30)	58.7
SQX	19.5	301.1 > 156 (15)	60	301.1 > 108 (25)	94.1
SNT	20.1	336.1 > 133.9 (25)	120	336.1 > 156 (5)	76.6
SMX-D4	18.6	258.1 > 96 (25)	70	258.1 > 160 (10)	62.5

Relative intensity of the analytical response of the confirmation transition / quantification transition

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Table 2: Extraction recovery, AR (%) and validation parameters of analysis of SAs in beef muscle using QuEChERS-LC-MSMS

SAs	AR[1] %	Accuracy A [2] (%)	$R^{2(3)}$	RSD r[4] %	RSD R[5] %	LOD[6] ($\mu g.kg^{-1}$)	LOQ[7] ($\mu g.kg^{-1}$)	CC_α[8] ($\mu g.kg^{-1}$)	CC_β[9] ($\mu g.kg^{-1}$)
SGN	53	111	0.995	11.7	34.3	1.7	5.8	109	118
SAA	70	105	0.996	4.7	18.4	11.7	39.2	106	113
SD	84	88	0.996	2.6	7.5	6.5	21.8	103	106
SIM	70	95	0.997	2.9	12.7	14.4	48.0	103	106
STZ	73	100	0.996	4.3	9.4	13.7	45.8	102	104
SP	81	99	0.998	2.3	2.4	14.2	47.5	103	106
SM	83	95	0.998	3.8	4.7	13.4	44.7	103	105
SME	82	92	0.997	2.5	8.7	14.5	48.4	103	107
SMZ	82	96	0.997	2.5	6.3	5.5	18.5	103	105
SMT	70	101	0.993	4.3	16.7	10.8	35.9	106	112
Da	54	97	0.992	8.7	9	0.5	1.8	0.5	0.6
SMP	75	98	0.998	3.9	7.1	10.6	35.3	103	105.6
SCP	75	99	0.999	4.1	5.8	5.1	17.0	105.5	111
SMX	81	97	0.999	2.5	4.9	2.4	8	102	105
SMM	78	98	0.999	2.6	9.5	5.3	17.6	102	104
SDO	84	91	0.997	2.6	6.2	10.4	34.8	101	103
SIX	77	100	0.998	2.3	4.0	12	40	103	106
SB	64	104	0.995	2.8	6.3	8.7	29.2	105	111
SCL	65	98	0.998	5.2	9.9	11.8	39.2	105	109
SNZ	73	94	0.997	5.6	6.9	7.1	24	105	110
SDM	70	99	0.998	6.6	7.6	13.4	44.6	102	104
SQX	60	96	0.997	9.6	11.7	15	49.7	105	111
SNT	42	71	0.960	17	47.8	-	-	103.5	107

[1]Absolute recovery (AR); [2] Accuracy (A); [3] Squared regression coefficient (R^2); [4] Relative standard deviation of intra-day precision (RSD r); [5] Relative standard deviation of inter-day precision (RSD R); [6] Limit of detection (LOD); [7] Limit of quantification (LOQ); [8] Decision limit (CC_α); [9] Detection capability (CC_β).

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Table 3: Results for the analyses of CRM (pig kidney) using QuEChERS extraction method, LC-MSMS and Orbitrap-MS

Analyte	C target (µg.kg-1) [1]	C (µg.kg-1) [2]		Z scores
SDO	63.4	MS/MS	62	-0.10
		Orbitrap	73	0.68
SMZ	68.7	MS/MS	44	-1.63
		Orbitrap	-	-
SP	120	MS/MS	106	-0.53
		Orbitrap	98	-0.81

[1] Calculated concentration obtained by FAPAS; [2] Calculated concentration obtained by our method;

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Table 4: Concentration of SAs in positive analysed samples

Sample number	Sample type	SDM		SMZ		SD		SQX	
		Concentration ($\mu g.kg^{-1}$)	Ion Ratio %	Concentration ($\mu g.kg^{-1}$)	Ion Ratio %	Concentration ($\mu g.kg^{-1}$)	Ion Ratio %	Concentration ($\mu g.kg^{-1}$)	Ion Ratio %
S4	BM	MS/MS 70.4 Orbitrap 62.5	58.1	-	-	-	-	-	-
S5	BM	MS/MS 63.0 Orbitrap 59.1	58.2	LOD<C<LOQ	95.4	-		-	-
S6	PM	MS/MS 53.0 Orbitrap 50.5	55.7	-	-	-	-	-	-
S10	SK	-	-	LOD<C<LOQ	83.5	-	-	-	-
S12	BM	-	-	LOD<C<LOQ	90.9	LOD<C<LOQ	98.8	-	-
S16	SM	-	-	LOD<C<LOQ	89.6	-	-	-	-
S17	SL	-	-	LOD<C<LOQ	78.8	-	-	-	-
S18	SM	-	-	25.6	79.5	-	-	-	-
S19	SK	-	-	LOD<C<LOQ	78.65	-	-	-	-
S20	SL	-	-	MS/MS 70.2 Orbitrap 66	80.77	-	-	-	-
S21	SM	-	-	LOD<C<LOQ	85.55	-	-	-	-
S22	SK	-	-	23.2	80.6	-	-	-	-
S25	SK	-	-	LOD<C<LOQ	89.0	-	-	-	-
S27	BL	-	-	LOD<C<LOQ	81.03	-	-	-	-
S30	CL	-	-	LOD<C<LOQ	66.5	-	-	LOD<C<LOQ	90.2
S33	PL	-	-	-	-	-	-	LOD<C<LOQ	95.3
S35	PK	-	-	-	-	-	-	LOD<C<LOQ	96.35

BM: Beef Muscle, PM: Pork Muscle, SK: Sheep Kidney, SM: Sheep Muscle, SL: Sheep Liver, BL: Beef Liver, CL: Chicken Liver, PL: Pork Liver, PK: Pork Kidney

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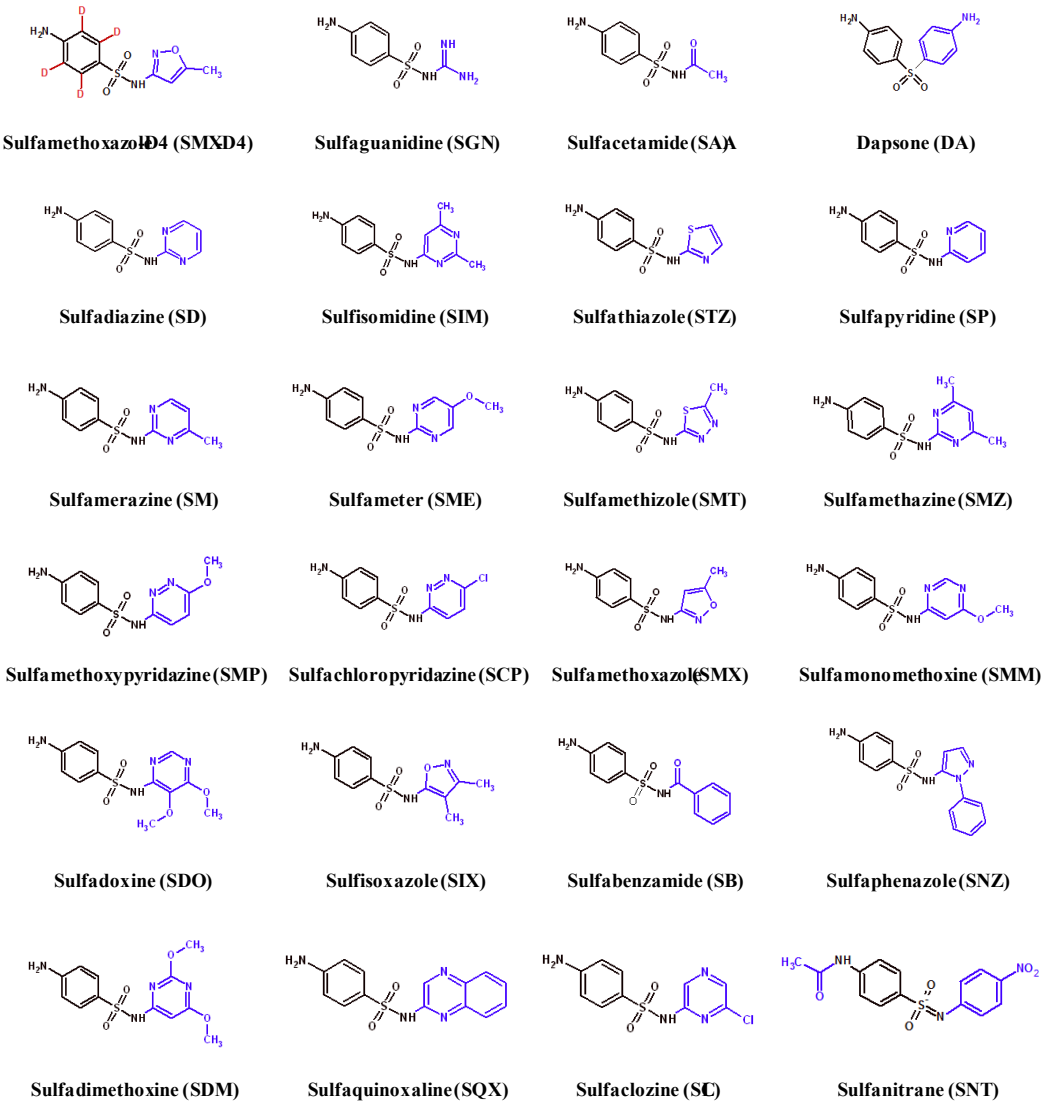

Fig.1: Structures of SAs antibiotics.

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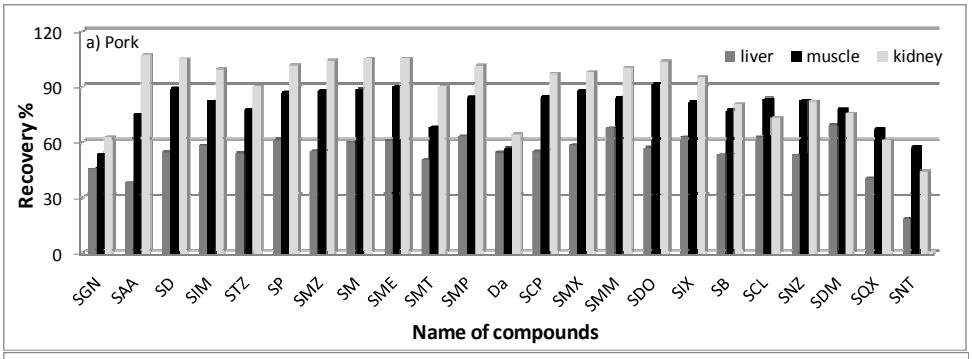

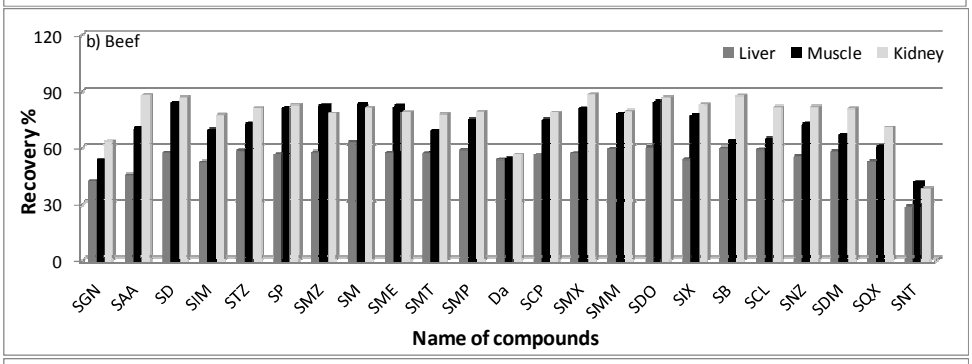

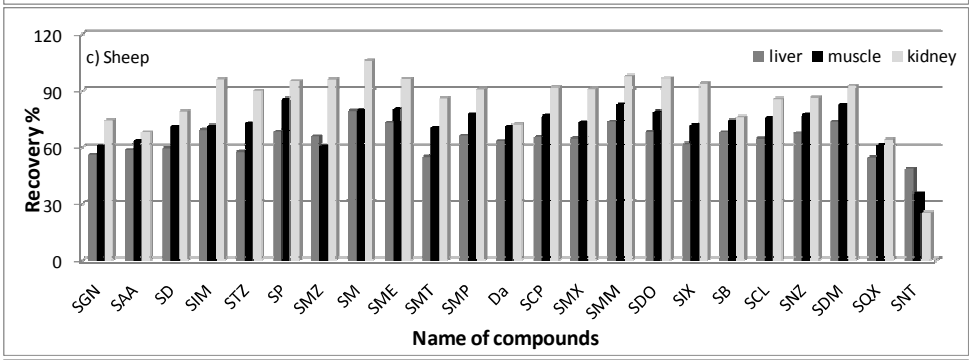

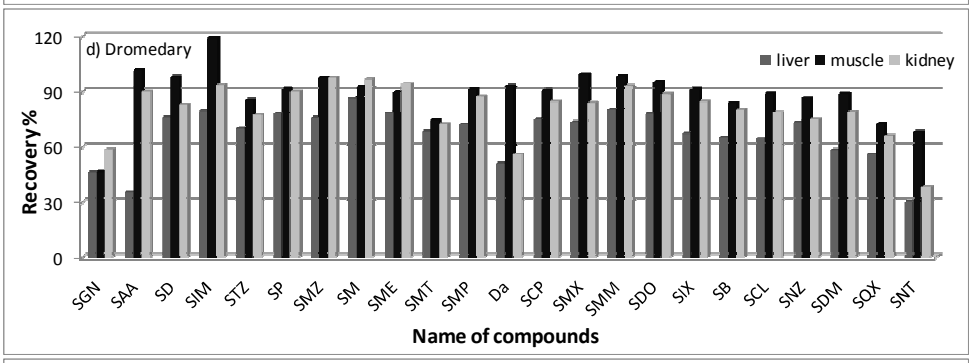

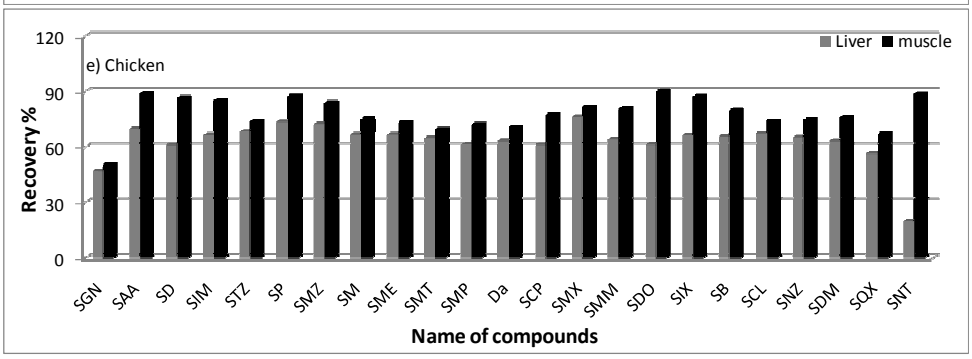

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Fig.2: Recovery values (%) obtained from a) pork samples, b) beef, c) sheep, d) dromedary and e) chicken with selected buffered QuEChERS method.

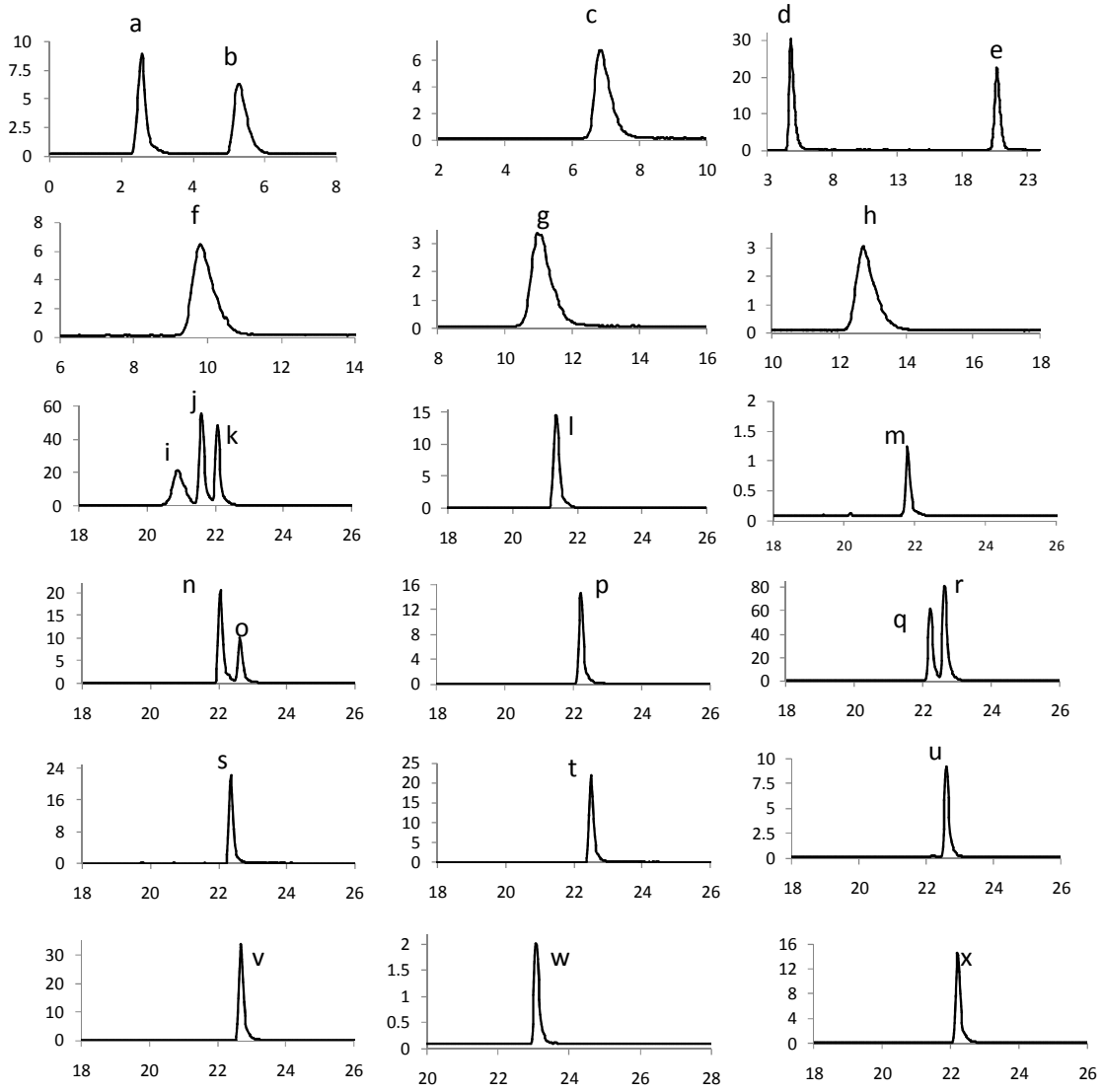

Fig.3: Total Ion Counts (TIC) of a beef muscle spiked with 23 sulphonamides at the fixed levels: 100 µg.kg^{-1} and 5 µg.kg^{-1} for SAs and dapsone, respectively. a) SGN, b) SAA, c) SD, d) SIM, e) SMZ, f) STZ, g) SP, h) SM, i) SME, j) SMP, k) SMM, l) SMT, m) Da, n) SCP, o) SCL, p) SMX, q) SDO, r) SDM, s) SIX, t) SB, u) SNZ, v) SQX, w) SNT and x) SMX-d4.

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Supplementary data

Appendix A

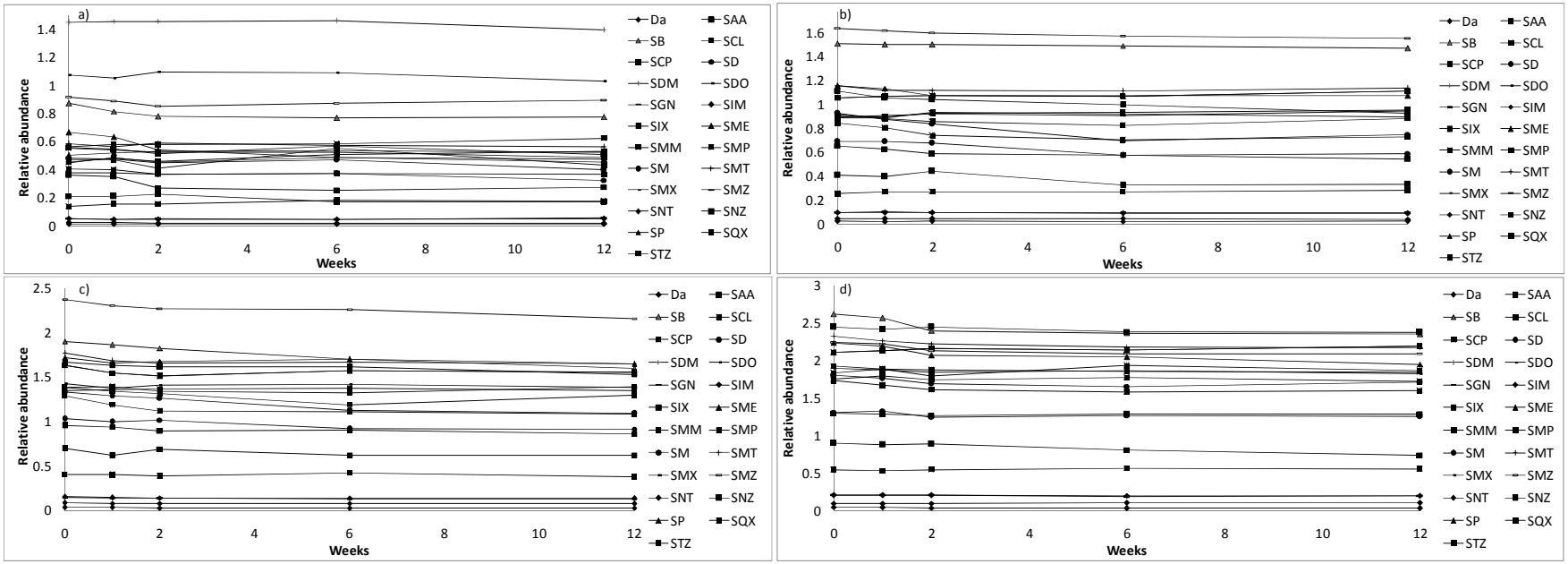

Fig.A1: Stability of SAs in spiked beef muscle at different concentrations a) 50 (1.25), b) 100 (2.5), c) 150 (3) and d) 200 (5) µg.kg^{-1} for respectively SAs and dapsone compounds.

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Appendix B

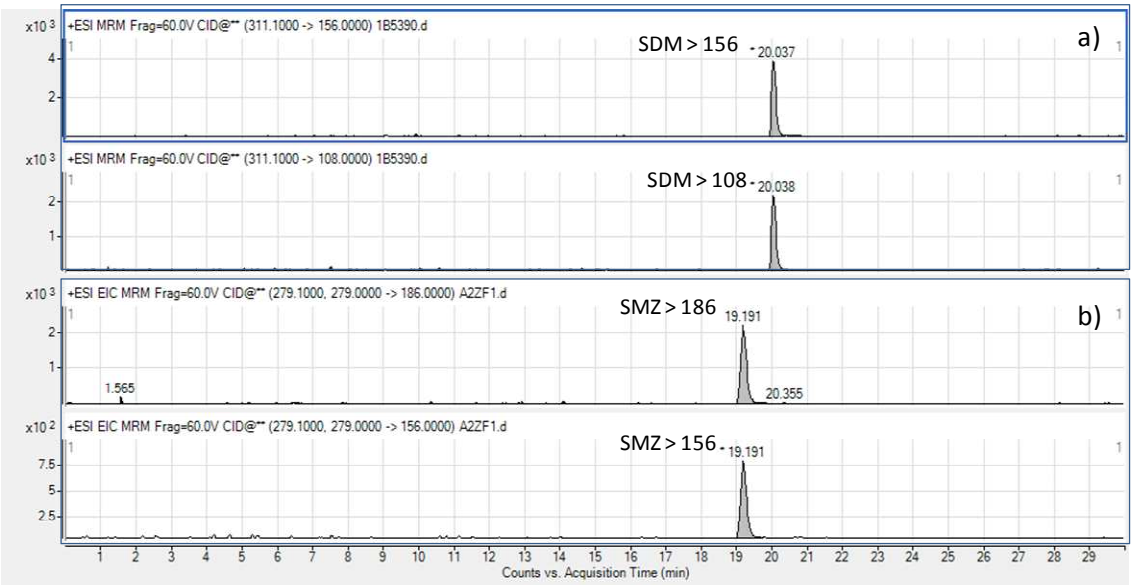

Fig. B1: LC-MS/MS chromatograms of some analysed samples (a) S5 containing SDM (59.1 µg.kg^{-1}) and (b) S20 containing SMZ (70.2 µg.kg^{-1}).

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Appendix C

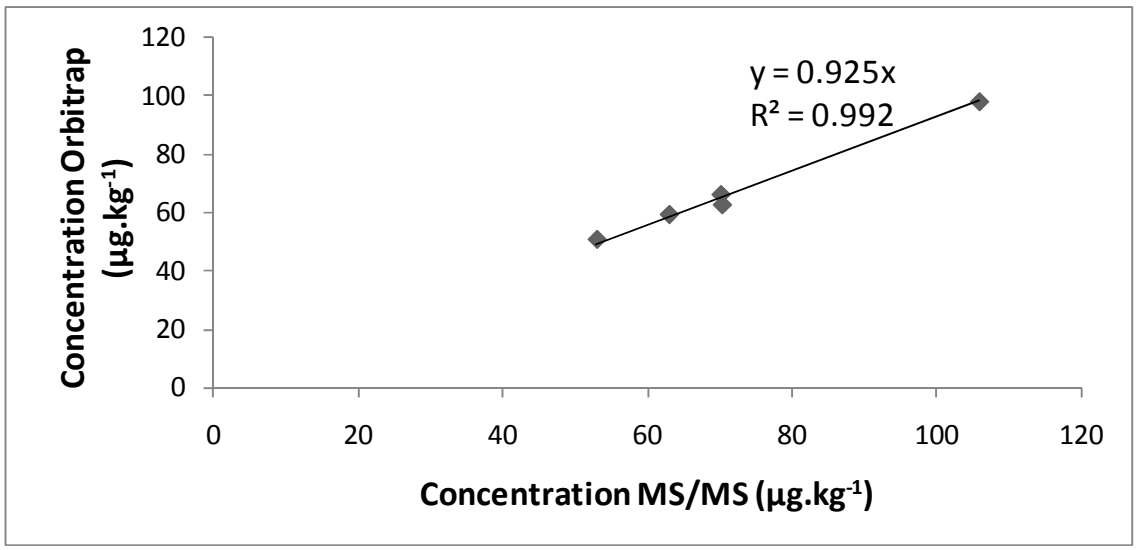

Fig. C1: Correlation graph of concentrations (µg.kg^{-1}) determined of studied compounds with triple quadrupole mass spectrometry (QqQ) and Orbitrap analyzers in meat samples.

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