NEW-ADVANCED-VERSATILE METHODOLOGY FOR SAMPLE PREPARATIONS FOR ANALYSIS OF 10 SULFONAMIDES IN MEAT USING LC-MS/MS

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INTRODUCTION

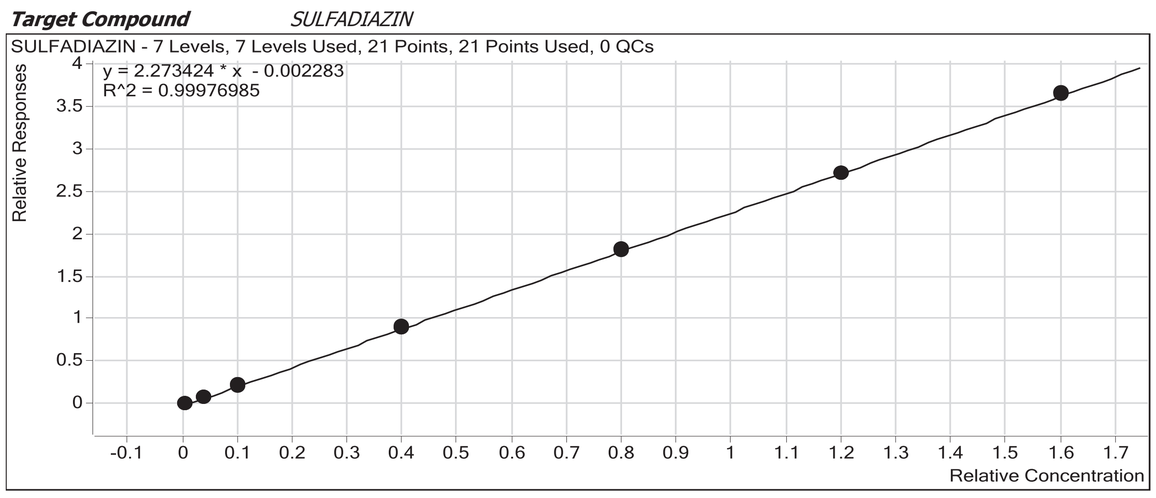
Sulfonamides are one of the oldest groups of pharmacologically active substances used in veterinary medicine. Sulfonamides are illegally used as additives in animal feed because they may have a growth-promoting effect.

Sulfonamides generate potentially serious problems in human health, such as allergic or toxic reactions. Furthermore, the main risk from the excessive use of antimicrobials in animals is that bacteria may develop resistance. In addition, some sulfonamides have been found to be potentially carcinogenic and this fact has become a cause for considerable debate in food safety.

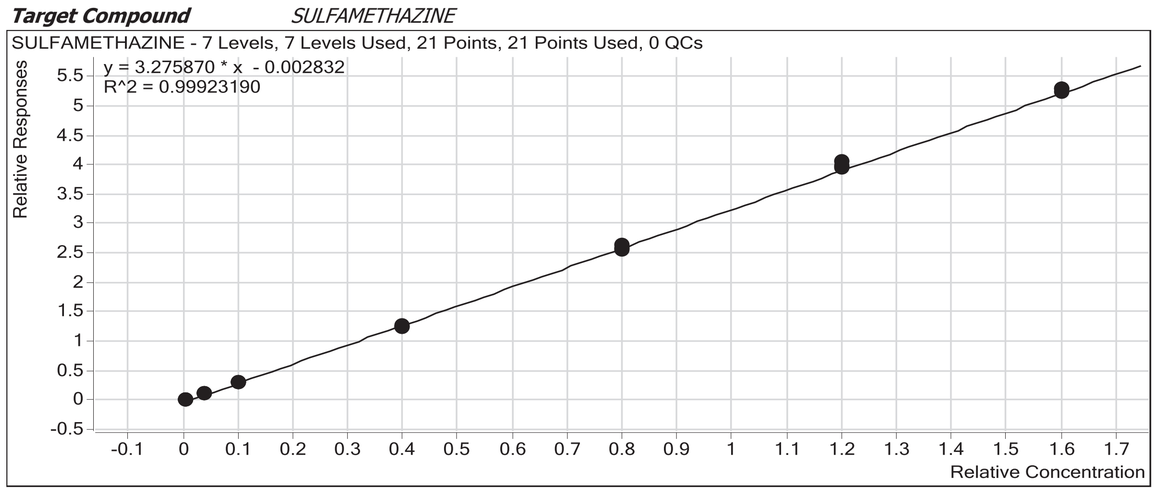
European Union, Canada and USA regulations have set the MRLs (maximum residue limits) of total sulfonamides of 100 µg kg-1 (ppb) in edible tissues.

RESULTS & DISCUSSION

Calibration curve of selected sulfonamides



Sulfadiazin calibration curve from 0.2 ng/ml to 40 ng/ml with 3 µl injection



Sulfamethazine calibration curve from 0.2 ng/ml to 40 ng/ml with 3 µl injection

Linearity, Quantitation Limit

Compound	R^2	LOQ (µg/kg)
Sulfadiazin	0.9994	2,12
Sulfathiazole	0.9996	1,96
Sulfapyridine	0.9998	2,35
Sulfamerazine	0.9994	2,34
Sulfamethazine	0.9995	1,77
Sulfachloropyridazine	0.9993	2,76
Sulfadoxin	0.9991	2,53
Sulfamethoxazole	0.9988	2,65
Sulfaquinoxaline	0.9993	2,56
Sulfadimethoxine	0.9993	2,00

Jasem® Method & Kit offers revolutionary benefits

Your Benefits Using a Jasem® Sample Preparation Kit

Fast and easy sample preparation time 20 minutes
(ref. method at least 2 hours)
Lower cost of sample preparation steps
Very sensitive method quantification limits 1.77-2.76 ppb
(ref. methods 50-200 ppb)
More selective LC-MS-MS method

Laboratory Workload & Cost comparison:
Reference Methods (R) vs. Jasem® Kits (J)

Milk:
R = 40 min. (sample prep) + 40 min. (run time) = 80 min.
J = 5 min. (sample prep) + 6,5 min. (run time) = 11,5 min.

Honey:
R = 60 min. (sample prep) + 19 min. (run time) = 79 min.
J = 16 min. (sample prep) + 6,5 min. (run time) = 22,5 min.

Meat (Cattle & Poultry):
R = 120 min. (sample prep) + 120 min. (run time) = 240 min.
J = 20 min. (sample prep) + 6,5 min. (run time) = 26,5 min.

JASEM® Method

This new method describes the determination of 10 different sulfonamide residues using simple extraction steps followed by liquid chromatography with electrospray ionization triple quadrupole mass spectrometry (LC-ESI-MS/MS) without the need for any clean-up.

This method has been studied in milk, meat (chicken, cattle) and honey matrixes. The LOQs of the whole method in these matrices are in the ranged 1,77 – 2,76 ng/ml which are below the MRLs specified in European Union, Canada and USA regulations.

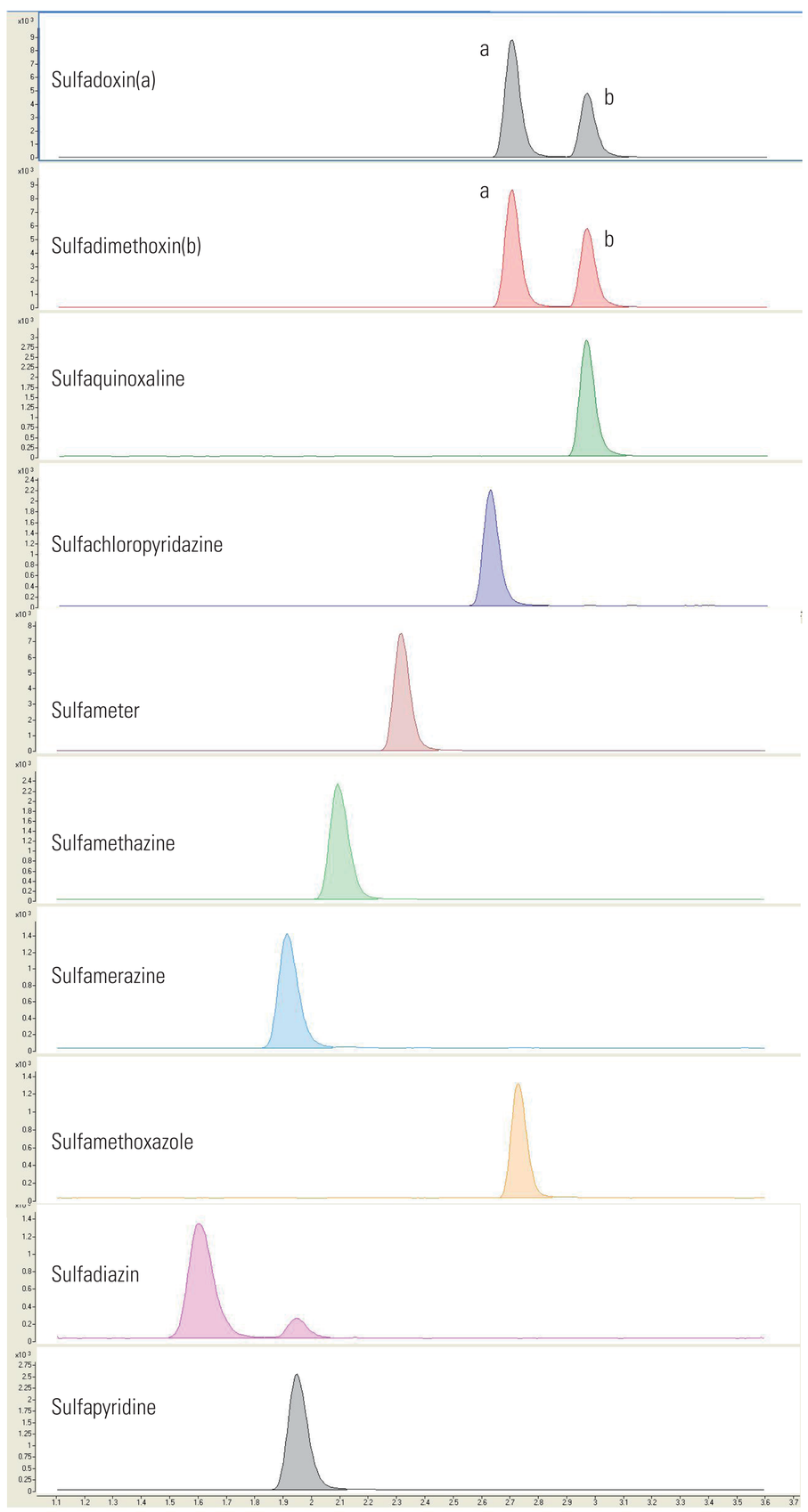
3 Defined Major Goals for our application

- Cost savings compared to existing methods
- Very easy & fast sample preparation
- Excellent repeatability
- Robust method

Jasem's technology focuses on improvement of the sample preparation part, since this analysis-part is till now taking dis-proportional long time in existing reference & house methods.

The Jasem® new sample prep method, with reference to its goals, is consisting of only one single extraction step and one evaporation step without solid-phase extraction SPE and it takes only 20 min. Also the instrument run time takes only 6,5 minutes (run to run).

Matrix effect and extracted ion chromatogram of spiked meat sample



Sample preparation for meat samples

Step 1

Weigh 5.0 g of minced meat into a 50 ml polypropylene centrifuge tube.

Step 2

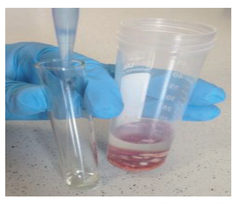
Add 62.5 µl of internal standard and 10 ml of Reagent 1 and vortex for 10 minutes vigorously.

Step 3

Centrifuge at 3000 rpm for 5 minutes.

Step 4

Pipet 1.0 ml from supernatant into a clean glass centrifuge tube. Add 1.5 ml Reagent 2 and dissolve the residue and vortex 1 minute.



Step 5

Filter out from 0.45 micron RC filters into HPLC vial.