# RESIDUES OF SULPHONAMIDES IN FOOD PRODUCING ANIMALS AND PRIMARY ANIMAL PRODUCTS

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## INTRODUCTION

In order to have better insight in the presence of sulphonamide residues in animal tissues and primary animal products, a comparative study of positive samples obtained by screening *(TLC)* and confirmative *(LC-MS/MS)* methods has been carried out.

## MATERIALS AND METHODS

According to the National Programme for monitoring certain substances and residues thereof in live animals and primary animal products, based on the regulations of the European Union (EU) (*Council Directive 96/23/EC)*, the samples are colected by authorized veterinary inspectors. Screening methods to determination of sulphonamide residues in all the samples was carried out by thin-layer chromatography *(AOAC, 1995)*. In case of detecting sulphonamide residues above the maximum residue limit *(MRL-0.10 mg/kg, EC/281/96)* in the examined samples, the confirmation was done by method of liquid chromatography with mass spectrometry *(LC-MS/MS*). The chromatography condition on LC-MS/MS was as follows: HPLC, Waters Alliance 2695, column X-Terra MS $C_{18}$ 2.1x50mm, 3.5 mm, mobile phase-1%HCOOH/ACN:1%HCOOH/$H_2O$ (40:60 v/v), flow-0,2 ml/min. Detector-Micromass Quattro Micro, Electrospray ionisation, positive mode, (ESI+), MRM (*monitoring recording mode*). TLC method was used for examination of 1301 samples, and 46 samples were sent to confirmation by LC-MS/MS method.

## RESULTS AND DISCUSSION

Table 2 shows the results of investigation obtained by screening *(TLC)* method, and Table 3 confirmative method by *(LC-MS/MS)*. The results obtained confirm that the sulphonamide residues pose a great problem to safety of meat and primary animal products in the food chain, with the highest incidence occurring in pork, followed by veal and poultry (NRA Review of sulphonamides, 2000) (Table. 2). Table 3 gives the results of sulphonamide investigation by confirmative *(LC-MS/MS)* method which shows that the presence of sulphonamide residues above the MRL is confirmed in 2 samples. Deviations in the results of investigations by screening *(TLC)* and confirmative *(LC-MS/MS)* methods, may be considered logical. The presence of isomers, metabolites, degradation products and endogenous substances in matrix pose a problem, especially for screening methods because not only the drugs are accumulated in liver but also the other ingredients and contaminants from food, which interfere with the compounds of interest. Presence of interfering substances in the fresh samples is lower. It is well known that in the homogenised samples stored at low temperatures (-8ºC, 2 months), enzymatic and microbiological processes lead to production of interfering substances (*Malish et al.,* 1992). It is considered that nicotinamide and caffeine are the substances which, in most cases, lead to the occurrence of interferences (*Malish,* 1986).

The results of our investigations show that in liver samples sulphadiazine and sulphadimidine are the most detected, while in milk the most detected are residues of sulphomethoxazole, which is mostly used locally in curing mastitis. Although systemic sulphonamides are distributed into milk in sub therapeutic quantities, these are still high enough concentrations to lead to the presence of residues in milk (*Roudaut et al.,* 1990; *Paulson et al.,* 1992; *Panel comment,* 1996).

**Table 1.** The mass of parents ions and daughter ions of the investigated sulphonamides

| Sulphonamides | m/z of parent ions | m/z of fragment ions |
|---|---|---|
| Sulphachloropyridazine | 285 | 156 |
| Sulphadiazine | 251 | 156 |
| Sulphaguanidine | 215.1 | 156 |
| Sulphadimidine | 279 | 186 and 124 |
| Sulphomethoxazole | 254.1 | 156 |
| Sulphamonomethoxine | 281 | 156 |

**Table 2.** The results of sulphonamides investigated by TLC method (mg/kg)

| Matrix | No. of samples with residues of sulphonamides in range | | | | |
|---|---|---|---|---|---|
| | **<0.02** | **0.02-0.05** | **0.05-0.10** | **³ 0.10** | **T o t a l** |
| Pork liver | 287 | 46 | 13 | 28 | 374 |
| Baby beef liver | 109 | 15 | 2 | 9 | 135 |
| Beef liver | 25 | 6 | | 4 | 35 |
| Chicken liver | 69 | 10 | 1 | 3 | 83 |
| Horse liver | 1 | | | | 1 |
| Eggs | 199 | | | | 199 |
| Milk | 288 | 57 | 9 | 2 | 356 |
| Fish | 118 | | | | 118 |
| **T o t a l** | **1096** | **134** | **25** | **46** | **1301** |

**Table 3.** The results of sulphonamides investigated by LC-MS/MS method (mg/kg)

| Matrix | No. of samples with residues of sulphonamides in range | |
|---|---|---|
| | **<0.10** | **>0.10** |
| Pork liver | 26 | 2 |
| Baby beef liver | 9 | - |
| Beef liver | 4 | - |
| Chicken liver | 3 | - |
| Milk | 2 | - |
| **T o t a l** | **44** | **2** |

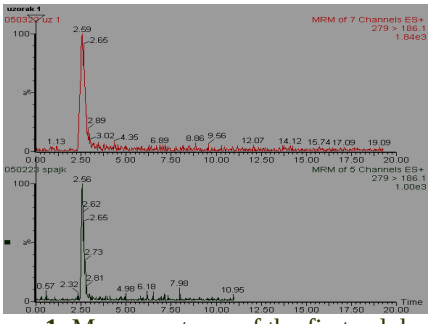

**Figure 1.** Mass spectrum of the first sulphadimidine transition in tested (a) and fortified sample (b)

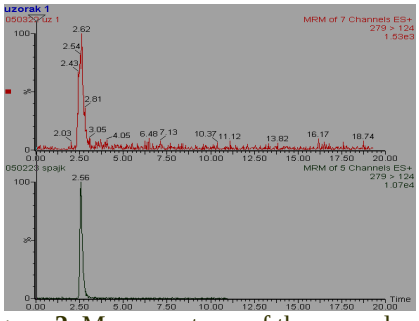

**Figure 2.** Mass spectrum of the second sulphad. transition in tested (a) and fortified sample (b)

## CONCLUSIONS

1. In order to prevent the presence of sulphonamide residues in animal tissues and primary animal products of animal origin, and by that possible adverse effect in humans, it is necessary in curing animals to use the prescribed doses of medicines and obey the prescribed carences.

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