

SULFONAMIDE RESIDUES DETERMINATION IN COMMERCIAL POULTRY MEAT AND EGGS

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ABSTRACT

The study was conducted to determine the residual level of sulfonamides in poultry meat and eggs. This drug is frequently used in poultry and suspected residues present in meat and eggs may be injurious to human health. A total of 30 egg samples, each consisting of 3 eggs, and 30 breast meat samples, collected randomly from sale points at different locations and poultry farms of Rawalpindi/Islamabad were used to detect the sulfonamide residues. These egg and meat samples were stored at 4°C and -20°C, respectively, until the time of analysis. Extraction of sulfonamides from eggs was performed using liquid-liquid extraction procedure with acetonitrile and n-hexane while acetonitrile was also used for meat samples followed by clean up with solid phase extraction columns (C18). Detection of sulfonamide residues were made by high performance liquid chromatography (HPLC) with UV detector set at 265 nm using C 18 column (25 cm×0.46, 5 µm) under isocratic conditions and using 0.01 M potassium di-hydrogen phosphate (KH₂PO₄) buffer and methanol (70:30 v/v) as a mobile phase with a flow rate of 1 ml/min. The limit of detection (LOD) was 0.02 µg/g and 0.025 µg/ml for meat and eggs, respectively. It was noted that 43% meat and 30% egg samples had detectable levels of sulfonamide residues whereas 23% meat and 10% egg samples exceeded recommended maximum residual level and were unfit for human consumption. The study revealed the presence of sulfonamide residues in poultry meat and eggs because of indiscriminate use of sulfonamides in commercial broilers & layers without observing withdrawal period of this drug.

Key words: Sulfonamide residue, withdrawal period, Layers, Broilers, Poultry meat & Eggs.

INTRODUCTION

Poultry meat has emerged as a good substitute for beef and mutton. However, due to lack of bio-security measures, prevalence of infectious diseases and subsequently indiscriminate drug usage, without observing withdrawal period, has made the poultry products unsafe for human health. In developed countries, federal agencies ensure the supply of residue free and wholesome products for human consumption. Consumers wish that their food supply be free from residues of pesticides, and drugs.

Sulfonamides drugs are most commonly used on poultry farms in Pakistan. These drugs can be easily absorbed and distributed through the body of the chicken, accumulate in various tissues and transferred into their products (Kan and Petz, 2000 and Weiss *et al.*, 2007). The recommended withdrawal periods if not observed before slaughtering of the medicated animals, the products obtained from such animals may be contaminated with residues of sulfonamides (Franco *et al.*, 1990 and McEvoy *et al.*, 1999). Sulfonamides in poultry are widely used for the treatment of Infectious Coryza, Pullorum disease, Fowl Typhoid and Coccidiosis (Giguere *et al.*, 2006). As a result, there is a concern that

the residues may be retained in the meat and eggs that present a potential risk to human health (Sutiak *et al.*, 2000, Kozarova *et al.*, 2002). These drugs or their metabolites left over in the body after their administration for a long time are termed as residues. After the treatment of infected animals with drugs, the residues of drugs are present at some level in edible products like milk, eggs and meat of treated animals. Drug residue concentrations vary considerably from tissue to tissue and are generally observed higher in tissues of storage such as liver and kidneys (Booth, 1973).

In order to decrease the potential risk to the consumer's health and to ensure the reduction of sulfonamide residues in edible tissues and eggs to an acceptable level, these substances must be administered only in recommended concentrations and their respective withdrawal times must be observed (Kozarova *et al.*, 2004). The maximum residue level (MRL) of sulfonamides in poultry tissues and eggs is 100 µg/kg (Council Regulation (EEC), 1990; Codex Alimentarius, 1996, FAO/WHO, 1992 and code of federal regulations, 1996). The purpose of the MRL is to limit the exposure of consumers to residues of medicines used in food animals, to concentrations that do not pose human health risk (Kennedy *et al.*, 2000).

The lack of bio-security measures, prevalence of diseases in poultry flocks, indiscriminate use of drugs without observing withdrawal period, drug residues especially sulfonamides retained in poultry meat and eggs may create hazards to human health in the country. In order to monitor the impact of these drug residues in poultry products, a research surveillance plan was designed to find out the residual levels in poultry meat and eggs by using High Performance Liquid Chromatography (HPLC) and to compare it with the MRL recommended by Codex Alimentarius Commission of the Slovak Republic (1996), FAO/WHO (1992) and Council Regulation, (EEC) (1990).

MATERIALS AND METHODS

Sample Collection and Analyses: A total of thirty samples of breast broiler meat were collected randomly from different selling outlets in and around Rawalpindi and Islamabad cities. About 50 g of each sample was cut aseptically from the breast meat and transferred to self-sealing colourless polythene bags. The bags were labelled and transported to National Veterinary Laboratories (NVL), Islamabad, Pakistan, under cold conditions in a foam box containing chiller packs. All the meat samples were stored at -20 °C until the time of analysis.

A total of thirty table egg samples, having three eggs per sample, were collected for sulfonamide residues analysis from Rawalpindi and Islamabad cities. Out of thirty samples, fifteen egg samples were collected randomly from different sale points and fifteen samples from different poultry farms within and around Rawalpindi and Islamabad. These samples were transported to NVL, Islamabad, Pakistan under cold conditions. The samples were stored in refrigerator at 4°C until the time of analysis.

The required mobile phase for the analyses of meat and egg samples was daily prepared and sulphonamide working standard solutions, needed for detection of sulphonamide residues in meat and eggs by using HPLC apparatus, were prepared in various dilutions.

Preparation and Extraction of Meat Samples: The chicken meat samples stored at -20°C were thawed overnight in a refrigerator. Meat samples (50 g) were cut into small pieces and blended at 20000 rpm in a tissue blender (IKA, M 20, Germany) for 2 minutes. Ten grams of blended meat tissue and 10 ml of deionized water were taken in 50 ml polypropylene centrifuge tube. The mixture was homogenized for 1.5 minutes using Ultra-Turrax T25 tissue homogenizer (IKA, Ultra-Turrax, T25 basic, Germany). Extraction method similar in principle to method described by Biswas *et al.*, (2007) was adopted for sample analysis.

The solid phase extraction (SPE) apparatus

(Lichrolut) was set up and Bond-Elute C18 cartridge column (3 ml Varian, USA) were fitted on the adapters of SPE apparatus. A 28 ml of universal bottles were placed under the column for receiving waste. The combined supernatant fluids were then passed through a Bond-Elute C18 cartridge column (3 ml Varian, USA) at a flow rate of 1 ml/min preconditioned with 5 ml of HPLC water followed by vacuum drying. After that the compounds were eluted with 3 ml of acetonitrile in universal bottles at a flow rate of 1 ml/minutes and then evaporated using rotary evaporator (BUCHI Rotavapor, R-200, Switzerland) at 45 °C. The residue was dissolved with 500 µL of an acetonitrile/water mixture (1:1) and filtered through a disposable syringe filter (Mini Sart RC4, 0.45 µm, Sartorius AG, Germany) into HPLC autosampler vials for HPLC analysis.

Preparation and Extraction of Egg Samples: A liquid-liquid extraction method similar in principle to methods described by Shaikh *et al.*, 1999, Sasanya *et al.*, 2005 and Howitz, 2000 was adopted for the analysis of egg samples. One millilitre of mobile phase and 8 millilitres of n-hexane were added into the flask to dissolve the residues. The combination of the residues, mobile phase and n-hexane was re-centrifuged at 4100RCF for 15 minutes at 4 °C after vigorous vortexing. The upper n-hexane layer was removed with Pasteur pipette and discarded. One gram of anhydrous NaCl (extra pure) was added to the lower aqueous layer, the mixture was vortexed and re-centrifuged. The aqueous layer was filtered using Whatman 0.45 µm nylon filter using a Buchner funnel and collected in a 14 ml conical centrifuge tube. The aqueous layer was again filtered through a disposable syringe filter (Mini Sart RC4, 0.45 µm Sartorius AG, Germany) and transferred to a HPLC vial (National Scientific Company, Japan) for HPLC analysis.

HPLC Determination: Analysis of the sulfonamide standards and extracted samples were conducted using a HPLC system equipped with HPLC column oven L-7300, detector L-7400, auto sampler L-7200, pump L-7100, vacuum degasser L-76610, interface module D-7000 and HSM software (Hitachi D-7000 series, Japan). The samples were filtered through disposable syringe filter (Mini Sart RC4, 0.45 µm, Sartorius AG, Germany); the filtrates were directly collected into HPLC sample vials. The sample vials were then placed in HPLC autosampler vial rack. A Teknokroma Mediterranea C 18 (5 µm 25 cm×0.46) column was used for the separation of the 5 sulfonamides using 0.01 M potassium dihydrogen phosphate buffer and methanol at 70:30, v/v as the mobile phase. The flow rate was fixed at 1.0 ml/min and analysis was performed at 35 °C. The injection volume was 15 µL and ultra violet detector wavelength of 265 nm was applied.

The optimal wavelength of detection:

Spectrophotometer was used to measure absorption spectrum of the mobile phase which showed absorption at 230 nm while sulfonamides standard solution showed maximum absorption at 250-275 nm. Therefore, UV at 265 nm was used for the detection of sulfonamide residues in poultry meat and eggs in this study. The sulfonamides selected in this study were sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxy-pyridazine and sulfamethoxazole (Table 1 & 2). When sulfonamides dissolved in mobile phase and scanned simultaneously, it revealed that mobile phase created no interference with the peaks of absorbance of sulfonamides.

A standard calibration curve for sulfonamides was obtained by running sulfonamide standard solutions (in triplicate) on HPLC and then plotting peak areas against concentrations in µg/ml and µg/g, respectively. For the curve, the best fit of the line was calculated by equation of line. Linearity was evaluated through the correlation coefficient. The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation:

$$Y = mx + b$$

Where, Y = Peak area, m = Slope, x = Concentration and b = Intercept

Limit of Detection (LOD): The present method provided the limit of detection of sulfonamides residues in meat and egg samples at 0.02 µg/g and 0.025 µg/ml, respectively which were below the MRL (0.1 µg/g).

RESULTS AND DISCUSSION

Status of Sulfonamide Residues in Commercial

Poultry Meat Samples: Mean concentration of sulfonamide residues in poultry meat samples collected from Rawalpindi and Islamabad are given in Table 3. Out of total 19 samples collected from Rawalpindi, 8 (42.10%) had detectable concentrations of sulfonamides, whereas out of these positive samples 5 exceeded recommended MRL. The sulfonamide residues of meat samples ranged from 0.02 to 0.8 µg/g. Out of total 11 poultry meat samples collected from Islamabad, 5 (45%) had detectable concentrations of sulfonamides, of 5 positive samples 2 exceeded recommended MRL. Meat samples collected from Islamabad had sulfonamide residues between 0.02 to 0.6 µg/g.

In total 30 poultry meat samples were analyzed for sulfonamide residues, out of which 43% (13 Nos) samples had detectable levels (0.02-2.0 µg/g) of sulfonamide residues (Fig. 1). Out of 13 positive samples, 7 (23.3%) exceeded recommended MRL (0.12-0.8 µg/g) and found to be unfit for human consumption. In poultry meat samples sulfonamide residues ranged in concentration from 0.02 to 0.8 µg/g.

The results obtained in the present study are in close agreement with Salem (2004) and Shaikh *et al.* (2000) who found sulfonamide residues above MRL level in chicken meat samples. They also used HPLC for the detection of sulfonamide residues as in this study and found similar results.

Status of Sulfonamide Residues in Poultry Egg

Samples: A total of 30 egg samples were analyzed for sulfonamide residues, out of which 9 (30 %) samples had detectable levels of sulfonamide residues (Table 4). Out of 09 positive samples, only 3 (10%) samples. 6 (20%) exceeded MRL (0.03-0.4 µg/g; Fig. 2), and were unfit for human consumption. The sulfonamide residue concentrations in egg samples ranged from 0.02 to 0.8 µg/ml.

The results of sulfonamide residues in egg samples are in line with Sasanya *et al.* (2005) who detected sulfonamide residues in 98% egg samples. Likewise, Furusawa (2001), Hussein *et al.* (2005) and Shaikh *et al.* (1999 & 2000) substantiated the results of this study that sulfonamide residual problem exists in most developing countries. However, the detection level was much higher than the present study.

Sulfonamides are widely used for therapeutic and prophylactic purposes in both human (Kim and Park, 1998) and animals (Schwarz and Dancla, 2001), sometimes being used as additives in animal feed because prolonged ingestion of sulfonamides may have a growth-promoting effect (Long *et al.*, 1990). If the proper withdrawal periods are not observed in broilers & layers after treatment, then meat and eggs of these birds may be contaminated with residual sulfonamides. In order to protect consumers from risks related to drug residues, maximum residue limits (MRL) have been established by law in many countries. The MRL of sulfonamides in poultry tissues and eggs is 100 µg/kg (Council Regulation, EEC) 1990; Codex Alimentarius, 1996, FAO/WHO, 1992, and Code of Federal Regulations, 1996) that must be observed and implemented in Pakistan. The present findings are based on egg and meat samples collected from Islamabad/Rawalpindi where technical know-how, extension services and the disease diagnostic laboratory facilities are frequently available to poultry farmers. The results could be different if compared with those poultry farms situated in far flung areas of the country where such facilities are very rare. Similar studies must be extended to such places where follow-up veterinary facilities are available at very low level in order to improve the health status of our people especially of rural masses.

The results of the present study suggest that detection of sulfonamide residues in poultry meat samples above the MRL is really a serious matter and an elaborative and very extensive work in this regard is required to quantify the sulfonamide residues in poultry

meat in other parts of the country. Besides, a campaign must be initiated to educate the farmers about the withdrawal period of drugs as well as the ill-effects of drug residues on human health.

Table 1: Average recoveries of sulfonamides at different spiking concentrations of meat ($\mu\text{g/g}$).

Spiked concentration ($\mu\text{g/g}$)	Recovery (%)				
	SDZ*	SMTZ*	SMXZ*	SMRZ*	SMPZ*
0.05	62-75	60-65	60-65	60-65	65-70
0.1	76-83	75-85	70-75	75-80	75-85
0.2	85-92	85-90	85-91	85-92	85-90

*Abbreviations are: SDZ = sulfadiazine, SMTZ= sulfamethazine, SMPZ=sulfamethoxy-pyridazine, SMXZ= sulfamethoxazole, SMRZ= sulfamerazine.

Table 2: Average recoveries of sulfonamides at different spiking concentrations of eggs ($\mu\text{g/ml}$).

Spiked concentration ($\mu\text{g/g}$)	Recovery (%)				
	SDZ*	SMTZ*	SMXZ*	SMRZ*	SMPZ*
0.05	60-70	65-70	65-75	64-72	65-70
0.1	75-82	73-83	68-76	73-78	75-80
0.2	85-90	82-88	82-88	85-90	82-88

*Abbreviations are: SDZ = sulfadiazine, SMTZ= sulfamethazine, SMPZ=sulfamethoxy-pyridazine, SMXZ= sulfamethoxazole, SMRZ= sulfamerazine.

Table 3: Concentrations of sulfonamide residues in poultry meat collected from Rawalpindi & Islamabad

Rawalpindi			Islamabad		
S. No.	Sample identity	Sulfonamide concentration mean ($\mu\text{g/g}$)	S. No.	Sample identity	Sulfonamide concentration mean ($\mu\text{g/g}$)
1	RPM-2	0.12 EMRL*	1	IDM-1	0.6 EMRL*
2	RPM-4	0.4 EMRL*	2	IDM-2	0.3 EMRL*
3	RPM-5	0.18 EMRL*	3	IDM4	0.06 BMRL**
4	RPM-9	0.52 EMRL*	4	IDM-7	0.08 BMRL**
5	RPM-14	0.8 EMRL*	5	IDM-9	0.03 BMRL**
6	RPM-16	0.04 BMRL**			
7	RPM-17	0.02 BMRL**			
8	RPM-19	0.06 BMRL**			

*EMRL: Exceeded maximum residue limit

**BMRL: Below Maximum Residue Limit

Table 4: Concentrations of Sulfonamide residues in poultry egg samples collected from Rawalpindi/ Islamabad.

Sale points			Poultry farms		
S. No.	Sample identity	Sulfonamide concentration mean ($\mu\text{g/ml}$)	S. No.	Sample identity	Sulfonamide concentration mean ($\mu\text{g/ml}$)
1	¹ RPE-1	0.4 EMRL*	1	PFE-3	0.3 EMRL*
2	¹ RPE-3	0.2 EMRL*	2	PFE-4	0.02 BMRL**
3	¹ RPE-5	0.03 BMRL*	3	PFE-9	0.02 BMRL**
4	² IDE-3	0.06 BMRL*	4	PFE-11	0.04 BMRL**
5	² IDE-5	0.07 EMRL*			

*EMRL: Exceeded maximum residue limit

**BMRL: Below Maximum Residue Limit

¹RPE: Rawalpindi Egg sample

²IDE: Islamabad Egg sample

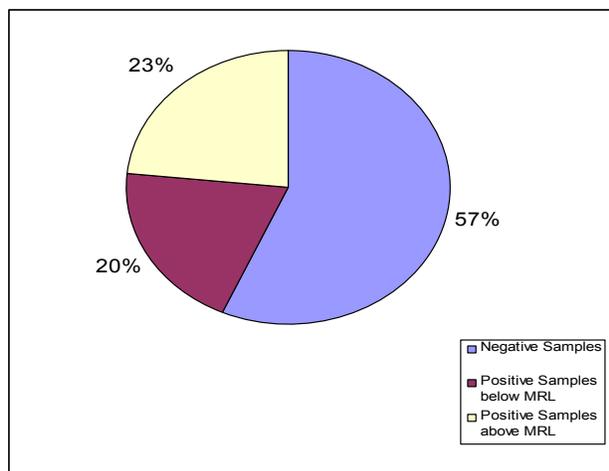


Fig. 1: Sulfonamide residue levels above & below MRL in Poultry meat.

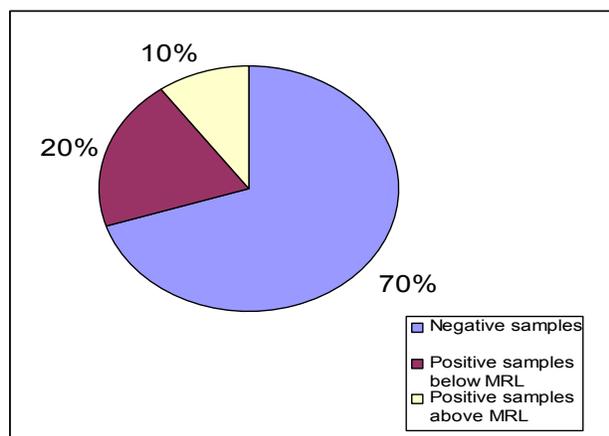


Figure 2: Sulfonamide residue levels found above and below MRL in Poultry eggs.

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