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DETERMINATION OF SULFONAMIDE, DAPSONE AND TRIMETHOPRIM RESIDUES IN EGGS BY LC-MS/MS TECHNIQUE

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Abstract

The analysis of sulfonamide and other drug residues is essential to increase consumer confidence in food products and to confirm that the product in question meets all the required conditions both in terms of drug residues and the quality and safety of these products. High-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS) was used for the determination of twenty sulfonamides, epson and trypzone sulfonamides. Control hen egg samples were analyzed and any interferences (signals, peaks) were controlled in the region of interest where the analytes under study are expected to elute. Control hen egg samples were then fortified to relevant concentrations with analytes.

Key words*: dapsone, eggs, sulfonamides, trimethoprim.*

INTRODUCTION

Sulfonamides are a category of antibiotics that act specifically on bacteria that cause bacterial infections, therefore they are considered broad-spectrum antibiotics, because they act on several types of bacteria (Grave et al., 2014). Substances such as trimethoprim and dapsone have similar effects to sulfonamides and can be administered together with other pharmacological substances (Sheridan et al., 2008). As for sulfonamides, they have a cumulative effect with trimethoprim and dapsone, but they can also have major negative effects on the human body, producing bacterial resistance, various allergies or carcinogenic effects (Gentili et al., 2005).Protecting consumer health is very important, especially from the point of view of contamination of eggs with sulfonamides, which end up in their content due to treatments carried out for infections in poultry (Wang et al., 2017). The main reactions that can be triggered by their presence in eggs are allergies, acute toxicity and other metabolic and intestinal imbalances (Shao et al., 2005). The consumption of eggs contaminated with certain sulfonamide residues can influence the development of bacterial resistance to antibiotics in the body, being considered a major problem internationally, also affecting the quality of medical treatments (Tian et al., 2013).

Compliance with existing legislation and regulations is very important, so EFSA and Codex Alimentarius have established certain maximum permissible limits (MRLs) for residues of veterinary drugs in products of animal origin (Tölgyesi et al., 2013).

Quality control and traceability in the food chain is an important step due to the determination of sulfonamides through veterinary and safety monitoring programs with the role of improving farm management (Dubreil-Chéneau et al., 2014). Egg quality is influenced by a number of factors, such as: practices used on the farm; drug treatments; hygiene and good practice standards, technology; storage conditions; feed; biological material (Kumar, 2019; Samman et al., 2009; Bologa et al., 2013).The aim of this study was to perform tests for the determination of sulfonamide, dapsone and trimethoprim residues in eggs using the LC-MS/MS technique.

MATERIALS AND METHODS

Samples

The analyzed samples were represented by eggs that were subjected to analysis for the

determination of sulfonamide, trimethoprim and dapsone residues.

Analysis of egg samples

The egg sample is mixed before weighing (2 g eggs) and placed in a 50 ml centrifuge tube. Add a 10 µl internal standard solution (sulfadimidine-13C6, sulfamethoxazole-13C6), with a concentration of 10 µg/ml.

Add 10 ml acetonitrile. Shake the sample until it is homogenized, using the shaker for 15 minutes. Then centrifuge the sample, at a speed of 3500 rpm, for 10 minutes. Prepare the supernatant and repeat the extraction with another 10 ml acetonitrile.

Then combine the extracts and evaporate to dryness, in a nitrogen stream, on a water bath at 50 degrees Celsius.

The residue is reconstituted in 20 ml acetate buffer solution pH 5.3 and shake at maximum speed for 15 minutes. Add 10 ml hexane and shake at maximum speed for 15 minutes. Centrifuge at a speed of 3500 rpm for 10 minutes, separate the organic phase and discard, and apply the aqueous phase to the conditioned SPE cartridges.

Sample purification

For purification, the SPE C18 Strata X column (500 mg/6 ml) was used, as follows: preconditioning with 10 ml methanol and 10 ml 0.2 mol/l acetate buffer, pH 5.3; passing the extract through gravity flow; washing with 10 ml water; drying the column by leaving it under vacuum for 5 minutes; elution with 10 ml acetonitrile.

The eluate is evaporated to dryness on a water bath at 50 degrees Celsius, under a nitrogen stream. The residue is taken up in 1000 µl of 0.1 N HCl. It is shaken for 15 minutes on a shaker at maximum speed and then ultrasonicated for 3 minutes. 30 µl of the egg sample is injected into the LC-MS/MS.

Liquid chromatography coupled with mass spectrometry

Chromatographic column used: Pursuit XRs Ultra C18, 100 x 2 mm/2.8 µm with a flow rate of 0.2 ml/min. Column temperature was 35°C, injection volume 30 µl. Mobile phase (component A: H_2O/ HCOOH 0.1%; component B: Acetonitrile/HCOOH 0.1% gradient A/B).

RESULTS AND DISCUSSIONS

Table 1 shows the analyzed components, the type of measurement and the level.

Table 1. Analyte (measuring) type and level

Compound	Analyte level (µg/kg)
	Chicken eggs (Cmin)
Sulfaguanidines	10*
Sulfacetamides	10*
Sulfonamides	10*
Sulfisomidine	10*
Sulfadiazines	10*
Sulfathiazoles	10*
Sulfapyridine	10*
Sulfamerazine	10*
Sulfamethazine	10*
Sulfamethizole	10*
Sulfamethoxypyridazine	10*
Sulfamonomethoxine	10*
Sulfameter	10*
Sulfachloropyridazine	10*
Sulfadoxine	10*
Sulfamethoxazole	10*
Sulfisoxazole	10*
Sulfabenzamide	10*
Sulfadimethoxine	10*
Sulfaquinoxalines	10*
Sulfaphenazole	10*
Sulfanitran	10*
Trimethoprim	10*
Dapsone	5*
Sulfadimidine - 13C6 (internal standard)	50
Sulfamethoxazole - 13C6 (internal standard)	50

A linear matrix calibration curve was obtained for each analyte of interest. For all substances of interest, correlation coefficients ≥ 0.99 and RSD<15% were obtained, as follows (Table 2).

Table 2. Correlation coefficients

Compound	Chicken eggs	
	Correlation coefficient	RSD (%)
Sulfaguanidines	0.993194	7.518
Sulfacetamides	0.994163	7.442
Sulfonamides	0.996967	7.317
Sulfisomidine	0.998723	6.610
Sulfadiazines	0.996330	10.62
Sulfathiazole	0.996202	6.842
Sulfapyridine	0.998336	6.615
Sulfamerazine	0.998790	6.431

Sulfamethazine	0.999857	2.348
Sulfamethizole	0.995961	7.656
Sulfamethoxypyridaz	0.997556	8.512
Sulfamonomethoxine	0.998534	8.049
Sulfameter	0.997324	8.403
Sulfacloropyridazine	0.996616	5.840
Sulfadoxin	0.998881	6.729
Sulfamethoxazole	0.999129	2.862
Sulfisoxazol	0.999525	6.972
Sulfabenzamide	0.997917	4.789
Sulfadimethoxin	0.996426	11.22
Sulfaquinoxaline	0.999319	10.35
Sulfaphenazole	0.996308	8.693
Sulfanitran	0.997694	7.721
Trimethoprim	0.998464	6.580
Dapsone	0.993902	10.33

For all substances of interest, the correlation coefficients and RSD obtained fall within the established acceptability limits. To establish the selectivity of the analyzed compounds, a chromatographic column of the following type was used:
Pursuit XRs Ultra C18, 100 x 2.0 mm with a particle diameter of 2.8 µm
Since the stationary phase is non-polar C18, and the mobile phase is a mixture of polar compounds (acetonitrile and formic acid 0.1%), the separation mechanism of the sulfonamides is reversed-phase chromatography.
Control samples of chicken eggs were fortified with decreasing concentrations of working standard, starting from the lowest concentration on the calibration curve, up to a concentration that the instrument could no longer detect.
The signal/noise ratio for each compound at a known concentration (Cmin) was calculated using the software (Table 3).

Table 3. The detection limits and quantification limits obtained

Compound	Chicken eggs			
	C_{min} (µg/kg)	S/Z	LOD (µg/kg)	LOQ (µg/kg)
Sulfaguanidines	10*	199	0.151	0.455
Sulfacetamides	10*	200	0.150	0.450
Sulfonamides	10*	15	2.000	6.000
Sulfisomidine	10*	3080	0.010	0.030
Sulfadiazines	10*	1420	0.021	0.063
Sulfathiazoles	10*	427	0.070	0.210
Sulfapyridine	10*	1432	0.021	0.063
Sulfamerazine	10*	1431	0.021	0.063
Sulfamethazine	10*	2104	0.014	0.042
Sulfamethizole	10*	559	0.054	0.161
Sulfamethoxypyridazine	10*	960	0.031	0.093
Sulfamonomethoxine	10*	763	0.039	0.117
Sulfameter	10*	469	0.064	0.192
Sulfachloropyridazine	10*	304	0.099	0.297
Sulfadoxin	10*	2699	0.011	0.033
Sulfamethoxazole	10*	575	0.052	0.156
Sulfisoxazol	10*	865	0.035	0.105
Sulfabenzamide	10*	214	0.140	0.420
Sulfadimethoxin	10*	1402	0.021	0.063
Sulfaquinoxaline	10*	357	0.084	0.252
Sulfaphenazole	10*	666	0.045	0.135
Sulfanitran	10*	17	1.765	5.295
Trimethoprim	10*	2437	0.015	0.045
Dapsone	5*	273	0.055	0.165

For all substances of interest, the detection limits and quantification limits obtained fall within the established acceptability limits.

Recovery
Recovery is the actual percentage of the concentration of a substance recovered during the analytical procedure. It is determined during validation if no certified reference material is available. The percentage recovery obtained for each component is accepted in the range of 80-120 %.
The egg control samples were previously analyzed and no residues of sulfonamides, dapsone and trimethoprim were detected.24 parts of the egg control material were selected and 6 parts were fortified at 0.5 x Cmin; 6 parts at 1 x Cmin; 6 parts at 1.5 x Cmin (minimum concentration) and 6 parts at 2 x Cmin. The samples were analyzed using the same method, the same reagent lots, the same LC-MS/MS equipment, and within a short time frame.The recovery for each compound in each sample was calculated using the following equation:

$$R = \frac{C_{P+Et} - C_P}{C_{Et}} \cdot 100$$

(%) where:
C_{P+Et} - concentration of the compound in the fortified sample;
C_P - concentration of the compound in the sample;
C_{Et} - theoretical concentration of the compound in the fortified sample.

The average recovery was calculated. The percentage recovery obtained for each component is in the range of 80-120% (Table 4).

Table 4. Recovery percentage for chicken eggs

Compound	Chicken eggs		
	Minimum recovery (%)	Average recovery (%)	Maximum recovery (%)
Sulfaguanidines	95.95	101.25	105.04
Sulfacetamides	96.61	100.90	106.54
Sulfonamides	95.08	100.47	104.32
Sulfisomidine	94.82	101.13	107.14
Sulfadiazines	96.32	101.54	107.27
Sulfathiazoles	96.42	100.76	104.53
Sulfapyridine	94.28	99.71	103.99
Sulfamerazine	95.19	99.63	102.61
Sulfamethazine	96.40	100.30	105.14
Sulfamethizole	96.22	101.93	106.24
Sulfamethoxypyridazine	95.48	99.22	102.86
Sulfamonomethoxine	95.76	10021	10385
Sulfameter	95.96	102.28	107.06
Sulfachloropyridazine	98.12	100.75	105.62
Sulfadoxin	95.19	100.26	104.53
Sulfamethoxazol	96.52	100.22	105.22
Sulfisoxazol	95.33	99.81	105.90
Sulfabenzamide	93.86	99.02	103.09
Sulfadimethoxin	96.91	100.46	105.52
Sulfaquinoxaline	95.12	100.67	105.85
Sulfaphenazole	95.49	100.45	104.66
Sulfanitran	96.49	99.97	103.95
Trimethoprim	95.36	101.20	106.22
Dapsone	96.20	100.81	105.08

For all substances of interest, the recovery percentages obtained fall within the established acceptability limits.

Repeatability

From the standard deviation of repeatability sr the "repeatability limit - r" is calculated which allows the analyst to decide whether the difference between repeated analyses of a sample, determined under repeatability conditions, is significant. For each component and for each concentration level it is expected that RSDr ≤ 20%. A set of 24 hen egg samples fortified with the analytes was prepared to produce equivalent concentrations of 0.5; 1; 1.5 and 2 times Cmin (minimum concentration). Six replicates were performed for each assay level. The samples were analyzed and the concentration detected in each sample was determined. The mean concentration, standard deviation, RSDr (%) and repeatability limit were calculated for the results from each series. The samples were analyzed using the same method, the same operator, the same reagent lots, the same LC-MS/MS equipment and within a short time frame. The repeatability limit (r) and RSDr for each series are calculated as follows:

$r = 2.8 \times sr$; $RSDr = sr/Xmed \times 100$ (%)

where:

sr - standard deviation of repeatability;

Xmed - mean concentration.

The experimental results and the calculation results for the mean concentration, standard deviation, RSDr (%) and repeatability limit for the results from each series obtained for hen eggs are as follows (Table 5).

Table 5. Mean concentration, standard deviation, RSDr (%) and repeatability limit for hen eggs

Compound	Chicken eggs				
	Concentration level (µg/kg)	Average concentration (µg/kg)	s_r	RSD_r (%)	r
Sulfaguanidines	5	5.048	0.143	2.84	0.402
	10	10.108	0.260	2.57	0.728
	15	15.242	0.349	2.29	0.979
	20	20.266	0.421	2.08	1.180
Sulfacetamides	5	5.086	0.143	2.82	0.402
	10	10.081	0.233	2.31	0.653
	15	15.125	0.317	2.10	0.887
	20	20.048	0.395	1.97	1.107
Sulfonamides	5	4.984	0.155	3.10	0.433
	10	9.964	0.272	2.73	0.763
	15	15.226	0.321	2.11	0.898
	20	20.214	0.404	2.00	1.132
Sulfisomidine	5	5.100	0.170	3.34	0.477
	10	9.932	0.281	2.83	0.787
	15	15.189	0.382	2.52	1.070
	20	20.390	0.501	2.45	1.401
Sulfadiazines	5	5.034	0.130	2.59	0.365
	10	9.981	0.246	2.47	0.690
	15	15.506	0.339	2.19	0.950
	20	20.460	0.264	1.29	0.740
Sulfathiazoles	5	5.008	0.123	2.46	0.345
	10	9.984	0.212	2.13	0.595
	15	15.298	0.255	1.67	0.715
	20	20.209	0.320	1.58	0.896
Sulfapyridine	5	4.932	0.173	3.51	0.485
	10	9.859	0.275	2.79	0.770
	15	15.004	0355	2.37	0.995
	20	20.315	0.427	2.10	1.196
Sulfamerazine	5	4.976	0.125	2.51	0.349
	10	9.760	0.208	2.13	0.582
	15	15.123	0.284	1.88	0.796
	20	20.116	0.338	1.68	0.947
sulfamethazine	5	4.927	0.140	2.83	0.391
	10	9.940	0.232	2.34	0.651
	15	15.378	0.218	1.42	0.611
	20	20.146	0.216	1.07	0.606
Sulfamethizole	5	5.013	0.153	3.05	0.428
	10	10.031	0.234	2.33	0.656
	15	15.619	0.278	1.78	0.778
	20	20.606	0.353	1.71	0.987
Sulfamethoxypyridazi	5	4.861	0.104	2.13	0.290
	10	9.822	0.191	1.94	0.534
	15	15.126	0.287	1.90	0.803
	20	20.122	0.345	1.71	0.965
Sulfamonomethoxine	5	4.960	0.119	2.40	0.334
	10	9.978	0.215	2.16	0.603
	15	15.236	0.265	1.74	0.741
	20	20.061	0.244	1.22	0.684

Sulfameter	5	5.086	0.145	2.85	0.406
	10	9.882	0.277	2.80	0.776
	15	15.665	0.277	1.77	0.777
	20	20.828	0.301	1.44	0.843
Sulfachloropyridazine	5	5.115	0.112	2.19	0.314
	10	10.091	0.165	1.63	0.461
	15	14.986	0.209	1.40	0.585
	20	19.981	0.247	1.24	0.692
Sulfadoxin	5	4.960	0.142	2.86	0.397
	10	10.008	0.274	2.74	0.768
	15	15.172	0.312	2.05	0.873
	20	20.123	0.323	1.60	0.904
Sulfamethoxazol	5	5.075	0.146	2.87	0.407
	10	10.043	0.232	2.31	0.648
	15	14.886	0.337	2.27	0.945
	20	19.944	0.401	2.01	1.123
Sulfisoxazol	5	4.974	0.174	3.51	0.488
	10	9.872	0.281	2.85	0.788
	15	14.913	0.374	2.51	1.048
	20	20.329	0.393	1.93	1.100
Sulfabenzamide	5	4.948	0.107	2.17	0.301
	10	9.720	0.198	2.04	0.554
	15	14.973	0.290	1.94	0.811
	20	20.021	0.305	1.52	0.855
Sulfadimethoxin	5	5.039	0.150	2.98	0.420
	10	9.967	0.244	2.45	0.682
	15	14.973	0.338	2.26	0.947
	20	20.313	0.368	1.81	1.032
Sulfaquinoxaline	5	5.109	0.178	3.48	0.498
	10	9.876	0.270	2.74	0.757
	15	15.301	0.392	2.56	1.096
	20	19.946	0.370	1.85	1.035
Sulfaphenazole	5	5.103	0.165	3.22	0.461
	10	9.878	0.234	2.37	0.655
	15	14.988	0.329	2.19	0.921
	20	20.214	0.389	1.93	1.090
Sulfanitran	5	5.015	0.132	2.62	0.368
	10	10.050	0.233	2.32	0.654
	15	15.050	0.315	2.09	0.883
	20	19.746	0.353	1.79	0.988
Trimethoprim	5	4.989	0.139	2.79	0.390
	10	10.050	0.262	2.61	0.735
	15	15.449	0.396	2.56	1.108
	20	20.301	0.294	1.45	0.823
Dapsone	2.5	2.511	0.073	2.89	0.203
	5	5.038	0.125	2.48	0.350
	7.5	7.559	0.169	2.23	0.473
	10	10.126	0.221	2.18	0.619

For all substances of interest, the relative standard deviation of repeatability, RSDr (%) obtained falls within the established acceptance limits.

Reproducibility

For each component and for each concentration level, RSDR ≤ 20% is expected. A set of 24 hen egg samples fortified with the analytes was prepared to produce equivalent concentrations of 0.5; 1; 1.5 and 2 times Cmin (minimum concentration).

Six replicates were performed for each assay level. The samples were analyzed and the concentration detected in each sample was determined. The mean concentration, standard deviation, RSD (%) and repeatability limit were calculated for the results in each series.

The samples were analyzed using the same method, by the same analyst, under the same working conditions, on the same equipment, with different reagent lots, on different days.

The first data set is the results of the repeatability test.

The standard deviation of reproducibility (sR), RSDR, RSDHorwitz and the reproducibility limit (R) were calculated for the results of the two sets of samples worked on different days, for each chosen concentration level. The reproducibility limit (R), RSDR and RSDHorwitz for each series are calculated:

$$R = 2.8 \times s_R;\ RSD_R = s_R/X_{med} \times 100\ (\%).$$

$$RSD_{Horwitz} = 2^{(1-0{,}5\log x_{med})}(\%)$$

where: sR - standard deviation of reproducibility; Xmed - mean concentration. The experimental results and calculation results for mean concentration, standard deviation, RSDR (%) and reproducibility limit for the results of each series obtained for hen eggs are as follows (Table 6).

Table 6. Mean concentration, standard deviation, RSDR (%) and reproducibility limit for hen eggs

Compound	Chicken eggs						
	Concentration level (µg/kg)	Average concentration (µg/kg)		S_R	RSD_R (%)	R	RSD_{Horw} (%)
		Series 1	Series 2				
Sulfaguanidine	5	5.048	5.022	0.152	3.02	0.426	1.57
	10	10.108	10.100	0.262	2.60	0.735	1.41
	15	15.242	15.083	0.376	2.48	1.052	1.33
	20	20.266	20.199	0.445	2.20	1.245	1.27
Sulfacetamide	5	5.086	5.046	0.155	3.06	0.434	1.57
	10	10.081	10.048	0.240	2.38	0.671	1.41
	15	15.125	15.245	0.334	2.20	0.935	1.33
	20	20.048	20.045	0.403	2.01	1.129	1.27
Sulfanilamide	5	4.984	5.103	0.170	3.37	0.475	1.57
	10	9.964	9.832	0.290	2.93	0.812	1.42
	15	15.226	14.965	0.378	2.50	1.057	1.33
	20	20.214	20.062	0.454	2.25	1.270	1.27
Sulfisomidin	5	5.100	5.039	0.188	3.71	0.526	1.57
	10	9.932	10.198	0.315	3.13	0.883	1.41
	15	15.189	14.992	0.401	2.66	1.124	1.33
	20	20.390	19.933	0.531	2.63	1.487	1.27
Sulfadiazine	5	5.034	4.940	0.146	2.94	0.410	1.57
	10	9.981	10.035	0.254	2.54	0.712	1.41
	15	15.506	15.190	0.367	2.39	1.028	1.33
	20	20.460	19.824	0.469	2.33	1.314	1.27
Sulfathiazole	5	5.008	4.969	0.146	2.93	0.409	1.57
	10	9.984	10.029	0.228	2.28	0.639	1.41
	15	15.298	15.075	0.280	1.85	0.785	1.33
	20	20.209	20.221	0.325	1.61	0.909	1.27
Sulfapyridine	5	4.932	4.888	0.180	3.68	0.505	1.57
	10	9.859	10.031	0.282	2.84	0.789	1.42
	15	15.004	15.127	0.379	2.51	1.060	1.33
	20	20.315	19.876	0.488	2.43	1.368	1.27
Sulfamerazin	5	4.976	5.042	0.129	2.57	0.360	1.57
	10	9.760	9.830	0.219	2.24	0.614	1.41
	15	15.123	15.158	0.287	1.90	0.803	1.33
	20	20.116	20.329	0.356	1.76	0.998	1.27
Sulfametazina	5	4.927	5.019	0.150	3.01	0.419	1.57
	10	9.940	9.893	0.247	2.49	0.693	1.41
	15	15.378	15.112	0.265	1.74	0.741	1.33
	20	20.146	20.176	0.242	1.20	0.677	1.27
Sulfamethizole	5	5.013	5.113	0.181	3.58	0.507	1.57
	10	10.031	9.940	0.257	2.57	0.718	1.41
	15	15.619	15.201	0.391	2.54	1.096	1.33
	20	20.606	20.083	0.469	2.30	1.313	1.27
Sulfamethoxypyridazine	5	4.861	4.971	0.137	2.79	0.385	1.57
	10	9.822	10.041	0.248	2.50	0.694	1.42
	15	15.126	15.080	0.325	2.15	0.911	1.33

	20	20.122	20.228	0.358	1.78	1.003	1.27
Sulfamonomethoxine	5	4.960	4.996	0.121	2.42	0.338	1.57
	10	9.978	9.984	0.225	2.26	0.631	1.41
	15	15.236	15.138	0.291	1.92	0.816	1.33
	20	20.061	20.181	0.252	1.25	0.707	1.27
Sulfameter	5	5.086	4.929	0.179	3.57	0.501	1.57
	10	9.882	10.100	0.302	3.02	0.846	1.41
	15	15.665	15.031	0.434	2.83	1.215	1.33
	20	20.828	20.193	0.454	2.21	1.270	1.27
Sulfachloropyridazine	5	5.115	5.047	0.114	2.24	0.318	1.57
	10	10.091	10.091	0.169	1.68	0.475	1.41
	15	14.986	15.218	0.243	1.61	0.679	1.33
	20	19.981	19.706	0.297	1.50	0.831	1.27
Sulfadoxin	5	4.960	4.989	0.147	2.96	0.413	1.57
	10	10.008	9.864	0.275	2.77	0.771	1.41
	15	15.172	14.988	0.329	2.18	0.922	1.33
	20	20.123	20.035	0.358	1.78	1.002	1.27
Sulfamethoxazol	5	5.075	5.006	0.152	3.01	0.425	1.57
	10	10.043	10.276	0.269	265	0.754	1.41
	15	14.886	15.121	0.368	2.45	1.030	1.33
	20	19.944	19.966	0.410	2.06	1.149	1.27
Sulfisoxazol	5	4.974	5.061	0.181	3.60	0.506	1.57
	10	9.872	9.986	0.298	3.00	0.835	1.42
	15	14.913	14.747	0.375	2.53	1.050	1.33
	20	20.329	20.164	0.430	2.12	1.204	1.27
Sulfabenzamide	5	4.948	5.020	0.120	2.41	0.337	1.57
	10	9.720	9.980	0.236	2.39	0.661	1.42
	15	14.973	15.188	0.306	2.03	0.856	1.33
	20	20.021	20.096	0.328	1.63	0.918	1.27
Sulfadimethoxin	5	5.039	4.911	0.164	3.30	0.460	1.57
	10	9.967	9.830	0.250	2.53	0.701	1.42
	15	14.973	15.069	0.348	2.32	0.974	1.33
	20	20.313	19.801	0.442	2.20	1.237	1.27
Sulfaquinoxaline	5	5.109	4.987	0.183	3.63	0.513	1.57
	10	9.876	9.966	0.275	2.77	0.770	1.41
	15	15.301	15.010	0.399	2.63	1.118	1.33
	20	19.946	20.104	0.403	2.01	1.128	1.27
Sulfaphenazole	5	5.103	4.992	0.180	3.57	0.504	1.57
	10	9.878	9.857	0.246	2.50	0.690	1.42
	15	14.988	15.080	0.360	2.40	1.008	1.33
	20	20.214	19.869	0.463	2.31	1.296	1.27
Sulfanitran	5	5.015	5.031	0.144	2.86	0.403	1.57
	10	10.050	10.056	0.243	2.41	0.679	1.41
	15	15.050	14.845	0.330	2.21	0.925	1.33
	20	19.746	19.841	0.363	1.83	1.017	1.27
Trimethoprim	5	4.989	5.006	0.141	2.81	0.394	1.57
	10	10.050	10.159	0.279	2.76	0.781	1.41
	15	15.449	15.315	0.402	2.61	1.125	1.33
	20	20.301	19.731	0.435	2.17	1.217	1.27
Dapsone	2.5	2.511	2.524	0.077	3.08	0.217	1.74
	5	5.038	5.025	0.136	2.70	0.380	1.57
	7.5	7.559	7.552	0.180	2.39	0.505	1.48
	10	10.126	10.098	0.232	2.29	0.649	1.41

For all substances of interest, the relative standard deviation of reproducibility, RSDR (%) obtained falls within the established acceptance limits.

Decision limit CCα

The CCα decision limit must be lower than the Cmin (minimum concentration) for dapsone, sulfonamides and trimethoprim. To determine the decision limit for sulfonamides, trimethoprim and dapsone, a set of 8 hen egg samples was prepared, fortified with the analytes, to produce equivalent concentrations of 0.5; 1; 1.5 and 2 times Cmin (minimum concentration). Two replicates were performed for each analysis level. The samples were analyzed and the decision limit was determined by the calibration curve method (α=1%), in accordance with ISO 11843. For the determination of sulfonamide, trimethoprim and dapsone residues, the decision limit was calculated, in accordance with ISO standard 11843, as follows:

$CC_\alpha = c_y + 2.33 \times s_R$,

where: sR - standard deviation of the intralaboratory reproducibility of the intercept; cy - concentration corresponding to the intercept y. Non-compliant samples are samples in which the concentration of sulfonamides, trimethoprim, respectively dapsone found exceeds CCα, for the confirmatory method (Table 7).

Table 7. The values obtained for the decision limit and CCα

Compounds	Chicken eggs
	CC_α (µg/kg)
Sulfaguanidines	0.26
Sulfacetamides	0.67
Sulfonamides	2.06
Sulfisomidine	0.43
Sulfadiazines	1.61
Sulfathiazoles	1.32
Sulfapyridine	2.01
Sulfamerazine	1.02
Sulfamethazine	1.00
Sulfamethizole	0.94
Sulfamethoxypyridazine	1.86
Sulfamonomethoxine	1.73
Sulfameter	1.03
Sulfachloropyridazine	1.03
Sulfadoxin	1.45
Sulfamethoxazol	0.93
Sulfisoxazol	1.72
Sulfabenzamide	1.89
Sulfadimethoxin	2.41
Sulfaquinoxaline	2.87
Sulfaphenazole	2.46
Sulfanitran	7.94
Trimethoprim	3.09
Dapsone	0.82

For all substances of interest, the values obtained for the CCα decision limits fall within the established acceptability limits.

Stability

The stability of the analyte or of the constituents of the matrix sample during storage or analysis must be controlled. A control chart was prepared on 20 readings on the same matrix, which shows the daily development of the values obtained for the compounds of interest, at the same fortification level (Cmin), by the same analyst on 20 different days. The results of the control chart (warning and control limits) are used in internal quality control. Chicken egg samples were fortified with the working standard at the Cmin level (minimum concentration), analyzed, and the results were entered into the control chart. The values obtained must fall within the limit: V(n) = mean ± 2 x SD

Where: mean - arithmetic mean of the series; SD - standard deviation of the series.
The results of the readings for the control sample must fall within the mean ± 2 x SD range, 3 values within the mean ± 3 x SD range, but not consecutive, or 2 consecutive values within the mean ± 3 x SD range and one value above the mean ± 3 x SD are accepted. At 11 consecutive increasing or decreasing values within the mean ± 2 x SD range, the control chart is redrawn, according to ISO 7870-2:2013. There may be situations in which in the analytical series (current samples) that are compared with the ranges in the initially established control chart, recoveries higher than the mean ± 3 x SD are recorded, provided that the values still fall within the accepted recovery range (80-120%).
The results obtained fall within the mean ± 2 x SD range.

Robustness

The analytical method must be tested under different experimental conditions that may include: variations in pH, temperature, source of reagents, centrifugation speed, vortexing time, stability of the derivatization product over time or as a function of temperature. Robustness testing was performed with minor parameter changes: Modification no. 1 (M1) – vortexing time in the extraction stage is changed from 15 minutes to 10 minutes; Modification no. 2 (M2) – changing the number of revolutions per minute of the centrifuge from 4000 rpm to 3500 rpm; Modification no. 3 (M3) – centrifugation time is changed from 10 minutes to 15 minutes; Modification no. 4 (M4) – in the purification phase, when washing the cartridges, the water flow is not allowed to be gravitational but is forced by applying pressure; Modification no. 5 (M5) – the column drying time is changed from 5 minutes to 10 minutes.
A series of chicken egg samples fortified at the Cmin level (minimum concentration) were processed, as follows: - 3 samples without modifying the working parameters (n01, n02, n03), - 5 samples with one working parameter modified (m1, m2, m3, m4, m5), 2 samples with two parameters modified at the same time (m6, m7). The Youden scheme was applied (Tables 8, 9).

Table 8. Testing the method under the influence of some factors

Factor value	Number of determination combinations (sample set)				
	1(n01)	2(n02)	3(n03)	4(m1)	5(m2)
A/a	A	A	A	a	A
B/b	B	B	B	B	b
C/c	C	C	C	C	C
D/d	D	D	D	D	D
E/e	E	E	E	E	E

Table 9. Testing the method under the influence of some factors

Factor value	Number of determination combinations (sample set)				
	6(m3)	7(m4)	8(m5)	9(m6)	10(m7)
A/a	A	A	A	A	A
B/b	B	B	B	b	B
C/c	c	C	C	c	C
D/d	D	d	D	D	d
E/e	E	E	e	E	e

A = readings in fortified samples, worked without changes in parameter M1; B = readings in fortified samples, worked without changes in parameter M2; C = readings in fortified samples, worked without changes in parameter M3; D = readings in fortified samples, worked without changes in parameter M4; E = readings in fortified samples, worked without changes in parameter M5; a = readings in fortified samples, worked with parameter M1 modified; b = readings in fortified samples, worked with parameter M2 modified; c = readings in fortified samples, worked with parameter; M3 modified; d = readings in fortified samples, worked with parameter M4 modified; e = readings in fortified samples, worked with parameter M5 modified.

Three samples with unchanged parameters (3 readings), 5 samples with a single modified parameter and 2 samples with two parameters modified at the same time (7 readings) were processed. The arithmetic mean of the readings from the samples with unchanged parameters and the arithmetic mean of the readings from the samples with modified parameters were calculated (Tables 10-12).

Table 10. Testing the method under the influence of some factors

Sample No.	M1 modifi-cation	M2 modifi-cation	M3 modifi-cation	M4 modifi-cation	M5 modifi-cation
n01	-	-	-	-	-
m1	+	-	-	-	-
m2	-	+	-	-	-
m3	-	-	+	-	-
m4	-	-	-	+	-
m5	-	-	-	-	+
n02	-	-	-	-	-
m6	-	+	+	-	-
n03	-	-	-	-	-
m7	-	-	-	+	+

"+" indicates the working parameters that have been modified. "-" indicates the working parameters that have not been modified.

Table 11. The readings obtained (n01, n02, n03, m1, m2)

Compound	Sample readings				
	n01	n02	n03	m1	m2
Sulfaguanidine	10.078	9.755	10.258	10.084	10.223
Sulfacetamide	9.890	9.672	9.678	9.906	10.301
Sulphanilamide	9.529	10.241	9.983	10.121	9.684
Sulfisomidine	9.622	10.343	9.652	10.105	10.088
Sulfadiazine	10.144	10.096	9.532	9.696	9.677
Sulfathiazole	9.866	10.052	9.463	9.855	9.555
Sulfapyridine	9.683	9.416	9.673	9.885	9.608
Sulfamerazine	9.438	9.694	9.575	9.855	10.133
Sulfamethazine	9.761	9.942	10.160	10.033	10.090
Sulfamethizole	10.019	10.185	10.217	10.062	10.150
Sulfamethoxypyridazine	9.983	10.029	9.769	10.092	10.054
Sulfamonomethoxine	9.930	10.001	9.893	10.083	9.882
Sulfameter	10.136	9.893	10.199	10.170	9.993
Sulfacloropyridazin	9.916	9.592	9.800	9.946	10.034
Sulfadoxine	10.113	9.780	9.964	9.970	9.727
Sulfametoxazol	9.672	10.129	9.751	9.886	9.886
Sulfisoxazol	9.716	10.011	9.935	9.657	9.855
Sulfabenzamide	9.473	9.674	10.048	9.867	9.931
Sulfadimetoxin	10.267	9.781	9.941	9.933	9.864
Sulfaquinoxalin	10.117	9.973	9.853	10.222	10.096
Sulfaphenazol	10.144	9.748	9.830	9.952	10.205
Sulfanitran	10.099	9.557	9.840	10.054	9.572
Trimethoprim	9.794	9.820	10.062	9.901	10.270
Dapsona	5.188	4.856	5.100	5.136	5.013

Table 12. The readings obtained (m3, m4, m5, m6, m7)

Compound	Sample readings				
	m3	m4	m5	m6	m7
Sulfaguanidine	10.229	9.960	10.093	9.932	10.022
Sulphanilamide	9.499	10.754	10.259	10.200	9.797
Sulfisomidine	9.656	10.139	10.089	10.053	9.880
Sulfadiazine	9.488	9.493	9.988	10.076	9.631
Sulfathiazole	9.430	9.861	9.990	10.127	10.097
Sulfapyridine	9.506	10.067	9.537	9.930	9.797
Sulfamerazine	10.225	9.752	9.699	9.712	9.923
Sulfamethazine	10.668	9.997	9.952	9.919	9.924
Sulfamethizole	9.817	10.193	10.389	10.309	9.774
Sulfametoxipiridazin	10.067	9.900	9.758	9.932	9.867
Sulfamonometoxin	10.073	9.760	9.894	10.001	9.895
Sulfameter	10.088	9.976	9.924	9.856	10.286
Sulfacloropyridazin	10.087	10.148	10.172	9.909	10.010
Sulfadoxin	10.023	10.010	9.890	10.288	10.150
Sulfametoxazol	9.980	9.700	9.707	10.021	9.768
Sulfisoxazol	9.826	9.974	9.628	10.298	9.963
Sulfabenzamida	9.935	10.087	9.605	10.063	10.090
Sulfadimetoxin	10.223	9.882	10.052	10.048	10.329
Sulfaquinoxalin	9.817	9.729	10.129	10.063	9.865
Sulfaphenazol	10.062	10.223	9.787	10.055	9.909
Sulfanitran	10.106	10.081	9.994	10.010	10.044
Trimethoprim	10.427	10.448	9.904	9.695	10.004
Dapsona	5.142	4.955	4.975	5.054	4.971

The difference between the average readings of samples with unchanged parameters and the average readings of samples with modified parameters was calculated and then the square of the differences was calculated (Tables 13-18).

$A_A=\sum(A_i)/9;$ $A_a=\sum(a_i)/1;$ $D_a=A_A-A_a=\sum(A_i)/9-\sum(a_i)/1;$ D_a^2

$A_B=\sum(B_i)/8;$ $A_b=\sum(b_i)/2;$ $D_b=A_B-A_b=\sum(B_i)/8-\sum(b_i)/2;$ D_b^2

$A_C=\sum(C_i)/8;$ $A_c=\sum(c_i)/2;$ $D_c=A_C-A_c=\sum(C_i)/8-\sum(c_i)/2;$ D_c^2

$A_D=\sum(D_i)/8;$ $A_d=\sum(d_i)/2;$ $D_d=A_D-A_d=\sum(D_i)/8-\sum(d_i)/2;$ D_d^2

$A_E=\sum(E_i)/8;$ $A_e=\sum(e_i)/2;$ $D_e=A_E-A_e=\sum(E_i)/8-\sum(e_i)/2;$ D_e^2

Table 13. The results obtained for A(A) A(a) D(a) D(a)2

Compound	**A(A)**	**A(a)**	**D(a)**	**D(a)2**
Sulfaguanidine	10.06	10.084	-0.02	0.0005
Sulfacetamide	9.98	9.906	0.07	0.0048
Sulphanilamide	9.99	10.121	-0.13	0.0161
Sulfisomidine	9.95	10.105	-0.16	0.0250
Sulfadiazine	9.79	9.696	0.10	0.0092
Sulfathiazole	9.83	9.855	-0.03	0.0008
Sulfapyridine	9.69	9.885	-0.19	0.0377
Sulfamerazine	9.79	9.855	-0.06	0.0037
Sulfamethazine	10.05	10.033	0.01	0.0002
Sulfametizol	10.12	10.062	0.05	0.0030
Sulfametoxipiridazin	9.93	10.092	-0.16	0.0266
Sulfamonometoxin	9.93	10.083	-0.16	0.0248
Sulfameter	10.04	10.17	-0.13	0.0172
Sulfacloropyridazin	9.96	9.946	0.02	0.0003
Sulfadoxin	9.99	9.97	0.02	0.0006
Sulfametoxazol	9.85	9.886	-0.04	0.0016
Sulfisoxazole	9.91	9.657	0.25	0.0649
Sulfabenzamide	9.88	9.867	0.01	0.0001
Sulfadimethoxine	10.04	9.933	0.11	0.0121
Sulfaquinoxaline	9.96	10.222	-0.26	0.0685
Sulfaphenazole	10.00	9.952	0.04	0.0019
Sulfanitran	9.92	10.054	-0.13	0.0173
Trimethoprim	10.05	9.901	0.15	0.0213
Dapsona	5.03	5.136	-0.11	0.0116

Table 14. The results obtained for A(B) A(b) D(b) D(b)2

Compound	A(B)	A(b)	D(b)	D(b)2
Sulfaguanidine	10.05	10.11	-0.06	0.0031
Sulfacetamide	9.93	10.12	-0.19	0.0343
Sulphanilamide	10.02	9.94	0.08	0.0065
Sulfisomidine	9.94	10.07	-0.13	0.0182
Sulfadiazine	9.76	9.88	-0.12	0.0139
Sulfathiazole	9.83	9.84	-0.01	0.0002
Sulfapyridine	9.70	9.77	-0.07	0.0054
Sulfamerazine	9.77	9.92	-0.15	0.0232
sulfamethazine	10.05	10.00	0.05	0.0025
Sulfamethizole	10.08	10.23	-0.15	0.0218

Sulfamethoxypyridazine	9.93	9.99	-0.06	0.0036
Sulfamonomethoxine	9.94	9.94	0.00	0.0000
Sulfameter	10.08	9.92	0.16	0.0254
Sulfacloropyridazin	9.96	9.97	-0.01	0.0002
Sulfadoxin	9.99	10.01	-0.02	0.0004
Sulfametoxazol	9.82	9.95	-0.13	0.0167
Sulfisoxazol	9.84	10.08	-0.24	0.0565
Sulfabenzamida	9.85	10.00	-0.15	0.0224
Sulfadimetoxin	10.05	9.96	0.10	0.0090
Sulfaquinoxalin	9.96	10.08	-0.12	0.0135
Sulfaphenazol	9.96	10.13	-0.17	0.0300
Sulfanitran	9.97	9.79	0.18	0.0327
Trimethoprim	10.05	9.98	0.06	0.0039
Dapsona	5.04	5.03	0.01	0.0000

Table 15. The results obtained for A(C) A(c) D(c) D(c)2

Compound	A(C)	A(c)	D(c)	D(c)2
Sulfaguanidine	10.08	10.02	0.05	0.0028
Sulfacetamide	9.94	10.08	-0.14	0.0197
Sulphanilamide	10.05	9.85	0.20	0.0386
Sulfisomidine	9.99	9.85	0.14	0.0183
Sulfadiazine	9.78	9.78	0.00	0.0000
Sulfathiazole	9.84	9.78	0.06	0.0041
Sulfapyridine	9.71	9.72	-0.01	0.0001
Sulfamerazine	9.76	9.97	-0.21	0.0440
Sulfamethazine	9.98	10.29	-0.31	0.0968
Sulfamethizole	10.12	10.06	0.06	0.0037
Sulfametoxipiridazin	9.93	10.00	-0.07	0.0046
Sulfamonometoxin	9.92	10.04	-0.12	0.0143
Sulfameter	10.07	9.97	0.10	0.0100
Sulfacloropyridazin	9.95	10.00	-0.05	0.0021
Sulfadoxin	9.95	10.16	-0.20	0.0420
Sulfametoxazol	9.81	10.00	-0.19	0.0354
Sulfisoxazol	9.84	10.06	-0.22	0.0482
Sulfabenzamida	9.85	10.00	-0.15	0.0231
Sulfadimetoxin	10.01	10.14	-0.13	0.0167
Sulfaquinoxalin	10.00	9.94	0.06	0.0034
Sulfaphenazol	9.97	10.06	-0.08	0.0070
Sulfanitran	9.91	10.06	-0.15	0.0234
Trimethoprim	10.03	10.06	-0.04	0.0013
Dapsona	5.02	5.10	-0.07	0.0054

Table 16. The results obtained for A(D) A(d) D(d) D(d)2

Compound	A(D)	A(d)	D(d)	D(d)2
Sulfaguanidine	10.07	10.05	0.02	0.0003
Sulfacetamide	9.96	9.99	-0.03	0.0008
Sulphanilamide	9.94	10.28	-0.34	0.1129
Sulfisomidine	9.95	10.01	-0.06	0.0034
Sulfadiazine	9.84	9.56	0.28	0.0757
Sulfathiazole	9.79	9.98	-0.19	0.0349
Sulfapyridine	9.65	9.93	-0.28	0.0769
Sulfamerazine	9.79	9.84	-0.05	0.0021
Sulfamethazine	10.07	9.96	0.11	0.0111
Sulfamethizole	10.14	9.98	0.16	0.0256
Sulfametoxipiridazin	9.96	9.88	0.08	0.0059
Sulfamonometoxin	9.97	9.83	0.14	0.0202
Sulfameter	10.03	10.13	-0.10	0.0097
Sulfacloropyridazin	9.93	10.08	-0.15	0.0216
Sulfadoxin	9.97	10.08	-0.11	0.0122
Sulfametoxazol	9.88	9.73	0.15	0.0210
Sulfisoxazol	9.87	9.97	-0.10	0.0106
Sulfabenzamida	9.82	10.09	-0.26	0.0697
Sulfadimetoxin	10.01	10.11	-0.09	0.0084
Sulfaquinoxalin	10.03	9.80	0.24	0.0561
Sulfaphenazol	9.97	10.07	-0.09	0.0087
Sulfanitran	9.90	10.06	-0.16	0.0251
Trimethoprim	9.98	10.23	-0.24	0.0585
Dapsona	5.06	4.96	0.09	0.0090

Table 17. The results obtained for A(E), A(e), D(e), D(e)2

Compound	A(E)	A(e)	D(e)	D(e)2
Sulfaguanidine	10.02	10.24	-0.22	0.0490
Sulfacetamide	9.95	10.06	-0.11	0.0124
Sulphanilamide	10.00	10.03	-0.03	0.0007
Sulfisomidine	9.96	9.98	-0.03	0.0007
Sulfadiazine	9.78	9.81	-0.03	0.0012
Sulfathiazole	9.78	10.04	-0.27	0.0715
Sulfapyridine	9.72	9.67	0.05	0.0029
Sulfamerazine	9.80	9.81	-0.01	0.0002
Sulfamethazine	10.07	9.94	0.13	0.0178
Sulfamethizole	10.12	10.08	0.04	0.0014
Sulfamethoxypyridazine	9.98	9.81	0.17	0.0275
Sulfamonomethoxine	9.95	9.89	0.06	0.0034
Sulfameter	10.04	10.11	-0.07	0.0044
Sulfacloropyridazin	9.93	10.09	-0.16	0.0262
Sulfadoxin	9.98	10.02	-0.04	0.0013
Sulfametoxazol	9.88	9.74	0.14	0.0198
Sulfisoxazol	9.91	9.80	0.11	0.0129
Sulfabenzamida	9.88	9.85	0.04	0.0014
Sulfadimetoxin	9.99	10.19	-0.20	0.0393
Sulfaquinoxalin	9.98	10.00	-0.01	0.0002
Sulfaphenazol	10.03	9.85	0.18	0.0322
Sulfanitran	9.91	10.02	-0.10	0.0108
Trimethoprim	10.05	9.95	0.10	0.0096
Dapsona	5.06	4.97	0.08	0.0068

For each compound, the standard deviation of the differences Di (SDi) was calculated with the formula:

$$S_{Di} = \sqrt{2 * \sum(Di^2)/5}$$

Table 18. The results obtained for D(a)2, D(b)2, D(c)2, D(d)2

Compound	D(a)2	D(b)2	D(c)2	D(d)2
Sulfaguanidine	0.0005	0.0031	0.0028	0.0003
Sulfacetamide	0.0048	0.0343	0.0197	0.0008
Sulphanilamide	0.0161	0.0065	0.0386	0.1129
Sulfisomidine	0.0250	0.0182	0.0183	0.0034
Sulfadiazine	0.0092	0.0139	0.0000	0.0757
Sulfathiazole	0.0008	0.0002	0.0041	0.0349
Sulfapyridine	0.0377	0.0054	0.0001	0.0769
Sulfamerazine	0.0037	0.0232	0.0440	0.0021
Sulfamethazine	0.0002	0.0025	0.0968	0.0111
Sulfamethizole	0.0030	0.0218	0.0037	0.0256
Sulfamethoxypyridazine	0.0266	0.0036	0.0046	0.0059
Sulfamonometoxin	0.0248	0.0000	0.0143	0.0202
Sulfameter	0.0172	0.0254	0.0100	0.0097
Sulfacloropyridazin	0.0003	0.0002	0.0021	0.0216
Sulfadoxin	0.0006	0.0004	0.0420	0.0122
Sulfametoxazol	0.0016	0.0167	0.0354	0.0210
Sulfisoxazol	0.0649	0.0565	0.0482	0.0106
Sulfabenzamida	0.0001	0.0224	0.0231	0.0697
Sulfadimetoxin	0.0121	0.0090	0.0167	0.0084
Sulfaquinoxalin	0.0685	0.0135	0.0034	0.0561
Sulfaphenazol	0.0019	0.0300	0.0070	0.0087
Sulfanitran	0.0173	0.0327	0.0234	0.0251
Trimethoprim	0.0213	0.0039	0.0013	0.0585
Dapsona	0.0116	0.0000	0.0054	0.0090

When SDi is significantly lower than the standard deviation of the method performed under reproducible conditions, the conclusion is that these factors, combined, have no effect on the result, even if each factor alone does not indicate a significant influence and that the method is sufficiently robust to the chosen modifications. The results obtained show that the method is sufficiently robust to the chosen modifications, since SDi is significantly lower than the standard deviation of the method performed under intralaboratory reproducible conditions (Table 19).

Table 19. D(e)2, SDi, SR for the analyzed compounds

Compound	D(e)2	S_{Di}	S_R
Sulfaguanidine	0.0490	0.15	0.26
Sulfacetamide	0.0124	0.17	0.24
Sulphanilamide	0.0007	0.26	0.29
Sulfisomidine	0.0007	0.16	0.32
Sulfadiazine	0.0012	0.20	0.25
Sulfathiazole	0.0715	0.21	0.23
Sulfapyridine	0.0029	0.22	0.28
Sulfamerazine	0.0002	0.17	0.22
Sulfamethazine	0.0178	0.23	0.25
Sulfamethizole	0.0014	0.15	0.26
Sulfametoxipiridazin	0.0275	0.17	0.25
Sulfamonometoxin	0.0034	0.16	0.23
Sulfameter	0.0044	0.16	0.30
Sulfacloropyridazin	0.0262	0.14	0.17
Sulfadoxin	0.0013	0.15	0.28
Sulfametoxazol	0.0198	0.19	0.27
Sulfisoxazol	0.0129	0.28	0.30
Sulfabenzamida	0.0014	0.22	0.24
Sulfadimetoxin	0.0393	0.18	0.25
Sulfaquinoxalin	0.0002	0.24	0.28
Sulfaphenazol	0.0322	0.18	0.25
Sulfanitran	0.0108	0.21	0.24
Trimethoprim	0.0096	0.19	0.28
Dapsona	0.0068	0.11	0.14

Table 20. The uncertainty values calculated for each compound

Compound	Chicken eggs			
	C_{min} (µg/kg)	Composite standard uncertainty, u_c (µg/kg)	Uncertainty extended, u_c (k=2) (µg/kg)	Final result (µg/kg)
Sulfaguanidine	10*	0.878	1.756	10 ± 1.756
Sulfacetamide	10*	0.828	1.656	10 ± 1.656
Sulphanilamide	10*	0.849	1.698	10 ± 1.698
Sulfadiazine	10*	0.804	1.608	10 ± 1.608
Sulfadiazine	10*	1.143	2.287	10 ± 2.287
Sulfathiazole	10*	0.786	1.573	10 ± 1.573
Sulfapyridine	10*	0.797	1.595	10 ± 1.595
Sulfamerazine	10*	0.749	1.497	10 ± 1.497
Sulfamethazine	10*	0.455	0.910	10 ± 0.910
Sulfamethizole	10*	0.862	1.724	10 ± 1.724
Sulfametoxipiridazin	10*	0.927	1.854	10 ± 1.854
Sulfamonometoxin	10*	0.882	1.764	10 ± 1.764
Sulfameter	10*	0.953	1.906	10 ± 1.906
Sulfacloropyridazin	10*	0.679	1.357	10 ± 1.357
Sulfadoxin	10*	0.804	1.608	10 ± 1.608
Sulfametoxazol	10*	0.509	1.019	10 ± 1.019
Sulfisoxazol	10*	0.836	1.671	10 ± 1.671
Sulfabenzamida	10*	0.616	1.231	10 ± 1.231
Sulfadimetoxin	10*	1.192	2.384	10 ± 2.384
Sulfaquinoxalin	10*	1.132	2.265	10 ± 2.265
Sulfaphenazol	10*	0.986	1.973	10 ± 1.973
Sulfanitran	10*	0.869	1.738	10 ± 1.738
Trimethoprim	10*	0.782	1.564	10 ± 1.564
Dapsona	5*	0.555	1.111	5 ± 1.111

The expanded measurement uncertainty for a factor k = 2 was calculated according to the Expanded Uncertainty Calculation Sheet – Chromatography, for each compound. The values of the calculated uncertainties for each compound, expressed in relation to the theoretical (added) concentration of the compound in the sample, are presented in the Table 20.

Varenin et al. (2016) uses liquid chromatography in tandem with mass spectrometry for the simultaneous determination of sulfonamides, trimethoprim and dapsone in muscle, egg, milk and honey. Park et al., 2024 uses the LC-MS/MS method with QuEChERS for the simultaneous determination of sulfonamides, trimethoprim, ormethoprim and dapsone in various zootechnical products. Heller et al., 2002 uses the LC-MS-MS method for sulfonamide residues in eggs and reports 5-10 ng/g (ppb) as the confirmation limit for each drug analyzed.

CONCLUSIONS

Given that all proposed performance parameters were achieved, the values obtained fall within the established acceptability requirements, it can be concluded that the method -Determination of sulfonamide, dapsone and trimethoprim residues by LC-MS/MS, can be used for the determination of sulfonamide, dapsone and trimethoprim residues in a liquid chromatography laboratory.

REFERENCES

Bologa, M., Pop, I.M., Albu A. (2013). Research on chemical composition of chicken egg from different systems of production (conventional and organic). *University of Agricultural Sciences and Veterinary Medicine Iasi*, 1-6.

Dubreil-Chéneau, E., Pirotais, Y., Verdon, E., & Hurtaud-Pessel, D. (2014). Confirmation of 13 sulfonamides in honey by liquid chromatography–tandem mass spectrometry for monitoring plans: Validation according to European Union Decision 2002/657/EC. *Journal of Chromatography A, 1339*, 128-136.

Gentili, A., Perret, D., & Marchese, S. (2005). Liquid chromatography-tandem mass spectrometry for performing confirmatory analysis of veterinary drugs in animal-food products. *Trends in Analytical Chemistry, 24*(7), 704-733.

Grave, K., Torren-Edo, J., Muller, A., Greko, C., Moulin, G., & Mackay, D. (2014). Variations in the sales and sales patterns of veterinary antimicrobial agents in 25 European countries. *Journal of Antimicrobial Chemotherapy*, *69*(8), 2284-2291.

Heller, D.N., Ngoh, M.A., Donoghue, D., Podhorniak, L., Righter, H., Thomas, M.H. (2002). Identification of incurred sulfonamide residues in eggs: methods for confirmation by liquid chromatography–tandem mass spectrometry and quantitation by liquid chromatography with ultraviolet detection. *Journal of Chromatography B, 744 (5)*39-52.

Kumar, P. (2019). Lyophilization: an important formulation technique. *International Journal of Research - Granthaalayah*, 7(9), 11-15.

Park, H., Hui-Seung, K. (2024). Simultaneous Determination of Sulfonamides, Trimethoprim, Ormethoprim and Dapsone in Livestock Products by LC-MS/MS with QuEChERS. *Mass Spectrom. Lett.*, *15*(4), 167-177.

Samman, S., Kung F.P., Carter, L.M., Foster, M.J., Ahmad, Z.I., Phuyal, J.L., Petocz, P. (2009). Fatty acid composition of certified organic, conventional and omega-3 eggs. *Food Chemistry, 116*, 911-914.

Shao, B., Dong, D., Wu, Y., Hu, J., Meng, J., Tu, X., et al. (2005). Simultaneous determination of 17 sulfonamide residues in porcine meat, kidney, and liver by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Analytica Chimica Acta, 546*(2), 174-181.

Sheridan, R., Policastro, B., Thomas, S., & Rice, D. (2008). Analysis and occurrence of 14 sulfonamide antibacterials and chloramphenicol in honey by solid-phase extraction followed by LC/MS/MS analysis. *Journal of Agricultural and Food Chemistry, 56*(10), 3509-3516.

Tian, W., Gao, L., Zhao, Y., Peng, W., & Chen, Z. (2013). Simultaneous determination of metronidazole, chloramphenicol and 10 sulfonamide residues in honey by LC–MS/MS. *Analytical Methods*, *5*, 1283-1288.

Tölgyesi, Á., Berky, R., Békési, K., Fekete, S., Fekete, J., & Sharma, V.K. (2013). Analysis of sulfonamide residues in real honey samples using liquid chromatography with fluorescence and tandem mass spectrometry detection. *Journal of Liquid Chromatography & Related Technologies*, *36*(8), 1105-1125.

Varenin, I., Bilandžić, N., Kolanović, B.S., Božić, D., Sedak, M., Đokić, M., Varga, I. (2016). Validation of a liquid chromatography-tandem mass spectrometry method for the simultaneous determination of sulfonamides, trimethoprim and dapsone in muscle, egg, milk and honey. *Food Additives & Contaminants*: *Part A, 33,* (4).

Wang, K, Lin, K.X., Huang, M. & Chen, M. (2017). A simple and fast extraction method for the determination of multiclass antibiotics in eggs using LC-MS/MS. *J. Agric. Food Chem., 65*, 5064-5073.