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Assessment of The Distribution and Concentration of Residual Antibiotics in Chicken Meat and Liver Samples Collected in Tehran by Liquid Chromatography and Tandem Mass Spectrometry

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ANTIBIOTICS are extensively used as therapeutic, prophylactic and growth promoting agents in the poultry industry. However, their widespread uses resulted in the presence of residues in poultry meat and offal potentially leading to public health hazards. The present research was done to assess the concentration of residual antibiotics in chicken meat and liver samples. Ninety chicken meat and liver samples were collected and transferred to laboratory. Presence and concentration of residual tetracyclines, sulfonamides and trimethoprim were assessed using the Liquid Chromatography-Tandem Mass Spectrometry. There were no detectableconcentrations of tetracyclines in all studied samples. Twenty-eight out of 90 (31.11%) raw meat and 31 out of 90 (34.44%) liver samples were positive for residual sulfonamides. Prevalence of positive meat and liver samples for residual sulfachloropyrazine, sulfadimethoxine and trimethoprim antibiotics were 16.66%, 5.55% and 8.88% and also16.66%, 5.55% and 11.11%, respectively. Sulfathiazole residue was only detected in 1.11% of chicken liver samples.Chicken liver samples had the higher concentrations of all detected residual sulfonamides. Sulfachloropyrazine had the highest concentration in raw chicken meat (20.8±1.88 µg/kg) and liver (24.4±1.54 µg/kg) samples, while sulfadimethoxine had the lowest (6.05±0.25 µg/kg and 9.26±0.36 µg/kg, respectively). All detected concentrations of residual sulfonamides were lower than Maximum Residue Limits (MRLs). Presence of residual antibiotics represents a serious public health treat regarding the occurrence of antibiotic resistant bacterial strains. LC-MS/MS has been introduced as a sensitive and specific technique for monitoring and surveillance of residual antibiotics in chicken samples.

Keywords: Antibiotics residues, Poultry, Meat, Liver, Liquid Chromatography-Mass Spectrometry.

Introduction

The chicken meat and further processed ready to eat chicken liver are rich source of essential amino acids, minerals and vitamins [1-4]. However, chicken meat and liver are not necessarily safe, as demonstrated by high prevalence of antibiotic resistant bacteria and high concentrations of residual chemical agents and especially antibiotics[5].

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Extensive use of antibiotics in poultry industry resulted in the presence of residuals in foodstuffs leading to a potential health hazards for consumers which include; carcinogenicity, mutagenicity, bone marrow toxicity and allergy as well as appearance of a resistant strains of pathogenic bacteria [5]. Moreover, extensive use of antimicrobial agents as growth stimulator has been banned by the European Union (EU) [6]. Additionally, oral administration of antimicrobial agents has been restricted by Veterinary Feed Directives (VFD) in the United State [7].

Tetracyclines are a family of compounds regularlyused due to their broad spectrum of activity as well as their low cost, compared with other antibiotics. Presently, there are over 20 tetracyclines available; however, tetracycline, chlortetracycline, oxytetracycline, and doxycycline are the most commonlyused in veterinary medicine[8]. The sulfonamides are a group of synthetic antimicrobial agents that are structural analogs of para-aminobenzoic acid (PABA). They are broad-spectrum antimicrobial agents which affects Gram-positive and many Gram-negative bacteria, toxoplasma and protozoal agents. Sulfabenzamide, sulfachloropyrazine, sulfadimethoxine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole,sulfaquinoxaline, sulfathiazole and sulfisoxazoleare the most commonly used sulfonamide antibioticsin both veterinary and medicine [9].Trimethoprim is a pyrimidine inhibitor of dihydrofolate reductase. It is a synthetic derivative of trimethoxybenzyl-pyrimidine with antibacterial and antiprotozoal properties.It is potentiated by sulfonamides and the trimethoprim-sulfamethoxazole combination is the form most often used. It is sometimes used alone as an antimalarial [5, 8-10].

Liquid chromatography (LC) has got a lot of benefits such as wide range detection and simultaneous quantitation of components based on their biological or chemical character [11]. Unit–mass resolution MS instruments does not provide enough selectivity in trace levels of residual antibiotics so that modern laboratories equipped by tandem Mass (MS/MS) capabilities to improve selectivity and consequently signal to noise ratio in their studied samples [11].

Regarding the high importance of residual antibiotics in chicken meat and liver, Maximum Residue Limits (MRLs) has been introduced by Food and Agriculture Organization (FAO) of the United States[12]. The present investigation was done to assess the concentrations of residual tetracyclines, sulfonamides and trimethoprimantibiotics in chicken meat and liver samples using Liquid Chromatography and Tandem Mass Spectrometry.

Materials and Methods

Chemicals

Reference standards of sulfonamides such as-sulfabenzamide (SBZ), sulfachloropyrazine (SCP), sulfadimethoxine (SDM), sulfameter (SME), sulfamethazine (SMZ), sulfamethizole (SMTZ), sulfamethoxazole (SMX),sulfaquinoxaline (SQX), sulfathiazole (STZ), and sulfisoxazole (SIX), trimethoprim (TRI) and tetracyclines such astetracycline (TET), oxytetracycline (OTC) andchlortetracycline (CTC) antibiotics were purchased from the Sigma company (>99% purity, Sigma Chemical Co. St.Louis, MO, USA). Sulfonamides, trimethoprim and tetracyclines stock solutions and internal standards were prepared by dilution of 1000 µg/mL of antibiotics in methanol (Merck, Germany). All stock standard solutions were then stored at -20 ◦C. High Performance Liquid Chromatography (HPLC)-grade acetonitrile and methanol were purchased from the Merck Company (Merck, Germany). Individual working standard mixtures were prepared daily by dilution of intermediate standards in water to achieve intended MRL. Water was also purified through the SG system (Germany).

Apparatus

Chromatographic separation, identification, and quantification were carried out by Perkin Elmerliquid chromatographic system (Flexar) with API 3200 triple quadrupole mass spectrometer (AB Sciex, Canada) equipped with a Turbo V Ione spray source in the positive mode. The curtain gas, ion source gas 1, ion source gas 2 and collision gas (all nitrogen) were set at 20, 50, 50 and 7 instrument units, respectively. The spray voltage was 5500 V, the heater temperature was 600 ◦C.The interface heater was turned on.Multi per reaction monitoring (MRM) and optimized potentials are shown in table 2.Two transitions per analyte were used for monitoring and confirmation. The analyst 1.6.2 software was used for acquisition, qualitative and quantitative of residual antibiotics. The instrument was provided with 100 vial capacity automaticsample management system.The separation was accomplished with a Pentaflurophenyl column, 150 mm×2 mm, 3µm, fitted with a 20×2 mm guard column of the same phase purchased from Macherey-Nagel (Germany). Column temperature was 30◦C.The mobile phase contained (A) acetonitrileand (B) 10mMammonium acetate (pH=3). Operated as a gradient from 0 to 2 min (90%A), 2-12 min (10% A) and re-equilibrium time were 3 min. The flow rate was 0.3mL/min. A total of 20 µl samples were injected in each time. A routine time of injection was adjusted to 15 min.

Sampling and sample preparation

From January to May 2018, a total of 90 chicken meat and also 90 chicken liver samples were collected from 13 different slaughterhouses located at Tehran, Iran using systematic random sampling procedure. Raw chicken meat samples were collected from the breast muscle. Samples (200 g) were collected under sterile hygienic conditions using sterile glass tubes. Targeted chickens which their meat and liver samples collected for this study were clinically healthy, and samples showed normal physical (color, odor, pH, and density) consistency. The samples were immediately transferred to laboratory in cooler with ice packs and were processed within an hour of collection. The samples were stored at -20°C until analysis.Concentrations of residual tetracyclines, trimethoprim and sulfonamides were studied using the method described by Granelli et al. [13]. Briefly, each sample was homogenized using a mill (Retsch-GM300-Germany). Three grams of the homogenized samples were transferred to a 50 mL falcon tube. Then, 200 µL EDTA (0.1M) were added to previous solution. Then, 10 µg/mL internal standards solutions (sulfamethazine phenyl $13C^6$ and sulfathiazole phenyl $13C^6$ and demeclocycline) (with concentration of 50 ng/g) were added to samples and allowed to stand in a dark for at least 15 min. Contents were then mixed well. Then, 15 mL of methanol (70%) was added into the contents and were shaken for about 10 min. Samples were then subjected to centrifuge at 4000 g for 5 min. The supernatant (100 µL) was diluted by 400 µL water in propylene vial. The sample was then mixed and injected to LC-MS/MS device. Each sample was first monitored by precursor ion and then judged by confirmation pair ions. A total of 10 ng of samples and standard solutions were injected to device.

Validation of analytical method

Table 1 represents the characters of method validation. The standard calibration curve was obtained by plotting concentration against average of the peak areas. Retention times of TC, OTC and CTC antibiotics were found to be 9.86, 9.80 and 11.10 min with recoveries of 82, 86 and 85%, respectively. Retention times of SCP, STZ, SDM and TRI antibiotics were 9.0, 7.5, 9.6 and 7.6 min with recoveries of 85, 78, 79 and 71%, respectively. Validation was performed according to method of Commission Decision 2002/657/EC. Negative samples of meat and liver were spiked with randomized amounts of 0.5, 1and 1.5 folds of MRLs of tetracyclines, sulfonamides and trimethoprimantibiotics.Evaluation of method validation was done by 6 times replication of each concentration in three days. Obtained results were used in order to validate test indexes. Test specificity was determined by analyzing 30 chicken meat and liver samples. Additionally, test recovery was evaluated by comparison of the determined amounts of antibiotics with blank sample. The linear cadence graph was drawn for each analyte with ranges of 2 to 750 µg/kg. The lowest acceptable regression was considered as 0.94. CCαand CCβ were set to level of β<5%. Limit of Detection (LOD) was defined for monitoring transition (S/N >3). Detected concentrations of antibiotics were compared with the standard MRLs [14]. For matrix effect evaluation, the peaks signal ratio of 100 ppb was compared betweenISTDs prepared in solvent and blank sample. We found no significant differences between them.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). Statistical analysis was done using the SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). One-way Analysis of Variance (One-way ANOVA) statistical method was used to assess any significant relationship for the concentrations of residual antibiotics between different samples. *P* value ≤0.05 was considered as statistically significant level.

Results

Concentrations of tetracyclines, trimethoprim and sulfonamides antibiotics

A total of 90 chicken breast meat and liver samples were analyzed for presence and concentrations of tetracyclines, trimethoprim and sulfonamides antibiotics using the LC and tandem mass spectrometry. Figure 1 represents the typical chromatogram of tetracyclines.

TABLE 1. Basic Information on component, regulatory limits and MS/MS conditions used for detection of residual antibiotics in chicken meat and liver samples.

Antibiotics	MW[a]	Formula	CAS No.	Lot No	Precursor ion (m/z)	Ion pairs for quantitation and confirmation (m/z)	DP[b]	EP[c]	CEP[d]	CE[e]	Retention Time (min)	LOD[f]	Recovery % (Standard deviation)	CCα[g]	CCβ[h]
SBZ*	276.057	C13H12N2O3S	127-71-9	#SZBE079XV	277	156	31	9	14.10	17	9.80	4	79.3(4.3)	111.50	123.00
						92	31	7.5	14.10	39					
SCP	284.013	C10H9ClN4O2S	102-65-8	#SZBA050XV	285	156	31	4	14.38	19	9.0	4	85.2(2.5)	113.60	127.20
						92	36	4	14.38	41					
SDM	310.074	C12H14N4O4S	122-11-2	#50813	311	156	41	3.5	15.29	23	9.6	4	79(4.5)	112.00	125.00
						92	70	10	15.29	50					
SME	280.063	C11H12N4O3S	651-06-9	#50624	281	156	36	4	14.24	21	8.50	4	73.9(1.9)	111.70	123.40
						108	36	4	14.24	39					
SMZ	278.084	C12H14N4O2S	57-68-1	#BCBD1654V	279	186	41	4.5	14.17	21	8.20	4	77(6.6)	113.00	125.00
						92	41	4	14.17	35					
SMTZ	270.025	C10H11N3O3S	723-46-6	#LRAA5713	270	156	36	5	13.86	17	9.80	4	92.7(4.1)	109.10	118.30
						92	36	4.5	13.86	37					
SMX	253.052	C10H11N3O3S	723-46-6	#SZBC341XV	254	156	36	5	13.30	19	9.30	4	82.2(8.1)	110.20	120.40
						108	36	5	13.30	35					
SQX	300.068	C14H12N4O2S	59-40-5	#SZBE339XV	301	208	46	3	14.95	17	9.90	4	83.8(3.8)	108.50	117.00
						146	46	3	14.95	17					
STZ	255.014	C11H12N4O3S	72-14-0	#SZBE203XV	256	156	36	5	13.37	29	7.5	4	78(3.0)	115.00	130.00
						92	36	5	13.37	41					
SIX	267.068	C12H14N4O2S	515-64-0	#MKBW7389V	268	156	36	5.5	13.79	17	9.40	4	81.4(1.6)	109.70	119.40
						92	36	5	13.79	39					
TRI	290.32	C14H18N4O3	738-70-5	#SZBE33XV	291	230	56	3.5	14.59	23	7.6	2	71(2.5)	56.90	64.20
						123	56	3.5	14.59	37					
TET	444.153	C22H24N2O8	60-54-8	#SZBE00XV	445	410	31	5	19.98	21	9.86	10	82(7.4)	109.20	118.40
						154	36	3.5	19.98	35					
OTC	460.148	C22H24N2O9	79-57-2	#SZBE42XV	461	426	36	6.5	20.54	21	9.80	10	86(3.1)	109.20	118.40
						443	36	7.5	20.54	19					
CTC	478.114	C22H23ClN2O8	57-62-5	#SZBE17XV	479	462	41	6.5	21.17	27	11.10	10	85(5.4)	108.60	117.20
						444	41	6	21.17	23					

[a] MW, Molecular weight, [b]DP, De clustering potential, [c] EP, entrance potential, [d] CEP, collision exit potential, [e] CE Collision energies, [f] LOD, Limit of Detection, CCα[g], decision limit, CCβ[h], detection capability.

*SBZ, Sulfabenzamide; SCP, Sulfachloropyrazinel SDM, Sulfadimethoxine; SME, Sulfameter; SMZ, Sulfamethazine; SMTZ, Sulfamethizole; SMX, Sulfamethoxazole; SQX, Sulfaquinoxaline; STZ, Sulfathiazole; SIX, Sulfisoxazole; TRI, Trimethoprim; TET, Tetracyclines; OTC,Oxytetracycline; CTC,. Chlortetracycline.

Figure 2 represents the typical chromatogram of sulfonamides and trimethoprim.

Figure 3 represents thetypical of SDM (a), TRI (b), SCP (c) and STZ (d) residual antibiotics chromatograms.

Table 2 represents the concentrations of tetracyclines, trimethoprim and sulfonamides antibiotics in chicken meat and liver samples.

There were no measurable amounts of tetracyclines in all studied samples. Results showed that 28 out of 90 (31.11%) raw meat and 31 out of 90 (34.44%) liver samples were positive for residual antibiotics. Prevalence of positive chicken meat samples for of residual SCP, SDM and TRI antibiotics were 16.66%, 5.55% and 8.88%, respectively. Prevalence of positive chicken liver samples for of residual SCP, SDM and TRI antibiotics were 16.66%, 5.55% and 11.11%, respectively. STZ residue was only detected in 1.11% of chicken liver samples.In raw chicken meat samples, the concentration of SCP was 20.80±1.88 µg/kg, while that of SDM was 6.05±0.25 µg/kg. Similarly, in raw chicken liver samples, the concentration of SCP was 24.40±1.54 µg/kg, while that of SDM was 9.26±0.36 µg/kg. Nine out of 90 (10%) liver samples and 7 out of 90 (7.77%) meat samples were simultaneously positive for SCP and TRI classes of antibiotics. All detected concentrations of residual antibiotics were lower than announced MRLs. There were no statistically significant differences for the concentrations of residual antibiotics between chicken meat and liver samples (*P*>0.05). Statistically significant differences were found between the concentrations of different antibiotics detected in meat and liver samples (*P* ≤0.05).

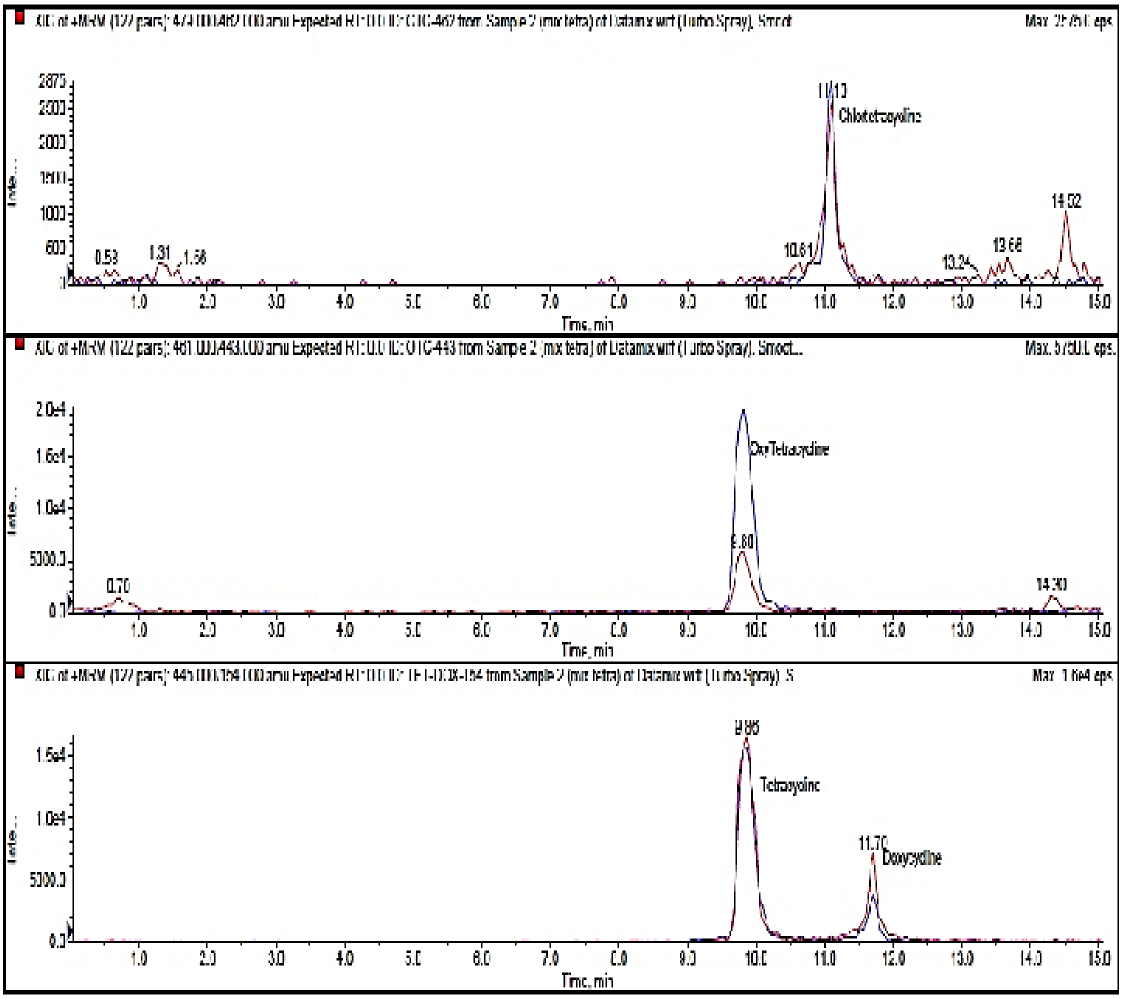

Fig. 1.Tetracyclines chromatogram.

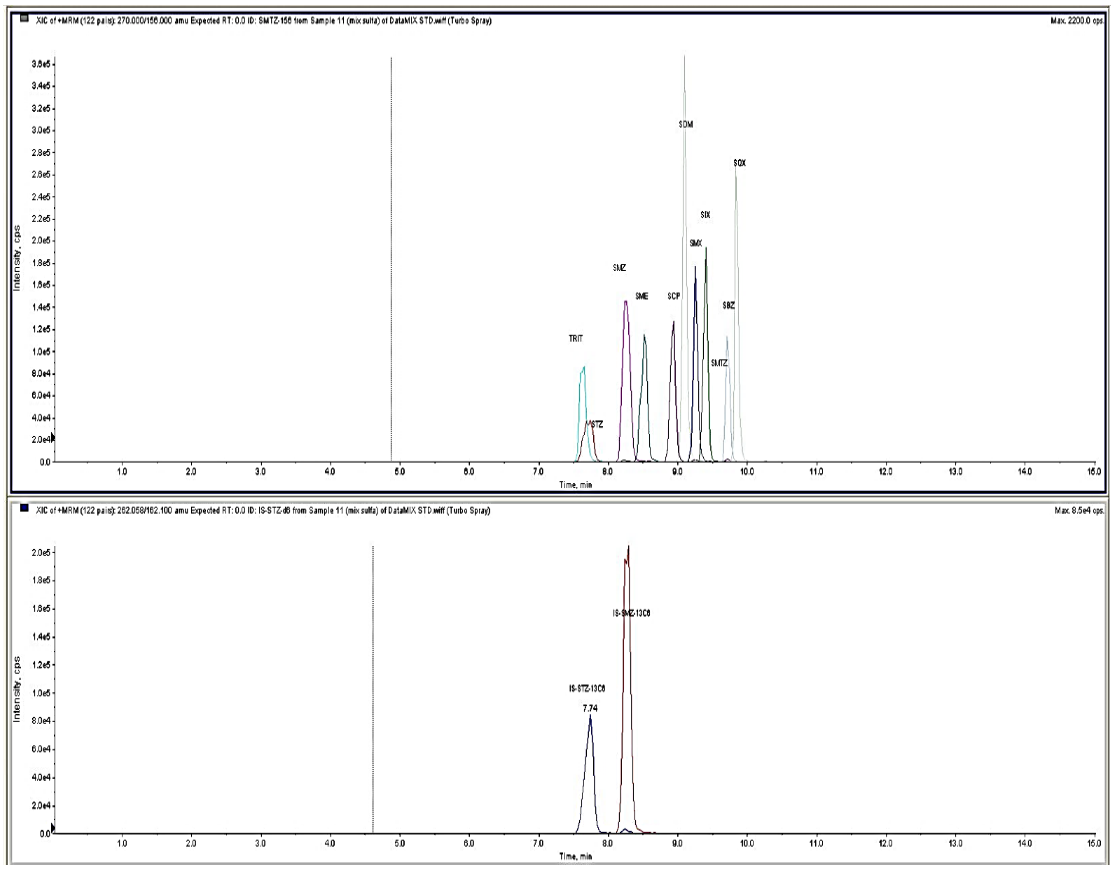

Fig. 2.Typical chromatogram of sulfonamides and trimethoprim.

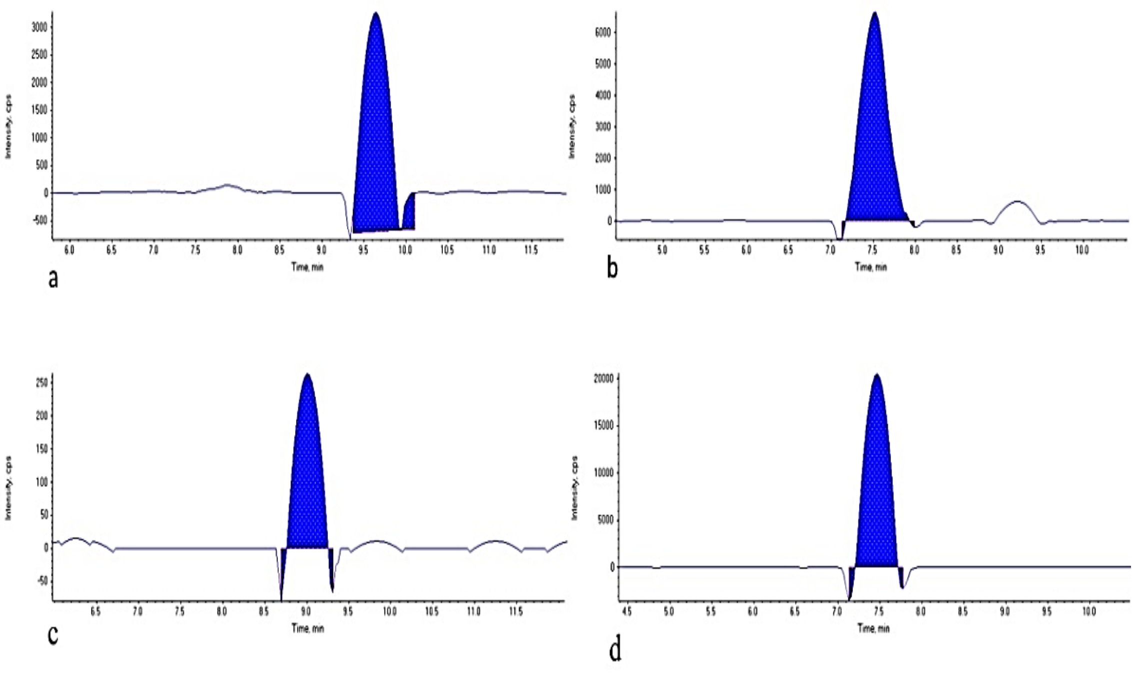

Fig. 3. Typical SDM (a), TRI (b), SCP (c) and STZ (d) chromatograms.

TABLE 2. Distribution and mean concentrations of residual antibiotics in chicken meat and liver samples.

Antibiotics	MRL** (µg/Kg)	Concentration (µg/kg)					
		Muscle			Liver		
		Detected	Range	Mean±SD******	Detected	Range	Mean±SD
SBZ	100 (M***, L****)	<LOD*****	-	-	<LOD	-	-
SCP	100 (M, L)	15 (16.66), <MRL	9.88-60.10	20.80±1.88 Aa	15 (16.66), <MRL	9.25-56.90	24.40±1.54 Aa
SDM	100 (M, L)	5 (5.55), <MRL	5.11-6.60	6.05±0.25 Ca	5 (5.55), <MRL	4.85-24.90	9.26±0.36 Ca
SME	100 (M, L)	<LOD	-	-	<LOD	-	-
SMZ	100 (M, L)	<LOD	-	-	<LOD	-	
SMTZ	100 (M, L)	<LOD	-	-	<LOD	-	-
SMX	100 (M, L)	<LOD	-	-	<LOD	-	-
SQX	100 (M, L)	<LOD	-	-	<LOD	-	-
STZ	100 (M, L)	<LOD	-	-	1 (1.11), <MRL	19.10	19.10±0.14 B
SIX	100 (M, L)	<LOD	-	-	<LOD	-	-
TRI	50 (M, L)	8 (8.88), <MRL	8.46-31.20	16.52±1.23 Ba	10 (11.11), <MRL	7.23-28.90	14.00±1.21 Ba
TET	100 (M) 300 (L)	<LOD	-	-	<LOD	-	-
OTC	100 (M) 300 (L)	<LOD	-	-	<LOD	-	-
CTC	100 (M) 300 (L)	<LOD	-	-	<LOD	-	-

*SBZ, Sulfabenzamide; SCP, Sulfachloropyrazinel SDM, Sulfadimethoxine; SME, Sulfameter; SMZ, Sulfamethazine; SMTZ, Sulfamethizole; SMX, Sulfamethoxazole; SQX, Sulfaquinoxaline; STZ, Sulfathiazole; SIX, Sulfisoxazole; TRI, Trimethoprim; TET, Tetracyclines; OTC, Oxytetracycline; CTC,. Chlortetracycline.
MRL, Maximum Residue Limits*M, Meat****L, Liver
*****LOD, limit of Detection******SD, Standard Deviation
Dissimilar capital letters in each column shows statistically significant differences about $P \leq 0.05$.
Dissimilar small letters in each ow shows statistically significant differences about $P \leq 0.05$.

Discussion

Over the past few decades, poultry have gone through tremendous growth; however, with the increase in production, the uses of certain drugs and feed additives have become crucial in order to prevent diseases, their treatment, and growth promotion. However, one of the drawbacks of excessive use of antimicrobial drugs is that they get accumulated in the tissues and organs of treated animals as residues and eventually become part of the food pyramid, hence excessive usage has been recognized as illegal and prohibited by the food regulatory and health authorities. Antibiotic residues are harmful chemical substances responsible for allergic and immunological reactions, mutations and cancers, bone marrow toxicity and occurrence of multi-drug resistant bacteria[15].

The present investigation was done to evaluate the distribution and concentration of tetracycline, trimethoprim and sulfonamide antibiotic residues in raw chicken meat and liver samples. We found that distribution of residual SCP, SDM and TRI antibiotics in chicken meat samples were 16.66%, 5.55% and 11.11%, respectively. Additionally, distribution of residual SCP, SDM and TRI antibiotics in chicken liver samples were16.66%, 5.55% and 11.11%, respectively.Distribution of STZ residue in chicken liver samples was 1.11%.Furthermore, the mean concentrations of SCP, SDM and TRI residual antibiotics in chicken meat samples were 20.8±1.88, 6.05±0.25 and finally 16.52±1.23 µg/kg, respectively. Moreover, the mean concentrations of SCP, SDM and TRI residual antibiotics in chicken liver samples were 9.25±56.90, 9.26±0.36 and finally 14.0±1.21 µg/kg, respectively. Additionally, the mean concentration of STZ in chicken liversamples was 19.1±0.14 µg/kg. In keeping with this, there were no detectable tetracycline residues in the studied samples. Tetracyclines are broad-spectrum antibiotics because they are active against both Gram-positive and -negative bacteria. Tetracycline supplemented feeds were extensively employed in the poultry industry to stimulate growth and egg production. Oxytetracycline is commonly used in livestock and poultry for prevention and treatment of various diseases. The main reason for the absence of tetracycline residues in studied samples is may be the fact that tetracyclines are not considered as an effective medication for controlling and treatment of poultry diseases in Iran. Additionally, its comprehensive use as growth promoting agent has been forbidden. The relevant organizations have imposed heavy fines for using tetracyclines in the poultry farms. Thus, it is not surprising that none of the studied samples were not harbored the tetracycline residues. Low concentration of residual tetracyclines have also been reported from South Africa (48.6±30.2 to 62.5±23.1 µg/kg) [16] and Turkey (17.2 to 19.9 µg/kg) [17]. Different organizations such as FAO, World Health Organization (WHO) and Food and Drug Administration (FDA) reported that the MRLs of tetracyclines in muscle, liver and kidney should be lower than 200, 300 and 600 µg/kg, respectively. Otherwise, Accepted Daily Intake (ADI) for human is recommended not to exceed than 3µg[18]. Thus, consumption of chicken meat and even liver samples studied in the presentresearch doesn't have any risk for transmission of antibiotics to humans. In keeping with this, higher concentrations of residual tetracyclines have been reported from Egypt [19], Bangladesh [20], Russia [21] and Iran [22].

Sulfonamides are synthetic antibiotics with a wide spectrum against most Gram-positive and many Gram-negative organisms. They are regularly used by veterinarians in chickens for therapeutic, prophylactic, or growth-promoting purposes and halt the growth of bacteria in animal production. They are also used to treat many kinds of infections caused by bacteria and certain other microorganisms such as infectious agents of digestive and respiratory tracts [9, 23]. Sulfonamides inhibit multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid in the folic acid metabolism cycle [9, 23]. Thus, it is widely used in veterinary science and especially poultry farms. Therefore, it is not surprising that some of the chicken meat and liver samples harbored considerable concentrations of sulfonamides, especially SCP, SDM, and STZ antibiotics. Trimethoprim was also detected in some chicken meat and liver samples of the present research. All detected concentrations of sulfonamides and trimethoprim were lower than MRLs announced by related organizations. Chitescu et al. [24] reported that the mean concentrations of sulfadiazine, SQX, SDM and SMX antibiotics in chicken samples collected from Romania were 97 to 312 µg/kg, 162 to 547 µg/kg, 115 to 456 µg/kg and 157 to 465 µg/kg, respectively which were higher than our findings. Mohameda et al. [25] reported that mean concentration of sulfonamide in samples collected from animals in Tanzania was 1320.9967±710.06372 µg/kg which was much higher than our record. Ramatla et al. [16] described that the mean concentrations of sulfonamides in chicken meat and liver samples collected from South Africa were 47.5±6.5 and 73.4±12.5 µg/kg, respectively which was relatively similar to our findings. The main reasons for absence of some kinds of antibiotic agents in examined samples are maybe their low prescription rate amongst the Iranian aviculture.

In keeping with the higher concentrations of detected residual antibiotics in chicken liver than meat samples, there were no statistically significant differences for detected concentrations between meat and liver samples. However, significantly higher concentrations of residual antibiotics in liver samples were reported previously [26-31]. Some kinds of antibiotics and especially tetracyclines, trimethoprim and sulfonamides diffuse throughout the body and found in highest concentrations in liver. Among the poultry tissues, liver contained the highest level of antibiotic residues in comparison to other samples. The order of sequences from the previous study was highest in liver followed by kidney, thigh muscles and breast muscle, respectively [20]. Liver is mainly act as detoxicated organ. Thus, higher concentrations of administrated antibiotics are neutralized and repelled by the liver during detoxification. Therefore, liver contained the highest level of antibiotic residues in comparison to other organs. Similar findings have been reported from Iran [32, 33], Bulgaria [34] and Egypt [35]. High prescription of antibiotic agents caused severe increase in the levels of antibiotic resistant-bacteria other than antibiotic residues [36-50].

Conclusion

The present study is the first report of detection of residual sulfonamides, especially SCP, SDM and STZ antibiotics in Iranian chicken meat and liver samples. Totally, 5.55 to 16.66% of chicken meat samples and also 1.11 to 16.66% of chicken liver samples harbored residual sulfonamides. Sulfonamides concentrationshad ranges from 6.05±0.25 to 24.40±1.54 µg/kg in chicken meat and liver samples which all were lower than acceptable MRLs. Trimethoprim concentrations had ranges from 14.00±1.21 to 16.52±1.23 µg/kg in chicken meat and liver samples which all were lower than acceptable MRLs. Higher concentrations of all detected sulfonamides and trimethoprim were also reported in chicken liver samples. Detection of residual antibiotics in chicken meat and liver samples even below the MRLs had a high importance with respect to occurrence of antibiotic resistance in bacterial strains. Using highly sensitive and specific LC-MS/MS is a practical approach for monitoring and surveillance of residual antibiotics in chicken meat and liver samples.

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Conflict of interest

Authors declare that they have no conflict of interest.

Funding statements

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