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Research Article

Sulfonamide Residue in the Broiler Chicken Circulating in Makassar

Muammar Fawwaz [1*], Rais Razak [1], Masdiana Tahir [1], Andi Permatasari [1], Andi Tenri Amula [1], Seniwati [2]

[1] Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Hasanuddin, Makassar, Indonesia.
* Corresponding Author. E-mail: muammar.fawwaz@umi.ac.id

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ABSTRACT: The use of sulfonamide related to drug residual in the product of broiler chicken, like meat and egg. This research aimed to determine the sulfonamides (sulfamerazine, sulfanilamide, and sulfadiazine) on the broiler chicken meat, breast, and egg from the three different slaughterhouses circulating in the City of Makassar. The sample was extracted by acetonitrile and acetone then concentrated. The extract and sulfonamides then analyzed by HPLC method using C_{18} reverse phased column, UV-Visible detector in the wavelength of 270 nm. The result of study shows that the chromatogram of sample breast and egg has similar retention time with sulfonamides as a standard reference. The breast sample contains sulfamerazine residues, sample B and C contains 0.014 and 0.005 % (w/w), respectively. The egg sample A and D contains sulfadiazine 0.045 and 0.108 % (w/w), respectively. Sample B contains sulfanilamide 0.053 % (w/w). Sample A and B is egg yolk, while sample D is egg whites. Based on the results obtained, this is not suitable for the rules set by JECFA of 0.1 mg/kg (10^{-5} % w/w) as well as the standard of SNI. It can be concluded that generally broiler chicken breast and egg circulating in the City of Makassar was contaminated by sulfonamide with high concentration.

KEYWORDS: Broiler chicken; HPLC; sulfamerazine; sulfanilamide; sulfadiazine.

1. INTRODUCTION

Sulfonamides regularly used by veterinarians in chickens for therapeutic, prophylactic and stop bacterial growth in animal production (Hela, 2003). The use of sulfonamide extensively as a result of it's their availability, and low cost has increased sulfonamide-resistant strains of bacteria (Cheong, 2010).

Use of sulfonamide continuously in chicken may result not only to prevent diseases but also in sulfonamide residues being present in the body of the chicken, and accumulate in various tissues, because this drug can be quickly absorbed and distributed (Kan, 2000; Weiss, 2007). As a consequence of its extensive usage, considerable attention has been paid to the potential risk of human health (Sutiak, 2000; Kozarova, 2002), due to their carcinogenic potency and possible antimicrobial resistance (Shao, 2005).

The withdrawal periods play an essential role to prevent contamination. Without observing withdrawal period, has made the poultry products unsafe for human health (Mehtabuddin, 2012). This study aims to determine the level of three sulfonamides, sulfamerazine, sulfanilamide, and sulfadiazine in chicken meat samples from slaughterhouses in the city of Makassar, Indonesia, by using High Performance Liquid Chromatography (HPLC). The result of study then compared with the Joint WHO / FAO Expert Committee on Food Additives (JECFA) and Indonesia National Standard (SNI) recommendation.

2. EXPERIMENTAL SECTION

2.1. Chemicals and standard solution

Acetonitrile and methanol (HPLC grade), whereas disodium hydrogen phosphate, and acetone were purchased from Merck (Darmstadt, Germany). Deionized water obtained through a Millipore-Q50 Ultrapure water system (Sartorius). The sulfonamide (sulfamerazine, sulfanilamide, and sulfadiazine) were purchased from Sigma (St. Louis, MO, USA). The stock solution (c = 50µg/mL) was prepared by dissolving 2.5 mg of all sulfonamide's standard with 50 mL of disodium hydrogen phosphate.

2.2. Sample collection

Total of samples consisted of four samples of meat, breast, egg yolk, and egg whites respectively were purchased from 4 slaughterhouses in the city of Makassar with random sampling method. The samples were immediately frozen to -20°C. Deep-frozen samples were kept at -20°C until analysis (Cheong, 2010).

2.3. Sulfonamide Extraction

The sulfonamide extraction was conducted out following the method described by Chitescu *et al.* (2017). Samples were cut into small portion and blended. An accurately weighed 10 g amount of sample was added 30 ml acetonitrile then homogenized with vortex for 1 minute, the solution was centrifuged at 3500 rpm for 10 minutes, the supernatant was stored in the container, while the precipitate was added 20 mL acetone and centrifuged at 3500 rpm for

10 minutes. The supernatant was fused with the previous supernatant and then evaporated at 40 ºC until forming a viscous extract (Chitescu, 2017).

2.4. HPLC analysis

The qualitative parameter is used retention time. By comparing the retention time of chromatogram of the sample solution with reference standard solution of sulfonamide in the same HPLC conditions (Fawwaz, 2016). Quantitative analysis of the sulfonamide standards (1.5; 2.0; 2.5; and 3.0 µg/mL) and samples (100 µg/mL) were conducted using an HPLC system equipped with HPLC column detector K-2501, pump K-1001 (Knauer 7125 series, Cotati-California, made in USA). The samples were held sonication for 20 minutes filtered through a disposable syringe filter (0.45 µm cellulose nitrate); the filtrates were directly infused into HPLC system. A Kromasil 100-5 C18 (150 x 4.6 mm) column was used for the separation of the sulfonamide that using disodium hydrogen phosphate solution 3 g/500 mL: methanol (75:25 v/v) as the mobile phase. The flow rate was fixed at 1.0 ml/min, and analysis was performed at room temperature. The injection volume was 60 µL, and ultraviolet detector wavelength of 270 nm was applied (Horii, 1990; Fawwaz, 2022).

2.5. Optimal wavelength detection

UV-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 270 nm. Therefore, it was used for the detection of sulfonamide residues in meat, breast, and egg in this study. A calibration standard curve for sulfonamide was obtained by running on HPLC and then plotting peak areas against concentrations. For the curve, the best fit of the line was calculated by the equation of line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept, and slope of the calibration curve was calculated. The best fit of data was determined by linear regression using the following equation: Y= bx + a, where:

Y = Peak area
b = Slope
x = Concentration
a = Intercept.

3. RESULTS AND DISCUSSION

Chicken is a popular food in Indonesian society. One of efforts to improve productivity by improving livestock health which is a priority in the poultry industry. Therefore, the use of drugs such as sulfonamide preparations for disease prevention and growth incentives is inevitable in optimizing poultry production. Uncontrolled use of drugs can cause residues in livestock products. Some of residues are carcinogenic, so it is very important for livestock product to meet food safety requirements (The European Agency for the Evaluation of Medicinal Product, 1995).

This study using HPLC method, which uses stationary phase is octadecyl-silica (ODS). It most widely used because of the ability to separate the compounds with various polarity. While the mobile phase used is disodium hydrogen phosphate solution, it means isocratic elution, with a wavelength of 270 nm UV detector, previously running on a spectrophotometer.

Table 1. Standard reference of sulfonamides and the residue in broiler chicken product

Conc. (µg/mL)	Sulamerazine		Sulfanilamide		Sulfadiazine		Conc. (% w/w)
	Area	Retention Time (min)	Area	Retention Time (min)	Area	Retention Time (min)	
1.5	93806	2.11	126581	1.81	110079	1.73	
2.0	126459	2.12	175105	1.83	154098	1.73	
2.5	160630	2.11	222014	1.83	189724	1.74	
3.0	190653	2.11	257372	1.84	233933	1.75	
Meat							
A, B, C	-	-	-	-	-	-	-
Breast							
A	94893	2.06	-	-	-	-	0.014
B	-	-	-	-	-	-	-
C	39445	2.07	-	-	-	-	0.005
Egg Yolk							
A	-	-	-	-	14966	1.70	0.045
B	-	-	7105	1.82	-	-	0.053
C, D	-	-	-	-	-	-	-
Egg Whites							
A, B, C	-	-	-	-	-	-	-
D	-	-	-	-	2401	1.70	0.108

Sulfonamide is used as a standard reference with various concentrations that can be seen in Table 1. The results of analysis on HPLC showed the same retention time of chromatogram between standard sulfonamides (Figure 1) with

samples, except meat in Figure 2 is not match with all standards. Chromatogram of sample breast and eggs in Figure 3 and 4 indicate there are some residuals of sulfonamides, which the detected sulfonamide type is sulfamerazine in breast, sulfadiazine, and sulfanilamide in egg (yolk and whites). The mean concentration of sulfonamide residues in breast and egg samples are given in Table 1. All of the detectable samples contain sulfonamide that exceeded the JECFA and SNI recommendation, which is not containing more than 0.1 mg/kg (10^{-5} % w/w) (Agency for Nasional Standarisation, 2000)

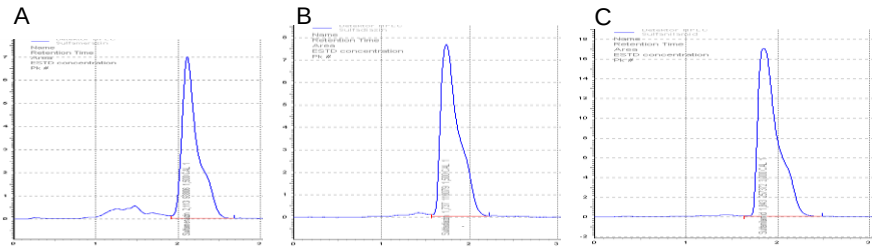

Figure 1. HPLC chromatogram of (A) sulfamerazine, (B) sulfadiazine, (C) sulfanilamide standard

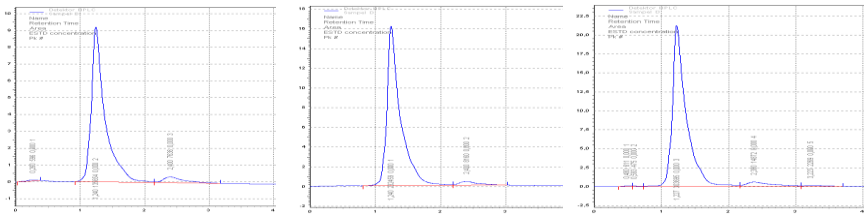

Figure 2. HPLC chromatogram of chicken meat

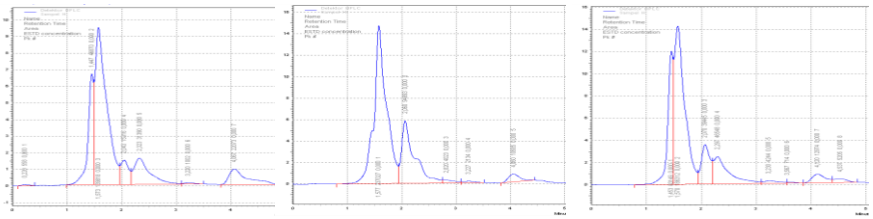

Figure 3. HPLC chromatogram of chicken breast

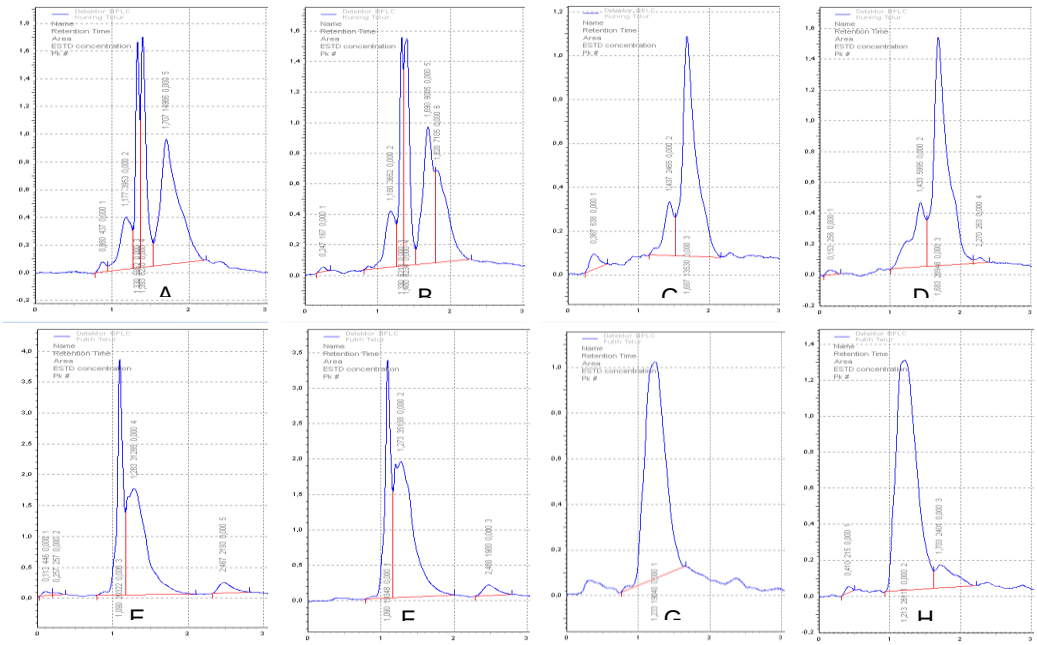

Figure 4. HPLC chromatogram of egg yolk (A,B,C,D) and whites (E,F,G,H)

These results showed that the use of sulfonamide in the farm is not well controlled, either the dosage used or the downtime of the drug before the chicken is taken to the slaughterhouse. Monitoring the use of sulfonamide is vital to avoid the presence of residuals in chicken products not only meat products but also eggs. After the downtime is exceeded, it is hoped that no drug residue or grade will be below the maximum limit value of the waste, so that the livestock product can be declared safe for consumption.

Sulfonamide downtime is 5-7 days from the data obtained from previous studies. Downtime is very important because it is the period of the last drug administration to cattle may be cut, or products such as meat, milk, and eggs should be consumed. After stopping time is exceeded, it is expected not be found drug residues or residues have been below the value of the maximum residue limit so that the public can declare livestock products safe for consumption (Aziz, 2008).

As a comparison to those reported in other countries, sulfonamides detected in chicken samples in Malaysia are considered low. The contamination rates of sulfonamides in the USA was published over 4% (Dey, 2003), while in Italy was smaller than 1%. Samples of poultry meat in Italy showed contamination of sulfaquinoxaline and sulfadiazine at 0.98-116.0 µg/kg and 0.64-21.0 µg/kg respectively (Weiss, 2007). Study on the drug residues, including sulfonamides, in poultry products in Nigeria showed contamination of 1% in eggs and 33.1% in broilers, 23.6% in slaughter and 4.8% in local chickens (Kabir, 2004). In Indonesia and other Asian countries, there was no sufficient report on sulfonamides occurrence for more realistic comparison (Cheong, 2010). However, consumers need to be conscious of the public health consequences of drug residues in chicken, and it might be useful to prepare guidelines for the uniform enforcement of residue control all over the country.

4. CONCLUSION

Study findings detect any evidence of misuse or abuse of the investigated drugs. Broiler chicken breast and egg samples were in violation of the regulation due to the presence of sulfamerazine, sulfanilamide, and sulfadiazine. This study clearly demonstrates that broiler chicken consumers in a part of Makassar are at high risk of exposure to high and non-tolerable drug residues of sulfonamide based on JECFA and SNI regulation. Therefore, need to perform comprehensive research to all parts of Makassar.

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