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COMPARATIVE EFFICACY OF FOUR DIFFERENT SOLID PHASE EXTRACTION CARTRIDGES USED TO DETERMINE TETRACYCLINE IN FOODS OF ANIMAL ORIGIN

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ABSTRACT

Four different types of Solid Phase Extraction (SPE) cartridges namely R-Biopharm (RB), Chromabond (CB), Isolute (IS) and Megabond (MB) were used in this study. Control and spiked samples of beef, chicken and shrimp were also used. Optical Density (OD) values of control and spiked samples were measured with the help of Enzyme Linked Immunosorbent Assay (ELISA) reader. Percent binding values for each SPE cartridges were calculated using OD values of respective control and spiked samples. Based on % binding values a comparative study of 4 different Solid Phase Extraction (SPE) cartridges was carried out. Analysis of variance indicated no significant differences among the % binding values considering different samples irrespective of SPE cartridges (P=0.821266, F = 0.201279) or different SPE cartridges irrespective of samples (P = 0.168119, F = 2.180932). From this study, it can be recommended that any of the four SPE cartridges available in the working area can be used for the analysis of tetracycline from foods of animal origin.

Key words: Solid phase extraction, ELISA, optical density, % binding

INTRODUCTION

The solid-phase extraction (SPE) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Analytical laboratories use different solid-phase extraction cartridges to concentrate and purify samples for analysis. Solid-phase extraction cartridge can be used to isolate analytes of interest from a wide variety of matrices including urine, blood, water, beverages, soil, and animal tissue (Guide to Solid Phase Extraction, 1998). Tetracyclines are broad spectrum antibiotics which are commonly used in veterinary medicine to prevent diseases and as an additive in foods of animal origin to promote growth (Carson *et al*., 1998; Moats, 2000; Schenck and Callery, 1998). These are widely used in dairy cattle, poultry and shrimp (Schenck and Callery, 1998; Tendencia and de la Pena, 2001). The use of antibiotics in animal feed helps increase the animal's ability to absorb feed and reach market weight on time. It may also counteract the effects of crowded living conditions and poor hygiene in intensive animal agriculture system (Environmental Media Services, 2000). Excessive use of these antibiotics may result in residues being present in various agricultural products such as meat, poultry, shrimp, honey, egg etc. Antibiotic residues remains in animal body even after slaughtering if there has been no enough time to their repel (Wilson *et al*., 2003). Residue of antibiotics in foodstuffs from animal origin could represent a hazard for the consumer of these products. To ensure food safety to consumers, EU, FAO/WHO and FDA have set safe levels for residue tetracyclines in milk (0.1 mg/kg) (Schenck and Callery, 1998), liver (0.3 mg/kg), egg (0.2 mg/kg) and muscle tissue (0.1 mg/kg) (Cooper *et al*., 1998). A sensitive and reproducible method named enzyme-linked immunosorbent assay (ELISA) were used in this case. The aim of the study was to compare the efficiency of several SPE cartridges for tetracycline determination from animal tissue by ELISA and to mark out optimal experimental conditions that can later be applied for screening and the quantification of the veterinary drug residue analysis.

MATERIALS AND METHODS

Sample Type

Control samples of muscle tissue of beef, chicken and shrimp were obtained from International Atomic Energy Agency (IAEA), Vienna, Austria. Tetracycline hydrochloride (21.6 mg) was taken in a 20ml volumetric flask and methanol was added up to the mark to prepare stock solution (1mg/ml). The stock solution was then diluted

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with distilled water to the desired concentration. Citric acid monohydrate (12.9g), Na_2HPO_4 (10.9g), EDTA sodium salt (37.2g) was taken in a 1000ml volumetric flask and distilled water was added up to the mark. The pH was adjusted with saturated citric acid. NaCl (9.0g), Na_2HPO_4 x $2H_2O$ (2.85g), NaH_2PO_4 x H_2O (0.55g), Tween 20 (1ml) was taken in a 1000ml volumetric flask and distilled water was added up to the mark. The pH was adjusted with sodium base. Methanol (100%) was added with 20mM oxalic acid (1.8 g/l) or oxalic acid dehydrate (2.522 g/l).

ELISA test kit and microplate reader

Ridascreen tetracycline ELISA test kit was purchased from R-Biopharm AG (Darmstadt, Germany), kit lot no. 14420, Art. No. R3503. Test kit contained microplate, tetracycline standard solution, tetracycline-conjugate, anti-tetracycline-antibody, red chromogen, stop solution, buffer and washing buffer. The microplate reader machine was Thermo Scientific Multiskan FC.

Solid Phase Extraction (SPE) cartridges

Four different types of SPE cartridges namely R-Biopharm (RB) (100mg), Machery-Nagel Chromabond C18ec (CB) (3ml/200mg), Biotage Isolute C18ec (IS) (6ml/1g) and Mega Bond Elute C18 (MB) (6ml/1g) were used.

Preparation of control and spiked samples

Eight control samples each from beef, chicken and shrimp were taken. The samples were homogenized with a rod homogenizer (Ultra turrax) and 5g each were taken into 50ml separate centrifuge tubes. Four of each from beef, chicken and shrimp were used as control. Five hundred µl oxytetracycline dihydrate solution (100µg/kg) was added in rest 4 tubes from beef, chicken and shrimp that were used as spiked samples.

Sample processing

Twenty five ml Mcllvaine buffer was added in each tubes, vortexed well and used for refrigerated centrifugation (4000g/15^{o}C/10min.). The supernatant was filtered into 50ml separate test tube using folded filter paper and the extraction procedure was repeated using same buffer for one more time. Again the supernatant was filtered into the respective tubes.

Solid Phase Extraction (SPE)

Supernatant of one control and one spiked from beef, chicken and shrimp were used for four different SPE cartridges. They were placed on to suction chamber connected to a pump and the pressure raised to 7 inches Hg. They were conditioned with 2ml methanol (100%), 2ml distilled water was added and allowed to pass. Five ml supernatant was put on the respective cartridges, 3ml distilled water was added and allowed to pass. Vacuum was reduced to 4 inches Hg with vessel controller. Two ml elution solution was added, eluents were collected in separate glass tubes and vortexed. Hundred µl eluent was taken into glass tube (12x75mm) and 400µl kit buffer was added. Fifty µl of this solution was taken for ELISA. It was done for all control and spiked samples.

ELISA test procedure

Fifty µl from each tubes were pipetted into corresponding wells. Fifty µl tetracycline antibody solution was added to each well. The plate was covered, mixed gently and incubated at room temperature for 1 hour without shaking. The microplate was washed with washer (Well wash, Thermo Scientific) 3 times with 250µl washing buffer. Hundred µl enzyme conjugate solution was added to each well. The plate was covered, mixed gently and incubated at room temperature for 15 minutes. Again the plate was washed as before. Substrate/chromogen solution of 100µl was added to each well. The plate was covered, mixed gently and incubated at room temperature for 15 minutes. Hundred µl stop solution was added to each well and mixed gently. Finally the measurement was made photometrically at 450nm.

RESULT AND DISCUSSION

Table 1 summarizes the OD values of control and spiked sample (beef, chicken and shrimp) of four different catridges. The % binding values were also calculated to interpret the result in Analysis of Variance which was used for comparing different SPE catridges that will be used for determination of tetracycline. The % binding

values showed not much variations for the four different cartridges. It was calculated using the following formula: % Binding = (OD of spiked/OD of control) x 100.

Table 1. Optical Density (OD) and % Binding values with 4 different cartridges for beef, chicken and shrimp (control and spiked)

SPE Cartridges	Beef			Chicken			Shrimp		
	OD values		% Binding	OD values		% Binding	OD values		% Binding
	*C	*S		*C	*S		*C	*S	
RB	1.566	0.968	61.81	1.620	0.984	60.74	1.577	0.964	61.12
CB	1.572	1.028	65.39	1.441	0.943	65.44	1.499	1.090	72.71
IS	1.304	0.815	62.50	1.323	0.893	67.49	1.203	0.737	61.26
MB	1.615	1.080	66.87	1.686	0.892	52.90	1.485	0.846	56.96

* C = Control, S = Spiked

Considering different samples irrespective of cartridges, analysis of variance indicated no significant differences (P=0.821266, F = 0.201279) among the % binding values (Table 2). Also considering different cartridges irrespective of samples, analysis of variance indicated no significant differences (P = 0.168119, F = 2.180932) among the % binding values (Table 2).

Table 2. Calculated P and F value from % binding value using ANOVA

Considering different samples with SPE cartridges constant	Considering different SPE cartridges with samples constant
P = 0.821266, F = 0.201279	P = 0.168119, F = 2.180932

However, it could be concluded that whatever SPE cartridges used, there will be no significant differences among the results. It is also recommended that the cartridges (especially among these four types) which are available in the working area would be used for the analysis of tetracycline in foods of animal origin.

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