Supercritical Fluid Extraction Compared with Solvent Method for Incurred Sulfamethazine in Chicken Eggs

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ABSTRACT

To obtain eggs containing "normally incurred" sulfamethazine (SMZ), 10 hens were fed a single dose of 75 mg/kg SMZ by capsule. The amount of SMZ in some of the eggs (n = 21) was determined by two extraction methods, supercritical fluid extraction (SFE) and solvent extraction. The mean SMZ values ranged from 0.10 to 0.78 ppm, with an overall mean of 0.32 ppm and no difference ($p>0.05$) was found between the methods. However, SFE provided a distinct advantage over other methods since the amount of sample manipulation and solvent use and disposal was minimal. Determination of SMZ in the normally incurred eggs over an 8-day period by SFE showed that levels reached a maximum after the first day, then declined slowly. However, SMZ exceeding 0.10 ppm still occurred 5 days after dosing.

Key Words: Supercritical fluid, extraction, eggs, sulfamethazine

INTRODUCTION

SULFONAMIDES ARE USED IN CATTLE, SWINE and poultry and sulfamethazine (SMZ) has the highest number of samples above the tolerance level (0.1 ppm) of all the sulfonamides (Matusik et al., 1990). Because of its widespread use and potential transfer into the food supply, there are health concerns about SMZ residues in foods. Such concerns include the acquisition of antimicrobial resistance that makes this drug less effective in treating humans (Franco et al., 1990), and its purported carcinogenicity (Littlefield, 1988). Because of the lack of effective screening methods for sulfonamides in eggs, and the need to reduce the use of organic solvents, we developed a supercritical fluid extraction (SFE) method for the isolation of sulfonamides, including SMZ, in fortified eggs (Pensabene et al., 1997). Recovery of SMZ from fortified eggs was 99.5% at the 0.10 ppm level, but binding characteristics in a normally incurred sample may be different than that in a fortified sample. The SMZ recovery from liver tissue was lower than from eggs, suggesting that the binding characteristics between the two substrates was different (Parks and Maxwell, 1994). Therefore, our objective was to determine the amount of SMZ present in eggs by both SFE and by a solvent extraction method, and the changes in levels of SMZ present in incurred eggs for up to 8 days post feeding.

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MATERIALS & METHODS

Eggs

Ten white leghorn hens were fed a single dose of 75 mg/kg SMZ by capsule. The eggs were collected over a period of 8 days after dosing. When the hen produced more than one egg per day, the eggs were labeled AM or PM. The entire egg from each hen was homogenized, ca. 40g, transferred to a plastic centrifuge tube, frozen, and shipped on dry ice from the FDA's Center of Veterinary Medicine, Laurel, MD, to the USDA's Eastern Regional Research Center, Wyndmoor, PA, by overnight freight. The egg samples were stored at -85°C until analyzed. Only eggs from hens that produced a single egg per day were used in the study.

SFE

Complete details have been described (Pensabene et al., 1997). Briefly, 1.0g of egg was mixed with Hydromatrix (Celite 566, Applied Separations, Allentown, PA) and added to an extraction vessel containing neutral alumina sorbent for trapping SMZ in-line. The sample was extracted at 40°C with supercritical CO_2 at 680 bar and a flow rate of 3.0 L/min (expanded gas), to a total volume of 120 L. SMZ was eluted, post SFE, using 4 mL of the HPLC mobile phase (65% phosphate buffer-35% methanol), followed by separation on a HPLC system using a C_{18} column and UV detection at 265 nm.

Solvent extraction

Details of this procedure have been described (Ikai et al., 1991). Briefly, 1.0g of egg was mixed with 2.0g of sodium sulfate and 10 mL ethyl acetate in a centrifuge tube, homogenized and centrifuged. The ethyl acetate was decanted and the extraction was repeated once more. The combined ethyl acetate extract was applied to a 3 mL Bakerbond SPE amino cartridge, (J.T. Baker, Phillipsburg, NJ) and the cartridge washed with 5.0 mL hexane. The SMZ was eluted from the cartridge with 4.0 mL acetonitrile-0.02M aqueous phosphoric acid (24:76), and determined by HPLC.

Statistical analysis

Data were analyzed by two-way ANOVA using the GLM procedure of the Statistical Analysis System PC software (SAS Institute, Inc. 1985). Significance was defined at $p \leq 0.05$.

RESULTS & DISCUSSION

THE ACCURACY OF THE SFE METHOD HAS been determined (Pensabene et al., 1997) by adding to egg samples known amounts of SMZ, sulfadimethoxine and sulfaquinoxaline at 0.05, 0.10 and 0.50 ppm. Recovery of SMZ at these levels was 86.7%, 99.5% and 99.3%, respectively. However, the potential variation in recovery due to sulfonamide-protein binding may be different with normally incurred samples. Therefore, 21 duplicate samples of homogenized whole egg from hens fed a sin-

Table 1—Comparison of SFE and solvent extraction methods for determining sulfamethazine in incurred eggs

Sample no.	Sulfamethazine, ppm[a] SFE	Solvent
1	0.17	0.18
2	0.17	0.17
3	0.27	0.27
4	0.28	0.28
5	0.17	0.14
6	0.20	0.21
7	0.18	0.21
8	0.28	0.29
9	0.21	0.19
10	0.12	0.12
11	0.29	0.30
12	0.10	0.10
13	0.10	0.10
14	0.34	0.35
15	0.19	0.23
16	0.72	0.78
17	0.58	0.60
18	0.38	0.39
19	0.44	0.43
20	0.72	0.71
21	0.71	0.72

[a]Average of duplicate determinations

Table 2—Analysis of variance of SFE and solvent extraction data

Source	Degrees of freedom	Sum of squares	Mean square	F value
Samples	20	3399994.08	169999.70	839.56[a]
Methods	1	453.33	453.33	2.24[b]
Sample × Method	20	9435.26	471.76	2.33[c]
Error	42	8504.45	202.49	
Total	83	3418387.12		

[a]$p < 0.01$
[b]No significant difference
[c]$p < 0.05$

Table 3—Sulfamethazine in incurred whole egg samples by SFE

Days after feeding	ppm	
	Range	Mean[a]
0	0.06 - 3.80	1.24
1	5.54 - 25.58	15.56[b]
2	11.58 - 21.50	13.94
3	0.38 - 0.72	0.59
4	0.17 - 0.34	0.23
5	0.10 - 0.29	0.17
6	0.04 - 0.14	0.09
7	0.04 - 0.11	0.06
8	N.D. - 0.04	0.02

[a]n=6 different hens
[b]n=2 hens

gle dose of SMZ were analyzed for SMZ by SFE and by a solvent extraction method, and quantitated using the same HPLC-UV detection conditions (Table 1). Prior to the start of the method comparison, a single egg sample from a randomly selected hen from each day was analyzed to determine the range of SMZ values to be expected. Thus, only eggs from day 3 to day 5 after feeding were used for comparison since the SMZ levels were in the range most likely to be encountered in normally incurred eggs. SMZ levels ranged from 0.10 to 0.72 ppm by SFE, and 0.10 to 0.78 ppm by solvent extraction, both with an overall mean of 0.32 ppm (Table 2). A highly significant difference was found among samples, as would be expected from eggs collected on different days, along with a significant sample by method interaction. No difference ($p > 0.05$) was found between the two methods of analysis. The standard deviation (SD) for SFE was 12.2 ppb with a coefficient of variation (CV) of 3.9% compared to SD 16.0 ppb and CV 5.0% for the solvent extraction method. Thus, this SFE method could isolate SMZ from the egg matrix without the need for organic modifiers as effectively as a solvent extraction method. Combs et al. (1997) extracted sulfonamides from egg using SFE, but because they used an extraction pressure of only 490 bar compared to 680 bar in our method, an organic modifier was required to obtain good recoveries.

To determine the residual levels of SMZ in eggs from hens fed a single dose, eggs from the same 6 hens/day for up to 8 days after withdrawal were analyzed by SFE (Table 3). SMZ levels were highest 1–2 days following administration of SMZ (range 5.54–25.58 ppm). By the third day after withdrawal, SMZ levels dropped from an average 13.9 ppm to 0.59 ppm. This decrease continued over the next several days until the average concentration of 0.02 ppm was detected on day 8. Factoring differences in dosage levels, these results confirmed previous studies examining SMZ transfer into eggs. Both Geertsma et al. (1987) and Paulson et al. (1983) dosed hens with a single oral dose of 100 mg/kg SMZ in gelatin capsules. Geertsma et al. (1987) detected maximum SMZ concentrations of 20.1 to 37 ppm, declining to less than 0.03 ppm seven days after dosage. Paulson et al. (1983), utilizing C^{14} Labeled SMZ, detected maximum levels of 33 ppm equivalents SMZ (whole egg basis), 32 to 56 h after dosing, which declined to detection level of 1 ppm, 8 days after dosing. Roudaut (1983) dosed hens with 1 g/L for 5 days in drinking water (corresponds to 81 mg/kg/day), and detected maximum concentrations ranging from 11 to 46 ppm within the dosing period and the first day after dosing, with a decline to 0.02 ppm 8 days after dosing.

CONCLUSIONS

COMPARISON OF THE SFE METHOD WITH A solvent extraction method showed that SFE could be used to effectively isolate SMZ from whole eggs, used less solvent, and required less sample manipulation than the solvent extraction method. This is important since EPA guidelines mandate reduction of solvent use. Also, SMZ results in incurred eggs were similar to results reported by other researchers, indicating that a level of 0.1 ppm SMZ could be detected in eggs 5 days after dosage.

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Ms received 6/9/97; revised 9/19/97; accepted 9/22/97.

We thank Judith M. Foster for technical assistance. Mention of brand or firm names does not constitute an endorsement above others of a similar nature not mentioned.