Extraction and Detection of Sulfamethazine in Spray-Dried Milk

SHAHANA MALIK and SUSAN E. DUNCAN
Department of Food Science and Technology

J. RUSSELL BISHOP
University of Wisconsin
Center for Dairy Research
Madison 53706

LARRY T. TAYLOR
Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg 24061

ABSTRACT

Processes that reduce moisture content of fluid milk may result in a high concentration of animal drug residues that are undetectable in the fluid milk on the basis of the same weights. The objectives were to determine the amount of sulfamethazine in spray-dried milk powder manufactured from fluid milk contaminated with sulfamethazine and to determine the effectiveness of supercritical fluid extraction as a means to extract sulfamethazine from dry milk powder. Fluid whole (3.25% fat) and skim milks with sulfamethazine added at concentrations of 5, 10, and 100 ppb were spray-dried. Based on total solids, observed concentrations were 493 and 523 ppb in skim and whole dry milk powders, respectively, compared with fluid milk containing 100 ppb of sulfamethazine as determined by HPLC. The increase in sulfamethazine concentration from fluid to dry milk was also measured quantitatively by a microbial receptor assay and an ELISA. Poor recoveries and variability in data were possibly due to binding of sulfamethazine to undetermined milk components. Dry milk powder with measured concentrations of sulfamethazine was treated with supercritical CO_2. Sulfamethazine was not detectable in the extracted dry milk powder by microbial receptor assay or ELISA.

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(**Key words**: drug residues, milk powder, sulfamethazine, supercritical fluid extraction)

Abbreviation key: **EIA** = enzyme immunoassay, **MRA** = microbial receptor assay, **SFE** = supercritical fluid extraction, **SMZ** = sulfamethazine.

INTRODUCTION

Publicity about animal drug residue contamination in fluid milk has alerted dairy manufacturers to the necessity of testing milk for contamination by residues other than β-lactams. Charm et al. (5) reported that 70% of market samples and tanker raw milk samples tested in the northeastern US were contaminated with sulfonamides >25 ppb; sulfamethazine (**SMZ**) was the major contaminant. Sulfamethazine is a suspected carcinogen (12), and concentrations >10 ppb of SMZ, the FDA-specified level of concern ("safe" level), guarantee regulatory action (9). Use of milk or dairy products with SMZ contamination >10 ppb in other food products is considered to be adulteration.

Manufacturing processes that reduce moisture content of fluid milk may result in high concentrations of previously undetectable animal drug residues. Surplus milk is frequently processed into various forms, such as dry milk powder and evaporated or condensed milk. Surplus milk is dried and later used during low production periods to standardize milk and to keep cheese production constant year round (17). Dry milk powder is used commonly in the manufacture of ice cream, yogurt, confectioneries, and desserts. Use of contaminated

dry milk powder results in an adulterated product.

Spray drying concentrates the solid matter in milk by removing moisture. Sulfamethazine, with a melting point range of 178 to 207°C (11), is not destroyed by heat or moisture (2). Hypothetically, undetectable SMZ in fluid milk would be concentrated by removal of moisture, as in the spray-drying processes. However, when the fluid source milk has no detectable drug residue, processed dairy products are not routinely tested for drug residues.

The objectives of this study were 1) to determine whether dry milk powder has greater SMZ concentrations, on a dry weight basis, than fluid milk contaminated with SMZ (from which the powder was manufactured), and 2) to determine the potential for removal of SMZ from contaminated dry milk powder using supercritical fluid extraction (**SFE**) by CO_2.

MATERIALS AND METHODS

Preparation of Samples

Commingled, uncontaminated raw milk was obtained from three first lactation cows previously untreated with antibiotics at the Virginia Polytechnic Institute and State University dairy farm. Milk was separated, a portion was retained for skim milk (.05% fat), and the remaining milk was standardized to 3.25% fat and homogenized. Sulfamethazine was added to skim and whole milk samples at 0 (control), 5, 10, and 100 ppb. Control and treatment samples were batch pasteurized at 63°C for 30 min and stored at 4°C for 1 to 4 d prior to drying. Samples were dried (Buchi mini-spray drier model 190 supplied by Brinkman Instruments, Westbury, NY) at an inlet temperature of 180 ± 2°C and outlet temperature of 100 ± 2°C.

Chemical Analyses

Analyses of fat and total solids were by standard methods (16). Qualitative and quantitative determination of SMZ were completed on fluid milk samples and on reconstituted (10%) dry milk powder because methodologies were not developed for dry samples.

The qualitative analysis of SMZ in samples was completed with an ELISA (Cite® sulfa trio test; IDEXX Corp., Portland, ME). This assay is sensitive to SMZ concentrations as low as 5 ppb (1).

The quantitative analysis of added SMZ in fluid milk and reconstituted dry milk powder was by HPLC, a rapid screening microbial receptor assay (**MRA**) (Charm II® penicillin assay Inc., Malden, MA), and a competitive enzyme immunoassay (**EIA**) method (LacTek® SMZ milk screening kit; Idetek, Inc., San Bruno, CA).

The quantitative analysis of SMZ by MRA was based on standard curves developed from standards included in the kit provided by the manufacturer. The MRA was modified based on recommendations from penicillin assays (1990, personal communication): 1) the rinse step was eliminated, and 2) the tube was drained and wiped dry with the cotton swab. The quantitative analysis of SMZ, by competitive EIA was based on a standard curve developed from SMZ standard dilutions and conducted following directions for the assay as described with the kit provided by the manufacturer.

The MRA and EIA were not developed as quantitative methods, but both provided numerical data that have potential application. As qualitative methods, MRA has a minimum level of detection of ≤5 ppb of SMZ, and EIA has a minimum level of detection of ≤10 ppb of SMZ (1).

Quantitative Analysis by HPLC. The extraction of SMZ followed the FDA-approved procedure of Weber and Smedley (20) with modifications. A 10-ml sample was extracted with 50 ml of chloroform:acetone (2:1, vol/vol) in a 125-ml separatory funnel. The solution was shaken (1 min), vented, shaken (1 min), and vented, and two layers were allowed to separate for 1 min. The process was repeated; 5 min were allowed for the second separation of layers. The extraction solution was drawn off and filtered. A second extraction of the sample using 25 ml of extraction solution was completed and added to the first extraction. The filter paper was rinsed twice with 5 ml of extraction solution, and total chloroform: acetone extract was evaporated to dryness on a rotary evaporator at 32 ± 2°C. One milliliter of .1 *M* potassium dihydrogen phosphate was ad-

ded to a pear-shaped flask and agitated vigorously for 1 min on a vortex mixer. Five milliliters of hexane were immediately added, and the solution was agitated again for 1 min. The layers were separated for a minimum of 15 min, and the aqueous layer was removed with a Pasteur pipet. The aqueous layer was filtered through an acrodisc (.2 μm; Gelman Sciences, Ann Arbor, MI) attached to a 1-ml syringe into a sample vial. The sample was capped and stored at 10°C until injection. A Waters HPLC (ALC Model M-6000 A; Waters Associates, Inc., Milford, MA) equipped with UV detector (Model 440; Waters Associates, Inc.) and Hewlett-Packard 3390A Integrator (Hewlett-Packard, Avondale, PA) were used for analyses of dilutions at 5, 10, 20, 40, and 100 ppb from the SMZ standard (Sigma Chemical Co., St. Louis, MO), and samples were extracted on a Phenomonex Bondclone 10 C-18 300- × 3.9-mm LC column (Torrance, CA) maintained at 35 ± 2°C with a Phenomonex Bondclone 10 C-18 guard column, 30- × 3.9-mm. The solvent system was a 76:24 (vol/vol) ratio of .1 *M* KH_2PO_3 to methanol at an isocratic flow rate of 1.5 ml/min with UV detection at 254 nm.

Supercritical Fluid Extraction

Supercritical fluid extraction of SMZ from 10 g of dry skim milk powder used CO_2 on an SFE screening system (Newport Scientific, Inc., Jessup, MD) with a 300-ml extraction vessel. Extraction conditions were 50°C and 387 kg/cm^2 (5500 psi). Supercritical CO_2 was passed for 20, 60, and 120 min through the sample of dry milk powder manufactured from fluid milk with SMZ added at 10 ppb, and the extract was trapped in a glass U tube. Dry milk samples from the extraction vessel were analyzed before and after SFE using EIA, MRA, and ELISA.

Calculation of SMZ Concentration in Dry Milk Powder

Approved methodology for direct detection of drug residues in dairy products is not available. Therefore, the SMZ concentration in dry milk powder was determined indirectly based on the concentration of SMZ in 10% reconstituted dry milk powder. The observed SMZ concentration (parts per billion) in the reconstituted milk was multiplied by the solids (grams) in the 10-g sample of powder to yield the calculated SMZ concentration in the dry milk powder.

Statistical Analyses

Standard curves for each of the quantitative SMZ procedures were obtained using the general linear models procedure (SAS Institute, Inc., Cary, NC) to obtain a best fit line. Paired *t* tests were used to compare calculated concentrations of SMZ in dry milk powder and in fluid milk from which powder was manufactured for whole and skim milks. Standard errors of means were used to determine variability among replications.

RESULTS AND DISCUSSION

Monitoring drug residue contamination in raw fluid milk is essential to maintaining a residue-free fluid milk supply for human consumption. Processed dairy products are not routinely tested for drug residues because the raw milk supply used for processing the product is routinely tested, and detected residues in the fluid milk must be at or below the FDA-specified level of concern (9).

In the US, "safe" levels for drug residues in processed dairy products are not identified because no AOAC-approved test methods exist for determination of drug residue concentration in the final product. No acceptable level of SMZ exists in processed dairy products, however, because SMZ is a suspected carcinogen. Legal action of the FDA based on detection of the residue in the final product is not possible without validated methods. Further investigation of a product may be warranted, however, when three different methods indicate drug residue contamination in the product because the probability of three false positive readings is remote (FDA Milk Safety Branch, 1993, personal communication).

During the drying process, inert compounds, such as SMZ, remain in the final product with the milk solids. Results are discussed based on comparisons only between recovered observed concentrations, not between those concentrations and concentrations (5, 10, and 100 ppb) originally added to the samples.

Analytical Detection of SMZ in Fluid and Dry Milks

HPLC. The modified HPLC method was used to measure quantitatively the SMZ added to skim and whole fluid products and in dry milk samples manufactured from the fluid products (Table 1). At an added SMZ concentration of 100 ppb, mean recovered concentrations of fluid and calculated concentrations in dry milk samples were significantly different ($P \leq .05$). The calculated SMZ concentrations in nonfat and whole dry powders were 492.9 and 522.7 ppb, respectively, compared with 51.6 and 74.6 ppb of SMZ in skim and whole fluid milk samples from which the powder was manufactured. The extraction method yielded mean recoveries of 52 and 75% for skim and whole fluid milk samples, respectively. The drying process did not increase recovery of SMZ, but the concentration in a given sample of dry milk powder was significantly greater than in fluid raw milk from which it was manufactured. Recoveries were low in previous work on SMZ determination in milk (20) and in swine liver (13) by the HPLC method.

Recovery of SMZ from fluid and reconstituted dry samples at 5 and 10 ppb was highly variable. A primary problem encountered with the modified HPLC method was the poor elution behavior of the extracted analyte at 10 ppb (20).

MRA. The MRA analyses for SMZ in skim and whole fluid and dry milk samples were much easier and less time-consuming to conduct than the HPLC method. At all concentrations of added SMZ, differences in concentration of recovered SMZ in fluid and dry milk samples were not significant ($P > .05$) because of high variability in results (Table 2). Variability in results could be attributed to the nonlinear standard curve at >20 ppb (not shown). Recoveries at 5 and 10 ppb ranged from 64 to 99% because the standard curve was linear at <10 ppb. At 100 ppb, recovery was 70% in skim fluid milk samples, but only 23.7% in whole milk. The higher fat content in the whole milk apparently interfered with the sensitivity of the assay at 100 ppb. The presence of inactive SMZ metabolites at higher SMZ concentrations may have increased variability, because inactive metabolites do not bind to receptor sites in cells and are not detected by the MRA (4). As with the HPLC method of evaluation, results from the MRA analyses demonstrated high SMZ concentrations in dry milk powder.

TABLE 1. Recovery of added sulfamethazine (SMZ) from fluid skim and nonfat dry milks and from fluid whole and dry milks by HPLC.

Concentration of added SMZ	Skim milk				Whole milk			
	Fluid[1]		Dry[2]		Fluid[3]		Dry	
(ppb)	(recovered ppb)							
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
5.0[4]	6.7	1.5	53.2	32.3	16.5		90.4	
10.0[5]	10.2		67.4		13.0	5.6	70.9	14.7
100.0[6]	51.6[a]	5.3	492.9[b]	40.0	74.6[a]	11.7	522.7[b]	30.1

[a,b]Mean comparisons were made by paired *t* test analyses on observed and calculated SMZ concentrations between fluid and dry products for skim and for whole milks, on observed SMZ concentrations in skim and whole fluid milks, and on calculated SMZ concentrations in skim and whole dry milks. Means within a row with different letters are significantly different ($P \leq .05$).

[1]Pasteurized skim fluid milk.

[2]10% Reconstituted for analyses. Results are reported as calculated SMZ in dry product. Calculated SMZ concentration (parts per billion) = (observed SMZ concentration in reconstituted product) × (grams of solids in 10 g of dry milk powder).

[3]Homogenized, pasteurized whole fluid milk.

[4]Mean and standard error of two replicates for skim fluid and dry milk samples. Whole milk samples based on a single value.

[5]Skim milk samples based on a single value. Mean and standard error of two replicates for whole fluid and dry milk samples.

[6]Mean and standard error of three replicates for skim and whole fluid and dry milk samples.

TABLE 2. Recovery of added sulfamethazine (SMZ) from fluid skim and nonfat dry milks and from fluid whole and dry milks by microbial receptor assay.

Concentration of added SMZ	Skim milk				Whole milk			
	Fluid[1]		Dry[2]		Fluid[3]		Dry	
(ppb)	(recovered ppb)							
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
5.0[4]	3.2[a]	3.1	28.2	25.9	3.2	1.5	21.7	16.6
10.0[5]	9.9	4.8	94.9	46.6	6.2	3.5	65.2	32.8
100.0[5]	70.0	47.0	805.8	422.8	23.7	9.7	188.0	89.6

[a]Mean comparisons were made by paired *t* test analyses on observed and calculated SMZ concentrations between fluid and dry products for skim and whole milks, on observed SMZ concentrations in skim and whole fluid milks, and on calculated concentrations in skim and whole dry milks. Means are not significantly different ($P \geq .05$).

[1]Pasteurized skim fluid milk.

[2]10% Reconstituted for analyses. Results are reported as calculated SMZ in dry product. Calculated SMZ concentration (parts per billion) = (observed SMZ concentration in reconstituted product) × (grams of solids in 10 g of dry milk powder).

[3]Homogenized, pasteurized whole fluid milk.

[4]Mean and standard error of two replicates for skim and whole fluid and dry milk samples.

[5]Mean and standard error of three replicates for skim and whole fluid and dry milk samples.

Quantitative EIA Method. The EIA method was equivalent in ease of use and time requirements to the MRA method and easier than the HPLC method. Quantitative data were also variable with the EIA method. The curve of ratio (a:b; a = absorbance of sample, and b = absorbance of control) versus concentration of SMZ was the standard for quantification of SMZ (not shown). Results were not very consistent because of the type of assay and its

TABLE 3. Recovery of added sulfamethazine (SMZ) from fluid skim and nonfat dry milks and from fluid whole and dry milks by competitive enzyme immunoassay.

Concentration of added SMZ	Skim milk				Whole milk			
	Fluid[1]		Dry[2]		Fluid[3]		Dry	
(ppb)	(recovered ppb)							
	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE	$\bar{X}$	SE
5.0[4]	9.6[a]	3.2	91.4[b]	3.7	8.6	2.1	41.8	21.0
10.0[5]	18.2[a]	1.5	162.8[b]	27.9	14.8[a]	2.4	147.1[b]	44.3
100.0[5]	103.5[a]	45.9	1229.4[b]	93.4	70.4[a]	20.4	624.4[c]	168.7

[a,b]Mean comparisons were made by paired *t* test analyses on observed and calculated SMZ concentrations between fluid and dry products for skim and whole milks, on observed SMZ concentrations in skim and whole fluid milks, and on calculated SMZ concentrations in skim and whole dry milks. Means within a row with different letters are significantly different ($P \leq .05$).

[1]Pasteurized skim fluid milk.

[2]10% Reconstituted for analyses. Results are reported as calculated SMZ in dry product. Calculated SMZ concentration (parts per billion) = (observed SMZ concentration in reconstituted product) × (grams of solids in 10 g of dry milk powder).

[3]Homogenized, pasteurized whole fluid milk.

[4]Mean and standard error of two replicates for skim and whole fluid and dry milk samples.

[5]Mean and standard error of three replicates for skim and whole fluid and dry milk samples.

relative lack of sensitivity (Table 3). Again, mean SMZ concentrations in dry milk at 5, 10, and 100 ppb were significantly higher ($P \leq .05$) than mean SMZ concentrations in fluid milks. Because milk is a complex mixture, it can react nonspecifically with the reagent to cause variability in the assay (6). The SMZ concentrations recovered were higher than those added to the milk, suggesting that another component in the milk may also compete for active sites.

Variability in recoveries can be attributed to the complexity of the milk system. Researchers using different biological systems observed variability in assessment of SMZ by EIA methods. Singh et al. (19) also observed recoveries of 97.6 to 112.5% (mean recovery, 103%) using the EIA method for determination of SMZ in swine plasma. In contrast, recoveries were 62 to 73% in a study using EIA to screen SMZ and its metabolites in swine blood (.01 ppm) (8). The discrepancy of recovery among different types of test methods could be explained by differences in sensitivity and detection time of each test and by the presence of inhibitors other than SMZ. Carlsson and Bjorck (3) observed the discrepancy of positive and negative results of two different tests.

Each of these methods (HPLC, MRA, and EIA) demonstrated that the apparent concentration of SMZ in dry milk made from contaminated fluid milk is much higher than the 10-ppb safe level. Recoveries lower than expected were possibly due to the binding effect of SMZ with milk components. Dried milk powder manufactured from fluid milk with SMZ concentrations at or below the safe level established by FDA will have high concentrations of SMZ.

Qualitative ELISA Method. This polyclonal antibody-based assay for detection of SMZ and its major metabolites in fluid milk was used as a rapid, subjective determination of presence of SMZ prior to HPLC analyses (Table 4). When added SMZ concentrations were at 5 ppb, the test was negative for all three replications of whole and skim fluid and reconstituted dry milk samples. At 10 ppb, the ELISA was inconclusive for both fluid and dry (10% reconstituted) whole and skim milks. The test was clearly positive for 100-ppb samples in all three replications and for all milk types. In contrast to our observations at 5 and 10 ppb of SMZ, Bishop et al. (1) reported positive ELISA results at 5 and 10 ppb of SMZ for raw whole milk samples.

Effect of Heating on SMZ Recovery

Comparison of the conflicting results for the ELISA analyses in the present study with those reported by Bishop et al. (1), the lower than expected recoveries, and the variability in results obtained by HPLC, MRA, and EIA methods all suggest that some type of interference may be responsible. Sheth et al. (18) reported that heat caused sulfathiazole to bind to reducing sugar and that it inhibited detection of the compound without altering the primary structure. A brief study was undertaken to determine whether heating of fluid milk samples with added SMZ affects SMZ recovery, as would occur during pasteurization (63°C, 30 min) and spray drying (inlet temperature of 180 ± 2°C; outlet temperature of 100 ± 2°C) (Table 5). The HPLC results demonstrated a decrease in recovery from 27.5 ppb in untreated milk to 18.5 ppb in pasteurized milk with SMZ added at 40 ppb. Decreases in recovery were similar for samples with SMZ added at 100 ppb. The binding of SMZ to proteins or reducing sugars present in heated milk samples or the binding of SMZ to both are possible explanations for the variability in recovery in the present study.

TABLE 4. Detection of added sulfamethazine (SMZ) in fluid[1] and reconstituted dry[2] milks by ELISA.

SMZ Concentration	Replicate		
	1	2	3
(ppb)			
0	–[3]	–	–
5	–	–	–
10	±[4]	±	±
100	+[5]	+	+

[1]Whole and skim milk.

[2]10% Reconstituted whole and skim milk.

[3]Sample spot on ELISA test was darker than control spot, indicating no detectable concentration of SMZ.

[4]Sample spot on ELISA test was the same as control spot, indicating that SMZ was present at a concentration at the limits of this method.

[5]Sample spot on ELISA test was lighter than control, indicating a detectable concentration of SMZ.

TABLE 5. Effect of pasteurization and freezing on recovery of added sulfamethazine (SMZ) from raw whole milk by HPLC and quantitative enzyme immunoassay (EIA).

Method	Concentration of added SMZ	Process		
		Untreated	Pasteurization	Freezing[1]
	(ppb)	(recovered ppb)[2]		
HPLC	40	27.5	18.5	ND[3]
	100	93.3	59.0	ND
EIA[4]	10	20.2	16.7	4.1
	100	57.6	65.7	51.5

[1]Samples frozen for 1 wk at -20°C.

[2]Based on single replication.

[3]No data available.

[4]All calculations based on control samples.

Sheth et al. (18) demonstrated the reaction of the N^4 aromatic group on the sulfonamide compound with reducing sugar to form a sugar-sulfonamide complex. Blanchflower and Rice (2) also suspected the cause of their poor recoveries to be the irreversible binding of SMZ to components in the feed matrix. Results by Epstein et al. (7), Giera et al. (10), and Parks (14) were similar for the effect of heating SMZ residues in meat. Sheth et al. (18) observed that higher heating temperature also increased the rate at which glucose was irreversibly bound to sulfonamide. In the present study, some lactose may have been irreversibly bound to SMZ at the high temperature (100°C) of the spray-drying process, and the SMZ-lactose compound may not have been extracted during the analytical procedure, resulting in decreased recovery.

The evidence by Sheth et al. (18) suggests that binding by reducing sugar is not permanent, but that free, active sulfonamides could be released from the complex by acidification or aqueous dilution. The dilution of dry powder for analyses may have permitted some lactose-bound SMZ to be released, providing another possible explanation for variability in recoveries. Heating milk samples contaminated with SMZ evidently can affect recovery of SMZ.

Extraction of SMZ by Supercritical CO_2

The SFE method has been used for industrial-scale separation and isolation of a variety of compounds in various systems. Ramsey et al. (15) found considerable potential for SFE in separation and isolation of trace concentrations of contaminants in foods. In the present study, SMZ was extracted from dry

TABLE 6. Extraction of sulfamethazine (SMZ) (10 ppb[1]) from skim milk powder[2] by supercritical CO_2[3] and detection in the residue using quantitative competitive enzyme immunoassay (EIA) and microbial receptor assay (MRA) and qualitative ELISA.

Extraction time	EIA	MRA	ELISA	
			Extraction vessel[4]	Final extract[5]
(min)	(extracted %)			
0	0	0	±[6]	NA[7]
20	7	56	–[8]	+[9]
60	55	77	–	+
120	100	97	–	+

[1]Calculated concentration of SMZ in dried powder prior to supercritical fluid extraction was 163 ppb by EIA and 94.6 ppb by MRA.

[2]10% Reconstituted.

[3]387 kg/cm² (5500 psi), 50°C.

[4]Analyses completed on final extraction vessel sample.

[5]Analyses completed on collected extract sample.

[6]Sample spot on ELISA test was the same as control spot, indicating that SMZ was present at a concentration at the limits of this method.

[7]Not applicable.

[8]Sample spot on ELISA test was darker than control spot, indicating no detectable concentration of SMZ.

[9]Sample spot on ELISA test was lighter than control, indicating a detectable concentration of SMZ.

skim milk powder contaminated with SMZ (163 ppb based on EIA analyses and 94.6 ppb by MRA, as manufactured from fluid milk with 10 ppb of added SMZ), using supercritical CO_2 at 120 min of processing to levels below minimum detection limits for the rapid assay methods (Table 6). Both EIA and MRA analyses were completed on the SMZ-extracted sample remaining in the extraction vessel. The SMZ concentration was reduced within 20 min of processing, but the concentration of SMZ in dry powder did not fall below the safe concentration of 10 ppb until extraction was completed for >60 min. The present study then demonstrates that SMZ can be extracted from dry milk powder using supercritical CO_2.

CONCLUSIONS

Processed dairy products, such as dry milk powder, in which moisture is removed and solids content is concentrated, may result in detectable SMZ when the dairy products are manufactured from fluid milk with low or undetectable animal drug residue contamination. The methods used in this study were developed for fluid milk; methodology for determination of drug residues also should be developed to provide direct detection of drug residues and to reduce variability in high solids dairy products. Interaction of SMZ and product components, such as lactose or proteins, during heat processing appears to affect recovery of SMZ. The SFE process, which is currently utilized in some food applications, has potential for extraction of SMZ in processing and analytical-scale applications.

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