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Article

Development of an Environmentally Friendly Extraction Method Using Smartphone-Based Digital Images for the Determination of Total Sulfonamides in Meat Samples

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Sulfonamides (SAs) are low-cost synthetic antimicrobials widely used in veterinary and human medicine to treat diseases and prevent infections. However, long periods of exposure to sulfonamides can cause adverse effects such as allergic reactions. This work aims to study dispersive solid-liquid microextraction as an alternative method for extracting total SAs in meat samples. The procedure uses a colorimetric reaction based on the formation of a pink compound (imine salt) to determine total sulfonamides (sulfamethazine, sulfadimethoxine, sulfathiazole) with digital measurements. A linear response was observed between 33-233 μg kg^{-1} for total sulfonamides, and the coefficient of variation (n = 11; 67 μg kg^{-1} of total SAs) and the limit of detection were estimated to be 0.63% and 10 μg kg^{-1}, respectively. For a 750 mg meat sample, 0.11 mg of 4-dimethylaminocinnamaldehyde, 2.60 mg of sodium dodecyl sulfate, and 275 μL of 1-butanol were consumed *per* sample, and consequently, generating only 335 μL of residue. Besides this, addition-recovery tests were performed, resulting in a 71-100% recovery range, indicating the trueness of the proposed method.

Keywords: antibiotics, digital image measurement, dispersive solid-liquid microextraction, green analytical chemistry, meat sample

Introduction

Antibiotics are composed of antibacterial molecules that destroy or inhibit the growth of microorganisms, block the synthesis of proteins and cell walls, break the structure of nucleic acids, and obstruct the main metabolic pathways.[1]

Sulfonamides (SAs) are polar synthetic compounds that belong to a group of antimicrobial veterinary drugs (Supplementary Information (SI) section, Table S1). Owing to their low cost and effectiveness, they are widely used in livestock for prophylactic and therapeutic purposes. In addition, they are also used for the treatment of gastrointestinal and respiratory diseases and as supplements in animal feeds.[2-4] These amphoteric compounds have a structure similar to *p*-aminobenzoic acid; therefore, they act competitively in organisms and prevent the reproduction of microorganisms in animals.[5]

These medications are administered to approximately 80% of farm animals, which raises a concern for human health due to our high consumption of products such as meat, milk, and eggs.[6] In addition, the overdose of these drugs can lead to its preservation in products of animal origin, causing potential threats to human health such as allergic reactions, pathogenic bacteria resistance, carcinogenic and mutagenic effects, hypersensitivity, nephropathy,[2,4,7] and risk of developing drug resistance.[8,9]

To protect public health from their toxic effects, regulatory authorities, such as European Union, World Health Organization, Food and Drug Administration, and Codex Alimentarius, have established the maximum residue limits (MRLs) for SAs (100 μg kg^{-1}) in foods of animal origin, including meat.[8-12]

Meats are complex matrices composed of various substances such as water (72-75%), nitrogen compounds (approximately 21%, including proteins and non-protein nitrogen compounds), lipids (2.5-15%), etc.[13,14] Additionally, because of the structural complexity, bipolar characteristic, and high steric impediment of SAs, the development of a new methodology that allows the selective extraction of these drugs from complex samples, such as meat, is quite challenging.[15]

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In order to achieve quantitative extraction of these compounds from complex food matrices, methods with high preconcentration factors and limits of detection below the MRL are required. Additionally, sample preparation that can eliminate any interference from the meat samples is highly recommended before the instrumental analysis. Research on innovative sample preparation methods and the use of novel materials to extract antibiotics from samples of animal origin have been widely published in recent years.[5,15-19]

Various procedures, including liquid-liquid extraction,[20] QuEChERS (quick, easy, cheap, effective, robust, and safe),[21] magnetic solid-phase extractions (MSPE),[4] liquid extraction on a solid support,[22] dispersive solid-phase extractions (DSPE),[23] and solid-phase extraction (SPE)[24] have been extensively reported for the extraction of antibiotics. However, these methods can be laborious because the large volume of organic solvent used results in exhaustive extractions that can cause contamination and loss of analyte.[25] Furthermore, most of these procedures, such as SPE, employ a high amount of sorbent and disposable polypropylene cartridges that must be preconditioned.[19]

Thus, it has complied with the principles of Green Analytical Chemistry (GAC) through the use of new miniaturized techniques, such as solid-phase microextraction (SPME), dispersive solid-phase microextraction (DSPME), liquid-liquid microextraction (LLME), and DLLME. They have been widely used, owing to the short analysis time required, good enrichment factors, as well as the small amount of sample and volumes of the extractor and dispersant solvent required.[26,27] However, some methods, such as DSPE and DSPME, present certain disadvantages as they are laborious and expensive techniques. In addition to the filtration and centrifugation steps, such techniques consist of adding a high-cost solid apparatus (adsorbent).[27,28] An alternative ecological and inexpensive method used for sample preparation is QuEChERS, which involves the extraction of analytes with an organic solvent and different salts to promote the salting-out effect followed by DSPE. However, there is a risk of extracting undesirable compounds concomitantly with the analytes of interest because it is a multi-residue technique, and its sorbent cannot be reused.[26,29]

Separation techniques, such as high-performance liquid chromatography coupled with ultraviolet detection (HPLC-UV) and mass spectrometry (MS), are also commonly used for the analyses. Although these techniques are time-consuming and costly, they can provide excellent analytical results.

Several studies[30-33] have reported the concomitant application of other drugs in the animal production chain in order to avoid suppressing or masking the detection of SAs residues. Nonetheless, SAs residues, even at very low concentrations, can cause severe damage to animal and human health, such as bacterial resistance, hypersensitivity reactions with fever, malaise and itching, suppression of enzymatic activity, alteration of the intestinal flora, disorders of the urinary tract, etc.[30-33] To increase the reliability of the results when there is a large amount of sample to be analyzed, a combination of confirmatory methods, such as liquid chromatography/tandem mass spectrometry (LC-MS)[7,34] and gas chromatography/tandem mass spectrometry (GC-MS),[35-37] has been employed.

Conventionally, numerous screening techniques for the determination of SAs residues in animal tissues such as LC-MS/MS,[38-40] HPLC[13,41-45] coupled with fluorescence (FL),[41] ultraviolet (UV),[46,47] or diode array detector (DAD),[48-50] capillary electrophoresis with UV-detector[51] or tandem mass spectrometry (CE-MS/MS),[52] fluorescence spectrometry and cyclic voltammetry,[53] and flow-through immunoaffinity chromatography[54] have been employed. Among these techniques, derivatization with fluorescamine has been receiving much attention as it can separate and detect various SAs and enhance the screening sensitivity of the procedure.[55] Furthermore, HPLC-UV is one of the most extensively used techniques in analytical chemistry; however, its extraction procedures require a thorough sample clean-up. Therefore, these techniques provide qualitative and quantitative analyzes with high precision, sensitivity, and satisfactory reproducibility, allowing multiresidue determination in complex matrices. However, they are costly and time-consuming as they require large amounts of toxic solvents and previous operational experience of the analyst;[7,38,55-58] thus, unsuitable for routine analysis.

To ensure food safety and quality must be developed extraction procedures that are simple, inexpensive, robust, reproducible, clean, and sensitive, using a low volume of reagents to reduce waste generation to the environment. Moreover, as an alternative to conventional chromatography, the detection of analytes using digital image measurements (DIM) has emerged as an increasingly viable and practical strategy.[59-78] DIMs are based on colorimetric analysis by scanning images on electronic devices such as smartphones and digital cameras. Compared to digital cameras, smartphones are easily operable, lightweight, portable, and widely used for image capture. The possibility of using free applications (APPs) in these devices allows the determination of analytes through the relationship between the image data obtained and the analytical concentration.[60-62,65] Specifically, color systems such as red, blue, green (RGB), mix of the three RGB curves, luminance, *quasi*-equal to blue (XYZ), cyan, magenta,

yellow, black (CMYK), hue, saturation, value (HSV), grayscale, and so on define a three-dimensional coordinate space where each color represents a single point. In the case of smartphones, mobile applications act as digital image meters, converting the measured colors into numerical data that can be treated as analytical information.[60-62,65] In food matrices, including those of animal origin, the use of DIM has been an economically viable alternative to guarantee safety and quality control.[59,60,62,64,65]

For the evaluation of microbial spoilage in ground meat without using antibodies, microspheres or any other reagents, a smartphone-based biosensor was developed as a preliminary screening tool.[63] In this study, a digital camera through a free APP installed on a smartphone was used as an optical detector to quantify the scattering intensities. An 880 nm near-infrared light emitting diode (LED) was irradiated perpendicularly to the ground meat surface. The scattering signals at various angles were evaluated using gyro sensor and digital camera from a smartphone.

Hosseinpour *et al.*[69] developed an APP to assess and predict the tenderness of meat from its fresh images. For this, a lighting algorithm was developed to obtain textural features. Then, the textural characteristics of the pre-processed image obtained were correlated with the instrumental data obtained using the measurement of the Warner-Bratzler shear force through the artificial neural network technique. At the end of the study, the developed APP proved to be an economically viable alternative and capable of predicting the tenderness values of the meat of the samples in a promising way.

Recently, Pereira *et al.*[79] proposed a system based on image analysis to evaluate the quality of bovine meat newly acquired in the market by the consumer. The developed system employed only a smartphone running an algorithm dedicated to quickly estimating meat quality. The data obtained were compared with the standard established by the AMSA (American Meat Science Association)[80] to establish a correlation between the color and the microbiological conditions. The results demonstrate that the proposed system could reliably estimate the actual condition of the meat by correlating the microorganisms and the measured color.

Since the evaluation and estimation of bovine meat yield, usually done by specialists, is expensive, time-consuming, and laborious, Wakholi *et al.*[81] developed a new image analysis system for predicting meat yield and quality with acceptable accuracy. The study aimed to combine image processing and statistical modeling to predict the main parameters of bovine carcass yield. From image data of 140 bovine carcass samples, it was possible to develop models that achieved good prediction performance for yield parameters. Furthermore, due to the current industrial trend in the classification of bovine meat carcass yield, the results achieved can serve as a basis for the online classification of a bovine carcass.

As presented, DIMs have been shown to be a viable, cheap, simple, and a fast alternative for determining different analytes in food, biological and environmental matrices, reaching low limits of detection and high reproducibility, with multiple samplings in a shorter time.[60-62,64] Although studies that determine SAs in foods of animal origin have been reported, few studies still explore the determination of total SAs in meat from dispersive microextraction with an environmentally friendly solvent and determination by DIMs.

We propose a miniaturized, fast, simple, and inexpensive analytical method based on dispersive solid-liquid microextraction with DIM determination. This methodology absolves the need for a sample-cleaning step for the simultaneous determination of three SAs in meat: sulfamethazine (SMZ), sulfadimethoxine (SDM), and sulfathiazole (STZ). The sulfonamides studied in this work are the main SAs recommended by the National Plan for the Control of Residues and Contaminants in Products of Animal Origin (PNCRC Animal-MAPA)[82] and by the Program for the Analysis of Residues of Veterinary Medicines in Foods of Animal Origin (PAMVet-ANVISA)[83] for monitoring in foods of animal origin.

The colorimetric reaction used for the determination of total SAs in meat was based on the formation of the pink imino salt (Schiff's base) with maximum absorption at $\lambda = 560$ nm[84-87] resulting from the condensation between the protonated amino group of the SAs with the carbonyl group of the 4-dimethylaminocinnamaldehyde (*p*-DAC) chromophore in acidic medium. This reaction was assisted by the presence of a surfactant and the addition of a disperser/extraction solvent (Figure S1, SI section). The digital measurements were performed using the G channel (green) of the RGB system, which represents the complementary color of the pink imino salt. The reflectance signals were analyzed using a free mobile application (Color Grab®) installed in a smartphone that was attached to a bottomless polystyrene box and positioned on an LED emergency light (rectangular). The addition-recovery tests, the limit of detection (LOD), and the coefficient of variation of the new method were evaluated for practical application.

Experimental

Chemicals and reagents

The solutions were prepared using ultrapure deionized water (Merck Millipore, Darmstadt, Germany; model

Synergy® Water Purification System) and analytical grade reagents (Sigma-Aldrich, St. Louis, MO, USA). A stock solution of total SAs (SMZ, SDM, and STZ) was prepared by dissolving 10 mg of each SA in 100 mL of ultrapure deionized water (18 μS cm^{-1}).

In the colorimetric reaction, a reagent solution was prepared by mixing *p*-DAC (10.65 mmol L^{-1}) in an acid medium (0.56 mol L^{-1} HNO_3) with the addition of sodium dodecyl sulfate (SDS) (0.15 mol L^{-1}). This study used acetonitrile, ethanol, methanol, and 1-butanol (Sigma-Aldrich, St. Louis, MO, USA) as the extraction solvents. Moreover, it evaluated seven bovine ground meat samples acquired from butcher shops in Piracicaba, SP, Brazil. Before the analyses, the samples were partitioned and frozen in a conventional (domestic) freezer.

Apparatus

The evaluation of the best method for dispersing the extractant was made throughout the sample. Different sample preparations were evaluated employing a vortex agitator (Genie 2, Scientific Industries Inc., Bohemia, NY, USA; model SI-0266), ultrasonic bath (Quimis, SP, Brazil; model Q335D2), or orbital shaker (Quimis, SP, Brazil; model Q225M). The phase separation was accelerated using a fixed-speed mini centrifuge (Crystal Technology & Industries, Inc., Addison, TX, USA; model MLX-106). Digital measurements utilizing the G channel from the RGB system were conducted using the Color Grab® mobile application (with flash on) on a Moto X Force smartphone (model XT1580) with a 21-megapixel camera. The resolution of the camera was 1440 × 2560 pixels. The bottomless polystyrene box (height 11 cm; width 8 cm; depth 14 cm) was adapted with a top opening for the vertical insertion of microtubes containing meat samples at 10.5 cm from the smartphone positioned on an LED emergency light (rectangular). A lamp with 30 high-brightness LEDs (1.5 W and 1.3 V) powered by a lithium battery (Kian, São Gonçalo, RJ, Brazil) was attached to the bottomless polystyrene box to maintain constant lighting during digital measurements. Digital measurements were performed in the center of the interest region, and the values obtained were treated by discounting the value of 255.

Procedure

Ground meat samples were weighed (750 mg) and placed into a microtube (Figure 1, step 1). The analytes were partitioned by adding 275 μL of 1-butanol (step 2), followed by 13 min of shaking using an orbital shaking table at 200 rpm (step 3), and 13 min of centrifugation using a mini centrifuge at 6000 rpm (step 4). Next, an aliquot of the supernatant (100 μL) containing the SAs was transferred to another microtube containing 60 μL of the previously prepared reagent solution (step 5). The samples were lightly shaken by hand to promote the colorimetric reaction and centrifuged for approximately 1 min for phase separation. Finally, the analytical signals were measured using digital images (step 6) to determine the total SAs using the G channel of the RGB system.

Results and Discussion

Optimization of the experimental parameters

Optimizing the chemical and physical parameters such as solvent and reagent solution volumes, sample mass, and the type and time of agitation and centrifugation were performed to achieve the highest analytical responses, minimize the volumes of organic solvent and reagent employed, and simplify the experimental procedures. Thus, the analysis conditions were optimized based on studies available in the literature.[84,87-89]

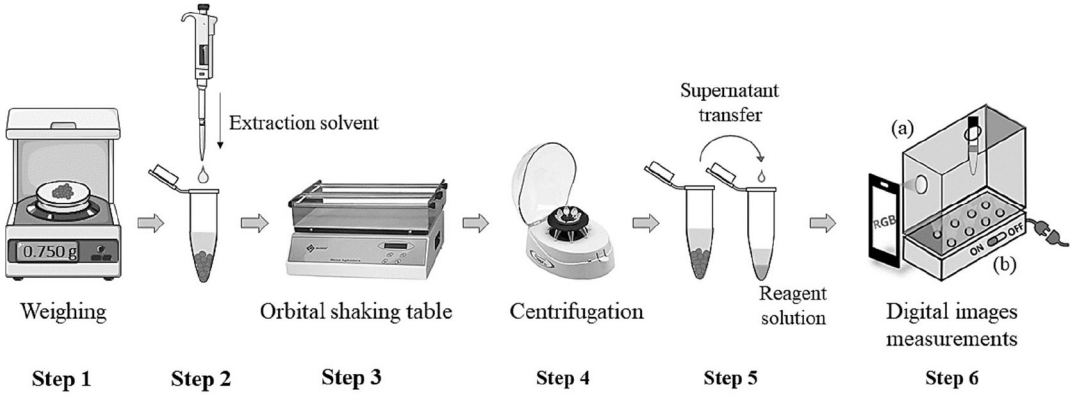

Figure 1. Steps involved in the SAs extraction and preconcentration procedure in bovine meat samples. (a) Bottomless polystyrene box (height: 11 cm; width: 8 cm; depth 14 cm); (b) emergency exit LED light.

Extraction solvent

To obtain better analytical responses with greater sensitivity in the determination of SAs, different solvents, such as acetonitrile, ethanol, and methanol, were evaluated to determine the best solvent and volume to be used with the reagent solution. Thus, a screening (without meat sample) was carried out with a fixed volume of SAs standard solution (1.0 mg kg^{-1}), varying the volumes of reagent solution and solvents. *F*-test results were used to determine the solvent that gave the best analytical response. Although all three solvents showed an increase in the analytical signal during the measurements, subsequent studies were conducted without acetonitrile, as this solvent altered the molar absorptivity of the compound formed, generating lower intensity in the analytical signal compared to the others.

To evaluate the extraction efficiency of SAs from meat samples, recovery tests were performed using ethanol, methanol, and 1-butanol as the extraction solvents. A standard SAs solution (1.0 mg kg^{-1}) was used for these tests to fortify the samples. The SAs were added directly to the sample with a micropipette. Subsequently, the samples were manually homogenized with a disposable micropipette tip and left to rest for 1 min before the extraction procedure with the solvents. These experiments tested two reagent solutions (A and B) of different concentrations. After extracting the SAs from 300 mg of fortified ground meat (1.0 mg kg^{-1}) with 300 µL of solvent, 100 µL aliquot of the supernatant was transferred to another microtube containing 60 µL of reagent solution A (3.04 mmol L^{-1} *p*-DAC, 0.16 mol L^{-1} HNO_3, and 0.043 mol L^{-1} SDS) or B (10.65 mmol L^{-1} *p*-DAC, 0.56 mol L^{-1} HNO_3, and 0.15 mol L^{-1} SDS). As a result, ethanol and methanol solvents did not result in significant and measurable analytical responses, even in both reagent solutions (A and B). Compared to 1-butanol, the analytical signals obtained by extraction with ethanol and methanol were very close to the analytical blank in this test, i.e., the sensitivity was reduced due to the change in molar absorptivity of the compound formed in the presence of these solvents. Therefore, the best extraction was observed in the analysis that used 300 µL of 1-butanol and 60 µL of reagent solution B, reaching 91.7 ± 0.2% recovery (n = 3).

These results determined the ideal volume of 1-butanol to be used in the analysis. Thus, different volumes in the range of 150-300 µL were evaluated by addition-recovery tests of total SAs from 300 mg of fortified ground meat (1.0 mg kg^{-1}). A recovery range of 77.8-119.4% was obtained for the different volumes of 1-butanol, and we found that the best volume of 1-butanol that should be used to extract the analytes was 275 µL, reaching 100.0 ± 0.6% recovery.

Furthermore, different amounts of meat samples were evaluated using the proposed procedure. In this study, 300 and 750 mg of fortified meat samples (1.0 mg kg^{-1}) were subjected to extraction using 275 µL of 1-butanol. Recovery of 91.8 ± 1.2% was obtained for the 750 mg meat sample, which was 1.3 times higher than that of the 300-mg meat sample.

Factorial designs

The use of chemometric tools related to factorial design is a useful analytical strategy that has contributed to improving analytical methods with greater sensitivity and sampling frequency.[90] The main application of a factorial design consists of screening (with all the variables) to select the most relevant variables of the analytical system under development. After selecting the most significant variables obtained in the screening, new experiments (factorial designs) must be performed to refine and optimize the proposed analytical procedure.[90]

The advantages of applying factorial design include: (*i*) fewer experiments performed compared to the conventional univariate procedure; (*ii*) saving of financial resources; (*iii*) obtaining results with more chemical and statistical reliability; (*iv*) the possibility of obtaining a mathematical model that allows predictions under untested conditions.[91,92]

To optimize the experimental physical parameters, factorial designs (screenings) were made to determine the optimum agitation mode, agitation time, and centrifugation time. From fortified samples (1.0 mg kg^{-1}), the ultrasonic, orbital and vortex agitation modes were evaluated for 5, 20 and 35 min at high-power agitation. As predicted, the ultrasound agitation was inefficient for extracting SAs in all samples. Therefore, excluding the ultrasound mode, a central composite design with axial points (2^3) was proposed to evaluate the agitation and centrifugation modes of the orbital (–1) and vortex (+1) machines for 5 (–1) and 20 min (+1), as listed in Table 1. Tests were conducted with 750 mg of fortified sample (1.0 mg kg^{-1}), 275 µL of 1-butanol and 60 µL of reagent solution B (n = 1).

The percentage of effects graph (Figure 2) shows the responses obtained from the interactions between variables: type of agitation (1), agitation time (2), and centrifugation time (3). The most significant interactions between variables were observed for agitation times (effect 2; 22.79%), centrifugation times (effect 3; 37.67%) and interaction between the agitation types and centrifugation times (effect 5; 22.79%).

Table 1. The proposed 2^3 factorial designs (screening)

Test	Agitation type	Agitation time / min	Centrifuge time / min
1	vortex (+)	20 (+)	20 (+)
2	vortex (+)	20 (+)	5 (–)
3	vortex (+)	5 (–)	20 (+)
4	vortex (+)	5 (–)	5 (–)
5	orbital shaking (–)	20 (+)	20 (+)
6	orbital shaking (–)	20 (+)	5 (–)
7	orbital shaking (–)	5 (–)	20 (+)
8	orbital shaking (–)	5 (–)	5 (–)

In parenthesis coded values.

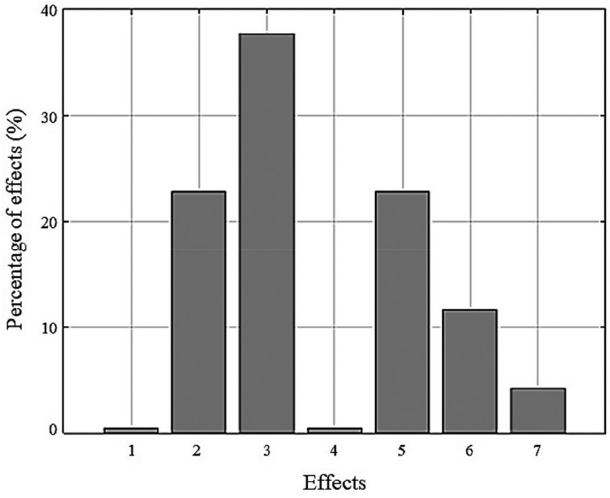

Figure 2. Percentage of effects in relation to variables and interactions, (1) type of agitation; (2) stirring time; (3) centrifugation time; (4) interaction 12; (5) interaction 13; (6) interaction 23 and (7) interaction 123.

The best results were observed using the orbital shaking table, which reached 100.0% recovery (n = 1). From the graph, a new 2^2 factorial design with three central points and four axial points was proposed to evaluate the agitation time (x_1) and centrifugation time (x_2) between 2 and 24 min (Table 2). The experimental design included eleven experiments that were performed at random to minimize errors.

The *F*-test was applied for the regression and residue to evaluate the lack-of-fit and pure error. The results demonstrated that the ratio between the calculated and tabulated *F* values remained > 10, and the lack of adjustment was < 1, indicating that the proposed model is acceptable. Based on the analysis of variance, it was possible to obtain and evaluate the regression coefficients. Except for the average and 2^2 interaction coefficients, all the others were insignificant. The significant coefficients were: 75.00 (average) and –15.60 (interaction 2^2).

The experimental data were correlated, and an empirical

Table 2. The proposed 2^2 factorial designs with three central and four axial points

Test	Agitation type	Agitation time / min	Centrifuge time / min
1	orbital shaking	5 (–)	5 (–)
2	orbital shaking	21 (+)	5 (–)
3	orbital shaking	5 (–)	21 (+)
4	orbital shaking	21 (+)	21 (+)
5	orbital shaking	13 (0)	13 (0)
6	orbital shaking	13 (0)	13 (0)
7	orbital shaking	13 (0)	13 (0)
8	orbital shaking	2 (1.41)	13 (0)
9	orbital shaking	13 (0)	2 (–1.41)
10	orbital shaking	24 (+1.41)	13 (0)
11	orbital shaking	13 (0)	24 (+1.41)

In parenthesis coded values.

relationship between the response and variables was expressed by fitting second-order polynomials (equation 1). From the results with the most significant coefficients, a response surface was obtained, and the quadratic regression model for the data obtained can be expressed as follows:

$$\text{Response} = 75.00 - 15.60x_2^2 \quad (1)$$

As demonstrated, the quadratic terms had the most significant influence on extraction efficiency. From the pre-established reaction conditions (750 mg of fortified sample (1.5 mg kg^{-1})), 275 µL of 1-butanol, and 60 µL of reagent-solution B and using equation 1, the optimum experimental conditions were found to be 13 min of orbital shaking and centrifugation (Figure 3). For validation, the central point was reproduced again (n = 3) and reached 84.6 ± 0.1% recovery.

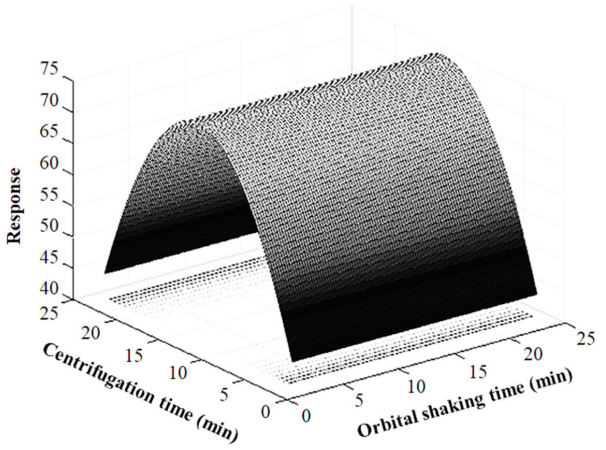

Figure 3. Optimization of the physical parameters: orbital shaking time (x_1) and centrifugation time (x_2). Experimental conditions: 750 mg of fortified sample, 275 µL of 1-butanol, and 60 µL of reagent solution B.

Analytical features

The analytical characteristics of the proposed procedure were estimated with optimized conditions, and the calibration curve was obtained for extraction from 750 mg of enriched meat and 275 µL of 1-butanol. A linear response was observed between 33 and 233 µg kg^{-1}, as expressed by the following equation: analytical signal = 0.33 + 0.03*C* (µg kg^{-1}) with R^2 (correlation coefficient) = 0.998. The coefficient of variation (n = 11; 67 µg kg^{-1} total SAs) and the limit of detection (LOD) were estimated to be 0.63% and 10 µg kg^{-1}, respectively. For 750 mg of sample, 0.11 mg of *p*-DAC, 2.60 mg of SDS, and 275 µL of 1-butanol were consumed, resulting in the generation of 335 µL of waste.

DIM has been an alternative to conventional analytical methods (such as chromatographic and spectrophotometric techniques) because of its economic, practical, fast, clean, accessible, reproducible, sensitive, and effective methodology.[60,62,65,93] A comparison between the analytical signals obtained by digital imaging and spectrophotometry was performed by adding 1.25 mL of standard solution, 2.25 mL *p*-DAC (2.66 mmol L^{-1}) in HNO_3 (0.14 mol L^{-1}), 500 µL of SDS (0.168 mol L^{-1}) and 1.25 mL of 1-butanol for extraction and obtaining calibration curves in the range between 50 and 500 µg kg^{-1} total SAs. The samples were vortexed (5 min at high power), centrifuged (17 min and 30 s at 4750 rpm) and first submitted to digital measurements. Subsequently, the supernatant phase of each test was transferred with a 1.0 mL microsyringe to a small-volume cuvette with a 1.0 cm path length. Spectrophotometric measurements were performed at the wavelength of maximum absorption of the imino salt (560 nm), as described in the literature.[84-87] Data measured by the smartphone (reflectance) and by the spectrophotometer (absorbance) were plotted (Figure 4).

In Figure 4 it was possible to compare the techniques used and verify the linearity described by the equation: Reflectance = 1.33 + 273.98Absorbance (R^2 = 0.998). Although measurements by digital images are less sensitive than spectrophotometric measurements, the use of the smartphone in the proposed procedure reached a limit of detection ten times lower than the MRL established by legislation.[82] Therefore, it can be concluded that the determination of analytes by measurements of digital images is applicable and can be used in analytical measurements as an economically viable alternative to spectrophotometry.

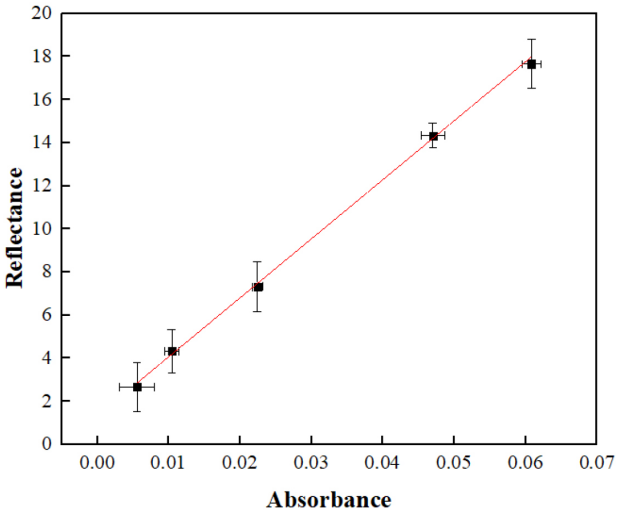

Figure 4. Comparison between analytical signals obtained by digital images and spectrophotometry.

Applications and validation

Seven meat samples were analyzed using the addition-recovery method. The proposed procedure was applied to determine the total SAs in meat after the samples were enriched with 66.7 and 100 µg kg^{-1}, resulting in 71.4 until 100.0% recovery, indicating the absence of matrix effects and the trueness of the procedure (Table 3).

Compared with other methods for determining total SAs in meat samples (Table 4), the analytical characteristics obtained using the proposed procedure were satisfactory, and the recovery values reached the expected range.

Table 3. Addiction-recovery experiment of SAs in bovine meat samples (n = 3)

Meat sample	Sulfonamides / (µg kg^{-1})					
	Spiked	Found	Recovery / %	Spiked	Found	Recovery / %
A	66.7	66.7	100.0 ± 0.5	100.0	81.8	81.8 ± 0.3
B	66.7	55.6	83.3 ± 0.3	100.0	72.7	72.7 ± 0.2
C	66.7	57.4	85.7 ± 0.1	100.0	72.7	72.7 ± 0.2
D	66.7	47.6	71.4 ± 0.2	100.0	81.8	81.8 ± 0.1
E	66.7	57.1	85.7 ± 0.4	100.0	90.9	90.9 ± 0.2
F	66.7	57.1	85.7 ± 0.4	100.0	88.9	88.9 ± 0.2
G	66.7	66.7	100.0 ± 0.2	100.0	88.9	88.9 ± 0.2

Table 4. Analytical features of microextraction methods for the determination of SAs in meat samples

Sample	Sample weight / g	Extraction and sample preparation	Solvent volume / mL	Extraction time / min	Technique	LOD / (μg kg^{-1})	Recovery / %	Reference
Pork, chicken, and meat samples	5.00	ACN and SPE-CE (Sep-Pak Alumina N, Oasis HLB)	30	35 + evaporation under N_2 gas	CE-DAD	5.0-10.0	80-97	16
Bovine, porcine, chicken muscle	5.00	Na_2SO_4, ACN, methanol and *n*-hexane	50	26	HPLC-MS/MS	0.03-3.0	70-110	94
Meat (muscle) of cattle and poultry	6.00	methanol, formic acid in ACN, and MSPD	1.80	90	HPLC-MS/MS	25.0-50.0	19-29	17
Poultry, meat, and pork muscle	10.00	acetone/ dichloromethane, acetic acid, and SPE (aromatic sulfonic acid)	50	15 + evaporation under N_2 gas	HPLC-MS/MS	56.2-66.5	90-110	5
Pork, meat, and chicken meat, meat tripe and pig liver	5.00	QuEChERS	15	60 + evaporation under N_2 gas	HPLC-MS/MS	0.01-0.03	87-100	7
Pork, meat, and mutton tissues	5.00	ACN	55	5 + evaporation under N_2 gas	HPLC-UV	6.5-11.0	82-94	33
Chicken and meat	2.00	phosphate buffer solution, PGE and aptamer-based biosensor	20	60	electrochemical biosensor	1.1×10^{-7}	93-103	18
Chicken, lamb, and meat	5.00	ACN, formic acid, and DSPE metallic organic structure	30	90	HPLC-UV	0.7-6.5	83-104	15
Ground meat	0.75	1-butanol	0.275	26	digital measurements	10.0	71-100	this work

ACN: acetonitrile; CE: capillary electrophoresis; DAD: diode array detector; DSPE: dispersive solid-phase extraction; HPLC: high-performance liquid chromatography; LOD: limit of detection; MS/MS: tandem mass spectrometry; MSPD: matrix solid-phase dispersion; PGE: pencil graphite electrode; QuEChERS: quick, easy, cheap, effective, rugged, and safe; SAs: sulfonamides; SPE: solid-phase extraction; UV: ultraviolet.

Furthermore, the LOD (10 μg kg^{-1} total SAs) is below the MRL (100 μg kg^{-1} total SAs in animal tissue) established by regulatory agencies.[82]

Meat sulfonamide preconcentration methods include SPE,[5] DSPE,[15] QuEChERS,[7] MSPD,[17] and others used for different animal tissues.[95-99] In some cases, sorbents such as neutral alumina,[16] aromatic sulfonic acids,[5] primary and secondary amine (PSA)[7] and sea sand[17] were used. Other studies have also synthesized new sorbents based on magnetic composites, such as organic metallic structures,[4,15] organic polymers[99] or electrochemical biosensors[18] for the purification and preconcentration stages.

SPE-based methods use expensive and rarely reusable adsorbent cartridges. Furthermore, developing these materials is laborious, reducing their applicability in laboratories in routine analysis. Although the extraction methods are highly efficient in partitioning the analytes, most of them employ exhaustive washes in the sample preparation step with large volumes of organic solvents such as *n*-hexane,[94] methanol,[17,94] acetonitrile,[15-17,33,94] or solvent mixtures such as acetone-dichloromethane-acetic acid[5] and acetic acid-acetonitrile[7] for partitioning SAs from meat samples. A study[33] have also reported the need to evaporate the solvent at elevated temperatures, under reduced pressure and constant nitrogen flow. However, according to Green Analytical Chemistry, the use of these solvents is strongly discouraged as they are potentially harmful to health and the environment. The proposed procedure stands out from the mentioned disadvantages as it does not require the sample clean-up step and uses only 275 μL of environmentally friendly organic solvent (1-butanol) to extract the analytes, generating 335 μL of residue *per* sample.

The analytical techniques most applied in the determination of SAs in meat use HPLC-UV[15,33] and HPLC-MS/MS.[5,7,17,94] Other studies also employ capillary electrophoresis systems equipped with a diode array detector (CE-DAD),[16] and electrochemical and spectroscopic techniques.[18] Although it is possible to separate the analytes individually, with high sensitivity and low limits of detection, these techniques are expensive, time-consuming, and require considerable operational experience from the analyst. Compared to other methods of analysis of SAs in foods of animal origin, the analytical procedure proposed has advantages such as the elimination of the sample clean-up step, the possibility of exploring digital measurements for the quantification of SAs in meat samples using an accessible and low-cost

device, i.e., a smartphone, the extremely low consumption of reagents (60 µL), the use of an environmentally friendly organic solvent (275 µL of 1-butanol *per* analysis), which generates only a few microliters of low-toxicity residue, and the short time required to perform the analysis (about 74 times less than that observed in the literature).[5,7,15-18,33,94] Furthermore, the sample clean-up step was eliminated and the recovery values were satisfactorily achieved within the range 71-100%. As a disadvantage, the proposed method has some significant limitations, such as: determining only the total amount of SAs without allowing the quantification of individual components, difficulty in automating the analyses, and lower sensitivity compared to conventional analytical techniques. Despite the limitations of the proposed method, the low LOD achieved (10 µg kg^{-1}) was 10 times below the MRL of total SAs (100 µg kg^{-1}) set by legislation.[12,83] Thus, the developed procedure proved to be a clean, practical, fast, sensitive, efficient, and economically viable alternative for determining total SAs in meat samples. These advantages make the proposed procedure significantly more ecological than those previously reported for fast routine analyses.

Conclusions

The proposed procedure for extracting and preconcentrating total SAs from meat samples using digital-image measurements proved to be an easy, fast, efficient, selective, and sensitive alternative compared to conventional methods. Further, the omission of the sample clean-up step is highly advantageous for routine analysis. In addition, it is cheaper, safer, and more environmentally friendly than previously reported methods because of the lower consumption of toxic reagents. Finally, the method proved to be sufficiently precise and accurate, thus providing an attractive alternative for detecting sulfonamide antibiotics in meat samples.

Supplementary Information

Supplementary information is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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