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A simple and rapid screening method for sulfonamides in honey using a flow injection system coupled to a liquid waveguide capillary cell

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ABSTRACT

A rapid and simple screening method was developed for the determination of sulfonamides in honey samples by flow injection analysis (FIA) coupled to a liquid waveguide capillary cell. The proposed method is based on the reaction between sulfonamides and *p*-dimethylaminocinnamaldehyde (*p*-DAC) in the presence of sodium dodecylsulate (SDS) in dilute acid medium (hydrochloric acid), with the reaction product being measured spectrophotometrically at λ_{max}=565 nm. Experimental design methodology was used to optimize the analytical conditions. The proposed technique was applied to the determination of sulfonamides (sulfaquinoxaline, sulfadimethoxine, and sulfathiazole) in honey samples, in a concentration range from 6.00×10^{-3} to 1.15×10^{-1} mg L^{-1}. The detection (LOD) and quantification (LOQ) limits were 1.66×10^{-3} and 5.54×10^{-3} mg L^{-1}, respectively. Positive and false positive samples were also analyzed by a confirmatory HPLC method. The proposed system enables the screening of sulfonamides in honey samples with a low number of false positive results, with fast response therefore offers a new tool for consumer protection.

1. Introduction

Veterinary drugs are widely used to treat or prevent diseases in animals, which can result in trace levels of drug residues in products of animal origin such as milk, meat, fish, eggs, and honey. Honey is considered to be a natural and healthy product of animal origin, and the addition of preservatives, additives, and other contaminants is not permitted. Among the contaminants encountered in beekeeping, the most important are substances used to control bee diseases. Antibiotics are employed in apiculture for the treatment of bacterial diseases, notably American and European foulbrood [1].

Sulfonamides constitute one of the antibiotic groups most widely used by beekeepers, due to their low cost. All sulfonamides inhibit the bacterial synthesis of folic acid, because their structure is analogous to that of *p*-aminobenzoic acid.

Contamination of foods with antibiotic residues poses risks to human health that include an increased resistance of bacteria to antimicrobial agents, allergic reactions, and possible carcinogenicity in humans. Regulatory agencies are responsible for ensuring that potentially harmful residues of these drugs are not present in honey or in products derived from honey. Nonetheless, in the European Union, maximum residue limits (0.1 mg kg^{-1}) have been established for sulfonamide compounds in various foodstuffs of animal origin [2,3], but not for honey, in which no antibacterial substances (including antibiotics and sulfonamides) are permitted. The discovery of any amount of these substances in honey disqualifies the product. However, other countries have set different limits for antibiotic residues in food. In Brazil, sulfonamides are regulated in accordance with the National Plan for the Control of Residues and Contaminants (Plano Nacional de Controle de Resíduos e Contaminantes, PNCRC) [4].

The use of sulfonamides in beekeeping has stimulated efforts to develop a simple and reliable method for the detection of these substances in honey. Numerous analytical methods have been reported for the determination of sulfonamides in honey, employing techniques such as thin-layer chromatography [5], colorimetry [6], enzyme immunoassay [7,8], and high performance liquid chromatography [9–15]. Sulfonamides are amphoteric chemicals whose solubility is pH dependent. The quantification of these compounds in complex matrices such as honey often requires laborious extraction procedures with an appropriate solvent followed by one or more clean-up processes [9,12–15], and recoveries can be low. These methods are suitable for confirmation but not for the screening of large numbers of samples.

A disadvantage of most of the analytical methods employed to measure sulfonamides in honey samples is that they require the use of large volumes of toxic solvents as extraction solvents and/or

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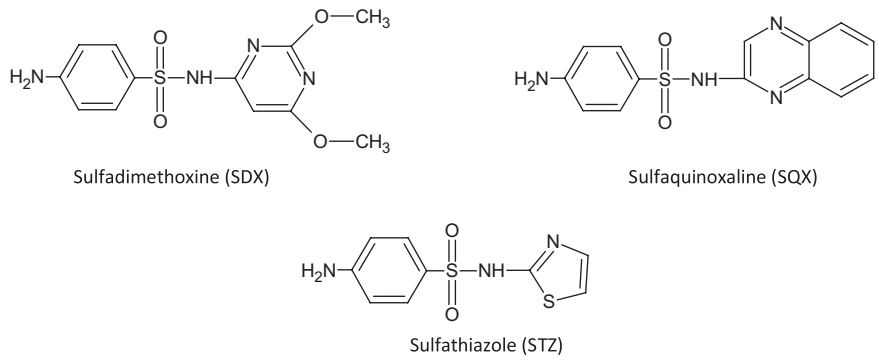

Fig. 1. Chemical structures of sulfonamides.

HPLC mobile phases [9,12,14,15], generating chemical wastes that contribute to environmental pollution. During the development of any new analytical method, the amounts and toxicities of the reagents used and the wastes produced are as important as any other analytical feature. Hence, there is a need to develop methods that are less harmful to humans and to the environment, and that comply with the twelve principles of green chemistry [16,17].

The aim of green analytical chemistry is to use analytical procedures that generate less hazardous waste and that are safer to use and more benign to the environment [18]. One way of achieving this goal is to develop screening methodologies, which can minimize the number of analyses required and therefore also reduce the amounts of reagents and solvents used. Screening methodologies are commonly employed to generate a binary Yes/No response, and act as a filter, avoiding the need to analyze a large number of samples by conventional analytical methods [19].

The aim of the present study was to develop a simple, rapid, and environmentally friendly screening system for the determination of the sulfonamides sulfadimethoxine (SDX), sulfaquinoxaline (SQX), and sulfathiazole (STZ) (Fig. 1) in honey samples, using a flow injection spectrophotometric procedure that employed a liquid waveguide capillary cell. The three sulfonamides were selected based on current Brazilian legislation [4].

Sulfonamides are known to enter condensation reactions with aromatics aldehydes. *p*-Dimethylaminocinnamaldehyde (*p*-DAC) has been proposed as a reagent for the spectrophotometric determination of sulfanilamide compounds in acetonitrile medium [20]. In aqueous solutions, the condensation of *p*-DAC with sulfanilamides yields a poorly colored soluble product [20]. The proposed method is based on the reaction between sulfonamides and *p*-dimethylaminocinnamaldehyde in a micellar medium, with the reaction product being measured spectrophotometrically at λ_{max}=565 nm. Experimental design methodologies were used to optimize the measurement conditions, and the proposed method was applied to the screening of sulfonamides in different honey samples.

2. Experimental

2.1. Apparatus

The flow injection system comprised an ASIA analyzer (Ismatec, Zürich, Switzerland) equipped with a variable speed (1–50 rpm) four-channel pump (Model 7610, Rheodyne, USA), Tygon® tubing (2.06 and 1.42 mm i.d.) for propelling the fluids, and a sample injection valve (Model 5041, Rheodyne, USA). Measurements of absorbance at 565 nm were carried out with a spectrophotometer (USB 4000 UV–vis, Ocean Optics) coupled via optical fibers (600 μm diameter) to a liquid waveguide capillary cell (LWCC, World Precision Instruments) with a flow path of 100 cm (inner volume 250 μL) and to a light source (Model LS-1-LL halogen lamp, Ocean Optics). Data acquisition employed SpectraSuite software (Ocean Optics).

The flow injection system is illustrated in Fig. 2. The solutions of sample and reagent (0.0052% (m/v) *p*-DAC, prepared in 2.43×10^{-2} mol L^{-1} HCl) were pumped using 1.42 mm i.d. Tygon® tubing, and the solution of 5.00×10^{-3} mol L^{-1} SDS, prepared in 2.43×10^{-2} mol L^{-1} HCl, was pumped using 2.06 mm i.d. tubing. The connection tubes, sample loop (120 cm), and reaction coil (80 cm) employed 0.8 mm i.d. polytetrafluoroethylene (PTFE) tubing. End-fittings and connectors were obtained from Omnifit (New York, USA).

The HPLC system consisted of a Shimadzu Prominence LC-20AT isocratic pump, an automatic injector, and an SPD-M20A photodiode array detector. A reversed-phase Inertsil ODS-3 (250 × 4 mm i.d., 5 μm) analytical column was employed (Supelco, Bellefonte, PA, USA).

2.2. Reagents and solutions

All the reagents employed were analytical grade and were used without any previous purification. Ultrapure water (18 MΩ cm, Milli-Q system, Millipore) was used to prepare the solutions. The HCl stock solution (1.00 mol L^{-1}) was prepared by appropriate dilution of concentrated acid (Mallinckrodt, Xalostoc, Mexico) in deionized water, and was standardized using a volumetric procedure. An aqueous 0.1 mol L^{-1} solution of sodium dodecyl sulfate (SDS) (Sigma, St. Louis, USA) was prepared weekly, and working solutions were prepared by dilution of the stock solution with deionized water. A 0.0052% (m/v) solution of *p*-dimethylaminocinnamaldehyde (*p*-DAC) (Aldrich, Milwaukee, USA) was prepared in 2.43×10^{-2} mol L^{-1} HCl and kept refrigerated for no more than one week. Stock aqueous standard solutions of the sulfonamides were prepared daily at concentrations of 100 mg L^{-1}. Individual and mixed working standard solutions (6.00×10^{-3} to 1.15×10^{-1} mg L^{-1}) of the three sulfonamides were prepared by diluting the stock solutions with water in the presence of HCl (2.43×10^{-2} mol L^{-1}) and SDS (5×10^{-3} mol L^{-1}).

2.3. Preparation of samples

Eight samples of orange and eucalyptus flower honey were used to evaluate the performance of the screening method. The samples were purchased from a local market and an apiary in the municipality of Araraquara (São Paulo State, Brazil).

The methodology used to prepare the samples did not employ any organic solvents and was compliant with the requirements of green chemistry [16]. A portion (2.5 g) of the sample was placed in

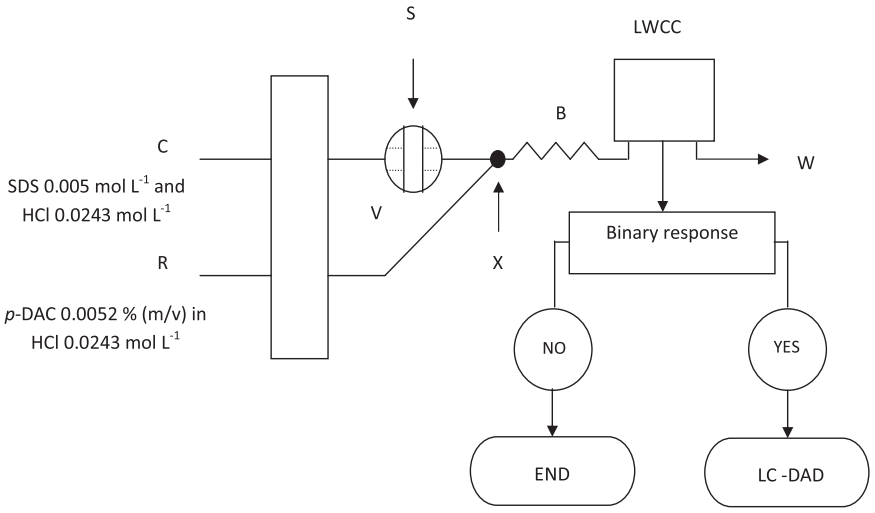

Fig. 2. Flow diagram of the proposed procedure. C: carrier solution; R: chromogenic reagent solution; S: sample loop (603 μL); V: injection valve; X: confluence point; RC: reaction coil (80 cm); W: waste.

a beaker and homogenized with 5.0 mL of deionized water in an ultrasonic bath for about 15 min. Next, this solution was filtered through a 0.45 μm filter (Millex-HV, Millipore) to remove the solid particles. The filtrate was then transferred to a 25 mL volumetric flask, and hydrochloric acid and sodium dodecyl sulfate were added to final concentrations of 2.43×10^{-2} and 5.00×10^{-3} mol L^{-1}, respectively, after completion with deionized water.

2.4. Experimental design

After identification of the significant parameters, the operational variables were optimized by multivariate analysis [21] using fractional factorial design (2^{6-3}) to obtain the best analytical conditions. The variables included were reactor length, sample loop size, flow rate, and the concentrations of *p*-DAC, HCl, and SDS. Matrix design was performed using Minitab 16 software, and optimization graphs were constructed using Statistica 8.0 software.

2.5. Screening procedure

A continuous flow system incorporating a liquid waveguide capillary cell (LWCC) was used (Fig. 2). Under optimized conditions, a sample volume (S) of 603 μL was injected into the carrier stream solution containing HCl and SDS (2.43×10^{-2} and 5.00×10^{-3} mol L^{-1}, respectively), pumped at a flow rate of 1.8 mL min^{-1}. The carrier solution then merged with a stream of the 0.0052% (m/v) *p*-DAC reagent prepared in 2.43×10^{-2} mol L^{-1} HCl, pumped at a flow rate of 1.5 mL min^{-1}. The mixture passed through the reaction coil (RC, 80 cm), which was kept at room temperature, and the product formed was carried to the detector flow cell, where the transient absorbance signal of the colored product was measured at 565 nm. The detector signal was previously adjusted to zero while pumping the carrier solution converged with the reagent stream, in the absence of sulfonamide. Peak height was used as the analytical signal, and its magnitude was proportional to the sulfonamide concentration.

2.6. Analytical curves

The system was calibrated for the sulfonamides using the selected reagent concentrations and operational conditions. A series of sulfonamide standard solutions (6, 10, 15, 40, 55, 70, 100, and 115 μg L^{-1}) were injected into the reagent stream, and the product formed was monitored continuously. A calibration graph was prepared by plotting peak height against sulfonamide concentration in the range 6.00×10^{-3} to 1.15×10^{-1} mg L^{-1}.

2.7. Study of interferences

An evaluation was made of possible interferences from the major compounds (glucose, fructose, and hydroxymethylfurfural) commonly present in honey. Solutions containing 0.1 mg kg^{-1} of sulfonamide and each of the major compounds at concentrations 10 times greater than that of sulfonamide were evaluated under the same conditions described in the screening procedure.

2.8. Reference method

An HPLC-UV technique that included sample pretreatment and solid-phase extraction, as recommended in the literature [9], was used as a reference analytical procedure. A linear relationship was obtained between the analytical signal and sulfonamide concentrations in the range 0.01 to 1.00 mg L^{-1}.

3. Results and discussion

Sulfonamides are primary aromatic amines that can react with *p*-DAC in an acidic medium (Fig. 3). The reaction is assumed to proceed by condensation of the protonated amino group with the carbonyl group of the reagent, producing an iminium salt [22].

In preliminary experiments, the sulfonamides were reacted with *p*-DAC in an acidic medium, in the presence or absence of the anionic SDS surfactant. The results revealed that the presence of SDS significantly enhanced the rate of the reaction that generated the colored product. The reaction was very slow in the absence of SDS. The combined benefits of the presence of the SDS micelles and the use of a liquid waveguide capillary cell resulted in a significant increase in the sensitivity of the reaction, and formed the basis of the proposed flow injection procedure.

3.1. Optimization of variables

3.1.1. Fractional factorial design: Screening of the factors

Investigations were carried out to establish the conditions that provided a maximum absorbance response at 565 nm, as well as a stable baseline and a compromise between the shape of the peak

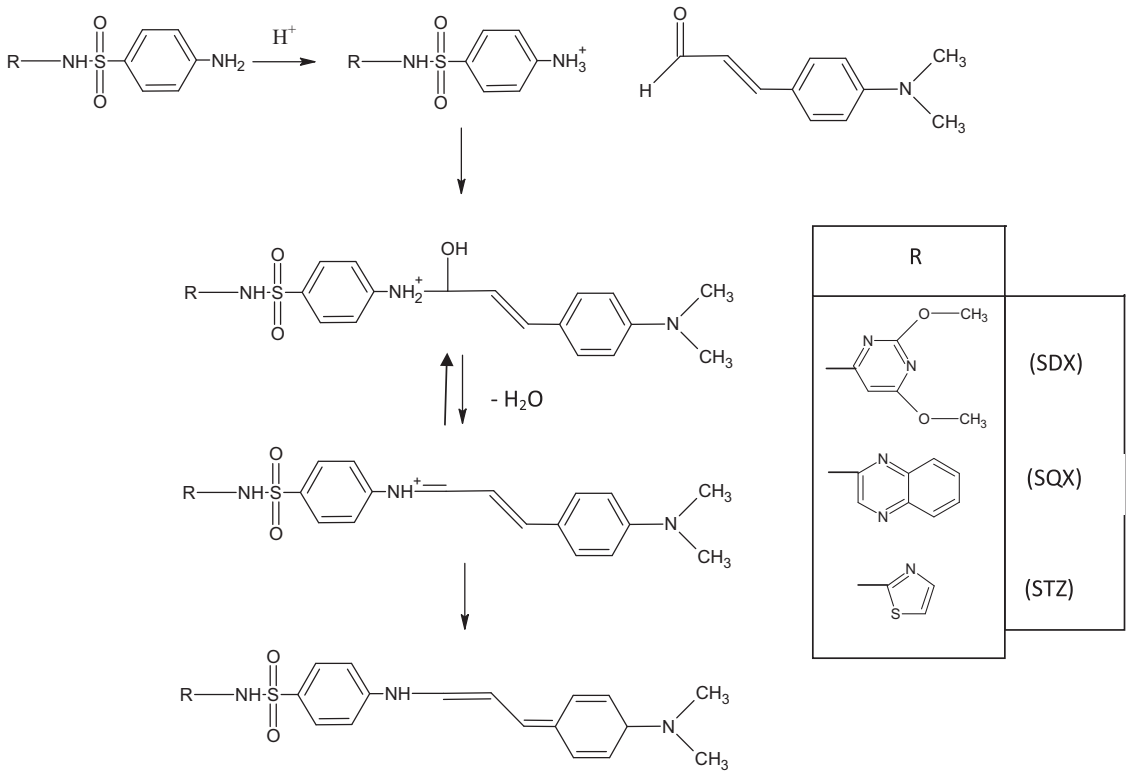

Fig. 3. Reaction between *p*-DAC and sulfonamides in acidic surfactant medium, producing a colored compound (Schiff base).

Table 1
Fractional factorial design matrix (2^{6-3}).

Expt.	Factors					
	$C_{p\text{-DAC}}$ (% m/v)[a]	C_{HCl} (mol L^{-1})[b]	C_{SDS} (mol L^{-1})[c]	Sample loop (μL)[d]	Reaction coil (cm)[e]	Flow rate (mL min^{-1})[f]
1	−1	−1	−1	+1	+1	+1
2	−1	+1	−1	−1	−1	+1
3	−1	−1	+1	−1	−1	+1
4	−1	+1	+1	+1	−1	−1
5	+1	−1	−1	+1	−1	−1
6	+1	+1	−1	−1	+1	−1
7	+1	−1	+1	−1	−1	+1
8	+1	+1	+1	+1	+1	+1

[a] −1 for 0.0011 and +1 for 0.0024.
[b] −1 for 0.020 and +1 for 0.050.
[c] −1 for 0.005 and +1 for 0.010.
[d] −1 for 402 and +1 for 603.
[e] −1 for 40 and +1 for 80.
[f] −1 for 1.5 and +1 for 2.0.

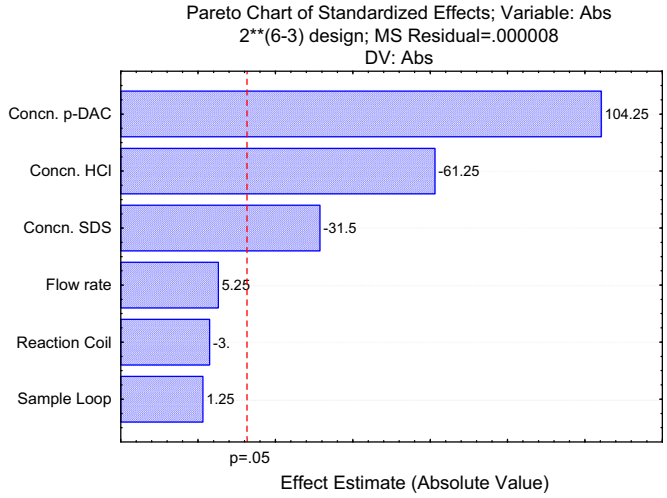

Fig. 4. Pareto chart for optimization using a 2^{6-3} fractional factorial design.

and the sampling rate. First, a 2^{6-3} factorial design was performed, which enabled a simultaneous evaluation of the factors that had an important effect on the reaction [21]. Table 1 presents the factorial design matrix, considering the different combinations of the factors and levels. For each factor, an upper (+1) and a lower (−1) level were selected, based on the preliminary experiments. Eight randomized experiments were performed (in triplicate). The sulfonamide concentration was kept constant at 5 mg L^{-1} in all experiments.

The individual effects of the various parameters, as well as their interactions, are illustrated in the form of a Pareto chart in Fig. 4. The length of each bar is proportional to the absolute value of the associated regression coefficient or estimated effect. The effects of all parameters and interactions were standardized (each effect was divided by its standard error). The order in which the bars are displayed corresponds to the order of the size of the effect. The chart includes a vertical line indicating the 95% statistical significance limit. An effect was therefore significant if the corresponding bar crossed this vertical line.

From Fig. 4, it is clear that the *p*-DAC concentration was the most significant factor, with a positive impact, indicating that the best results were obtained when this factor was adjusted to a high level (+1). The individual effect of the HCl concentration was also significant, with a negative impact. The individual effect of the SDS concentration was significant, but to a lesser extent. The SDS concentration was therefore set at 0.005 mol L^{-1} (the lowest concentration level studied). The other variables (reaction coil and sample loop size, and flow rate) did not show any significant

Table 2
Central composite design for the variables *p*-DAC and HCl.

Expt.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Conc. *p*-DAC[a]	−1	+1	−1	+1	0	0	0	$-\sqrt{2}$	$+\sqrt{2}$	0	0	0	0	0
Conc. HCl [b]	−1	−1	+1	+1	0	0	0	0	0	$-\sqrt{2}$	$+\sqrt{2}$	0	0	0

[a] −1 for 0.0020, +1 for 0.0050, $-\sqrt{2}$ for 0.0014, $+\sqrt{2}$ for 0.0056, and 0 for 0.0035 (%, m/v).
[b] −1 for 0.0050, +1 for 0.0200, $-\sqrt{2}$ for 0.0019, $+\sqrt{2}$ for 0.0230, and 0 for 0.0125 (mol L^{-1}).

influence on the reaction, so fixed values were selected that enabled the reaction to proceed while minimizing the consumption of reagents.

3.1.2. Central composite design

Based on the results obtained in the full factorial design, a central composite design [21] was carried out with the variables that showed the greatest influence on the reaction. In this case, the objective was to identify the best conditions in terms of the concentrations of the two most influential variables, namely the concentrations of *p*-DAC and HCl. The two variables were coded using five levels, with four central points (Table 2).

Fig. 5 shows the three-dimensional response surface graph obtained by fitting the experimental data. A statistically significant quadratic model, accounting for 89.0% of the variance, was fitted to the data. The quadratic regression model is given by the equation

$$Z = -13.836 + 0.43784\,x - 0.003325\,x^2 + 0.01965\,y - 0.00002045889\,y^2 - 0.00012915\,xy,$$

where Z is the response factor corresponding to the absorbance value, and the factors x and y are the *p*-DAC and HCl concentrations, respectively. From the fitted surface, the critical values found for the *p*-DAC and HCl concentrations were 0.0052% (m/v) and 0.0243 mol L^{-1}, respectively.

The optimized values of the variables were therefore a *p*-DAC concentration of 0.0052% m/v, HCl concentration of 0.0243 mol L^{-1}, SDS concentration of 0.0050 mol L^{-1}, 603 μL sample loop, 80 cm reaction coil, carrier/sample flow rate of 1.8 ml min^{-1}, and reagent flow rate of 1.5 ml min^{-1}.

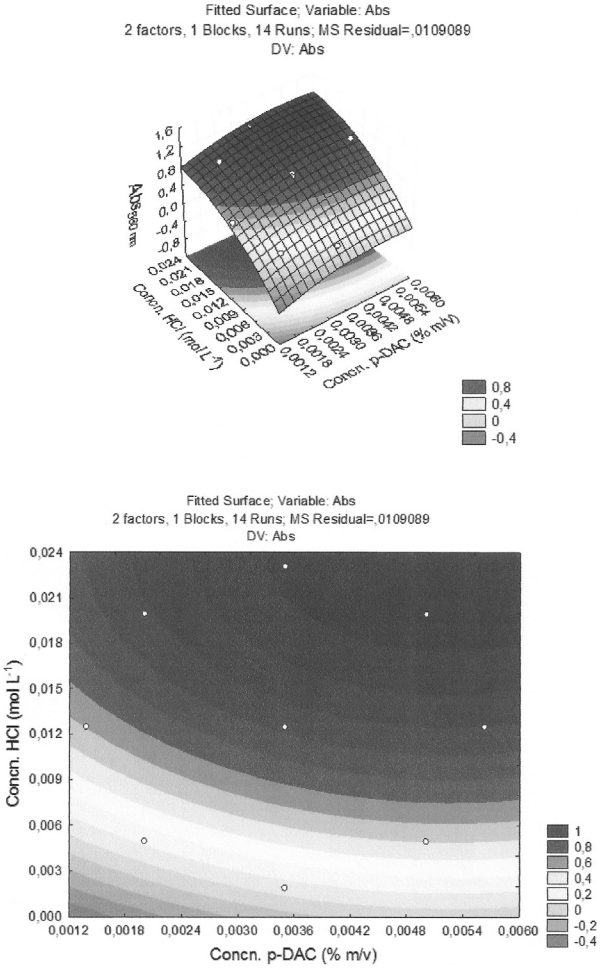

Fig. 5. Response surface obtained for absorbance values as a function of *p*-DAC and HCl concentrations.

3.2. Stability of the reaction product

The stability of the absorbance at 565 nm was determined by performing measurements every 5 min over a period of 1 h. The results demonstrated that there was no significant change in the absorbance value with time.

3.3. Analytical characteristics of the proposed method

3.3.1. Analytical curve, LOD, and LOQ

Use of the strategies to increase sensitivity (employing micellar media and LWCC) enabled analytical curves to be constructed in a concentration range from 6.00×10^{-3} to 1.15×10^{-1} mg L^{-1} (6–115 ppb), for all three sulfonamides. Linear relationships were obtained between the absorbance values and the sulfonamide concentrations. The limits of detection (LOD) and quantification (LOQ) were determined according to the IUPAC [23] recommendations: LOD = $3S_b/b$ and LOQ = $10S_b/b$, where S_b is the standard deviation of blank measurements ($n = 10$) and b is the slope of the linear dynamic range. The sensitivity of the method was consistent with the maximum permitted contaminant level for honey samples in Brazil (100 ppb) [4]. Table 3 lists the parameters (figures of merit) of the proposed procedure. The results indicated that the proposed system was sufficiently sensitive for screening of the three sulfonamides in honey samples.

3.3.2. Study of interferences

A variation of the signal exceeding ± 5% in the determination of sulfonamide, due to the presence of major compounds commonly present in honey (Section 2.7) was considered to be indicative of interference. No interferences in the proposed method were observed for up to around 10-fold excesses of glucose, fructose, and hydroxymethylfurfural. The percentage of sulfonamide found in the spiked solutions was in the range of 95.8 to 101%.

3.3.3. Standard addition and recovery

Recovery assays were carried out to evaluate accuracy and to detect possible matrix interferences. Samples of two different honey types were spiked with 80, 100, and 115 μg kg^{-1} of sulfaquinoxaline, and each sample was analyzed three times. The results obtained are presented in Table 4. The percentage

Table 3
Figures of merit of the proposed method.

Compound	Linear range	R[a]	Linear equation	LOD (μg L^{-1})	LOQ (μg L^{-1})	Wavelength
SQX	6–115 μg L^{-1}	0.9999	$A=-0.0302+0.0153C_{SQX}$	1.74	5.81	565 nm
SDX		0.9998	$A=0.0175+0.0160C_{SDX}$	1.66	5.54	
STZ		0.9998	$A=-0.0211+0.0134C_{STZ}$	1.99	6.65	

[a] Linear correlation coefficient.

Table 4
Results of recovery of sulfonamide added to honey samples.

Sample	Added value (μg kg^{-1})	Found value (μg kg^{-1})	Recovery (%)[a]
Orange honey	80	74	92.5 ± 2.2
	100	96	96.0 ± 3.7
	115	112	97.4 ± 5.7
Eucalyptus honey	80	89.6	112 ± 0.5
	100	88	88.0 ± 1.0
	115	111	96.5 ± 1.5

[a] = Average ± standard deviation (SD), $n=3$.

recoveries were between 88 and 112%, indicating good accuracy and an absence of matrix effects.

3.4. Application to honey samples

The efficiency of the proposed screening methodology was evaluated by analyzing three groups of samples. In the first group, eight honey samples were spiked with individual sulfonamides. In the second group, samples were spiked with combinations of two sulfonamides, and in the third group, a combination of the three sulfonamides was employed. Three sulfonamide concentration levels were used: 80 μg kg^{-1} (below the limit stipulated in legislation), 100 μg kg^{-1} (at the stipulated limit), and 115 μg kg^{-1} (above the stipulated limit). The screening test classified the samples as either "below the tolerance limit" (negative response) or "exceeding or close to the tolerance limit" (positive response). Positive and false positive results required confirmatory analysis by the chromatographic method [17,24,25].

A cut-off concentration of 100 μg kg^{-1} was used, since this is the limit value for sulfonamide residues in honey stipulated in current Brazilian legislation [4]. A false negative was produced when a negative response was obtained for a honey sample solution containing the sulfonamide at a level above the cut-off value. A false positive occurred when a positive response was obtained for a honey sample solution containing the sulfonamide at a level below the cut-off value.

The results obtained for samples spiked with the individual sulfonamides at different concentrations are shown in Table 5. A small number of false positive results were recorded. The occurrence of a low number of false negative results is very important in screening methods, since only false positive results are normally checked using a confirmatory method.

The second and third steps of application of the screening method for the determination of sulfonamides involved spiking the samples with combinations of two and three sulfonamides, respectively. The objective was to determine the behavior of each sulfonamide in the presence of another. Orange flower and eucalyptus flower honeys were used, and the sulfonamide combinations were SDX/STZ, SDX/SQX, STZ/SQX, and STZ/SQX/SDX.

For the orange flower honey, combinations of two sulfonamides at 80 μg kg^{-1} gave negative results in all cases. Combinations at 100 μg kg^{-1} showed one false positive result, for 50 μg kg^{-1} SDX+ 50 μg kg^{-1} SQX. The combinations at 115 μg kg^{-1} all gave positive results. The SDX/STZ/SQX combination showed negative results at a concentration of 80 μg kg^{-1}, a false positive result for the combination 33.33 μg kg^{-1} SDX+33.33 μg kg^{-1} STZ+33.33 μg kg^{-1} SQX, and a positive result at a concentration of 115 μg kg^{-1}.

Table 5
Results obtained for spiked honey samples.

Sample	80 μg kg^{-1}			100 μg kg^{-1}			115 μg kg^{-1}		
	SQX	STZ	SDX	SQX	STZ	SDX	SQX	STZ	SDX
1[a]	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
2[b]	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
3[b]	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
4[a]	(−)	(−)	(−)	(F_+)	(F_+)	(F_+)	(+)	(+)	(+)
5[a]	(−)	(−)	(−)	(F_+)	(F_+)	(F_+)	(+)	(+)	(+)
6[b]	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)
7[a]	(−)	(−)	(−)	(F_+)	(−)	(−)	(+)	(+)	(+)
8[b]	(−)	(−)	(−)	(−)	(−)	(−)	(+)	(+)	(+)

Note: (−) Negative, (+) Positive, (F_+) False positive.
[a] Orange honey.
[b] Eucalyptus honey.

For the eucalyptus flower honey, all combinations of two sulfonamides at 80 μg kg^{-1} gave negative results. Combinations at 100 μg kg^{-1} showed one false positive, for 50 μg kg^{-1} SDX+ 50 μg kg^{-1} SQX. All combinations at 115 μg kg^{-1} gave positive results. The SDX/STZ/SQX combination showed a negative result at 80 μg kg^{-1}, a negative result at 100 μg kg^{-1}, and a positive result at 115 μg kg^{-1}.

Samples that gave positive and false positive results in the proposed screening method were analyzed using the HPLC technique, which is the official method for determination and quantification of sulfonamides in honey [9]. Table 6 lists the positive and false positive results obtained for the spiked honey samples using the proposed screening method, together with the concentrations measured by HPLC.

4. Conclusions

A new methodology for the screening of sulfonamide residues in honey samples is presented. Advantages of the technique include its high sensitivity (low detection limit), operational simplicity, and speed. It is environmentally friendly and does not require clean-up steps or complicated sample treatments. The good accuracy of the procedure, together with an absence of matrix effects, indicates that it could provide a valuable tool for fast screening and detection of sulfonamides at the concentration levels established by Brazilian legislation [4], as well as the lower values stipulated elsewhere. This screening methodology can act as a filter, minimizing the number of samples submitted for full analysis and substantially reducing costs and analysis times. It can help to avoid possible environmental damage caused by the disposal of solvents required in the confirmatory method (HPLC). The screening system proposed here contributes to the evolution of green analytical technologies.

Table 6
Positive and false positive results obtained for the spiked honey samples using the proposed screening method, and concentrations measured by HPLC.

Compound	Sample	Fortification level (total concentration, μg kg^{-1})	Screening result	HPLC result (μg kg^{-1})
Individual spiked samples[a]				
SQX	Sample 4	100	False positive	90.00 ± 0.01
	Sample 5	100	False positive	92.35 ± 0.01
	Sample 7	100	False positive	97.70 ± 0.01
	Sample 1	115	Positive	112.25 ± 0.01
	Sample 2	115	Positive	110.60 ± 0.01
	Sample 3	115	Positive	114.85 ± 0.01
	Sample 4	115	Positive	114.30 ± 0.01
	Sample 5	115	Positive	112.65 ± 0.01
	Sample 6	115	Positive	112.85 ± 0.01
	Sample 7	115	Positive	114.35 ± 0.01
	Sample 8	115	Positive	111.20 ± 0.01
STZ	Sample 4	100	False positive	95.70 ± 0.01
	Sample 5	100	False positive	96.35 ± 0.01
	Sample 1	115	Positive	110.50 ± 0.01
	Sample 2	115	Positive	110.75 ± 0.01
	Sample 3	115	Positive	112.30 ± 0.01
	Sample 4	115	Positive	110.45 ± 0.01
	Sample 5	115	Positive	113.45 ± 0.01
	Sample 6	115	Positive	114.85 ± 0.01
	Sample 7	115	Positive	114.30 ± 0.01
	Sample 8	115	Positive	112.55 ± 0.01
SDX	Sample 4	100	False positive	94.70 ± 0.01
	Sample 5	100	False positive	96.35 ± 0.01
	Sample 1	115	Positive	110.50 ± 0.01
	Sample 2	115	Positive	113.70 ± 0.01
	Sample 3	115	Positive	110.70 ± 0.01
	Sample 4	115	Positive	111.20 ± 0.01
	Sample 5	115	Positive	112.85 ± 0.01
	Sample 6	115	Positive	110.55 ± 0.01
	Sample 7	115	Positive	113.05 ± 0.01
	Sample 8	115	Positive	113.65 ± 0.01
Combination spiked samples (two and three sulfonamides)				
Orange honey, Sample 4	SDX + SQX	100	False positive	97.34 ± 0.01
	SDX + STZ	115	Positive	111.36 ± 0.01
	SDX + SQX	115	Positive	110.25 ± 0.01
	STZ + SQX	115	Positive	112.09 ± 0.01
	SDX + SQX + STZ	115	Positive	114.02 ± 0.01
Eucalyptus honey, Sample 3	SDX + SQX	100	False positive	98.29 ± 0.01
	SDX + STZ	115	Positive	105.30 ± 0.01
	SDX + SQX	115	Positive	109.65 ± 0.01
	STZ + SQX	115	Positive	107.49 ± 0.01
	SDX + SQX + STZ	100	False positive	95.14 ± 0.01
	SDX + SQX + STZ	115	Positive	113.57 ± 0.01

[a] Orange honey (Samples 1, 4, 5, and 7); Eucalyptus honey (Samples 2, 3, 6, and 8).

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