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A spectrophotometric flow injection system for streptomycin determination in veterinary samples

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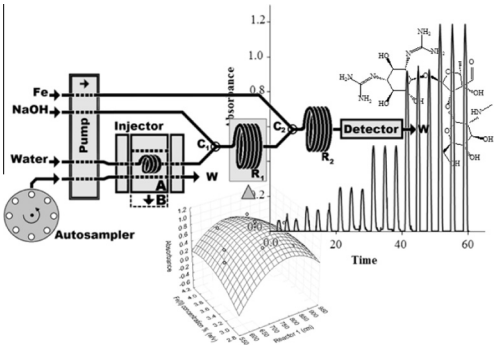
HIGHLIGHTS

- A FIA for streptomycin determination with good analytical performance was proposed.
- Pharmaceuticals is important both for the animal itself and for its meat consumers.
- The FIA presented LOD of 18 mg L^{-1} and analytical frequency of 36 readings per hour.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work a spectrophotometric flow injection analysis system for streptomycin determination in veterinary samples, is being proposed. The method is based on streptomycin alkaline hydrolysis that forms guanidine, followed by the reaction with Fe(II). The colored product has absorption peak at 520 nm. To evaluate and optimize the system parameters, chemometrics tools, such as factorial design, Pareto chart and Doelhert design, were used. The veterinary samples are diluted in water and introduced in the FIA system, therefore no sample preparation is required. The optimized system presented: linear range of 60 up to 1000 mg L^{-1}, limit of detection of 18 mg L^{-1} and sampling rate of 36 readings per hour. The precision was checked and the CV for veterinary sample readings were always less than 6.5%. The accuracy was studied by comparison with chromatographic method, thus, five samples of pharmaceutical veterinary were determined by HPLC and by the proposed method, and the results are in agreement (t-test, $p = 0.05$).

Introduction

The streptomycin is an antibiotic initially studied by Schaltz et al. in 1943, who isolated it from a group of fungi *Streptomices griseus* [1,2]. The streptomycin's action is based on inhibition of ribosomal protein that leads cells to death [2,3]. The quality control of these veterinary samples is important because inadequate dosages can cause allergic reaction, as well as bacteria resistance increase [1].

Among the methods for streptomycin determination there are: ICP-MS [1], capillary electrophoresis [4], HPLC [5], spectrofluorimetry [6] and UV–Vis spectrophotometry [7]. For routine analysis the UV–Vis spectrophotometric determination can be very useful due to its simplicity and low cost. Additionally, the accomplishment between spectrophotometry and FIA is very common and in general it improves the method's performance [8,9]. The main advantages of FIA system are: high sampling rate, better precision,

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low susceptibility to contamination, exploration of some reactions' kinetic, lower reagent/sample consumption and, therefore, lower analysis cost [10].

In this work, it is proposed a FIA system for streptomycin determination based on alkaline analyte hydrolysis, followed by guanidine reaction generated with Fe(II). The reaction's product is determined by spectrophotometer at 520 nm. To evaluate what were the significant parameters of the FIA system, initially a screening employing a factorial design was done, after the Doelhert design is employed to found the optimum values for each parameter [11,12]. The multivariate tools are advantageous because the optimization is done with fewer experiments, and the results are more informative, e.g., it is possible to verify if there is interaction among the variables, and the final optimized values in general are more trustworthy [12].

Experimental

Reagents, solutions and samples

All solutions are prepared with deionized water Milli-Q system (Millipore, Bedford, MA, USA). All glasses are soaked in 10% (v/v) nitric acid for, at least, 24 h to decontamination and after rinsed with deionized water. The streptomycin sulfate, standard solution, is purchased from Sigma Aldrich (Germany); sodium hydroxide and ammonium iron sulfate from Vetec (Brazil). For HPLC analysis the reagents: triethylamine (0.35% v/v), phosphoric acid (0.136% v/v), sodium sulfate (0.71% w/v) (solvent solution), are purchased from Vetec (Brazil), and acetonitrile 18% (v/v) from (Aldrich, Milwaukee, WI, USA).

For interferent studies a binary solution containing the analyte and several common antibiotics such as: Benzylpenicillin potassium, Benzylpenicillin procaine, and Benzylpenicillin benzatine, all of them purchased from Sigma Aldrich (Germany), were checked.

The samples were purchased in local market, and diluted in water for determinations. In FIA system were used the diluted samples resulting in 2.00 g per liter. In the HPLC determination the dilutions were of 0.025 g per liter.

Apparatus

A UV–Vis spectrophotometer (BIOSPECTRO SP-220) in FIA system was used. An eight-channel peristaltic pump (Ismatec IPC-08, Glattzbrugg, Switzerland), provided with Tygon tubes to propel the fluids, and polyethylene tubes of 0.8 mm internal diameter to conduct the fluids, were used. A lab-made autosampler and commutator [13] to FIA system were also used. The reactor coil R1 (see Fig. 1) was kept at 90 °C in boiling water (Quimis Q218-1). The chromatographic determinations were done with a HPLC (Shimadzu) model LC 20A. Table 1 shows experimental conditions adopted for chromatographic determinations [14].

FIA manifold for streptomycin determination

Fig. 1 has shown the manifold of FIA system to streptomycin determination. It can be seen that the sample loop (362 cm) is introduced in the system and receives the sodium hydroxide at confluence C1 and, in the reactor R1 at 90 °C, occur the hydrolysis of streptomycin. In the confluence C2 the sample receives the Fe(II) solution, which reacts with the guanidine in reactor R2, resulting in a colored product, which is determined by spectrophotometer at 520 nm [15,16]. All process is controlled by a personal computer via software written in Visual Basic® language [13].

Optimization of FIA system

Five variables were checked initially in the proposed FIA system: NaOH concentration, Fe(II) concentration, sample loop, reactor R1 and reactor R2. A screening to verify if the effect of each variable is significant employing a fractional factorial design (2^{5-1}) consisting in sixteen experiments were done. The values for each variable studied in fractional factorial plan were shown in Table 2. A Pareto chart for visualization of all variables effects was also done [17]. The significant variables verified in the Pareto chart were set to the next optimization step, i.e., Doelhert design (Table 3). All statistical multivariate calculus and plots were done employing a Statistica® software package (StatSoft, Tulsa, USA).

Results and discussion

System optimization

Pareto chart provided from factorial design is shown in Fig. 2. The R2 variable presented a low effect, probably due to the fast reaction between guanidine and iron, thus the 100 cm to this reactor was adopted.

The variable R1 presented a significant effect. It could be seen that when the length of this reactor was changed from 250 to 500 cm there was an increase in analytical signal. This fact can be attributed to the better interaction between sample/NaOH reagent, also to the temperature effect on the reaction, both contributing to the streptomycin's hydrolysis.

The sample loop provoked a positive effect indicating that better signal at higher loop (377 cm) occurred due to higher sample volume and lesser dispersion into the system.

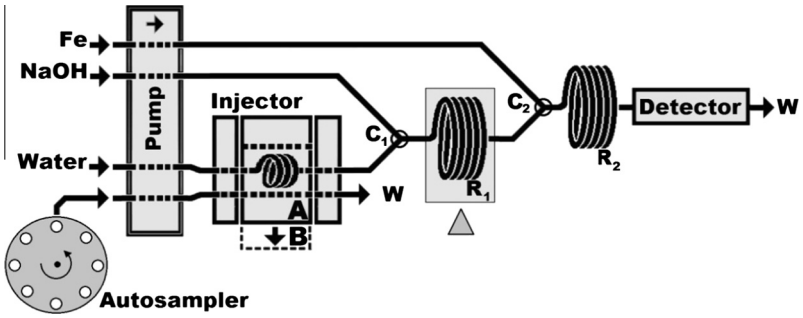

Fig. 1. Manifold of flow system for streptomycin determination. C1 and C2 are confluence streams; NaOH concentration is 0.5 mol L^{-1} (2.4 mL min^{-1}); R1: warmed reactor 753 cm; Δ: thermal bath 90 °C; Fe(II) 3.8% (w/v) (2.6 mL min^{-1}) and R2: reactor for guanidine complexation (100 cm).