Simple and clean determination of tetracyclines by flow injection for monitoring and quality control in veterinary drug industrial production

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Introduction

Tetracyclines are known antimicrobial compounds that present low cost and constitute one of the most widely used antibiotic groups, not only in human medicine for the treatment of infectious diseases but also in the stockbreeding sector as preventive and curative drugs or as an additive in animal feed[1].

The quality control of veterinary pharmaceuticals commercially available is an issue of relevant importance at industrial scale, since inadequate dosage can cause allergic reaction and may increase the number of infections by resistant strains.

Numerous analytical methods which are not in accordance with the principles of green chemistry have been reported for tetracyclines determination in pharmaceuticals.

Thus, the aim of the present study was to develop an environmentally safer analytical methodology for direct quantification of tetracycline (TC) and oxytetracycline (OTC) in veterinary pharmaceuticals, using continuous flow injection analysis with spectrophotometric detection.

Results and discussion

The proposed method is based on the diazotization reaction between the studied tetracyclines and the diazotized sulfanilic acid in a basic medium, resulting in the formation of intense orange azo compounds with a maximum absorption at 434 nm. Initially, various reagents were tested as possible basic medium for the diazotization reaction under study, being more suitable the sodium acetate.

Analytical conditions were optimized using experimental designs that involve different physical and chemical variables of the flow system.

Under the optimal experimental conditions, excellent linear relationship (r^2>0.99) in the concentration range of 1 to 40 µg mL^{-1} were obtained between the measured absorbance values and concentrations of tetracyclines. Detection and quantification limits were 0.40 and 1.35 µg mL^{-1} for TC and 0.95 and 3.18 µg mL^{-1} for OTC, respectively. These results indicate that the proposed flow methodology has adequate sensitivity and linearity to determine TC and OTC in veterinary drugs. The effects of possible interferences were also evaluated and the average recoveries obtained for pharmaceuticals samples ranged from 98.87 to 101.87% for TC and 98.98 to 101.65% for OTC. These results indicate the absence of matrix effects and a good accuracy of the flow methodology proposed.

Finally, six tetracyclines formulations for veterinary use were analyzed by the proposed methodology and by an HPLC confirmatory method. According to the obtained results, the calculated t values did not exceed the theoretical values to 95% confidence level (t = 4.303), indicating that there was no significant difference between both methods[2].

Table 1. Determination of tetracycline and oxytetracycline in commercial veterinary drugs.

Samples	Labeled content	Proposed method [d]	Reference method [d]	*t*-Teste
A - TC	10 [a]	11.03	11.14	3.787
B - TC	1 [b]	1.12	1.07	3.883
C - TC	10 [c]	12.11	12.26	1.750
D - OTC	20 [a]	22.00	21.45	2.199
E - OTC	5.5 [c]	6.04	6.42	3.418
F - OTC	20 [a]	22.04	21.79	0.980

[a] g in 100 mL , [b] g in 7 g, [c] g in 100 g, [d] Average

The recommended procedure substantially reduces the cost and time of analysis and avoids possible environmental damage caused by the disposal of the solvents required for the reference method (HPLC). Thereby, this technique is considered a valuable tool for quality control and monitoring in industrial production of veterinary pharmaceuticals with tetracyclines as active ingredient.

Conclusions

A simple and environmentally safer method to determine tetracycline and oxytetracycline through continuous flow injection analysis in veterinary pharmaceuticals was developed and validated. The flow injection technique proposed is considered as an evolution of Green Analytical Chemistry, contributing to the establishment of methodological tools to prevent and reduce the harmful effects of analytical activities.

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