UDC: 636.09:[615.33.074:543.422.3

COMPARISON OF SPECTROPHOTOMETRIC AND COMPLEXOMETRIC – SPECTROPHOTOMETRIC ASSAY FOR DETERMINATION OF OXYTETRACYCLINE IN VETERINARY DRUGS

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ABSTRACT

Two parallel methods for assay determination of oxytetracycline (OTC) in a final pharmaceutical product were optimized and compared. Both methods include solid-liquid extraction; the first one represents a simple extraction procedure; and the second employed Zirconium (IV) ion to obtain yellow-colored complex with OTC (1:4), whose composition was previously determined with application of the spectrophotometric method of Job. Water was chosen as a solvent in both of the cases, because of its availability; and because it is more ecological and cheaper solvent. The assay was determined UV-spectroscopically, with a VARIAN Carry Win 50 UV/Visible spectrophotometer, in a 1-cm cell at wavelength range 190−500 nm, with resolution 0.5 nm and scan rate of 300 nm/min.

The linear calibration function was established in the concentration range 1 to 40 μg/mL of OTC in respect of both cases (without and with Zr(IV) application). Of the analyzed data the following results for parameters were obtained linearity (both cases $R^2 > 0.999$), accuracy (without Zr - Recovery = 92.0−94.0 %; with Zr – Recovery = 97.0−100.0 %), sensitivity (LOD = 0.27 μg/mL; LOQ = 0.89 μg/mL without; and LOD = 0.58 μg/mL and LOQ = 1.96 μg/mL Zr(IV) addition) and precision (RSD ≤ 2.0 %) in the respective linear concentration ranges.

Both of the presented methods offer the simplicity needed for testing a large numbers of samples, but adding Zirconium is crucial in the assay determination procedure in order to exclude the possible interferences of the excipients or other active ingredients (in case of combined dosage forms) in the tested specimens, which improves the sensitivity, accuracy and the precision of the method.

Key words: UV-VIS spectroscopy, Oxytetracycline -Zirconium complex, method optimization and validation

INTRODUCTION

Since their isolation from different *Streptomyces* species in the late 1940s and early 1950s, the tetracyclines are widely and commonly used broad spectrum antibiotics in humans, to treat different diseases (acne and skin infections; systemic infections of the respiratory, urinary and gastrointestinal tract etc.), and also have a common and important application in veterinary medicine.

Tetracyclines, generally act as bacteriostatic antibiotics, by inhibiting the protein synthesis by reverse binding the 30S ribosomal subunits of susceptible organisms, and preventing access of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex. Tetracyclines also are believed to reversibly bind to 50S ribosomes and additionally alter cytoplasmic membrane permeability in susceptible organisms. In high concentrations, tetracyclines can also inhibit protein synthesis by mammalian cells [1].

Due to its wide antibacterial spectrum, Oxytetracycline (OTC) is a common antibiotic used to treat different food-producing animals such as cattle, pigs, sheep and polutrty, as well as in dogs and cats and fish. Usually it is administrated orally with feed dosage rate of 25−700 mg/kg [1, 2].

Quality assurance and control of OTC in the veterinary products is important in order to prevent overdosage/toxicity or lack of the therapeutical effect; and usually performed with validated and standardized analytical methods. The official valid United States Pharmacopoeia monographs for OTC (and OTC hydrochloride) and British Pharmacopoeia, current edition, state High Performance Liquid Chromatography with UV-detection, as an analytical procedure for quantification of our analyte of interest. Other HPLC techniques include Photodiode array, MS or electrochemical detection. Also throughout the literature there are other methods reported for OTC determination, such as the ones based on microbiological assay which is a procedure with limitations (pH-dependence, low sensitivity, low stability and time consumption). Metal-chelate affinity chromatography, TLC with UV- or FL-detection, UV/FL-spectrophotometry (with or without derivations) are also reported and employed; as well as electrophoresis [2, 3, 4].

Spectrophotometric determination of tetracyclines with uranyl acetate, among which is OTC as well, was reported as well as by using sodium molibdate as analytical reagent [5, 6].

The aim of our study was to develop a simple, accurate, fast, sensitive and economical method for determination of OTC in veterinary drugs that can be readily

used in every day practice in the analytical and quality control laboratories. That is why two parallel methods of OTC determination in a final pharmaceutical (veterinary) product were optimized, then validated, compared and evaluated.

MATERIALS AND METHODS

Apparatus and spectrophotometric conditions

For content determination of OTC, two spectrophotometric methods were developed, which include "VARIAN Carry Win 50" UV-VIS-spectrophotometer, 1-cm quartz cell at wavelength range 190 - 500 nm, with resolution 0.5 nm and scan rate of 300 nm/min.

Chemicals and Reagents

The Oxytetracycline hydrochloride standard (98.1 %) was supplied from Sigma Aldrich. The water that was used as a solvent was de-ionized water supplied form the Faculty of Veterinary Medicine – Skopje, Institute for Food. Zirconium and Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph. Eur, are supplied from Merck.

Commercial veterinary formulation

Two combined veterinary drugs (soluble oral powders) were tested: Neosulfox P where OTC represents 4 % of the powder (1 g powder contains 100 mg of Sulfadimidine, 60 mg Neomycin sulphate, 40 mg Oxytetracycline Hydrochloride) and Geomycin, where OTC represents 5 % of the powder (1 g powder contains 50 mg Oxytetracycline in form of hydrochloride and 35 mg Chlorhexidine digluconate).

Preparation of Standard Stock Solutions of OTC:

• OTC Standard Stock Solution:

20 mg of Oxytetracycline hydrochloride RS precise weight is transferred into 20 ml volumetric flask, then 12 mL of Methanol is added, mechanically shaken to achieve complete dissolution (sonicated if necessary) and filled up to the mark with the same solvent; concentration of about 1 mg/mL Oxytetracycline RS.

Then by applying suitable dilutions with water, deionized, calibration standard solutions for the range of the method were prepared, i.e. from 1 to 40 µg /mL.

• Zirconium – OTC (1:4) Standard Stock Solution:

The basic stock solution is prepared identically as previously mentioned with the difference when making the dilutions into suitable concentration range, i.e. from 1 to 40 µg/mL, 100 µL of the Zirconium(IV) solution is added to produce yellow colored complex. The solvent is water, de-ionized.

Test solution(s) preparation

Of the previously fine powdered granulates of the Neosulfox P and Geomycin, a quantity of the homogenous powder equivalent to 5 mg OTC hydrochloride and OTC respectively is weighed and transferred quantitatively into 50 mL volumetric flask. 30 mL water, deionized is added and the sample is sonificated about 20 minutes and mechanically shaken 15 minutes and then filled up to the mark with the same solvent.

The test solution then is filtered, discarding the first 10 ml of the filtrate.

The filtrate is then used for preparation of suitable dilutions for further testing.

Procedure

Both methods include solid-liquid extraction; the first one represents a simple extraction procedure; and the second emloyes Zirconium(IV) ion to obtain yellow-coloured complex with OTC (1:4), whose composition was previously determined with application of the spectrophotometric method of Job.

When performing the analysis with Zirconium, all the test solutions preparation steps are the same as previously, with a difference in the final dilutions, when Zr(IV) solution is added and then filled to the mark with water.

Data analysis

For determination of the statistical parameters, the Microsoft Office Excel is used.

RESULTS AND DISCUSSION

All of the analytical validation parameters for this proposed method were determined according to the ICH guidelines. A parallel summary for both veterinary drugs of the obtained validation parameters are presented in Table 1a and 1b. The comparison between the spectral curves of OTC standard solutions without and with Zirconium(IV) ion is presented in addition (Figure 1) [5, 8, 9].

1. LINEARITY& RANGE

The Linearity of the both spectroscopic methods was determined at ten concentration levels ranging from 1-40 µg Oxytetracycline hydrochloride RS/mL (1, 2, 3, 5, 10, 12, 15, 20, 30 and 40 µg/mL) and calibration curve was constructed for both cases by plotting the respective concentrationsvs. detector's response i.e. absorption maxima. Linear correlation in the above mentioned concentration range was confirmed with the coefficient of correlation of R^2 =1.000 in case of simple extraction without, and R^2 = 0.9998 in the case with Zirconium(IV) addition.

2. ACCURACY/ Analytical recovery

The Accuracy of the method with the study of analytical recovery was performed and confirmed at three concentration levels (5 -15 µg /mL) for both cases, by spiking the various pre-analyzed sample formulations of OTC with known quantities of OTC standard solution, and then analyzing the mixtures by the proposed method. The recovery results were found to be in the range of 92.0 - 94.0 % without and 97.0-100.0 % with Zirconium(IV) addition.

3. SENSITIVITY

Both the Limit of Detection (LOD) determination at lowest concentration giving response and Limit of Quantification (LOQ) determination were estimated from the standard deviation of the response and the slope, based on the data of the calibration curve. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.27 µg/mL and 0.89 µg/mL without; and 0.58 µg/mL and LOQ = 1.96 µg/mL, respectively, with Zirconium(IV) addition. The LOD and LOQ showed that both of the methods are sensitive for OTC determination.

4. PRECISION/ Repeatability

The method repeatability was performed using 9 de-

terminations covering the specified range of the procedure i.e. three concentrations with three replicates each. The obtained RSD is lower than 2.0 %, i.e. Geomycin 1.69 % without and 1.06 % with Zirconium(IV) addition and for Neosulfox P, 1.22 % and 1.49 % respectively. The calculated RSD value for Neosulfox P according to Horwitz [8] is 4.80 % and for Geomycin 4.60 %.

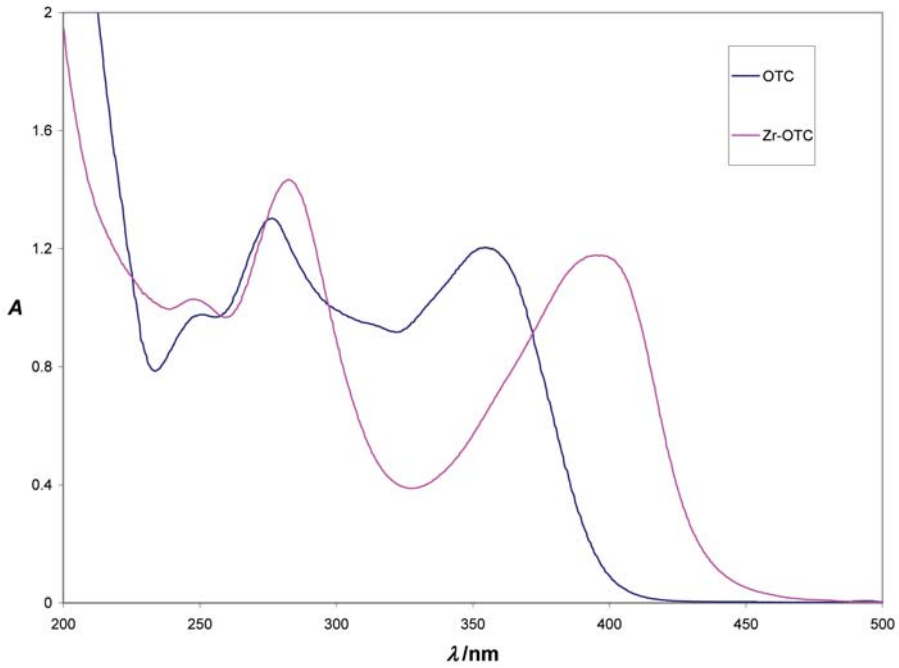

Figure 1. *Comparison between the UV spectrum of OTC standard solutions without (λmax = 275 and 350 nm) and with Zirconium(IV) ion is presented (λmax. = 280 and 395 nm)*

It can be noted that the spectral curves of OTC-Zr-complex are more defined than the ones of OTC solely. For results evaluation in both cases the data obtained from λmax. = 350 nm for OTC and 395 nm for OTC-Zr were used.

Table 1a. *Validation summary for NEOSULFOX P, without and with Zirconium(IV) introduction into the standard/test solution.*

Analytical technique		UV-Spectrophotometry	
Type of apparature for validation		UV – VIS Spectrophotometer VARIAN, Carry Win 50	
Validation parameters	**Acceptance criteria**	**Results**	
		Without Zirconium(IV)	With Zirconium(IV)
RANGE:	min. acceptable 80 – 120 %	1 to 40 µg/mL of OTC Oxytetracycline hydrochloride RS / mL	1 to 40 µg/mL of OTC Oxytetracycline hydrochloride RS / mL
LINEARITY: Correlation coefficient R^2:	≥ 0.9900	R^2 =1.0000	R^2 =0.9998
SENSITIVITY: LOD		0.27 µg/mL	0.58 µg/mL
LOQ		0.89 µg/mL	1.96 µg/mL
ACCURACY: Recovery:	95 – 105 %	Recovery = 93.6 %	Recovery = 97.02 %
PRECISION: (method repeatability)	RSD≤ 4.80 %	s = 0.1117 RSD = 1.22 %	s = 0.1517 RSD = 1.49 %

Table 1b: *Validation summary for GEOMYCIN, without and with Zirconium(IV) introduction into the standard/test solution.*

Analytical technique		UV-Spectrophotometry	
Type of apparature for validation		UV – VIS Spectrophotometer VARIAN, Carry Win 50	
Validation parameters	**Acceptance criteria**	**Results**	
		Without Zirconium(IV)	With Zirconium(IV)
RANGE:	min. acceptable 80 – 120 %	1 to 40 µg/mL of OTC Oxytetracycline hydrochloride RS	1 to 40 µg/mL of OTC Oxytetracycline hydrochloride RS
LINEARITY: Correlation coefficient R^2:	≥ 0.9900	R^2 =1.0000	R^2 =0.9998
SENSITIVITY: LOD		0.27 µg/mL	0.58 µg/mL
LOQ		0.89 µg/mL	1.96 µg/mL
ACCURACY: Recovery:	95 – 105 %	Recovery = 92.18 %	Recovery = 97.21 %
PRECISION: (method repeatability)	RSD≤ 4.60 %	s = 0.1240 RSD = 1.69 %	s = 0.0970 RSD = 1.06 %

CONCLUSION

Comparing the results obtained with statistical evaluation from both proposed methods for spectrophotometric content determination of Oxytetracycline in the combined veterinary drugs, it can be concluded that the method where Zirconium(IV) ion is added, is more selective, sensitive, accurate and reproducible, than the one that only includes simple extraction procedure with water, because when the OTC-Zr complex is made the absorption maxima excludes the possible additive effects and interferences of the other active compounds, as well as the excipients within the formulation.

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СПОРЕДБА НА СПЕКТРОФОТОМЕТРИСКИ И КОМПЛЕКСОМЕТРИСКО-СПЕКТРОФОТОМЕТРИСКА АНАЛИЗА ЗА ОПРЕДЕЛУВАЊЕ НА ОКСИТЕТРАЦИКЛИН ВО ВЕТЕРИНАРНИ ЛЕКОВИ

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АПСТРАКТ

Оптимизирани се и споредени два паралелни метода за определување на окситетрациклин (OTC) во финален фармацевтски производ. И двата метода вклучуваат цврсто-течна екстракција; првиот претставува едноставна екстракциона постапка а вториот метод вклучува цирконium (IV) јон за добивање на жолто обоен комплекс со OTC (во однос 1:4), чиј состав беше претходно утврден со примена на спектрофотометрискиот Job-метод. И во двата случаи, како растворувач беше користена водата заради нејзината достапност, ниската цена и занемарливиот ефект врз екологијата. Анализата беше направена со УВ-спектроскопија, со VARIAN Carry Win 50 UV/Visible спектрофотометар, во 1-cm кварцна ќелија во област на бранови должини од 190-500 nm, со резолуција 0,5 nm и брзина на скенирање 300 nm/min.

Со двата метода (без и со Zr(IV) јон) конструирана е калибрациона права во концентрациски опсег од 1-40 μg/mL OTC. Од анализираните податоци беа добиени следниве резултати за параметрите на линеарност (и во двата случаја R^2> 0,999), точност (без Zr – аналитички принос = 92,0-94,0%; со Zr(IV) - аналитички принос = 97,0-100,0%), осетливост (LOD = 0,27 μg/mL ; LOQ = 0,89 μg/mL , без Zr(IV) и LOD = 0,58 μg/mL и LOQ = 1,96 μg/mL со Zr(IV)) и прецизност (RSD ≤ 2,0%) во соодветниот линеарен концентрациски опсег.

Двата претставени метода ја нудат потребната едноставност за тестирање на голем број на примероци. Додавањето на цирконium-јон е од клучно значење во постапката за анализа на OTC, со цел да се исклучат можните пречки од ексципиентите или другите активни состојки (во случај на комбинирани дозирани форми) во тестираните примероци, со што се подобрува осетливоста, точноста и прецизноста на методот.

Клучни зборови: УВ-ВИС спектроскопија, окситетрациклин-цирконium комплекс, оптимизација и валидација