

Development and Validation of a Spectrofluorimetric Method for the Assay of Tetracycline in Capsules

Lucien Murhula Namegabe^{1*}, Serigne Omar Sarr^{2,3}, Yérím Mbagnick Diop^{2,3}

¹Department of Galenic Pharmacy, Official University of Bukavu, Bukavu, Democratic Republic of the Congo

²Analytical Chemistry and Food Sciences Laboratory, Cheikh Anta Diop University, Dakar, Senegal

³National Medicines Control Laboratory, Dakar, Senegal

Email: *murhulan@yahoo.fr

How to cite this paper: Namegabe, L.M., Sarr, S.O. and Diop, Y.M. (2018) Development and Validation of a Spectrofluorimetric Method for the Assay of Tetracycline in Capsules. *American Journal of Analytical Chemistry*, 9, 162-170.

<https://doi.org/10.4236/ajac.2018.93014>

Received: February 7, 2018

Accepted: March 12, 2018

Published: March 15, 2018

Copyright © 2018 by authors and Scientific Research Publishing Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

The purpose of this study is to develop and validate a method for the analysis of tetracycline capsules by spectrofluorimetry. A pH 9 borate buffer was used as diluent of tetracycline after reaction with magnesium salt at the excitation wavelength of 372 nm and 516 nm of emission. A linear response was observed between 0.25 µg/mL and 1.5 µg/mL with a correlation coefficient (R) of 0.9998. The detection and quantification limits found are 0.0125 µg/mL and 0.0412 µg/mL respectively. The proposed method proved trueness with a recovery between 99.88% and 101.10%. The relative standard deviations of repeatability and intermediate precision found $\leq 2.88\%$ reflected a good precision of the method. The proposed method is therefore valid within the limits of 90% to 110%. The proposed method was applied to the quality control of 9 tetracycline samples from market and gave results in accordance with the pharmacopoeia standards.

Keywords

Validation, Spectrofluorimetry, Tetracycline

1. Introduction

Tetracycline hydrochloride (THC) is a broad-spectrum polyketide antibiotic produced by the genus *Streptomyces*. It exerts a bacteriostatic effect on bacteria by reversibly binding the 30S ribosomal subunit of bacteria and blocking the binding of tRNA to the ribosome acceptor site. It also binds to some extent to

the bacterial 50S ribosomal subunit and can alter the cytoplasmic membrane causing leakage of intracellular components of bacterial cells [1].

A large number of analytical techniques for the determination of tetracycline and its degradation products have been reported, particularly in biological fluids and pharmaceuticals such as spectrophotometry [2] [3] which is of limited utility due to its non-specificity. More efficient separative methods, such as capillary electrophoresis [4] and high performance liquid chromatography [5] [6] using UV and fluorescence detectors require a lot of time, proven expertise and a very high operating cost. This limits their use in routine quality control and especially in developing countries with limited resources. The aim of this study is to validate a spectrofluorometric method for tetracycline assay in capsules by using the accuracy profile approach [7]. This method has proved to be simple, sensitive, fast, efficient and cheap.

2. Material and Method

2.1. Materials

2.1.1. Apparatus

A Perkin Elmer luminescence spectrometer model LS 45[®] (Perkin Elmer instruments, Massachusetts, USA) connected to a Fujitsu Siemens computer loaded with the FLwinlab[®] application software was used.

2.1.2. Reagent

The following reagents and chemicals of analytical grade (except specific indication) were used:

Potassium dihydrogenphosphate (KH_2PO_4), hydrogenphosphatedisodium (Na_2HPO_4), boric acid, Sodium hydroxide both from sigma-aldrich, Tetracycline hydrochloride (**Figure 1**) was provided by the European Pharmacopeia.

Purified water was produced in situ with a Milli-Q Ultrapure Water System (Millipore, Molsheim, France).

2.2. Method

2.2.1. Analytical Parameters

Optimum reaction conditions have been studied such as: The solvent, Effect of magnesium sulphate, Stability of tetracycline in water and Influence of pH.

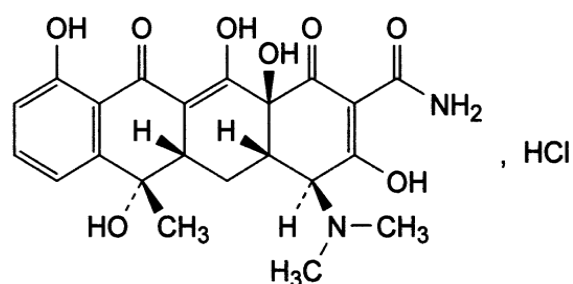


Figure 1. Structure of tetracycline hydrochloride.

2.2.2. Preparation of Solutions for the Determination of the Calibration Curve

10.00 mg of THC are dissolved in 100 mL of distilled water to obtain the standard solution (SS). 0.5-1-1.5-2-2.5 and 3 mL of the SS are transferred respectively into the 200 mL volumetric flasks and diluted to the mark. 5 mL of the pH9 buffer solution are added to each 5mL of dilution followed by 2.5 mL of the MgSO₄ solution (0.75 M).

2.2.3. Accuracy Profile

Accuracy is the total error after the sum of systematic error (trueness) and random errors (precision). It is represented by an accuracy profile for the area of measures (75% - 125%). Accuracy of the method is established through the accuracy profile described by Feinberg.

The accuracy profile approach allows to determine simultaneously the recovery and precision of the method using standard and validation solutions as previously described [8].

1) Preparation of Standard Solution

Three solutions of concentration of 0.75-1-1.25 µg/mL are prepared. 5 ml of each dilution, 5 mL of the pH 9 buffer solution and 2.5 mL of the MgSO₄ solution (0.75 M) are mixed in a conical tube. The reading of the fluorescence intensity is carried out after 25 min at 372 nm of excitation and 516 nm of emission.

2) Preparation of Test Solution

The reference sample for validation is reconstituted from the active ingredient (tetracycline) and the matrix (Lactose, Mannitol, magnesium stearate and starch), thus the stock solution is made of 10 mg of tetracycline and 10 mg of the matrix dissolved in 100 mL of water and filtered. Solutions of concentrations of 0.75-1-1.25 µg/mL are prepared. 5 mL of the buffer solution at pH 9 and 2.5 mL of the MgSO₄ solution (0.75 M) are added to 5 mL of each solution. The reading of the fluorescence intensity is carried out after 25 min at 372 nm of excitation and 516 nm of emission.

2.2.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection is estimated by diluting a solution of known concentration until the lowest near detectable signal is different from that of blank, while the limit of quantification is 3.3 times the limit of detection [9]. They were determined as specified in the ICH (International Conference on Harmonisation) protocol [10].

2.2.5. Application of the Method

A test sample of 10 mg of tetracycline is carried out on the powder emptied from 5 capsules (taking into account the average weight). After dissolution in 100 mL of distilled water, homogenization with ultrasound for 10 minutes and filtration, 2 mL of the filtrate are diluted to 200 mL to obtain a solution of 1 µg/mL of THC. To 5 mL of the 1 µg/mL solution are added 5 mL of Borate buffer at pH 9 and 2.5 ml of the 0.75 M MgSO₄ solution. The reading of the fluorescence inten-

sity is carried out after 25 min at 372 nm of excitation and 516 nm of emission.

3. Results

3.1. Study of the Optimal Reaction Conditions

3.1.1. Effect of Solvent on Fluorescence Intensity

A solution with a cloudy appearance is obtained when the magnesium sulfate is put into the THC solution in basic medium. This solution is unusable due to the formation of insoluble compounds. On the other hand, in the water a weak signal is observed as it appears in **Table 1**.

3.1.2. Influence of pH

Signals are stronger with basic buffers than acidic buffers (**Figure 2**). An excellent and stable signal is observed at pH 9. The use of MgSO_4 at pH 5.6 helped to boost the fluorescence of tetracycline dissolved in water (non fluorescent). The complex formed between THC and Mg gave a good stability in function of time.

3.2. Study of Selectivity

The comparison of data found during the analysis of THC pure product and when it is with the other substances which accompany it in galenic presentation (the excipients) gave two spectra (**Figure 3** and **Figure 4**).

3.3. Graph Calibration

Employing the conditions described in the procedure, a calibration curve was drawn in **Figure 5**.

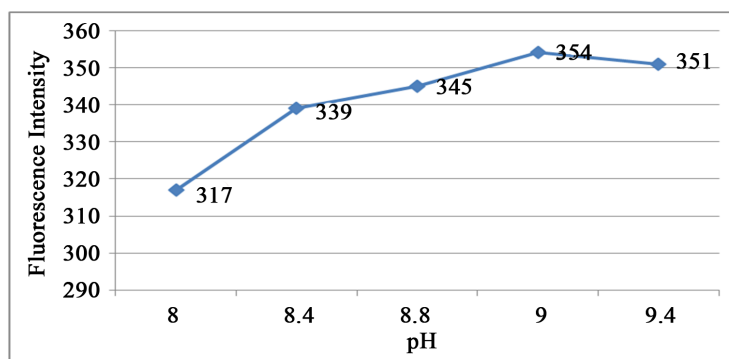


Figure 2. Effect of pH on the fluorescence intensity.

Table 1. Effect of magnesium sulfate on the bypass.

Settings	NaOH 0.5 µg/mL	H ₂ O 5 µg/mL
	MgSO ₄	MgSO ₄ à pH 5.6
λ _{ex}	-----	380 nm
λ _{em}	-----	520 nm
FI*	-----	103
Observation	Non exploitable	stable

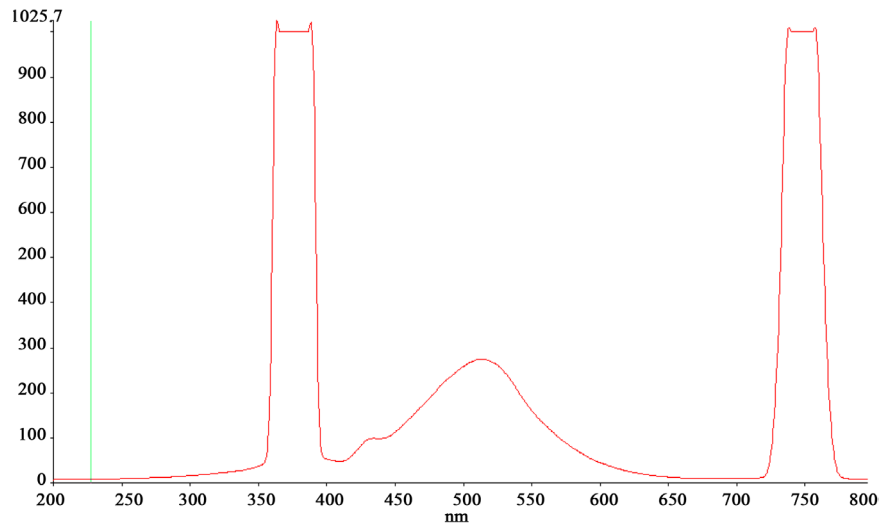


Figure 3. Spectrum of pure TCH.

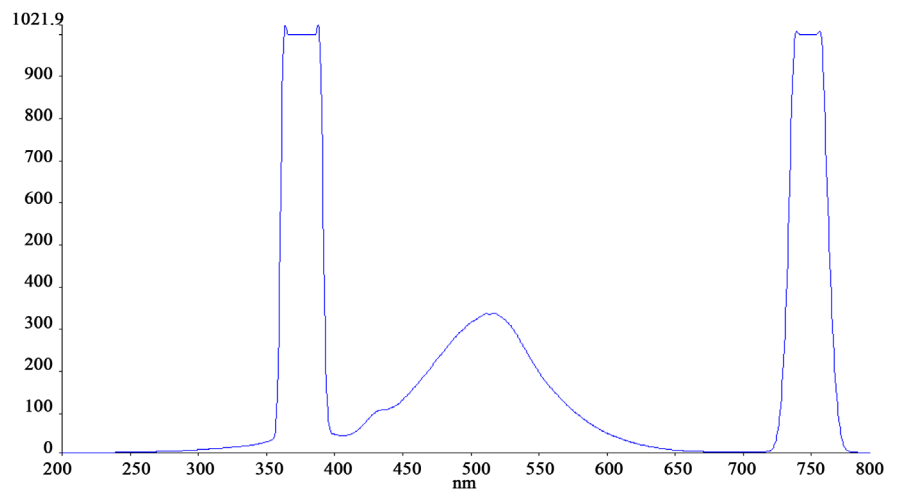


Figure 4. Spectrum of TCH in the matrix.

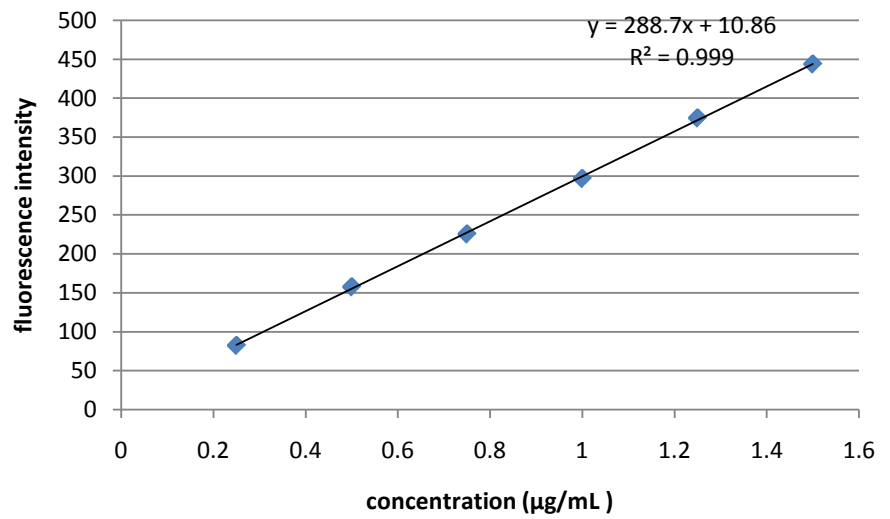


Figure 5. TCH Calibration Curve.

The equation of regression way $y = 289.11 + 10.448x$ with a correlation coefficient (R) of 0.9998.

3.4. Accuracy Profile

The recovery data, precision and confidence interval are shown in **Table 2** and **Figure 6**. For trueness, recoveries were between 99.88% and 101.10%. For intra-day and inter-day precision the RSD (relative standard deviation) between 1.57% and 2.88% were lower than 5%.

3.5. Application of the Validated Method for the Determination of TCH in Pharmaceutical Preparations

To make the application of the method effective, it has been used for the direct determination of TCH in pharmaceutical preparations. Nine lots of THC capsules were separately selected for quantitative determination of THC. The THC content in samples collected from the market is determined qualitatively by the validated method and results are presented in **Table 3**.

4. Discussion

Although Tetracycline is soluble in aqueous solutions, water and methanol, it is far from stable in these solvents. Most often, it is transformed into Epitetracycline hydrochloride (ETC). This epimerization reaction is reversible. In the presence of divalent cations the reaction is not made [11].

Dissolved in water, no signal was found for TCH; on the other hand dissolved in water in the presence of Mg at a pH of 5.6, a signal was found at 380 nm of

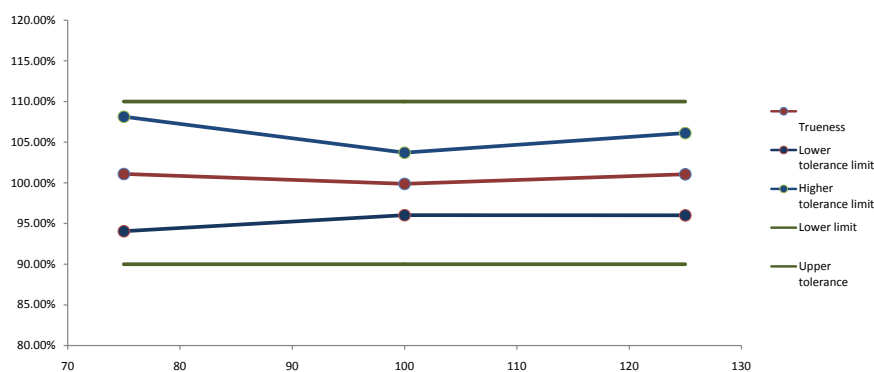


Figure 6. Diagram of the accuracy profile of the proposed method.

Table 2. Summary data for establishing the accuracy profile.

concentration	Trueness	Repeatability RSD	Intermediate precision RSD	Tolerance	Lower tolerance limit	Higher tolerance limit	Lower limit	Upper tolerance
75%	101.10%	2.88%	2.88%	7.04%	94.06%	108.13%	90%	110%
100%	99.88%	1.57%	1.57%	3.84%	96.04%	103.72%	90%	110%
125%	101.07%	1.80%	2.07%	5.06%	96.01%	106.13%	90%	110%

T = 2.45; ddl = 6 ; alpha risk = 0.05.

Table 3. Results of samples assay.

Sample	1	2	3	4	5	6	7	8	9
Mean +/- SD (%)	99.20 ± 0.91	101.90 ± 0.54	99.00 ± 2.02	103.10 ± 2.83	98.50 ± 0.96	108.20 ± 1.26	105.30 ± 0.63	104.00 ± 0.46	94.50 ± 1.02

Mean: mean concentration ; SD: standard deviation.

excitation and 520 nm of emission (**Table 1**). This coincided with the results found by Rodríguez *et al.* [12] using methanol as a diluent and other divalent cations selective for tetracycline [13] [14].

It turns out that pH 9 is the one that gave the highest signal (**Figure 2**). THC is more fluorescent under these conditions, i.e. stable at pH > 8 [12].

Thus, for the analysis of TCH, distilled water (used as diluent), Borate at pH 9 (buffer) and MgSO₄ 0.75 M (not only to boost fluorescence but also to keep the stability of the TCH) are retained in this proposed method.

Linearity was established by the least squares regression method of the calibration curve. This standard curve was obtained in the range of 0.25 to 1.5 µg/mL. The correlation coefficient is greater than 0.9998. The results show an excellent correlation between the fluorescent intensity and the concentration of the analyte. These values are in accordance with ICH specification [10].

The trueness recoveries were between 99.88% and 101.10%. For intra-day and inter-day precision the relative standard deviation (RSD) between 1.57% and 2.88% were lower than 5%.

These values below 5% indicate a good precision confirmed by the accuracy profile (**Figure 6**) which clearly shows the good quantitative method performance throughout the validation field [15].

The student test was used at 5% risk and at a degree of freedom of 6, the found t is 2.45, thus, the confidence interval found per level varies from 3.84 to 7.04%. These values are in accordance with ICH specifiable accepted limit for tetracycline content varies from 90 to 125% for the American Pharmacopoeia [5] for our method the limit or interval of validity fixed is between 90% - 110%. With regard to the profile drawn (**Figure 6**), we note that the lower and upper bounds found vary from 94.06% to 108.13%. This method is therefore valid in this concentration range.

Limits of detection and quantification are determined based on signal-to-noise ratios, comparing signals measured from samples of known concentrations of THC with the signal from the blank. Experimentally, the detection limit (LD) found is 0.0125 µg/mL and the limit of quantification (LOQ) is 0.0412 µg/mL. This LOQ obtained is approximately 35 times lower than that (1.739 µg/mL) reported for the spectrophotometric method [3]. It is close (0.026 µg/mL) to that reported for HPLC [6].

The content of our samples is quantitatively determined. The results found vary from 94.5% to 108.5%. Margins of (100 ± 10)% have been set as the acceptable limit for the method used. The results of the samples met the official acceptance criteria.

5. Conclusions

The present work demonstrates that the method is fair, faithful, selective and sensitive. The linear response function was found for a range of concentrations ranging from 0.25 to 1.5 µg/mL with a correlation coefficient of 0.9998.

Considering its low limit of detection and quantification, its sensitivity and selectivity, the method seems competitive with respect to titration and spectrophotometry which are reported as other analytical techniques. Although high performance liquid chromatography has a similar sensitivity, the method developed gives the advantage of being easy, fast, less expensive and avoids the tedious extraction procedure, the use of expensive and sometimes toxic organic solvents.

The validated spectrofluorimetry method was successfully applied in the direct determination of THC in 9 batches of pharmaceutical preparations.

It is therefore usable as a technique for routine analysis of tetracycline in pharmaceutical preparations.

References

- [1] Drug Bank, Tetracycline (2016). <http://www.drugbank.ca/drugs/DB00759>
- [2] Clarke (1986) Clarke's Isolation and Identification of Drugs in Pharmaceuticals. 2nd Edition, Pharmaceutical Press, London.
- [3] Hadi, H. and Fadhil, G. (2014) Sensitive Spectrophotometric Determination of Tetracycline Hydrochloride in Dosage Forms Using Sodium Nitroprusside and Hydroxylamine Hydrochloride. *Journal of Al-Nahrain University*, **17**, 53-58. <https://doi.org/10.22401/JNUS.17.3.07>
- [4] Kowalski, P. (2008) Capillary Electrophoretic Method for the Simultaneous Determination of Tetracycline Residues in Fish Samples. *Journal of Pharmaceutical and Biomedical Analysis*, **47**, 487-493.
- [5] United States Pharmacopoeia (2016). http://www.pharmacopeia.cn/v29240/usp29nf24s0_m81820.html
- [6] Hussien, E. (2014) HPLC Method Validation for Modernization of the Tetracycline Hydrochloride Capsule USP Monograph. *Bulletin of Faculty of Pharmacy*, **52**, 239-244. <https://doi.org/10.1016/j.bfopcu.2014.08.002>
- [7] Feinberg, M. (2010) Interpretation of the Accuracy Profile. Le Cahier des techniques de l'INRA, Special Volume, 45-60.
- [8] Feinberg, M. (2006) Approche globale et harmonisée de la validation. *Spectra Analyse* No. 249.
- [9] Recueil International des méthodes d'analyses (2016) Résolution OENO 7/2000. <http://www.oiv.int/public/medias/2750/oiv-ma-as1-10fr.pdf>
- [10] ICH Validation of Analytical Procedures (1997) Text and Methodology. *International Conference of Harmonization*, **62**, 27463-27467. www.ich.org/products/guidelines/quality/quality-single/article/validation-of-analytical-procedures-text-and-methodology.html
- [11] Florer, K. (1984) Analytical Profiles of Drug Substances. Academic Press, Cambridge, Vol. 13, 602-603, 613-616.
- [12] Rodríguez, N., Ortiz, C., Herrero, A. and Sarabia, L. (2007) Performance Character-

ristics According to Commission Decision 2002/657/EC in the Fluorimetric Determination of Tetracycline in the Absence and in the Presence of Magnesium. *Luminescence*, **22**, 518-526. <https://doi.org/10.1002/bio.993>

- [13] Abdel-hady, M., Barary, H. and Mahgdub, H. (1985) Spectrofluorimetric Assay of Tetracycline and Anhydrotetracycline in Combination. *Tafanto*, **32**, 1153-1155.
- [14] Caswell, A. and Hutchison, J. (1971) Selectivity of Cation Chelation to Tetracyclines: Evidence for Special Conformation of Calcium Chelate. *Biochemical and Biophysical Research Communications*, **43**, 625-630. [https://doi.org/10.1016/0006-291X\(71\)90660-7](https://doi.org/10.1016/0006-291X(71)90660-7)
- [15] ORA LABS (2014) Validation and Verification Guidance for Human Drug. Analytical Method, Appendix 1, 17-19.