Development and Validation of Spectrophotometric Methods for Determination of Thiamphenicol in Capsule Forms

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ABSTRACT: A simple and direct spectrophotometric method is developed for the determination of Thiamphenicol (THIA) in pharmaceutical formulations. The optimum conditions for the analysis of phosphate buffer at pH 2.0 of drug are studied. Under the optimum conditions, the drug could be assayed in the concentration range 1.10^{-5}-6.10^{-5} M. Detection and quantification limits are calculated.

Key Words: *Spectrophotometric determination; drug.*

Tiamfenikol'ün Kapsül Formlarından Spektrofotometrik Tayin Yönteminin Geliştirilmesi ve Geçerliliği

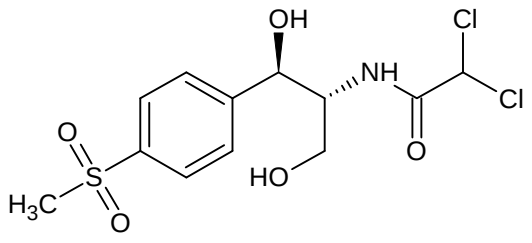
ÖZET: Bu çalışmada Tiamfenikol'ün farmasötük formlarından basit ve direkt spektrofotometrik tayin yöntemi geliştirilmiştir. Bu çalışma için en uygun çalışma ortamı fosfat tamponu (pH=2) seçilmiştir. Optimum şartlar altında çalışma aralığı 1.10^{-5}-6.10^{-5} M olup, yakalama alt sınırı ve tayin alt sınırı hesaplanmıştır.

Anahtar Kelimeler: *Spektrofotometrik tayin, ilaç*

1. Introduction

Thiamphenicol (also known as thiophenicol and dextrosulphenidol) is an antibiotic. It is the methyl-sulfonyl analogue of chloramphenicol and has a similar spectrum of activity, but is 2.5 to 5 times as potent. Like chloramphenicol, it is insoluble in water, but highly soluble in lipids. It is used in many countries as a veterinary antibiotic, but is available in China and Italy for use in humans. Its main advantage over chloramphenicol is that it has never been associated with aplastic anaemia.[1]

Different methods for analysis of the THIA were reviewed. The structural formula of this drug was illustrated in Scheme 1.



Scheme 1. Structure of THIA

Reviewing the literature revealed that several methods have reported for the analysis of this drug. Whether in pharmaceutical preparations or in biological fluids. Among these methods are spectrophotometry [2,3], chromatography [4,5] and electrochemical methods [6]. These methods noted in the literature, particularly the chromatographic techniques are time consuming, costly and require expertise. A simple and precise UV-spectrophotometric method can be highly applicable for routine analysis of bulk, formulations and dissolution samples. The aim of the present study is to improve a simple, precise, accurate and economic analytical method with a better detection range for the estimation of THIA in bulk and pharmaceutical formulations. No extraction, derivatization or evaporation step, no complexation agent and no harmful chemicals are involved in the suggested method, in that connection decreasing time and the error in quantitation.

To our awareness, there is no direct UV spectrophotometric method for determination of this drug in pharmaceutical compounds for phosphate buffer media in literature. Hence, the objective of the present study is the development of basic and sensitive analytical methods for the determination of THIA in pharmaceutical preparations. The method was validated to determine the dosages form.

2. Experimental

2.1. Apparatus

Perkin-Elmer Lambda 45 UV-vis double beam spectrophotometer with a slit width of 2 nm was used. The absorbance values were measured using 1 cm quartz cells.

2.2. Reagents

THIA and dosage forms (Urfamycin) capsule (500 mg) were kindly provided by Bilim Pharmaceutical Co. (Istanbul, Turkey). All chemicals for preparation of

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buffers such as H_3PO_4, NaH_2PO_4 and Na_2HPO_4 were reagent grade (Merck or Sigma). Stock solutions of THIA ($1x10^{-3}$ or $1x10^{-4}$M) were prepared in doubly distilled water. Phosphate buffers (0.2M, pH 2.0–12.0) were used in this study. Standard solutions were prepared by dilution of the stock solution with selected buffer solutions containing THIA in the concentration range of $1x10^{-5}$ to $6x10^{-5}$M. The calibration equation drug analysis was constructed absorbans against THIA concentration.

2.3. Preparation of standard and quality control solutions

An aqueous primary stock solution of 10^{-3}M of THIA was prepared in water. All measurements were performed at 25 ^{o}C. The standard solutions were prepared by the suitable dilutions of the primary stock solution with ultra pure water to obtain working standards in the concentration range of 1.10^{-5}–6.10^{-5}M. The absorbances of these solutions were then fitted in the calibration curve to calculate the accuracy and precision of the method.

3. Method Validation

3.1. Linearity

The method was validated according to International Conference on Harmonization Q2B guidelines [7] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte [8-11].

3.2. Precision and accuracy.

The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as RSD % for a statistically significant number of replicate measurements [12]. The intermediate precision was studied by comparing the assays on three different days and the results were documented as standard deviation and RSD %. Accuracy is the percent of analyte recovered by assay from a known added amount. Data from nine determinations over three concentration levels covering the specified range were obtained. The repeatability of the method was determined by assaying six sample solutions of the highest test concentration [13].

3.3. LOD and LOQ.

In this study, LOD and LOQ were based on the standard deviation of response and the slope of the corresponding curve using following equations [12];

$$LOD = 3s/m; \quad LOQ = 10s/m$$

Where s, the noise estimate, is the standard deviation of the absorbance of the sample, m is the slope of the related calibration graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability [12]. The values of LOD and LOQ were given in Table 1. Extraction of active ingredient. The five capsules were accurately weighed and emptied as so completely as possible. The emty capsules were weighed again, and the differences were given as the total amount of the five capsules contents. The combined contents of the capsules were thoroughly ground to a fine powder. A sufficient amount of this powder for preparing a stock solution of 1.10^{-3} M accurately weighed and transferred into a 25 ml calibrated flask and volume was completed with distilled water for THIA. Appropriate dilutions were made and the samples were subjected to UV analysis.

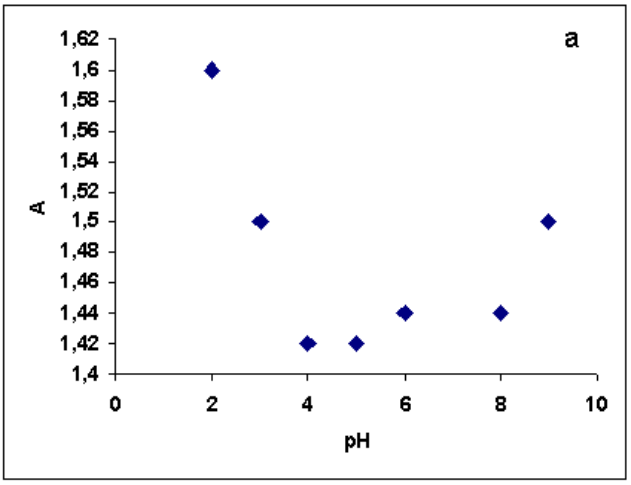
3.4. Recovery experiment from dosage forms

Recovery of the analytes of interest from a given matrix can be used as a measure of the accuracy or the bias of the method. The same range of concentrations as employed in the linearity studies were used. To study the accuracy, precision and reproducibility of the proposed methods and to check the interference from the excipients used in the dosage forms, recovery experiments were carried out using the standard addition method. These studies were performed by addition of known amounts of pure THIA to the pre-analysed dosage forms and the mixtures were analysed by the proposed techniques. After parallel analyses, the recovery results were calculated using the related calibration equations.

4. Results and Discussion

4.1. Analytical parameters of developed method

The development of simple, rapid, sensitive and accurate analytical method for routine quantitative determination of samples will reduce unnecessary tedious sample preparations and cost of materials and labor. The effects of pH of the phosphate buffer on the reaction of THIA in aqua media were examined by varying the pH from 2.0 to 12. Phosphate buffers generally gave a higher and more stable product yield and the best yields were obtained at pH 2.0 (Fig 1a). The pH effects on the absorbance and wavelength have been given in the Fig. 1a,b. The maximum wavelength is not been changed by pH (Fig 1b).



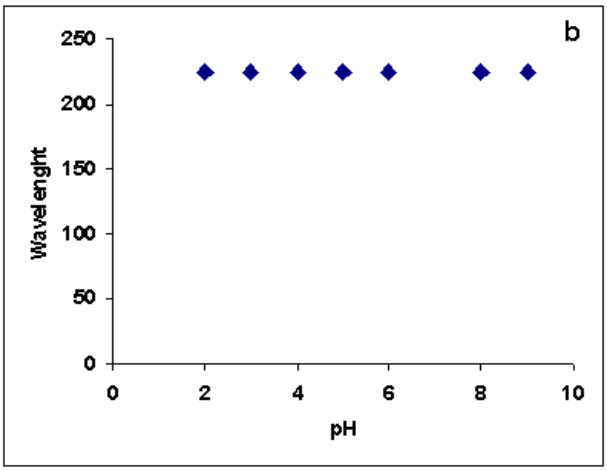


Fig.1. The effects of pH of the phosphate buffer on the reaction of THIA

THIA is UV absorbing molecules with specific chromophores in their structures that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations by UV spectrophotometric method. The absorption spectra of THIA in Phosphate buffer is illustrated in Fig. 2 . Calibration curve data were constructed in the range of expected concentrations of 1.10^{-5} to 6.10^{-5} M for THIA. Beer's law was obeyed over this concentration range. The regression equations were found; y= $1.4119.10^{4}$ x + 0.0986 for THIA (r= 0.9988). The stock solutions and working standards were made in phosphate buffer pH 2.0 media. The λ_{max} of the drug for analysis was determined by taking scans of the drug sample solutions in the entire UV region (225 nm).

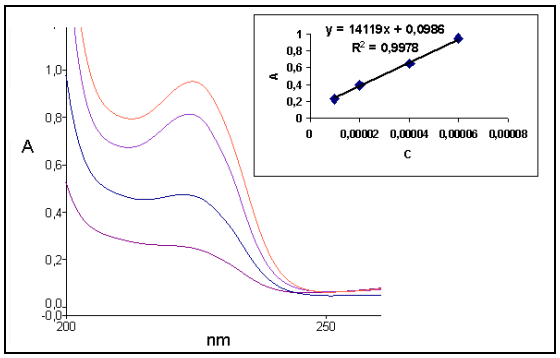


Fig.2. The absorption spectra and calibration graph of THIA in Phosphate buffer.

The characteristics of the calibration plots are summarized in Table 1 and the analytical characteristics and necessary validation parameters for UV techniques for THIA.

Table 1: Regression data of the calibration lines for quantitative determination of THIA by UV method.

	THIA
Measured wavelength (λmax as nm)	225
Linearity range (mol/L)	1.10-5-8.10-5
Slope	1.41x104
Absorbance range	0.23-0.95
Intercept	0.098
Correlation coefficient (r)	0.997
LOD (mol/L)	3.24x10-6
LOQ (mol/L)	1.08x10-5
Repeatability of absorbance (RSD%)	1.45
Repeatability of wavelength (RSD%)	0.19
Reproducibility of absorbance (RSD%)	1.01
Reproducibility of wavelength (RSD%)	0.02

Performing replicate analyses of the standard solutions was used to assessed the accuracy, precision and reproducibility of the proposed methods. The selected concentration within the calibration range was prepared in phosphate buffer pH 2.0 and analyzed with the relevant calibration curves to determine intra-day and inter-day variability. The intra and inter-day precision were determined as the RSD %. The precision, accuracy and reproducibility results given in Table 1 demonstrate a good precision, accuracy and reproducibility.

The commercial dosage form showed 106.58 % recovery by this method, which were within the specified limits of content uniformity. Moreover, the UV method offers a cost effective and time saving alternative to other methods for example colorimetric, complexometric and chromatographic of analysis.

The proposed methods can be successfully applied for THIA assay in dosage forms without any interference. The assay showed the drug content of this product to be in accordance with the labeled claim (Table 2). The recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy of the method. In order to check the accuracy and precision of the developed method and to prove the absence of interferences by excipients, recovery studies were carried out using the standard addition technique.

Recovery studies were carried out after the addition of known amounts of the pure drug to various pre-analyzed formulation of drug. The application of this procedure is explained in Experimental Section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of drug in capsules (Table 2). These results reveal that the method has adequate precision and accuracy, and consequently, can be applied to the determination of drug forms in pharmaceuticals without any interference from the excipients.

Table 2: Assay results from THIA dosage forms and mean recoveries in spiked dosage forms.

	THIA
Product	Urfamycin
Labeled claim (mg)	500
Amount found (mg)	532.40
RSD %	1.36
Bias %	6.48
Added (mg)	40
Found (mg)	42.63
Recovery %	106.58

5. Conclusions

The presented study proposes a simple, inexpensive, precise and accurate method for the determination of THIA in pharmaceutical preparation. The proposed methods were advantageous over other reported extractive spectrophotometric, colorimetric and chromatographic methods. The most striking advantage of the spectrophotometric method is the sensitivity, which surpasses the sensitivity of some of the previously reported methods. A important advantage of the spectrophotometric method is that it can be applied for the determination of individual compounds in a multi component mixture. Unlike the gas chromatographic and HPLC procedures, the instrument is simple and is not of high cost. In addition, the harmful chemicals using in the extractive spectrophotometric methods are not used in this method. In summary, the proposed method can be used for the drug analysis in routine quality control.

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References

1. en.wikipedia.org/wiki/ Thiamphenicol.
2. Shen JZ., Xia X., Jiang HY., et al., Journal of Chromatography B-Analytical Technologies in The Biomedical and Life Sciences, 877, 14-15, 1523-1529, 2009.
3. Tian DF., Feng F., Li Y., et al., Journal of Pharmeceutical and Biomedical Analysis, 48, 3, 1015-1019, 2008.
4. Chen HX., Chen H., Liao L., Ying J., Huang JL., Journal of Chromatographic Science, 48, 6, 450-455.
5. Fernandez- R., Consentino MO., Lopez MAB. Mochon MC., Talanta, 81, 3, 871-880, 2010.
6. Codognoto L., Winter E., Doretto KM., Monteiro GB. Rath S., Microchimaca Acta, 28, 10, 1819-1831, 1995.
7. Golcu A., Doğan B., and Ozkan S.A., Analytical Letters, 38, 1913, 2005.
8. Golcu A., and Ozkan S.A., Die Pharmazie, 61,760, 2006.
9. Golcu A., Journal of Analytical Chemistry, 63, 6, 538-543, 2008.
10. Laviron E., Rouillier L., Degrand C., J. Electroanal. Chem, 112, 1, 11, 1980.
11. C.M. Riley, T.W. Rosanske (Eds.). Development and Validation of Analytical Methods. New York: Elsevier Science Ltd., 1996.
12. Golcu A., Doğan B., and Ozkan S.A., Analytical Letters, 42, 689, 2009.
13. Muslu H., Golcu A., and Ozkan S.A., Current Analytical Chemistry, 6, 299-309, 2010.