DEVELOPMENT AND VALIDATION OF AN UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF THIAMPHENICOL IN DOSAGE FORM

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A novel UV spectrophotometric method has been developed for the determination of thiamphenicol in a solid dosage form. The spectrophotometric detection was carried out at an absorption maximum wavelength of 224 nm using water as solvent. The method was validated for specificity, linearity, precision, accuracy, robustness, and limit of detection (LOD) and limit of quantitation (LOQ). The detector response for thiamphenicol was linear in the selected concentration range of 5 to 25 μg/ml with a correlation coefficient of 0.9975. The precision (RSD) among six sample preparations was 0.65 and 1.50 % for analysts 1 and 2, respectively. The average recovery was 99.91 ± 0.65%. The LOD and LOQ were respectively 0.59 and 1.99 μg/ml. The results demonstrated that the excipients in thiamphenicol soft capsules did not interfere with the spectrophotometric determination of the drug. Using the Youden and Steiner approach, the method proved to be robust. The proposed method can be conveniently employed for routine analysis of thiamphenicol in bulk drug and soft capsules.

Keywords: *thiamphenicol, spectrophotometry, validation, dosage form.*

РАЗРАБОТКА И ВАЛИДАЦИЯ УФ-СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА ОПРЕДЕЛЕНИЯ ТИАМФЕНИКОЛА В ЛЕКАРСТВЕННОЙ ФОРМЕ

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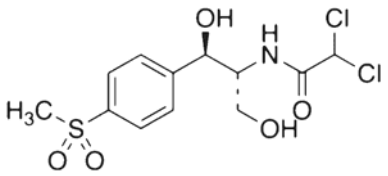
Разработан новый УФ-спектрофотометрический метод определения тиамфеникола в твердой лекарственной форме. Спектрофотометрическое детектирование проведено на длине волны максимального поглощения 224 нм с использованием воды в качестве растворителя. Метод проверен на специфичность, линейность, точность, надежность, а также на предел обнаружения (LOD) и предел количественного определения (LOQ). Отклик детектора на тиамфеникол линейный в диапазоне концентраций 5–25 мкг/мл с коэффициентом корреляции 0.9975. Точность (RSD) среди шести проб препаратов 0.65 и 1.50% у аналитиков 1 и 2. Средний процент восстановления 99.91 ± 0.65%; LOD и LOQ 0.59 и 1.99 мкг/мл. Показано, что вспомогательные вещества в мягких капсулах тиамфеникола не влияют на результаты применения метода. Использование подхода Йодена и Штейнера способствовало повышению надежности метода.

Ключевые слова: *тиамфеникол, спектрофотометрия, валидация, лекарственная форма.*

Introduction. Thiamphenicol, (D(+)-threo-2-di-choloroacetamido-1-(4-methylsulfonylphenyl)propane-1,3-diol)



is an antimicrobial substance structurally related to chloramphenicol [1], which was first introduced in 1951 [2]. Thiamphenicol has a wide spectrum of action against gram-positive, gram-negative, and anaerobic bacteria involved in upper and lower respiratory infections, bacterial prostatitis, and sexually transmitted infections [3].

Thiamphenicol binds to the 50S subunits of the 70S ribosomes to block peptidyl transferase, hence inhibiting the extension of peptide chain and synthesis of bacterial protein [3]. The administration of thiamphenicol to rats showed hematological toxicity only in relatively high doses [4, 5]. Nevertheless, thiamphenicol for humans, livestock, and aquaculture is still used because of its reliable antibiotic effectiveness and low cost [6]. Currently, the methods and techniques described in the scientific literature for the determination of thiamphenicol in honey, meat, seafood, milk, egg, plasma, and urine include immunoassay [7], liquid chromatography tandem mass spectrometry (LC-MS/MS) [8–11], gas chromatography (GC) [12–14], and capillary electrophoresis (CE) [15]. Despite the fact that thiamphenicol has largely been used and commercialized for more than 60 years, few publications regarding analysis of thiamphenicol in pharmaceutical formulations and bulk drug have been reported in the scientific literature [16, 17]. The UV spectrophotometry method presents notable applications in pharmaceutical analysis as being a suitable method for the quantification of drugs in formulations, and there can be found in the scientific literature lots of examples of the UV spectrophotometry application in pharmaceutical analysis [18–21]. Accordingly, the aim of the present study was to develop and validate an analytical methodology by UV spectrophotometry for qualitative and quantitative determination of thiamphenicol in bulk drug and soft capsules. An extraction method of thiamphenicol from its pharmaceutical formulation was first developed since the dosage form consisted of an oily suspension of the drug.

Experimental. Thiamphenicol was used as standard chemical substance and was acquired from Sigma-Aldrich® (USA). Glitisol® capsules (Zambon, São Paulo, Brazil) labeled as containing thiamphenicol in 500 mg/capsule were obtained commercially. This drug was used as received, and solutions were freshly prepared everyday to be used as working standards. All reagents were of analytical grade (Merck®, Germany). Doubly distilled water was used throughout. All substances and reagents were kept protected from light throughout the whole procedure.

A Hitatchi-U2900 spectrophotometer with a data processing system (UV-visible ChemStation software) was used for all absorbance measurements. UV spectra absorbance of reference and sample solutions were recorded in 10 mm quartz cells at 224 nm.

Stock solution of thiamphenicol (100 μg/ml) was prepared by dissolving 10 mg of thiamphenicol in a 100 ml volumetric flask. The solution was sonicated for 10 min and brought to the mark with distilled water; 3 ml of this solution was scanned from 400 to 200 nm with an UV spectrophotometer. Thiamphenicol showed maximum absorption at 224 nm, and water was used as blank.

Method of extraction for thiamphenicol and thiamphenicol sample solutions. Twenty capsules were individually weighed for extraction of thiamphenicol. An accurately weighed quantity containing 1500 mg of thiamphenicol was dissolved in chloroform and centrifuged for 5 min at 3000 rpm. The supernatant was discarded, and the remaining chloroform was withdrawn by evaporation at 70°C for 50 min in a water bath (Fig. 1). From the obtained powder, 500 mg was weighed and transferred to a 500 ml volumetric flask; 300 ml of distilled water was added, and the whole shaken for 20 min and brought to the mark to give a nominal concentration of 1000 μg/ml. Sample solutions were prepared in triplicate at the final concentration of 12 μg/ml and analyzed.

The process of validation aimed to demonstrate the reliability of the chosen method to achieve the results. The validity of the proposed method was tested in regard to specificity, linearity, precision, accuracy, robustness, and limit of detection (LOD) and limit of quantification (LOQ) according to the International Conference on Harmonization (ICH) guidelines [22].

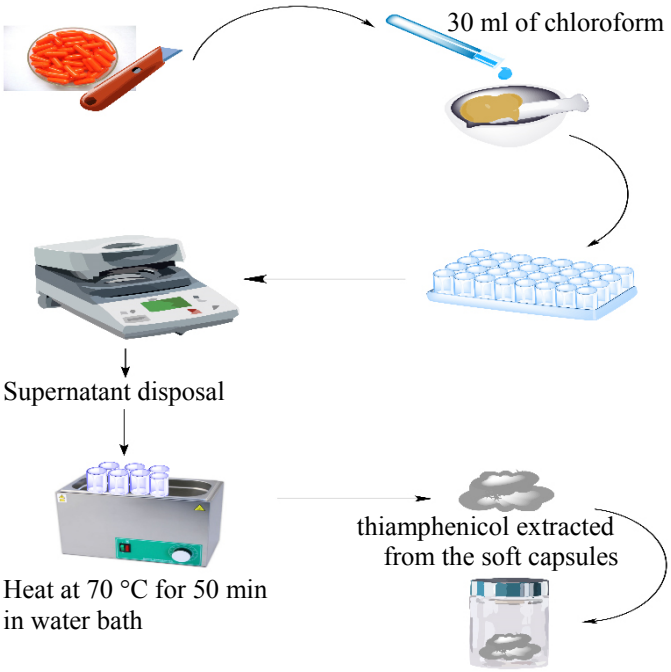


Fig. 1. Extraction of thiamphenicol from soft capsules.

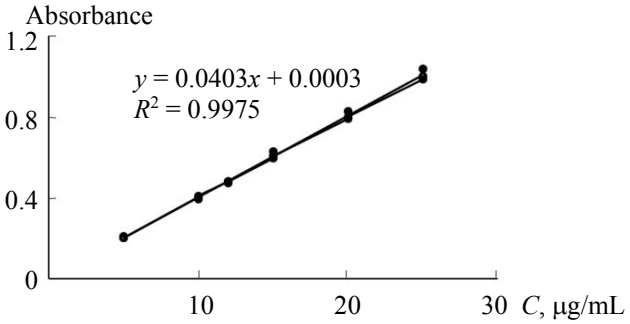


Fig. 2. Analytical curve for thiamphenicol from standard solutions in the range 5 to 25 µg/ml.

Specificity is the ability of the method to accurately measure a compound in the presence of other components such as impurities, degradation products, and matrix components. Interference of the excipients (soy oil and lecithin soy) in the capsules was evaluated. A wavelength scan was performed, and the absorption of the excipients at 224 nm was analyzed.

Linearity was determined by plotting concentration against corresponding absorbance. The analytical curve was defined in the concentration in which the intensity of the spectrophotometer response was linearly proportional to the concentration of the analyzed substance, obeying Beer's law according to the equation

$$A = aC + b, \quad (1)$$

where A is the absorbance; C is sample concentration; a is slope of the curve; and b is y-intercept of the curve. The analytical curve was made by analyzing six different concentrations of standard solutions, prepared on the same day. The range of solutions varied from 5 to 25 µg/ml (Fig. 2). Linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The correlation coefficient (r) indicated the linearity of the method. Analytical curves were made in triplicate on three consecutive days.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to ICH guidelines [22]. Intra-day and inter-day precisions were determined by analyzing the samples of thiamphenicol at the concentration of 12 µg/ml. The analyses took place on three consecutive days with two analysts. Each analyst prepared six sample solutions on each day. The precision was expressed as relative standard deviation (RSD).

Accuracy was determined by recovery test. Recovery tests were performed by adding known amounts of the standard solution to the sample solutions followed by analyses using the proposed method. Method accuracy was tested (% recovery and % R.S.D. of individual measurements) by analyzing samples of thiamphenicol at three different levels using three preparations for each level.

To a thiamphenicol sample solution of 400 μg/ml, different amounts of thiamphenicol standard solutions (3.0, 5.0, and 7.0 μg/ml) were added, yielding solutions at the final concentrations of 15, 17, and 19 μg/ml, respectively. The assay was performed in triplicate.

The percentage of recovery (R) of the added pure drug was calculated as suggested by the Association of Official Analytical Chemists International [23]:

$$R = [(C_F - C_U)/C_A] \times 100, \quad (2)$$

where C_F is the total drug concentration measured after standard addition; C_U is drug concentration in the sample solution (before standard addition); and C_A is drug concentration added to sample solutions.

The robustness evaluation was performed using the method proposed by Youden and Steiner [24]. Seven parameters were selected (wavelength, sonication time, volume of chloroform, centrifugation length, rotation speed, evaporation length, and evaporation temperature), and small variations were induced in the nominal values of the method (Table 1). Eight runs were performed to determine the influence of each parameter on the final result. To determine the influence of variation of each parameter on the final result, the mean of the four values corresponding to nominal conditions (designated by uppercase letters) was compared to the mean of the four values corresponding to the altered conditions (designated by lower case letters) (Table 2). The values obtained from the equations

$$\text{Effect } C/c = (s + u + w + y)/4 - (t + v + x + z)/4, \quad (3)$$

$$\text{standard value} = S\sqrt{2}, \quad (4)$$

where S is standard deviation of the eight levels (s, t, u, v, w, x, y, z) of the sample analyzed in the test, were compared for the interpretation of the test.

The value found in Eq. (3) must be smaller than the value determined at Eq. (4) for the effect to be interpreted as not significant. All statistical analysis was calculated using spreadsheet programs and Origin 8.0 software.

TABLE 1. Analytical Parameters and Variations for the Robustness Evaluation of the Method

Parameter	Nominal condition (upper case letters)	Variation (lower case letters)
Wavelength	224 nm (A)	223 nm (a)
Time in sonicator	25 min (B)	23 min (b)
Volume of chloroform	30 ml (C)	28 ml (c)
Centrifugation length	5 min (D)	4.5 min (d)
Rotation speed	800 g (E)	750 g (e)
Evaporation length	50 min (F)	45 min (f)
Evaporation temperature	70º C (G)	65° C (g)

TABLE 2. Factorial Combination of the Analytical Parameters for Robustness Evaluation by Youden and Steiner's Test

Parameter	Factorial combination							
	1	2	3	4	5	6	7	8
Wavelength	A	A	A	A	a	a	a	a
Time in sonicator	B	B	b	b	B	B	b	b
Volume of chloroform	C	c	C	c	C	c	C	c
Centrifugation length	D	D	d	d	d	d	D	D
Rotation speed	E	e	E	e	e	E	E	E
Evaporation length	F	f	f	F	F	f	F	F
Evaporation temperature	G	g	g	G	g	G	G	G
Result	s	t	u	v	w	x	y	z

N o t e. C: Upper case letters, nominal conditions, c: Lower case letters, altered conditions.

Results and discussion. *Method development and optimization.* Different media were investigated to develop a suitable UV-spectrophotometric method for the analysis of thiamphenicol in capsules. For the selection of media, the criteria employed were the sensitivity of the method, ease of sample preparation, solubility of the drug, cost of solvents, and applicability of the method in the routine analyses of the pharmaceutical industry. The proposed UV method allowed for a rapid and economical quantitation of thiamphenicol in capsules without any time-consuming sample preparation. Moreover, the spectrophotometric methods involve simple instrumentation compared to other instrumental techniques. An extraction method of thiamphenicol from its pharmaceutical formulation was developed since thiamphenicol capsules consisted of an oily suspension of the drug. The λ_{max} of thiamphenicol in distilled water was found to be 224 nm. This wavelength was used for all measurements.

Linearity. The analytical curves were constructed from six points covering the concentration ranges 5–25 μg/ml. Beer's law was obeyed over the concentration range. The regression equation obtained was $y = 0.0403x + 0.0003$, where y and x are thiamphenicol absorbance and concentration in μg/ml, respectively. The linearity was demonstrated by the high value of the correlation coefficient ($r^2 = 0.9975$) and the small value of the y-intercept of the regression equation.

LOD and LOQ of this method were determined from the standard deviation of the response of a known concentration of thiamphenicol as per ICH [22]. The LOD calculated from three times the noise level of the response was 0.59 μg/ml. The LOQ calculated from ten times the noise level of the response was 1.99 μg/ml.

Intra-day and inter-day precision. The RSD of the six measurements from the thiamphenicol sample was found to be 0.65% for analyst 1 and 1.50 % for analyst 2. The percentage contents are presented in Table 3 for the three days of analyses. The method was applied for the assay of commercial soft-gel capsules containing 500 mg of thiamphenicol. Each sample was analyzed in triplicate after extracting the drug as mentioned in assay sample preparation in the experimental section (see Method of thiamphenicol extraction and thiamphenicol sample solutions). The results exhibited in Table 3 are in close agreement with the labeled content. Assay results, expressed as the percentage of the label claim, were found to be 97.15 % (RSD 0.95%), between different analysts.

The recovery values obtained were 99.91 ± 0.65% for thiamphenicol in dosage form. This result confirmed the accuracy of the method. The percentages of the recovery test are presented in Table 4.

TABLE 3. Statistical Data Obtained from the Analysis of Capsule Samples (500 mg) by Using the Proposed Method

Analyst	Day	Content (%)± RSD* Intra-day	RSD (%) Inter-day
1	1	96.80 ± 0.60	0.65
	2	96.91 ± 0.62	
	3	97.30 ± 0.59	
2	1	96.30 ± 0.21	1.50
	2	96.62 ± 0.62	
	3	98.98 ± 0.59	

* Mean value of three determinations.

TABLE 4. Recovery Data of Thiamphenicol Standard Solution Added to the Samples Analyzed by Using the Proposed Spectrophotometric Method

	Sample concentration (μg/ml)	Theoretical concentration added (μg/ml)	Theoretical final concentration (μg/ml)	Found experimental concentration (μg/ml)	Recovery (%) Mean ± RSD*
R1	12.0	3.0	15.0	2.98	99.59 ±1.40
R2	12.0	5.0	17.0	5.04	100.89 ± 0.55
R3	12.0	7.0	19.0	6.94	99.24 ± 0.0

* Mean value of three determinations.

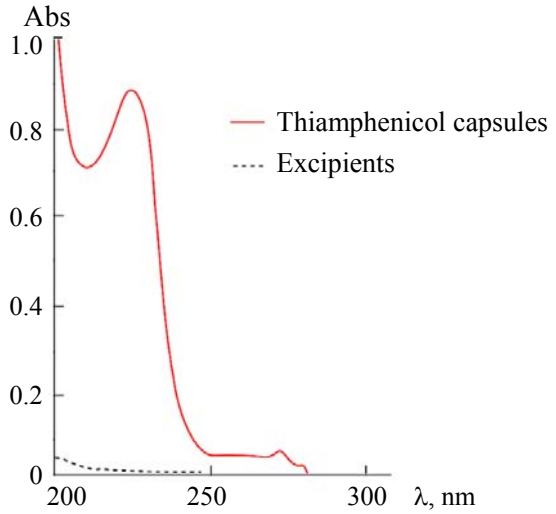


Fig. 3. Overlapping spectra of thiamphenicol capsules and excipients from 200 to 400 nm.

The UV-spectrum of thiamphenicol had no interference in the presence of the excipients used in the dosage form (Fig. 3). Therefore, the proposed analytical method is specific for determination of thiamphenicol in pharmaceutical formulation.

The results of robustness obtained in the experiments are shown in Table 5. The mean values of the nominal were subtracted from the values of the altered conditions. The standard value was calculated, and the results were compared. For the seven parameters, the results of the effects were less than standard value ($s\sqrt{2}$ = 1.42). Therefore, the results achieved with this method were not significantly affected by the slight modifications from the nominal conditions (Table 5).

TABLE 5. Youden and Steiner's Test Results

Parameter	Content (%)*	Standard value ($s\sqrt{2}$)
Wavelength	100.18 – 101.28 = –1.14	
Time in sonicator	100.00 – 101.26 = –1.25	
Volume of chloroform	101.60 – 100.45 = 1.14	
Centrifugation length	100.22 – 101.04 = –0.81	1.42
Rotation speed	100.72 – 101.01 = –0.29	
Evaporation length	100.67 – 101.26 = 0.58	
Evaporation temperature	100.41 – 101.26 = –0.81	

*Difference between the mean values obtained from nominal conditions and from altered conditions.

Conclusion. In this study, the developed and validated UV-spectrophotometric method for the determination of thiamphenicol in pharmaceutical formulation has the advantage of being fast, cheap, simple, highly accurate and precise, specific, and robust. These advantages support the application of this method in the routine analysis of thiamphenicol in capsules.

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