SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF CHLORAMPHENICOL, DEXAMETHASONE SODIUM PHOSPHATE, AND TETRAHYDROZOLINE HCl IN THEIR PURE AND OPHTHALMIC DOSAGE FORMS

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Chloramphenicol (CHL), dexamethasone sodium phosphate (DSP), and tetrahydrozoline HCl (THZ) are co-formulated for conjunctivitis treatment. The ternary mixture could not be simultaneously determined because of the overlap of the zero order absorption spectra. Herein, simple and validated UV spectrophotometric techniques have been developed for the determination of CHL, DSP, and THZ in their pure and ophthalmic dosage forms. Meanwhile, only CHL was directly determined at 284.0 nm in the range 4.0–36.0 μg/mL, while DSP and THZ were determined using single or double divisor derivative ratio spectrophotometric methods. For the single divisor derivative ratio-zero crossing spectrophotometric method (SDDR-ZC), 4.0 μg/mL CHL was used as a single divisor, where DSP and THZ were detected at 272.0 and 239.0 nm, respectively. Both DSP and THZ showed linearity ranges of 4.0–32.0 μg/mL for DSP and 3.0–24.0 μg/mL for THZ, whereas for the double divisor derivative ratio spectrophotometric method (DD-DR), (12.0 μg/mL CHL and 12.0 μg/mL THZ) and (12.0 μg/mL CHL and 12.0 μg/mL DSP) were used as double divisors for the quantitative assessment of DSP and THZ, respectively. Both DSP and THZ showed a linearity range of 4.0–32.0 μg/mL, and they were detected at 258.0 and 237.0 nm, respectively. The developed techniques were successfully applied for the determination of the three drugs in their dosage form. The proposed techniques were validated showing no significant differences when statistically compared to a reported HPLC method.

Keywords: *chloramphenicol, dexamethasone sodium phosphate, tetrahydrozoline HCl, single divisor, double divisor.*

СПЕКТРОФОТОМЕТРИЧЕСКИЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ ХЛОРАМФЕНИКОЛА, ДЕКСАМЕТАЗОНА НАТРИЯ ФОСФАТА И ТЕТРАГИДРОЗОЛИНА HCl В ИХ ЧИСТЫХ И ОФТАЛЬМОЛОГИЧЕСКИХ ЛЕКАРСТВЕННЫХ ФОРМАХ

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Хлорамфеникол (CHL), дексаметазон натрия фосфат (DSP) и тетрагидрозолин HCl (THZ) входят в состав препаратов для лечения конъюнктивита. Тройную смесь невозможно определить одновременно из-за сильного перекрытия их спектров поглощения нулевого порядка. Разработаны простые и проверенные УФ-спектрофотометрические методы определения CHL, DSP и THZ в их чистых и офтальмологических лекарственных формах, причем только CHL непосредственно определялся при 284.0 нм в диапазоне 4.0–36.0 мкг/мл, в то время как DSP и THZ определены с помощью спектрофотометрических методов отношения производных с одним или двумя делителями. Для спектрофотометрического метода пересечения нуля и отношения производных с одним делителем

(SDDR-ZC) 4.0 мкг/мл CHL использовали в качестве единственного делителя, где DSP и THZ детектировались при 272.0 и 239.0 нм. DSP и THZ показали линейность в диапазонах 4.0–32.0 и 3.0–24.0 мкг/мл. Для спектрофотометрического метода двойного делителя отношения производных (DD-DR), (12.0 мкг/мл CHL и 12.0 мкг/мл THZ) и (12.0 мкг/мл CHL и 12.0 мкг/мл DSP) использовались в качестве двойных делителей для количественной оценки DSP и THZ. DSP и THZ показали линейность в диапазоне 4.0–32.0 мкг/мл и обнаружены при 258.0 и 237.0 нм. Разработанные методики успешно применены для определения трех лекарственных препаратов в их лекарственной форме. Методы прошли валидацию, показав отсутствие значительных различий при статистическом сравнении с методом ВЭЖХ.

Ключевые слова: *хлорамфеникол, дексаметазон натрия фосфат, тетрагидрозолин HCl, одинарный делитель, двойной делитель.*

Introduction. Conjunctivitis and keratitis are bacterial infections related to perceived health risks, with severe eye pain, blurring of vision, and extreme photosensitivity as major symptoms [1]. The most frequent drug classes used for curing conjunctivitis are antibacterial, anti-inflammatory, and sympathomimetic drugs. Chloramphenicol (CHL) demonstrates bacteriostatic action effective against Gram-negative and Gram-positive bacteria (Fig. 1a) [2]. CHL has benefits of being cheap and more readily available than other antibiotics [3]. Dexamethasone sodium phosphate (DSP) is a water-soluble and inorganic ester known for anti-inflammatory activity (Fig. 1b). DSP is frequently used to cure disorder relevant to adrenal cortex insufficiency [4, 5]. Tetrahydrozoline hydrochloride (THZ) (Fig. 1c) has a sympathomimetic action, so it is used as a conjunctival decongestant [6, 7]. Ocuphenicol-D® eye drops are available on the market, each one mL contains 5.0 mg CHL, 1.0 mg DSP, and 0.25 mg THZ. It is used to treat conjunctivitis, acute and chronic infection, and inflammation of the uvea anterior.

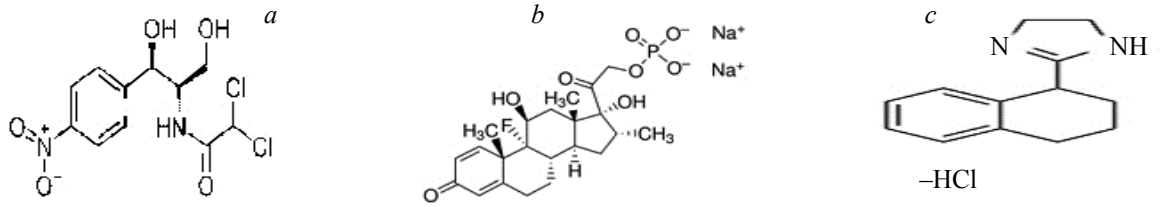

Fig .1. Chemical structure of (a) Chloramphenicol, (b) dexamethasone sodium phosphate, and (c) tetrahydrozoline hydrochloride.

Different spectrophotometric techniques were found to determine this mixture in its pharmaceutical dosage form [8, 9]. Moreover, spectrophotometry methods were used to determine any mixture in a dissolution test and determine multicomponent dosage form in presence of ompurities [10, 11]. Also, four chromatographic methods were reported for the determination of this mixture [12–15]. Most of the reviewed methods need pre-separation, much time, and excessive data treatment. Therefore, the aim of this work is to analyze this challenging ternary mixture depending on simpler spectrophotometric techniques with no need of prior separation or sophisticated data manipulation. CHL could be easily determined in zero order spectra, but two spectrophotometric methods were developed to determine DSP and THZ. The first method was the single divisor derivative ratio spectra-zero crossing method (SDDR-ZC) dependent on the first derivative of the ratio spectra after being divided on a single divisor. The second one was the double divisor derivative ratio spectra method (DD-DR) dependent on the derivative of the ratio spectra for a ternary mixture after division by using a ‘double divisor.’

Experimental. A JASCO dual beam (Tokyo, Japan) UV-visible spectrophotometer model V-630 was used. The software bundle used was spectra II manager. The spectral slit width was 2 nm, and the scan speed was 1000 nm/min. CHL and DSP standards were kindly provided by Egyptian International Pharmaceutical Industries Co. (E.I.P.I.CO.) (Cairo, Egypt), while THZ was furnished by Orchidia Company (Cairo, Egypt). Their purity was found to be 99.47% for CHL, 99.36% for DSP and 100.63% for THZ, according to the official method [15]. Ocuphenicol-D® eye drops (Batch No. 8529007) claim to have 5.0 mg of CHL, 1.0 mg of DSP, and 0.25 mg of THZ per mL and are manufactured by Alexandria Company for Pharmaceuticals and Chemical industries, Alexandria, Egypt. All the chemicals and solvents used were of analytical grade and used without further purification. Methanol HPLC grade was bought from Merck (Darmstadt, Germany).

Stock standard solutions: (1.0 mg/mL) of each of CHL, DSP and THZ were separately prepared in methanol HPLC as a solvent. Working standard solutions: (40.0 μg/mL, each) were processed freshly by diluting relevant aliquots in the same solvent.

Into an array of 10 mL volumetric flasks, aliquot concentrations of CHL, DSP, THZ were transferred from their working standard solutions (40.0 μg/mL, each) to develop five different mixtures of the three drugs in various ratios. Methanol was used to complete the required volume.

Construction of calibration curve. Into three isolated sets of 10 mL volumetric flasks, aliquot concentrations from CHL, DSP, and THZ working standard solutions (40.0 μg/mL, each) were transferred accurately. The volumes were then brought to the mark with methanol. In the range 200.0–400.0 nm, the prepared solutions were scanned, and their absorption spectra were then stored for further manipulation.

Zero order absorption spectra (D^0) for Chloramphenicol. CHL can be determined precisely from zero order spectra where the two other drugs showed no absorbance. The linearity of CHL was observed at a wavelength of 284.0 nm; then the corresponding concentration of CHL and the regression equation were computed.

Single divisor derivative ratio-zero crossing spectrophotometric method (SDDR-ZC). The stored absorption spectra of DSP and THZ were divided by the 4.0 μg /mL CHL spectrum. Then the scaling factor 10 and $\Delta\lambda = 4$ nm were used, the obtained ratio spectra were derivatized to the first derivative (^1D), and the peak amplitudes were detected at 272.0 nm for DSP and 239.0 nm for THZ.

Double divisor derivative ratio spectrophotometric method (DD-DR). For DSP, the stored zero order absorption spectra were divided by the sum of the absorption spectra of 12.0 μg /mL CHL and 12.0 μg/mL THZ, while for THZ the stored zero order absorption spectra were divided by the sum of the absorption spectra of 12.0 μg/mL CHL and 12.0 μg/mL DSP. ^1D was then calculated for the obtained ratio spectra of DSP and THZ. The peak amplitudes of the processed ^{1}D were detected at the coincidence points 258.0 and 237.0 nm for DSP and THZ, respectively.

The prepared laboratory mixtures spectra were scanned in the range 200.0–400.0 nm, and the concentration of each drug was calculated separately using the above-mentioned methods.

Into a 25 mL volumetric flask, 1 mL was accurately transferred from Ocuphenicol-D®, and the volume was brought to the mark with the same solvent. The prepared solution (1 mL) was transferred into a 10 mL volumetric flask to reach concentrations of 20.0 μg/mL CHL, 4.0 μg/mL DSP, and 1.0 μg/mL THZ. The obtained solution was spiked with 3.0 μg THZ, and the volume was brought to the mark with methanol. Since the concentration of THZ in the mixture was very low, it had to be increased. This was done by adding a fixed volume of standard THZ to be analyzed. Then we subtracted the THZ concentration before calculating the claimed concentration of the drug. The concentration of each drug was determined using its reciprocal regression equation according to the already mentioned procedures.

Result and discussion. Drug efficacy and safety are the keys to good quality in pharmaceutical practice. To assure these criteria, an assay of drugs in both the pure powder and the pharmaceutical preparation should be done to ensure the right quantity of the active ingredients before trailing to further steps. The absorption spectra of the three drugs (CHL, DSP, and THZ) were found to strongly overlap in the region 215.0–350.0 nm. Therefore, direct spectrophotometry could determine CHL only, while DSP and THZ could not be determined in zero order spectra (Fig. 2). This work presents the development and verification of UV

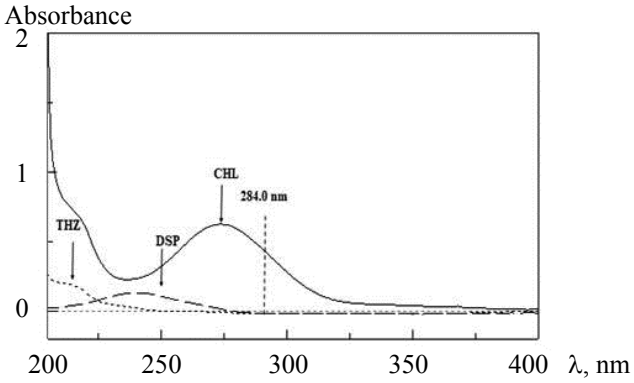

Fig. 2. Zero order absorption spectra of chloramphenicol, dexamethasone sodium phosphate, and tetrahydrozoline HCL, with methanol as a solvent.

spectrophotometric techniques for the determination of CHL, DSP, and THZ in their pure and pharmaceutical forms using single and double divisor derivative ratio spectra methods. This was followed by a statistical comparison between the suggested methods and a reported HPLC method [14].

Direct spectrophotometric method for the *determination of CHL (^0D).* CHL could be determined directly in zero order absorption spectra without any interference and absorbance of DSP and THZ. The calibration curve of CHL was plotted between the concentration and the absorbance at a wavelength of 284.0 nm, and the regression equation was then computed.

Single divisor derivative ratio spectra-zero crossing method (SDDR-ZC). The determination of two other compounds which could not be determined directly in their ternary mixtures was processed by measurement of the amplitude at the zero-crossing points in the derivative ratio spectra [17, 18]. The absorption spectra of DSP and THZ were divided by a standard spectrum of CHL, and the first derivative of the ratio spectra was calculated.

The selected divisor should have high sensitivity and low noise [19]. For selecting the single divisor of the appropriate concentration, some single divisor concentrations were tested, such as 4.0, 8.0, and 12.0 μg/mL CHL. Finally, 4.0 μg/mL CHL was found to be the optimum single divisor regarding sensitivity and specificity in determining DSP and THZ in the presence of CHL. The stored spectra of DSP and THZ were divided by the spectrum of the standard solution of 4.0 μg/mL CHL, and the ratio spectra were obtained in the region 215.0–320.0 nm; then the ^1D of the ratio spectra was computed. A calibration curve for each of DSP and THZ was plotted using the peak amplitudes at 272.0 and 239.0 nm, respectively, where both drugs showed zero crossing points to each other (Fig. 3).

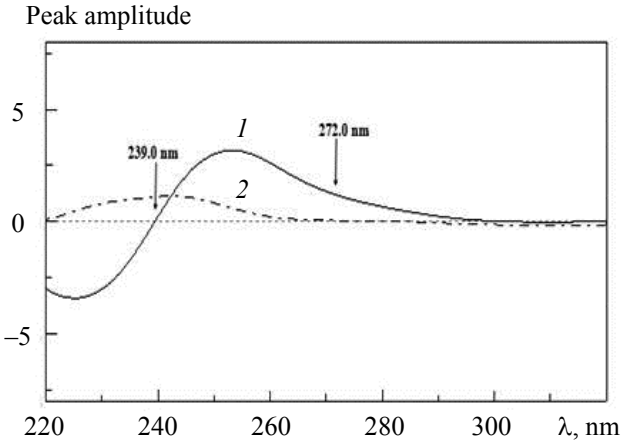

Fig. 3. First derivative of the ratio spectra of DSP (*1*) and THZ (*2*), with 4.0 μg/mL CHL as a divisor.

Double divisor derivative ratio spectra method (DD-DR). DD-DR is an easy to spread, sensitive, and very cheap method. This method depends on the division of the absorption spectrum of the mixture by using a 'double divisor' (sum of the other two drugs spectra); then the first derivative of the ratio spectrum is measured. The calibration graphs are obtained by measuring the amplitudes at a coincident point [20, 21]. The selected double divisor should be characterized by minimum noise and maximal sensitivity. For selecting an appropriate double divisor, different concentrations were tested. It was found that 12.0 μg/mL CHL +12.0 μg/mL THZ, and 12.0 μg/mL CHL +12.0 μg/mL DSP were the best double divisors to be used for accurate determination of DSP and THZ, respectively. For DSP, the absorption spectra of the solutions in methanol were recorded in the range 215.0–350.0 nm and divided by the chosen double divisor to obtain ratio spectra. The ^1D of the obtained ratio spectra was then computed. The concentration of DSP was obtained by measuring the peak amplitude at 258.0 nm, which showed the coincident point of the ^1D of the ratio spectra of 12.0 μg /m DSP and the different laboratory-prepared mixtures containing the same concentration of DSP (Fig. 4a). Similarly, 12.0 μg/mL CHL + 12.0 μg/mL DSP were used as a double divisor for THZ. THZ was determined at its coincidence point of 237.0 nm (Fig. 4b).

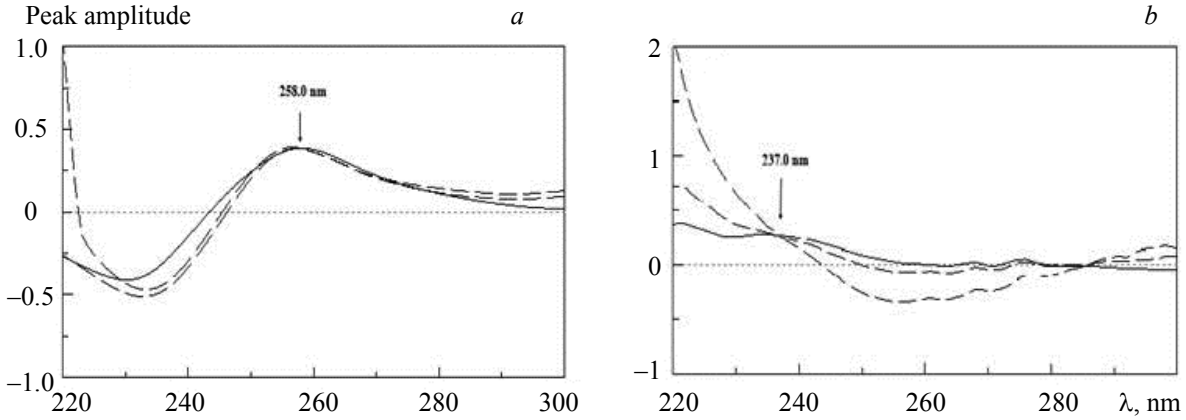

Fig. 4. The coincident spectra of the ^1D of the ratio spectra of (a) 12.0 μg/mL pure DSP (____) and different lab mixtures (- - - -) containing12.0 μg/mL DSP (using 12.0 μg/mL CHL +12.0 μg/mL THZ as a double divisor) and (b) 12.0 μg/mL pure THZ (___) and different lab mixtures (- - - -) containing 12.0 μg/mL THZ (using 12.0 μg/mL CHL+12.0 μg/mL DSP) as a double divisor.

Method validation. The suggested spectrophotometric methods were validated according to the ICH guidelines, namely linearity, range, accuracy and precision [22]. The linear regression evidence for the calibration curves showed great linear relationships (Table 1). The accuracy was proved by calculating the % recovery for the three replicates of the three various concentrations inside the linearity range for CHL, DSP, and THZ. The precision was assessed by measuring the three concentration levels intradaily and interdaily for both repeatability and intermediate precision Table 1. The specificity of the proposed spectrophotometric methods was estimated by the analysis of laboratory mixtures that include various ratios of CHL, DSP, and THZ. Gratifying results were obtained, indicating the great selectivity of the methods (Table 2).

TABLE 1. Assay Parameter and Validation Data Result Sheet Obtained by Applying the Proposed Spectrophotometric Methods

Parameter	^0D method	SDDR-ZC method		DD-DR method	
	CHL	DSP	THZ	DSP	THZ
Wavelength, nm	284.0	272.0	239.0	258.0	237.0
Calibration range (μg/mL)	4.0–36.0	4.0–32.0	3.0–24.0	4.0–32.0	4.0–32.0
Slope	0.0293	0.0489	0.0925	0.03413	0.0236
Intercept	0.0051	–0.0252	–0.0596	–0.0108	–0.0337
Correlation coefficient (*r*)	0.9997	0.9996	0.9995	0.9996	0.9990
Accuracy [a] (mean± SD)	100.25±1.542	99.81±1.544	101.14±0.528	101.32±0.602	99.91±1.066
Precision [b] (RSD%)					
Repeatability	0.998	0.613	0.696	0.740	0.337
Intermediate precision	1.861	0.116	0.660	1.099	1.783
LOD, μg/mL [c]	0.720	0.683	0.655	0.682	0.850
LOQ, μg/mL [c]	2.183	2.072	1.987	2.067	2.578

[a] Average of three different concentrations repeated three times within the day.

[b] Precision was evaluated by measuring the response of three concentrations of each drug (10.0, 16.0, and 20.0 μg/mL for CHL; 10.0, 14.0, and 22.0 μg/mL for DSP; 10.0, 16.0, and 20.0 μg/mL for THZ) three separate times on the same day (repeatability) and on three different days (intermediate precision).

[c] Calculated from equation LOD = 3.3 (S.D/S), LOQ = 10 (S.D/S), where S.D is the residual standard deviation of the slope and S is the slope for the proposed methods.

TABLE 2. Analysis (Recovery, %) of Laboratory Prepared Mixtures by Applying the Proposed Spectrophotometric Methods

Claimed concentration taken, μg/mL			^0D method	SDDR-ZC method		DD-DR method	
CHL	DSP	THZ	CHL	DSP	THZ	DSP	THZ
18.00	8.00	12.00	100.89	99.87	99.88	101.11	99.55
18.00	12.00	12.00	98.19	101.20	100.35	98.24	101.20
20.00	4.00	1.00*	98.78	100.86	100.87	101.05	100.41
18.00	10.00	10.00	98.17	100.10	98.38	98.64	101.35
18.00	12.00	10.00	99.93	98.42	101.31	98.15	98.77
Mean (%)			99.19	100.09	100.16	99.44	100.25
SD			1.187	1.077	1.128	1.508	1.096
RSD			1.197	1.076	1.126	1.516	1.093

* Same ratio in dosage form after subtraction of spiked THZ concentration (3.0 μg/mL).

Application of the proposed methods for the analysis of Ocuphenicol-D® eye drops. The concentration of THZ was increased due to its low concentration in dosage form as the THZ calibration range started from 3.0 μg/mL in all the proposed methods. CHL, DSP, and THZ were successfully quantified in their ophthalmic solution (Ocuphenicol-D®) using the proposed methods. Statistical comparison was carried out between two pharmaceutical dosage forms. As a result, the standard addition technique was not used to assess the validity of the methods (Table 3).

TABLE 3. Statistical Comparison of the Results Obtained from the Proposed Methods and the Reported Method

Value	CHL		DSP			THZ		
	^0D method	reported method [a]	SDDR-ZC method	DD-DR method	reported method [a]	SDDR-ZC method	DD-DR method	reported method [a]
Mean (%)[b]	100.07	100.25	99.32	96.76	101.33	95.61	98.65	99.97
SD	0.271	0.300	1.635	0.704	1.66	0.600	0.940	0.896
n	3	3	3	3	3	3	3	3
Variance	0.073	0.090	2.672	0.495	2.772	0.359	0.882	0.802
Student's *t*-test (2.78) [c]	1.125	–	0.670	0.238	–	0.142	0.577	–
F value [c] (19)	1.231	–	1.042	5.592	–	2.231	1.109	–

[a] HPLC method: using C_{18} column and mobile phase consisting of acetonitrile:phosphate buffer ((30:70, v/v) at a flow rate of 1.0 mL/min and detection at 230.0 nm) [13].

[b] Average of three experiments.

[c] Figures between parentheses represent the corresponding tabulated values of *t* and *F* at $P = 0.05$.

Statistical comparison. Both *F*-test and *t*-test were achieved. The proposed techniques for the determination of CHL, DSP and THZ were statistically compared to the reported HPLC method (Table 3) [14]. No significant statistical difference was observed between the proposed and reported methods.

Conclusions. Single divisor derivative ratio spectra-zero crossing and double divisor ratio spectra derivative methods were precisely used for the determination of a ternary mixture of CHL, DSP, and THZ without preliminary separation. The methods mentioned above were found to be rapid, smooth, and sensitive for the analysis of the three drugs in pure and pharmaceutical forms with high accuracy and specificity. Statistical comparison between the proposed and a reported method showed no clear difference, which proves their sensitivity. Furthermore, the suggested three methods were more suitable and less problematic than the reported ones for the determination of the studied mixture of drugs. The proposed methods were validated according to the ICH guidelines used for the determination of CHL, DSP, and THZ with highly accurate and precise results.

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