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Spectrophotometric determination of Dalacin phosphate from formulations using reduce atomic absorption of calcium ion by phosphate

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Abstract

A simple, sensitive and accurate method for spectrophotometric determination of Dalacin-phosphate has been established. The method is based on the reduction of atomic absorption calcium ion by phosphate. Because, there is a phosphate mole in every Dalacin mole structure and with measurement of phosphate, Dalacin is beyond measurement too. Therefore, the reaction between acidic hydrolysis products of antibiotics with the mixture of calcium ion evaluated for the spectrophotometric determination of mentioned drug in formulations. Experimental parameters related to the performance of atiomic absorption spectrometry (AAS), such as concentration of calcium ion, reaction time and temperature were investigated and optimized. Under the optimized conditions, the limit of detection ($3\sigma_B$) and relative standard deviation (RSD, n=4) were 1.75 μg mL^{-1} and 1.4 %, respectively. The results obtained agreed with those obtained by the USP method.

Keywords: Dalacin phosphate; Atomic absorption spectrometry; Pharmaceutical preparation.

1. Introduction

Dalacin [7(S)-chloro-7-deoxylincomycin] is a lincosamide antibiotic. It is synthesized from microbially fermented lincomycin by replacing a hydroxyl group at the 7-position of lincomycin by a chlorine group, that significantly increases its activity. The effect of clindamycin, which is primarily bacteriostatic, is exerted by its binding to the 50S ribosomal subunit and the consequent inhibition of bacterial protein synthesis [1].

It is active against aerobic Gram-positive and anaerobic bacteria, mycoplasmas, and some protozoa. In companion animal medicine, clindamycin is mainly used in the treatment of diseases like staphylococcal skin infections and osteomyelitis, periodontal disease, bacterial prostatitis, toxoplasmosis, and neosporosis [2]. Dalacin is launched in formulations for either oral (as clindamycin hydrochloride), or parenteral (as Dalacin - phosphate) administration. The molecular weight of Dalacin - phosphate is 504.96 and its structural formula is represented in Fig. 1.

Due to the vital importance of antibiotic drugs in biological fluids and pharmaceutical preparations, there were many methods to determine Dalacin with spectrophotometry [3],

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capillary electrophoresis [4], gas chromatographic [5] and high-performance liquid chromatography (HPLC) with MS [6, 7], UV [8-10], electrochemical [11] and chemiluminescence (CL) [12, 13]. However, these methods are less specific (microbiological) and accuracy, more time and reagent consuming due to sample preparation [5, 14] or not widely accessible. But, the extensive development of the pharmaceutical field requires more rigorous analytical method in control of drugs. Therefore, in this study we report the reduction atomic absorption of calcium ion by phosphate to development a simple, sensitive, low cost and reliable spectrophotometric method for the indirect determination of Dalacin in medicinal product as capsule, solution and gel. of course, there is a phosphate mole in every Dalacin mole structure and with measurement of phosphate, Dalacin is beyond measurement too. The proposed method is not only adopting official methods [3, 15] but also is very fast, simple, accurate and inexpensive in comparison to various reported methods such as HPLC.

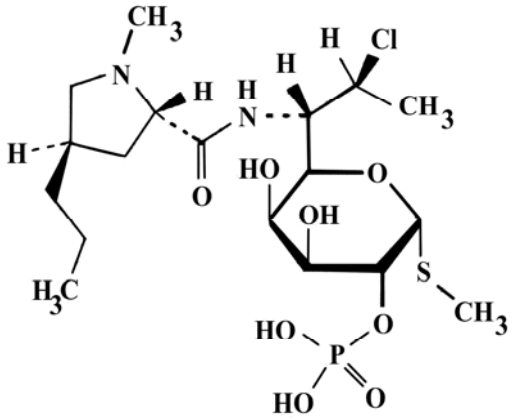

Fig. 1. Structural formula of Dalacin-phosphate.

2. Experimental

2.1. Reagents and chemicals

All of the chemicals used in this study were of the highest purity available from Merck or Fluka and used without further purification. Triply distilled water was used throughout. Reagent grade Dalacin phosphate and gel (1%) Dalacin phosphate obtained from Hakim pharmaceutical company (Tehran-Iran), capsule (150 mg) Dalacin phosphate obtained from Yasmin pharmaceutical company, Solution (1%) Dalacin phosphate obtained from PaK pharmaceutical company (Tehran-Iran).

2.2. Apparatus

An atomic absorption spectrometer Philips model PU 9100X was used for all measurements. A calcium hollow cathode lamp (Hamamatsu Photonics, Japan) was used under the following operating conditions: Wavelength 422.7 nm, lamp current 12 mA, slit width 100 μm and sensitivity 3 times. A COOLNICS THERMOBATH model CTE-21 was used for the temperature adjustment at 60±2 ºC.

2.3. Standard solutions

In order to make 20 μg mL^{-1} of phosphate, 0.145 g KH_2PO_4 will be poured in to a 100 mL volumetric flask and diluted to the mark with water and 2 mL of that will be taken and poured in a 100 mL flask and volumized to have 20 μg mL^{-1} phosphate solution produced. Also, to make 30 μg mL^{-1} Ca^{2+} solution, 0.59 g $Ca(NO_3)_2$ will be poured in a 100 mL volumetric flask and

diluted to the mark with water to have a 1000 μg mL^{-1} Ca^{2+} solution. Then 3 mL of the obtained solution will be taken and poured in to a 100 mL volumetric flask, and then complete to volume with distilled water to make 30 μg mL^{-1} of Ca^{2+}.

2.4. General recommended procedures

2.4.1. Spectrophotometric determination of Dalacin

Transfer 10 mL of 30 μg mL^{-1} calcium ion into 100 mL volumetric flasks then add accurate volumes of Dalacin solution containing suitable amounts of the drug. Complete to volume with distilled water and mix well. After 5 minute at 60 ºC, absorptions will be read by atomic absorption spectrometry at λ=422.7 nm and molar absorptivity = 4×10^4 L mol^{-1} cm^{-1}. Alternatively, derive the corresponding regression equation.

2.4.2. Sample preparation solutions

An accurately weighed amount of Dalacin gel or powdered Dalacin capsule or specific volume of Dalacin solution was dissolved in water into a 100 ml calibrated flask containing a few drops of 0.1 mol L^{-1} Hydrochloric acid and diluted to the mark with water, and the recommended procedures for the determination of Dalacin were followed.

3. Results and discussion

There is a phosphate mole in every Dalacin mole structure and with measurement of phosphate, Dalacin is beyond measurement too. Therefore, the concentration of Dalacin-phosphate was found to be proportional to the measured absorbance of calcium ion by atomic absorption spectrometry (AAS) in order to reduce atomic absorption of calcium ion by phosphate in pharmaceutical preparations. Experimental parameters that might affect to the performance of the method and AAS signal were investigated and optimized, by utilizing the univariant method for simplifying the optimization procedure.

3.1. Effect of calcium ion concentration:

The indirect determination of Dalacin-phosphate is related to the concentration of calcium ion. Therefore, the calcium ion concentration on the reaction with standard phosphate ion was studied over the range of 10-50 μg mL^{-1} calcium ion concentration.

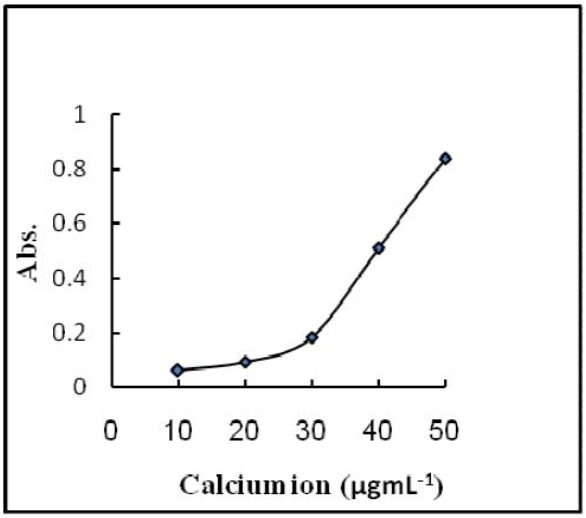

Fig. 2. Effect of calcium ion concentration on the reaction with 20 μg mL^{-1} phosphate ion measured at room temperature after 5 minute.

The results illustrated in Fig. 2 show that the suitable and best absorbance was observed at 30 μg mL^{-1} calcium ion concentration. Therefore, 30 μg mL^{-1} calcium ion concentrations were chosen as the most suitable concentration of calcium ion.

3.2. Effect of time

In order to study the effect of time, samples were assayed at different time intervals at room temperature. Results are shown in Fig. 3. It was found that a 5 minute time interval gave the best signal.

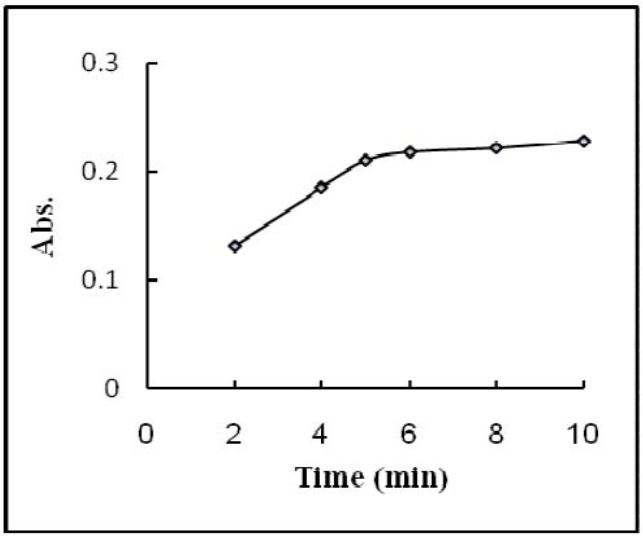

Fig. 3. Effect of time on the reaction between 30 μg mL^{-1} calcium ion and 20 μg mL^{-1} phosphate ion measured at room temperature.

3.3. Effect of temperature

Fig. 4 shows the effect of temperature on the reaction of calcium ion with phosphate. As can be seen from the figure, the best signal was obtained at 60 ºC temperature. With considerable improvement in linear concentration range and maximum sensitivity occurred in comparison to room temperature, so, a temperature of 60 ºC was selected as the optimum temperature.

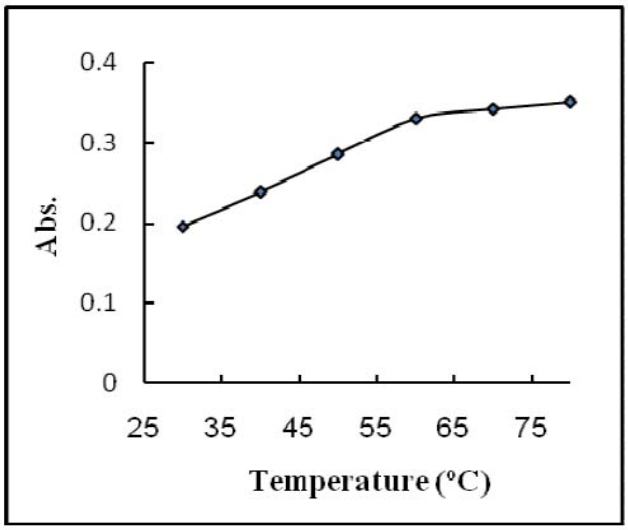

Fig. 4. Effect of temperature on the reaction between 30 μg mL^{-1} calcium ion and 20 μg mL^{-1} phosphate ion measured after 5 minute.

3.4. Evaluation of the method performance

To evaluate the practical utility of the proposed method, linear range, reproducibility and limit of detection were investigated using standard solutions of phosphate under the optimal conditions under the optimum conditions; the results are summarized in Table 1. Good linearity was observed, with the square of the correlation coefficient (R^2) >0.999 in the range studied. The limits of detection (LOD), estimated on the basis of three times the standard deviation of the peak absorbance for ten blank and quantification limits were 1.75 and 1.8 μg mL^{-1} respectively. The proposed method was applied to the determination of Dalacin-phosphate in pharmaceutical preparations. The concentration of Dalacin was calculated using the corresponding regression equation. The results obtained are given in Table 2. Statistical analysis of the results obtained by the proposed and titrimetric methods [3] revealed no significant difference in the performance of the two methods regarding accuracy and precision are revealed by the student´s t-test and F-test, respectively.

Table 1
Linearity, regression equation, correlation coefficient, reproducibility, detection limit and quantitation limit for indirect determination of Dalacin-phosphate by the proposed method.

Compound	LR [a]	Regression equation	R^2 [b]	RSD (%) [c]	LOD [d]	LOQ [e]
Dalacin-phosphate	2-120	y= -0.0582x+0.9483 [f]	0.999	1.40	1.75	1.8

a. Linear Range (μgmL^{-1})
b. Correlation coefficient
c. Relative standard deviation; mean value for four replicate analyses
d. Limit of detection (μgmL^{-1}); estimated on the basis of three times the standard deviation of the peak absorbance for ten blank
e. Limit of quantitation (μgmL^{-1}).
f. y= Absorbance, x= Volume (standard phosphate, 20 μgmL^{-1})

We have also evaluated the accuracy of the proposed method by performing experiments on the samples prepared from dosage forms and pure drugs. The mean percent recoveries obtained from three replicate measurements were found to be 99.9 with an RSD% between 0.8-1.5 percent.

Table 2
Determination of Dalacin-phosphate in its formulation.

Sample	Label	% Recovery ± SD [a]	
		Proposed method	Official method
Capsules	150 mg	146.8±3.1 mg	148.6±1.6 mg
		t=0.82, F=3.75 [b]	
Gel	1% (w/w)	0.99±0.05%	1.02±0.11 %
		t=0.85, F=4.84 [b]	
Solution	1% (w/w)	1.02±0.04 %	1.08±0.09 %
		t=2.11, F=5.06 [b]	

a. Standard deviation, average of three replicate measurements.
b. Tabulated values of t and F are 2.78 and 19.00 at 95% confidence level.

4. Conclusions

The above results obtained from proposed method showed that the method is comparable to the official USP method. The proposed spectrophotometric method is simple, fast and inexpensive, does not require any toxic organic solvents, and is precise and accurate. This method was satisfactory for the determination of Dalacin phosphate in drug formulations such as capsules (150 mg), gels (1%) and solutions (1%) without considerable interference. The student′s t-test and F-test values for the 95% confidence level did not exceed the theoretical values of 2.78 and 19.00 for t- and F-tests, respectively, indicating no significant difference between the accuracy and precision of the two methods. Therefore, this makes the method applicable for the determination of Dalacin - phosphate.

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