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A simple and sensitive spectrophotometric method for estimation of diethylene triamine penta acetic acid (DTPA) in topical gel formulations

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ABSTRACT

A simple, sensitive, economical and reproducible UV-Spectrophotometric method was developed for the estimation of DTPA in topical gel formulations. The method is based on NaFeDTPA complex formation by the reaction of DTPA with ferric chloride in 0.1N HCl. Optimum conditions for the reaction were investigated and absorbance was read at λ_{max} at 272 nm. Bears law was obeyed in the range of 5µg/mL-50µg/mL with correlation of 0.9998. The detection and quantitation limits were found to be 0.8701 and 2.6366 µg/mL respectively. The proposed method was successfully applied for the determination of DTPA in topical gel formulations. Accuracy was examined through recovery studies. The results show that the procedure is accurate, precise and reproducible (relative standard deviation < 1 %), while being simple, cost effective and less time consuming, which proves the suitability of the proposed method for the estimation of DTPA in topical gel formulations.

Keywords: DTPA, U.V Spectrophotometric method, Topical gel.

INTRODUCTION

Diethylene Triamine Penta Acetic Acid (DTPA) (Figure 1) is a well known chelating agent and approved as decontaminating agent for number of radionuclides and poisons [1]. It binds to form water soluble, stable complexes with radionuclides and poisons. It is approved by U.S. FDA for chelation of three radioactive materials: plutonium, americium and curium [2,3]. Calcium DTPA and Zinc DTPA are well known forms which make water soluble complexes with curium, americium and plutonium, to remove from the body through urine. Calcium DTPA is about 10 times more effective than Zinc DTPA for chelating plutonium, americium and curium when given immediately after contamination [3-8]. Literature suggests that there is no simple, rapid and sensitive spectrophotometric method for the estimation of DTPA in topical gel formulations.

However various methods based on HPLC [9], HPTLC and other chromatographic techniques have been reported for the estimation of DTPA in different formulations, but these are time consuming, costly and require expertise [9, 10-18]. The aim of the present study was to develop and validate a simple, sensitive, accurate and reproducible U V spectrophotometric method for routine analysis of DTPA in topical gel formulations.

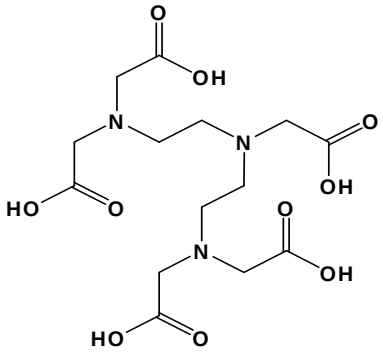

Figure 1: Structure formula of DTPA

MATERIALS AND METHODS

Chemicals and reagents

DTPA (Merck Ltd. Mumbai, India) gel formulations containing DTPA were prepared in-house. Topical gel contains excipients like carbopol (Qualikems, Vododara), methyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi), propyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi) and triethanolamine (Fisher scientific, Mumbai).

Apparatus

A shimadzu model 1601 UV-VIS spectrophotometer with 1cm quartz cells at the wavelength ranges of 200-400 nm was used for spectrophotometric analysis. The absorbance of DTPA in the selected medium was determined and the validation parameters were calculated (Table 1).

Procedure for Calibration curve and sample preparation

Stock solution of 1mg/mL of DTPA was prepared in 1M sodium hydroxide solution. Different concentrations of DTPA (5-50 µg/mL) were prepared by transferring the aliquots of stock solution in 10 mL standard volumetric flasks containing 1mL ferric chloride solution (500µg/mL in 0.1N HCl) and volumes were made up with 0.1N HCl solution.

Sample was prepared by dissolving 5 g topical gel (5% w/v DTPA) in 200mL distilled water using mechanical stirrer, sonicated and filtered. Aliquot equivalent to 1.25 mg of DTPA was taken and mixed with 1mL of ferric chloride solution, suitably diluted with 0.1N HCl to get a concentration of 25µg/mL and analyzed at 272 nm.

Specificity and selectivity

DTPA solutions (25µg/mL) were prepared separately in the selected media with and without common excipients used in the formulation. All solutions were scanned in range of 200 to 400 nm and checked for any change in the spectrum.

Linearity and accuracy

To establish linearity of the proposed method, a series of DTPA solutions (5-50µg/mL) were prepared from stock solution and analyzed. The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, different levels of drug concentrations i.e. lower concentration (LC), intermediate concentration (IC) and higher concentration (HC) were prepared from independent stock solutions and analyzed (Table 2). To provide an additional support to the accuracy of the developed assay an additional method was used which involved the addition of different concentrations of DTPA (12.5, 25 and 37.5 µg/mL) to a pre-analyzed formulation sample and the total concentration was determined using the proposed method (n = 5). The accuracy was calculated as % recovery = [*Ct*/ (*Ca*+*Cs*)] x 100, where *Ct* is the total drug concentration measured after standard addition; *Cs* is drug concentration in the formulation sample; *Ca* is drug concentration added to the formulation (Table 3).

Precision

Repeatability was determined at three different levels of drug concentrations, prepared from independent stock solutions and analyzed in triplicates three different times in a day and studied for intra-day variation (Table 2).

The intermediate precision was determined by inter-day variation. The estimation was followed for three different days to study inter-day variation. One set of different levels of the concentrations was reanalyzed using the UV–VIS spectrophotometer. The percent relative standard deviation (% R.S.D.) of the predicted concentrations from the regression equation was taken as precision (Table 3).

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for DTPA by the proposed method were determined from calibration standards. LOD and LOQ were calculated by using the formula as 3.3 σ/S and 10 σ/S respectively, Where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n = 5) (Table 1).

RESULTS AND DISCUSSION

The λ_{max} of DTPA in solution was found to be 272nm. Calibration curve was obtained by using linear regression equation. The developed method was found to be linear in the range of 5–50 µg/mL with correlation coefficient (r^2) of 0.9998 (Figure 2).

Specificity and selectivity

The UV-spectras of DTPA alone and with excipients were found to be similar which indicates no effect of excipients on the absorption of DTPA. Therefore it can be said that the proposed analytical method is specific and selective for DTPA estimation in topical gel formulations.

Linearity

The linearity range for DTPA was found to be 5–50 µg/mL with r^2 value of 0.9998 (Table 1). The low values of the standard error (S.E.) of slope and intercept indicated high precision of the proposed method. The quality of the fit of the regression equations was supported by the high regression coefficient values.

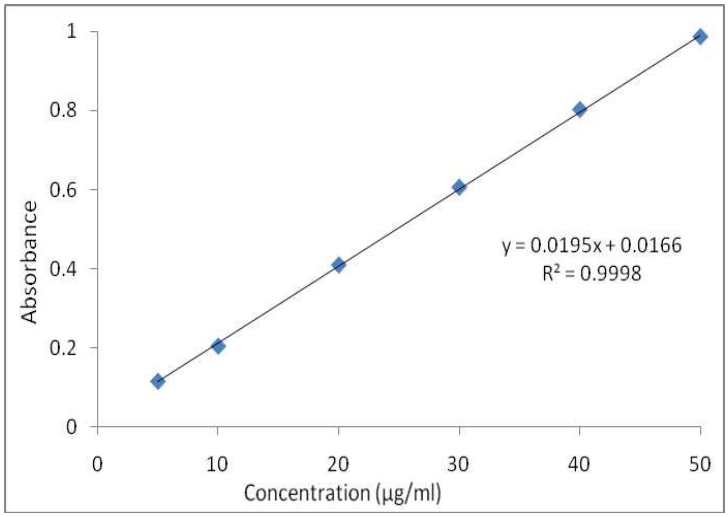

Figure 2: Calibration curve of DTPA

Table 1: Optical characteristics, statistical data and validation parameters

Parameters	**Data**
Optical characteristics	
$E_{1\%, 1cm}$	2.06 x 10^{-2}
Regression analysis	
Slope	0.0195
Intercept	0.0166
Regression coefficient (r^2)	0.9998
Validation parameters	
Linearity (µg/mL)	5-50 µg/mL
Limit of detection (LOD) (µg/mL)	0.8701
Limit of quantification (LOQ) (µg/mL)	2.6366

Accuracy

The excellent mean % recovery values, close to 100%, and their low standard deviation values (S.D < 1) indicated high accuracy of the analytical method. The validity and reliability of the proposed method was assessed by the recovery studies and is summarized in table 1. Further, validity and reliability of the proposed method was accessed via recovery studies by the standard addition method (Table 3). These results revealed that any minor change in the drug concentration in solutions could be accurately determined by the proposed analytical method.

Table 2: Accuracy and precision data for the developed method (n = 5)

Level	**Estimated concentration[a] (µg/mL)**			**Mean % recovery (±S.D)**	**Accuracy[b] (%)**
	Range	**Mean (±S.D)**	**% R.S.D.**		
LC (20µg/mL)	19.67-20.14	19.83 (±0.18)	0.9083	99.19 (±0.90)	-0.85%
IC (25µg/mL)	24.56-25.09	24.73 (±0.21)	0.856	98.92 (±0.85)	-1.08%
HC (30µg/mL)	29.79-30.23	29.99 (±0.15)	0.159	99.98 (±0.53)	-0.033%

[a]estimated concentration of DTPA was calculated by linear regression equation.

[b]accuracy is given in % relative error = [100 × [(predicted concentration – nominal concentration)/ nominal concentration].

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability was found in the range 19.67 µg/mL -30.23 µg/mL at all given levels of DTPA concentrations (Table 4). In intermediate precision study, % R.S.D. values were found to be less than 1% in all

the cases. R.S.D. values for the proposed analytical method were well within the acceptable range, indicating that the method has an excellent repeatability and intermediate precision. Moreover LOD and LOQ for DTPA were found to be 0.8701 and 2.6366 µg/mL respectively.

Table 3: Standard addition of DTPA for accuracy (n = 5)

Drug in formulation (µg/mL)	Pure drug added (µg/mL)	Total drug found (µg/mL) (±S.D)	% Recovery (±R.S.D)	Accuracy (%)
25	0	24.41 (±0.15)	-	-
25	12.5	36.91 (±0.29)	98.42 (±1.21)	-1.58%
25	25	49.41 (±0.27)	98.80 (±1.17)	-1.18%
25	37.5	61.91 (±0.20)	99.05 (±1.05)	-0.95%

Table 4: Results of intermediate precision and repeatability (n = 5)

Concentration (µg/mL)	Estimated concentration			
	Intermediate precision (µg/mL) (% R.S.D.)			Repeatability (µg/mL) (% R.S.D.)
	Day 1	Day 2	Day 3	
20	19.79 (0.79)	19.53 (0.91)	20.08 (1.12)	19.79 (0.97)
25	24.41 (0.81)	24.12 (0.74)	24.66 (0.84)	24.39 (0.79)
30	29.09 (0.49)	29.77 (0.56)	29.14 (0.63)	29.33 (0.56)

CONCLUSION

It is concluded that the developed method for estimation of DTPA in topical gel formulations is a simple, sensitive, cost effective, precise and reproducible. It showed acceptable linearity and accuracy. The proposed method is highly sensitive therefore it could be used for routine analysis of DTPA in topical gel formulations.

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