Original Article

Simple spectrophotometric method for estimation of disodium edetate in topical gel formulations

Abstract

A simple, sensitive, cost-effective and reproducible UV-spectrophotometric method has been developed and validated for the estimation of disodium edetate in topical gel formulations. Solution of disodium edetate reacts with ferric chloride to form complex in 0.1 N HCl giving λ_{max} at 270 nm. Beer's law was obeyed in the concentration range of 5–50 µg/mL ($r^2 = 0.9997$). The limit of detection and limit of quantitation were found to be 1.190 and 3.608 µg/mL, respectively. The results show that the procedure is accurate, precise, and reproducible (relative standard deviation < 1%), while being simple and less time consuming. The study concluded that the UV-spectrophotometric method could be used for the quantification of disodium edetate in pure form as well as in pharmaceutical formulations.

Key words: Disodium edetate, method validation, UV-spectrophotometric method

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INTRODUCTION

Disodium edetate is a disodium salt of ethylenediamine tetra acetic acid (EDTA) [Figure 1]. It is a white crystalline powder, soluble in water.[1-6] It forms stable and water-soluble complexes with various heavy metals such as arsenic, mercury, antimony, and gold and can also be used in the treatment of metal poisoning as decontaminating agent.[1,2] It has been approved by United State Food and Drug Administration for the treatment of heavy metal poisoning and radioactive contamination, as decorporating agent, examples are mercury, plutonium, curium, cobalt, and americium.[5,7-11] Chelation therapy using disodium edetate is medically accepted treatment for lead poisoning and digoxin toxicity.[12-15] Various methods such as thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and high pressure thin layer chromatography (HPTLC) have been reported for the estimation of disodium edetate in different formulations,[16-19] but they are time consuming, costly, and require expertise.[20-23] However, literature suggests that there is no simple, rapid, and sensitive method for the estimation of disodium edetate in topical gel formulations. The current study investigated the feasibility of developing a UV-spectroscopic method for the estimation of disodium edetate in pharmaceutical formulations.

MATERIALS AND METHODS

Reagents, chemicals, and instruments

Disodium edetate (Merck Ltd. Mumbai, India) gel formulations were prepared in-house. Topical gel contains excipients such as carbopol (Qualikems, Vododara, India), methyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi, India), propyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi, India), and triethanolamine (Fisher scientific, Mumbai, India). All chemicals and reagents used were of the analytical grade.

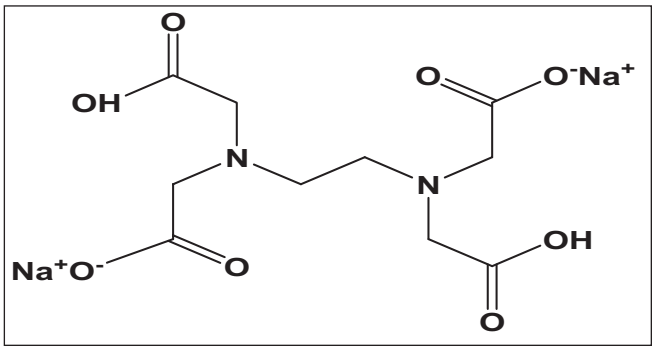

Figure 1: Structural formula of disodium edetate

Table 1: Optical characteristics, statistical data of the regression equations and validation parameters for disodium edetate (n = 5)

Parameter	Data
Optical characteristics	
$E_{1\%, 1\ cm}$	1.90 ×10^{-2}
Regressionanalysis	
Slope	0.0191
Intercept	0.0013
Regression coefficient (r^2)	0.9997
Validation parameters	
Linearity (µg/mL)	5–50
Limit of detection (µg/mL)	1.190
Limit of quantification (µg/mL)	3.608

The spectrophotometric measurements were carried out using UV-VIS spectrophotometer (Shimadzu model 1601) (Shimadzu Analytical Pvt. Ltd, Mumbai, India) with a diode array detector (DAD) (190–1100 nm). The absorbance of disodium edetate in the selected medium was determined and the validation parameters were calculated [Table 1].

Procedure for calibration curve and sample preparation

The estimation of disodium edetate by UV-spectrophotometry is based on the reaction between Na_2H_2EDTA with $FeCl_3$ that leads to the formation of the NaFeEDTA complex which absorbs light at 270 nm. Different concentrations of disodium edetate (5–50 µg/mL) were prepared by transferring the aliquots of the stock solution (1 mg/mL) in 10 mL standard volumetric flasks containing 1 mL ferric chloride solution (500 µg/mL of 0.1 N HCl). Volumes were made up with 0.1 N HCl. Sample was prepared by dissolving 5 g topical gel (5% w/v disodium edetate) in 200 mL distilled water using mechanical stirrer, sonicated, and filtered. Aliquot equivalent to 1.25 mg of disodium edetate was taken and mixed with 1 mL of the ferric chloride solution (500 µg/mL of 0.1 N HCl), suitably diluted with 0.1 N HCl to get a concentration of 25 µg/mL and analyzed at 270 nm.

Specificity and selectivity

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

Linearity, accuracy, and precision

To establish linearity of the proposed method, a series of disodium edetate solutions (5–50 µg/ml) were prepared from the stock solution and analyzed. The accuracy of the method is the closeness of the measured value to the true value. To determine the accuracy, 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. Accuracy was assessed as the percentage relative error and mean % recovery [Table 2]. To provide an additional support to the accuracy of the developed assay another additional method was used, which involved the addition of different concentrations of disodium edetate (12.5, 25, and 37.5 µg/ml) to a preanalyzed formulation sample and the total concentration was determined using the proposed method (n = 5). The accuracy was calculated as percentage recovery = [Ct/ (Ca+Cs)] × 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 formulation [Table 3].

Repeatability was determined by using different levels of drug concentrations from independent stock solutions and analyzed in triplicates, three different times in a day and studied for intraday variation.

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

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) for disodium edetate by the proposed method were determined using 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].

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

Level (µg/mL)	Range (µg/mL)	Mean (±S.D)	R.S.D (%)	Mean % recovery (±S.D)	Accuracy[a] (%)
20 (LC)	19.78–20.34	19.97 (±0.23)	1.15	99.89 (±1.15)	-0.11
25 (IC)	24.36–24.92	24.62 (±0.21)	0.85	98.48 (±1.04)	-1.51
30 (HC)	29.83–30.29	30.05 (±0.17)	0.56	100.16 (±0.84)	0.16

[a] Accuracy is given in % relative error = [{(predicted concentration – nominal concentration)/ nominal concentration} ×100].

Table 3: Standard addition of disodium edetate in formulation for accuracy (n = 5)

Drug in formulation (µg/mL)	Pure drug added (µg/mL)	Total drug found (µg/mL) (±S.D)	Mean % Recovery (±S.D)	Accuracy[a] (%)
25	0	24.65 (±0.17)	-	-
25	12.5	37.15 (±0.26)	99.06 (±1.26)	-0.93
25	25	49.65 (±0.23)	99.3 (±1.15)	-0.7
25	37.5	62.15 (±0.20)	99.44 (±0.99)	-0.56

[a] Accuracy is given in % relative error = [{(predicted concentration – nominal concentration)/ nominal concentration} × 100].

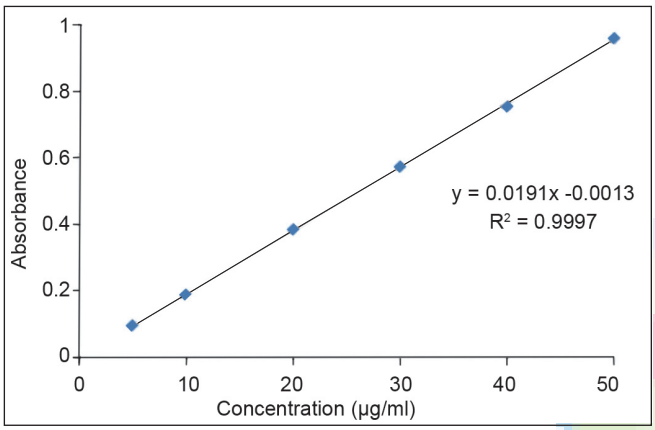

Figure 2: Calibration curve of disodium edetate

RESULTS AND DISCUSSION

The λ_{max} of disodium edetate in solution was found to be 270 nm by scanning the sample solutions in entire UV region. The developed method was found to be linear in the range of 5–50 µg/mL, where Beer's law was well obeyed. Calibration curve was constructed by using linear regression equation. The regression equation was originate $y = 0.0191x - 0.0013$. The correlation coefficient (r^2) of the regression curve was found to be 0.9997 [Figure 2]. All the validation parameters for disodium edetate are listed in Table 1.

Specificity and selectivity

The UV spectras of disodium edetate alone and with excipients were found to be similar, indicated no effect of excipients on the absorption of disodium edetate. Hence, it can be said that the proposed analytical method is specific and selective for the estimation of disodium edetate in topical gel formulations.

Linearity, accuracy, and precision

The linearity range for disodium edetate was found to be 5–50 µg/mL with r^2 value of 0.9997 [Table 1]. The quality of fit of the regression equations was supported by the high-regression coefficient values [Table 1]. For accuracy, recovery studies were carried out and the percentage recovery was found in the range of 98.48–100.16. The excellent mean % recovery values, close to 100% and their low-standard deviation values (SD < 1) indicated high accuracy of the analytical method. The validity and reliability of the proposed method was assessed by the recovery studies and was summarized in Table 1. Further, the validity and reliability of the proposed method were also accessed by the standard addition method [Table 3]. These results revealed that any minor change in disodium edetate concentration in solutions could be accurately determined by the proposed analytical method. The low values of the standard error (SE) of slope and intercept [Table 1] indicated high precision of the proposed method. Precision was also determined by studying the repeatability and intermediate precision. Repeatability was found in range of 19.96–29.92 µg/mL at all the given levels of disodium edetate concentrations [Table 4]. In an intermediate precision study, %RSD values were found to be less than 2% in all the cases. The RSD values found were well within the acceptable range indicating that the proposed method has an excellent repeatability and intermediate precision [Table 4]. These results also suggested that the proposed method may be considered validated in term of precision.

Limit of detection and limit of quantitation

LOD and LOQ of calibration curve were calculated which was based on the standard deviation (σ) of y-intercept of regression line and slope (S) of the

Table 4: Precision data for the developed method

Concentration (µg/mL)	Estimated concentration (n = 5)			
	Intermediate precision (µg/mL) (% R.S.D)			Repeatability (µg/mL) (% R.S.D)
	Day 1	Day 2	Day 3	
20	19.93 (0.96)	19.86 (1.12)	20.05 (1.07)	19.96 (1.18)
25	24.19 (0.83)	24.83 (0.88)	24.95 (0.79)	24.65 (0.84)
30	29.89 (0.56)	30.14 (0.63)	29.74 (0.55)	29.92 (0.61)

calibration curve at the levels approximating the LOD and LOQ, LOD = 3.3 σ/S and LOQ = 10 σ/S respectively. LOD and LOQ of calibration curve were found to be 1.190 and 3.608 µg/mL, respectively for disodium edetate [Table 1].

CONCLUSIONS

It is concluded from the performed study that the developed UV-spectrophotometric method for the estimation of disodium edetate in topical gel formulations, is a simple and cost-effective method. Results also showed good precision and reproducibility. It showed acceptable linearity and accuracy. The proposed method is found to be highly sensitive; therefore, it could be used for routine analysis of disodium edetate in topical gel formulations.

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