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Original Article

DEVELOPMENT AND VALIDATION OF A GRADIENT HPLC METHOD FOR QUANTIFICATION OF EDETATE DISODIUM IN LYOPHILIZED INJECTABLE DRUG PRODUCT

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ABSTRACT

Objective: The present study was aimed to validate a developed reversed phase gradient high-performance liquid chromatography method for the quantitative determination of Edetate Disodium in the lyophilized injectable drug product.

Methods: The amount of total Edetate disodium was analysed by HPLC assay using Edetate disodium USP as a reference standard. Injectable product was dissolved in acetone and Edetate disodium is separated out from API and then dissolved in water. Analysis was carried out using ferric chloride as a precolumn derivatizing reagent and YMC Pack ODS-A, 5 µm column with mobile phase as a mixture of tetrabutylammonium bromide buffer pH 2.8 and acetonitrile as the solvent, water used as diluent. The Edetate disodium quantified by U. V. wavelength at 254 nm.

Results: The method was suitably validated with respect to specificity, linearity, precision, accuracy and solution stability, using this method the average recovery from spike sample is 98.2%, with a relative standard deviation of<3%. The minimal quantifiable level was 1.5 µg/ml. The results show that the procedure is accurate, precise and reproducible.

Conclusion: In the present study an attempt has been made to develop and validate the analytical method for lyophilised injectable formulations and to generate the scientific database for formulation and evaluation of various lyophilised injectable containing Edetate disodium.

Keywords: HPLC, Edetate disodium, Ferric chloride, Method validation

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INTRODUCTION

Disodium edetate is a disodium salt of ethylenediaminetetraacetic acid (EDTA). It is a white crystalline powder, soluble in water. It forms stable and water-soluble complexes with various metal ions and also used in the treatment of metal poisoning as a decontaminating agent [1, 2]. EDTA in its disodium salt or calcium disodium salt form is frequently used in pharmaceuticals because of its stability, compatibility and low toxicity a relatively nontoxic chelating agent can be defined as a substance whose molecules can form several bonds to a single metal ion that is used to remove divalent metal ions, aid the action of preservatives and antibacterial, and stabilize the action of antioxidants [3]. Its stability, compatibility, and low toxicity account for its wide use in ophthalmic and contact lens care solutions. Chelation therapy using EDTA is medically accepted treatment for lead poisoning and digoxin toxicity.

There are several methods presently available to measure Disodium Edetate. These include colorimetry [4, 5], photometry [6], spectrophotometry [6-8], titration [9-14], TLC [15, 16], GC [17, 18], HPLC [19, 20] and ion chromatography [21-23]. However some of these methods require extensive sample workup, including the use of hazardous derivatizing reagent, some are not sufficiently sensitive to permit reproducible quantitation at normal sample concentrations, and others are neither technically nor economically feasible for routine use in most analytical and quality assurance laboratories. The gas chromatographic methods always include time-consuming derivatization steps, in which EDTA is converted into methyl, ethyl, propyl and butyl esters to obtained volatility. Although many of the chromatographic methods offer the advantage of being specific, most require large sample volumes in order to maintain reasonable accuracy and reproducibility.

As EDTA does not contain any chromophoric group, it is very difficult to determine EDTA by direct UV detection. The aim of this study describes the development and validation of the pre-column derivatization analytical method with direct UV-detection for the quantitative determination of Disodium Edetate in the lyophilised injectable drug product. Also to show that the study has advantages over some techniques as mentioned in above references, like here EDTA response is measured by direct UV detection with enhanced sensitivity and method is simpler, highly reproducible, specific and accurate, compare to using complex techniques like titrimetric, spectrophotometric, capillary electrophoresis or GC technique. The method has been optimized with respect to reaction time and derivatization reagent volume and suitably validates for its intended use. The applicability of the method is illustrated for several typical samples.

MATERIALS AND METHODS

Tetrabutylammonium bromide was purchased from SIGMA-ALDRICH. Acetonitrile and Methanol were purchased from Merck. Purified water was obtained from Milli-Q water system (Millipore Corp). The lyophilized injectable product was obtained from precise chromatography for development purpose. Disodium Edetate (fig. 1) was purchased from Merck. Ferric chloride was purchased from Merck. All chemicals were used as such and provided by the manufacturer, no further purification has been done. All the other chemicals were of analytical grade. Magnetic stirrer, cyclomixer, microcentrifuge bought from Eppendorf Equipment's Pvt. Ltd. was used. HPLC from Waters, used in the analysis of the drug. Bath sonicator from PCI Analytics brand was used.

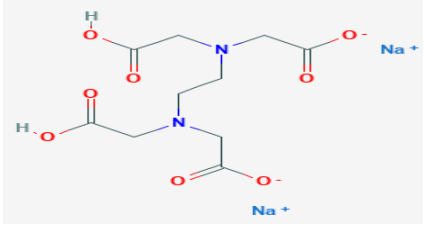

Fig. 1: Structure of disodium edentate

Chromatographic system and condition

The HPLC system consists of a water 2489 module, including quaternary pump, auto-sampler with thermostat, column oven, coupled with a multiple wavelength or diode array UV detectors. YMC Pack C18 (4.6 mm 250 mm, 5µ) analytical columns were used for method development and validation. The Empower 3 chromatographic Software was used for data acquisition and processing. The mobile phase A is consisted of a buffer pH 2.8 prepared by dissolving 1 gm. of tetrabutylammonium bromide in 1000 ml of water and mixed; pH of the buffer was adjusted to 2.8 with the diluted phosphoric acid solution. The mobile phase B consists of buffer and acetonitrile in the ratio of (10:90). The wavelength selected at 254 nm, injection volume kept as 20 µl. Sampler cooler was kept at 5 °C. The analytical column theoretical plate number and tailing factor of the analytes under different chromatographic conditions were calculated using USP methods. Binary solvent gradient was applied at a flow rate of 1.0 ml min-1 and programmed as follows: 82% mobile phase A and 18% mobile phase B at 0 min to 15 min., progressing linearly at 10 % mobile phase A and 90 % mobile phase B at 16 min, followed by the hold in the mobile phase A to 10% and mobile phase B to 90 % at 20 min., finally returning to the initial gradient and flow at 21 min and maintained at this composition and flow for 10 min in the total time of 30 min of analysis.

Preparation of ferric (III) chloride and blank solution

Ferric chloride (III) stock solution is prepared by transferring about 65 mg of Ferric (III) chloride anhydrous into 200 ml amber colour volumetric flask Added 80 ml of water and dissolved. Amber colour flask was taken to avoid the oxidation. Make up the volume with water and mix. Accurately transfer 5 ml of water into 25 ml of volumetric flask and add 5 ml of Ferric (III) chloride solution. Keep the flask at room temperature for 30 min. and make up the volume with water and mixed well.

Preparation of edetate disodium standard stock solution

A standard stock solution at 0.1 (mg/ml) was prepared by dissolving about 20 mg of Disodium Edetate into 200 ml volumetric flask, added about 120 ml of diluent, sonicate to dissolve. Makeup to the mark with diluent and mix well.

Preparation of lyophilized injectable product sample (stock)

1. Randomly selected the five vials of lyophilized injectable product and removed the flip off seal.

2. Removed the air from the vials by inserting the needle into the center of the rubber stopper.

3. Carefully transferred content from five vials into the clean and dry beaker. Rinsed the individual five vials with 2 ml of acetone along with its stopper.

4. Transferred entire content from beaker to centrifuge tube. Rinsed the beaker with about 2-3 ml of acetone and transferred the rinsed solution in the centrifuge tube. Centrifuge the solution for 10 min. at 5000 RPM. Sediment mass was observed at the bottom of the centrifuge tube.

5. Decanted the supernatant solution without disturbing the sediment mass. Added 10 ml of water into the centrifuge tube and sonicate to dissolve the sedimented mass. Transferred the content into 50 ml volumetric flask by using a funnel. Rinsed the centrifuged tube with 7-8 ml of water for 3 more times and transferred to 50 ml volumetric flask. Made up the volume with water and mixed.

Derivatization procedure for standard and sample solution

Each 5.0 ml of standard and sample stock solution is transferred to a 25 ml of separate volumetric flask and 5.0 ml of ferric chloride is added to it, the solutions are kept at room temperature for 30 min. on the bench top and further diluted with diluent and injected on HPLC.

Specificity and selectivity

To assure the specificity of the proposed method, the Edetate disodium solution is prepared in diluent with and without excipients used in the lyophilised product. Also blank and impurity degraded at various conditions, All samples injected in HPLC and checked for its interference (table 1).

Table 1: Specificity for edetate disodium

S. No.	Name of the solution	No. of injection	Retention time
1	Blank	1	NA
2	Placebo	1	NA
3	Standard solution (Disodium Edetate)	1	8.313
4	Sample solution (API)	1	19.219
5	Related impurity-1	1	19.484
6	Related impurity-2	1	19.306

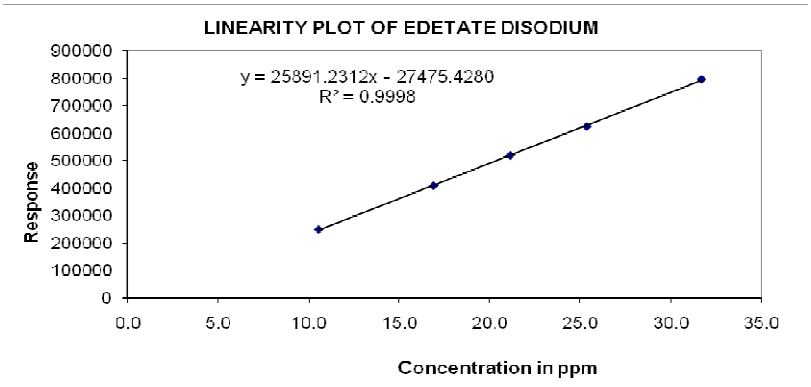

Fig. 2: Linearity plot edetate disodium

Linearity, accuracy and precision

To establish the linearity of the proposed method, a series of edetate disodium solution (10-30 µg/ml) were prepared from the stock solution and analyzed. The relationship between EDTA concentration and detector response was evaluated to confirm linearity. EDTA standards at five different concentrations encompassing a minimum of 80–120% of the expected concentration of EDTA in the lyophilized product were included in the study (fig. 2, table 2).

Table 2: Linearity concentration and response for edetate disodium

S. No.	Target level (%)	Concentration (µg/ml)	Area-1	Area-2	Average area
1	50	10.0	249402	248652	249027
2	80	16.0	409653	407488	408571
3	100	20.0	518878	519446	519162
4	120	24.0	625175	625337	625256
5	150	30.0	799186	795434	797310
Slope					25891.2
Correlation coefficient					0.99988
R^2					0.99975
% Y-intercept					-27475.43

The accuracy of the method is the closeness of measured value to true value. To determine the accuracy, different levelsofedetate disodium prepared by weighing and adding it to the placebo and analysed. The accuracy was calculated as the percentage recovery (table 3).

Table 3: Accuracy for edetate disodium

S. No.	Target level	Average area	Amount drug added (µg/ml)	Amount of drug found (µg/ml)	% Recovery
1	50% sample-1	236665	2.626	2.504	95.35
2	100% sample-2	478071	5.074	5.058	99.68
3	100% sample-3	482472	5.166	5.104	98.8
4	100% sample-1	468848	5.126	4.96	96.8
5	150% sample-2	733740	7.749	7.762	100.2
Mean					98.2
SD					2.039
%RSD					2.08

The precision of the analytical method was evaluated by system precision (table 4) and repeatability (intra-day) (table 5) and intermediate precision (inter-day) (table 6). The repeatability was done by analysing three samples of lyophilised injectable product (duplicate injection of each). The same process was repeated on the second day to assess intermediate precision using three freshly prepared sample solutions. The precision was measured by the % RSD of the duplicate injections for each of the three samples.

Table 4: Precision-system precision for Edetate disodium

S. No.	Standard area response (day-1)	Standard area response (day-2)
1	538589	536588
2	532506	528879
3	531503	531003
4	534242	526599
5	533621	531108
6	522727	53228
Mean	532198	531077.3
SD	5240.7	3368.2
%RSD	0.98	0.63

Table 5: Method precision (M. P.)-Intra-day

S. No.	Sample name	Area	Average area	Amount of mg/ml	% Drug assay
1	M. P. Sample 1-1	487708	487310	0.968	96.8
	M. P. Sample 1-2	486912			
2	M. P. Sample 2-1	490987	489693	0.973	97.3
	M. P. Sample 2-2	488399			
3	M. P. Sample 3-1	485793	483763	0.961	96.1
	M. P. Sample 3-2	483763			
Mean					96.7
SD					0.60
%RSD					0.62

Table 6: Method precision (I. P.)-inter-day

S. No.	Sample name	Area	Average area	Amount of mg/ml	% Drug assay
1	I. P. Sample 1-1	503767	501838	0.959	95.9
	I. P. Sample 1-2	499908			
2	I. P. Sample 2-1	496790	497290	0.955	95.5
	I. P. Sample 2-2	497789			
3	I. P. Sample 3-1	505098	505098	0.970	97.0
	I. P. Sample 3-2	498909			
Mean					96.1
SD					0.78
% RSD					0.81

RESULTS AND DISCUSSION

The developed method was observed to be linear in the range of 10-30 µg/ml. The calibration curve was constructed by using the linear regression equation. The regression equation was originating y =25891.2312x-27475.4280. The correlation coefficient (r^2) of the regression curve was found to be 0.9998.

In specificity experiment, no peak is observed at the retention time of Edetate Disodium peak from blank, placebo, degraded sample and related impurities. Hence it can be said that the proposed analytical method is specific and selective for the determination of edetate disodium.

The average percentage recoveries of EDTA in the spiked samples at three different levels ranged from 95.4% to 100.2% with an overall average of 98.2%. The percentage of relative standard deviation (%RSD) for the triplicate measurements at each level was less than 3%. Which demonstrated a high level of accuracy.

Precision was also determined by repeatability and intermediate precision. It was found that the system precision was 0.98% on day-1 and 0.63% on day-2. The method precision was 0.62% on day-1 and 0.81% on day-2 these data demonstrated the acceptable precision of the method. The RSD value found is well within the acceptable range indicating that the proposed method may be considered validated in term of precision.

CONCLUSION

The simplicity, specificity, and versatility of this method have been shown through experiments conducted on the lyophilised injectable product. The method was validated and shown to be precise, accurate, and reproducible. The method should be readily adaptable to the analytical needs of the pharmaceutical and cosmetics industry. The proposed method is found to be highly sensitive; therefore it could be used for routine analysis for determination of Disodium Edetate content.

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AUTHORS CONTRIBUTIONS

The entire author contributed equally

CONFLICT OF INTERESTS

Declare none

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