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A Sensitive Method for Determination of Residual EDTA in Diuretic Drugs, Furosemide by Reversed Phase High Performance Liquid Chromatography

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ABSTRACT

A sensitive method for the determination of residual EDTA by high-performance liquid chromatographic method with UV detection has been developed. The analysis was performed in gradient mode on a reversed phase C18 column, 150 mm x 4.6 mm x 5 µm. Gradient mobile phase consisted of a 25mM tetrabutyl ammonium hydrogen sulphate, in water as mobile phase A and acetonitrile as mobile phase B. The linear gradient conditions 0-7 minutes 2%B; 7-10 minutes 80%B; 10-15 minutes 80%B, 15-17 minutes 2%B and 17-32 minutes2%B was set for the determination of residual EDTA . Chromatograms were recorded by using flow rate 1.0 mL/minute and the injection volume was 25 µL. The detection wavelength was set at 258 nm. The proposed liquid chromatographic method was successfully applied to the determination of residual EDTA in furosemide. A linear calibration graph was obtained for 25% to 150%of specification limit (200 ppm) with a correlation coefficient of 0.9999 for EDTA. The quantification limit for EDTA in furosemide drug substances was found to be 50 ppm. The recovery for EDTA was found to be within the acceptance criteria (75-125%) from QL level to 150% of the specification limit. Limit of quantitation was found to be 50ppm. The precision of the method at 100% level for EDTA was within the acceptance criteria. The method developed in this study is sensitive and selective and can be applied to routine quality control

Keywords : Routing, non-repudiation, Byzantine failure, MANET, Security, Authentication, Integrity, Non-repudiation, Confidentiality, Key and Trust Management(KTM).

I. INTRODUCTION

EDTA is a prescription medicine, given by injection into the vein (intravenously) or into the muscle (intramuscularly). Intravenous EDTA is used to treat lead poisoning and brain damage caused by lead poisoning; to evaluate a patient's response to therapy for suspected lead poisoning; to treat poisonings by radioactive materials such as plutonium, thorium, uranium, and strontium; for removing copper in patients with Wilson’s disease; and for treating high levels of calcium. EDTA is also used intravenously for heart and blood vessel conditions including irregular heartbeat due to exposure to chemicals called cardiac glycosides, “hardening of the arteries” (atherosclerosis), chest pain (angina), high blood pressure, high cholesterol, and blood circulation problems such as intermittent claudication and Raynaud's syndrome. Other intravenous uses include treatment of cancer, rheumatoidarthritis, osteoarthritis, an eye condition called macular degeneration, diabetes, Alzheimer's disease, multiple sclerosis, Parkinson's disease, and skin conditions.

EDTA is also used intramuscularly for lead poisoning and related brain damage. EDTA is sometimes used as an ointment for skin irritations produced by metals such as chromium, nickel, and copper. Eye drops containing EDTA are used to treat calcium deposits in the eye.

In foods, EDTA bound to iron is used to “fortify” grain-based products such as breakfast cereals and cereal bars.

EDTA is also used in calcium and sodium compounds to preserve food; and to promote the color, texture, and flavor of food [1].

In manufacturing, EDTA is used in calcium and sodium compounds to improve stability in pharmaceutical products, detergents, liquid soaps, shampoos, agricultural chemical sprays, oil emulsion devices, contact lens cleaners and cosmetics. It is also used in certain blood collection tubes used by medical laboratories.

Ethylenediminetetraacetic acid is a powerful chelating agent, forming stable complexes with most metal ions. EDTA is a chemical that binds and holds on to (chelates) minerals and metals such as chromium, iron, lead, mercury, copper, aluminum, nickel, zinc, calcium, cobalt, manganese, and magnesium. When they are bound, they can't have any effects on the body and they are removed from the body. Due to its ability to sequester metal ions, EDTA is widely used in medicine, chemical industry, food technology, agriculture and pharmaceutical technology. EDTA in its disodium salt or calcium disodium salt form is frequently used in pharmaceuticals because of its stability, compatibility and low toxicity. The most common use of EDTA in analytical chemistry is in complexometric titrations [2,3]. In analytical techniques EDTA is being used in the ligands for the complexation of metals, which enables for the chromatographic separations [4].

Administering EDTA intravenously and intramuscularly is effective for treating lead poisoning and brain damage caused by lead exposure. The calcium disodium form of EDTA is approved by the U.S. Food and Drug Administration (FDA) for these uses. Treatment with calcium disodium EDTA improves symptoms of lead poisoning such as abdominal pain, fatigue, constipation, and loss of appetite. It also seems to slow progression of kidney failure in patients who have had long-term lead poisoning. However, EDTA does not seem to be effective for diagnosing lead poisoning. Emergency treatment of life-threatening high calcium levels (hypercalcemia), when given intravenously. The disodium form of EDTA is approved by the FDA for this use, but healthcare providers generally prefer other methods of treatment that are less likely to cause kidney side effects.

EDTA is safe when used as a prescription medicine, as eye drops, and in small amounts as a preservative in foods however the safety of large amounts is unknown [5]. EDTA can cause nausea, vomiting, diarrhea, headache, low blood pressure, skin problems, and fever. Nebulizer solutions containing disodium EDTA as a preservative can cause the breathing tubes to narrow in some people with asthma. The size of the dose determines the amount of the narrowing. EDTA might make heart rhythm problems worse. EDTA might interfere with blood sugar control because it can interact with insulin. EDTA can decrease serum calcium levels, making hypocalcemia worse. EDTA can bind with potassium and increase the amount of potassium that is flushed out in the urine. This might cause potassium levels to drop too low, especially in people who have low levels to begin with. EDTA can bind with magnesium and increase the amount of magnesium that is flushed out in the urine. This might cause magnesium levels to drop too low, especially in people who have low levels to begin with. EDTA might make liver disease worse.

EDTA can harm the kidney and might make kidney disease worse. EDTA doses should be reduced in patients with kidney disease. There is some concern that EDTA might increase the risk of seizure in people with epilepsy or in people who tend to have seizures. EDTA can cause severe decreases in blood levels of calcium, and this can cause a seizure [6].

EDTA can decrease blood sugar. Insulin is also used to decrease blood sugar. By taking EDTA along with insulin can cause serious decreases in your blood sugar. So closely monitoring of blood sugar is necessary and accordingly the dose of insulin might need to be changed. As interaction of EDTA with insulin and Warfarin is major so as per literature it is advisable not to take in combination. Warfarin (Coumadin) is used to slow blood clotting. EDTA has been reported to decrease the effectiveness of warfarin (Coumadin). Decreasing the effectiveness of warfarin (Coumadin) might increase the risk of clotting. It is unclear why this interaction might occur. Be sure to have your blood checked regularly. The dose of your warfarin (Coumadin) might need to be changed [7, 8].

Water pills (Diuretic drugs) interacts with EDTA: Large amounts of EDTA can decrease potassium levels in the body. "Water pills" can also decrease potassium in the

body. Taking EDTA along with "water pills" might decrease potassium in the body too much.

Some "water pills" that can deplete potassium include chlorothiazide (Diuril), chlorthalidone (Thalitone), furosemide (Lasix), hydrochlorothiazide (HCTZ, HydroDiuril, Microzide), and others. Hence the caution shall be taken when it will be used in combination.

Furosemide is a potent diuretic (water pill) that is used to eliminate water and salt from the body. The onset of action after injection is five minutes and the duration of diuresis is two hours. The diuretic effect of furosemide can cause depletion of sodium, chloride, body water and other minerals. EDTA is being used in the synthesis of Furosemide. Therefore the quantification of residual EDTA is essential as per regulatory requirement [9, 10, 11].

There are several analytical methods have been proposed for the determination of EDTA in wide variety of sample matrices. They include spectrophotometry [12], electrochemistry [13], differential pulse polorography [14], voltametric determination [15], amperometry [16], capillary electrophoresis [17] and chromatography [18, 19]. Among these HPLC (ion pair or ion exchange retention mechanism) appear to be the prevailing techniques, despite the fact that EDTA lacks volatility and exhibits low UV/visible absorptivity [20, 21]. The gas chromatographic methods always include a time consuming derivatization steps, in which EDTA is converted into methyl, ethyl, propyl and butyl esters to obtain volatility [22, 23].

The present study deals the development and validation of HPLC method for determination of EDTA in Furosemide drug substance utilizing trivalent iron for complex formation and its subsequent determination based on ion pair chromatography. EDTA being UV inactive was first complexed with trivalent iron resulting in the formation of strong UV active complex. In order to retain the EDTA-Fe+++ complex on to a C18 reverse phase column, tetra butyl ammonium hydrogen sulfate was used as ion pairing agent. A well separated peak was obtained for EDTA-Fe+++ complex on a C18 column with an acetonitrile and 25mM tertiary butyl ammonium hydrogen sulfate mobile phase (1 ml/min).A gradient pump program was used with 2% acetonitrile for an initial 7 min which was increased linearly to 80% over 10 min and then maintained isocratic for 8 min, the acetonitrile ratio finally being returned to 2% in 1 min.

In this paper, the development and validation of a reproducible RP-HPLC method intended to quantify ethylenediaminetetraacetic acid content in Furosemide for routine laboratory use are described, according to International Conference of Harmonisation (ICH) guidelines [24, 25].

II. EXPERIMENTAL

2.1 Instrumentation

HPLC analysis was carried out using a Agilent 1200 Series HPLC system (Agilent, USA), which is equipped with a degasser, a quaternary pump, an auto injector, a temperature controlled column compartment and photo diode array detector connected to EZ-Chrome software. An Agilent XDB C18, 150 mm x 4.6 mm x 5 □m from Agilent technologies USA was used. The column temperature was maintained at 25ºC. The standard and samples solutions are analysed using gradient elution mode using 25mM tetra butyl ammonium hydrogen sulphate (A) and acetonitrile (B). The linear gradient conditions were 0-7 minutes 2%B; 7-10 minutes 80%B; 10-15 minutes 80%B, 15-17 minutes 2%B and 17-32 minutes2%B. The flow rate was set at 1.0 mL/minute and the injection volume was 25 µL. The detection wavelength was set at 258 nm.

2.2 Reference substance, reagents and chemical

Furosemide drug substance samples were obtained from DK Pharma Chem Pvt Ltd, Mumbai. EDTA di-sodium salt, Hydrochloric acid, Tetra butyl ammonium hydrogen sulphate, Ferric Chloride Hexahydrate and Acetonitrile (HPLC grade) was purchased from Merck Chemicals India Pvt Ltd. HPLC grade water used of a Millipore, Milli-Q water purification system (Millipore) Milford, MA, USA.

2.3. Chromatographic Conditions

Gradient mobile phase consisted of a 25mM tetrabutyl ammonium hydrogen sulphate, in water as mobile phase A and acetonitrile as mobile phase B. The gradient profile was Time - %B, 0-7min 2%, 10-15min 80%, 17-32min 2%. The Mobile phase was filtered and degassed through membrane filter of 0.45 µm porosity under vacuum. HPLC column Zorbax XDB C-18,150 mm x

4.6 mm, 5□m analytical column was used as stationary phase. A constant flow rate of 1.0mL/min was employed throughout the analysis. Variable UV-Vis detector was set at 258 nm. All pertinent analyses were made at 25□C and volume of solution injected on to the column was 25 µl. The diluents blank was used by transferring 5mL of Acetonitrile and 50mL of 1mM ferric chloride into 100mL volumetric flask, sonicate for 10min and make up to the mark with 25mM tetrabutyl ammonium hydrogen sulfate.

2.4. Samples

The Furosemide drug substance was obtained from DK Pharma Chem Pvt. Ltd, Mumbai -A Furosemide API Manufacturer Company.

2.5. Solution preparation

2.5.1. EDTA disodium standard solution

Accurately weighed 20 mg of EDTA di-sodium in to a 100 mL volumetric flask, transferred 5 mL of acetonitrile and 50 mL of 1 mM ferric chloride solution, sonicate for 10 minutes and make up to the mark with 25 mM tetra butyl ammonium hydrogen sulphate. The solution was allowed to stand for 15 minutes. This is EDTA di-sodium stock solution.

Transferred 1.0 mL of EDTA di-sodium standard stock solution in to a 100 mL standard flask, added 5 mL of acetonitrile and 50 mL of 1 mM ferric chloride solution, sonicate for 10 minutes and make up to the mark with 25 mM tetra butyl ammonium hydrogen sulphate. The solution was allowed to stand for 15 minutes and then filtered through 0.45 µm membrane filter and 25 µL was injected.

2. 6. Estimation from drug substance

Accurately weighed 1000 mg of furosemide sample in to 100 mL volumetric flask, transferred 5 mL of acetonitrile and 50 mL of 1 mM ferric chloride solution, sonicate for 10 minutes and make up to the mark with 25 mM tetra butyl ammonium hydrogen sulphate. The solution was allowed to stand for 15 minutes.

Six test preparations of drug substances were prepared of 10.0 mg/mL concentration and mixed. About 1000mg of test sample was weighed accurately into a 100 ml volumetric flask, about 10 mL of diluent was added, shaken to dissolve the sample and diluted to volume with diluent and mixed. The solution was filtered through 0.45µm membrane filter and 25µl was injected directly on to the column.

2.7. Quantitation

Peak areas were recorded for all peaks. Peak areas were taken into account to quantitate the EDTA disodium content in drug substance by using the following formula:

$$\text{Content of EDTA (Di-Sodium in ppm)} = \frac{Peak\ area\ of\ EDTA\ disodium\ in\ sample}{Peak\ area\ of\ EDTA\ disodium\ in\ stanadrad} \times \frac{Concentration\ of\ EDTA\ disodium\ in\ \mu g/mL}{Concentration\ of\ sample\ in\ g/mL}$$

III. RESULTS AND DISCUSSION

3.1. Chromatography

HPLC method development started with the search for the suitable column and mobile phase. Chromatographic system comprising0.05M sodium dihydrogen orthophosphate : acetonitrile (80 : 20 v/v), as mobile phase at a constant flow rate of l.0mL/min, Luna C-18 HPLC column, 250 mm x 4.6 mm, 5□m analytical column as stationary phase and detector wavelength at 258nm resulted in no peak elution even after 60 minutes of run time. Mobile phase consisting of 0.05M aqueous ammonium dihydrogen phosphate and acetonitrile in the ratio (80:20) v/v, were tried in isocratic conditions on the InertsilODS-1, 250 mm x 4.6 mm, 5□m to obtain symmetrical peak shapes, clear separation of the signal peaks from the solvent front peaks.

After investigation of following two chromatographic system containing 25mM tetra butyl ammonium hydrogen sulphate and acetonitrile as mobile phase (90 : 10) at a constant flow rate of l.0mL/min, on Agilent HPLC column XDB C-18, 150mm x 4.6mm, 5□m and50mM aqueous diammonium hydrogen phosphate and acetonitrile as mobile phase (80 : 20) on Waters C-18, 250mm x 4.6 mm, 5□m HPLC column at constant flow rate of 1.0mL/min and detector wavelength at 258nm resulted in peak elution at about 2.6 minutes which is very close to peaks of blank diluent. First investigation resulted in EDTA disodium peak eluted very close to blank peak (peak from diluent) as shown figure 1.

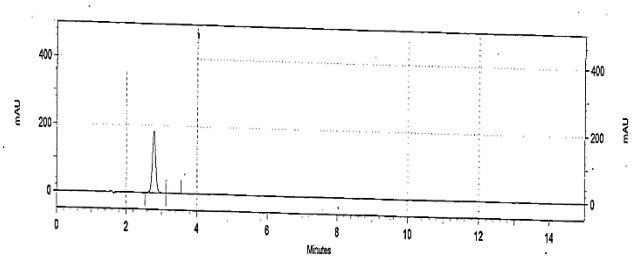

Fig 1.
Chromatogram of EDTA disodium showing first investigated chromatographic column: Waters C-18, 250 mm x 4.6mm, 5µm, mobile phase: 50mM diammonium hydrogen phosphate and acetonitrile (80:20) v/v, flow rate 1ml/min. Detector wavelength: 258 nm

Further, in order to develop a suitable and robust LC method for the determination of EDTA disodium by UV detection different mobile phases and columns were employed (table 1) to achieve the best signal response and retention time.

Finally, the mobile phase consisting of 25mM tetrabutyl ammonium hydrogen sulphate and acetonitrile with gradient programme, at a constant flow rate of 1.0 mL/min and detector wavelength set at 258 nm, using Agilent XDB, C-18, 150mm x 4.6 mm, 5 m HPLC column was found to be appropriate, allowing good signal response of EDTA disodium.

3.2 Optimization of HPLC

The gradient mobile phase use can affect the analyte's retention time as well as the detection sensitivity. No peak elution of Furosemide was observed due to isocratic use of mobile phase as 25mM tetrabutylammonium hydrogen sulphate and acetonitrile (90:10), so the gradient use of mobile phase was chosen for the determination of EDTA disodium and Furosemide elution with appropriate retention time.

Finally gradient mobile phase chromatographic conditions were optimized to have good retentions and detection response for EDTA di-sodium and elution of Furosemide peak. The EDTA di-sodium is eluted at retention time at about 5.6 minutes and Furosemide at about 14.5 minutes, the well resolved peaks between EDTA di-sodium and Furosemide is shown in figure 2. Therefore, gradient mobile phase 25mM tetrabutyl ammonium hydrogen sulphate and acetonitrile was chosen for estimation of EDTA disodium in Furosemide drug substance, typical chromatogram of test solution is shown in Figure. 3.

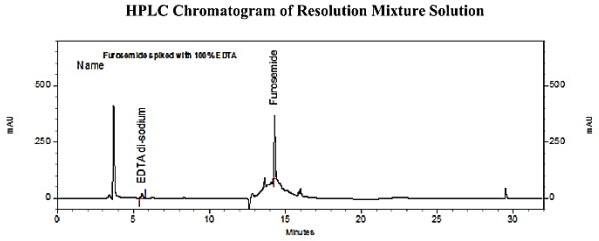

Fig 2.
Effect of gradient mobile phase on peak elution of ETDA di-sodium and Furosemide on Agilent XDB C-18, 150 x 4.6 mm, 5µm, mobile phase: 25mM tetrabutyl ammonium hydrogen sulphate, and acetonitrile.

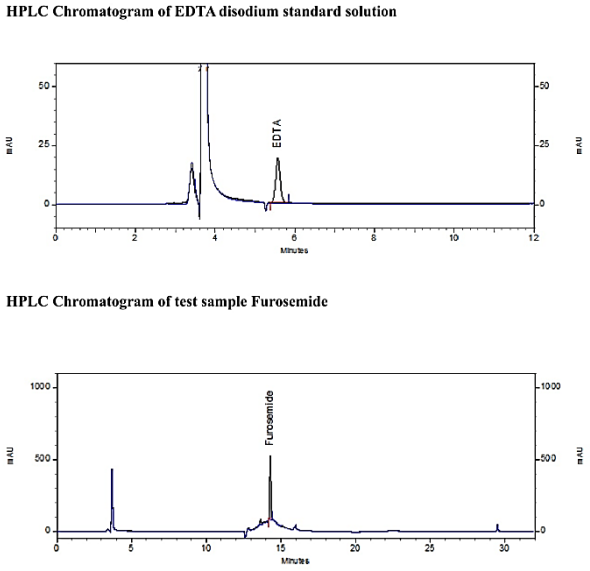

Fig 3.
A typical chromatograms of EDTA di-sodium standard solution and test sample by proposed methods column: Agilent XDB C-18, 150 x 4.6 mm, 5µm, gradient mobile phase: A-25mM Tetra butyl ammonium hydrogen sulphate and B-acetonitrile, flow rate 1ml/min, λ set at 258 nm.

3.3. Method validation

Test method for the determination of EDTA disodium was validated to include the essential demands of International conference on Harmonization (ICH) guidelines. Parameters like specificity, linearity, accuracy, precision, range and system suitability were examined.

3.3.1. Specificity

No interferences were observed due to obvious presence of mobile phase.

3.3.2. Linearity

Peak areas versus concentration in milligram per milliliter were plotted for EDTA disodium at the concentration range between LOQ level to150 percent of target level. EDTA disodium showed linearity in the

range of 0.5-3.0µg/mL with a correlation coefficient (r2) of 0.9999.

3.3.3. Accuracy

Accuracy of the proposed HPLC determination was evaluated from the EDTA disodium content determination results of the components. Accuracy was done by performing the samples and calculated the peak area responses of different samples by component recovery method.

Stock solution

Stock Solution was prepared by dissolving accurately weighed about 19.95mg of EDTA disodium in portions mobile phase and diluted to produce 100 mL solution. Appropriate portions of stock solution were spiked into blank diluent to produce concentrations of 25, 50, 75, 100, 125 and 150% of target level. Mean recovery of spiked samples was 103.9 % for EDTA disodium (Table 2).

3.3.4. Precision

Instrumental precision was determined by five replicate determinations of standard solution and the relative standard deviation was 0.2% for EDTA disodium.

Method precision or intra-precision was performed by preparing six different samples involving different weighing. Each solution was injected in under same conditions and peak area responses for each solution were taken. Corrections in area were made for each weight taken to prepare six sample solutions. The contents of EDTA disodium in all six sample solutions were calculated. The EDTA disodium content in all six preparations was found nil.

Intermediate precision was performed by analyzing the samples by two different instruments. Standard solution and six different samples at 100 percent target level were prepared. The EDTA disodium content in all 12 preparations was found nil.

3.3.5. System suitability

System suitability tests were performed to chromatograms obtained from standard solutions to check the relative standard deviation of five replicate injections. Results obtained from five replicate injections of standard solution observed relative standard deviation less than 2.

3.4. Application of the proposed method

In-house prepared samples were evaluated for content of EDTA disodium. The method gave reproducible results for all the samples tested for EDTA disodium. The probable degradation products of test sample and EDTA disodium did not interfere with the estimation of the component. The EDTA disodium content, of the test samples are summarized in Table 3.

IV. CONCLUSION

A sensitive HPLC method based on UV detection has been developed and validated for determination of EDTA disodium in Furosemide drug substance. The method is simple, rapid, specific, accurate (error ±4.9%), precise, (RSD <2.0%) robust and linear (r2=0.9999). The described method is suitable for routine analysis and quality control for determination of EDTA disodium content in Furosemide drug substance and drug product.

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Table 1. Employed mobile phases, columns and elution time during the investigation of EDTA

Column	Mobile phase	Elution time
Luna C-18 column, 250 mm x 4.6 mm, 5µm	0.05M sodium dihydrogen orthophosphate : acetonitrile (80 : 20 v/v)	*No peak eluted
Inertsil ODS-1, 250 mm x 4.6 mm, 5µm	0.05M ammonium dihydrogen phosphate : acetonitrile, (75 : 25 v/v)	*No peak eluted
Waters, C-18, 250 mm x 4.6 mm, 5µm	50mM aqueous diammonium hydrogen phosphate : acetonitrile, (80 : 20 v/v)	** Peak eluted close to blank peak
Agilent XDB, C-18, 150 x 4.6 mm, 5µm.	25mM Tetrabutyl ammonium hydrogen sulphate and acetonitrile (90:10)	** Peak eluted close to blank peak
Agilent XDB, C-18, 150 x 4.6 mm, 5µm.	25mM Tetrabutyl ammonium hydrogen sulphate and acetonitrile (Gradient programme)	About 5.6 minutes.

***** No peak EDTA disodium eluted after 60 minutes time

****** EDTA disodium peak eluted near very close to blank peak (peak from diluents)

Table 2. Accuracy data (analyte recovery): EDTA

	Amount std. added(µg/mL)	Amount std. recovered (µg/mL)	Determined (% of target level)	Recovered (%)	Bias (%)
1	0.527*	0.561	25.07	102.3	2.3
2	2.108*	2.125	104.7	104.7	4.7
4	3.204*	3.222	154.3	104.9	4.9

*Average of three determination.

Table 3: Application of the developed HPLC method for the determination of EDTA content in Furosemide drug substance and drug product

Sr. No.	Test sample	ETDA Content found to be
1	Furosemide Drug substance	Nil
2	Lasix ® Furosemide tablets	Nil
3	Furosemide tablets	Nil