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Development of a simple RP-HPLC method for the determination of EDTA residue in pharmaceutical clean-in-place (CIP) applications

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Abstract

A reversed-phase method was developed for the determination of EDTA (ethylenediaminetetraacetic acid) in a clean-in-place detergent through HPLC connected with UV or PDA detector. The EDTA signal was well above the detection limit and no interference was observed from water sample or any other swab and rinse diluent. The purpose of this study is to develop a simple, cost-effective reversed-phase HPLC method for the identification and quantification of EDTA in the cleaning solution. This method may contribute to further development and validation of residue detection from cleaning solutions used in pharmaceutical manufacturing. A HPLC system connected with UV detector and a column, Hamilton PRP-X100, 250 mm long x 4.1 mm internal diameter, 5 µm-thick was used to develop this method. This method is specific and precise for EDTA determination. The %RSD for six replicate injections was found to be within 10.0%. This method can be applied to cleaning validation samples and should be validated according to ICH guidelines before being used to analyze cleaning validation samples.

Keywords: HPLC; EDTA; Cleaning Validation; Method Development; Reversed Phase

1. Introduction

CIP-100 cleaner is specially formulated to meet the unique cleaning demands found in the pharmaceutical, biotechnology, cosmetic, and dietary supplement industries, as well as other sectors requiring an effective detergent. CIP 100 detergent contains approximately 1% - 5% EDTA in its composition.

EDTA is a chelating agent that prevents detergent from binding with trace mineral elements that are present in water. It can break the bond of Cu-1,2,4-triazole through strong interactions between O16, H2, H36 and copper sulfate for 1,2,4-triazole removal [1].

The Food and Drug Administration (FDA) enforces on cleaning process and the agency published a guideline where they specified that no detergent residues should remain after the cleaning process [2]. It is particularly impossible to prove that equipment is completely clean and devoid of contaminant. However, it is possible to prove that the residual contaminants are below a certain allowable level [3]. Therefore, a specific and precise method is required to determine residue levels after cleaning.

Detecting detergent residues challenging task in cleaning validation. However, there are several techniques were developed by scientists throughout the years. W. Resto developed and validated an ion chromatographic method for

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detecting traces of CIP-100 detergent [2]. Atomic absorption spectroscopy (AAS) techniques have also been developed for determining residual active pharmaceutical ingredients (APIs) in cleaning validation samples [4]. Additionally, a derivatized HPLC method was validated for the determination of disodium EDTA in Meropenem drug substances [5].

The first step in cleaning equipment is selecting of an appropriate cleaning agent for the type of residue to be removed. Once this is determined, an analytical strategy must be devised to assess the amount of cleaning agent residue left on the cleaned surface. This assessment is important, because if a cleaning agent effectively removes the drug product residue but leaves behind its own residue, one type of contamination is simply exchanged with another, and the equipment has not been cleaned effectively [6].

This method is developed considering the simplicity, cost-effectiveness of analytical process and identification of very low concentration of EDTA.

2. Material and methods

2.1. Material

The source of reagents and chemicals used in this study from the following sources: Copper (II) Sulphate pentahydrate ($CuSO_4.5H_2O$) (Merck, Germany), Isopropyl alcohol (IPA) (Merck, Germany), Sulfuric Acid (RCI Labscan Ltd., Thailand), Methanol (Merck, Germany).

2.2. Methods

2.2.1. Instrumentation

A high-performance liquid chromatography equipped with UV or PDA detector (Brand: Waters, USA) and a column, Hamilton PRP-X100, 250 mm long x 4.1 mm internal diameter, 5 µm-thick were used. Analytical balance: SARTORIOUS CPA224S.

2.2.2. Chromatographic condition

Column	4.1 mm x 250 mm, 5 µm, Hamilton PRP-X100
Wavelength	254 nm
Flow rate	1.0 mL /min.
Oven temperature	35 °C
Inject volume	100 µl
Needle wash	Water: Methanol (5:95)
Seal wash	Water: Methanol (95:5)

2.2.3. Mobile phase

Transfer 6 mL of 1N Sulfuric acid into a 1 litter volumetric flask containing approximately 500 mL of purified water. Add approximately 125 mg of copper sulfate pentahydrate, 100 mL of methanol, and 10 mL of isopropyl alcohol. Make volume up to the mark with purified water and mix properly.

This mobile phase should be degassed using vacuum filtration through a 0.45µm nylon filter.

2.2.4. Standard solution

Prepare 20 ppm CIP 100 cleaner solution in Purified water.

- Stock-1: Weigh accurately and transfer approximately 500 mg of CIP-100 detergent into a 100 mL clean and dried volumetric flask. Make up the volume to the mark with purified water and mix well.
- Stock-2: Transfer 10 mL of the above solution (Stock-1) into a 250 mL clean and dried volumetric flask. Make up the volume to the mark with purified water and mix well.

Final standard solution (20 ppm): Transfer 10 mL of the above solution (Stock-2) into a 100 mL clean and dried volumetric flask. Make up the volume to the mark with purified water and mix well.

2.2.5. Sample Solutions

Diluent for Swab sample and Rinse sample: Purified water.

- Preparation of diluent with swab stick (Swab blank): Place two swab sticks into a 10 mL of purified water at the time of sampling and sonicate for 5 minutes. Filter the solution through a 0.2 µm PTFE filter, discard the first 2 to 3 mL, and transfer the aliquot into an HPLC vial.
- Preparation of Swab sample: After sampling from the equipment, sonicate for 5 minutes. Filter the solution through a 0.2 µm PTFE filter, discard the first 2 to 3 mL, and transfer the aliquot into an HPLC vial.
- Preparation of Rinse sample: After sampling from the equipment, sonicate for 5 minutes. Filter the solution through a 0.2 µm PTFE filter, discard the first 2 to 3 mL, and transfer the aliquot into an HPLC vial.

3. Results and discussion

This analytical method was developed for the determination of EDTA to identify and quantify trace amounts of the cleaning agent remaining as residue on cleaned equipment. All chemicals used in this study were of reagent grade. CIP-100 reagent was used as a standard to identify EDTA in the sample solution, as it contains 1% to 5% EDTA. A specificity and precision study were performed to demonstrate that the method is specific and precise. From the precision study, it was observed that this method gives reproducible result. The %RSD found from the six-replicate injections was less than 10.0.

3.1. Specificity

Purified water as a diluent, a diluent with swab stick, a standard solution, a swab sample, and a rinse sample were injected to check for any interference with the EDTA peak. The retention time of EDTA was found to be approximately 5.0 minutes. All the samples were prepared and injected into the chromatographic system to confirm the retention time and check for interference from any other peaks.

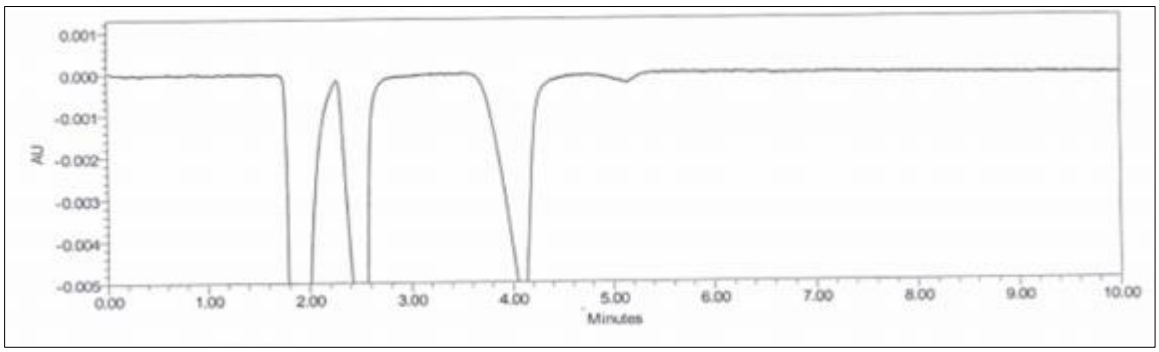

Figure 1 Chromatogram of Diluent (Purified water)

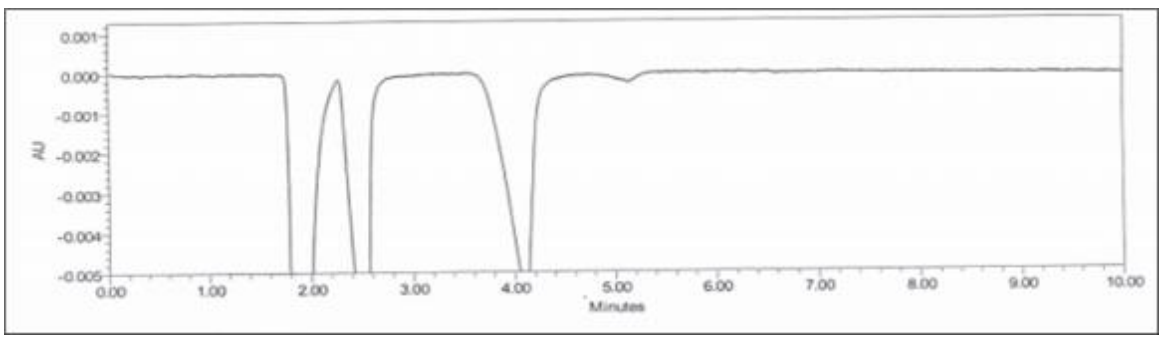

Figure 2 Chromatogram of Diluent with swab stick

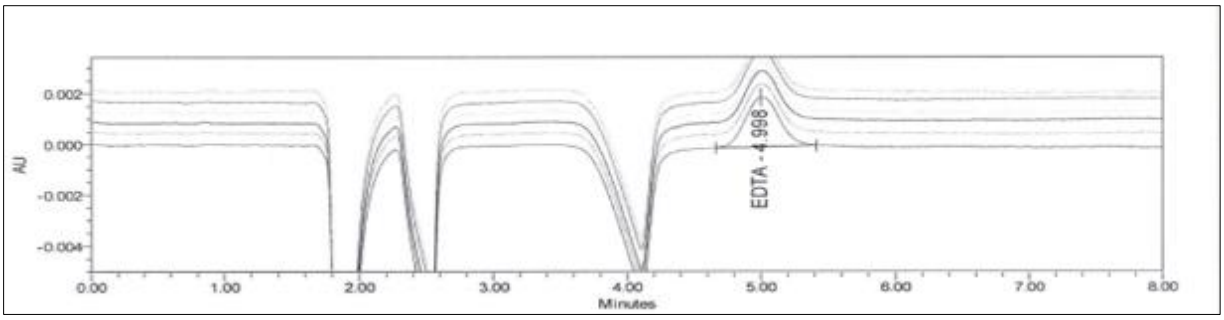

Figure 3 Chromatogram of standard solution

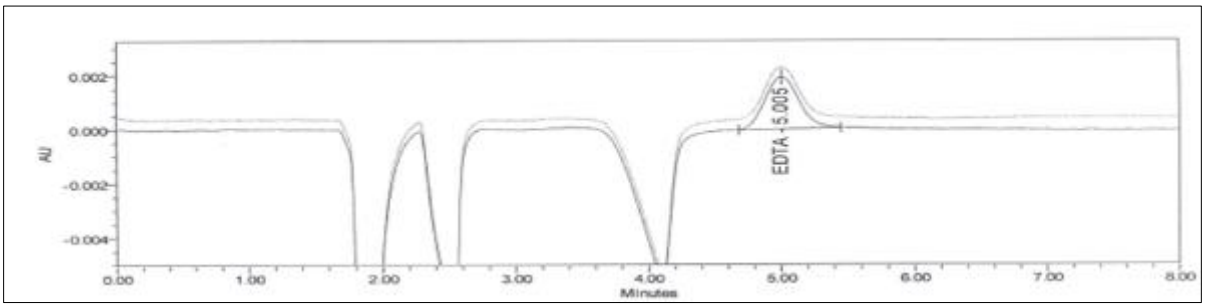

Figure 4 Chromatogram of Sample solution (swab sample & rinse sample)

3.2. Precision Study

As a part of this study, system precision was evaluated. The standard solution was injected six times, and the chromatograms were observed. **Table 1** represents the system precision result. The %RSD of the peak areas of EDTA was found to be below 10.0. **Table 2** represents the %RSD of retention time (RT), confirming the suitability of the method.

Table 1 System precision data (%RSD of areas)

SN.	Name of the molecule	%RSD of area
1	EDTA	4.3

Table 2 %RSD of RT from the precision data

SN.	Name of the molecule	%RSD of RT
1	EDTA	0.4

3.3. System suitability

The tailing factor of the principal peak was taken as the system suitability criterion. The system suitability criteria required that the tailing factor of the EDTA peak should not be more than 1.5, and the result showed that it was found well within the acceptance criteria. The result is presented in **Table 3**.

Table 3 System suitability data

SN.	System suitability parameter	Result
1	Tailing factor of EDTA	1.1

3.4. LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) have been established by calculating the signal-to-noise ratio according to ICH guidelines. The LOD and LOQ concentration were presented in **Table 4**.

Table 4 LOD and LOQ concentration

SN.	Name of the molecule	LOD	LOQ
1	EDTA	1.5 ppm	4.5 ppm

List of Abbreviations

- RT: Retention time
- API: Active pharmaceuticals ingredient
- AAS: Atomic absorption spectroscopy
- ICH: International Council for Harmonisation of Technical Requirements for Pharmaceuticals and Human Use
- LOD: Limit of detection
- LOQ: Limit of quantification
- µm: Micrometer
- ppm: Parts per million
- RSD: Relative standard deviation
- EDTA: Ethylenediaminetetraacetic acid
- HPLC: High performance liquid chromatography
- CIP: Clean-in-place
- FDA: Food and Drug Administration
- IPA: Isopropyl alcohol
- UV: Ultra-violet
- PDA: Photo diode array

4. Conclusion

The proposed RP-HPLC method was developed for the identification and quantification of traces of CIP-100 detergent after cleaning the production equipment. This method has been evaluated for specificity, precision, and LOD & LOQ and proved to be convenient and effective for the cleaning validation samples. LOD data show that this method can detect very low concentrations of EDTA. This method is fast, cost-effective, and convenient for pharmaceutical quality control laboratory or any other food and drug manufacturing companies. Additionally, it can serve as study material for students learning about method development. This method can be used to determine the residue content of EDTA in the equipment after cleaning with CIP-100 detergent. This method is precise and specific, and should be validated according to the ICH guidelines before being used to release the commercial products.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors disclose no conflict of interest regarding the publication of this paper.

Authors' Contribution

The research was designed and performed by Sreekanta Nath Dalal. Md Jabir Rashid and Md Sakil Amin were observed and reviewed the article.

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