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Development and validation of an HPLC Method for Determination of EDTA in cleaning validation samples

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Abstract

A simple, sensitive, and reliable High Performance Liquid Chromatography (HPLC) method was developed and validated for the determination of Ethylenediaminetetraacetic acid (EDTA) in cleaning validation samples. The method aims to ensure effective monitoring of residual EDTA on pharmaceutical manufacturing equipment to prevent cross-contamination and maintain product quality. Chromatographic separation was achieved using a Hamilton PRP X-100 (150 × 4.6 mm, 10 µm) column with a mobile phase consisting of sulfuric acid, copper sulfate, methanol, and isopropyl alcohol. The flow rate was maintained at 0.8 mL/min, and detection was carried out at 240 nm with a run time of 8 minutes. The method was validated according to standard regulatory guidelines by evaluating parameters such as system precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, specificity, solution stability, and intermediate precision. The system precision showed excellent reproducibility with a %RSD of 0.8%. The method exhibited strong linearity over a concentration range from LOQ to 300%, with a correlation coefficient of 0.9999. The LOD and LOQ were found to be 0.0121 µg/mL and 0.0242 µg/mL, respectively, indicating high sensitivity. Recovery studies for both swab and plate methods were within acceptable limits (80–120%), with average recoveries around 100%. The method demonstrated good specificity with no interference at the retention time of EDTA and showed stability of solutions up to 72 hours. The validated method is precise, accurate, and robust, making it suitable for routine cleaning validation studies in pharmaceutical industries to ensure compliance with quality standards.

Keywords: Cleaning Validation; HPLC; EDTA; Method Validation

1. Introduction

Cleaning validation plays a vital role in the pharmaceutical industry to ensure that manufacturing equipment is adequately cleaned and free from residues of active pharmaceutical ingredients (APIs), excipients, and cleaning agents.[1] This process is essential to prevent cross-contamination, which can compromise product quality, safety, and efficacy. Regulatory authorities require validated cleaning procedures supported by reliable analytical methods to confirm that residue levels are within acceptable limits.[2,3] Ethylenediaminetetraacetic acid (EDTA) is a widely used chelating agent in pharmaceutical formulations and processing due to its ability to bind metal ions. Despite its usefulness, residual EDTA left on equipment surfaces after cleaning may interfere with subsequent manufacturing processes and affect product stability. Therefore, it is important to develop a sensitive and accurate analytical method for the detection and quantification of EDTA in cleaning validation samples. High Performance Liquid Chromatography (HPLC) is a powerful and widely accepted analytical technique used for cleaning validation because of its high sensitivity, specificity, precision, and reproducibility. It is particularly suitable for detecting trace levels of residues collected through swab sampling methods.[4] The use of appropriate chromatographic conditions enables effective separation and accurate quantification of analytes like EDTA. The present study focuses on the development and validation of an HPLC method for the determination of EDTA residues from equipment surfaces using swab sampling.[5] The method validation was

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performed in accordance with standard guidelines, evaluating key parameters such as system precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, specificity, solution stability, and intermediate precision.[6-8] The objective of this study is to establish a robust, reliable, and reproducible method that can be routinely applied in cleaning validation to ensure compliance with regulatory requirements and maintain high standards of pharmaceutical quality.[9-11]

2. Materials and Methods

Drugs and chemicals EDTA Reference standard procured from TLC pharmaceutical standards. HPLC grade Methanol, Isopropyl alcohol, Copper sulphate, Sulfuric acid and Milli-Q water, obtained from Merck.

2.1. Instrumentation

Instrumentation A waters HPLC E-2695 instrument equipped with Empower software. Column configuration is Hamilton PRP X-100, 150 x 4.6 mm 10 µm. Sartorius electronic balance was used to weigh the materials.

2.2. Mobile Phase preparation

2.2.1. Preparation of 1M H_2SO_4

Transferred 5.6 mL of Sulfuric acid into a 100 mL Volumetric flask. Diluted to volume with water. Mixed well.

2.2.2. Mobile Phase

Transferred 6 mL of 1M H_2SO_4, 125 mg of Copper sulfate,100 mL of Methanol and 10 mL of Isopropyl alcohol into a 1000 mL volumetric flask containing 500 mL of water. Dilute to volume with water and mixed well. Filtered through 0.45µm membrane.

2.2.3. Diluent

Water

2.3. Standard preparation

Weighed accurately and transferred about 32 mg of EDTA RS into a 50 mL volumetric flask. Added 30 mL of diluent sonicated to dissolve and diluted to the volume with diluent and mixed well. Further transferred 5.0 mL of above stock solution into a 50 mL volumetric flask. Pipette out 2.5 mL Intermediate stock standard solution into a 50 mL volumetric flask (2.5µg/mL).

2.4. Preparation of Swab Sample

Placed an adequate number of swabs into a beaker and added acetonitrile to immerse the swabs. Sonicated for about 10 minutes and drained the acetonitrile. Squeeze the swabs with parafilm. Repeated this procedure two more times and in the final step finger squeezed the individual swab with parafilm. Placed the swabs into a beaker and added diluent to immerse the swabs. Sonicated for about 20 minutes and drain the diluent. Squeezed the swabs with parafilm. Repeated this procedure two more times and in the final step finger squeezed the individual swab with parafilm**.**

2.5. Blank Preparation

Placed one pretreated swab in a suitable test tube. Pipetted 10.0 mL of the diluent into the same test tube. Closed the test tube and vortex for about three minutes and filled up the vial with a clear liquid.

2.6. Swabbing Procedure

One test tube will be prepared for each sampling site. One additional test tube will be prepared and used as a blank. (The swab will be included in a test tube but not used to sample). Placed one pretreated swab into each test tube. Pipette 10.0 mL of the diluent into *each test tube. Labelled the test tubes and specify the particular piece of equipment to be swabbed. Area on each plate to be swabbed will consistently be 100 cm^2 (i.e., 4”x 4” inch = 100 cm^2). FirmLy squeezed the swab along the sides of the test tube and swab the sampling site two times in a same manner. Swabbing should be unidirectional, from left to right starting from the left top corner. Returned the swab to its respective test tube. Closed each test tube and vortex for about three minutes. Fill up the HPLC vial with a clear liquid.

2.7. Chromatographic conditions

Hamilton PRP X-100, 150 x 4.6 mm 10 µm column was utilized for chromatographic separation. The mobile phase composed with 6 mL of 1M H_2SO_4, 125 mg of copper sulfate,100 mL of Methanol and 10 mL of Isopropyl alcohol, at flow rate of 0.8 mL/min with run time of 8 minutes. Detection wavelength at 240 nm. Retention time of EDTA was found to be 6 minutes. Injection volume 100 µl, Column oven thermostatically controlled with 55°C, Sampler temperature maintained at ambient.

2.8. Method Validation

2.8.1. System Precision

Standard solution of EDTA was prepared as per the method and injected. Relative standard deviation (RSD) for peak area responses of EDTA from six (6) replicate injections of the standard solution were calculated and reported. The RSD from six replicate injections of the standard solution preparation should be NMT 6.0 % and Tailing factor should be NMT 2.0

2.8.2. Linearity

Prepared Standard solutions in different concentrations From LOQ level to 300 % as per the specification limits**.** Intercept and Slope calculated for each element from linearity curve. The correlation coefficient should be NLT 0.97 over the concentration range.

2.8.3. Limit of Detection (LOD), Limit of Quantitation (LOQ) and Precision at LOQ

Standard solutions of EDTA were serially diluted and injected to determine limit of detection (LOD) and limit of quantitation (LOQ). The concentration of LOQ was calculated signal to noise ratio of 8. The concentration of LOD was calculated signal to noise ratio of 4.

2.8.4. Recovery study

Plate recovery was performed by spiking known concentrations of EDTA on 10 cm x10 cm plate in triplicate, at 50%,100% and 150% of targeted concentrations (i.e 1.385 µg/mL,2.77 µg/mL and 4.155 µg/ mL, respectively). The plates were air-dried. The recovery should be between 80%-120%.

2.8.5. Solution stability

Plate Recovery and swab recovery sample solutions (100% recovery samples) were prepared and injected into the system at Initial, 24 hours, 48 hours and 72 hours. The Recovery must be between 80%-120%. The difference between initial results should be between NMT 10%.

2.8.6. Specificity

To verify the specificity of the cleaning verification method, diluent, plate blank preparations were injected into the chromatographic system for identification and impurity interference. No peak should be observed at main peak.

2.8.7. Intermediate Precision

The intermediate precision was performed by a second analyst on a different day using a different HPLC system by spiking known concentrations of EDTA solution on 10 cm x 10 cm stainless steel plates, at 100% of the targeted concentrations. The plate was then air-dried using pre-treated swabs, plate was swabbed and collected in 10 mL diluent to give 100% of the standard solution concentration (i.e. 2.75 µg/mL). The samples were injected in HPLC, and percent recovery was calculated. Recovery must be between 80%-120%.

3. Results and Discussions

3.1. System Precision

Table 1 System Precision

Injection No	Peak Area	USP Tailing
1	232146	1.1
2	233512	1.1
3	230696	1.1
4	232580	1.1
5	233991	1.1
6	229179	1.1
Mean	232017	1.1
%RSD	0.8	

3.2. Linearity

Table 2 Linearity

Linearity standard	Level %	Peak Area
1	LOQ	1732
2	40	90462
3	80	184892
4	100	230066
5	120	277044
6	200	463188
7	300	699893
Correlation Coefficient	0.9999	

3.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Table 3 LOD and LOQ

Name	Concentration (µg/mL)	Peak Area	S/N ratio
LOD	0.0121	1293	4
LOQ	0.0242	1732	8

3.4. Recovery study

Table 4 Recovery from Swab

Sample	% Recovery Level	% Recovered	% Mean Recovery
1	50%	110	105
2	50%	104	

3	50%	101	
4	100%	103	100
5	100%	100	
6	100%	98	
7	150%	98	98
8	150%	98	
9	150%	99	
Average Recovery			101

Table 5 Recovery from Plates

Sample	% Recovery Level	% Recovered	% Mean Recovery
1	50%	101	100
2	50%	100	
3	50%	100	
4	100%	99	99
5	100%	98	
6	100%	100	
7	150%	100	100
8	150%	100	
9	150%	99	
Average Recovery			100

3.5. Solution stability

Table 6 Solution stability of Plate Recovery

Time (hours)	Area	% Recovered
Initial	229012	99
24	227866	98
48	231613	100
72	219275	97

Table 7 Solution stability of Swab Recovery

Time (hours)	Area	% Recovered	% Difference
Initial	237199	103	NA
24	226257	98	2.0
48	231211	100	0.0
72	228498	101	1.0

3.6. Specificity

No interference at main peak RT

3.7. Intermediate Precision

Table 8 System Precision study

Injection No	Analyst-1 Peak Area	Analyst-2 Peak Area
1	232146	213528
2	233512	214003
3	230696	213397
4	232580	216955
5	233991	216521
6	229179	214321
Mean	232017	214788
%RSD	0.8	0.7
USP Tailing	1.1	1.0

Table 9 Plate Precision study

Injection No	Analyst-1 Peak Area	Analyst-2 Peak Area
1	229012	203693
2	228481	203631
3	232097	201033
	% Recovery	
	Analyst-1	Analyst-2
1	99	95
2	98	95
3	100	94
Mean	99	0.7

4. Conclusion

The present study describes the development and validation of a simple, sensitive, and reliable HPLC method for the determination of EDTA in cleaning validation samples. The method was carried out using a Hamilton PRP X-100 column with a mobile phase consisting of sulfuric acid, copper sulphate, methanol, and isopropyl alcohol, and detection at 240 nm. Validation parameters were evaluated as per standard guidelines, including system precision, linearity, LOD, LOQ, recovery, solution stability, specificity, and intermediate precision. The system precision showed excellent reproducibility with a %RSD of 0.8%. The method exhibited strong linearity from LOQ to 300% concentration levels with a correlation coefficient of 0.9999. The LOD and LOQ values were found to be 0.0121 µg/mL and 0.0242 µg/mL, respectively, indicating high sensitivity. Recovery studies for both swab and plate methods were within acceptable limits (80–120%), with average recoveries close to 100%, confirming the accuracy of the method. Solution stability results demonstrated that samples were stable up to 72 hours with minimal variation. The specificity study confirmed that there was no interference at the retention time of EDTA, and intermediate precision results showed consistency

between different analysts and instruments. Overall, the developed method meets all validation criteria and is suitable for routine use in cleaning validation to ensure effective monitoring of EDTA residues and compliance with pharmaceutical quality standards.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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