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Research Article

CLEANING VALIDATION METHOD FOR RESIDUAL ESTIMATION OF AMC AND RUTIN ON SURFACE OF PHARMACEUTICAL MANUFACTURING EQUIPMENT WITH SWAB SAMPLING TECHNIQUE BY USING HPLC UV METHOD

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ABSTRACT

Introduction: Cleaning validation helps in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals. Cleaning validation involves using an analytical instrument to perform quantitative analysis of residues in manufacturing equipment.

Objective: The objective of this research is to develop and validate a single reverse phase-high performance liquid chromatography (RP-HPLC) method for determination of AMC-Rutin in Vitamin K tablets.

Methods: AMC-Rutin estimated using Phenomenex Synergi 4µ Polar-RP 80A column at 1.2 mL/min flow rate at 354 nm. The mobile phase consists of a mixture of acetonitrile: methanol: pH 4.0 buffer (15:10:75, v/v/v).

Results: Recoveries were found to be in the range of 89.0% to 100.1% with R.S.D below 2.0% at three concentration levels. Residual concentration was found to be linear in the range of 0.0075 to 0.1652 µg/mL for AMC and 0.0829 to 1.2435 µg/mL for Rutin. The LOD and LOQ for AMC and Rutin were found to be 0.00250 & 0.02763, and 0.0075 & 0.0829 µg/mL, respectively.

Conclusion: A simple, precise and accurate method was developed and subsequently validated for simultaneous estimation of AMC-RUT residues on surface of manufacturing equipment by RP-HPLC. The validated method was found to be simple, selective and sensitive for demonstration of cleaning validation of AMC and Rutin residues on the stainless steel surface and manufacturing equipment. This method can be used to determine trace levels of AMC-RUT residues in production equipment area to confirm efficiency of cleaning procedure in pharmaceutical industries to avoid cross contamination.

Keywords: AMC-Rutin, Residual estimation, Swab sampling, Cleaning validation, RP-HPLC/UV.

INTRODUCTION

Cleaning validation helps in the pharmaceutical field to avoid potential clinically significant synergistic interactions between pharmacologically active chemicals. Cleaning validation involves using an analytical instrument to perform quantitative analysis of residues in manufacturing equipment. The test method of analytical are used to generate data to establish an identity, potency, purity, and overall quality of drug substance and drug product. A well-developed test method can control not only quality of product but also speed development process by shortening development time for raw material vendor selection, qualification and formulation screening. Further, a well-developed method can enhance an efficiency for downstream product launch and routine release tests. The analytical methods are stakeholders of product development by providing accurate and reliable data to support formulation, packaging, process development, characterization and process controls, stability and release, pharmacokinetics and bioequivalence, and regulatory filing.

As per US-FDA guidelines, there are two general types of sampling that have been found acceptable: The most desirable direct sampling from surface of equipment by using swab and use of rinse solution. The challenges for cleaning validation are encountered especially when developing an adequate sampling procedure and sensitive analytical methods capable of detecting traces of active pharmaceutical ingredients, which are likely to remain on the surface of the equipment after cleaning. The HPLC with UV detection is used to monitor the efficiency of the cleaning methods due to its high sensitive, selective and automation characteristics [1-2].

Vit-K tablets [Adrenochrome Monosemicarbazone 330mcg-Menadione Sodium Bisulphite 10mg-Rutin 50mg-Dibasic Calcium Phosphate 125mg], under the generic name of Styptovit-K (trade name: Haemostyptic tablets). It is used to treat conditions such as hemmorhoids and varicose veins. It is intended to help slow or stop bleeding.

AMC (Fig.1a) is also called as Carbazochrome; Adrenoxyl; Cromosil. AMC is control oozing from raw surfaces and micro vessel bleeding. AMC is a pigment obtained by the oxidation of adrenaline (epinephrine).

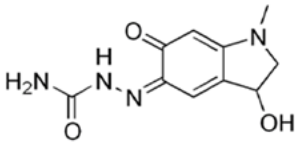

Fig. 1a: The chemical formula of AMC

Rutin (RUT) (Fig. 1b) is a citrus flavonoid glycoside foud in buckwheat, the leaves and peptide of rheum species and asparagus. Rutin would offer some protection against cancer.

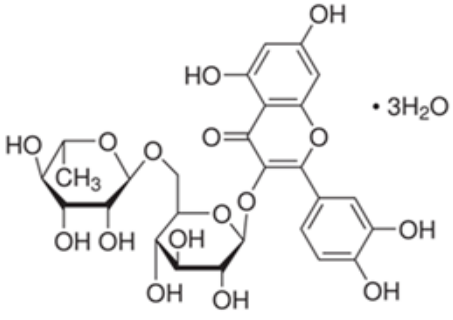

Fig. 1b: The chemical formula of Rutin trihydrate

The aim of this study is to develop and validate novel RP-HPLC method for determination of AMC-RUT residues on equipments at production area and to confirm the efficiency of cleaning procedure. The effectiveness of cleaning process has to be confirmed by cleaning validation, which involves sampling and testing for acceptable residue on pharmaceutical manufacturing equipment at production área. The validation procedure of method was followed guidelines of ICH and USP 36.

A literature survey revealed that no validated cleaning method for AMC-RUT is to be found. Hence, we have been developed a RP-HPLC method for the estimation of trace level residue of AMC-RUT on swab and rinse solution collected from manufacturing surfaces and production area after cleaning of the equipments [3-34]. The developed analytical method was validated with respect to specificity, linearity, precision, accuracy, limit of detection (LOD) and quantification (LOQ). These studies were performed in accordance with established ICH guidelines.

MATERIAL AND METHODS

Chemicals and reagents

AMC and RUT working standards were supplied by Dr. Reddy's Lab. Ltd (Hyderabad, India). Placebo mixtures were prepared in laboratory using US Pharmacopoeia grade excipients. HPLC grade acetonitrile and methanol, analytical grade triethylamine and glacial acetic acid were purchased from Merck (Mumbai, India). Swabs for sampling were purchased from ITW Texwipe. Deionized water purified using a Milli Pore Milli Q System (Waters), was used to prepare the mobile phase solutions.

Chromatographic equipment and conditions

The development and validation work was performed on Agilent 1200 series HPLC system consists of UV/Visible detector, degasser, quaternary pump and auto sampler system. The output signal was monitored and processed the output by using Empower 2 software. The analytical columns used to achieve chromatographic separation were Synergi I.D.,4µm particle size Polar-RP 80A^0, 250 x 4.6 mm, purchased from Phenomenex Inc. and The pH of the solutions was measured by a pH meter (Make: Thermo). The semi microbalance (Make: Sartorious), Bandelin Sonorex sonicator, Heraeus Biofuge Stratos Centrifuge and Stainless steel plates (4 cm × 4 cm) were used during development study. Glassware used were of 'A' grade and were soaked overnight in a mixture of chromic acid and sulphuric acid, rinsed through double distilled water and dried in a hot air oven. The mobile phase for both the AMC and Rutin in Vitamin K tablets residue validation methods was made by first preparing a pH 4.0 buffer (Dissolved 1.0mL of triethylamine in a 1000mL of water and adjusted pH of the solution to 4.0 with diluted glacial acetic acid). The mobile phase consists of a mixture of pH 4.0 buffer, methanol and acetonitrile in ratio of 75: 10: 15, *v/v/v* respectively. The mobile phase flow rate is 1.2 mL/min with 35°C column temperature, 50µL injection volume, and UV detection at 354 nm. The total run time of the chromatogram was 12 min. and UV detection wavelength for Rutin was changed to 354 nm to achieve similar signal strengths for the two actives due to the absorption minimum in the UV spectrum for Rutin at approximately 286 nm (Fig. 2) and the 330 mcg of AMC and 10 mg of Rutin in Vitamin K tablets.

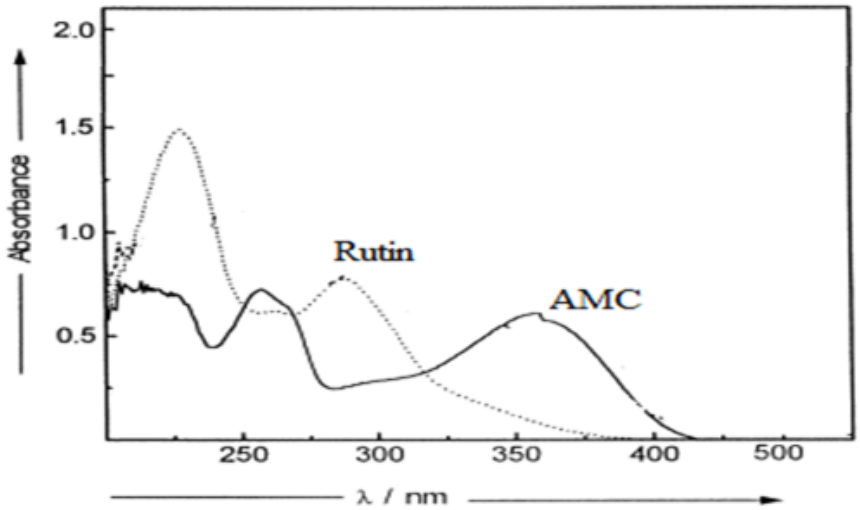

Fig. 2: The UV spectrum of AMC and Rutin

Preparation of standard solution

Standard stock solutions were prepared by weighing about 50.0 mg each of AMC and Rutin standard into 100 mL of volumetric flask, made up to volume with methanol (AMC-Rutin: 500 µg/mL). Transferred 1.0 mL of AMC and 10 mL of Rutin stocks into 50 mL of volumetric flask and made up to volume with methanol. Pipette 1.0 mL of this solution to 100 mL with methanol. The final concentration of solution was 0.10 µg/mL for AMC and 1.0 µg/mL for Rutin. The methanol was used as a diluent.

Preparation of samples

Mixed stock solution of (above) AMC-RUT of 1.0 mL was spiked on surface of cleaned and dried stainless-steel (4 cm × 4 cm) plate and then allowed to evaporate the solvent (approximate time was 10 min). The surface of S.S plate was wiped with the first cotton swab soaked with methanol, passing it in various directions to remove the residues from the stainless steel. The other dry cotton swab was used to wipe the wet surfaces. The swabs were placed in a 25mL screw cap test tube containing 10 mL of diluent. The negative swab control was prepared in the same way as sample, using swabs, which have not been in contact with the test surface. Subsequently, the test tubes were shaken for 10 min on vertex cyclo mixture apparatus followed by sonicated for 10 min in ultrasonic bath. Squeezed the swabs and filtered the solutions through 0.45µm Nylon 66 hydrophilic membrane filter and solutions were analyzed by HPLC. Rinse-sampling was performed with extraction solvent for decron cloth. The volume of the rinsing liquid for sampling point was 10 mL for 625 cm^2 surface.

Collection of swab samples from manufacturing area

Swab samples from different locations within the manufacturing equipment and relevant area were submitted to the laboratory for analysis of AMC and Rutin residues. These samples were prepared and analyzed as described in sample preparation.

RESULTS AND DISCUSSION

Establishment of acceptance criteria for cleaning limits

The acceptable limit for the drug residue would be ensured the absence of cross contamination for subsequent batches manufactured in affected equipment. FDA's guidance for determining residue limits requires a logical, practical, achievable and verifiable determination practice.

The basic principle of cleaning verification is that the patient should not take more than 0.1 % of the standard therapeutic dose (effective dose). The calculation formula is based on the dosage criteria is as follows;

$$MAC = \frac{\mathbf{STD} \times \mathbf{SBS}}{\mathbf{SF} \times \mathbf{LWD}}$$

Where, **'MAC'** is the maximum allowable carryover, **'STD'** is the minimal daily dose (active weight) of previous product, **'SF'** is a safety factor (10000), **'SBS'** is the smallest batch size of the subsequent product and **'LWD'** is the maximum daily dose (product weight) of the following product. An additional criterion is the 10 ppm (part per million) or µg/mL limit. According to this criterion not more than 10 ppm of the previously manufactured product is allowed to appear in the subsequent product. If the value, which is obtained from the calculation based on the dosage criterion, is greater than 10 ppm, then the 10 ppm criterion is applicable. The acceptable limit for residues (**LSA**) is expressed in µg/dm^2.

$$LSA\ (\mu g/dm2) = \frac{\mathbf{MAC\ ppm}\ (\mu g)}{\mathbf{SA}(dm2)}$$

and $$LSA\ (\mu g/16dm2) = \mathbf{LSA}\ (\mu g/dm2) \times \mathbf{S}\ (dm2)$$

LSA is the acceptance limit per unit area, calculated basis of equipment surface area and the most stringent MAC, **SA** is the sampling area of equipment in common between one product and the subsequent product, expressed in dm^2, **S** is the swab area (16dm^2). On the basis of aforementioned discussion the acceptance limit for the residue of AMC and Rutin were 0.1101 and 0.8290 µg/mL. No, interference was found at the retention time of the AMC and Rutin, this indicates that the method is specific for the quantification of analyte.

Optimization of chromatographic conditions

The wavelength for detection was selected by scanned known concentration of AMC and Rutin solutions separately, in UV Visible spectrophotometer. The UV spectra has been shown wavelength maxima at 354 nm for AMC and 286 nm for Rutin. Based on the low dose and low response at 286nm of AMC, the wavelength has been finalised to 354 nm.

The different types of stationary phases [C8, C18 (Hypersil BDS, Inertsil, X-Terra)] were tried for the getting good peak shapes and sharp peaks but compared to all stationary phases 250 x 4.6mm, 4µm, Phenomenex, Synergi 4µ Polar-RP 80A was given very sharp and symmetric peak shapes. The mobile phase selection different types of buffer solutions (like phosphate buffer, triethylamine buffer and acetate buffers) were tried during the method development and finalised triethylamine buffer with pH 4.0 with the ration of acetonitrile and methanol. All other chromatographic parameters such as column temperature of 35°C, injection volume 50 µL, flow rate of 1.2 mL/min and run time of 12 minutes were finalized during development study (Fig. 3).

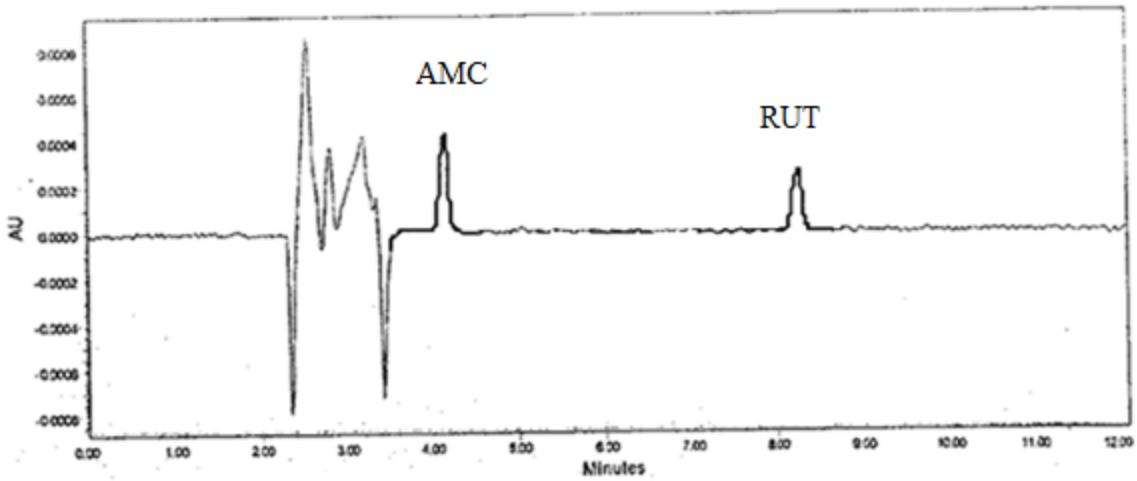

Fig. 3: LC chromatogram of AMC & Rutin at quantification level

Optimization of sample preparation

The cotton swabs were spiked with different quantities of AMC and Rutin and then placed in glass test tubes. After an addition of different solvents and their mixtures (water, methanol and acetonitrile), the tubes were sonicated for different times (5, 10, 20 and 30 minutes) and the solutions were analyzed by HPLC. The optimum conditions were achieved with Methanol as a diluent by sonicating 10 minutes and then followed by 5 minutes shaking. In all the cases, the best results were obtained using two cotton swabs (first wetted with diluent and second dry).

Method validation

The method validation can be defined by (ICH) as "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

The method validation is an integral part of the method development; it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality ,purity , and potency of the drug substances and drug products. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose.

The analytical method validation has been performed as per the USP <1225> and ICH guidelines. The method validation parameters were as follows: precision, accuracy, limit of detection and quantification, linearity, range, solution stability and specificity.

System suitability

All the target analytes (AMC-RUT) can be resolved, the requirements for column performance was well-established, the instrument characteristics such as sensitivity and precision re-established, and system reproducibility was established.

Precision

The precision of the test method was evaluated by repeatability study. The repeatability was determined by analyzing the six replicate samples of extraction-recovery. In the precision study the percentage R.S.D. of injection repeatability for AMC and Rutin was found to be 1.5,1.0, 1.3 for AMC and 1.8, 0.9, 0.7 for Rutin (SS plate, glass plate and decron cloth) respectively, at the concentration levels of 0.1101 and 0.8290 µg/mL which is in the acceptable ranges. The results are listed in Table 1.

Accuracy

The accuracy of the method was determined in triplicate by spiking all surfaces with known amount AMC-RUT. The accuracy study of the test method was carried out in triplicate using the three concentration levels of the test method concentration (0.1101 µg/mL for AMC and 0.8290 µg/mL for Rutin), i.e. at 50 %, 100 % and 150 % level. Worst case placebo solutions that contained excipients for all formulations were utilized in this experiment. Individual recoveries of AMC-RUT ranged from 89.0 to 100.1%. The mean recoveries for each component at each level and the respective R.S.D. are shown in Table 2.

Limits of Detection (LOD) and Quantification (LOQ)

The limit of detection (LOD) represents, concentration of analyte that would yield a signal-to-noise ratio of 3. LOD for AMC-RUT was found to be 0.00250 µg/mL and 0.02763µg/mL. The limit of quantification (LOQ) represents, concentration of analyte that would yield a signal to noise ratio of 10. LOQ for AMC-RUT were found to be 0.0075 µg/mL and 0.0829µg/mL. The precision study also carried out at LOQ level by injecting six individual preparations of sample solution (i.e. for AMC-RUT). The % RSD of AMC-RUT at LOQ level was 3.5 and 4.5. The results were listed in Table 3.

Linearity

The method should operate in linear response range of detector. Although, linearity is usually obtainable, occasionally linearity can not be met due to nature of detector used. In such cases, a multiple point calibration curve should be established and used for quantization.

The linearity of test method was performed by using six different concentration levels of AMC-RUT. i.e. LOQ level to 150% of analyte concentrations. The linear regression analysis of AMC-RUT were constructed by plotting peak area of analytes (y) versus analytes concentration in (x) axis. The calibration curves (n = 6) were linear in range of 0.0075 µg/mL to 0.1652 µg/mL for AMC and 0.08291 µg/mL to 1.2435 µg/mL for Rutin with a correlation coefficient of more than 0.9999 for both molecules. The slope, y-intercept and correlation coefficient were calculated and summarized in Table 4.

Range

The linearity, accuracy and precision results were considered as range parameter.

Stability of analytical solutions

The stability of AMC-RUT in swab matrix and standard solution were established. The spiked samples and standard solution were stored on bench top at room temperature and analyzed against freshly prepared standard solution and the solutions were stable up to 24 hours on the bench top at room temperature.

Specificity

The method has to be able to separate the target analyte from mother components and the method can be quantitated this analyte without ambiguity. The specificity of an analytical method is an ability of test method to determine an analyte response in presence of additional components such as impurities, degradation products and matrix. The solution of analytical placebo (containing all excipients without AMC-RUT) was prepared according to sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients, standard solutions, and commercial pharmaceutical preparations including AMC-RUT were analyzed by developed method. No, interference was observed due to blank, stainless still plate, glass plate, and decron cloth surfaces and placebo solutions.

Table 1: Precision of AMC and Rutin

Prep. No.	% AMC			% Rutin		
(n)	SS Plate	Glass Plate	Decron Cloth	SS Plate	Glass Plate	Decron Cloth
Prep.1	98.2	98.4	98.2	98.4	98.6	97.1
Prep.2	98.4	100.0	100.4	97.7	98.0	97.7
Prep.3	99.4	99.4	101.1	100.0	97.1	95.7
Prep.4	101.7	98.7	100.9	96.4	96.3	96.4
Prep.5	101.4	101.1	98.8	96.5	96.8	96.5
Prep.6	98.7	98.7	101.4	95.3	97.1	97.1
Mean	99.6	99.4	100.1	97.5	97.3	96.7
Std. dev	1.5	1.0	1.3	1.7	0.8	0.7
R.S.D (%)	1.5	1.0	1.3	1.7	0.9	0.7

Table 2: Accuracy of AMC and Rutin

Spike level	Amount added in µg/mL		Amount found		% Recovery	
	AMC	Rutin	AMC	Rutin	AMC	Rutin
50%-1	0.0551	0.4145	0.0551	0.3872	100.1	93.4
50%-2	0.0551	0.4145	0.0512	0.3880	93.0	93.6
50%-3	0.0551	0.4145	0.0509	0.3849	92.5	92.8
100%-1	0.1101	0.8290	0.1007	0.7798	91.4	94.1
100%-2	0.1101	0.8290	0.0982	0.7769	89.2	93.7
100%-3	0.1101	0.8290	0.1002	0.7798	91.0	94.1
150%-1	0.1652	1.2436	0.1487	1.2278	90.0	98.7
150%-2	0.1652	1.2436	0.1515	1.2056	91.7	96.9
150%-3	0.1652	1.2436	0.1537	1.2380	93.1	99.6

Table 3: Precision at Limit of Quantification level

Prep. No.	% Residue at Limit of Quantification level	
	% AMC	% Rutin
Prep.1	101.5	98.3
Prep.2	99.1	91.7
Prep.3	96.9	87.6
Prep.4	95.1	88.6
Prep.5	91.6	89.1
Prep.6	96.4	94.4
Mean	96.8	91.6
Std. dev	3.4	4.1
R.S.D (%)	3.5	4.5

Table 4: Linearity of AMC and Rutin

Conc. in %		Conc. in µg/mL		Area	
AMC	**Rutin**	**AMC**	**Rutin**	**AMC**	**Rutin**
7	10	0.0075	0.0829	1648	2560
50	50	0.0551	0.4145	11009	12858
75	75	0.0826	0.6283	16538	19305
100	100	0.1101	0.8290	22037	25742
125	125	0.1376	1.0363	27538	32605
150	150	0.1652	1.2435	33040	38605
Correlation coefficient				0.9999	0.9999
Regression coefficient				0.999	0.999
y-intercept				99.07327	-99.716
Slope				199311.04	31237
Bias at 100% response level				0.45	-0.39

CONCLUSION

The goal of this work achieved by separating and quantitating both the components in Vit-K (Styptovit K) tablets and its application to residue method by using HPLC. The proposed method for quantitative determination of AMC-RUT residue on production area equipments is efficient and sensitive. The validation studies shown that HPLC-UV method is rapid, linear, precise, accurate, rugged and robust. The recoveries obtained from stainless steel plate, glass plate and decron cloth surfaces were more than 85 % and there is no interference from the cotton swabs. The overall procedure can be used as part of a cleaning validation program in pharmaceutical manufacture of AMC-RUT in Vit-K tablets.

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