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Method development and validation by chromatographic method for determination of erythromycin in pharmaceutical dosage form

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Abstract
A simple, precise, accurate and reproducible UPLC method was developed for quantitative determination of erythromycin in cleaning validation swab samples and pharmaceutical formulations. Water Acquity UPLC System equipped with quaternary gradient pump, auto sampler, column oven, and photodiode array detector was used for method development and method validation. UPLC column BEH C18, (50 mm x 2.1 mm, 1.7 µm) thermostated at 50°C was used for separation. Mobile Phase-A comprising of a mixture of Buffer solution (2.84gm of Disodium hydrogen phosphate in 1000mL water, pH adjusted to 8.50 ± 0.05 using diluted Orthophosphoric acid solution and thereafter solution filtered through 0.22 µm PVDF filter) and Methanol in the ratio of 35:65 (% v/v) respectively and Mobile Phase-B comprising of Methanol was used. The flow rate and injection volume was 0.5 mL·min−1 and 7.0 µL respectively. The analysis was carried out under the gradient conditions as time (min)/A (v/v): B(v/v); T_0/100:00, $T_{1.9}$/100:00, $T_{2.2}$/50:50, $T_{3.7}$/50:50, $T_{4.0}$/100:00, and $T_{6.0}$/100:00. The developed method was validated with respect to specificity, linearity, limit of detection and quantification, accuracy and precision. Results of all validation parameters were within the limits as per ICH guidelines.

Keywords: cleaning validation, erythromycin, residues, swab analysis, UPLC

Introduction
Erythromycin is a bacteriostatic antibiotic macrolide. Erythromycin is used in the treatment of infections caused by susceptible strains of microorganisms in the diseases like Respiratory tract infections, Acute pelvic inflammatory, Primary syphilis, Intestinal amebiasis. Erythromycin interferes with aminoacyl translocation, preventing the transfer of the tRNA bound at the A site of the rRNA complex to the P site of the rRNA complex. Without this translocation, the A site remains occupied, thus the addition of an incoming tRNA and its attached amino acid to the nascent polypeptide chain is inhibited. This interferes with the production of functionally useful proteins, which is the basis of this antimicrobial action.
The primary structural features of erythromycin A are a 14-member lactone ring, an amino sugar at C5, and a sugar attached to the lactone at C3. Its empirical formula is $C_{37}H_{67}NO_1$, which corresponds to a molecular weight of 733.94 g/mol. Its solid oral dosage form is available as a tablets which contains erythromycin equivalent to 250 mg and 500 mg.

Material and Method
Instrumentation: Ultra Performance Liquid Chromatography. (Waters Acquity), Waters BEH C_{18} column (50 mm x 2.1 mm, 1.7 µm), column detection of drug carried by PDA detector at data processing was carried out by Empower 3 software, weighing balance, pH meter, Sonicator.

Chemicals and reagents
Acetonitrile (HPLC grade), Ortho Phosphoric Acid, Di-sodium hydrogen phosphate and Methanol (HPLC Grade), Water (prepared by using Millipore Milli-Q Plus water purification system) and Swabs (Texwipe). Liquinox cleaning agent was used for cleaning of equipment surfaces after batch manufacturing.

Solution Preparation
Preparation of Standard solution
Preparation of standard solution-1 (2000 ppm)
Weigh accurately about 100 mg of Erythromycin standard and transfer into a 50 mL

volumetric flask, add about 35 mL of diluent and sonicate to dissolve it. Dilute to volume with diluent and mix well.

Preparation of standard solution-2 (400 ppm)
Pipette 10.0 mL of standard solution-1 and transfer into a 50 mL volumetric flask. Dilute to volume with diluent and mix well.

Preparation of standard solution (8 ppm)
Pipette 2.0 mL of standard solution-2 and transfer into a 100 mL volumetric flask. Dilute to volume with diluent and mix well.

Swab pre-treatment
Take swabs and dip them into an appropriate container containing about 50 mL of Acetonitrile. Shake for about 2 minutes, drain excess of Acetonitrile by pressing swabs against the wall of the container.

Blank swab preparation
Open the cap of the glass vial containing blank swab. Let the glass vial remain opened for 30 minutes. Add 10.0 mL of diluent into it and shake it for 2 minutes. Filter the blank swab solution through 0.2 µm Nylon filter. Discard the first 3 mL of filtrate.

Preparation of sample solution
Open the cap of the glass vial containing swab. Let the glass vial remain opened for 30 minutes. Add 10.0 mL of diluent into it and shake it for 2 minutes. Filter the sample solution through 0.2 µm Nylon filter. Discard the first 3 mL of filtrate.

Chromatographic condition
The method was developed using an Acquity UPLC BEH C18, (50 mm x 2.1 mm, 1.7 µm) with an gradient mobile phase containing a mixture of 0.02 M dihydrogen phosphate, pH adjusted to 8.50 ± 0.05 with diluted ortho-phosphoric acid and methanol (35:65 v/v). The mobile phase was filtered through 0.22 µm PVDF filter. The flow rate of the mobile phase was 0.5 mL/min. The column temperature was maintained at 50 °C and the eluted compounds were monitored at the wavelength of 210 nm. The sample injection volume was 7.0 µl.

Results and Discussion
1) System Suitability
The system suitability test was used to ensure that the UPLC system and procedures are adequate for the analysis performed. Parameters of this test were column efficiency (number of theoretical plates), asymmetry of chromatographic peak, and reproducibility as RSD of peak area of six injections of standard solution. During performing the system suitability test, in all cases relative standard deviation (RSD) of the peak areas was ≤10.0%, the number of theoretical plates per column was 2000, and the USP tailing factor was ≤2.0.

2) Specificity
Specificity is the ability of the analytical procedure to assess unequivocally the analyte in the presence of components which may be expected to be present. Analyze each of single injection of diluent, blank swab solution, swab extracted with respective diluent, swab extracted after swiping the stainless steel coupon surface and swab extracted after treating with detergent solution. It was observed that no interference was observed at retention time of Erythromycin peak in the chromatograms obtained from diluent, blank swab solution, swab extracted with diluent and stainless steel coupon surface swab sample solution and swab treated with detergent solution. Results are shown in fig No. 1,2,3,4 & 5 and Table No 1,2,3,4 & 5

3) Linearity
Linearity of the method was studied by analysing standard solutions at eight different concentration levels ranging from 0.5 to 2.0 µg/mL. The calibration curve was constructed by plotting the response area against the corresponding concentration injected, using the least square method. The correlation coefficient for erythromycin observed is 0.9995(Limit: NLT 0.9900). The high value of the correlation coefficient indicated good linearity. The linearity data meets the acceptance criteria indicates that the method is linear within the concentration range from LOQ to 200% of critical limit level concentration.

4) Limit of Detection and Quantification
The LOD and LOQ were determined, by injecting a series of dilute solutions of analyte with known concentrations. The precision study was also carried out at the LOD and LOQ levels by injecting six replicates of erythromycin preparation. Calculated the %RSD of the peak area and found <3.1% at the LOQ concentration and <1.5% at the LOD concentration. Results are shown in Table No 6.

5) System Precision
The precision of the chromatographic method, reported as RSD, was estimated by measuring time-dependent intermediate precision on six replicate injections of standard solution. The % RSD value was 0.4 (Limit NMT 10.0%) and illustrated the good precision of the chromatographic system. Results are shown in Figure No 7 and Table No 8.

6) Method Precision
The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same sample under the prescribed condition.
The precision of the chromatographic method, reported as RSD, was estimated by spiking swab sample solution at 20%, 100% & 150% of critical limit level concentration (CLLC). The % RSD values was 1.7, 0.3 and 0.2 (Limit NMT 10.0%) at 20%, 100% & 150% respectively. The observed %RSD values illustrated the good precision of the analytical method. Results are shown in Table No 9.

7) Accuracy
The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the procedure was assessed by comparing the analyte amount determined *versus* the known amount spiked at three different surfaces (SS equipment surface, Silicon rubber surface and Acrylic sheet surface) at concentration levels (20%, 100% and 200%) with three replicates for each concentration. Results are shown in Figure No 8, 9 & 10 and Table No 10, 11 & 12.

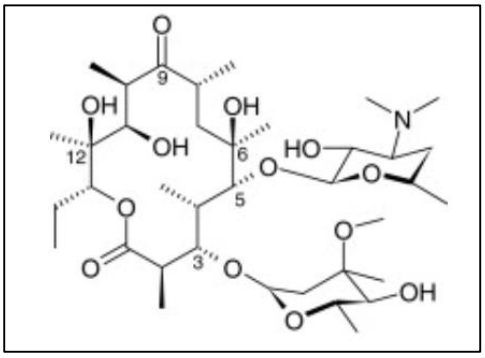
8) Stability of analyte solution

The stability of the erythromycin in the swab matrix and standard solution was tested. The spiked sample and standard solution were stored at 2 °C to 8 °C temperature for 75 and 76 hours respectively. All the samples were injected into the UPLC system after different time intervals against freshly prepared standard solution. Sample and standard solution were stable up to 75 hours and 76 hours respectively. No changes in the chromatography of the stored samples were found, and no additional peak was registered when compared with the chromatograms of the freshly prepared samples. Results are shown in Table No 13.

9) Application of developed UPLC method on Drug Product

The analytical method developed and validated for the cleaning sample assessment is also explore for the assay test of drug product.

Chromatographic conditions were Acquity UPLC BEH C18, (50 mm x 2.1 mm, 1.7 µm) with an gradient mobile phase containing a mixture of 0.02 M dihydrogen phosphate, pH adjusted to 8.50 ± 0.05 with diluted ortho-phosphoric acid and methanol (35:65 v/v). The mobile phase was filtered through 0.22 µm PVDF filter. The flow rate of the mobile phase was 0.5 mL/min. The column temperature was maintained at 50 °C and the eluted compounds were monitored at the wavelength of 210 nm. The sample injection volume was 7.0 µl. Results are shown in Table No 14.



Erythromycin

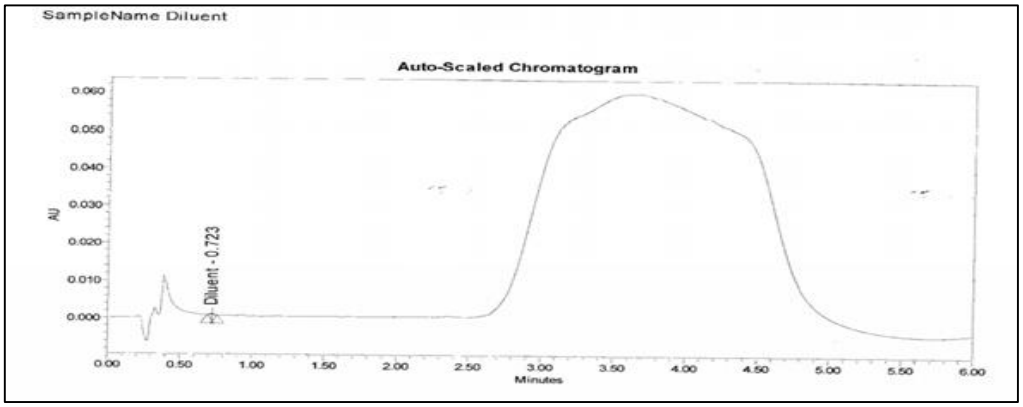


Fig 1: Typical chromatogram for Diluent

Table 1: Results of Specificity (Diluent)

	Name	RT
1	Diluent	0.72

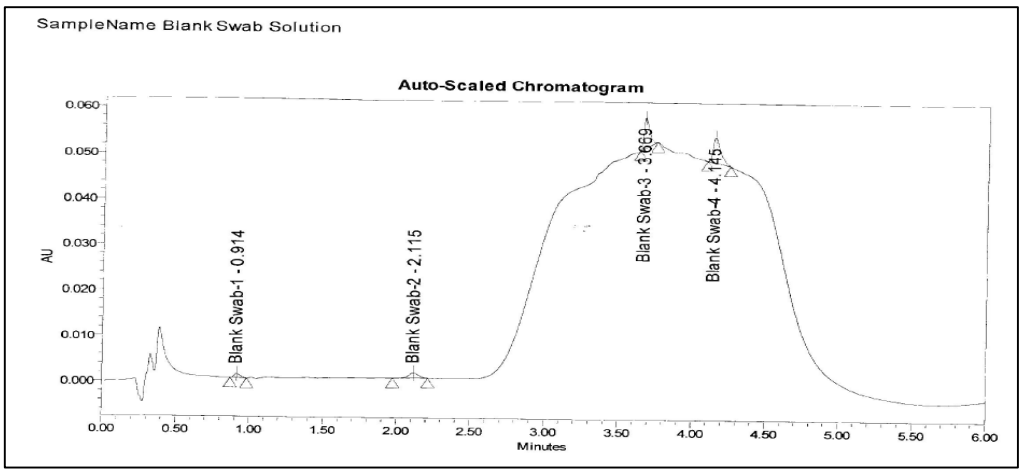


Fig 2: Typical chromatogram for Blank swab solution

Table 2: Results of Specificity (Blank Swab Solution)

	Name	RT
1	Blank Swab-1	0.91
2	Blank Swab-2	2.12
3	Blank Swab-3	3.67
4	Blank Swab-4	4.14

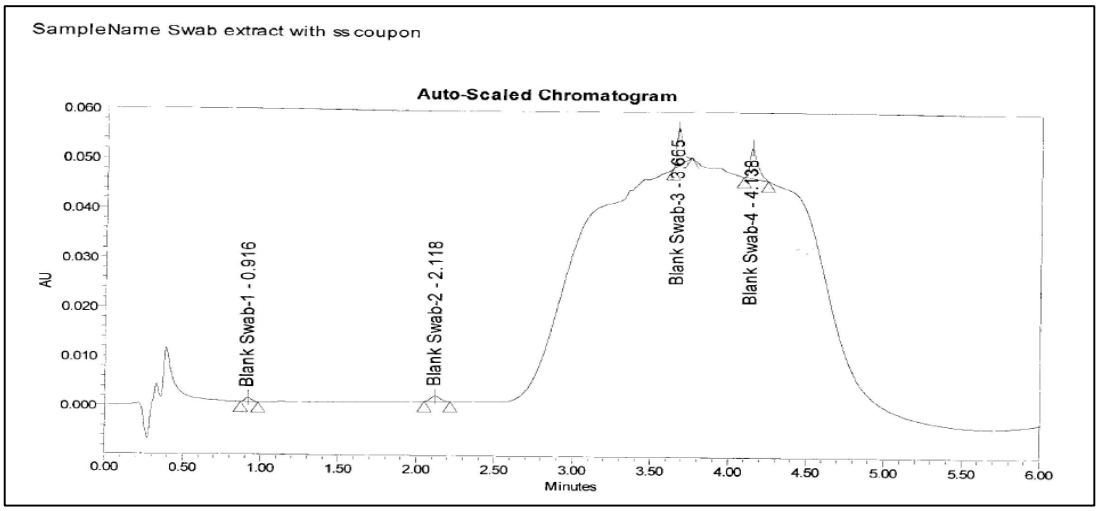


Fig 3: Typical chromatogram for Swab extract after swiping SS coupon surface

Table 3: Results of Specificity (SS coupon surface)

	Name	RT
1	Blank Swab-1	0.92
2	Blank Swab-2	2.12
3	Blank Swab-3	3.67
4	Blank Swab-4	4.14

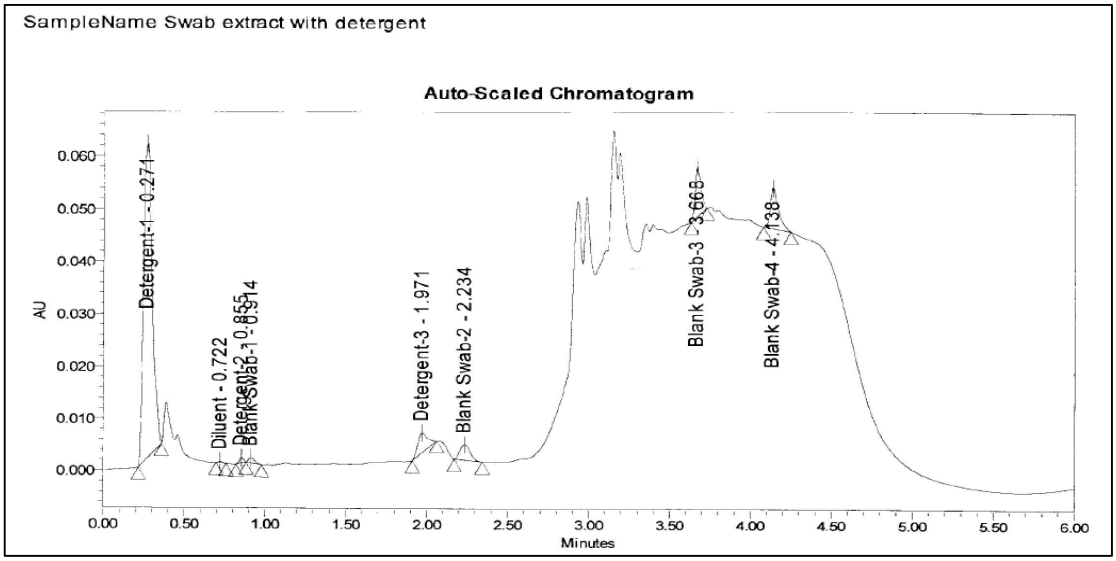


Fig 4: Typical chromatogram for Swab extract after treating with detergent liquinox

Table 4: Results of Specificity (Swab extract with detergent)

	Name	RT
1	Detergent-1	0.27
2	Diluent	0.72
3	Detergent-2	0.86
4	Blank Swab-1	0.91
5	Detergent-3	1.97
6	Blank Swab-2	2.23
7	Blank Swab-3	3.67
8	Blank Swab-4	4.14

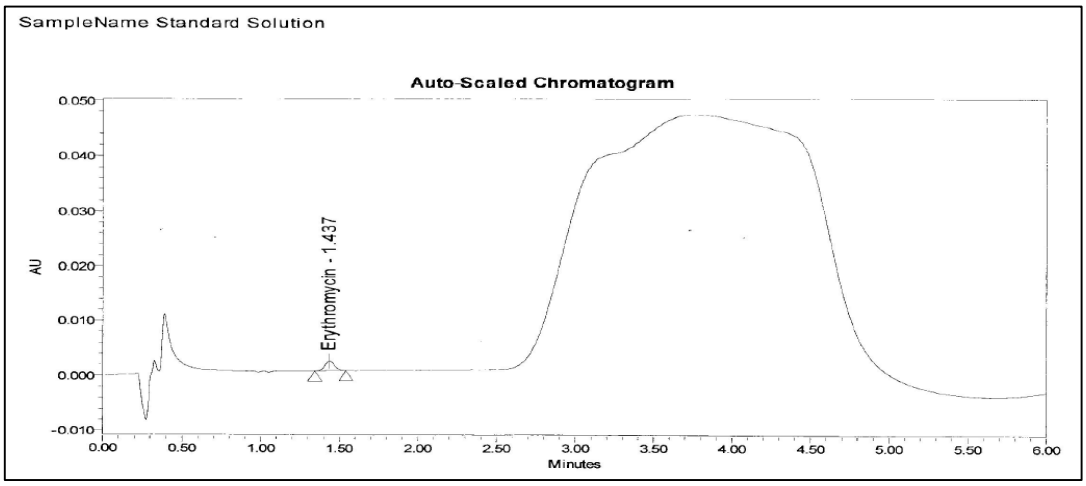


Fig 5: Typical chromatogram for erythromycin standard solution

Table 5: Results of Specificity (Standard Solution)

	Name	RT
1	Erythromycin	1.44

Table 6: LOD & LOQ data for Erythromycin

Component Name	LOD (µg/mL)	LOD (µg/inch^2)	% RSD
Erythromycin	0.153	0.170	1.530

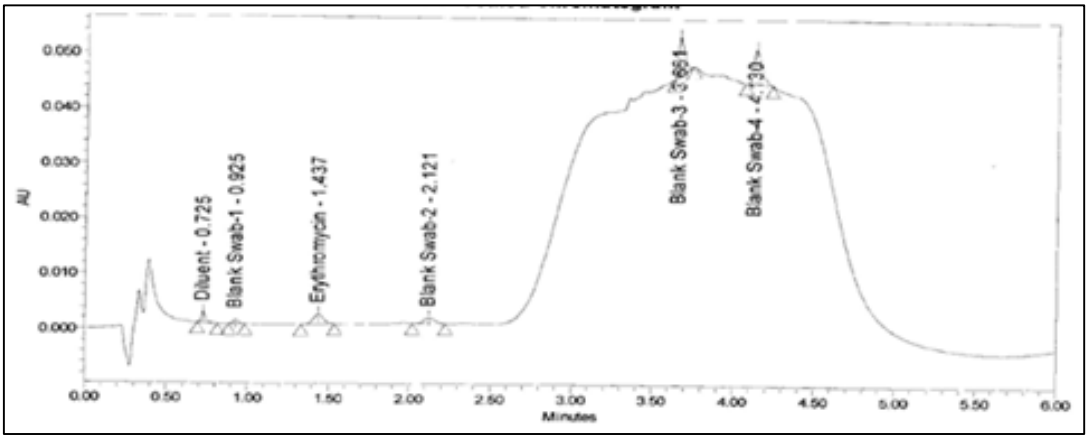


Fig 7: Typical chromatogram for System Precision

Table 8: Results of System Precision

	Name	RT
1	Diluent	3500
2	Blank Swab-1	1807
3	Erythromycin	7706
4	Blank Swab-2	4991
5	Blank Swab-3	18187
6	Blank Swab-4	22108

Table 9: Results of Method Precision

Spiking Level	% RSD
20 %	1.7
100 %	0.3
150 %	0.2

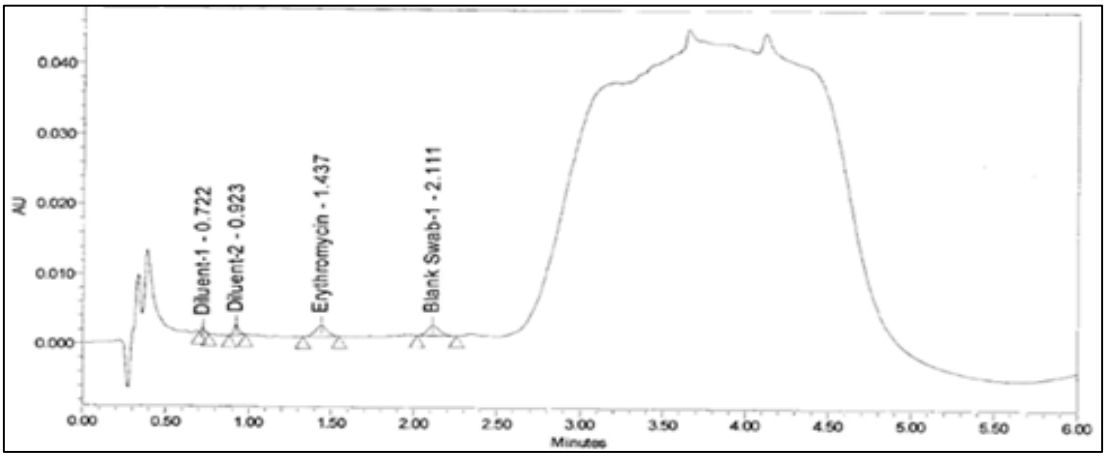


Fig 8: Typical chromatogram for Swiping from SS equipment surface

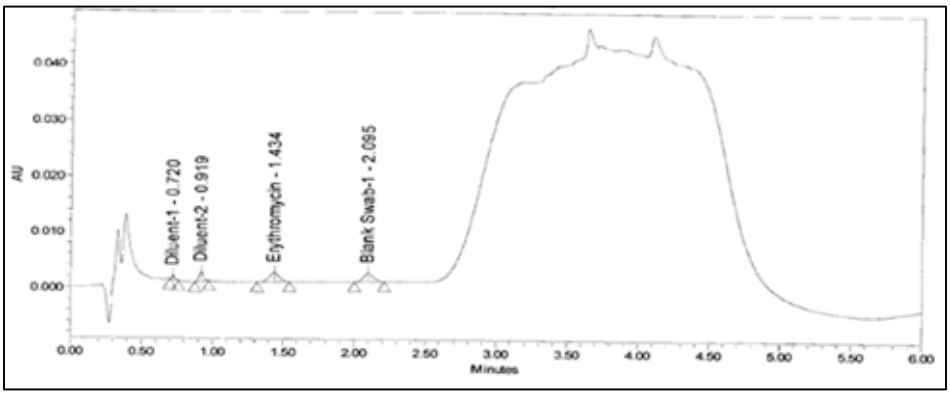


Fig 9: Typical chromatogram for Swiping from Silicon Rubber surface

Table 10: Accuracy data for Swiping from SS equipment surface

	Name	RT
1	Diluent-1	0.72
2	Diluent-2	0.92
3	Erythromycin	1.44
4	Blank Swab-1	2.11

Table 11: Accuracy data for Swiping from Silicon Rubber surface

	Name	RT
1	Diluent-1	0.72
2	Diluent-2	0.92
3	Erythromycin	1.43
4	Blank Swab-1	2.09

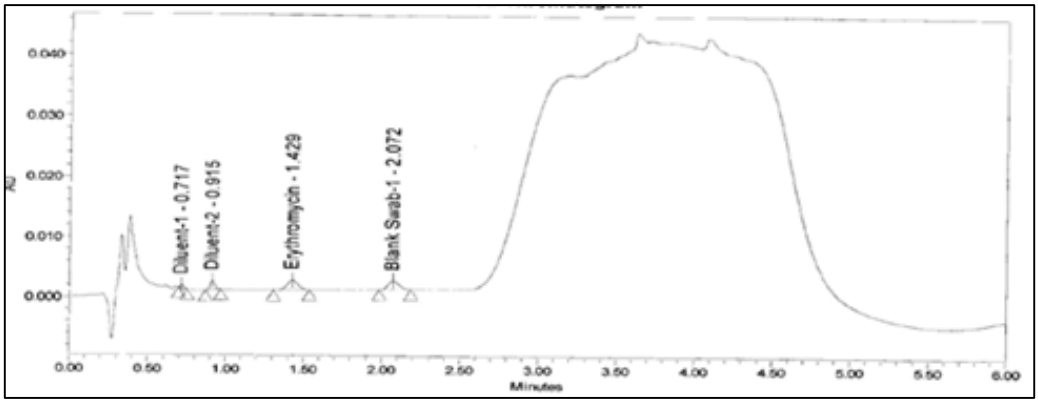


Fig 10: Typical chromatogram for Swiping from Acrylic surface

Table 12: Accuracy data for Swiping from Acrylic surface

	Name	RT
1	Diluent-1	0.72
2	Diluent-1	0.91
3	Erythromycin	1.43
4	Blank Swab-1	2.07

Table 13: Results for Stability of analyte solution

Standard Solution		Sample Solution	
Time Interval	**% Difference**	**Time Interval**	**% Difference**
Initial	N/A	Initial	N/A
28 hours	1.3	27 hours	1.9
52 hours	2.9	51 hours	10.7
64 hours	5.7	63 hours	2.6
76 hours	2.6	75 hours	6.4

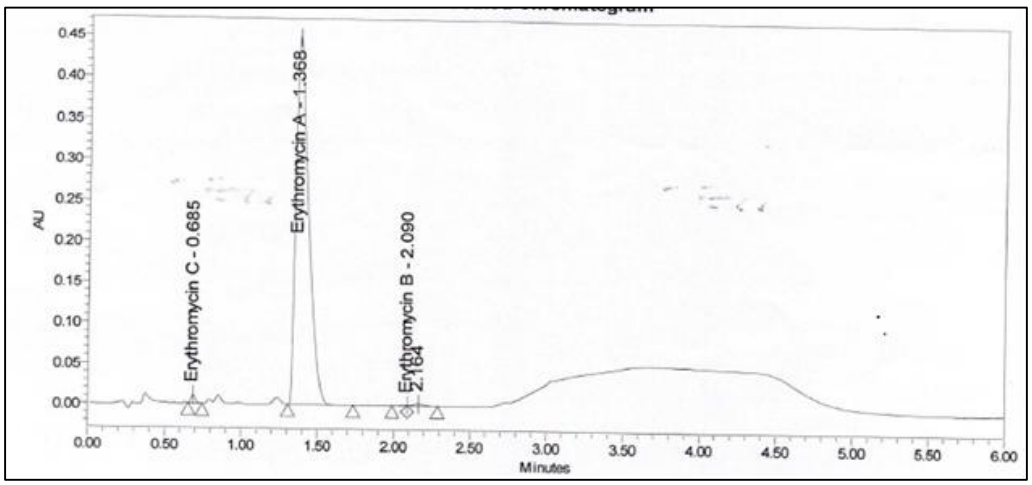


Fig 11: Typical chromatogram for Standard Solution

Table 14: Results for Standard Solution.

	Name	RT
1	Erythromycin C	0.69
2	Erythromycin A	1.37
3	Erythromycin B	2.09

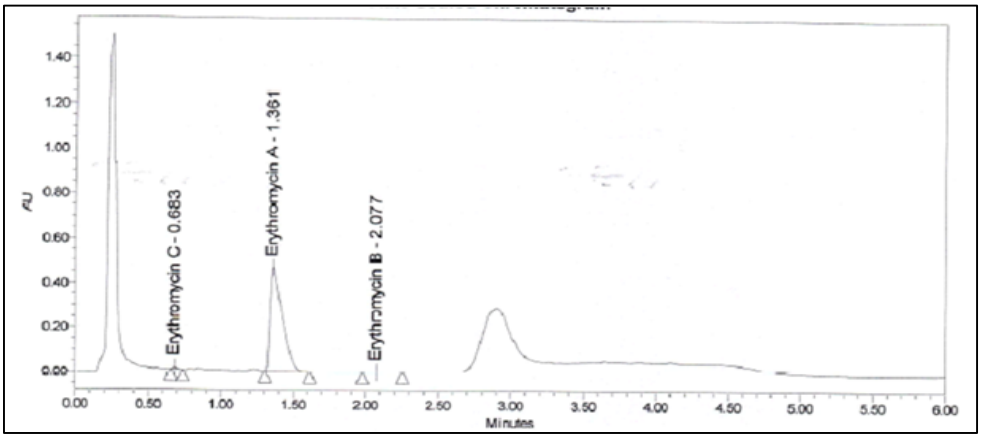


Fig 12: Typical Chromatogram of Sample Solution

Table 15: Results for Sample Solution

	Name	RT
1	Erythromycin C	0.68
2	Erythromycin A	1.36
3	Erythromycin B	2.08

Table 16: Results for Erythromycin Delayed Release Tablets 250mg

Precision set	% Assay	Acceptance Criteria
Set 1	104	The %RSD of assay results obtained from six sample preparation should be not more than 2.0.
Set 2	104	
Set 3	104	
Set 4	103	
Set 5	103	
Set 6	103	
Mean	103.5	
%RSD	0.5	

Conclusion

UPLC method of analysis of swab samples for traces of erythromycin is simple, precise and accurate method. 4 fold reduction in analysis time is observed by using UPLC (6 minutes run time) method as compared to HPLC (32 minutes run time) method. The analytical procedure is valid and suitable for determination of residue of Erythromycin in swab samples from equipment surfaces. The results obtained for the drug product assay found very consistent and precise, with a very short runtime of 6 minutes which indicate that the method can also be used for the assay test of Erythromycin Delayed Release Tablets 250 mg.

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