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# Stability-Indicating HPLC Method for the Simultaneous Estimation of Erythromycin and Sulfafurazole in Bulk and Oral Suspension.

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**ABSTRACT**

Forced degradation study was effectively applied for the development of a stability-indicating HPLC method for simultaneous determination of erythromycin and sulfafurazole in oral suspension in the presence of its degradation products. The method showed enough separation of erythromycin and sulfafurazole from their degradation products. Separation was achieved on an Inertsil BDS C18, 5 µm, 250 x 4.6 mm id column at 27±1 $^{o}$C by using the mobile phase methanol- 0.01 M dipotassium hydrogen phosphate (80:20, *v/v*) at a flow rate of 1 mL/min and UV detection at 254 nm. The method was validated in terms of system suitability, linearity, precision, accuracy, specificity, robustness, ruggedness and specificity. The linearity of the proposed method was in the range of 10-70 µg/mL ($R^2$ = 0.9990) for erythromycin and 30-210 µg/mL ($R^2$ = 0.9995) for sulfafurazole. Stress testing of erythromycin and sulfafurazole was carried out according to the International Conference on Harmonization guideline Q1A (R2). The drugs were subjected to acid, base, oxidation, thermal and photo degradation conditions. There were no interfering peaks from excipients or degradation products due to variable degradation conditions. Degradation products produced as a result of forced degradation studies did not interfere with the detection of erythromycin and sulfafurazole and the method can thus be considered stability indicating.

**Keywords:** erythromycin, sulfafurazole, oral suspension, stability indicating, HPLC, forced degradation

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## INTRODUCTION

Erythromycin belongs to macrolide antibiotics group of drugs. Chemically, it is known as (3R*, 4S*, 5S*, 6R*, 7R*, 9R*, 11R*, 12R*, 13S*, 14R*)-4-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo - hexopyranosyl)oxy] - 14 - ethyl - 7,12,13 - trihydroxy - 3,5,7,9,11,13 - hexamethyl - 6 - [[3,4,6 - trideoxy - 3 - (dimethylamino) - β - D - xylo -hexopyranosyl] oxy] oxacyclotetradecane- 2, 10-dione. It is prescribed for the treatment of acute bacterial infections like respiratory infections, urine infections, skin infections and mouth infections caused by erythromycin sensitive bacteria such as S*treptococcus pyogenes, Streptococcus pneumoniae, Mycoplasma pneumoniae, Staphylococcus aureus, Neisseria gonorrhoeae* etc., [1,2] Erythromycin kills the infection causing bacteria by decreasing the production of main proteins required for the survival of the bacteria. The bacterial protein synthesis was inhibited by binding of erythromycin to the bacterial 50S ribosomal subunit [3,4].

Erythromycin is official in United States Pharmacopoeia (USP) [5] and British Pharmacopoeia (BP) [6]. The USP and BP describe a HPLC method and microbial assay for the analysis of erythromycin in raw material and finished products, respectively. Several researchers have been reported the determination of the erythromycin in biological samples and/or pharmaceutical preparations. These include UV spectrophotomtry [7,8], visible spectrophotometry [9-13], spectrofluorometry [14,15], HPLC with UV detection [16,17], HPLC with chemiluminescence detection [18], HPLC with diode array detection [19], capillary electrophoresis with electrochemiluminescence detection [20], HPLC–ESI-MS [21], microbiological methods [22] and potentiometry [23].

Sulfafurazole, also known as sulfisoxazole, belongs to sulfonamide category of antibiotics. Chemically, it is known as 4-amino-N-(3,4-dimethyl-1,2-oxazol-5-yl) benzenesulfonamide. Sulfafurazole is a broad spectrum antibiotic and inhibits the growth and replication of a wide range of Gram-negative and Gram-positive bacteria. Sulfafurazole is used in treatment of different types of infections caused by bacteria like bladder infections, ear infections, meningitis etc [24,25]. Sulfafurazole acts by interfering with the bacterial synthesis of folic acid, purines and pyrimidines. This leads to cell growth arrest and cell death [26].

Sulfafurzole is official in US pharmacopeia which describes a titration method for the estimation of sulfafurazole using 0.1 N lithium methoxide in toluene as titrant in dimethylformamide medium [27]. Many researchers have developed methods for sulfafurazole determination in biological samples as well as in pharmaceutical preparations and these methods have been reviewed. The methods used for sulfafurazole quantification include spectrophotometric [28], UPLC with photodiode array detector [29], HPLC with UV detector [30,31], HPTLC [32] and chlorocoulometric [33] procedures.

In childrens, the combination of erythromycin and sulfafurazole is used in the treatment of middle ear infections [34,35]. However, to the best of our knowledge, there was no report on the stability-indicating assay for the simultaneous determination of erythromycin and sulfafurazole. Therefore, the present study describes the development and validation of a stability-indicating HPLC method for quantitative determination of erythromycin and sulfafurazole simultaneously in the presence of their forced degradation products.

## EXPERIMENTAL

### Materials

Erythromycin and sulfafurazole reference standards were obtained as gift samples from Aurobindo Laboratories Pvt. Ltd (Hyderabad, India). Generic version oral suspension labeled to contain 200 mg of erythromycin and 600 mg of sulfisoxazole per 5 mL was obtained from the local market. HPLC grade methanol was purchased from Merck India Limited (Mumbai, India) Analytical grade dipotassium hydrogen phosphate**,** hydrochloric acid, sodium hydroxide and hydrogen peroxide were from Sdfine-Chem limited (Mumbai, India). Milli-Q-water was used throughout the process.

### Instrumentation and Chromatographic conditions

HPLC apparatus consisted of Shimadzu HPLC class LC series equipped with two LC-10 AT, VP pumps and variable wavelength programmable UV detector. The HPLC data were recorded and processed using LC

solution soft ware. The chromatographic separations were performed on an Inertsil C18, BDS column (250 x 4.6 mm, i.d., particle size 5 µm). The column temperature was maintained at 27±1°C. Separations were carried out in isocratic mode using a mobile phase consisted of methanol and 0.01 M dipotassium hydrogen phosphate (80:20, *v/v*). The mobile phase was filtered by a millipore membrane filter paper, degassed by ultrasonic bath 15 min prior to its use. The flow rate of the mobile phase was 1 mL/min, and the sample injection volume was 20 µl. The UV detector was set at 254 nm.

**Preparation of standard and sample solutions**

An Accurately weighed quantity of erythromycin (40 mg) and sulfafurazole (120 mg) reference standards was transferred to a 100 mL volumetric flask and dissolved in 100 mL of mobile phase. This solution is used as stock standard solution. The working standard solutions of erythromycin and sulfafurazole were prepared by appropriate dilution of the stock standard solution in mobile phase at the concentration of 40 µg/mL and 120 µg/mL, respectively.

Five mL of oral suspension equivalent to 200 mg of erythromycin and 600 mg of sulfafurazole was transferred to a 100 mL volumetric flask. A 25 mL of mobile phase was added, the contents of the flask were sonicated for 10 minutes. The volume was diluted to 100 mL with the same solvent and filtered through a millipore membrane filter. This solution was diluted with the mobile phase to give a concentration of 40µg/mL and 120µg/mL of erythromycin and sulfafurazole, respectively.

**General assay procedure:**

Working standard solutions equivalent to 10 to 70µg/mL erythromycin and 30 to 210 µg/mL sulfafurazole were prepared by appropriate dilution of the stock standard solution with the mobile phase. Prior to injection of the drug, the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. Twenty µl of each solution was injected onto the column in triplicate and the peaks were determined at 254 nm. The calibration curves were constructed for erythromycin and sulfafurazole by plotting the peak area *vs* concentration. The concentration of the analytes was calculated either from the corresponding calibration curve or from the corresponding regression equation.

**Assay of oral suspension**

Twenty µl of the sample solution (40µg/mL of erythromycin and 120µg/mL of sulfafurazole) was injected into the HPLC system. The chromatograms and peak areas of the analytes were determined at 254 nm. The nominal concentration of analytes in the test sample was calculated by either from the corresponding calibration curve or from the corresponding regression equation.

**Stress degradation studies:**

The International Conference on Harmonization guideline is followed to elucidate the inherent stability characteristics of erythromycin and sulfafurazole [36]. For this purpose, the stress degradation studies were performed on the erythromycin and sulfafurazole using the proposed method.

**Acid degradation:**

Five mL of oral suspension equivalent to 200 mg of erythromycin and 600 mg of sulfafurazole and 5 mL of 0.1 N HCl were added in 100 mL volumetric flask. The flask was kept at 80 °C reflux condition for 2 hrs and neutralized with sufficient volume of 0.1 N NaOH. Cool the solution to room temperature and dilute to the volume with mobile phase.

**Alkali hydrolysis**

Aliquot of 5 mL of oral suspension (200 mg - erythromycin and 600 mg - sulfafurazole) was transferred to a 100 mL volumetric flask. The suspension was mixed with 5 mL of 0.1 N sodium hydroxide. The prepared solution was subjected to reflux at 80 °C for 2 hrs. The sample was cooled to room temperature and

neutralized with an amount of acid equivalent to that of the previously added. The resulting solution was diluted to the volume with mobile phase.

**Oxidative degradation**

Five mL of oral suspension equivalent to 200 mg of erythromycin and 600 mg of sulfafurazole and 5 mL of 20% $H_2O_2$ were added in 100 mL volumetric flask. The flask was kept at 80 °C reflux condition for 2 hrs. Cool the solution to room temperature and dilute to the volume with mobile phase.

**Thermal degradation**

Five 5 mL of oral suspension (200 mg - erythromycin and 600 mg - sulfafurazole) was transferred to a 100 mL volumetric flask. The flask was kept at 105°C in hot air oven for 2 hrs. Cool the suspension to room temperature and the volume was made up to the mark with mobile phase.

**Photo degradation**

Five 5 mL of oral suspension (200 mg - erythromycin and 600 mg - sulfafurazole) was transferred into a petri dish and exposed to sun light for 24 hrs. Then, the suspension was transferred to a 100 mL volumetric flask and was made up to the mark with mobile phase.

After degradation, all stress degraded samples were appropriately diluted with mobile phase to give a final concentration of 40µg/mL and 120µg/mL of erythromycin and sulfafurazole, respectively. Filter the solution with millipore membrane filter paper and 20 µl of the sample was injected into the HPLC system.

## RESULTS AND DISCUSSION

**Optimization of chromatographic conditions**

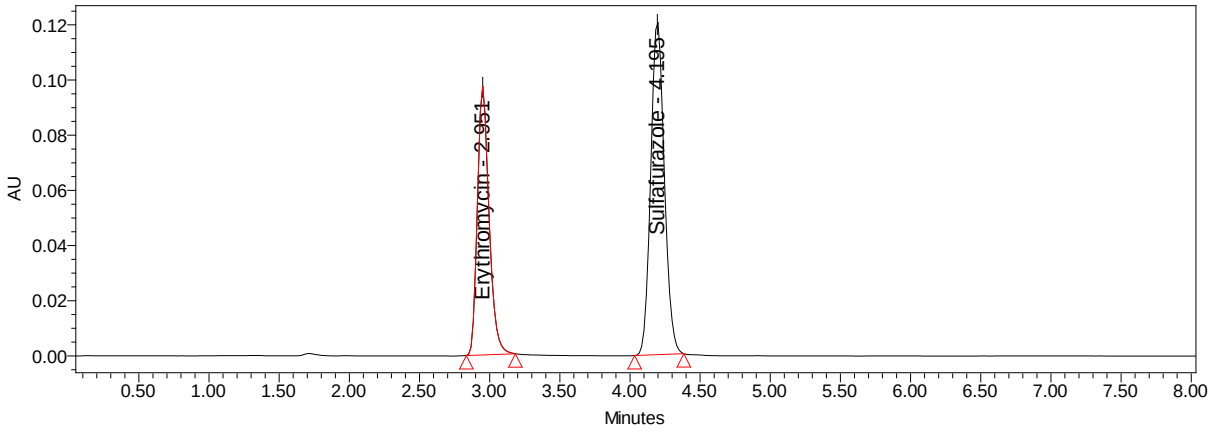


**Figure 1: Chromatogram of erythromycin and sulfafurazole under optimized conditions**

The main aim of the present investigation is to develop a rapid, cost-effective, precise and sensitive HPLC with UV detection method for the simultaneous estimation of erythromycin and sulfafurazole. The primary goal in developing this stability-indicating HPLC method is to achieve the optimum resolution between the erythromycin, sulfafurazole and its degradation products. To develop a stability-indicating method, two HPLC analytical columns, Zorbax Eclipse plus C8 column (250 x 4.6 mm; 5 µm particle size) and Inertsil BDS C18 column (250 mm x 4.6 mm; 5 µm particle size) were tested during method development. Different composition of mobile phases containing 100% methanol, methanol–acetonitrile (*v/v*) and methanol-0.01 M dipotassium hydrogen phosphate (*v/v*) in different ratios were tried so as to obtain appropriate composition of mobile phase. This challenge was met by using methanol - 0.01 M dipotassium hydrogen phosphate (80:20, *v/v*) where optimum resolution and good symmetric peaks were observed by using Inertsil BDS C18 column

(250 mm x 4.6 mm; 5 µm particle size) analytical column in isocratic mode at a flow rate of 1 mL/min and column at ambient temperature. Under the above optimized conditions, the retention time reported was 2.951 min for erythromycin and 4.195 min for sulfafurazole (Figure 1).

**Method validation:**

The method was validated as per ICH guidelines in terms of system suitability, linearity, LOD, LOQ, accuracy, precision, specificity, ruggedness and robustness.

System suitability tests were carried out on freshly prepared standard solution of erythromycin (40µg/mL) and sulfafurazole (120µg/mL) to check the various parameters such as retention time, peak area, USP plate count, resolution and USP tailing (Table 1). The results are found to be within the acceptance limit. The resolution was more than 3, USP plate count were more than 2000, USP tailing was less than 2.0, % RSD of retention time and peak area was less than 2.0 for the erythromycin and sulfafurazole peaks.

**Table 1: System suitability parameters**

| Parameters | Value | | Recommended limits |
|---|---|---|---|
| | Erythromycin | sulfafurazole | |
| **Retention time** | 2.994 (%RSD – 0.386) | 4.191 (%RSD – 0.640) | RSD ≤2 |
| **Peak area** | 677206.8 (%RSD – 1.071) | 3680071 (%RSD – 0.167) | RSD ≤2 |
| **USP plate count** | 6108.8 | 8890.6 | > 2000 |
| **USP tailing factor** | 1.154 | 0.889 | ≤ 2 |
| **Resolution** | - | 9.654 | > 3 |

The linearity of the proposed method was demonstrated by preparing and analyzing the working standard solution at seven different concentrations of erythromycin (10, 20, 30, 40, 50, 60, 70µg/mL) and sulfafurazole (30, 60, 90, 120, 150, 180, 210µg/mL). The calibration curve was constructed for erythromycin and sulfafurazole by plotting the peak area versus concentration. From the calibration curve coefficient of correlation, intercept and slope were calculated. The results were shown in Table 2. The results demonstrate an excellent correlation between the peak area and concentration of analytes in the concentration range of 10-70µg/mL (erythromycin) and 30-210µg/mL (sulfafurazole).

**Table 2: Linearity and sensitivity data of the proposed method**

| Parameter | Erythromycin | Sulfafurazole |
|---|---|---|
| Linearity (µg/mL) | 10-70 | 30-210 |
| Regression equation (y* = m x** + c) | y = 16472x + 7772 | y = 30318x - 37039 |
| Slope (m) | 16472 | 30318 |
| Intercept (c) | 7772 | -37039 |
| Correlation coefficient ($R^2$) | 0.9990 | 0.9995 |
| LOD (µg/mL) | 0.254 | 1.047 |
| LOQ (µg/mL) | 0.769 | 3.172 |

*peak area
** Concentration of analyte in µg/mL

The limit of detection (LOD) and limit of quantitation (LOQ) for erythromycin and sulfafurazole was calculated using relative standard deviation of the response and slope of the calibration curve. The results are shown in Table 2. The values indicate the adequate sensitivity of the proposed method.

Precision was established for both system and method at a concentration of 40 µg/mL and 120 µg/mL erythromycin and sulfafurazole, respectively. System precision was determined by six replicate injections of

working standard solution injected into the HPLC system. Method precision was determined by the six suspension sample preparations injected to the HPLC system. The results are summarized Table 3. The relative standard deviation was found to be <2, indicating the precision of system and method.

**Table 3: Results of precision**

| System precision | | | Method precision | | |
|---|---|---|---|---|---|
| **Erythromycin** | | | | | |
| **Amount of drug (µg/mL)** | **Peak area** | **Statistical Analysis** | **Amount of drug (µg/mL)** | **Peak area** | **Statistical Analysis** |
| 40 | 674562 | **Mean:** 676597 **SD:** 6832.6229 **%RSD:** 1.009 | 40 | 675368 | **Mean:** 680321 **SD:** 8776.0810 **%RSD:**1.289 |
| 40 | 672951 | | 40 | 674528 | |
| 40 | 672365 | | 40 | 676328 | |
| 40 | 677351 | | 40 | 675172 | |
| 40 | 689996 | | 40 | 683684 | |
| 40 | 672357 | | 40 | 676851 | |
| **Sulfafurazole** | | | | | |
| 120 | 3684943 | **Mean:** 3688417 **SD:** 2479.5520 **%RSD:** 0.672 | 120 | 3679704 | **Mean:** 3681960 **SD:** 3660.2824 **%RSD:** 0.994 |
| 120 | 3690150 | | 120 | 3686846 | |
| 120 | 3690212 | | 120 | 3682530 | |
| 120 | 3689505 | | 120 | 3681073 | |
| 120 | 3685543 | | 120 | 3676667 | |
| 120 | 3690150 | | 120 | 3684943 | |

**Table 4: Results of accuracy**

| Spiked level (%) | Amount of drug | | % Recovery | Statistical Analysis of % Recovery |
|---|---|---|---|---|
| | Added (µg/mL) | Found (µg/mL) | | |
| **Erythromycin** | | | | |
| 50 | 20 | 19.95 | 99.75 | **Mean:** 99.55 **SD:** 1.262 **%RSD:** 1.267 |
| | 20 | 20.14 | 100.70 | |
| | 20 | 19.64 | 98.20 | |
| 100 | 40 | 39.95 | 99.87 | **Mean:** 100.08 **SD:** 0.215 **%RSD:** 0.215 |
| | 40 | 40.12 | 100.30 | |
| | 40 | 40.03 | 100.07 | |
| 150 | 60 | 59.84 | 99.73 | **Mean:** 100.45 **SD:** 0.857 **%RSD:** 0.853 |
| | 60 | 60.84 | 101.40 | |
| | 60 | 60.14 | 100.23 | |
| **Sulfafurazole** | | | | |
| 50 | 60 | 60.15 | 100.25 | **Mean:**99.89 **SD:**0.310 **%RSD:** 0.311 |
| | 60 | 59.86 | 99.77 | |
| | 60 | 59.80 | 99.67 | |
| 100 | 120 | 119.88 | 99.90 | **Mean:** 99.94 **SD: 0.140** **%RSD: 0.140** |
| | 120 | 120.12 | 100.10 | |
| | 120 | 119.80 | 99.83 | |
| 150 | 180 | 180.12 | 100.11 | **Mean: 100.00** **SD: 0.128** **%RSD: 0.128** |
| | 180 | 179.76 | 99.86 | |
| | 180 | 180.06 | 100.03 | |

To confirm the accuracy of the proposed method, standard addition technique was applied. Different amounts of standard working solution were added to suspension sample solution in three different

concentration levels (50%, 10% and 150%) and were assayed by the proposed method. The percent recoveries of the added sample solutions were calculated. The results of the recovery study were shown in Table 4. The average percent recoveries indicate good accuracy of the method.

The method robustness was determined by studying the effect of slight changes on the peak area of the analytes. Four factors were selected from the proposed method to be examined in the robustness: the mobile phase composition, flow rate, column temperature and detection wave length. Results are shown in Table 5. It was observed that none of these variables had a significant effect (% RSD <1%) on the peak areas of the investigated drugs. Therefore, the developed method is considered robust.

**Table 5: Results of robustness**

| Parameter | Value | Erythromycin | | Sulfafurazole | |
|---|---|---|---|---|---|
| | | Pek area | Statistical Analysis | Peak area | Statistical Analysis |
| **Flow rate (mL/min)** | 0.9 | 674225 | **Mean:** 673798 **SD:** 1065.7143 **%RSD:** 0.167 | 3663157 | **Mean:** 3681224 **SD:** 16754.0579 **%RSD:** 0.455 |
| | 1.0 | 672585 | | 3696248 | |
| | 1.1 | 674584 | | 3684267 | |
| **Temperature ($^{o}$C)** | 25 | 676258 | **Mean:** 672452 **SD:** 3453.7487 **%RSD:** 0.514 | 3662842 | **Mean:** 3670311 **SD:** 12096.2197 **%RSD:** 0.329 |
| | 27 | 669517 | | 3663824 | |
| | 29 | 671582 | | 3684267 | |
| **Mobile phase ratio (*v/v*)** | 78:22 | 673281 | **Mean:** 676014 **SD:** 2625.1337 **%RSD:** 0.388 | 3663284 | **Mean:** 3662981 **SD:** 262.5458 **%RSD:** 0.071 |
| | 80:20 | 678516 | | 3662845 | |
| | 82:18 | 676245 | | 3662815 | |
| **Wavelength (nm)** | 253 | 678513 | **Mean:** 677330 **SD:** 1103.8977 **%RSD:** 0.162 | 3663842 | **Mean:** 3663546 **SD:** 609.5252 **%RSD:** 0.166 |
| | 254 | 676327 | | 3662845 | |
| | 255 | 677152 | | 3663951 | |

For ruggedness of the proposed method was established by analyzing 40 µg/mL of erythromycin and 120 µg/mL of sulfafurazole by two different analysts, columns and systems using similar chromatographic conditions. As shown in Table 6, the results are in acceptable range that is %RSD values are less than 1%. The results showed no statistical differences between different analysts, columns and systems suggesting that the developed method is rugged.

**Table 6: Results of ruggedness**

| Parameter | Erythromycin | | | | Sulfafurazole | | | |
|---|---|---|---|---|---|---|---|---|
| | Taken (µg/mL) | Found (µg/mL) | % Recovery | % RSD | Taken (µg/mL) | Found (µg/mL) | % Recovery | % RSD |
| Analyst I | 40 | 39.99 | 99.99 | 1.011 | 120 | 119.94 | 99.95 | 0.072 |
| Analyst II | 40 | 40.01 | 100.02 | 0.502 | 120 | 119.91 | 99.92 | 0.101 |
| Column I | 40 | 40.08 | 100.20 | 0.652 | 120 | 120.14 | 100.12 | 0.562 |
| Column II | 40 | 39.96 | 99.90 | 0.562 | 120 | 119.89 | 99.91 | 0.351 |
| System I | 40 | 40.12 | 100.30 | 0.254 | 120 | 120.09 | 100.07 | 0.482 |
| System II | 40 | 40.09 | 100.22 | 0.268 | 120 | 119.95 | 99.96 | 0.219 |

So as to establish whether the proposed method was stability-indicating or not, erythromycin and sulfafurazole was exposed to different ICH prescribed stress conditions such as acidic, basic, oxidative, thermal and photo degradation conditions. The results of the degradation studies are presented in Table 7. Comparison of the two drugs showed that erythromycin is more stable as compared to sulfafurazole in acidic and photo degradation conditions. Whereas erythromycin is more degraded in alkali, oxidative and thermal degradation conditions than sulfafurazole. A number of degradation products were produced under acidic (2 degradation peaks with retention times 4.017 and 4.239 min), alkali (1 degradation peak with retention time 4.527 min), oxidative (1 degradation peak with retention time 3.683 min), photolytic (1 degradation peak with retention time 1.705 min) and thermal (1 degradation peak with retention time 1.02 minutes) degradation conditions. The proposed HPLC method effectively separated the degradants produced from erythromycin and

sulfafurazole peaks (Figures 2, 3, 4, 5, 6). Therefore, the developed method is to be considered highly specific for intended use and also confirms the stability indicating power the developed method.

**Table 7: Results of degradation studies**

| Condition | Erythromycin (40 ug/mL) | | Sulfafurazole (120 ug/mL) | |
|---|---|---|---|---|
| | % Recovery | % Degraded | % Recovery | % Degraded |
| Acid degradation (0.1N HCl) | 89.52 | 10.48 | 85.62 | 14.38 |
| Alkali degradation (0.1N NaOH) | 95.64 | 4.36 | 97.98 | 2.02 |
| Oxidative degradation (30% hydrogen peroxide) | 98.67 | 1.33 | 99.51 | 0.49 |
| Photo degradation (exposure to sunlight) | 97.68 | 2.32 | 97.54 | 2.46 |
| Thermal degradation (105 $^{o}$C) | 92.55 | 7.45 | 93.62 | 6.38 |

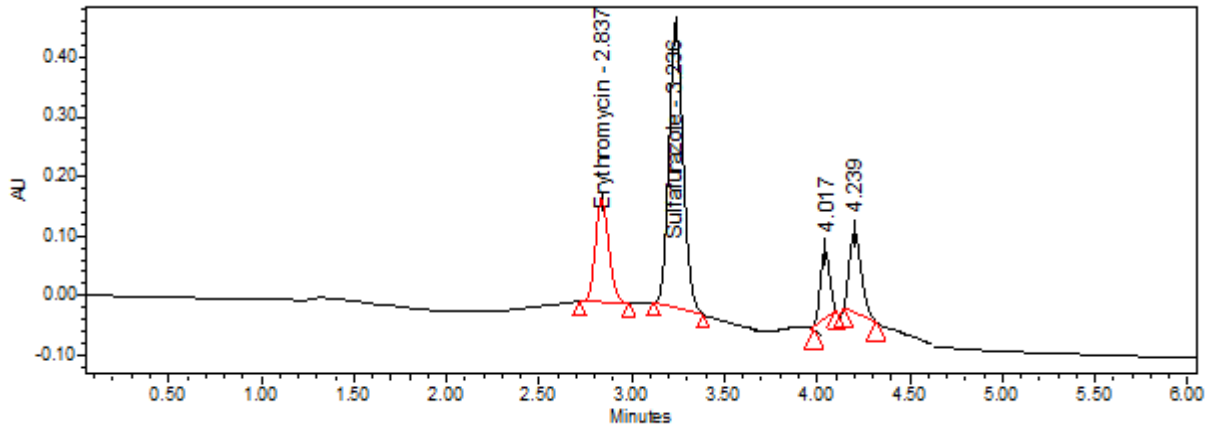


**Figure 2: Chromatogram of erythromycin and sulfafurazole after acid degradation**

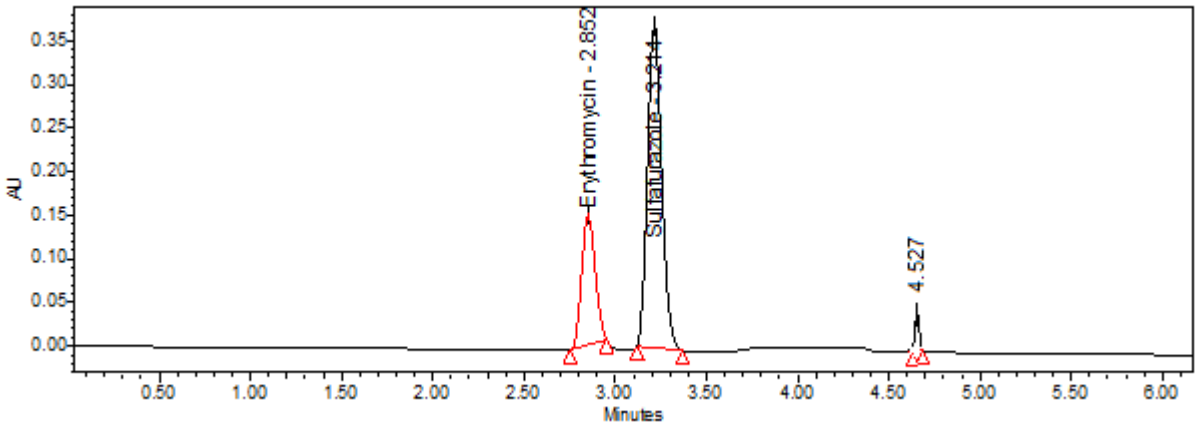


**Figure 3: Chromatogram of erythromycin and sulfafurazole after alkali degradation**

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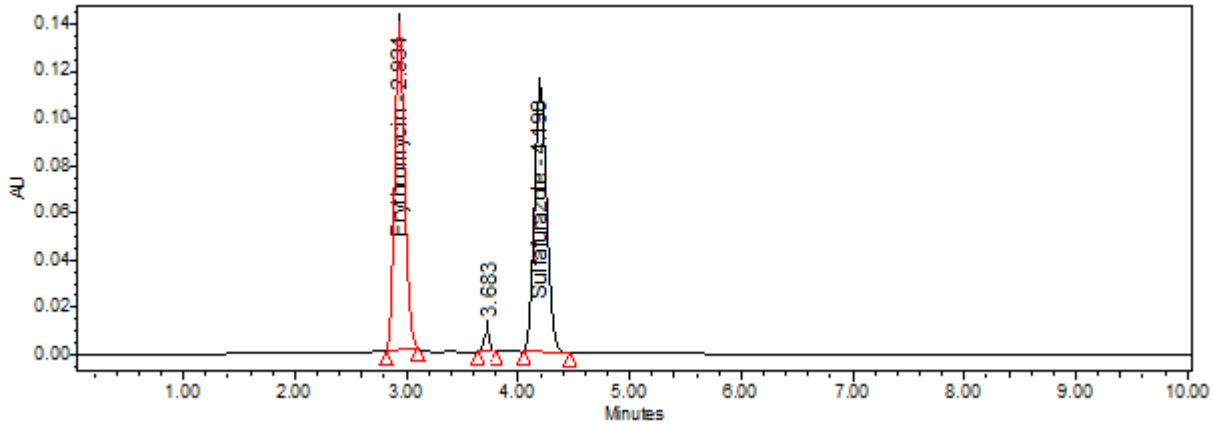


**Figure 4: Chromatogram of errthromycin and sul erythromycin and sulfafurazole after oxidative degradation**

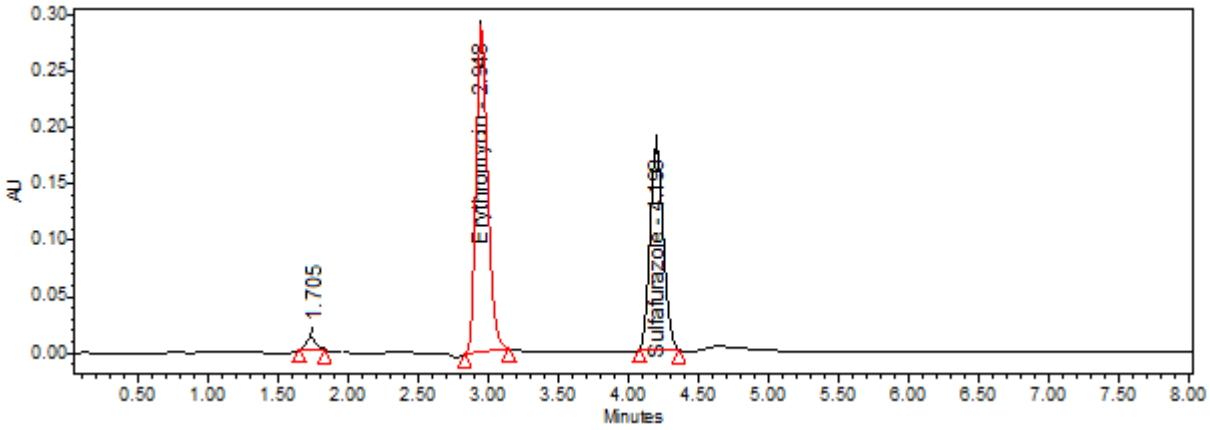


**Figure 5: Chromatogram of erythromycin and sulfafurazole after photo degradation**

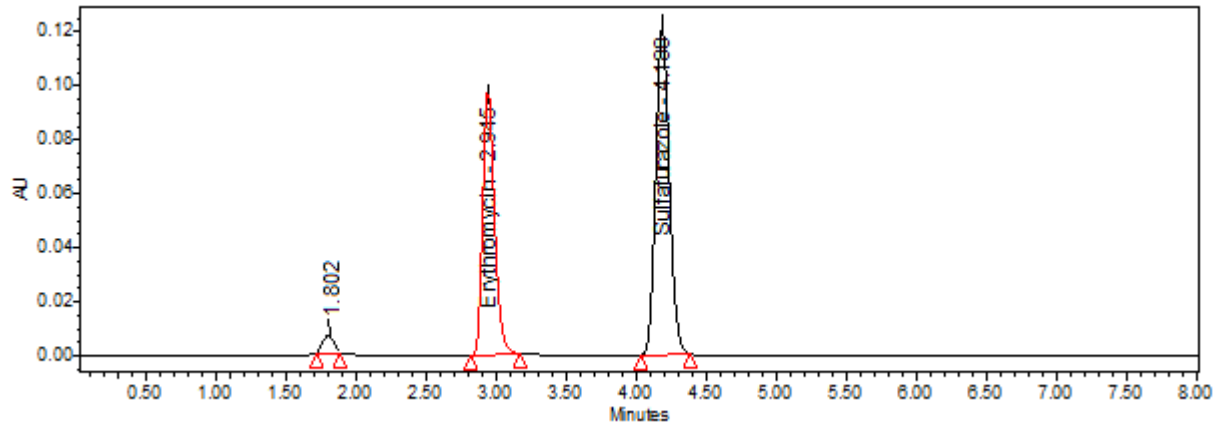


**Figure 6: Chromatogram of erythromycin and sulfafurazole after thermal degradation**

**Application of the method to the analysis of erythromycin and sulfafurazole in suspension:**

The developed and validated method was applied for the simultaneous determination of erythromycin and sulfafurazole in a commercially available oral suspension (labeled to contain 200 mg - erythromycin and 600 mg – sulfafurazole per five mL). Assay results are summarized in Table 8. Good agreement between the total value as claimed by the manufacturer and the developed HPLC method was

obtained. It was found that no excipients present in the oral suspension interfered with the assay of erythromycin and sulfafurazole, indicating the method suitability to be used for routine quality control work.

**Table 8: Analysis of analysis of erythromycin and sulfafurazole in suspension**

| Analyte | Labeled claim (mg/5mL) | Found (mg) | Mean | % Recovery | % RSD |
|---|---|---|---|---|---|
| Erythromycin | 200 | 200.15 | 199.91 | 99.95 | 0.105 |
| | 200 | 199.76 | | | |
| | 200 | 199.82 | | | |
| Sulfafurazole | 600 | 599.95 | 600.04 | 100.01 | 0.131 |
| | 600 | 600.06 | | | |
| | 600 | 600.12 | | | |

## CONCLUSION

A simple, sensitive, precise and accurate stability indicating HPLC method with UV detector is described for simultaneous determination of erythromycin and sulfafurazole in bulk and in oral suspension. The developed HPLC method was validated by testing its system suitability, linearity, limit of detection, limit of quantitation, accuracy, precision, robustness, ruggedness and specificity. The method is good enough to resolve the peaks of erythromycin and sulfafurazole from the degradation products produced during forced degradation studies. In the proposed method the retention time of erythromycin and sulfafurazole was very low (less than 5 min) which enabled the estimation of a number of samples in a short time without any interference from the excipients or degradation products. As a result, it is concluded that the proposed method could be a useful method for quality control laboratories

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