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Novel Method for the Estimation of Minoxidil and Aminexil in Marketed Formulation

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Abstract
The goal is to provide a simple, accurate, and sensitive HPTLC technique for estimating Minoxidil and Aminexil in topical solutions. Silica gel 60 F254 pre-coated TLC aluminium plates were used as the stationary phase in the procedure. Methanol: water (5:5) was the solvent system in use. At 254 nm, densitometric analysis was performed. The results showed that the Rf values for Aminexil and Minoxidil were 0.88 and 0.69, respectively. According to the findings, the scanning wavelength was 254 nm and was linear between 2 and 10 µg/spot for minoxidil and 6–14 µg/spot for aminexil. The %RSD was less than 2%. Minoxidil and Aminexil I were shown to have regression coefficients of 0.9983 and 0.9993, respectively. A simple, accurate, precise, and sensitive HPTLC approach is designed and validated in compliance with ICH criteria for measuring minoxidil and aminexil in combination in topical solution.

Keywords: Aminexil, Minoxidil, HPTLC, Validation, ICH guidelines.

1. Introduction

Minoxidil, also known as 6-peridin-1-yl-pyrimidine-2,4-diamine-3-oxide, is a potent vasodilator that lowers blood pressure by directly lowering peripheral resistance. It is applied topically to treat hair loss. For both men and women with pattern baldness, minoxidil is clinically efficient in encouraging hair growth and halting hair loss. For ongoing maintenance of existing hair follicles and the protection of existing hair, minoxidil must be used indefinitely. Females with androgenic alopecia are treated with a 2% alcoholic solution. Androgenic alopecia in the males can be treated by using a 5% alcoholic solution [1].

Aminexil also known as Kopexil (2,4diamino pyrimidine-3-oxide), is a varied form of Minoxidil which does not have the side effects like Minoxidil due to the structural modification. It fights against the root stiffening and is used as a genuine anti-hair loss innovation. Hair loss is mainly due to the root deterioration which is seen both in men and women [7] . During the growing stage, Aminexil successfully acts on the deep root structure to improve the volume of hair. Because Kopexil revitalizes the hair roots, the endurance of healthy hair growth is evident. Kopexil is used for the treatment of fibrosis condition (constriction of blood vessels and reduction in the life of hair follicle) of hair roots [1].

According to a review of the literature, a number of analytical techniques have been documented for the HPLC determination of minoxidil and aminexil in the combination medication [2]. However, there were no reports on the simultaneous HPTLC estimate of Aminexil and Minoxidil. This led to the development of a better, easy, efficient and economical analytical method.

2. Materials and Methods

Chemicals and reagents:

Methanol of HPLC grade was acquired from Rankem in Gujarat, India. Water of HPLC grade was acquired from Merck Limited in Mumbai, India. MIN and AMI standard drugs were obtained from Yarrow Chem products and Micro Labs limited (Bangalore, India) respectively. Marketed formulation HAIRBLESS (topical solution form), manufactured by Lifestar was obtained from local pharmacy.

Instrumentation and Chromatographic conditions:

The MIN and AMI samples were spotted in 6 mm bands using a Camag Linomat automatic TLC sample applicator (Switzerland) and a Camag microlitre syringe (100 µL) (Hamilton, Switzerland) on a precoated silica gel aluminium plate 60 F254 with a thickness of 0.2 mm. [4] (10 cm × 10 cm). Methanol and water were combined in a 5:5 ratio to form the mobile phase. The development took place in a 20 cm x 10 cm Camag twin trough glass chamber from Muttenz, Switzerland, and the mobile phase was fully saturated. The chamber was saturated with the chosen mobile phase for 20 minutes around room temperatures. The chromatogram run's extent was roughly 85 mm. Following development, the TLC plate was allowed to air dry before densitometric scanning (slit-width, 6 x 0.3 mm) was performed with a Camag TLC scanner III in the absorbance mode at 254 nm by estimating MIN and AMI using winCATS software. The light source was a deuterium lamp (D2) lamp.

Standard stock solution preparation:

Accuracctely weighed 10 mg of standard MIN was added to the 5 ml methanol, dissolved completely and later methanol was used to make up the volume to 10 ml. Similarly 10 mg of standard AMI was added to 5 ml methanol, dissolved completely and later methanol was used to make up the volume to 10 ml. The concentration of the AMI and MIN stock solutions was 1 mg/ml. (1000 µg/1000 µl or 1 µg/1 µl).

Preparation of sample solution:

HAIRBLESS topical solution, a commercial product from LIFESTAR consists of 5% of MIN and 2% of AMI as per label claim. The amount of solution which is equivalent to 10mg of MIN was dissolved in 5ml of Methanol with five minutes of sonication. Filtration was done on the sonicated solution, methanol was used to make up the volume to 10ml. The sample solution concentration's ultimate volume was in the ratio 10:4 of MIN and AMI respectively.

Analytical method validation:

Linearity and range:

MIN and AMI standard solutions were used for ensuring linearity The calibration curves were created while the concentration range for MIN was 2–10 µg/spot and for AMI was 6–14 µg/spot. Peak areas versus concentrations were constructed to create the calibration curves.

Method precision:

Standard MIN and AMI solutions were used to test the sample's repeatability and intra and inter day precision. Three replicas of the spots were observed at varying concentrations: 2, 6, and 10 µg/spot for MIN and 6, 8, and 10 µg/spot for AMI.

Specificity studies:

To verify the high level of specificity, a representative three-dimensional HPTLC chromatogram was produced using diluents, mobile phase, standard MIN, standard AMI, and the sample solution made up of MIN and AMI. By comparing the two spectra, the peak purity at three distinct levels—that is, The peak start, apex, and end positions of the spot of MIN and AMI were examined. [4].

Limit of detection (LOD) and quantification (LOQ):

Using a formula based on the response's standard deviation and slope, the limits of detection (LOD) and quantification (LOQ) were determined.

$LOD = 3.3\sigma / S$ (1)

$LOD = 10\sigma / S$ (2)

where,

σ : standard deviation of the response

S : slope of the calibration curve

Slope (S) can be estimated using the information derived from the analyte calibration curve. By using the response's standard deviation, one can estimate σ.[3]

Accuracy (Recovery):

To determine the percentage recovery of the drug at various levels in the formulations, the formulation samples were spiked with the standard MIN and AMI by 80, 100, and 120%. The mixtures were then subjected to analysis using the HPTLC method that was developed. Three repetitions of the experiment were made.

Robustness:

The suggested TLC-densitometric methods' robustness was assessed in order to assess the impact of minor, intentional modifications to the chromatographic conditions during the determination of the cited drug.

Analysis of the marketed formulation:

Five replicates of the standard the solution were then applied to the TLC plate with 2 and 6 µg/spot of MIN and AMI, respectively. Development and scanning were then conducted. In the analysis, the excipient's risk and potential for interference were examined.

3. Results and Discussion

Method optimisation:

In the present study, for development of the proposed method, various combinations of mobile phase by trial and error method were used. Mobile phase consisting of methanol:water(5:5) showed good separation and Rf of 0.69 and 0.88 for MIN and AMI respectively in Figure 3.

Linearity and Range:

The suggested method's linearity was tested between 2 and 10 µg/spot for MIN and 6 and 14 µg/spot for AMI. The linearity of the concentration plot on the x-axis against the peak area on the y-axis is displayed in the linear regression analysis results for the calibration graphs (Table 1). For MIN (Fig. 4), the correlation coefficient (r2) was 0.9982, and for AMI (Fig. 5), it was 0.9993. As shown in Figures 6 and 7, the results showed that the method used is linear in the concentration range of 2–10 µg/spot for MIN and 6–14 µg/spot for AMI.

Method precision:

The suggested method's precision was tested by identifying the MIN and AMI standard solutions. Three concentrations of three replicates of the spots of 2, 6 and 10 µg/spot for MIN and 6, 8 and 10 µg/spot for AMI were spotted to check precision of the method. The suggested method's excellent precision is demonstrated by the repeatability and intra- and inter-day variation of MIN and AMI being less than 2% (Table 2 and 3).

Limit of detection (LOD) and Limit of quantification (LOQ)

A formula was used to calculate the limit of detection and limit of quantification based on response stand-

ard deviation and slope. The LOD and LOQ for MIN and AMI were 0.0051 and 0.0156µg/spot, and 0.0063 and 0.019µg/spot, respectively, indicating that small amounts of the compounds can be accurately estimated.

Specificity studies and Selectivity:

Comparing the two spectra allowed us to assess peak purity at three different levels: peak start, peak apex, and peak end positions of the MIN and AMI spots. By checking and comparing peak purity of the MIN and AMI, the spectra at peak start, peak apex and peak end positions (Fig. 8) are found to be similar which show that the method is specific for the proposed method. The method's selectivity was further investigated by creating many laboratory-prapared mistures of the drugs at different concentrations within the specified linearity limits. The suggested technique was followed in the analysis of the laboratory-prepared mixtures. The above table's recovery values, RSD%, and Er% values were all acceptable, confirming the procedures' selectivity.

Accuracy (Recovery):

The percentage recovery of the drug at different levels in the formulations was checked by spiking the formulation samples with the standard MIN and AMI by 80, 100 and 120% and the mixtures were analyzed by the developed HPTLC method which showed a recovery of 99-101% of MIN and AMI respectively (Table 5). Good accuracy of the suggested methods and no interference from formulation recipients were indicated by the excellent mean recoveries and standard deviation.

Robustness:

The chamber saturation temperature, chamber saturation time, and changes in the mobile phase ratio (4.5:5.5) and (5.5:4.5) were all purposefully changed in the optimized methods. Changes in the aforementioned responses of the drugs under analysis were recorded and computed using the modified parameters for methanol: water. Since the values in the modified parameters fell within the permitted range, the techniques were shown to be reliable.

Analysis of the marketed formulation:

On the pre-coated aluminum TLC plate, three duplicates of the standard solution were applied with 2 and 6 µg/spot of MIN and AMI, respectively. Development and scanning were then conducted. The amount of drug in the sample was calculated, and the potential for excipient interference in the analysis was examined. None of the formulation excipients or other contaminants interfered with the formulation sample (Fig. 9). The fact that the peaks of MIN and AMI do not conflict with one another indicates that the suggested approach is unique. For MIN and AMI, the drug content was determined to be 100.17% and 99.67%, respectively.

4. Conclusion

A validated HPTLC method was developed to quantitatively estimate minoxidil and aminexil in pharmaceutical formulations. The developed HPTLC method showed good resolution between MIN and AMI. After validation, the technique was determined to be straightforward, sensitive, precise, and accurate. The suggested approach can be used to pharmaceutical formulations for the estimation and measurement of MIN and AMI. As a result, methods developed for MIN and AMI estimate and quantification in pharmaceutical formulations are thought to be reliable, sensitive, specific, rapid, and straightforward. They can be effectively employed for routine analysis at authorised testing facilities, academic institutions, and industry quality control divisions.

References

1. Rizwana I, Prakash VK, Mohan KG. Simultaneous Estimation of Minoxidil and Aminexil In Bulk and Pharmaceutical Formulations by RP-HPLC Method. Oriental journal of chemistry 2015;31(1):277-84.
2. Rudrapal M, Surya N, BVVS, Sridhar N, Raghavendra M. Development and Validation of RP-HPLC Method for Simultaneous Estimation of Minoxidil and Aminexil in Topical Formulation. Asian Journal Of Chemistry 2016;28(1):157-60.
3. International Conference on Harmonization, (2005) Guidelines on "Validation of Analytical procedures: Text & Methodology", ICH Q2(R1) 6-13.
4. Patel N, Meshram D. Development and validation of analytical method for simultaneous determination of minoxidil and finasteride in pharmaceutical dosage form by RP- HPLC method. IJPSR 2015;6(11):4882-5.
5. Patel PD, Surti NI, Upadhyay UM. Development and Validation of Stability Indicating Method for Minoxidil and Finasteride in its Pharmaceutical Dosage Form. IJPRS 2015;4(2):221-38.
6. Messenger AG, Rundegren J. Minoxidil: mechanisms of action on hair growth. British Journal of Dermatology;2004 150:186–94.

Tables:

Table 1: Linearity data, Rf and Scanning wavelength

Sample	**Regression Coefficient**	**Slope**	**Intercept**	**Detection Limit**	**Quantification Limit**	**Retardation Factor (Rf)**	**Scanning Wavelength**
MIN	0.9982	2407.4	7004.3	0.0051 µg/spot	0.0156µg/spot	0.69	254nm
AMI	0.9993	1367.6	3586.1	0.0063 µg/spot	0.019µg/spot	0.88	254nm

Table 2: Intra-day precision results

Drug / Parameters	Minoxidil			Aminexil		
Concentration (ug/ml)	2	6	10	6	10	14
Mean Area	11659.09	21737.87	30689.99	4804.52	7756.76	10390.23
SD	227.48	138.63	189.39	79.69	121.56	41.419
%RSD	1.95	0.63	0.61	1.658	1.567	0.398
SEM	131.34	80.036	109.34	46.014	70.18	23.91

Table 3: Inter-day precision results

Drug / Parameters	**Minoxidil**			**Aminexil**		
Concentration (ug/ml)	2	6	10	6	10	14
Mean Area	11761.43	21646.75	30740.39	4823.17	7786.2	10397.02

SD	70.598	107.94	34.168	76.23	64.53	54.048
%RSD	0.6002	0.4986	0.1111	1.580	0.828	0.519
SEM	40.760	62.317	19.72	44.012	37.258	31.204

Table 4: LOQ and LOD results

Drugs	**Minoxidil**	**Aminexil**
Limit of Detection	0.0051 µg/spot	0.0063 µg/spot
Limit of Quantification	0.0156 µg/spot	0.019 µg/spot

Table 5: Identification of bulk laboratory-prepared mixtures of the drugs under analysis using the suggested HPTLC techniques.

Nominal Value (ug/spot)		**Found +/- SD (ug/spot)**		**RSD (%)**		**Er (%)**	
MIN	AMI	MIN	AMI	MIN	AMI	MIN	AMI
2	6	2.0014 ± 0.0078	6.0416 ± 0.0712	0.3897	1.1784	0.07	0.693
4	8	4.0356 ± 0.0058	7.9959 ± 0.0092	0.1437	0.1150	0.89	0.051
6	10	5.9986 ± 0.0108	9.9986 ± 0.0221	0.1800	0.2210	0.02333	0.014
8	12	8.0082 ± 0.0215	12.0849 ± 0.0854	0.2684	0.7066	0.1025	0.707
10	14	10.0857 ± 0.0482	14.0784 ± 0.0452	0.4779	0.3210	0.857	0.560

Table 6: Recovery studies.

Drug	**Minoxidil**			**Aminexil**		
Amount of drug present (µg/spot)	2	2	2	6	6	6
Amount of drug added (µg/spot)	1.6	2.0	2.4	4.8	6.0	7.2
Percentage recovery	98.6	100.32	101.59	98.80	100.40	99.82
Total	100.17%			99.67%		

Table 7: Robustness evaluation for determination of the analyzed drugs using the proposed HPTLC methods

Selected parameters	**MIN**		**AMI**	
	Peak Area	**Rf**	**Peak Area**	**Rf**
Chamber saturation time (10-20-30 min)	21700 +/- 81.24	0.69 +/- 0.01	7780 +/- 18	0.89 +/- 0.03

Chamber saturation temperature (25-30-35)	21588 +/- 58.36	0.69 +/- 0.02	7698 +/- 29	0.88 +/- 0.02
Mobile phase ration (4.5:5.5) and (5.5:4.5) Methanol : water	21630 +/- 67.55	0.68 +/- 0.02	7715 +/- 30	0.88 +/- 0.02

Figures:

Figure 1: Minoxidil

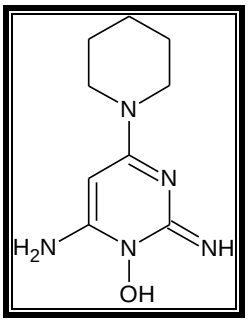

Figure 2: Aminexil

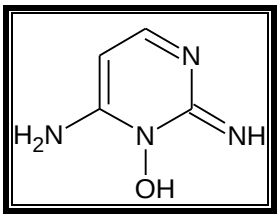

Figure 3: Standard chromatogram showing Rf of MIN and AMI

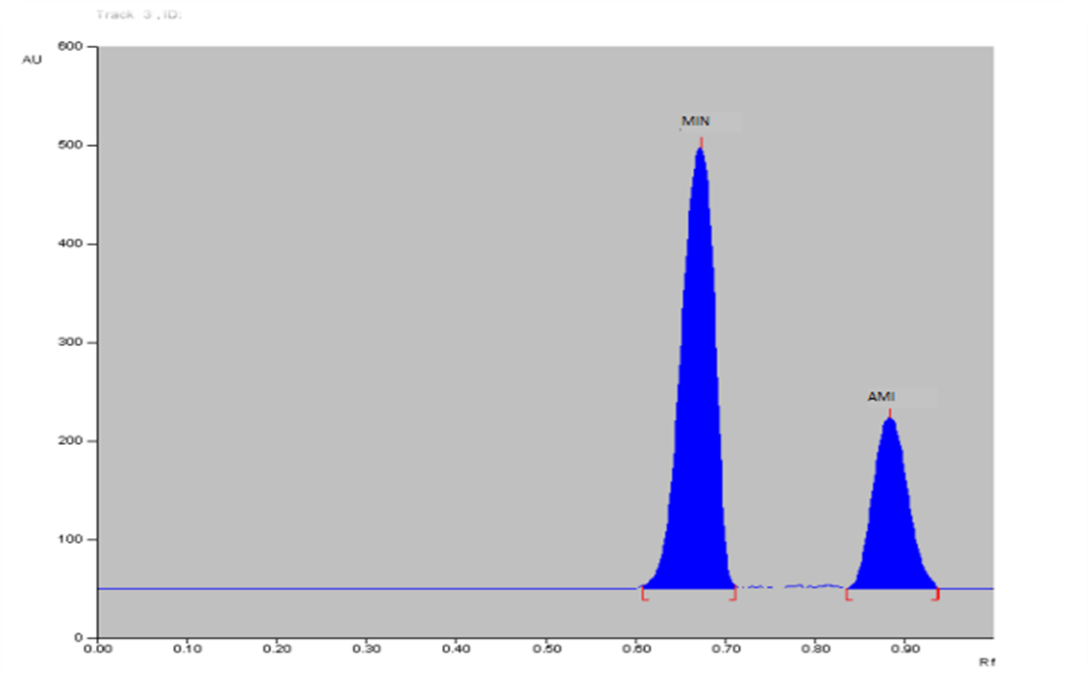

Figure 4: Calibration plot of MIN

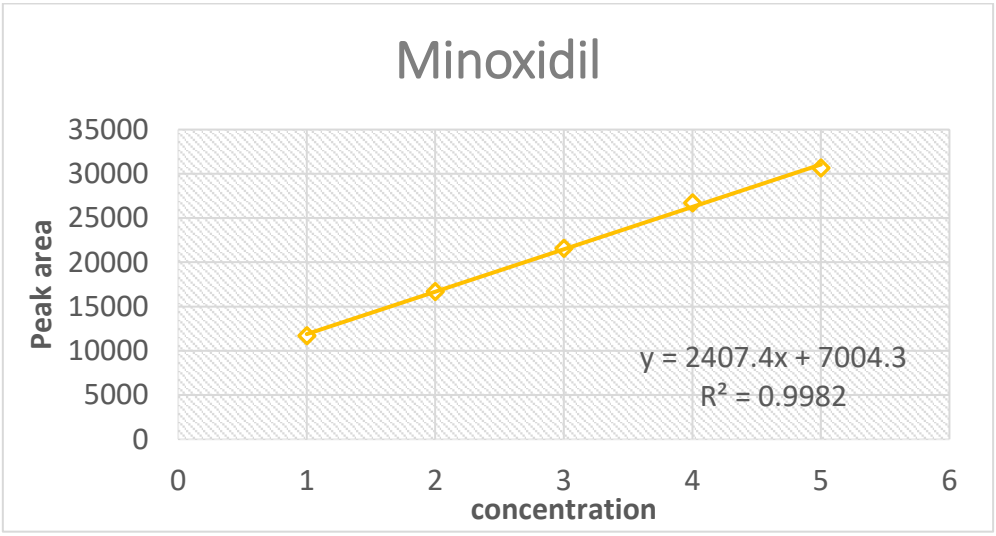

Figure 5: Calibration plot of AMI

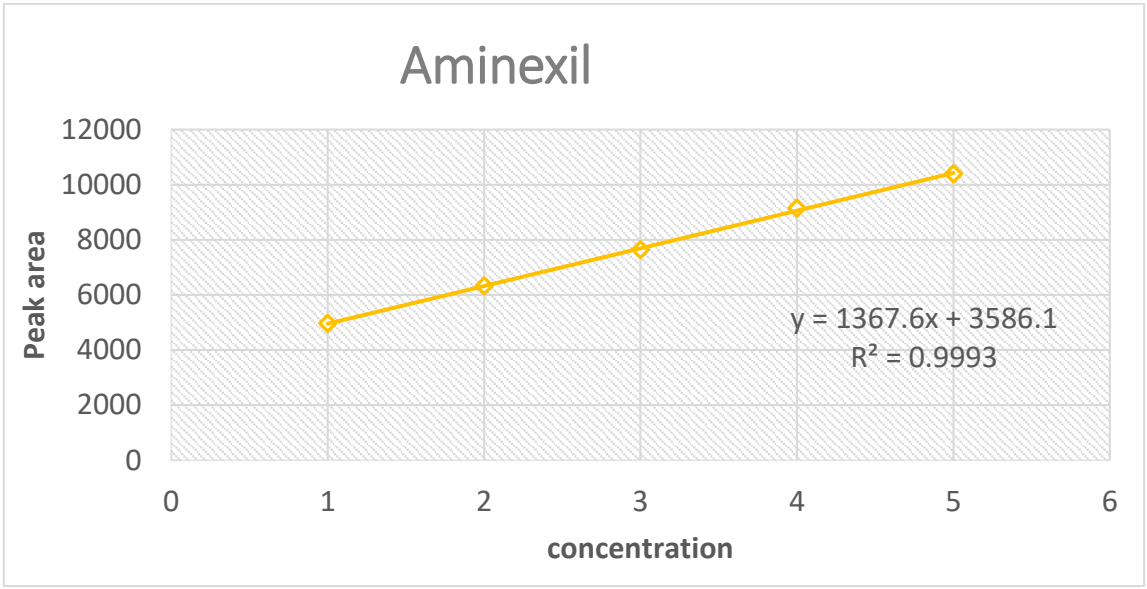

Figure 6: 3D chromatogram showing linearity of MIN

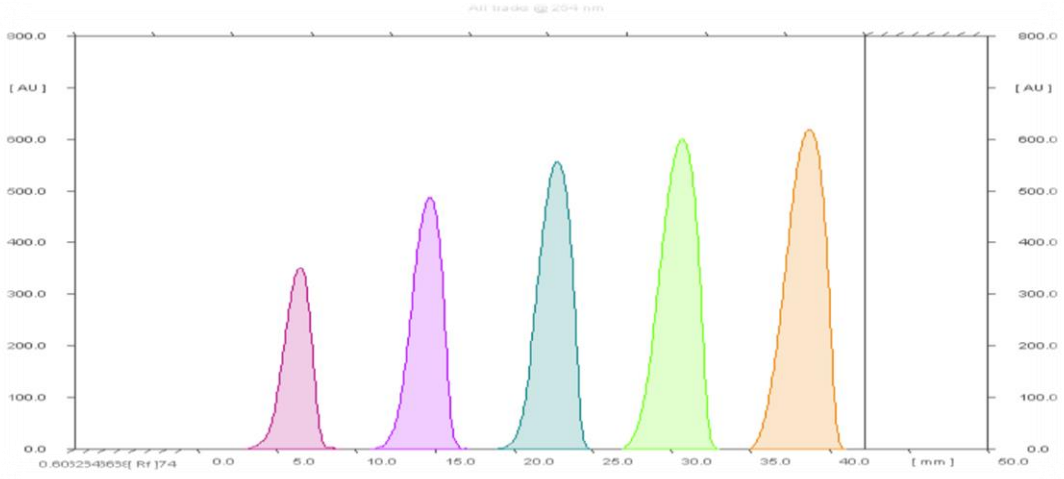

Figure 7: 3D chromatogram showing linearity of AMI

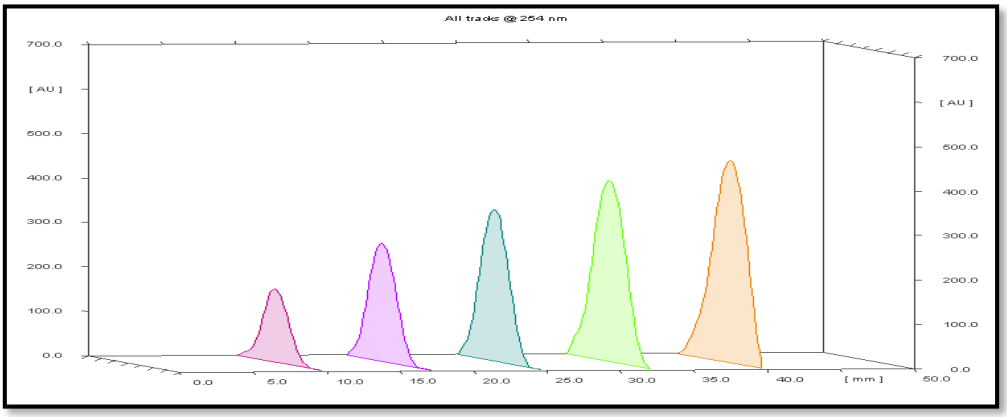

Figure 8: Specificity spectra of MIN

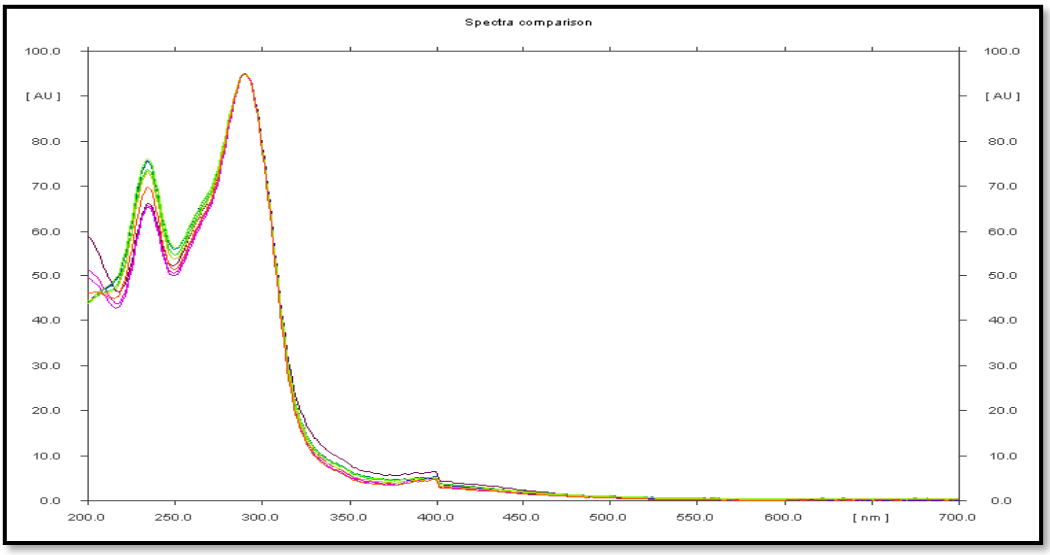

Figure 9: 3D chromatogram showing comparison of MIN and AMI (STANDARD & SAMPLE)

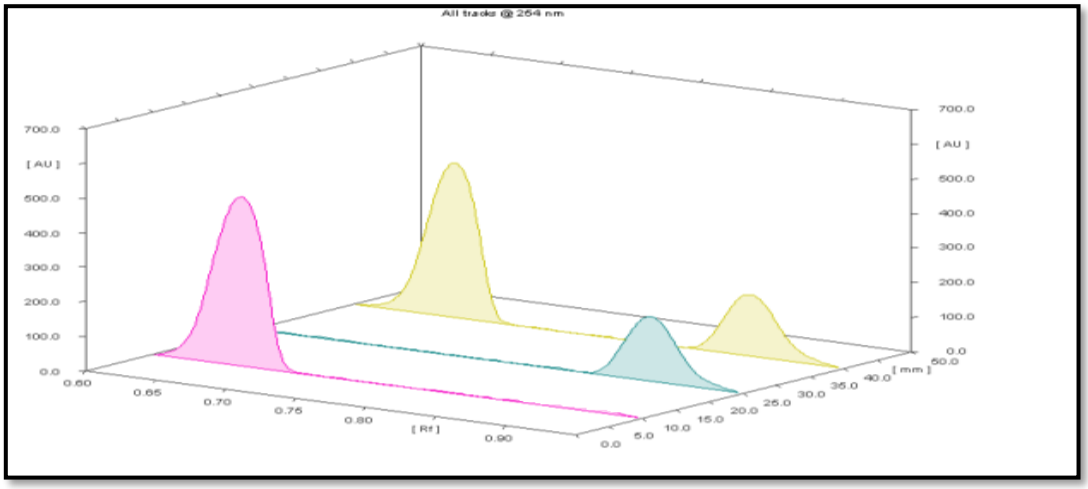

Figure 10: Overlay spectra comparison of MIN and AMI