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SIMULTANEOUS DETERMINATION OF LENVATINIB AND EVEROLIMUS BY HPTLC METHOD

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ABSTRACT

HPTLC Method was developed and validated for Lenvatinib (LEN), a tyrosine kinase inhibitor and mTOR inhibitor Everolimus (EVE) used to treat Renal cancer. Chromatographic separation of Lenvatinib and Everolimus in synthetic mixture was achieved on Silica gel aluminium plate 60F-254 (20 × 10 cm with 250 µm thickness) using mobile phase, Chloroform: Toluene: Methanol: Glacial Acetic Acid [4: 4: 2: 0.1 v/v/v/v]. Validation was performed at 254 nm wavelength. The calibration curve was found to be linear in the concentration range of 280 – 1400 and 100 – 500 ng/band for LEN and EVE, respectively. Well resolved bands were obtained with Rf value 0.8 for Lenvatinib and 0.25 for Everolimus with 99.74 and 99.72% recovery, respectively. The method is simple, rapid, accurate, precise, reproducible, and economic as per ICH guidelines and can be used for routine quantitative analysis of Lenvatinib and Everolimus combination.

KEYWORDS: Lenvatinib, Everolimus, HPTLC, Anti-Cancer drugs.

INTRODUCTION

A cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Cancer is the uncontrolled growth of abnormal cells anywhere in the body. These abnormal cells are called as cancer cells, Malignant cells or tumor cells. These cells can infiltrate normal body tissues. Many cancers and the abnormal cells that compose the cancer

tissue are further identified by the name of the tissue that the abnormal cells originated from (for example, breast cancer, lung cancer, and colorectal cancer, Renal Cancer). Anything that may cause a normal body cell to develop abnormally potentially can cause cancer. Some cancer causes remain unknown while other cancers have environmental or lifestyle triggers or may develop from more than one known cause.

Carcinoma, A type of Cancer that begins in the skin or in tissues that line or cover internal organs -- "skin, lung, colon, pancreatic, ovarian cancers," epithelial, squamous and basal cell carcinomas, melanomas, papillomas, and adenomas.[1-3] Mainly from renal epithelium renal cancer arises and it is known as renal cell carcinoma. Clear cell, papillary type 1 and 2 and chromophobe tumors are types of renal cell carcinoma.[4] Renal cystic disease occurs due to haemo-dialysis also consider as a risk factor.[5] Most renal masses are confined to one organ. During diagnosis a haematogenic dissemination causes metastasis of tumor. Lungs, bone and brain are the most frequent distant sites of metastasis in which adrenal gland, contralateral kidney and liver might not be involved.[6]

Table 1: Treatment of Renal Cell Carcinoma.[7]

Approved first line and second line of drugs for treatment of RCC.	**Drugs class**
Bevacizumab, Nivolumab	Monoclonal anti body
Sunitinib, Pazopanib, Sorafenib, Axitinib, ***Lenvatinib***	Tyrosine kinase Inhibitor
Temsirolimus, ***Everolimus***	mTOR inhibitor

Lenvatinib Mesylate ($C_{22}H_{23}ClN_4O_7S$, Chemically known as 4-[3-chloro-4-(cyclopropylcarbamoylamino)phenoxy]-7-methoxyquinoline-6-carboxamide;methanesulfonic acid as shown in figure 1) is a synthetic, orally available inhibitor of vascular endothelial growth factor receptor 2 (VEGFR2, also known as KDR/FLK-1) tyrosine kinase with potential antineoplastic activity. E7080 blocks VEGFR2 activation by VEGF, resulting in inhibition of the VEGF receptor signal transduction pathway, decreased vascular endothelial cell migration and proliferation, and vascular endothelial cell apoptosis.[8-10]

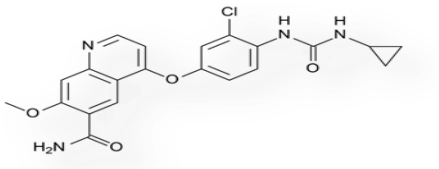


Figure 1: Structure of Lenvatinib.

Everolimus ($C_{53}H_{83}NO_4$, chemically known as (1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*, 24*E*,26*E*,28*E*,30*S*,32*S*,35*R*)-1,18-dihydroxy-12-[(2*R*)-1-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone as shown in figure 2) is a mTOR inhibitor that binds with high affinity to the FK506 binding protein-12 (FKBP-12), thereby forming a drug complex that inhibits the activation of mTOR. This inhibition reduces the activity of effectors downstream, which leads to a blockage in the progression of cells from G1 into S phase, and subsequently inducing cell growth arrest and apoptosis. Everolimus also inhibits the expression of hypoxia-inducible factor, leading to a decrease in the expression of vascular endothelial growth factor. The result of Everolimus inhibition of mTOR is a reduction in cell proliferation, angiogenesis, and glucose uptake.[11-13]

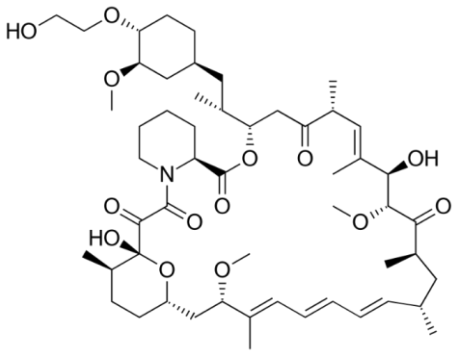


Figure 2: Structure of Everolimus.

Literature review for both the drugs reveals that no any analytical methods have been reported for the simultaneous estimation of Lenvatinib and Everolimus drug combination. The present study was aimed to develop and validate Simple, Economical, Precise, Reproducible, Accurate HPTLC method for the simultaneous estimation of Lenvatinib and Everolimus in their synthetic mixtures according to ICH guidelines.

MATERIAL AND METHOD

2.1 Materials

Lenvatinib and Everolimus bulk drug was procured from the manufacturer for the analytical work purpose. Analytical Grade toluene, Methanol, Chloroform, glacial acetic acid was used. As stationary phase Silica gel aluminum plate 60F-254 (20 × 10 cm with 250 µm thickness) was used.

2.2 Instrumentation

HPTLC system comprising of CAMAG Linomat 5 sample applicator, Coupled with Camag Hamilton Bonaduz schwets syringe, UV chamber with dual wavelength lamps nad CAMAG TLC scanner 4 controlled by vision CATS software (CAMAG) was used for the application and detection of spots respectively. Silica gel aluminum plate 60F-254 (20 × 10 cm with 250 µm thickness) HPTLC plates was used for the chromatographic separation of the drugs with CAMAG twin-trough developing chamber for the development.

2.3 Chromatographic conditions

Spotting was done using Camag Linomat 5 sample applicator (CAMAG, Switzerland) and Camag Hamilton Bonaduz microlitre syringe (100 µl) on HPTLC aluminium plates precoated with silica gel 60 F_{254} (20 cm × 10 cm with 250 µm thickness; Sigma-Aldrich). The plates were prewashed with methanol for 30 min in a CAMAG twin trough glass chamber closed with lid. The plates were activated at 50^0C for 10 min. The samples were spotted in the form of narrow bands having length of 8 mm. The application position X and Y were kept at 8 mm and 20 mm, respectively, to avoid edge effect. The distance between the two bands was 20 mm. Bands were applied at a constant rate of 15 nL/s using a nitrogen aspirator. Linear ascending development of chromatogram was carried out in a CAMAG twin trough glass chamber saturated with the mobile phase for 25 min and chromatogram run was kept up to 50 mm. Following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Spectro densitometric analysis of the separated components was carried out using CAMAG TLC Scanner 4 in the reflectance–absorbance mode at 254 nm using a deuterium lamp. The slit dimension used was 6.0 mm × 0.3 mm and sensitivity was kept at auto mode. Scanning speed was 100 nm/s. Evaluation was achieved by linear regression of the peak area response against amount of drug by using vision CATS (CAMAG) software for peak area measurement and data processing.

2.4 METHOD

2.4.1 Preparation of standard stock solution

Drug was accurately weighed to make the standard stock solution 2800 & 1000 µg/mL for LEN and EVE, respectively in 10 mL volumetric flask. Add 5 mL methanol into it and stir well to make the drugs soluble properly. Makeup the volume up to the mark to give standard stock Solution.

2.4.2 Preparation of Standard stock solution of LEN and EVE mixture

280 mg of Lenvatinib and 100 mg of Everolimus was accurately weighed and transferred into 100 mL volumetric flask. Drugs were dissolved with 10 mL of Methanol with rigorous shaking and followed by sonicator for 5 min or till get completely dissolved and then diluted up to the mark with methanol which gives standard stock solution of LEN and EVE mixture of 2800 µg/mL and 1000 µg/mL, respectively.

2.4.3 Selection of detection Wavelength

These drug solutions were scanned in the UV-region of 200-400 nm and the spectra were recorded to get maximum of analytes in mobile phase. LEN and EVE were scanned in UV in which both the drugs show reasonably good response at 275 nm. But during selection, observed that TLC plate was readable in UV scanning So that **254 nm** was considered.

2.4.4 Validation of Method[14–15]

I. Linearity

Analysis was performed on Precoated silica gel aluminium Plate 60F–254 (20 × 10 cm with 250 µm thickness) (E. Merck) pre-washed with methanol and then dried for 30 minutes at 50°C. From the Stock A, appropriate aliquots were spotted on the TLC plate under nitrogen stream using Desaga Applicator, AS30 win to final concentration range 280 – 1400 ng/band and 100 – 500 ng/band for LEN and EVE, respectively.

II. Precision

a. Repeatability

Repeatability of the method was performed by six different solutions of same concentration prepared from single stock solution and were analyzed containing 840 & 300 ng/band of LEN and EVE, respectively.

b. Precision

Intraday & Interday precision of the proposed method was performed by analysing 3 concentration (in ng/band) levels i.e. low, medium and high in triplicates on the same day for Intraday study (with the interval of 2 hour) and on 3 different days for Interday study.

III. Accuracy (Recovery study)

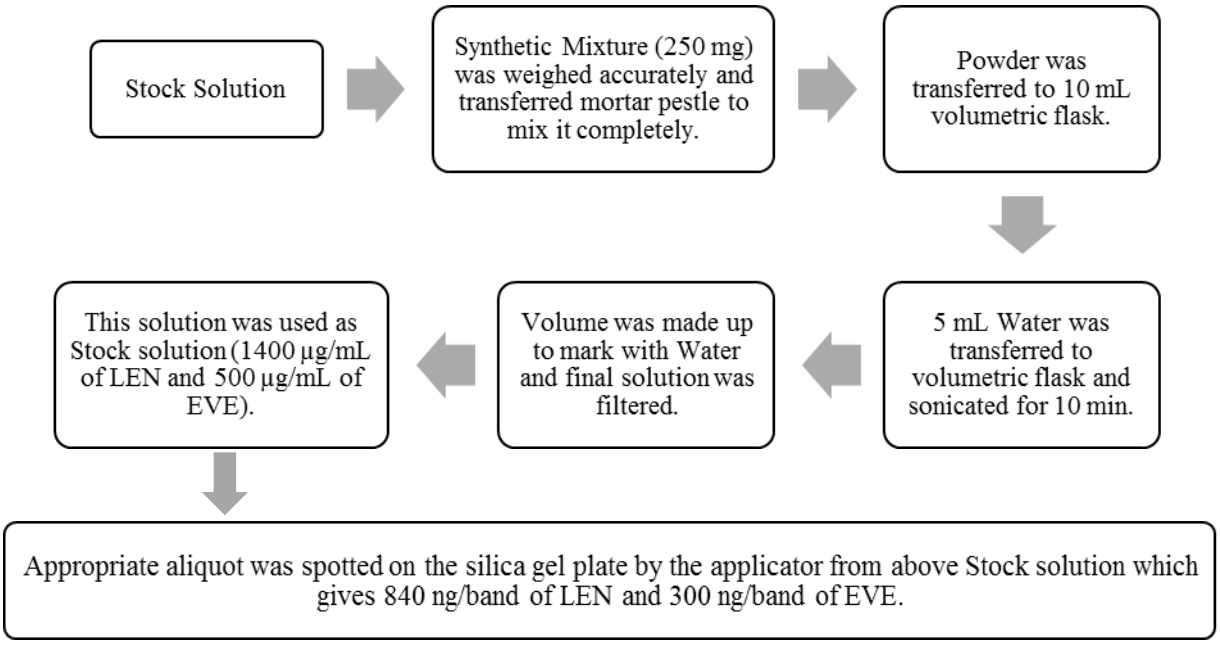
Accuracy was determined by performing recovery studies by spiking different concentration of drug to pre-analyzed sample solution of 500 ng/band for LEN and 200 ng/band of EVE.

With different levels i.e., 80%, 100% and 120% the experiment was performed in triplicate. The result was evaluated in terms of % Recovery.

IV. Assay of Synthetic Mixture of LEN and EVE

Synthetic Mixture was prepared as per the conventional drug combinations as below for single dose formulation.

Lenvatinib	Everolimus	MCC	Talc
14 mg	5 mg	50 mg	Qty Sufficient up to 250 mg



From recorded Peak area and concentration, % purity was calculated for LEN and EVE.

V. Robustness

Robustness of proposed method was evaluated by deliberately changing parameters like Mobile phase composition and Saturation Time.

Table 2: Optimized Conditions for the estimation of LEN and EVE.

Parameters	Optimized Conditions
TLC Plate	Silica gel aluminum plate 60F-254 (20 × 10 cm with 250 µm thickness)
Mobile Phase	Chloroform: Toluene: Methanol: Glacial Acetic Acid [4: 4: 2 v/v, pH was adjusted with 0.1 mL of Glacial Acetic Acid]
Wavelength	254 nm
Saturation Time	25 min
Rf Value	LEN – 0.8, EVE – 0.25

2.4.5 RESULT AND DISCUSSION

A. Linearity

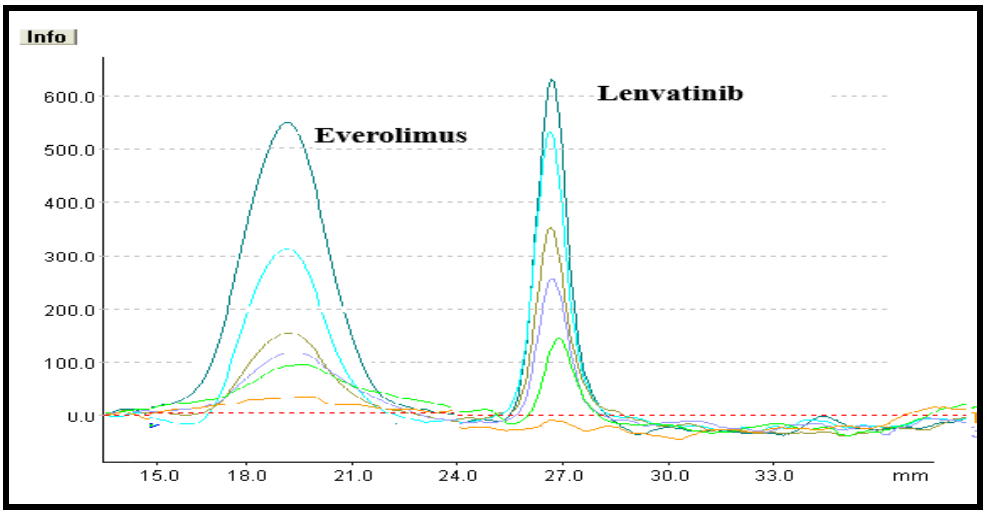


Figure 3: Overlain chromatogram of LEN (280 – 1400 ng/band) and EVE (100 – 500 ng/band).

Table 3: Linearity data of LEN & EVE.

Sr no.	LEN			EVE		
	Conc. (ng/band)	Mean of Area ± SD (n=5)	% RSD	Conc. (ng/band)	Mean of Area ± SD (n=5)	% RSD
1	**280**	136.33 ± 1.53	**1.12**	**100**	67.67 ± 0.58	**0.85**
2	**560**	262.33 ± 1.53	**0.58**	**200**	177.67 ± 1.53	**0.86**
3	**840**	386.67 ± 5.03	**1.30**	**300**	279.00 ± 3.00	**1.08**
4	**1120**	512.00 ± 8.54	**1.67**	**400**	380.00 ± 4.58	**1.21**
5	**1400**	642.33 ± 7.64	**1.19**	**500**	489.67 ± 9.87	**1.89**

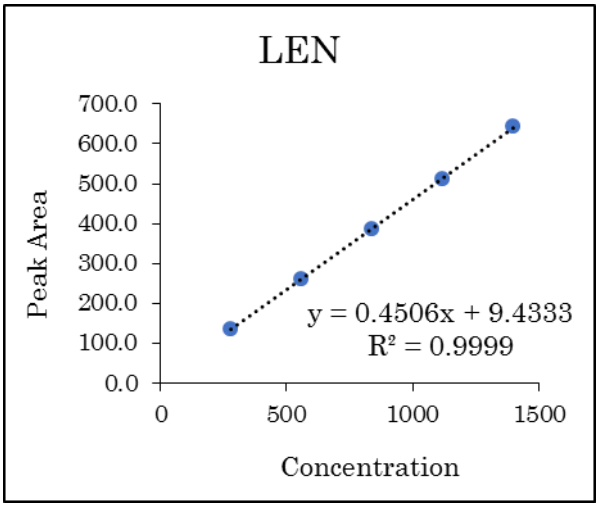


Figure 4: Calibration Curve of LEN.

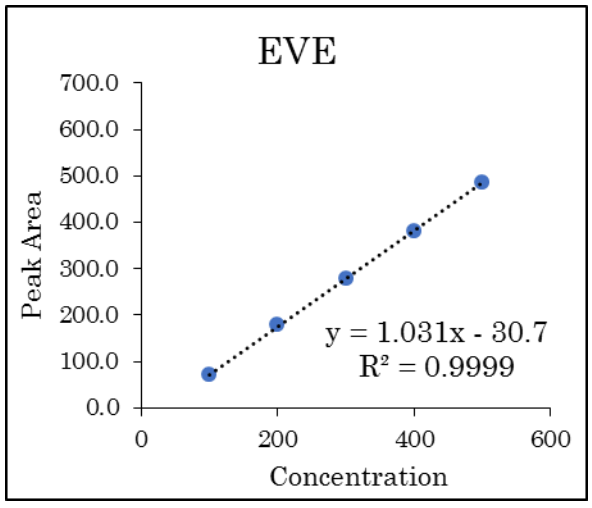


Figure 5: Calibration curve of EVE.

DISCUSSION

Linear correlation was obtained between absorbance vs. concentration of LEN and EVE in concentration range of **280 – 1400 & 100 – 500 ng/band,** respectively. The linearity of calibration curve was validated by high value of correlation coefficient of regression which was 0.999 for both the drugs.

B. Precision

Table 4: Precision data of LEN and EVE.

Repeatability			
Drug	**Concentration (ng/band)**	**Mean ± SD**	**%RSD**
LEN	840	386.50 ± 4.64	**1.20**
EVE	300	278.33 ± 3.72	**1.34**

Drug	**Concentration (ng/band)**	**Intraday**		**Interday**	
LEN	**280**	135.67 ± 1.53	**1.13**	135.33 ± 1.53	**1.13**
	840	387.34 ± 4.16	**1.07**	388.00 ± 3.61	**0.93**
	1400	643.67 ± 6.66	**1.03**	642.33 ± 5.51	**0.86**
EVE	**100**	72.00 ± 1.00	**1.39**	73.00 ± 1.00	**1.37**
	300	278.33 ± 4.04	**1.45**	278.33 ± 2.52	**0.90**
	500	484.67 ± 5.13	**1.06**	482.33 ± 4.51	**0.93**

Discussion: The lower RSD values of Precision study for LEN and EVE respectively, reveals that the proposed method is precise.

C. Accuracy

Table 5: Pre-analysed conc. of LEN & EVE.

Drug	**Conc. (ng/band)**	**Mean area**	**Pre-analysed conc. (ng/band) ± SD**	**%RSD**	**Mean Conc. obtained (%) ± SD**
LEN	500	235	500.60 ± 1.00	0.43	100.12 ± 0.44
EVE	200	176	200.48 ± 1.94	0.97	100.24 ± 0.97

Table 6: Recovery study of LEN & EVE (n=3).

Level (%)	**LEN**					**EVE**				
	Std conc. spiked	**Total conc. taken**	**Mean % Recovery at diff. levels ± SD**	**Overall Mean % Recovery (%± SD)**	**% RSD**	**Std conc. spiked**	**Total conc. taken**	**Mean % Recovery at diff. levels ± SD**	**Overall Mean % Recovery (%± SD)**	**% RSD**
80	400	900	100.24 ± 1.40	99.74 ± 1.16	**1.16**	16	36	99.62 ± 1.26	99.72 ± 1.03	**1.03**
100	500	1000	99.47 ± 0.32			20	40	99.58 ± 1.22		
120	600	1100	99.91 ± 1.24			24	44	99.96 ± 1.02		

Discussion: Obtained results reveals that % recovery of LEN and EVE are within acceptance criteria given in ICH i.e., 98-102%.

D. Limit of Detection and limit of quantification.

Table 7: LOD and LOQ for LEN and EVE (n=3).

Drug	SD	Mean	LOD	LOQ
LEN	0.55	0.450	4.03	12.22
EVE	2.19	1.03	7.02	21.27

Discussion: LOD was found to be **4.03 ng/band** and **7.02 ng/band** respectively and LOQ was found to be **12.22 ng/band** and **21.27 ng/band** for LEN and EVE respectively. The proposed method can detect and quantify small amount of drugs precisely. So, it was concluded that the proposed method is very sensitive in nature.

E. Analysis of LEN and EVE in Synthetic Mixture: (Assay)

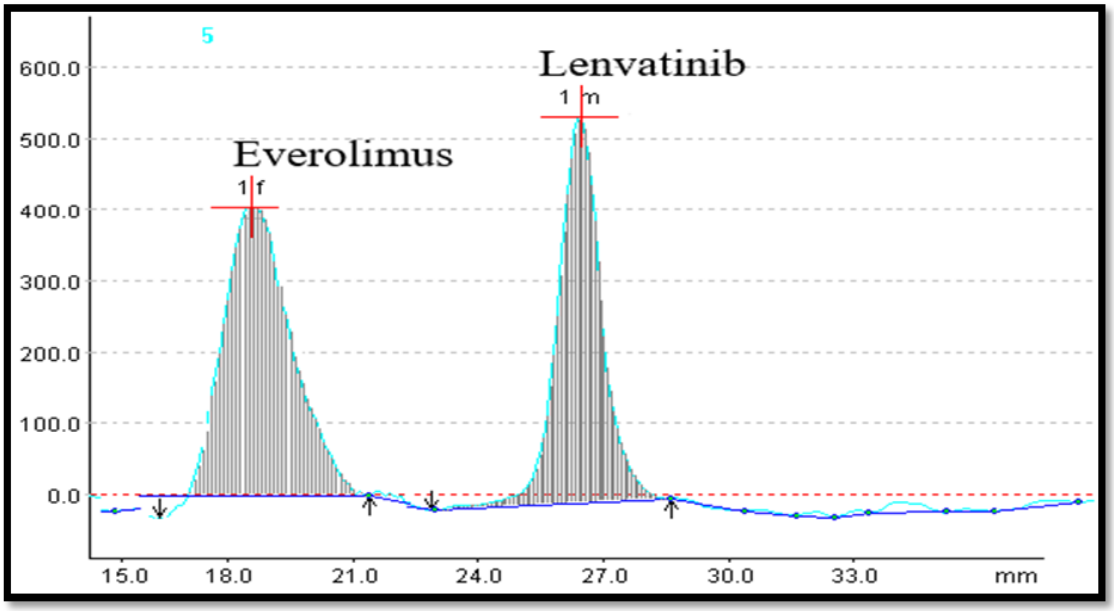


Figure 6: Chromatogram of Synthetic Mixture of LEN and EVE (840 & 300 ng/band).

Table 8: Analysis of LEN and EVE (n=6)

Drug	Label claim (mg)	Conc. Taken (ng/band)	% Purity ± SD	% RSD	Label Claim found
LEN	14	840	100.06 ± 1.04	1.04	14.01 mg
EVE	5	300	100.24 ± 1.25	1.25	5.01 mg

Discussion: Formulation claims to contain 14 mg LEN and 5 mg EVE. %Assay of LEN and EVE was found in an acceptance limit so this method could be used for analysis of this combination.

F. Robustness

Table 9: Robustness Study of LEN and EVE (840 & 300 ng/band).

Parameter	Peak Area			
	LEN		EVE	
Optimized Condition				
Mean ± SD	387.33 ± 1.53		279 ± 3.00	
RSD	**0.39**		**1.08**	
Saturation Time (± 2 min)	**+ 2 min**	**-2 min**	**+ 2 min**	**-2 min**
Mean ± SD	387.00 ± 2.00	386.33 ± 2.52	280.33 ± 3.06	279.33 ± 3.51
RSD	**0.52**	**0.65**	**1.09**	**1.26**
Mobile Phase Composition (± 0.2 mL)	***3.8:4.2:2***	***4.2:3.8:2***	***3.8:4.2:2***	***4.2:3.8:2***
Mean ± SD	388.33 ± 1.53	383.67 ± 1.53	278.67 ± 2.52	276.33 ± 1.53
RSD	**0.39**	**0.40**	**0.90**	**0.55**

Discussion: The study suggested that all the parameters have no significant influence on the determination. Results indicates that the selected factors remained unaffected by small variations and % RSD was less than 2 %, which demonstrates that the proposed method was robust.

Table 10: Result summary of HPTLC method for LEN and EVE Combination.

Parameters	LEN	EVE
Concentration range (ng/band)	280 – 1400	100 – 500
Slope	0.45	1.03
Intercept	9.43	-30.70
Correlation coefficient (R^2)	0.999	0.999
Precision (% RSD)	< 2.0	
Accuracy (% Recovery) ± SD	99.74 ± 1.16	99.72 ± 1.03
LOD (ng/band)	4.03	7.02
LOQ (ng/band)	12.22	21.27
%Assay ± SD	100.06 ± 1.04	100.24 ± 1.25
Robustness (% RSD)	< 2.0	

CONCLUSION

This study reports a simple, fully validated HPTLC protocol for the quantification of the Lenvatinib and Everolimus in synthetic mixture. It demonstrates that the method can accurately quantify the drug content of the synthetic mixture without any interference of excipients. All the data which was obtained from the different validation parameters indicated that the method is precise and accurate hence method can be applied for the routine analysis of the combination of the drug combination.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

1. Mathur, G. 2015. Cancer: A Review. Academic Journal of Cancer Research, 8(1): 1-9.
2. Bordoloi, D. 2021. Introduction and basic Concepts of Cancer. Cancer cells chemoresistance and Chemosensitization.
3. Kumari. M. 2020. Cancer Notes. 1-15.
4. Moch, H. The 2016 WHO classification of tumours of the urinary system and male genital organs part-A: renal, Penile, and testicular tumours, 70(1): 93-105.
5. MacDougall, ML. 1987. Renal adenocarcinoma and acquired cystic disease in chronic hemodialysis patients, 9: 166-171.
6. Bianchi, M. 2012. Distribution of metastatic sites in renal cell carcinoma: a population-based analysis, 23: 973–80.
7. Amparo, S. 2017. Systematic Treatment of Renal Cell Cancer: A Comprehensive Review. Cancer Treatment Reviews, 60: 77-89.
8. "Drug profile of Lenvatinib", Jan 2023 https://go.drugbank.com/drugs/DB09078
9. "Drug profile of Lenvatinib", Jan 2023 https://pubchem.ncbi.nlm.nih.gov/compound/11237762#section=Chemical-Disease-Co-Occurrences-in-Literature
10. "Drug profile of Lenvatinib", Jan 2023 https://en.wikipedia.org/wiki/Lenvatinib
11. "Drug profile of Everolimus", Jan 2023 https://go.drugbank.com/drugs/DB01590
12. "Drug profile of Everolimus", Jan 2023 https://pubchem.ncbi.nlm.nih.gov/compound/6442177#section=Therapeutic-Uses
13. "Drug profile of Everolimus", Jan 2023 https://en.wikipedia.org/wiki/Everolimus
14. Chauhan, A. 2015.Analytical Method Development and Validation: A Concise Review. Analytical & Bioanalytical Techniques, 6(1): 1-5.
15. Lavanya, G. 2013. Analytical Method Validation: An Updated Review. IJPSR, 4(4): 1280-1286.