Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://saudijournals.com/

Separation and Quantitation of Rapamycin, Termsirolimus Regio Isomer (Monoester) and Termsirolimus Diester in Termsirolimus by Normal Phase HPLC

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Original Research Article

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Article History
Received: 12.09.2018
Accepted: 26.09.2018
Published: 30.09.2018

DOI:
10.36348/sjmps.2018.v04i09.013

Abstract: Normal-Phase High Performance Liquid Chromatography method was developed for determination of Rapamycin, Temsirolimus Regio isomer (monoester) and Temsirolimus diester in Temsirolimus drug and pharmaceutical formulations. The separation was accomplished on YMC Pack SIL (250 x 4.6 mm, particle size 5µm) column under isocratic mode. The column oven temperature was set at 35° C and Auto sampler temperature used at 5°C. The mobile phase is a mixer of n-hexane, ethanol and trifluoroacetic acid in the ratio of 90:10:0.01 and PDA detector set at 280 nm used for detection. The retention times of Rapamycin, Temsirolimus peak, Temsirolimus monoester and Temsirolimus diester peaks are 12.29, 21.24, 33.11 and 51.91 min. respectively. The method has been fully validated and is linear. Results of analysis are validated statistically and by recovery studies. During the process development of Temsirolimus, three process impurities (Rapamycin. Temsirolimus monoester and Temsirolimus diester) are detected by high performance liquid chromatography (HPLC). This method offers advantages over using photodiode-array UV detection (LC-PDA) for the determination of peak purity, namely components with similar UV spectra can be distinguished, the molecular mass of the impurity can be determined, and structural data can be obtained by using LC-MS. The result of studies shows that the proposed normal phase-HPLC method is found to be precise, linear, accurate, rugged, selective, specific, and robust. Hence this method may be used for the routine analysis in bulk drug and in its pharmaceutical dosage forms.

Keywords: Temsirolimus, Rapamycin, Temsirolimus Regio isomer, Temsirolimus diester, Process impurities, Normal phase Liquid chromatography, Validation.

INTRODUCTION

Renal cell carcinoma (RCC) is the most common malignancy of the kidney and accounts for 2%-3% of all adult cancers [1]. Although surgical resection can be curative in localized disease, prognosis of advanced renal cell carcinoma is very poor with a 5-years survival rate of 5%-10%. Immunotherapy with interferon-α has produced modest survival benefice in clinical trials [2-7]. High dose interleukin-2, though active in highly selected patients, is associated with severe toxicity [8, 9]. Phase III studies since 2007 have emphasized the importance of targeting angiogenesis through Vascular Endothelial Growth Factor Receptor (VEGFR), tyrosine kinase inhibition with sunitinib [10] and sorafenib [11] or direct VEGF inhibition with bevacizumab in combination with IFN [12, 13]. These anti angiogenic agents have demonstrated improved overall survival (sunitinib) [14] or progression free survival (Sorafenib [15] and bevacizumab/IFN) [16-17] for patients with advanced RCC. The mammalian target of rapamycin (mTOR), a member of the phosphatidyl inositol 3 kinase family, is a multifunctional serine-threonine kinase that acts as central regulator of cell growth, proliferation and apoptosis [18, 19]. It modulates the expression and stability of hypoxia-inducible factor (HIF)-1α, which regulates expression of VEGF. Temsirolimus (known as cci-779) is a potent and selective inhibitor of mTOR. It has demonstrated its efficacy as first line immunotherapy in poor prognosis metastatic RCC in comparison with IFN [20]. It is a derivative of sirolimus and is sold as Torisel TM, 25 mg/ml concentrate and diluent for an infusion solution in the treatment of advanced RCC. The recommended dose of Temsirolimus is 25 mg infused over a 30 to 60-minute period once weekly. Temsirolimus (sirolimus-42-[2,2-bis-(hydroxymethyl)]-propionate) is an ester analog of rapamycin, a natural macrolide antibiotic with antifungal, antitumor, and immunosuppressive activities. Temsirolimus has demonstrated significant inhibition of tumour growth both in vitro and in vivo. It binds to the cytoplasmic protein FKBP, forming a complex that antagonizes the mammalian target of rapamycin (mTOR) signalling pathway [21] which consequently inhibits many of the downstream process affected by mTOR kinase activity, including transcriptional and translational control of important cell cycle regulators, resulting in cell cycle arrest [22].

Organic non-polar impurities Rapamycin, Temsirolimus monoester (Ts monoester) and Temsirolimus diester (Ts diester) are process-related impurities that may be present in the Temsirolimus drug substance. Their profiles are influenced by the choice of synthetic route; the quality of starting materials, reagents and solvents, the reaction conditions, the work-up and final purification and the design of process equipment. Since impurities can have safety and efficacy implications, they are the subject of considerable attention by both the manufacturer and regulatory agencies [23–25]. Thus, an analytical method to go together with process design is needed to detect and identify these impurities. In addition, the importance of qualifying impurity profiles is also relevant to the development scientists to ensure consideration given to the impurities present in batches being used in safety studies.

Historically normal-phase separation was the earliest form of HPLC. Normal-phase separations have been given less attention, recently, due to the belief that it is complicated and unpredictable. But normal-phase chromatography is a powerful tool for the separation of positional isomers that are difficult to separate by reverse-phase mode. Due to rigid surface in comparison with the more flexible carbon chains of reversed-phase stationary phases the analytes are affected by well-defined steric interaction with polar groups. Rapamycin, TS monoester, TS diester and Temsirolimus (TS) are non-polar, poorly water soluble, non-ionizable and have similar structures like positional isomer compounds preferably analysed by normal phase chromatography. No normal phase analytical method for determination of Rapamycin, TS monoester and TS diester in Temsirolimus drug substance is reported in literature [26-28].

The purpose of this work is to develop and validate a highly sensitive, specific and reproducible method for the determination of Rapamycin, TS monoester and TS diester that may be present in Temsirolimus drug substance in a single normal phase chromatographic run. During the process development of Temsirolimus, three process impurities (Rapamycin. Temsirolimus monoester and Temsirolimus diester) were detected by high performance liquid chromatography (HPLC) [29]. This method offers advantages over using photodiode-array UV detection (LC-PDA) for the determination of peak purity, namely components with similar UV spectra can be distinguished, the molecular mass of the impurity can be determined, and structural data can be obtained by using LC-MS. This could potentially improve the efficiency of the analysis and reduce laboratory supply costs associated with revalidating and testing of methods for individual impurities.

EXPERIMENTAL

Reagents and chemicals

The water used to prepare the solutions has been purified by a Milli-Q system (Millipore). n-Hexane (HPLC grade), Methanol (HPLC grade) and Acetonitrile (HPLC grade) were purchased from Rankem (Ranbaxy India). Trifluoro acetic acid (HPLC grade), Acetic acid (HPLC grade), Ethanol (HPLC grade) and ammonia (HPLC grade) were purchased from Sigma-Aldrich Ltd. Rapamycin, Temsirolimus monoester, Temsirolimus diester and Temsirolimus drug sample were obtained from Hetero drugs Limited Hyderabad, India as gift samples.

High Performance Liquid Chromatography (analytical)

A Waters HPLC system equipped with Alliances 2695 series low pressure quaternary gradient pump along with photo diode array detector and auto sampler has been used for the analysis of samples. The data was collected and processed using Waters Empower 3 software. The YMC PACK SIL (250 X 4.6 mm, 5µm, YMC, America, Inc) column was employed for the separation of impurities from Temsirolimus. The column eluent was monitored at 280 nm. A simple isocratic Normal-Phase HPLC method was optimized for the separation of impurities from Temsirolimus active pharmaceutical ingredient, where the mobile phase ratio was a mixture of n-Hexane, ethanol and trifluoroacetic acid in a ratio of 90:10:0.01, v/v. Chromatography was performed at column oven temperature 35˚C with a flow rate of 1.5 mL min^{-1}, auto-sampler temperature 5°C and injection volume 10 µL. The chromatographic run time is 60 min.

Mass spectrometry (LC-MS/MS)

The LC-MS analysis was carried out on AB Sciex Instruments 4000 Q TRP Linear Ion Trap Quadrupole LC/MS/MS Mass Spectrometer connected with Waters HPLC system equipped with Alliances 2695 series with PDA detector. The YMC PACK SIL (250 X 4.6 mm, 5µm, YMC, America, Inc) column was used for the separation. A simple isocratic Reverse-phase HPLC method was optimized for the separation of impurities from Temsirolimus active pharmaceutical ingredient where the mobile phase ratio was a mixture of water, methanol, acetonitrile and trifluoroacetic acid in a ratio of 15:70:15:0.01, v/v. Chromatography was performed at column oven temperature 35˚C at a flow rate of 1.5 mL min^{-1}, auto-sampler temperature 5°C and injection volume 10 µL. The chromatographic run time was 60 min. After chromatographic separation the mobile phase was directly introduced into the mass spectrometer via electro-spray ionization (ESI) source operating in the positive and negative mode. Identification was performed using Q1 MS (Q1) and product ion (MS2) scan types in order to optimize all the mass parameters. The curtain gas reached 20 psi.

The ion spray voltage was set at 4500V and temperature at 475 ˚C. The nebulizer gas (GS1) and turbo gas (GS2) were 45 and 55 psi. The declustering potential (DP) and entrance potential (EP) were 25 and 10 V. Data acquisition was carried out by analyst 1.4.2 software on a DELL computer.

Preparation of Diluent

Mixture of n-Hexane, ethanol and acetic acid in the ratio of 85:15:0.01

Preparation of system suitability solution

This solution was prepared using previously prepared impure mixture of Temsirolimus monoester and Temsirolimus diester (0.15% of each impurity spiked with respect to Temsirolimus) of all three process-related impurities at the concentration of 1 mg/mL using mobile phase and injected into the system. The system suitability solution chromatogram is shown in Figure-1.

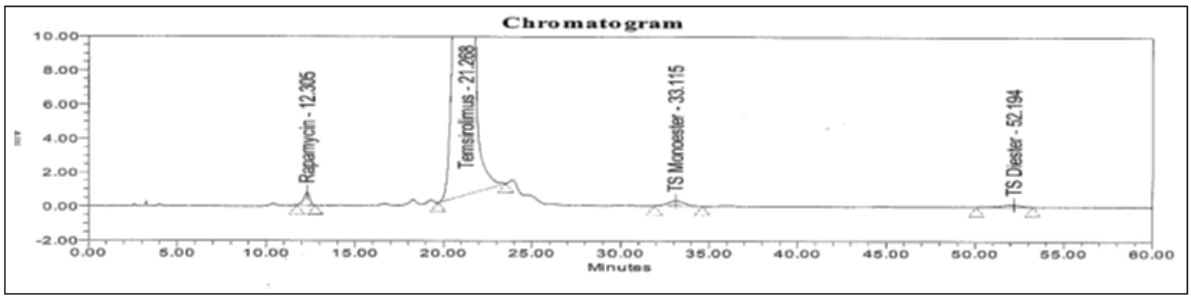


Fig-1: Chromatogram for System suitability solution

Preparation of standard solution

0.0015 mg/mL solution of Temsirolimus, working standard, is prepared using mobile phase and injected into the system.

Preparation of sample solution

1 mg/mL solution of Temsirolimus sample was prepared using mobile phase and injected into the system.

RESULTS AND DISCUTION

Method Development

Separation of process of process-related impurities

All the three impurities detected were characterized using LC-MS/MS, FT-IR and NMR experimental data. Of these three process impurities, one impurity was previously known impurity Rapamycin, while other two process impurities were found to be novel and were characterized as Rapamycin, Temsirolimus Monoester and Temsirolimus Diester [30].In order to develop a suitable and robust LC method for the determination of Rapamycin, TS monoester and TS diester, different mobile phase compositions were utilised to achieve the best separation, during the development of the method, reverse phase stationary phase (C-18) with water and acetonitrile in the ratio of 30:70(v/v) as a mobile phase is studied and found not selective enough compared to normal phase separation, because Rapamycin, TS monoester, TS diester and Temsirolimus are chemically hydrophobic molecules with very low water solubility (2.6 µg/mL) and no ionizable functional groups over a wide pH range. Rapamycin, TS monoester, TS diester and Temsirolimus are non-polar and have similar structures of positional isomer compounds preferably analysed by normal phase chromatography. Among YMC-Pack SIL, YMC-Pack SIL - 06, YMC-Pack Diol-NP, YMC-Pack CN, YMC-Pack PVA- SIL, YMC-Pack NH2 and YMC-Pack PA-G columns, YMC-Pack SIL column was selected, since this column is suitable for separation of organic-soluble compounds using non-polar mobile phase and separation of positional isomers or compounds with similar structures that are difficult to separate in reversed-phase mode. SIL with pore size 60 Å has greater adsorption properties than SIL with pore size 120 Å due to its larger specific surface area, and is generally useful for separating of compounds with similar structures with a macro cyclic compound. The composition of the mobile phase was next examined using an initial isocratic method. As a preliminary guide to the selection of the mobile phase, the system suitability solution was injected into chromatographic system and elution was studied using mobile phase comprising tertiary mixture of n-hexane, ethanol and trifluoroacetic acid varying ratios, finally optimized the mobile phase composition ratio n-hexane: ethanol: trifluoroacetic acid (90:10:0.01), which was found to give good peak shape and retention time without sacrificing resolution. The critical pair under these conditions was Rapamycin and Temsirolimus. Further optimization was performed by varying column temperature. As would be expected, a decrease in retention was observed with increasing temperature. However, no significant improvements in resolution were observed with changes in temperature. If the temperature is kept lower than 25˚C resulted in high back pressure while resolution of several other impurities was sacrificed at temperature above 40˚C. As a result, a column temperature of 35˚C was chosen for further method development. Detection was performed at 280 nm for low noise level and baseline consistency purpose. All the anticipated impurities were expected to

absorb at this wavelength and therefore can be detected. Mobile phase of n-hexane: ethanol: trifluoroacetic acid (90:10:0.01% v/v) at a flow rate of 1.5 ml/min, YMC Pack SIL(250 x 4.6)mm, 5.0 µm was found to be good for the separation of Rapamycin, TS monoester, TS diester and Temsirolimus. One dimensional (1D) liquid chromatography (LC) has been widely adopted in the separation and identification of pharmaceutical compounds. However, this technique has proven to have limitations in selectivity and peak capacity when analysing chemically complex samples. Therefore, the use of multidimensional LC-MS can serve as an additional analytical tool providing enhanced peak capacity and selectivity and thus superior assessment of peak purity and impurity identification. Therefore, this method discusses the application LC-MS as a tool for peak purity assessment and as a technique that can be used to identify unknown impurities when using MS compatible chromatographic methods needed for demonstrating selectivity of the LC method and stability indicating nature as well, hence 2D-HPLC/MS provides orthogonality as well as a means of performing mass spectral analysis on the impurities and main chromatographic peak. At least the key predictive samples should be screened by both photodiode array and LC-MS mass detector. The samples and spiked samples were analysed in the system with PDA detector and LC-MS system to evaluate peak purity of Rapamycin, TS monoester, TS diester and Temsirolimus. Purity angle of Temsirolimus and impurities were found to be less than purity threshold and no additional mass at Temsirolimus and its impurities peak, hence no interference was found at know impurities and Temsirolimus peak. So Temsirolimus and known impurities peaks were spectrally pure. The purity data of known impurities and Temsirolimus peak indicates that the peaks are homogeneous and no co-eluting peaks indicating specificity of the method, hence this method was found to be stability indicating method. The chromatograms showing the separation of Rapamycin, TS monoester and TS diester in real sample and the real sample spiked with 0.15% w/w of Rapamycin, TS monoester and TS diester are shown in Figure 2. The LC/MS data gave the molecular ions of the impurities. The HPLC retention times (RT) and the structures are shown in Table-1.

Quantification of Rapamycin, TS monoester and TS diester

Known concentration of standard solution (0.0015mg/ml) was used for quantification of Rapamycin, Temsirolimus monoester and Temsirolimus diester in Temsirolimus sample (1.0 mg/ml). Not more than 0.12% w/w of Rapamycin, TS monoester and TS diester were found in Temsirolimus API.

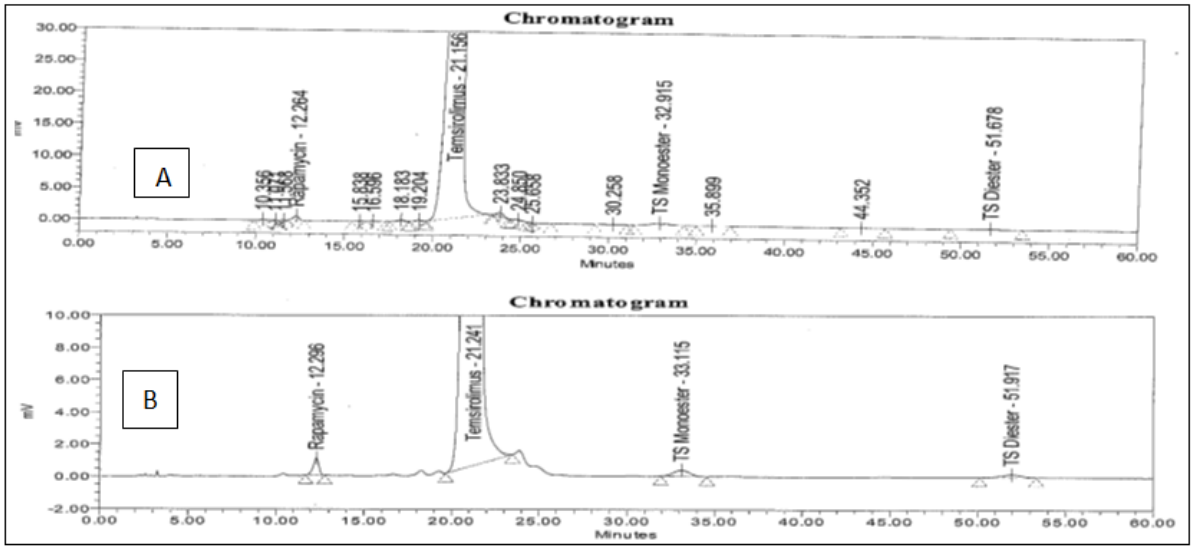
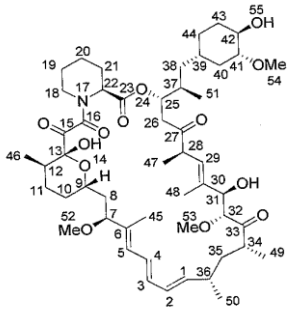
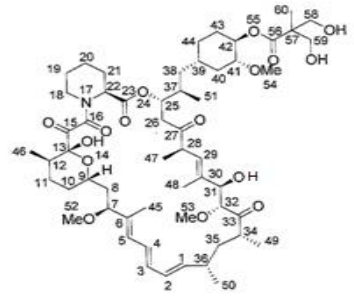
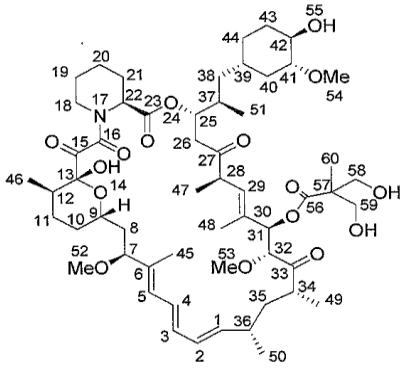
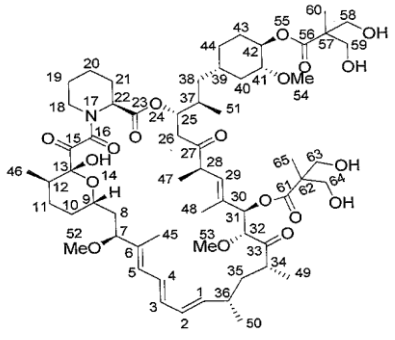


Fig-2: A - Chromatogram of real sample, B - the real sample spiked with 0.15% w/w of Rapamycin, TS monoester and TS diester

Table-1: Name of compound, Retention time, molecular weight and atom numbering of the impurities

Name	RT(HPLC)(min)	Structure	m/z
Rapamycin	12.29		913
Temsirolimus	21.24		1030
TS Monoester	33.11		1030
TS Diester	51.91		1146

Analytical Method Validation

Validation of the optimized normal phase HPLC method was done with respect to various parameters, as required under ICH guideline Q2 (R1) [30-31). This validation study covered selectivity, specificity, linearity, precision (system precision, method precision and intermediate precision), accuracy as recovery, range, stability in analytical solution and robustness.

Specificity

Blank, System suitability solution, standard solution, sample solution, spiked sample solution and individual impurity solutions were injected into system. No interference was observed from blank and placebo at retention time of Rapamycin, TS monoester, TS

diester and Temsirolimus peak. The purity Rapamycin, TS monoester, TS diester and Temsirolimus peak were found to be less than purity threshold in PDA detector analysis and no additional mass was observed at retention time of Rapamycin, TS monoester, TS diester and Temsirolimus peak in LC-MS analysis. The purity data of Temsirolimus peak indicates that the peak is homogeneous; hence the analytical method is specific.

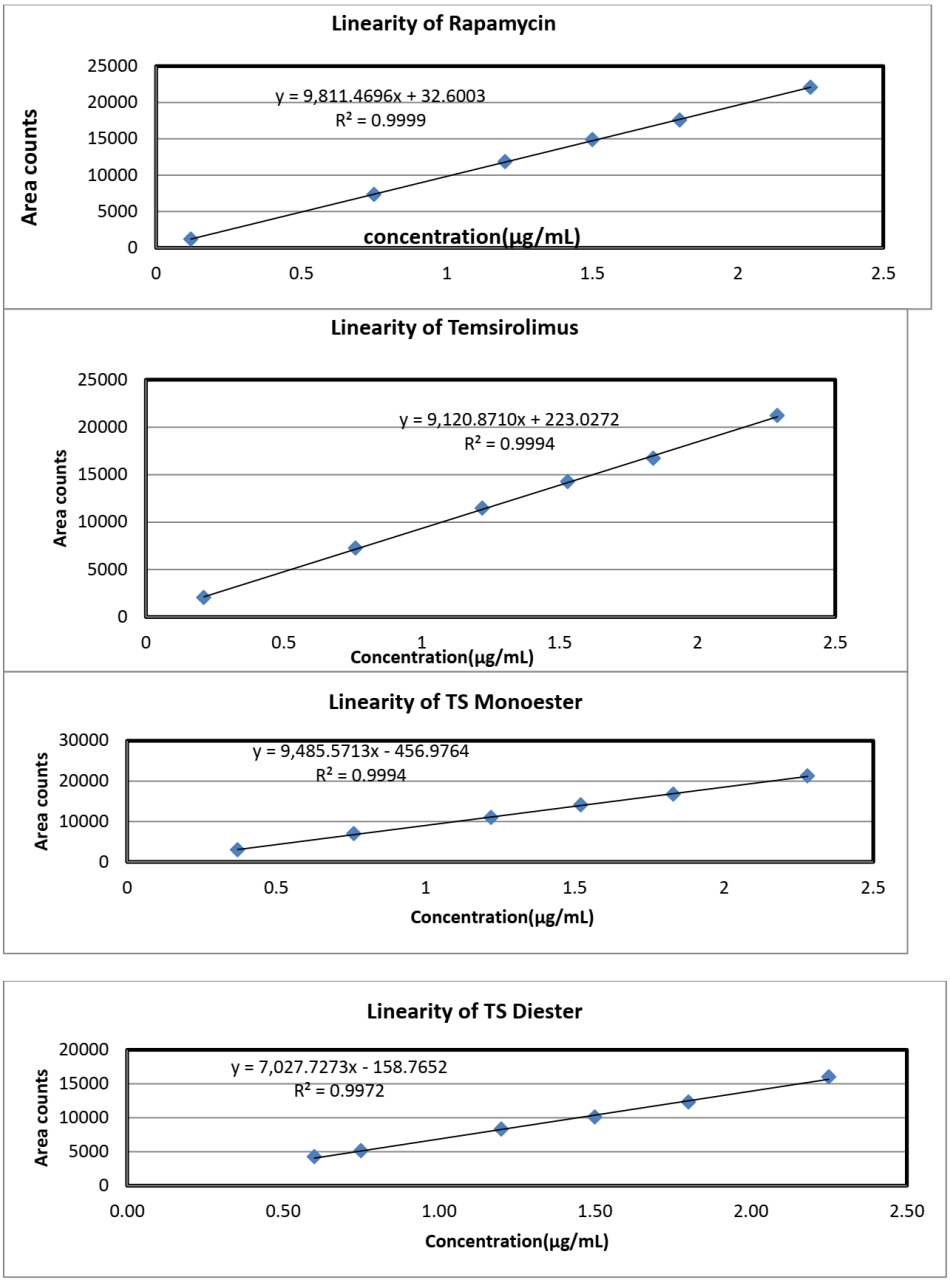


Fig-3: Calibration curve for Rapamycin, Temsirolimus, TS monoester and TS diester

Linearity

Linearity was established over specified range of the LOQ to the 150% of the specification limit (0.15% w/w) of individual components. Each sample solution was injected in triplicate. Standard solutions at six different concentration levels of Rapamycin (0.12, 0.75, 1.20, 1.50, 1.80 and 2.25µg/mL), Temsirolimus (0.27, 0.76, 1.22, 1.53, 1.84 and 2.29µg/mL), TS monoester (0.37, 0.76, 1.22, 1.52, 1.83 and 2.28µg/mL) and TS diester (0.60, 0.75, 1.20, 1.50, 1.80 and 2.25µg/mL) are injected into HPLC and their respective areas are noted. The respective concentrations were plotted against respective mean area counts to draw Linearity graph and correlation coefficient was calculated. The correlation coefficient for Rapamycin, Temsirolimus, TS monoester and TS diester were found to be 0.9999, 0.9997, 0.9997 and 0.9986 showing good linearity. All the calibration curves are shown in Figure-3.

Accuracy (as recovery)

The accuracy was performed by spiking respective impurity standards with Temsirolimus sample at LOQ, 50%, 100% and 150% of specification level. The solution was prepared in triplicate at each level and injected. The % recoveries and mean % recoveries of Rapamycin, TS monoester and TS diester were calculated. The individual % recoveries and mean % recoveries were found within 80.0%-120.0% at LOQ to 150% levels. Hence method is accurate. Accuracy results are shown in Table 2.

Table-2: Recovery results for Rapamycin, TS monoester and TS diester

Impurity Name	Spike level (%)	Amount added(mg)	Amount recovered(mg)	%Recovery	Mean % Recovery
Rapamycin	LOQ-1	0.00122	0.00125	102.5	101.9
	LOQ-2	0.00122	0.00121	99.2	
	LOQ-3	0.00122	0.00127	104.1	
	50% -1	0.00748	0.00771	103.1	100.6
	50%- 2	0.00748	0.00771	103.1	
	50% -3	0.00748	0.00715	95.6	
	100%-1	0.01495	0.01472	98.5	98.5
	100% -2	0.01495	0.01458	97.5	
	100% -3	0.01495	0.01486	99.4	
	150%-1	0.02164	0.02164	96.5	97.3
	150%-2	0,02176	0.02176	97.0	
	150%-3	0.02206	0.02206	98.4	
TS Monoester	LOQ-1	0.00374	0.00367	98.1	98.4
	LOQ-2	0.00374	0.00378	101.1	
	LOQ-3	0.00374	0.00359	96.0	
	50% -1	0.00759	0.00801	105.5	100.5
	50%- 2	0.00759	0.00764	100.7	
	50% -3	0.00759	0.00723	95.3	
	100%-1	0.01518	0.01370	90.3	90.6
	100% -2	0.01518	0.01351	89.0	
	100% -3	0.01518	0.01402	92.4	
	150%-1	0.02277	0.01974	86.7	88.0
	150%-2	0.02277	0.02010	88.3	
	150%-3	0.02277	0.02027	89.0	
TS Diester	LOQ-1	0.00623	0.00582	93.4	97.6
	LOQ-2	0.00623	0.00598	96.0	
	LOQ-3	0.00623	0.00645	103.5	
	50% -1	0.00751	0.00723	96.3	88.6
	50%- 2	0.00751	0.00628	83.6	
	50% -3	0.00751	0.00645	85.9	
	100%-1	0.01503	0.01386	92.2	90.8
	100% -2	0.01503	0.01253	83.4	
	100% -3	0.01503	0.01456	96.9	
	150%-1	0.02254	0.02070	91.8	96.4
	150%-2	0.02254	0,2099	93.1	
	150%-3	0.02254	0.02354	104.4	

Precision

System precision was performed by injecting six replicate injections of standard solution of Temsirolimus. The % RSD of six injections of standard solution was found to be 0.9%. Method precision was performed by analysing six sample preparations, spiked with Rapamycin, TS monoester and TS diester at specification level as per test procedure. The % RSD of six results of Rapamycin, TS monoester and TS diester were found to be 6.3, 3.6 and 4.0, respectively. Intermediate precision was performed by analysing six sample preparations, spiked with known Rapamycin, TS monoester and TS diester at specification level as per test procedure by a different analyst, on different days, on a different instrument, using a column of different serial no. The overall % RSD of twelve results of rapamycin, TS monoester and TS diester was found to be 5.6, 4.8 and 8.6 respectively. The results are shown in table-3.

Table-3: Precision results for Rapamycin, TS monoester and TS diester

	Precision			Intermediate Precision		
Sample preparation	Rapamycin (%w/w)	Monoester (%w/w)	Diester (%w/w)	Rapamycin (%w/w)	Monoester (%w/w)	Diester (%w/w)
1	0.16	0.22	0.15	0.15	0.21	0.12
2	0.18	0.22	0.16	0.15	0.20	0.14
3	0.16	0.21	0.15	0.15	0.22	0.14
4	0.16	0.20	0.15	0.15	0.20	0.14
5	0.15	0.22	0.15	0.16	0.21	0.12
6	0.16	0.22	0.14	0.16	0.20	0.13
Mean	0.16	0.22	0.15	0.15	0.21	0.13
%RSD	6.3	3.6	4.0	3.3	3.8	7.7
Overall Mean				0.16	0.21	0.14
Overall %RSD				5.6	4.3	8.6

Limit of Quantitation and Limit of Detection

The limit of quantitation(LOQ) and Limit of Detection(LOD) values of Rapamycin, Temsirolimus, TS monoester and TS diester were determined based on calibration curve plotted between concentration of respective components (Prepared below 50% of specification limit) and their respective responses. The respective LOD and LOQ of impurities were calculated from the residual standard deviation obtained from calibration curve. Precision at limit of quantization and verification of limit of detection value were performed and chromatograms are shown in Fig-4. The % RSD of LOD for Rapamycin, Temsirolimus, TS monoester and TS diester were found to be 14.4, 15.8, 4.7 and 5.9 respectively. The % RSD of LOQ for Rapamycin, Temsirolimus, TS monoester and TS diester were found to be 2.5, 4.9, 6.8 and 2.3 respectively. The results are shown in Table-4.

Table-4: LOD and LOQ values for Rapamycin, Temsirolimus, Monoester and Diester

Component	Concentration LOD(%w/w)	Concentration LOD(µg/mL)	Concentration LOQ(%w/w)	Concentration LOQ(µg/mL)
Rapamycin	0.006	0.06	0.012	0.12
Temsirolimus	0.008	0.08	0.027	0.27
TS monoester	0.012	0.12	0.037	0.37
TS diester	0.027	0.27	0.060	0.60

Analytical Solution stability

The standard solution is stable up to 17 hours at 5°C with cumulative % RSD of 2.5. The sample solution is unstable. It should be freshly prepared and injected. The spiked sample solution is stable up to 12 hours at 5°C with cumulative %RSD of 4.1.

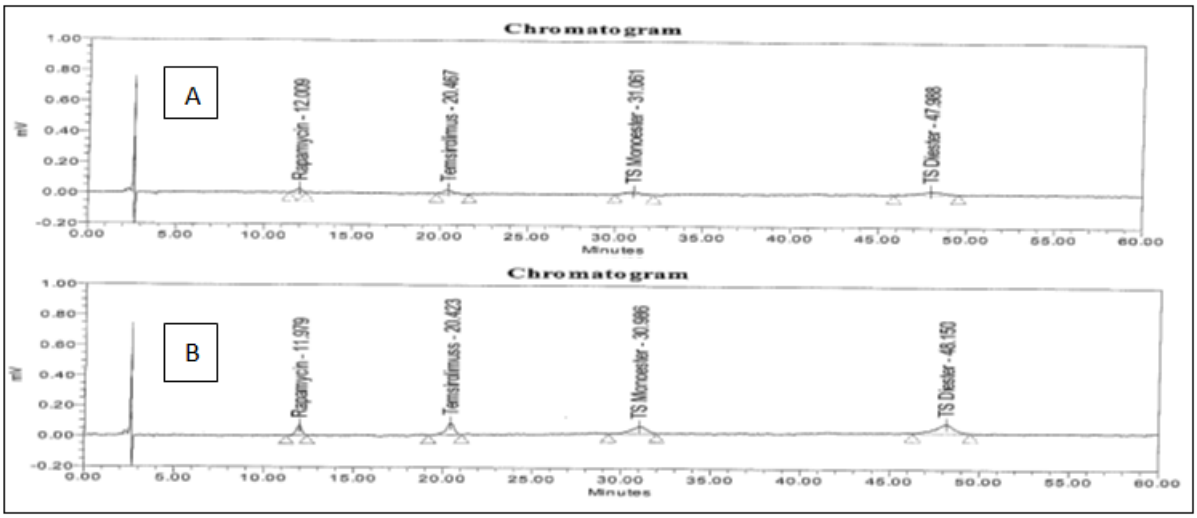


Fig-4: A - Chromatogram for LOD solution, B - Chromatogram for LOQ solution

Robustness

The robustness of the HPLC method for the determination of Rapamycin, Temsirolimus, TS monoester and TS diester in Temsirolimus was established by varying analytical conditions one at a time from the test method. System suitability parameters were monitored. The results are shown in Table-5. The system suitability parameters complied in every condition. The method was found to be robust.

Table-5: Results for robustness

Varying analytical conditions	System suitability parameter	Acceptance Criteria	Results
Change in column (ARD/145/15)	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.42
	USP tailing factor for Temsirolimus.	< 1.5	0.88
	% RSD of standard injections.	<10.0	0.9
Chang In column (ARD/147/15)	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.62
	USP tailing factor for Temsirolimus.	< 1.5	0.72
	% RSD of standard injections.	<10.0	4.8
Change in flow rate to 1.3 ml/min instead of 1.5ml/min	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.42
	USP tailing factor for Temsirolimus.	< 1.5	0.81
	% RSD of standard injections.	<10.0	1.5
Change in flow rate to 1,7 ml/min instead of 1.5ml/min	Resolution between Rapamycin and Temsirolimus.	> 8.0	10.98
	USP tailing factor for Temsirolimus.	< 1.5	0.82
	% RSD of standard injections.	<10.0	1.8
Change in detector wavelength to 277 nm instead of 280nm	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.33
	USP tailing factor for Temsirolimus.	< 1.5	0.79
	% RSD of standard injections.	<10.0	0.6
Change in detector wavelength to 283 nm instead of 280nm	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.29
	USP tailing factor for Temsirolimus.	< 1.5	0.78
	% RSD of standard injections.	<10.0	0.6
Change in column temperature to 33˚C instead of 35˚C	Resolution between Rapamycin and Temsirolimus.	> 8.0	10.78
	USP tailing factor for Temsirolimus.	< 1.5	0.80
	% RSD of standard injections.	<10.0	1.8
Change in column temperature to 37˚C instead of 35˚C	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.57
	USP tailing factor for Temsirolimus.	< 1.5	0.80
	% RSD of standard injections.	<10.0	1.1
Change in n-Hexane: Ethanol: TFA (88:12:0.01 instead of 90:10:0.01)	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.43
	USP tailing factor for Temsirolimus.	< 1.5	0.86
	% RSD of standard injections.	<10.0	2.4
Change in n-Hexane: Ethanol: TFA (92:08:0.01 instead of 90:10:0.01	Resolution between Rapamycin and Temsirolimus.	> 8.0	11.07
	USP tailing factor for Temsirolimus.	< 1.5	0.84
	% RSD of standard injections.	<10.0	2.8

CONCLUSION

This is a simple analytical method for quantitative estimate of Rapamycin, Temsirolimus monoester and Temsirolimus diester in Temsirolimus drug substance. With great detection power come great possibility – minimize the risk of unexpected co-elutions or components and confirm trace components with the analytical confidence of mass detection. Enhance the analytical value and productivity of each analysis. One dimensional (1D) liquid chromatography (LC) has been widely adopted in the separation and identification of pharmaceutical compounds. However, this technique has proven to have limitations in selectivity and peak capacity when analysing

chemically complex samples. Therefore, the use of multidimensional LC-MS can serve as an additional analytical tool providing enhanced peak capacity and selectivity and thus superior assessment of peak purity and impurity identification. This is a simple, cost effective, time saving and very effective means of enhancing the chromatographic detection of the compound. The present paper describes the development of new normal phase HPLC method for quantisation of Rapamycin, TS monoester and TS diester in Temsirolimus and its validation. The method was found to be selective, sensitive, precise and accurate for quantisation of Rapamycin, Temsirolimus monoester and Temsirolimus diester. This method can be used for the routine analysis as well as for stability studies to evaluate content of Rapamycin, TS Monoester and TS Diester in pharmaceutical quality control.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. This work and this article do not contain any studies with animals or human participants performed by any of the authors.

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