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RP-HPLC Method Development and Validation for the Estimation of Trelagliptin in Pure and Pharmaceutical Dosage Form

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Abstract: ***An accurate, sensitive, precise, simple isocratic reverse phase HPLC (RP-HPLC) method has been developed and validated for quantification of Trelagliptin in bulk and pharmaceutical tablet dosage forms. With acetonitrile as the organic solvent, the best separation was achieved on a 250 mmx 4.6 mm i.d, 5µ-particle size Inertsil®-Octadecyl-silyl-3V-Reverse-Phase-C_{18}-column with 0.02M Dipotassium hydrogen orthophosphate in water pH: 2.5 with Orthophosphoric acid: Acetonitrile (40:60v/v) in the isocratic mode of elution as mobile phase solvent at a speed of 1.0 ml/ min. UV detection was at 210 nm. Retention time of Trelagliptin was 12.8 minutes. With a correlation coefficient of about 0.9974, peak-response was obtained as function of concentration over the range of 80 to 240 µg/ ml for Trelagliptin. Trelagliptin was shown to have a percentage assay of 110.89 %. Trelagliptin had a limit of detection (LOD) of 0.2 µg/ mL and a limit of quantification (LOQ) of 0.6 µg/ ml. The presence of excipients in the formulation Zafatek had no effect on the assay method. The procedure is appropriate for use in QC- laboratories since it is economical and precise.***

Keywords: Trelagliptin, Zafatek, RP-HPLC, Isocratic, Dipotassium hydrogen Orthophosphate.

1. Introduction

Trelagliptin a novel dipeptidyl peptidase-4 inhibitor with the IUPAC name 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl} methyl)-4-fluorobenzonitrile is indicated in the control of type 2 diabetes mellitus. Its molecular formula is $C_{18}H_20FN_5O_2$ with molecular weight 357.4g/mol [1].

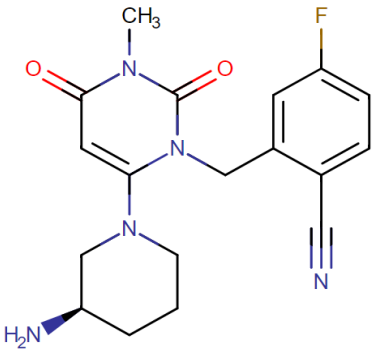

Figure 1: Structure of Trelagliptin

A prescription medication known by the brand name Zafatek, Trelagliptin varies from the parent compound Alogliptin due to the presence of 5-F substitution on cyanobenzyl group. Trelagliptin forms a non-covalent reversible bond with DPP-4 through competitive inhibition. It acts by inhibiting dipeptidyl peptidase- 4 to prevent the inactivation of incretin hormone (release of insulin) and increases the concentration of GLP-1 (glucagon like peptide) [2]. Trelagliptin is a crystalline solid soluble in organic solvents like DMSO and DMF upto 1-2mg/ml and in phosphate buffer saline pH 7.2 up to 1mg/ml [3]. Literature revealed that few spectroscopic and chromatographic techniques for the determination of Trelagliptin in oral fixed dosage form have been published [4-11]. Furthermore, no official or preliminary monograph on this analyte has been published in any of the compendial pharmacopoeias. The goal of this study was to develop a most simple, accurate, precise and efficient RP-HPLC method to estimate Trelagliptin in unit dosage forms for oral administration. The validation of the devised approach is also addressed in this study, as per ICH standards [12].

2. Experimental

Chemicals and Reagents:

- 99%, Trelagliptin pure was acquired from RN Laboratories Pvt. Ltd., Mumbai, India.
- Rankem-Fine-Chemicals of HPLC- Grade- Acetonitrile
- Orthophosphoric acid, 85% (v/v), Qualigen-Fine chemicals.
- Dipotassium hydrogen Orthophosphate, Qualigen-Fine chemicals.
- HPLC Grade water, Rankem-Fine chemicals.

Chromatographic-Instrument:

Quantitative RP- HPLC was carried out on a Waters 2996 high-performance liquid chromatograph with a PDA detector module, which included an automated injector with a 20 microliters injection volume and a quadra-pump. The column utilized was a Reverse Phase Inertsil Octa Decyl-S-3V-C_{18} column (250mmx 4.6 mm internal diameter with particle size 5µm). EMPOWER Software was installed on the HPLC equipment. The column temperature was adjusted to 25^0C and eluted over 24.0 minutes at a mobile solvent speed of 1.0 ml/

min under isocratic conditions. The mobile phase used was 0.02M Dipotassium hydrogen Orthophosphate in water pH:2.5 with Orthophosphoric acid: Acetonitrile (60:40 v/v). It was degassed and filtered via 0.45µm Nylon membrane filters before use. For the analyte, UV detection at 210 nm was used as wavelength of detection with a PDA detector. Elution solvent was used as diluent to make the standard dilutions. Trelagliptin was eluted at 12.8 minutes.

Preparation of the Primary Standard Drug solutions: To make the primary standard stock solution, 200 mg of Trelagliptin was dissolved in a volumetric flask (100ml) and diluted with the mobile phase, sonicated for 15 minutes and diluted up to 100ml with the diluent to get the primary standard stock solution containing 2000 µg/ ml of Trelagliptin.

Preparation of Working Standard Drug Solution: After adding 5 ml of the primary working standard solution to the 50-ml volumetric flask, the flask was filled with 50 ml of the diluent. This resultant mixture, which includes 200 µg/ml of Trelagliptin, was suitable for use as a working standard solution. The stock solutions were kept in a cool, dark place that was controlled at four degrees Celsius.

Sample Preparation: After measuring the weight of each individual tablet, the average weight of twenty Zafatek® pills was calculated. Crushing the tablets into a powder form obtained a sample containing 200-mg of Trelagliptin, which was then weighed, shifted to a 100ml pre-calibrated-measuring flask, and dissolved in a blend of 0.02M Dipotassium hydrogen Orthophosphate in water pH: 2.5 with Orthophosphoric acid: Acetonitrile (60:40 v/ v). After being sonicated in the diluent and strained via Whattman 41 filter paper, the resultant primary working sample solution has 2000 200 µg/ml of Trelagliptin. After quantitatively transferring 5ml of the filtrate to a 50ml pre-calibrated-measuring flask, the diluents were added to bring the volume of the solution to 50 ml. This served as a working testing solution having 200 µg/ml of Trelagliptin. The stock solution was kept in a dark place at 4 degrees centigrade.

3. Results and Discussion

The purpose of this research was to create a chromatographic technique for the quantifiable determination of fixed-dose of Trelagliptin.

Optimized Chromatographic Conditions:
Elution solvents: 0.02 M Dipotassium hydrogen orthophosphate in water pH: 2.5 with Orthophosphoric acid: Acetonitrile (60:40 v/v)
Elution mode: Isocratic
Column: Inertsil ODS C-18-3V (250 x 4.6mm, 5µm particle size)
Flow rate: 1.0 ml/ min
Injection volume: 20 µl
Detector: Photo diode array (PDA)
Wavelength (λ_{max}): 210nm
Column temperature: Ambient
Diluent: 0.02M Dipotassium hydrogen Orthophosphate in water pH: 2.5 with Orthophosphoric acid: Acetonitrile (60:40 v/v)
Run time: 24 minutes
Retention time: 12.8 mins

Linearity: Aliquots of Trelagliptin working stock solutions was placed in various 10ml volumetric flasks and the volume was made up to the 10ml with the mobile phase, yielding in final strengths of 80-240 µg/ml (Table 2). The peak areas and retention times of each of these drug solutions (loaded at 20µL) were measured thrice in the column. Using a PDA-detector set at 265 nm, a linearity-graph was generated by plotting peak areas-vs- Trelagliptin concentrations in µg/ml.

Accuracy: The accuracy of the method was found by evaluating the drug recovery using the standard-spiking method. To assess if the analyte contained in the formulation caused positive or negative interventions, known amounts of the drug equivalent to 12 percent standard drug solution was added to 80 percent, 100 percent and 120 percent of the target test concentrations of the formulation. Each set-of-addition was replicated thrice at each dilution level. The results were compared to a competent reference standard after extraction of sample preparation. The percentage of analyte recovered by the assay was used to assess the accuracy. Table-3 shows the results of accuracy investigations on standard solution and process-related impurity; recovery measurements suggest that the procedure was accurate.

Precision: Quality-control samples in 100 % (w/v) dilution were used to assess intraday and inter-day precision. On the same day, six replicates of the target concentrations were examined for intra-day variation, and six replicates were examined for inter-day variation on three different days. The method's repeatability was indicated by the low RSD value (1%). (Table-4)

Limits of Detection and Quantification: The method's LOD was set at the lowest concentrations of active pharmaceutical component with a signal-to-noise (S/N) ratio of around 3. (LOD). The lowest active therapeutic medication concentration that can be assessed with acceptable precision and accuracy while maintaining a signal-to-noise (S/N) ratio of roughly 10 (LOQ) was also determined.

Method Applicability: The newly created method was evaluated by applying it to pharmaceutical tablets for the estimation of Trelagliptin.

Optimization of Chromatographic Conditions:
An isocratic RP- HPLC procedure for assaying the active ingredients was developed due to lack of an easy, economical, reproducible, and quick-to-use method for the determination of Trelagliptin concentrations in formulary matrices. The effect of various HPLC technique variables was examined on the result of the study to optimize the chromatographic parameters, various proportions of CH_3CN-KH_2PO_4-H_3PO_3, CH_3CN-H_2O, and CH_3CN: *O*-H_3PO_3 buffer were tested. After

several early investigatory tests, 0.02M Dipotassium hydrogen Orthophosphate in water pH:2.5 with Othophosphoric acid: Acetonitrile binary system at the proportion of (60:40 v/ v) was chosen over other mobile phases because it resulted in improved resolution of active component. This procedure gave the good detection of analyte after multiple exploratory & investigatory trail runs. The active pharmaceutical analyte had excellent UV sensitivity and was interference-free at 210 nm. The analyte peak was highly defined and showed no incidence of tailing under these conditions. The set of conditions previously noted in this article were chosen for additional validation after considering the entire body of data acquired from this extensive study.

Method Validation Tests:
Method precision (RSD percent), method accuracy (recovery percent & % RSD,), linearity range (r^2) and LOD & LOQ were explored as recommended method validation characteristics.

Linearity: With a correlation coefficient of 0.9974, the graph of chromatographic-peak areas of the analyte versus respective concentration was shown to be linear in the band of 80-240 µg/ml for Trelagliptin (Table 2). The least square fit data of linear regression analysis derived from the measurements is given in Table 1. Trelagliptin is y = 56491x-302508. Table 1 presents the regression parameters for this technique that include slope, intercept, and % RSD. These findings suggest that there was a significant correlation (Fig 3).

Accuracy: Individual recovery of analyte at 80 %-dilution level on w/v basis, 100 %-dilution level on w/v basis and 120 %-dilution level on w/v basis of prescribed concentrations was 95.33 % to 118 % for Trelagliptin demonstrating the method's accuracy. The % RSD was usually less than 1% in these data, demonstrating that the technique seems to be very accurate and generates consistent results (Table 3).

Precision: Table 4 summarizes the intraday and interday fluctuation in precision analysis. The method's repeatability is indicated by the low RSD value (less than-1%). These results show that the approach has a high level of precision and repeatability, both within a single analytical run and across multiple runs.

Limit-of-Detection & Limit-of-Quantifications:
Trelagliptin has a limit of detection (LOD) of 0.2 µg/ml and a limit of quantification (LOQ) of 0.6 µg/ml. These numbers illustrate the method's high sensitivity, which is essential in most investigations, as well as the fact that it can be used to detect and quantify the analyte over a wide concentration range.

Specificity: The Retention time for Trelagliptin was determined to be 12.8 minutes, according to the representative chromatogram given in Figure 2. When the pharmaceutical tablet matrices were evaluated, no indication of excipient interference signal was observed in the respective retention time of the chromatogram. It indicates that the analyte was not disturbed of probable merging peaks. As a result, this technique can be employed with certainity.

Table 1: Regression analysis & Operating-System Suitability Results

Study-Parameter	Trelagliptin
Retention Time (min)	12.8
Peak areas	10548737
Percentage of peak areas	98.39
USP-Tailing	1.14
Theoretical Plates	10701.62
Resolution	21.11
Linear range in µg/ ml	80-240
Limit-of-Detection in µg/ ml	0.2
Limit-of-Quantification in µg/ml	0.6
Correlation-Coefficient (r^2)	0.9974
Assay-in-Percentage (%)	110.89

Table 2: Summary of the standard calibration Curve for Linearity experiment

Calibration Standard Dilution Level	Concentration of Trelagliptin (µg/ ml)	Peak Area
40 %	80	4260181
60 %	120	6385279
80%	160	8637292
100 %	200	11292764
120 %	240	13104603

Table 3: Accuracy evaluation by Spike-analysis method

Accuracy study at 80% target level	Injection Number	Trelagliptin	
		Standard Soln.	Spiked Soln.
Zafatek® tablet dosage form solution at 80% level was spiked with 12% of standard solution of API	1	8424185.59	9655065.44
	2	8502336.09	9614151.29
	3	8486889.18	9596625.46
	Mean area	8470809	9631847
	Std. Dev	35607	29461.00
	% RSD	0.39	0.25
	% Recovery		95.33%
80% of the target concentration is equivalent to 160 µg/ ml in the mobile phase as diluent.			
Accuracy study at 100% target level	Injection Number	Trelagliptin	
		Standard Soln.	Spiked Soln.
Zafatek® tablet dosage form solution at 100% level was spiked with 12% of mixed standard solution of API's	1	10504069.08	11701346.76
	2	10570168.72	11773819.76
	3	10472457.12	11737856.86
	Mean area	10558893	11763834
	Std. Dev	12728	7759
	% RSD	0.98	**0.12**
	%Recovery		104.16
100% of the target concentration is equivalent to 200 µg/ ml in the mobile phase as diluent.			
Accuracy study at 120% target level	Injection Number	Trelagliptin	
		Standard Soln.	Spiked Soln.
Zafatek® tablet dosage form solution at 120% level was spiked with 12% of mixed standard solution of API's	1	12460431.02	14246664.35
	2	12944796.56	14294438.47
	3	12776466.23	14075461.92
	Mean area	12733208	14159368
	Std. Dev	28996	27305
	% RSD	0.19	0.18
	%Recovery		118.0
120% of the target concentration is equivalent to 240 µg/ ml in the mobile phase as diluent.			

Table 4: Evaluation of precision with-in-day and day-to-day analysis

Intra-Day Precision study of 100% standard dilution containing 200 µg/ ml of Trelagliptin			Inter-Day Precision study of 100% standard dilution containing 200 µg/ ml of Trelagliptin	
S. No	Trelagliptin		Trelagliptin	
	Ret. time	Peak area	Ret. time	Peak area
1	13.233	10436333.60	12.947	10322096.62
2	13.161	10478781.28	12.923	10351304.13
3	13.098	10416144.11	12.900	10392331.18
4	13.051	10460532.29	12.899	10421984.06
5	13.023	10513260.36	12.868	10370306.84
6	12.973	10418056.58	12.857	10424412.68
Average	13.090	10455670	12.899	10387099
Std. Dev	0.095	28020	0.034	13383
% RSD	0.73	**0.33**	0.26	0.11

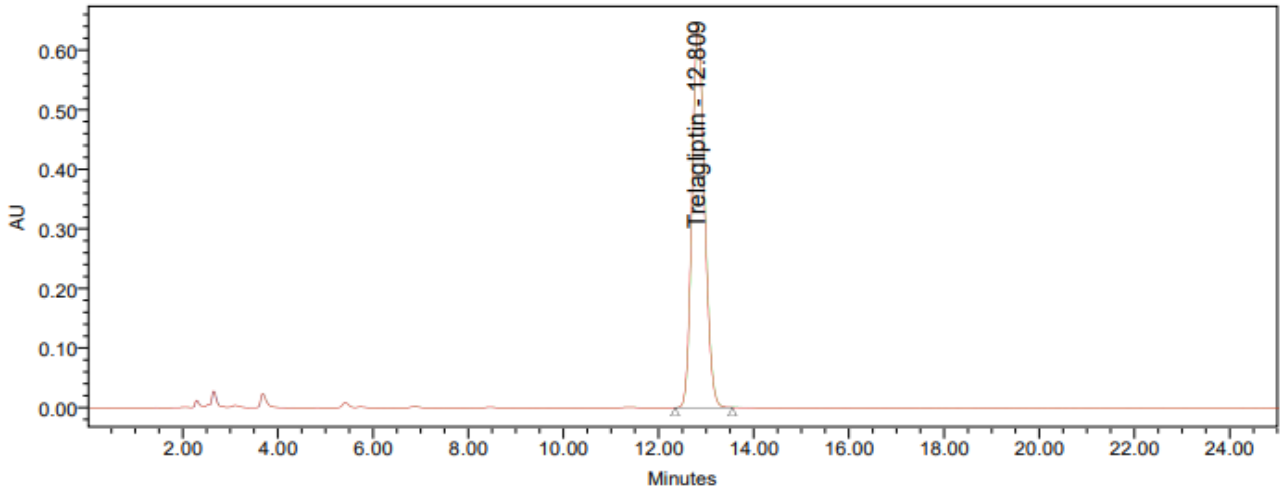

Figure 2: Chromatogram of Trelagliptin 200µg/mL analyzed by optimized Isocratic RP-HPLC method

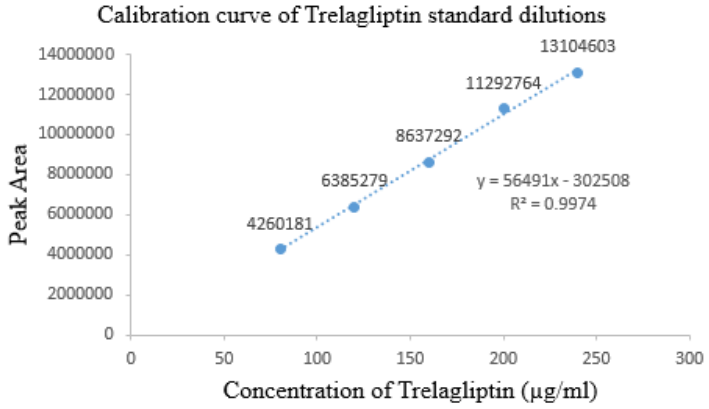

Figure 3: Linearity graph of Trelagliptin standard solutions

4. Conclusion

In this study, an economical, efficient and commonly available HPLC method for the analysis of Trelagliptin in pharmaceutical matrices was devised. This method's key advantages are its significantly reduced cost and ease of operation. All these features are critical in operation, especially when analyzing a large number of samples. The validation experiments demonstrated that the procedural approach has a large calibration concentration range, adequate precision & accuracy, and practically reliable sensitivity. The method can be used for regular analysis in formulation QC-studies and allows for a straightforward, selective, sensitive, and specific assessment of Trelagliptin.

References

[1] https://pubchem.ncbi.nlm.nih.gov/compound/15983988.
[2] Deepthi Ramesh, Balaji Gowrivel Vijayakumar, Tharanikkarasu Kannan. Therapeutic potential of uracil and its derivatives in countering pathogenic and physiological disorders. European Journal of Medicinal Chemistry. Volume

207, 1 December 2020, 112801. https://doi.org/10.1016/j.ejmech.2020.112801.
[3] https://cdn.caymanchem.com/cdn/insert/18504.
[4] M. M. Eswarudu, P. Siva Krishna, K. Sai Varsha, K. Sowmya Sri, P. Shafiya, S. K. Abjul, P. Prathyusha, P. Ravi Sankar, P. Srinivasa Babu. Spectrophotometric Method Development and Validation for the Estimation of Trelagliptin Succinate in Bulk and Pharmaceutical Dosage Form. Sch Acad J Pharm. May, 2024; 13(5): 132-138. DOI: 10.36347/sajp. 2024. v13i05.001.
[5] Shereen Mowaka, Nermeen Ashoush, Mariam M. Tadros, Bassam M. Ayoub. Investigation of Pharmacokinetic Parameters of Trelagliptin in Egyptian Volunteers using Sensitive LC-MS/MS: A Comparative Study with a Japanese Population. Journal of Analytical Methods in Chemistry Volume 2021, Article ID 9664099, 9 pages. https://doi.org/10.1155/2021/9664099.
[6] K. Durga Malleswar et al. A Validated Chiral HPLC Method for the Enantiomeric Purity of Trelagliptin. Indo American Journal of Pharmaceutical Research.2019:9(11). 2019. DOI NO.: 10.5281/zenodo.3559498.
[7] Maria A. Attallah, Shereen Mowaka, Ehab F. Elkady, Marwa Fouad, Bassam Ayoub. Analysis and bio-analysis of Omarigliptin, Trelagliptin and Alogliptin: Applied to biological samples and degradation kinetic study. Microchemical Journal Volume 148, July 2019, Pages 253-261. https://doi.org/10.1016/j.microc.2019.05.010.
[8] Shereen Mowaka, Bassam M. Ayoub, Mostafa A. Hassan, Wafaa A. Zaghary. Different Spectrophotometric Methods for Simultaneous Determination of Trelagliptin and Its Acid Degradation Product. Journal of Analytical Methods in Chemistry. Volume 2018, Article ID 7370651, 7 pages. https://doi.org/10.1155/2018/7370651.
[9] Zhiqiang Luo, Xinjing Chen, Guopeng Wang, Zhibo Du, Xiaoyun Ma, Hao Wang, Guohua Yu, Aoxue Liu, Meng wei Li, Wei Peng, Yang Liu. Development of a validated HPLC method for the quantitative determination of trelagliptin succinate and its related substances in pharmaceutical dosage forms. European Journal of Pharmaceutical Sciences. Volume 111, 1 January 2018, Pages 458-464. https://doi.org/10.1016/j.ejps.2017.10.028.
[10] Dr. Ajit Anerao, Vikas Mundekar, Satish John, Nitin Pradhan. Development and validation of related substances method by hplc for analysis of Trelagliptin succinate. World journal of pharmacy and pharmaceutical sciences. Volume 5, Issue 8, 1844-1858. DOI: 10.20959/wjpps20168-7509.
[11] Xiao-xia Hu, Tian Lan, Zhe Chen, Cheng-cheng Yang, Peng-fei Tang, Ling-jing Yuan, Guo-Xin Hu, Jian-Ping Cai. A rapid and sensitive UHPLC–MS/MS assay for the determination of trelagliptin in rat plasma and its application to a pharmacokinetic study. Journal of Chromatography B. Volumes 1033–1034, 15 October 2016, Pages 166-171. https://doi.org/10.1016/j.jchromb.2016.08.024.
[12] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q2B: Guideline on Validation of Analytical Procedure/ Methodology, 1996.

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