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CHROMATOGRAPHIC METHOD FOR CONCURRENT QUANTITATION OF VILDAGLIPTIN AND PIOGLITAZONE HYDROCHLORIDE: GREENNESS EVALUATION BY AGREE AND GAPI TOOL

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ABSTRACT
The RP-HPLC technique has been developed and proven to be a cost-effective, precise, and accurate technique for the concurrent quantitation of Pioglitazone hydrochloride and Vildagliptin. The eco-friendliness of the proposed approach has been analyzed by the AGREE calculator and the GAPI tool. Agilent Eclipse XBD C18 column (5 µm, 150 × 4.5 mm) was utilized with an eluent consisting of 20mM dipotassium hydrogen phosphate and acetonitrile in gradient mode at 1 mL/min. The drugs were recorded at 210 nm. As per ICH standards, the method was validated. Good level linearity was achieved within the concentration span of 25 - 75 µg/mL, and 7.5 – 22.5 µg/mL for VIL and PIO individually. The accuracy results were within the permissible limit of % relative standard deviation (% RSD), i.e., below 2, and the % recovery results was within the limit. This method can be applied on a routine basis to determine VIL and PIO simultaneously in quality control laboratories. The method developed is eco-friendly and operator-friendly.

Keywords: Pioglitazone, Vildagliptin, RP-HPLC, Validation, Greenness, AGREE, GAPI.
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INTRODUCTION

High blood sugar levels characterize diabetes mellitus, or simply diabetes, a metabolic disorder. The most widespread disease in the world today is diabetes. Recent research shows that Type 2 diabetes (T2D) is increasing in society. This disease is treated by the ingestion of drugs like dipeptidyl peptidase-4 (DPP-4), biguanides, thiazolidinediones, or secretagogues (sulphonylureas/glinides).[1] Vildagliptin (VIL) is classified as a DPP-4 inhibitor, as shown in Fig.-1(a). Glucagon-like peptide-1 (GLP-1) and glucose-dependent gastric inhibitory polypeptide (GIP) are subsequently disturbed due to this inhibition.[2] Pioglitazone hydrochloride (PIO), as illustrated in Fig.-1(b), is a thiazolidinedione oral antidiabetic. It works by activating the peroxisome proliferator-activated receptor (PPAR). It reduces hepatic gluconeogenesis and increases tissue sensitivity to insulin.[3] In order to enhance analyst safety and minimize their harmful contribution to the environment, scientists are increasingly resorting to green analytical chemistry (GAC). GAC's primary objective is to reduce or exclude the application of dangerous chemicals in analytical procedures in an effort to enhance terrestrial and health results without compromising technique performance.[4] This research utilizes two forms of greenness evaluation methods, according to the 12 principles of green chemistry: AGREE and the green analytical procedure index (GAPI).

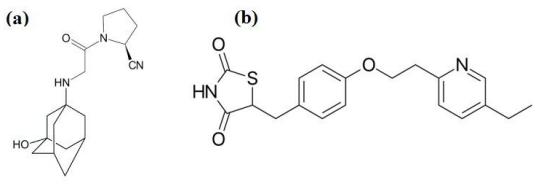

Fig.-1: Structure of (a) VIL and (b) PIO

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AGREE allows variable weighting of 12 important green chemistry principles for a personalized greenness evaluation. The measurement, which presents the result as a colored chart, is designed to be simple to understand.[5] The colour may range from dark green (1) to dark red (zero). In the GAPI tool, a pictogram makes use of a colour scheme (yellow, green, and red) to classify the ecological value of each phase in an analytic process. The colour green is an indication of a safe way, whereas the colour red denotes an ecologically unfavourable one.[6] The combo of VIL (50 mg) and PIO (15 mg) was approved in May 2023 for the treatment of T2D.[7] An Extensive literature search identified that very few chromatographic[8-10] and UV[11] techniques have been published for the concurrent determination of VIL and PIO. Up to Date, only a few RP-HPLC methods have been reported for the concurrent quantitation of VIL and PIO, as well no eco–friendly evaluation of the method using greenness tools has been conducted. In the reported method,[9] ion pairing reagent was utilized for separation, and an individual tablet of PIO and VIL was utilized for assay, while in the present approach, ion pairing reagent was not used, and a fixed dose combination was utilized for assay, and gradient mode was utilized with the flow rate of 1 mL/min. In the reported method,[11] no assay findings were reported for the quantitation of both drugs in the dosage form. As per the above aspects, the purpose of the current research is to establish a simple, specific, sensitive, and accurate RP-HPLC method that would be capable of quantifying the concentration of VIL and PIO in tablets, as well as their greenness assessment. Therefore, AGREE and GAPI tools were used in a greenness profile evaluation to find out the eco-friendliness of the recent HPLC approach. The validation of the current approach was accomplished according to ICH recommendations.[12]

EXPERIMENTAL

Materials and Methods

VIL (Purity: 99.9 %) and PIO (Purity: 98.9 %) were procured as gift samples from Century Pharmaceuticals Ltd, Vadodara, Gujarat, and Abhilasha Pharma, Pvt. Ltd., Ankleshwar, Gujarat, respectively. HPLC grade solvents like Dipotassium hydrogen phosphate (K_2HPO_4), Methanol (MeOH), Potassium dihydrogen phosphate (KH_2PO_4), and Acetonitrile (ACN) were bought from Rankem Pvt. Ltd, Mumbai.

Instruments and Chromatographic Conditions

A Shimadzu LC 2010 CHT HPLC system with a PDA detector was utilized. LC solution (software) was used to acquire data. The separations were accomplished on a Kromasil C18 column (5µm, 150 mm x 4.6 mm) at 25 °C, and the eluent was monitored at 210 nm. The eluent utilized is a mixture of buffer (20 mM dipotassium hydrogen phosphate) and ACN in a gradient mode at 1 mL/min. 30 µL was set as the injection volume.

Preparation of Buffer

Precisely weigh & transfer 3.48 g of dipotassium hydrogen phosphate in 1 L of water. Stir well and sonicate for 10 min, followed by vacuum filtration through a 0.45 µm filter.

Preparation of Eluent

Buffer and ACN were utilized in a gradient program.

Preparation of Diluent

MeOH was utilized as a diluent for the further dilutions.

Preparation of Standard Solution

50 mg VIL and 17 mg PIO (equivalent to 15 mg Pioglitazone) were transferred to a 10 mL volumetric flask (VF) individually. The drugs were solubilized by sonication and further diluted with MeOH to the mark. Aliquot 1 mL filtrate in 10 mL VF and mark with MeOH to get the standard stock solutions containing VIL (500 µg/mL) and PIO (150 µg/mL), respectively. To get a working standard solution, additional dilutions were made using VIL (50 µg/mL) and PIO (15 µg/mL).

Sample Preparation

20 PIOZ-V tablets were tested for weight and powder. A powder sample of quantity equivalent to VIL (50 mg) and PIO (15 mg) was placed in a 10 mL VF, shaken for 15 min, and diluted with MeOH. Filter

using a 0.45µ µm Millipore filter. Aliquot 0.1 mL filtrate in 10 mL VF and make up volume using MeOH, which resulted in VIL (50 µg/mL) and PIO (15 µg/mL).

Validation of the HPLC Approach

System Suitability Study

30 µl of binary mixture having VIL (50 µg/mL) and PIO (15 µg/mL) was injected into the HPLC apparatus six times in an attempt to evaluate the parameter. The variables such as tailing factor (T_f), theoretical plates (TP), resolution (R_s), and retention time (R_t) were calculated.

Specificity and Selectivity

To ensure the specificity of a developed method, a chromatogram of the placebo was recorded. Additionally, it was compared with the spectra of the sample solution and the solution standard, including VIL (50 µg/mL) and PIO (15 µg/mL). The chromatogram was checked for the interference of excipients at R_t of the analytes. Selectivity was determined using peak purity to verify that the chromatographic peak was due to one analyte.

Linearity

Calibration curve solutions of VIL (25 - 75 µg/mL) and PIO (7.5 – 22.5 µg/mL) were obtained by diluting the standard stock solution. Aliquot (0.5, 0.75, 1, 1.25, and 1.5 mL) was pipetted from the solution (stock) in a series of VF (10 mL), and volume was made up using diluent. The graphical curve (calibration) was prepared by plotting response (peak area) against concentration (n=3).

Repeatability

Repeatability was evaluated by repetitive scanning (n=6) of a binary mixture (50 µg/mL VIL, and 15 µg/mL PIO) without varying the considerations of the current approach. Data were recorded, and % RSDs were computed.

Intermediate Precision

Solutions containing VIL (25, 50, and 75 µg/mL) and PIO (7.5, 15, and 22.5 µg/mL) were scanned thrice within a day (Intraday) and thrice on 3 successive days (Interday), and % RSD were computed.

Sensitivity

The parameter was calculated by estimating the values of the limit of detection (LOD) and the limit of quantitation (LOQ). They were assessed by substituting the mean of slope and the standard deviation of Y-intercepts in the equation as per ICH recommendations.

Accuracy

The standard addition method was used to determine accuracy. Standard solution was spiked at 50%, 100%, and 150% of the label claim into a pre-analyzed sample containing 50 µg/mL VIL, and 15 µg/mL PIO. Suitable dilutions were performed to ensure that the final concentration was linear. The experiment was conducted three times.

Robustness

The Robustness was conducted by studying a binary mixture by altering different parameters such as Flow rate (FR) of mobile phase (± 0.2 mL/min), Temperature (± 5 ℃), and Detection wavelength (DL) (± 2 nm). Measurement was taken three times at every level. Results were calculated.

Solution Stability

The solution stability of a solution comprising VIL (50 µg/mL) and PIO (15 µg/mL) was evaluated by allowing the solution to stand in a tightly capped VF at ambient temperature for 24 h. It was analyzed at an interval of 6 h till 24 h. The obtained outcomes were matched with the freshly prepared solution. The solution stability was ascertained by comparing the peak area.

Greenness Assessment

The AGREE and GAPI were employed to assess the suggested chromatographic technique. AGREE is a user-friendly program consisting of software that is simple to use. This value varies from 0.0 (worst score) to 1.0 (best score) and is rounded to two decimals and plotted in the middle of the graph. The GAPI tool

was utilized to compute the greenness of the analytical procedure. The intensity of environmental deterioration is represented by the three colors (green, yellow, and red).

RESULTS AND DISCUSSION

Method Development and Optimization

The current work was carried out to establish an RP-HPLC method for the concomitant quantitation of PIO and VIL in tablets. To enhance the efficiency and resolution of the system, several chromatographic parameters were optimized, like, column temperature, flow rate, detection wavelength, analytical column, and mobile phase composition. Two analytical columns-Kromasil C18 (5 µm,150 mm × 4.6 mm) and Kromasil C8 (5 µm,100 mm × 4.6 mm)-were evaluated, and based on system suitability parameters including R_t, TP, T_f, and R_s, the Kromasil C18 column was finalized for the analysis. A series of mobile phase mixtures incorporating water, methanol, acetonitrile, dipotassium hydrogen phosphate (20 mM), and potassium dihydrogen phosphate (20 mM) buffer in various ratios was also investigated to evaluate the best separating conditions. During the use of Methanol and Water in different proportions, improper peak shape of both drugs was observed. Then Buffer: Methanol in varying ratios was attempted, which also causes an improper shape of both the drugs. Organic Phase was replaced with ACN, and buffer (20 mM potassium dihydrogen phosphate) was used as eluent, and attempted in various ratios and pH of the buffer was also fixed as 2.5 using OPA, which produces the chromatogram with a single drug only. Finally, the ratio of Buffer (pH 2.5) and ACN was optimized in the gradient mode, but VIL shows poor peak shape, as well it eluting under the void volume. Therefore, the buffer was switched to dipotassium hydrogen phosphate, and the ratio was optimised in a gradient mode. Last but not least, buffer (20mM dipotassium hydrogen phosphate) and ACN was finalized as eluent at 1 mL/min in gradient mode because it gave a chromatogram (Fig.-2) with good peak shape of VIL and PIO and free from tailing, and TP was within the limit. Quantitation of analytes was done at 210 nm, as both drugs show maximum absorbance. Column temperature was set at 25 °C, and 30 µL was finalized as the injection volume. The R_t of PIO and VIL was found at 4.880 and 7.990 min individually.

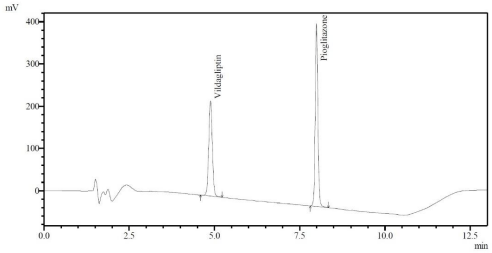

Fig.-2: HPLC Chromatogram of PIO and VIL

Method Validation

System Suitability

The outcomes (Table-1) acquired from the response (chromatogram) of solution comprising PIO (15 µg/mL) and VIL (50 µg/mL) demonstrated that the variables including R_s (> 2), TP (> 2000), T_f (< 2) and % RSD (< 2) of six injections were inside the limit.

Table-1: Outcomes of System Suitability

Limit (n = 6)	VIL (Average ± SD), % CV	PIO (Average ± SD), % CV
R_t	4.880 ± 0.003, 0.06	7.990 ± 0.08, 1.05
TP	7321.5 ± 1.516, 0.02	194717 ± 28.51, 0.01
T_f	1.17 ± 0.009, 0.77	1.16 ± 0.008, 0.69
R_s	--	12.6 ± 0.040, 0.32

Specificity and Selectivity

According to Fig.-3, it was determined that there was no interference from diluent and excipients at the Rt of VIL and PIO. It reflects that the process is specific in the determination of both analytes in the

synthetic mixture. The parameter (selectivity) was deduced from the peak purity values (VIL: 0.9998 and PIO: 0.9999) to ensure the homogeneity of the peak.

Linearity

VIL demonstrated a linear response across 25–75 µg/mL, having a correlation coefficient (R^2) of 0.9991, while PIO exhibited linearity in the 7.5–22.5 µg/mL range with an R^2 value of 0.9992, confirming a strong correlation between analyte concentration and detector response. Detailed outcomes of linearity are tabulated in Table-2.

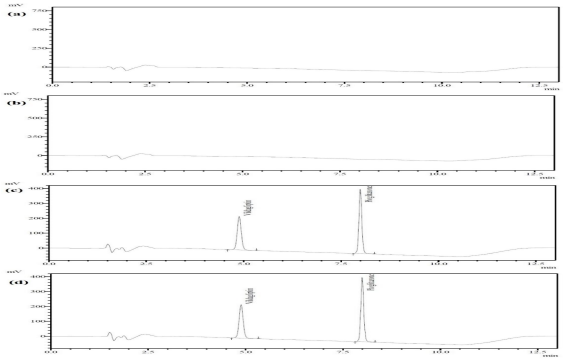

Fig.-3: Chromatogram of (a) Eluent, (b) Placebo, (c) Solution (Standard) of VIL (50 µg/mL) & PIO (15 µg/mL), (d) Solution (Sample) of VIL (50 µg/mL) & PIO (15 µg/mL)

Precision

Repeatability

The % CV values for repeatability of VIL (50 µg/mL) and PIO (15 µg/ mL) are mentioned in Table-2. Since % CV is < 2, it confirms that the current method is repeatable.

Intermediate Precision

Table 2 indicates the results with moderate precision. The CV percentage in all determinations was below 2%, which demonstrates that the method is precise for contemporaneous drug quantification.

Accuracy

APIs were added to the pre-analyzed sample at 3 levels (50, 100, and 150 %). The outcomes were represented in Table-2.

Robustness

To assess the robustness of the suggested approach, divergences from method attributes were carried out. According to the results presented in Table-2, it was confirmed that there were no crucial differences in the results (peak area) due to the method parameter change. Additionally, the % RSD of all the determinations was less than 2 %, validating the method's robustness.

Table-2: Validation Parameter Summary

S. No.	Parameter		VIL	PIO
1	Specificity		No interference	No interference
2	Range for linearity (µg/ml)*		25-75	7.5-22.5
3	Equation of Regression		y = 43632x - 164118	y = 188020x - 37166
4	Coefficient of determination (R^2)		0.9991	0.9992
5	Precision (% RSD)	Repeatability#	0.06	0.28
		Intraday*	0.13-0.39	0.49-0.80
		Interday*	0.65-1.22	0.41-0.90
6	Accuracy (% recovery) *		98.2-101.6	98.4-101.7
7	Robustness (% RSD)	Change in DL (± 2 nm) *	(+) 0.26, (-) 0.25	(+) 1.52, (-) 0.57
		Change in FR (± 0.2 mL/min) *	(+) 0.51, (-) 0.29	(+) 0.11, (-) 0.22
		Change in Column Temperature (± 2 ºC) *	(+) 0.22, (-) 0.29	(+) 0.16, (-) 0.28

* Mean of 3 determinations, # Mean of 6 determinations

Sensitivity

The LOD values for VIL and PIO were 0.53 µg/mL and 0.11 µg/mL, respectively. The LOQ values for VIL and PIO were 1.60 µg/mL and 0.33 µg/mL, respectively.

Solution Stability

No visible change in the amount of drug was observed, and the experimental results indicated a difference of less than 2%. Thus, the drug solution stability was determined for a day. The outcomes were summarized in Table-3.

Table-3: Solution Stability Outcomes

	VIL (50 µg/mL)		PIO (15 µg/mL)	
Time (h)	Peak Area	% Stability	Peak Area	% Stability
0	1650528	99.99	2314920	99.95
6	1634578	99.03	2309845	99.78
12	1619989	98.14	2289076	98.88
18	1609923	97.53	2278990	98.44
24	1606678	97.34	2278990	98.38

Assay of Synthetic Mixture

The average % purity of PIO and VIL in lab blend was determined to be 99.3 % ± 0.665 and 101.8 % ± 0.529, respectively. The % RSD of VIL and PIO in the assay was determined to be 0.67 and 0.53 % respectively. As per the label claim, the percentage assay was considered acceptable.

Greenness Assessment

The suggested method's greenness was evaluated utilizing the AGREE tool. The result of AGREE having a score of 0.73 (Fig.-4(a)). Section 11 of the AGREE score indicates red color because ACN is used. ACN is combustible, volatile, and poisonous (ICH Q3C (R8)).[13] By using the GAPI tools for the proposed method, eleven green fields (11), four yellow fields, and no red fields were found (Fig.-4(b)). Yellow pentagrams are used to represent the process type that involves trash generation and filtering phases.

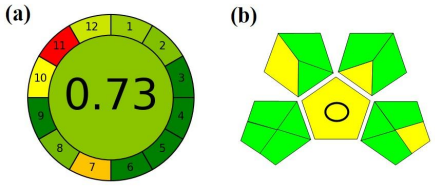

Fig.-4: AGREE (a) and GAPI (b) Pictogram for the Current Approach

CONCLUSION

A novel, consistent, linear, precise, and specific RP-HPLC approach for the concurrent determination of VIL and PIO in tablets is described in this research. ICH recommendations were also employed to validate the method, and the data confirmed the proposed method's selectivity, linearity, sensitivity, precision, and accuracy. With the use of the AGREE and GAPI, the current approach was ecologically sustainable. The proposed method measures API at reduced concentration levels and optimally discriminates all drugs having desirable R_s. As a result, quality control labs can use the recommended method for routine analysis. It is convenient and beneficial if the regression values, % RSD, and standard deviations are appropriate for the concurrent quantitation of both drugs in a formulation. The proposed approach permits VIL and PIO to be routinely assessed in quality control laboratories.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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