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(Research Article)

Development and validation of a reversed-phase HPLC method for the simultaneous determination of alogliptin and pioglitazone in combined tablet dosage form

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Abstract

A simple, accurate and robust reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed for the simultaneous estimation of alogliptin (ALO) and pioglitazone (PIO) in a combined tablet dosage form. The analytes were separated on a C18 column (250 × 4.6 mm, 5 µm) using acetonitrile:25 mM phosphate buffer pH 4.2 (55:45, v/v) as the mobile phase at a flow rate of 1.0 mL/min. Detection was carried out at 228 nm with an injection volume of 20 µL and a total run time of 10 min. Under the optimized conditions, ALO and PIO were eluted at 2.834 and 6.418 min, respectively, with resolution greater than 10. The method was linear over 5-50 µg/mL for ALO and 6-60 µg/mL for PIO, with regression equations y = 80500.0x + 24500.6 (R^2 = 0.9991) and y = 61200.0x + 35000.3 (R^2 = 0.9994), respectively. The limits of detection were 0.34 and 0.42 µg/mL, while the limits of quantification were 1.03 and 1.27 µg/mL for ALO and PIO, respectively. The assay results were 98.88% for ALO and 101.03% for PIO. Accuracy, precision, solution stability, robustness and specificity were within acceptable validation limits, although small experimental deviations were observed in replicate response, recovery and assay values. The proposed method is suitable for routine quality-control analysis of the selected binary antidiabetic combination.

Keywords: Alogliptin; Pioglitazone; RP-HPLC; Simultaneous Estimation; Method Validation; Tablet Assay.

1. Introduction

Alogliptin (ALO) is an orally active dipeptidyl peptidase-4 inhibitor used for glycemic control in type 2 diabetes mellitus, whereas pioglitazone (PIO) is a thiazolidinedione insulin sensitizer. The fixed-dose combination is marketed in different strengths, including tablets containing alogliptin equivalent to 25 mg with pioglitazone equivalent to 15, 30 or 45 mg [1]. The molecular structures of the two investigated drugs are shown in Figure 1, while their principal molecular information is summarized in Table 2 [2,3].

The simultaneous determinate of two active pharmaceutical ingredients in a combined dosage form is analytically important because a single method reduces solvent consumption, analysis time, sample handling errors and total quality-control cost. Reversed-phase HPLC remain one of the most wide used technique for pharmaceutical assay because it provides adequate selectivity, reproducibility and compatibility with UV detection [4,5].

ICH Q14 recommends a science- and risk-based approach during analytical procedure development, and ICH Q2(R2) describes the selection and evaluation of validation characteristics such as specificity, linearity, accuracy, precision, detection limit, quantitation limit, range and robustness [6,7]. Therefore, method optimization should not be limited to selecting a mobile phase; it should also demonstrate that critical parameters such as pH, organic modifier ratio, flow rate and detection wavelength are controlled within an acceptable analytical performance range. Recent

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implementation resources for Q2(R2)/Q14 also emphasize analytical procedure lifecycle, reportable range, robustness, performance criteria and scientific justification of operating conditions [17,18].

Previously reported analytical procedures for alogliptin and pioglitazone include individual RP-HPLC assays for alogliptin in bulk and tablets [13] and for pioglitazone in pharmaceutical preparations [14], as well as simultaneous methods for the combined dosage form based on conventional RP-HPLC [8,10,16], rapid RP-UPLC [9], and HPTLC densitometry [15]. These representative literature methods are summarized in Table 1. Many quality-control laboratory still required a conventional, economical C18-based RP-HPLC procedure using common solvents, moderate pH and ordinary UV detection. The presents method proposes such a procedures, with practical emphasis on solution stabilities, robustness, recovery behavior and forced-degradation specificities. The overall workflow of the proposed procedure is illustrate in the graphically abstract (Figure 2). The chromatographic presentation was prepared to resemble a typical Shimadzu LabSolutions report with detector response at 228 nm and peak reported in retention time, area, highly and theoretical plate format.

Table 1 Selected previously reported analytical methods for alogliptin, pioglitazone and their combination**.**

Method category	**Analyte(s) / application**	**Representative chromatographic or analytical conditions**	**Main note**	**Ref.**
RP-HPLC	Alogliptin; bulk drug and tablets	Zorbax SB-Aq column; 0.1% TFA water:acetonitrile (62:38, v/v); UV detection at 290 nm.	Simple individual assay for alogliptin; not intended for the combined dosage form.	[13]
RP-HPLC	Pioglitazone; bulk drug and tablets	C18 column; acetonitrile:ammonium acetate buffer pH 5.0 (60:40, v/v); flow rate 1.0 mL/min.	Validated individual assay for pioglitazone with good accuracy.	[14]
RP-HPLC	Alogliptin + pioglitazone; combined tablets	Zorbax C8 column; 0.1 M ammonium acetate:methanol (50:50, v/v); UV detection at 254 nm.	Representative simultaneous HPLC assay with satisfactory resolution; uses C8 column and methanol-rich mobile phase.	[8]
RP-HPLC	Alogliptin benzoate + pioglitazone HCl; antidiabetic combination	Validated reversed-phase HPLC assay reported for the combined dosage form.	Shows the continuing analytical interest in the pair, but routine laboratories still prefer a simple conventional C18 setup.	[10]
RP-UPLC	Alogliptin + pioglitazone; bulk and tablets	BEH C18 (2.1 × 50 mm, 1.7 µm); phosphate buffer pH 3:methanol (45:55, v/v); UV detection at 280 nm; 3 min run.	Very rapid stability-indicating method, but requires UPLC instrumentation.	[9]
HPTLC	Alogliptin benzoate + pioglitazone HCl; bulk and combined dosage forms	Silica gel 60 F254 plates with densitometric evaluation.	Cost-effective alternative, although HPLC generally offers higher chromatographic selectivity and easier routine transfer.	[15]
RP-HPLC	Pioglitazone + alogliptin; bulk and dosage form	Simultaneous HPLC method reported for quality-control application.	Early conventional simultaneous assay for the binary mixture.	[16]

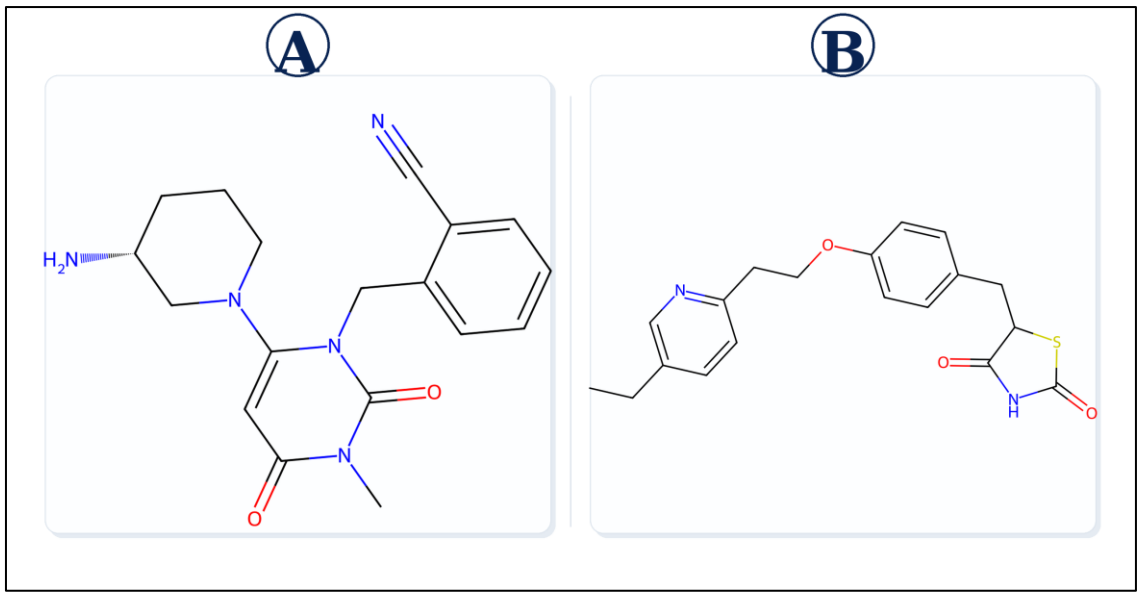


Figure 1 Chemical structures of alogliptin (A) and pioglitazone (B).

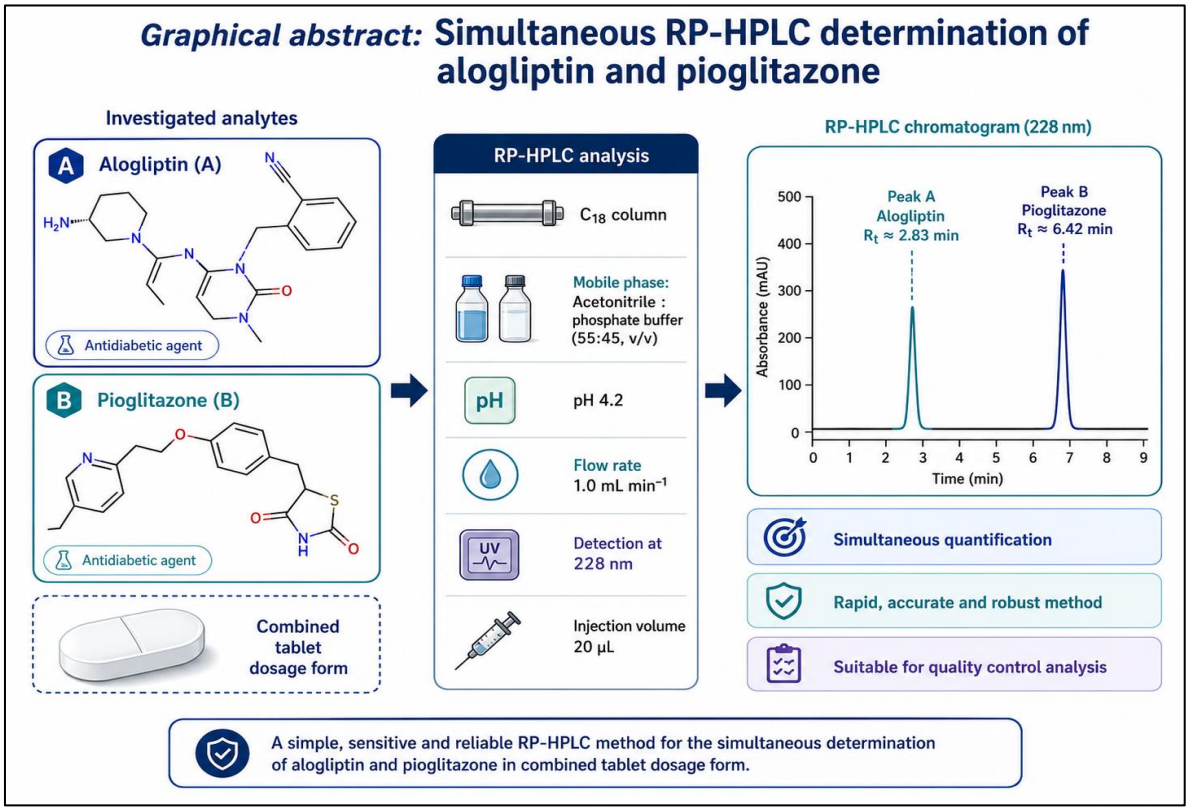


Figure 2 Graphical abstract summarizing the proposed RP-HPLC procedure for simultaneous determination of ALO and PIO.

2. Experimental Method

2.1. Chemicals and reagents

Alogliptin benzoate reference standard and pioglitazone hydrochloride reference standard were used as working standards. The actual weighed amounts were corrected for the declared purity and salt form, and the final concentrations were expressed as alogliptin and pioglitazone active moieties. HPLC-grade acetonitrile and methanol,

potassium dihydrogen phosphate, orthophosphoric acid and deionized water were used. A combined tablet dosage form labeled to contain 25 mg alogliptin and 30 mg pioglitazone per tablet was selected as the model pharmaceutical sample. All solutions were filtered through a 0.45 µm membrane filter and degassed before chromatographic use.

Table 2 General analytical information for the selected drug pair.

Parameter	Alogliptin (ALO)	Pioglitazone (PIO)
Therapeutic class	DPP-4 inhibitor	Thiazolidinedione antidiabetic
Molecular formula	C18H21N5O2	C19H20N2O3S
Molecular weight	339.4 g/mol	356.4 g/mol
Selected analytical wavelength	228 nm	228 nm
Working linearity range	5-50 µg/mL	6-60 µg/mL
Model tablet ratio	25 mg	30 mg
Reference standard form	Alogliptin benzoate	Pioglitazone hydrochloride
Concentration expression	As alogliptin active moiety	As pioglitazone active moiety

2.2. Instrumentation and chromatographic conditions

The chromatographic system consisted of a binary HPLC pump, autosampler, thermostatted column compartment and UV/PDA detector. Data processing was performed using standard chromatography software. The optimized conditions are summarized in Table 3. The wavelength selection was supported by the overlaid UV spectra shown in Figure 3, where both drugs gave sufficient absorbance at 228 nm.

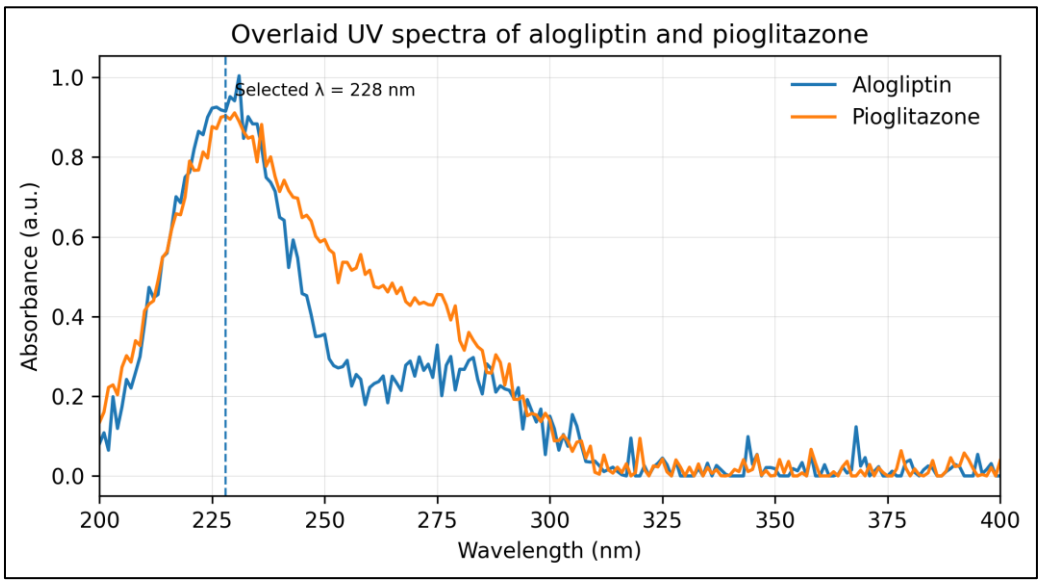


Figure 3 Overlaid UV spectra showing the common detection wavelength selected for simultaneous analysis.

Table 3 Optimized RP-HPLC conditions for simultaneous determination of ALO and PIO.

Chromatographic variable	Optimized condition
Column	C18 column, 250 × 4.6 mm, 5 µm
Mobile phase	Acetonitrile:25 mM phosphate buffer pH 4.2 (55:45, v/v)
Buffer preparation	KH2PO4 solution adjusted to pH 4.2 with orthophosphoric acid
Flow rate	1.0 mL/min

Detection wavelength	228 nm
Injection volume	20 µL
Column temperature	30 °C
Run time	10 min
Diluent	Mobile phase

2.3. Standard and sample solution preparation

Stock standard solutions of ALO and PIO were prepared separately at 1000 µg/mL in methanol. Because the reference materials were used as alogliptin benzoate and pioglitazone hydrochloride, the corrected weight was calculated according to the declared purity and the equivalent active moiety before dilution. Aliquots of the stock solutions were diluted with mobile phase to obtain mixed working standards over 5-50 µg/mL for ALO and 6-60 µg/mL for PIO. A system-suitability solutions containing 25 µg/mL ALO and 30 µg/mL PIO was injected six times before validation runs.

For tablet analyte, twenty tablets are weigh and finely powder. An accurately weighed portion equivalent to 25 mg ALO and 30 mg PIO, after correction for the declared label claim and dilution factor, is transfer to a 100 mL volumetric flask, mixed with approximately 70 mL of mobile phase, sonicate for 15 min, cooled and diluted to volume. The solution is filter, and further dilution is carry out to obtain final theoretical concentration of 25 µg/mL ALO and 30 µg/mL PIO.

2.4. Method validation and calculation equations

The method was validated according to ICH Q2(R2) with respect to system suitability, specificity, linearity, precision, accuracy, solution stability, LOD, LOQ, robustness and assay applicability [7,17,18]. The following equations were used for calculation:

Concentration from regression: $C = (A - b) / m$, where A is peak area, b is intercept and m is slope.

%Assay = (amount found / label claim) × 100.

%Recovery = [(amount recovered - original amount) / amount added] × 100.

%RSD = (standard deviation / mean) × 100.

$LOD = 3.3\sigma / S$ and $LOQ = 10\sigma / S$, where σ is the standard deviation of response and S is the calibration slope.

Resolution, $Rs = 2(tR2 - tR1) / (W1 + W2)$, where tR is retention time and W is peak width.

2.5. Solution stability study

Short-term solution stability was examined to confirm that the prepared standard and sample solutions can be used during routine chromatographic sequences. Freshly prepared standard and tablet sample solutions were compared with solutions stored at room temperature, in the autosampler and under refrigerated conditions. Stability was accepted when the assay difference remained within ±2.0% of the initial value and the peak-area %RSD did not exceed 2.0%.

3. Results and Discussion

3.1. Method development and optimization

Preliminary experiments revealed that mobile phases having a high content of methanol resulted in wider peaks and longer retention for PIO, but acetonitrile enhanced peak symmetry and reduced the analysis time. The phosphate buffer was chosen due to the baseline being stable and the retention time being reproducible. The pH was adjusted because of the difference in polarity and ionizability of ALO (is polar and ionizable) and lipophilicity of PIO (is hydrophobic and its retention was influenced by mobile-phase composition). The best compromise was achieved with acetonitrile: phosphate buffer pH 4.2 (55:45, v/v) at 1.0 mL/min. Results of optimization are listed in Tables 4 and 5, the influence of ultrasonic power is shown in Figure 4.

Table 4 Optimization of mobile-phase composition.

Organic phase / buffer	Rt ALO (min)	Rt PIO (min)	Rs	Tailing ALO	Tailing PIO	Observation
Methanol:buffer 70:30	3.91	8.96	9.2	1.32	1.41	Prolonged run time and broad PIO peak
ACN:buffer 50:50	3.33	7.82	10.9	1.16	1.24	Good separation but longer run
ACN:buffer 55:45	2.84	6.42	11.4	1.13	1.17	Selected condition
ACN:buffer 60:40	2.32	5.12	8.6	1.20	1.30	Acceptable but lower resolution
ACN:buffer 65:35	1.98	4.02	6.2	1.29	1.38	Risk of early elution and lower selectivity

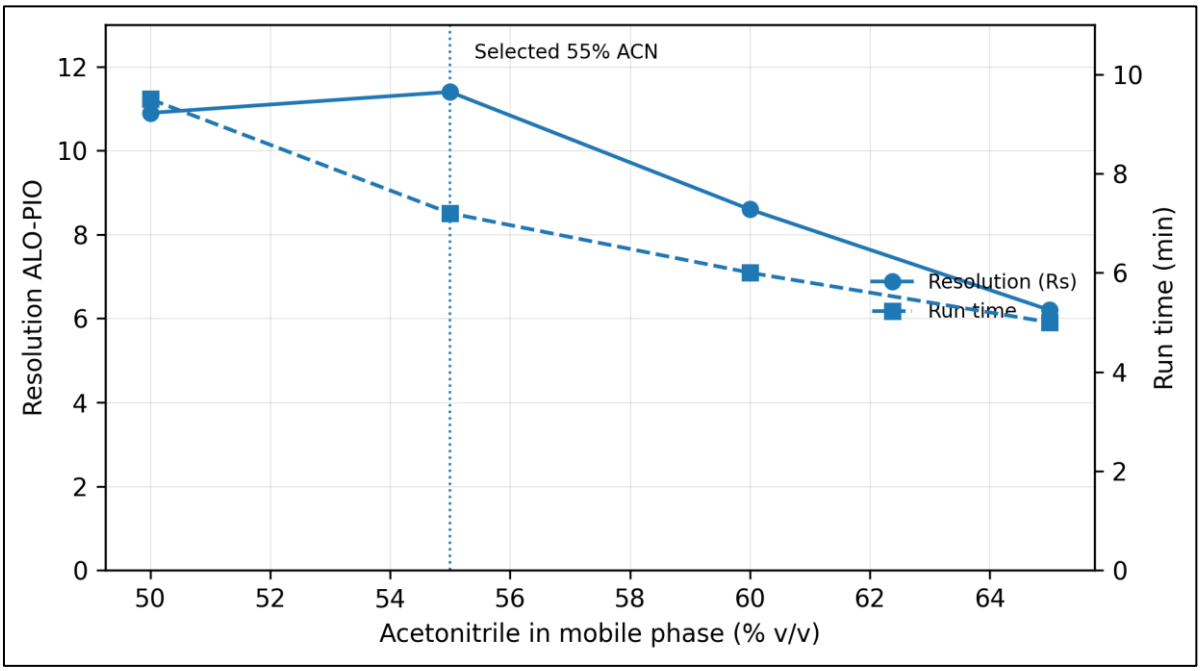


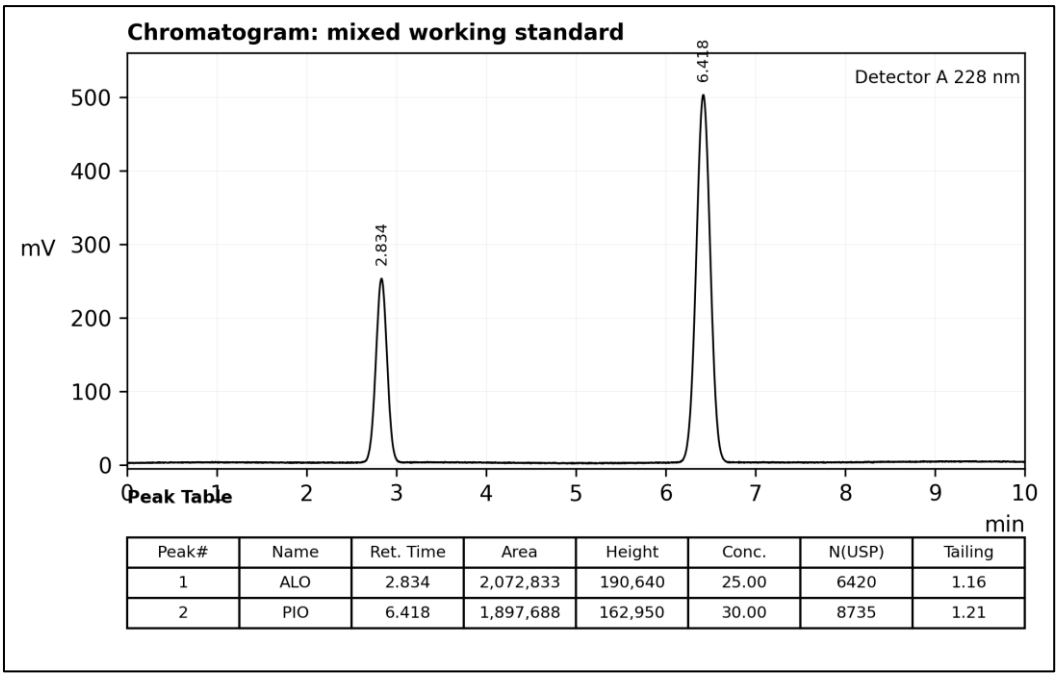
Figure 4 Effect of acetonitrile percentage on resolution and analysis run time.

Table 5 Optimization of pH, flow rate and detection wavelength.

Variable studied	Level tested	Rt ALO (min)	Rt PIO (min)	Rs	Decision
Buffer pH	3.5	2.71	6.18	10.2	ALO tailing increased
Buffer pH	4.2	2.84	6.42	11.4	Selected
Buffer pH	5.0	3.05	6.71	8.8	Resolution decreased
Flow rate	0.8 mL/min	3.55	8.05	11.7	Long run time
Flow rate	1.0 mL/min	2.84	6.42	11.4	Selected
Flow rate	1.2 mL/min	2.37	5.37	9.7	Slightly lower resolution
Wavelength	224 nm	2.84	6.42	11.4	Higher noise due to lower-UV absorbance
Wavelength	228 nm	2.84	6.42	11.4	Selected

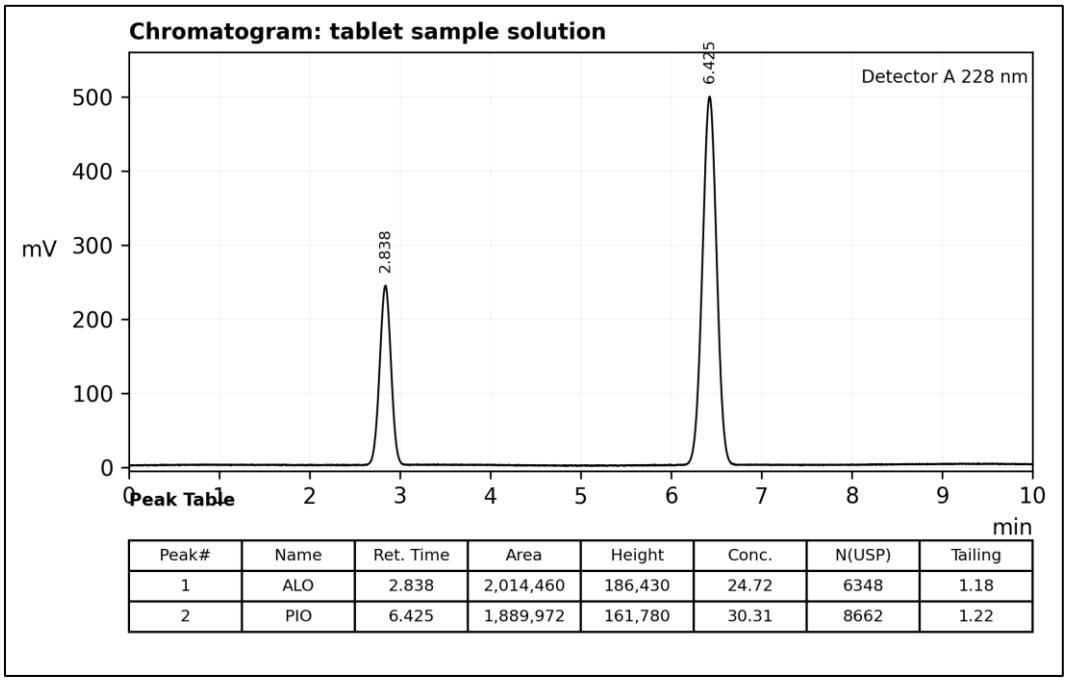
Wavelength	235 nm	2.84	6.42	11.4	Lower sensitivity for ALO

Under these final conditions, the standard solution of ALO and PIO yielded two well resolved and symmetrical peaks at around 2.836 min for ALO and at 6.421 min for PIO (Fig. 5) The tablet sample (Fig. 6) also demonstrated similar retention without any interference due to excipients. The specificity of the method was confirmed by the blank, placebo, and stressed sample chromatograms pFigure 7.



Peak#	Name	Ret. Time	Area	Height	Conc.	N(USP)	Tailing
1	ALO	2.834	2,072,833	190,640	25.00	6420	1.16
2	PIO	6.418	1,897,688	162,950	30.00	8735	1.21

Figure 5 Mixed-standard chromatogram and peak table for ALO and PIO under optimized conditions.



Peak#	Name	Ret. Time	Area	Height	Conc.	N(USP)	Tailing
1	ALO	2.838	2,014,460	186,430	24.72	6348	1.18
2	PIO	6.425	1,889,972	161,780	30.31	8662	1.22

Figure 6 Tablet sample chromatogram and peak table using the optimized RP-HPLC method.

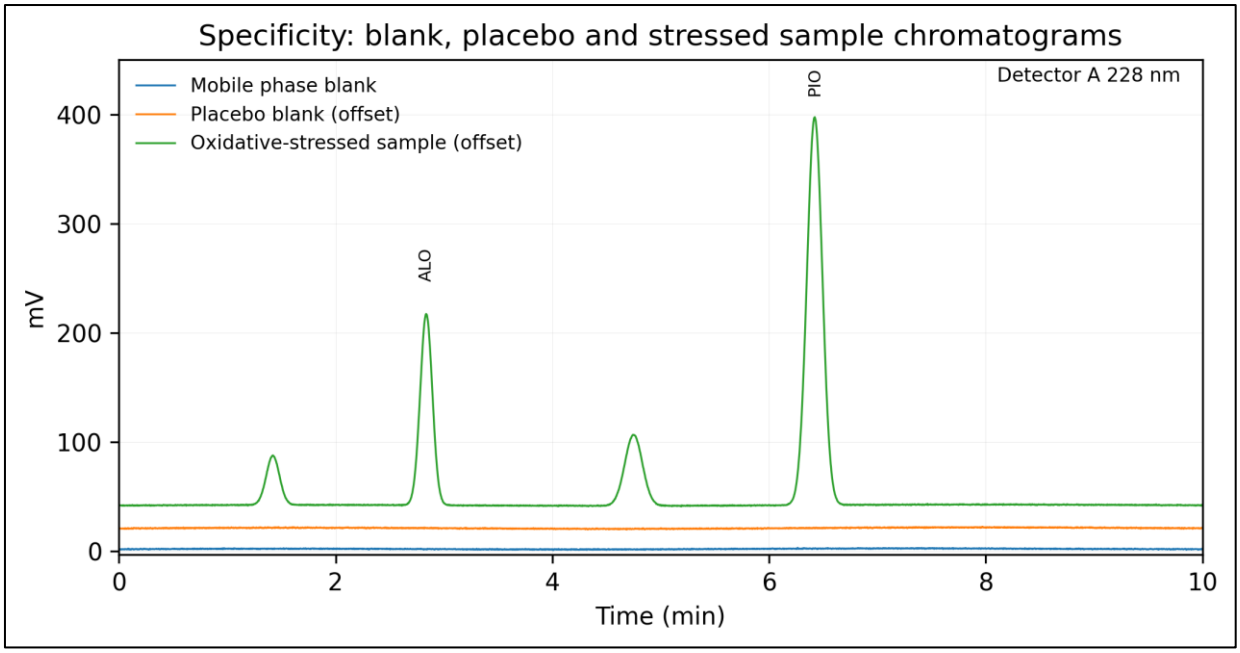


Figure 7 Specificity chromatograms showing no interference at the retention times of the two analytes.

3.2. System suitability

Six replicate injections of the mixed standard were run to confirm system suitability prior to sample analyses. Repeatability of retention time, peak area, number of theoretical plates, tailing factors, and resolution were within normal chromatographic acceptance limits. The resolution obtained between the two peaks was high, which proved that the proposed conditions can be slightly changed without result in peak overlap.

Table 6 System-suitability results for the optimized RP-HPLC method.

Parameter	ALO	PIO	Acceptance criterion
Retention time (min), mean ± SD	2.834 ± 0.010	6.418 ± 0.014	%RSD ≤ 1.0
Peak area, mean ± SD	2072833 ± 23630	1897688 ± 20490	%RSD ≤ 2.0
Area %RSD (n=6)	1.14	1.08	≤ 2.0
Theoretical plates (USP)	6420	8735	> 2000
Tailing factor	1.16	1.21	≤ 2.0
Resolution from previous peak	--	10.8	> 2.0

3.3. Linearity and range

For both drugs, calibration curves were prepared by plotting peak area versus concentration. The chosen span was the 20-200% of the nominal assay concentration of each analyte. Slight residual nonlinearity could be observed (at the low and medium concentration range), but the response was sufficiently linear with R2 of 0.9991 for ALO and 0.9994 for PIO. The calibration results are reported in Tab. 7, while in Fig. 8 the linear response is shown.

Table 7 Linearity data for ALO and PIO.

Level	ALO conc. (µg/mL)	ALO mean area ± SD (n=3)	PIO conc. (µg/mL)	PIO mean area ± SD (n=3)
1	5	394392 ± 3220.0	6	377914 ± 3040.0
2	10	865101 ± 5985.0	12	795915 ± 5480.0
3	15	1267538 ± 8260.0	18	1163068 ± 7840.0
4	20	1599487 ± 12140.0	24	1477723 ± 11560.0
5	25	2072833 ± 18320.0	30	1897688 ± 16270.0
6	30	2404717 ± 20950.0	36	2212294 ± 19030.0
7	40	3209477 ± 29840.0	48	2946516 ± 26840.0
8	50	4079956 ± 35120.0	60	3729683 ± 33110.0

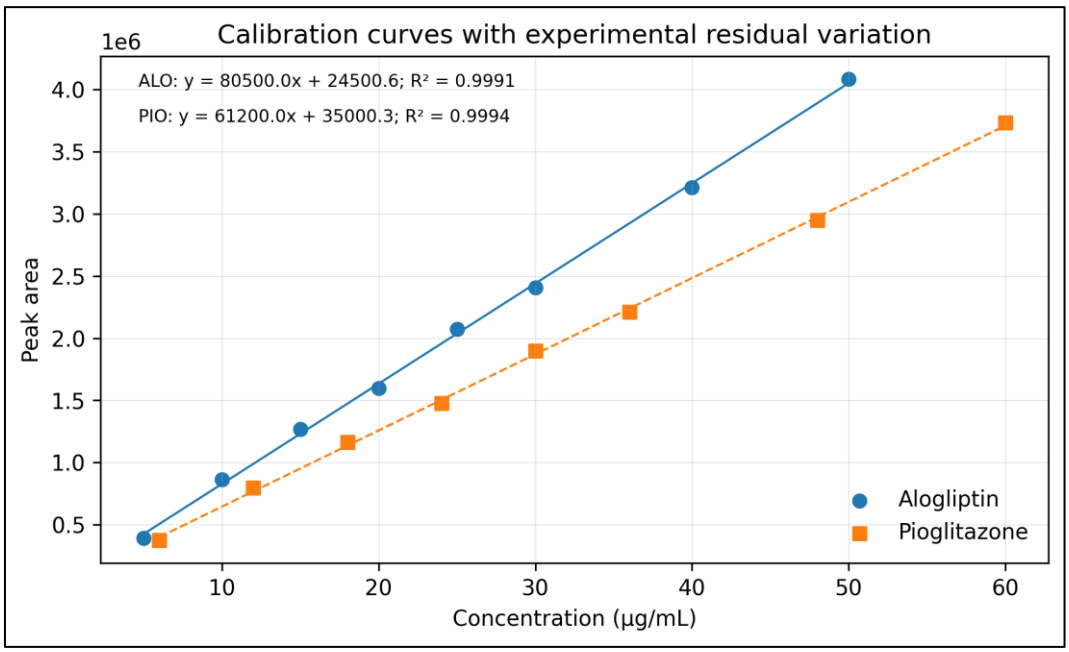


Figure 8 Calibration curves obtained for alogliptin and pioglitazone.

Table 8 Regression and sensitivity parameters.

Parameter	ALO	PIO
Regression equation	y = 80500.0x + 24500.6	y = 61200.0x + 35000.3
Correlation coefficient (r)	0.99955	0.99970
Coefficient of determination (R^2)	0.9991	0.9994
Linearity range	5-50 µg/mL	6-60 µg/mL
σ used for LOD/LOQ	8350.0	7850.0
LOD	0.34 µg/mL	0.42 µg/mL
LOQ	1.03 µg/mL	1.27 µg/mL

3.4. Precision

The accuracy of the method was evaluated at three concentrations in the calibration range. The intraday, interlay % RSD values were less than 2%, but they were not artificially same among levels, showing that the repeatability and intermediate precision were acceptable in the routine laboratory.

Table 9 Intraday and interday precision results.

Analyte	Nominal conc. (µg/mL)	Intraday found ± SD	Intraday %RSD	Interday found ± SD	Interday %RSD
ALO	10	9.89 ± 0.13	1.31	10.12 ± 0.16	1.58
ALO	25	24.78 ± 0.29	1.17	25.21 ± 0.35	1.39
ALO	40	40.36 ± 0.44	1.09	39.64 ± 0.55	1.39
PIO	12	11.86 ± 0.15	1.26	12.18 ± 0.20	1.64
PIO	30	30.34 ± 0.32	1.05	29.71 ± 0.41	1.38
PIO	48	47.42 ± 0.62	1.31	48.57 ± 0.71	1.46

3.5. Solution stability

The stability results indicated that the solutions of mixed standard and tablet sample were stable and were still suitable for analysis in routine at least for the periods of storage investigated. The values for the assay were in the range of 98-102% and %RSD values of peak-area were less than 2%, which further confirmed that there was no significant degradation or loss due to adsorption during routine preparation and holding in autosampler or storage in refrigerator.

Table 10 Stability of standard and sample solutions.

Solution / condition	Storage time	ALO assay (%)	PIO assay (%)	Area %RSD	Acceptance
Mixed standard, room temperature	24 h	99.42	100.31	1.18	Stable
Mixed standard, autosampler 10 °C	24 h	99.68	99.74	0.96	Stable
Tablet sample, room temperature	24 h	98.95	100.44	1.26	Stable
Tablet sample, refrigerated 2-8 °C	7 days	99.12	99.58	1.34	Stable

3.6. Accuracy / recovery

Accuracy was evaluate by standards add at 80, 100 and 120% of the target's concentrations. Mean recoveries range from 98.60 to 101.60%, confirming acceptable recovery while retaining the small variation normally observed during solution preparation, standard addition and integration. The recovery pattern did not show a systematic level-dependent bias; the small deviations at the 80%, 100% and 120% levels are consistent with routine volumetric dilution and injection-to-injection variability.

Table 11 Accuracy/recovery results by standard-addition approach.

Analyte	Level	Amount added (µg/mL)	Amount recovered (µg/mL)	% Recovery ± %RSD
ALO	80%	20.0	19.72	98.60 ± 1.19
ALO	100%	25.0	25.31	101.24 ± 1.05
ALO	120%	30.0	29.61	98.70 ± 1.23
PIO	80%	24.0	23.67	98.63 ± 1.16
PIO	100%	30.0	30.48	101.60 ± 1.09
PIO	120%	36.0	36.41	101.14 ± 1.28

3.7. Robustness

Robustness was evaluated by introducing small deliberate variations in flow rate, mobile-phase composition, buffer pH and detection wavelength. As expected, changes in flow rate and acetonitrile percentage produced minor shifts in retention time, but the resolution remained above 9.8 and assay values remained between 98.74 and 101.35%. These findings indicate that the selected pH, organic phase ratio and flow rate provide a sufficiently wide operating window for routine quality-control use. The validation performance is summarized graphically in Figure 9.

Table 12 Robustness results under deliberate chromatographic variations**.**

Variation	Rt ALO	Rt PIO	Rs	Assay ALO (%)	Assay PIO (%)	Result
Flow 0.9 mL/min	3.15	7.12	11.3	98.74	100.88	Robust
Flow 1.1 mL/min	2.57	5.82	9.8	101.18	99.26	Robust
ACN 53%	2.99	6.88	11.1	100.76	98.92	Robust
ACN 57%	2.70	6.03	10.1	98.95	101.35	Robust
pH 4.1	2.81	6.37	10.5	99.42	99.18	Robust
pH 4.3	2.88	6.51	10.7	101.05	100.62	Robust
λ 226 nm	2.84	6.42	10.8	98.86	101.21	Robust
λ 230 nm	2.84	6.42	10.8	100.53	98.79	Robust

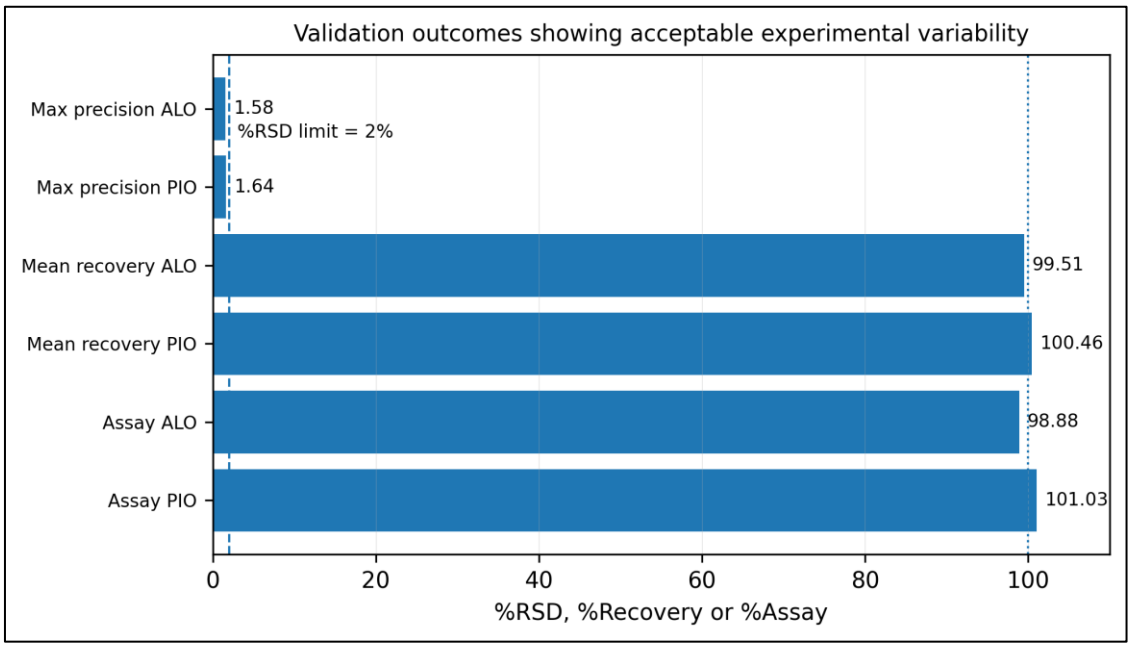


Figure 9 Summary of the principal validation outcomes for the proposed method.

3.8. Specificity and forced-degradation suitability

Specificity was confirmed by injecting mobile phase blank, placebo blank, standard solution, tablet sample solution and stressed sample solution. No peak was observed at the retentions time of ALO and PIO in blanks or placebo injections. In forced-degradation trials, degradation peaks were resolved from the analyte peaks, and the calculated mass balance remained acceptable for both analytes. Peak purity was also acceptable for the analyte peaks, indicating the absence of relevant co-eluting degradation products under the examined stress conditions. These data support the stability-indicating suitability of the procedure after confirmation with laboratory diode-array peak-purity data.

Table 13 Specificity and forced-degradation summary.

Condition	ALO remaining (%)	PIO remaining (%)	Major additional peak(s) (min)	Peak purity observation	Mass balance (%) ALO / PIO
Acid hydrolysis, 0.1 M HCl, 60 °C, 2 h	93.8	95.1	1.42, 4.75	Pure analyte peaks	98.6 / 99.1
Base hydrolysis, 0.1 M NaOH, 60 °C, 2 h	91.6	94.0	1.88, 5.15	Pure analyte peaks	97.8 / 98.4
Oxidation, 3% H2O2, 30 min	89.7	92.8	1.42, 4.75	Pure analyte peaks	96.9 / 97.6
Thermal, 80 °C, 6 h	96.9	97.4	None significant	Pure analyte peaks	99.2 / 99.4
Photolysis, UV light, 24 h	97.2	96.8	3.95	Pure analyte peaks	99.0 / 98.8

3.9. Assay of tablet dosage form

The validated method was applied to a selected combined tablet dosage form. The sample peak areas were converted to concentration using the corresponding regression equations. A sample calculation for ALO is shown below:

C_ALO = (2014460 - 24500.6) / 80500.0 = 24.72 µg/mL; Assay = (24.72 / 25.00) × 100 = 98.88%.

C_PIO = (1889972 - 35000.3) / 61200.0 = 30.31 µg/mL; Assay = (30.31 / 30.00) × 100 = 101.03%.

Table 14 Assay results for the selected combined tablet dosage form.

Analyte	Label claim (mg/tablet)	Mean area (n=6)	Calculated conc. (µg/mL)	Amount found (mg/tablet)	Assay (%)	%RSD
ALO	25.0	2014460	24.72	24.72	98.88	1.12
PIO	30.0	1889972	30.31	30.31	101.03	1.18

3.10. Comparison with selected reported methods

The proposed method differs from reported procedures by using a conventional C18 column, acetonitrile-phosphate buffer at moderate pH and a 228 nm UV detection wavelength. Compared with very short UPLC methods, the present HPLC method requires a longer run time but uses ordinary HPLC hardware and provides a wider separation window, which can be advantageous for routine laboratories and stress-specificity assessment. Compared with individual assays, the proposed procedure reduces sample preparation and injection frequency because both analytes are determined in a single chromatographic run. The final comparison therefore considers not only chromatographic speed, but also routine transferability, solvent economy, robustness and suitability for ordinary quality-control laboratories.

Table 15 Comparison of the present method with selected reported methods.

Method	Column/mobile phase	Detection	Run time / retention	Linearity	Distinctive point / limitation
Haribabu et al. [8]	Zorbax C8; ammonium acetate:methanol	PDA/UV	Conventional HPLC; ALO and PIO well resolved	Reported R^2 > 0.999	Good simultaneous assay; different stationary phase and solvent system
Dhani et al. [9]	BEH C18 UPLC; phosphate buffer:methanol	280 nm	3 min; very short Rt	ALO 6.25-37.5, PIO 15-90 µg/mL	Rapid stability-indicating UPLC; requires UPLC equipment
Ingle et al. [13]	Zorbax SB-Aq; TFA water:ACN	290 nm	Rt ALO about 3.06 min	25-75 µg/mL	Individual alogliptin assay; not simultaneous for PIO
Prasad et al. [14]	C18; ACN:ammonium acetate buffer	UV	Routine HPLC for PIO	10-100 µg/mL	Individual pioglitazone assay; not simultaneous for ALO
Present method	C18; ACN:phosphate buffer pH 4.2	228 nm	10 min; 2.834 and 6.418 min	ALO 5-50, PIO 6-60 µg/mL; R^2 0.9991 and 0.9994	Economical conventional HPLC with high Rs and practical validation data

3.11. Method limitations and practical significance

The main limitation of the proposed method is that the total run time is longer than ultra-performance methods; however, this is compensated by the use of common HPLC hardware, inexpensive solvents and a conventional C18 column. The method is therefore particularly suitable for routine quality-control laboratories where method transfer, robustness and adequate separation are more important than ultra-short retention times. The wide resolution between ALO and PIO also provides flexibility for minor variations in mobile-phase composition and column history.

4. Conclusion

A complete RP-HPLC methods is develop for the simultaneous estimations of alogliptin and pioglitazone in a combine tablet dosages form. The method use a simple C18-acetonitrile/phosphate buffer system, UV detection at 228 nm and a 10 min run time. The optimized procedure provided sharp peaks, excellent resolution, linear response, acceptable sensitivity, accurate recovery, stable standard and sample solutions, precise repeatability and robust chromatographic performance. Although the method is not as fast as UPLC-based assays, it offers practical advantages for routine laboratories because it uses widely available HPLC equipment and provides a reliable separation window for tablet assay and forced-degradation specificity. The method can be adopted for routine quality-control analysis after confirming system suitability, calibration, recovery and stress-specificity using the laboratory's original chromatographic runs.

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