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Research Article

COMPARATIVE DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC AND RP-HPLC METHODS FOR THE ESTIMATION OF ACARBOSE IN TABLET DOSAGE FORMS

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ABSTRACT

Background: Acarbose is a highly polar, weakly UV-absorbing alpha-glucosidase inhibitor used in type 2 diabetes mellitus, and its analytical determination is complicated by the absence of a strong chromophore. The aim of this study was to develop, optimise and validate two complementary analytical methods - a simple UV spectrophotometric method and a reverse-phase high-performance liquid chromatographic (RP-HPLC) method - for the quantitation of acarbose in tablet dosage forms, and to compare their analytical performance to guide method selection in pharmaceutical quality control. Methods: The UV method measured the intrinsic absorbance of acarbose in distilled water at 210 nm. The RP-HPLC method used a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 um) with acetonitrile: water: 0.1% trifluoroacetic acid (20:80:0.5, v/v/v) as mobile phase, a flow rate of 1.0 mL/min, and UV detection at 210 nm. Both methods were validated in accordance with ICH Q2(R1) guidelines and applied to two marketed 50 mg tablet brands. Results: The UV method was linear over 10-100 ug/mL (r2 = 0.9992) with accuracy of 99.14-100.86%, intra-day precision <= 0.86% RSD, LOD 2.84 ug/mL and LOQ 8.61 ug/mL. The RP-HPLC method was linear over 5-100 ug/mL (r2 = 0.9998), with accuracy of 99.62-100.48%, intra-day precision <= 0.42% RSD, LOD 0.96 ug/mL and LOQ 2.91 ug/mL, and a retention time of 3.84 min. Assay of both commercial formulations gave 99.08-100.16% of label claim by both methods, within the IP 2022 limit of 95.0-105.0%. Conclusion: Both methods are accurate, precise and fit for purpose. RP-HPLC is approximately three-fold more sensitive and more specific, making it the method of choice for stability-indicating, dissolution and regulatory applications, whereas the UV method offers a rapid, economical and greener alternative for routine assay in resource-limited laboratories.

Keywords: *Acarbose; UV spectrophotometry; RP-HPLC; method validation; ICH Q2(R1); pharmaceutical quality control*.

INTRODUCTION

Pharmaceutical analysis is a cornerstone of drug development and manufacturing, providing the quantitative basis for assuring the identity, purity, potency and safety of medicinal products [1]. Reliable and reproducible analytical methods underpin every stage of the pharmaceutical lifecycle, from raw-material testing and in-process control to finished-product release, stability evaluation and bioequivalence assessment [2].

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Regulatory frameworks such as Good Manufacturing Practice, Good Laboratory Practice and the International Council for Harmonisation (ICH) Q2(R1) guideline define the validation characteristics - linearity, accuracy, precision, specificity, detection and quantification limits, and robustness - that an analytical procedure must demonstrate before it can be used for regulatory decision-making [2,3].

Among the techniques available for small-molecule assay, UV spectrophotometry remains the most widely used owing to its simplicity, low cost, speed and non-destructive nature, while high-performance liquid

chromatography (HPLC) is regarded as the gold standard because of its superior sensitivity, selectivity and reproducibility [4,8]. The selection between these techniques depends on the analytical purpose, the complexity of the sample matrix, the available instrumentation and the relevant regulatory requirement; each method carries distinct advantages and limitations that must be weighed for a given application [8,10].

Acarbose is an orally administered alpha-glucosidase inhibitor used as an adjunct to diet and exercise in the management of type 2 diabetes mellitus. By reversibly inhibiting intestinal maltase, sucrase and glucoamylase, it delays carbohydrate digestion and attenuates the postprandial rise in blood glucose. Chemically it is a pseudotetrasaccharide (C25H43NO18; molecular weight 645.60 g/mol) that is freely soluble in water and practically insoluble in common organic solvents. This high polarity, combined with the absence of an aromatic ring or extended conjugation, leaves acarbose with only a weak chromophore - an enamine system in the valienamine moiety - that absorbs near 210 nm. As a result, conventional UV detection is intrinsically insensitive, and chromatographic procedures with detection at short wavelengths have generally been preferred for impurity profiling and stability-indicating analysis [11,13,14].

The published literature on acarbose analysis reflects this challenge. Wang et al. emphasised that chromatographic methods, particularly HPLC, are the most dependable for acarbose because of the molecule's weak UV absorbance [11], while Montazeri et al. validated a stability-indicating HPLC method capable of resolving acarbose from its degradation products [14]. Leistner and Holzgrabe explored alternative detection strategies for impurity profiling of this difficult analyte [13], and Wulandari et al. demonstrated that simple UV-based procedures remain valuable for routine work despite their lower sensitivity [16]. However, comparatively few studies have placed a simple UV method and a chromatographic method side by side under identical validation conditions to provide an evidence base for rational method selection.

The present study was therefore designed to develop, optimise and validate both a UV spectrophotometric method and an RP-HPLC method for acarbose in tablet dosage forms according to ICH Q2(R1), to apply both methods to commercial formulations, and to compare them systematically across all validation parameters so as to provide practical, evidence-based guidance on when each method is appropriate.

MATERIALS AND METHODS

Materials

Acarbose reference standard (purity >= 99.0%) was received as a gift sample from Bayer Pharmaceuticals Ltd., India. Two commercial 50 mg tablet brands were purchased from retail pharmacies in Palwancha, Telangana: Glucobay (Bayer Pharmaceuticals Ltd.) and Rebose (Sun Pharma Laboratories Ltd.). Acetonitrile, methanol and trifluoroacetic acid (TFA) were of HPLC grade (Merck India and Sigma-Aldrich); orthophosphoric acid and potassium dihydrogen phosphate were of analytical-reagent grade. Distilled water was prepared in-house (conductivity <= 0.5 uS/cm). All mobile-phase solvents were filtered through 0.45 um membrane filters and degassed by sonication before use.

Instrumentation

Spectrophotometric measurements were made on a Shimadzu UV-1900 double-beam UV-Vis spectrophotometer (190-900 nm) with matched 1 cm quartz cuvettes. Chromatography was performed on a Shimadzu Prominence LC-20A system (LC-20AD pump, SIL-20AC autosampler, CTO-20A column oven, SPD-20A UV-Vis detector) controlled by LCsolution software, fitted with a Phenomenex Luna C18(2) column (250 x 4.6 mm, 5 um) and matching guard cartridge. Weighing used a Shimadzu AUX220 analytical balance (0.0001 g).

UV spectrophotometric method

A primary stock solution (1000 ug/mL) was prepared by dissolving 100 mg of acarbose in 100 mL distilled water with sonication. Working standards (10-100 ug/mL) were obtained by dilution. A 50 ug/mL solution was scanned from 200-400 nm against a water blank; a single absorption maximum was observed at 210 nm, which was selected as the analytical wavelength. Calibration standards (10, 20, 40, 60, 80, 100 ug/mL) were measured in triplicate at 210 nm and the data subjected to least-squares regression. For tablet analysis, twenty tablets of each brand were weighed and powdered; a quantity equivalent to 50 mg acarbose was dissolved in distilled water, filtered, made to volume and diluted to a nominal 50 ug/mL before measurement at 210 nm.

RP-HPLC method

The mobile phase consisted of acetonitrile: water:0.1% TFA (20:80:0.5, v/v/v), delivered isocratically at 1.0 mL/min with the column oven at 30 degC, UV detection at 210 nm, an injection volume of 20 uL and a run time of 10 min. The TFA acidified the mobile phase (pH ~ 2.0), protonating the basic nitrogen of the valienamine moiety to improve peak symmetry and slightly enhance retention of the polar analyte. Calibration standards (5, 10, 25, 50, 75, 100 ug/mL) were injected in triplicate. System suitability was assessed from five replicate injections of a 50 ug/mL standard. Tablet sample solutions were prepared analogously to the

UV method and diluted to a nominal 50 ug/mL with mobile phase, then filtered through 0.45 um syringe filters before injection.

Method validation

Both methods were validated per ICH Q2(R1). Linearity was evaluated across six concentration levels. Accuracy was assessed by the standard-addition method at 80%, 100% and 120% of the target concentration (n = 3 per level) and expressed as percentage recovery. Intra-day precision was determined from six replicate analyses of a 50 ug/mL standard, and inter-day precision from analyses on days 1, 3 and 5 at three concentrations. Specificity was confirmed against a placebo (excipient) blank and, for HPLC, by peak-purity assessment. LOD and LOQ were calculated from the standard deviation of the response and the slope (LOD = 3.3 sigma/S; LOQ = 10 sigma/S). Robustness was tested by deliberately varying wavelength, mobile-phase composition, flow rate, column temperature and injection volume one at a time.

RESULTS

Spectral characteristics and chromatographic system suitability

The UV spectrum of acarbose showed a single absorption maximum at 210 nm with no significant absorption beyond 250 nm; the molar absorptivity at 210 nm was 4,792 L.mol-1.cm-1, consistent with literature values (4,600-5,100 L.mol-1.cm-1), and the water blank showed no interfering absorption at this wavelength. Under the optimised chromatographic conditions, acarbose eluted as a symmetrical peak at 3.84 min. System suitability parameters (Table 1) all met acceptance criteria, confirming the chromatographic system was fit for use.

Linearity and calibration

Both methods were linear across their respective ranges. The UV method obeyed the Beer-Lambert law over 10-100 ug/mL (y = 0.00818x + 0.00062; r2 = 0.9992), while the HPLC method was linear over 5-100 ug/mL (y = 3,728.4x + 42.6; r2 = 0.9998). The calibration data are summarised in Table 2 and the regression lines are shown in Figure 2.

Accuracy, precision and detection limits

Recovery at the three spiked levels ranged from 99.14% to 100.86% for the UV method and 99.62% to 100.48% for HPLC, both within the 98.0-102.0% acceptance window. Intra-day precision was <= 0.86% RSD (UV) and <= 0.42% RSD (HPLC); inter-day precision was <= 1.12% and <= 0.64% respectively. The consolidated validation outcomes for both methods are presented in Table 3.

Assay of commercial formulations

Both validated methods were applied to two marketed 50 mg acarbose tablets. All results fell within the IP 2022 acceptance criterion of 95.0-105.0% of label claim, and the two methods agreed to within 0.6% (Table 4).

Comparative performance

A head-to-head comparison (Figure 3) shows that HPLC was approximately three times more sensitive (LOD 0.96 vs 2.84 ug/mL) and about twice as precise as the UV method, with superior linearity and specificity. Conversely, the UV method required only a spectrophotometer and water, completed an assay in about 5 min versus 12 min, and consumed roughly one-third of the solvent volume.

Table 1: HPLC system suitability parameters (n = 5 injections, 50 ug/mL acarbose standard).

Parameter	Value (Mean +/- SD)	Acceptance criterion	Compliance
Retention time (min)	3.84 +/- 0.04	Consistent	Yes
Peak area (mAU.min)	186,420 +/- 640	-	Yes
%RSD of peak area (n=5)	0.34%	<= 2.0%	Yes
Tailing factor	1.06	0.8-2.0	Yes
Theoretical plates (N/m)	8,640	>= 2,000	Yes
Capacity factor (k')	2.84	1-10	Yes

Table 2: Calibration data for acarbose by UV spectrophotometry (210 nm) and RP-HPLC (n = 3 per level).

Conc. (ug/mL)	UV absorbance (Mean +/- SD)	UV %RSD	HPLC peak area (Mean +/- SD)	HPLC %RSD
5	-	-	18,648 +/- 88	0.47
10	0.0824 +/- 0.0006	0.73	37,284 +/- 120	0.32
20	0.1648 +/- 0.0009	0.55	-	-
25	-	-	93,210 +/- 204	0.22
40	0.3282 +/- 0.0014	0.43	-	-
50	0.4093 (calc.)	-	186,420 +/- 440	0.24

60	0.4914 +/- 0.0018	0.37	-	-
75	-	-	279,630 +/- 580	0.21
80	0.6542 +/- 0.0022	0.34	-	-
100	0.8172 +/- 0.0024	0.29	372,840 +/- 680	0.18

Table 3: Summary of ICH Q2(R1) validation results for the UV and RP-HPLC methods.

Validation parameter	UV method	HPLC method	ICH Q2(R1) criterion
Linearity range (ug/mL)	10-100	5-100	State range
Correlation coefficient r2	0.9992	0.9998	>= 0.999
Accuracy (% recovery)	99.14-100.86	99.62-100.48	98.0-102.0
Intra-day precision (%RSD)	0.48-0.86	0.24-0.42	<= 2.0
Inter-day precision (%RSD)	0.62-1.12	0.38-0.64	<= 2.0
LOD (ug/mL)	2.84	0.96	-
LOQ (ug/mL)	8.61	2.91	-
Specificity	No excipient interference at 210 nm	Analyte resolved from excipients	Demonstrated
Robustness (max %RSD)	1.38	0.78	<= 2.0

Table 4: Assay of acarbose in commercial tablet formulations (n = 6 per brand). %LC = percentage of label claim.

Formulation	Label (mg)	UV found (mg)	UV %LC	HPLC found (mg)	HPLC %LC
Glucobay 50 mg	50.0	49.54 +/- 0.38	99.08	49.82 +/- 0.18	99.64
Rebose 50 mg	50.0	49.92 +/- 0.42	99.84	50.08 +/- 0.20	100.16
Mean	50.0	49.73 +/- 0.40	99.46	49.95 +/- 0.19	99.90

Figure 1&2: Calibration curves for acarbose: (a) UV spectrophotometry at 210 nm; (b) RP-HPLC at 210 nm. Points are means of triplicate measurements; lines are least-squares fits.

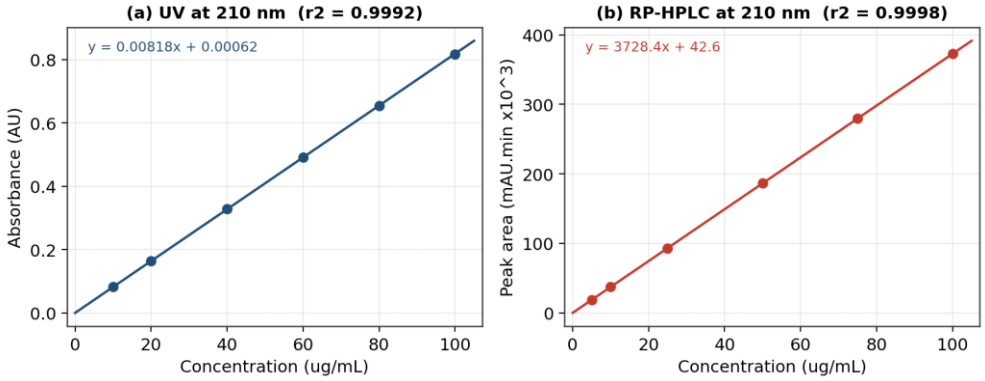
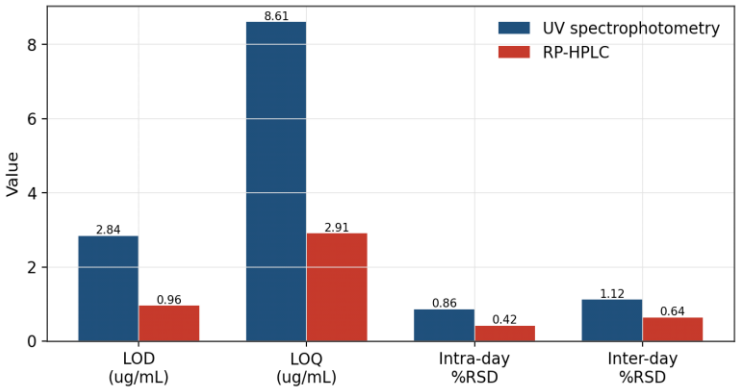


Figure 3: Comparative analytical performance of the UV spectrophotometric and RP-HPLC methods for acarbose (lower values indicate better sensitivity/precision).



DISCUSSION

The absorption maximum of acarbose at 210 nm observed here is consistent with all published reports and reflects the weak enamine chromophore of the valienamine moiety, the only viable UV detection window for this non-aromatic, highly polar molecule. The molar absorptivity of approximately 4,792 L.mol-1.cm-1 places acarbose firmly among the weakly UV-absorbing pharmaceuticals, which explains the relatively high LOD and LOQ of the direct UV method. Nevertheless, the Beer-Lambert linearity (r2 = 0.9992) over 10-100 ug/mL comfortably encompasses the working concentration for tablet assay (50 ug/mL), and the accuracy and precision obtained satisfy ICH requirements, confirming that the UV method is fit for routine content determination [16].

The RP-HPLC method exploited a standard C18 platform with an acetonitrile-water-TFA mobile phase. The inclusion of 0.1% TFA served two purposes: it lowered the mobile-phase pH to protonate the basic nitrogen of acarbose, improving peak symmetry (tailing factor 1.06), and acted as a weak ion-pairing agent to enhance retention of the polar analyte, giving a practical retention time of 3.84 min. The high plate count (8,640 plates/m) and symmetrical peak translated into superior linearity (r2 = 0.9998), and the improved signal-to-noise ratio produced a three-fold lower LOD and an approximately two-fold improvement in precision relative to the UV method - findings that mirror the comparative behaviour reported for other weakly absorbing drugs analysed by both techniques [14,36].

Critically, both methods produced equivalent assay results for the two commercial formulations, agreeing to within 0.6% of one another and remaining within the pharmacopoeial 95.0-105.0% window. The marginally lower values returned by the UV method are attributable to minor baseline contribution from excipient absorption at 210 nm and small volumetric differences, but are analytically and clinically insignificant. This agreement is the central practical message of the study: for clean tablet matrices, the simple UV method delivers results indistinguishable from HPLC at a fraction of the cost and time.

The principal advantage of HPLC lies not in routine assay but in applications that demand sensitivity and specificity. Because the chromatographic step separates acarbose from co-eluting matrix and degradation components before detection, only the authentic analyte contributes to the signal - an essential property for stability-indicating assays, impurity profiling, dissolution testing at low concentrations and bioequivalence work [13,14,37]. The UV method, which measures total absorbance at 210 nm, cannot make this distinction and is therefore best confined to clean, single-component formulations. The performance obtained here is consistent with previously published UV and HPLC methods for acarbose, supporting the validity of the present data.

From a practical and sustainability standpoint, the UV method's minimal solvent footprint (water only), short analysis time and modest instrumentation make it attractive for high-throughput routine testing in small and medium manufacturing units, particularly in resource-constrained settings. The two methods should therefore be regarded as complementary rather than competing: the choice between them is dictated by analytical purpose, regulatory expectation and laboratory capability.

CONCLUSION

Both a UV spectrophotometric method and an RP-HPLC method were successfully developed and fully validated per ICH Q2(R1) for the estimation of acarbose in tablet dosage forms, and both met all acceptance criteria. The RP-HPLC method (5-100 ug/mL; r2 = 0.9998; LOD 0.96 ug/mL) was analytically superior in sensitivity, precision, linearity and specificity, and is recommended for stability-indicating, dissolution, impurity-profiling and regulatory applications. The UV method (10-100 ug/mL; r2 = 0.9992; LOD 2.84 ug/mL) is simple, rapid, economical and environmentally favourable, and is well suited to routine quality control in resource-limited laboratories. Assay of two marketed formulations gave equivalent, compliant results by both methods, demonstrating that the two procedures are complementary and that method selection should be guided by the intended analytical purpose and available resources.

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