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Research Article

A NOVEL VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF ALBIGLUTIDE IN BULK

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ABSTRACT

A simple, selective, linear, precise and accurate RP-HPLC method was developed and validated for rapid assay of Albiglutide in bulk form. Isocratic elution at a flow rate of 1.0ml/min was employed on a symmetry C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: phosphate Buffer (PH-5.5) 85:15(V/V). The UV detection wavelength was 246nm and 20µl sample was injected. The retention time for Albiglutide was 6.01 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Albiglutide in bulk.

Keywords: Albiglutide, RP-HPLC, UV detection, recovery, precise.

INTRODUCTION

Albiglutide is a long-acting glucagon-like peptide-1 (GLP-1) analog that has been developed by Novo Nordisk for the treatment of type2 diabetes. Albiglutide a new, long-acting GLP-1 mimetic generated by genetic fusion of a DPP-IV-resistant GLP-1 dimer (97% homologous to native human GLP-1) to human serum albumin (HSA), was developed to provide an extended half-life and allow less frequent dosing than exenatide, the currently available GLP-1 mimetic.2 Albiglutide decreased glycemic excursions and stimulated insulin secretion in pre-clinical studies.3,4 This study provides the fi rst assessment of short-term safety/ tolerability and demonstrates the extended half-life and glucose-lowering ability of albiglutide in healthy humans.

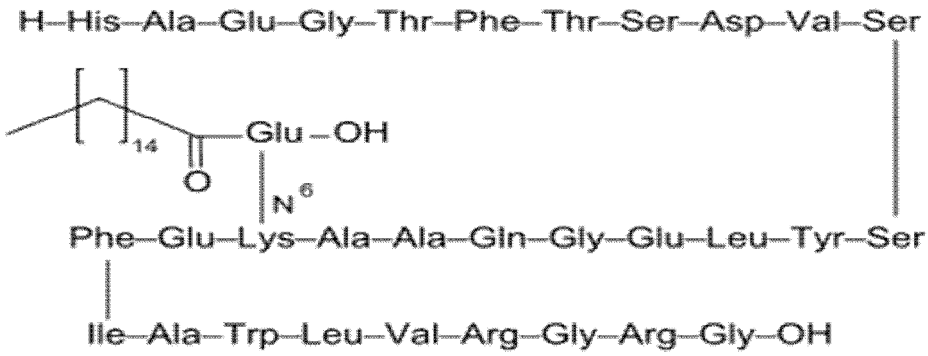

Fig. 1: Structure of Albiglutide

EXPERIMENTAL

Chemicals and Reagents

HPLC grade, Methanol and phosphate Buffer (PH-5.5)

Instrumentation and Analytical Conditions

The analysis of drug was carried out on a Waters HPLC 22695 series consisting 4 pump, Auto sampler, equipped with PDA detector,thermostat column connected with waters (alliance) Empower software,Inertsil ODS C18 column (250x4.6mm, 5µm in particle size). Isocratic elution with Methanol: phosphate Buffer (PH-5.5) 85:15(V/V) was used at a flow rate of 1.0ml/min. The mobile phase was prepared freshly and degassed by sonicating for 5 min before use.

Stock and Working Standard Solutions

10mg of Albiglutide working standard was accurately weighed and transferred into a 10ml volumetric flask containing HPLC grade Methanol as the diluent. It was sonicated, dissolved completely and made volume up to the mark with the same solvent. 1ml of the above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with HPLC grade Methanol. The contents were mixed well and filtered through 0.45µm nylon filter paper and 60ppm was prepared finally. The calibration curve was plotted with the seven concentrations of the 30 ppm-90 ppm working standard solutions. Calibration solutions were prepared daily and analyzed immediately after preparation.

Validation Procedure

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, stability and system suitability. Standard plots were constructed with five concentrations in the range of 30 ppm to 90 ppm prepared in triplicates to test linearity. The peak area of Albiglutide was plotted against the concentration to obtain the calibration graph. The linearity was evaluated by linear regression analysis that was calculated by the least square regression method. The precision of the assay was studied with respect to both repeatability and intermediate precision. Repeatability was calculated from seven replicate injections of freshly prepared Albiglutide test solution in the same equipment at a concentration value of 100% (60 ppm) of the intended test concentration value on the same day. The experiment was repeated by assaying freshly prepared solution at the same concentration additionally on two consecutive days to determine intermediate precision. Peak area of the Albiglutide was determined and precision was reported as %RSD. Method accuracy was tested (% recovery and %RSD of individual measurements) by analyzing sample of Albiglutide at three different levels in pure solutions using three preparations for each level. The results were expressed as the percentage of Albiglutide recovered in the samples. Sample solution short term stability was tested at ambient temperature (20±10^{0}C) for three days.

RESULTS AND DISCUSSION

Optimization of the Chromatographic Conditions

Proper selection of the stationary phase depends up on the nature of the sample, molecular weight and solubility. Among C8 and C18, C18 column was selected. Non-polar compound is very attractive with reverse phase columns. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of HPLC grade Methanol: phosphate Buffer (PH-5.5) 85:15(V/V) was selected as mobile phase and the effect of composition of mobile phase on the retention time of Albiglutide was thoroughly investigated. The concentration of the HPLC grade Methanol: phosphate Buffer (PH-5.5) were optimized to give symmetric peak with short run time (Fig.3).

Validation of Method

Linearity

Five points graphs was constructed covering a concentration range 3-18ppm (Three independent determinations were performed at each concentration). Linear relationships between the peak area signal of Albiglutide the corresponding drug concentration was observed. The standard deviation of the slope and intercept were low. The statistical analysis of calibration is shown in Table 1.

System Suitability

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits (Table.3). The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it

could be used for the rapid and reliable determination of Albiglutide in bulk form. The results are furnished in Table 3.

Robustness

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures. This indicated the reliability of the proposed method during its routine application for the analysis of Albiglutide.

Ruggedness

Ruggedness was also tested by applying the proposed methods to the assay of Albiglutide using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (RSD) did not exceed 2%. The proposed method gave satisfactory results with Albiglutide in bulk.

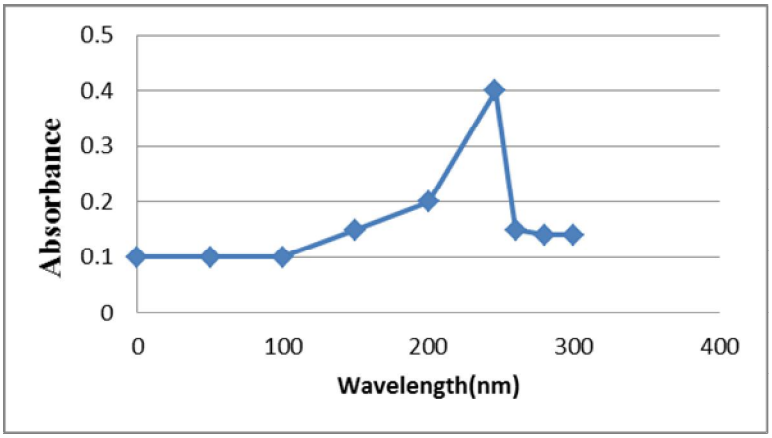

Fig. 2: Absorption spectrum of Albiglutide

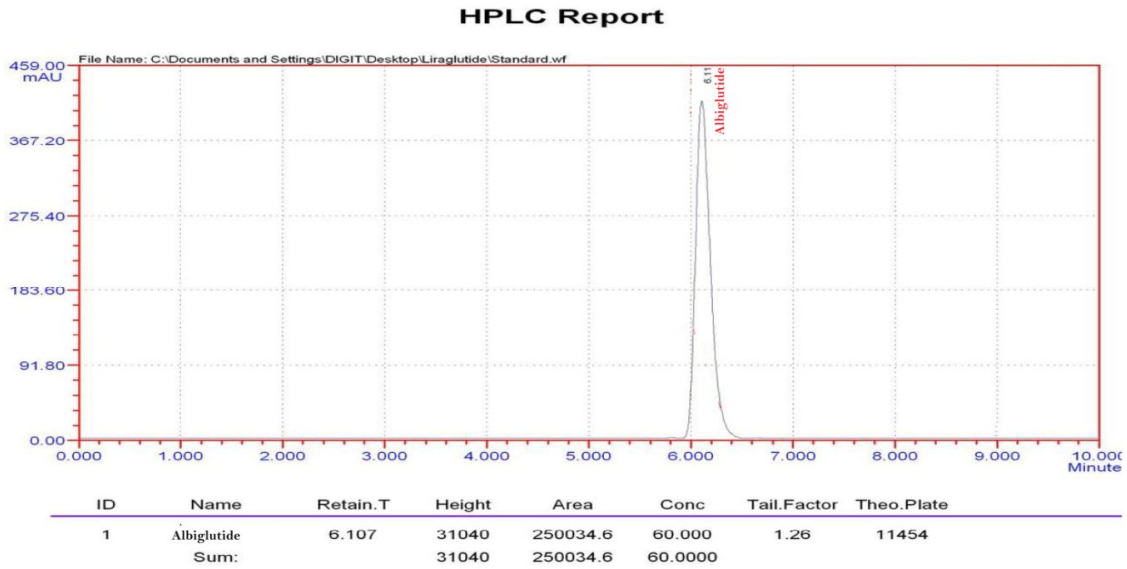

Fig. 3: chromatogram of Albiglutide standard

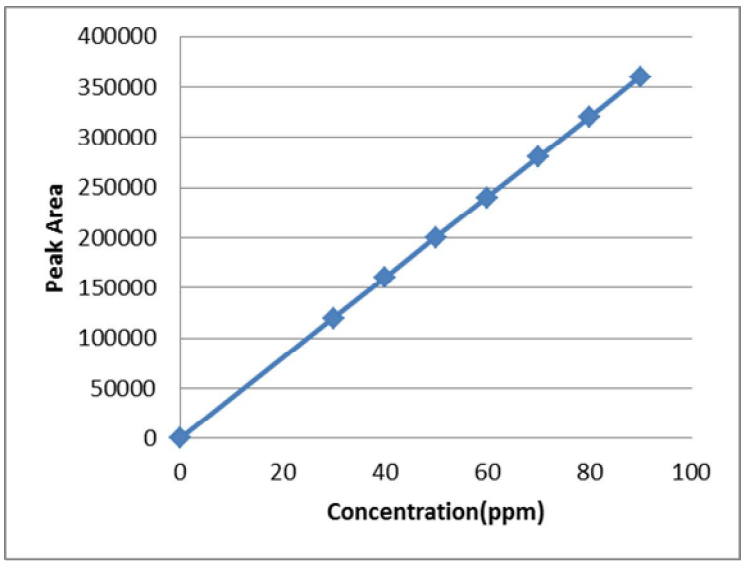

Fig. 4: Standard calibration curve for analysis of Albiglutide at 246nm

Table 1: Linearity of Albiglutide

S. No.	Concentration in ppm	Area
1	30	125796
2	40	160123
3	50	205632
4	60	246951
5	70	289860
6	80	321961
7	90	361258
Range 30-60ppm	Slope Intercept Correlation coefficient	4216 -633 0.999

Table 2: System stability parameters

S. No.	Parameters	Values
1	λ max (nm)	246nm
2	Beer's law limit (ppm)	30 – 90 ppm
3	Correlation coefficient	0.999
4	Retention time	6.11
5	Theoretical plates	11453
6	Tailing factor	1.26
7	Limit of detection	0.06ppm
8	Limit of quantification	0.2 ppm
9	Slope	4216
10	Intercept	-633
11	Accuracy	99.31
12	R.S.D.	1.13

Table 3: Chromatographic condition

Mobile phase	Methanol: phosphate Buffer (85:15(V/V).
PH	5.5
UV detection	246nm
Analytical column	C_{18} Inertsil ODS
Flow rate	1.0 ml/min
Temperature	ambient
Injection volume	20µl
Runtime	10 min
Retention time	6.11 min

CONCLUSION

A validated RP-HPLC method has been developed for the determination of Albiglutide in Bulk form. The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 10 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of Albiglutide in Bulk form. The limit of detection for Albiglutide was found to be 0.06 ppm and the limit of quantification was found to be 0.2 ppm. It proves the sensitivity of method.

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REFERENCES

1. Matthews JE, Stewart MW, De Boever EH, et al. (December 2008). "Pharmacodynamics, Pharmacokinetics, Safety, and Tolerability of Albiglutide, a Long-Acting Glucagon-Like Peptide-1 Mimetic, in Patients with Type 2 Diabetes". J. Clin. Endocrinol. Metab. 93 (12): 4810–4817. doi:10.1210/jc.2008 1518. PMID 18812476.
2. Baggio et al. (2008). "Glucagon-like Peptide-1 Analogs Other Than Exenatide".
3. "Phase III clinical trials of Albiglutide".
4. Holst JJ, Physiol Rev 2007; 87:1409–1439
5. Lam S, et al. Cardiol Rev 2006; 14:205–211
6. Baggio L, et al. Diabetes 2004; 53:2492–2500
7. Bloom M, et al. Diabetes Care 2003; 52(Suppl1): Abstract A112
8. UK UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–853
9. A Action to Control Cardiovascular Risk in Diabetes Study Group. Gerstein HC, Miller ME, Byington RP, Goff DC, Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH, Jr, Probstfield JL, Simons-Morton DG, Friedewald WT.: Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545–2559
10. ADVANCE Collaborative Group. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F.: Intensive blood

glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med2008;358:2560–2572

11. Abraira C, Duckworth WC, Moritz T.: VADT Group. Glycaemic separation and risk factor control in the Veterans Affairs Diabetes Trial: an interim report. Diabetes Obes Metab 2009;11:150–156
12. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA.: 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577–1589
13. American Diabetes Association. Standards of medical care in diabetes—2009. Diabetes Care2009;32(Suppl. 1):S13–S61.