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Development and validation of analytical technique for the evaluation of insulin glargine by RP-HPLC

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Abstract

A rapid, exact, trustworthy, and repeatable RP-HPLC method was created for the determination of insulin glargine. Adjusting pH 2.5 with sodium dihydrogen phosphate anhydrous buffer is used as the mobile phase-A in the most recent validated gradient RP-HPLC analytical technique for detecting insulin glargine: acetronitrile R1, buffer solution (7:93 V/V) and mobile phase-B: buffer solution, acetronitrile R1 (43:57 V/V). Waters, synergi 4 µm fusion-RP 80 A° 250 × 3 mm (phenonmenox), column: size l = 0.125 m, ϕ =3.0 mm with a flow rate of 0.55 ml/min, samples were allocated. It was found that 214 nm is the wavelength at which ultraviolet detection occurs. The technique was evaluated in the presence of phenol and m-cresol, and these are present in low concentrations in commercial insulin glargine preparation as preservatives, as well as for the purpose of research on insulin glargine and its desamido degradation product. These preservatives are present in commercial insulin glargine preparations as well. The method's linearity over the measured concentration between 12 to 18 mg/ml was found, with coefficient regression r^2 = 0.998. During accuracy tests, it was revealed that the mean recovery was around 100.35 per cent. A relatively inexpensive, dependable, accurate, linear, and quick RP-HPLC technique was created and verified in accordance with ICH guidelines requirements. This strategy has been demonstrated to be reliable, and it can currently be used to routinely assess insulin glargine.

1. Introduction

In both clinical trials with type 1 and type 2 diabetes patients and preclinical glucose clamp tests, insulin glargine (21A-Gly30Ba-L-Arg-30Bb-L-Arg human insulin) has demonstrated considerable advantages. Similar to NovoSol basal, insulin glargine works by bringing the insulin's isoelectric point to a neutral pH (7.0); due to the homogeneous nature of insulin glargine and the absence of some of the negative effects associated with insulin suspensions (Hamidli *et al*., 2022; Oliva, *et al*., 2000).

Compared to NPH, long-lasting consequences of insulin glargine, more predictable pharmacokinetics and a peak-less action profile, according to research on the drug's absorption in individuals with type 1 and type 2 diabetics in addition to healthy volunteers (Mounika *et al*., 2021; Amisha *et al*., 2021; Divya *et al*., 2021). In a crossover investigation with healthy individuals, no variations in insulin glargine absorption were seen when the drug was injected into the leg, arm, or abdomen.In patients with type 1 and type 2 diabetes, there have been temporary (4 weeks) and for a long time (28 weeks) clinical safety and efficacy research analyzing insulin glargine to NPH human insulin (De souza von zuben*et al*., 2020; Rajeshwari *et al.,* 2013; Gourdy *et al*., 2021).These studies have demonstrated that for glycemic management, as determined by HbA1c and fasting glucose levels in plasma, once-daily insulin glargine is frequently less likely to result in nocturnal hypoglycemia than once-daily and twice-daily NPH regimens.

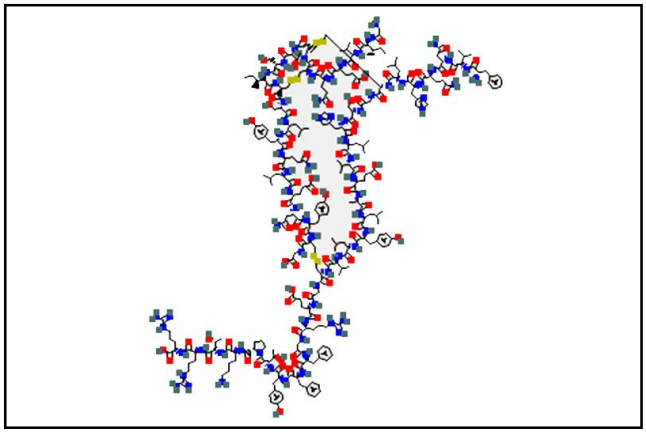

Figure 1: Molecular formula $C_{267}H_{404}N_{72}O_{78}S_6$.

Compared to NPH and lente insulins, insulin glargine has the disadvantage of not being able to be combined with other insulins (like lispro). Insulin glargine has been evaluated for safety and at least equally secure as NPH in terms of the occurrence and challenging occurrences that diabetes people may experience. Studies on the immunogenicity of insulin glargine in comparison to conventional human insulin demonstrate no greater formation of antibodies. Additionally, *in vitro* research demonstrates that insulin glargine's

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growth-promoting activity in muscle cells *via* the IGF 1 receptor is identical to that of native human insulin (Janez *et al*., 2020; Legg *et al*., 2019; Agin*et al*., 2007). To substantiates the positive effects of insulin glargine observed in diabetic subjects in carefully controlled research, follow-up information is crucial. In order to demonstrate the therapeutic advantages of insulin glargine, improvements in total medical results as assessed by glycemic management, the probability of hypoglycemia, and higher lifestyle quality are required. In the near future, therapeutic choices are anticipated to be expanded by long-acting insulin analogues, enhancing diabetes patients well-being. Patients with type 2 diabetes will be more likely to accept insulin therapy, if doctors are enthusiastic about it (Hazra *et al*., 2021; Farog *et al.,* 2016; Kristl *et al*., 2021).

2. Materials and Methods

2.1 Drugs and chemicals

The unprocessed insulin glargine (100 IU/ml) was supplied by Sigma Aldrich in the United States, while the final product may be purchased from the local pharmacy. Methanol of an HPLC grade, acetonitrile of an HPLC grade, water of an HPLC quality, anhydrous sodium dihydrogen phosphate of an analytical grade, orthophosphoric acid solution of an analytical grade, and sodium chloride were all available for use from the Central Raw Material Storage at Regenix Biosciences Ltd.

2.2 Instrumentation

Shimadzu Corporation of Japan's HPLC 2030 plus-prominence I series, which consists of a pump and UV detector, was subjected to analysis. Synergi 4 µm fusion - RP 80A 250*3 mm (phenonmenox) was used for separations and data gathering and analysis were done using lab solution software. All weighing operations for this study were carried out on the SHIMADZU AUX-120 analytical balance. The samples were ultrasonically treated using an ultrasonicator from ENERTECH Electronics Pvt. Ltd. in India.

2.3 Preparation of buffer solution

Weigh about 20.7 g of anhydrous sodium dihydrogen phosphate in 1000 ml of water, adjust with orthophosphoric acid to a pH of 2.5 and filter the solution in 0.45 micron filter.

2.4 Preparation of mobile phase A

Weigh about 18.4 g of sodium chloride add 250 ml of buffer solution (pH 2.5) in to a 1000 ml flask, dissolve the material with buffer solution and add 250 ml of acetonitrile dilute up to the mark with water and mixed well.

2.5 Preparation of mobile phase B

Weigh about 3.2 g of sodium chloride add 250 ml of buffer solution (pH 2.5) in to a 1000 ml flask, dissolve the material with buffer solution and add 650 ml of acetonitrile dilute up to the mark with water and mixed well.

Table 1: Mobile phase composition

Time (min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0	0.55	96	4
20	0.55	83	17
30	0.55	63	37
33	0.55	96	4
40	0.55	96	4

2.6 Preparation of 0.01 M hydrochloric acid solution

Take 0.85 ml of hydrochloric acid to a 1000 ml standard flask, stir well to dissolve with water, and then dilute with water to the desired strength.

2.7 Standard solution preparation

15 mg of insulin glargine should be weighed into a 10 ml standard flask. The material should be dissolved in 1.5 ml of 0.01 M hydrochloric acid, then diluted with water to the appropriate strength and thoroughly mixed.

2.8 Sample solution preparation

Take two sample vials, make 2 ml of the sample solution into a 5 ml standard flask, diluted with water to the prescribed level, and thoroughly mixed.

2.9 Chromatographic conditions

Table 2: Optimization of chromatographic conditions

Instrument	HPLC
Column name	A stainless steel column measuring 25 cm by 3.0 mm and filled with porous silica and spherical end-capped octadecylsilane (4 µm) or equivalent
Pump mode	Gradient
Flow rate	0.55 ml/min
Detector wavelength	214 nm
Column oven temperature	35°C
Injection volume	5 µl
Sampler cooler	2-10ºC

3. Results

3.1 Method validation

3.1.1 System suitability test

Retention time and peak area values derived from the system suitability study were determined to be within acceptable ranges. Results are shown in Table 3.

Table 3: System suitability test

Parameters	Insulin glargine
Peak area	15240658
Retention time	26.483 min

3.1.2 Specificity

The specificity study discovered no diluents interference despite the fact that they were all recognized impurities at the time the peak was retained. It was displayed in the chromatogram Figure 2.

3.1.3 Linearity

To determine linearity and develop a calibration curve, a graph was made between the measured peak areas and concentrations. A linear relationship was discovered for the concentration range of 12 to 18 mg/ml, with a slope of 75126, an intercept of 92752, and a correlation coefficient of $r^2 = 0.998$. The regression equation developed during the linearity evaluation was $y = 92752x + 75126$. In Figure 3, the calibrated curve is displayed. Table 4 presents the analysis results.

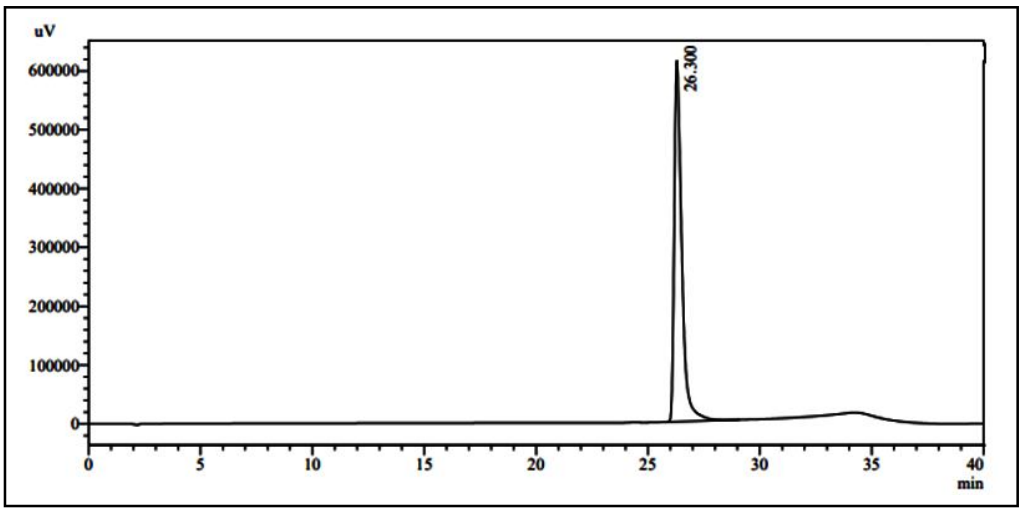

Figure 2: Specificity studies for insulin glargine.

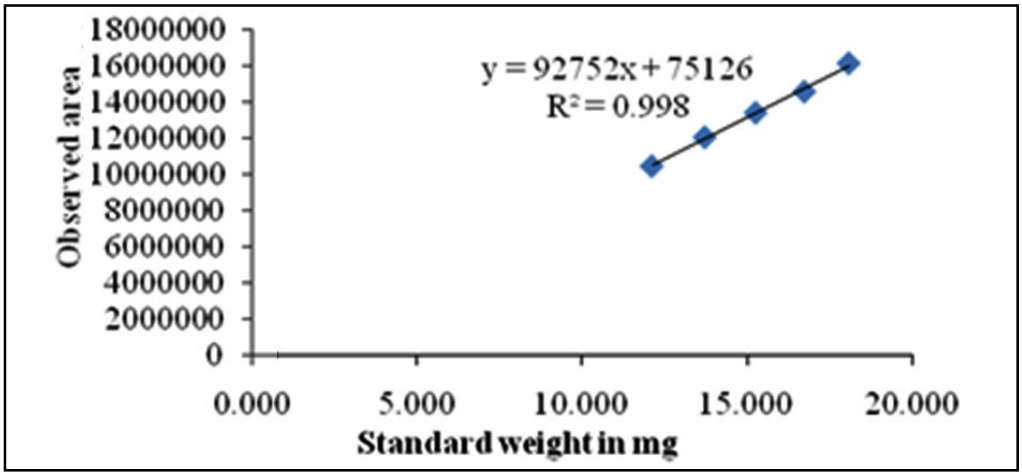

Figure 3: Linearity studies for insulin glargine.

Table 4: Linearity response of insulin glargine

Concentration (%)	Peak area
80	10479753
90	12067262
100	13416800
110	14582700
120	16142895
r^2	0.998
Slope (C)	75126
Intercept (m)	92752

3.1.4 Accuracy

When pre-analyzed data were combined with a known amount of the reference drug and exposed to the suggested HPLC method, a known proportion of the standard drug was recovered, measured in terms of per cent recovery studies at three distinct levels: 80%-120%. It was found that the average recovery rate was 99.70%. RSD as a percentage was measured to be 0.81. It was found that the RSD value in per cent was less than 2%. That confirmed that the strategy was sound. The reports are shown in Table 5.

Table 5: Accuracy studies for insulin glargine

Concentration (%)	% Recovered	Mean	% RSD
80	99.20		
90	99.20		
100	100.60	99.70	0.81
110	101.80		
120	98.20		

3.1.5 Precision

Insulin glargine estimate variance within and across days revealed that the % RSD was less than 2%. These low RSD values demonstrate the method's high degree of precision. The reports are shown in Table 6.

Table 6: Precision studies for insulin glargine

S.No.	Sample ID	Content (% of label claim)
1	Sample 1	106.8110 (106.8%)
2	Sample 2	107.6800 (107.7%)
3	Sample 3	107.7480 (107.7%)
4	Sample 4	107.0520 (107.1%)
5	Sample 5	103.6010 (103.6%)
6	Sample 6	103.1150 (103.1%)
	Mean	106.00 mg
	RSD (NMT 2.0%)	1.96

3.1.6 Robustness

The robustness analysis found that minor modifications in temperature, pH, and flow rate had no impact on the selected parameters. It was found that the retention period was typical and significant. The procedure was trustworthy as a result. The robustness results are given in Table 7.

Table 7: Robustness studies for insulin glargine

Parameters	Conditions	Retention time
High flow rate (ml/min)	1.1 ml/min	26.383
Low flow rate (ml/min)	0.9 ml/min	27.425
High PHL	3.0	26.533
ow PH	2.0	26.417
High column temperature	41°C	26.225
Low column temperature	39°C	26.117

4. Discussion

System appropriateness studies are important in liquid chromatographic procedures, according to the US Pharmacopoeia. We determined the area, retention duration, and quantity of theoretical plates for common solutions. Chromatograms were obtained after a standard solution was injected into the apparatus.

The skill to appropriately evaluation of the analyte when there are potential elements is known as specificity. Common examples include impurities, degradants, matrix, and other compounds. There were three options made: blank, standard, and sample. The chromatograms have been recorded following the addition of solutions to the apparatus. HPLC instrument heading waters, synergiphenonmenox, 4 m fusion-RP 80 A°250 x 3 mm, pump mode 1.0 ml/min isocratic flow rate injection volume: 10 µl; column pressure: 400 kgf/cm^2; sampler cooler: 2 to 10°C; detector wavelength: 214 nm; column oven temperature: 40°C.

Each of the six separate 10 ml volumetric flasks containing the normal stock solutions of the sample received 0.40, 0.45, 0.50, 0.55, and 0.60 ml of the linearity solutions, which were then diluted to 10 ml with diluents to produce 40 µl/ml, 45 µl/ml, 50 µl/ml, 55 µl/ml, and 60 µl/ml, respectively. Chromatograms have been captured at 214 nm after each solution injection. The concentration spectrum seen above follows Beer's law and is linear. By plotting the peak regions vs. concentration, the calibration was created.

By incorporating the drug standard at concentrations of 80, 100, and 120 per cent into a predefined pill solution and performing per cent recovery trials, the method's accuracy was examined.

Precision was estimated by intra- and inter-day precision studies. On the same day, at a concentration of 4 µl/ml, six analyses of an identical sample were performed to look for variations in the results. Inter-day accuracy was studied for three days in a succession.

A system's robustness is its capacity to survive subtle or deliberate changes to chromatographic parameters such as pH, flow rate (0.1 ml/min), and temperature (°C). After injecting 10 µl of the solution into the chromatographic apparatus under each condition, chromatograms were recorded. We looked at how the device suitability considerations affected things. The results obtained were within the limits.

Acceptance criteria

Assay: 95.0 % - 105.0 % of the label claim, method precision: RSD shall be NMT 2.0 % in six sample preparations, linearity: correlation coefficient NLT 0.98, accuracy: recovery NLT 98.0 % to NMT 102.0 %, robustness: no impact on the retention period.

5. Conclusion

According to ICH standards, a novel, quick, and verified RP-HPLC technique for determining insulin has been reported in this work. All metrics fall within the ranges suggested by those recommendations for pharmaceutical formulations, demonstrating the specificity, accuracy, and robustness of this approach. The method's validation demonstrates that it is accurate, repeatable, and linear. It may be applied to assay analysis in quality control procedures for both final formulations and raw materials. Therefore, a simple analytical, robust, and gradient RP-HPLC technique for determining insulin glargine was developed.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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