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Simultaneous estimation of Insulin glargine and it's excipients in formulation using LC-UV-MS/MS

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1. Overview

Simultaneous estimation plays a very important role in biopharmaceutical industry as it is very feasible and time saving. For the multi component analysis various techniques like spectrophotometric techniques (UV-VIS, IR, NMR and mass spectrometry) and chromatographic techniques (Thin Layer Chromatography, High Performance Liquid Chromatography, Ultra-High Performance Liquid Chromatography, High Pressure Thin Layer Chromatography and Gas Chromatography) is used. These techniques provide high degree of specificity and selectivity and further provide the high degree of assurance that these techniques fit for the simultaneous estimation of the biopharmaceutical dosage form.

Chromatographic and spectrophotometric techniques together develop new hyphenated techniques which are useful for the simultaneous estimation of active component, excipients, contaminants, and impurity profiling. The simultaneous analytical analysis provides specificity, high throughput, and assurance for the identification of various entities in the biopharmaceutical formulation. The main objective behind the analytical estimation is to provide the assurance that the particular formulation contains the equal amount of active ingredient and excipients as mentioned in the label.

In this poster, an LC-UV-MS/MS method has been developed for the simultaneous determination of insulin glargine and its excipients in formulation .

2. Introduction

Insulin glargine is a recombinant human insulin analogue that does provide 24-hour duration of action in most, but not all, patients with type 1 diabetes mellitus. It differs from human insulin in that the amino acid asparagine at position A21 is replaced by glycine, and two arginine are added to the carboxy (C)-terminus of the B chain. In the injection solution at pH 4, insulin glargine is completely soluble. However, it has low solubility at neutral pH.

After injection into the subcutaneous tissue, the acidic solution is neutralized, leading to the formation of micro precipitates from which small amounts of insulin glargine are slowly released; this results in absorption over a period of approximately 24 hours with no pronounced peak. Insulin glargine thus simulates the basal production of insulin. In other respects, its mechanism of action is similar to that of human insulin, and on a molar basis its glucose-lowering effects are similar to those of human insulin when given intravenously.

Product release and comparability testing is a crucial part of ensuring drug product quality and safety. Regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require lot release tests for each biopharmaceutical batch. This testing is performed at all stages of drug development like raw materials, unprocessed bulk, drug substance and final formulation. Lot Release Testing relies on previously established specifications to demonstrate product identity, purity, and potency. Similarly, comparability testing is aimed at identifying whether changes in the manufacturing process lead to downstream changes in product quality. [1] [2]

A LC-UV-MS/MS method was developed for the detection and quantitation of insulin glargine and its excipients namely m-cresol, glycerol, and polysorbate 80 in final formulation. (refer Figure 1-4).

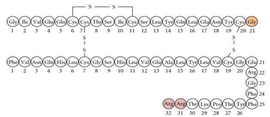

Figure 1. Insulin glargine chemical structure

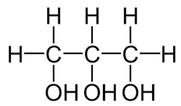

Figure 2. Glycerol chemical structure

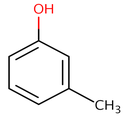

Figure 3. M-cresol chemical structure

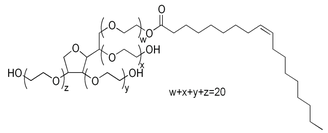

Figure 4. Polysorbate 80 chemical structure

3. Methods

3-1. Standard solutions preparation

Stock solutions: The stock solutions for all the four compounds namely insulin glargine, m-cresol, glycerol and polysorbate 80 were prepared in water at concentration of 5000 ppm separately.

Linearity Standards: The linearity standards were prepared in mixture at concentrations (insulin glargine 10ppb, 75ppb, 250ppb, and 500ppb), (glycerol 500ppb, 1000ppb, 1500ppb, and 2500ppb), (m-cresol 200ppb, 500ppb, 750ppb, and 1000ppb) and (polysorbate 80 1ppb, 25ppb, 50ppb, and 100ppb) respectively in 1% formic acid in water

3-2. Sample preparation Methods

The insulin glargine formulation used for the analysis was Lantus® insulin glargine (rDNA origin injection, 100 units/mL). The formulation sample was diluted with 1% formic acid and injected to perform absolute quantification of insulin glargine and excipients using linear calibration curve.

3-3. LC-MS/MS analysis

Insulin glargine along with its excipients were analyzed using Ultra High Performance Liquid Chromatography (UHPLC) Nexera XS coupled with SPD-40, a UV-visible spectrometer and LCMS-8060, a triple quadrupole mass spectrometer from Shimadzu Corporation, Japan (Figure 5 & 6).

LCMS-8060, sets a new benchmark in triple quadrupole technology with an unsurpassed sensitivity (UFsensitivity), ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

Insulin glargine, glycerol and polysorbate 80 were ionized in positive mode using Electro Spray Ionization (ESI) and quantified using Multiple Reaction Monitoring (MRM) mode on LC-MS/MS. The ionization efficiency of m-cresol was very less and hence simultaneously quantified using UV-visible spectrometer connected in series.

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Figure 5. Nexera XS with LCMS-8060 triple quadrupole mass spectrometer

Figure 6. SPD-40 (UV-visible spectrometer)

Table 1. Instrument parameters

UHPLC condition (Nexera XS system)	
Column	Shim-pack Velox C18 (150 mm x 4.6 mm, 5 micron) (P/N :227-32012-04)
Mobile phase	A: 0.1% Formic acid in water; B: 0.1% Formic acid in acetonitrile
Flow rate	0.5 mL/min
Gradient program (B %)	0-4 min →20(%); 4-10 min → 20-90(%); 10-13 min→90(%); 13-13.1 min→90-20 (%); 15 min →STOP
Injection vol.	1 µL
Column temperature	40 ^{0}C
MS Parameters (LCMS-8045)	
MS interface	ESI
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 5 L/min
MS temperatures	Desolvation line- 300 ^{0}C; Heating block- 200 ^{0}C; Interface- 300 ^{0}C

Table 2 Detection conditions (MRM transitions and UV-visible wavelength)

Sr.No.	Compound name	CAS Number	Absorption wavelength (nm)	MRM transition
1	Insulin glargine	160337-95-1	--	1011.50 > 1179.00
2	Glycerol	56-81-5	--	93.20 > 74.90
3	M-cresol	108-39-4	254	--
4	Polysorbate 80	9005-65-6	--	782.70 > 782.70 (SIM mode)

4. Results

4.1 MRM chromatograms and linearity

The calibration curves for all four compounds were found to be linear. Representative chromatograms of insulin glargine, glycerol, m-cresol and polysorbate 80 is shown in Figure 7.

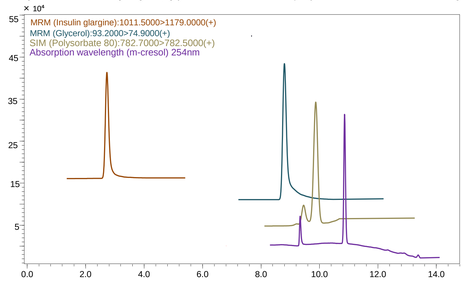

Figure 7. Representative MRM/SIM/UV chromatogram of insulin glargine and excipients

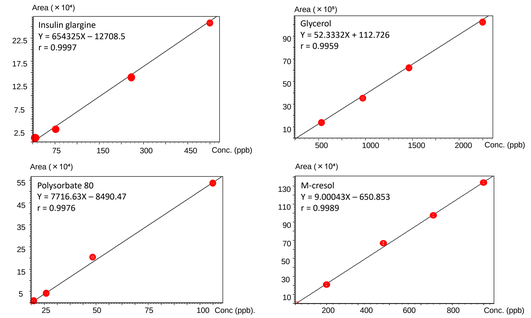

Figure 8. Calibration curves

The linear calibration curves are plotted for all the compounds using weighting method $(1/X^2)$.

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Table 3 Repeatability results using standard mixture

	Insulin glargine	Glycerol	Polysorbate 80	M-cresol
Concentration (ppb)	75	1000	25	500
% RSD (n=6)	**1.58**	**3.73**	**1.94**	**0.20**

Table 4 Quantitation results in Lantus formulation

	Insulin glargine	Glycerol	Polysorbate 80	M-cresol
Label claim (mg/mL)	3.63	20	0.02	2.7
Back calculated concentration (mg/mL)	3.61	19.10	0.018	2.73

5. Discussion and Conclusion

- A single LC-UV-MS/MS method has been developed for the simultaneous quantitation of insulin glargine and its excipients in formulation Lantus by using the UHPLC (Nexera XS), UV (SPD-40) and MS (LCMS-8060) systems connected in series.
- A robust MRM based method along with UV-visible detection gives quick result in a single analysis for all the compounds in a formulation.
- The wide dynamic range of Shimadzu LC-MS accommodates all the compounds of different concentration range in a single analysis.
- This simple and quick method can be applied for release test and comparability test in QC department.

6. References

[1] Guidance for Industry, Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, J:\IGUIDANC\5544fnlcln2.doc 4/15/2005.
[2] ICH Topic Q 6 B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products, September 1999 CPMP/ICH/365/96.

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