# Quantitation of Pegylated Drug Conjugate Interferon Alfa-2b in Human Serum using QTRAP® 6500 System

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## INTRODUCTION

One of the most effective adaptable and acceptable procedure for stabilization of therapeutic protein is PEGylation which improves the shelf life. PEGylated therapeutics proteins are known for its stability and some of them are commercially available. There is a demand for developing sensitivities and selective LC-MS/MS based method for the quantitation which will help to understand the pharmocokinetics of these moleucles. In this study we have developed MRM based quantitation method in human serum for Interferon Alfa-2b which is PEGylated therapeutic protein drug conjugate. In this method surrogate peptides were generated using trypsin digestion from the therapeutic protein. Various sample preparation and cleanup parameters were evaluated and optimized for achieving the highest sensitivity for PEGylated Interferon Alfa-2b (PEG-IFN)

## MATERIALS AND METHODS

**PEGylated Interferon Alfa-2 b (PEG-IFN)**

PEGylated Interferon Alfa-2 b was provided by Lupin Bioresearch Center, Pune Maharasthra, India, at the concentration of 5ng/ul, subsequently diluted as per the study reported for quantitation.

### LC-MSMS Conditions:

**LC gradient run for Information dependent Acquisition (IDA) to select surrogate peptide**

A Shimadzu Nexra LC system with Xbridge dC18, 100x 2.1 mm, 3.5µm column at 40°C with an elution gradient of 5-35% acetonitrile (0.1% formic acid) with a 35 min gradient was used at a flow rate of 300µL/min. The injection volume was set to 10µL. Mobile Phase A : Water :01.% Formic Acid Mobile Phase B : ACN 01.% Formic Acid

### Gradient for MRM

A Shimadzu Nexra LC system with Xbridge dC18, 100x 2.1 mm, 3.5µm column at 45°C with a mobile phase gradient A (Water with 01.% FA ) and B (Methanol 0.1% FA) was used at a flow rate of 400µL/min. The injection volume was set to 30µL. Gradient is listed in Table No: 1

**Extraction of PEGylated proteins from Serum**

Most of the proteins in serum samples were precipitated with water-miscible organic solvents and protein of interested (PEG-IFN) were collected in supernatant by centrifugation.

### LC-MS/MS Conditions:

An SCIEX QTRAP® 6500 LC/MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe was used for quantitation. QTRAP based IDA workflow was used to identify a surrogate and sensitive peptide of PEGylated Interferon Alfa-2b and sequence confirmation of the same done by ProteinPilot™ 5.0 . Skyline software was used to identify the correct precursor and its fragments and the acquisition method was automatically created and exported to Analyst® 1.6 software for the respective peptide. Source and compound related parameters were optimized to achieve highest sensitivity. Three MRM transitions were selected for quantitation and identification of PEG-IFN in serum.

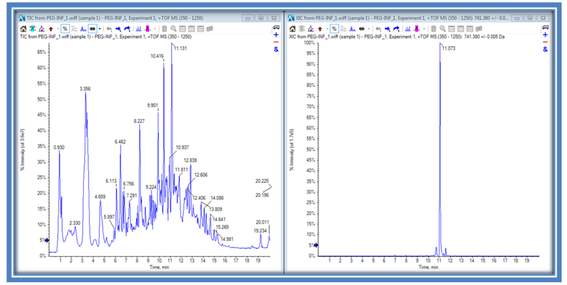
| Time(min) | Flow (µl/min) | % A (0.1% FA in Water) | % B (0.1% FA in MeOH) |
|---|---|---|---|
| **0.01** | 400 | 80 | 20 |
| **0.50** | 400 | 80 | 20 |
| **15.0** | 400 | 40 | 60 |
| **16.0** | 400 | 20 | 80 |
| **18.0** | 400 | 20 | 80 |
| **18.5** | 400 | 80 | 20 |
| **20.0** | 400 | 80 | 20 |

**Table 1: LCMS gradient table for seperation of Peptides in matrix used for MRM**

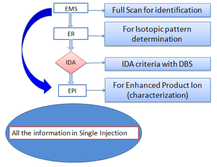
**ProteinPilot™ Software**

SCIEX QTRAP® 6500

IonDrive™ Turbo V Source, IonDrive™ QJet Ion Guide, IonDrive™ High Energy Detector



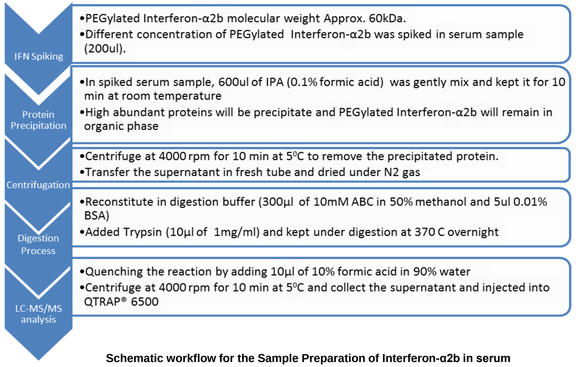
**Information Dependent Acquisition (IDA) with XIC of selected peptide**



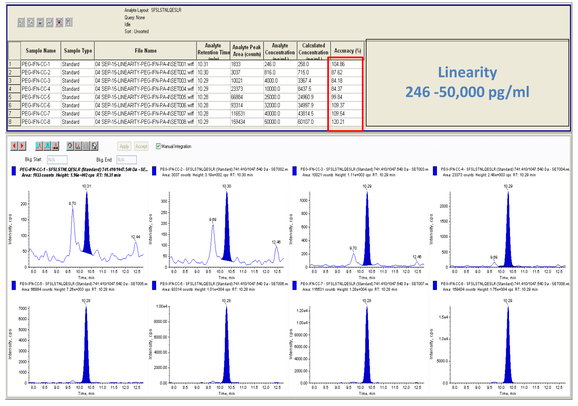

**IDA Method Criteria**

| Source Parameters | | Compound | |
|---|---|---|---|
| CUR | 30 | DP | 85 |
| IS | 5500 | EP | 10 |
| TEM | 500 | CE | 32 |
| GS1 | 55 | CXP | 12 |
| GS2 | 50 | *Dwell (ms)* | 400 |
| CAD | 06 | | |

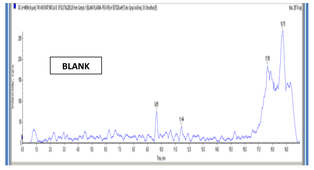
**Source and compound Parameters for MRM**

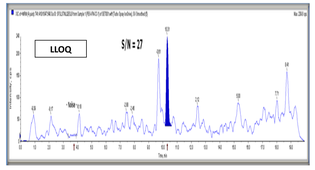



**Schematic workflow for the Sample Preparation of Interferon-α2b in serum**

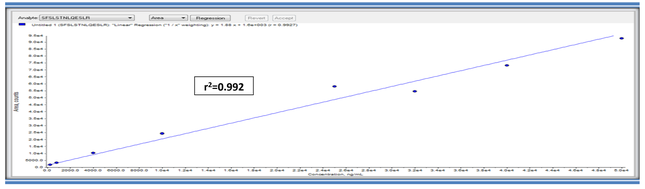



**Linearity of extracted PEG-IFN peptide in the range of 246 pg/ml-50,000 pg/ml in serum**




**Chromatograms of blank matrix and LLOQ**




**Calibration curve of peptide "SLSTNLQESLR" m/z 741.38$^{2+}$>1047.5**

## CONCLUSION

- Highly specific and robust method is developed for PEGylated drug conjugate Interferon Alfa-2b.
- Doubly charge peptide were selected for better and robust ionization for MRM m/z 741.412+=> 1047.54
- Liquid liquid Extraction (LLE) based method was optimized for the quantitation of PEG-IFN in serum matrix
- Linearity within a concentration range of 246-50,000 pg/ml of PEG-IFN was achieved in serum matrix for quantitation
- All signal responses were linear in the tested concentration range, represented by regression coefficients r2 0.992
- The limit of quantification (LOQ) was determined for target peptide in serum was estimated as the lowest concentration measured with % CV< 20%.

### REFERENCES

1. Rinat R. et al.,International Journal of Mass Spectrometry 312 (2012) 135– 143.
2. Wu S, et al,Rapid Commun Mass Spectrom. 2011;25:281-90.
3. Yang Z, et al., J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877:1737-42.
4. Long Yuan and Mingshe Zhu, Mater Methods 2015;5:133

### TRADEMARKS/LICENSING