INTRODUCTION

One of the most effective adaptable and acceptable procedure for stabilization of therapeutic protein is PEGylation which improve the half-life. PEGylated therapeutic proteins are known for their stability and some of them are commercially available. There is a demand for developing methodologies and select LC-MS/MS based method for the quantitation which will help to understand the pharmacokinetics of these molecules. In this study we have developed MRM based quantitation method in human serum for Pegylated Drug Conjugate Interferon Alfa-2b (PEG-IFN) which is a PEGylated therapeutic drug conjugate. In this method surrogate peptides were generated using tryptic 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) was provided by Lupin Bioresearch Center, Pune Maharasthra, India. The concentration of 5 ng/ul, subsequently diluted as per the study reported for quantitation.

LC-MSMS Conditions:

- Phase A: Water : 0.1% FA
- Phase B: Methanol : 0.1% FA
- Injection volume was set to 30 µL
- The injection volume was set to 3 µL

Gradient timeline for Optimization of MRM acquisition method was automatically created and exported to Analyst 1.6 software for the respective MS/MS conditions.

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CONCLUSION

A highly specific and robust method is developed for Pegylated drug conjugate Interferon Alfa-2b.

The limit of quantification (LOQ) was determined for target peptide in serum was estimated as the lowest concentration level with acceptable precision (RSD% < 14%).

The linearity within a concentration range of 246-50,000 pg/ml of PEG-IFN was achieved in serum matrix for quantitation.

Liquid liquid Extraction (LLE) based method was optimized for the quantitation of PEG-IFN in serum matrix.

REFERENCES