WP 681

Simultaneous quantitation of 7 excipients in biological formulation by using LC-MS/MS

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1. Overview

In today's pharmaceutical development, excipients are an integral part of pharmaceutical products, used in multiple ways, and have defined functional roles. Excipients are used in biological formulations for several critical roles which include solubility enhancement, process, and shelf-life stability enhancement, controlling pH, and maintaining preferred stable conformation. Change in excipient or excipient level in a formulation should be notified to FDA with justification for change[1]. (Generally, the change should not exceed 10 % of the originally approved formulation) [1]. The selection and composition of individual excipients are key to the efficacy of any biological formulations which demands analytical testing.

2. Introduction

The word excipient is derived from the Latin excipere, meaning 'to except', which is simply explained as 'other than'. An excipient is a pharmacologically inert substance by itself, but when used in combination with an active ingredient it can provide several benefits. Excipients tend to degrade over a period and can react with the active biological component leading to change in the efficacy of the drug and adverse reactions Biologics are therapeutics that can be based on proteins, peptides, viruses, viral vectors, or nucleic acids. They require a defined environment to remain intact and functional throughout the manufacturing and formulation process, as well as during long-term storage. On the basis of their functions, excipients can be categorized as binders, lubricants, cosolvents, fillers, disintegrates, surfactants, antimicrobials, preservatives, etc. General excipients added in biopharmaceuticals are Tween 80 Tween 20, Span 60, Arginine, Glycine, Trehalose D-Mannitol. These diverse molecules exhibit different physico-chemical properties and are difficult to analyse simultaneously in a single run (Figure 1 for structure of Trehalose, Arginine and Tween 80). This poster describes simultaneous quantitation of multiple excipients in biological formulation by using LC-MS/MS 8045.

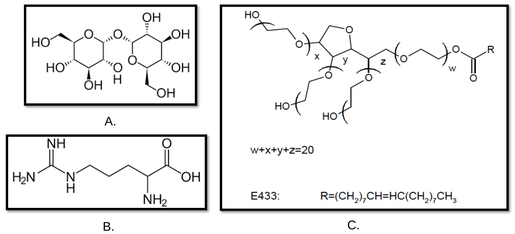

Figure 1: Structure of Trehalose(A), Arginine (B), Tween 80(C)

3. Methods

3-1. LC-MS/MS analysis

For this study, For this study commercially available excipient standards and biological formulations (Leuprolide acetate and Renocrit) were purchased from a local vendor. Stock solutions of all excipients were analysed in scan mode. . Further, steps such as precursor ion selection, Multiple Reaction Monitoring (MRM) optimization at different Collision Energies (CE), and voltage optimization were performed using Shimadzu's LabSolutions auto MRM optimization feature to obtain MRMs and their optimum CEs (Table 2). An LC method (Table 1) was developed with an aim to separate all excipients under study which was achieved using Shimadzu make Shim pack GISS C8, 75 mm x 4.6 mm I.D. and 3 µm LC column. For quantitation, six-point linearity for 7 excipients were plotted. The limit of quantitation (LOQ) were determined based on S/N ratio. The S/N ratio & % RSD at LOQ are shown in Table 3.

Figure 2: Shimadzu Nexera™ X3 UHPLC coupled with an LCMS-8045 Triple quadrupole.

3-2. Analytical conditions

Table 1. Instrument parameters for LC-MS/MS

HPLC System	: Nexera™ X3
Column	: Shim pack GISS C8, 75 mm x 4.6 mm I.D. and 3 µm (P/N :227-30167-04)
Column Oven	: 45 ° C
Mobile Phases	: A-0.1% Formic acid in LC-MS grade water B-0.1% Formic acid in LC-MS grade acetonitrile
Flow Rate	: 0.4 mL/min
Gradient program (B%)	: 0-3 min → 5 (%); 3-12 min → 5-100 (%); 12-20 min → 100 (%); 20-21 min → 100- 5 (%); 25 min → STOP.
Injection Volume	: 20 µL
LC-MS System	: LCMS™-8045
Ionization source	: ESI
LC-MS Températures	: Interface: 270° C Desolvation Line: 220° C Heater Block: 220° C
LC-MS Gas Flows	: Nebulizing Gas: 3 L/min Drying Gas: 10 L/min

Table 2: MRM transitions for 7 excipients

Compound	Precursor m/z	Product m/z	Polarity	CE
Span 60	431.4	431.4	Positive	-9
Tween 80	782.8	782.8	Positive	-11
Tween 20	648.4	648.4	Positive	-10
Arginine	175.0	70.0	Positive	-30
Glycine	75.9	30.1	Positive	-14
Trehalose	360.1	85.1	Positive	-29
D-mannitol	181.1	101	Negative	15

3-3. Sample preparation

Samples were suitably diluted with diluent to fit under calibration curve range.

Centrifuge the sample sample at 12000 rpm for 5 mins at 5°C

Collect the supernatant and inject in LC-MS/MS

4. Results and Discussion

Figure 3 depicts the calibration curve and standards (Representative chromatograms of 3 compounds)

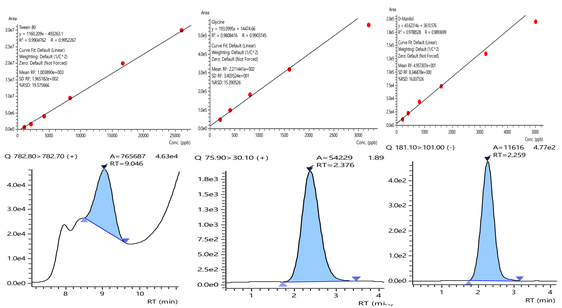

Figure 3: a) Calibration curves b) Chromatograms of 1 ppm for Tween 80, 0.2 ppm for Glycine, and D-Mannitol as representative compounds

Table 3: Coefficient of determination for calibration curves, repeatability of area ratios for LOQ solution and S/N ratio for LOQ solution (Conc. expressed are as such)

Sr. No.	Compound	r^2	CC Range (ppm)	LL0Q		
				Conc. (ppm)	% RSD (n=6)	S/N
1	Span 60	0.988	1.0 to 26.0	1.00	6.9	119
2	Tween 80	0.990	1.0 to 26.0	1.00	4.9	1306
3	Tween 20	0.990	1.0 to 26.0	0.90	2.3	273
4	Arginine	0.995	0.04 to 1.0	0.40	3.8	1910
5	Glycine	0.980	0.2 to 5.0	0.20	4.4	437
6	Trehalose	0.985	0.2 to 5.0	0.18	2.1	450
7	D-Manitol	0.980	0.2 to 5.0	0.18	7.2	673

The summary of all 7 Excipients detected are included in Table 4.

Table 4: Sample summary

Biological Formulations	Concentration in ppm						
	Span 60	Tween 80	Tween 20	Arginine	Glycine	Trehalose	D-Manitol
Renocrit 4000 IU	ND	4.0	ND	ND	2.5	ND	ND
Luprolide DEPOT 3.75 mg	ND	1.5	ND	ND	ND	ND	2.5

Note:- Renocrit contains Tween 80 and Glycine as excipients and Leuprolide acetate contains Tween 80 and D-Mannitol as excipients.
(ND- Not detected)

5. Conclusion

- Quantitation of 3 excipients (Tween 80, Glycine, D-Manitol) in 2 biological formulation (Renocrit and Leuprolide acetate) and samples was successfully demonstrated on Shimadzu LCMS-8045
- Repeatability for all excipients were found to be less than 10 %.
- Simultaneous quantitation of multiple excipients in a single run was successfully demonstrated with UFMS (Ultra Fast Mass Spectrometer) technology of Shimadzu which helps low-level determination in complex biological formulations.

6. References

[1] INTERNATIONAL PHARMACEUTICAL EXCIPIENTS COUNCIL OF INDIA (IPEC INDIA)
[2] Biologic excipients: Importance of clinical awareness of inactive ingredients Published: June 25, 2020 (https://doi.org/10.1371/journal.pone.0235076)