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Development and Validation of a Sensitive UHPLC-MS/MS Method for Teduglutide Analysis in Human Plasma on LCMS-8060RX

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1. Introduction

Teduglutide is a synthetic GLP-2 analog used for treating Short Bowel Syndrome (Refer fig.1 for structure of Teduglutide). Accurate quantification in human plasma is essential for bioequivalence studies of generic formulations[1]. Shimadzu has developed and partially validated a robust UHPLC-MS/MS method for sensitive and precise measurement of teduglutide in plasma using Shimadzu LCMS-8060RX integrated with Nexera X2 UHPLC system.

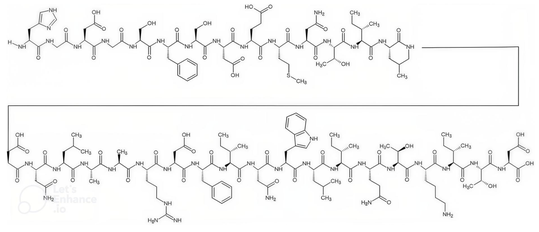

Fig. 1 Structure of Teduglutide

2. Methods

Sample Preparation:

Calibration standards (0.5–100.0 ng/mL) and QC samples (0.5–50.0 ng/mL) were prepared in K_2EDTA human plasma. For each sample, 500 µL plasma was mixed with 600 µL methanol, vortexed for 10 minutes, and centrifuged at 13,700 rpm for 10 minutes at 4 °C. The resulting supernatant was transferred to pre-labeled vials and pretreated with 700 µL of 5 % aqueous ammonia, then vortexed for 30 seconds.

Samples were loaded onto conditioned Mixed mode Anion Exchange, 1 mg 30 CC solid-phase extraction (SPE) cartridges, washed sequentially with 0.5 mL of 5 % ammonia in methanol:water (1:1, v/v) and 0.5 mL of 20 % acetonitrile in water containing 0.05 % formic acid. Teduglutide was eluted with 0.2 mL of 0.3 % formic acid in acetonitrile:water (1:1, v/v). Finally, 50 µL of the eluate was injected into the LCMS-8060RX system for analysis.

3. LC-MS/MS Conditions

LCMS-8060RX coupled with Nexera™ X2 UHPLC system (Shimadzu Corporation), was used to acquire the data in MRM mode. The instrumental conditions used during the analysis were presented below in Table 1.

Table 1 Instrument Parameters for analysis of Teduglutide

UHPLC condition (Nexera™ X2)	
Column	Shim-pack™ Scepter C18-120 column (1.9 µm particle size, 100 × 3.0 mm, P/N: 227-31013-03)
Mobile phase	A: 0.1 % formic acid in water, B: 0.1 % formic acid in acetonitrile
Flow rate	0.5 mL/min
Elution mode	Gradient
Column temp	50 ℃

MS parameters (LCMS-8060RX)	
MS interface	Electro Spray Ionization (ESI)
Nitrogen gas flow	Nebulizing gas- 3 L/min; Drying gas- 10 L/min
Zero air flow	Heating gas- 10 L/min
MS temp	Desolvation line- 250 °C; Heating block- 400 °C; Interface- 300 °C

4. Results

4.1 Selectivity:

Method demonstrated excellent selectivity with minimal interference (<5.68 %) across six plasma lots (refer Table 2). Chromatograms of extracted blank and extracted LLOQ (0.5 ng/mL) are shown in Figure 2.

Table 2 Selectivity

Lot no.	Area in blank matrix	LLOQ area	% Interference
V13717	134	3147	4.26
V8651	172	3686	4.67
V10821	108	2966	3.64
V11808	203	3576	5.68
V5006	136	3481	3.91
V1992	149	3048	4.89

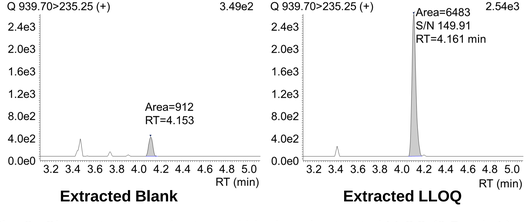

Fig. 2 Chromatograms of extracted blank and extracted LLOQ (0.5 ng/mL)

4.2 Linearity:

Eight-point calibration curve (0.5–100.0 ng/mL) with 1/x² weighting showed excellent linearity (r > 0.99) as shown in fig.3.

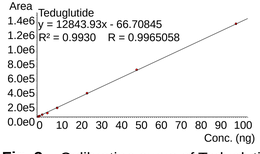

Fig. 3 Calibration curve of Teduglutide

4.3 Precision and Accuracy:

Results of precision and accuracy showed excellent precision and accuracy across all quality control levels, with intra-day and inter-day accuracy ranging from 100.19 % to 105.40 % and coefficient of variation below 10.73 %.Results are summarized in Table 3.

Table 3 Precision and Accuracy

QC level	Intra-day (n=6)			Inter-day (n=18)		
	Mean Conc.	% Accuracy	% CV	Mean Conc.	% Accuracy	% CV
LLOQ QC (0.50 ng/mL)	0.52	104.67	10.05	0.51	102.00	10.10
LQC (2.50 ng/mL)	2.50	100.19	10.70	2.59	103.61	10.73
MQC (10.00 ng/mL)	10.14	101.38	9.04	10.28	102.78	10.13
HQC (50.00 ng/mL)	51.83	103.65	6.45	52.70	105.40	7.78

4.4 Recovery:

Teduglutide exhibited consistent and reproducible results (refer Table 4 & Table 5) with global recovery of 97.83 % (RSD: 2.43 %)

Table 4 Recovery

Sr.No.	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample	Ext- Sample	PE-Sample
	LQC-2.50 ng/mL		MQC-10.00 ng/mL		HQC- 50.00 ng/mL	
1	3147	3667	13810	13946	64220	64529
2	3686	3382	14093	14609	66653	64941
3	2966	3675	14263	14005	66917	65065
4	3576	3382	14288	14156	65860	65330
5	3481	3415	13177	13662	65225	67948
6	3048	3320	13035	14197	66962	67025
AVERAGE	**3,317**	**3,474**	**13,778**	**14,096**	**65,973**	**65,806**
STD DEV	**301.53**	**156.06**	**549.33**	**314.99**	**1,093.34**	**1,358.58**
% RSD	**9.09**	**4.49**	**3.99**	**2.23**	**1.66**	**2.06**
% Recovery	**95.50**		**97.74**		**100.25**	

Note: Read Ext-Sample as extracted sample and PE-Sample as post extracted sample

Table 5 Global Recovery

QC level	Recovery
LQC (n=6)	95.50
MQC (n=6)	97.74
HQC (n=6)	100.25
Mean	**97.83**
SD	**2.38**
% RSD	**2.43**

4.5 Matrix Effect:

No significant matrix effects were observed for Teduglutide across six plasma batches (matrix factor: 0.96 % at LQC, 0.98 % at HQC). Refer Table 6 for results of Matrix effect experiment.

Table 6 Matrix Factor

Teduglutide	AQ-sample	PE-sample	Matrix factor	Teduglutide	AQ-sample	PE-sample	Matrix factor
LQC-2.50 ng/mL	124433	134804	0.92	HQC-50.00 ng/mL	2517814	2652070	0.95
	124646	134604	0.93		2673177	2664337	1.00
	139293	136714	1.02		2188901	2681205	0.82
	127380	135098	0.94		2515429	2662994	0.94
	117592	133638	0.88		2702115	2644779	1.02
	144428	132784	1.09		2991359	2607556	1.15
Mean			**0.96**	Mean			**0.98**
SD			**0.08**	SD			**0.11**
% RSD			**7.90**	% RSD			**11.10**

Note: Read AQ-Sample as aqueous sample and PE-Sample as post extracted sample

4,6 Carry-over effect:

No carryover was observed after injection of highest calibrator

5. Conclusion

We developed and partially-validated an efficient UHPLC-MS/MS method for measuring Teduglutide concentrations in human plasma. The method exhibited robust analytical performance with a linear range of 0.5–100.0 ng/mL and high recovery rates (>97.83 %), while maintaining precision and accuracy within acceptable limits throughout the method validation[1].

Reference
1) ICH M10 Bioanalytical Method Validation and Study Sample Analysis. Step 4, May 2022.(accessed 12-01-2025)

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