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Quantitative Determination of Rapid Acting Insulin Analog- Insulin Aspart with Direct measurement in human plasma by LC-MS/MS.

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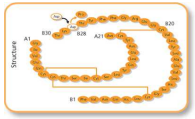
Introduction:

Rapidly acting insulin analogues have been developed, which after subcutaneous administration to a larger extent simulate the postprandial insulin profile for endogenous insulin secretion than for human insulin preparations. The rapidly acting insulin aspart is structurally identical to human soluble insulin except for one amino acid, as proline is substituted with aspartic acid in amino acid position 28 of the B-chain. The effect of these modifications is a reduction of the tendency of the insulin molecules to self-associate into hexamers. This accounts for a faster onset of action after subcutaneous administration and leads to a maximal effect (after 45–60 min.) with a shorter duration of action (around 5 hr) as compared to soluble human insulin. Studies in patients with diabetes have shown that the new rapidly acting insulin analogues have pharmacokinetic and pharmacodynamic advantages as mealtime substitution, regarding flexibility and hypoglycaemic events, compared to human insulin preparations.

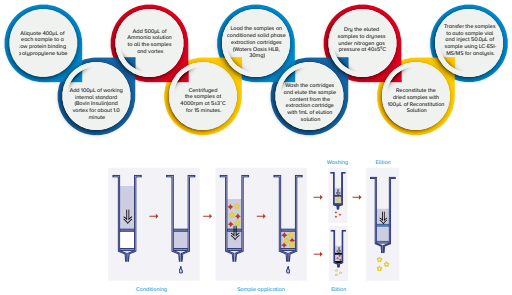
Insulin is one of the oldest and, perhaps, best known, and to this day remains the primary treatment for diabetes. In addition to recombinant human insulin, a number of closely related analogs with altered PK have been developed, result¬ing in several long-, fast- and intermediate-acting versions. Insulin glulisine and insulin aspart are two widely prescribed fast-acting analogs.

Here, Selective, Sensitive and reproducible Determination of Rapid Acting Insulin analog, Insulin Aspart was carried out in human plasma using solid phase extraction technique to enhance the precise and reproducible results at sensitive level. Method is used to determine Insulin Aspart involved solid phase extraction. The assay employed gradient elution program on sub Acquity CSH C18 1.7µ, (2.1mm × 50mm) column followed by tandem mass spectrometric detection in electro spray positive ionization mode. Method is successfully validated for plasma samples analysis of Insulin Aspart. The results of the present work demonstrated that our bio analytical LC-MS/MS method is rapid, sensitive, selective and reliable for the quantitative analysis of Insulin Aspart. The validated method is suitable for analysis for pharmacokinetic study for direct measurement of Insulin Aspart 100U/mL in Healthy subjects.



Molecular Formula: C256H381N65O79S6
Molecular Weight: 5826g/mol
PI value: 5.1

Sample Preparation:



LC and Mass Parameters:

Column	Acquity CSH C18, 1.7µ (2.1 × 50mm)
Mobile Phase	0.1% v/v Acetic Acid in water and 0.1% v/v Acetic acid in Acetonitrile with gradient program
Column oven temperature	40±5°C
Auto sampler temperature	5±3°C
Volume of injection	50.0µL
Detector	Shimadzu-8060 triple-quadrupole mass spectrometer
Run time	23.0 Minutes

Insulin Aspart	Bovine insulin
Q1 971.6 (m/z) with multiple selective daughter ions 1139.6, 1110.4, 1133.3 and 661.1 (m/z)	Q1 956.2 (m/z) with Q3 1121.0 (m/z)

Validation Experiments:

The matrix stability was evaluated by using freshly prepared duplicate calibration curve standard and six sets of freshly prepared batch qualifying QCs along with six sets of stability samples (HQC and LQC levels) using K2EDTA human plasma.

Precision and Accuracy of Intra-batch & Inter-batch

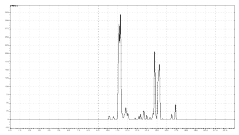
Level	Nominal Concentration (ng/mL)	Intra-batch			Inter-batch		
		Mean Concentratio found (ng/mL) a	Accuracy (%)	CV (%)	Mean Concentration found (ng/mL) b	Accuracy (%)	CV (%)
LLOQ QC	0.100	0.106	106.0	6.6	0.102	102.0	7.8
LQC	0.300	0.331	110.3	2.7	0.324	108.0	8.0
MQC	1.500	1.591	106.1	2.3	1.534	102.3	4.1
HQC	3.750	3.876	103.4	3.4	3.664	97.7	5.4

CV: Coefficient of variation
a Mean of six replicates at each concentration
b Mean of six replicates for three precision and accuracy batches

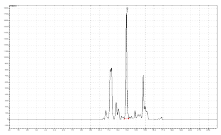
Specificity

The specificity of the method was established using blank samples (without spiking with drug or internal standard) and LLOQ samples which are prepared from six different donor K2EDTA human plasma lots. All the plasma lots were found free from Drug (with respect to <20% of LLOQ response) and internal standards interference (with respect to <5% of mean ISTD response of accepted CCs & QCs samples).

Chromatogram of STD BL:



Chromatogram of LLOQ:



Matrix Effect by evaluating Matrix Factor	HQC	LQC
Matrix Factor for Analyte (Mean)	1.22	2.00
ISTD Normalized Matrix Factor (Mean)	1.09	0.88
% CV of ISTD Normalized Matrix Factor	1.33	3.42

Recovery of Analyte & Internal Standard

Level	Insulin Aspart			Bovine Insulin		
	% Mean Recovery	Overall CV (%)	Global Average Recovery (%)	% Mean Recovery	Overall CV (%)	Global Average Recovery (%)
HQC	18.5	11.1	16.5	45.7	6.5	43.6
MQC	16.5			44.6		
LQC	14.4			40.3		

Stability experiment

Stability experiment Condition	Level	Mean Concentration found (ng/mL)a	CV (%)	% Bias
Bench Top (BT) stability 20 Hours at Ambient Temperature	HQC	3.494	4.4	-6.7
	LQC	0.283	10.6	-5.7
Stability of Extract (SE) 148 hours at 5±3°C	HQC	3.786	2.5	1.0
	LQC	0.339	5.0	13.0
Stability of Extract (SE) 19 hours at Ambient Temperature	HQC	3.793	2.3	-0.3
	LQC	0.272	14.1	-9.3
Stability of Dry extract (DE) 147 hours at -20±5°C	HQC	3.770	3.2	0.5
	LQC	0.317	3.5	5.7
Freeze Thaw Stability 5 Cycles at freezing temperature of -20±5°C	HQC	3.508	8.7	-6.4
	LQC	0.312	4.8	4.0
Freeze Thaw Stability 5 Cycles at freezing temperature of -78±8°C	HQC	3.673	6.1	-2.0
	LQC	0.308	8.1	2.7
Long-Term Stability of Analyte in Matrix 33 Days at -20±5°C	HQC	3.789	6.7	1.0
	LQC	0.325	5.8	8.3
Long-Term Stability of Analyte in Matrix 36 Days at -78±8°C	HQC	3.678	4.0	-1.9
	LQC	0.331	3.9	10.3

a Mean of six replicates at each concentration

Stability of Analyte in Blood	HQC	LQC
For 02 hrs. at wet ice bath (below10°C) and Ambient Temperature with respect to zero hour.	% Mean Stability within 92.00-99.00% and Precision within 5.0%	

Selectivity of Method in Presence of Concomitant Medication Drugs		
Results Summary	% Mean Bias	
In Presence of Acetaminophen, Caffeine, Cetirizine, Domperidone, Diclofenac, Ibuprofen, Nicotine and Ranitidine	CME HQC	CME LQC
	0.58 to 9.83	-11.12 to 0.32

Conclusion:

The bio analytical methodology for Insulin Aspart can be highly useful for the clinical trial samples with precision, accuracy and high throughput. Data processing was done by using the LIMS software which gives the highest data integrity during the method validation. This method involved solid phase extraction technique in sample cleaning by gradient chromatographic. The validated method was found to be specific, sensitive, accurate, precise and reproducible and successfully and there is no any cross reactivity with endogenous human insulin. This validated method is suitable for direct measurement of Insulin Aspart in Human plasma in Healthy subjects with Insulin Aspart 100U/mL Euglycemic clamp study.

Acknowledgements:

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Reference:

US FDA - Bioanalytical Method Validation Guidance for Industry, May 2018

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