BIO-ANALYTICAL METHOD BASED ON MALDI-MS ANALYSIS FOR THE CIGB-300 ANTI-TUMOR PEPTIDE QUANTIFICATION OF IN HUMAN PLASMA

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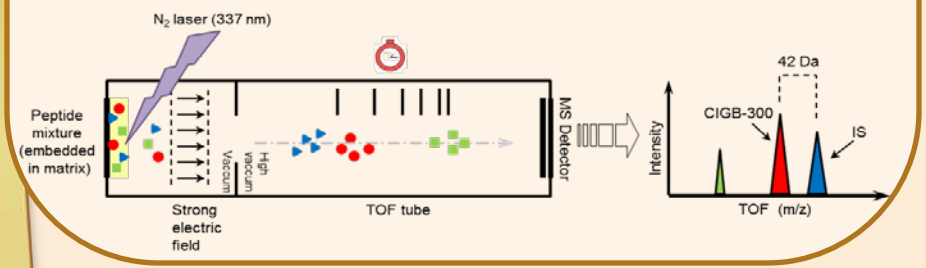
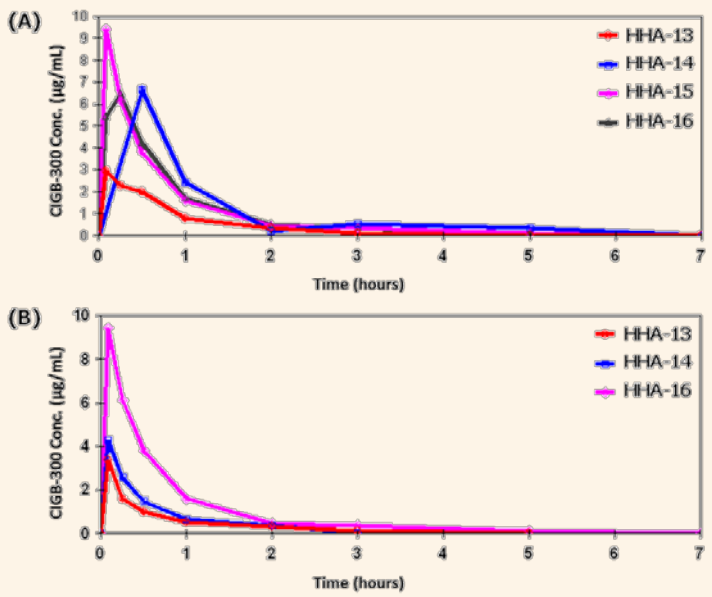
Abstract

A fully validated bio-analytical method based on Matrix-Assisted-Laser-Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) was developed for the quantification in human plasma of an anti-tumor peptide CIGB-300. An analogue of this peptide, acetylated at the N-terminal end, was used as internal standard to perform the absolute quantification. Acid treatment allowed an efficient precipitation of plasma proteins as well as a high recovery (approximately 80 %) of the intact peptide. No other chromatographic step was required for the sample processing before MALDI-MS analysis. The spectra were acquired in linear positive ion mode to ensured maximum sensitivity. The lower limit of quantification (LLOQ) was established at 0.5 µg/ml, which is equivalent to 160 fmol of the analyzed peptide. The calibration curve showed a linear trend from 0.5 to 7.5 µg/mL, with an r2 > 0.98, and permitted the quantification of high concentrated samples, evaluated by dilution integrity test. All parameters assessed during five validation batches, met the FDA guidelines for industry. This method was successfully applied to the analysis of clinical samples obtained in a phase I clinical trial following intravenous administration of CIGB-300 (1.6 mg/kg of body weight dose). Regarding pharmacokinetics parameters, there were only statistically differences ($p < 0.05$) between Cmax and AUC for ELISA and MALDI-MS analytical methods.



Method application

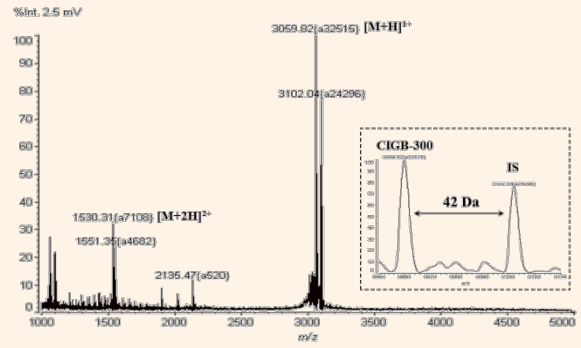
PLASMATIC CONCENTRATION OF CIGB-300 *vs.* TIME COURSE



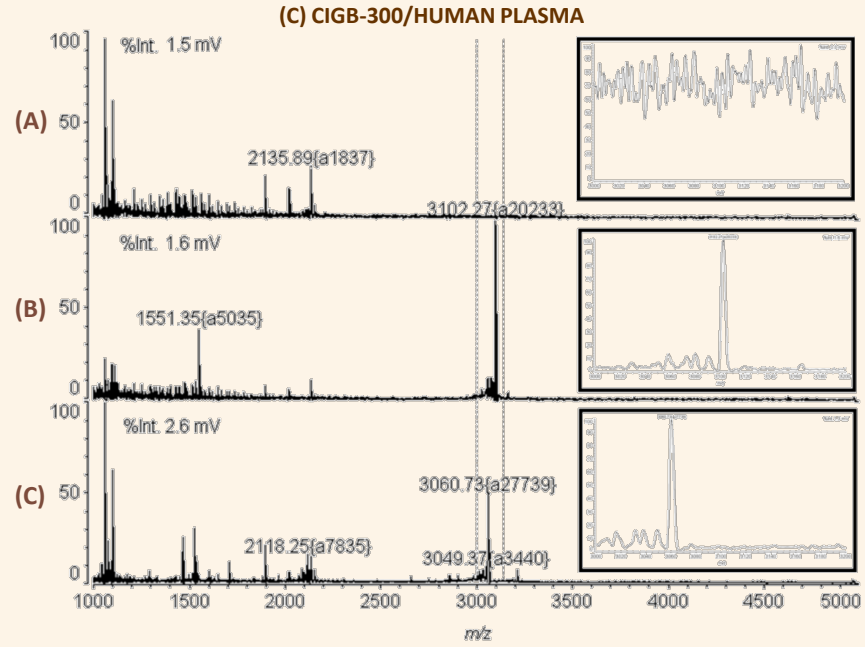
PK PARAMETERS	CIGB-300 I.V. INFUSION		
	MALDI-TOF MS		ELISA
	FIRST DOSE	FIFTH DOSE	FIRST DOSE
T max (h)	0.23 ± 0.20	0.08 ± 0.00	0.12 ± 0.09
Cmax (µg/mL)	6.36 ± 2.66	5.69 ± 3.25	14.77 ± 2.70**
λ	0.90 ± 0.25	0.99 ± 0.22	0.92 ± 0.17
$T_{1/2}\lambda$ (h)	0.82 ± 0.26	0.72 ± 0.15	0.75 ± 0.26
AUC (µg/mL·h)	5.39 ± 1.84	3.58 ± 2.32	11.45 ± 1.84*
MRT (h)	0.91 ± 0.30	0.77 ± 0.11	0.92 ± 0.50
CL/kg (L/h·Kg)	0.34 ± 0.17	0.33 ± 0.30	0.15 ± 0.05
Vss/kg (L/Kg)	0.52 ± 0.50	0.24 ± 0.19	0.13 ± 0.05

Method development and validation

MALDI-MS ANALYSIS OF CIGB-300/IS (1:1) MIX



MALDI-MS ANALYSIS OF (A) HUMAN PLASMA, (B) IS/HUMAN PLASMA AND (C) CIGB-300/HUMAN PLASMA



VALIDATION PARAMETERS	ACCEPTANCE CRITERIA	RESULTS
Matrix Interferences (MF)	MF≈1	1.14
Lower Limit Of Quantization [LLOQ]	CV ≤ 20%	≤ 6.61%
Calibration curves	$R^2 \geq 0.98$	≥ 0.9848
Repeatability	CV ≤ 15%	≤ 6.90%
Variability between days	CV ≤ 15%	≤ 8.34%
Stock solution sample stability	Δ Conc. ≤ 10%	≤ 5.29%
Long Term Sample Stability (CV%) [p]	CV ≤ 15%	≤ 7.68% [p ≥ 0.1939]
Freeze-Thaw Sample Stability (CV%) [p]	CV ≤ 15%	≤ 8.39% [p ≥ 0.1000]
Bench top Sample Stability (CV%) [p]	CV ≤ 15%	≤ 8.02% [p ≥ 0.1000]
Processed Sample Stability/ - 20 C (CV%) [p]	CV ≤ 15%	≤ 9.66% [p ≥ 0.2503]
Processed Sample Stability/ R.T. (CV%) [p]	CV ≤ 15%	≤ 5.10% [p ≥ 0.1157]
Recovery (%) [CV%]	[CV ≤ 15%]	51.96-58.58 [≤ 13.2%]

Conclusions

1. A method based on MALDI-TOF MS to quantify CIGB-300 in human plasma was developed and fully validated.
2. The 42 Daltons mass shift between the analyte and its IS was sufficient for absolute quantification of the CIGB-300.
3. No liquid chromatography step was required previous to MS analysis because there were not interferences and recovery, after plasma processing, was very high.
4. The method was successfully applied to the analysis of CIGB-300 in clinical samples from pharmacokinetic study with this peptide after intravenous infusion.

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