ANAQUANT

SOLUTIONS FOR BIOANALYSIS

How LC-MS can bring specificity for biologics bioanalysis? Applied to insulin analogues and metabolites.

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INTRODUCTION

Over the past years, large biomolecules, also called Biologics or Biotherapeutics, gained interest in the development of new drug entities. Through the different development phases, most of biological measurements have been performed using ligand-binding assays (LBAs). Even if this analytical tool is considered as the gold standard for biologics bioanalysis, quantification by immuno-based assays might suffer from cross-reactivity coming from a lack of specificity.

Since the beginning of this century, LC-MS in targeted analysis (SRM/MRM/PRM - Selected Reaction Monitoring/Multiple Reaction Monitoring/Parallel Reaction Monitoring) has emerged as an alternative tool for biologics analysis thanks to its high specificity and multiplexing capability.

This application note presents the use of LC-MS in targeted mode for a specific multiplex analysis of 2 insulin analogues and their respective metabolites.

INSULIN analogues AS BIOTHERAPEUTICS

Insulin is used for glucose regulation as the primary treatment for diabetes. Beside insulin, few analogues have been developed to speed-up or reduce the in-vivo bioavailability. Thus, insulin analogues represent a major class of biotherapeutics and their detection is essential for therapeutic development and clinical research.

ISSUE

In development phases, pharmacokinetic-pharmacodynamic (PKPD) *in vitro* analysis is performed to evaluate parameters such as drug timelife, metabolism or therapeutic window determination.

To improve high throughput analysis and minimize the number of samples, several biotherapeutics can be analyzed within the same sample using a multiplex analytical method.

Insulin analogues can differ from each other by only few amino acids (Figure 1). Because of this slight difference, LBAs assays are not able to detect and quantify specifically these analogues. Thanks to its mass selectivity, LC-MS assays bring more specificity in multiplex analysis. Moreover, within the same analysis the 2 metabolites M1 and M1' will also be analyzed and quantified.

This application note will present the multiplex LC-MS analysis of 2 commercial insulin analogues (lispro and glargine).

Human insulin
GIVEQCCTSICSLYQLENYCN
FVNQHLCGSHLVEALYLVCGERGFFYTPKT
Glargine
GIVEQCCTSICSLYQLENYCG
FVNQHLCGSHLVEALYVLCGERGFFYTPKTRR
Lispro
GIVEQCCTSICSLYQLENYCN
FVNQHLCGSHLVEALYVLCGERGFFYTKPT

Figure 1: Human insulin and 2 analogues, glargine and lispro, sequences.

SAMPLE PREPARATION

Sample preparation is described in Figure 2.

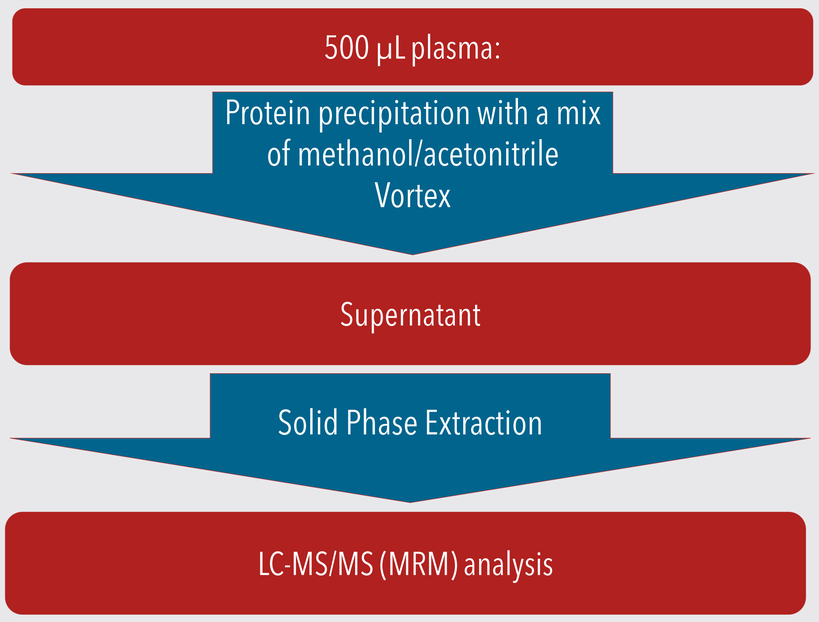

Figure 2: Sample preparation steps for the SPE protocol.

RESULTS

The method validation was performed with 3 replicates each day over 3 separate days. Figure 3 shows the calibration curves for the 2 analogues. The intra- and inter-day precision and accuracy are shown in Table 1. The limit of detection (LOD) for each analog was determined at 100 pg/mL.

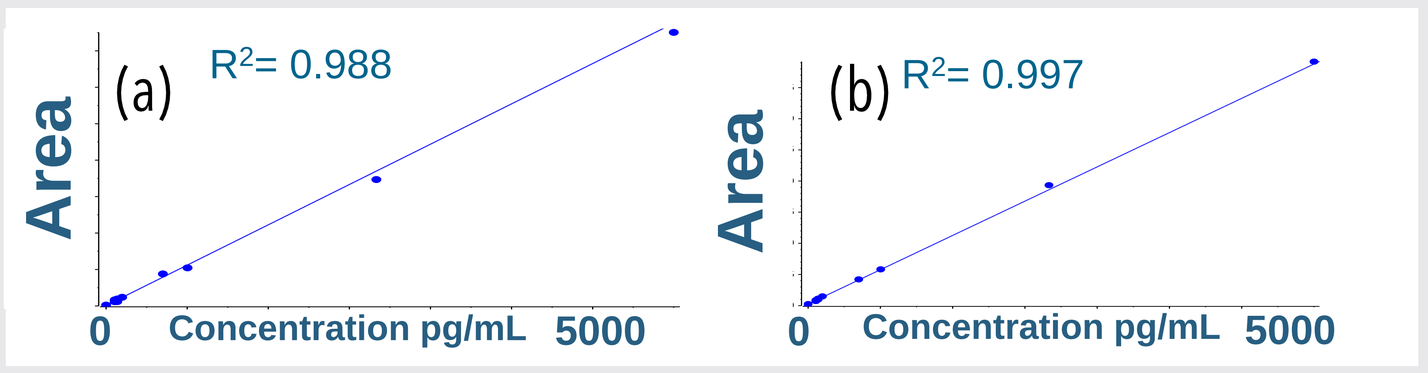

Figure 3: Calibration curves for the quantification of Glargine (a) and Lispro (b) in plasma.

Lispro				Glargine			
Nominal Concentration (ng/mL)	n	Precision (%) [a]	Accuracy (%) [b]	Nominal Concentration (ng/mL)	n	Precision (%) [a]	Accuracy (%) [b]
100	12/12	17%	98%	100	12/12	18%	103%
140	12/12	11%	101%	140	12/12	20%	101%
200	4/4	10%	99%	200	4/4	10%	100%
700	4/4	5%	105%	700	4/4	9%	100%
1000	4/4	4%	99%	1000	4/4	5%	99%
3333	4/4	1%	104%	3333	4/4	5%	98%
7000	4/4	6%	98%	7000	4/4	5%	96%

[a] Expressed as R.S.D. (relative standard deviation) (SD./Mean) x 100
[b] Expressed as: (Determined concentration / Nominal concentration) x 100

Table 1: Inter-day variation statistic parameters for lispro and glargine analogues.

Thanks to this multiplex method, in vitro PKPD analysis of the 2 analogues has been performed in serum. The 2 molecules have been incubated at 37°C over 2h. Figure 4 shows the clearance of the 2 analogues and the simultaneous detection of the 2 main metabolites of each analog (M1 for glargine and M1' for lispro).

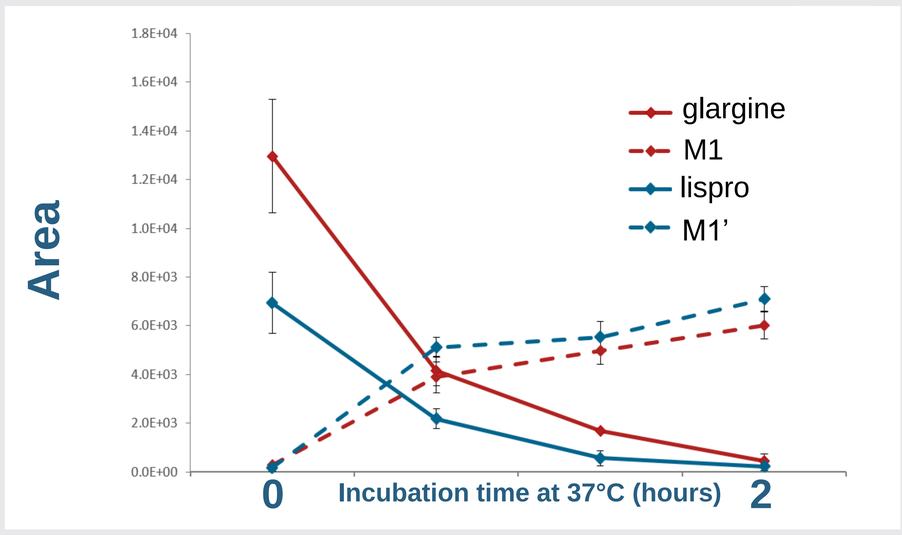

Figure 4: PKPD analysis of lispro and glargine analogues and their metabolites (M1 and M1').

CONCLUSION

This application note presents a reliable quantification method of glargine and lispro, but also their metabolites, by multiplex LC-MS analysis. While immunoassays can not specifically detect each analog within the same sample, LC-MS shows specific detection and quantification for *in vitro* biologics PKPD analysis.