Bioanalytical challenges with BioChaperone® BC structures: polyanionic amphiphilic polymers to quantify parent and truncated metabolite in rat, dog, and human plasmas

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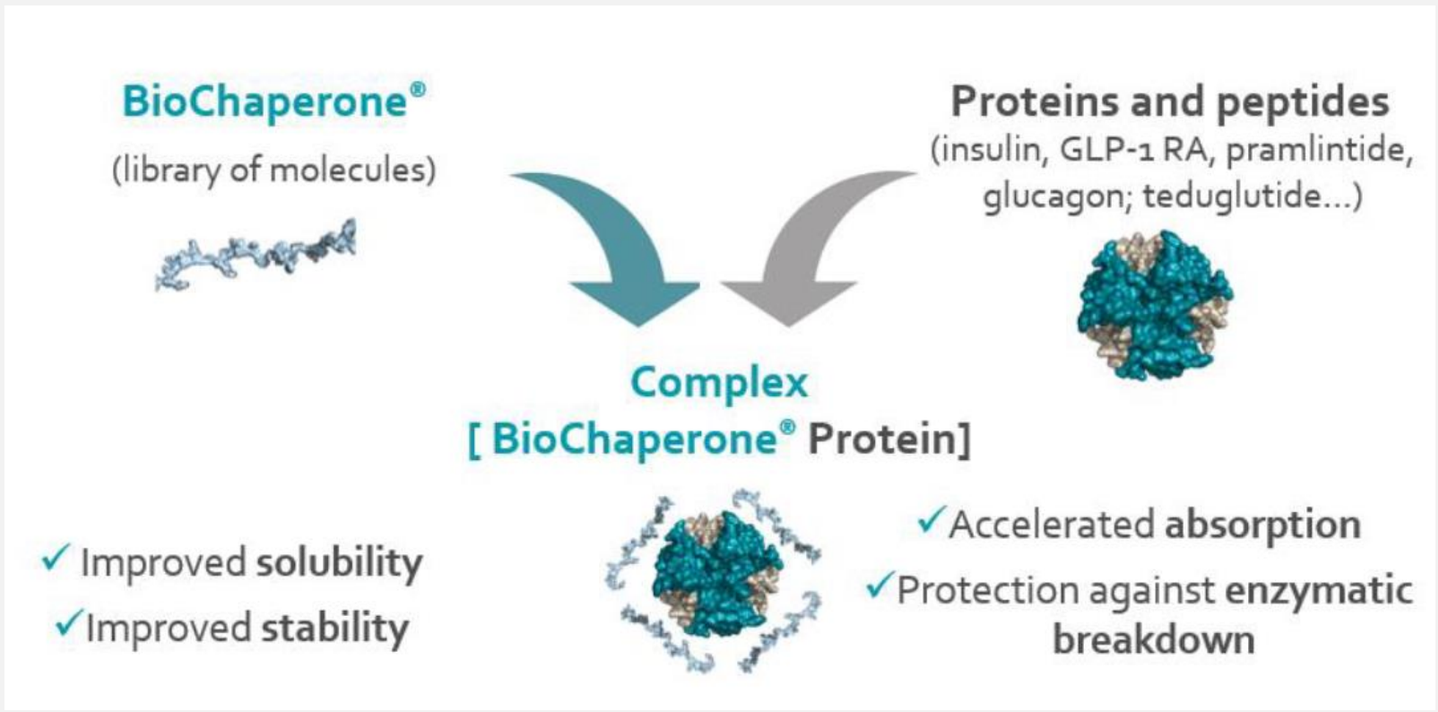
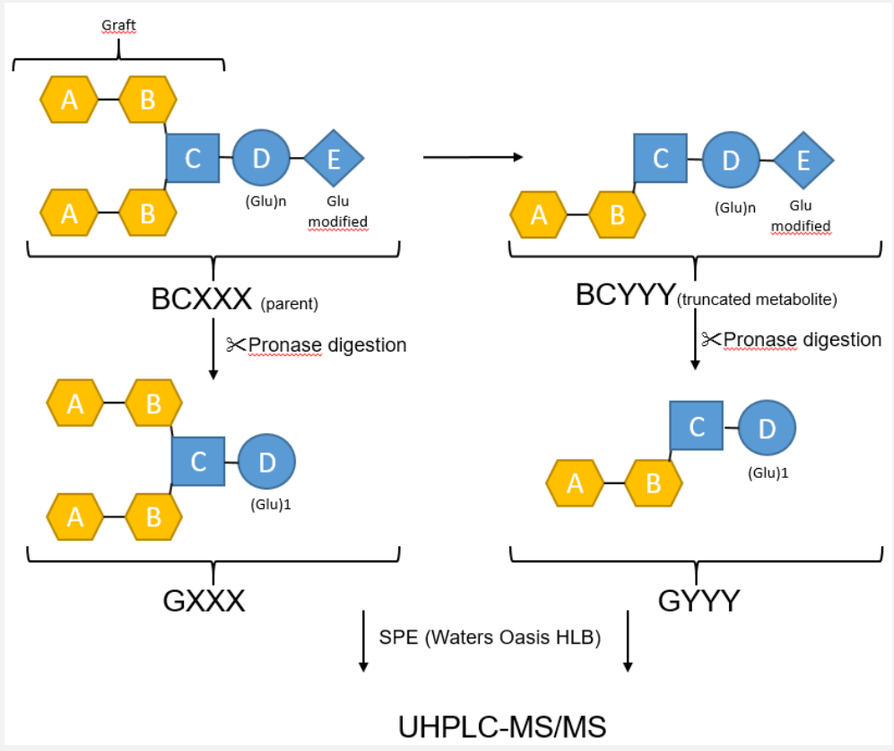
1 CONTEXT & OBJECTIVES

We wish to develop and validate LC-MS/MS methods for the quantitation of both BioChaperone® **BCXXX** (Parent) and its truncated metabolite **BCYYY** in Sprague-Dawley rat, Beagle dog and human plasma following the current international guidelines for industry [**1, 2**].
Prior to validation and because of the particular structure of BioChaperone® molecules, several steps need to be optimize such as Pronase (Protease from *Streptomyces griseus*) digestion, extraction, injection solvent chromatography and mass spectrometry conditions. Since the BioChaperone® molecules have a MW not compatible with conventional triple-quadrupole system mass range, a digestion coupled to SPE is mandatory.





These novel polymers, oligomers, and innovative small molecules, called BioChaperone® have the property of spontaneously combining with certain therapeutic proteins. This non-covalent combining helps increase the solubility and efficacy of the therapeutic protein and protects it from enzymatic breakdown.

2 RESULTS

Conditions of digestion were optimized with 50 µL of Pronase (20g/L) without SDS for 16 hours at 50°C and reaction was quenched with a dilution buffer with 6M guanidine chloride. The parent and truncated metabolite were extracted by SPE (Waters Oasis HLB 30 mg) prior to LC-MS/MS. The bioanalytical methods in rat, dog, and human plasma were all linear ($1/X^2$ weighting factor) over 20.0 to 500 ng/mL range for the parent **BCXXX** (Deuterated BCWWW-d27 as IS) and 50.0 to 5000 ng/mL range for the metabolite **BCYYY** (Deuterated BCZZZ-d27 as IS).

Matrices	BCXXX: Within-run precision and accuracy					Between-run precision and accuracy			
	Conc. (ng/mL)	20.0	60.0	250	400	20.0	60.0	250	400
K2 EDTA Human plasma (H)	CV (%)	16.6	14.5	12.2	12.0	11.7	14.8	12.1	12.3
	Accuracy (%)	101	94.7	98.3	101	86.4	92.7	91.1	98.6
K2 EDTA SD rat plasma (R)	CV (%)	17.2	10.2	11.8	9.30	13.4	11.7	9.40	14.8
	Accuracy (%)	104	99.4	107	114	101	97.1	103	103
K2 EDTA Beagle Dog plasma (D)	CV (%)	12.4	13.4	8.70	12.5	12.0	11.4	14.6	12.4
	Accuracy (%)	92.9	101	93.8	109	97.6	102	102	107

Matrices	BCYYY: Within-run precision and accuracy					Between-run precision and accuracy			
	Conc. (ng/mL)	50.0	150.0	2500	4000	50.0	150.0	2500	4000
K2 EDTA Human plasma (H)	CV (%)	12.5	2.90	4.30	8.60	15.3	7.10	7.50	9.90
	Accuracy (%)	117	96.1	89.5	90.1	105	98.0	93.2	94.6
K2 EDTA SD rat plasma (R)	CV (%)	5.60	3.60	4.50	2.60	15.4	4.90	10.2	12.4
	Accuracy (%)	108	107	104	103	105	100	103	102
K2 EDTA Beagle Dog plasma (D)	CV (%)	15.4	4.90	10.2	12.4	9.60	9.00	6.10	8.30
	Accuracy (%)	105	100	103	102	105	105	105	104

Product	Q1 mass	Q3 mass	Time	DP	CE	CXP	EP
	(amu)	(amu)	(ms)	(volts)	(volts)	(volts)	(volts)
GXXX (Parent)	596.0	308.2	120	180	21	28	10
GYYY (Metabolite)	754.3	300.2	120	176	41	20	10
GWWW (IS for P)	622.5	335.2	120	131	21	28	10
GZZZ (IS for M)	781.6	300.2	120	246	47	22	10

Type	API 5500
Ionisation source	Turbo V
Ionisation conditions	
Parameter	**Value**
Polarity	positive mode
Collision gas pressure	7 psi
Curtain gas pressure	20 psi
Ion Source Gas 1	60 psi
Ion Source Gas 2	20 psi
Temperature	700°C
Ion Spray Voltage	5500 V

BCXXX (Parent) Stabilities in matrices (Human H, Rat R & Dog D)	
Freeze and thaw cycles at < -65°C:	3 cycles (H, R, D)
Short term stability at RT:	475 min (H), 470 min (R) & 315 min (D)
Long term stabilty at < -65°C:	169 days (H), 214 days (R) & 134 days (D)
In blood:	1 h at RT (H, R, D)
Extracted samples	
On the autosampler at 5±4°C:	811 min (H), 874 min (R) & 909 min (D)
In injection solvent at 5±4°C:	94.1 h (H), 93.6 h (R) & 118.8 h (D)

BCYYY (Metabolite) Stabilities in matrices (Human H, Rat R & Dog D)	
Freeze and thaw cycles at < -65°C:	3 cycles (H, R, D)
Short term stability at RT:	475 min (H), 470 min (R) & 445 min (D)
Long term stabilty at < -65°C:	169 days (H), 214 days (R) & 115 days (D)
In blood:	1 h at RT (H, R, D)
Extracted samples	
On the autosampler at 5±4°C:	883 min (H), 874 min (R) & 909 min (D)
In injection solvent at 5±4°C:	76.3 h (H), 93.6 h (R) & 162.8 h (D)

Stock & Working Solutions Stabilities

Stock solution stability	BCXXX	41 days at < -65°C	BCYYY	41 days at < -65°C
	BCWWW (IS)	41 days at < -65°C	BCZZZ (IS)	41 days at < -65°C
Working solution stability	BCXXX	to be prepared daily	BCYYY	to be prepared daily
	BCWWW (IS)	to be prepared daily	BCZZZ (IS)	to be prepared daily

3 CONCLUSION

The detection and the quantification of **BCXXX** (Parent) and **BCYYY** (metabolite) were carried out in the multiple reaction monitoring mode using internal standard calibration. The assay was linear over 20.0 to 500 ng/mL for **BCXXX** and over 50.0 to 5000 ng/mL for **BCYYY** concentration range in human, rat and dog plasmas. A good within-run and between-run reproducibility's have been proven. **BCXXX** and **BCYYY** were confirmed stable in different stress conditions in each corresponding matrix.

The preliminary pronase digestion coupled to SPE did not impact the bioanalytical method validation of **BCXXX** (Parent) and **BCYYY** (metabolite). All data demonstrated that the method was selective, specific, precise, accurate, and capable of producing reliable results.

In New Zealand rabbit K_2EDTA plasma, the assay was linear ($1/X^2$ weighting factor) over 500 to 50000 ng/mL for **BCXXX** concentration range.

[1]Guideline on bioanalytical method validation. Committee for medicinal products for human use. Document reference: 21 July 2011. EMEA/CHMP/EWP/192217/2009 Rev.1 Corr.2.1
[2]U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for industry: bioanalytical method validation. May 2018