A Novel Trap-and-Elute LC-MS/MS Method to Quantify Acyl Ghrelin and Des-Acyl Ghrelin in Human Plasma

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Introduction

The “hunger hormone” Ghrelin is a biomarker that attracts increasing attention as a potential anti-obesity therapeutic target, since it stimulates food intake and is involved in long-term body weight regulation. Ghrelin can be detected in its active acylated (octanoyl modification on Ser3) and its inactive des-acylated form.

Nowadays, ELISA or RIA assays are commonly used for quantification of Ghrelin, but the development of specific antibodies that can distinguish active and inactive Ghrelin is challenging. Here we present an LC-MS/MS method that allows for selective quantification of acyl Ghrelin and des-acyl Ghrelin in human plasma in the range of 50.0 - 2500 pg/mL.

Sample collection, Standard (STD) / Quality Control (QC) sample preparation and extraction procedure

Acyl Ghrelin can rapidly get enzymatically degraded in plasma. Therefore, blood collection was done in the presence of protease inhibitors (P800 tubes from BD) and plasma samples were acidified with 1% formic acid.

Unknown samples, QC Low, Mid, High

- Blood collection in EDTA/P800 tubes
- Centrifugation
- Plasma acidification
- Storage at -80 °C

STDs, Blanks, STD0s, QC LLOQ, QC S

Surrogate matrix: water, 0.1% human serum albumin, 0.1% formic acid

Extraction procedure

- 600 µL of sample
- 60 µL of internal standard (IS)
- Precipitation with MeOH
- Centrifugation and transfer of supernatant
- Evaporation until dryness
- Reconstitution in water, 0.1% formic acid
- Centrifugation and transfer of supernatant
- Storage at 5 °C until injection

Figure 1: Workflow of clinical sample collection, QC and STD sample preparation and extraction procedure.

LC-MS/MS conditions

Samples were loaded on a C4 trapping column and were eluted using in-line dilution on a C18 analytical column for chromatographic separation. As a mass detector, a TQ 6500 instrument operating in the MRM mode with positive electrospray ionization was used.

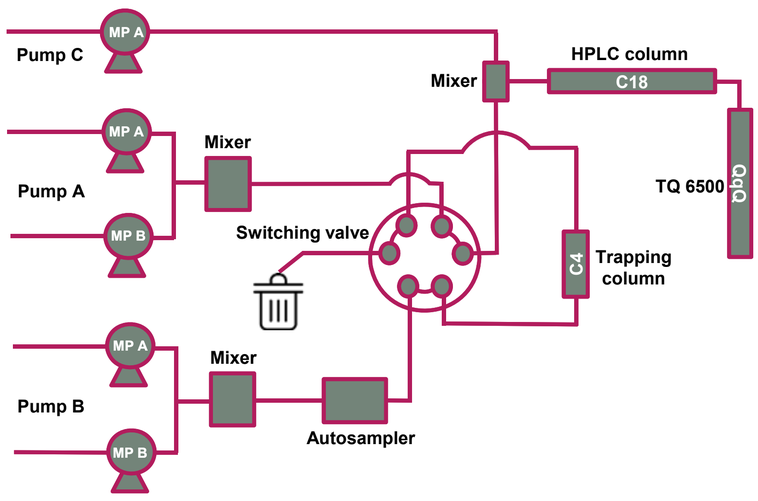


Figure 2: Schematic representation of the chromatographic trap-and-and elute system.

Chromatographic conditions	
UHPLC	AB Sciex Exion LC system
Analytical column Trapping column	Waters XSelect CSH C18, 100 × 2.1mm, 2.5 µm Thermo Hypersil GOLD C4, 10 × 2.1mm, 5 µm
Mobile phase A (MP A) Mobile phase B (MP B)	Water/Acetic acid 98:2 (v/v) Acetonitrile/Acetic acid 98:2 (v/v)
Flow rate Column temperature Injection volume	0.4 (analytical column) 1.0 (trapping column) mL/min 50 °C 100 µL
Total run time	6 min
MS/MS conditions	
Mass spectrometer	SCIEX TQ 6500
Source/Polarity	ESI / Positive
Followed MRM transitions (Charge state)	Human acyl Ghrelin: m/z 482.8 (+7) → m/z 535.8 Human des-acyl Ghrelin: m/z 464.5 (+7) → m/z 517.7 Rat acyl Ghrelin (IS): m/z 474.7 (+7) → m/z 523.2 Rat des-acyl Ghrelin (IS): m/z 399.7 (+8) → m/z 450.5

Table 1: Chromatographic conditions and MS/MS parameters.

Results

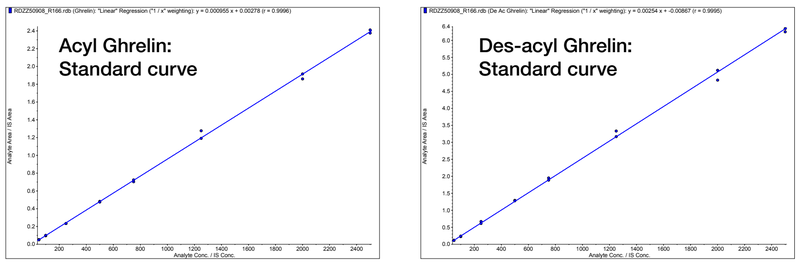
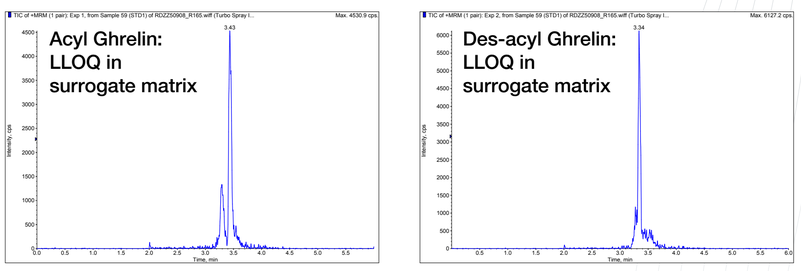


Figure 3: Calibration curve from 50.0 to 2500 pg/mL, linear regression, 1/x weighting factor for acyl Ghrelin (left) and des-acyl Ghrelin (right).



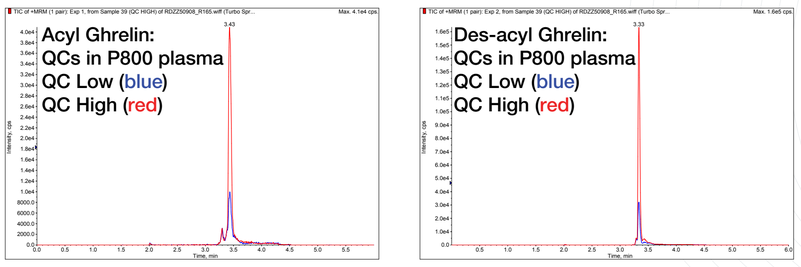


Figure 4: Typical chromatograms of the LLOQ in surrogate matrix (top), QC Low (blue) and QC High (red) in P800 plasma (bottom) for acyl Ghrelin (left) and des-acyl Ghrelin (right).

Intra-batch and inter-batch precision and accuracy results for acyl Ghrelin		QC LLOQ 50.0 pg/mL	QC S 150 pg/mL	QC Low 500 pg/mL	QC Mid 1000 pg/mL	QC High 1800 pg/mL
Run 1	Accuracy (%) CV (%) N	99.8 7.8 6	99.3 3.5 6	90.4 3.6 6	83.0 4.2 6	78.3 2.8 6
Run 2	Accuracy (%) CV (%) N	96.2 3.2 6	95.3 4.1 6	87.0 5.6 6	80.1 3.0 6	76.7 3.0 6
Run 3	Accuracy (%) CV (%) N	102.2 6.8 6	94.0 1.9 6	79.8 5.1 6	72.2 4.4 6	69.4 3.0 6
Inter-batch Precision and Accuracy	**Accuracy (%) CV (%) N**	**99.4 6.5 18**	**96.0 4.0 18**	**85.8 7.0 18**	**78.4 7.0 18**	**75.0 6.2 18**

Intra-batch and inter-batch precision and accuracy results for des-acyl Ghrelin		QC LLOQ 50.0 pg/mL	QC S 150 pg/mL	QC Low 500 pg/mL	QC Mid 1000 pg/mL	QC High 1800 pg/mL
Run 1	Accuracy (%) CV (%) N	109.6 8.1 6	104.0 7.1 6	81.8 3.3 6	88.6 4.5 6	87.2 4.0 6
Run 2	Accuracy (%) CV (%) N	103.4 4.7 6	100.0 4.6 6	78.8 5.1 6	83.5 3.3 6	85.6 3.0 6
Run 3	Accuracy (%) CV (%) N	108.2 12.1 6	104.0 3.3 6	77.6 3.4 6	90.3 4.4 6	89.4 5.6 6
Inter-batch Precision and Accuracy	**Accuracy (%) CV (%) N**	**107.0 8.8 18**	**102.7 5.3 18**	**79.4 4.4 18**	**87.5 5.2 18**	**87.2 4.5 18**

Table 2: Intra- and inter-run precision and accuracy of three independent runs for acyl Ghrelin (top) and des-acyl Ghrelin (bottom).

Conclusions

To our knowledge this is the first LC-MS/MS method which permits to selectively quantify acyl- and des-acyl Ghrelin in human plasma with an LLOQ of 50.0 pg/mL for both analytes. This allows for getting more insights into the complex role of this hormone in the regulation of hunger and metabolism.