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LC-MS/MS quantitation of insulin, glucagon, somatostatin, and C-peptide

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ABSTRACT

An LC-MS method using multiple reaction monitoring for quantitation of insulin, C-peptide, glucagon, and somatostatin secretion from human islet populations is described. A 5 min separation was developed using a 2.1 × 30 mm (i.d. x length) C18 column. The method was used to measure secretions from static incubations of human islets from 2 donors. Insulin and C-peptide matched well with reported values of these hormones.

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DISCLAIMER:

Reproduction of the method is highly dependent on having a well-conditioned LC column. We have found that a brand new column does not provide the same separation as one that has been subjected to numerous separations. It is highly recommended to condition a new column with repeated injections (minimum 150) using high concentrations (> 400 nM) of all analytes and IS.

ABSTRACT

An LC-MS method using multiple reaction monitoring for quantitation of insulin, C-peptide, glucagon, and somatostatin secretion from human islet populations is described. A 5 min separation was developed using a 2.1 × 30 mm (i.d. x length) C18 column. The method was used to measure secretions from static incubations of human islets from 2 donors. Insulin and C-peptide matched well with reported values of these hormones.

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Chemicals and Reagents

LC grade water (H_2O)

LC grade acetonitrile (ACN)

Somatostatin-14, ([ring-D_5] Phe^6) somatostatin-14 (somatostatin IS) - BaChem

C-peptide trifluoroacetate salt, SILu^{TM}Pep C-peptide (([$^{13}C_6$]Leu^{26+30})-C-peptide) (C-peptide IS) - BaChem and Sigma

Glucagon, ([$^{13}C_6$]Leu^{14})-glucagon (1-29) trifluoroacetate salt (glucagon IS) - Sigma and BaChem

Human insulin, SILu^{TM}Prot insulin, human (([$^{15}N_7$] (all amino acids excluding Thr^{30} in β chain)) labeled insulin) (insulin IS) - Sigma

Bovine serum albumin (BSA)

Balanced Salt Solution (BSS) was used in certain experiments and was composed of 25 mM tricine, 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, and 2.4 mM $CaCl_2$adjusted to a pH of 7.4 and contained different amounts of glucose as described in the text

Materials and Instrumentation

Low adsorption autosampler vials

Halo Peptide column - ES-C18, 2.1 X 30 mm, 2.7 µm, 160 Å

Thermo Scientific^{TM} Vanquish^{TM} Flex UHPLC system with a Split Sampler Module^{TM}

Thermo Scientific^{TM} TSQ Quantis^{TM} triple quadrupole mass spectrometer.

Preparation and Analysis of Calibration Standards

Solubilize somatostatin, C-peptide, glucagon, insulin, and all IS according to vendor protocols to a concentration of 1 mg mL^{-1} using:

Somatostatin - H_2O
Somatostatin IS - H_2O
C-peptide - 8.7 M acetic acid
C-peptide IS - 8.7 M acetic acid
Glucagon - 0.05 M acetic acid

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Glucagon IS - H_2O
Insulin - 0.01 M HCl
Insulin IS - 0.35 M acetic acid

These stock solutions were stored at -20 °C for long term storage or 4 °C for short term storage.

All water and ACN contain 0.1% formic acid (FA) unless otherwise stated.

Hydrolyzed BSA (Hyd-BSA) should be made according to a published protocol.[1]

Final solvent composition of Hyd-BSA is 30:70 ACN:H_2O (v:v).

Calibration curves with BSS

Dilute analyte and IS stock solutions to 2 µM and 200 nM, respectively (with the exception of insulin IS which was diluted to 50 nM), with Hyd-BSA.

Further dilute analyte solutions with BSS containing 1 mM glucose to concentrations of 10, 20, 100, 200, and 400 nM.

Dilute 5 µL of these solutions to 100 µL via addition of 35 µL IS and 60 µL Hyd-BSA.

Final concentrations of the analytes are 0.5, 1, 5, 10, and 20 nM and IS concentration is 10 nM.

Human Islet Experiments - Obtained from ProdoLabs

Static Incubation of Islets

Wash 250 IEQ with BSS with 1 mM glucose

Place islets into a microcentrifuge tube with 50 µL of BSS with 1 mM glucose

Incubate at 37 °C and 5% CO_2 for 2 hrs

Remove 3 x 5 µL aliquots from the microcentrifuge tube

Add 15 µL of BSS with 51 mM glucose to the islets to bring the final concentration of glucose to 16 mM and final volume back to 50 µL.

Place islets back in the incubator at 37 °C and 5% CO_2 for 2 hrs

Remove 3 x 5 µL aliquots from the microcentrifuge tube

Dilute all 6 aliquots to a final volume of 100 µL with IS and Hyd-BSA as described in "Calibration Curves with BSS" section

Islet Lysis

Remove remaining BSS (except the last 10 µL) from the tube that contained islets.

Lysis is performed using an acid ethanol mixture as described previously.[2]

Transfer lysate to a low adsorption vial.

Dry completely with nitrogen gas.

Reconstitute dried solute with Hyd-BSA to make lysate stock solution.

Determine LC-MS response of stock solution.

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Dilute stock solution appropriately to ensure response of analytes fit within calibration range (multiple dilutions may be necessary).

Maintain final sample volumes for these lysate samples at 100 µL including addition of described IS volume (35 µL)

LC-MS Experiments

Mobile phases (MP): H_2O with 0.1% FA (MPA) and ACN with 0.1% FA (MPB)

Injection volume: 5 µL

MP flowrate: 0.2 mL min^{-1}

Column temperature: 30 °C

Optimized gradient: 21.3% MPB and held for 1 min. The MP composition underwent a linear change to 45.9% MPB over 4.55 min. The last 8 min of the method switched between 65% MPB and 21.3% MPB every 2 min.

Operate mass spectrometer in selected reaction monitoring mode (SRM)

Electrospray settings: spray voltage of 3500 V, sheath gas setting of 4.09 L min^{-1}, an auxiliary gas setting of 6.4 L min^{-1}, ion transfer tube temperature of 325 °C, and a capillary temperature of 350 °C

Transitions used were:

A	B	C
	Transition	Collision Energy (eV)
Somatostatin	819 --> 221	40.6
Somatostatin IS	822 --> 221	41.7
C-peptide	1007 --> 927	22.9
C-peptide IS	1011 --> 939	22.4
Insulin	968 --> 1129	20.6
Insulin IS	979 --> 1148	18.3
Glucagon	871 --> 1084	24.2
Glucagon IS	873 --> 1004	26.4

MS transitions and collision energies used.

If desired, directly infuse each analyte and IS (no Hyd-BSA in solution) to develop other transitions.

SRM dwell time - 50 ms

Daily calibration curves for quantitation of islet secretion and lysate samples using the low (0.5 nM) and high (20 nM) calibrants are recommended.

Data Analysis

Plot average ratio of analyte peak area to IS peak area at each analyte concentration from three runs to build calibration curves.

Fit data with linear trendlines.

Utilize to interpolate concentrations of analyte from islet and lysate samples.

The concentrations of each hormone in each of the fractions were then converted to pmol by accounting for dilutions.

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The amounts of each hormone secreted can be further described by:

a) Reporting secretion amounts as a percent of total hormone:

Calculate the moles of peptides secreted from islets and islet lysate by determining concentrations from the analyzed samples and taking into account the total dilution performed.

Calculate the percentage of each hormone release, with respect to the total content of that hormone, per minute of incubation time at each glucose condition.[3]

b) Convert the measured values to pg mL^{-1} and normalize to IEQ and incubation time.

Use the average measured values in the 5 µL aliquots and extrapolate to the total amount in the 50 µL tube.
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