Glucagon levels were robustly quantified using a high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) system. Data was acquired with Analyst software, with a dwell time of 100 milliseconds. Samples were ionized by ESI in positive ion mode with an ISV of 5500 V, CAD gas was set to high, and MS/MS was used. An Eksigent 425 LC system was used with an Eksigent Halo C18, 45% H2O (A) and in ACN (B). Samples were diluted (50 μL/sample) and 10-fold diluted (50 μL/sample) with a mobile phase of 0.1% formic acid in H2O (A) and in ACN with 10% acetic acid (50 μL/sample). Glucagon was added to each sample. Glucagon was extracted from plasma using a strong anion exchange column, followed by desalting/cleanup and trap alignment with the QTRAP® 6500 system. The LC/MS/MS system was developed: 1) a conventional LC method for high-throughput and sensitivity and 2) a microflow LC method for limited sample volumes. We developed a traditional microflow LC method that utilized the QTRAP® 6500 LC/MS/MS system to develop a highly sensitive and robust assay. The purpose of this study was to develop a highly sensitive and robust LC-MS/MS method that measures glucagon levels in human plasma for accurate assessments of glucose homeostasis. Herein, we describe an LC-MS/MS method that measures glucagon levels in human plasma for accurate assessments of glucose homeostasis. The conventional LC method for high-throughput and sensitivity and the microflow LC method for limited sample volumes were developed.

**ABSTRACT**

Glucagon is a peptide hormone produced by alpha cells in the pancreas, involved in glucose homeostasis. Typically, glucagon levels are low in healthy adults, but during certain pathophysiological states, glucagon can be elevated. A highly sensitive and robust LC-MS/MS method for monitoring glucagon levels in human plasma is needed for accurate assessments of glucose homeostasis.

**INTRODUCTION**

Glucagon is a hormone produced by the alpha cells in the pancreas, involved in glucose homeostasis. Typically, glucagon levels are low in healthy adults. However, during certain pathophysiological states, glucagon can be elevated. A highly sensitive and robust LC-MS/MS method for monitoring glucagon levels in human plasma is needed for accurate assessments of glucose homeostasis.

**MATERIALS AND METHODS**

**Sample Preparation**

Samples were diluted (50 μL/sample) and 10-fold diluted (50 μL/sample) with a mobile phase of 0.1% formic acid in H2O (A) and in ACN with 10% acetic acid (50 μL/sample). Glucagon was added to each sample. Glucagon was extracted from plasma using a strong anion exchange column, followed by desalting/cleanup and trap alignment with the QTRAP® 6500 system. The LC/MS/MS system was developed: 1) a conventional LC method for high-throughput and sensitivity and 2) a microflow LC method for limited sample volumes. We developed a traditional microflow LC method that utilized the QTRAP® 6500 LC/MS/MS system to develop a highly sensitive and robust assay.

**Conventional Flow:**

A Dionex™ Prominence LC system was used with an IonPac™ AS19-PG 4 mm x 25 cm column. The mobile phase was 0.1% formic acid in H2O (A) and H2O/formic acid/water/acetonitrile (40:1:5:49 (B)). Samples were diluted (50 μL/sample) and 10-fold diluted (50 μL/sample) with a mobile phase of 0.1% formic acid in H2O (A) and in ACN with 10% acetic acid (50 μL/sample). Glucagon was added to each sample. Glucagon was extracted from plasma using a strong anion exchange column, followed by desalting/cleanup and trap alignment with the QTRAP® 6500 system. The LC/MS/MS system was developed: 1) a conventional LC method for high-throughput and sensitivity and 2) a microflow LC method for limited sample volumes. We developed a traditional microflow LC method that utilized the QTRAP® 6500 LC/MS/MS system to develop a highly sensitive and robust assay.

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