Introduction

The peptide hormone glucagon plays an important role in homeostasis of glucose concentrations in the blood and serves as a biomarker for pathologies such as diabetes or pancreatic cancer. Reliable assays for determination of glucagon levels in biological samples are therefore important in order to gain a better understanding of the pathology and treatment of such diseases. Over the years there have been numerous glucagon assays available that mainly employ an immunometric approach. However, these methods may suffer from cross-reactivity, lack of specificity and sensitivity for determination of low glucagon concentration levels. Recent advances in MS instrumentation and the amenability of glucagon for analysis by LC-MS/MS have brought these new methods to the forefront to support quantification of endogenous glucagon levels in the low pg/mL range. Here we present a sensitive and robust fully automated LC/MS/MS method that was developed and validated according to FDA and EMA guidelines allowing for quantification of glucagon from 20.0 to 2000 pg/mL in human plasma.

Sample Collection and Standard (STD) / Quality Control (QC) Sample Preparation

Unknown samples / QC samples

Steady solutions in the low pg/mL range

Transfer of plasma into polypropylene tubes containing 33% aq. formic acid

Addition of 15 µL (13C6)-Glucagon

Centrifugation within 30 min of addition

Loading onto the LC column using a positive injection procedure

Centrifugation within 30 min of centrifugation

Vaporization until dryness

Resolubilization with 2.5 % NH4OH in Water

Injection volume

Mean measured (pg/mL)

Accuracy (%)

Bias (%)

Calibration curve from 20.0 to 2000 pg/mL

Results

Table 1: Chromatographic conditions and MS/MS parameters using SCIEX TQ 6500 and 6500+ instruments.

Table 2: Intra- and inter-run precision and accuracy of three independent runs measured on SCIEX TQ 6500 and 6500+ instruments.

Table 3: Cumulated freeze/thaw and bench top stability results could be shown in P800 plasma. In addition stability in the autosampler was for at least 36 hours at 5°C.

Table 4: Long-term stability at -40°C in charcoal stripped EDTA plasma and in P800 plasma.

Table 5: Matrix effect determination at QC Low level.

Table 6: Matrix effect determination at QC High level.

Conclusion

The validated dynamic range of this method allows for quantification of endogenous as well as doxorubicin glucagon in human plasma.

Sample stability could be shown during the entire bioanalytical process.

The robustness of the developed method allowed for analysis of more than 2000 clinical samples with a run success rate of 93%. Incurred sample manysis (IS) was successful with 94% matching rate.

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