Method Development for Quantifying Meglitinides- LC-MS/MS

Type 2 Diabetes mellitus (T2DM) characterised by hyperglycaemia is a global public health concern that accounts for about 90% of diabetes cases (Diabetes UK, 2017). This has had an impact on healthcare economics. Life style intervention is the foundation of good glycaemic control. However, treatment with antidiabetic drugs alongside life style intervention may be required to achieve the desired glycaemic control. Unfortunately, the use of antidiabetic drugs is associated with hyperglycaemia. Therefore, there is a clinical demand for assays to measure antidiabetic drugs in the investigation of hyperglycaemia. The use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has the potential to overcome these issues faced with other methods e.g. immunoassays.

The inclusion of hypoglycaemic drugs testing in cases of unknown origin is vital as it would help with differential investigation of drug induced hyperglycaemia from other potential causes requiring further investigation e.g. insulinoma.

This poster focuses on one of the major classes of hypoglycaemic drugs, Meglitinide (drug analogues nateglinide & repaglinide).

Aim
To develop an LC-MS/MS method for the quantification of Meglitinides (nateglinide and repaglinide) in human blood and harmonise the Meglitinide method with the current method used for the detection of Sulphonylureas. The LC-MS/MS is also a versatile method for expanding the range of drug compounds to be analysed.

Method development
MS initial tuning:
- The first practical step MS initial tuning- mass spectral settings set to operate in positive ion mode APCI
- The mass spectrometer parameters such as gas flow and ion voltage were manually tuned to replicate those of Sulphonylurea method
- This allowed for the creation of optimum conditions of ionisation and sensitivity for the repaglinide, nateglinide (Meglitinides) and the internal standard (IS)
- HPLC optimization:
  - Liquid chromatography (LC) optimization was a critical part of developing the assay because this affected the selectivity and sensitivity
  - The mobile phase gradient was set using the Sulphonylurea assay gradient & the ion transitions were imported from the tuning file
  - The retention time of all three analytes were observed as nateglinide 5.55 - 5.60minutes, repaglinide 5.75-5.83minutes and IS 5.75 - 5.83minutes

MS/MS optimization:
- The optimisation of the ion source parameters was dependent on the LC conditions and vital for assay sensitivity and reproducibility
- Precursor ion and product ion mass (MRM transitions) for all 3 analytes were obtained from literature review (Zhang et al., 2011 and Hess et al., 2011).

Sample preparation:
- Standards were prepared by the addition of the appropriate volume of stock solution to ‘blank’ pooled serum to give the following concentrations: 6.1, 1.82, 5.49, 1.646, 4.938, 1.482, 4.444, 1.333, and 4 (g/L) for nateglinide and repaglinide

Sample extraction:
- A simple liquid-liquid extraction method was applied in this project for the extraction of the Meglitinides and IS from the serum matrix
- Blank serum was used to draw a calibration curve- nine concentrations of repaglinide and nateglinide ranging from 6.1 g/L to 0.006g/L. The linearity was then evaluated by a weighted (1/concentration) least squared regression analysis.

Accuracy Imprecision:
- The intra-day accuracy for this method was evaluated by analysing QC samples at three levels in three replicates over two days (Coefficient Variation)
- Matrix factor and extraction efficiency:
  - To determine the matrix effect a matrix factor (MF) was quantified for each compound.
  - Specificity/selectivity:
    - Specificity/Selectivity of the analytes was also carried out at the beginning of the method development by confirming the presence of the analytes during initial MS tuning, the monitoring of multiple mass transitions during MS optimization and observing the retention time of analyte during LC optimization see figure 6.

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Results

Figure 3 and 4. show the calibration curves normalised to the internal standard for nateglinide and repaglinide respectively. The calibration curves show good linearity over the working concentration ranges with correlation coefficients (R²) >0.999 for both repaglinide and nateglinide. However at the lower end of the calibration curve concentration liquid chromatography-tandem mass spectrometry (LC-MS/MS) is also a versatile method for expanding the range of drug compounds to be analysed.

A: A graph to highlight the amount of repaglinide being detected respectively. The calibration curves show good linearity over nine concentrations of repaglinide and nateglinide ranging from 6.1 g/L to 0.006g/L. The linearity was then evaluated by a weighted (1/concentration) least squared regression analysis.

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Conclusion
The mobile phase gradient, LC optimization and the liquid-to-liquid extraction process was successfully adopted from that of the Sulphonylurea group. The project was successful in developing a LC-MS/MS method that was selective and sensitive to nateglinide, repaglinide and IS. The IS used in this project used repaglinide labelled with deuterium for the correction of the matrix effects. The IS was also used to help correct for any variation during the sample processing. This project demonstrated unexpected results for the deuterated labelled IS (deuterated IS) see Figure 5 and Figure 7. The calibration data at low concentration demonstrated a regression of no better than R²=0.6 for both target analytes. Future work can be built from this project on method validation. However an observation of isobaric interference from the internal standard particularly impacted the quantification of repaglinide. Possible deuterium labelled IS exchanged the deuterium for hydrogen therefore compromising the stability of the IS during the project.

Development of method was successful however, further studies are required for method validation.

References