Measurement of Pancreatic Polypeptide and its Peptide Variant in Human Serum and Plasma by Immunocapture-Liquid-Chromatography-Tandem Mass Spectrometry. Reference Intervals and Practical Assay Considerations.

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Pancreatic polypeptide (PP) is secreted by pancreatic islets after ingestion of food. Meal composition is associated with concentration of PP released in circulation. PP can be elevated in blood of patients with endocrine pancreatic tumors, supporting its use as a biomarker. We developed an Immunocapture-Liquid Chromatography-

pancreatic tumors PP concentrations in excess of 1,000 pg/mL were found in 15-74% of patients [13].

depleted from PP and PP3-36 using PP antibody were prepared spiking known standard concentration of PP and PP3-36 to contain 5, 10, 20, 30 and 40 pg/µL of each analyte to evaluate the LLOQ and LOD of the method analyzed over a 4 days period in three replicates each per day. The lowest concentration for which precision was within 15% and accuracies were within 20% of the expected value, was set as the lower limit of quantitation. Limit of detection was determined as the lowest concentration at which the peaks of the analytes were present in both mass transitions at the expected retention time and signal to noise ratio for the quantitative mass transition was ≥ 5. Samples for linearity test were pools of serum samples (remaining aliquots of patient samples submitted to ARUP laboratories for testing) depleted from PP using anti-peptide antibody and spiked with a defined concentration of PP and PP3-36 for seven levels total: 50, 250, 1000, 2500, 5000, 50000 pg/mL. The samples were aliquoted in tubes, stored at –70°C and thawed prior to the analysis. They were analyzed in duplicates over a period of 5 days. The highest concentration at which precision was within 10% and accuracy within 20% of the expected values was considered to be the Upper Limit of Linearity (ULOL) of the method. Method recovery was determined with 11 serum samples analyzed as is and spiked with 650 pg/mL of PP and PP3-36. Samples were tested in duplicate. Difference between the observed and expected concentrations gave a measure of recovery of the sample preparation. Ion suppression was evaluated using post column infusion method [19]. A serum sample spiked with 50 pg of PP and other with PP3-36 were processed and injected into a flow of analyte with 1000 ng/mL at flow rate of 7 µL/min. Presence of potential interferences was evaluated by monitoring ratios of primary and secondary MRM transitions for PP and PP3-36; the acceptability range for the ratio was ± 30% (0.7-1.3) based on comparison of the quantitative results using the two MS/MS transitions. Effects of icterus (bilirubin), hemolysis and lipemia were assessed in serum spiked with PP and PP3-36 mixed with the interfering substances: red blood cells (86 mg/L), lipids (320 mg/L) and bilirubin (173 mg/L). Method

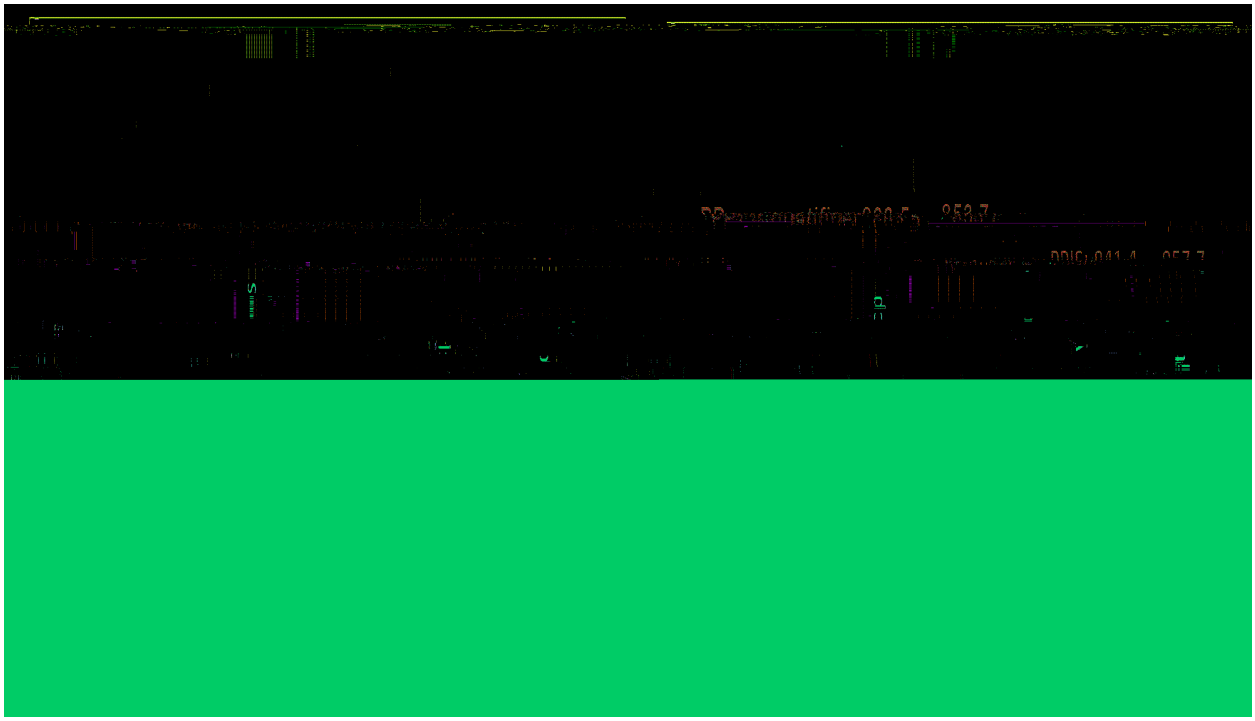

Figure 1: Chromatograms of the analytes and internal standard in serum sample, depleted of target peptides, spiked with 100 pg/mL of PP, PP3-36 and PPIS. Primary (m/z: 837.3→953.3) and secondary (m/z 837.3→411.4) mass transitions of PP and primary (m/z: 803.5→853.7) and secondary (m/z: 803.5→197.2) mass transitions of PP3-36. Primary and secondary mass transitions for PPIS (m/z: 841.4→957.7, 841.4→418.2).

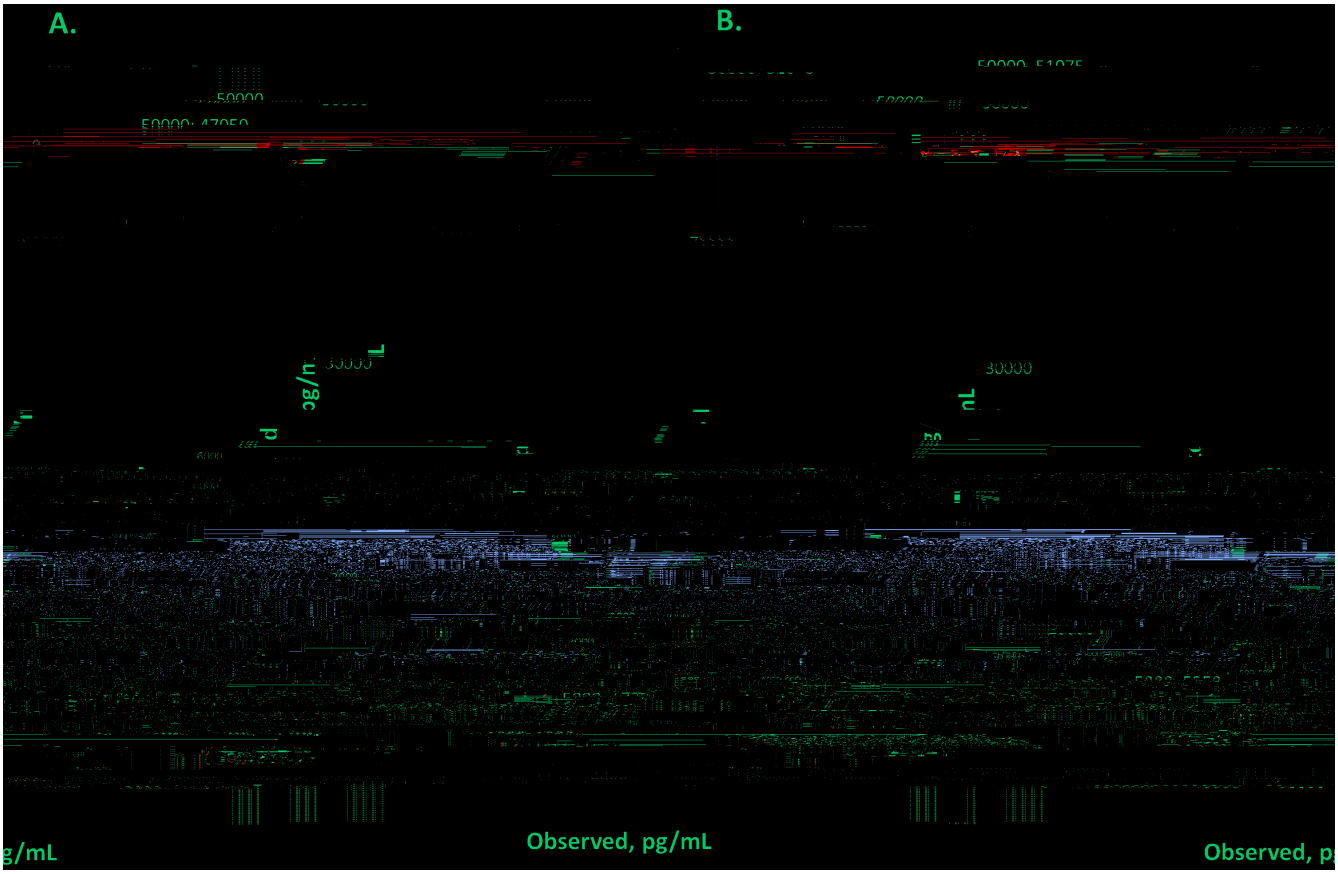

Figure 2:

4	500	508.3	7.2	17.6	19
5	1000	1080.3	7.2	13.4	15.2

Table 2: Inter and intra-assay imprecision for PP3-36*. *Three replicates per run during five days Carryover observed was <0.7% (Supplementary Table 4).

The presence of PP3-36 in blood together with the intact hormone PP1-36 follow the same expression of PYY and NPY, members of the peptide family circulating with two major forms: intact (PYY1–36 and NPY1-36) and truncated (PYY3–36 and NPY3-36). The truncated form is the product of cleavage of the amino terminus Tyr-Pro by the enzyme dipeptidyl peptidase-IV (DPP-IV) able to target proline-rich proteins and polypeptides [5,21] cleaving the peptide bond following a penultimate N-terminus proline, suggesting similar mechanism could generate the fragment PP3-36 from the intact PP hormone. We found enzyme inhibitors for DPP-IV, the most probable generator of PP3-36, and neprilysin, a metallopeptidase which cleave peptides at the amino side of hydrophilic residues did not exerts action on PP during sample processing demonstrating that the variant PP3-36 is of endogenous origin.

We used BSA solution as a blocking agent to passivate active sites in the flow path reducing peptide losses in the flow path of the HPLC; the HPLC column also required a preliminary treatment with BSA to reach an appropriate performance. Other approaches evaluated separately to reduce peptide losses in the HPLC system were addition of 5% of DMSO in mobile phases [22] and addition of 0.001% of Polyethylene glycol [23] or 0.03% CHAPS [24] in the acid elution buffer. The above approaches led to adverse results with undetectable peptides analytes. DMSO can enhance number of charges in the mass spectrometer electrospray ionization chamber [25] decreasing the selected charged precursor ion. Additional enhancement of the sensitivity was achieved through use of acetic acid (versus formic acid) as mobile phase additive modifiers (Supplementary Figure 7).

The method was linear (for both PP and PP3-36) in the range of 10 to 50000 pg/mL (Figure 2).

However the signal of PPIS at PP concentrations >1000 pg/mL was reduced (Supplementary Figure 8), suggesting a signal suppression by PP. The assay precision is acceptable for diagnostic purposes, and the LOQ afforded was six times lower than the commercial RIA method. No interference was observed in over 150 patient samples analyzed during the method evaluation.

agreement between both methods was reasonably good (Supplementary Figure 9).

Meal composition results indicated that consumption of carbohydrates alone provides weak stimuli for secretion of PP, compared to the meals rich in protein and fat, or a combination of carbohydrate and fat (Figure 4).

We did not observe a relationship with release of PP with obesity [26], BMI, and body fat percentage; PPT concentrations were comparable in the fasting and postprandial samples of obese and non-obese subjects (Supplementary Figure 6).

Conclusions

In summary, we have developed a direct LC-MS/MS method for determination of PP and PP3-36 in serum and plasma using affinity immunoenrichment without enzyme digestion. The fragment PP3-36 is endogenously produced and present in circulating blood. Other earlier reported truncated forms of PP were not detected. The method has been fully validated according to CLSI guidelines and applied to