

# Diagnosis of Carcinoid Tumors in the Small Intestine by LC-MS/MS analysis of 5-hydroxyindoleacetic acid (5-HIAA) in Serum.

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## Introduktion

Carcinoid tumors are neuroendocrine tumors (NET), mainly occurring in intestines. In most cases the neoplastic cells produce serotonin, which is metabolised to 5-hydroxy-indoleacetic acid (5-HIAA). In Sweden, 50-100 new cases are discovered annually. For diagnosis, determination of urinary output of 5-HIAA for 24 hours have been used for over 40 years. Determination of 5-HIAA in serum has been introduced in the later years as the sampling is more convenient for the patient.

## Objective

To establish an automated method for quantitation of 5-HIAA in serum using LC-MS/MS.

## Methods

Sample preparation were carried out by a pipetting robot (Hamilton MicrolabSTARlet) equipped with a vacuum station and nitrogen evaporator. D<sub>5</sub>-5-HIAA was used as internal standard. The amount of 5-HIAA was determined by reversed phase chromatography and tandem mass spectrometry (Waters, Acquity-Xevo TQ). A gradient from 5 to 35% acetonitrile in 0.2% formic acid was used to separate the analyte on an Acquity UPLC HSS T3, 2,1x150cm column. Positive electrospray ionization and monitoring of the transition from 192 to 146 M/Z (loss of CH<sub>2</sub>O<sub>2</sub>) was used for quantification.

## Conclusions:

Quantitation of 5-HIAA in serum is a useful and reliable method for diagnosis of intestinal carcinoid tumors. The method is less laborious than analysing 5-HIAA in urine, and for the patients much more comfortable.

## Sample preparation with robot

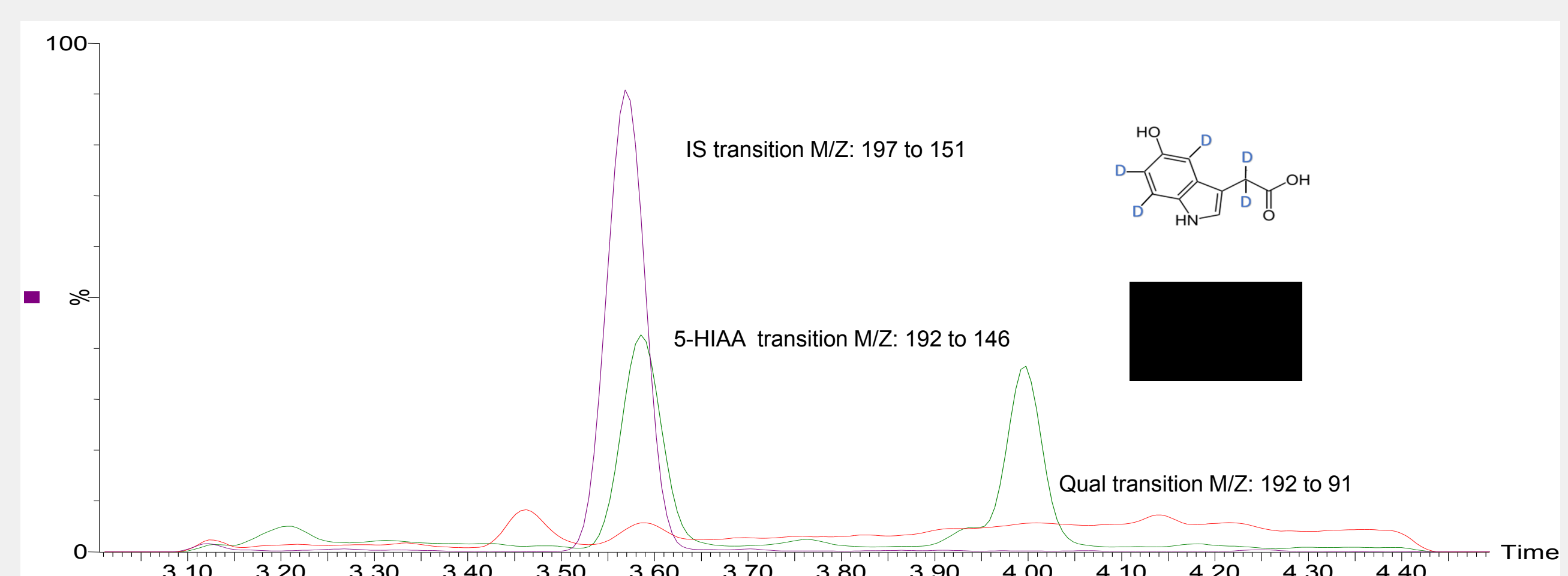
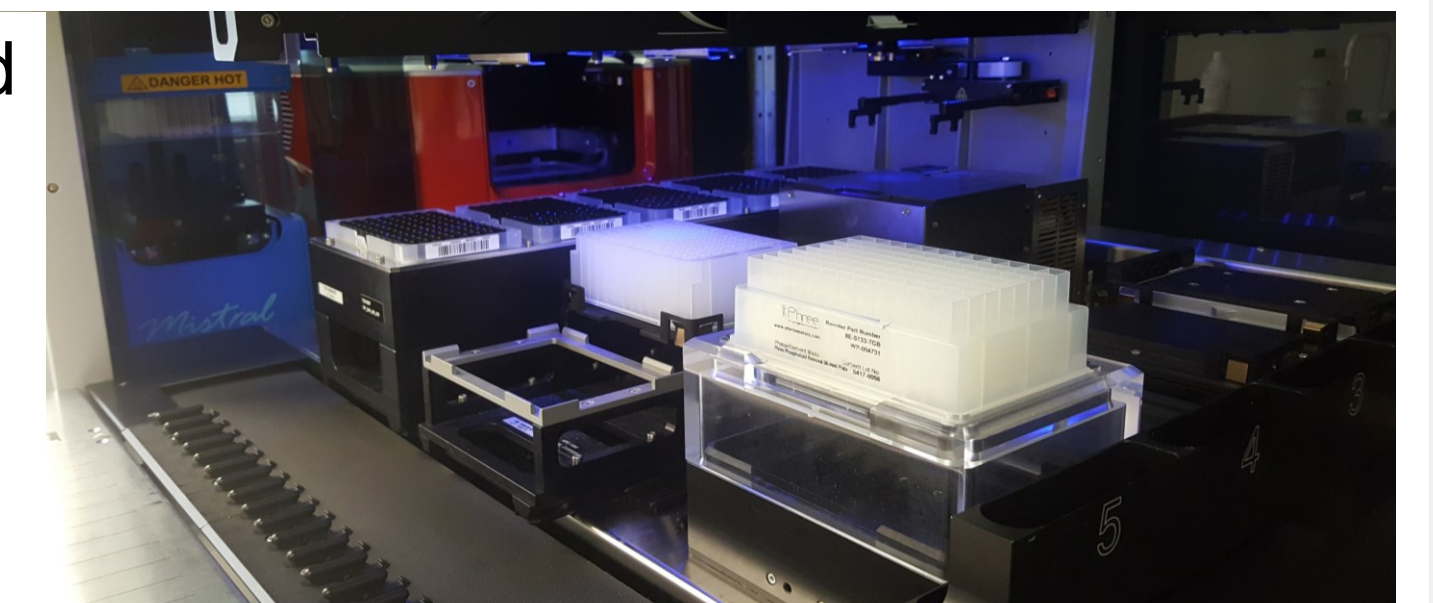
50 µL serum and 40µL IS solution was mixed 5min. in a Phree phospholipid removal plate (Phenomenex)

450 µL acetonitrile was added, mix 5min

Sample was eluted with vacuum.

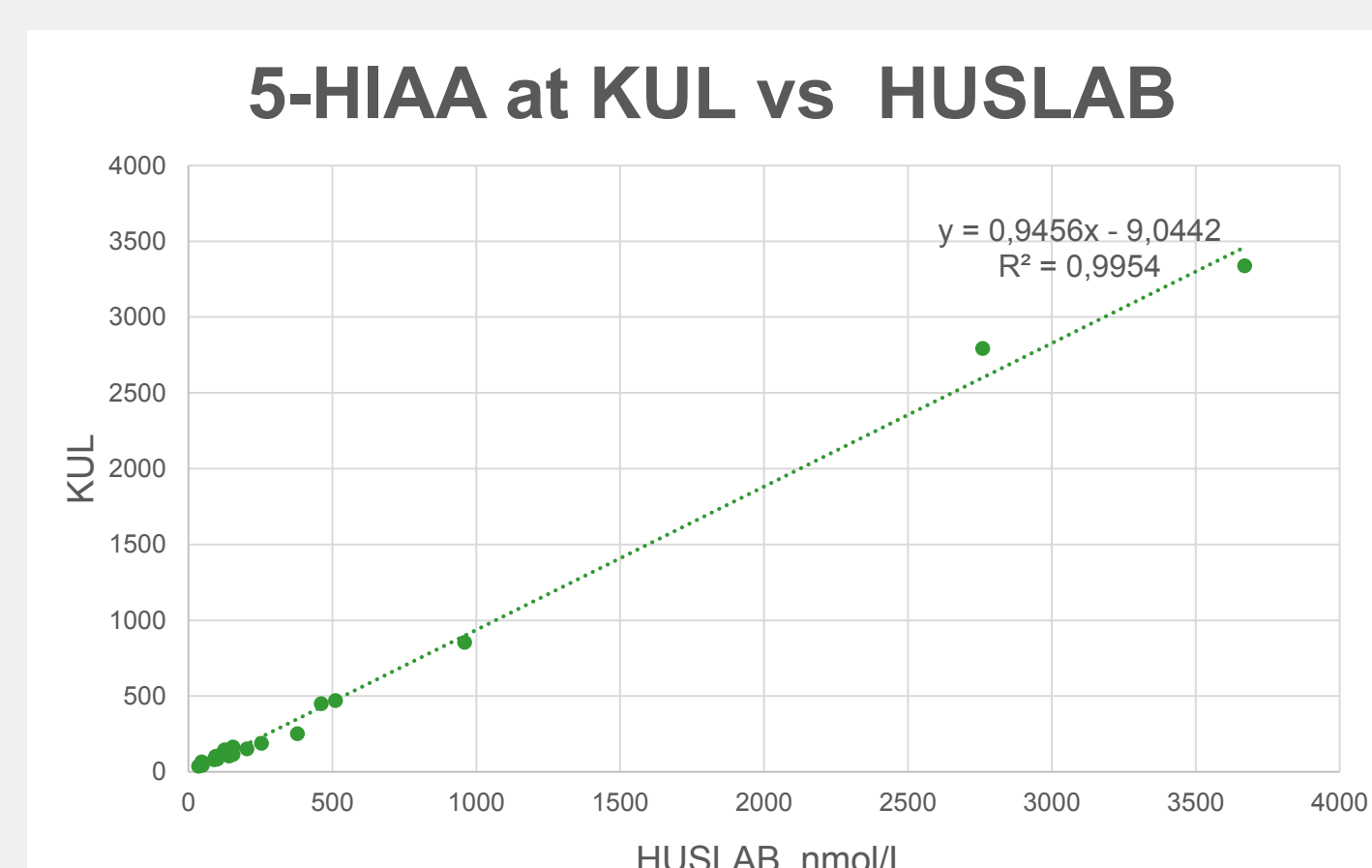
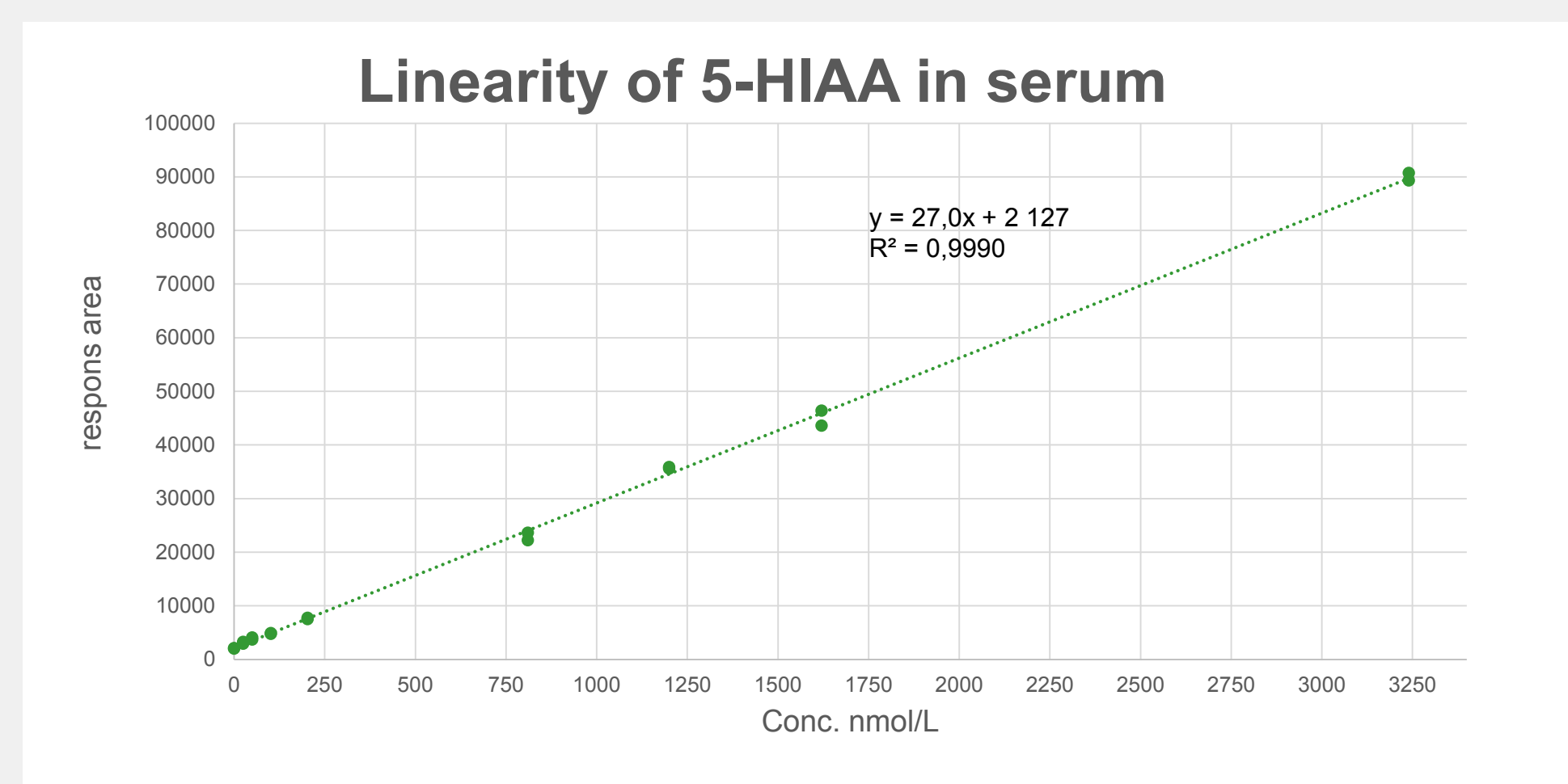
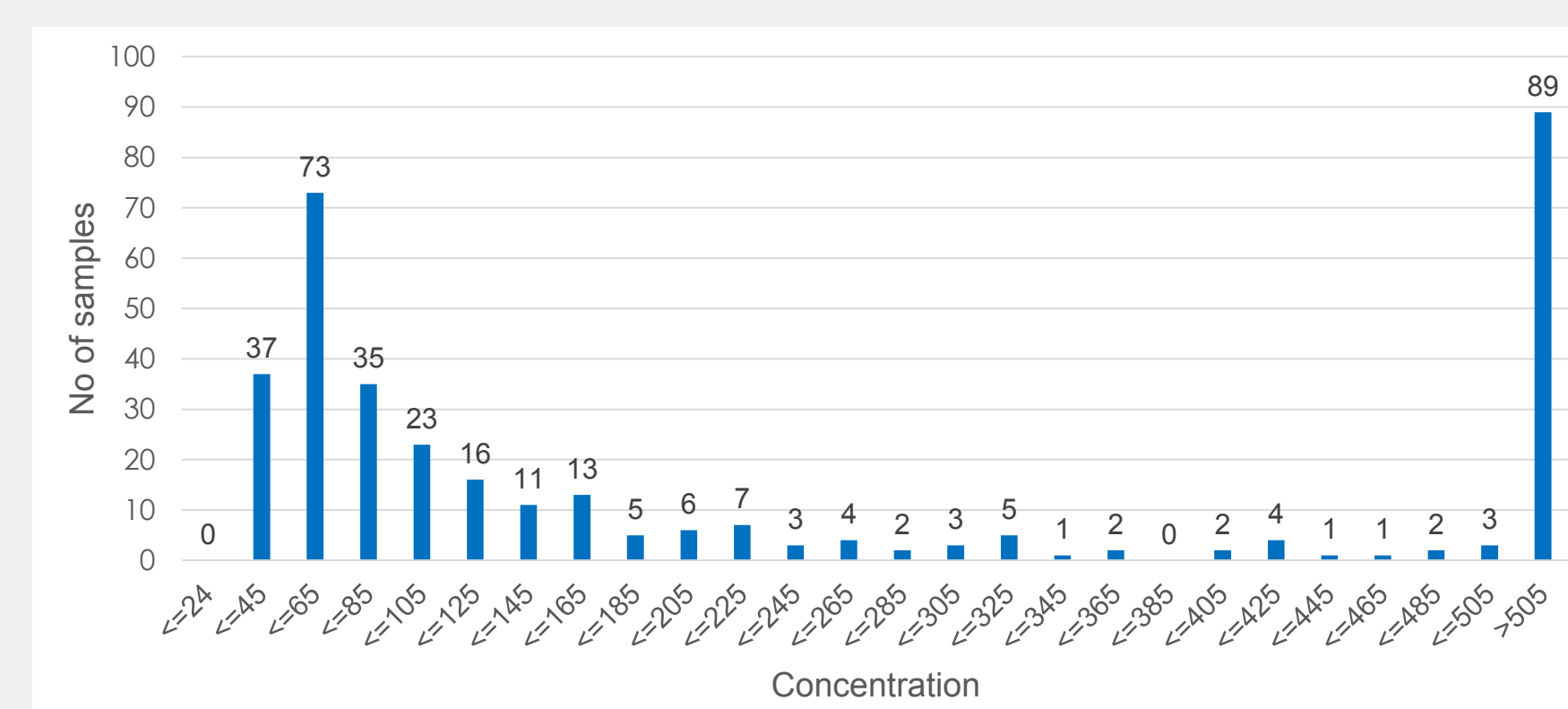
Acetonitrile was evaporated with N<sub>2</sub>.

160µL 0.2% formic acid was added.



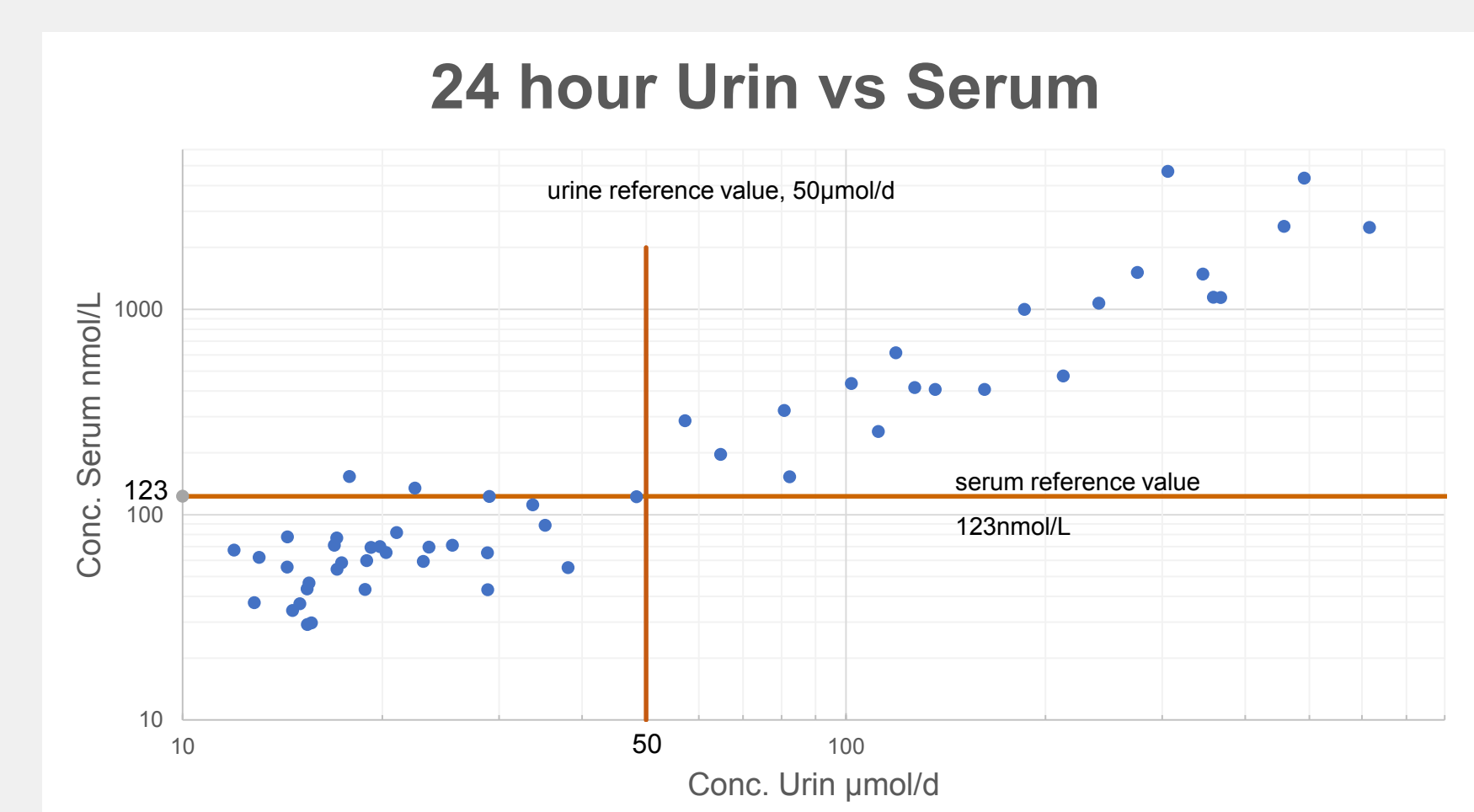
## Results

**Total CV:** 6.5% / 690 nmol/L  
7,4% / 146 nmol/L (Controls made of pooled serum with addition of 5-HIAA).  
**Limit of quantification:** 25 nmol/L (Lowest concentration with CV less than 20%).  
**Linearity range:** 25 – 3600 nmol/L.  
**Accuracies:** -4.8 to 2.6% (Standard addition experiments in serum).



Comparison experiments with 5-HIAA assayed at HUSLAB (Helsinki University Hospital) showed acceptable agreement.

Frequency of samples with different concentrations analysed from Jan. 19 to Aug. 19 in 2019. The highest values found were 9,000-10,000 nmol/L, and these patients all had NET tumors with metastatic spreading.



Comparison of the concentration of 5-HIAA in 24 h urine and serum sampled, within one week. The graph is extracted from anonymised patient data. There may be discrepancies from the normal sampling procedure and the patient may have been treated in different ways between the sampling occasions. Generally, most patients having pathologically elevated serum concentrations also showed increased excretion of urinary 5-HIAA during 24 hours. In some cases with only slightly elevated serum levels of 5-HIAA (up to 200 nmol/L), urinary excretion of 5-HIAA was not elevated.

## References

Tohmola N, Itkonen O, Sane T, Markkanen H, Joenväärä S, Renkonen R, E. Analytical and preanalytical validation of a new mass spectrometric serum 5-hydroxyindoleacetic acid assay as neuroendocrine tumor marker. Clin. Chim. Acta 2014;428:38-43.