Semi-quantitative Determination of Tramadol in Oral Fluid Utilizing Homogeneous Enzyme Immunoassay (HEIA): Comparison with ELISA

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Objective

Tramadol, a CNS depressant and analgesic is used in the treatment of moderate to severe pain. The objective was to determine whether semi-quantitative data collected with HEIA has a strong correlation with routine immunoassay, ELISA.

Relevance

Since the main issues in clinical practice are the time and expense of sample processing, the development of a rapid semi-quantitative method for the screening of pain management drugs is necessary.

Methodology

• Oral fluid samples collected in the Immunalysis Quantisal™ device were screened at a cut-off concentration of 50µg/L using ELISA and HEIA.

• Controls for ELISA and HEIA were diluted 1+3 with Quantisal™ buffer to achieve neat oral fluid concentration.

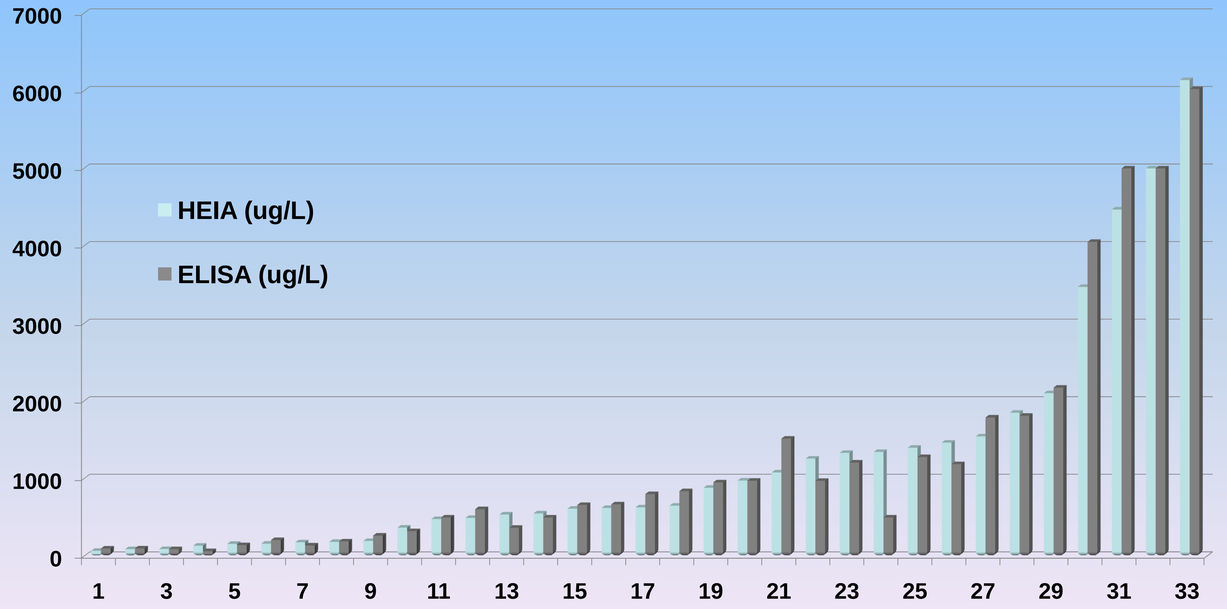
•Positive samples were extracted using a previously published and validated solid phase extraction and analyzed using GC-MS.[1]

Validation

Both assays: Calibration standards: 25, 50, 100, 200, 500µg/L

HEIA: Instrument: Olympus AU400e; Sample volume: 25µL; R1 and R2 volumes: 100µL
Primary wavelength: 340nm; secondary wavelength: 410nm Measuring points 15th -19th rotation.
Inter-day precision was performed over 20 days with 2 replicates per day (N=40)
Coefficient of variation (CV): 150µg/L and 250µg/L - 0.65% and 0.64% respectively.

ELISA: Sample volume: 10µL.
Intra-assay precision CV% for 150µg/L and 250µg/L - 5.35% and 4.05%, respectively
Linearity r^2=0.9794 for ELISA semi-quantitative curve.



Results

• Thirty-three (33) oral fluid specimens previously found to be positive for tramadol using GC-MS were analyzed using HEIA and ELISA in the semi-quantitative mode.

• Comparison of the data with the quantitative GC-MS data showed samples in the linear range of the assay to be within +/-20% of the confirmed concentration. Samples outside the upper limits of the curve and causing maximum absorbance readings were later diluted 1:10 or 1:100 and re-plated to fit within the standard curve range.

• Samples with screen values above the upper limits of the curve could be further diluted prior to extraction to eliminate the need for re-analysis, ultimately decreasing extraction costs and increasing throughput.

Conclusion

• Tramadol can be determined in oral fluid specimens using semi-quantitative screening modes on routine chemistry analysers or ELISA platforms.

• Both assays were rapid and simple to operate, showing a high degree of quantitative correlation with GC-MS.

Reference

Moore C, Rana S, Coulter C. Determination of meperidine, tramadol and oxycodone in human oral fluid using solid phase extraction and gas chromatography-mass spectrometry. *J Chromatogr B. Biomed Applns* 2007; 850: 370 - 375

Oral Fluid

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