Development and validation of a novel homogeneous immunoassay for the detection of tapentadol in urine

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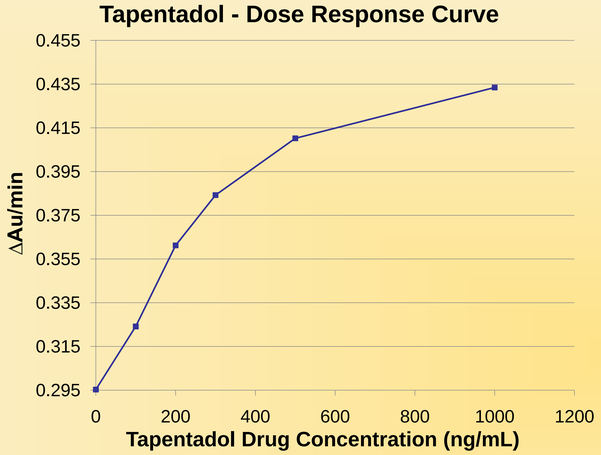
Introduction and Objective

The objective of this project was to validate a new high throughput homogeneous enzyme immunoassay (HEIA) for the rapid detection of tapentadol in human urine. Tapentadol is a new and potent opioid analgesic that was approved by the FDA in 2008 for relief of moderate to severe acute pain in patients 18 years and older. Tapentadol is 18 times less potent than morphine and the exact mechanism of action is unknown. However, its analgesic efficacy is believed to be two mechanisms, one as a μ-opioid agonist and the other as the inhibition of norepinephrine reuptake. Since the drug is fairly new to the commercial market, to our knowledge there is no commercially available immunoassay screening method for this drug. For this reason, it is valuable to develop and validate a tapentadol HEIA to detect tapentadol in urine.

Advantages

1. Ready to use reagents suitable for high throughput instruments
2. Targeting the parent drug and O-glucuronide metabolite
3. Highly sensitive and specific with low cross reactivity with amphetamines and tramadol

Results and Discussion



Precision: Daily Calibration Required

The precision was determined by assaying calibrators and controls for 4 days, 2 runs per day in replicates of 10 (N=80). The results are summarized in table below.

Conc. ng/mL	Mean Conc. (ng/ml)	C.V.%
Intra-day Precision (n = 20)		
150 ng/mL (Control LOW)	145.5	6.3
200 ng/mL Calibrator	205.7	4.1
250 ng/mL (Control HIGH)	254.7	3.9
Inter-day Precision (n = 80)		
150 ng/mL (Control LOW)	149.0	6.5
200 ng/mL Calibrator	208.8	4.6
250 ng/mL (Control HIGH)	256.2	3.8

Cross Reactivity:

The table below represents related drugs that have cross reactivity with the EIA assay.

Analytes	Analyte Conc. (ng/mL)	Tapentadol equivalents (ng/mL)	Cross Reactivity (%)
Tapentadol	200	200	100
Tapentadol Glucuronide	800	200	25
N-desmethyl tapentadol	10,000	200	2

The table below represents unrelated drugs that could yield screening positive results at high concentrations.

Analytes	Analyte Conc. (ng/mL)	Tapentadol equivalents (ng/mL))	Cross Reactivity (%)
Chlorpromazine	40,000	200	0.5
Cyclobenzapine	100,000	200	0.2
Doxepin	100,000	200	0.2
Imipramine	50,000	200	0.4
Tramadol	50,000	200	0.4
Trimipramine	30,000	200	0.7

Authentic specimens:

A total of 191 urine specimens previously confirmed with LC-MS/MS by external reference laboratories were analyzed using the HEIA assay at a cut off of 200ng/mL; the confirmation cut off was 50ng/mL. Due to the cross reactivity with both parent drug and its major metabolites, the 200ng/mL screening cutoff concentration correlates well with confirmation method and at the same time eliminates potentially false positive results.

		LC-MS/MS (unhydrolyzed) +	LC-MS/MS (unhydrolyzed) -
HEIA	+	72	2*
	-	2**	115

The sensitivity, specificity, and accuracy were calculated to be 97%, 98%, and 98% ,respectively.
*False positive by HEIA Screening
** 32ng/ml & 65ng/ml assayed by tapentadol HEIA; 72ng/ml & 52ng/ml by LC-MS/MS analysis.

Summary

A high throughput HEIA has been developed for the detection of tapentadol and one of its major metabolites in human urine which correlates well with LC-MS/MS. This is the first report of a homogeneous immunoassay for tapentadol

References

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2. J.A. Bourland, A.A. Collins, S.A. Chester S.Ramachandran, R.C.Backer. Determination of tapentadol (Nucynta®) and N-desmethyltapentadol in authentic urine specimens by ultra-performance liquid chromatography-tandem mass spectrometry. *J. Anal. Toxicol*. **34:** 450-457(2010).

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