Monitoring Naltrexone Compliance in Urine by Enzyme Immunoassay and GC-MS

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Abstract

Naltrexone is a narcotic antagonist belonging to the opioid class of drugs. It binds to the same receptors in the brain as heroin and alcohol and hence can be administered as a treatment for the addiction of heroin and alcohol in rehabilitation programs. Naltrexone is non-addicting and therapy depends on regular intake of naltrexone by subjects, every 2-3 days. It is metabolized in the liver, mainly to 6β-naltrexol and to a minor extent to 2-hydroxy-3-methoxy-6β-naltrexol and 2-hydroxy-3-methoxy-naltrexone, which are then further metabolized to the corresponding glucuronides. The plasma half-life of naltrexone is about 4 hours and for 6β-naltrexol about 13 hours.

Objective

The main objective of this study was to develop a sensitive and specific enzyme immunoassay technique and GC-MS confirmation method for the detection and quantification of both the parent drug naltrexone, as well as the major metabolite 6β-naltrexol in human urine. Other groups have developed LC-MS procedures for detection of naltrexone in urine and GC-MS methods for plasma. This newly developed immunoassay and GC-MS method is unique, as it can detect low levels of naltrexone without cross-reacting with oxycodone, which is a common problem that toxicologists face.

Methods

For the enzyme-linked immunosorbent assay (ELISA), naltrexone specific IgG was coated on microtiter plates and HRP labeled naltrexone was used as the conjugate in a competitive binding ELISA . 10 µL of diluted urine specimens (1:10 dilution in phosphate buffered saline) were used in the assay.

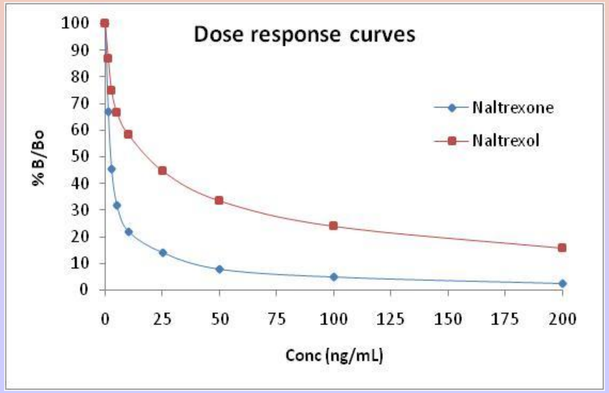
For GC-MS confirmation, urine specimens (0.1 mL) were extracted with SPE columns, followed by derivatization with BSTFA. Calibration curves for naltrexone and 6β-naltrexol were prepared from 0-200 ng/mL in synthetic urine, using deuterated naltrexone as the internal standard.

Analysis*:** Agilent 6890N GC-MS in electron impact mode. Ions monitored: Naltrexone: ***m/z 557, 542, 425; 6β-Naltrexol: ***m/z*** 559, 544, 372; Naltrexone d_3: ***m/z*** 560, 545.

Assay Performance

The ELISA dose response curves for naltrexone and 6β-naltrexol, were plotted from 0-200 ng/mL in synthetic urine, with a cutoff at 25 ng/mL. The limit of detection of both parent drug and metabolite was 1 ng/mL.

For confirmation by GC-MS, the limit of detection was found to be 0.1 ng/mL and the limit of quantification was 1 ng/mL. The curve was found to be linear from 0-200 ng/mL.



Cross-reactivity

Compound	*Conc (ng/mL)*	*Naltrexone equivalents (ng/mL)*	*% Cross-reactivity*
Naltrexone	50	50	100
6β-Naltrexol	100	10	10
Naloxone	100	15	15
Codeine	50,000	<1	ND*
Diacetylmorphine	20,000	<1	ND
Ethyl morphine	10,000	<1	ND
Hydrocodone	10,000	<1	ND
Hydromorphone	10,000	<1	ND
Meperidine	20,000	<1	ND
Methadone	50,000	<1	ND
Morphine	50,000	<1	ND
Nalorphine	10,000	<1	ND
Oxycodone	10,000	5	0.05
Propoxyphene	50,000	<1	ND
Fluoxetine	10,000	<1	ND
Levorphanol	10,000	<1	ND

** ND = Not detected*

Accuracy and Precision

ELISA	*GC-MS*	
	+	-
+	**19**	**0**
-	**0**	**16**

Sensitivity: 100%

Specificity: 100%

Accuracy: 100%

Precision:

Intra-assay (*n=6)*: ELISA and GC-MS <5%

Inter-assay (*n=30)*: ELISA and GC-MS <10%

Summary

The described methods are highly sensitive and specific for the detection of naltrexone and its major metabolite 6β-naltrexol in urine, for compliance within a heroin or alcohol treatment program. The assay can detect very low to high levels of naltrexone, without detecting high oxycodone levels.

References

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Disclosure: Immunalysis Corporation manufactures and distributes the immunoassay described in this presentation