Development and validation of a highly sensitive homogenous immunoassay for the detection of Δ⁹-THC in oral fluid using the Quantisal™ collection device

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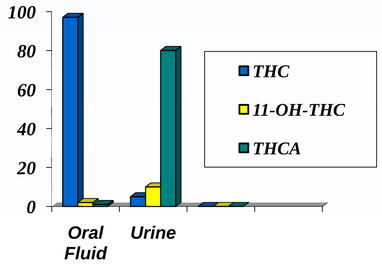
Abstract

Oral fluid is a useful biological specimen to detect recent drug usage, and is included as a specimen type in the proposed Federal guidelines for workplace drug testing. While it has advantages over urinalysis such as observed collection and difficulty of adulteration, oral fluid contains lower concentrations of drugs and sample volume is limited. Commercially available urine THC immunoassays are designed specifically for the THC metabolites, Δ9-carboxy-THC and are not sensitive enough for the detection of the parent drug which is predominantly detected in oral fluid. We developed an improved and highly sensitive homogeneous immunoassay for the detection of THC in oral fluid. This assay was validated with 77 oral fluid specimens previously analyzed using GC-MS. The accuracy was greater than 90% when using 8ng/mL of Δ9-THC as the cutoff concentration.

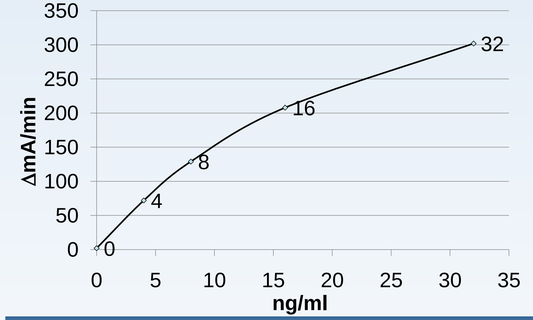
THC metabolic profile

After smoking marijuana the major component in oral fluid is parent drug Δ9-THC , not 9-carboxy-THC (normally less than 1ng/mL in oral fluid) (1,2)



Assay Profile

The assay working range is 0-32ng/mL with the detection limit at 2ng/mL. The cutoff concentration was set at 8ng/mL neat oral fluid (equal to 2ng/mL diluted concentration obtained by the Quantisal™ device).



Cross-reactivity

Commonly abused other drugs or unrelated drugs at a concentration of 20,000ng/mL showed no cross-reactivity or interference with the assay. More importantly, the assay shows broad cross-reactivity with a wide range of cannabinoids.

	Cross Reactivity (%)
Δ9-THC	100
11-hydroxy-THC	60
11-Nor-9 Carboxy THC	90
Cannabinol	40
Cannabidiol	<1

Assay Characteristics

1. Precision:

The precision of the assay at 4, 8, 12 and 16ng/mL was less than 20% CV.

ng/mL (n=15)	*SD*	*Average*	*%CV*
4	*0.8*	*3.4*	*18.8*
8 (cutoff)	*1.5*	*8.9*	*17.2*
12	*1.6*	*14.2*	*11.2*
16	*2.6*	*18.8*	*13.7*

2. Accuracy

The assay was further challenged with oral fluid specimens previously confirmed by GC-MS. The results indicated that the newly developed THC HEIA assay correlated well with both GC-MS and ELISA results as showed in the following table.

	GC/MS		*ELISA*	
HEIA	*+*	*-*	*+*	*-*
+	*34*	*4*	*33*	*2*
-	*3*	*36*	*4*	*38*

Agreement with GC/MS:
Sensitivity: 34/(34+3) = 92%
Specificity: 36/(34+4) = 90 %

Summary

The described assay is precise, specific and sensitive, and is suitable for the screening of oral fluid specimens collected with the Quantisal™ device at a cut-off concentration of 8ng/mL of Δ⁹-THC. The Quantisal device allows THC to be recovered from the collection pad in excess of 80% (3).

In addition, this assay is compatible with most commercial chemistry analyzers as only 20µl of diluted oral fluid sample is needed.

The detection limit is 2ng/mL. Further improvement of this assay to achieve 4ng/mL as the cutoff concentration is in progress.

References

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2. *C. Moore, W. Ross, C. Coulter, et al. Detection of the marijuana metabolite 11-nor-delta-9-tetrahydrocannabinol-9-carboxy acid in oral fluid specimens and its contribution to positive results in screening assays, J. Anal. Toxicol. 30: 413-418 (2006)*
3. *O. Quintela, D. J. Crouch, D. Andrenyak, Recovery of drugs from Immunalysis Quantisal oral fluid collection device. J Anal Toxicol 30: 614-616 (2006).*

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