A Rapid Instrumented Fluorescence Immunoassay for the Detection of Tetrahydrocannabinols

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Keywords: Marijuana; saliva; fluorescence immunoassay; drugs of abuse; immunosensor.

Abstract

A rapid instrumented fluorescence immunoassay for the detection and quantitation of tetrahydrocarnnabinols (THC) in oral fluids has been developed for use in testing for marijuana abuse. The lower detection limits for (*l*)-Δ9-THC and (*l*)9-Carboxy-11-nor-Δ9-THC was found to be 1.5 ng/mL and 5.5 ng/mL, respectively. Having a total assay reaction time of less than 10 minutes, the assay is ideally suited for use in point-of-care testing of saliva THC by law enforcement and in the workplace.

Introduction

Our laboratory is currently developing a unique product that will provide immediate, on-site assay results for drugs of abuse in oral fluids. The assay method is based on the flow immunosensor technology that is licensed from the Department of the United States Navy[1]. This technology, when used in conjunction with LifePoint's patented technology[2] for oral fluid collection allows for the development of an instrument-based system that will have broad application for non-invasive, on-site diagnostics. The use of oral fluids as the specimen of choice for drugs of abuse testing offers a number of advantages over other bodily fluids, particularly in that it can be used to detect the presence of intact tetrahydrocannabinol (THC) and cocaine. The detection of these intact drugs in oral fluid can be used to provide current status information on the abused drug[3].

Materials and Methods

All chemicals except those specified are from Sigma Chemical Company

Assay principle

The Laminar Flow Fluorescence Immunoassay utilizes an antibody-fluorescent labeled tracer complex in which the antibody is bound to the solid phase through a covalent bond. The resulting reagent is packed into a microcolumn in each assay channel of the Laminar Flow Fluorescence Immunoassay Instrument. The tracer is an analyte (THC) labeled with a cyanine

dye, Cy5. The affinity of the antibody for the tracer is equal to or lower than that for the analyte. When a sample solution containing the analyte passes through the assay reagent in the micro column, the analyte displaces the tracer at the binding site of the antibody. The displacement of flourophore-labeled THC from the immobilized antibody by saliva specimens containing THC produces a fluorescent signal. The fluorescence intensity of the solution passing the column is proportional to the concentration of the analtye in the sample solution and is read in the detection cell of the fluorescence instrument.

Preparation of Reagent for Laminar Flow Fluorescence Immunoassay

4 mM of sodium periodate was added to a 50/50 suspension of Sephacryl 1000 beads (Pharmacia 17-0476) in 0.001M sodium acetate buffer, pH 4.4. After 20 minutes incubation at room temperature while roller-mixing the suspension, the oxidized resin was filtered and washed with 10 volumes of deionized water, 3 times. THC monoclonal antibody (1um) in 1M sodium bicarbonate buffer, pH 9.5 was added to the oxidized resin followed by the addition of 5mM ascorbic acid to the mixture. The reaction was allowed to proceed overnight at ambient temperature while roller-mixing. Equal volume of blocking buffer, 1M sodium bicarbonate buffer containing 0.1M lysine, and 5mM of ascorbic acid was added to the suspension. Roller-mixing was continued at room temperature for another 4 hours. The resin was washed 10 times with 10 resin volumes of 0.01M sodium phosphate buffered saline (PBS), pH 7.3 containing 0.05% Tween 20. The suspension of antibody-coupled Sephacryl beads was filtered 1 time and one resin volume of 1uM Cy5 labeled THC (tracer) was added to the resin. The suspension was roller-mixed at room temperature for 2 hours before it was drained and washed with 15 volumes of PBS. The washed resin was re-suspended in an equal volume of PBS containing 0.2% bovine serum albumin and 0.05% sodium azide.

Reagent assay resin (28ul) was filled into each micro polystyrene column with an inner diameter of 2mm and length of 10mm. Each column was then fitted into one of the five flow channels of the Laminar Fluorescent Immunoassay Instrument (LifePoint, Inc.).

Sample Preparation

Equal volumes of saliva of 11 healthy volunteers were pooled. The pooled saliva was centrifuged at 1500g for 15 minutes and supernatant was recovered. (*l*)9-Carboxy-11-nor-delta-9-THC (Altech 01422) or (*l*)-delta-9-THC (Research Triangle Institute 5754-51d) was spiked into the pooled normal human saliva to provide the appropriate concentration of drug for testing.

Laminar Flow Fluorescence Immunoassay

Five saliva samples were assayed simultaneously using five separate flow channels. The assay process including sampling and post-washing was controlled by an automatic system supported by Labview software (National Instruments, Inc). Saliva sample (250ul) was applied to each channel at a flow rate of 50uL/minute followed by 150uL of PBS containing 0.2% BSA and 0.05% NaN_3 at the flow rate of 150uL/minute. The average intensity of the fluorescence signal was used to determine the drug concentration in the sample.

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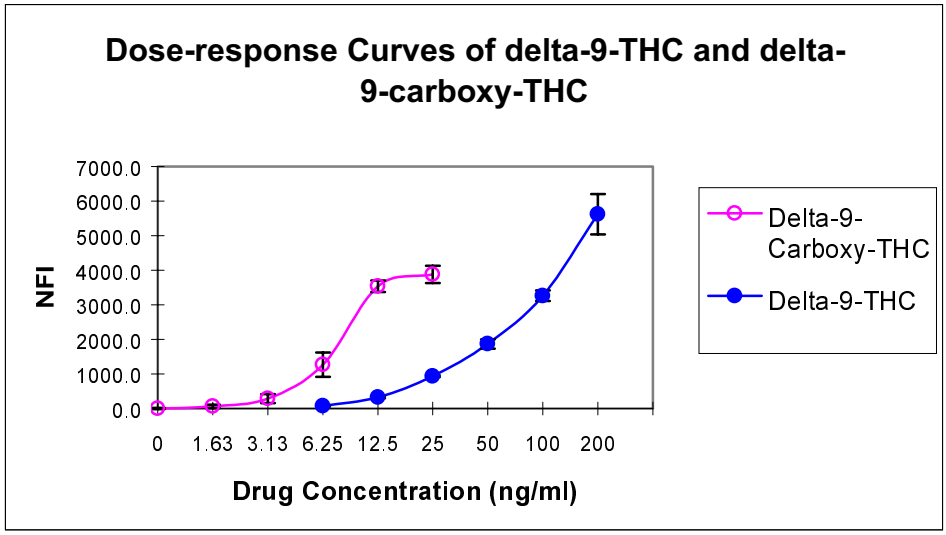
The cross-reactivity of the THC assay to cocaine, phencyclidine, morphine, amphetamine, methamphetamine, as well as ethanol was tested using saliva specimens spiked with each of the these analytes.

Results

Dose-Response curves of the THC Assay

The dose-response curves for (*l*)-Δ9-THC and (*l*)9-Carboxy-11-nor-Δ9-THC are shown in Figure I. The net fluorescence intensity (NFI) is the intensity obtained using a positive drug sample minus the fluorescence intensity of the zero-drug specimen, which is the average fluorescence intensity of twelve replicates of the zero-drug sample.

Figure I. Dose-response curves for (*l*)-Δ9-THC and (*l*)9-Carboxy-11-nor-Δ9-THC



Limit of Detection for (*l*)-Δ9-THC and (*l*)9-Carboxy-11-nor-Δ9-THC

Using the dose-response curves and the value of 3 times the standard deviation of twelve zero-drug specimens it was found that the limit of detection (LOD) for (*l*)-Δ9-THC and (*l*)9-Carboxy-11-nor-Δ9-THC is 5.5 ng/mL and 1.5 ng/mL, respectively.

Cross-reactivity

Saliva samples spiked separately with cocaine, amphetamine, phencyclidine, morphine, codeine, methamphetamine and ethanol were each tested as unknown samples and the degree of cross-reactivity of each was calculated by dividing the equivalent THC concentration (derived from the (*l*)-Δ9-THC dose-response curve) by the concentration of the cross-reactant tested (Table 1). The results show that there is no significant cross reactivity when any of these analytes is tested in the THC assay.

Substance	Concentration Tested (ng/ml)	Cross-Reactivity
cocaine	5000	<0.1%
morphine	5000	<0.1%
amphetamine	5000	<0.1%
methamphetamine	5000	0.12%
phencyclidine	2000	<0.3%
codeine	2000	<0.3%
ethanol	0.5%	<0.12%

Table 1. Cross-reactivity test results.

Discussion

Currently, the predominant rapid test assay format for drugs of abuse screening uses lateral flow membrane technology with urine as a specimen. Many such membrane chromatographic assays are commercially available for THC. Most of these tests have a limit of detection for □9-carboxy-THC of 50ng/mL. Study conducted in our laboratory showed that the sensitivity of these tests for □9-THC is very poor. The laminar flow fluorescence immunoassay represents an improvement over these methods with an LOD of 5.5 ng/mL and 1.5 ng/mL for □9-THC and □9-carboxy-THC, respectively and provides a means for the rapid detection of THC in saliva samples. Furthermore, the instrumented fluorescence immunoassay system in development at LifePoint will detect and provide quantitative results for cocaine, THC, opiates, PCP and amphetamines simultaneously in a saliva sample. Such a system will be ideal for use in law enforcement and work place testing for drugs of abuse.

References

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