



## Detection of $\Delta$ 9-tetrahydrocannabinol ( $\Delta$ 9-THC) in human urine by Solid Phase Extraction and HPLC.

Chetna Mittal, PhD, Asha Oroskar, PhD, , Anil Oroskar, PhD  
Orochem Technologies Inc. Lombard, IL, 60148

### Abstract

A rapid method for the simultaneous confirmatory analysis of forensically relevant cannabinoids, Delta(9)-tetrahydrocannabinol 9 THC by means of high-performance liquid chromatography in human urine samples is developed . Sample clean-up was performed by Orochem's anionmixed mode solid-phase extraction and the HPLC separation was carried out using a Reliasil Phenyl column (150 x 4.6 mm). Quantitation was achieved by the addition of internal standard in urine. Recoveries for 9-THC from Urine samples is 75-80%.

### Introduction

For "Work place drug testing", "Driving under influence of drugs" and for Forensic cases, urine samples are analyzed for 9 TCH.  $\Delta$  9-Tetrahydrocannabinolic acid A (  $\Delta$  9-THCA-A) is the precursor of  $\Delta$  9-tetrahydrocannabinol (  $\Delta$  9-THC) in hemp plants. The ability to concentrate Delta-9 THC, ( $\pm$ )-11-nor-9-Carboxy- $\Delta$ 9-THC (Racemic mixture) and ( $\pm$ )-11-Hydroxy- $\Delta$ 9-THC are explored here as to whether the Orochem Mixed mode anion exchange was an effective single sorbent. We found that the Orpheus mixed anion exchange showed broad selectivity and very good capacity for each of the three varieties. For HPLC-analysis, urine samples were prepared by solid-phase extraction. Orochem's anion mixed mode (200mg 3cc) columns were used for SPE. HPLC analysis was performed using Reliasil Phenyl column and Mobile phase with acetonitrile and water (60:40).

### Chemicals and materials

9-THC reference standard was obtained from Cerilliant Inc.(USA). All other chemicals were of the highest analytical grade and were obtained from Sigma. De ionized water was purchased from VWR. Solid-phase extraction (SPE) columns (Orochem's C8-NH<sub>2</sub> mixed mode,200mg 3cc) were used for extraction from Urine samples.

### Sample preparation

At the beginning of the sample preparation process, serial dilutions of standard solution of 9-THC were prepared in deionized water then aliquots of 30  $\mu$ l internal standard (IS) solution (30  $\mu$ g/ml of 9-THC) was added to each sample (1 ml of urine). After addition of 1 ml 10mM Sodium Hydroxide and vortex-mixing for 30 s, the samples were kept for 15 minutes at 60° C for urine hydrolysis. Then 1 ml of glacial acetic acid is added to each sample and transferred to SPE columns, which had been preconditioned as follows: 2 ml of methanol and of then 2 ml of 0.1 M acetic acid each at a flow rate of 2 ml/min. The samples were then applied to the SPE columns slowly and rinsed through the sorbent bed at a flow rate of 1 ml/min. The SPE columns were washed with 1 ml 0.1 M acetic acid first and acetonitrile/water (40 : 60, v : v) each at a flow rate of 1 ml/min . Elution was then performed with 1.0 ml acetonitrile at a flow rate of 1 ml/min.

### Sample preparation (cont'd)

The extracts were evaporated to dryness at 40 °C under a stream of nitrogen using Orochem's Evaporator. For HPLC analysis, the residue was reconstituted in 200 µl of the LC mobile phase (solvent Acetonitrile: Water, 60: 40, v: v), and 25 µl were injected into the HPLC

Detection of the THC was performed at 40 °C on a Reliasil 5 µ Phenyl column (150 × 4.5mm) (Orochem Technologies Inc., USA) Using Mobile phase of acetonitrile (solvent A) and Water. The flow rate was 1.00 ml/min and wavelength was 220nm.

### Results and Conclusion-

The above data shows the effective use of anion mixed mode SPE, with HPLC detection for the extraction and quantification of THC from Urine samples at higher concentration. SPE methods can be used effectively for sample cleanup and concentrating the sample up to desired range by evaporation ( Orochem's Evaporator) before HPLC testing. Very low concentration of 9THC and its metabolites can be detected by GC/MS with the Orochem Phenyl HPLC column proving to be highly effective in showing selectivity for all of the compounds. Future work is to be conducted to devise a single method to simultaneously demonstrate the identification of each of the compounds under the same set of SPE, and HPLC conditions.

## Protocol for Extraction

Condition / Sample Transfer Sequence							
Volume (ul)	Vacuum Levels, Vacuum Duration (inches Hg), (seconds)				(Step #)	Fluid	Reagent (R) / Line Flush (LF)
	1	2	3	LLD			
3000	2.0,40	2.2,15	0.0,0	On	(1)	Methanol	(R)
3000	2.7,65	2.8,20	0.0,0	Off	(2)	0.1 M Acetic Acid	(R)
3000	2.7,70	2.8,25	0.0,0	On	(3)	SAMPLE TRANSFER	
Wash I Sequence							
Volume (ul)	Vacuum Levels, Vacuum Duration (inches Hg), (seconds)				(Step #)	Fluid	Reagent (R) / Line Flush (LF)
	1	2	3	LLD			
3000	2.7,60	2.8,10	0.0,0	Off	(1)	0.1 M Acetic Acid	(R)
3000	2.8,60	2.9,30	0.0,0	Off	(2)	Acetonitrile:DI Water 40:60	(R)
Elution I Sequence							
Volume (ul)	Vacuum Levels, Vacuum Duration (inches Hg), (seconds)				(Step #)	Fluid	Reagent (R) / Line Flush (LF)
	1	2	3	LLD			
1000	2.0,45	2.3,30	0.0,0	Off	(1)	Acetonitrile	(R)

### Column Engineering and Orochem Technologies Inc.,

331, S. Eisenhower Lane, Lombard, IL 60148  
Telephone: 630-916-0225 Fax: 630-916-0250

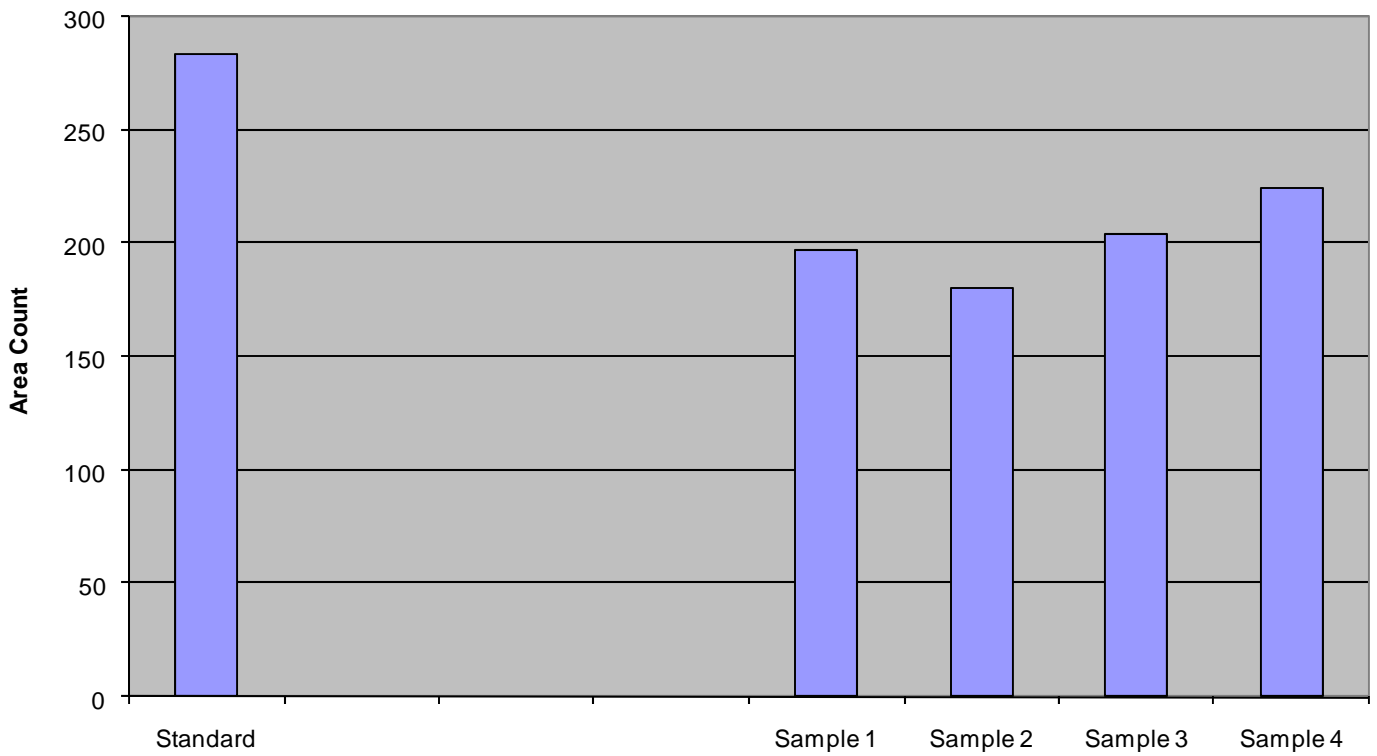
### Orochem India Pvt Ltd.,

Plot 304 TTC Rabale, MIDC, NaviMumbai—400701 India  
Telephone: +91-22-27603570/69 Fax: +91-22-2-7603571

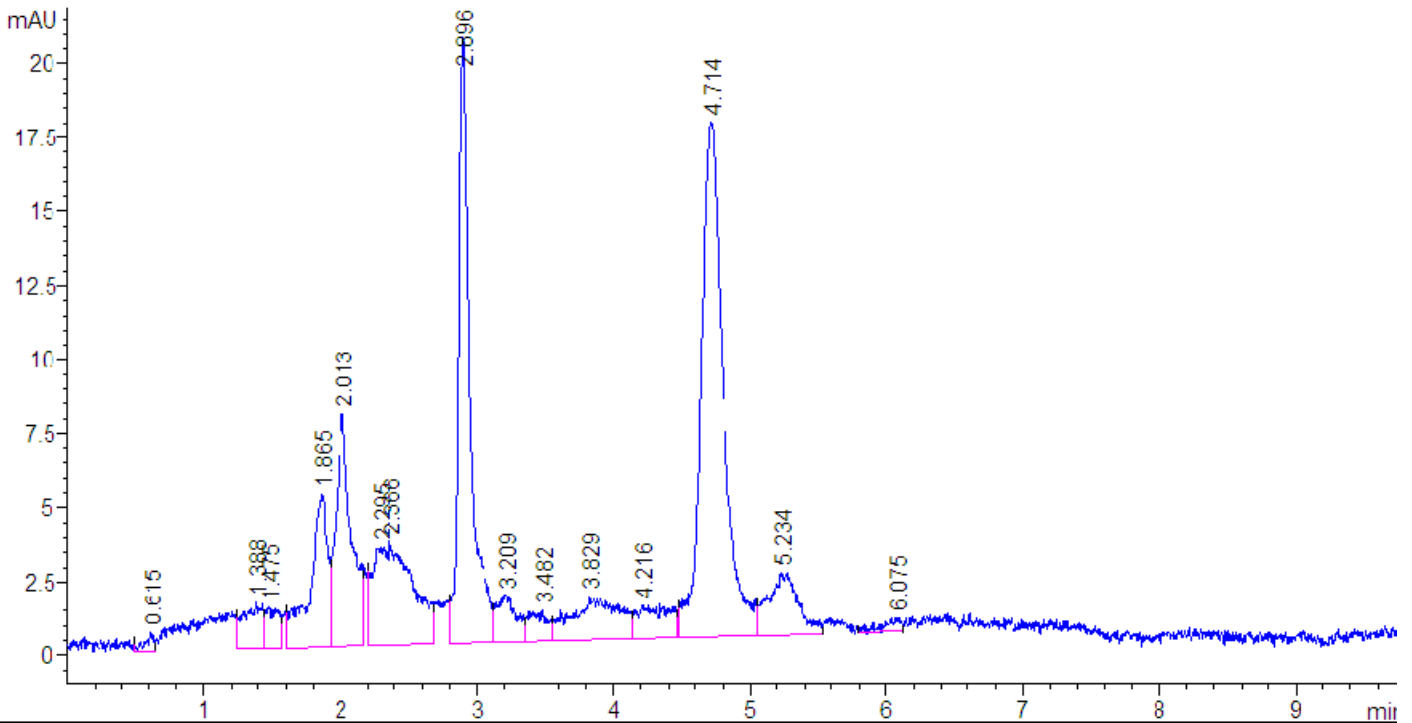
Email: sales@orochem.com www.orochem.com

# THC-COOH

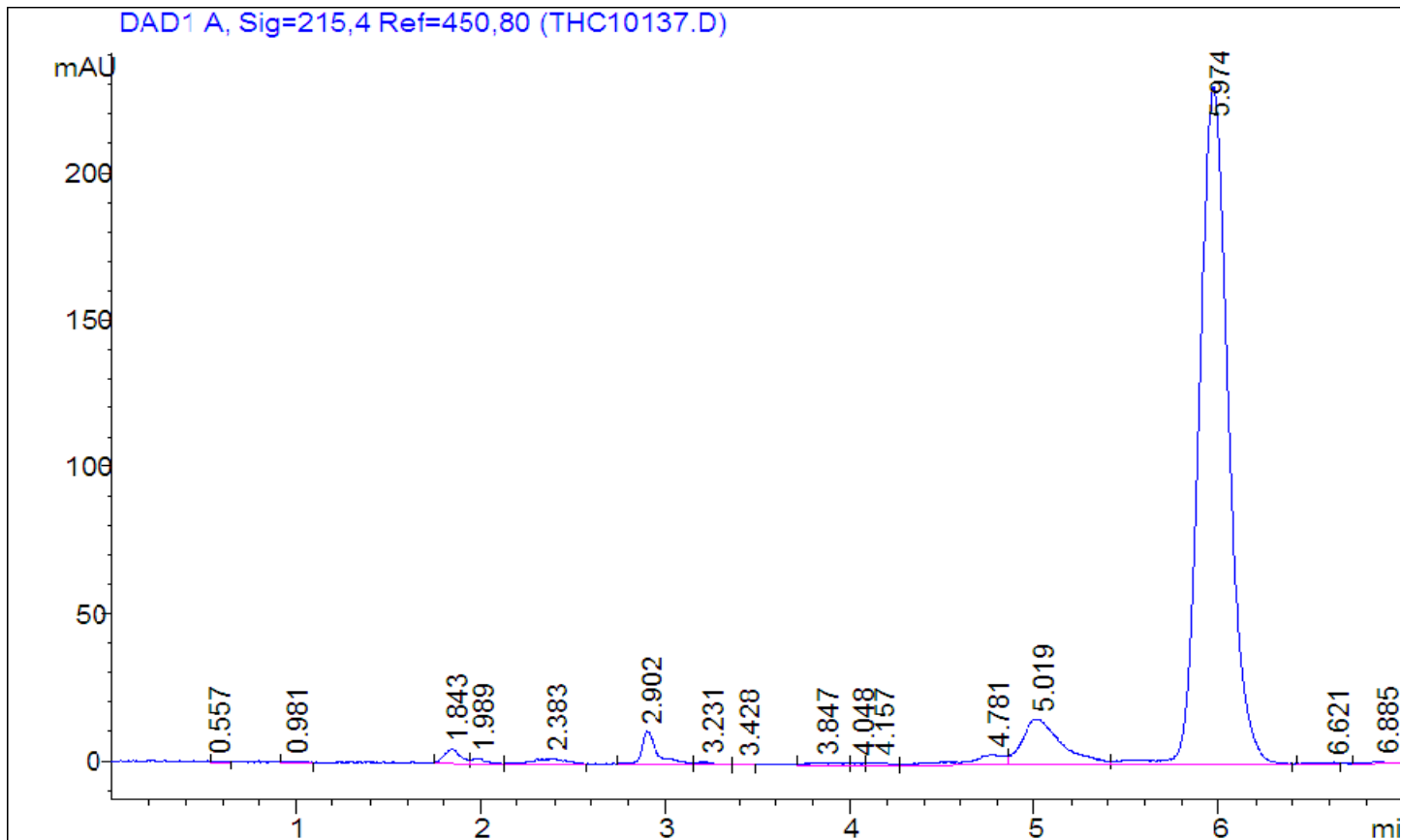
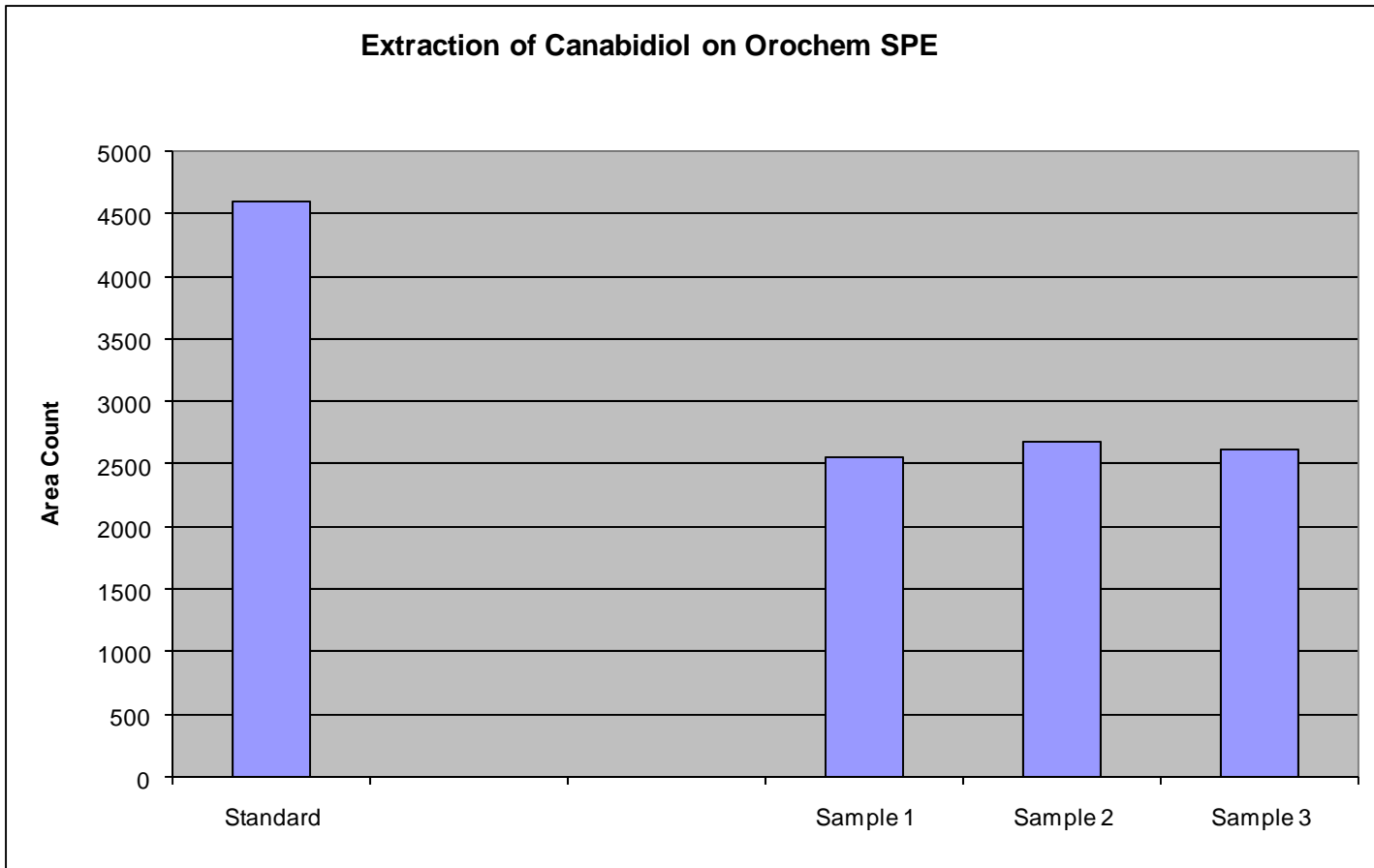
## Extraction of 11-Nor-9 Carboxy-Delta-9THC



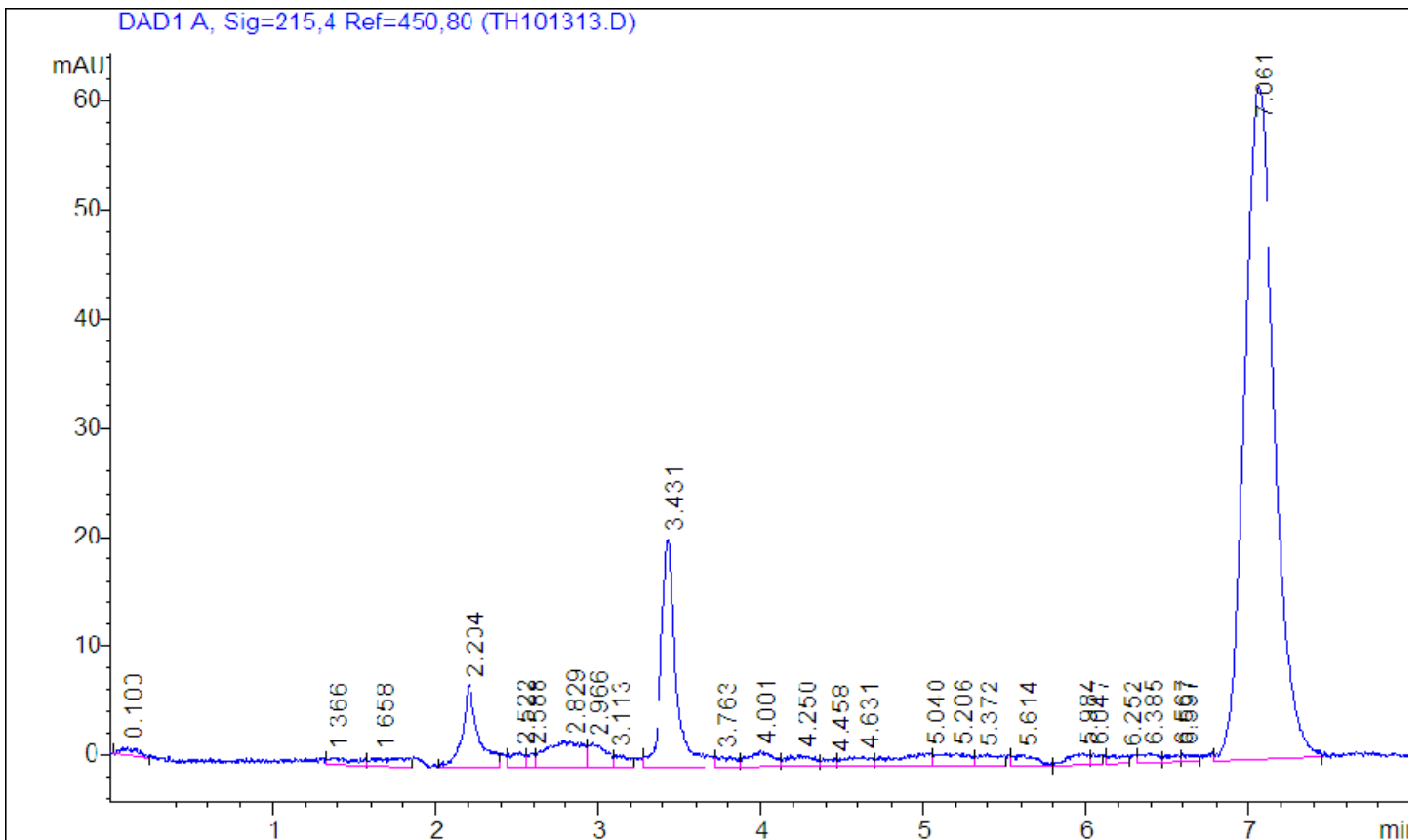
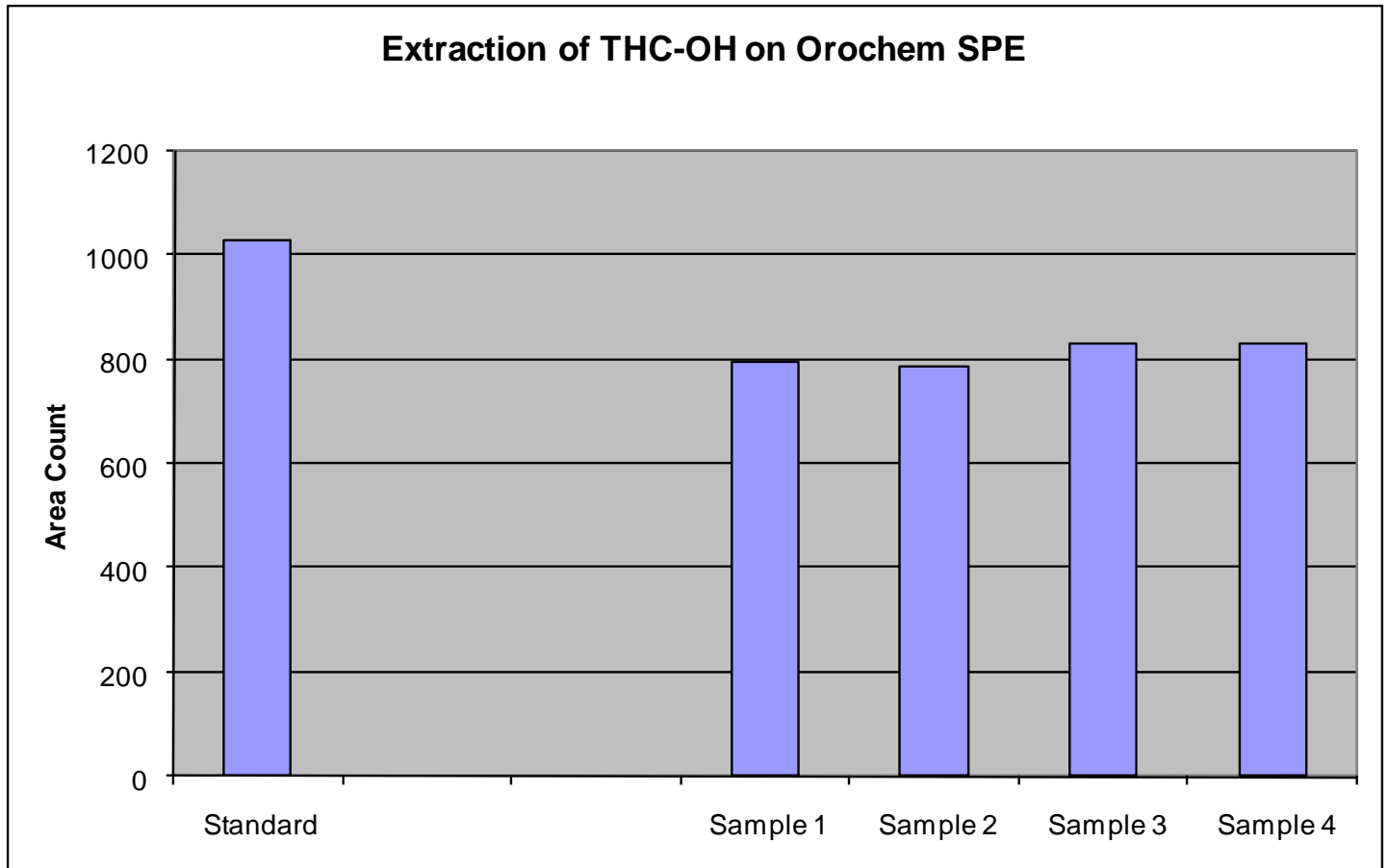
DAD1 A, Sig=215,4 Ref=450,80 (THC10\*33.D)



# Canabidiol

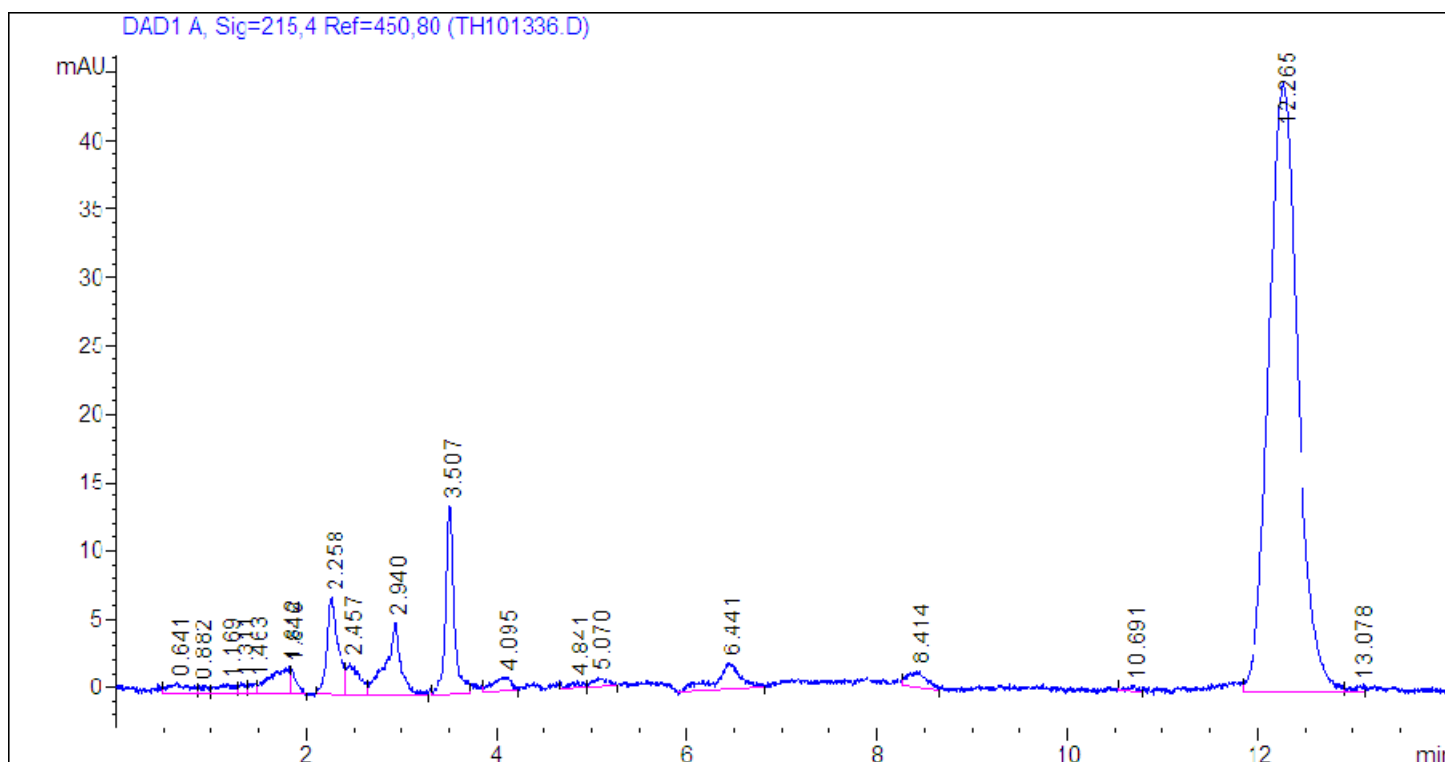


# THC-OH



# Cross-Talk Analysis

## Sample THC-COOH



## THC-COOH Blank

