K46 The Detection of 11-nor-Δ9-THC9-Carboxylic Acid (THC-COOH) in Hair and Urine

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After attending this presentation, attendees will understand how to analyze 11-nor-Δ^9-THC-9-carboxylic acid (THC-COOH) in hair using a two dimensional gas chromatographic system coupled to a single quadropole mass selective detector

The detection of marijuana in hair at meaningful concentrations has currently been limited to analysis using triple quadrupole mass analyzers. This presentation will impact the forensic community and/or humanity by describing the application of two dimensional gas chromatography to a toxicological problem, allowing the analysis of drugs and metabolites at extremely low levels. The modifications can be applied for many different applications in toxicology

Methods: Tetrahydrocannabinol (THC) is the active ingredient in marijuana and is generally administered orally or by smoking, resulting in euphoria and hallucinations. Since its main metabolite, 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), is acidic, its incorporation into the hair shaft is not as extensive as that of more basic drugs such as cocaine or methamphetamine. Hence, the detection of marijuana metabolite, THC-COOH, in hair is extremely difficult, due to the very low levels incorporated and the sensitivity of detection. Even though the use of two-dimensional chromatography has been applied for many years in the oil and petroleum industry, its application to forensic toxicological problems was first described in 2003, and coupling to mass spectrometry for the detection of drugs of abuse was reported for the first time in 2004. The approach to the problem of inadequate detection levels using a single stage quadrupole mass spectrometer was to make sufficient small improvements over the entire assay, so that the final required detection limit could be routinely achieved.

Gas Chromatography - Two dimensional GC: The application of a prior separating column to the assay allowed the background associated with the hair extract to be spread out over a longer time frame. Once the analyte retention time on the first column had been determined, the pressure switch (Dean's switch) was turned on to divert the flow, and turned off 0.4 minutes later. This created a narrow "window" of the effluent from the first column containing the analyte to be passed to the analytical column with minimal background. The second analytical column was of a different polarity than the first and provided a further separation of the analyte from potential interferences.

Cryogenic Focusing: The fraction from the first column was selectively transferred to the analytical column where a cryogenic trap focused the peak of interest. The column was cooled as the analyte entered, effectively "cold-trapping" the drug. The focuser was then heated quickly allowing the peak of interest to advance through the analytical column and enter the mass spectrometer. This resulted in a much sharper chromatographic peak, producing an improved signal to noise ratio.

Mass Spectrometry: Chemical ionization provided a more specific and selective ionization of analytes than electron impact ionization, by enhancing the signal and lowering noise generated by potential interferences. The greatest potential gains were found in applying electron capture chemical ionization (ECCI) using ammonia as the reagent gas. The low gas pressure provided sufficient fragmentation to allow the monitoring of two ions for the drug and internal standard. The modifications described were necessary to in order to analyze samples at the proposed Federal guideline cut-off of 0.05 pg/mg.

Our study enrolled 156 subjects, all of whom admitted to recent marijuana use. Each subject provided a urine sample and a hair specimen taken from the head at the time of interview. Information on drug use, including time of last use, frequency of use, ethnicity, age, sex and hair color were recorded for each subject. Hair samples were analyzed at Immunalysis Corporation; urine samples were analyzed by a reference laboratory.

Results: Of the 156 specimens collected, 46 (29%) of the samples were positive using hair, urine or both. Eight (5.1%) were positive using urine only, nine (5.7%) were positive via hair only, twenty-eight (17.9%) were positive in both matrices, and the remaining 71% were negative. One sample (# 50) had no data for the urinalysis.

The frequency of use reported by the subjects ranged from as high as 10 times per day (subjects 50 and 151) to as infrequently as 3 times per week (subjects 19, 58, 134, 152 and 154). Overall, there appeared to be very little correlation between the self-reported use of marijuana and the concentrations detected in hair or urine.

Summary: The detection of THC-COOH in hair can be achieved at a similar positivity rate to urine when a low enough detection limit is used. Using a modified GC/MS system, THC-COOH was identified in hair at the level of 0.05 pg/mg, as mandated in the proposed Federal guidelines.

 ** Presenting Author*

Sample ID	*Hair result (pg/mg)*	*Urine result (ng/mL)*
1	**Negative**	129
2	0.42	352
4	0.41	427
10	0.29	**Negative**
17	1.22	598
18	2.51	405
19	**Negative**	135
24	0.12	**Negative**
25	0.18	262
26	0.45	181
29	0.16	**Negative**
30	0.95	522
35	0.13	**Negative**
37	0.88	634
39	0.1	**Negative**
49	0.18	312
50	0.89	Data unavailable
51	0.62	407
57	0.24	489
58	**Negative**	297
65	**Negative**	307
69	0.68	613
72	0.49	505
79	0.45	**Negative**
81	0.12	**Negative**
96	**Negative**	465
97	1.12	592
99	0.11	**Negative**
100	0.52	397
103	1.04	686
109	0.15	500
110	0.44	299
114	**Negative**	136
122	0.99	530
125	**Negative**	463
126	**Negative**	200
129	0.56	810
130	0.59	772
131	0.3	472
134	0.2	**Negative**
138	1.08	621
139	0.23	70
146	0.35	277
151	0.95	586
152	0.22	149
154	1.16	234

THCA, Two-Dimensional GC, Hair Analysis

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