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Determining Cannabinoids in Blood Using Electron Capture Negative Chemical Ionization with the 5973 CI MSD

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Application Brief

A 1995 survey of illicit drug users indicated that marijuana was used by 77 percent of Americans age 12 and older, making it the most frequently abused drug. It was then estimated that 4.7% of the American population—9.8 million people—were current cannabis abusers.[1] Moreover, the use of marijuana by youths aged 12 to 17 has more than doubled between 1992 and 1995.

In 1996, California passed the Medical Use of Marijuana Initiative (Proposition 215), which allows using marijuana by prescription for medical conditions; similar legislation is pending in other states. From medical, pharmacological, forensic, and law enforcement perspectives, the analysis of cannabinoids in biological matrices is a subject of considerable and growing interest.

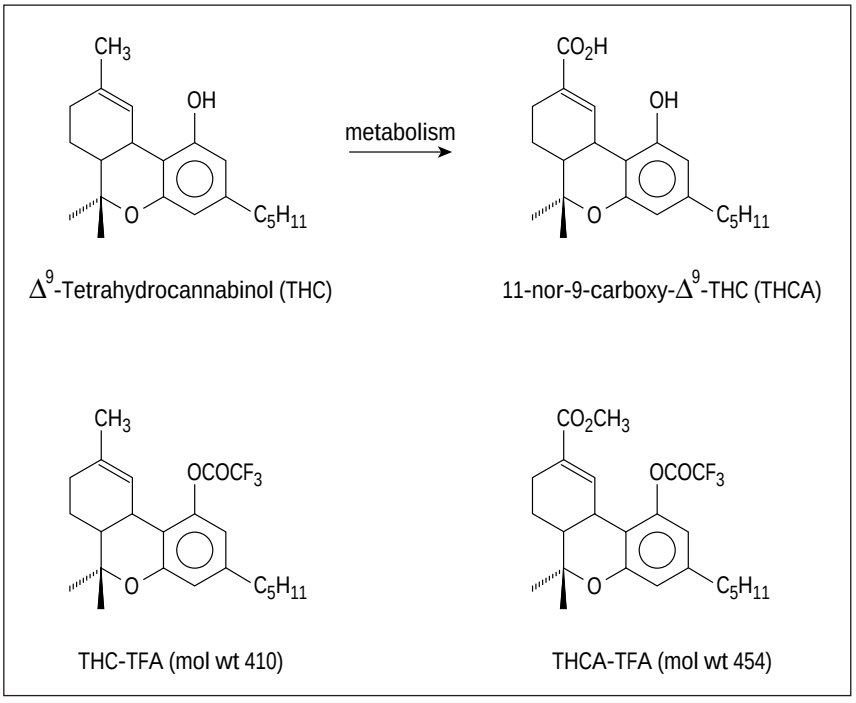

Figure 1. Structures of THC, the major THC metabolite, THCA, and their trifluoroacetyl derivatives.

Chemical ionization provides a less energetic and potentially more selective method for detecting and quantifying analytes in complex matrices than electron impact ionization mass spectrometry. Electron capture negative chemical ionization (ECNCI) exploits the electrophilic (electron-"loving") nature of particular atoms or molecular arrangements in the same way that electron capture detectors have in the past. The advantage is that mass spectrometric data are generated which lend an additional degree of selectivity and certainty to the analysis.

The active agent in marijuana is the Δ^9-tetrahydrocannabinol (THC) which is rapidly metabolized in humans primarily to the 11-*nor*-9-carboxy-Δ^9-tetrahydrocannabinol (THCA). There are several approaches to detecting THC and THCA in human fluids,[2] but the most definitive is GC/MS. In 1983, Foltz, et al.[3] developed a method for extracting THC and THCA from blood and then derivatizing them to produce analytically suitable compounds. In this approach, THC and THCA are extracted from serum samples using solid-phase extraction cartridges. Trideuterated THC

and THCA are added to the serum as recovery surrogates prior to extraction. The extracted THC is converted to the trifluoroacetyl derivative (THC-TFA) using trifluoroacetic anhydride; the THCA is first methylated, then treated with trifluoroacetic anhydride to produce the trifluoroacetyl derivative (THCA-TFA). The structural details are shown in Figure 1.

Recently, Stonebraker et al.[4] have created an elegant automated form of this method that reduces the manual labor involved, accelerates the time-to-analysis, and improves the accuracy, precision and recoveries over the original manual method. The trifluoroacetyl derivatives, THC-TFA and THCA-TFA, have molecular weights of 410 and 454 amu, respectively, and the trideuterated THC recovery surrogates have molecular weights that are 3 amu higher. The ECNCI mass spectra of the surrogates, similar to those of the natives, show that very little fragmentation occurs, a feature typical of NCI (Figure 2). Worth noting is the absence of a CF_3CO_2 fragment from the derivatizing agent in the mass spectra. Such a fragment would appear as a peak at 113 amu and usually indicates difficulties with source inertness or other design or method parameters.

Monitoring the molecular ions of the native and deuterated surrogates provides a sensitive method for determining the presence of THC and THCA at low levels. A typical extracted ion chromatogram is shown in Figure 3, method parameters are provided in Table 1. By injecting standards created in blood, calibration curves were generated for both

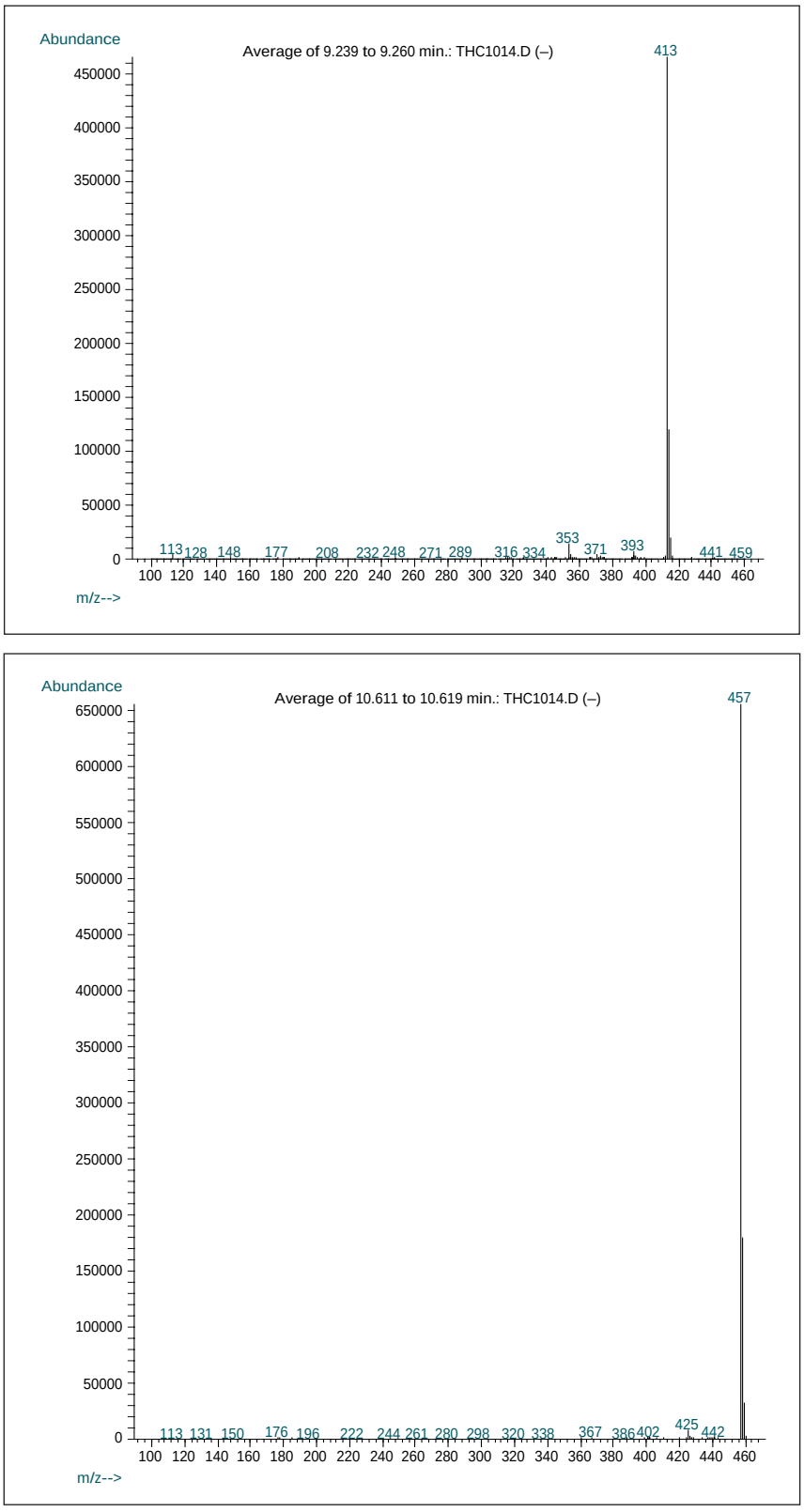

Figure 2. Electron capture negative chemical ionization mass spectra of the trideuterated THC (upper) and THCA (lower) recovery surrogates as their trifluoroacetyl derivatives.

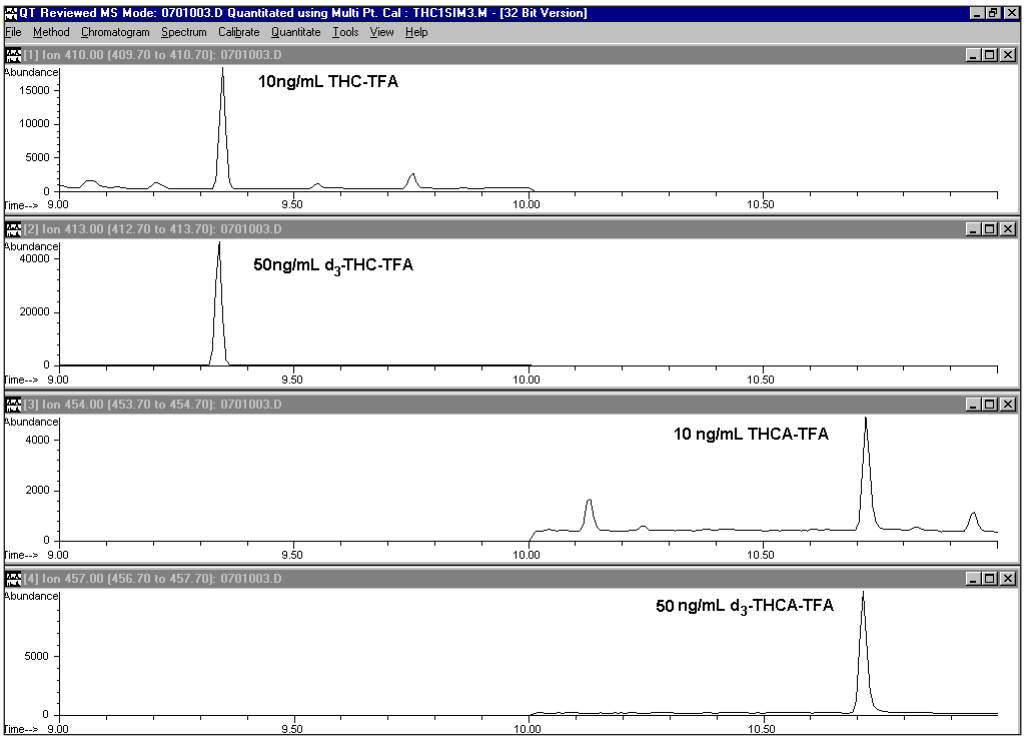

Figure 3. Extracted ion chromatograms of the THC-TFA, d_3-THC-TFA, THCA-TFA, and d_3-THCA-TFA in Blood.

Table 1. GC/MS SIM Parameters

GC PARAMETERS		MS PARAMETERS	
Oven	100°C for 1 min 20°C/min to 290°C Hold for 2.50 min	Source Temperature Quadrupole Temperature	150°C 106°C
Injection	Pulsed Splitless 250°C	Reagent Gas	Methane 2.0 mL/min
Flow Parameters: Helium Carrier Gas with Electronic Pressure Control	25 psi for 1.5 min then 1.2 mL/min or 41 cm/sec	Solvent Delay EM Voltage	8.00 min 1800 Volts
GC Column	HP 5MS 30 m, 0.25 mm diameter 250 micron film	SIM Groups	0.6 amu resolution 200 msec dwell
Transfer Line	295°C	THC / d_3 ions	410.0 amu 413 amu
		THCA / d_3 ions	454.0 amu 457.0 amu

THC and THCA using the deuterated surrogates as internal standards, each at 50 ng/mL (Figure 4). The curves are very linear over the range of concentrations from 0.5 ng/mL to 50 ng/mL for both THC-TFA and THCA-TFA.

Electron capture negative chemical ionization produces minimal response from matrix interferences and, when combined with the high sensitivity and reproducibility of the 5973 CI MSD, an approach to determining THC and THCA in blood with detection limits below 0.5 ng/mL.

If the automated methodology for the determination of THC and THCA in blood described here produces analytical results superior to earlier approaches, performing it with the 5973 CI MSD adds still another level of analytical refinement. Its impressive NCI sensitivity and reproducibility makes it possible to reliably determine THC and THCA at detection limits of better than 0.5 ng/mL.

Acknowledgments

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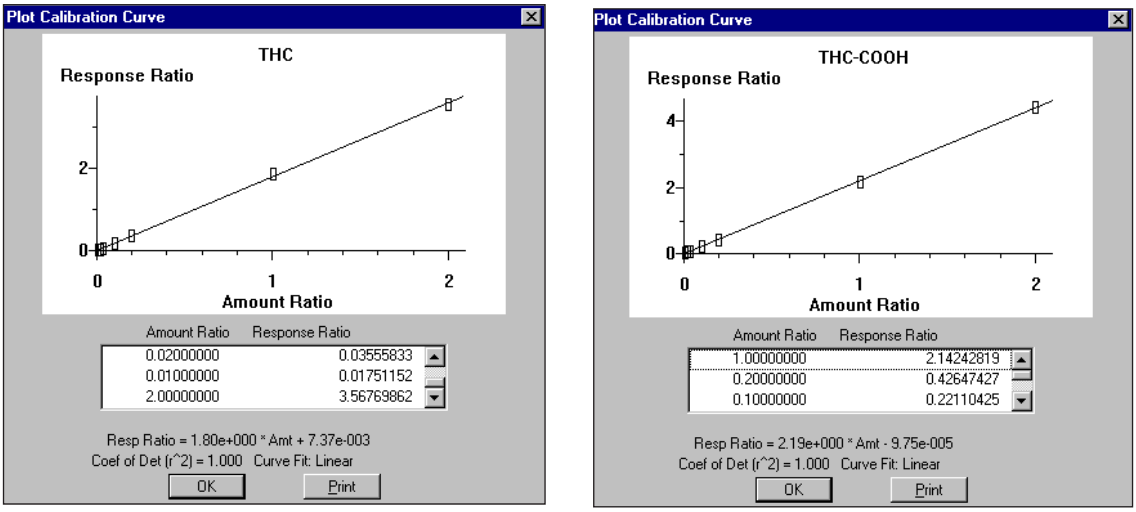

Figure 4. Calibration plots for THC-TFA and THCA-TFA concentrations from 0.50 ng/mL to 50 ng/mL. Note R^2 = 1.000 for both compound calibration curves.

References

1. Unknown, *National Household Survey on Drug Abuse, Advance Report #18*. **1997**, SAMSA, Office of Applied Studies, http://www.samhsa.gov/oas/nhsda/nhsdafls.htm.

2. R. L. Hawks, *The Analysis of cannabinoids in biological fluids*. **1982**, Rockville, Md. [Washington, D.C., Dept. of Health and Human Services, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute on Drug Abuse, Supt. of Docs., U.S. G.P.O., distributor].

3. R. Foltz, K. McGinnis, and D. Chinn, *Quantitative Measurement of delta-9-Tetrahydro-cannabinol and two major metabolites in physiological specimens using capillary gas chromatography negative ion chemical ionization as spectrometry*. Biomedical Mass Spectrometry, **1983**. 10(5): p. 316–323.

4. W. E. Stonebraker, T. C. Lamoreaux, M. Bebault, S. A. Rasmussen, B. R. Jepson, and B. K. Beck, *Robotic solid phase extraction and GC/MS analysis of THC in Blood*. American Clinical Laboratory, 1998. **In press.**

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