Confirmation of THC in Oral Fluids Using High-Resolution 2-D GC/MS

Application Note

Forensic Toxicology

Authors

Dean F. Fritch, Ph.D., DABFT, DABCC-TC
Orasure Technologies, Inc.
150 Webster Street
Bethlehem, PA 18015
USA

Bruce D. Quimby, Ph.D.
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Oral fluids are being used as an alternative matrix to urine for drug testing. Oral fluid is considered to be less invasive and much more difficult to adulterate than urine samples. Samples of oral fluid are typically screened by an ELISA immunoassay method. Those found to be positive must be further analyzed with a mass spectrometry confirmation technique.

The confirmation technique must be able to detect the drugs of abuse (DOA) in oral fluids down to concentrations lower then those used in urine testing. For example, tetrahydrocannabinol (THC) must be measured down to 0.5 ng/mL of diluted oral fluid when collected with the Intercept® oral fluid collector. The analysis is complicated by interferences from the complex sample matrix. For these samples, techniques like gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS) and liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) have been used. The higher level of selectivity afforded by a secondary mass spectral step is used to overcome interference problems.

GC/MS has the required sensitivity to confirm THC but lacks the selectivity over matrix interferences. This application describes a two-dimensional (heart cutting) GC/MS system where THC is heartcut from a nonpolar DB-1ms column to a polar DB-17ms column. An air-cooled focusing trap is used to improve resolution and sensitivity. Coupling high-resolution two-dimensional (2-D) GC with a standard benchtop quadrupole gas chromatography/mass selective detector (GC/MSD) provides the required selectivity and sensitivity for THC confirmations.

Introduction

GC/MS with a quadrupole mass spectrometer is a widely used analytical technique. The selectivity, sensitivity, cost effectiveness, and ability to use library searching for identification have made this the instrument of choice for many years. There are some types of samples, however, where matrix interferences prevent sucessful analysis of the desired analytes. For these samples, techniques like GC/MS/MS and LC/MS/MS have been used. The higher level of selectivity afforded by a secondary mass spectral step is used to overcome interference problems. For many analyses, especially those with a limited number of analytes, the use of a two-dimensional (heart cutting) GC with a standard quadrupole MS can be a simpler and less expensive alternative.

This application describes a two-dimensional (heart cutting) GC/MS system. The instrument configuration is a standard quadrupole GC/MS

system to which a Deans switch and air-cooled focusing trap have been added. The first GC column is typically a nonpolar DB-1ms and the second column a polar DB-17ms. Upon injection into the GC, the analytes separate on the first column. The Deans switch is time programmed to heart cut the elution time range of the analyte(s) from the first column onto the second column, where they are focused by the air-cooled trap. Upon thermal desorption in the second column, the analytes are further separated from the matrix compounds that co-eluted with them on the first column. The focusing trap is used to improve both resolution and sensitivity. The two-dimensional (2-D) GC separation is used in place of a secondary mass spectrometric operation. At the end of analyte elution, the carrier gas in the column can be reversed to backflush unwanted heavy sample components out the split vent in the inlet. This saves analysis time and reduces the need for column trimming and replacement. Since only a small portion of the injected sample enters the MS ion source, source cleaning is reduced as well. For limited numbers of analytes (typically five or fewer), high-resolution 2-D GC/MS can be a suitable alternative to MS/MS techniques.

The detection of drugs of abuse in oral fluids serves as a good example of where high-resolution 2-D GC/MS can be used.

Oral fluid is increasingly being used as an alternative matrix to urine in testing for recent drug exposure and impairment. The technique offers several advantages, including ease of collection, minimization of adulteration, and lowering costs for collections, scheduling, and lost time.

One challenge presented by oral fluid testing is in the confirmation of positive screen results. For example, confirmation is required down to 0.5 ng/mL of THC in oral fluid. The determination of THC at this level is complicated by interferences from nondrug compounds in the matrix that chromatographically overlap with analytes and contain ions with the same m/z values. Due to this problem, techniques such as LC/MS/MS and GC/MS/MS are often used for confirmation. This application demonstrates that high-resolution 2-D GC/MS can be used to analyze for THC in oral fluids. The extremely high chromatographic resolution afforded by the 2-D approach resolves matrix interferences from the THC. This results in detection levels comparable to MS/MS techniques.

Experimental

GC/MSD Configuration

The GC/MSD configuration used is shown in Figure 1. The system comprises:

G1540N	6890N Network GC System with options:
	652 Oral fluids analysis kit (includes Cryo-Trap, PCM and 15M DB-1MS, 15M DB-17MS columns)
	112 Split/splitless with EPC (112)
	201 MSD interface (201)
	211 Capillary FID with EPC (211)
	888 Microfluids dean switch
	002 (240 V fast oven power supply) or 003 (198 to 231 V fast oven power supply)
G3243A	5975B inert MSD/DS perf turbo EI bundle
G3397A	Ion gauge/controller for use with 5975 MSD
G2913A	7683B Autoinjector module
G2614A	7683 Autosampler tray module

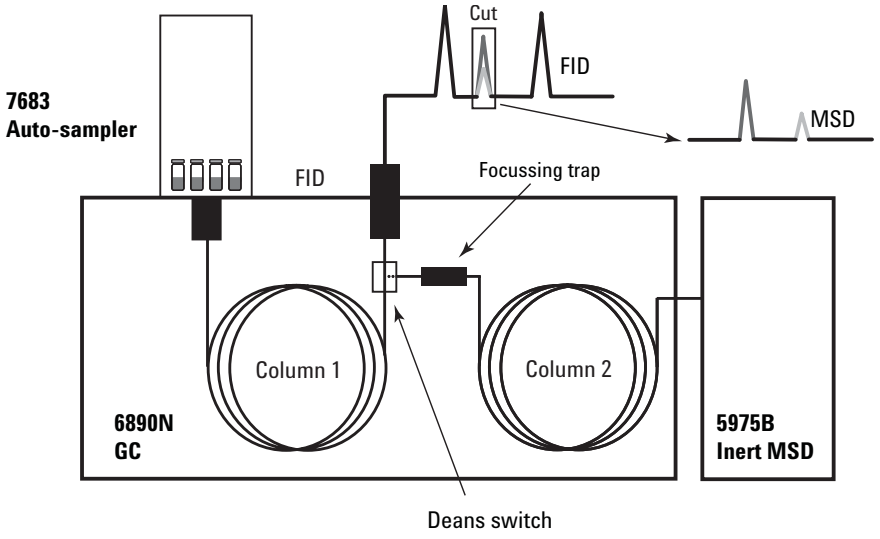

Figure 1. Hardware configuration of 2-D heartcutting GC/MSD system.

The gas chromatograph and mass spectrometer operating conditions are listed in Table 1.

Table 1. Gas Chromatograph and Mass Spectrometer Conditions

GC	
Agilent Technologies 6890N 7683 autoinjector and tray	
Autoinjector	
Sample washes	0
Sample pumps	0
Injection volume (µL)	4
Syringe size (µL)	10
Preinjection solvent A washes	0
Preinjection solvent B washes	0
Post-injection solvent A washes	10
Post-injection solvent B washes	10
Viscosity delay (s)	2
Plunger speed	Slow
Preinjection dwell (min)	0
Post-injection dwell (min)	0
Front Inlet	
Type	EPC split/splitless
Mode	Constant pressure
Inlet temp (°C)	250
Injection type	Pulsed splitless
Pulse pressure (psig)	45
Pulse time (min)	0.5
Purge time (min)	1
Purge flow (mL/min)	50
Pressure, nominal (psig)	26.59
Gas saver	Off
Gas type	Helium
Back Inlet	
Type	PCM/focusing trap
Initial temp (°C)	300
Initial time (min)	5.3
Ramp rate 1 (°C/min)	799
Final temp 1 (°C)	100
Final hold 1 (min)	2
Ramp rate 2 (°C/min)	799
Final temp 2 (°C)	300
Final hold 2 (min)	10
Oven	
Voltage (VAC)	240
Initial oven temp (°C)	130
Initial oven hold (min)	0.5
Ramp rate 1 (°C/min)	35
Final temp 1 (°C)	250
Final hold 1 (min)	0
Ramp rate 2 (°C/min)	10
Final temp 2 (°C)	280
Final hold 2 (min)	2.5
Equilibration time (min)	0.5
Column 1	
Inlet	Split/splitless (front)
Type	DB-1 ms
Agilent part number	122-0112
Length (m)	15
Diameter (mm)	0.25
Film thickness (µm)	0.25
Column 2	
Inlet	Deans switch (back)
Type	DB-17 ms
Agilent part number	122-4712
Length (m)	15
Diameter (mm)	0.25
Film thickness (µm)	0.25
FID	
Temperature (°C)	250
Hydrogen flow (mL/min)	50
Air flow (mL/min)	450
Mode: Constant makeup flow	Constant makeup flow
Nitrogen makeup flow (mL/min)	45
Data rate (Hz)	10
Deans Switch	
FID restrictor length (m)	0.31
FID restrictor id (mm)	0.10
Carrier gas supply	PCM of cryotrap
Deans pressure (psig)	19.60
THC cut time start (min)	6.33
THC cut time end (min)	6.44
MSD	
Agilent technologies	5975B inert MSD
Solvent delay (min)	4
Tune file	Atune.U
Mode	SIM
EM voltage	Atune voltage
Quad temp (°C)	150
Source temp (°C)	230
Transfer line temp (°C)	280
Acquisition mode	SIM
Dwell time (msec)	10
THC-TMS SIM ions	371, 386, 303
THC-D3-TMS SIM ions	374, 389, 303
Post-Run Backflush Conditions	
Post time (min)	3
Oven temperature (°C)	300
Column 1 pressure (psig)	1.0
Column 2 pressure (psig)	65

Oral Fluid Sample Collection and Preparation

Oral fluid samples were collected from 20 volunteers in a drug clinic using the Intercept® oral fluid collector (OraSure Technologies). Collected samples consisted of ~400 µL of saliva diluted with 800 µL of preservative buffer.

Samples were screened for THC using the Intercept Micro-Plate EIA Screen from OraSure Technologies, Inc. (OTI). Samples found positive for THC were prepared as TMS derivatives as described below and analyzed with the 2-D GC/MSD system.

Calibration standards were prepared with the same procedure as oral fluid samples except that Oral Fluid Diluent (OTI) was used to simulate the sample matrix.

Sample preparation for 2-D GC/MSD consists of:

- Dilute 400 µL sample in 4 mL of 50 mM phosphoric acid
- Add deuterated THC internal standard (2 ng/mL)
- Wash column with 500 µL methanol
- Add diluted sample to column (Varian SPEC DAU 30 mg)
- Wash with 2 mL 50/50 (methanol/water)
- Dry 2 minutes
- Elute with 1mL 78/20/2 (methylene chloride/isopropanol/ammonia)
- Dry and derivatize with 25 µL BSTFA+1%TMCS at 70 °C for 15 minutes
- Add 25 µL acetonitrile

Standards of THC and deuterated THC-D3 were purchased from Cerilliant. BSTFA+1%TMCS was purchased from Pierce.

Deans Switch Operation

The Deans switch is a fluidic device used to heart cut peaks from the first column to the second. For analyses involving trace levels of drug analytes detected with a mass spectrometer, severe requirements are placed on components directly in the sample path. These requirements are: absence of any air leaks; inertness of surfaces contacted by samples; minimum dead volume; ease of use; and reliability over time. The Agilent microfluidic Deans switch meets all these requirements. A more detailed description of the device and its application is given in reference 1.

Figure 2a. shows a diagram of the Deans switch with the solenoid valve turned off. With the valve in this position, the effluent from the DB-1 ms column is pushed through the restrictor to the FID. When the solenoid valve is turned on as in Figure 2b, the column effluent is now pushed to the DB-17 ms column. Heart cutting a peak from the first column to the second is thus accomplished by time programming the solenoid valve to turn on just before elution of the peak and turn off just after elution of the peak.

A second use of the Deans switch is to backflush the first column. At the end of the run, the oven temperature is raised to 300 °C, the pressure in the split/splitless inlet is dropped to 1 psig, and the PCM pressure is raised to 65 psig. This change in inlet pressures causes the flow of carrier gas to reverse through column 1. This reverse flow

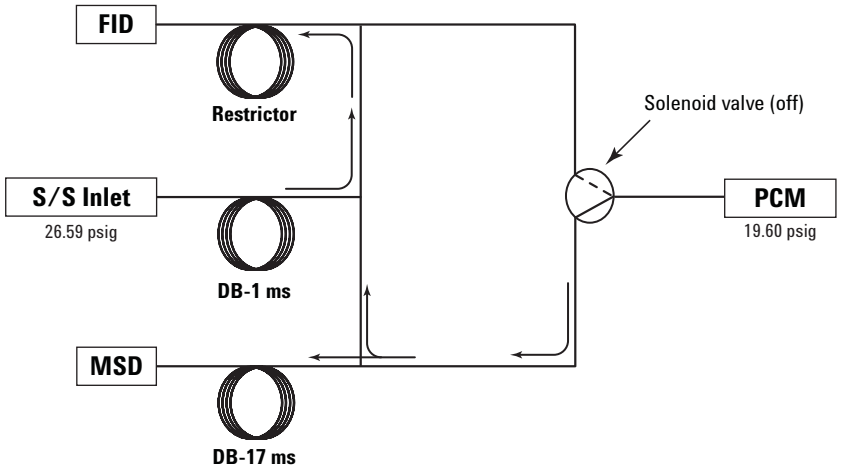

Figure 2a. Deans switch valve in no cut position. Column 1 effluent goes to FID.

backflushes any heavy materials at the head of column 1 out through the split vent trap. Backflushing increases the life of the column and results in cleaner chromatographic baselines. The backflushing mode is shown in Figure 2c.

Temperature Ramps and Cut Times

In determining the parameters for a 2-D chromatographic method, the oven temperature program is established first. The initial oven temperature is chosen to be the highest value that does not result in broadened, misshapen analyte peaks. Standards prepared at 10 ng THC/milliliter were run with initial temperatures ranging from 100 °C to 150 °C while monitoring the peak shape on the FID (no cut). For this method, 130 °C was found to perform well. The temperature ramp from 130 °C up to 250 °C was chosen to be the highest possible that would not cause oven control warnings. A ramp of 35 °C/minute was chosen. To increase the resolution of the THC from other matrix components on the first column, the oven ramp is reduced to 10 °C per minute about two minutes before elution of the THC. After elution of the THC the temperature is held isothermal at 280 °C.

Figure 3a shows the first column FID chromatogram of a volunteer sample. A large number of matrix components are clearly evident. Figure 3b is an expanded view of the elution region of THC. Also shown in Figure 3b is the chromatogram from a 10

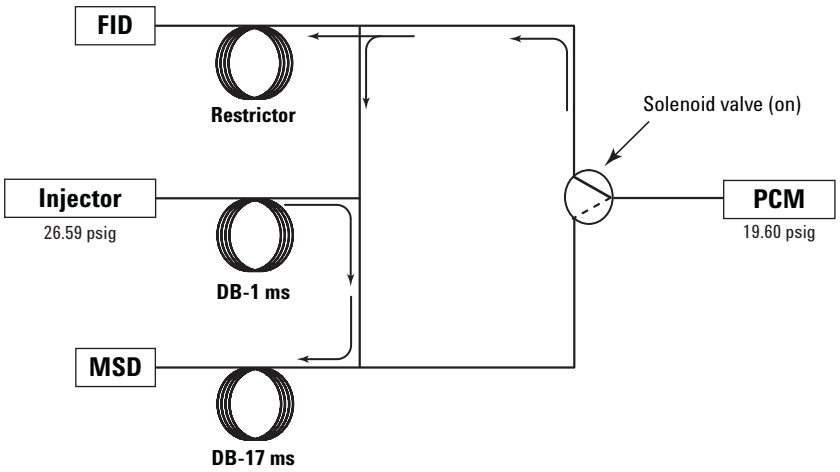

Figure 2b. Deans switch valve in cut position. Column 1 effluent goes to column 2.

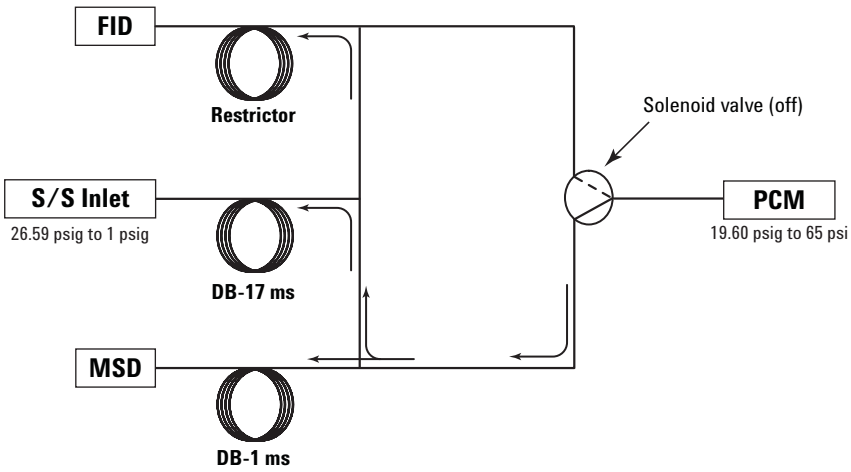

Figure 2c. Backflushing column 1. After the last analyte elutes from column 2, program inlet pressure down to 1 psig and program the PCM to 60 psi to backflush heavies out split vent.

ng/mL THC standard. The cut time for THC is chosen to start immediately before the THC peak and end immediately after it. In this example the cut time range was 6.33 minutes to 6.44 minutes.

After the cut time is determined, the focusing trap temperature program is chosen. The trap is initially held at 300 °C (that is, no trapping) and is programmed to cool to 100 °C at about one to two minutes before the cut time. This is to ensure that the trap is at 100 °C when the cut is made. After the cut is finished, the trap is then programmed back to 300 °C at its maximum rate (799 °C per minute). This desorbs the trapped components. Normally, desorption is set to start at about 0.2 minutes after the end of the cut. In this example, desorption starts approximately 1 minute after the cut.

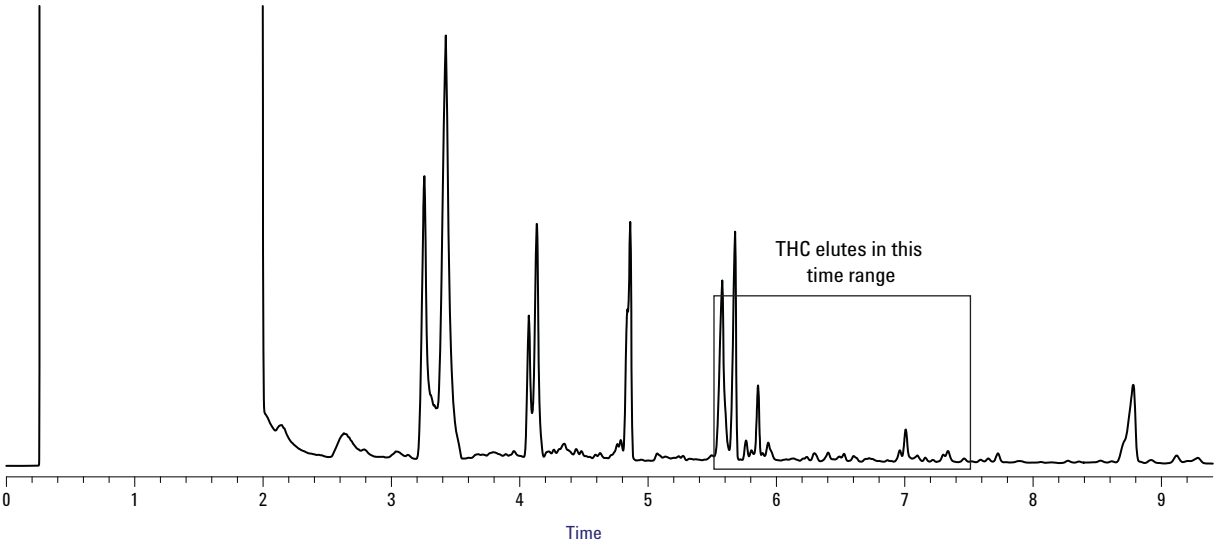

Figure 3a. First column FID chromatogram of volunteer sample showing complexity of matrix (no cut to second column).

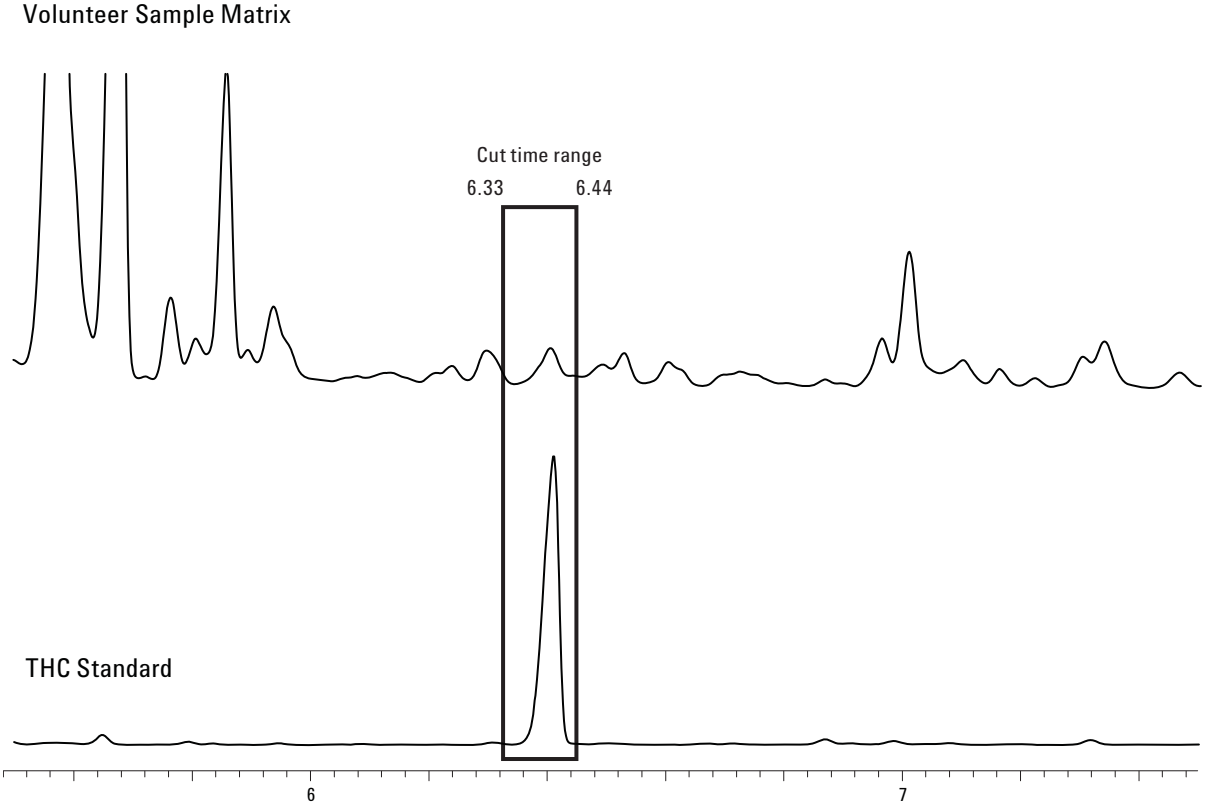

Figure 3b. Expanded view of THC elution range from Figure 3a.

Figure 4 is a timing diagram showing the relationship between the trap temperature, the oven temperature, and the Deans switch valve.

Results and Discussion

Figure 5a shows the SIM chromatograms for the internal standard (THC-D3) spiked at 2 ng/mL. The retention time of the internal standard is very close to that of THC, being only 0.002 minutes earlier. Since the retention times are so close, cut times chosen for the THC will also work well for the internal standard.

Figure 5b shows the SIM chromatograms from an unextracted THC standard prepared at the cutoff level of 0.5 ng/mL. The cutoff level is the concentration of THC in a saliva sample below which the sample is considered to be negative. The sample in Figure 5b is prepared by derivatizing the THC directly without going through the sample cleanup procedure. It is used as a reference for measuring the recovery from the sample preparation procedure.

A cutoff level standard prepared in surrogate saliva and taken through the entire sample preparation procedure is shown in Figure 5c. Comparison of the response here to that of the unextracted standard in 5b shows the recovery to be 70% or greater. It also shows that there are no significant sample preparation artifacts in the retention time range of the THC that would interfere with quantitation at the cutoff level.

A volunteer sample found to be positive for THC is shown in Figure 5d. The measured concentration of 0.64 ng/mL is just above the cutoff level. There are no significant interferences evident in the retention time region of the THC.

A negative volunteer sample is shown in Figure 5e. The concentration of THC was found to be below the lowest calibration level and was estimated to be 0.17 ng/mL THC.

Figure 6 shows the THC calibration curve from the MSD ChemStation. Acceptable linearity was found over the calibration range of 0.2 to 32 ng/mL of THC. The plot in Figure 6 shows the range bracketing the cutoff level.

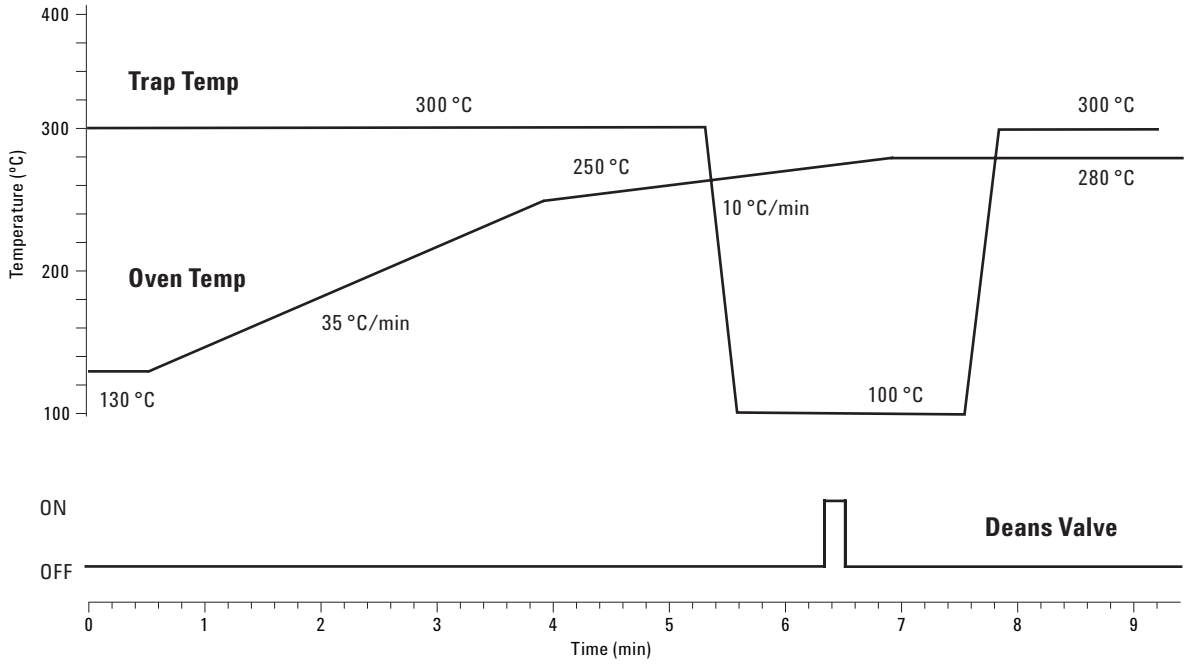

Figure 4. Temperature and valve timing for THC analysis.

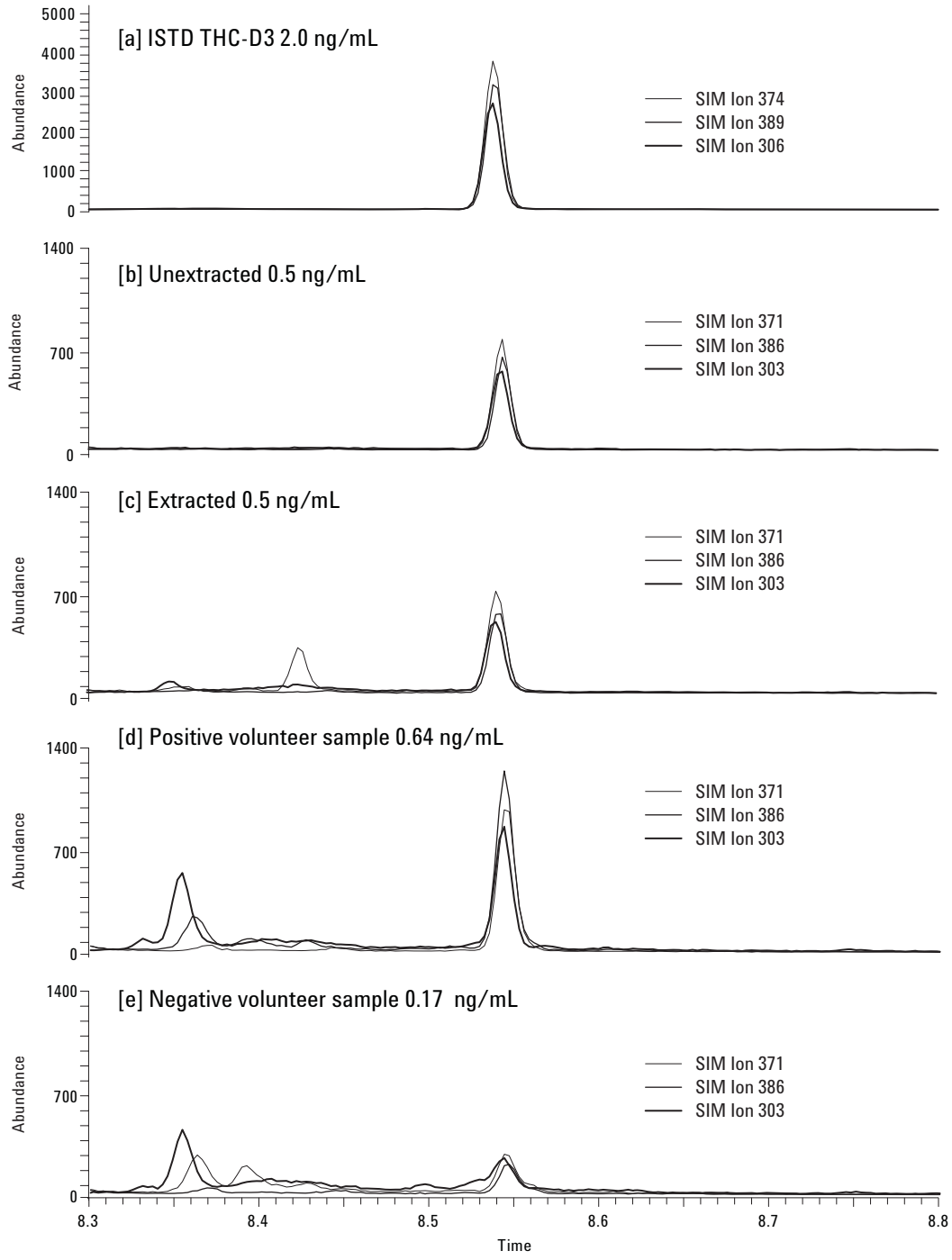

Figure 5. SIM ion chromatograms from a) internal standard THC-D3 at 2 ng/mL, b) unextracted THC cutoff at 0.5 ng/mL, c) extracted THC cutoff at 0.5 ng/mL, d)positive volunteer THC sample at 0.64 ng, and e) negative volunteer sample at 0.17 ng/mL.

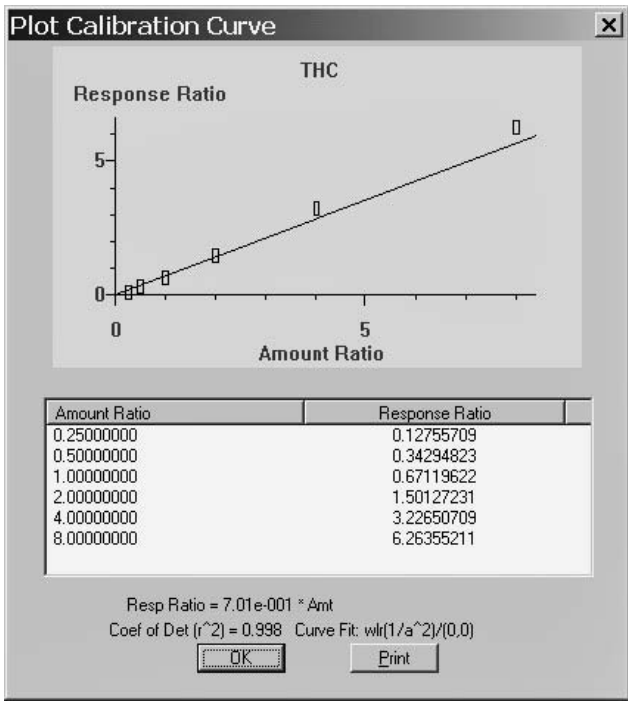

Figure 6. THC calibration curve.

The analytical performance results for the method are summarized in Table 2. The data are based on three runs at each level. The row LOD is the limit of detection. Row LOQ is the limit of quantitation. The column labeled Mean is the average concentration measured for three runs of a standard at that level when calculated using the calibration curve. The column STDEV is the standard deviation from mean, and %CV is the percent coefficient of variation. The column %Accuracy represents the agreement between the known and measured values, with 100% being perfect agreement.

Table 2. Calibration Results for THC

	ng/mL	Mean*	STDEV	%CV	%Accuracy
LOD (0.2X)	0.1	0.22	0.03	12.86	220.00
LOQ (0.4X)	0.2	0.29	0.01	3.94	146.67
Cutoff (X)	0.5	0.46	0.08	16.58	91.33
2X	1	1.03	0.04	4.23	103.00
4X	2	1.95	0.04	2.07	97.67
8X	4	4.08	0.17	4.17	102.08
Pos control	2	1.86	0.06	2.99	93.00
Recovery			70 %		
Linearity			0.2–32 ng/mL		
Carryover			None up to 32		

*3 runs

Table 3 presents the results of comparing the EIA screening results of with those of the 2-D GC/MS method. In the left-hand section in Table 3 is a matrix showing the distribution of results for the 20 volunteer samples. Four samples were found to be positive by both techniques as shown in the upper left-hand quadrant of the matrix. One sample was found to be negative by GC/MS but positive by EIA. Since GC/MS is considered to be the reference technique, the sample is considered to be a false positive for EIA. The lower left-hand quadrant shows that no samples were found to be positive with GC/MS and negative with the EIA. That is, no false negatives were found for EIA. The remaining 15 samples were found to be negative by both techniques.

Table 3. Comparison of EIA and 2-D GC/MS Results

		GC/MS +	GC/MS –
EIA	+	4	1
	–	0	15

	Deans switch	Package insert
N	20	200
Sensitivity	100%	97.62%
Specificity	93.75	91.67
Confirm cutoff	0.5	0.5

The right-hand portion of Table 3 represents a comparison of the sensitivity and specificity of the GC/MS method with the EIA screening technique. For this comparison, sensitivity is defined as the number of true positives divided by the sum of the true positives and the false negatives. True positives are defined as those samples that were found to be positive by both techniques. Specificity is defined as the number of true negatives divided by the sum of the true negatives and the false positives.

The data in the rightmost column of Table 3 is from a study of 200 samples provided with the package insert for the Intercept EIA kit. The present results show good agreement between the 2-D GC/MS technique and the 200-sample study used for the package insert.

Conclusions

High-resolution 2-D GC/MS can be used as a confirmatory technique for oral fluid drug screening for THC. Samples collected in Intercept® collectors had no major interferences that prevented analysis. Suitable results were obtained with 0.4 mL sample volume corresponding with the "withdrawn" SAMHSA Guideline cutoff of 2 ng/mL THC.

Note: The cutoff of 0.5 ng/mL THC was used in this application to compensate for the dilution of oral fluid that occurs when collecting samples with the Intercept collector, which contains a preservative buffer.

Sensitivity and specificity data between Intercept EIA kits and 2D GC/MS on 20 volunteers from a drug clinic were similar to that published in the package inserts.

References

1. James D. McCurry, "Using a New Gas Phase Micro-Fluidic Deans Switch for the 2-D GC Analysis of Trace Methanol in Crude Oil by ASTM Method D7059," Agilent Technologies publication 5989-1840EN

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