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# Rapid Multidimensional GC Analysis of Trace Drugs in Complex Matrices

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Drugs, Urine, Blood, Dean‘s Switch, MACH, Fast GC/MS

## Abstract
Reduction in analysis time is an important goal for easing the burden of large sample sets associated with routine screening of blood samples and sample sets produced from drug metabolism studies.

This study focuses on reduction in analysis time for simultaneous detection of delta-9-THC, 11-OH-THC, and THC-COOH in whole blood and urine extracts. This was achieved using the GERSTEL MACH fast GC system combined with an Agilent GC-MSD. Addition of the MACH system allows three independently heated temperature zones for multidimensional chromatography using Agilent Capillary Flow Technology Dean’s Switches along with fast heating/cooling rates. A novel pre-column approach protects the analytical columns in independent temperature zones and adds a high level of robustness. The Agilent Capillary Flow Technology Dean’s Switch allows a combination of heart-cutting multidimensional GC and back flushing to reduce the amount of unwanted background components.

Standard analysis time was reduced from 15 minutes to less than 11 minutes for blood samples and from 15 to less than 8 minutes for urine samples. Two Agilent Capillary Flow Technology Deans Switches in tandem were used for this analysis. Three independently programmed pressure zones were used in conjunction with three independent heated zones. The MS was operated in the EI mode.

## Introduction

Many recent advances in gas chromatographic methods have concentrated on reduction in analysis time and improved resolution capability. Advances in pneumatics control, temperature ramping, column dimensions, and electronics, among others, enable faster chromatographic separations. Reducing analysis time improves laboratory efficiency, service, and capability.

Newer chromatographic techniques for drugs of abuse have included use of two-dimensional gas chromatography (2D-GC) to resolve matrix interference and improve detection limits in hair, oral fluid, blood, and plasma specimens. 2D-GC provides advanced opportunity in resolving interferences and should be applicable to other complex matrices.

MACH (Modular Accelerated Column Heater) technology (GERSTEL, Inc.) incorporates standard capillary GC columns into external low thermal mass column modules, allowing independent temperature control of multiple columns. The technology also allows fast ramping and rapid cooling, resulting in faster analysis times.

The objective of this study was to evaluate application of independently controlled MACH capillary columns in a two-dimensional configuration to simultaneous quantification of delta-9-tetrahydrocannabinol (delta-9-THC) and metabolites, 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC), and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). A pre-column was integrated into the system to protect analytical columns and reduce maintenance. Pneumatic control of flow and heart cutting was accomplished with tandem microfluidic Dean's switches. Independent temperature control of the secondary capillary column enables refocussing of analytes to enhance sensitivity.

The MACH 2D-GC instrument configuration decreased run times and achieved resolution of analyte from complex matrix components. The method can potentially be applied to detection and quantification of other drugs in various complex matrices such as blood, urine, meconium, among others.

## Experimental

*Reagents.* THC, 11-OH-THC, and THC-COOH and deuterated internal standards were purchased from Cerilliant. All chemicals were ACS reagent grade and solvents were HPLC grade. N,O-bis(Trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) (Pierce Biotechnology) and Clean Screen solid-phase extraction (SPE) columns (part no. ZSTHC020, 200 mg of sorbent, 10 mL tube volume) (United Chemical Technologies) were utilized in specimen preparation.

Sodium acetate buffer (pH 4.0 + 0.1) was prepared with 2.0 M sodium acetate and 2.0 M acetic acid. 0.1 M Potassium phosphate buffer was prepared by adjustment of 0.1 M monobasic potassium phosphate to pH 6.8 with 0.1 M dibasic potassium phosphate. The elution solvent was hexane:ethyl acetate, 80:20 by volume. Working b-glucuronidase (20,000 units/mL) was prepared by dilution of stock enzyme with 0.1 M pH 6.8 phosphate buffer. Blank blood and matrices were verified to be drug free prior to preparation of calibrators and controls.

*Specimen Preparation.* One mL of quality control sample or calibrator was combined with 25 uL working internal standard and 1 mL 0.1 M pH 6.8 phosphate buffer. Proteins were precipitated with 2.0 mL cold acetonitrile, added in 0.5 mL increments, with vortexing. Tubes were centrifuged at 1800g for 10 minutes to pellet protein. Supernatants were decanted into tubes containing 3.0 mL 2 N pH 4.0 sodium acetate buffer and vortex mixed.

*Solid-Phase Extraction and Derivatization.* SPE columns were conditioned with 1 mL elution solvent (hexane:ethyl acetate, 80:20), 3 mL methanol, 3 mL deionized water, and 2 mL 0.1 N HCl. Buffered supernatants were added to conditioned columns. Columns were washed with 3 mL distilled water and 2 mL 0.1 N HCl/acetonitrile (70:30) and dried by vacuum for 10 minutes. After priming the sorbent bed with 0.2 mL hexane, analytes were eluted with 5 mL elution solvent into 10 mL centrifuge tubes containing 0.5 mL absolute ethanol. Eluates were dried under nitrogen at 40°C.

Dried extracts were reconstituted in 25 mL BSTFA, capped, derivatized at 70°C for 30 min and transferred to autosampler vials.

*Instrumentation.* Agilent Technologies 6890 Gas Chromatograph with Flame Ionization Detector, Agilent Technologies 5973 EI/CI Mass Spectrometer, Agilent Technologies Microfluidics Dean‘s Switch, GERSTEL MACH Fast GC Modular Accelerated Column Heater.

*GC parameters.*

| | |
|---|---|
| GC Inlet: | splitless, He, $P_i$ = 44.44 psi<br>250°C |
| GC Oven: | 280°C, held for duration |
| FID: | 250°C |
| Aux 3 press: | 41.2 psi |
| Aux 4 press: | 29.2 psi |
| MSD: | transfer 250°C<br>quad 150°C<br>source 250°C |

*Urine extract chromatography.*

| | |
|---|---|
| Guard Column: | 1 m DB-1 (Agilent),<br>$d_i$ = 0.15 mm $d_f$ = 1.2 µm |
| MACH Module 1: | 15 m DB-17MS (Agilent),<br>MACH format<br>$d_i$ = 0.25 mm $d_f$ = 0.25 µm<br>100°C (4.83 min); 800°C/min;<br>210°C; 25°C/min;<br>300°C (0.5 min) |
| MACH Module 2: | 15 m DB-1MS (Agilent),<br>MACH format<br>$d_i$ = 0.25 mm $d_f$ = 0.25 µm<br>100°C (1.2 min); 800°C/min;<br>230°C; 25°C/min;<br>300°C (5 min) |

*Whole blood extract chromatography.*

| | |
|---|---|
| Guard Column: | 1 m DB-1 (Agilent),<br>$d_i$ = 0.15 mm $d_f$ = 1.2 µm |
| MACH Module 1: | 15 m DB-17MS (Agilent),<br>MACH format<br>$d_i$ = 0.25 mm $d_f$ = 0.25 µm<br>100°C (4.83 min); 800°C/min;<br>200°C; 10°C/min; 260°C;<br>50°C/min; 300°C |
| MACH Module 2: | 15 m DB-1MS (Agilent),<br>MACH format<br>$d_i$ = 0.25 mm $d_f$ = 0.25 µm<br>100°C (1.2 min); 800°C/min;<br>230°C; 25°C/min;<br>300°C (7.6 min) |

*MSD SIM mode ions.*

| | |
|---|---|
| THC : | 386, 371, 303 |
| d3-THC : | 389, 374 |
| 11-OH-THC : | 371, 474, 459 |
| d3-11-OH-THC : | 374, 477 |
| THC-COOH : | 371, 488, 473 |
| d3-THC-COOH : | 374, 491 |

## Results and Discussion

Figure 1 shows a picture of the instrument used for this study. The pre-column capillary is housed in the GC oven. Short pieces of fused silica transfer capillaries connect the MACH ovens, on the oven door, to the Microfluidic Dean‘s switch inside the oven.

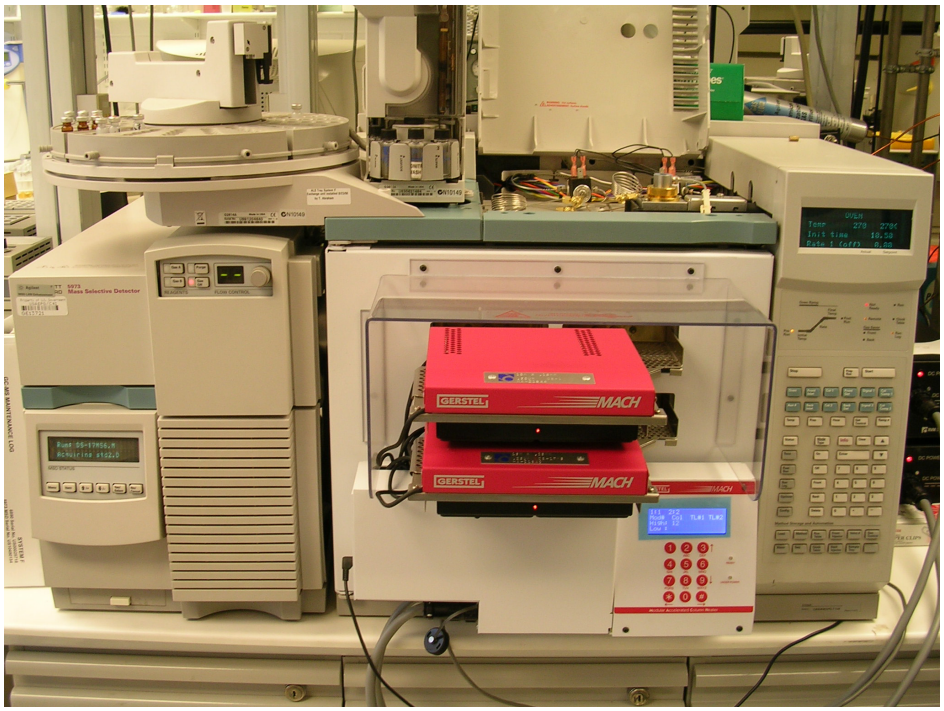


**Figure 1.** Photograph of instrument used for this study.

Figure 2 shows the flow diagram for this configuration. Sample is introduced into the inlet, analytes undergo an initial separation on a 1m x 0.15mm x 1.2 mm DB-1 guard column. Analytes, as a group, are cut over to the first MACH GC column, pressure at the inlet is dropped, and unwanted sample components are back flushed from the pre-column through the split vent. Initial chromatographic separation occurs on a 15 m x 0.25 mm x 0.25 mm DB-17MS primary MACH column. Following separation on the primary column, the Dean’s switch valve is programmed to divert individual “cuts” from the effluent from the primary column to the secondary GC column. Final chromatographic resolution of analyte is performed on a 15m x 0.25 mm x 0.25 mm DB-1MS secondary MACH column.

Analytes were detected using a bench top quadrupole mass spectrometer. All three columns have independent temperature and pressure control for optimizing separation of analytes from matrix. GERSTEL MACH ovens use low thermal mass technology for fast heating and cooling, keeping run times short.

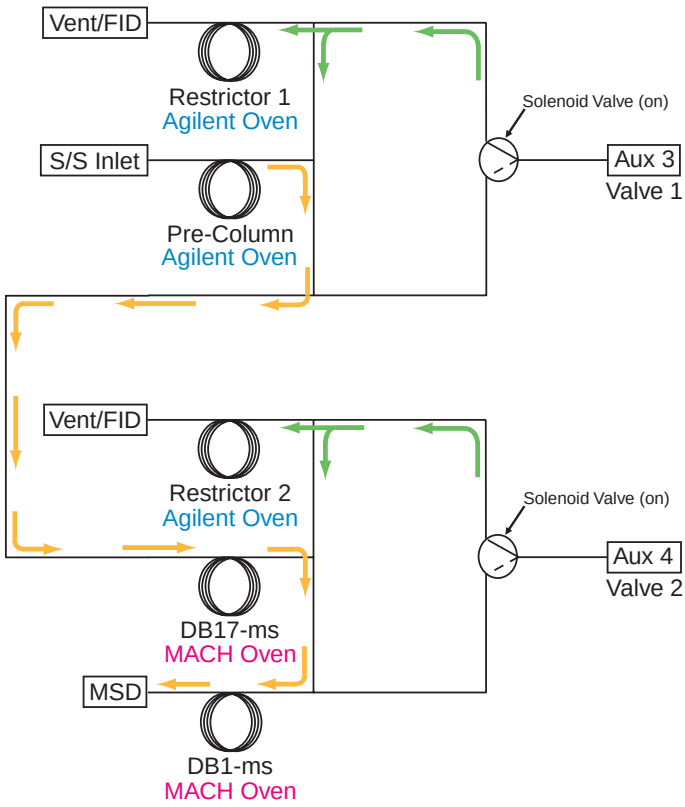


**Figure 2.** Flow diagram of dual Dean‘s switch.

Figure 3 shows a comparison of chromatograms of a urine extract from a conventional 2-D run and from a run on the current MACH system. Run time is reduced from 15 minutes to less than 8 minutes.

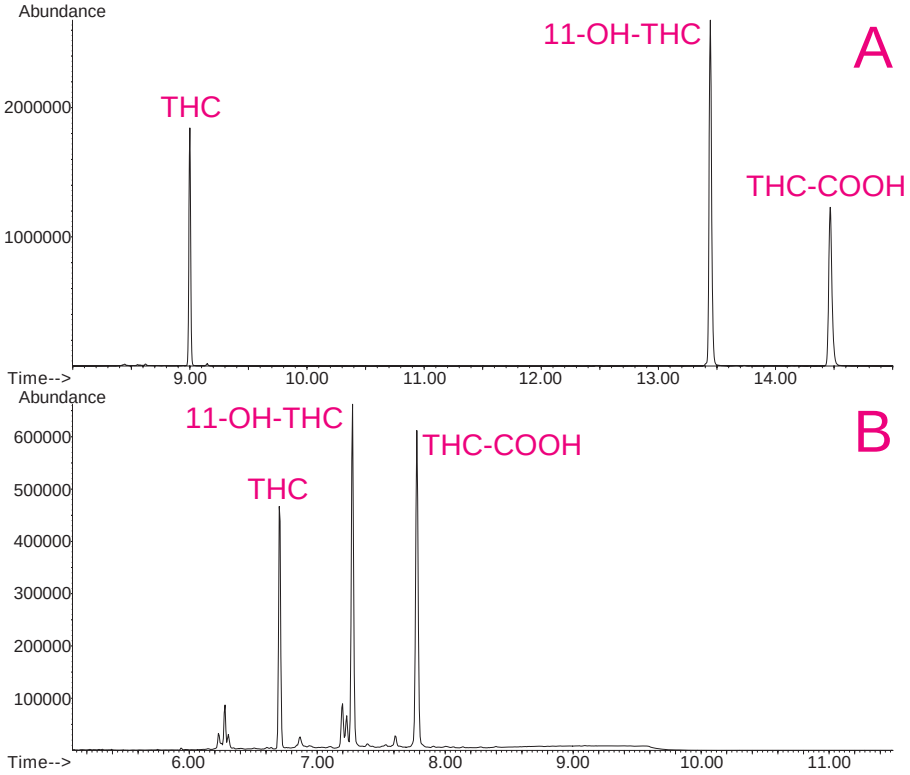


**Figure 3.** Comparison of TIC‘s from standard 2-dimensional (A) and MACH methods (B) for urine extract showing reduction in run time.

Figure 4 shows extracted ion chromatograms (EIC‘s) for THC and THC-COOH in a urine extract using the MACH/Dean‘s switch configuration. Both show excellent separation from background. Replicate injections (n = 5) of a 0.25 ng/mL standard of delta-9-THC, 11-OH-THC, THC-COOH, d3-delta-9-THC, d3-11-OH-THC, and d3-THC-COOH gave %CV‘s of <0.07% for retention time reproducibility and < 5.5% for area count reproducibility.

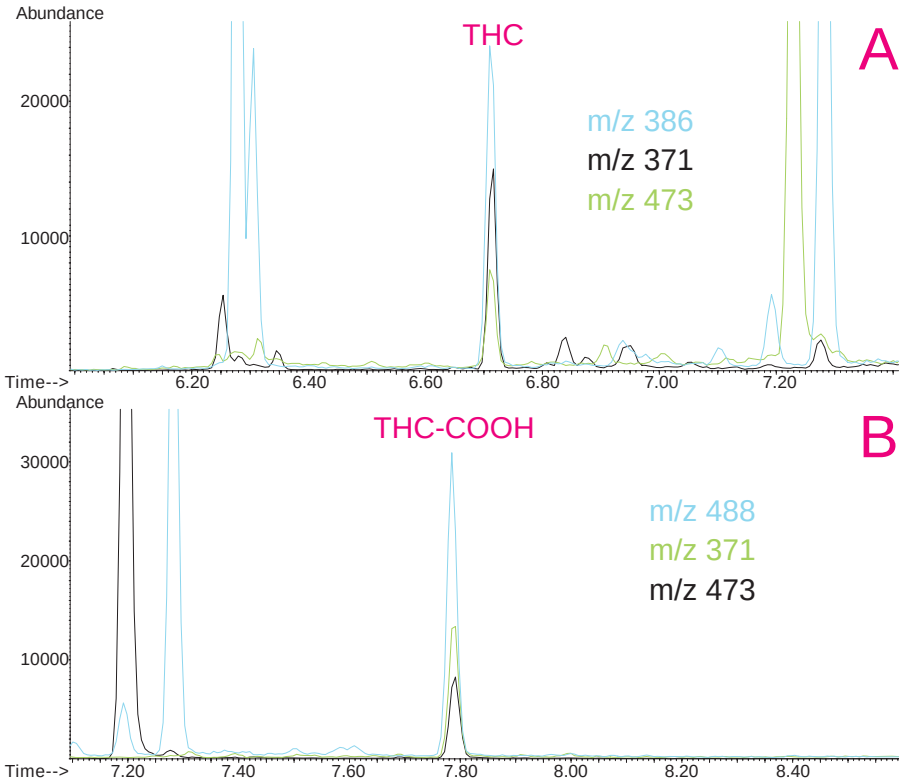


**Figure 4.** EIC‘s of THC (A) and THC-COOH (B) in urine extract showing resolution from background.

Figure 5 shows a comparison of chromatograms of a whole blood extract from a conventional 2-D run and from a run on the current system. Run time is reduced from 15 minutes to less than 11 minutes.

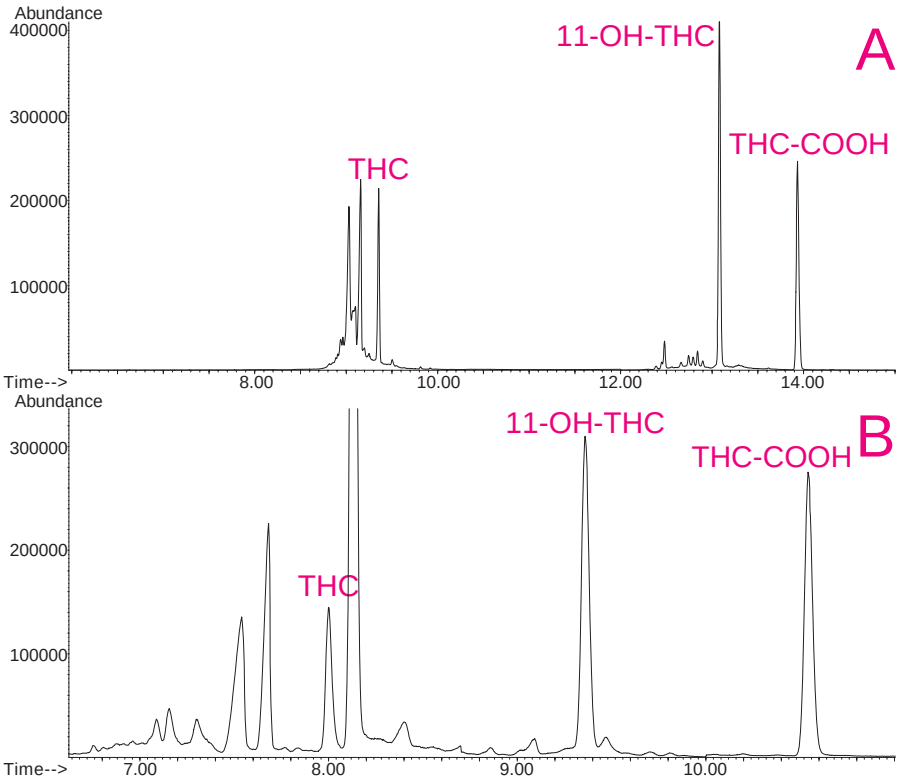


**Figure 5.** Comparison of TIC‘s from standard 2-dimensional (A) and MACH methods (B) for blood extract showing reduction in run time.

Figure 6 shows extracted ion chromatograms (EIC‘s) for THC and THC-COOH in a blood extract using the MACH/Dean‘s switch configuration. Both show excellent separation from background.

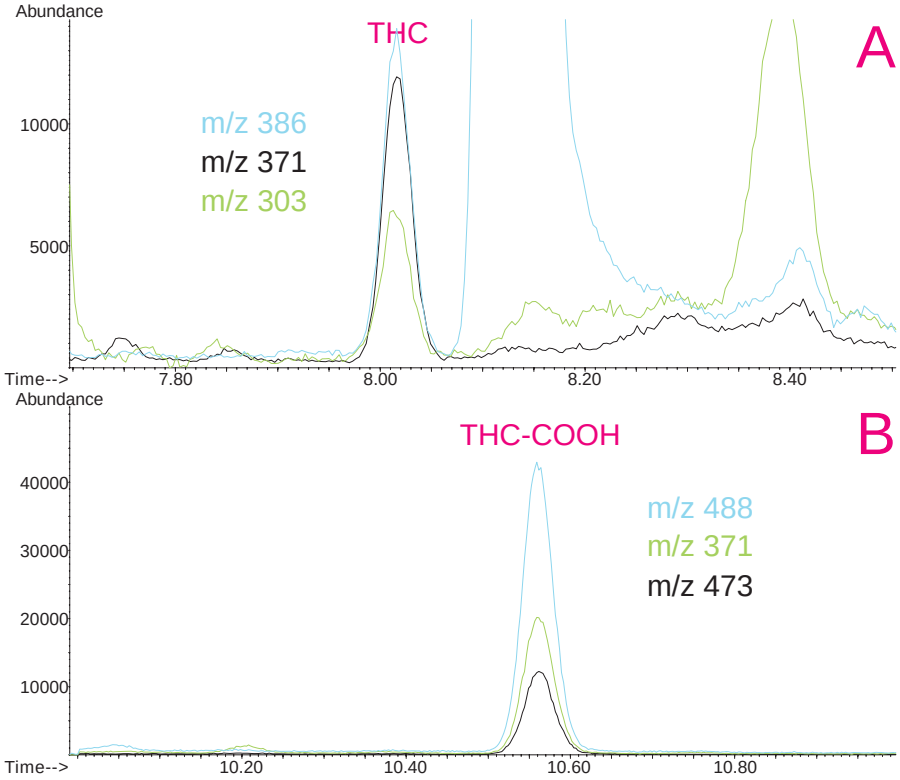


**Figure 6.** EIC‘s of THC (A) and THC-COOH (B) in whole blood extract showing resolution from background.

Figure 7 shows a calibration curve for THC-COOH in whole blood extracts over the range 0.5 - 25 ng/mL. The coefficient of determination was > 0.997 for all three analytes.

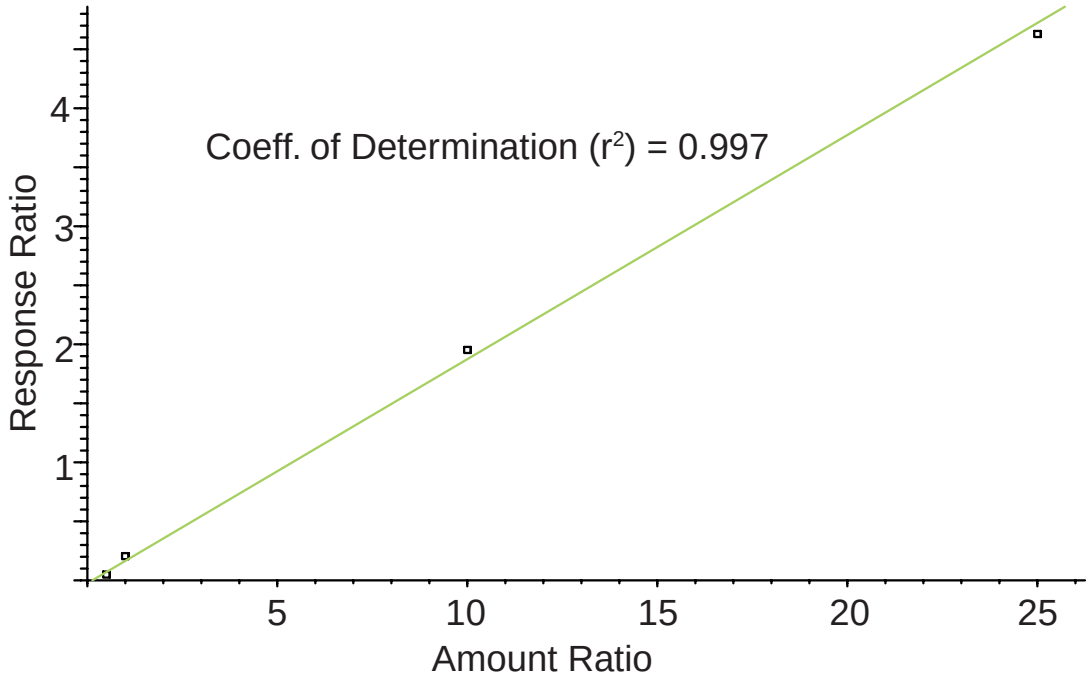


**Figure 7.** Calibration curve for THC-COOH in whole blood extracts.

## Conclusions

This study shows the ability to reduce runtime for delta-9-THC, 11-OH-THC, and THC-COOH in whole blood and urine extracts using a combination of dual microfluidic Dean's switches and MACH fast GC modules. The ability to back flush combined with multiple heart cutting reduces the amount of background materials reaching the mass spectrometer. This serves to reduce instrument maintenance and enhance detection of analytes. Precision and linearity of this analysis were excellent even with reduced analysis time. The combination of reduced run time and lower maintenance makes this setup suitable for high through put applications.

## References

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