

Optimal Quantification of THC in Blood



Application Note

AN0014

INTRODUCTION

The number of regular cannabis users in the world is estimated at 183 million people^[1]. It is illegal to drive a vehicle whilst under the influence of cannabis. The active ingredient in cannabis is delta9-Tetrahydrocannabinol (Δ^9 -THC). Common side effects includes the feeling of euphoria, hallucinations, relaxation and even sedation. Post ingestion, Δ^9 -THC is metabolised into the inactive ingredient Tetrahydrocannabinolic Acid (THCA/ THC-COOH).

Suspected drug drivers are often subjected to a screening test of cannabinoids in saliva. A positive result must then be confirmed by the presence of Δ^9 -THC in a blood sample; by gas chromatography. Quantification of THCA can indicate long term use of the drug. Drug detection limits are set by government laws and vary from country to country. The limits in the United Kingdom and Canada is 2ng/mL whereas in France the limit is 1ng/mL.

EXPERIMENTAL

A SCION 456 GC coupled with the SCION Single Quad Mass Spectrometer was configured for cannabinoid analysis in blood.

Calibration standards were prepared with a range of 0.5ng/mL to 10ng/mL for Δ^9 -THC and 2.5ng/mL to 50ng/mL for THC-COOH, with 20 μ L of internal standard (Δ^9 -THC-D3 and THC-COOH-D3).

Biological samples were prepared using the following method. 20 μ L of internal standard was added to 1mL of blood sample. 200 μ L of acetic acid (10%) and 5mL of hexane/ethyl acetate (9-1 V/V) were added before being centrifuged for 10 minutes at 4000rpm. The organic phase was removed and evaporated at 50°C. The dry extract was then derivatised with 50 μ L of BSTFA+1%TCMS for 40 minutes at 80°C. 2 μ L was injected into the split/splitless injector.

Analytical conditions can be found in Table 1.

Table 1. Analytical Conditions of the GC-MS

Conditions	
S/SL Injector	280°C, splitless, 2 μ L
Column	SCION-5MS 15m x 0.25mm x 0.25 μ m
Oven	50°C (hold 0.10min) 15°C/min to 260°C (15.30 mins) 20°C/min to 300°C (18 mins)
Carrier Gas	Helium, 1mL/min
Source	250°C
Transfer Line	310°C
Mode	Positive, 70eV
THC Ions	371, 386
THC-COOH Ions	371, 473

RESULTS

The calibration curves for both THC and THC-COOH can be found in Figures 1 and 2. The linearity for both cannabinoids were excellent.

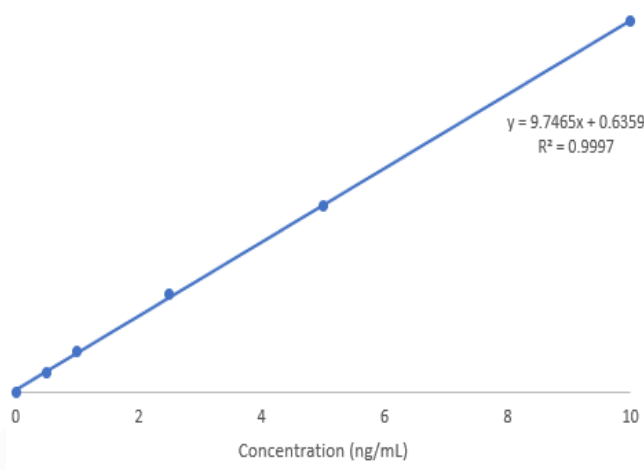


Figure 1. Δ^9 THC Calibration Curve

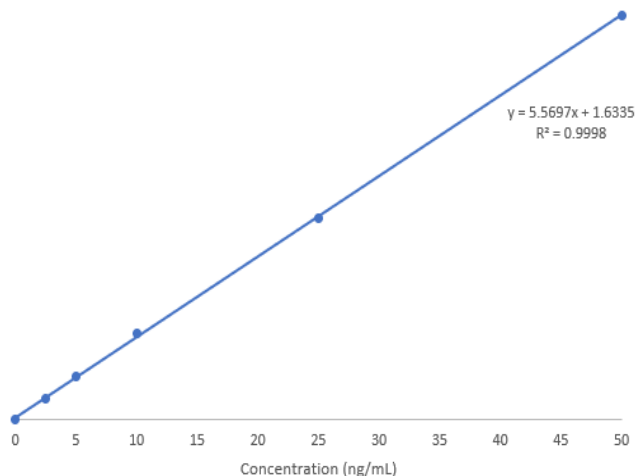


Figure 2. THC-COOH Calibration Curve

The sensitivity of the SCION SQ MS is excellent, thus exceeding the lowest quantification limit set in France (1ng/mL), for Δ 9-THC. The limit of detection for Δ 9-THC is 0.2ng/mL with the limit of quantification 0.5ng/mL whereas the limit of quantitation of THC-COOH is 2.5ng/mL.

Reproducibility (n=10) and repeatability (n=5) of the system were tested using consecutive injections of Δ 9-THC at both 0.20ng/mL and 0.5ng/mL plus THC-COOH at 2.5ng/mL. Analysing the sample spiked at the limit of quantification shows the high reliability and robustness of the results. Table 2 details the performance of the SCION GC-MS.

Table 2. Reproducibility and repeatability of Δ 9-THC and THC-COOH

Analyte	n	Concentration (ng/mL)	RSD (%)
Δ 9-THC	10	0.5	6.8
Δ 9-THC	5	0.20	3.8
THC-COOH	5	2.5	5.5

Figure 3 shows the chromatogram from seven consecutive injections, of 0.5ng/mL Δ 9-THC, which were then overlaid in the Mass Spectrometry Work Station software.

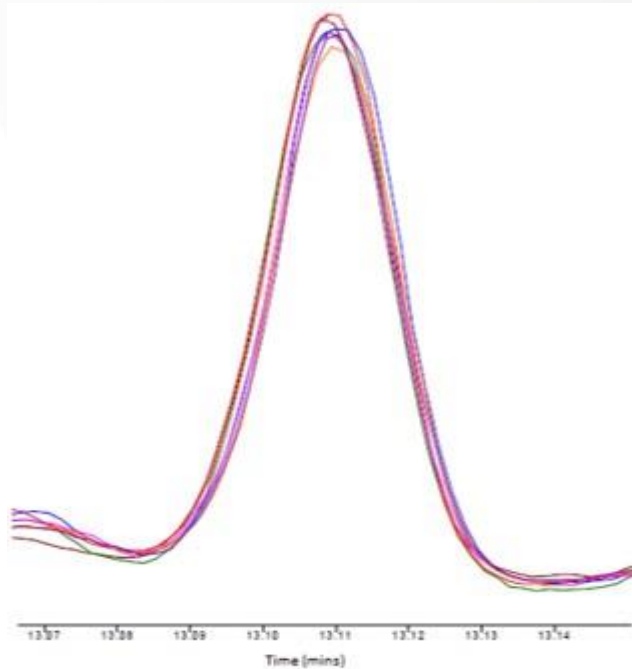


Figure 3. Overlay of 7 consecutive injections of 0.5ng/mL Δ 9-THC

A blood sample was obtained from a cannabis smoker. The cannabinoids were extracted from the plasma and ran using the same method. Figure 4 shows the total ion chromatogram (TIC) of both Δ 9-THC and THC-COOH.

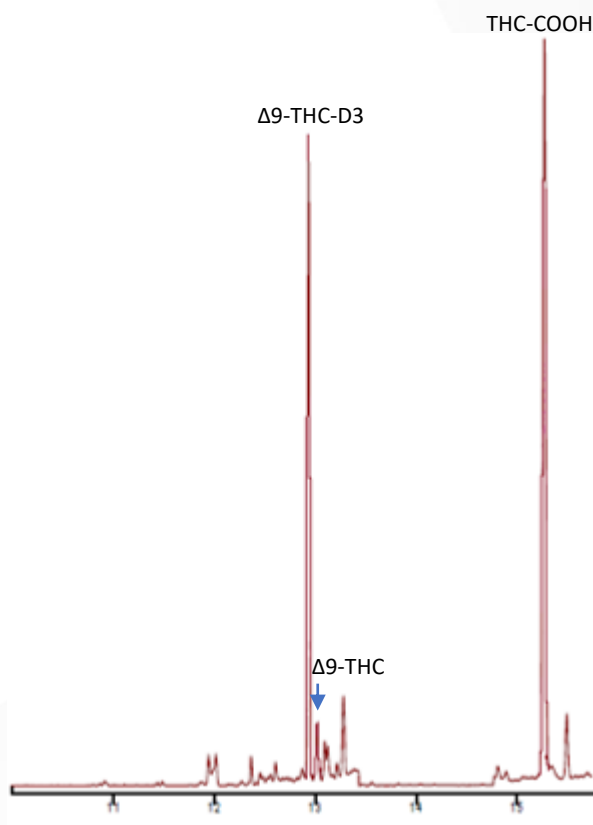


Figure 4. TIC of blood sample from cannabis user

CONCLUSION

The analysis of cannabinoids from biological matrices is made easy with the SCION GC-MS. The highly sensitive and robust instrument has exceptional limit of detection for both Δ^9 -THC and THC-COOH. With an LOD of 0.2ng/mL and LOQ of 0.5ng/mL, for Δ^9 -THC, these limits exceed the requirements specified by the laws in UK, Canada and France. Additionally, the cannabinoid analyser is capable of detecting THC-COOH at concentrations as low as 2.5ng/mL, with excellent repeatability for both cannabinoids.

REFERENCES

[1] United Nations Office on Drugs and Crime (2017). *Statistics and Trends in Illicit Drugs*. [online] United Nations, p.1. Available at: https://www.unodc.org/wdr2017/field/WDR17_Fact_sheet.pdf [Accessed 11 Dec. 2018].