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# Development of a GC-MS forensic profiling method for Cannabis resin and characterization of target analytes

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Master thesis, Molecular Technologies

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## SUMMARY

The main goal of this thesis was the characterization of targeted analytes in Cannabis resin and the development of a validated gas chromatography - mass spectrometry (GC-MS) method, based upon an existing flame ionization detector (FID) method.

A general isolation protocol, a GC-MS method and sample preparation procedure were generated. It was possible to isolate and characterize seven compounds from Cannabis resin seizures. The sample preparation as well as the analytical run time could be shortened. The method was successfully validated and qualified to be implemented at the Swedish National Forensic Centre (NFC) for chemical profiling of Cannabis resin seizures.

## INTRODUCTION

*Cannabis sativa* L. has been used for several hundred years, mainly due to its psychoactive effects, and is today the most frequently abused drug in the western world. The plants active components have grown interest also from a pharmaceutical point of view. Historically, the analytical focus used to be set on the main active substance $\Delta^9$-Tetrahyrocannabinol (THC), but the identification of further compounds became important in understanding the pharmacological properties of *Cannabis* [1]. Especially the terpenes have gained attention due to their use in fingerprinting [2].

In forensics, comparative investigations based on the chemical profile can be used to track drug organizations and trading routes or to compare different seizures for use as evidence in courts of law [3]. Such comparison measurements of Cannabis resin seizures are made at NFC in Linköping, utilizing a GC-FID method. To gain more knowledge of the components of a Cannabis resin profile, increase the specificity of the method and acquire supplementary forensic information, a GC-MS method was developed and optimized.

## RESULTS

Through the application of vacuum liquid chromatography (VLC), flash column chromatography (FC) and preparative high performance liquid chromatography (HPLC), it was possible to separate terpenes and cannabinoids. In total, seven pure fractions were gained and characterized by GC-MS, $^1$H and $^{13}$C nuclear magnetic resonance (NMR). The compounds could be identified as β-caryophyllene, α-humulene, cannabicycloic acid (CBLA), THC and its analogue acid (THCA), cannabidiol (CBD) and cannabidiolic acid (CBDA).

Additionally, the analytical sample preparation was optimized. Through the change of solvent, a reduction of preparation time from previously 2 h to 20 min could be achieved.

Further, the GC method was investigated in more detail. Several stationary phases were tested and the effect of different polarities on the separation evaluated. Figure 1 displays the concept of testing, describes the polarities and indicates applied dimensions.

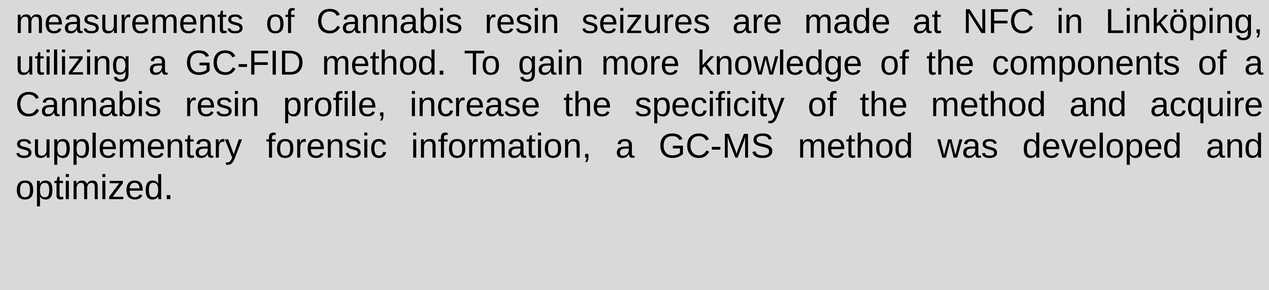


**Figure 1.** Concept for the column testing. First, the dimensions were reduced, followed by the investigation of different column polarities.

To compare the separation on different columns, two different types of temperature program ramps were used (7°C/min and 15°C/min) to analyze real samples. The column of choice was selected by the criteria separation power, resolution and overall visual assessment of the chromatograms. The DB-1701 column was found to be most suited for the further optimization.

All important GC parameters such as injection volume or average velocity were examined and optimized. Additionally, some of the important MS settings like the number of A/D samples were investigated. Further, the application of extracted ion chromatogram (EIC) versus total ion chromatogram (TIC) was tested. It could be shown that using EIC can help in the differentiation and categorization of various seizure samples, illustrated in Figure 2.

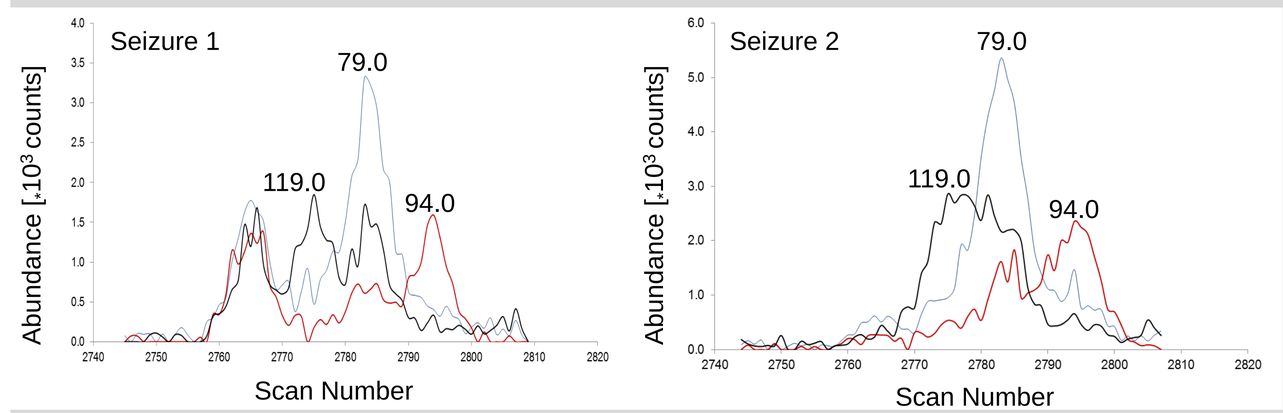


**Figure 2**. Comparison of two different seizure samples in the EIC mode. Used ions for this mode possess $^m/_z$ values of 79.0 (blue line), 94.0 (red line) and 119.0 (black line).

The analysis time could be shortened from 53.0 min to 40.5 min, although a larger temperature range is covered and several, characteristic terpenes are now displayed, which were not visible using the GC-FID method. Figure 3 shows a Cannabis resin profile analyzed by the developed method. The new region is indicated with the black box. The final method was tested for recovery, carryover, reproducibility and linearity, resulting in acceptable values for all validation parameters.

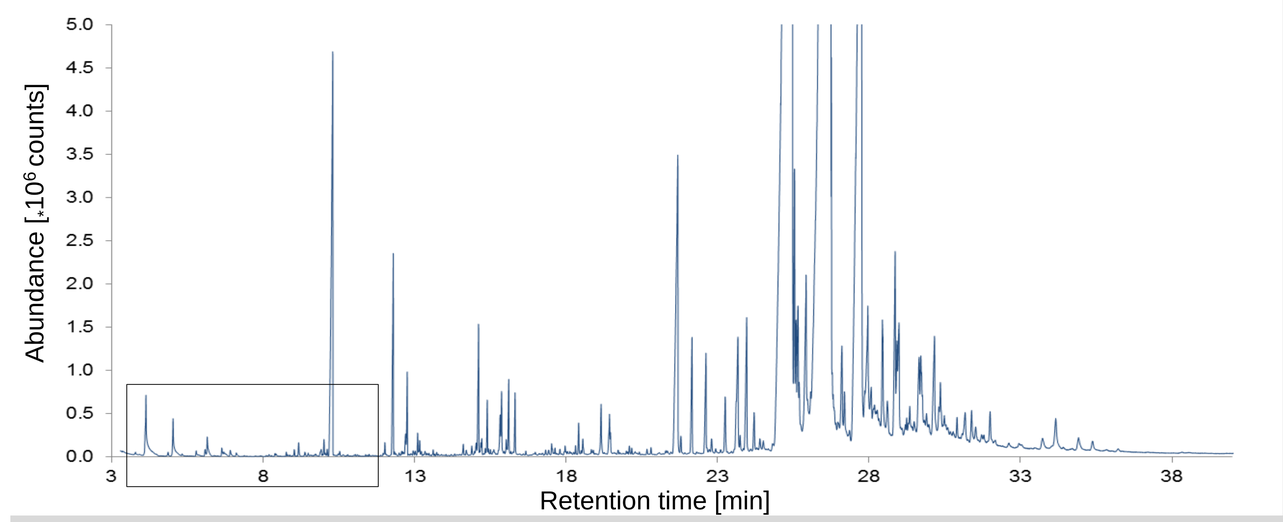


**Figure 3**. Cannabis resin profile sample analyzed with the old and the developed method

## CONCLUSION

It was possible to isolate several compounds out of hashish seizures and to identify these substances. These isolates were used as reference material during the GC-MS method optimization. The currently used GC-FID method could successfully be adopted to the MS and all important parameters were investigated and optimized. The analysis time was reduced from 53.0 min to 40.5 min and the sample preparation shortened from 2 h to 20 min.

Furthermore, it was possible to introduce EIC in the evaluation of different seizure types to support the assessment of drug samples. The method was successfully validated and is thus qualified to be implemented at the NFC for chemical profiling of hashish seizures.

## REFERENCES

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