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VILNIUS UNIVERSITY
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Enhancing Gas Chromatographic Analysis: a Novel Cryo-Enrichment Module for GC Analysis

DOCTORAL DISSERTATION

Natural Sciences,
Chemistry (N 003)

VILNIUS 2023

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FIZINIŲ IR TECHNOLOGIJOS MOKLSŲ CENTRAS

Audrius Sadaunykas

Dujų chromatografinės analizės tobulinimas: inovatyvus krio-koncentravimo modulis GC analizei

DAKTARO DISERTACIJA

Gamtos mokslai,
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For English speakers: thank you for reading this, hope you enjoy it.

ABBREVIATIONS

GC – gas chromatography

ppm – parts per million

ppb – parts per billion

FID – flame ionization detector

DC – direct current

MS – mass spectrometry

m/z – mass-to-charge ratio

SPE – solid-phase extraction

SPME – solid-phase microextraction

SBSE – stir bar sorptive extraction

LOD – limit of detection

LOQ – limit of quantification

LLOQ – lower limit of quantification

ULOQ – upper limit of quantification

CRM – certified reference materials

RSD – relative standard deviation

R^2 – coefficient of determination (square of the product-moment correlation coefficient)

ICH – International Council for Harmonisation

FDA – U.S. Food and Drug Administration

N_2 – liquid nitrogen

C8 – octane

C9 – nonane

C10 – decane

HPLC – high-performance liquid chromatography

MeOH – methanol

ACN – acetonitrile

DEHP – bis(2-ethylhexyl) phthalate

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INTRODUCTION

Gas chromatography (GC) stands as a versatile and widely embraced analytical method with a market size reported to be 3.0 billion US dollars and is expected to reach 3.9 billion by 2029 [1]. Its fundamental principle of separation is well-suited for effectively separating virtually any mixture of components with reasonable volatility. GC analysis became a routine tool for quality control and integration into production processes [2]. Although other techniques, such as infrared and near-infrared spectroscopy [3], are currently used for these purposes, gas chromatography has two distinct advantages. First, it has superior selectivity, which is crucial when dealing with compounds having similar spectroscopic properties. Second, it can quantify compounds present at both high and very low concentrations (ppm or sub ppm levels [4]) in complex mixtures.

Gas chromatography has had a significant impact on the analysis of organic trace components and continues to be widely used in this field. Trace components are relevant in many areas, e.g. in food [5, 6], fragrance [7], bulk production chemicals [8-10] and, last but not least, environmental chemistry [11]. Tighter product specifications, extended legislation, and increased cost efficiency often drive the development of new methods, necessitating faster analysis and better detectability and quantitative accuracy. A typical gas chromatographic analysis of trace components involves several steps, such as sample preparation and or analyte enrichment, sample introduction, separation, and detection. One of the analyte enrichment methods is cryo-enrichment.

Cryo-enrichment in gas chromatography is a powerful technique employed to enhance the separation and detection of volatile compounds in complex mixtures. Unlike traditional gas chromatography, this approach incorporates a distinctive strategy by lowering the temperature of both the sample and the chromatographic column to exceptionally low levels, often plunging below the boiling or even melting points of the target analytes. This cooling process results in a reduction of the compounds volatility, prompting them to efficiently condense onto the stationary phase of the column.

The focus of this thesis revolves around the development of a custom-made analyte enrichment method aimed at enhancing system efficiency and sensitivity. We constructed and evaluated multiple prototypes of a cryo-enrichment module designed for diverse applications. The cryo-enrichment process yielded impressive results, significantly augmenting the efficiency and sensitivity of gas chromatography methods. Nevertheless, it is essential to

acknowledge that this technique and its associated prototype exhibit limitations in terms of analyte working range and ease of operation.

Aim and tasks of the doctoral dissertation

The aim of this doctoral dissertation is to advance the efficiency and sensitivity of gas chromatography through the development and application of a novel analyte enrichment technique. By enhancing the separation and detection capabilities of GC, this research seeks to contribute to the broader field of analytical chemistry.

- To develop an innovative analyte enrichment technique that efficiently concentrates trace components from intricate mixtures, thereby elevating the sensitivity of GC analysis.
- To optimize analyte enrichment parameters.
- To evaluate the performance of the enrichment technique.
- To demonstrate the practical applicability of the enrichment technique in diverse fields.

Scientific novelty

The scientific novelty of this thesis lies in the development and exploration of a novel analyte enrichment technique to improve gas chromatography. While gas chromatography itself is a well-established and widely utilized analytical technique with diverse applications, the proposed custom analyte enrichment technique represents a unique advancement. This technique aims to enhance the efficiency and sensitivity of gas chromatography systems by addressing the challenges associated with detecting and quantifying trace components, a crucial aspect in various fields. By introducing this innovative enrichment method, the thesis seeks to contribute to the ongoing evolution of gas chromatography technology, enabling researchers and analysts to achieve higher levels of accuracy and detection capability in the analysis of complex mixtures, and ultimately advancing the ability to meet increasingly stricter analytical requirements in diverse industries.

Statements to be defended

1. The cryo-enrichment module is compatible for integration with gas chromatography systems.
2. The cryo-enrichment module enhances separation and analysis efficiency by focusing analyte peaks.
3. By accumulating multiple injections within the cryogenic zone and eluting them as a unified peak, the cryo-enrichment module increases system sensitivity.
4. The cryo-enrichment module demonstrates adaptability for diverse gas chromatography applications.

1. LITERATURE REVIEW

1.1. Gas chromatography

Gas chromatography (GC) is a highly precise analytical method used for separation, identification and quantification of volatile compounds [12]. The equipment used in gas chromatography typically comprises (Figure 1): a source of continuous flow of carrier gas, a pressure and flow regulator for the carrier gas, an inlet system for the material to be analyzed, a chromatographic column and a detector for registering the separated components [13]. These components will be described further paragraphs.

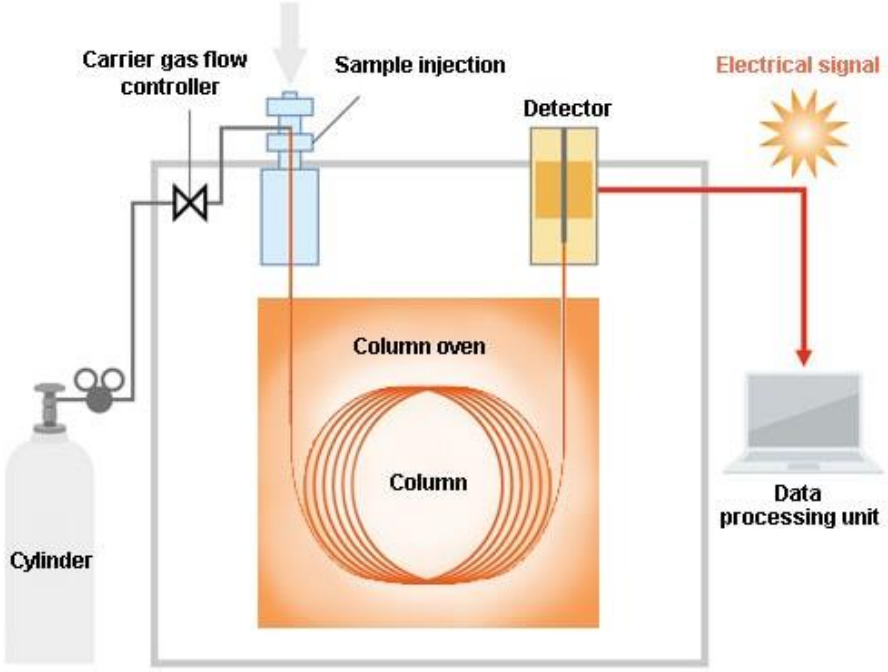

Figure 1. Gas Chromatograph [14].

The carrier gas, usually helium, hydrogen, or nitrogen [15], is fed continuously through the chromatographic column at a constant flow rate. The purity of the gas used is recommended to be at least 99.995 % [16].

Capillary gas chromatography columns, which are usually made of quartz, glass or steel, are the most commonly used type of column. To protect the column from degradation, the outside of the quartz capillary is covered with a flexible polymer. The column itself is coated on the inside with a stationary phase, which allows the separation of the different components of

the test mixture. The most commonly used stationary phases include squalane ($C_{30}H_{62}$), polysiloxanes, alcohols, esters, and nitriles. Polysiloxanes are typically preferred as the stationary phase, as they can be easily modified and adapted to various compounds [17, 18].

The movement of analytes through the column is achieved through the flow of the carrier gas and the temperature of the oven in which the column is placed. The temperature of the oven is carefully chosen to ensure that all the analytes move through the column, and a temperature programming mode can be used for more complex mixtures.

The sample introduction system (injector) (Figure 2) comprises an automated syringe sampling system and an evaporator [19]. The sample is injected into the vaporizer with a syringe, where it is rapidly vaporized and transported to the column by the gas flow. The evaporator operates under precise thermostatic control. To achieve optimal performance, it is advisable to set the temperature at 20-30°C above the boiling point of the analyte with the highest vaporization temperature.

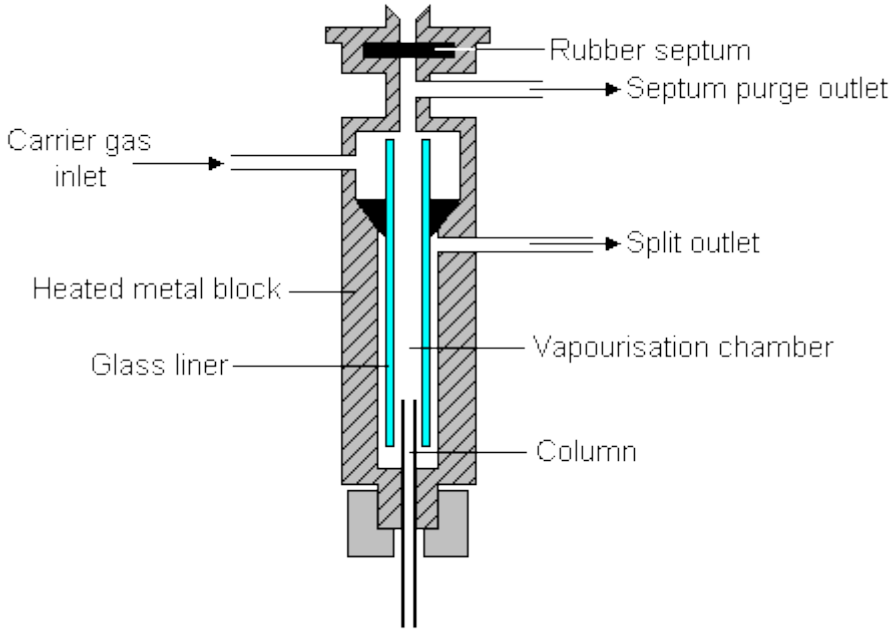

Figure 2. Injector [20].

The flame ionization detector (FID) (Figure 3) has been chosen as the main detector for this thesis. An important benefit of the FID is its remarkable consistency in response, regardless of variations in temperature or gas flow rate, which significantly enhances its overall reliability. Furthermore, the FID

has a high sensitivity and a wide range of possible analytes, making it a versatile choice for various types of analyses [21, 22].

To measure the electric current that flows through an ionized gas between two direct current (DC) electrodes, the FID uses a flame that burns a mixture of hydrogen and air. When the analytes enter the flame, they become ionized, leading to a decrease in the resistance between the electrodes and a subsequent change in current, which is recorded by the detector [23]. To ensure that incoming analytes do not condense, the detector is thermostatically controlled. The temperature in the detector is usually maintained at 20˚C higher than the maximum temperature of the chromatographic analysis in the chromatograph to avoid condensation of the analytes.

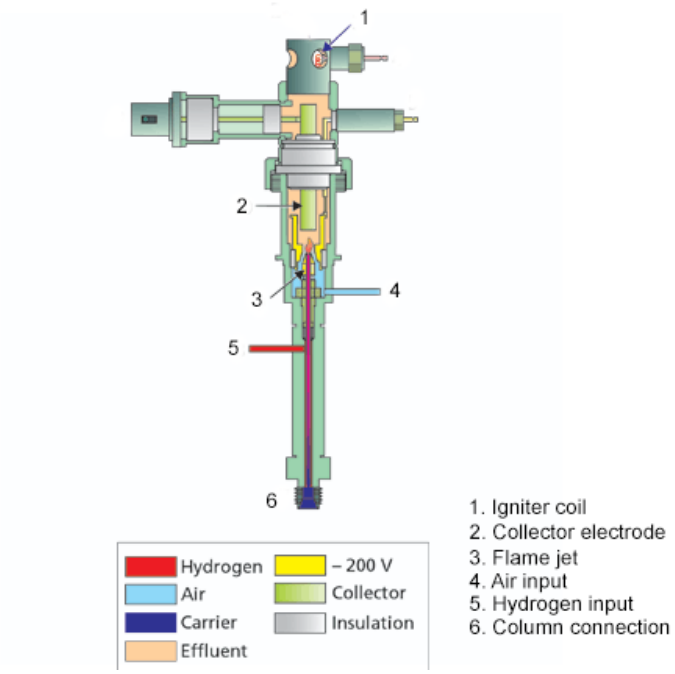

Figure 3. Flame ionization detector [24].

A single quadrupole mass spectrometer (MS), used in part of this thesis, operates based on the principle of mass-to-charge ratio (m/z) separation using a single set of quadrupole rods (Figure 4). The main components of a single quadrupole mass spectrometer for GC include the ion source, quadrupole mass filter, and ion detector.

Ionization: The GC column separates the compounds in the sample mixture, and the eluting compounds are introduced into the mass

spectrometer via the ion source. In the ion source, the compounds are ionized, typically by electron impact or chemical ionization techniques, resulting in the formation of positively charged ions.

Quadrupole Mass Filter: The ionized ions are then directed into the quadrupole mass filter, which consists of four parallel metal rods. These rods are maintained at specific radio frequency and direct current voltages. The combination of these voltages creates a quadrupole electric field. Ions with specific m/z can pass through the quadrupole and reach the detector.

Mass Selection: The quadrupole mass filter acts as a mass-selective filter. By adjusting the RF and DC voltages applied to the rods, the quadrupole allows only ions with a specific m/z ratio to pass through, while other ions are transmitted out of phase and do not reach the detector.

Ion Detection: The ions that successfully pass through the quadrupole mass filter are detected by an ion detector. Commonly used detectors in single quadrupole GC-MS systems include electron multipliers or Faraday cups.

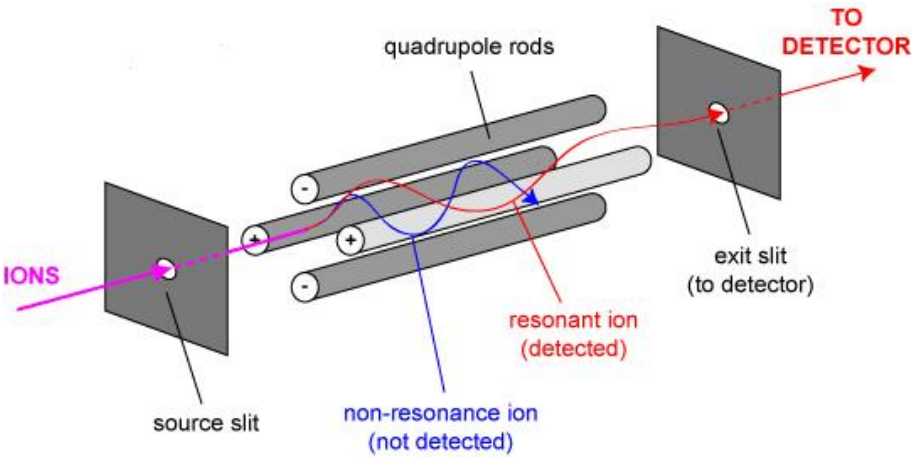

Figure 4. Single quadrupole mass spectrometer [25].

1.2. Peak Broadening

In recent decades, there has been a significant increase in the number of complex mixtures that need to be analyzed, consisting of several dozen or even hundreds of compounds. This has presented a challenge for specialists in separating multicomponent mixtures, particularly in industries such as oil,

food, and biotechnology. As a result, there has been a growing need to improve gas chromatography techniques in order to enhance the separation of compounds and the efficiency of analysis.

One way to improve peak capacity, or the number of components that can be separated, is to increase the duration of the analysis. However, this approach can be time-consuming. Alternatively, peaks can be narrowed to enable the separation of more components in a shorter amount of time. To achieve narrower analyte peaks, it is important to understand the factors that influence the spread of analyte peaks in the chromatogram.

Analyte peaks spread due to two groups of processes: those occurring in the column and those occurring before and after the column [26, 27]. In order to model the peak shape, the Gaussian model is commonly used, which represents the cumulative peak broadening as the sum of the broadening processes occurring both in and outside the column.

$$\sigma_{peak}^2 = \sigma_{system}^2 + \sigma_{column}^2 \quad (1)$$

here σ_{system}^2 is peak broadening caused by the processes outside the column, and σ_{column}^2 is broadening caused in the column.

1.2.1. Peak Broadening in column

In gas chromatography, the broadening of peaks in the chromatographic column during analysis decreases the column's efficiency. The equation of peak propagation was first described by J.J. van Deemter in 1956:

$$H = A + Bu + C \times u \quad (2)$$

where A is representing Eddy diffusion, B representing molecular diffusion, C representing resistance to mass exchange between phases, and u representing the linear velocity of gas flow [28].

As solute molecules pass through the column, they travel through different paths that differ in length (Figure 5). Because of this difference in path length, solute molecules entering the column at the same time, exit the column at different times. The result is band broadening. Eddy diffusion is no longer relevant in modern capillary gas chromatography columns, as they are not usually filled with solid sorbent particles.

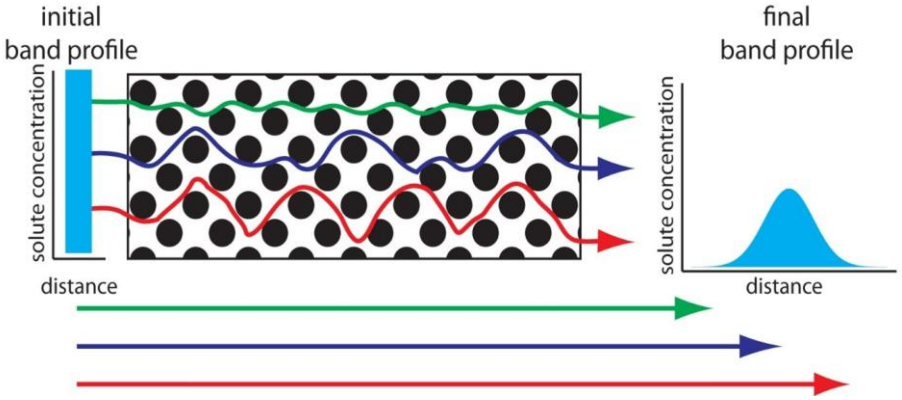

Figure 5. Eddy diffusion (multipath) broadening in chromatography [29].

The second factor for band broadening is the solute's longitudinal diffusion in the mobile phase. Solute molecules are constantly in motion, diffusing from regions of higher concentration to regions of lower concentration. The result is an increase in the solute's band width. The effect of longitudinal diffusion on a solute's band broadening. Two horizontal cross sections through the column and the corresponding concentration versus distance profiles are shown (Figure 6), with (a) being earlier in time. The red arrow shows the direction in which the mobile phase is moving.

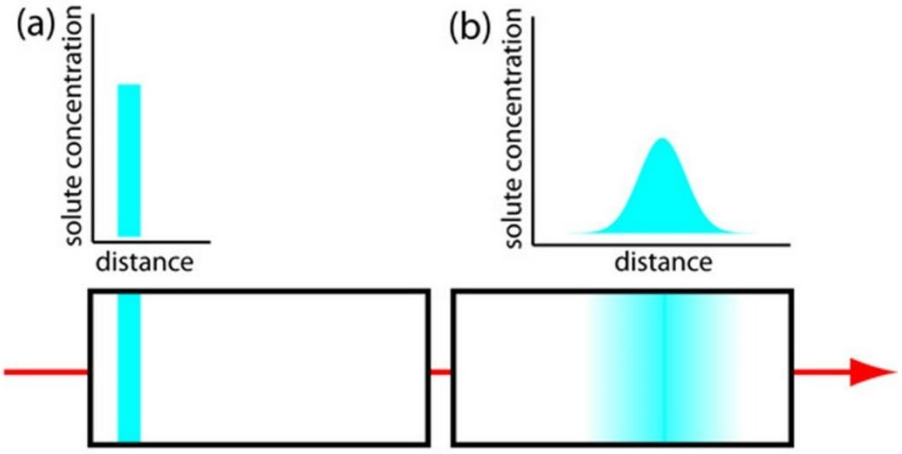

Figure 6. Longitudinal (molecular) diffusion broadening in chromatography [30].

As the solute passes through the column it moves between the mobile phase and the stationary phase. This movement between phases is called mass

transfer. Band broadening occurs if the solute's movement within the mobile phase or within the stationary phase is not fast enough to maintain an equilibrium partitioning of solute between the two phases (Figure 7). On an average, solute molecules in the mobile phase move further down the column before passing into the stationary phase. Solute molecules in the stationary phase, on the other hand, take longer than expected time to move back into the mobile phase.

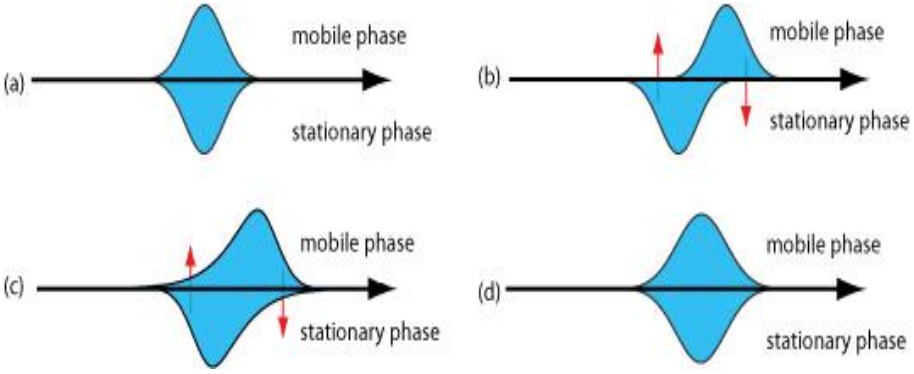

Figure 7. Mass transfer broadening in chromatography [31].

1.2.2. Peak broadening outside column

To enhance the separation efficiency of gas chromatography, it is necessary to minimize peak broadening that occurs before or after the column. The extent of peak broadening depends on the design characteristics of the chromatograph. The evaporation chamber of the vaporizer has a larger volume compared to the column, leading to longitudinal diffusion, which slows down the entry of the sample into the column. To mitigate this, reducing the evaporator chamber's volume, using a narrower diameter column or employing gas flow split mode is recommended (Figure 8).

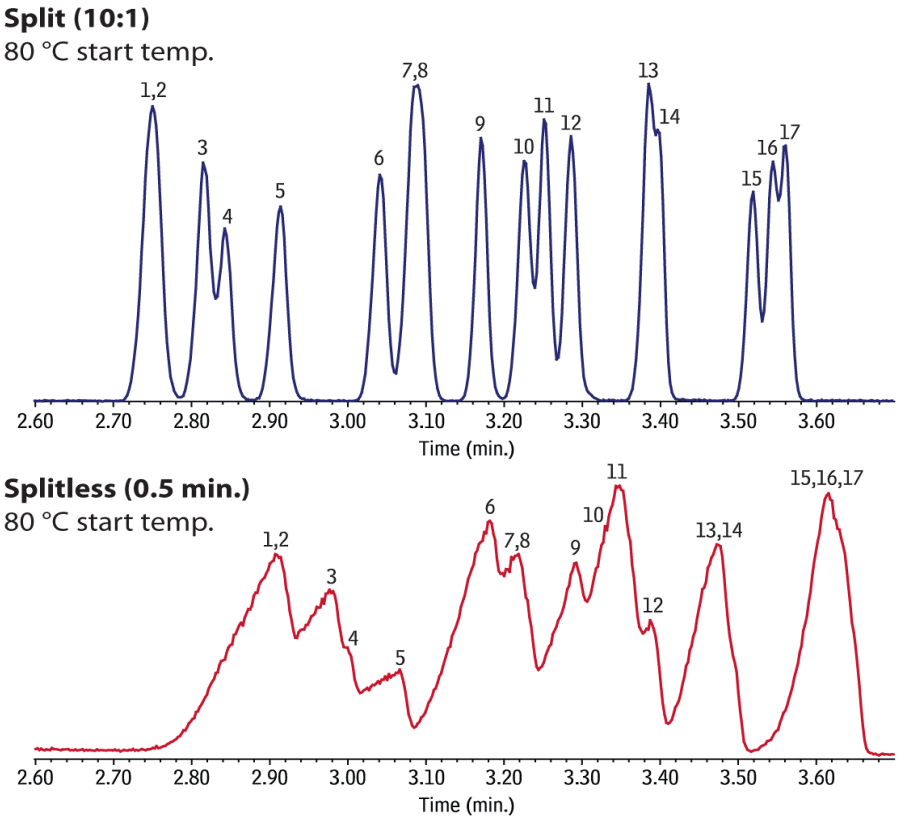

Figure 8. Peak shape comparison using split (10:1) and splitless injection [32].

Split injection became a standard option for majority of GC methods. With split injection method, a syringe injects the sample into the GC inlet, while the split vent is opened. The carrier gas flow is typically high, causing a majority of the injected sample to be diverted out of the system through the split vent, while the remaining portion enters the chromatographic column for separation (Figure 9). This injection method greatly reduces longitudinal diffusion but sacrifices systems sensitivity, by limiting the amount of analyte, that is injected.

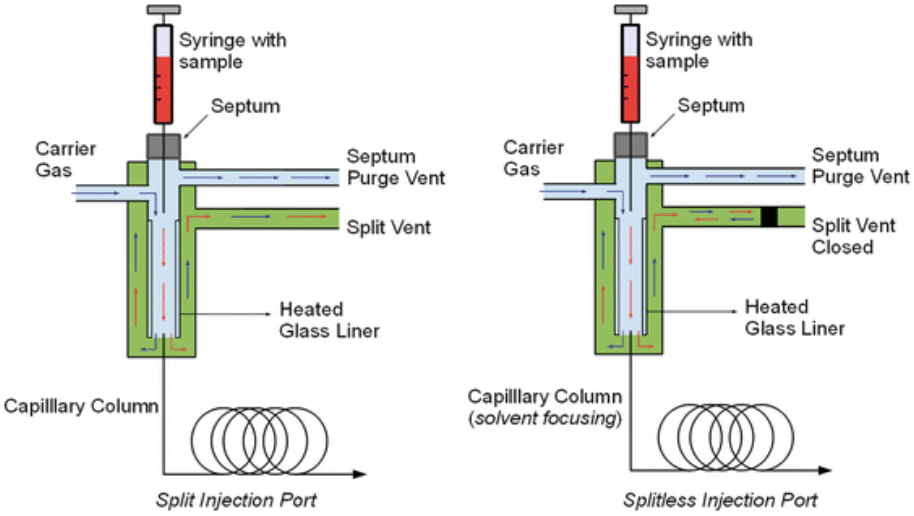

Figure 9. GC injector in split and splitless injection modes [33].

Similarly, peak broadening occurs in the detector due to the cell volume being larger than the column. To counteract this, reducing the detector cell volume is the conventional approach but with modern detectors, this approach has reached a limit. To address this, sometimes additional gases are used to feed the detector, promoting the movement of analytes through the detector cell and minimizing peak broadening.

1.3. Enrichment techniques

In a number of disciplines, including flavor [34], pheromone chemistry [35], volatiles from construction materials [36], contaminants in food [37, 38], and environmental chemistry [39], gas chromatography is a crucial separating tool. Relevant substances are frequently found in really low amounts (ppb, ppt, and lower). Direct analysis is not an option in many situations since it would require an excessively high sample volume. Additionally, the sample matrix is frequently incompatible with GC since it could contain sizable amounts of non-volatile material or be naturally non-volatile (such as soil or building materials). This necessitates moving the analytes to a matrix that is more GC-friendly. Ideally, this stage should also involve sample enrichment [40, 41]. The limitations of various techniques are examined, and a few popular concentration techniques are briefly described in this chapter.

1.3.1. Liquid-liquid extraction

One of the earliest methods of enrichment still in use today is liquid extraction. The extraction procedure is predicated on the creation of an equilibrium where the analytes are divided between two immiscible liquids, typically an organic solvent and water (Figure 10).

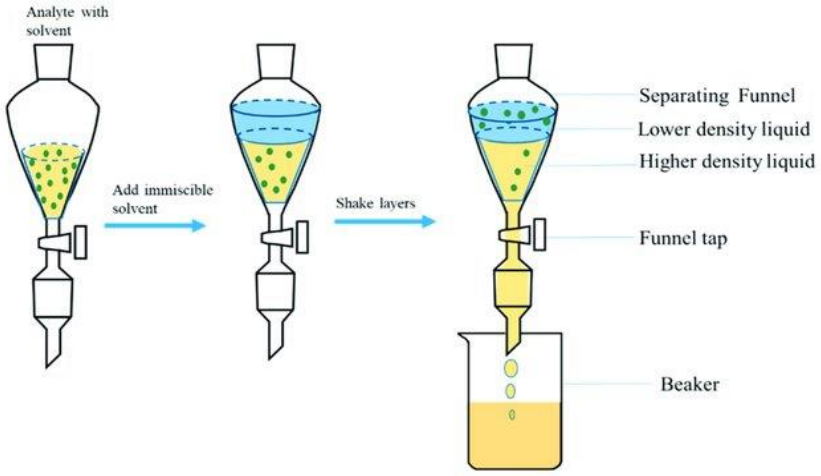

Figure 10. Liquid-liquid extraction [42].

The extraction yield can be enhanced by increasing the volume of solvent or by repetitive extraction with small portions of solvent. A powerful means to improve the selectivity of the extraction is to utilize a combination of solvents. Liquid-liquid extraction is quite laborious, but fully automated methods using segmented flow extraction combined with GC have been reported [43, 44]. However, there is a tendency to replace liquid-liquid extraction by other enrichment methods. This is related to the inconvenience of handling solvents, which is a negative factor when personal safety aspects are considered.

1.3.2. Concentration by evaporation

Concentration by evaporation is a sample preconcentration technique widely employed in gas chromatography for enhancing analyte concentration by solvent removal and sample volume reduction [45]. This technique proves particularly valuable when dealing with large volume samples or when the

analyte of interest is present in low concentrations. Evaporation can be achieved through reduced pressure techniques, such as vacuum evaporation [46], by utilizing nitrogen blow-down [47] or rotary evaporation methods [48]. As the solvent evaporates, the remaining residue in the vessel becomes enriched with the target analytes. The resulting residue is then reconstituted in a suitable GC-compatible solvent, and the dissolved analytes are subsequently injected into the GC system for separation and detection. Concentration by evaporation provides a means to increase the sensitivity of GC analysis, especially when working with trace-level analytes.

1.3.3. Adsorption-based techniques

Adsorption-based extraction, commonly known as solid-phase extraction (SPE), is a pivotal technological advancement extensively employed in trace analysis [49-51]. In this method, the sample is typically passed through a cartridge containing an adsorbent material to enrich the analytes [52]. The captured analytes can be desorbed by extracting them with a small volume of organic solvent after sampling. A simpler desorption method involves thermally desorbing the analytes by heating the adsorbent in the presence of an inert gas flow. This method can then be coupled with gas chromatography (GC) to cryotrap and analyze the analytes. The thermal desorption procedure offers the advantage of clear chromatographic peaks without significant interference from solvent peaks, making it well-suited for volatile substance analysis. Adsorption have found successful applications in a wide range of fields, including clinical examination of bodily fluids [53], analysis of environmental contaminants in air and water [54-56], among others. The strong molecular interactions between adsorbents and analytes result in high partition coefficients and significant sample capacities. Additionally, an appropriate adsorbent minimizes water retention [57].

However, SPE is accompanied by several limitations. Prior to desorption, it is necessary to remove residual water from the trap, typically achieved through a nitrogen flow [58]. Unfortunately, this drying process may result in substantial losses of trapped volatiles. The catalytic activity of adsorbents further restricts the technique's applicability in enriching reactive analytes [59]. Catalytic activity may already manifest at the low temperatures during sample collection [60]. Furthermore, there are two factors that hinder the use of thermal desorption. First, thermal desorption is primarily suitable for thermostable analytes due to the high temperatures required to release the molecules in the first place [61, 62]. Second, when polymer-based adsorbents

are employed, heating can generate degradation products, especially in the presence of oxygen and water [57, 63, 64]. These degradation products can significantly interfere with the analysis, posing a serious concern.

1.3.4. Sorption based techniques

In order to overcome some of the disadvantages associated with the use of adsorbents, extraction techniques based on sorption have been developed [65, 66]. The principle of sorption is based on the dissolution of analytes into an immobilized liquid phase, typically employed in GC as a stationary phase, and does not rely on the temporary storing of the analytes on a limited number of adsorptive sites. The degree of interaction between extraction phase and analyte is not affected to the same extent by variations in concentration of the analytes or matrix constituents [67-69]. In fact, the process of sorption is in a sense similar to liquid-liquid extraction, without using solvents.

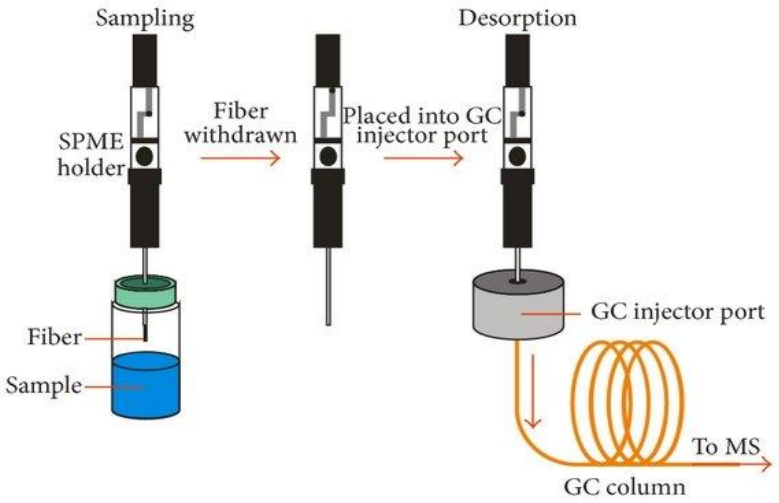

Figure 11. Analysis with solid phase microextraction-gas chromatography [70].

Solid-phase microextraction (SPME) is a powerful sample preparation technique widely used in GC for the extraction and enrichment of volatile and semi-volatile compounds. It was introduced in 1990 by Pawliszyn et al. [71]. SPME offers several advantages such as simplicity, rapidity, and versatility, making it an invaluable tool in various fields including environmental analysis, food and flavor analysis, pharmaceutical analysis, and forensic

sciences. The technique involves the use of a fiber coated with a stationary phase, typically polydimethylsiloxane (PDMS) or a combination of PDMS and other selective materials, which selectively extracts analytes from the sample matrix in the headspace or through direct immersion. The analytes are adsorbed onto the fiber, and upon desorption, they are introduced into the GC system for separation and analysis (Figure 11).

Furthermore, advancements in SPME have led to the development of novel techniques such as stir bar sorptive extraction (SBSE), which combines SPME principles with a magnetic stir bar for enhanced analyte extraction. SBSE has been successfully applied in the analysis of various sample matrices, including water, beverages, and biological fluids [72]. The sample enrichment device consists of a magnetic stir bar covered by a piece of PDMS tubing. Liquid samples are extracted by placing the device in a flask or vial containing the sample. A magnetic stirrer is used to set the stir bar in rotation, and this enhances the mass transport in the system and thus increases the extraction rate. After an extraction has been carried out, the stir bar device is manually removed from the sample container, optionally rinsed with pure water and finally dried by wiping it with a tissue [73]. Finally, the analytes are desorbed either thermally using a designated desorption unit [74] or by extraction with a suitable solvent [75] (Figure 12).

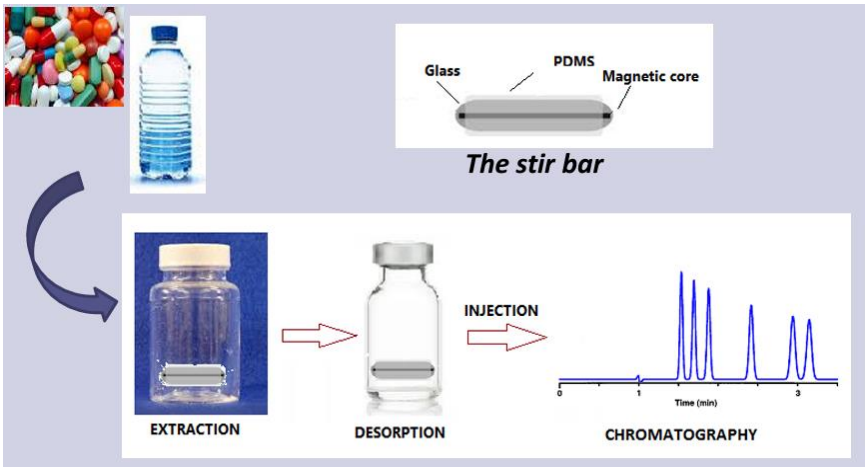

Figure 12. Main steps of stir bar sorptive extraction [76].

1.3.5. Cryogenic concentration

Cryo-enrichment is a sample preconcentration technique that utilizes low temperatures to enhance the enrichment of volatile and semi-volatile

compounds in GC analysis. This technique involves cooling the sample or the sample inlet system to cryogenic temperatures, causing the analytes of interest to condense and concentrate, while non-volatile matrix components remain in the gas phase or are removed. Cryo-enrichment offers several advantages, including increased sensitivity, improved peak resolution, and reduced interference from matrix components, making it a valuable tool in various fields, including environmental analysis, food and flavor analysis, and forensic sciences. Major part of this thesis is focused on cryo-enrichment and this topic therefore is described in more detail.

1.3.5.1. Thermoelectric cooling system

The thermoelectric cooling system is characterized by its simplicity and low maintenance requirements since it doesn't rely on the addition of refrigerant. It works by utilizing Peltier effect to create a heat flux at the junction of two different types of materials [77]. However, its major drawback lies in its relatively limited cooling capacity. Standard commercial refrigeration devices can achieve temperatures as low as -30˚C, which falls short when it comes to concentrating more volatile organic compounds.

In a 2005 study by M. Libardoni [78], a refrigeration unit capable of cooling the column junction to -32˚C was employed. The analytes were subsequently vaporized through sudden heating of the junction. To provide clarity, Figure 13 presents a schematic of the focusing system utilized for two-dimensional chromatography.

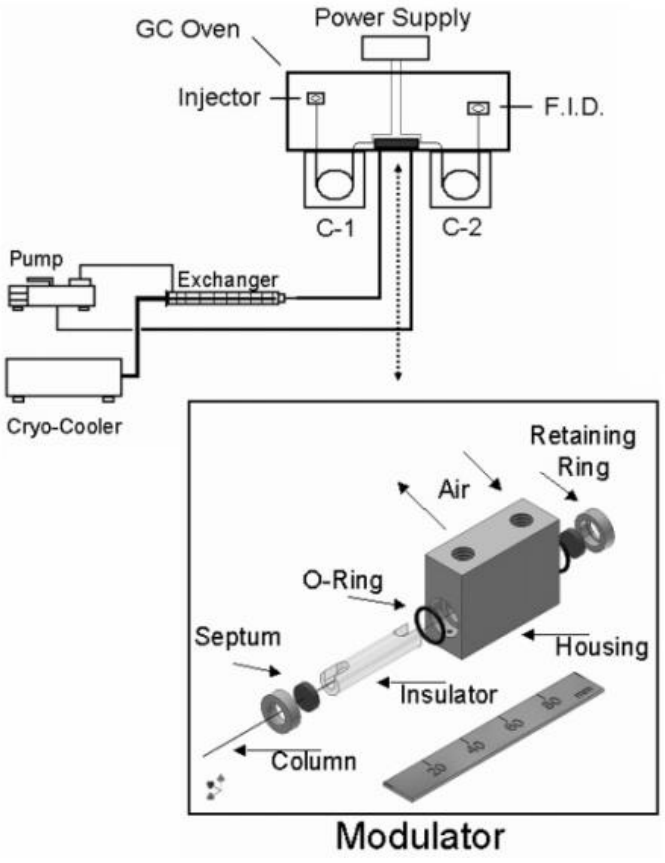

Figure 13. Schematic diagram of a column refrigeration system based on a thermoelectric cooling.

During the research, an interesting observation was made regarding the impact of varying the length of the frozen joint on the widths of the peaks, particularly the n-octane peak. For cryogenic junction lengths of 6, 8, and 15 cm, the peak widths were measured at 72, 61, and 137 ms, respectively. As depicted in Figure 14, it is evident that the 6 cm junction was too short, resulting in incomplete retention of the analyte and consequently causing peak distortion. On the other hand, the 8 cm junction proved to be adequate for retaining the entire compound, while the 15 cm junction led to broadening of analyte peaks due to diffusion and insufficient low temperature.

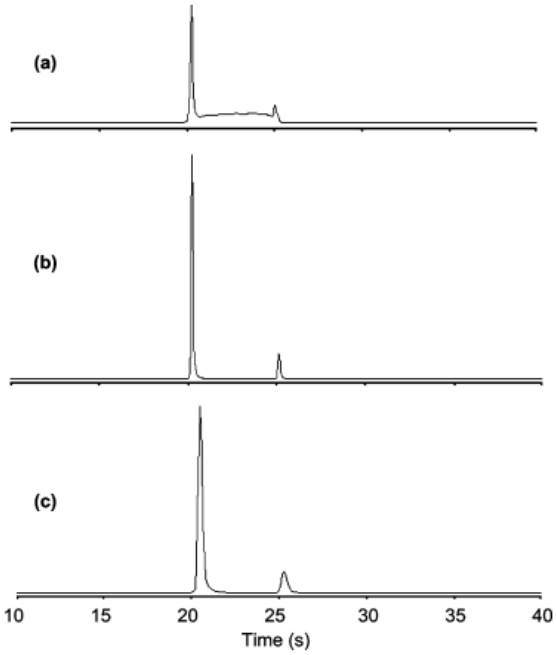

Figure 14. Octane peaks at different lengths of the frozen zone: a) 6 cm, b) 8 cm, c) 15 cm.

One drawback of this design lies in the limited cooling zone of the column, which imposes restrictions on the injection volume. In the study, a 1 µl injection was utilized with a flow split ratio of 1:100. The achieved system sensitivity was approximately 10 parts per billion (10 ppb).

1.3.5.2. Compressed fluid system

One of the most effective methods for column cooling is utilizing compressed liquid, as it allows for rapid cooling and achieves extremely low temperatures (approximately -200 ˚C). However, a drawback of this method is the significant consumption of cooling agents. Nevertheless, it remains one of the most commonly employed approaches for cryo-enrichment.

Italian researchers successfully utilized cryo-focusing in their study on volatile cyclic acetals emitted from a rubber factory, aiming to identify compounds at sub-ppb concentrations [79]. Thermal desorption technology was utilized for sample collection and introduction into the gas chromatograph. Air in the vicinity of the factory was pumped through thermal

desorption tubes, some of which were filled with a sorbent to capture volatile organic substances. The volatile compounds were introduced into the system by desorbing them from the sorbent at high temperatures (Figure 15). However, thermal desorption resulted in peak broadening, which was mitigated by implementing a cooling trap and flow splitting. Nevertheless, the analytes still entered the chromatograph as a band with a spread. To address this, a liquid nitrogen system was employed to focus the peaks and enhance separation.

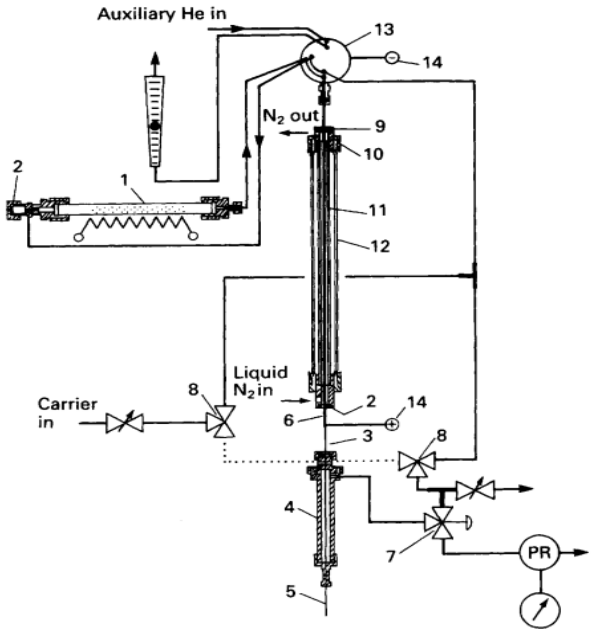

Figure 15. Schematic diagram of a column cooling system based on N_2.

Once the analytes entered the cold zone, they were effectively focused. Upon sudden heating, they evaporated, allowing for subsequent chromatographic analysis of the mixture. This technology enabled the detection of compounds at concentrations of approximately 1 ppt by mass.

1.3.5.3. Cooled gas system

Cooled gas system is using gas, that have been cooled to cryogenic temperatures to cool down part of the GC column inside or outside the GC system.

The chilled nitrogen gas system was used by American researchers from the University of Washington [80]. Its operating principle is a 6 cm long metal MXT-5 (stationary phase dimethyl polysiloxane) column installed behind the gas chromatograph sample inlet system, whose 3 cm section is cooled with nitrogen gas. The nitrogen gas is cooled by passing it through a spiral tube that is placed in a liquid nitrogen container. Cold nitrogen gas reaches the cooled part of the column through a Teflon tube. Once the analytes have entered this zone, they are focused and then dislodged by heating a part of the metal column. Heating occurs by passing an electric current through a metal column (Figure 16). Due to the internal resistance of the metal, the column heats up very quickly, the analytes evaporate and are transported by the gas flow to the traditional capillary gas chromatography column, where their separation takes place later.

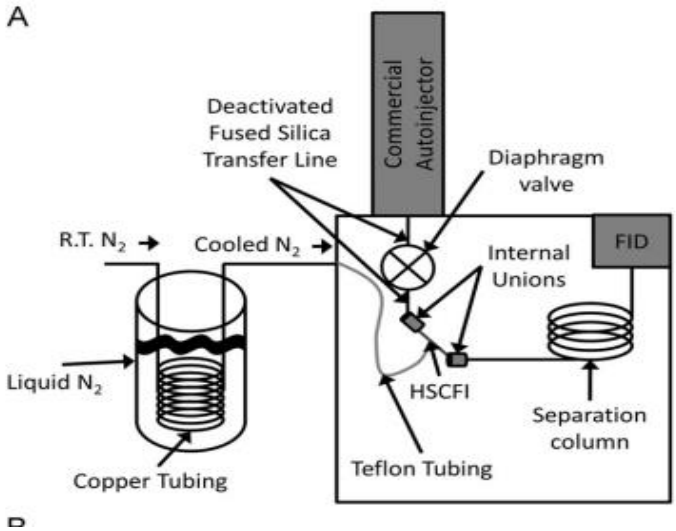

Figure 16. Schematic diagram of a column refrigeration system based on cooled gas

This system was used to analyze a gasoline sample. The separation of all gasoline components took 200 s, and the width of one peak varies from 400 to 480 ms. Using an average peak area of 440 ms, the calculated peak capacity is 460 or 140 peaks per minute. In comparison, using a classical chromatographic system, with a 1:200 flow split, the peak area is ∼2 s. Peak capacity ~30 peaks per minute. A cold gas cooled sample focusing system increased the resolution by a factor of 4.7.

1.3.5.4. On-column cryo-enrichment

Column focusing, unlike analyte focusing, has received relatively limited research attention. In most cases, samples are concentrated during their preparation before undergoing chromatographic analysis. However, on-column concentration offers a more convenient approach as it eliminates the need for additional steps in sample preparation. The concentration of the sample on the column is achieved by cooling a section of the column to a temperature below the melting point of the analyzed compound. Sung-Tong Chin et al. [81] accomplished this by employing a moving column cooling system, which they applied to two-dimensional chromatography (Figure 17).

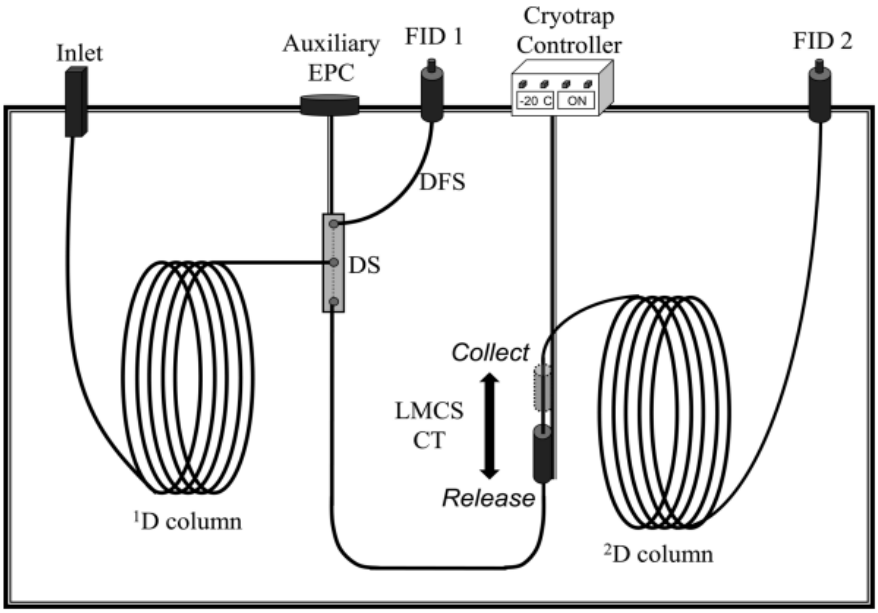

Figure 17. Column concentration system for analytes.

In this approach, analytes are injected into the first column and travel towards the FID detector. However, at the desired time, the flow can be switched to the initially cooled second column using a Dean switch [82]. The analytes come to a halt upon entering the cold zone. Injections are repeated until the desired amount of analytes reaches the cold point. Subsequently, the analysis in the second column commences by reversing the direction of the refrigeration device against the gas flow and gradually increasing the temperature of the gas chromatograph oven. The frozen analytes are re-

evaporated and separated in the second column before reaching the FID detector.

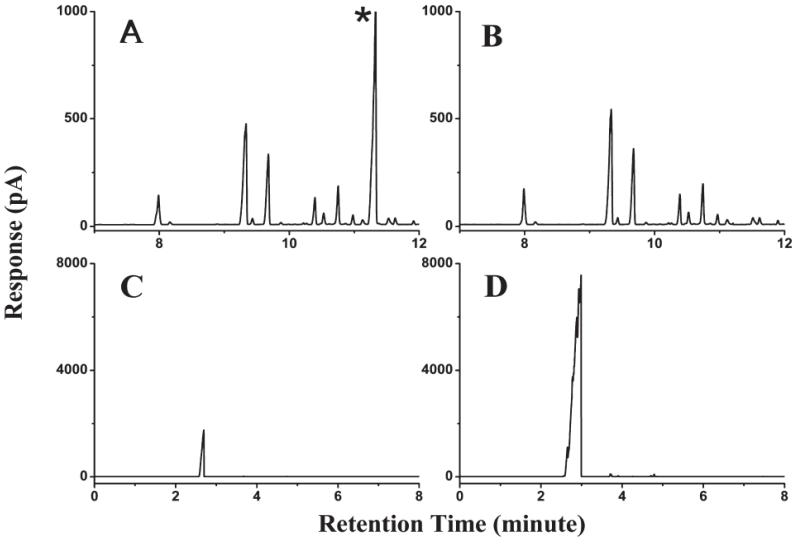

Figure 18. Chromatograms of 0.2% Peppermint oil sample: A – Full chromatogram with FID 1; B – Chromatogram with FID 1 and cut menthol peak; C – FID 2 chromatogram when a single injection is accumulated; D – FID 2 chromatogram after accumulating fifteen injections.

In the study, menthol was chosen as the analyte from a 0.2% solution of peppermint oil in acetone. A 1 µl injection volume with a 1:20 gas flow split was utilized. After 15 injections, the peak area reached 89% of the area of the first injection multiplied by 15 (Figure 18). Up to 50 injections were measured using a standard menthol solution. However, the concentration of menthol in the standard solution was approximately 40 times lower than in the oil sample. It was observed that the number of injections that can be held is determined by the internal capacity of the refrigerated section of the column. When this capacity is exceeded, the column becomes overloaded, resulting in analyte loss and pronounced peak asymmetry. To enhance the peak shape of the analytes and increase the number of injections that can be held, the authors recommend either freezing a longer section of the column or employing a column with a thicker stationary coating layer.

1.4. Measured compounds

This chapter will provide a more detailed description of certain compounds that were measured using the cryo-enrichment module throughout this thesis.

1.4.1. Terpenes

The term "terpenes" is derived from turpentine, also known as "pine resin." Turpentine, a fragrant balsam obtained from cut pine wood, contains compounds initially referred to as terpenes. These natural compounds, composed of isoprene subunits mainly sourced from plants, are traditionally known as terpenes [83]. In scientific literature, you may also come across "terpenoids," a class of secondary metabolites derived from terpenes, often rich in cyclic structures and oxygen, both stemming from isopentenyl and dimethylallyl diphosphates [84].

Terpenes, the largest group of phytochemicals, encompass over 100 compounds identified in hemp, responsible for the diverse scents and flavors in different cannabis strains. Terpenes are categorized based on the number of repeating 5-carbon units, including monoterpenes (C-10), sesquiterpenes (C-15), diterpenes (C-20), and triterpenes (30 carbon atoms) [85, 86]. Terpene production and distribution within the plant are influenced by factors such as extraction methods, environmental conditions, and plant maturity. Monoterpenes dominate the volatile terpene profile and include D-limonene, β-myrcene, α- and β-pinene, terpinolene, and linalool. Cannabis extracts also feature abundant sesquiterpenes like β-caryophyllene. Triterpenes can be found in hemp roots, fibers, and seed oil, with compounds such as friedelin, β-amyrin, and cycloartenol [85].

Monoterpenes are isoprene dimers with the molecular formula $C_{10}H_{16}$. They are mildly volatile and possess pleasant fragrances, serving as the foundation for plant essential oils. Monoterpenes can be categorized into two primary structural groups: acyclic, featuring an open carbon chain (e.g., myrcene, ocimene), and cyclic, which may consist of single cycles (like limonene) or multiple cycles (as seen in pinenes).

Sesquiterpenes are C15-terpenes composed of three isoprene units. These compounds can exist as hydrocarbons or can take on oxygenated forms, which include lactones, alcohols, acids, aldehydes, and ketones [87].

Currently, only a small fraction of terpenes has been thoroughly investigated. Hemp stands out as a prominent source of these compounds.

Terpenes and terpenoids exhibit a wide array of biological and pharmacological activities, including antifungal, antiviral, anticancer, anti-inflammatory, antihyperglycemic, antiparasitic, antioxidant, and antimicrobial effects. Bellow, you can find examples of some significant properties of terpenes found in this plant:

β-Caryophyllene: This terpene possesses gastroprotective, analgesic, anticancer, antifungal, antibacterial, antidepressant, anti-inflammatory, antiproliferative, antioxidant, anxiolytic, analgesic, and neuroprotective properties.

α-Pinene: Known for its antibacterial, antioxidant, anti-inflammatory, bronchodilator, antiseptic, and gastric protective qualities.

1.4.2. Propiconazole

Fungicides are a vital class of chemical agents used in agriculture, horticulture, and forestry to combat fungal diseases that can devastate crops and plants. These compounds are specifically designed to inhibit the growth and reproduction of fungi, which are responsible for a wide range of plant diseases. Fungicides work by disrupting the fungal life cycle, primarily targeting processes such as spore germination, mycelial growth, and the formation of fungal cell walls [88]. They can be applied through various methods, including foliar sprays, soil drenches, and seed treatments, depending on the specific needs of the crop and the nature of the fungal threat. Fungicides are an essential tool for farmers and growers in safeguarding the health and productivity of their crops, ultimately contributing to food security and the overall sustainability of agriculture.

It's important to note that the use of fungicides requires careful consideration and responsible application. Also, governments around the world are limiting the type of fungicides and their quantities used in agriculture and their residues in food products. For example propiconazole, recently was banned in EU [89]. EU commission set propiconazole allowed limit to zero, but it is clear that there is no valid way to measure absolute zero in analytical chemistry. Propiconazole allowed amount (0,1 mg/kg; 0,1 ppm, sum of all isomers) was tied to LC-MS/MS method LOD [90, 91].

Propiconazole is a widely used systemic fungicide known for its effectiveness in preventing and controlling fungal infections in various crops, including cereals, fruits, and ornamental plants. It belongs to the class of triazole fungicides and acts by inhibiting the biosynthesis of ergosterol, a vital component of fungal cell membranes [92]. By disrupting this key process,

propiconazole hinders the growth and reproduction of a broad spectrum of plant-pathogenic fungi. Its versatility and efficacy in managing diseases like powdery mildew, rusts, and leaf spot have made it a valuable tool in modern agriculture. Moreover, propiconazole is recognized for its systemic nature, allowing it to be absorbed by plants and translocated throughout their vascular systems, offering prolonged protection against fungal pathogens.

1.5. Vapor-compression refrigeration

Vapor-compression refrigeration is a highly efficient and widely utilized refrigeration technology based on the thermodynamic principles of phase change cooling [93]. This method involves the cyclic process of compressing and condensing a refrigerant gas to extract heat from a low-temperature environment and reject it to a higher-temperature medium. The refrigerant undergoes phase transitions between its liquid and vapor states within a closed-loop system, comprising four main components: the compressor, condenser, expansion valve, and evaporator (Figure 19).

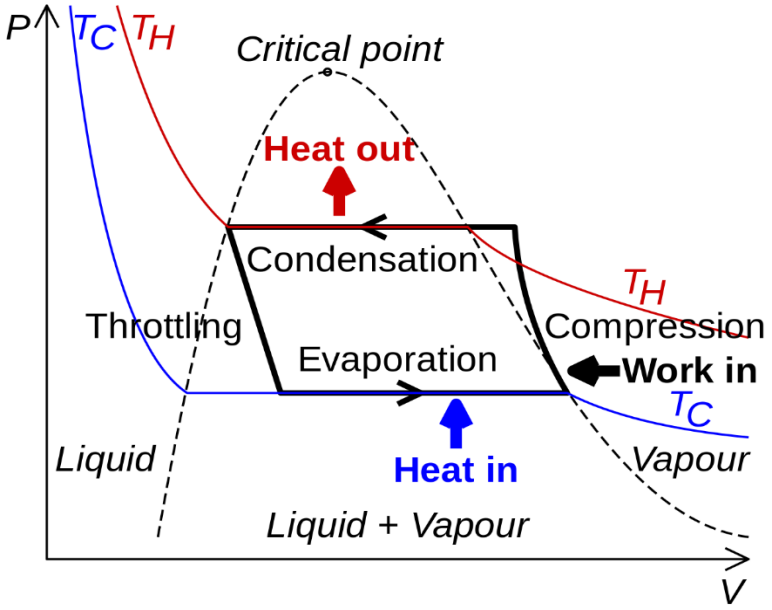

Figure 19. A representative pressure–volume (P-V) diagram for a refrigeration cycle [94].

The refrigeration cycle begins in the evaporator, where the low-pressure, low-temperature liquid refrigerant absorbs heat from the surrounding

environment, such as the indoor air in cooling applications. As a result, the refrigerant undergoes vaporization, changing into a low-pressure vapor. This process occurs at a constant temperature, allowing the refrigerant to efficiently extract heat from the refrigerated space while keeping the temperature constant.

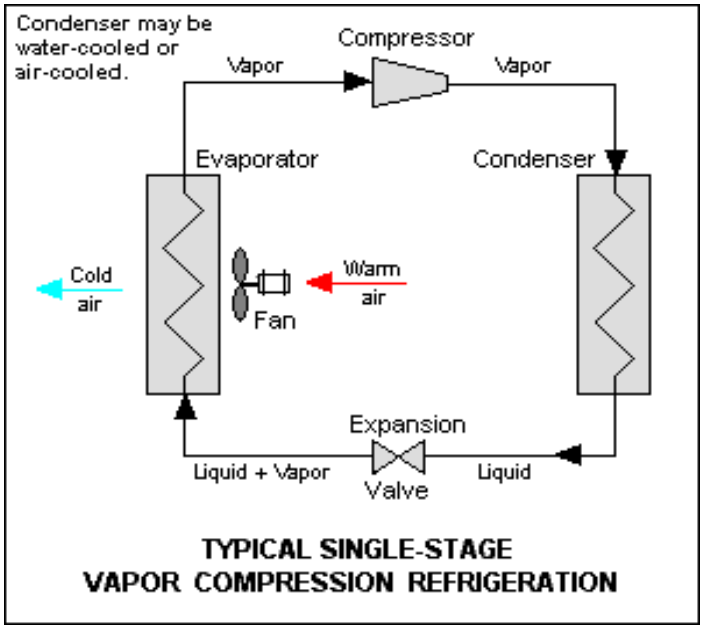

Figure 20. Vapor compression refrigeration [95].

The vaporized refrigerant is then compressed by the compressor, increasing its pressure and temperature. The compression process raises the refrigerant's enthalpy, which corresponds to the energy added to the system. The high-pressure, high-temperature vapor is then conveyed to the condenser, where it releases heat to the surrounding environment, typically through air or water cooling. As the refrigerant undergoes condensation, it transitions back to a high-pressure liquid state, effectively transferring the heat absorbed from the evaporator to the external environment.

Following condensation, the high-pressure liquid refrigerant passes through the expansion valve, which reduces its pressure rapidly. As a result, the refrigerant's temperature and enthalpy decrease substantially, allowing it to enter the evaporator again as a low-pressure liquid. The cycle then repeats, continually removing heat from the refrigerated space and dissipating it to the ambient environment through the condenser.

This method allows to reach temperatures up to around -40 ºC. To cool down to lower temperatures two-stage vapor-compression refrigeration is used. Where second stage is cooled by the first ant that allows cooled to reach temperatures of -70 ºC and lower [96].

Vapor-compression refrigeration has become a cornerstone technology in various sectors, including air conditioning, refrigeration, and industrial cooling. Its efficiency, adaptability, and ability to maintain stable temperature conditions make it the preferred choice for a wide range of applications. Ongoing research and development efforts aim to enhance the performance and environmental sustainability of vapor-compression refrigeration systems, contributing to the advancement of modern refrigeration technologies.

1.6. Chemical Metrology

Chemical metrology [97, 98] occupies a pivotal role within the realm of analytical science, offering a comprehensive and structured approach to establishing accurate and consistent measurements of chemical composition and properties. At its core, chemical metrology operates on the principles of measurement traceability, ensuring that the values obtained from instruments or experiments are ultimately linked back to internationally recognized and standardized measurement units. This requires the use of certified reference materials and primary measurement standards, which act as anchor points in the measurement process. Through a procedure of calibration against these standards, analytical instrument results are compared to a reliable scale, enabling the determination of chemical quantities with a high degree of accuracy.

In combination with traceability, chemical metrology places significant emphasis on the assessment of measurement uncertainty. Quantifying the potential variability and error associated with measurements is a must to discover the reliability and confidence of the reported results. This requires a systematic evaluation of all possible sources of uncertainty, encompassing instrumental fluctuations, environmental conditions, operator variability, and inherent limitations of the measurement process.

Chemical metrology actively promotes interlaboratory proficiency testing and collaborative initiatives, whereby different laboratories engage in comparative studies to validate and demonstrate the robustness of their measurement methodologies. This iterative process not only refines measurement protocols but also bolsters the credibility of scientific findings by substantiating their reproducibility across diverse settings.

The significance of chemical metrology is pronounced in various fields: from environmental monitoring and food safety assessment to pharmaceutical analysis and industrial quality control. By underpinning the accuracy and credibility of chemical measurements, chemical metrology ensures that scientific research and industrial applications alike rest upon a robust and universally agreed-upon framework of precision and trueness.

Chemical metrology not only describes the accuracy of the measurement devices and methods that are used in the industry. It also covers the development and testing of new type of measurement procedures or instrumentation. Major part of this thesis was done, while working in department of metrology and all the research was completed following the principles of metrology (accuracy, precision, traceability, reproducibility and uncertainty evaluation).

1.7. Method Validation

The purpose of any analytical method is to provide consistent, reliable, and accurate data. For this reason, the performances and the limitations of the method, as well as the external influences which may modify these features, must be determined prior to its use [99].

The validation consists in the determination of well-defined quality parameters: selectivity, specificity, calibration curve, linearity, calibration range, accuracy, precision, recovery, uncertainty, limit of detection, limit of quantification (LOQ), decision limit, detection capability, robustness, stability, system suitability [100, 101].

The following parameters are set to validate the method:

- linearity of the calibration curves (limits and correlation coefficient),
- limit of detection (LoD),
- limit of quantification (LoQ),
- accuracy of the measurement,
- precision of the result.

1.7.1. Linearity of the calibration curves

The linearity is first evaluated visually by plotting the average values of peak area versus the analyte amount. But for proper evaluation, these data are treated by least-square linear regression to calculate the constants of the calibration curve and evaluate the quality of the linear relationship (the

determination coefficient, R^2, and the residual sum of squares). This method aims to minimize the difference between the experimental and the calculated peak area in the tested values. A minimum of five calibration points at increasing concentrations, equally spaced, is recommended. Each calibration level should be calculated by three independent replicates. Using this statistical method, the calibration points are "naturally" weighted by the concentration. A y-intercept significantly different from 0 indicates a bias and should be further studied. A R^2 close to 1 indicates adequate linearity, whereas a R^2 close to 0 indicates the total absence of proportionality. A calibration curve is accepted at $R^2 > 0.990$ [102].

The calibration range is the interval between the minimum (lower limit of quantification, LLOQ) and the maximum (upper limit of quantification, ULOQ) amount in samples, in which the analytical procedure provides quantitative results with a suitable level of linearity, accuracy, and precision. It is normally established by the linearity studies. The LLOQ depends on the sensitivity of the method and the ULOQ depends on the saturation of the extraction step and the detector.

Even if the logical process will be to determine the entire calibration range, the evaluation of the linearity over a range spanning 50–150% of the expected concentration or the maximum residue limit (MRL) in real samples is usually enough. Anyway, the minimum acceptable range must be taken depending on the scope of the analysis [103].

1.7.2. Limit of detection (LOD)

In chromatographic methods, the noise is the oscillation of the baseline of the chromatogram at the retention time of the analyte, when injected a blank sample. When a signal near the background noise is obtained, it must be decided if it corresponds to random responses of the blank or to the presence of the analyte. The limit of detection (LOD) is a statistical value that establishes the minimum concentration that provides a signal that can be reliably differentiated from the background noise. The International Council for Harmonisation (ICH) guideline has clearly defined several useful approaches to calculate the LOD [104]:

- Visual evaluation: Injection of samples containing decreasing known concentrations of the analyte. LOD is the minimum concentration providing a distinguishable peak area.
- Signal to noise (S/N): The minimum concentration providing a peak height three times the baseline noise.

- Standard deviation, the 3.3s criterion: The LOD is calculated as 3.3 times the standard deviation of the blank (s0) divided by the slope of the calibration curve. The s0 is the average noise ratio of the background signal (width of the baseline) at the time window in which the analyte is expected, taken with >20 replicates, or as the standard deviation of the y-intercept.

1.7.3. Limit of quantification (LOQ)

The LOQ is the lowest concentration that can be quantitatively determined with accuracy and precision. The quantification of the analyte in the LOD–LOQ range is possible, but with a too high associated uncertainty. Thus, the reported confidence interval would be uninformative. Thus, the result must be simply reported as "concentration between LOD and LOQ." The LOQ would be reasonably close to the LLOQ. The LOQ must be under the minimum concentration expected in a real sample.

As for LOD, LOQ should be determined in matrix sample. ICH [104], EUROCHEM [105], and U.S. Food and Drug Administration (FDA) [106] guidelines have proposed different approaches for the determination of the limit of quantification:

- Visual evaluation: Analyze a series of samples with decreasing concentrations by six replicates. The relative standard deviation (RSD, %) is plotted versus the concentration. The RSD normally increases at lower concentrations. The LOQ is the amount that corresponds to the previously defined required precision [105].
- Signal to noise: The minimum concentration providing a peak height 10 times the baseline noise [104].
- Standard deviation, the 10s criterion: Same as LOD, but 10 times the standard deviation of the blank (s0) divided by the slope of the calibration curve [104].
- Calibration curve: The LOQ is taken at the LLOQ level [106].

1.7.4. Accuracy of the measurement

Accuracy and trueness, while with a different meaning [99], are interchangeably used in the literature [107], and "accuracy" is the preferred term (Figure 21). Accuracy/trueness is the closeness between the concentration provided by the analytical assay (calculated from the peak area

through the calibration curve) and the true value [108]. The "found concentration" is taken as the average of several measurements to minimize the effect the random errors. The accuracy shows the extent to which the systematic errors affect the result, and is quantitatively stated in terms of bias. Thus, it has been highlighted as the most crucial aspect of any analytical procedure [102]. The following materials are recommended, indicated in decreasing order of appropriateness:

- Certified reference materials (CRM) close to the sample: the concentration has been accurately determined by an accredited laboratory.
- Spiked sample: A blank sample is fortified with a known amount of the analyte. In this case, the accuracy is determined as recovery.
- Standard solutions: Not recommended, as the results do not include the matrix effect.
- Real samples analyzed using a reference reliable method: Applicable only if standards are not available.

The obtained values of bias and variability are compared with those established as acceptance level, in order to determine if the differences between the replicates and between the found concentration and the true value are significant.

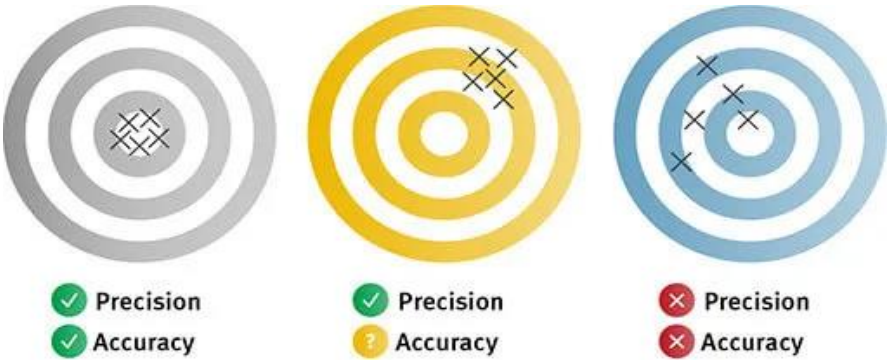

Figure 21. Accuracy and precision comparison [109].

1.7.5. Precision of the result

The precision is defined as the closeness of agreement between the detector responses obtained by several individual measurements of a homogeneous sample, under stipulated conditions [108]. In chromatography, it is the similarity between the values of peak area obtained from independent

analyses of homogeneous aliquots. The precision is provided as dispersion or variability, and quantified through the RSD of the detector response. This value is always positive (the sign of the deviation is neglected), and an RSD close to 0 means an excellent precision. The variability is due to the random errors through the method.

1.7.6. Uncertainty

The uncertainty in measurement refers to the fluctuations in the obtained results. It is a parameter associated with the measured quantity (measurand) that characterizes the dispersion of values that can reasonably be attributed to the measurement [110]. In chromatographic analysis, uncertainty represents the maximum deviation between the measured concentration and the true value, obtainable when analyzing a sample at a specific significance level (α). It quantifies the level of doubt regarding the obtained concentration. The result of an analytical measurement is expressed as a confidence interval (found concentration ± uncertainty), which indicates the region around the routine analysis within which the true value lies with a probability of $(1 - \alpha)$ % [102]. The uncertainty is calculated as the standard deviation of the measured concentration multiplied by the *t*-student factor corresponding to the significance level α with ν degrees of freedom for a two-tailed test.

The total variance in the measurement results arises from random errors introduced by various factors in the method. These factors include: incomplete or imperfect definition of the measure, imprecise values of physicochemical constants, storage conditions, sampling, sample preparation, chemical purity, matrix effects, environmental conditions, equipment calibration, operator effects, reference value errors, blank correction, limitations in the analytical instrumentation's discrimination or resolution, approximations and assumptions made in the measurement method, and statistical treatment of data, among others [111].

A low uncertainty value indicates that the experimental procedure was meticulously conducted, following sound principles. However, it may not detect biases in the method. The estimation of uncertainty is essential to assess the utility and adequacy of the result based on the analysis's stated purpose. A larger confidence interval increases the probability of containing the true value but provides less information. It is important to note that uncertainty is related to precision but represents a distinct concept. Precision relates to the variability of detector response, while uncertainty reflects the variability of the concentration in the sample.

2. TOOLS AND VALIDATION

2.1. Equipment used

1. Perkin Elmer Autosystem XL GC (Perkin Elmer, USA):

   Gas chromatography (GC) system manufactured by Perkin Elmer, one of the leading companies in the field of analytical instrumentation and life sciences. System used in this thesis contained an autosampler for precise and accurate automated sample injection, pneumatic pressure control, capillary injector and flame ionization detector.
2. Perkin Elmer Autosystem XL GC with Perkin Elmer TurboMass Upgrade Mass Spectrometer (Perkin Elmer, USA):

   Gas chromatography (GC) system coupled with single quadrupole mass spectrometer manufactured by Perkin Elmer. It is one of the most widely used mass analyzers due to its simplicity, cost-effectiveness, and versatility. Single quadrupole mass spectrometers are commonly integrated into gas chromatography systems to identify and quantify compounds present in complex mixtures.
3. GC column: Agilent DB-5MS 30m x 0.250 mm, 0.25 µm (Agilent Technologies Inc., USA).
4. Domnick Hunter 40H-MD H_2 Gas Generator (Domnic Hunter, England).
5. JUN-AIR OF302-15B air compressor (JUN AIR Inc., USA)
6. SP Scientific FC100 Flexi-Cool™ chiller (SP Industries, USA).
7. Fluke 50D K/J Thermometer (Fluke, USA).
8. Laboratory mill (Standart, China).
9. Analytical scales Kern Sohn 770-60, weighing limit 0,00001 g - 210 g, (KERN, Germany).
10. Bandelin Sonorex RK Sonication bath (BANDELIN electronic GmbH & Co, Germany).

2.2. Materials

Table I. Materials used in this thesis

Name	**CAS**	**Concentration/purity**	**Supplier**	**Country/ Region**
Dichloromethane	75-09-2	>99 %	Mavis	Germany
Octane	111-65-9	99,46 ± 0,30%	Merc	Germany

Nonane	111-84-2	99,53 ± 0,30%	Merc	Germany
Decane	124-18-5	99,54 ± 0,30%	Merc	Germany
n-Hexane	110-54-3	>99 %	Honeywell	Germany
α-Pinene	80-56-8	>99 %	Sigma-Aldrich	USA
β-Caryophyllene	87-44-5	>98 %	Sigma-Aldrich	USA
Methanol	67-56-1	>99 %	Sigma-Aldrich	France
Acetone	67-64-1	>99 %	Sigma-Aldrich	Israel
Acetonitrile	75-05-8	>99 %	VWR BDH	France
Pentane	109-66-0	>99 %	Honeywell	Germany
Isoprene	78-79-5	>99 %	Sigma-Aldrich	USA
Chloroform	67-66-3	99,28 ± 1,00%	Dr. Ehrenstorfer	Germany
Propiconazole	60207-90-1	>98,0 % (Sum of isomers)	Sigma-Aldrich	Germany
(±)-Linalool	78-70-6	>95.0	Fluka	Germany
Bis(2-ethylhexyl) phthalate (DEHP)	117-81-7	99,71 ± 0,71 %	Dr. Ehrenstorfer	Germany

2.3. Validation parameters

Later in the thesis terpene compounds (α-pinene and β-caryophyllene) were analyzed and method for terpene analysis in hemp had to be validated. Calculation of validation parameters is described here.

Linearity was evaluated by calculating R^2 of the linear regression in the selected range of the calibration curve. It was decided that there is no point in evaluating linearity outside the range of the calibration curve.

Limit of detection (LOD) and limit of quantification (LOQ) were both determined from the signal to noise (S/N) of the diluted lowest calibration point for both analytes and interpolated to the point where S/N is 3,3 for LOD and 10 for LOQ.

$$LOD = 3{,}3 \times (Peak\ height)/(noise\ height) \quad (4)$$

$$LOQ = 10 \times (Peak\ height)/(noise\ height) \quad (5)$$

Accuracy was determined by calculating the recovery of the method. Recovery (accuracy) was calculated by measuring analyte concentration in spiked and non-spiked samples.

$$Recovery\ \% = (c_{non\text{-}spiked\ sample} + c_{spike})/(c_{spiked\ sample}) \times 100\ \% \quad (6)$$

Precision was assessed through the computation of the relative standard deviation (RSD). The standard deviation and mean were derived from a minimum of ten successive measurements taken from an identical sample.

$$RSD = (Standard\ deviation)/(Mean) \times 100\ \% \quad (7)$$

Uncertainty quantification was conducted by aggregating the uncertainties stemming from precision; standard reference materials, and the calibration of scales employed in both the preparation of standard solutions and samples. Notably, the contribution of scale error to the overall uncertainty was determined to be less than 3% of the total uncertainty value. Consequently, this component was deemed inconsequential and thus omitted from the subsequent calculations.

$$U = k \times C_X \times \sqrt{u^2(r) + u^2(C_Z)} \quad (8)$$

k – coverage factor (k=2)

C_x – measured concentration of the analyte

u(r) - relative standard uncertainty of the sample measurement

$u(C_Z)$ - relative standard uncertainty of the certified reference material

While not part of validation procedure, peak resolution (R) was calculated in some parts of this thesis. It represents ability to separate two peaks in a numerical value.

$$R = \frac{t_{R2} - t_{R1}}{\frac{1}{2}(W_1 + W_2)} \quad (9)$$

tR_1, tR_2 – retention time for each peak

W_1, W_2 – width of each peak

3. DEVELOPMENT AND RESULTS

3.1. Oven cooling

Prior to more advanced cryo-enrichment techniques, simpler oven cooling prototype was built. Idea was to improve upon thermal management of the standard GC system, which relied mostly on the heater coil and a fan. Heater coil is used to heat the oven and the fan is used to improve heat transfer from heater to the air inside the oven. While cooling, small hatch opens in the side of the oven letting in fresh air and expelling hot air into the lab. This process is slow and is limited by ambient temperature. Ideally GC operator has to choose temperature that are at least 10 ºC above the ambient temperatures, because cooling further is slow and impractical.

Standard GC system was improved upon by adding custom made cooling apparatus (Figure 22). It was based on the evaporative cooling principle and consisted of outside cooling unit and a thin metal evaporator inside the GC oven. Unit outside the GC houses compressor, condenser and thermal expansion valve. Compressed refrigerant is cooled down in condenser and travels inside the GC through isolated transfer line where it expands inside the evaporator and cools down the GC oven. Then refrigerant is transported back to compressor, where it performs the cycle again.

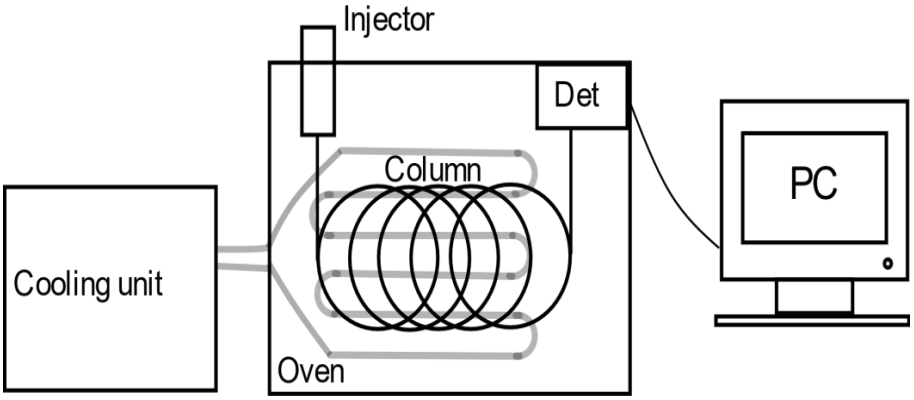

Figure 22. Gas chromatography systems with oven cooling add-on.

Addition of the cooling unit greatly improved the cooling performance of the GC oven. Time required to cool down the oven from 200 ºC to 40 ºC decreased significantly (from 8:20 min to 1,56 min) (Figure 23). This reduction in system cooling time can greatly increase throughput of the laboratory hence improving analysis time and saving costs.

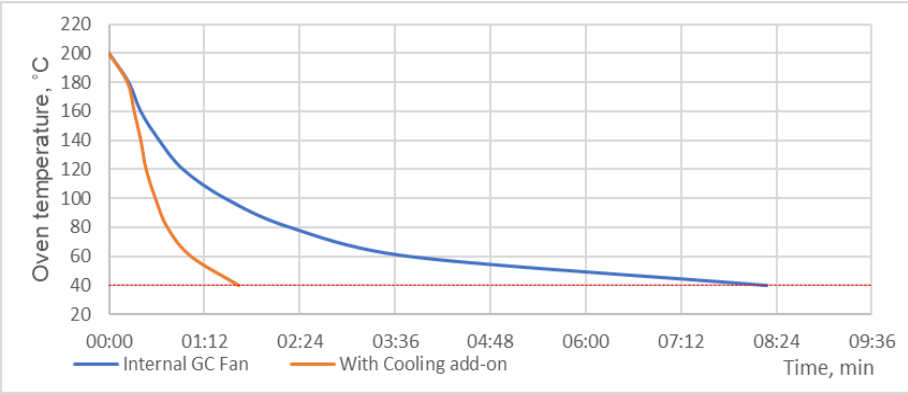

Figure 23. Gas chromatograph oven cooling time from 200 ºC to 40 ºC without and with oven cooling add-on.

Cooling unit not only reduced the oven cooling time, but also allowed for better separation and increased capabilities of the GC system to measure higher volatility compounds. For this demonstration, we carefully selected a gas mixture primarily composed of isobutane and its isomers, along with various other impurities. Without oven cooling add-on, the analysis of this mixture could only be started at a relatively high temperature of 35 ºC in the GC oven. However, at this starting temperature, considerable challenges in achieving any notable separation of the components within the mixture were encountered. By drastically reducing the starting temperature, analysis could be initiated at a relatively low temperature of -10 ºC. With the aid of the cooling unit, the gas mixture's components exhibited improved resolution, leading to the detection of four distinct peaks in the chromatogram (Figure 24).

Table II. GC conditions.

Injector, °C	Oven, °C	Detector, °C	H_2 flow, ml/min	Split ratio
150	-10 (0 min) → 10 °C/min → 100 (1min)	150	1	1:10

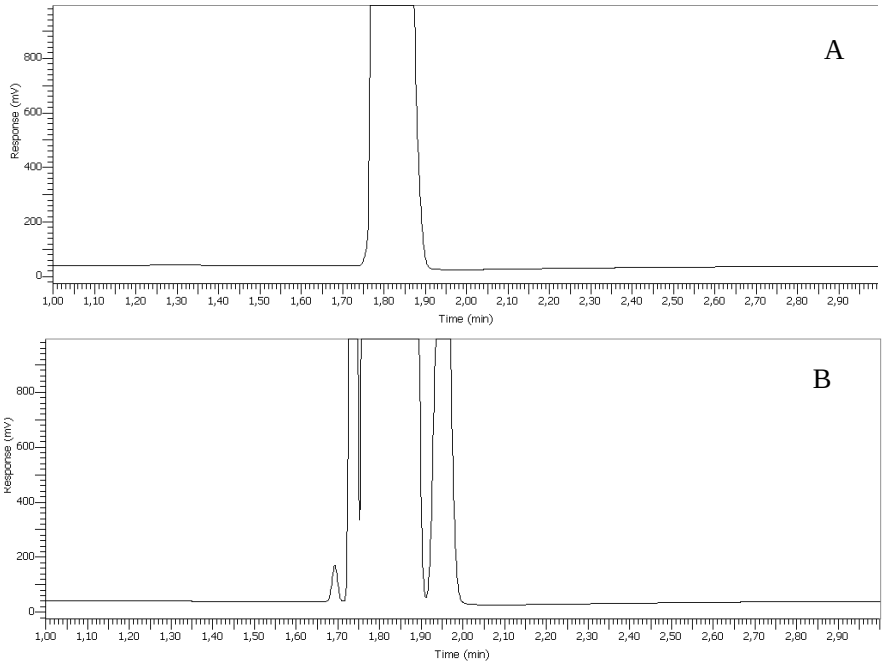

Figure 24. **A** Chromatogram performed starting at 35 ºC. **B** Chromatogram performed starting at -10 ºC.

Though improvements to the GC methods are clear this research was abandoned due to moving to the on-column cryo-cooling, which allowed to achieve even lower temperatures and increased system sensitivity.

3.2. Development of cryo-enrichment module

3.2.1. First prototype

It has been discussed in the introduction, that there are various methods to perform sample or analyte enrichment. However, for this thesis, the on-column cryo-enrichment technique was chosen as an external module for existing GC systems. The design of this module is original and comprises four aluminum rings (Figure 25) , 7 cm in diameter. Within these rings, two nichrome heaters (200W) and a cavity for the capillary GC column are housed. The rings are securely held within a thermally insulated body and connected to the GC system through a heated transfer line. The entire cryo-enrichment module is positioned on top of a liquid nitrogen (N_2) dewar. To ensure proper cooling, N_2 is directed onto the metal rings via an internal heater within the

dewar. Each heater's temperature is independently controlled, and the temperatures are monitored using thermal couples.

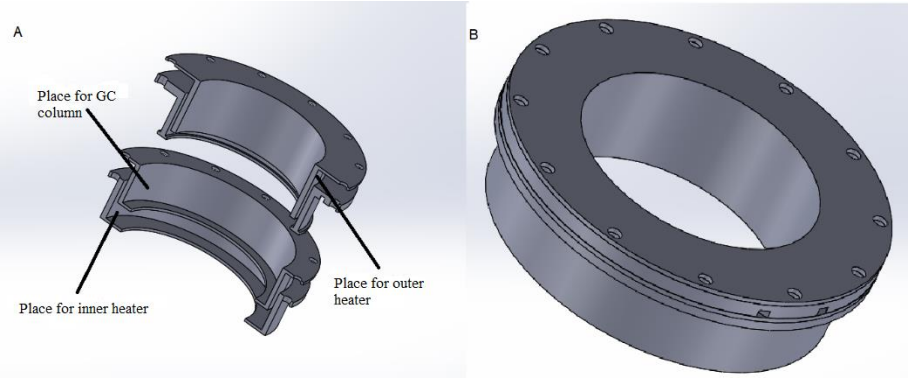

Figure 25. The aluminum ring body is designed to wrap the capillary column and heaters: A – cross-section of the aluminum rings unstacked, B – the rings stacked on top of each other.

The column is routed from the gas chromatograph to the refrigeration zone and back via a continuously heated transfer line. At one end, the column is connected to the injection system, then it traverses through the cryo-enrichment module before returning to the gas chromatograph oven. Within the oven, rest of the column is housed where the chromatographic separation occurs. Finally, the column is connected to a flame ionization detector (Figure 26).

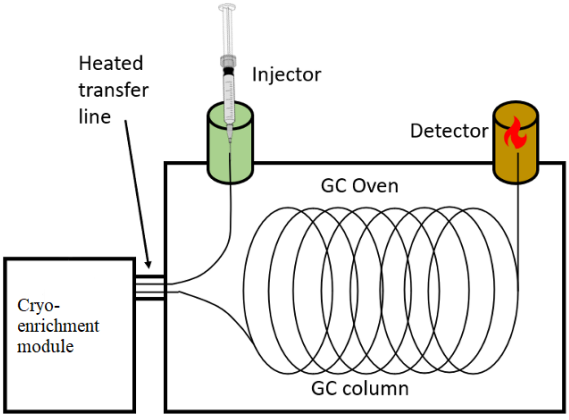

Figure 26. Schematic of the enrichment system connected to the gas chromatograph.

3.2.2. Testing first prototype

Purpose of the prototype was to test the principle of on-column cryo-enrichment. Module was paired with Perkin Elmer, Autosystem XL gas chromatography system with Agilent DB-5ms, 30 m, 0,25 mm ID, 0,25 µm. Mixture of alkanes (octane, nonane, decane) in dichloromethane was used to test the system. Module was tested for both analyte focusing and analyte enrichment.

3.2.2.1. Cryo-focusing

Focusing of the peaks is an act of making the peaks narrower and taller. Testing of focusing performance was completed by cooling down cryo-enrichment module to -60 ºC before analysis and then injecting (1 µl, 1:10 split injection) analyte mixture into heated injector (temperature kept at 150 ºC). Analyte mixture was propelled forward in column by 1 ml/min flow of helium gas and was predicted to condensate and become stationary once it reached cryo-enrichment module. After 2 minutes cryo-enrichment module was switched to heating mode and rapidly (~2 ºC/sec) heated to around 120 ºC. Vaporized analytes were made mobile again and moved along the column (oven temperature kept at 80 ºC) towards FID detector (temperature kept at 150 ºC).

Table III. GC conditions.

Injector, °C	Oven, °C	Detector, °C	H_2 flow, ml/min	Split ratio	Cryo temp, °C	Transfer line, °C
150	80	150	1	1:10	-60 → 120	100

Results indicate that cryo-focusing gives narrower and taller peaks, thus increasing the efficiency and sensitivity of the method. Also, solvent was not retained in the cryofocusing device (Figure 27).

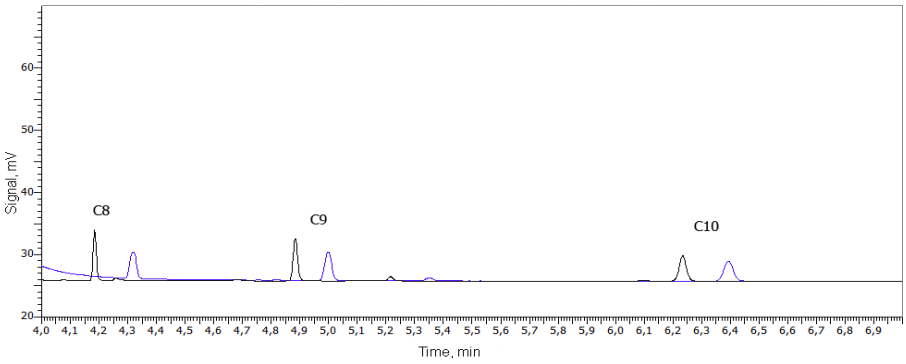

Figure 27. Comparison of focused and unfocused chromatograms. Analytes: octane (C8), nonane (C9), decane (C10). Blue for analysis without focusing, black for analysis with focusing.

Cooled column length influence on focusing performance was evaluated by comparing peak height after analysis of the same mixture at different cooled column lengths. It was found that longer cooled column lengths produced taller and narrower peaks. Peak height increased up to 1,83 times for octane and the heavier analyte focusing was slightly less noticeable: 1,47 times for nonane and 1,27 times for decane. Resolution between the peaks of octane and nonane increased by 2,2 times (7,2 to 15,8).

3.2.2.2. Cryo-enrichment

Enrichment performance was evaluated similarly to focusing performance test. Analyte mixture was injected n (see table IV) number of times into GC system with cryo-enrichment module cooled down to -60 ºC. Then after two minutes cryo-enrichment module was heated up to 120 ºC. It was anticipated that injected analytes would be retained in cooled part of the column and after heating it up, their analyte area would increase corresponding to the number of injections made of the system.

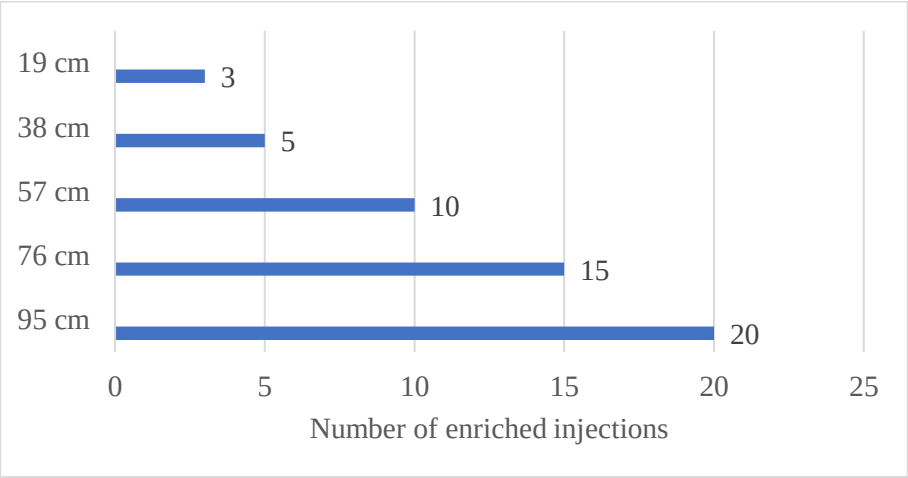

Figure 28. Cooled length of the column influence of the number of injections retained.

Figure 28 illustrates the examination of how the length of the cooled column affects the retained number of injections. The results revealed that utilizing 95 cm of cooled column at -60 ºC allowed for the retention of up to 20 injections, leading to a peak area enrichment of up to 20 times. Octane and nonane displayed narrow and tall peaks, while peak splitting was prominent in decane peak (Figure 29).

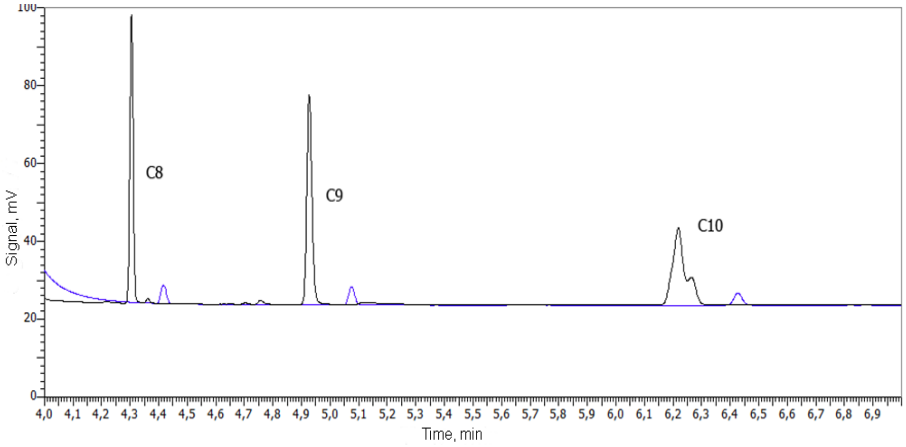

Figure 29. Comparison of chromatograms of 10-times enriched (black) and non-concentrated (blue) chromatograms. Analytes: octane (C8), nonane (C9), decane (C10).

This phenomenon might be attributed to the sequential injections and the heat introduced to the cooled segment of the column. Following each

injection, the influx of heat and solvent into the cryo-enrichment module propelled the analytes along the column. Those analytes with higher volatility were carried further forward, while the less volatile ones exhibited decreased susceptibility to movement. The compound decane, being less volatile, experienced non-uniform displacement, leading to the formation of distinct concentrated zones along the column. Upon re-elevating the temperature of the cryo-enrichment device, these split peaks were reintroduced into the GC system. It is worth noting that the observed asymmetry could potentially be attributed to uneven cooling throughout the column.

The impact of temperature on enrichment performance was as a critical factor affecting both the shape and width of peaks. At temperatures of -30 ºC and -45 ºC, the compound nonane exhibited asymmetry. Similarly, decane displayed asymmetry down to -90 ºC, but as the temperature decreased further, its peak became narrower and more symmetric (Figure 30). This observed peak asymmetry might be attributed to the same heat influence entering the cryo-enrichment device as discussed earlier. Dichloromethane started to exhibit retention at -60 ºC, and its retention further increased with decreasing temperatures.

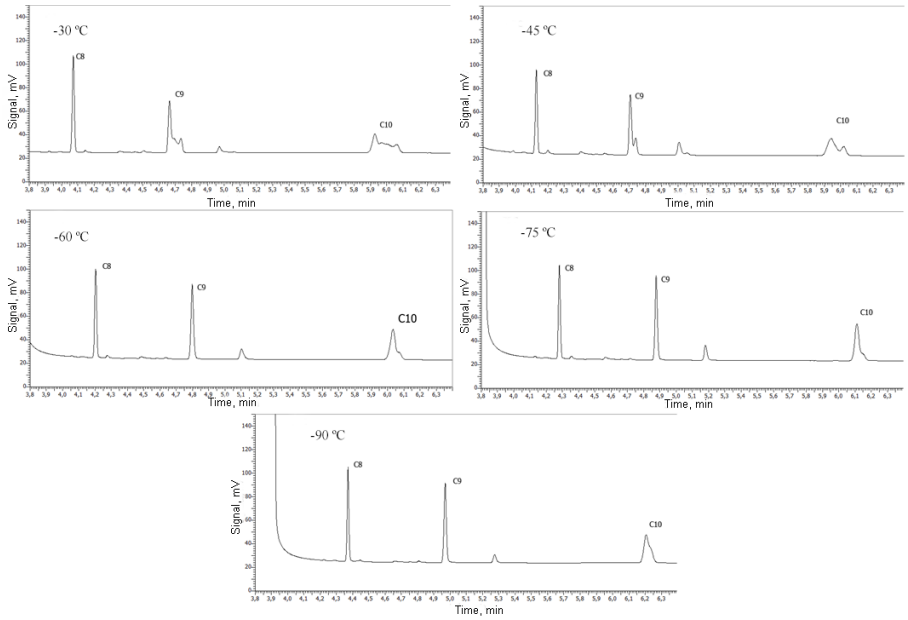

Figure 30. The temperature dependence of the peak shape in the cooled zone of the column. Analytes: octane (C8), nonane (C9), decane (C10).

3.3. Improved prototype

The previous prototype underwent significant improvements through the adoption of an evaporative two-stage cooling system, replacing the cumbersome and costly liquid nitrogen method. This shift was motivated by the wish to eliminate the need for expensive coolant and the constant hassle of refilling it thus making the module easier to operate. However, achieving the capability to cool an object to cryogenic temperatures within minutes, while using an evaporative cooler for analysis, presented a challenge. This was primarily due to the fact that commercially available coolers designed for desk or under-desk placement lacked the necessary cooling capacity.

Finding a suitable solution demanded careful consideration, as opting for a large-scale evaporative cooler proved both financially and physically impractical. Instead, we devised an innovative approach by modifying the design of the aluminum column holder and the entire module. This reimagining allowed us to use the evaporative cooling system without the need for excessive size or investment. By customizing the column holder and module design, we were able to optimize the cooling process while maintaining efficiency and cost-effectiveness.

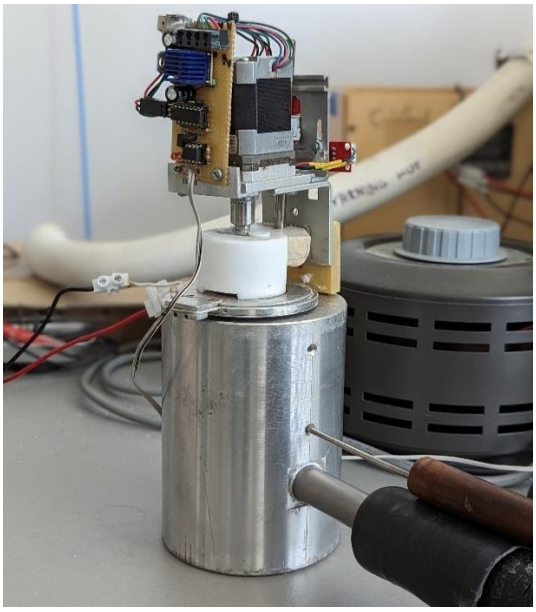

Figure 31. Custom built cryo-enrichment module without the insulating case.

This decision to refine the aluminum column holder and the module not only addressed the cooling capacity limitations but also enhanced the overall

performance and usability of the prototype. The revised design allowed for seamless integration of the commercially available evaporative cooling system, enabling rapid and precise temperature control during the analysis phase.

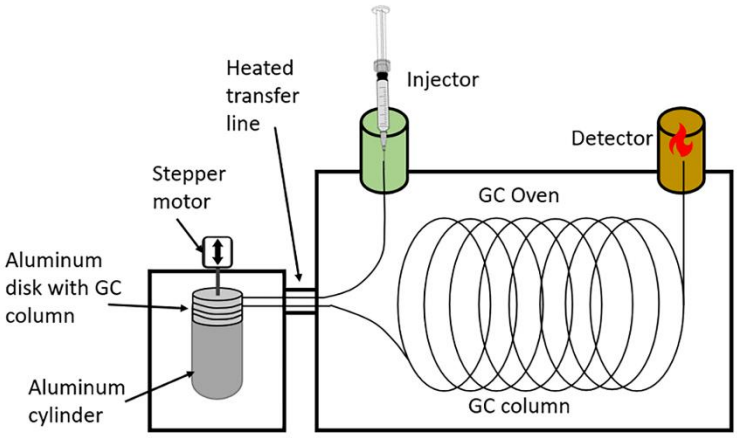

Figure 32. Schematic of cryo-enrichment prototype system connected to gas chromatograph.

Revised module consisted of two aluminum parts. The first component was a single, precisely sized (10x13 cm) aluminum cylinder which is cooled by SP Scientific FC100 Flexi-Cool™ immersion cooler down to cryogenic temperature (Figure 31). The second component, a column holder (7 cm diameter), was an aluminum plate, machined with grooves designed to hold the nichrome wire heater (200 W) and a side groove for column to wrap around (Figure 33). Stepper motor situated above the column holder precisely manipulated column holder position: either pressed against the aluminum cylinder or approximately 2 millimeters above it. This dynamic manipulation facilitated the necessary contact between the column holder and the cooled surface during the cooling phase, while permitting swift separation for rapid heating. 95 cm of column was winded inside the column groove.

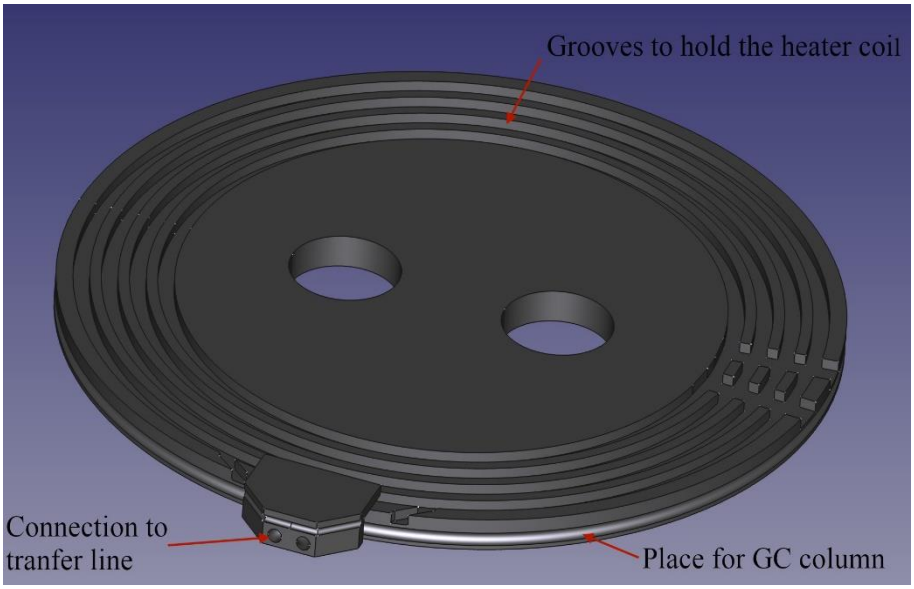

Figure 33. Aluminum column holder drawing.

The improved design, featuring a separation mechanism and a smaller form factor for the column holder, resulted in a halving of the heating time from -60 °C to 150 °C. This substantial reduction translated into a one-minute heating duration, effectively doubling the efficiency compared to the previous two-minute duration. To ensure optimal insulation and temperature stability, the entire module, with the exception of the motor, was insulated with polystyrene and glass wool. Module was connected to GC via the heated transfer line to ensure desired temperature conditions throughout the analytical process. Column holders' position is controlled with a custom-made controller (Figure 34).

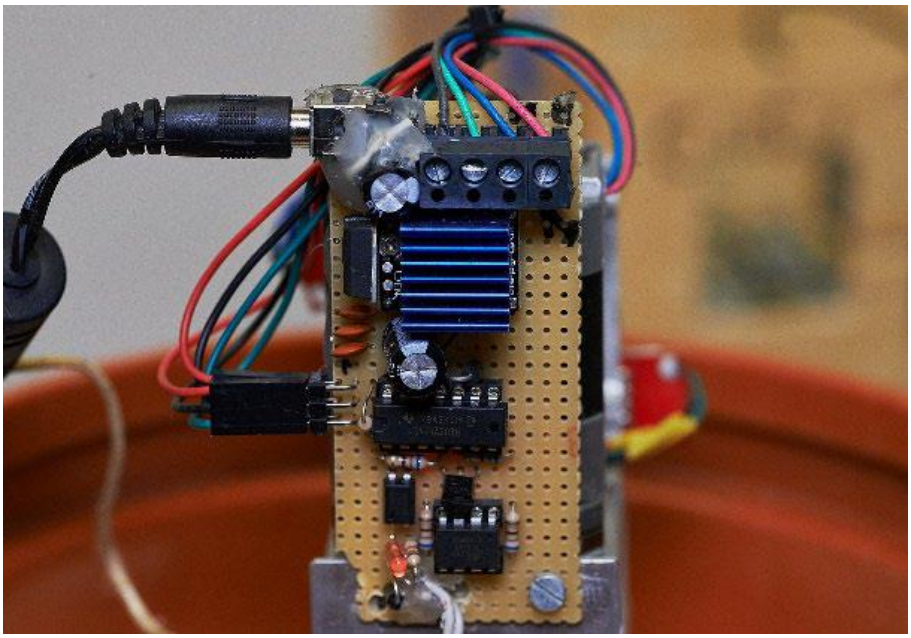

Figure 34. Custom built cryo-enrichment module column holder controller.

3.3.1. Testing of the new prototype

The decision to transition from helium to hydrogen as the carrier gas for the enhanced prototype was driven by the aim to eliminate dependence on gas lines. This choice was motivated by the existing use of a hydrogen gas generator to provide hydrogen gas to the FID detector. Consequently, this same gas generator could effortlessly fulfill the role of supplying carrier gas to the system. However, as the system began operating at cryogenic temperatures and particularly during extended operation, an issue emerged wherein the gas flow would cease. It was deduced that disturbances in the gas flow was caused by the ice plug forming in the capillary column. Gas from the generator had some level of moisture in them and over time formed a plug. To combat that, a gas filter was designed and built. It consisted of two (26 x 5 cm) cylinders filled with sorbent (Figure 35). First with silica gel and another with carbon molecular sieve. With this filter in place ice plug problems never occurred again.

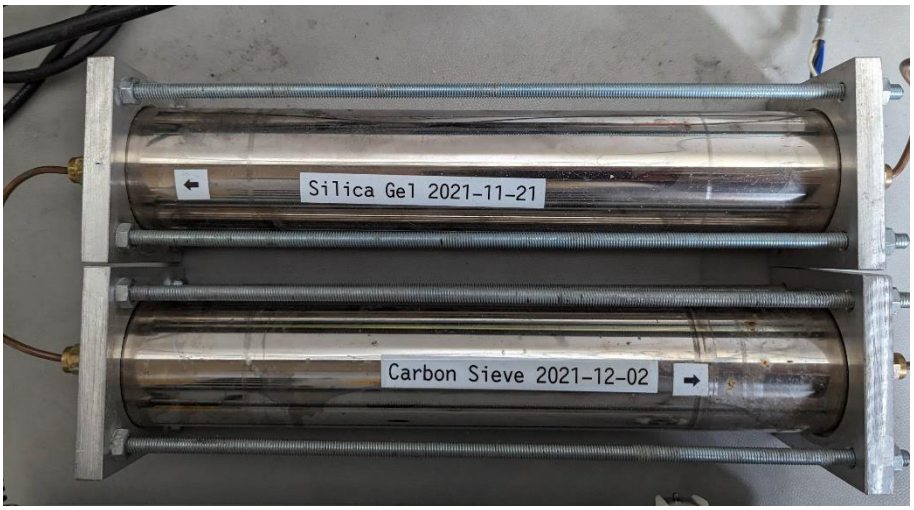

Figure 35. Photo of the custom H_2 carrier gas drying filter.

Once the gas flow problem was solved, it was time to evaluate the performance of the new module. Mixture of octane, nonane and decane was injected, peak shapes and enrichment performance were tested.

Table IV. GC conditions.

Injector, °C	Oven, °C	Detector, °C	H_2 flow, ml/min	Split ratio	Cryo temp, °C	Transfer line, °C
150	50 (3 min) → 6 °C /min → 100 (2 min)	150	1	1:10	-60 → 120	150

Peak shape improved drastically (Figure 36). Because of the uniform cooling and faster heating, there was no longer doubling of the decane peak.

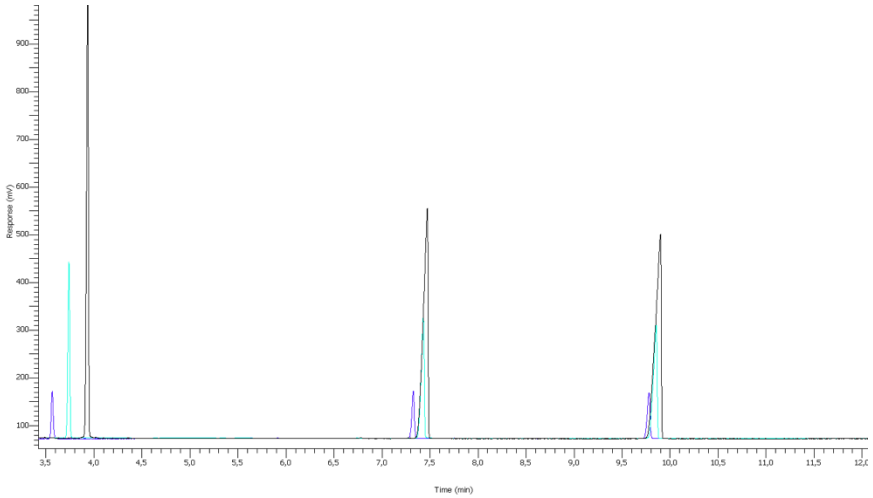

Figure 36. Comparison of chromatograms: 1 time enriched (dark blue), 5 times enriched (light blue), 10 times enriched (black).

Splitless injection mode with cryo-enrichment module was tested. In splitless mode sample is injected into the inlet, but no flow splitting is present. Because of that all the sample is transferred into the column, but this process takes some time and causes massive peak broadening. Cryo-enrichment module should solve this by focusing peaks after the injection. And our findings confirm that: peaks are unresolvable. Octane peak disappears completely, while nonane and decane peaks are present, but are heavily overlapping with the solvent peak. While in the splitless injection with cryo-enrichment peaks are well separated (Figure 37).

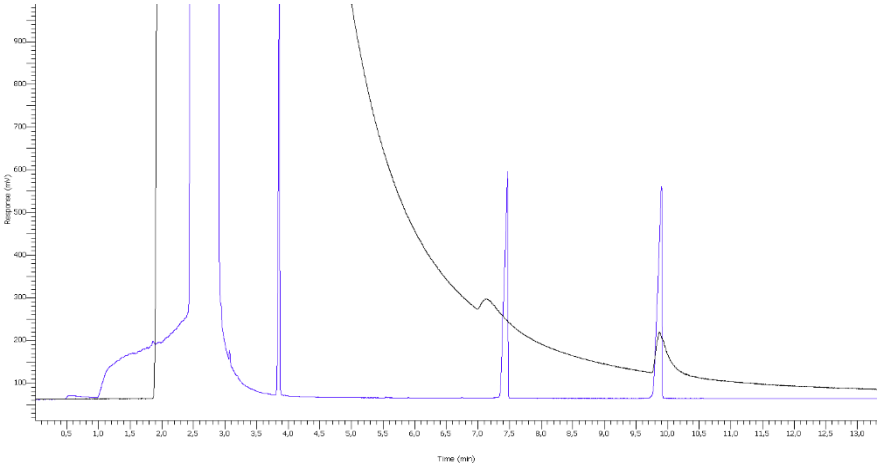

Figure 37. Chromatogram of octane, nonane, decane in pentane. Splitless without cryo-enrichment (black), splitless with cryo-enrichment (blue).

For chromatography expert question should arise: why should 10 injections with split be made instead of 1 in splitless mode? To answer this question, comparison of two different approaches was made (Figure 38). The results show that 10 injections with split produce narrower and taller peaks, while also providing better resolution between solvent peak and octane peaks. Though the difference is marginal, resolution increased 20 %.

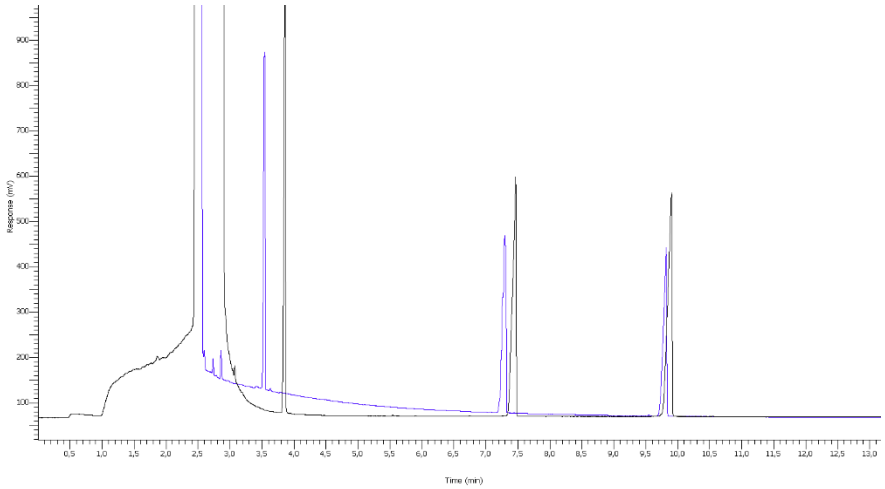

Figure 38. Enriched 10 times injection chromatogram (black), enriched splitless chromatogram (blue).

Additional measurements were conducted using a wider range of analytes to assess the performance of the module when analyzing different substances. The selection of analytes was based on their volatility, ranked from most volatile to least as follows: isoprene, chloroform, α-pinene, and Bis(2-ethylhexyl) phthalate (DEHP).

The results indicated that isoprene exhibited excessive volatility, making it challenging for the cryo-enrichment module to consistently collect multiple injections. This resulted in doubled and broadened peaks with varying peak areas. In contrast, DEHP showed strong retention within the cryo-enrichment module. Even after heating the module to 250°C, peaks were not reliably registered. Among the chosen analytes, α-pinene yielded the most reliable results, with narrow and consistent peaks. More detailed information about α-pinene will be provided in subsequent chapters. However, measuring chloroform proved to be challenging because it did not separate effectively from the solvent, dichloromethane, resulting in overlapping peaks. To address this issue, a decision was made to dissolve a smaller quantity (ppm) of dichloromethane in chloroform. This adjustment allowed for the separation of

peaks after cryo-enrichment. Nevertheless, after 10 injections, some doubling and deformation of the dichloromethane peak were observed (Figure 39).

Based on these findings, it can be inferred that the cryo-enrichment module has a limited working range. The lower limit appears to be attributed to inadequate cooling (-60 °C), while the upper limit is constrained by the maximum heating temperature (250 °C).

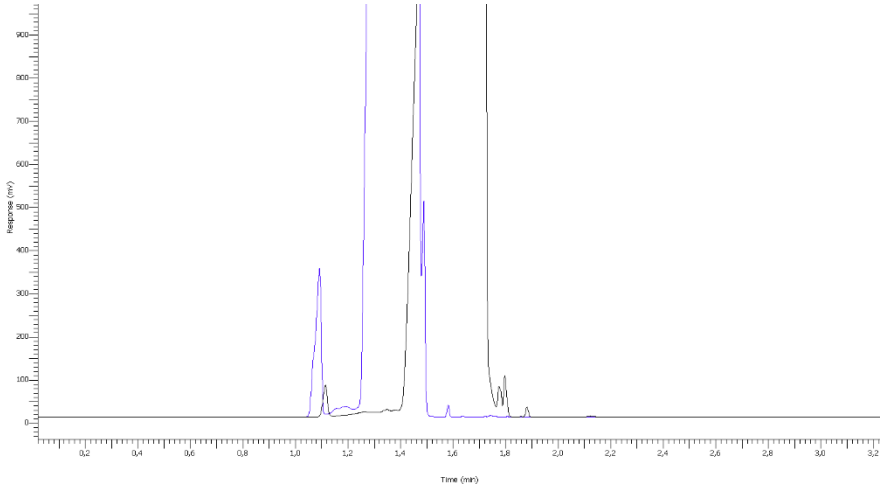

Figure 39. Overlapped chromatograms comparing dichloromethane and chloroform mixture: 1x (black) and 10x (blue).

3.3.2. Measuring terpenes using on-column cryo-enrichment

Real world application for measuring compounds in biomass matrix was developed. Terpene compounds (α-pinene and β-caryophyllene) were chosen as targets in dried hemp (*Cannabis Sativa*), because they are present in the hemp and they have been shown to possess antioxidant, medicinal properties [112, 113].

3.3.2.1. Developing the method

The need for measuring α-pinene and β-caryophyllene in hemp arose and it was decided to adapt this method to GC with cryo-enrichment module. First, method was developed for measuring with GC-MS. Mass spectrometer allowed for easier peak identification and also served as a point of comparison to GC-FID coupled with cryo-enrichment prototype.

Proper extraction of the terpenes was crucial to the reliability of the method. After evaluating methods in the literature, it was decided that sonication with an organic solvent would be quickest and easiest option of extraction.

Hemp material was grinded into a fine powder using a mill. All extractions were carried out in 50 mL plastic vials. Extract was filtered through 0,45 µm polytetrafluoroethylene membrane syringe filter into 2 mL amber glass chromatographic vial.

After identifying terpenes: α-pinene, β-caryophyllene in the extract of fibrous hemp, a study was conducted to determine how the extraction of these compounds depends on the nature of the extracting solvent. Extraction from hemp was carried out for 15 min in an ultrasonic bath with the following solvents: MeOH, n-hexane, acetone and ACN, at a temperature of 30±1 °C. From the peak areas of the compounds presented in Figure 40, it can be seen that the highest peak area of terpenes was obtained with n-hexane.

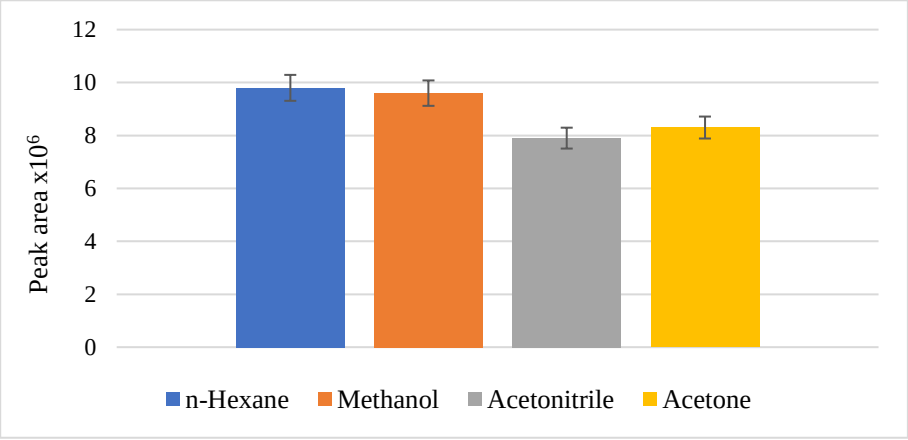

Figure 40. Peak area of terpenes obtained by extraction of hemp with different solvents (1.00±0.03 g of dry hemp, 10.00±0.30 ml extracting solvent, 15 min extraction, temperature 30±1 °C) (n = 3).

After choosing the extracting solvent, temperature influence studies were carried out, the purpose of which was to check at which temperature it would be optimal to carry out the extraction in order to obtain the maximum yield of caryophyllene derivatives.

Extraction temperature influence was evaluated by incubating the extraction mixture at 30±1 °C, 40±1 °C, 50±1 °C and 60±1 °C for 15 minutes. The results are presented in Figure 41.

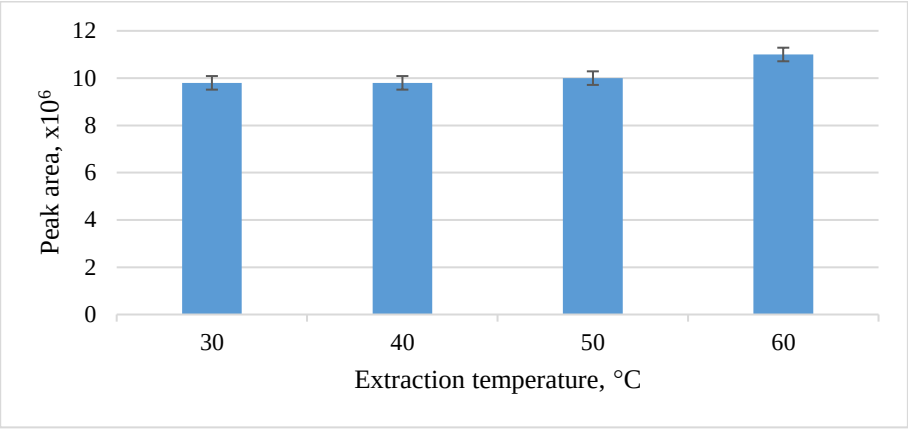

Figure 41. Dependence of the peak areas of terpenes on the extraction temperature (1±0.03 g dry hemp, 10.00±0.30 ml n-hexane, 15 min extraction) (n = 3).

From the bar graph in Figure 41, it can be seen that the amount of terpenes in the hemp extract varies slightly and all values are within a margin of error with increasing temperature. Additional studies were also carried out to verify why larger peak areas are obtained at 60 °C. After additional weighing, it was observed that the solvent had evaporated. Therefore, was decided to carry out extractions in 30 °C.

In the next phase, the quantities of terpenes extracted during 5, 10, 15, and 20 minute extractions were compared. The findings are presented in Figure 42.

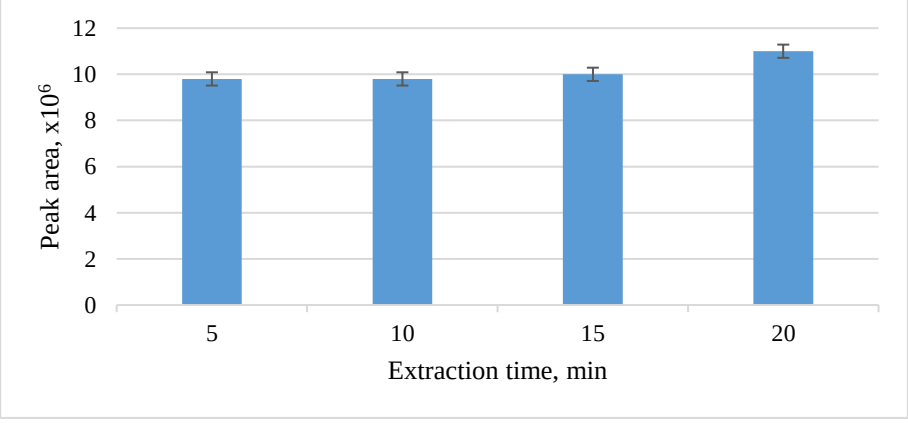

Figure 42. Dependence of the peak areas of terpenes on the extraction temperature (1±0.03 g dry fibrous hemp, 10.00±0.30 ml n-hexane, 15 min extraction) (n = 3).

The extracted amounts of terpenes do not differ significantly. Additional tests were carried out, which aimed to check why larger peak areas are obtained at 20 min time. After weighing measurements, it was observed that the solvent had evaporated. Therefore, an extraction time of 5 minutes was chosen. Optimization results displayed in Table V.

Table V. Optimized conditions for terpene extraction.

Extraction parameter	**Result**
Solvent	n-hexane
Solvent amount, ml	10,00±0,30
Temperature, °C	30±1
Time, min	5

3.3.2.2. GC and MS conditions

Identification and quantitative analysis of terpenes was performed using a Perkin Elmer Autosystem XL gas chromatography system with a Perkin Elmer TurboMass Upgrade mass spectrometer, column - Agilent DB-5MS 30m x 0.250 mm, 0.25 µm. Helium was used as carrier gas. The optimization of analysis conditions was a crucial step in achieving accurate results.

Optimized analysis conditions: injector temperature 300 °C, column temperature 60 °C, injection volume – 1 µl. The temperature in the gas chromatograph was raised gradually during programming: from 60 °C (1 min) to 300 °C, at a rate of 10 °C/min. The total analysis time of one sample is 30 min. The mass spectrometry detector conditions are given in Table VI:

Table VI. Mass spectrometer parameters.

Ionization source temperature, °C	Transfer line temperature, °C	Analysis time, min	Scan speed, s	Mass scan interval, m/z
300	300	30	0,1	50-350

Analysis was performed on α-pinene and β-caryophyllene standard mixture in total ion chromatogram mode (Figure 43).

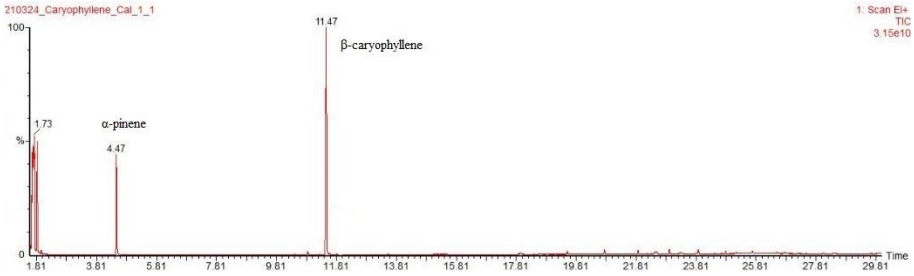

Figure 43. Chromatogram of α-pinene and β-caryophyllene.

Mass spectra of α-pinene and β-caryophyllene displayed in Figure 44.

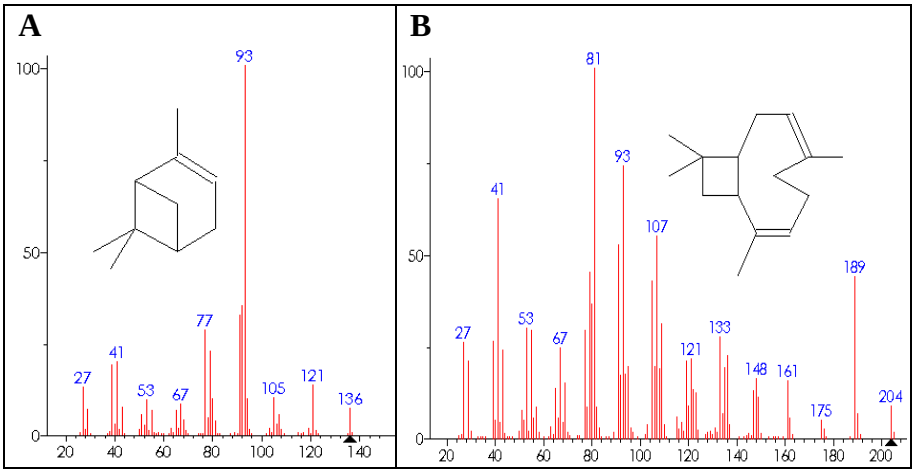

Figure 44. α-pinene and β-caryophyllene mass spectrum.

GC-MS method for analyzing α-pinene and β-caryophyllene was validated and validation data is displayed in the Table VII.

Table VII. Method validation with GC-MS.

Analyte	RSD	U	Recovery	U	Linearity coef.	Linear equation	LOD	LOQ
	%	±	%	±	R^2		ppm	ppm
α-Pinene	5,4	0,2	92,6	3,4	0,9965	y = 0,0053x–0,0126	0,28	0,83
β-Caryophyllene	5,7	0,2	111,5	5,1	0,9936	y = 0,0041x–0,0228	0,30	0,91

3.3.2.3. Analysis using cryo-enrichment module

Dried hemp (*Cannabis sativa*) samples were prepared as described in the previous section. Standard calibration curves were prepared in the range of 0.2 - 204 ppm for α-pinene and 0.1 - 128 ppm for β-caryophyllene.

To find optimal analysis conditions for terpenes, measurements were completed using different GC and cryo-enrichment module parameters. It was determined that 10 consecutive sample injections showed the best peak symmetry and sufficient sensitivity, while providing ample repeatability (Figure 45). During sample injections GC oven temperature was kept at 150 °C to make sure all analytes pass into the cryo-enrichment module, which was maintained at -60 °C. After all injections were made, oven was cooled down to 50 °C and column holder was raised above aluminum block by stepper motor. Temperature in the cryo-enrichment column holder was rapidly raised to 150 °C. Both injector and detector temperatures were kept at 200 °C during the whole analysis. Injection volume was 1 µl and carrier gas (H_2) flow was set to 1 ml/min with split value of 10 ml/min. GC oven temperature program: 50 °C for 3 minutes, then raised to 200 °C with the rate of 10 °C/min.

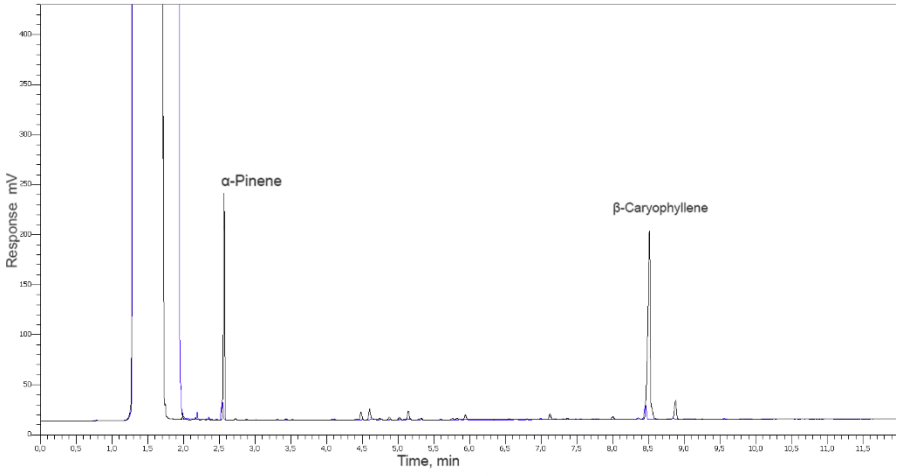

Figure 45. Chromatograms of α-pinene and β-caryophyllene standards standard GC (blue) and using cryo-enrichment module (black).

Table VIII. Data of method validation.

Analyte	RSD	U	Recovery	U	Linearity coef.	Linear equation	LOD	LOQ
	%	±	%	±	R^2		ppb	ppb
α-Pinene	6,7	0,3	95,3	2,4	0,9963	y = 18675x + 52469	0,35	1,05
β-Caryophyllene	6,8	0,3	105,7	3,1	0,9972	y = 54595x + 78699	0,23	0,68

Method was successfully validated (see table VIII) and was found to be fit for purpose for the analysis of α-pinene and β-caryophyllene in the biomass of *Cannabis Sativa* (Figure 46).

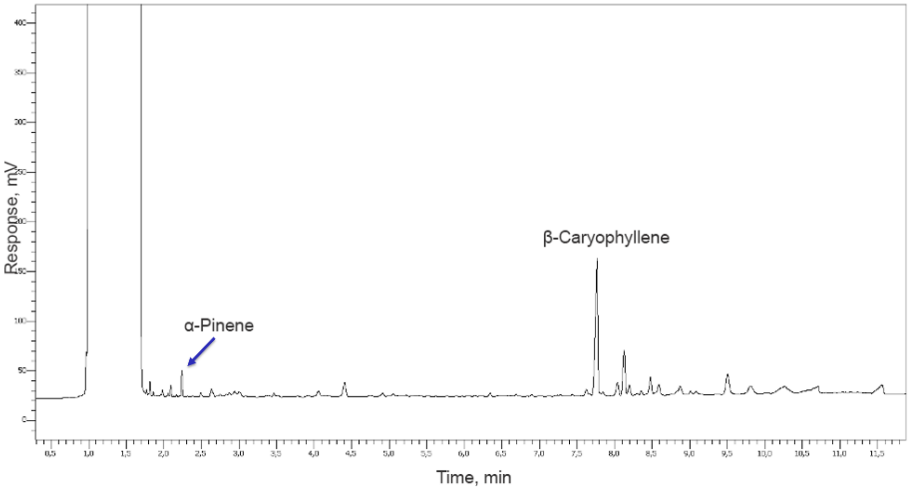

Figure 46. Chromatogram of *Cannabis Sativa* sample biomass extract.

Measurement precision was evaluated by calculating the relative standard deviation (RSD) values. A standard solution was prepared and measured 10 times to assess the precision of the method. The resulting RSD values for α-pinene and β-caryophyllene were 6.7% and 6.8%, respectively. These results demonstrate that the method exhibits a high degree of precision, with only a small deviation in the measured concentrations of the analytes.

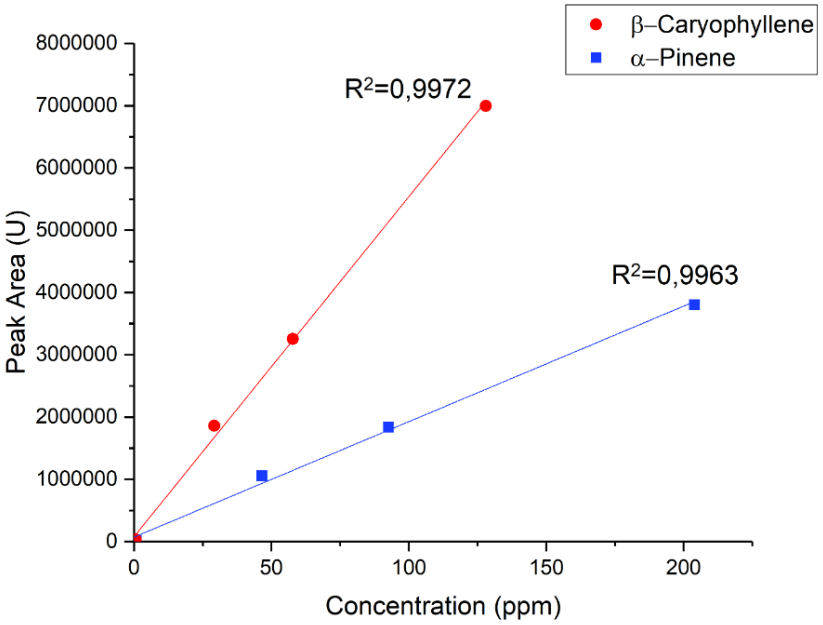

Figure 47. Calibration curve of *α*-pinene and *β*-caryophyllene.

Trueness was determined by calculating the recovery of the method. Recovery (trueness) was calculated by measuring analyte concentration in spiked and non-spiked samples. Five samples were spiked and prepared from the same hemp batch. Results were compared with non-spiked hemp sample. Recovery was found to be 95,3 ± 2,4% and 105,7% ± 3,1% for *α*-pinene and *β*-caryophyllene respectively. Method exhibited good performance, as recoveries for both of the compounds were in the 70–120 % range, which is recommended by DG-SANCO method validation guidelines [114]. Overall, recovery was demonstrated to be satisfactory for the analysis of *α*-pinene and *β*-caryophyllene in *Cannabis Sativa*.

Linearity was assessed by analyzing standard solutions of five different concentrations, each of which was measured three times. The concentration versus peak area for both standards was interpolated to obtain calibration curves (Figure 47). Linearity was determined by R^2 factor from calibration curve. The resulting calibration curve, shown in Figure 47, demonstrated linearity in the range of 0.2 - 204 ppm for *α*-pinene and 0.1 - 128 ppm for *β*-caryophyllene, with correlation coefficients of 0.9963 and 0.9972, respectively.

LOD and LOQ were determined from signal to noise ratio (S/N) of the diluted lowest calibration point for both analytes and interpolated to the point where S/N is 3,3 for LOD and 10 for LOQ.

Values determined are 0,35 ppb LOD, 1,05 ppb LOQ for α-pinene and 0,23 ppb LOD, 0,68 ppb LOQ for β-caryophyllene. LOD and LOQ values indicate that the method is highly sensitive, with the ability to detect and quantify very low concentrations of terpene analytes.

From what has been shown above, we can conclude that cryo-enrichment module is working as expected and is providing precise results for measuring chosen analytes. The main advantage for cryo-enrichment is improved method sensitivity and ability to measure low concentration samples. Compared to quadrupole GC-MS, method sensitivity increased around 1000 times (0,3 ppm to 0,23 ppb).

3.4. Additional applications

3.4.1. Calibration

The recommended approach for generating calibration curves in gas chromatography involves employing uniform-volume injections with diverse standard solution concentrations. Although, a single calibration solution with adjustable injection volumes might be considered for calibration, it would be inadequate in terms of precision. Challenges associated with the adoption of varying injection volume calibration include:

Nonlinear Response. GC systems might not exhibit a linear response across a wide range of injection volumes. Changing the injection volume can lead to nonlinearity in detector response, making it difficult to accurately quantify compounds. This may be caused by the inaccuracy of the autosampler.

Column Overloading. Injecting larger volumes might lead to column overload, causing distorted peak shapes, tailing, and overlapping peaks. Overloading the column with too much sample can result in poor separation and inaccurate quantification.

Instrument Limitations. Some GC instruments might have limitations on the range of injection volumes they can handle accurately. Using injection volumes outside the instrument's optimal range can lead to inaccurate results.

We tried to solve these problems with varying injection volume calibration by using cryo-enrichment prototype. With our method autosampler injection volume stayed the same, but the number of injections varied.

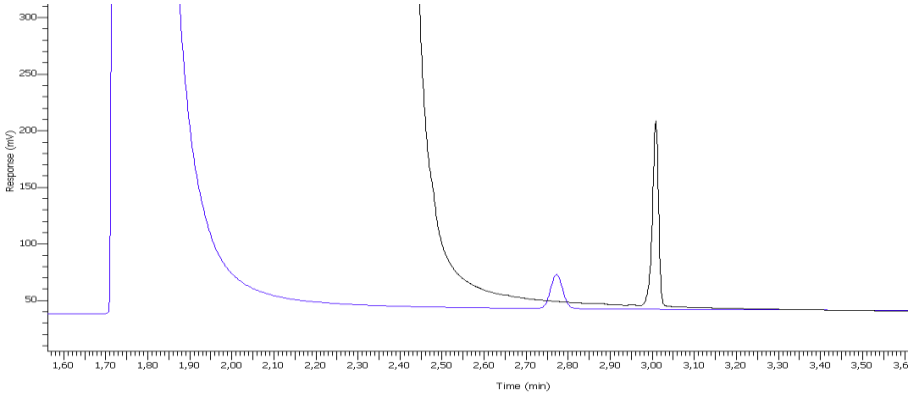

Figure 48. Overlapped chromatograms comparing nonane peaks after one (blue line) and five (black line) injections.

Standard solution of nonane in pentane (100 ppm) was prepared (Figure 48). Analyte was retained and enriched in the module for a five-point calibration. Enrichment level corelating with the number of enriched injections.

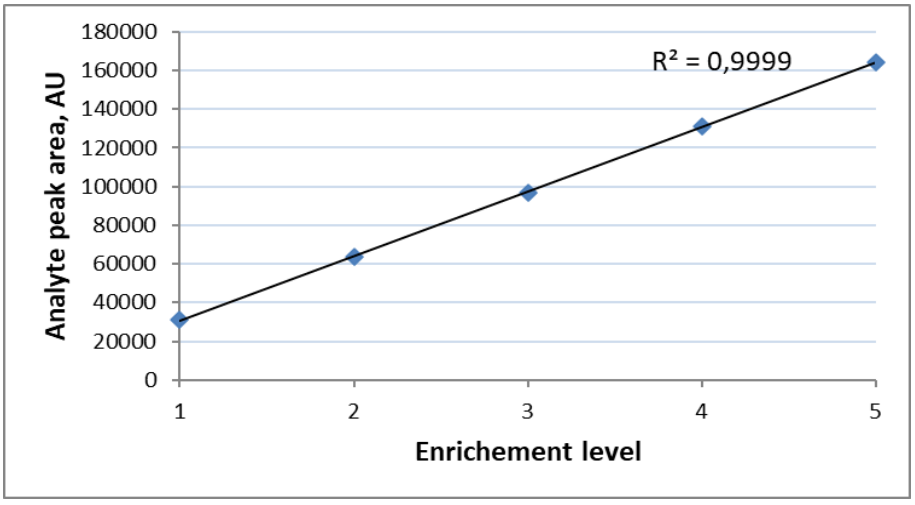

Figure 49. Peak areas at different enrichment levels.

The data presented in Figure 49 demonstrates a high degree of calibration precision. The implementation of this method offers substantial advantages, mainly by considerably simplifying the work required for the preparation of

calibration solutions intended for GC analysis. Secondly, the risk attributed to potential human errors is notably diminished, contributing to an overall enhancement in the reliability and accuracy of the analytical process.

3.4.2. Analysis of impurities

In analytical chemistry, the quantification of impurities in CRMs or other materials is a meticulous process. Advanced techniques such as high-performance liquid chromatography, gas chromatography-mass spectrometry, inductively coupled plasma-optical emission spectroscopy (ICP-OES), titration, infrared spectrometry, nuclear magnetic resonance and etc. are employed to identify and measure impurities, often at trace levels [115]. Calibration against standards, careful sample preparation, and data validation ensure accurate results. CRMs serve as crucial benchmarks for impurity measurements, guaranteeing the reliability of analytical methods and enabling result comparability across laboratories. This is essential for maintaining quality control in various industries that rely on precise impurity analysis.

An impurity analysis was conducted using a standard reference material of (±)-Linalool. The manufacturer (Fluka) specifies that the standard has a purity of over 95.0% as determined by gas chromatography (GC) testing. To perform the analysis, a Linalool solution in hexane was injected into the GC system ten times, utilizing a cryo-enrichment module under the conditions outlined in the table IX.

Table IX. GC conditions.

Injector, °C	Oven, °C	Detector, °C	H_2 flow, ml/min	Split ratio	Cryo temp, °C	Transfer line, °C
200	80 (2 min) → 10 °C /min → 180 (2 min)	200	1	1:10	-60 → 180	180

The results revealed the presence of the primary peak corresponding to (±)-Linalool and three smaller peaks, suggesting the presence of impurities (Figure 50). To verify that these impurities did not originate from the solvent or the system, the same measurement was repeated using only hexane. Upon integrating all the peaks, the calculated purity of (±)-Linalool was found to be 97.7%, thus confirming the manufacturer's assay.

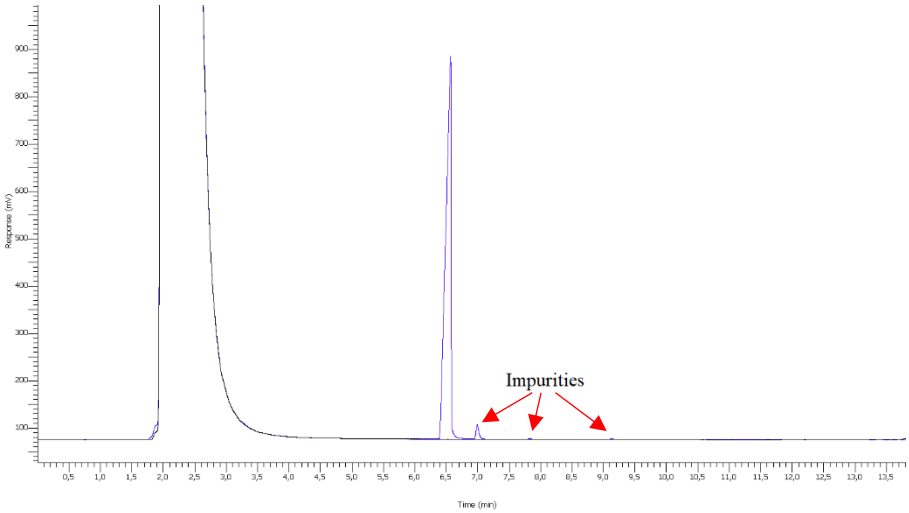

Figure 50. Overlapped chromatograms comparing (±)-Linalool standard reference material in hexane (blue) with only hexane (black).

3.4.3. Fungicide analysis

The importance of food quality has increased in the recent years and reliable measurements has become a serious challenge. Over the past decade, there is a noticeable increase in the use of fungicides worldwide due to the ever-increasing population and rapid urbanization [89]. Hence, it is necessary to monitor and control amounts these substances.

As described in 1.4.2 chapter propiconazole allowed amount (0,1 mg/kg; 0,1 ppm, sum of all isomers) was tied to LC-MS/MS method LOD [90, 91]. While LC-MS/MS is an accurate and reliable method it is also expensive to run and maintain. If propiconazole analysis could be adapted to GC-FID with similar or greater sensitivity, it would make monitoring cheaper and more efficient.

To test cryo-enrichment module performance when analyzing propiconazole standard in pentane, a method was developed. GC and module conditions stated in table X.

Table X. GC conditions.

Injector, °C	Oven, °C	Detector, °C	H_2 flow, ml/min	Split ratio	Cryo temp, °C	Transfer line, °C
270	100 (4 min) → 25 °C /min → 260 (6 min)	270	1	1:10	-60 → 250	250

During the testing phase, propiconazole was concentrated up to 10 times using a cryo-enrichment module (Figure 51), enabling the achievement of a limit of detection (LOD) as low as 0.04 ppm. This LOD surpasses the sensitivity requirements set by the European Commission for propiconazole control. The utilization of GC in conjunction with the cryo-enrichment module proved to be an effective approach for propiconazole analysis. However, it is worth mentioning that propiconazole exhibited a reluctance to elute easily, necessitating the heating of the cryo-enrichment module to temperatures as high as 250 °C. Due to the extended cooling times required at such elevated temperatures, this analysis method may not be practical for routine laboratory use.

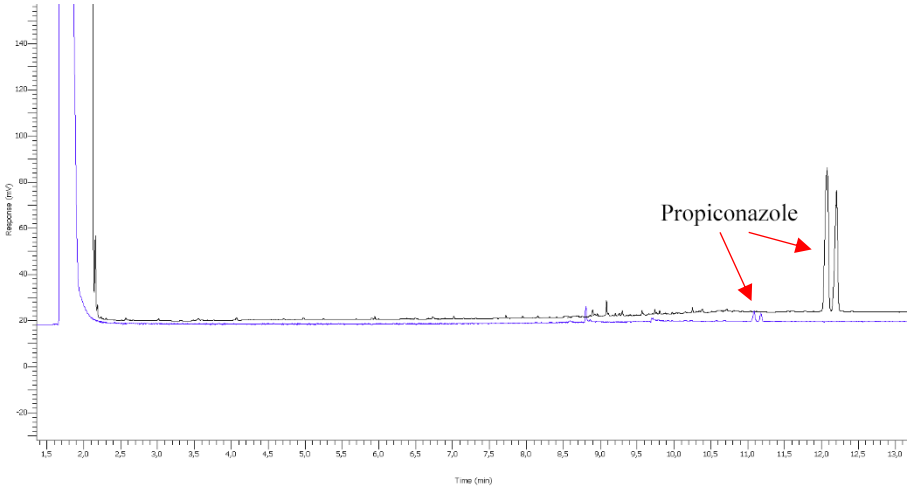

Figure 51. GC chromatograms of propiconazole isomer standard in pentane. Blue single injection enrichment, black 10 injection enrichment.

3.5. Limitations of cryo-enrichment module

While cryo-enrichment module shows promising results like focusing of the peaks and increased sensitivity. However, it is as any other method not without any drawbacks and limitations. Bellow you can see module limitations and drawbacks:

- Module has a limited analyte working range. A subset of compounds employed for the experimental assessment of the module's performance exhibited either non-retention or failed to elute entirely within the module. This could have been caused by the temperature control limitations. While dichloromethane was retained in the

module at -60 °C it showed a minor peak shoulder (see figure 40). Isoprene was not retained properly at -60 °C and was eluting slowly throughout the enrichment process. For less volatile molecules like DEHP modules maximum temperature of 250 °C is not enough. DEHP did not elute after heating the column holder. Module analyte working range can be connected to the analyte vapor pressure (Figure 52). By increasing thermal range, the module's operational scope can be extended to encompass analytes with varying vapor pressure characteristics, thereby enhancing its analytical versatility.

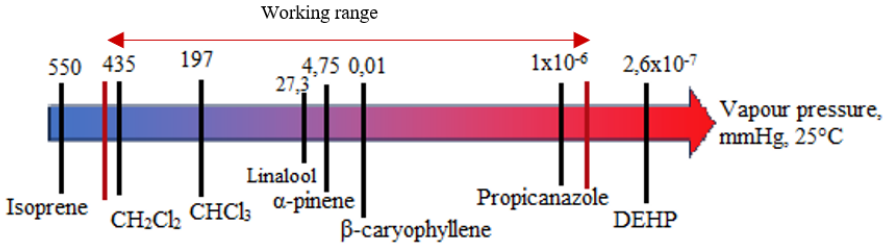

Figure 52. Visualization of analyte vapour pressures used in cryo-enrichment module testing, from highest to lowest (mm Hg, 25 °C) [116].

- Long-term stability of the module was not tested. The enrichment module stability and reproducibility might degrade over time due to factors like wear and tear of equipment, changes in column performance due to high degree of temperature variation.
- Due to module cooling and heating time and also time taken up by multiple injections, system sample throughput is lowered. This module might not be viable option in a high sample throughput application.
- The cooling time of the system to reach working temperature of -60°C is 3-4 hours. This limitation necessitates that the operator plan ahead for system use or leave the system on, which results in unnecessary electricity consumption.
- Using the cryo-enrichment module requires special training and it is not convenient to install or change column. Column is easy to break. Future iterations of the module should move to permanently installed metal column in the module, which system operator can easily attach to the injector and to a selected GC column inside the GC system.

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CONCLUSIONS

- The module has demonstrated the capability to enhance analysis resolution and efficiency by focusing peaks, resulting in narrower and taller peaks. Peak resolution increased up to 2,2 times.
- Extensive experimentation with varying cooled peak lengths has revealed that the most optimal outcomes are achieved when cooling 95 cm of the column length. Sensitivity increased up to 20 times.
- The module's capacity to accommodate a range of analytes is constrained by the working temperature range. Some analytes were not retained adequately within the cryo-enrichment module due to insufficient cooling, while others were retained excessively due to insufficient heating.
- Module is proven to work with multiple applications: method calibration, impurities analysis, fungicide analysis and method was validated for measuring terpenes in *Cannabis Sativa* biomass.
- Module is in a proof of concept and initial development stage. It is not yet ready for commercial applications.

SANTRAUKA

ĮVADAS

Dujų chromatografija (angl. GC) yra vienas plačiausiai naudojamų analizės metodų chemijos laboratorijose ir pramonėje [2]. GC rinkos dydis šiuo metu siekia 3,0 milijardus JAV dolerių, ir prognozuojama, kad iki 2029 m. šis sektorius išaugs iki 3,9 milijardų dolerių [1]. Nors analizėje gali būti naudojami ir kiti metodai, pavyzdžiui, infraraudonųjų ir artimųjų infraraudonųjų spindulių spektroskopija, tačiau dujų chromatografija turi kelis pranašumus. Pirma, GC pasižymi dideliu selektyvumu, kuris yra itin svarbus dirbant su junginiais, turinčiais panašias spektroskopines savybes. Antra, GC gali kiekybiškai įvertinti tiek didėles, tiek mažas junginių koncentracijas įvairiuose mišiniuose (galima matuoti ppm arba net žemesnes nei ppm koncentracijas) [4].

GC iki šiol išlieka itin populiarus ir plačiai naudojamas analizuojant šiuos junginius. Mažos koncentracijos junginių analizė yra svarbi daugelyje sričių, pvz. maisto [5, 6], kvepalų [7], chemijos [8–10] pramonės ir aplinkos chemijoje [11]. Sugriežtinus gamybos reikalavimus, išplėtus teisės aktus ir siekiant sumažinti sąnaudas, atsiranda poreikis naujų metodų kūrimui. Dėl šių priežasčių reikalinga greitesnė analizė, didesnis jautris ir tikslumas. Įprasta labai mažos koncentracijos komponentų dujų chromatografinė analizė apima kelis etapus, tokius kaip: mėginio paruošimas ir (arba) analitės koncentravimas, mėginio įvedimas, atskyrimas ir aptikimas. Vienas iš analičių koncentravimo būdų yra krio-koncentravimas.

Krio-koncentravimas dujų chromatografijoje yra metodas, naudojamas siekiant pagerinti lakiųjų junginių atskyrimą ir aptikimą. Skirtingai nuo tradicinės dujų chromatografijos, šio metodo principas, sumažinti tiek mėginio, tiek chromatografinės kolonėlės temperatūrą iki kriogeninės temperatūros, dažnai žemiau analičių virimo ar net lydymosi taškų. Dėl šio proceso sumažėja junginių lakumas, todėl jie efektyviai kondensuojasi į stacionarią kolonėlės fazę arba ant jos paviršiaus.

Šio baigiamojo darbo metu buvo sukurtas unikalus analičių koncentravimo modulis, kuriuo siekiama padidinti sistemos efektyvumą ir jautrumą. Sukonstravome ir įvertinome kelis krio-koncentravimo modulio prototipus, skirtus įvairioms reikmėms. Krio-koncentravimo procesas pademonstravo įspūdingus rezultatus, ženkliai padidindamas dujų chromatografijos metodų efektyvumą ir jautrumą. Nepaisant to, svarbu

paminėti, kad ši technika ir su juo susijęs modulio prototipas turi apribojimų, susijusių su matuojamų analičių intervalu ir naudojimo patogumu.

Darbo tikslas ir uždaviniai

Pagrindinis šios daktaro disertacijos tikslas – tobulinti dujų chromatografijos efektyvumą ir jautrumą kuriant ir pritaikant naują analitės koncentravimo techniką.

- Sukurti analitės krio-koncentravimo technika pagrįstą prototipą, kuris efektyviai fokusuoja ir koncentruoja analites, taip padidindamas GC analizės jautrį.
- Optimizuoti analitės krio-koncentravimo parametrus.
- Įvertinti krio-koncentravimo technikos efektyvumą.
- Išbandyti praktinį krio-koncentravimo technikos pritaikomumą įvairiose srityse.

Mokslinis naujumas

Nors dujų chromatografija yra įsitvirtinusi ir plačiai naudojama analizės technika, kurią galima pritaikyti įvairiems tikslams, sukurtas analičių krio-koncentravimo modulis išplečia dujų chromatografijos galimybes. Šiuo metodu siekiama padidinti dujų chromatografijos sistemos efektyvumą ir jautrumą, sprendžiant problemas, susijusias su mažos koncentracijos komponentų aptikimu ir kiekybiniu nustatymu. Darbe siekiama prisidėti prie nuolatinės dujų chromatografijos technologijos tobulinimo, leidžiančios tyrėjams ir analitikams pasiekti aukštesnes tikslumo ir aptikimo galimybes analizuojant įvairius lakiuosius junginius.

Ginamieji teiginiai

1. Krio-koncentravimo modulis yra suderinamas su dujų chromatografijos sistema.
2. Krio-koncentravimo modulis padidina atskyrimo ir analizės efektyvumą, fokusuodamas analitės smailes.
3. Sukaupdamas daugybę injekcijų krio zonoje ir sukoncentruodamas jas į vieną smailę, krio-koncentravimo modulis padidina sistemos jautrumą.
4. Krio-koncentravimo modulis gali būti pritaikomas įvairioms dujų chromatografijos reikmėms.

4. LITERATŪROS APŽVALGA

Krio-koncentravimas - tai metodas, kai žema temperatūra naudojama lakiųjų ir pusiau lakiųjų junginių koncentracijai dujų chromatografijos analizėje padidinti. Metodas yra pagrįstas mėginio arba mėginio įleidimo sistemos atšaldymu iki kriogeninės temperatūros. To pasekoje, analitės yra sufokusuojamos ir sukoncentruojamos atšaldytoje kolonėlės dalyje. Krio-koncentravimas turi keletą privalumų, įskaitant didesnį jautrumą, geresnę smailės skiriamąją gebą ir mažesnę matricos komponentų interferenciją. Krio-koncentravimo sistemas, aprašytas literatūroje, galima suskirstyti į kelias kategorijas.

Termoelektrinės aušinimo sistemos yra paprastos ir nereikalauja daug priežiūros, tačiau jų aušinimo pajėgumas yra ribotas. 2005 m. tyrime [78] buvo naudojamas šaldymo įrenginys, galintis atšaldyti kolonėlės dalį iki -32˚C, todėl analitė buvo nevisiškai sulaikoma ir smailės iškraipomos.

Kitas metodas – suslėgto skysčio sistema [79], kuri leidžia greitai atšaldyti ir pasiekti itin žemą temperatūrą. Ši sistema pasižymi didele šaldymo galia ir efektyvumu. Tačiau tam reikia sunaudoti daug šaldymo priemonių, o tai nėra praktiška.

Aušinamų dujų sistemose iki kriogeninės temperatūros atšaldytos dujos naudojamos GC kolonėlės daliai atvėsinti GC sistemos viduje arba išorėje. Atšaldytų azoto dujų sistemą naudojo amerikiečių mokslininkai iš Vašingtono universiteto, kuri leido atskirti visas benzino sudedamąsias dalis per 200 s [80].

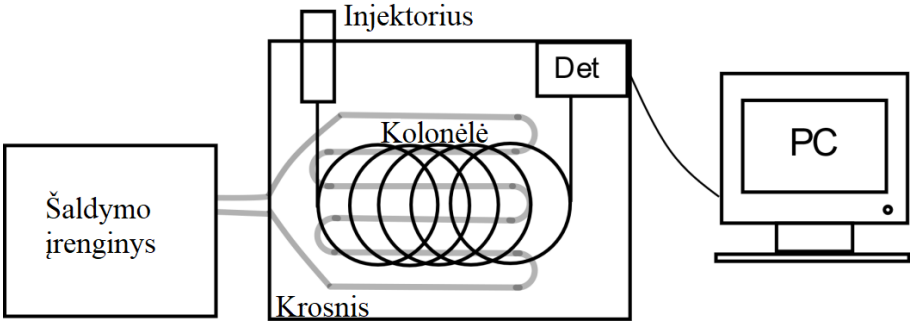
Fokusavimas kolonėlėje – tai metodas, kai mėginiai sutelkiami kolonėlėje, atšaldant kolonėlės dalį iki žemesnės temperatūros nei analizuojamo junginio lydymosi temperatūra. Šis metodas yra patogesnis už analitės koncentravimą klasikiniais metodais (garinimas, ekstrakcija, adsorbcija), nes nereikia atlikti papildomų mėginio paruošimo etapų. Sung-Tong Chin ir kt [81], pritaikė judančios kolonėlės aušinimo sistemą dvimatėje chromatografijoje, kai analitės injektuojamos į pirmąją kolonėlę ir keliauja link FID detektoriaus. Analizė antrojoje kolonėlėje pradedama keičiant šaldymo įrenginio kryptį prieš dujų srautą ir palaipsniui didinant dujų chromatografo krosnelės temperatūrą. Autoriai rekomenduoja užšaldyti ilgesnę kolonėlės atkarpą arba naudoti kolonėlę su storesniu stacionariosios dangos sluoksniu, kad būtų pagerinta analičių smailės forma ir padidintas įpurškimų skaičius.

5. PROTOTIPO KŪRIMAS IR TYRIMŲ REZULTATAI

5.1. Krosnies šaldymas

Prieš tiriant pažangesnę krio-koncentravimo technologiją, buvo sukurtas nesudėtingas krosnies aušinimo prototipas, skirtas pagerinti standartinės dujų chromatografijos sistemos šiluminį valdymą. Standartinė GC sistema yra pagrįsta kaitinimo spirale ir ventiliatoriumi temperatūros kontrolei, tačiau ji negali veiksmingai atvėsinti krosnies iki žemesnės nei aplinkos temperatūros.

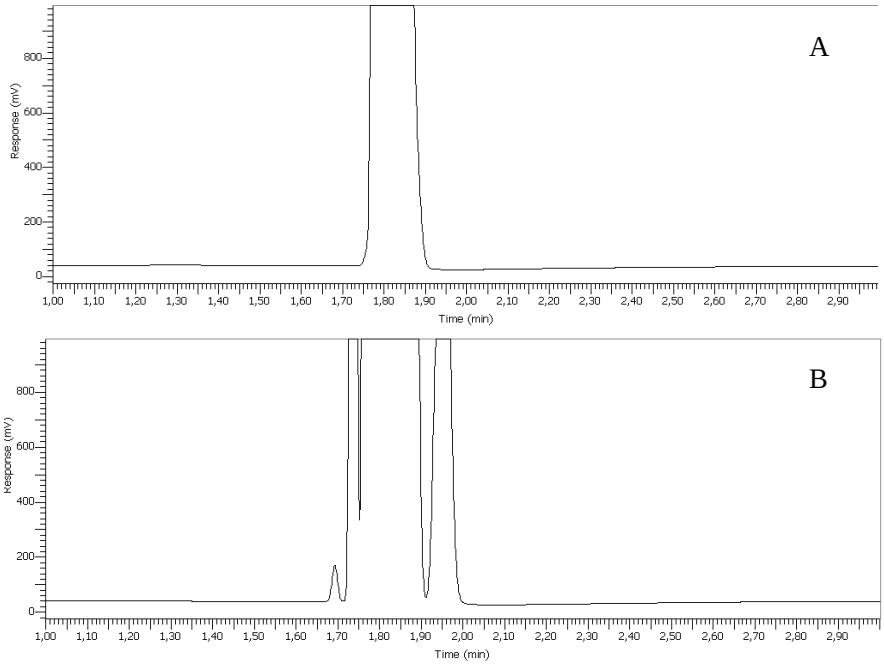
Siekiant išspręsti neefektyvaus šaldymo problemą, į GC sistemą buvo integruotas individualus šaldymo įrenginys, pagrįstas šilumos siurblio principu. Įrenginį sudarė: išorinis komponentas su kompresoriumi, kondensatoriumi, šiluminio plėtimosi vožtuvu ir vidinis komponentas – plonas metalinis garintuvas, patalpintas GC krosnies viduje (1 pav.). Suslėgtas šaltnešis cirkuluoja per sistemą, efektyviai šaldydamas GC krosnį.



1 pav. Dujų chromatografijos sistema su krosnies aušinimo priedu.

Naudojant šaldymo įrenginį ženkliai pagerėjo GC krosnies aušinimo charakteristikos. Atvėsimo nuo 200 ºC iki 40 ºC laikas sutrumpėjo nuo 8 minučių ir 20 sekundžių iki vos 1 minutės ir 56 sekundžių. Šis aušinimo laiko sutrumpėjimas padidino sistemos mėginių pralaidumą, pagerino analizės greitį.

Be to, aušinimo įrenginys leido geriau atskirti ir išplėtė GC sistemos galimybes matuoti didesnio lakumo junginius. Sistemai testuoti buvo pasirinktas dujų mišinys, daugiausia sudarytas iš izobutano ir jo izomerų bei priemaišų. Atliekant analizę be šaldymo priedo (pradinė temperatūra 35 °C) ir su šaldymo priedu (pradinė temperatūra -10 °C), buvo pastebėtas didelis skirtumas junginių atskyrime. Prie žemesnės temperatūros dujų mišinys buvo atskirtas į kelias smailes (2 pav.).

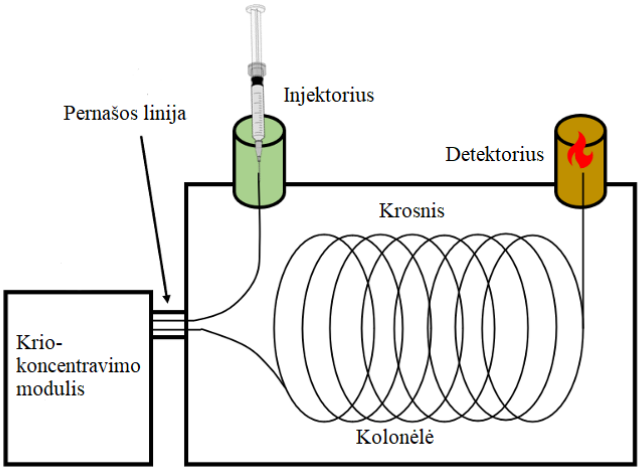


2 pav. **A** Chromatograma, atlikta pradedant nuo 35 °C temperatūros. **B** Chromatograma, atlikta pradedant nuo -10 ºC temperatūros.

Nepaisant šių aiškių GC metodo patobulinimų, tyrimai buvo nutraukti ir buvo pereita prie kolonėlės krio-šaldymo, kuris leido pasiekti dar žemesnę temperatūrą ir padidinti sistemos jautrį.

5.2. Pirmasis krio-koncentravimo prototipas

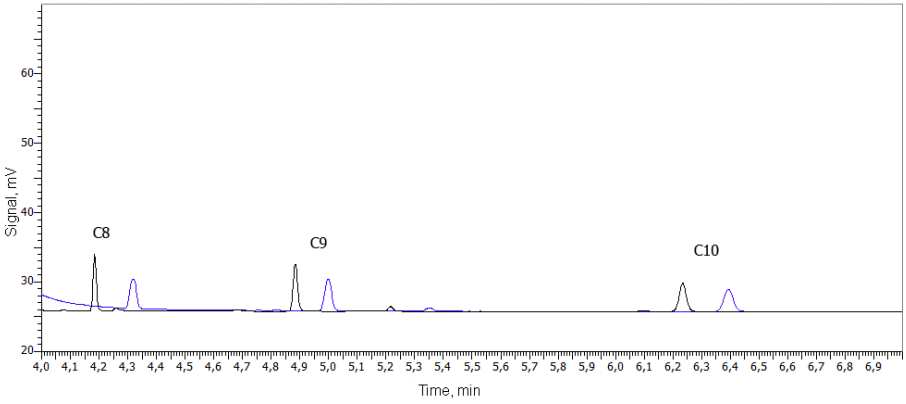
Disertacijoje aptariami įvairūs mėginių ar analičių koncentravimo metodai, daugiausia dėmesio skiriant kriogeninio koncentravimo kolonėlėje metodui. Šio darbo metu pagamintas GC modulis, sudarytas iš keturių aliuminio žiedų, kuriuose integruoti nichromo vielos šildytuvai ir kapiliarinė GC kolonėlė. Šie žiedai yra termiškai izoliuoti ir prijungti prie GC sistemos per šildomą perdavimo liniją, o modulis pastatytas ant skysto azoto diuaro (3 pav.). Skysto azoto srautas reguliuojamas laboratorijoje sukurto kaitintuvo pamerkto į diuarą pagalba. GC kolonėlė iš injektoriaus vedama per krio-koncentravimo modulį ir atgal į dujų chromatografo krosnį, kur vyksta chromatografinis atskyrimas ir analitės pasiekia liepsnos jonizacinį detektorių.



3 pav. Krio-koncentravimo sistemos, prijungtos prie dujų chromatografo, schema.

5.2.1. Krio-fokusavimas

Atlikome krio-koncentravimo modulio gebėjimo sulaikyti ir sufokuosuoti analites tyrimus. Modulis atšaldytas iki -60 ºC prieš įleidžiant 1 µl analičių mišinio (oktanas, nonanas, dekanas ištirpinti dichlorometane) į 150 ºC temperatūros įkaitintą injektorių. Helio dujų srautas 1 ml/min, srauto dalijimas 1:10. Analitėms pasiekti modulį ir ten kondensuotis, buvo skirta 2 minutės ir tada modulis buvo greitai įkaitintas iki 120 ºC, kad analitės vėl taptų judrios ir galėtų judėti išilgai kolonėlės (krosnies temperatūra 80 ºC) link FID detektoriaus (temperatūra 150 ºC).



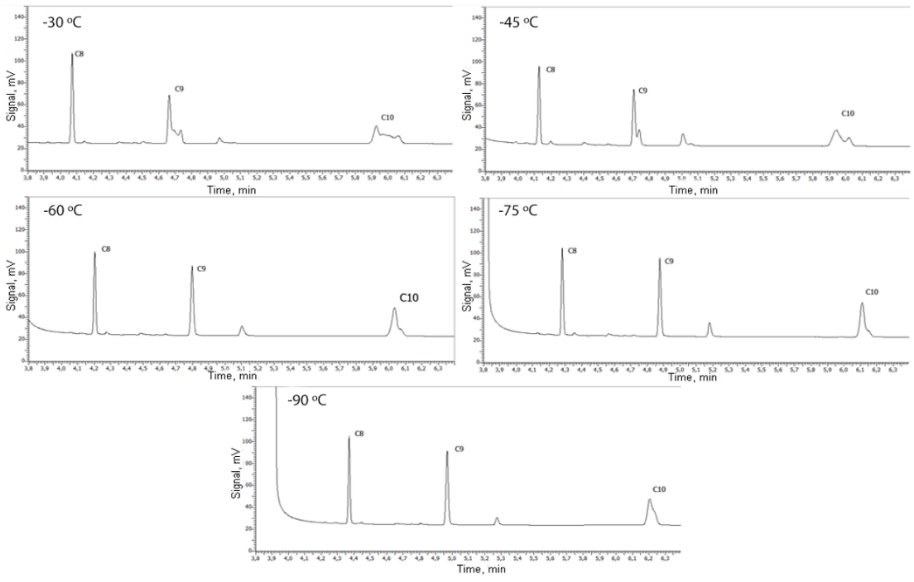
4 pav. Fokusuotų ir nefokusuotų chromatogramų palyginimas. Analitės: oktanas (C8), nonanas (C9), dekanas (C10). Mėlyna spalva - analizė be fokusavimo, juoda - analizė su fokusavimu.

Iš rezultatų matyti (4 pav.), kad dėl krio-fokusavimo smailės tapo siauresnės ir aukštesnės, o tai padidino metodo efektyvumą ir jautrumą. Oktano smailės aukštis padidėjo iki 1,83 karto, o sunkesnių analičių (nonano ir dekano) smailės aukštis padidėjo mažiau (atitinkamai 1,47 ir 1,27 karto). Skiriamoji geba tarp oktano ir nonano smailės padidėjo 2,2 karto (nuo 7,2 iki 15,8).

5.2.2. Krio-koncentravimas

Tyrime buvo įvertintas sukurto modulio analičių koncentravimo efektyvumas, kelis kartus įleidžiant analičių mišinį į GC sistemą. Visų injekcijų metu modulyje buvo palaikoma -60 ºC temperatūra. Po dviejų minučių krio-koncentravimo modulis buvo įkaitintas iki 120 ºC. Rezultatai parodė, kad įleistos analitės buvo sulaikytos šaltojoje kolonėlės dalyje, o analitės plotas didėjo tiek kartų, kiek buvo atlikta injekcijų.

Tiriant šaldomos kolonėlės ilgio įtaką krio-koncentravimui gauta, kad efektyviausias koncentravimas vyko, kai šaldomos kolonėlės dalies ilgis buvo 95 cm. Naudojant 95 cm ilgio -60 ºC temperatūroje atšaldytą kolonėlę, buvo galima sulaikyti iki 20 injekcijų, todėl smailės plotas padidėjo iki 20 kartų. Oktano ir nonano smailės buvo siauros ir aukštos, o dekano smailė pasižymėjo asimetrija.



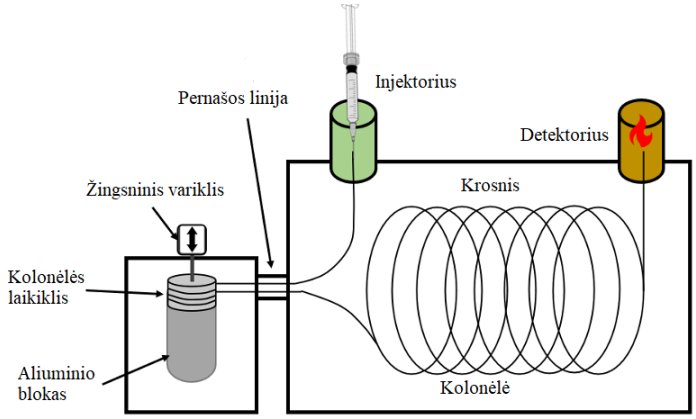
5 pav. Smailės formos priklausomybė nuo temperatūros šaldomoje kolonėlės zonoje. Analizuojamos medžiagos: oktanas (C8), nonanas (C9), dekanas (C10).

Darome prielaidą , kad pastebėta dekano smailės asimetrija pasireiškė dėl nuoseklių injekcijų ir jų įnešamos šilumos į šaldomą kolonėlės segmentą bei netolygios temperatūros kontrolės. Po kiekvienos injekcijos tirpiklis patekęs į šaldomą kolonėlės segmentą įneša šilumos, dėl kurios analitės pajuda šaldoma kolonėlės dalimi. Dekanas, kuris labiau linkęs kondensuotis, nei nonanas ar oktanas, netolygiai pasiskirsto šaldomoje kolonėlės dalyje.

Nustatyta, kad temperatūra yra esminis veiksnys, turintis įtakos smailės formai ir pločiui. Nonanas pasižymėjo asimetrija esant -30 °C ir -45 °C temperatūrai, o dekanas - iki -90 °C, tačiau jo smailė tapo siauresnė ir simetriškesnė toliau mažėjant temperatūrai. Dichlormetano sulaikymas prasidėjo nuo -60 ºC ir didėjo mažėjant temperatūrai.

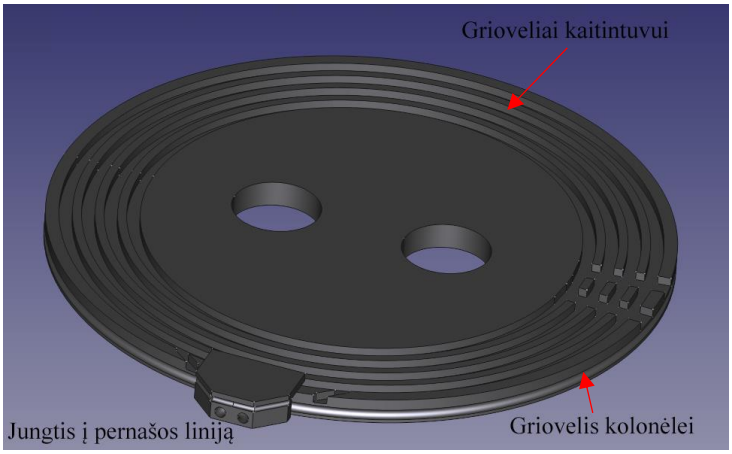
5.3. Patobulintas prototipas

Atsižvelgiant į prieš tai atliktus tyrimus ir ju rezultatus, siekdami pašalinti prieš tai buvusio modulio trūkumus jį patobulinome. Pirmiausia buvo atsisakyta skysto azoto aušinimo sistemos, ją pakeičiant dviejų pakopų garinamojo šaldymo sistema., Tai padaryta siekiant atsisakyti sąnaudinių azoto dujų ir supaprastinti darbą GC operatoriui. Tačiau šios sistemos trūkumas yra ribota atšaldymo galia – neužtenka galios greit atšaldyti modulį iki -60 °C. Siekiant išspręsti šį trūkumą, buvo pakeista aliumininio kolonėlės laikiklio ir viso modulio konstrukcija. Kolonėlės laikiklio dydis buvo sumažintas siekiant pagreitinti šaldymą ir kaitinimą (7 pav.). Visas šaldymo sistemos darbas buvo sutelktas į daug didesnės terminės masės (nei kolonėlės laikiklis) aliuminio bloką. Blokas ir laikiklis atskiriami kaitinimo metu, taip pagreitinant kaitinimą ir išlaikant modulį šaltame stovyje.



6 pav. Krio-koncentravimo modulio prototipo, prijungto prie dujų chromatografo, schema.

Pakeistą modulį sudaro dvi aliuminio dalys: šaldomas aliuminio blokas (10x13 cm) ir kolonėlės laikiklis su grioveliais nichromo vielos šildytuvui (7 pav). Kolonėlės laikiklio padėtį valdo žingsninis variklis, todėl šaldymo metu jis liečiasi su šaltu bloko paviršiumi, o kaitinimo metu greitai pakyla 1-2 mm virš bloko paviršiaus. Šie konstrukcijos patobulinimai kaitinimo nuo -60 °C iki 150 °C laiką sutrumpino nuo dviejų iki vienos minutės. Modulis yra termiškai izoliuotas, siekiant užtikrinti temperatūros stabilumą. GC prie modulio prijungtas per šildomą perdavimo liniją, o kolonėlės laikiklio padėtis valdoma valdikliu prijungtu prie GC sistemos.

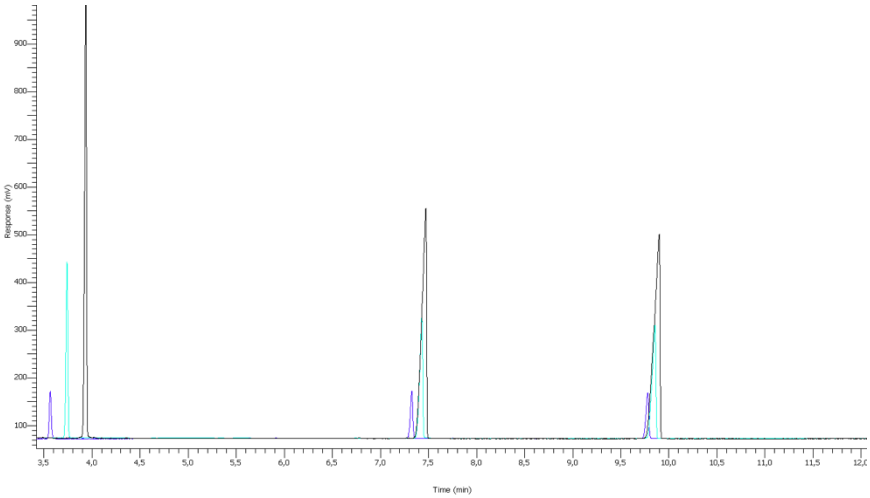


7 pav. Aliuminio kolonėlės laikiklio brėžinys

5.3.1. Patobulinto prototipo tyrimai

Siekiant pašalinti priklausomybę nuo dujų linijos, buvo pakeistos nešančios dujos: iš helio į vandenilį. Tačiau esant kriogeninei temperatūrai kolonėlėje pradėjo formuotis ledo kamščiai, dėl drėgmės esančios dujose. Šiai problemai išspręsti buvo sukonstruotas dujų filtras su silikageliu ir anglies molekuliniu sietu.

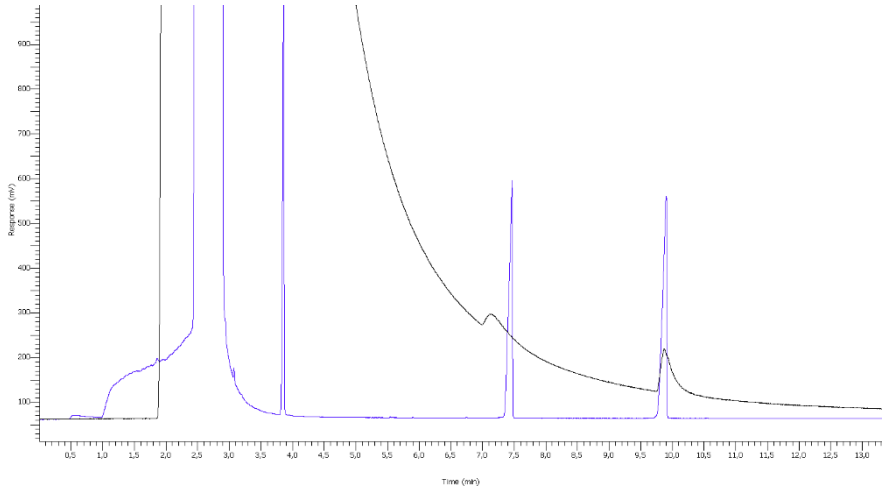
Išsprendus dujų srauto problemą, naujojo modulio veikimas buvo įvertintas naudojant oktano, nonano ir dekano mišinį. Dėl tolygaus aušinimo ir greitesnio kaitinimo smailių formos susiaurėjo, o dekano atveju panaikintas smailių padvigubėjimas (8 pav.).



8 pav. Chromatogramų palyginimas: 1 injekcija (tamsiai mėlyna), 5 injekcijos (šviesiai mėlyna), 10 injekcijų (juoda).

Išbandytas nedalomos injekcijos režimas (angl. splitless injection mode) su krio-koncentravimo moduliu. Naudojant šį režimą mėginys įleidžiamas į injektorių, tačiau srautas neskaidomas. Dėl šios priežasties mėginio patekimo į kolonėlę laikas prailgėja ir sukelia didžiulį smailės išsiplėtimą. Buvo numanoma, kad krio-koncentravimo modulis turėtų išspręsti šią problemą fokusuodamas smailes po injekcijos. Analizuojant mėginį be krio-koncentravimo modulio buvo pastebėta, kad oktano smailė visiškai išnyksta, o nonano ir dekano smailės išlieka, tačiau stipriai persidengia su tirpiklio smailėmis. Tuo tarpu atliekant injekciją be padalijimo su modulio smailės yra gerai atskiriamos (9 pav.).

Galima kelti klausimą: kodėl reikia atlikti 10 įpurškimų su padalijimu, o ne 1 įpurškimą be padalijimo? Siekiant atsakyti į šį klausimą, buvo palyginti du skirtingi metodai. Rezultatai atskleidžia, kad 10 įpurškimų su padalijimu sukuria siauresnes ir aukštesnes smailes, taip pat užtikrina geresnę skiriamąją gebą tarp tirpiklio smailės ir oktano smailės. Nors skirtumas reliatyviai nedidelis, skiriamoji geba padidėjo 20 %.



9 pav. Oktano, nonano, dekano pentane chromatogramos be srauto dalinimo. Be krio-koncentravimu (juoda), su krio-koncentravimu (mėlyna).

Siekiant įvertinti krio-koncentravimo modulio veikimą su skirtingomis medžiagomis, buvo atlikta daugiau matavimų. Analitės buvo pasirinktos pagal jų lakumą (nuo labiausiai lakių iki mažiausiai lakių): izoprenas, chloroformas, α-pinenas ir Bis(2-etilheksil) ftalatas (DEHP). Izoprenas pasižymėjo pernelyg dideliu lakumu, dėl to kilo sunkumų nuosekliai renkant kelias injekcijas iš eilės, o smailės buvo dvigubos ar išsiplėtusios. DEHP buvo per stipriai sulaikomos modulyje, o smailės nebuvo patikimai registruojamos net įkaitinus modulį iki 250 °C temperatūros. Iš visų analizuojamų medžiagų α-pinenas davė patikimiausius rezultatus su siauromis ir aukštomis smailėmis. Atskiriant chloroformą nuo tirpiklio dichlormetano kilo sunkumų, nes smailės persidengė. Siekiant išspręsti šią problemą tirpiklis ir analitė buvo sukeisti. Chloroforme buvo ištirpintas mažesnis dichlormetano kiekis ir smailės atsiskyrė. Vis tik, po 10 injekcijų buvo pastebėtas tam tikras dichlormetano smailės padvigubėjimas ir deformacija. Šie rezultatai atskleidžia, kad krio-koncentravimo modulio veikimo intervalas yra ribotas, apatinė riba - nepakankamas šaldymas (-60 °C), o viršutinė riba - maksimali 250 °C kaitinimo temperatūra.

5.3.2. Terpenų analizė naudojant krio-koncentravimą

5.3.2.1. Metodo kūrimas

Sukurtas α-pineno ir β-kariofileno matavimo kanapėse metodas. Iš pradžių buvo sukurtas GC-MS metodas, kuris leido lengviau identifikuoti

smailes mėginiuose ir buvo panaudotas kaip palyginimo taškas lyginant su GC-FID metodu. Teisingi terpenų ekstrakcijos iš kanapių biomasės parametrai buvo labai svarbūs metodo patikimumui. Atlikus literatūros apžvalgą, ekstrakcijos metodu pasirinkta sonikacija organiniame tirpiklyje. Kanapių medžiaga buvo smulkiai sumalta, o ekstrakcija atlikta 50 ml plastikiniuose buteliukuose. Ekstraktai buvo filtruojami ir surenkami į 2 ml gintarinio stiklo chromatografinius buteliukus. Siekiant nustatyti ekstrahavimo tirpiklio įtaką terpenų ekstrakcijai iš kanapių, buvo įvertinti keturi tirpikliai (metanolis, n-heksanas, acetonas ir acetonitrilas). Didžiausias terpenų smailės plotas buvo gautas iš n-heksano. Vėliau buvo įvertinta temperatūros įtaka ekstrakcijai, kuri buvo atliekama 15 min. veikiant skirtingoms temperatūroms (30±1 °C, 40±1 °C, 50±1 °C ir 60±1 °C). Gauta, kad terpenų kiekis didėjant temperatūrai nežymiai kito. Pastebėta, kad didesni smailės plotai 60 °C temperatūroje atsirado dėl tirpiklio garavimo, todėl nuspręsta ekstrakciją atlikti 30 °C temperatūroje. Lyginant ekstrakcijos trukmę (5, 10, 15 ir 20 minučių), išgautų terpenų kiekiai reikšmingai nesiskyrė. Tolesnis tyrimas atskleidė, kad didesni smailės plotai, po 20 min. ekstrakcijos, atsirado dėl tirpiklio garavimo, todėl pasirinktas 5 min. ekstrakcijos laikas.

I Lentelė. Optimizuotos terpenų ekstrakcijos sąlygos.

Ekstrakcijos parametras	**Rezultatas**
Tirpiklis	n-heksanas
Tirpiklio tūris, ml	10,00±0,30
Temperatūra, °C	30±1
Laikas, min	5

5.3.2.2. GC-MS sąlygos

Terpenų (*α*-pineno ir *β*-kariofileno) identifikavimas ir kiekybinė analizė atlikta naudojant "Perkin Elmer Autosystem XL" dujų chromatografijos sistemą su "Perkin Elmer TurboMass Upgrade" masės spektrometru. Naudota chromatografinė kolonėlė Agilent DB-5MS (30 m x 0,250 mm, 0,25 µm), o nešančiosios dujos - helis. Analizės sąlygų optimizavimas buvo labai svarbus žingsnis siekiant patikimo ir tikslaus metodo. Optimizuotos tokios analizės sąlygos: injektoriaus temperatūra - 300 °C, kolonėlės temperatūra - 60 °C, įpurškimo tūris - 1 µl. Dujų chromatografo temperatūra buvo palaipsniui didinama nuo 60 °C (1 min.) iki 300 °C 10 °C/min. greičiu. Bendra vieno

mėginio analizės trukmė buvo 30 minučių. Masių spektrometrijos detektoriaus sąlygos išsamiai aprašytos II lentelėje.

II lentelė. Masių spektrometro parametrai.

Jonizacijos šaltinio temperatūra, °C	Pernašos linijos temperatūra, °C	Analizės laikas, min	Skenavimo greitis, s	Masių skenavimo intervalas, m/z
300	300	30	0,1	50-350

Šių terpenų analizės GC-MS metodas buvo validuotas, valdiavimo duomenys pateikti III lentelėje.

III lentelė. Metodo validavimo rezultatai.

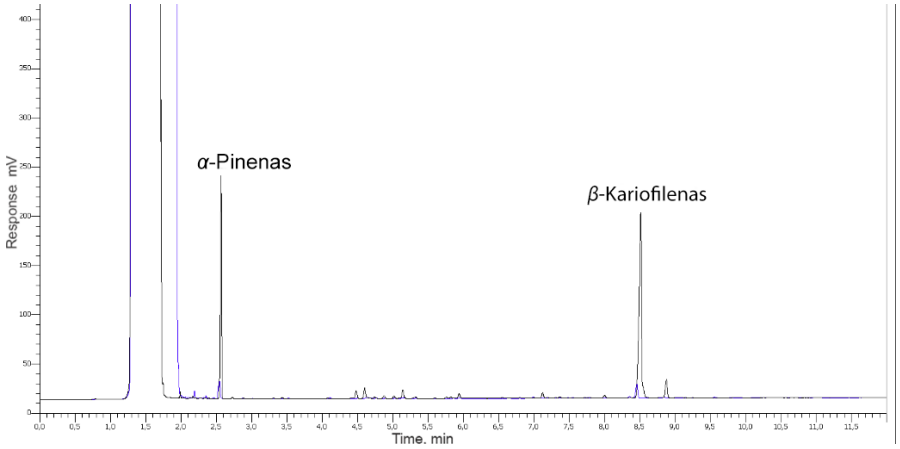
Analitė	SSN	U	Išgava	U	Koreliacijos koef.	Tiesės lygtis	LOD	LOQ
	%	±	%	±	R^2		ppm	ppm
α-Pinenas	5,4	0,2	92,6	3,4	0,9965	y = 0,0053x– 0,0126	0,28	0,83
β-Kariofilenas	5,7	0,2	111,5	5,1	0,9936	y = 0,0041x– 0,0228	0,30	0,91

5.3.2.3. Analizė naudojant krio-koncentravimą

Sukurtas ir patvirtintas *α*-pineno ir *β*-kariofileno identifikavimo ir kiekybinės analizės metodas džiovintų kanapių (*Cannabis sativa*) mėginiuose (10 pav.). Buvo parengtos standartinės kalibravimo kreivės *α*-pineno 0,2-204 ppm ir *β*-kariofileno 0,1-128 ppm koncentracijos intervalais.

Nustatyta, kad 10 iš eilės atliktų mėginio injekcijų užtikrina geriausią smailės simetriją, jautrumą ir pakartojamumą. Mėginio injekcijos metu GC krosnies temperatūra buvo 150 °C, siekiant kad visos analitės patektų į modulį, kuriame buvo palaikoma -60 °C temperatūra. Po visų injekcijų krosnis buvo atvėsinta iki 50 °C, o kolonėlės laikiklis pakeltas. Temperatūra kriogeninio koncentravimo kolonėlės laikiklyje buvo greitai padidinta iki 150 °C. Visos analizės metu injektoriaus ir detektoriaus temperatūra buvo palaikoma 200 °C temperatūroje. Injekcijos tūris buvo 1 µl, o nešančiųjų dujų (vandenilis) srautas buvo 1 ml/min, o padalijimo vertė - 10 ml/min. GC krosnies temperatūros programa buvo sudaryta iš 3 minučių išlaikymo 50 °C

temperatūroje, po to palaipsniui didinant temperatūrą iki 200 °C 10 °C/min greičiu.



10 pav. *α*-pineno ir *β*-kariofileno standartų chromatogramos. GC be koncentravimo (mėlyna spalva) ir naudojant krio-koncentravimo modulį (juoda spalva).

Metodas buvo sėkmingai validuotas, o tai įrodo, kad jis tinka *α*-pineno ir *β*-kariofileno analizei *Cannabis Sativa* biomasėje (IV lentelė). Įvertinus tikslumą, *α*-pineno santykinio standartinio nuokrypio (RSD) vertės buvo 6,7 %, o *β*-kariofileno - 6,8 %, o tai rodo aukštą tikslumo lygį. Tikrumas buvo nustatytas pagal išgavą - *α*-pineno išgava buvo 95,3 % ± 2,4 %, o *β*-kariofileno - 105,7 % ± 3,1 %, t. y. neviršijo rekomenduojamo 70-120 % intervalo.

Tiesiškumas buvo patvirtintas kalibravimo kreivėmis, kurių R^2 vertės *α*-pinenui buvo 0,9963, o *β*-kariofilenui - 0,9972, atitinkamai 0,2-204 ppm ir 0,1-128 ppm koncentracijos ribose.

Nustatytos aptikimo (LOD) ir kiekybinio įvertinimo (LOQ) ribos: *α*-pineno LOD vertė - 0,35 ppb, *β*-kariofileno - 0,23 ppb, o LOQ vertė - 1,05 ppb *α*-pineno ir 0,68 ppb *β*-kariofileno. Šios vertės rodo didelį jautrumą nustatant ir kiekybiškai įvertinant labai mažas terpenų analitės koncentracijas.

IV lentelė. Metodo validavimo rezultatai.

Analitė	SSN	U	Išgava	U	Koreliacijos koef.	Tiesės lygtis	LOD	LOQ
	%	±	%	±	R^2		ppb	ppb
α-Pinenas	6,7	0,3	95,3	2,4	0,9963	y = 18675x + 52469	0,35	1,05
β-Kariofilenas	6,8	0,3	105,7	3,1	0,9972	y = 54595x + 78699	0,23	0,68

Apibendrinant buvo nustatyta, kad krio-koncentravimo modulis veikia taip, kaip tikėtasi, ir užtikrina tikslius pasirinktų analičių matavimo rezultatus. Svarbiausias krio-koncentravimo modulio privalumas - padidėjęs metodo jautrumas, kuris, palyginti su kvadrupoline GC-MS, yra ~1000 kartų didesnis (0,3 ppm → 0,23 ppb).

5.4. Papildomi pritaikymai

5.4.1. Kalibracija

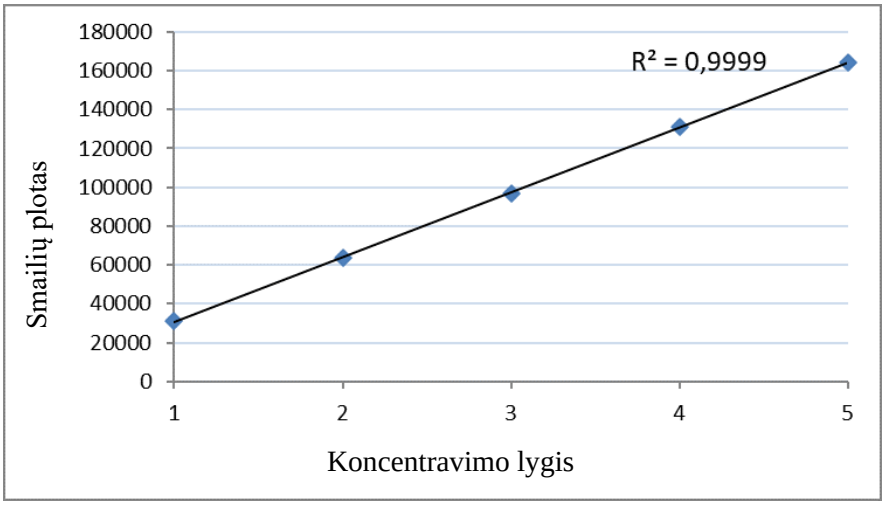
Dujų chromatografijoje kalibravimo kreivės paprastai sudaromos naudojant vienodo tūrio injekcijas su kintančia standartinio tirpalo koncentracija. Nors gali būti patogu naudoti vieną kalibravimo tirpalą su reguliuojamu įpurškimo tūriu, toks metodas gali būti netinkamas tikslumo požiūriu. Su kintamo tūrio injekecijos kalibravimu susiję keli iššūkiai:

1. Nelinijinis atsakas: GC sistemos gali neturėti linijinio atsako plačiame įpurškimo tūrio intervale. Keičiant įpurškimo tūrį, detektoriaus atsakas gali būti netiesinis, todėl sudėtinga tiksliai kiekybiškai nustatyti junginius. Šį netiesiškumą gali lemti auto-injektoriaus netikslumai.

2. Kolonėlės perkrovimas: Injektavus didesnį mėginio tūrį, kolonėlė gali būti perkrauta, todėl iškraipoma smailės forma, atsiranda uodegos ir persidengiančios smailės.

3. Instrumento apribojimai: Kai kurie GC prietaisai gali turėti apribojimų, susijusių su injekcijos tūrių, kuriuos jie gali tiksliai apdoroti, intervalu. Naudojant injekcijos tūrius, kurie nepatenka į optimalų prietaiso intervalą, rezultatai gali būti netikslūs.

Siekiant išspręsti šias problemas, buvo bandoma taikyti kitokį metodą, naudojant krio-koncentravimo prototipą. Taikant šį metodą, injekcijos tūris išliko pastovus, tačiau svyravo injekcijų skaičius. Paruoštas standartinis nonano tirpalas pentane (100 ppm). Analitė buvo sulaikoma ir praturtinama modulyje penkių taškų kalibravimui, o praturtinimo lygis koreliavo su praturtintų injekcijų skaičiumi.



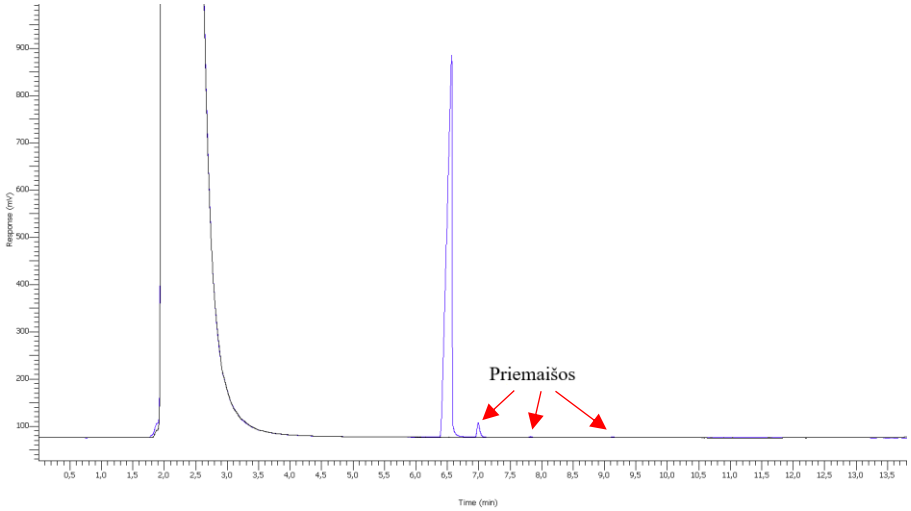
11 pav. Smailių plotai esant skirtingiems koncentravimo lygiams

Pasiektas didelis kalibravimo tikslumas, vizualinę reprezentaciją galima matyti 11 paveiksle. Šio metodo pritaikymas suteikia didelių privalumų, visų pirma supaprastina kalibracinių tirpalų ruošimą GC analizei. Be to, jis sumažina žmogiškųjų klaidų riziką, todėl padidėja bendras analizės proceso patikimumas ir tikslumas.

5.4.2. Priemaišų analizė

Analitinėje chemijoje kiekybinis priemaišų nustatymas sertifikuotuose pamatinėse medžiagose (CRM) yra daug tikslumo reikalaujantis procesas. Siekiant nustatyti ir išmatuoti priemaišų kiekį taikomi tokie pažangūs metodai kaip didelio efektyvumo skysčių chromatografija, dujų chromatografija ir masės spektrometrija, induktyviai surištos plazmos optinė emisijos spektroskopija (ICP-OES), titravimas, infraraudonųjų spindulių spektrometrija, branduolių magnetinis rezonansas ir kt. [116]. CRM yra labai svarbūs matavimų etalonai, užtikrinantys analizės metodų patikimumą ir leidžiantys palyginti skirtingų laboratorijų rezultatus. Tai labai svarbu siekiant užtikrinti kokybės kontrolę įvairiose pramonės šakose, kuriose svarbi tiksli priemaišų analizė.

Priemaišų analizė buvo atlikta naudojant standartinę etaloninę medžiagą (±)-linalolį. Gamintojas (Fluka) nurodo, kad standartinės medžiagos grynumas, nustatytas atliekant dujų chromatografijos (GC) bandymus, yra daugiau kaip 95,0 %. Analizei atlikti linalolio tirpalas heksane buvo dešimt kartų injektuotas į GC sistemą.



12 pav. (±)-linalolio standartas heksane (mėlyna) ir tik heksanas (juoda), išmatuota naudojant krio-koncentravimo modulį.

Chromatogramoje matosi kelios smailės, pagrindinė, atitinkanti (±)-linalolą, ir trys mažesnės, rodančios, kad yra priemaišų (12 pav.). Norint patikrinti, ar šios priemaišos atsirado ne iš tirpiklio ar sistemos, tas pats matavimas buvo pakartotas naudojant tik heksaną. Apskaičiuotas (±)-linalolo grynumas yra 97,7 %, taip patvirtinant gamintojo tyrimą.

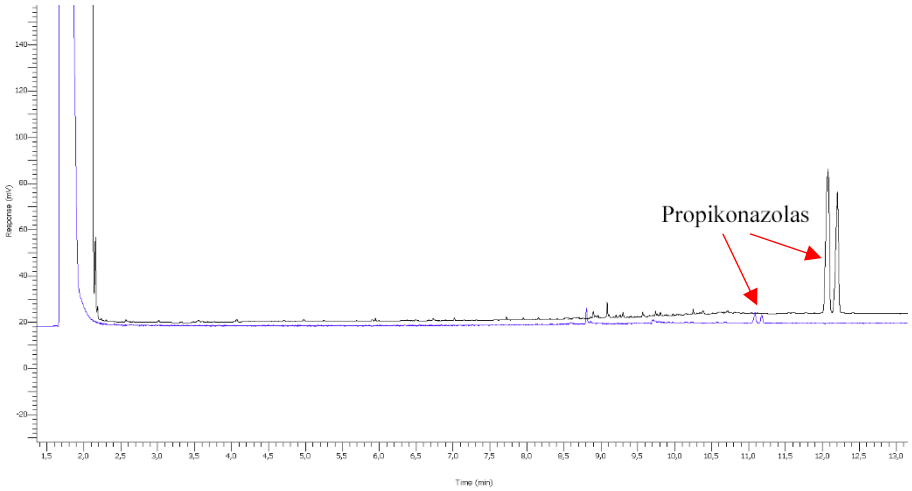
5.4.3. Fungicidų analizė

Maisto kokybės svarba labai išaugo dėl plačiai paplitusio pesticidų, herbicidų ir fungicidų naudojimo, kurį lemia didėjantis gyventojų skaičius ir urbanizacija. Siekiant užtikrinti maisto saugą, tapo būtina stebėti ir kontroliuoti šių medžiagų kiekį, pavyzdžiui ES neseniai buvo uždraustas fungicidas propikonazolas. ES Komisija nustatė nulinę propikonazolo tolerancijos ribą, nors pripažįstama, kad absoliutaus nulio neįmanoma išmatuoti analitinėje chemijoje. Leistinas propikonazolo kiekis (0,1 mg/kg; 0,1 ppm, visų izomerų suma) buvo susietas su LC-MS/MS metodo LOD. Nors LC-MS/MS metodas yra tikslus ir patikimas, tačiau jis yra sudėtingas ir brangus. Pritaikius propikonazolo analizę GC-FID, kurios jautrumas būtų panašus arba didesnis, analizė taptų pigesnė.

Siekiant įvertinti krio-koncentravimo modulio veikimą analizuojant propikonazolo standartą pentane, buvo sukurtas GC metodas (13 pav.). Atliekant bandymus, krio-koncentravimo modulyje propikonazolas buvo

koncentruotas iki 10 kartų, o LOD siekė 0,04 ppm. Šis LOD viršija Europos Komisijos nustatytus propikonazolo kontrolės jautrumo reikalavimus.

Atliekant propikonazolo analizę, GC derinys su krio-koncentravimo moduliu pasirodė esąs veiksmingas. Tačiau verta pažymėti, kad propikonazolas pasižymi aukšta virimo temperatūra, todėl reikėjo kaitinti krio-koncentravimo modulį iki 250 °C temperatūros. Dėl aukštos kaitinimo temperatūros, prailgėja šaldymo laikas, todėl šis analizės metodas gali būti mažiau praktiškas įprastiniam laboratoriniam naudojimui.

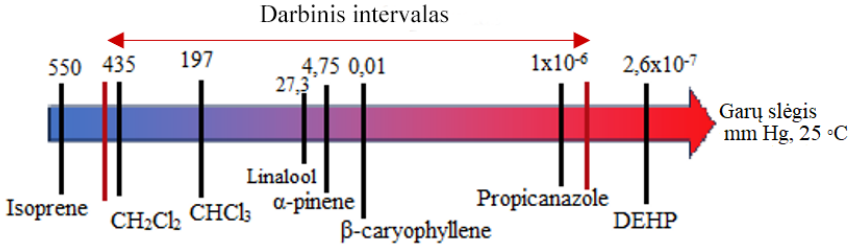


13 pav. Propikonazolo izomerų standarto pentane GC chromatogramos. Mėlyna viena injekcija, juoda - 10 injekcijų.

5.5 Modulio ribotumai

Nors krio-koncentravimo modulis duoda daug žadančių rezultatų, jis turi tam tikrų trūkumų ir ribotumų:

- Modulis turi ribotą analičių matavimo intervalą (14 pav.). Kai kurie išbandyti junginiai modulyje nebuvo sulaikomi arba buvo sulaikomi per stipriai. Šį ribotumą galima sieti su temperatūros kontrolės apribojimais, pavyzdžiui, mažiau lakių molekulių, tokių kaip DEHP, nebuvo galima tinkamai analizuoti, nes modulio maksimali 250 °C temperatūra buvo nepakankama. Išplėtus modulio temperatūrinį intervalądia, būtų galima išplėsti jo veikimo galimybes, kad būtų galima tirti įvairesnes analites, kurių garų slėgio charakteristikos skiriasi, ir taip padidinti jo analitinį universalumą.



14 pav. Analizuojamų medžiagų sočiųjų garų slėgio, naudoto atliekant krio-koncentravimo modulio bandymus, vizualizavimas nuo didžiausio iki mažiausio (mm Hg, 25 °C)[116]

- Nebuvo įvertintas modulio ilgalaikis stabilumas ir atkuriamumas. Laikui bėgant tokie veiksniai kaip įrangos nusidėvėjimas ir kolonėlės našumo pokyčiai dėl temperatūros svyravimų gali turėti įtakos stabilumui ir atkuriamumui.
- Dėl laiko, reikalingo moduliui atšaldyti ir pašildyti, taip pat dėl daugkartinių injekcijų, sumažėja sistemos našumas. Todėl šis modulis gali būti netinkamas laboratorijoms, kurioms reikia didelio mėginių pralaidumo.
- Ilgas sistemos paruošimo darbui laikas. Sistemai reikia 3-4 valandų, kad pasiektų -60 °C darbinę temperatūrą. Dėl šio apribojimo reikia iš anksto planuoti sistemos naudojimą arba palikti sistemą įjungtą, todėl be reikalo sunaudojama elektros energija.
- Sistemos naudojimas ir priežiūra yra sudėtinga. Norint ja naudotis reikia papildomu mokymu. Taip pat, detaliu keitimas ar aparato valymas gali reikalauti papildomu pastangų. Ateities modulio iteracijose galėtų būti apsvarstyta galimybė modulyje visam laikui įmontuoti metalinę kolonėlę, tai smarkiai supaprastintų operatoriaus darbą.

IŠVADOS

- Modulis padidina analizės efektyvumą, fokusuojant smailes, todėl smailės tampa siauresnės ir aukštesnės. Smailių skyra padidėjo iki 2,2 karto.
- Krio-koncentravimo modulis yra efektyvus sistemos jautrio didinimui. Atlikus išsamius eksperimentus su skirtingais šaldomos kolonėlės dalies ilgiais matyti, kad optimaliausi rezultatai pasiekiami šaldant 95 cm kolonėlės ilgio. Pasiektas iki 20 kartų didesnis jautris.
- Modulio gebėjimą apdoroti įvairias analites riboja darbinės temperatūros intervalas. Kai kurios analitės krio-koncentravimo modulyje nebuvo sulaikytos dėl nepakankamai žemos temperatūros, tuo tarpu kitos buvo per ilgai sulaikomos dėl nepakankamo kaitinimo.
- Patikrinta, kad modulis veikia įvairiose srityse (sertifikuotų pamatinių medžiagų priemaišų analizė, fungicidų analizė, metodo validavimui terpenams *Cannabis Sativa* biomasėje matuoti).
- Modulio prototipas veikia, tačiau jis dar nėra paruoštas komerciniam naudojimui. Pasiektas 5 MTEP (mokslinių tyrimų ir eksperimentinės plėtros) vystymo etapas.

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PUBLICATIONS

The main results of the doctoral thesis were published in 2 scientific publications (P: 1, 2) and 6 conference reports (C: 1-6).

Publications in thesis topic

P1. **Audrius Sadaunykas**, Audrius Zolumskis, Audrius Markevičius, Evaldas Naujalis. On-column cryofocusing and analyte enrichment device for gas chromatography systems. Chemija, Vol 31, No 4, 2020, DOI: 10.6001/chemija. v31i4.4326.

P2. **Audrius Sadaunykas**, Audrius Zolumskis, Simonas Balčiūnas, Evaldas Naujalis. Enhancing Chromatographic Analysis: A Novel Cryo-Enrichment Module for GC Analysis of Terpenes in *Cannabis Sativa*, Journal of Chromatographic Science, 2023, DOI: 10.1093/chromsci/bmad079

Other publications

OP1. Greta Bikelytė, Martin A.C. Härtel, Thomas M. Klapötke, Burkhard Krumm, **Audrius Sadaunykas**. Experimental thermochemical data of CWA simulants: Triethyl phosphate, diethyl methylphosphonate, malathion and methyl salicylate. The Journal of Chemical Thermodynamics, Vol 143, 2020.

OP2. Adrian Vicent Claramunt, **Audrius Sadaunykas**, Simonas Balčiūnas, Birutė Knašienė, Audrius Zolumskis, Evaldas Naujalis. Profiling of volatile organic compounds for environment discrimination in Vilnius City. Chemija, Vol 33, No 1, 2022.

OP3. Adrian Vicent Claramunt, **Audrius Sadaunykas**, Birutė Knašienė, Evaldas Naujalis. Determination of pyridine and furfuryl alcohol in breath after coffee consumption. Chemija Vol 33, No 3, 2022.

Conferences

C1. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Analyte focusing and enrichment on gas chromatography column. Open Readings 2020, Vilnius, Lithuania. *Canceled due to COVID-19*.

C2. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Gas chromatography oven cooling add-on prototype. Open Readings 2021, Vilnius, Lithuania.

C3. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Analičių fokusavimo ir koncentravimo ant dujų chromatografinės kolonėlės galimybių tyrimai. FizTech2021, Vilnius, Lithuania, 2021.

C4. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Development and testing of cryofocusing add-on for gas chromatography. Open Readings 2022, Vilnius, Lithuania.

C5. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Analičių fokusavimo ir koncentravimo ant dujų chromatografinės kolonėlės pritaikymas matuojant terpenų koncentracijas mėginiuose. FizTech2022, Vilnius, Lithuania, 2022.

C6. **Audrius Sadaunykas**, Audrius Zolumskis, Evaldas Naujalis. Cryo-focusing in GC analysis: A new approach for measuring terpenes in *Cannabis Sativa*. Open Readings 2023, Vilnius, Lithuania.

Other conferences

OC1. Miglė Bartkutė, **Audrius Sadaunykas**, Evaldas Naujalis. HPLC method development and validation for main cannabinoids determination in hemp biomass and oil. Open Readings 2021, Vilnius Lithuania

REFERENCES

[1] IMARC Group, Gas Chromatography Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023-2028. 2022.

[2] Richard Villalobos Raymond Annino, Process Gas Chromatography: Fundamentals and Applications : On-line Analysis for Process Monitoring and Control. 1992, Research Triangle Park, NC: Instrument Society of America.

[3] Jerome Workman, et al., Process Analytical Chemistry. Analytical Chemistry, 2001. **73**(12): p. 2705-2718.

[4] Sandra Patrick, Chromatographic Methods -Sample Introduction in capillary Gas Chromatography, ed. H.F. Vertrieb. Vol. 1. 1985, Heidelberg.

[5] Farid Ahmed, Analysis of polychlorinated biphenyls in food products. TrAC Trends in Analytical Chemistry, 2003. **22**: p. 170-185.

[6] Steven J. Lehotay and Jana Hajšlová, Application of gas chromatography in food analysis. TrAC Trends in Analytical Chemistry, 2002. **21**(9): p. 686-697.

[7] Arian van Asten, The importance of GC and GC-MS in perfume analysis. TrAC Trends in Analytical Chemistry, 2002. **21**(9): p. 698-708.

[8] W. Czerwiński and A. Stepień, Capillary gas chromatography of impurities in cyclohexanone. Journal of Chromatography A, 1982. **241**(1): p. 57-60.

[9] W. Luitjen, Gas Chromatography in the chemical and pharmaceutical industries. Analusis, 1998. **26**(1): p. M24-M27.

[10] Yuan-yuan Zhao, et al., The determination of impurities in caprolactam by capillary gas chromatography-mass spectrometry. Microchemical Journal, 2001. **69**(3): p. 213-217.

[11] Donald L. Fox, Air Pollution. Analytical Chemistry, 1997. **69**(12): p. 1-14.

[12] Robert L Grob and Eugene F Barry, Modern practice of gas chromatography. Fourh Edition. 2004: John Wiley & Sons.

[13] Gurleen Kaur and Sahil Sharma, Gas Chromatography-A Brief Review. Inter J Inf Comp Scie, 2018. **5**: p. 125-131.

[14] Richard Evers, Development of a Liquid Chromatography Ion Trap Mass Spectrometer Method for Clinical Drugs of Abuse Testing with Automated On-Line Extraction Using Turbulent Flow Chromatography. 2014.

[15] Daniel C Harris, Quantitative chemical analysis. 2010: Macmillan.

[16] Piet de Coning John Swinley, A Practical Guide to Gas Analysis by Gas Chromatography. 1st ed. 2019.

[17] JOLANTA Liesienė and G Buika, Skysčių ir dujų chromatografijos pagrindai. Mokomoji Knyga. Kaunas: Technologija, 2012.

[18] Raymond PW Scott, Introduction to analytical gas chromatography, revised and expanded. 1997: CRC press.

[19] Konrad Grob, Injection techniques in capillary GC. Analytical chemistry, 1994. **66**(20): p. 1009 A-1019 A.

[20] Gas Chromatography. Sheffield Hallam University; Available from: https://teaching.shu.ac.uk/hwb/chemistry/tutorials/chrom/gaschrm.htm.

[21] Charles Harold Hartmann, Gas chromatography detectors. Analytical Chemistry, 1971. **43**(2): p. 113A-125a.

[22] Douglas A Skoog, F James Holler, and Stanley R Crouch, Instrumental analysis. Vol. 47. 2007: Brooks/Cole, Cengage Learning Belmont.

[23] István Halasz and Werner Schneider, Quantitative gas chromatographic analysis of hydrocarbons with capillary column and flame ionization detector. Analytical Chemistry, 1961. **33**(8): p. 978-982.

[24] John V Hinshaw, The flame ionization detector. LCGC North America, 2005. **23**(12): p. 1262–1272-1262–1272.

[25] Paul Gates. Quadrupole Mass Analysis. University of Bristol, School of Chemistry 2014; Available from: https://www.chm.bris.ac.uk/ms/quadrupole.xhtml.

[26] John Calvin Giddings, Unified separation science. 1991: Wiley New York.

[27] Thomas Wenzel, Collaborative group learning in remotely taught analytical chemistry courses. Journal of Chemical Education, 2020. **97**(9): p. 2715-2718.

[28] JJ Van Deemter, FJ Zuiderweg, and A van Klinkenberg, Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. Chemical Engineering Science, 1956. **5**(6): p. 271-289.

[29] Thomas WENZEL, Eddy Diffusion (Multipath) Broadening in Chromatography. Chemistry LibreTexts, 2016.

[30] David Harvey. CHM 331 Advanced Analytical Chemistry 1. Available from: https://chem.libretexts.org/@api/deki/files/187045/Figure12.19.png?revision=1.

[31] Girijan Menon. Band Broadening in Chromatography. 2016; Available from: https://www.linkedin.com/pulse/band-broadening-chromatography-girijan-menon/.

[32] RESTEK. Optimizing Splitless Injection. Available from: https://www.restek.com/global/en/pages/chromatogram-view/GC_EV1190/.

[33] Alberto Sánchez-Guijo, Michaela F. Hartmann, and Stefan A. Wudy, Introduction to Gas Chromatography-Mass Spectrometry, in *Hormone Assays in Biological Fluids*, M.J. Wheeler, Editor. 2013, Humana Press: Totowa, NJ. p. 27-44.

[34] Saskia M Van Ruth, Methods for gas chromatography-olfactometry: a review. Biomolecular Engineering, 2001. **17**(4-5): p. 121-128.

[35] Graeme R Jones and Neil J Oldham, Pheromone analysis using capillary gas chromatographic techniques. Journal of Chromatography A, 1999. **843**(1-2): p. 199-236.

[36] Katja Elke, et al., Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID-a new feasible approach for the detection of an exposure to indoor mould fungi? Journal of Environmental Monitoring, 1999. **1**(5): p. 445-452.

[37] Henryk H Jeleń, et al., Determination of geosmin, 2-methylisoborneol, and a musty-earthy odor in wheat grain by SPME-GC-MS, profiling volatiles, and sensory analysis. Journal of Agricultural and food Chemistry, 2003. **51**(24): p. 7079-7085.

[38] Johan Schnürer, Johan Olsson, and Thomas Börjesson, Fungal volatiles as indicators of food and feeds spoilage. Fungal Genetics and Biology, 1999. **27**(2-3): p. 209-217.

[39] FJ Santos and MT Galceran, Modern developments in gas chromatography–mass spectrometry-based environmental analysis. Journal of Chromatography A, 2003. **1000**(1-2): p. 125-151.

[40] Ronald E Majors, An overview of sample introduction and sample preparation of volatile organic compounds. LC GC, 1999. **17**(9S): p. S7-S13.

[41] Roger M Smith, Before the injection—modern methods of sample preparation for separation techniques. Journal of chromatography A, 2003. **1000**(1-2): p. 3-27.

[42] Sarem Targuma, Patrick Njobeh, and Patrick Ndungu, Current Applications of Magnetic Nanomaterials for Extraction of Mycotoxins, Pesticides, and Pharmaceuticals in Food Commodities. Molecules, 2021. **26**: p. 4284.

[43] Elisabet Fogelqvist, Mikael Krysell, and Lars Goeran Danielsson, On-line liquid-liquid extraction in a segmented flow directly coupled to on-column injection into a gas chromatograph. Analytical chemistry, 1986. **58**(7): p. 1516-1520.

[44] J Roeraade, Automated monitoring of organic trace components in water: I. Continuous flow extraction together with on-line capillary gas chromatography. Journal of Chromatography A, 1985. **330**: p. 263-274.

[45] Victor David Serban C. Moldoveanu, Modern Sample Preparation for Chromatography. 2nd ed. 2021: Elsevier.

[46] Jonas Mechelke, et al., Vacuum-assisted evaporative concentration combined with LC-HRMS/MS for ultra-trace-level screening of organic micropollutants in environmental water samples. Analytical and Bioanalytical Chemistry, 2019. **411**(12): p. 2555-2567.

[47] Yukio Kemmochi, et al., Centrifugal concentrator for the substitution of nitrogen blow-down micro-concentration in dioxin/polychlorinated biphenyl sample preparation. Journal of Chromatography A, 2002. **943**(2): p. 295-297.

[48] Nataliya Kulikova, et al., Evaporation as a Method for Obtaining Plant Concentrates. Food Processing: Techniques and Technology, 2023. **53**: p. 335-346.

[49] I Liška, Fifty years of solid-phase extraction in water analysis–historical development and overview. Journal of Chromatography A, 2000. **885**(1-2): p. 3-16.

[50] Colin F Poole, Ajith D Gunatilleka, and Revathy Sethuraman, Contributions of theory to method development in solid-phase extraction. Journal of Chromatography A, 2000. **885**(1-2): p. 17-39.

[51] Earl Michael Thurman and Margaret S Mills, Solid-phase extraction: principles and practice. Vol. 16. 1998: Wiley New York.

[52] William T Sturges and James W Elkins, Use of adsorbents to collect selected halocarbons and hydrohalocarbons of environmental interest from large air volumes. Journal of Chromatography A, 1993. **642**(1-2): p. 123-134.

[53] A. J. Louter, et al., Automated on-line solid-phase extraction-gas chromatography with nitrogen-phosphorus detection: determination of benzodiazepines in human plasma. J Chromatogr B Biomed Sci Appl, 1997. **689**(1): p. 35-43.

[54] C. Aguilar, F. Borrull, and R. M. Marcé, Determination of pesticides in environmental waters by solid-phase extraction and gas chromatography with electron-capture and mass spectrometry detection. Journal of Chromatography A, 1997. **771**(1): p. 221-231.

[55] E. Ballesteros and M. J. Parrado, Continuous solid-phase extraction and gas chromatographic determination of organophosphorus pesticides in natural and drinking waters. J Chromatogr A, 2004. **1029**(1-2): p. 267-73.

[56] Toshiro Yamashita, et al., Determination of chlordane in air by gas chromatography-mass spectrometry with selected ion monitoring. Journal of Chromatography A, 1993. **657**(2): p. 405-411.

[57] V Camel and M Caude, Trace enrichment methods for the determination of organic pollutants in ambient air. Journal of Chromatography A, 1995. **710**(1): p. 3-19.

[58] P. J. M. Kwakman, et al., Determination of organophosphorus pesticides in aqueous samples by on-line membrane disk extraction and capillary gas chromatography. Chromatographia, 1992. **34**(1): p. 41-47.

[59] Barbara Zielinska, et al., Potential for artifact formation during Tenax sampling of polycyclic aromatic hydrocarbons. Journal of Chromatography A, 1986. **363**(2): p. 382-386.

[60] M P Ligocki and J F Pankow, Assessment of adsorption/solvent extraction with polyurethane foam and adsorption/thermal desorption with Tenax-GC for the collection and analysis of ambient organic vapors. Anal. Chem.; (United States), 1985. **57:6**: p. Medium: X; Size: Pages: 1138-1144 2016-05-30.

[61] P.A. Waeger H. Rothweiler, C. Schlatter, Comparison of Tenax TA and Carbotrap for sampling and analysis of volatile organic compounds in air. Atmospheric Environment, 1991. **Part B**(Urban Atmosphere 25B): p. 231.

[62] F. David E. Baltussen, P. Sandra, H.-G. Janssen, C.A. Cramers, "Sorption tubes packed with polydimethylsiloxane. A new and promising technique for the preconcentration of volatiles and semivolatiles from air and gaseous samples. J. High Resolut. Chromatogr., 1998. **21**: p. 332.

[63] Erik Baltussen, et al., Retention model for sorptive extraction–thermal desorption of aqueous samples: application to the automated analysis of pesticides and polyaromatic hydrocarbons in water samples. Journal of Chromatography A, 1998. **805**(1-2): p. 237-247.

[64] Per Clausen and Peder Wolkoff, Evaluation of Automatic Thermal Desorption - Capillary GC for Determination of Semivolatile Organic Compounds (SVOCs) in Indoor Air. Journal of High Resolution Chromatography, 1997. **20**: p. 99-108.

[65] E. Baltussen, C. Cramers, and P. Sandra, Sorptive sample preparation – a review. Analytical and Bioanalytical Chemistry, 2002. **373**(1): p. 3-22.

[66] S. Blomberg and J. Roeraade, A technique for coating capillary columns with a very thick film of cross-linked stationary phase. Journal of High Resolution Chromatography, 1988. **11**(6): p. 457-461.

[67] Erik Baltussen, et al., Equilibrium Sorptive Enrichment on Poly(dimethylsiloxane) Particles for Trace Analysis of Volatile Compounds in Gaseous Samples. Analytical Chemistry - ANAL CHEM, 1999. **71**.

[68] Erik Baltussen, et al., A New Method for Sorptive Enrichment of Gaseous Samples: Application in Air Analysis and Natural Gas Characterization. Journal of High Resolution Chromatography, 1997. **20**: p. 385-393.

[69] Juergen Poerschmann, Tadeusz Górecki, and Frank-Dieter Kopinke, Sorption of Very Hydrophobic Organic Compounds onto Poly(dimethylsiloxane) and Dissolved Humic Organic Matter. 1. Adsorption or Partitioning of VHOC on PDMS-Coated Solid-Phase Microextraction FibersA Never-Ending Story? Environmental Science & Technology, 2000. **34**(17): p. 3824-3830.

[70] Kamila Schmidt and Ian Podmore, Current Challenges in Volatile Organic Compounds Analysis as Potential Biomarkers of Cancer. Journal of Biomarkers, 2015. **2015**.

[71] Catherine L. Arthur and Janusz Pawliszyn, Solid phase microextraction with thermal desorption using fused silica optical fibers. Analytical Chemistry, 1990. **62**(19): p. 2145-2148.

[72] Erik Baltussen, et al., Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: Theory and principles. Journal of Microcolumn Separations, 1999. **11**(10): p. 737-747.

[73] V. M. León, et al., Analysis of 35 priority semivolatile compounds in water by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry. I. Method optimisation. J Chromatogr A, 2003. **999**(1-2): p. 91-101.

[74] N. Ochiai, et al., Simultaneous determination of preservatives in beverages, vinegar, aqueous sauces, and quasi-drug drinks by stir-bar sorptive extraction (SBSE) and thermal desorption GC-MS. Anal Bioanal Chem, 2002. **373**(1-2): p. 56-63.

[75] Bita Kolahgar, Andreas Hoffmann, and Arnd Heiden, Application of stir bar sorptive extraction to the determination of polycyclic aromatic hydrocarbons in aqueous samples. Journal of chromatography. A, 2002. **963**: p. 225-30.

[76] Ursula Telgheder, Nabil Bader, and Nessma Alshelmani, Stir bar sorptive extraction as a sample preparation technique for chromatographic analysis: An overview. 2018. **1**.

[77] F. J. DiSalvo, Thermoelectric cooling and power generation. Science, 1999. **285**(5428): p. 703-6.

[78] M. Libardoni, J. H. Waite, and R. Sacks, Electrically heated, air-cooled thermal modulator and at-column heating for comprehensive two-dimensional gas chromatography. Anal Chem, 2005. **77**(9): p. 2786-94.

[79] Vincenzo Cocheo, et al., Identification by cryofocusing–gas chromatography–mass spectrometry of odorous cyclic acetals emitted from a polyester resin plant. Analyst, 1991. **116**(12): p. 1337-1342.

[80] R. B. Wilson, et al., High-speed cryo-focusing injection for gas chromatography: reduction of injection band broadening with concentration enrichment. Talanta, 2012. **97**: p. 9-15.

[81] S. T. Chin, B. Maikhunthod, and P. J. Marriott, Universal method for online enrichment of target compounds in capillary gas chromatography using in-oven cryotrapping. Anal Chem, 2011. **83**(17): p. 6485-92.

[82] Peter Boeker, et al., Comprehensive Theory of the Deans' Switch As a Variable Flow Splitter: Fluid Mechanics, Mass Balance, and System Behavior. Analytical Chemistry, 2013. **85**(19): p. 9021-9030.

[83] Eberhard Breitmaier, Terpenes: Importance, general structure, and biosynthesis. Terpenes: Flavors, fragrances, pharmaca, pheromones, 2006. **1**: p. 1-3.

[84] Kosina S. M. Louie K. B., Hu Y., Otani H., de Raad M., Kuftin A. N., Mouncey N. J., Bowen B. P. ir Northen T. R. , Mass Spectrometry for Natural Product Discovery. In Comprehensive Natural Products III 2020: Elsevier Ltd.

[85] Christelle M Andre, Jean-Francois Hausman, and Gea Guerriero, Cannabis sativa: the plant of the thousand and one molecules. Frontiers in plant science, 2016: p. 19.

[86] Lumír Ondřej Hanuš and Yotam Hod, Terpenes/terpenoids in cannabis: are they important? Medical Cannabis and Cannabinoids, 2020. **3**(1): p. 25-60.

[87] Ekaterina Proshkina, et al., Terpenoids as potential geroprotectors. Antioxidants, 2020. **9**(6): p. 529.

[88] SC Knight, et al., Rationale and perspectives on the development of fungicides. Annual review of phytopathology, 1997. **35**(1): p. 349-372.

[89] FAOSTAT, Food and agriculture data for all FAO regional groupings. 2019.

[90] DE (BAuA), Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products. Product assesment report of a biocidal product for national authorizarion applications. 2021.

[91] European Commision, COMMISSION REGULATION (EU) 2021/155 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for carbon tetrachloride, chlorothalonil, chlorpropham, dimethoate, ethoprophos, fenamidone, methiocarb, omethoate, propiconazole and pymetrozine in or on certain products in *Official Journal of the European Union*. 2021, Official Journal of the European Union.

[92] European Food Safety Authority, et al., Peer review of the pesticide risk assessment of the active substance propiconazole. Efsa Journal, 2017. **15**(7): p. e04887.

[93] Y. V. C. Rao, An introduction to thermodynamics. 2004: Universities Press.

[94] Shengan Zhang, Kaiyu Li, and Guilian Liu, An efficient hydrogen liquefaction process integrated with a solar power tower and absorption precooling system. Clean Technologies and Environmental Policy, 2022. **25**: p. 1-27.

[95] Poonam Patil Jadhav, Cooling Techniques for a Spindle of Machine Tool. International Journal Of Engineering And Computer Science, 2016.

[96] Qiulin Wang, et al., Thermodynamic performance comparison of series and parallel two-stage evaporation vapor compression refrigeration cycle. Energy Reports, 2021. **7**: p. 1616-1626.

[97] Amctb No Analytical Methods Committee, Chemical metrology. Analytical Methods, 2016. **8**(46): p. 8119-8122.

[98] M. Valcárcel, Metrology in Chemistry, in *Quality in Chemical Measurements: Training Concepts and Teaching Materials*, B. Neidhart and W. Wegscheider, Editors. 2001, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 89-107.

[99] Bertil Magnusson, The fitness for purpose of analytical methods: a laboratory guide to method validation and related topics (2014). 2014, Eurachem.

[100] Commision Decision, 657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C (2002) 3044). Off. J. Eur. Communities, 2002: p. 8-36.

[101] Ludwig Huber, Validation and qualification in analytical laboratories. 2007: CrC Press.

[102] James Miller and Jane C Miller, Statistics and chemometrics for analytical chemistry. 2018: Pearson education.

[103] Pedro Araujo, Key aspects of analytical method validation and linearity evaluation. Journal of chromatography B, 2009. **877**(23): p. 2224-2234.

[104] Steven Walfish, Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. BioPharm International, 2006. **19**(12): p. 1-6.

[105] EURACHEM, Guide to Quality in Analytical Chemistry An Aid to Accreditation. 2016.

[106] US Food and Drug Administration, Bioanalytical method validation. Guidance for industry, 2001.

[107] MJ Ruiz-Angel, et al., Are analysts doing method validation in liquid chromatography? Journal of Chromatography A, 2014. **1353**: p. 2-9.

[108] M Thompson, SLR Ellison, and R Wood, Harmonized guidelines for single-laboratory validation of methods of 507 analysis (IUPAC Technical Report). Pure Appl. Chem, 2002.

[109] ChemoMetec A/S. Revealing the precision of your manual cell counts. Available from: https://chemometec.com/perfect-precision-manual-cell-counting/.

[110] Stephen LR Ellison and Alex Williams, Eurachem/CITAC guide: quantifying uncertainty in analytical measurement. LGC, Teddington, 2012.

[111] A Gustavo González, M Angeles Herrador, and Agustín G Asuero, Practical digest for evaluating the uncertainty of analytical assays from validation data according to the LGC/VAM protocol. Talanta, 2005. **65**(4): p. 1022-1030.

[112] L. C. Assis, et al., β-Caryophyllene protects the C6 glioma cells against glutamate-induced excitotoxicity through the Nrf2 pathway. Neuroscience, 2014. **279**: p. 220-31.

[113] S. Ojha, et al., β-Caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of Parkinson disease. Mol Cell Biochem, 2016. **418**(1-2): p. 59-70.

[114] European Commission DG-SANCO, Method validation and quality control procedures for pesticide residues analysis in food and feed No. SANCO/12495/2011. 2012.

[115] Jürg Wüthrich and Michael Weber, Building-up a Double Accredited CRM Production Laboratory in an Industrial Environment: A Description. CHIMIA International Journal for Chemistry, 2009. **63**: p. 632-636.

[116] National Center for Biotechnology Information. PubChem Compound Summary. Available from: https://pubchem.ncbi.nlm.nih.gov.

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