Accelerating Techniques for Whisky Maturation

Effect of heat, iron and light on the maturation process

MASTER'S THESIS
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Contents

Preface

This paper is written as a master thesis from the Department of Food Technology, Engineering and Nutrition at Lund University. The part of the material (new make spirit, commercial samples, and wood) used in this project was supported by EtOH Spirits, a company focusing on developing and improving the methods of accelerating the aging of spirits in Copenhagen. Since 2017, EtOH Spirits started to cooperate with students to investigate the effects of various technologies on spirit maturation. This project is based on the achievement of EtOH Spirits to investigate how heat, light, and iron affect the aging of spirits.

Acknowledge

Abstract

The maturation of whisky is a long and complex process, the extremely long waiting time causes a large cost for new whisky distilleries, and without sufficient funding, it is hard to start a whisky business. To produce satisfying whisky products within the short term, accelerating the reaction during aging provides a promising approach. Various chemical reactions take place during this stage, this project aims to investigate how heat, light, and iron catalyst affect these reactions. A temperature chamber was used to perform heat treatment. The source of light was a plasma lamp that simulate solar light. Ferrous sulfate was chosen as the source of iron.

Also, Ultraviolet-Visible Spectroscopy was utilized to collect the absorbance of each sample to evaluate the effect of these treatments on the content of total phenolic compounds. On the other hand, to evaluate the impact of these treatments on whisky maturation, the contents of six phenolic compounds (gallic acid, vanillic acid, vanillin, caffeic acid, trans-ferulic acid, and trans-cinnamic acid) derived from the wood were considered a key indicator since these phenolic compounds make an important contribution to the development of flavor and aroma of whisky. And HPLC-DAD was chosen as the analytical method for identifying and quantifying these phenolic compounds.

The results found a good correlation between the heat treatment and the generation of phenolic compounds, but the current results are insufficient to conclude that the light treatment can improve the maturation of whisky. In addition, the iron catalyst does not show any beneficial behavior, and the high concentration of iron even decreased the content of phenolic compounds.

1. Introduction

As one type of popular distilled alcoholic beverage, the value of whisky is realized by more and more consumers in recent years, and the demand for whisky is also increasing in the global market. However, according to The Scotch Whisky Regulations, the whisky must be matured in oak casks at least for three years, and commonly it requires at least 10-12 years to produce a bottle of whisky with good quality. The cost of long-term storage and maintenance causes a heavy burden on the whisky producer. To find a solution, the interest in developing new methods to accelerate the process of maturation is increasing in the alcoholic beverage industry. EtOH Spirits is such a company trying to produce high quality spirits in mere days. Through the collaboration with students from the University of Copenhagen, they found some correlation between the generation of esters and some treatments including heating, the addition of organic acid as well as ultrasound (Yding & Raditya, 2019). Some techniques have already been applied in their spirits production, to gain more experience and methods to improve the quality of their products, this project focus on investigating the potential of light, heat, and iron catalyst for improving the characteristic of whisky. To evaluate the effect of these treatments on the chemical profile, the content of certain phenolic compounds was chosen as the main indicator. And in this experiment, Ultraviolet-Visible and High-Performance Liquid Chromatography- Diode-Array Detection were utilized as chemical analysis tools for identifying and quantifying these compounds to monitor the spectral development of the new make spirited treated with varying intensity of light, the concentration of iron (II) as well as temperature setting in 14 days. Also, a comparison between the samples and commercial whisky is necessary to be performed to evaluate the similarity of the profile between the treated samples and common products.

2. Theoretical Background

2.1 Whisky Overview

Whisky is one type of distilled alcoholic beverage made from grain. As one of the most popular spirit drinks, whisky distilleries are distributed all over the world, especially in Ireland, Japan, Canada, the USA as well as Scotland, each region has its regulation or definition of whisky. Ireland is widely regarded as the origin of whisky, the most widely accepted story is that the monks brought spirits distillation techniques to Ireland in the 6th century. After that, distillation techniques were brought to Scotland by Irish monks, and the development of whisky started in these two lands. (Russell and Stewart, 2014).

In Sweden, the native whisky distilleries are trying to create fantastic Swedish whisky. The production process of Swedish whisky is similar to Scotch whisky. The preferred ingredient is barleys instead of other grains, part of barleys is imported from Scotland, but local barleys also account for a large proportion in the manufacturing. In addition, like Scotch whisky, ex-bourbon and ex-sherry barrels are commonly used in

the maturation of Swedish whisky, and Swedish oak is also applied to provide special flavor in some conditions. Currently, almost 20 distilleries which have whisky production lines are active in Sweden, Mackmyra Distillery and High Coast Distillery are the most exceptional brands among them. As the oldest active whisky distillery in Sweden, Mackmyra was founded in 1999 and launched its first batch of whisky after 7 years. The founding time of High Coast Distillery is in 2010, which is a bit later, but it is still a well-known brand as well as Mackmyra in the international whisky market. Some other distilleries also have whisky production lines, but they are less known internationally or whisky is not included in their core products (The Scotch Whisky Experience team, 2019; whiskybase, n.d.).

2.2 Wood Type Selection

Numerous types of oak wood have been used for the maturation of spirits or wine, American white oak and European white oak are the most popular choices for cooperage. Cellulose (45-50%), hemicellulose (22-25%), and lignin (23-32%) are the main component of white oak, and commonly white oak contains various extractable acids, carbohydrates, and phenolic compounds (SINGLETON, 1974). In America, at least 7 oak species are utilized for the maturation of bourbon, *Quercus alba* predominates among them (Russell and Stewart, 2014). After finishing the maturation of bourbon, the majority of casks are transported to Scotland and sold to local distilleries. While in Europe, European white oak, particularly *Quercus robur*, is the major wood species for sherry barrel production (Russell, 2003). But from 1986, the Spanish government required all sherry wine shall be bottled in Spain, the export of sherry casks was totally limited. Therefore, American white oak is exported to Spain as the replacement for European white oak to produce sherry cask (González Gordon and Doxat, 1990). The main differences between American white oak and European white oak are that American white oak can provide a stronger oak flavor per unit of tannin, but the level of extractable solids and phenol is normally higher in European white oak (SINGLETON, 1974).

2.3 Phenolic Compounds in Whisky

Oakwood tissue mainly contains three types of macromolecular components, cellulose, hemicellulose, and lignin. Cellulose is the major compound in wood (40-50%), which is a linear chain polymer composed of several glucose units. These glucose units are bound by the β (1→4)-glycosidic bonds by removing the water between two glucose units. The linkage of sugar monomers leads to the formation of the microfibrils, which contribute to the mechanical properties of wood. Hemicellulose is the second largest composition of wood, which is one type of heteropolymer and consists of several types of sugar monomers. And hemicellulose is associated with cellulose, but usually, it has a lower molecular weight than cellulose. In oak wood, the hemicellulose is mainly composed of xylose, accounting for 15 to 30% of the dry weight (Fengel and Wegener, 1984; Manzoni et al., 2008). Lignin (15-30% of dry weight) is located between cell walls or in intercellular regions, which is the polymers consisting of numerous phenolic compounds. It plays a key role in

binding the microfibrils between cell walls and it is also essential for preventing the degradation of the many other compounds locked in lignin linkages (George, et al., 2005). Distinguished from cellulose and hemicellulose, lignin is highly branched with a three-dimensional structure. The phenylpropane units substituted with hydroxyl and methoxyl groups are the main reason causing the formation of this complicated structure. Also, coniferyl and sinapyl alcohols are the two essential precursors producing lignin, the reactions between these two groups have an important effect on the synthesis of various flavor or aroma substances, especially for compounds having aromatic rings and side chains (Monties, 1987). Figure 1 gives the chemical structures of some interested phenolic compounds.

vanillin   vanillic acid   gallic acid

caffeic acid   trans-ferulic acid   trans-cinnamic acid
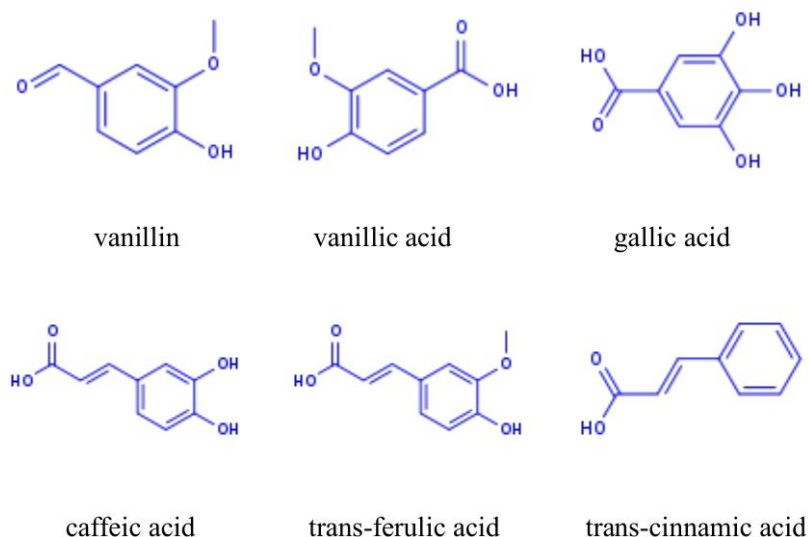
Figure 1. Chemical structures of interested phenolic compounds (www.reaxys.com, n.d.)

Phenolic compounds are one of the most abundant compounds in the plant, which comprise at least one aromatic ring with attached hydroxyl groups. Not only do phenolic compounds provide important and characteristic flavor and aroma, but also act as an antioxidant (Alañón et al., 2011). Also, these compounds normally are divided into three classes: volatile phenols, phenolic acids, and ellagitannins. The structure of volatile phenols and phenolic acids are similar, they all comprise an aromatic ring with hydroxyl substituents. In whisky, the majority of them can be divided into two types, hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids like gallic, syringic, salicylic, and vanillic acids are transformed from benzoic acid. As for hydroxycinnamic acids, ferulic and sinapic acids are the most abundant forms presented in the whisky (Zhang et al., 2015). While ellagitannins have a more complex structure, it is one type of water-soluble polyphenol formed due to the oxidative linkage of galloyl groups in β-1,2,3,4,6-pentagalloyl glucose (Zhang et al., 2015). Another difference is that ellagitannins have more impact on taste rather than the aroma. And the concentrations of some common phenolic compounds in whisky are presented in Figure 2.

beverage	gallic acid	vanillic acid	syringic acid	syringaldehyde	vanillin	ellagic acid
whiskies						
single-malt Scotch (23)	1.29 ± 0.19	0.21 ± 0.03	0.85 ± 0.11	1.00 ± 0.14	0.47 ± 0.06	10.04 ± 1.60
blended Scotch (12)	0.81 ± 0.15	0.34 ± 0.09	0.54 ± 0.23	1.23 ± 0.43	0.32 ± 0.09	5.09 ± 1.22
Canadian rye (19)	0.57 ± 0.09	0.21 ± 0.03	0.98 ± 0.15	1.14 ± 0.15	0.38 ± 0.05	6.09 ± 0.71
American bourbon (12)	1.28 ± 0.19	0.64 ± 0.17	2.06 ± 0.25	4.44 ± 0.49	0.94 ± 0.12	11.68 ± 2.10

Figure 2. The concentrations of some common phenolic compounds in whisky (mg/L) (Goldberg et al., 1999)

During the maturation, several phenolic compounds are derived from the breakdown of lignin. Normally the degradation of lignin occurs through two pathways, the hydrolysis of lignin by ethanol and water, or the thermal treatment. For the barrels without thermal treatment, hydrolysis by ethanol and water is the main pathway to break the lignin and produce low molecular weight phenolic compounds, such as syringaldehyde and vanillin. But since the solubility of lignin is minor, only a small proportion of phenolic compounds is extracted in whisky through this route (Conner, Paterson and Piggott, 1992; Nishimura et al., 1983). Thermal treatment including charring and toasting is another method to favor the degradation of lignin. In the cooperage, high temperature treatment is applied to modify the chemical and physical properties of the barrel, leading to the occurrence of various hydrothermolysis and pyrolysis reactions. These reactions partially result in the degradation of some compounds in oak, not only the readily hydrolyzed ellagitannins but also the more stable substances, in particular lignin and hemicelluloses (Matricardi and Waterhouse, 1999; Frangipane, Santis and Ceccarelli, 2007). A light toasting can facilitate the formation of tannins but reduce the level of aromatic compounds. The medium toasting can cause a partial degradation of oak and favor the generation of phenolic (especially vanillin) and furanic aldehydes. Also, heavy toasting leads to a higher degree of degradation and forms volatile phenols, which contribute to the smoky and spicy flavor of the products (Jackson, 2017). Compared with toasting, charring is much rougher heat treatment, the internal surface of the barrel is burned to become crisp and black, with more ash residue generated. The level of some compounds including vanillin, coniferaldehyde, sinapaldehyde, acetosyringone, and their derivatives is increased, contributing to the formation of vanilla flavor. Thus, the whisky matured in the charred cask commonly has a much deeper color and some characteristics like smooth, vanilla, sweet, spicy, fruity as well as floral. Eugenol has a clove-like aroma and its formation is also affected by the thermal treatment. However, it has been discovered that it is derived from glycosidic precursors instead of lignin, the final content of eugenol principally depends on the concentration of it or its precursors in the barrel (Nonier, de Gaulejac Nicolas Vivas and Vitry, 2005). In addition, charring and toasting also alter the composition and variety of the polyphenols in the oak. Ellagitannins, such as castalagin, vescalagin, grandinin, and roburins A–E or their derivatives are generated during the heat treatment and maturation (Russell and Stewart, 2014). But another research has investigated the concentration of a variety of ellagitannins in bourbon and find only the concentration of ellagic acid is higher than the taste recognition threshold (Glabasnia and Hofmann, 2006). For the whisky matured in the uncharred cask, the hydrolysis products like gallic acid are the most abundant content, and the compounds naturally present in the oak (syringaldehyde and vanillin) have a greater influence on the flavor. In the United States, the whisky labeled as bourbon is required to be matured in a new charred oak cask, therefore normally bourbon has distinguished vanilla and sweet flavor characteristics. In Scotland, toasting and charring are used for the reactivation of the

exhausted barrels to extend their life. (CLYNE et al., 1993; Martinez et al., 1996). Therefore, thermal treatment plays a key role in the process of cask manufacturing, not only does it facilitate the generation of thermolysis products derived from lignin, such as vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, vanillic acid as well as syringic acids, but also break the physical structure of the wood to accelerate the rate of extraction of flavor and aroma compounds during the aging.

2.4 Temperature

Maturation temperature is an essential parameter to control the quality of the spirits. Another popular spirit rum, which commonly is matured in a tropical hotter climate, has a similar production process as whisky. Although tropical temperature causes 10-12% evaporation rates per year, much higher than Scotland (2-4% per year), it has been investigated that higher temperature also contributes the faster maturation (Ashok et al., 2017). Meanwhile, high temperature has the effect of destroying the cell structure of oak wood, helping release the phenolic compounds from cells. Also, the thermal treatment can lower the activation energy of reactions (Russell and Stewart, 2014). And in 2019, a small team from the University of Copenhagen also cooperated with EtOH Spirits, their study also found that temperature can accelerate the aging of whisky (Yding & Raditya, 2019).

2.5 Photodegradation of Lignin

It is well known that photodegradation induced by light has a significant impact on the wood material. One research gives evidence that solar radiation, especially UV radiation (280–400 nm) and shortwave visible light (400-550nm) plays an important role in the reduction of organic compounds in wood (Austin, Méndez and Ballaré, 2016). Another research shows that UV radiation and shortwave visible light are responsible for 55% and 45% of photochemically induced carbon dioxide emissions in plant litter (Brandt, Bohnet and King, 2009). Light absorbing chromophores including a-carbonyl, biphenyl, and ring conjugated double bond structures are the primary reason for the occurrence of photodegradation in lignin. When light is absorbed by chromophores, free radicals are produced in this process. As a consequence, free radicals react with oxygen and result in the change in microscopic structure and chemical composition of wood (Hon and Shiraishi, 2000). And one research found lignin has much higher light absorbance than that of cellulose in the blue and green light region, and the blue and green component had a much larger impact than the UV component in the solar radiation (Austin, Méndez and Ballaré, 2016). On the other hand, the depth of the photodegrading is also affected by the wavelength of light, longer wavelength light, like blue or green light, can penetrate the wood to a deeper region beyond the accessibility of UV radiation (Kataoka et al., 2007). Also. one patent owned by LOST SPIRITS TECHNOLOGY LLC mentioned the application of a variety of lamps with 1,000,000 to 4,500,000 lux hours during the maturation of spirit showed significant effects on improving the quality of spirits (Davis, 2021).

2.6 Iron Catalysis

The catalysis of iron on oxidation has been investigated in several alcoholic beverage manufacturing. Iron can act as a catalyst to accelerate the reaction between oxygen and phenolic compounds to change the character of alcoholic beverages (CLARK and SCOLLARY, 2002). Reactions between ground state oxygen and some organic compounds are not able to happen, thus oxygen is required to be excited to the single state. Meanwhile, metal ion like iron is capable of donating and accepting electrons, with the presence of reduced transition iron ion (ferrous), the reactivity of molecular oxygen is improved. In addition, the process of electron transition reduces the oxygen to the forms of hydroperoxide radical and hydrogen peroxide. The reaction between hydrogen peroxide and ferrous can form hydroxyl radical, which can oxidize almost all the organic compounds in wine or spirits (Danilewicz, 2003; Oliveira et al., 2011). Figure 3 gives more precise processes of catalysis. However, the addition of iron also may cause spoilage to the wine or whisky. Some reactions occurring in some steps of wine production can induce turbidity, which may affect the aroma and flavor of the wine. Also, a paper investigated the effects of iron on the oxidation of wine and a correlation between iron and oxidation rate was found (Rousseva, 2014). On the other hand, during the maturation of whisky, even the concentration of iron higher than10 mg/L can cause virtually black to whisky (Russell and Stewart, 2014).
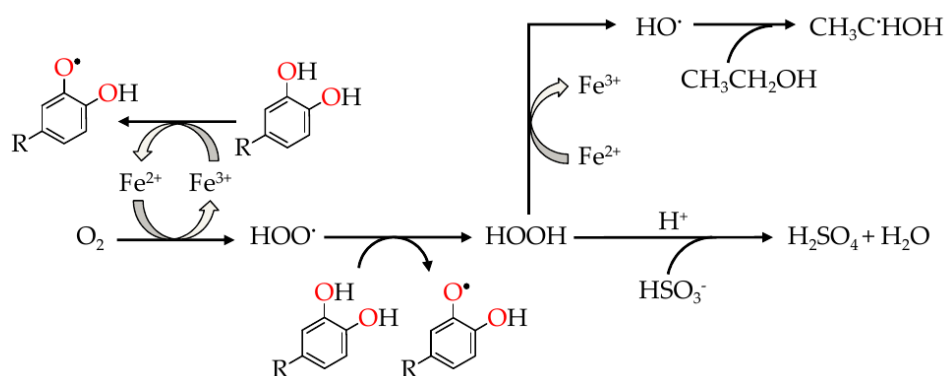
Figure 3. Processes of the catalysis of iron (Elias and Waterhouse, 2010)

2.7 Ultraviolet-Visible Spectroscopy

As a cheap and simple analytical technique, UV-Visible spectroscopy is commonly applied in the measurement of absorbance. The principle of UV-Visible spectroscopy depends on the π bonding and conjugated double bonds. The absorbance at the wavelength of 280 nm is chosen since most phenolic compounds have a distinct absorption around 280 nm due to the presence of an aromatic ring. Also, the absorption of the major components in whisky, like water, ethanol, and organic acids cannot be detected between the wavelengths of 200 and 600nm (Joshi et al., 2019).

2.8 HPLC-DAD

During the maturation, various nonvolatile compounds are extracted into a new make spirit or formed, especially phenolic compounds, High-Performance Liquid Chromatography- Diode-Array Detection (HPLC-DAD) is a suitable method to identify and quantify these compounds. Since all the nonvolatile compounds are mixed in the spirit, HPLC is capable of separating them. Depending on the polarity, phenolic compounds could be classified into non-polar phenolics and polar phenolics. Also, since most phenolic compounds contributing to flavor are monomers, reverse-phase HPLC could be applied for the separation by using a gradient solvent system to elute the compounds. In addition, the system must contain at least one acidic solvent based on the properties of the ion (Beer et al., 2004). On the other hand, a characteristic peak at 280 nm can be observed for several phenolic compounds since these substances have a strong absorption band at 280 nm. By using UV-vis spectroscopy, the spectra of these phenolic substances and their derivatives could be collected for classification and quantification (Zhang et al., 2015; Rosa Lidia Solís-Oviedo and Pech-Canul, 2019).

3. Materials and Methods

3.1 Chemicals and Reagents

Standards: Vanillin (Sigma-Aldrich), Gallic acid (Sigma-Aldrich), Vanillic acid (≥97.0%, FLUKA), Caffeic acid (99%, Sigma-Aldrich), trans-Cinnamic acid (97%, Sigma-Aldrich) and trans-Ferulic acid (99%, Sigma-Aldrich).
Iron (II) sulfate heptahydrate was purchased from MERCK. Acetonitrile, Acetic acid, and Methanol were supplied by VWR International. Each standard was dissolved into 80% methanol to obtain a 1 mg/mL standard solution.

3.2 Sample Preparation

The project aims to investigate the effects of heat treatment, light treatment, and the addition of iron on the maturation of whisky. The oak wood stave used in the experiment is derived from a charred ex-bourbon barrel (American oak), provided by EtOH Spirits. The 6mm of the inner portion (the side touching bourbon) of the wood stave was removed by a saw and cut into small chips (0.8cm×0.8cm×0.6cm), an intense toffee and vanilla aroma was smelled during the sawing. The new-make whisky (62.1% ABV) was also provided by EtOH Spirits, it mixed the wood chips with the ratio of 1.2g chips per 100 mL new-make whisky. 9 groups of samples were prepared, and each group had 3 replicates (27 samples). All the samples contained 0.6g chips and 50 mL new-make whisky and were filled into 100 mL glass bottles. Also, two commercial products, Been Apart (42% ABV) and Hafnium (42% ABV), from EtOH Spirits was involved as reference.

3.3 Artificial Solar Light Treatment

A plasma lamp was applied on 4 groups, 2 of them were exposed to the light for 8h and 2 of them were exposed to the light for 24h. Each sample was placed as a circle around the highest intensity point of the light on the ground, the illuminance and irradiance on the position of each sample were around 127,000 lux and 430 W/m-2, which means 6 samples reached 1,016,000 lux hours exposure and the other 6 samples reached 3,048,000 lux hours exposure. Figure 4 gives the information on the wavelength range of the light. Also, since the lamp could increase the temperature of the samples during the light exposure, the blank and the group that shall be stored at room temperature were placed into a paper box, and the other groups were placed into the temperature chamber which was set as 27 Celsius to keep the same temperature as the light treatment group and avoid the disturbance of the light from the environment. The groups treated with 8h light exposure were also transferred into the temperature chamber after finishing the light exposure.
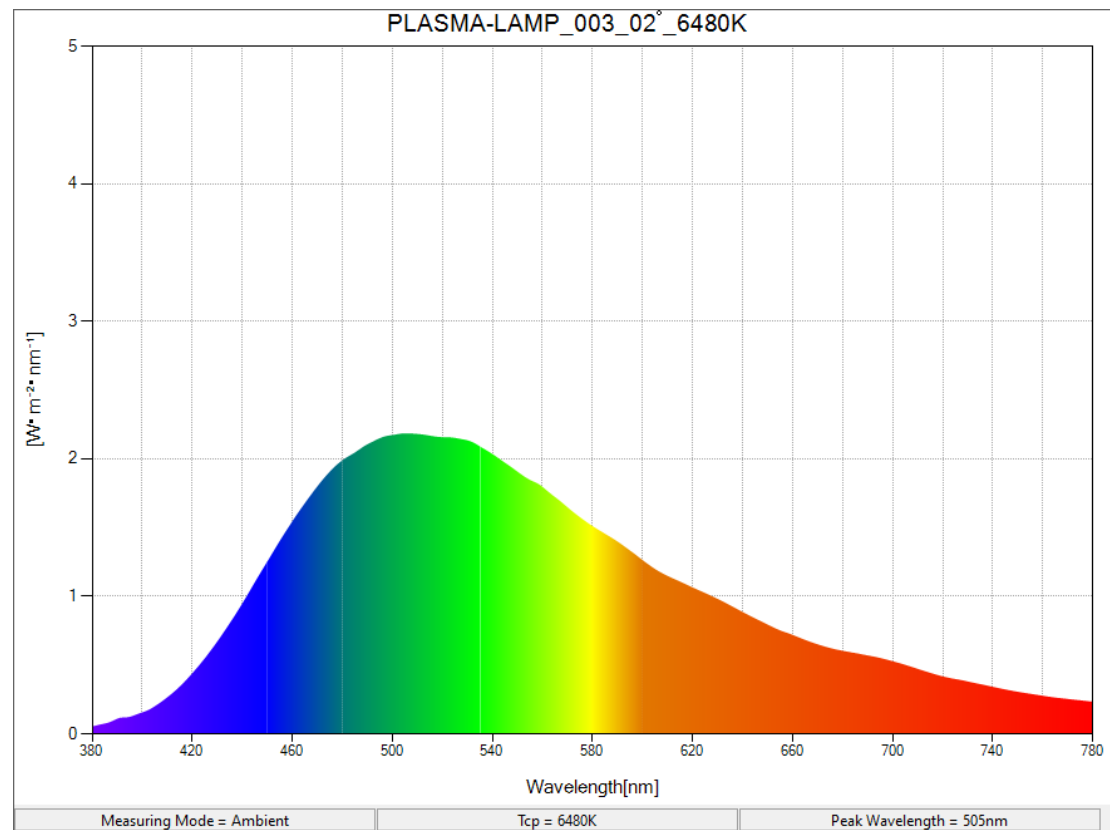
Figure 4. the wavelength rage of the plasma lamp

3.4 Addition of Iron

After finishing the light treatment, the iron was added into 5 groups in the form of iron (II) sulfate heptahydrate (278.01 g/mol) with three different concentrations (6, 12, 24 mg/L iron), and 2 groups of them had been exposed to light before.

3.5 Heat Treatment

To accelerate the extraction and the reaction between the compounds, the heat treatment was utilized during the maturation. 8 groups of samples were stored in the middle of the temperature chamber which was set to 65 Celsius for 14 days. The choice of temperature was according to the parameter from the pervious diploma work cooperated with EtOH Spirits (Yding & Raditya, 2019). The rest groups (T23, control, commercial samples) were stored in a paper box at room temperature (23 Celsius). On the 7th day, 2 mL were taken from each group and stored in tubes. Also, after finishing the heat treatment, the wood chips were taken from the bottles to end the further extraction. All the bottles and tubes containing samples were stored in a cooling room (4 Celsius) to mitigate further reaction. The code of each group was presented in Table 1.

NW (control)	Containing new make spirit
T23 (1,2,3)	Containing new make spirit and oak chips, 23 Celsius maturation
T65 (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation
T65L8 (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, 8h light treatment
T65L24 (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, 24h light treatment
T65LI (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, low iron concentration
T65MI (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, medium iron concentration
T65HI (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, high iron concentration
T65L8MI (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, 8h light treatment, medium iron concentration
T65L24MI (1,2,3)	Containing new make spirit and oak chips, 65 Celsius maturation, 24h light treatment, medium iron concentration
Been Apart	Provided by EtOH Spirits, matured in Burgundy Brandy and Rivesaltes Vins doux naturel barrels for 10 days
Hafnium	Provided by EtOH Spirits, matured in Sherry barrels for 9 days

Table 1. The code of each group

3.6 UV-VIS Spectroscopy Measurement

0.1 mL whisky from each group was diluted in 9.9 mL 62.1% (v/v) ethanol. After that, a 1 mL sample was taken and transferred into a quartz cuvette. The spectrum of each sample was measured by a Varian® Cary 50 Bio UV-Vis Spectrophotometer (Agilent Technologies Sweden AB, Sundbybergs, Sweden) between the wavelength range 260 and 650 nm with 1 nm intervals, the 62.1% (v/v) ethanol was also scanned as control.

3.7 HPLC-DAD analysis

6 phenolic compounds including gallic acid, vanillin, vanillic acid, caffeic acid, trans-cinnamic acid, and trans-ferulic acid were identified and quantified by HPLC. The development of this method was according to the research relevant to the determination of phenolic compounds by UHPLC (Schwarz et al., 2009). The HPLC system consisted of a pump (LC-20ADsp), autosampler (SIL-20AC), column oven (CTO-20AC), and diode array detector (SPD-M20A). A reversed-phase C18 ODS1 5 µm column (4.0 mm× 150 mm, Waters Spherisorb) was selected for the HPLC equipment. To obtain the calibration curves, three different concentrations (0.0035, 0.002646, 0.001764 mg/mL) of the chemical standard of each compound are made. And two mobile phases, A (3% acetonitrile, 1% acetic acid, and 96% Milli-Q water) and B (85% acetonitrile, 2% acetic acid, and 14% Milli-Q water) were prepared. Also, 50 µL of each sample was taken and diluted with 983 µL Milli-Q water to keep level of organic phase same as mobile phase A. After that, the diluted samples were centrifuged by a spin (6.5 cm diameter) with 13.4×10^3 rpm for 10 min. 10 µL of each standard and 20 µL of each sample were determined as injection volumes. And the gradient elution conditions were applied as follows: flow rate, 1 mL/min; temperature of oven, 40 °C; 0 min, 100% A; 5 min, 90% A; 10 min, 90% A; 20 min, 60% A; 25 min, 0% A. The column was washed with 100% B for 5 minutes and equilibrated with 100% A for 10 minutes. The identification of each compound was based on the retention time and UV–Visible spectra of standards.

3.8 Data Collection and Treatment

The absorbance of each sample at 280 nm was measured and processed by Cary WinUV Software (Agilent) and saved in Excel form. The visualization of chromatograms and spectrum was achieved by LabSolutions LC (SHIMADZU). The concentration of each identified compound was calculated based on the area of a specific peak. Analysis of variance (ANOVA) was run by Excel on data.

4. Results and Discussion

4.1 Visual observations

After 14 days of maturation, the change of appearance of each group was presented in Figures 5, 6, and 7. The control group maintained almost clear, the color of T23 became gold or amber. The influence of light on the color of samples did not be observed by the eyes but obviously, the heat treatment darkened the samples to tawny or auburn. The virtually black could be observed within 1 hour after adding the iron (II) sulfate heptahydrate and transferring the samples into the temperature chamber even for the lowest iron dosage. The higher dosage of iron was added, the darker color was formed, and the black deposition was also found in the sample with the highest iron dosage. It is hypothesized that the formation of black color may due to the reaction between iron and polyphenolic compounds, which produced dark color chelation complexes (Preedy, 2013). On the other hand, the appearance of the samples without iron addition had no significant difference from commercial whisky, but since whisky with a very dark color is not common in the market, it is not clear whether the samples added with iron are acceptable for consumers.

Figure 5. The appearance of samples after 14 days maturation (From left to right: NW, T23, T65, T65L8, T65L24, T65LI)
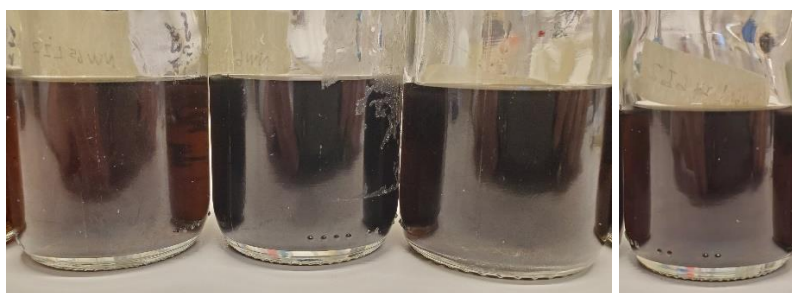
Figure 6&7. The appearance of samples after 14 days maturation (From left to right: T65MI, T65HI, T65L8MI, T65L24MI)

4.2 UV-VIS Spectroscopy results

ANOVA was applied to the absorbance data at 280 nm to investigate whether a significant difference exists between the samples with different treatments. The absorbance data at 280 nm of each sample can be found in Table 2. The data of T65L8 3 (7 days), T65L24 1 (7 days), T65L24MI 2 (7 days), and T65L24 1 (14 days) were excluded due to the high deviation.

	7 days	14 days
NW		0.08
T23 1	0.13	0.16
T23 2	0.13	0.17
T23 3	0.12	0.17
T65 1	0.33	0.34
T65 2	0.29	0.34
T65 3	0.28	0.34
T65L8 1	0.28	0.34
T65L8 2	0.30	0.33
T65L8 3	0.22	0.34
T65L24 1	0.29	0.34
T65L24 2	0.33	0.37
T65L24 3	0.32	0.35
T65LI 1	0.31	0.34
T65LI 2	0.33	0.29
T65LI 3	0.30	0.35
T65MI 1	0.32	0.38
T65MI 2	0.30	0.34
T65MI 3	0.31	0.34
T65HI 1	0.22	0.27
T65HI 2	0.27	0.31
T65HI 3	0.27	0.29
T65L8MI 1	0.33	0.37
T65L8MI 2	0.32	0.34
T65L8MI 3	0.31	0.34
T65L24MI 1	0.29	0.31

T65L24MI 2	0.30	0.34
T65L24MI 3	0.30	0.32

Table 2. The absorbance data at 280 nm after 7 and 14 days

From the data in Table 3&4, it could be found that the heat-treated group had a significant difference from the room temperature group. On the 7th and 14th day of maturation, the content of the average total phenolic compounds in the T65 group was much higher, and the absorbance of the T23 group was almost only half of the heat-treated group. But it was also noticed that the rate of the increase of absorbance of the T65 group was only relatively higher in the first 7 days, in the later 7 days, the rate of the increase of absorbance of the T65 group lowered to the same level as the T23 group.

SUMMARY (7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T23	3	0.377993	0.125998	2.88E-06		
T65	3	0.896915	0.298972	0.000675		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.04488	1	0.04488	132.3961	0.000326	7.708647
Within Groups	0.001356	4	0.000339			
Total	0.046236	5				

SUMMARY (14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T23	3	0.498909	0.166303	9.37E-05		
T65	3	1.025347	0.341782	1.95E-05		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.04619	1	0.04619	815.8672	8.94E-06	7.708647
Within Groups	0.000226	4	5.66E-05			
Total	0.046416	5				

Table 3&4. ANOVA test for investigating the effects of heat treatment after 7 and 14 days

Based on the data in Table 5&6, a significant difference was found between the T65, T65L8, and T65L24 groups after 14 days of maturation, but on the 7th day, the significant difference was not presented. The average content of the total phenolic compounds of the T65 group was slightly lower than that of the T65L24 group, but

also a bit higher than that of the T65L8 group, no matter in 7 or 14 days. The different results might be explained by the insufficiency of the duplicates or other reasons, like instrument malfunction or sample error. Whether the 8h or 24h light treatment with certain intensity influences on increasing the phenolic compounds content in the high temperature environment is still a doubt.

SUMMARY (7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	0.896915	0.298972	0.000675		
T65L8	2	0.575625	0.287812	0.000149		
T65L24	2	0.646302	0.323151	0.000118		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001321	2	0.000661	1.635023	0.302723	6.944272
Within Groups	0.001616	4	0.000404			
Total	0.002938	6				

SUMMARY (14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	1.025347	0.341782	1.95E-05		
T65L8	3	1.005314	0.335105	3.29E-05		
T65L24	2	0.719835	0.359917	5.75E-05		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000759	2	0.000379	11.67822	0.013056	5.786135
Within Groups	0.000162	5	3.25E-05			
Total	0.000921	7				

Table 5&6. ANOVA test for investigating the effects of light treatment after 7 and 14 days

Based on the data in Table 6&7, a significant difference can be found between the T65, T65LI, T65MI, and T65HI groups. However, the average total phenolic compound contents of T65, T65LI, and T65MI groups were similar, much higher than that of the T65HI group. The current results were not possible to prove the positive effects of iron on increasing the phenolic compounds content in the high temperature environment. Also, the high concentration of iron showed an adverse influence on the phenolic compounds content of the whisky.

SUMMARY (7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	0.896915	0.298972	0.000675		
T65LI	3	0.937869	0.312623	0.000263		
T65MI	3	0.933157	0.311052	7.73E-05		
T65HI	3	0.761071	0.25369	0.000797		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006861	3	0.002287	5.049174	0.029843	4.066181
Within Groups	0.003624	8	0.000453			
Total	0.010485	11				

SUMMARY (14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	1.025347	0.341782	1.95E-05		
T65LI	3	0.976112	0.325371	0.001069		
T65MI	3	1.056047	0.352016	0.000474		
T65HI	3	0.874342	0.291447	0.000348		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.006328	3	0.002109	4.414755	0.041328	4.066181
Within Groups	0.003822	8	0.000478			
Total	0.01015	11				

Table 7&8. ANOVA test for investigating the effects of the addition of iron after 7 and 14 days

Based on the data in Table 9&10, a significant difference was not found between the T65, T65L8, T65L24, T65L8MI, T65L24MI, and T65MI groups. The total phenolic compounds content of the groups with combined treatment (T65L8MI, T65L24MI) had no significant difference from the other groups treated only with light or iron.

SUMMARY (7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	0.896915	0.298972	0.000675		
T65L8	2	0.575625	0.287812	0.000149		
T65L24	2	0.646302	0.323151	0.000118		
T65MI	3	0.933157	0.311052	7.73E-05		
T65L8MI	3	0.95574	0.31858	6.24E-05		
T65L24MI	2	0.587816	0.293908	3.01E-05		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00224	5	0.000448	2.093374	0.158316	3.481659
Within Groups	0.001926	9	0.000214			
Total	0.004166	14				

SUMMARY (14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T65	3	1.025347	0.341782	1.95E-05		
T65L8	3	1.005314	0.335105	3.29E-05		
T65L24	2	0.719835	0.359917	5.75E-05		
T65MI	3	1.056047	0.352016	0.000474		
T65L8MI	3	1.058116	0.352705	0.000289		
T65L24MI	3	0.978424	0.326141	0.000247		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.002134	5	0.000427	2.151943	0.134486	3.203874
Within Groups	0.002182	11	0.000198			
Total	0.004316	16				

Table 9&10. ANOVA test for investigating the combined effects of the addition of iron and light treatment after 7 and 14 days

4.3 HPLC-DAD

More than 20 compounds were detected by using HPLC-DAD and six types of phenolic compounds were applied as standards, three of them (gallic acid, vanillic acid, and vanillin) were identified in the samples based on the spectra and retention time. The information on the spectra and retention time of samples and standard solution can be found in Appendix 1. The identified compounds were quantified by plotting calibration curves. The plotting of calibration curves was according to the concentrations of each compound in the standard solution and the peaks in the chromatogram. Appendix 2 was the calibration curves of the gallic acid, vanillic acid,

and vanillin respectively. The equations for calculating the concentration of each compound in the samples were obtained by describing the correlation between the concentration gradient of the compound in the standard solution and the peak area of each compound. The actual concentration of gallic acid, vanillic acid, and vanillin in each undiluted sample was given in Table 11.

	vanillin	gallic acid	vanillic acid
CONTRL	0.54	0.71	2.16
7T23 1	0.68	3.27	1.98
7T23 2	1.02	4.72	2.17
7T23 3	0.79	5.61	2.04
7T65 1	1.79	8.35	2.41
7T65 2	1.62	7.17	2.18
7T65 3	2	6.99	2.29
7T65L8 1	2.05	6.61	2.21
7T65L8 2	1.48	5.87	2.08
7T65L8 3	1.54	4.68	2.32
7T65L24 1	1.6	4.36	2.25
7T65L24 2	2.09	7.53	2.11
7T65L24 3	1.85	6.5	2.18
7T65LI 1	1.98	6.83	2.34
7T65LI 2	2.11	7.78	2.54
7T65LI 3	1.8	6.02	2.07
7T65MI 1	2.2	6.59	2.29
7T65MI 2	1.99	5.97	2.41
7T65MI 3	1.88	7.88	2.12
7T65HI 1	1.95	4.77	2.29
7T65HI 2	2.02	6.42	2.25
7T65HI 3	1.61	3.58	2.11
7T65L8MI 1	2.09	7.61	2.52
7T65L8MI 2	1.92	7.35	2.15
7T65L8MI 3	2.48	7.6	2.31
7T65L24MI 1	1.71	7.14	2.11
7T65L24MI 2	2.02	5.25	2.08
7T65L24MI 3	2.21	7.48	2.39
14T23 1	0.7	4.29	2.1
14T23 2	1.12	5.46	2.07
14T23 3	0.96	7.68	2.06
14T65 1	2.01	7.24	2.26
14T65 2	2.27	6.59	2.18
14T65 3	2.29	5.76	2.26
14T65L8 1	2.52	6.03	2.49
14T65L8 2	1.77	6.09	2.2
14T65L8 3	1.78	4.38	2.11
14T65L24 1	2.18	4.65	2.2

14T65L24 2	2.59	7.55	2.24
14T65L24 3	2.43	6.48	2.17
14T65LI 1	2.37	6.38	2.35
14T65LI 2	2.46	7.75	2.14
14T65LI 3	2.31	6.42	2.45
14T65MI 1	3.06	7.4	2.52
14T65MI 2	2.59	6.49	2.32
14T65MI 3	2.28	7.27	2.42
14T65HI 1	2.41	3.96	2.35
14T65HI 2	2.79	5.9	2.44
14T65HI 3	2.18	4.19	2.17
14T65L8MI 1	2.6	7.46	2.22
14T65L8MI 2	2.54	7.81	2.31
14T65L8MI 3	3.06	7.01	2.25
14T65L24MI 1	2.11	7.19	2.18
14T65L24MI 2	2.77	7	2.46
14T65L24MI 3	3.08	7.28	2.58
R1(Been Apart)	1.64	5.81	1.14
R2(Hafnium)	0.76	1.09	1.03

Table 11. the concentration of gallic acid, vanillic acid and vanillin (mg/L) in each sample

Figure 8 showed the chromatogram of T65L24MI 3 (14 days), the peaks of gallic acid, vanillic acid, and vanillin appeared at around 2.8 min, 7.3 min, and 9.5 min respectively, and the retention time of these compounds was the same as that in the standard solution. On the other hand, around 7.7 min, 13.5 min, and 17.8 min, no peak was found in these time ranges, which means caffeic acid, trans-ferulic acid, and trans-cinnamic acid did not present in the samples or their concentrations were under the detection limit. Besides, by comparing the spectra of gallic acid, vanillic acid, and vanillin of samples and standard solution, the similarity of the maximum absorbance was observed. The chromatograms of the rest sample were presented in Appendix 4.
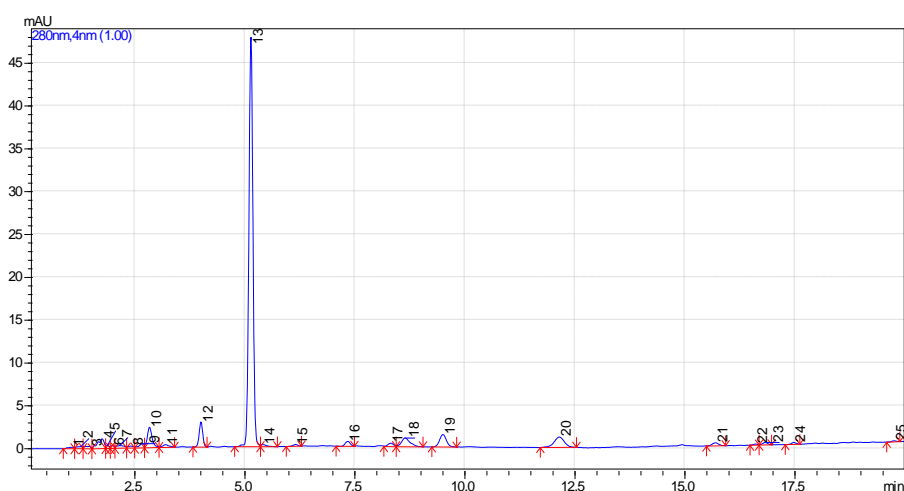
Figure 8. The chromatogram of T65L24MI 3 (gallic acid (10), vanillic acid (16), vanillin (19))

Also, the ANOVA tests were used for evaluating the difference in the contents of each compound in the full data. The data of T65L8 3 (7 days), T65L24 1 (7 days), T65L24MI 2 (7 days), and T65L24 1 (14 days) were excluded due to the high deviation.

The descriptor of gallic acid: astringent, puckering astringency (Miller, 2020). Assuming that samples were diluted to 40% ABV, the content of gallic acid of the single-malt Scotch and American bourbon from Figure 2 was only around 30% of that in heat treated samples (14 days). Also, the ANOVA test in Appendix 3 showed the concentration of gallic acid in each group had a significant difference. The concentration of gallic acid in all the samples increased in the first 7 days, the gallic acid contents of heat-treated groups were much higher than that of T23. However, the gallic acid contents of the majority of heat treated groups maintained stability or decreased after 14 days. On the other hand, the gallic acid contents of the T65HI group were much lower than other heat-treated groups.

The descriptor of vanillic acid: astringent, vanilla, sweet (Miller, 2020). Assuming that samples were diluted to 40% ABV, the content of vanillic acid of the single-malt Scotch and American bourbon from Figure 2 was only around 15% and 45% of that in heat treated samples (14 days). Also, the ANOVA test in Appendix 3 showed the concentration of vanillic acid in each group had no significant difference. The concentration of vanillic acid in each group was similar no matter what treatment was applied, and the contents of vanillic acid also did not vary with time.

The descriptor of vanillin: vanilla-like, sweet (Miller, 2020). Assuming that samples were diluted to 40% ABV, the content of vanillin of the single-malt Scotch and American bourbon from Figure 2 was only around 30% and 60% of that in heat treated samples (14 days). Also, the ANOVA test in Appendix 3 showed the concentration of vanillin in each group had a significant difference. The content of vanillin in each group increased sharply in the first 7 days, but unlike the gallic acid, the content of vanillin kept raising after 7 days at a slower rate. Also, the heat was the only treatment that caused a significant influence on the content of vanillin, even the high centration of iron did not change the production and extraction of vanillin.

5. Conclusion

The purpose of this project is to investigate the correlation between several treatments and the formation of phenolic compounds during whisky maturation. The maturation of whisky is a complex process, many chemical reactions occur in this stage, and the generation of phenolic compounds is an essential part of them. Oak chips treated with 65 ℃ indeed released an abundance of phenolic compounds like vanillin after 7 days, but the effect of heat treatment will start to decay after that. Besides, the excessively long period of treatment can reduce the content of some compounds like gallic acid. Also, the significant change in the concentration of any target substances cannot be observed with light exposure (between 380 to 780 nm) over 24h, one reason could be that the short wavelength visible light or ultraviolet from the lamp is not sufficient to affect the degradation of wood. On the other hand, the addition of iron does not show

any beneficial behavior on the maturation of whisky in this project, and the high concentration of iron seems to cause spoilage of the quality of the whisky.

Three compounds (gallic acid, vanillin, and vanillic acid) were identified by HPLC-DAD, the other three substances (caffeic acid, ferulic acid, and cinnamic acid) were not detected or the concentrations of them were too low to be detected. For gallic acid, adequate heat treatment shows a positive influence while excessively long periods of heating or a high dosage of iron addition have an adverse effect. The level of the vanillin in the samples seems only to be affected by heating in this project, while the concentration of vanillic acid did not vary with time during the 14 days of maturation.

On the other hand, the samples in the project are still distinct from the commercial products. The application of light is still potential, the impact of light is likely to be observed if the proportion of shortwave visible light or exposure time is increased. But the addition of iron was not positively correlated to compounds of interest analyzed in this project. And we observed the potential to control the color level, but unlike red wine with deep color, the iron ion can change the color of whisky completely, which may be not acceptable to some potential consumers. The powder of iron or iron oxides could be a better option.

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Appendix 1

the spectra and retention time of the samples and standard solution

The spectra and retention time of gallic acid in standard solution:
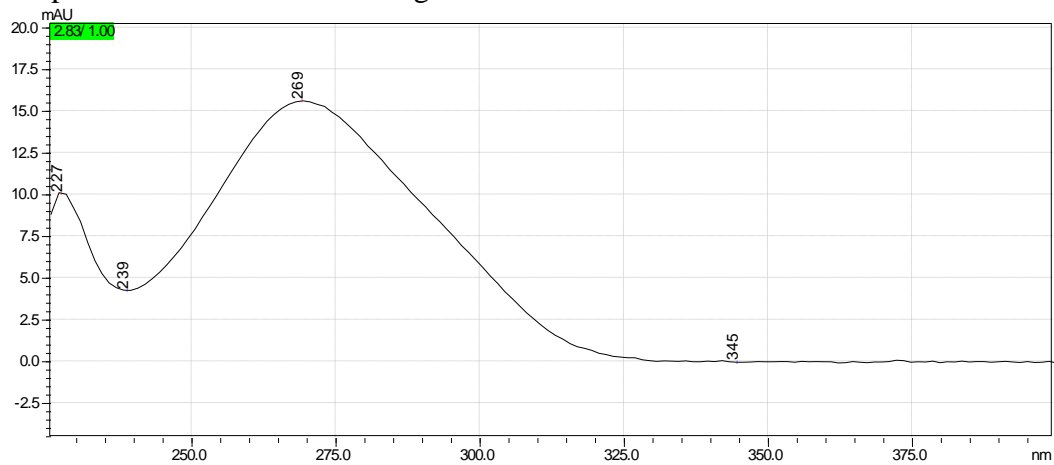
The spectra and retention time of vanillic acid in standard solution:
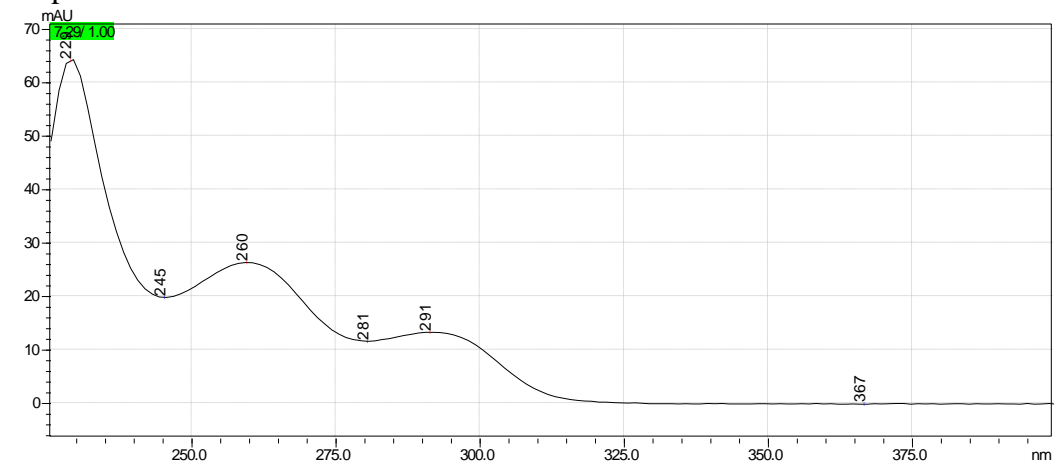
The spectra and retention time of vanillin in standard solution:
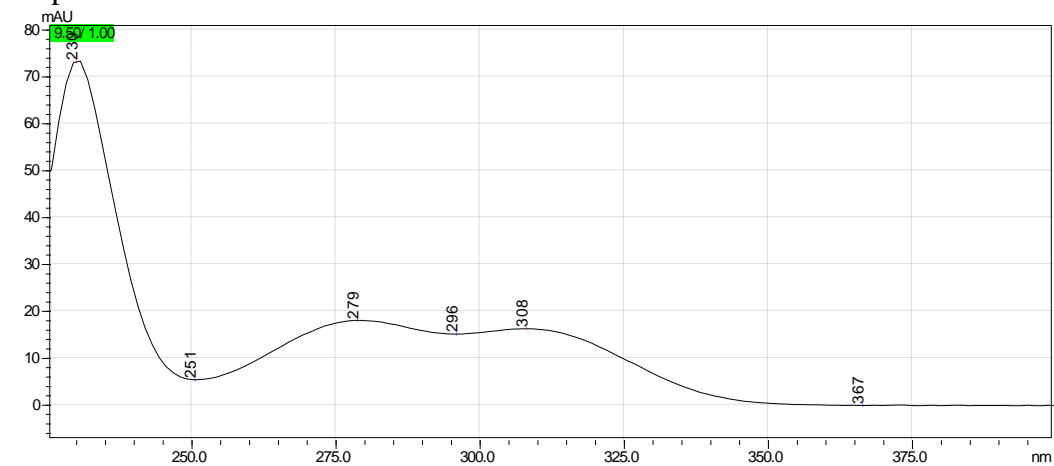
The spectra and retention time of caffeic acid in standard solution:
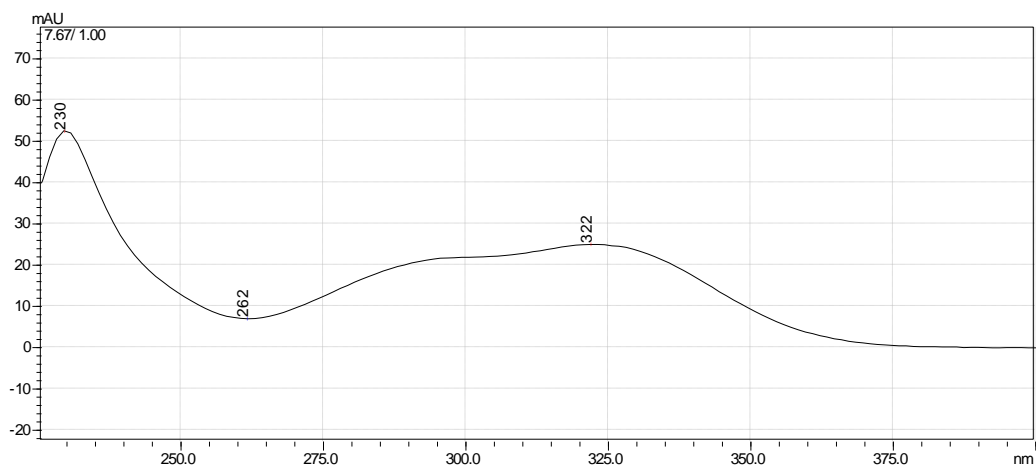
The spectra and retention time of trans-ferulic acid in standard solution:
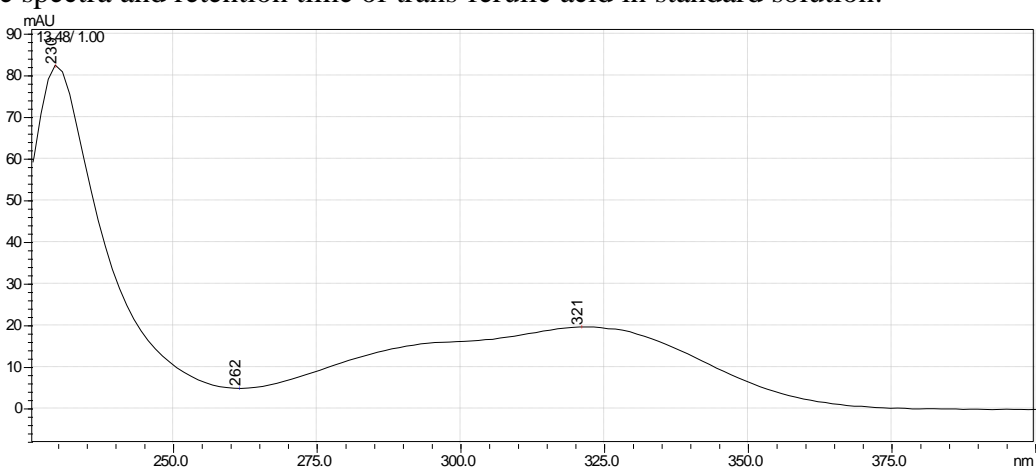
The spectra and retention time of trans-cinnamic acid in standard solution:
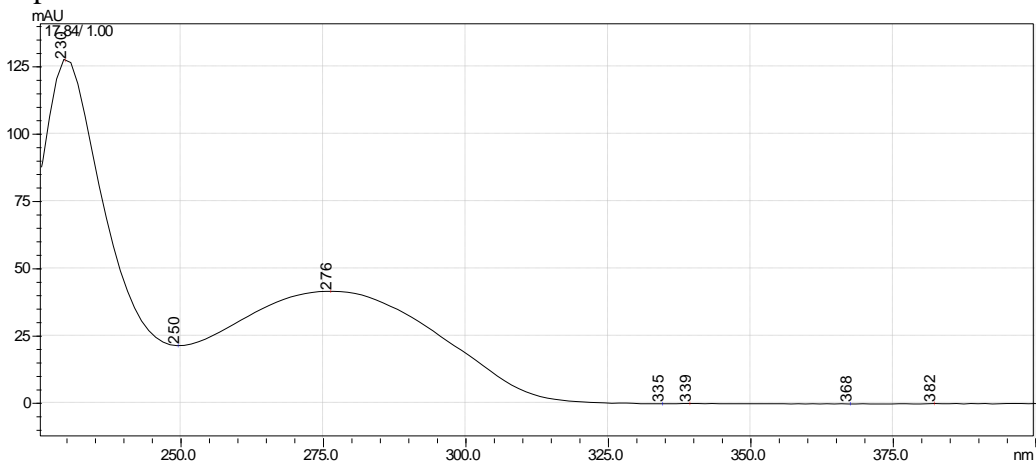
The spectra and retention time of gallic acid in T65L24MI 3 (14 days):
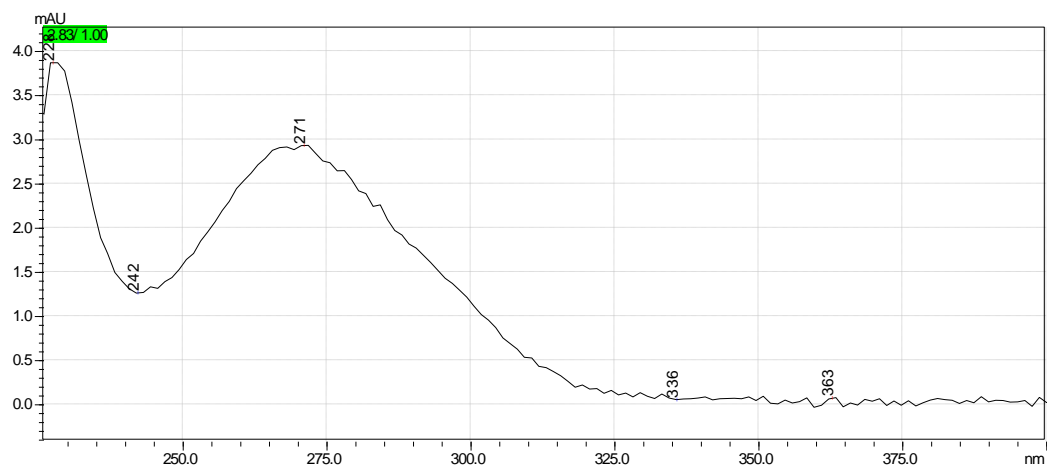
The spectra and retention time of vanillic acid in T65L24MI 3 (14 days):
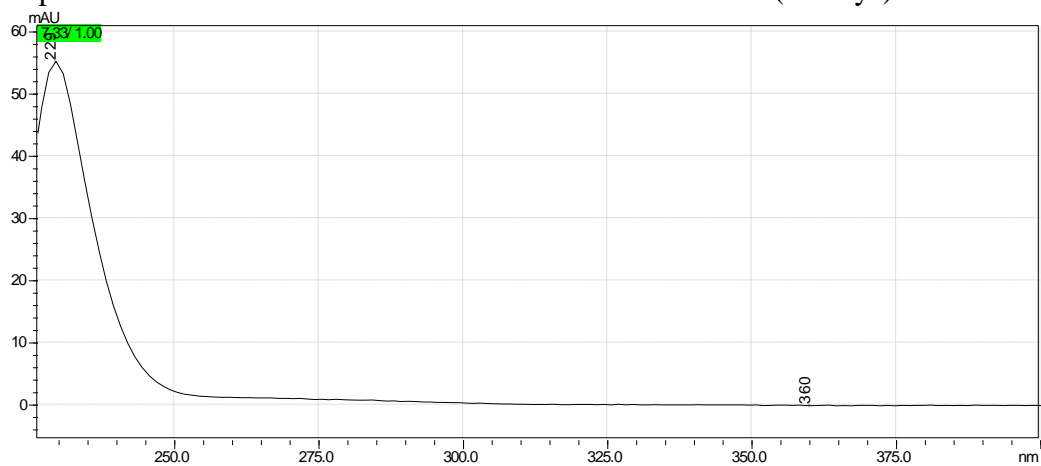
The spectra and retention time of vanillin in T65L24MI 3 (14 days):
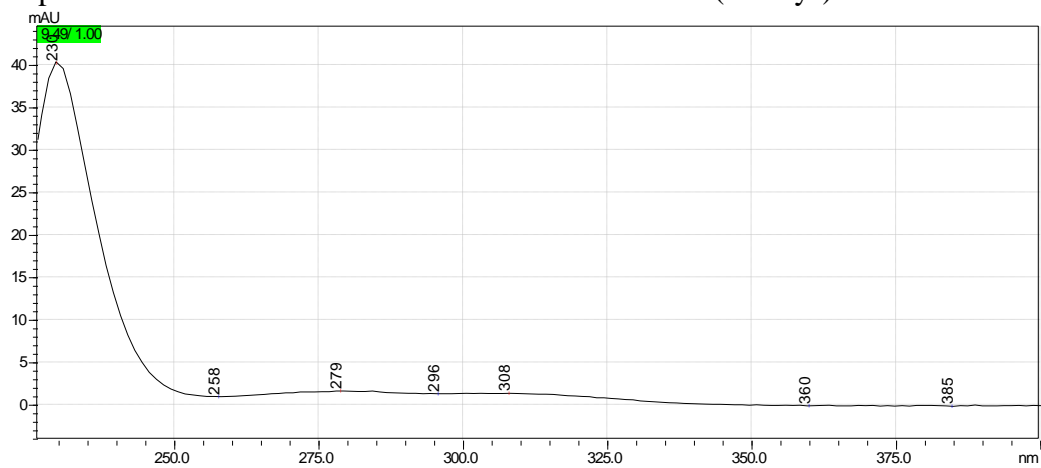
Appendix 2
the calibration curves of the gallic acid, vanillic acid, and vanillin

gallic acid

$y = 27{,}378{,}558.1127x - 879.2316$
$R^2 = 0.9785$

vanillic acid

$y = 23{,}076{,}006.4288x - 1{,}059.2370$
$R^2 = 0.9896$

vanillin

$y = 50{,}824{,}967.4846x + 583.3357$
$R^2 = 0.9846$
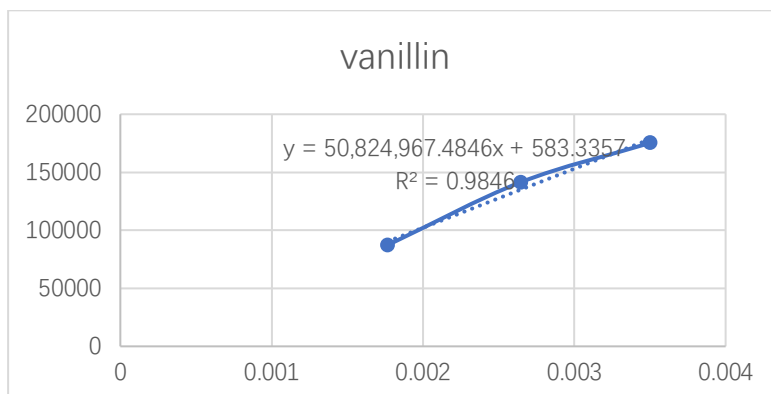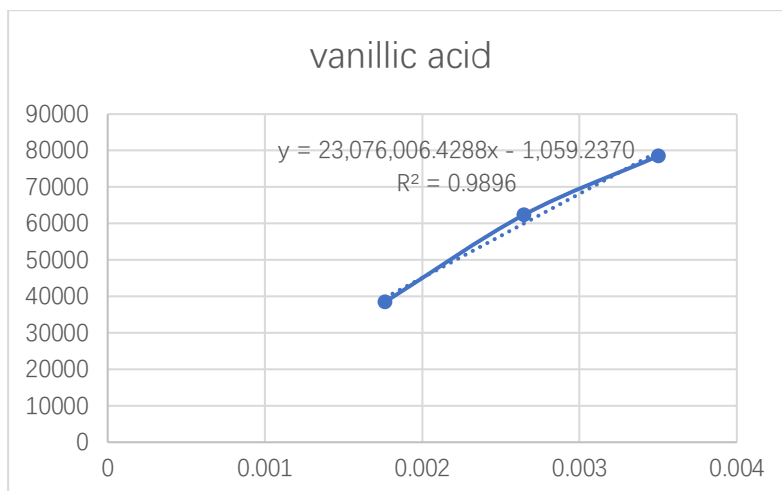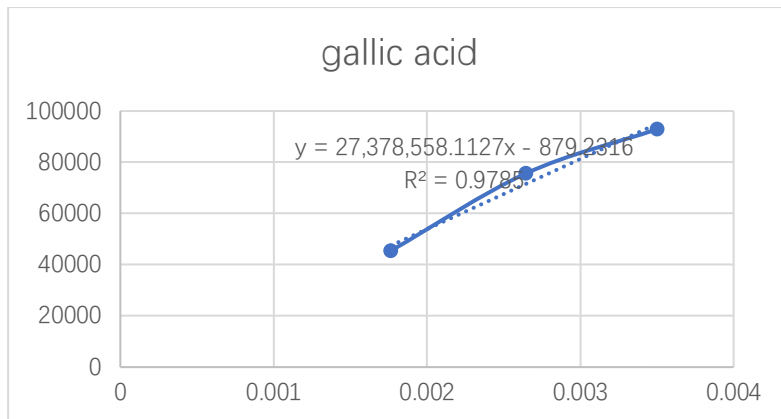
Appendix 3
the ANOVA test tables of each compound

SUMMARY (gallic acid 7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		

T23	3	13.59783	4.53261	1.401475		
T65	3	22.515	7.505	0.5407		
T65L8	2	12.48112	6.240558	0.276461		
T65L24	2	14.02791	7.013953	0.535083		
T65LI	3	20.62234	6.874112	0.770321		
T65MI	3	20.43882	6.812939	0.947394		
T65HI	3	14.76705	4.922351	2.024517		
T65L8MI	3	22.55986	7.519954	0.021158		
T65L24MI	2	14.61763	7.308815	0.058658		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27.90896	8	3.488619	4.260879	0.007617	2.640797
Within Groups	12.28134	15	0.818756			
Total	40.19029	23				

SUMMARY (gallic acid 14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T23	3	17.43556	5.811855	2.958653		
T65	3	19.59332	6.531107	0.548169		
T65L8	3	16.49574	5.49858	0.941823		
T65L24	2	14.03274	7.016368	0.578392		
T65LI	3	20.55325	6.851084	0.61204		
T65MI	3	21.15664	7.052212	0.243524		
T65HI	3	14.04724	4.682412	1.122103		
T65L8MI	3	22.28096	7.426986	0.16291		
T65L24MI	3	21.4681	7.156033	0.021358		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19.88562	8	2.485703	3.062197	0.02496	2.547955
Within Groups	13.79955	17	0.811738			
Total	33.68518	25				

SUMMARY (vanillic acid 7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		

T23	3	6.178844	2.059615	0.009627		
T65	3	6.878164	2.292721	0.012599		
T65L8	2	4.288799	2.1444	0.007934		
T65L24	2	4.291306	2.145653	0.002183		
T65LI	3	6.95995	2.319983	0.055964		
T65MI	3	6.825565	2.275188	0.021711		
T65HI	3	6.644043	2.214681	0.009202		
T65L8MI	3	6.981035	2.327012	0.035755		
T65L24MI	2	4.502463	2.251232	0.037271		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.187998	8	0.0235	1.045663	0.446609	2.640797
Within Groups	0.337102	15	0.022473			
Total	0.5251	23				

SUMMARY (vanillic acid 14 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T23	3	6.235158	2.078386	0.000403		
T65	3	6.702864	2.234288	0.002175		
T65L8	3	6.812583	2.270861	0.039618		
T65L24	2	4.413649	2.206825	0.002355		
T65LI	3	6.936583	2.312194	0.024619		
T65MI	3	7.26592	2.421973	0.009712		
T65HI	3	6.960711	2.320237	0.018662		
T65L8MI	3	6.785142	2.261714	0.00176		
T65L24MI	3	7.228407	2.409469	0.041973		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.258755	8	0.032344	1.962361	0.115552	2.547955
Within Groups	0.280201	17	0.016482			
Total	0.538956	25				

SUMMARY (vanillin 7 days)						
Groups	*Count*	*Sum*	*Average*	*Variance*		
T23	3	2.477229	0.825743	0.029817		

T65	3	5.415544	1.805181	0.036656		
T65L8	2	3.53296	1.76648	0.161851		
T65L24	2	3.939717	1.969858	0.030652		
T65LI	3	5.890389	1.963463	0.02476		
T65MI	3	6.080221	2.02674	0.026164		
T65HI	3	5.580133	1.860044	0.047359		
T65L8MI	3	6.491836	2.163945	0.084989		
T65L24MI	2	3.920998	1.960499	0.125573		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.600967	8	0.450121	8.258417	0.000265	2.640797
Within Groups	0.817567	15	0.054504			
Total	4.418535	23				

SUMMARY (vanillin 14 days)						
Groups	Count	Sum	Average	Variance		
T23	3	2.777586	0.925862	0.044566		
T65	3	6.5697	2.1899	0.025496		
T65L8	3	6.070851	2.023617	0.183292		
T65L24	2	5.021293	2.510647	0.013905		
T65LI	3	7.143363	2.381121	0.005665		
T65MI	3	7.924115	2.641372	0.151985		
T65HI	3	7.378682	2.459561	0.095465		
T65L8MI	3	8.198497	2.732832	0.079119		
T65L24MI	3	7.948464	2.649488	0.245387		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	7.370566	8	0.921321	9.345948	6.64E-05	2.547955
Within Groups	1.675855	17	0.09858			
Total	9.046421	25				

Appendix 4
the chromatogram of each sample

The chromatogram of control group:
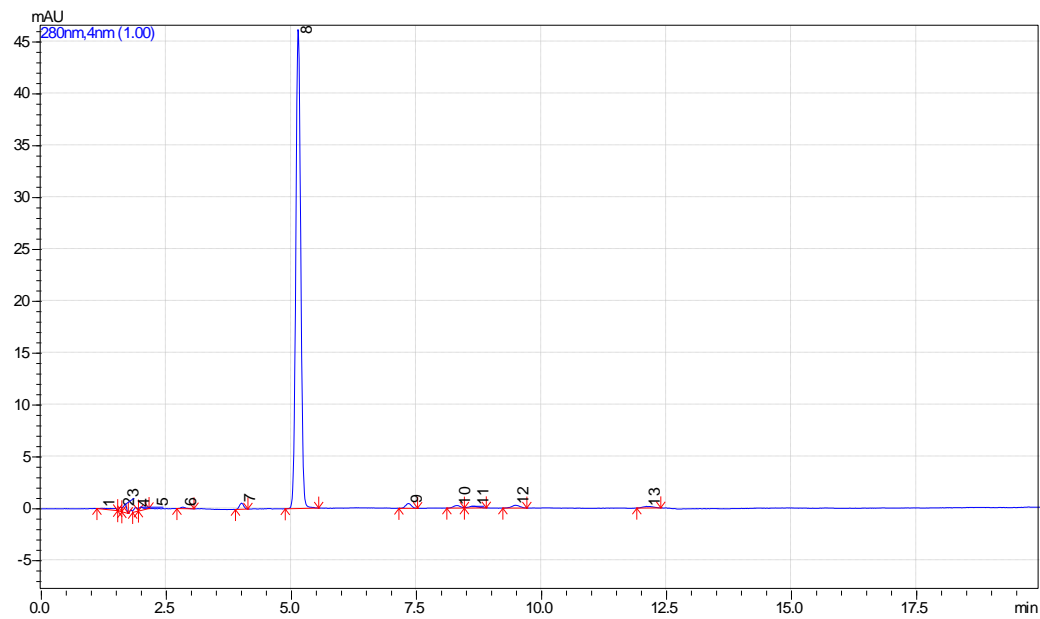
The chromatogram of T23 1 (7days):
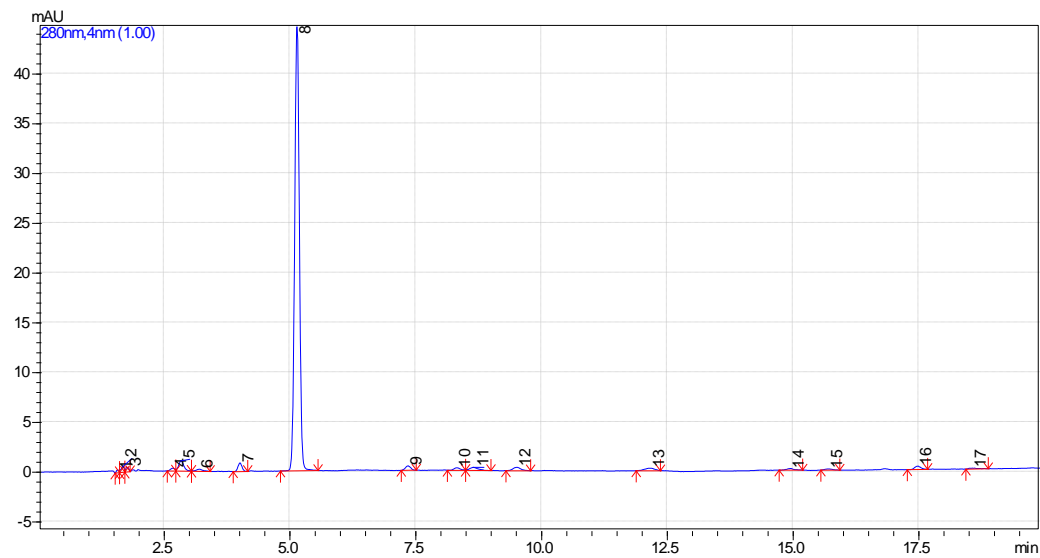
The chromatogram of T23 2 (7days):
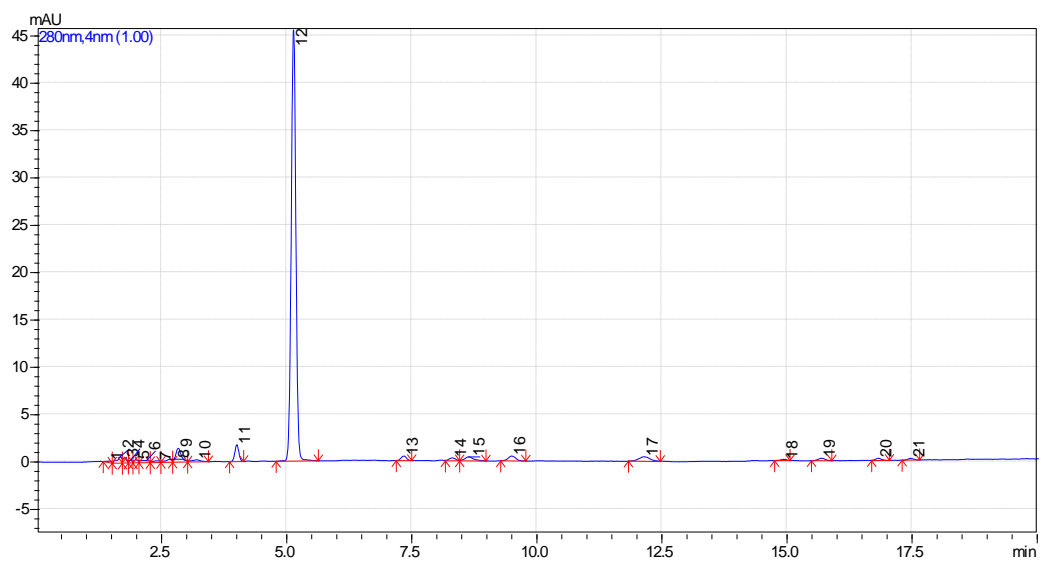
The chromatogram of T23 3 (7days):
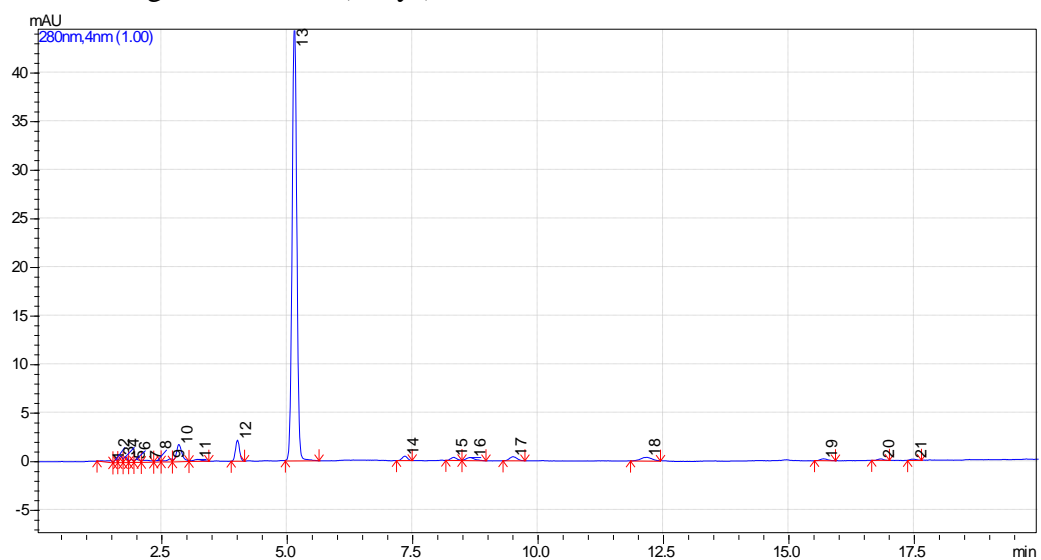
The chromatogram of T65 1 (7days):
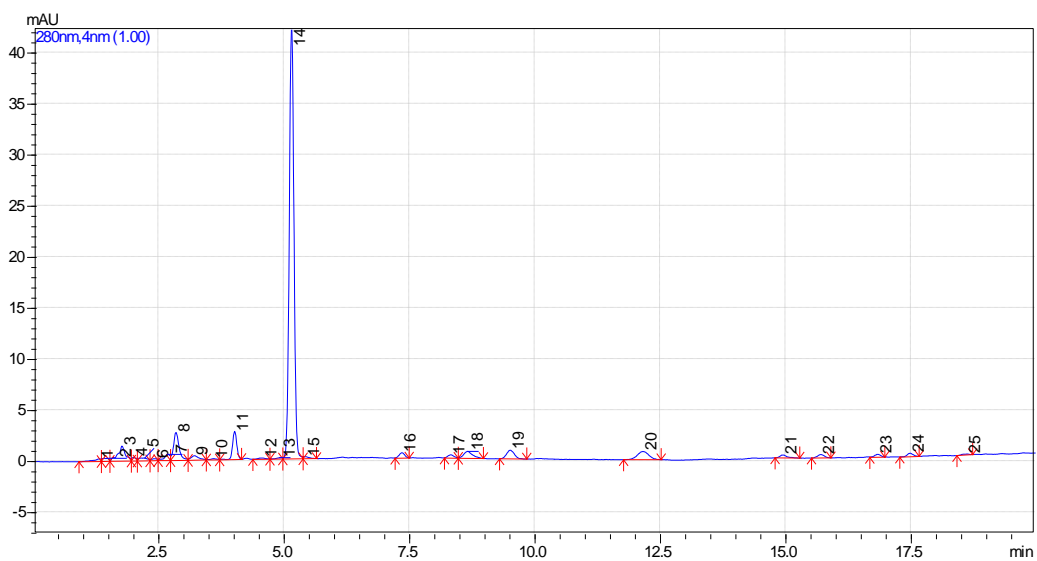
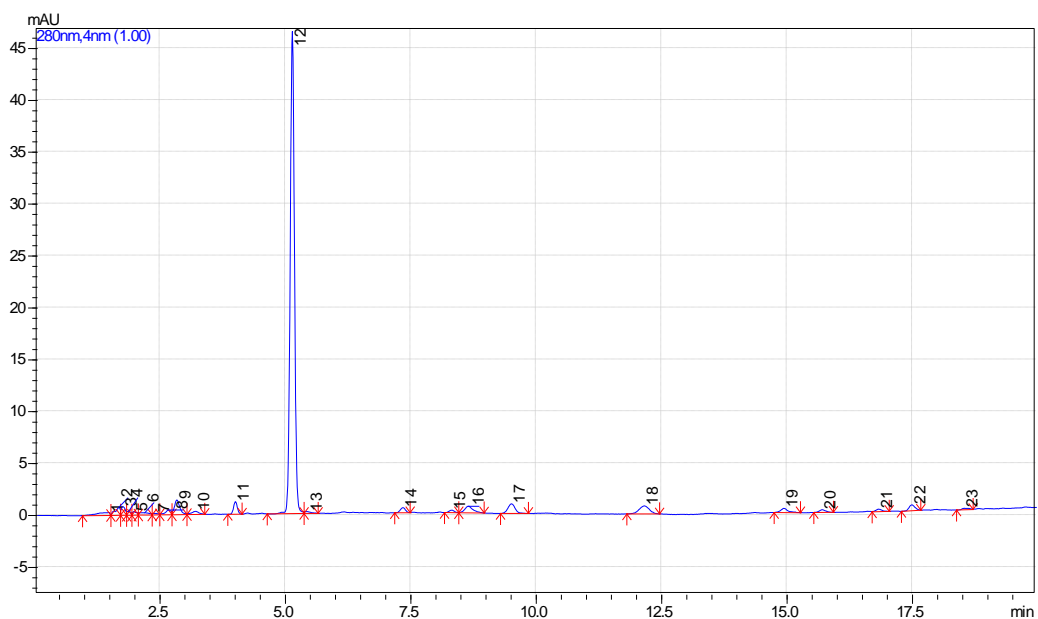The chromatogram of T65 2 (7days):
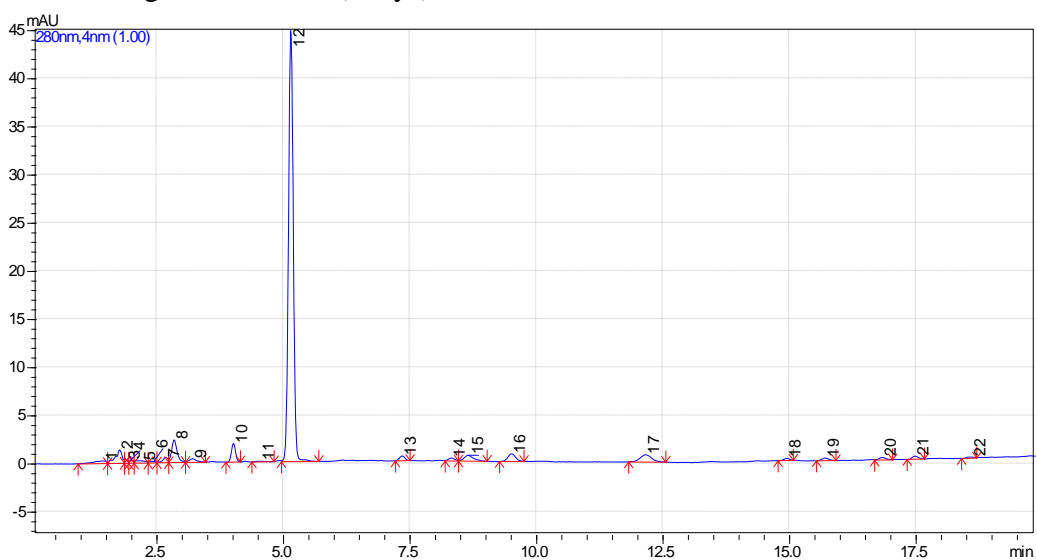
The chromatogram of T65 3 (7days):
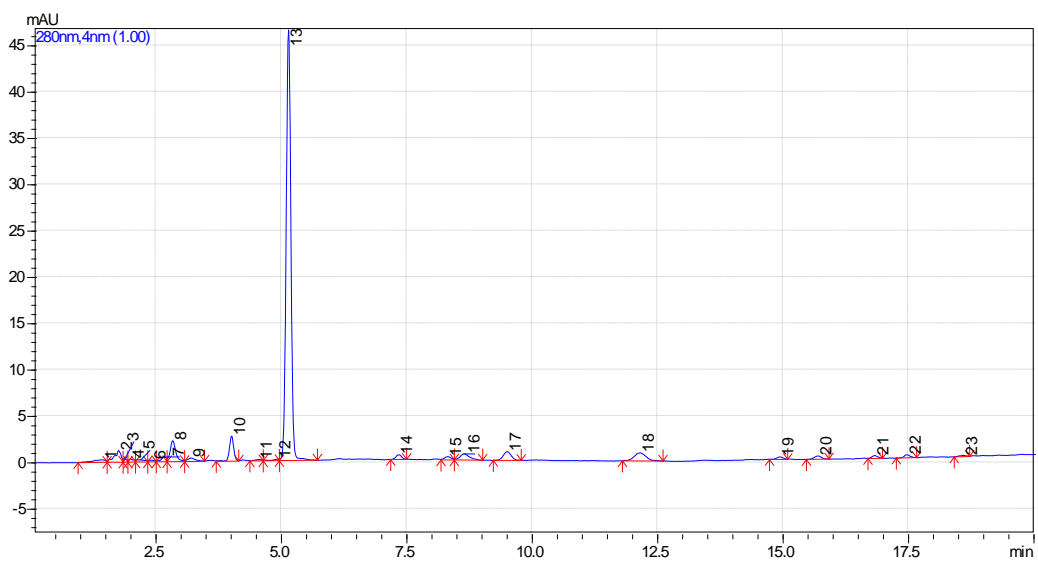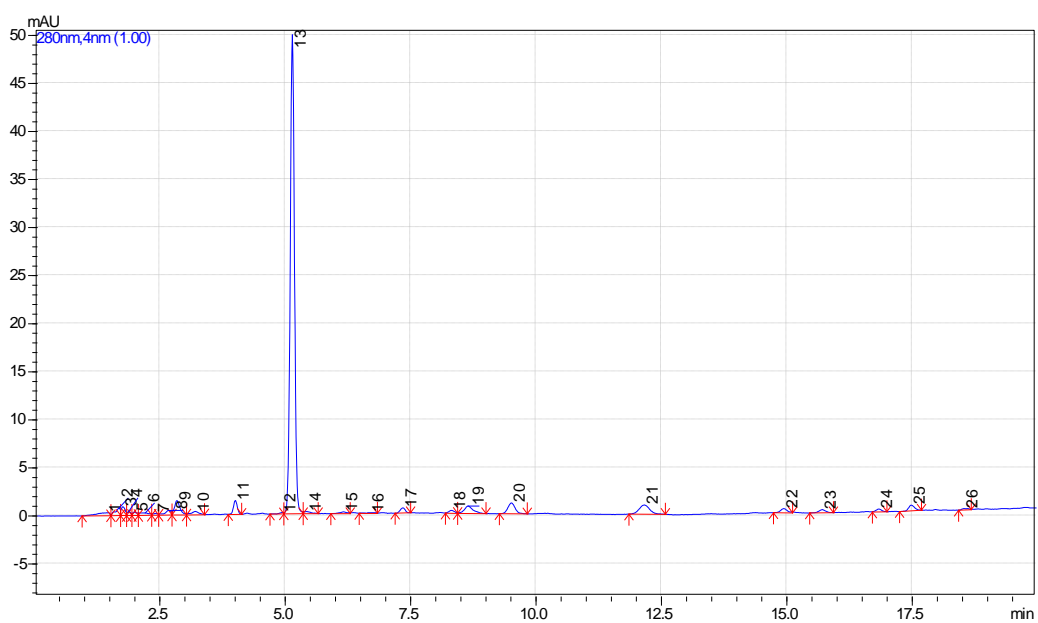
The chromatogram of T65L8 1 (7days):
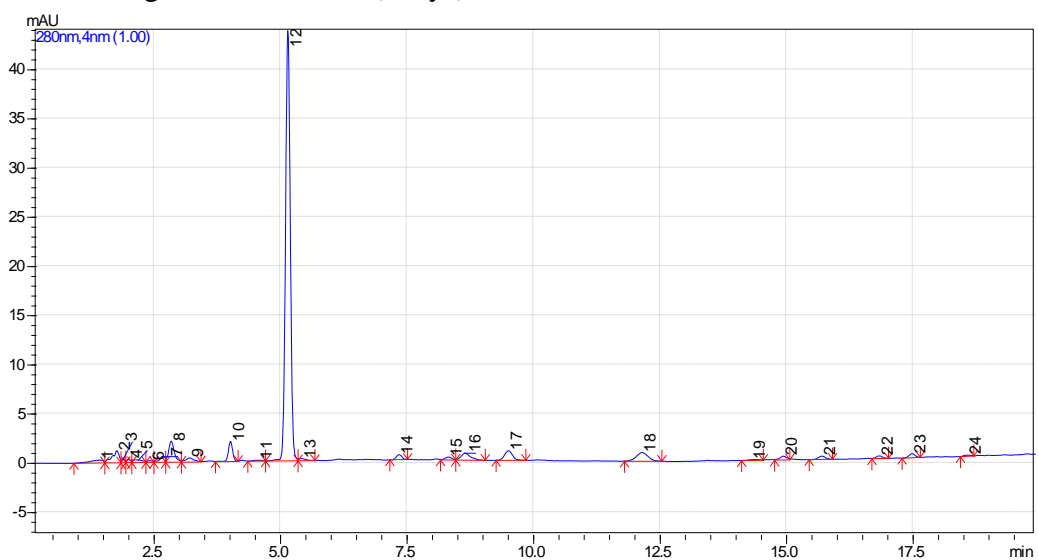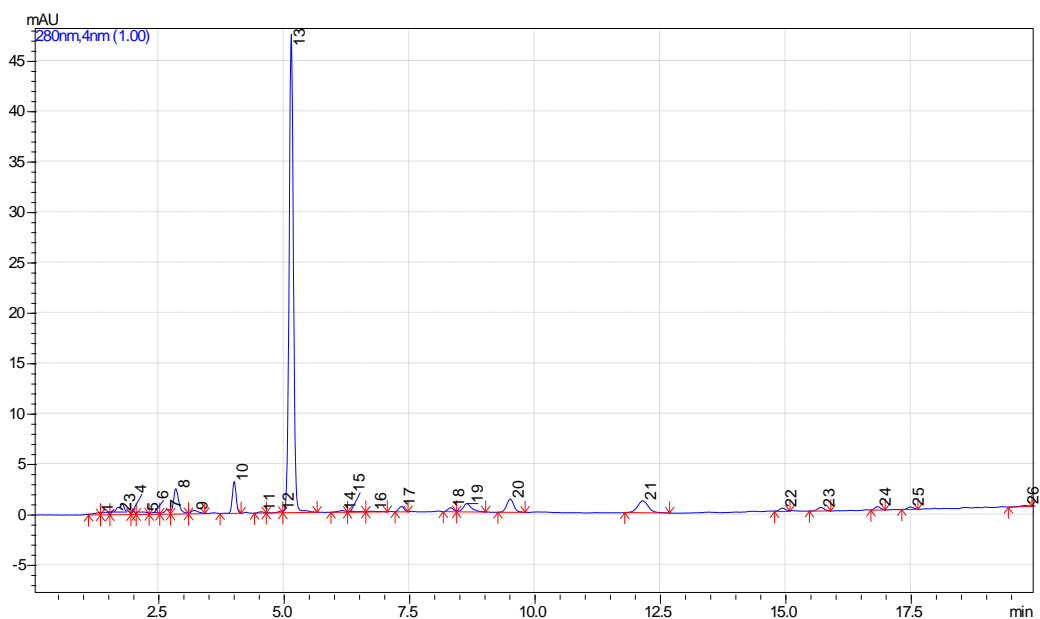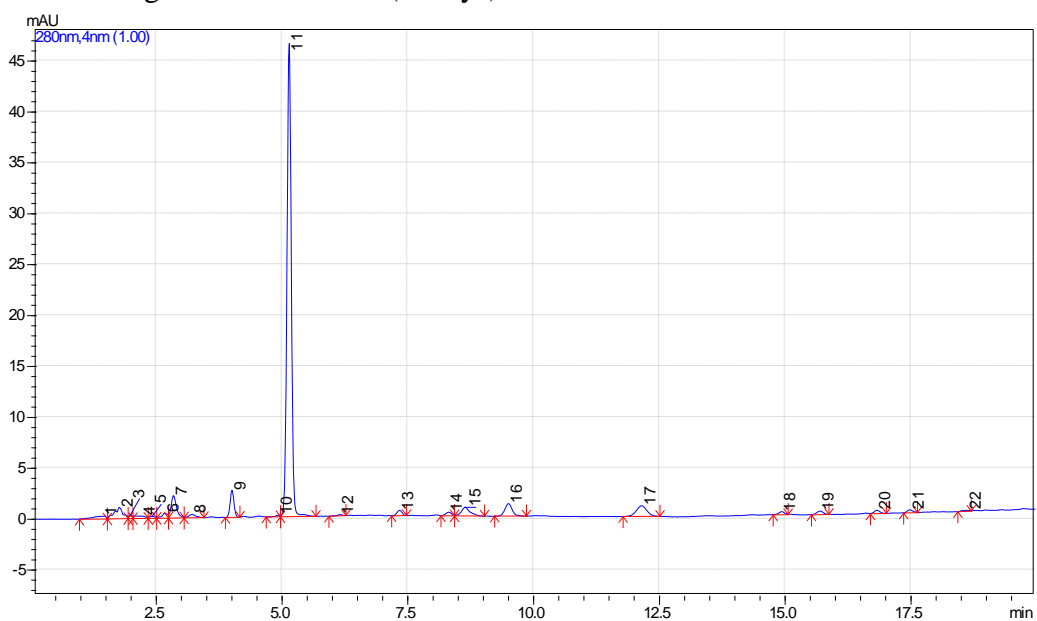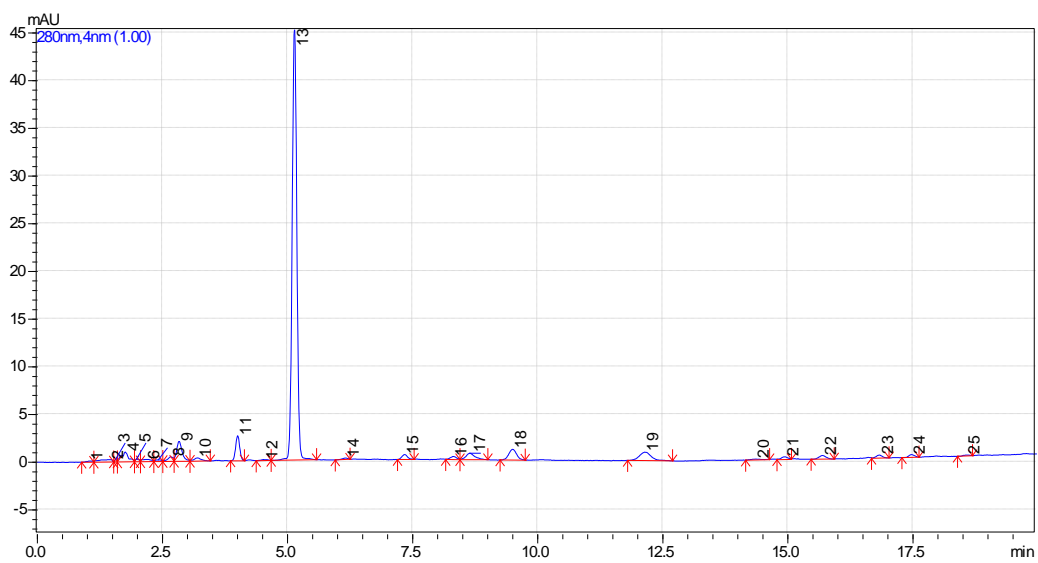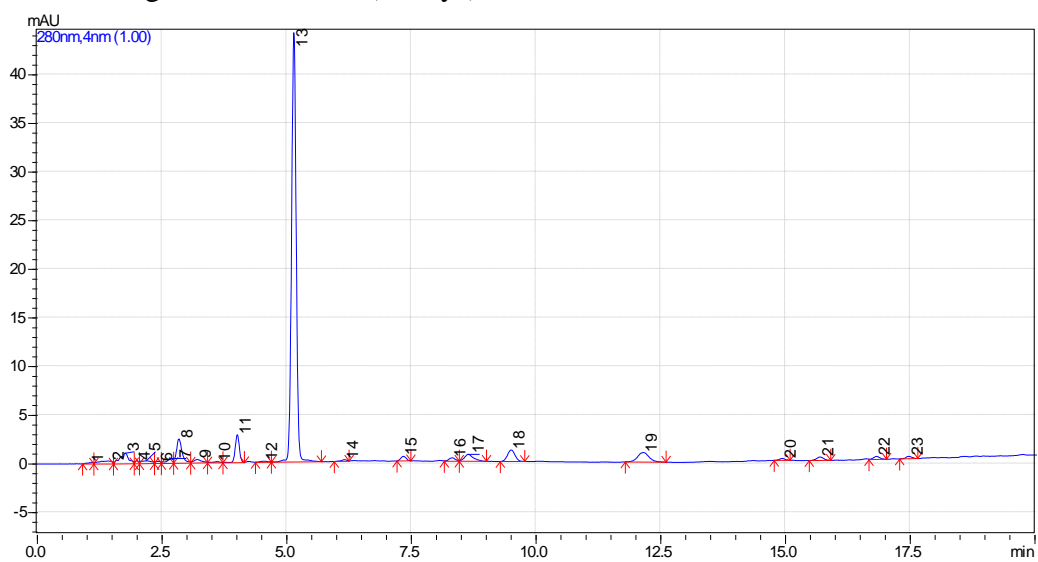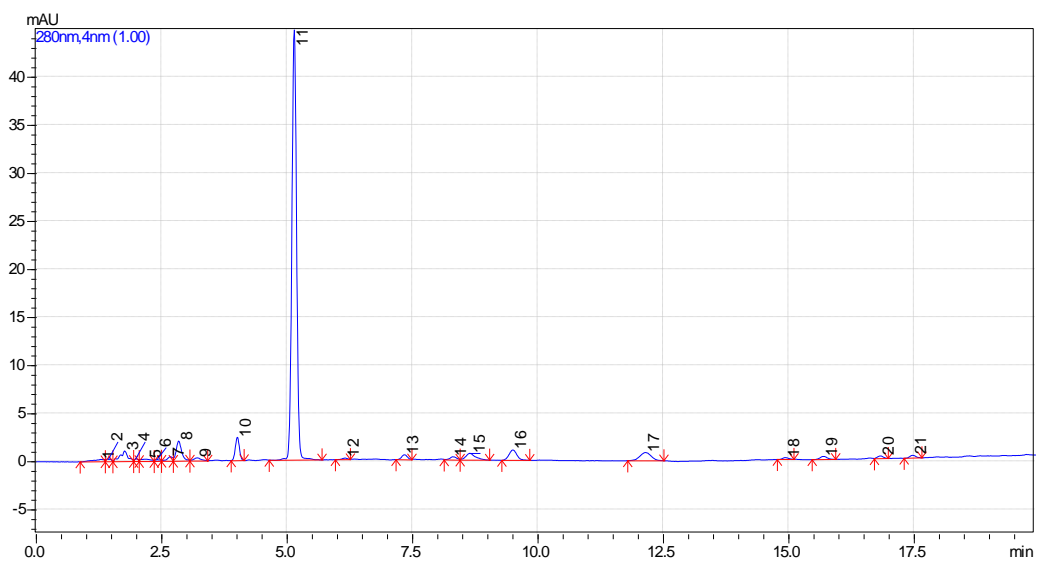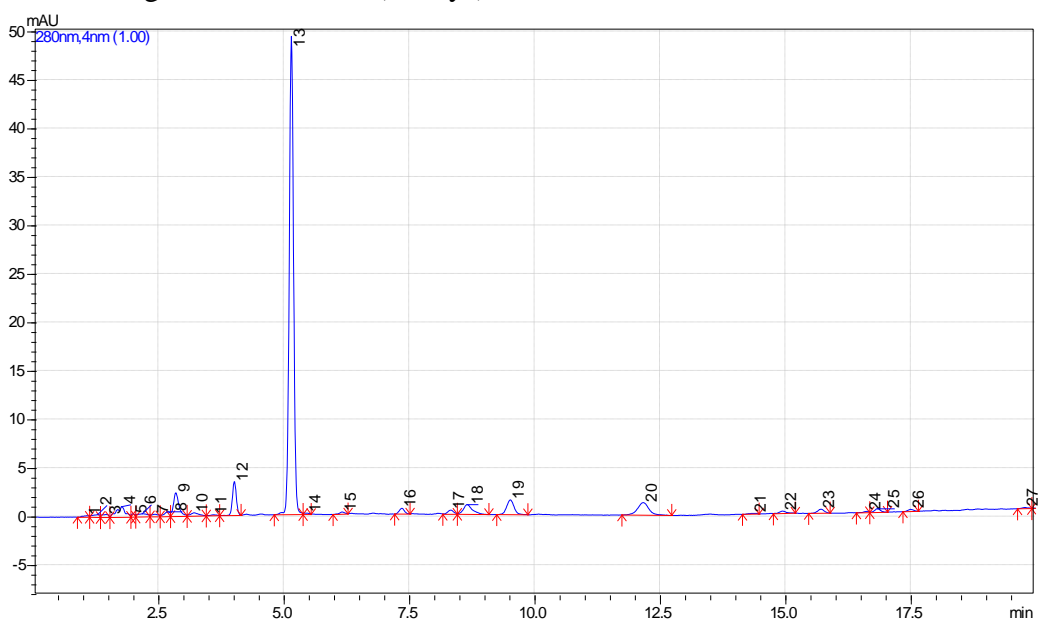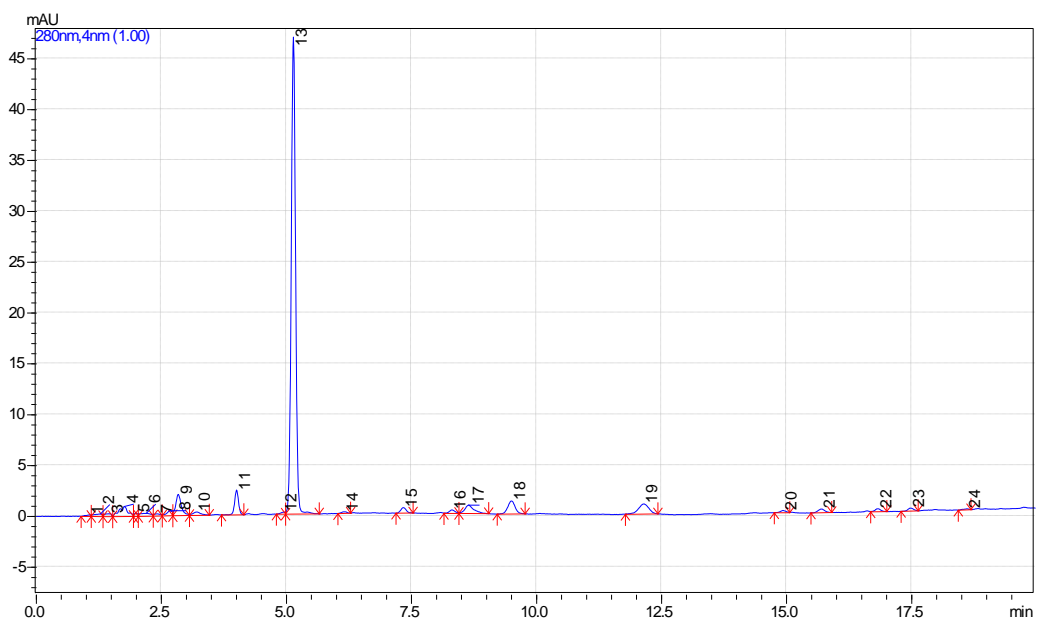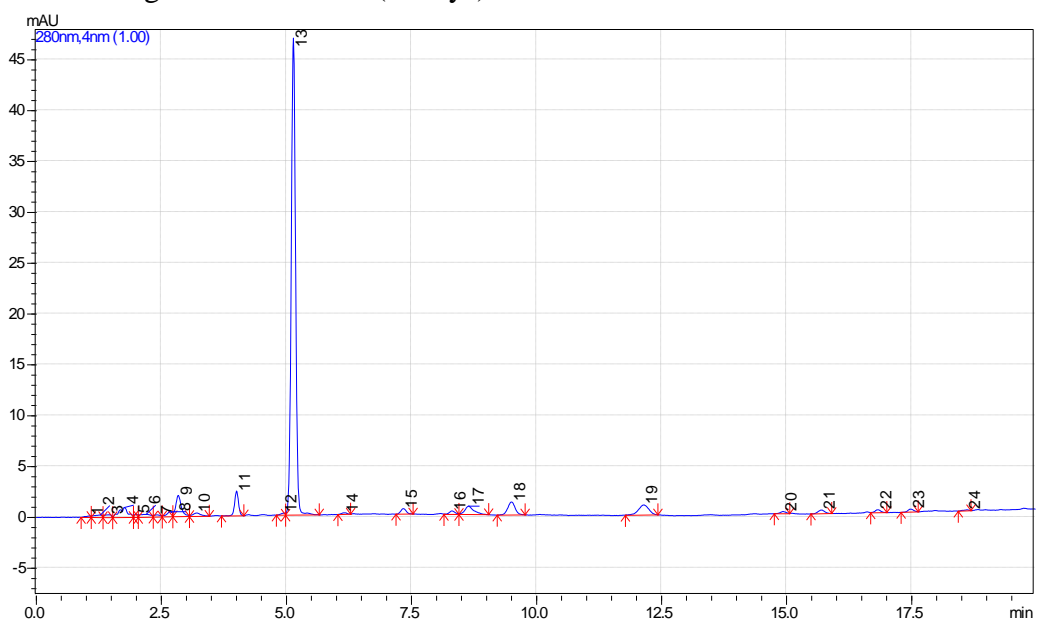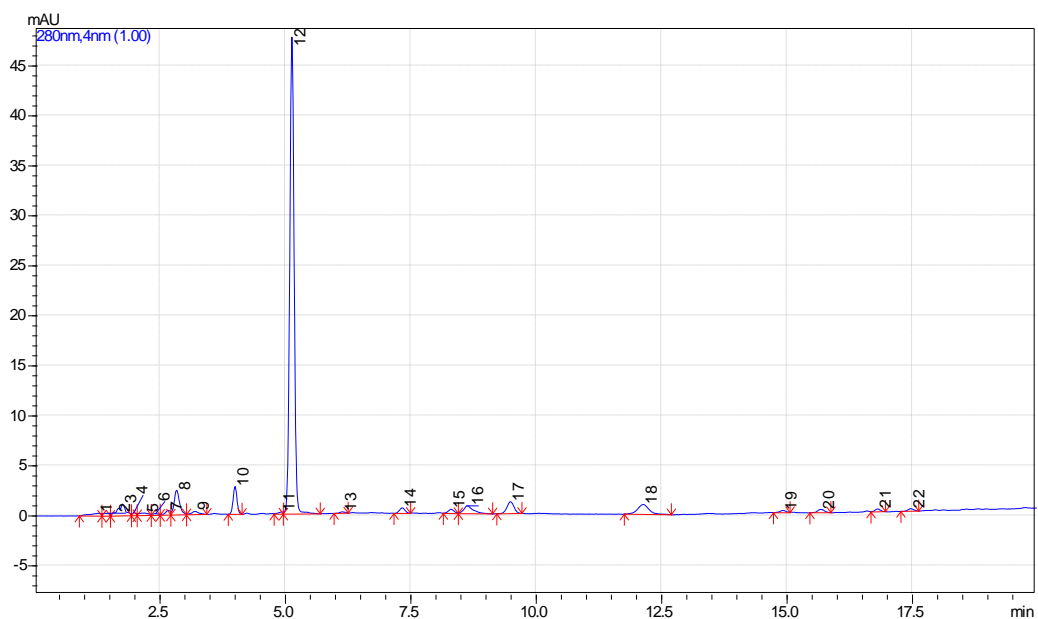

The chromatogram of T65L8 2 (7days):
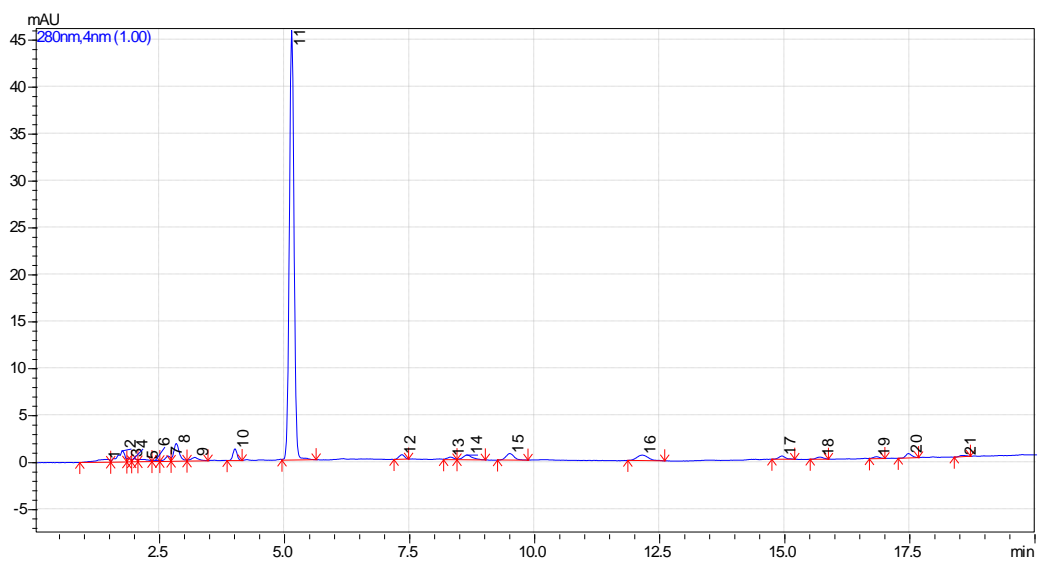
The chromatogram of T65L8 3 (7days):
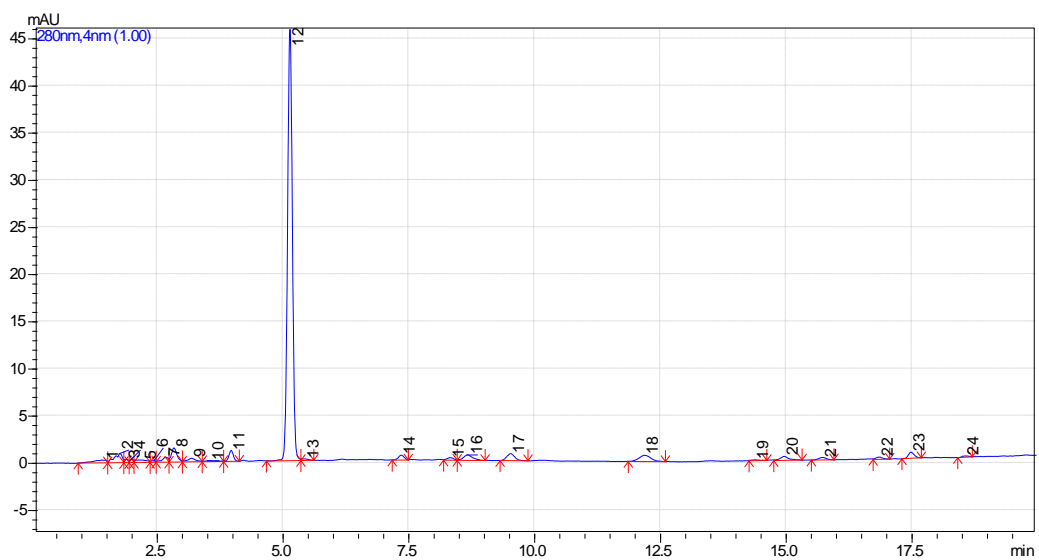
The chromatogram of T65L24 1 (7days):
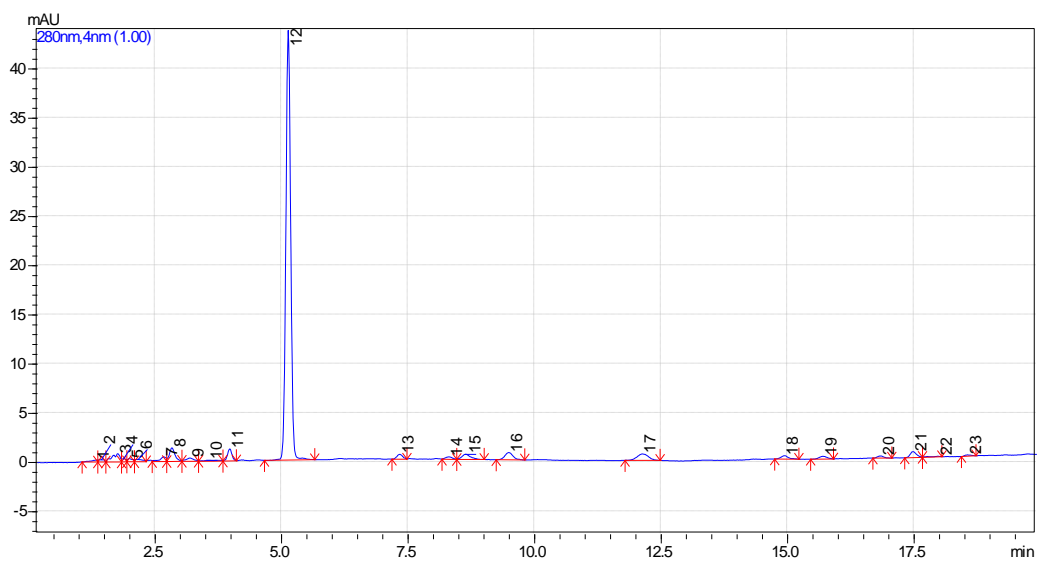
The chromatogram of T65L24 2 (7days):
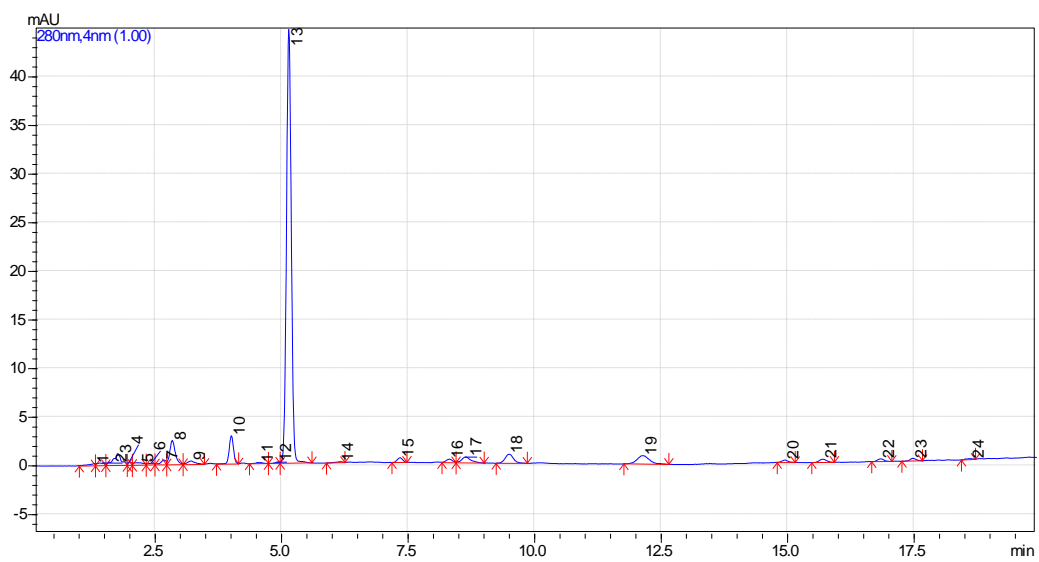
The chromatogram of T65L24 3 (7days):
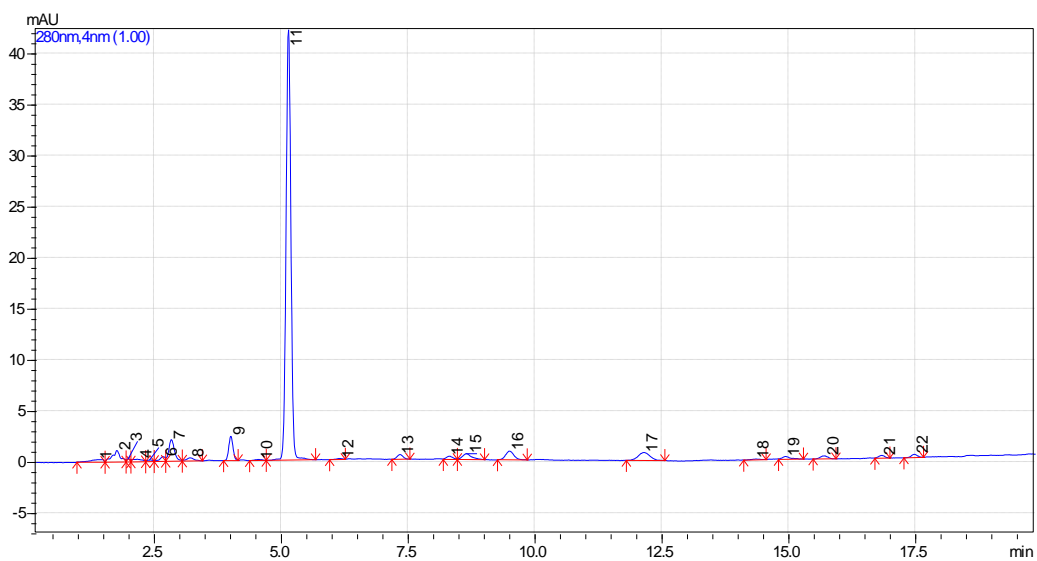
The chromatogram of T65LI 1 (7days):
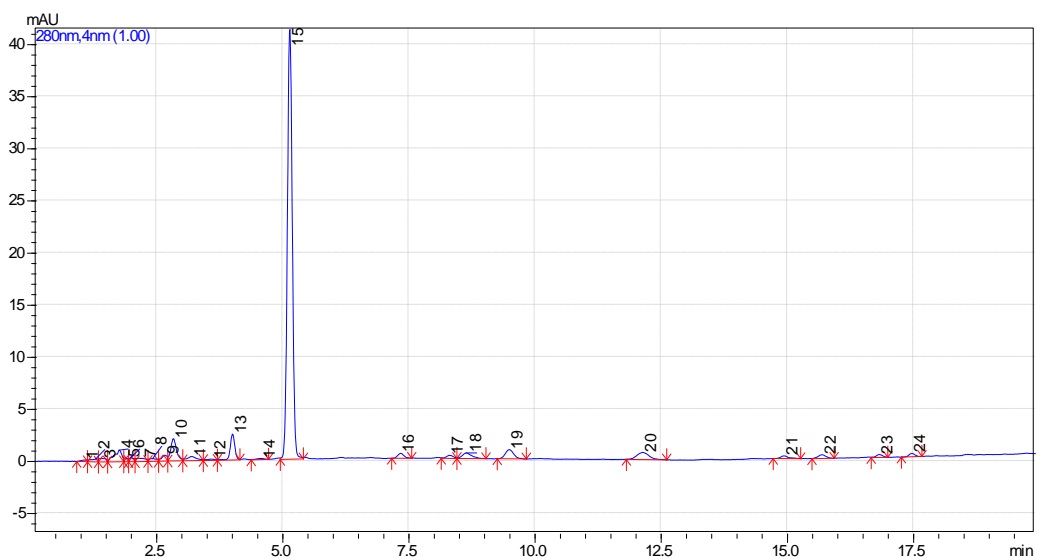
The chromatogram of T65LI 2 (7days):
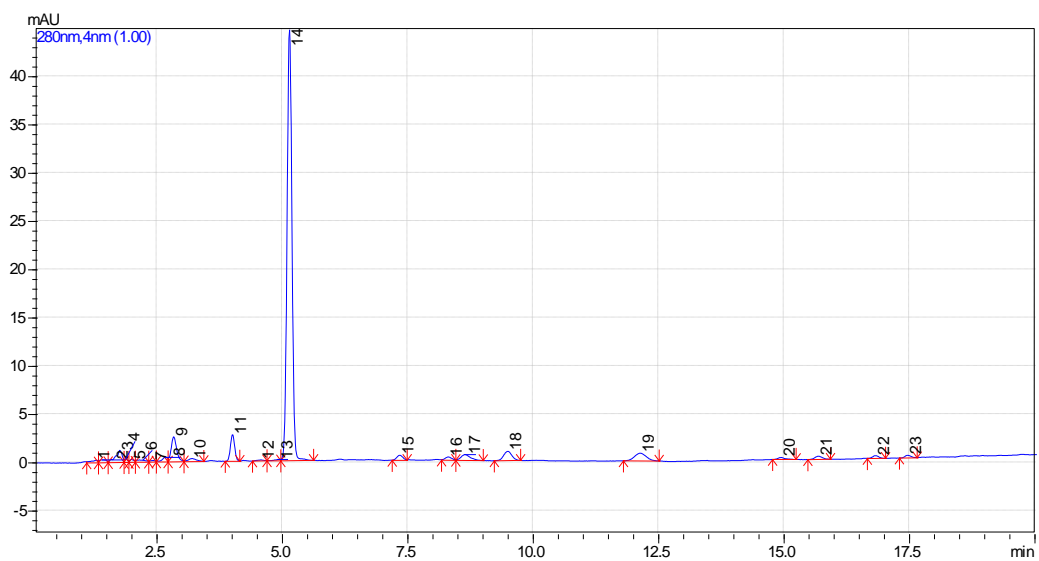
The chromatogram of T65LI 3 (7days):
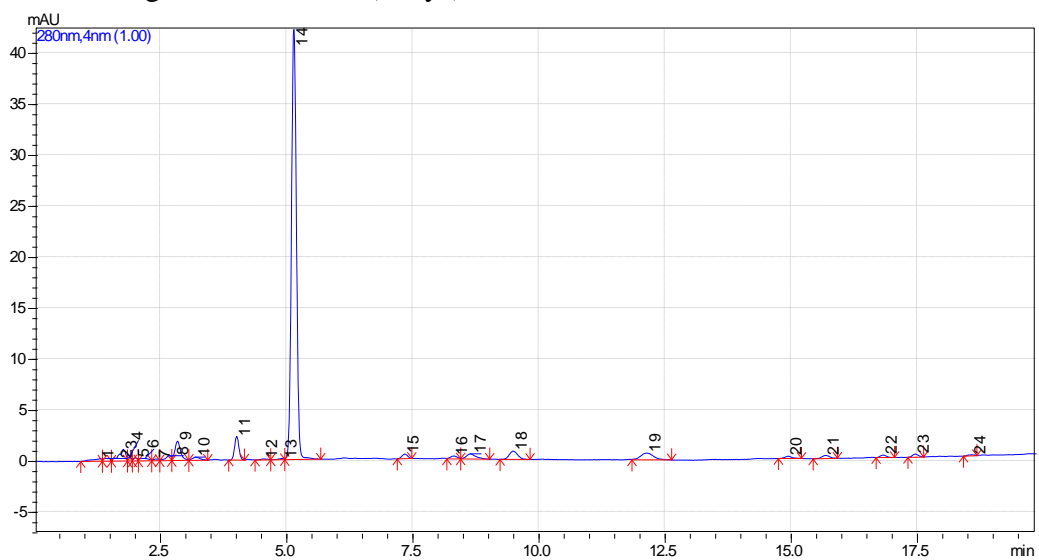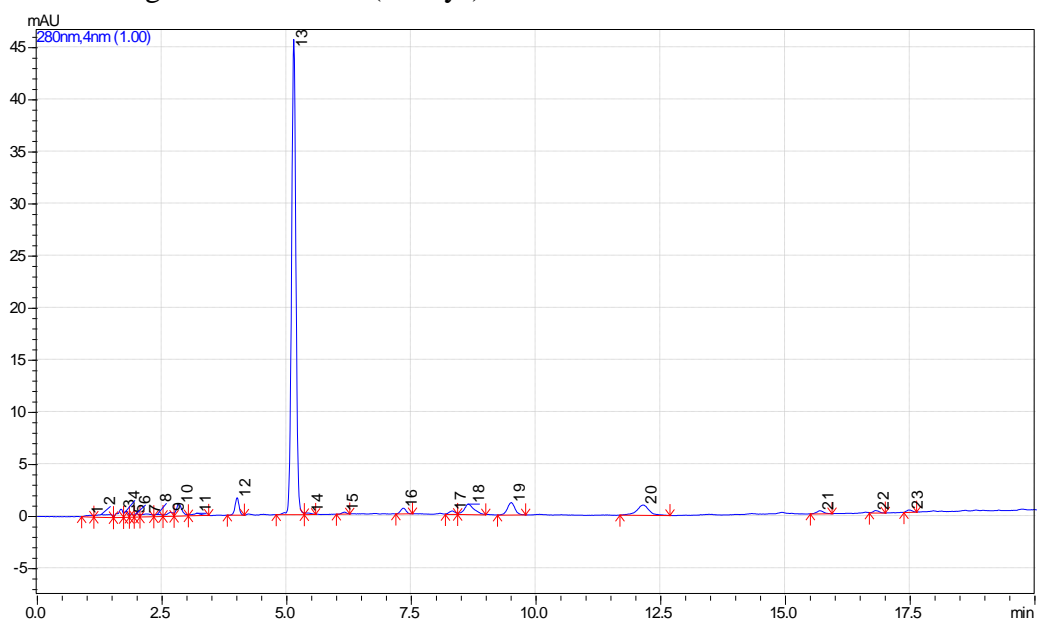

The chromatogram of T65MI 1 (7days):
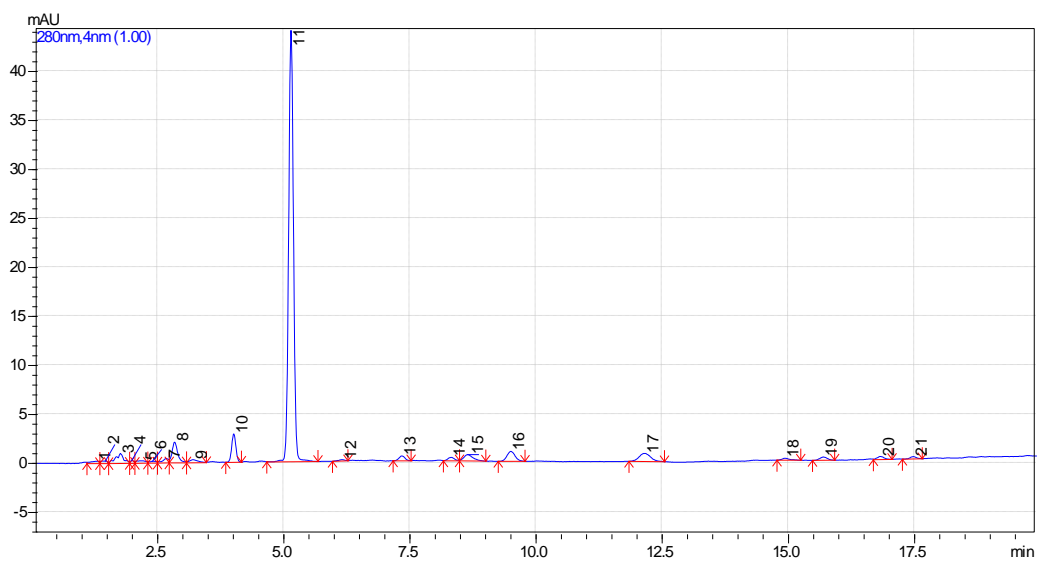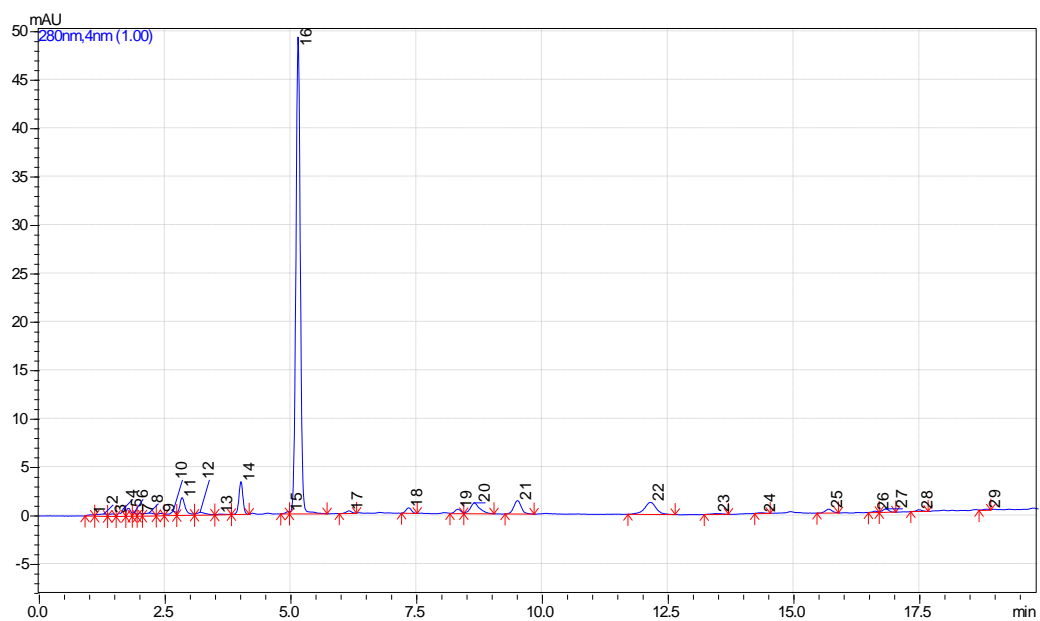
The chromatogram of T65MI 2 (7days):
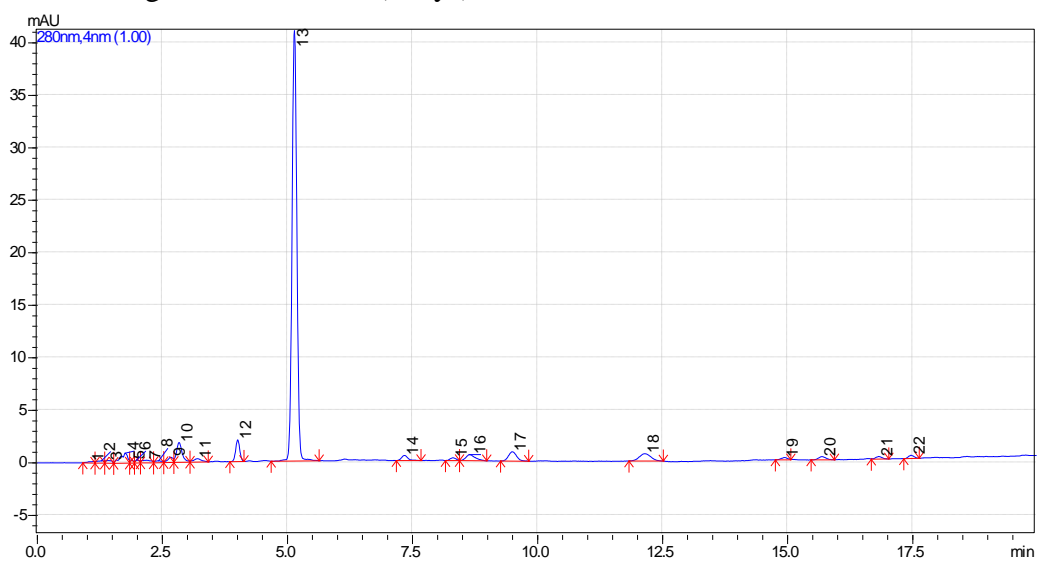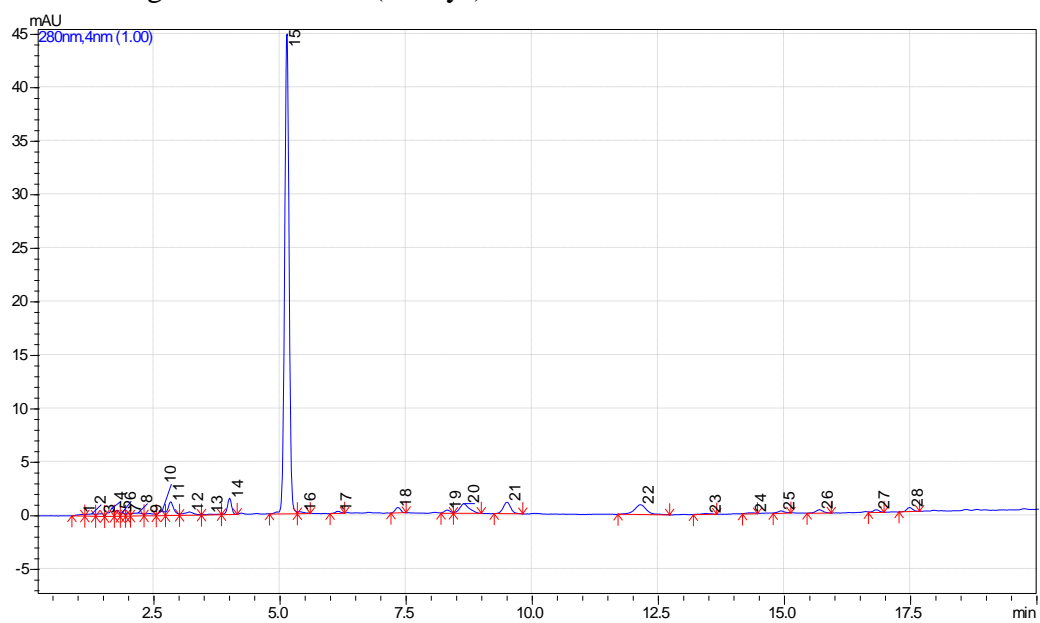

The chromatogram of T65MI 3 (7days):
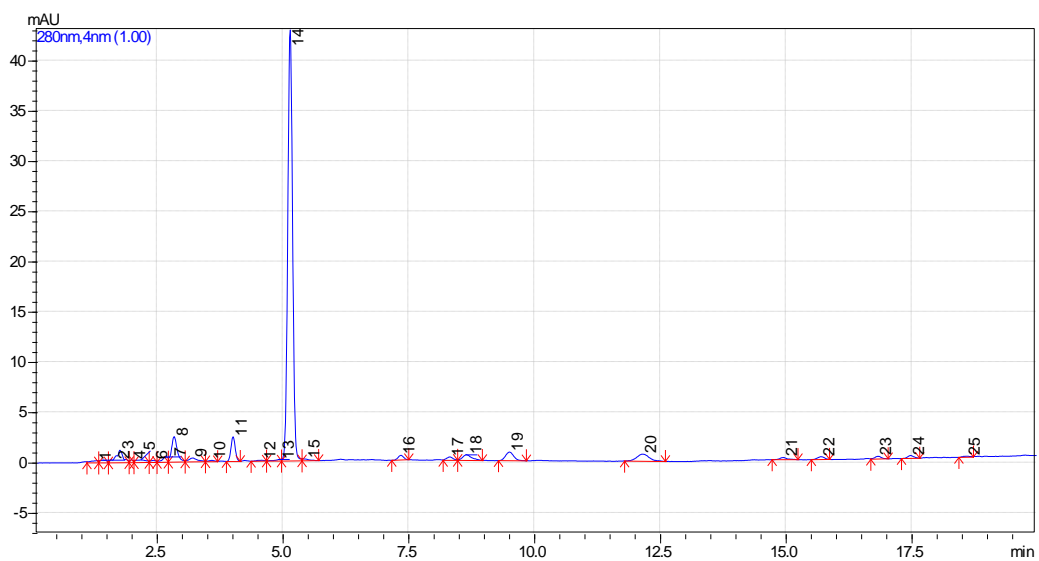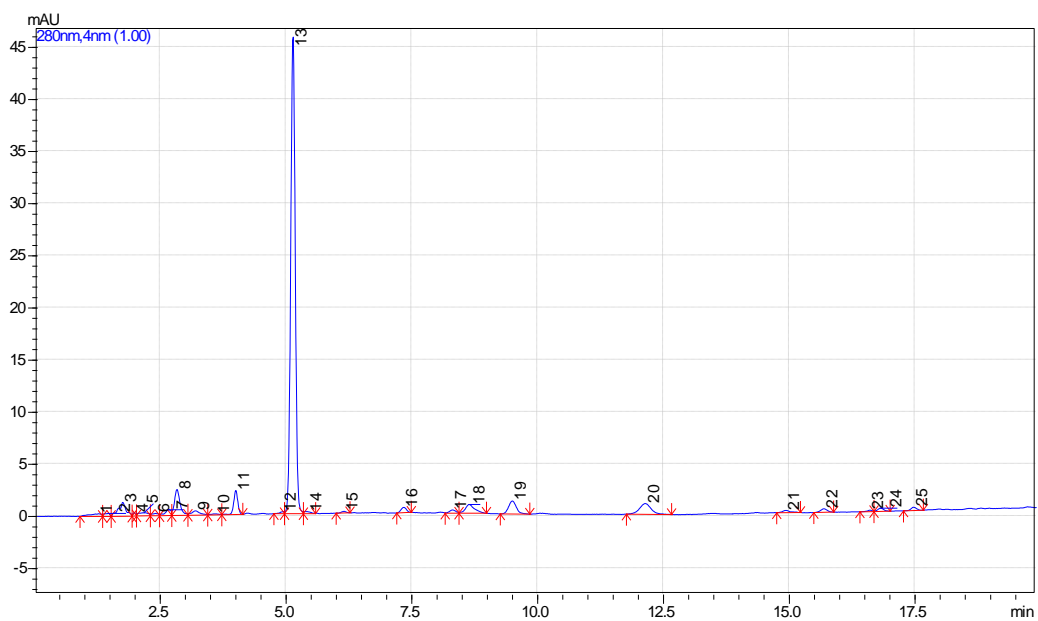
The chromatogram of T65HI 1 (7days):
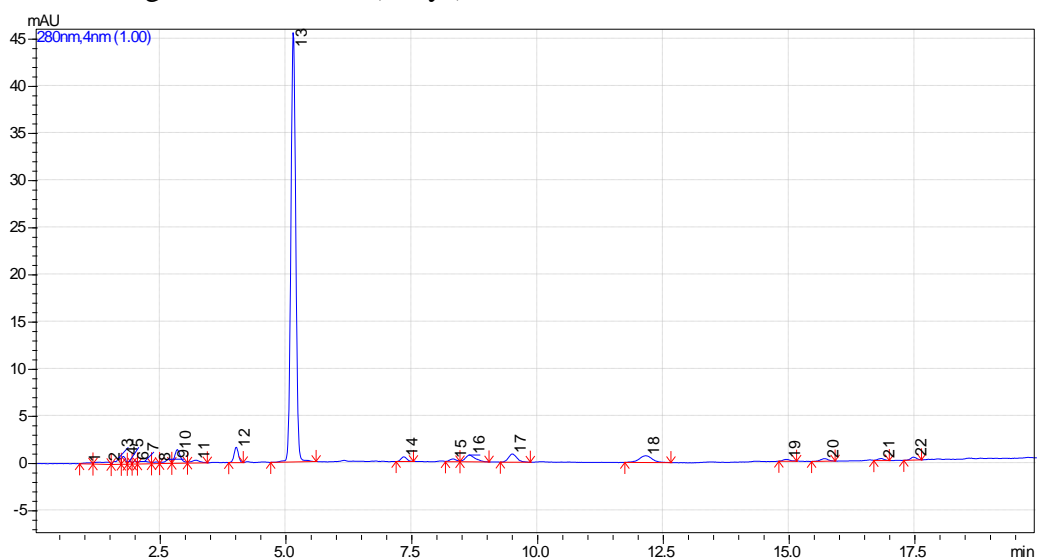
The chromatogram of T65HI 2 (7days):
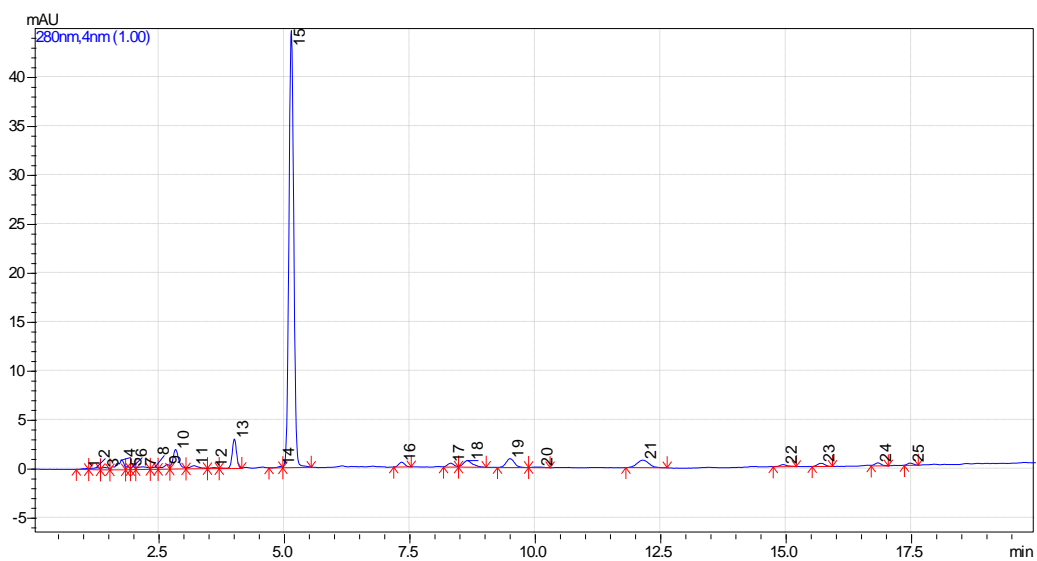
The chromatogram of T65HI 3 (7days):
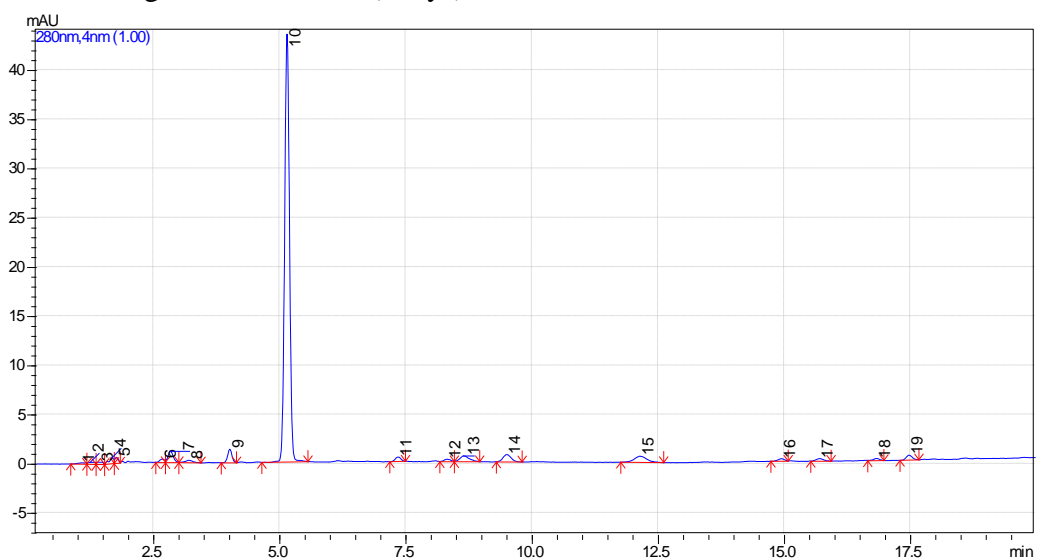
The chromatogram of T65L8MI 1 (7days):
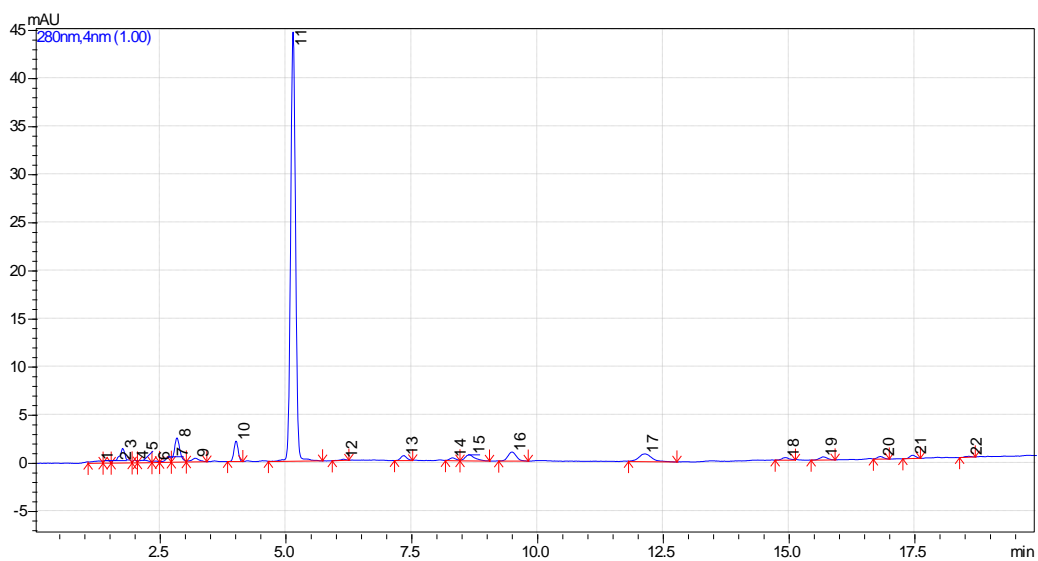
The chromatogram of T65L8MI 2 (7days):
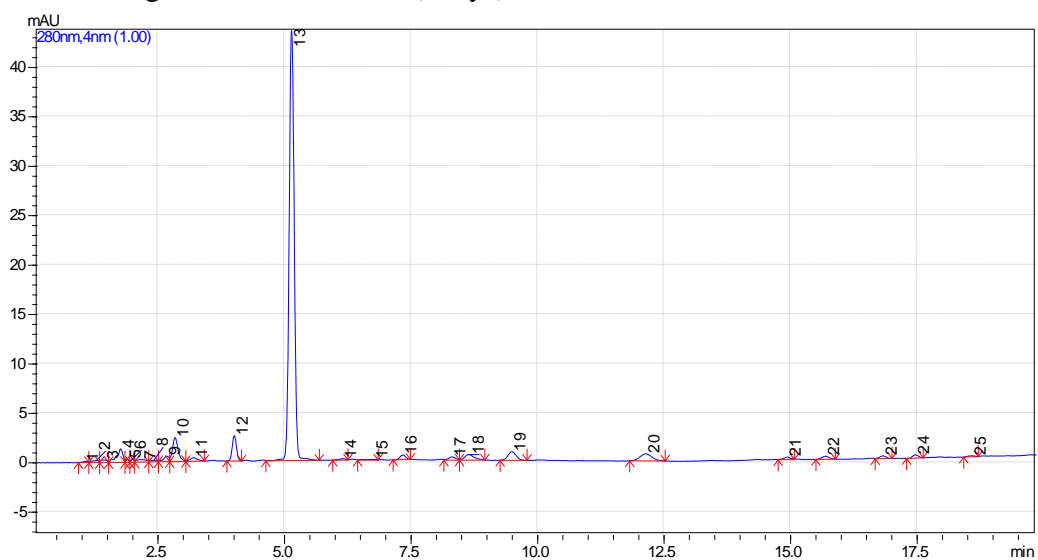
The chromatogram of T65L8MI 3 (7days):
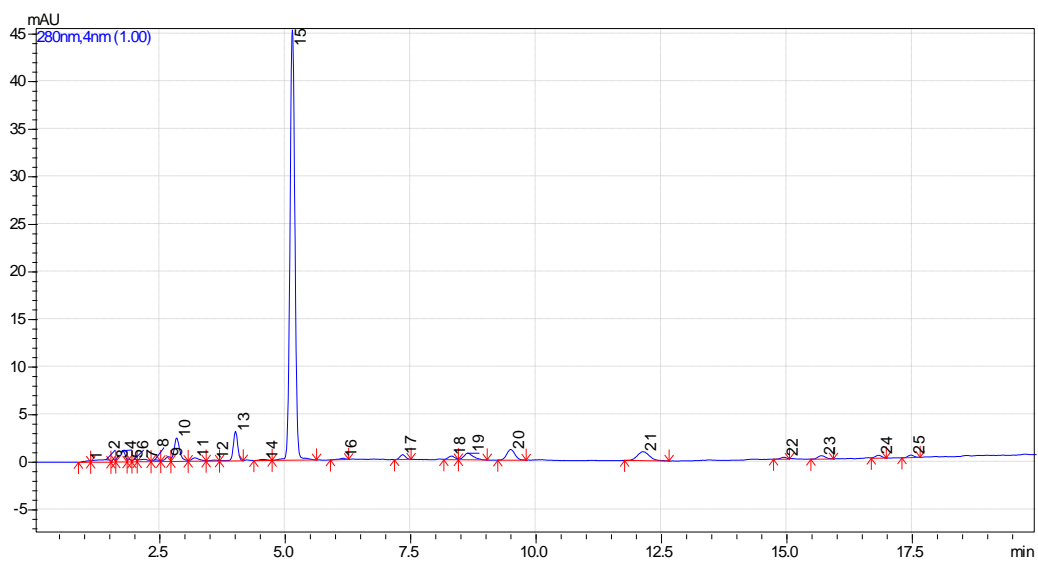
The chromatogram of T65L24MI 1 (7days):
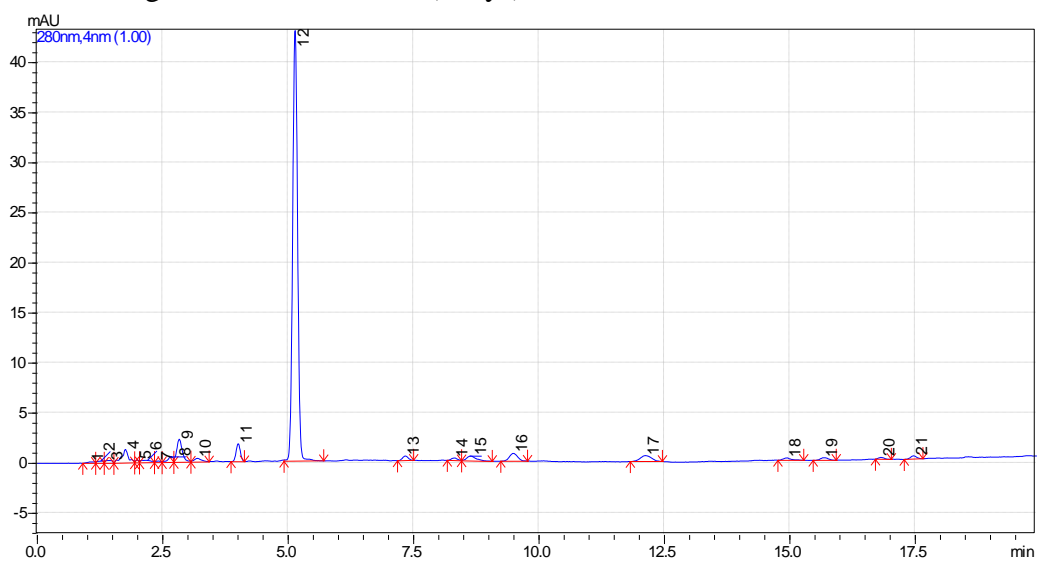
The chromatogram of T65L24MI 2 (7days):
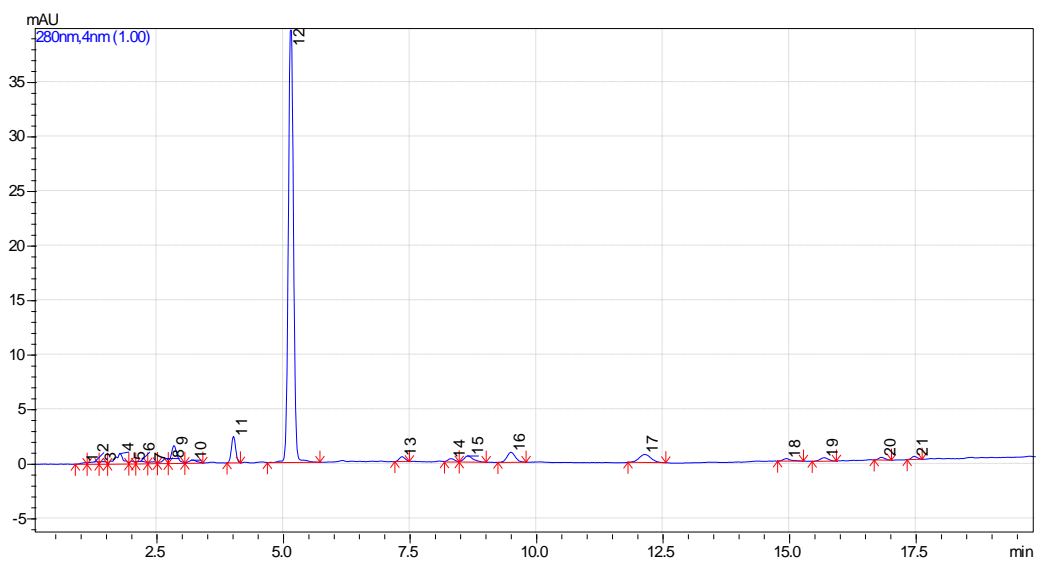
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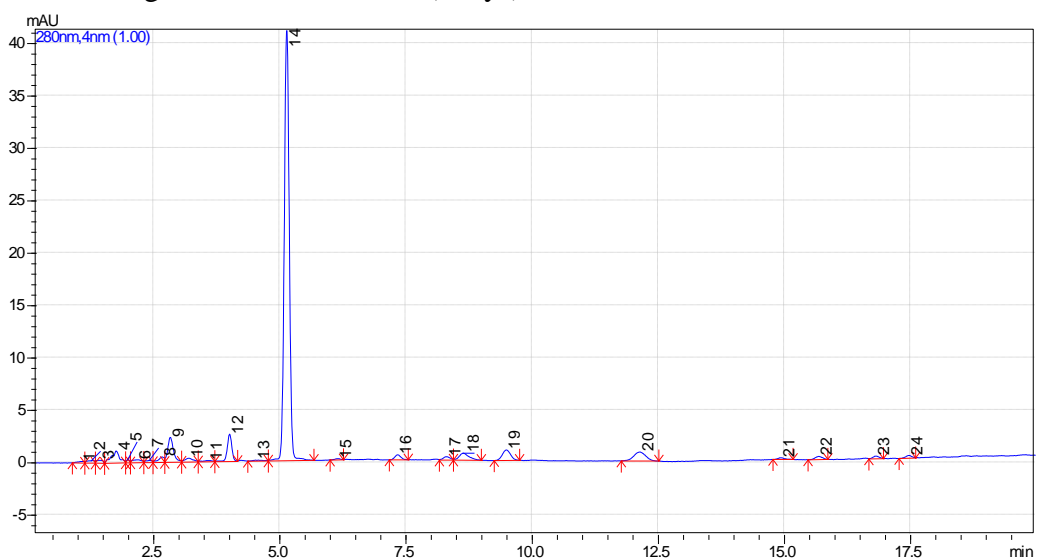
The chromatogram of T23 1 (14days):
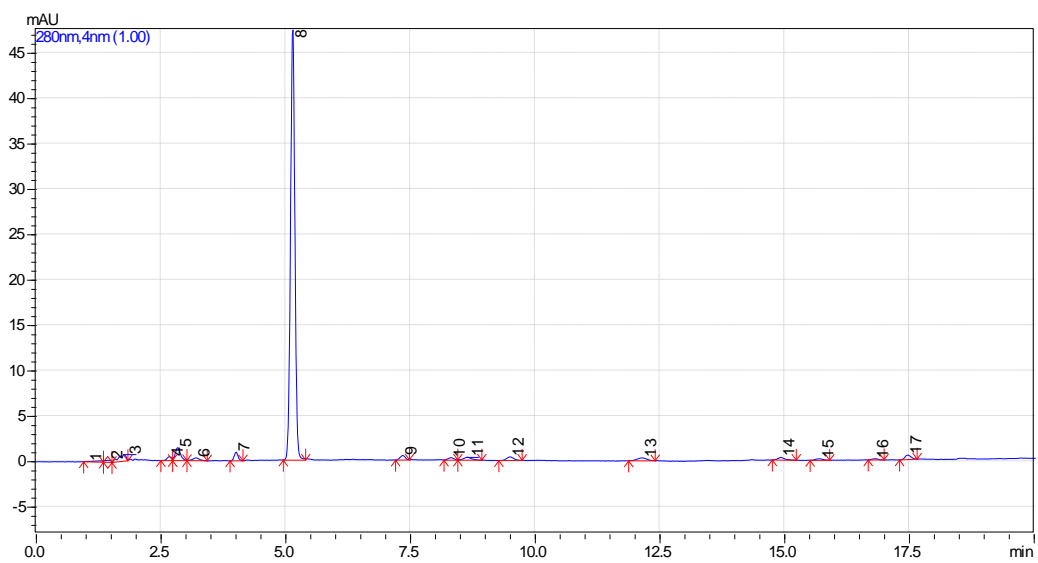
The chromatogram of T23 2 (14days):
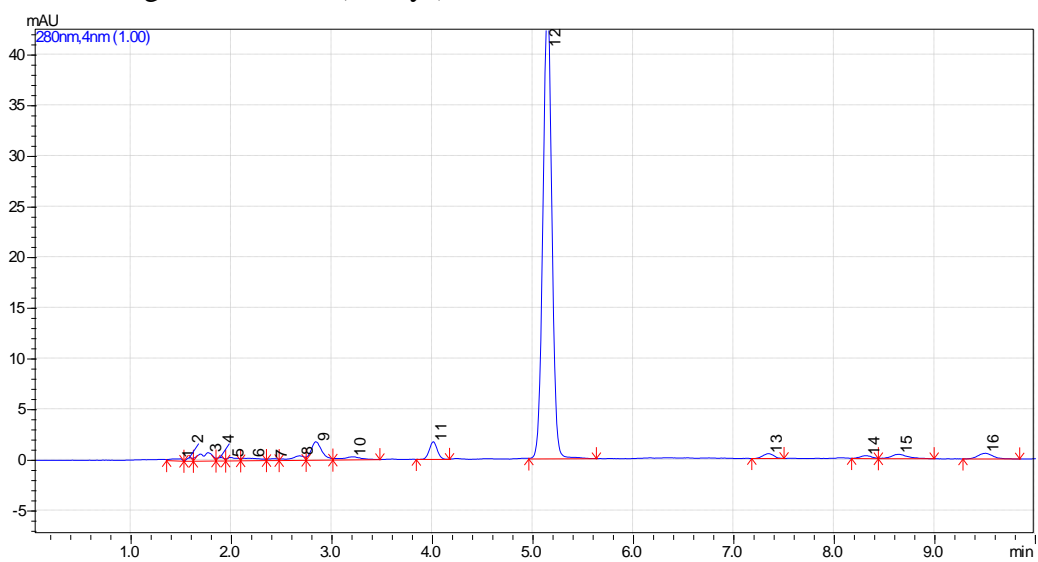
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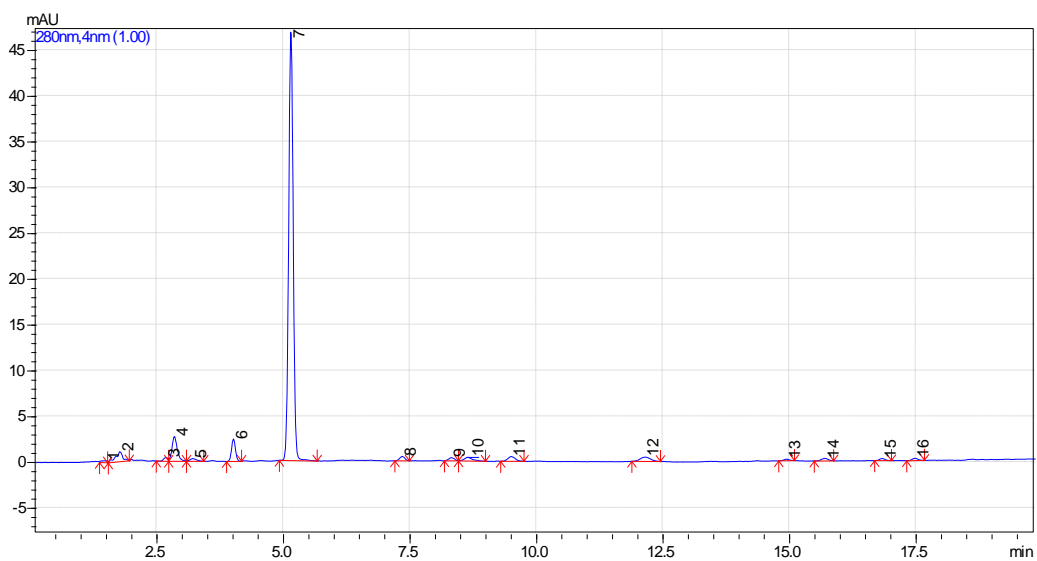
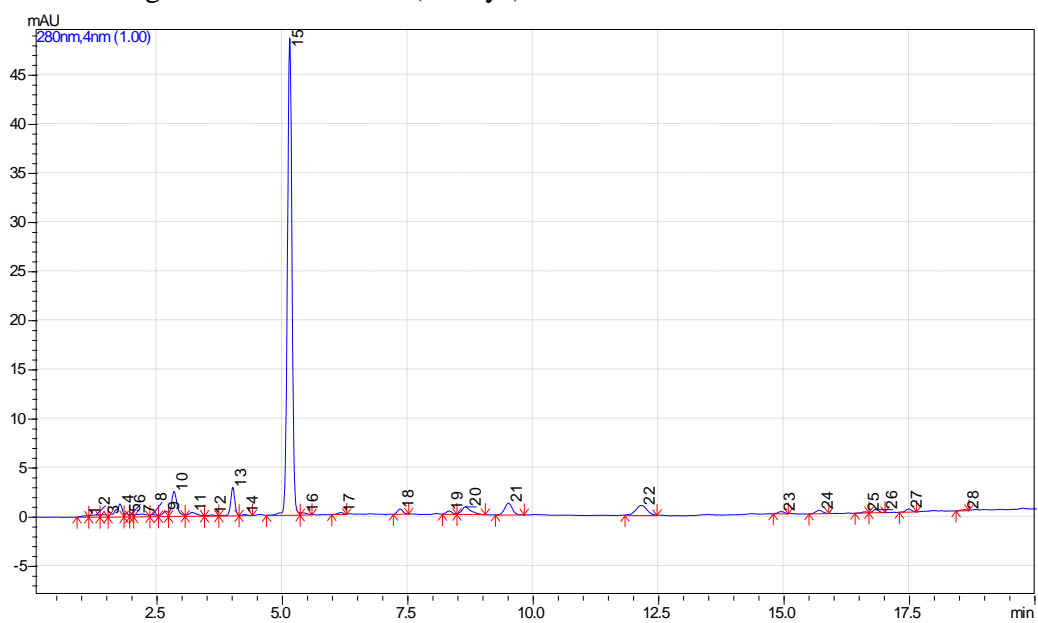The chromatogram of T65 1 (14days):
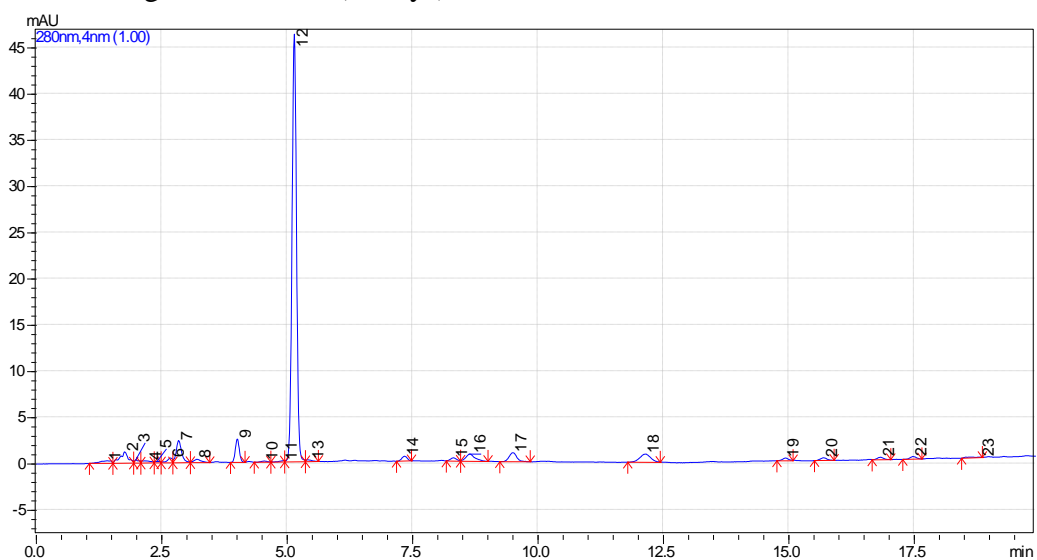
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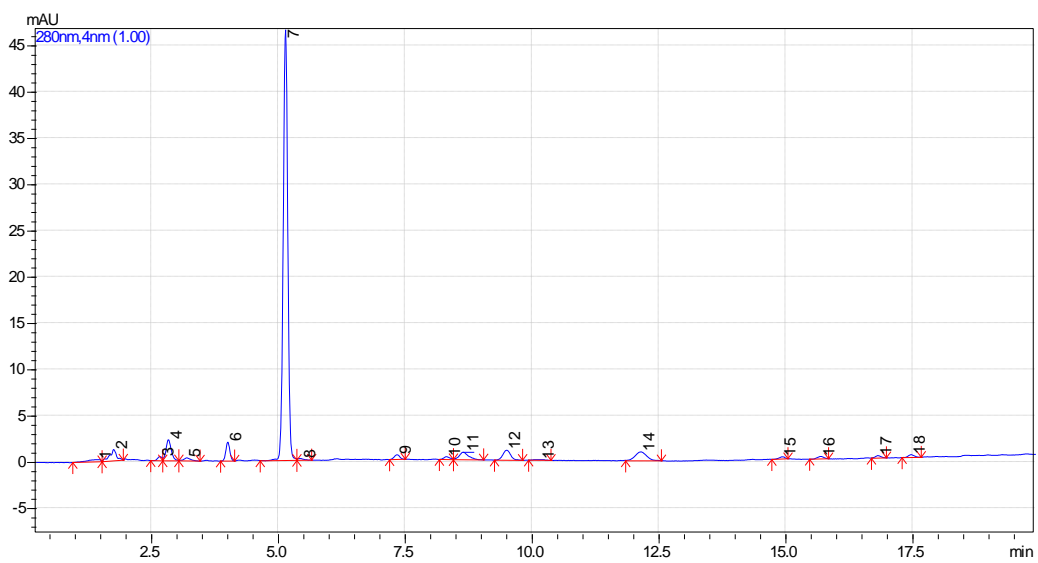
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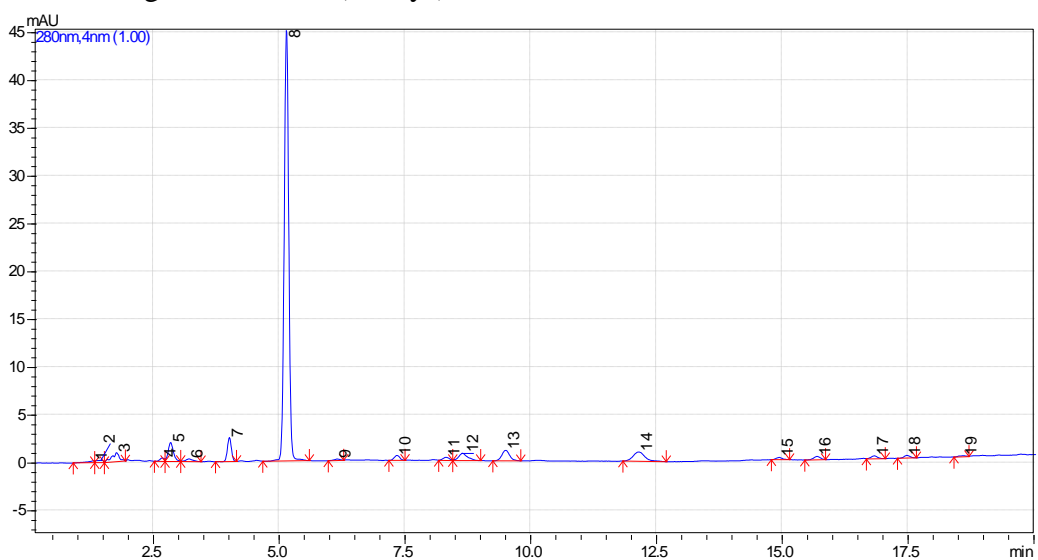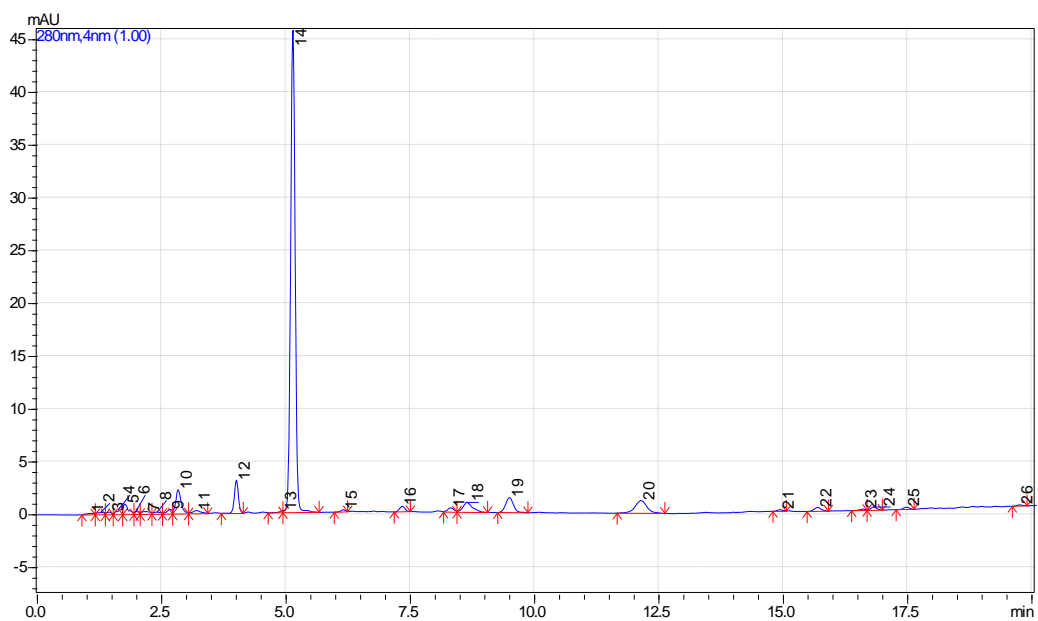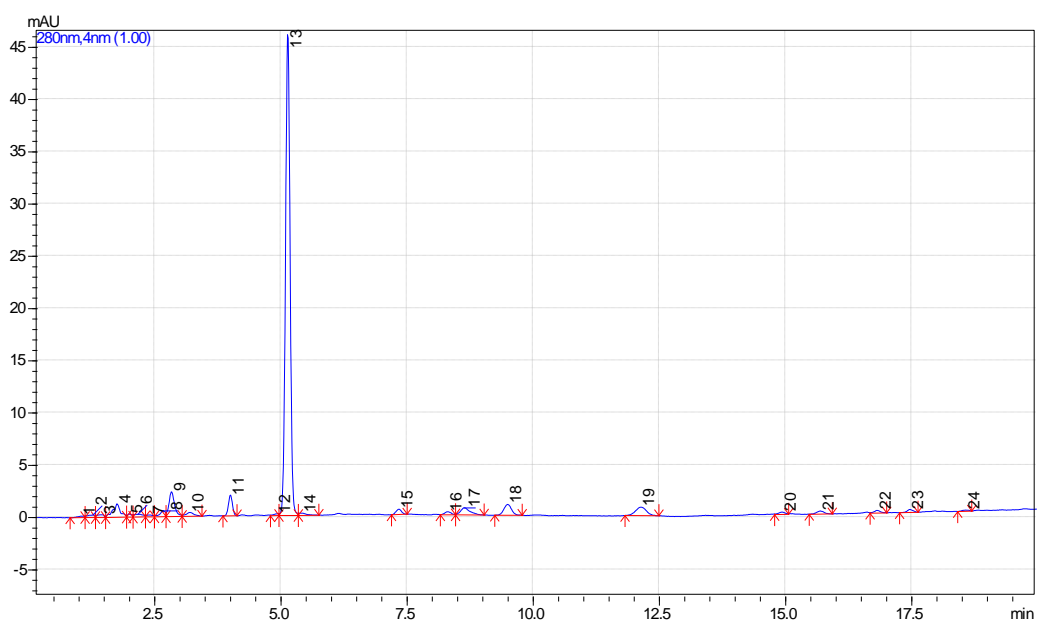

The chromatogram of T65L8 1 (14days):
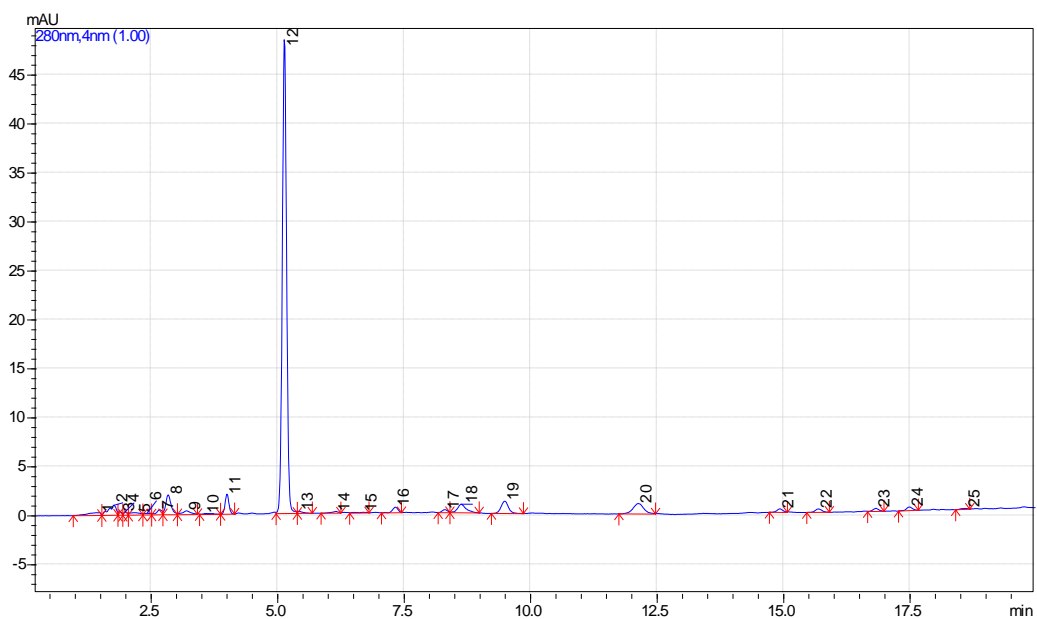
The chromatogram of T65L8 2 (14days):
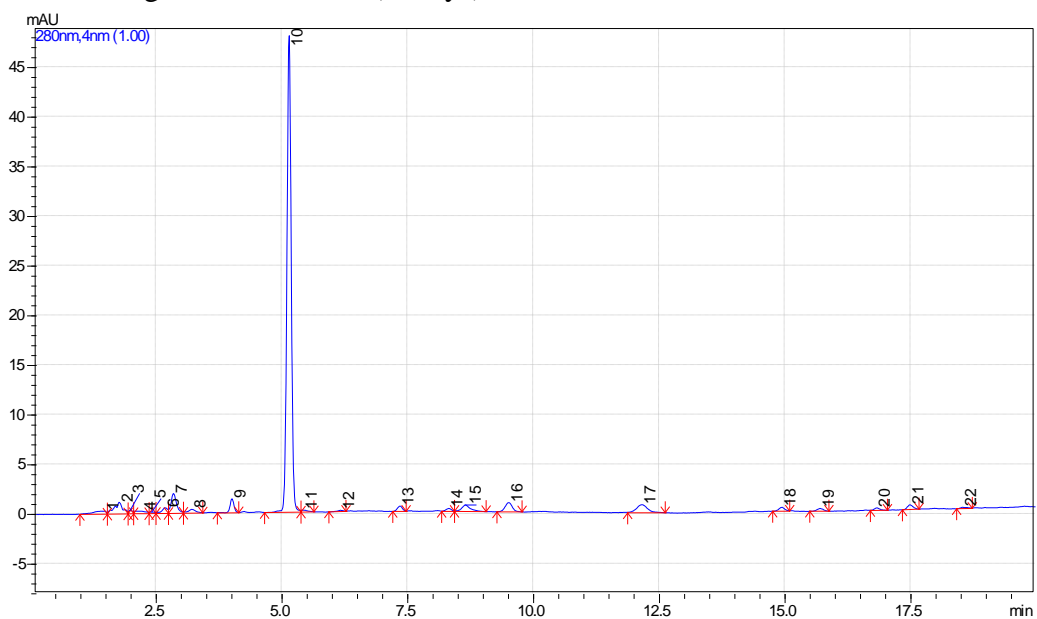
The chromatogram of T65L8 3 (14days):

The chromatogram of T65L24 1 (14days):

The chromatogram of T65L24 2 (14days):

The chromatogram of T65L24 3 (14days):

The chromatogram of T65LI 1 (14days):

The chromatogram of T65LI 2 (14days):

The chromatogram of T65LI 3 (14days):

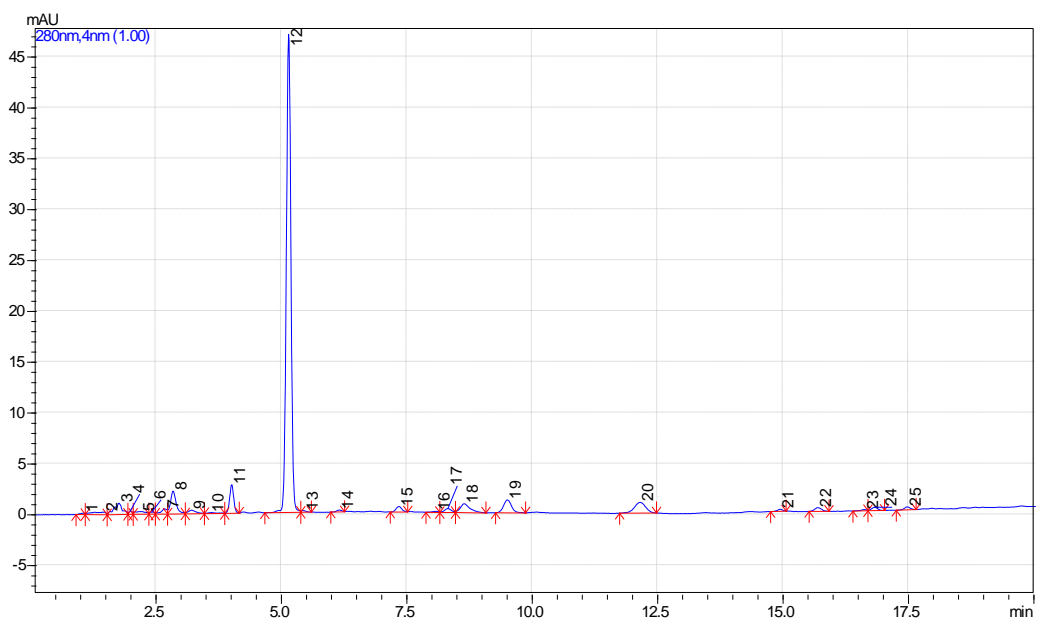The chromatogram of T65MI 1 (14days):

The chromatogram of T65MI 1 (14days):

The chromatogram of T65MI 2 (14days):

The chromatogram of T65MI 3 (14days):

The chromatogram of T65HI 1 (14days):

The chromatogram of T65HI 2 (14days):

The chromatogram of T65HI 3 (14days):

The chromatogram of T65L8MI 1 (14days):

The chromatogram of T65L8MI 2 (14days):

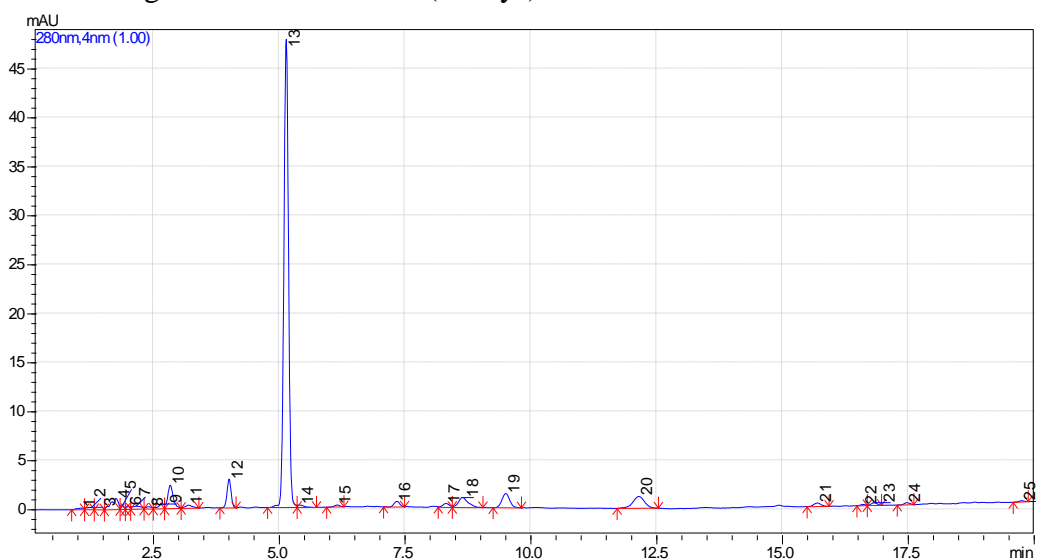The chromatogram of T65L8MI 3 (14days):

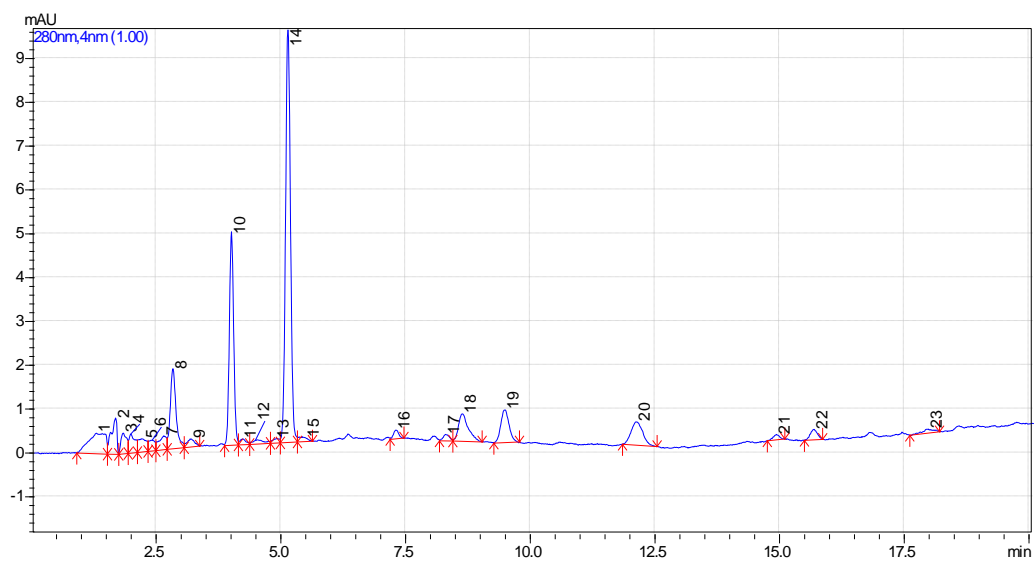The chromatogram of T65L24MI 1 (14days):

The chromatogram of T65L24MI 2 (14days):

The chromatogram of T65L24MI 3 (14days):

The chromatogram of Been Apart:

The chromatogram of Hafnium: