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DEVELOPMENT OF DERIVATIZATION-BASED LC-MS METHOD FOR ANALYSIS OF LIGNIN COMPONENTS

Master's Thesis (30 EAPs)
Applied Measurement Science

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ABBREVIATIONS

3MC	3-Methoxycatechol
4MC	4-methylcatechol
APCI	Atmospheric Pressure Chemical Ionization
APPI/MS	Atmospheric Pressure Photoionization Mass Spectrometry
CID	Collision-Induced Dissociation
DC	Direct Current
DEEMM	Diethyl ethoxymethylenemalonate
DMP	2,6-dimethoxyphenol
ESI	Electrospray Ionization
ESI-HR-TOF/MS	Electrospray Ionization High-Resolution Time-of-Flight Mass Spectrometry
FA	Ferulic Acid
HPLC	High-Performance Liquid Chromatography
HPLC-ESI-MS/MS	High-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry
HR-TOF	High-Resolution Time-of-Flight
IV	Isovanillin
LC/UV	Liquid Chromatography with UV Detection
LC-MS/MS	High-Performance Liquid Chromatography/Tandem Mass Spectrometry
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometric
MS 1	First Quadrupole mass analyzer
MS 2	Third Quadrupole mass analyzer
MS/MS	Tandem Mass Spectrometry
MS^n	Multiple-stage Tandem Mass Spectrometry
m/z	Mass-to-charge Ratio
PVDF	Polyvinylidene Difluoride
QqQ	Triple Quadrupole
QqToF	Quadrupole Time-of-Flight
QqToF-MS	High-Resolution Quadrupole Time-of-Flight Tandem Mass Spectrometry

RPLC	Reverse-Phase Liquid Chromatography
SA	Syringic Acid
SIM	Selected Ion Monitoring
TIC	Total Ion Chromatogram
TMPP-AcPFP	S-pentafluorophenyl tris(2,4,6-trimethoxyphenyl)phosphonium acetate bromide
UHPLC-ESI-MS/MS	Ultra High-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry
VA	Vanillic Acid

1. INTRODUCTION

Lignin can be found in plant cell walls. Lignin is the second most abundant biopolymer in nature after cellulose. Its role in providing structural integrity to plants, contributing to tissue stiffness, acting as an antiseptic, and imparting hydrophobic properties underscores its vital functions in vascular plants. Lignin has three primary monomers, which are called monolignols, namely p-coumaryl (H), coniferyl (G) and sinapyl alcohols (S). The monolignols have a phenolic moiety in its structure. Lignin is a byproduct of wood processing, paper, and biomass industries. The byproduct of lignin is over 50 million tons per year (Evtuguin & Amado, 2003). Nowadays, it is of interest to turn lignin into something more valuable by transforming lignin into valuable aromatic and phenolic chemicals. It holds promise as a potential feedstock for renewable liquid fuels to foster a greener and more sustainable future.

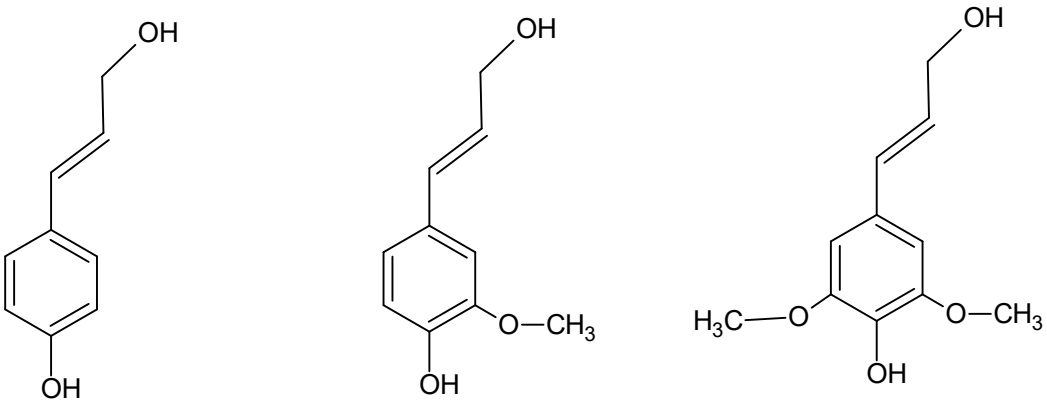
Modern mass spectrometry techniques play a crucial role in lignin analysis, providing detailed insights into the structure and composition of lignocellulosic materials. High-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI) emerges as a widely utilized method due to its milder ionization conditions and ability to ionize involatile compounds, enabling sensitive detection of target compounds. Techniques like Ultra High-Performance Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (UHPLC-ESI-MS/MS) and electrospray ionization high-resolution time-of-flight mass spectrometry (ESI-HR-TOF/MS) have further expanded the capabilities for analyzing lignin and its degradation products (Andrianova et al., 2018; Banoub et al., 2007; Kiyota et al., 2012; Owen et al., 2012).

This project aims to develop a derivatization-based LC-MS method to detect phenolic compounds for lignin structure elucidation. The study seeks to selectively detect phenolic compounds by applying precursor ion scan mode or neutral loss scan mode during LC-MS analysis of derivatized phenolic compounds, shedding light on their correlation with lignin.

2. LITERATURE REVIEW

2.1 Lignin: structure, biosynthesis, and sustainable applications

Lignocellulosic material is a part of plant cell walls, which consists of cellulose, hemicellulose, and lignin (Alherech et al., 2021). Lignin is the second most abundant biopolymer in nature after cellulose. It is vital in providing structure and strength to plants. It serves essential functions in vascular plants, contributing to tissue stiffness, acting as an antiseptic, and providing hydrophobic properties (Evtuguin & Amado, 2003). Lignin biosynthesis is the process that produces lignin by involving the radical coupling of three monomers, which are namely p-coumaryl (H), coniferyl (G) and sinapyl alcohols (S). Figure 1 shows the structure of the monomers. The building blocks of lignin can be called monolignols (H, G, and S). A radical coupling process leads to the formation of lignin which gives unique characteristics to plant cell walls. Lignocellulosic material is crucial for building strong plant cell walls. The complex way plants create lignin is key to their strength and protection (Kiyota et al., 2012).



p-coumaryl alcohol (H) coniferyl alcohol (G) sinapyl alcohol (S)

Figure 1. Structure of the three main lignin monomers: p-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S).

Furthermore, exploring the application of lignin in the industry reveals that its global production as a byproduct in wood processing industries exceeds 50 million tons per year, positioning it as a vast and renewable organic resource (Evtuguin & Amado, 2003). Readily acquired cheaply from paper and biomass industries, lignin is gaining more attention for transforming into valuable aromatic compounds in perfume industries, shifting towards more sustainable practices (Song et al., 2022). Lignin can be a renewable carbon source, such as liquid fuels and chemicals, for a more sustainable future (Andrianova et al., 2018).

2.2 Modern mass spectrometry in lignin analysis: techniques and applications

LC-MS/MS with an ESI ion source is widely used because it works at lower temperatures to protect thermal sensitive compounds and produces less background noise, which makes it easier to detect specific compounds (Santa et al., 2007). A few examples of the characterization of lignin are presented below.

UHPLC-ESI-MS/MS is a technique used to analyze soluble lignin in sugarcane, identifying and quantifying its components (monomers, dimers, trimers, and tetramers) by comparing them with known standards from the library. This provides insights into the structure of sugarcane lignin, with the relative abundance of these components determined by comparing the areas of their corresponding chromatographic peaks (Kiyota et al., 2012).

The ESI-HR-TOF/MS method has been used to analyze mono-, di-, and trine lignin model compounds and kraft alkali lignin. This approach detects multiply charged lignin species and targets higher molecular weight species. Using ion mobility ESI-HR-TOF/MS further validates this capacity, opening up new possibilities for characterizing lignin, lignocellulose, and their degradation products, which is crucial for developing renewable feedstocks (Andrianova et al., 2018).

A technique combining high-performance liquid chromatography with multiple-stage tandem mass spectrometry (HPLC-MS^n) analysis has been developed. This HPLC-MS^n method allows for the direct analysis of complex mixtures, offering molecular-level structural information for individual components. Stable deprotonated analytes are produced by adding sodium hydroxide as a dopant in negative-ion-mode ESI. When combined with HPLC-MS^n, this methodology enables the identification and structural characterization of components in natural lignin degradation mixtures (Owen et al., 2012).

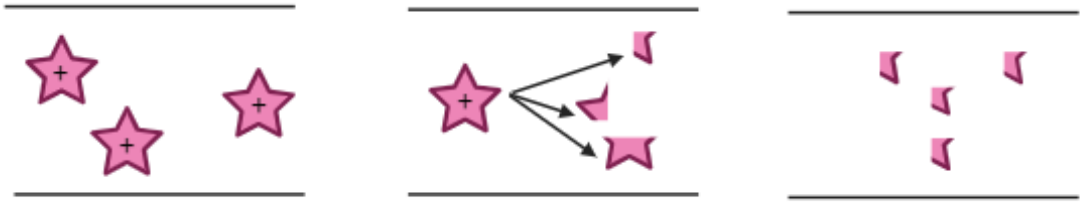
In addition, atmospheric pressure photoionization mass spectrometry (APPI/MS) has been used to explore the molecular structure of lignin derived from wheat straw. This method reveals specific oligomeric ions in both positive and negative ion modes, and their exact molecular structures and arrangements can be determined through high-resolution quadrupole time-of-flight tandem mass spectrometry (QqToF-MS) (Banoub et al., 2007).

In this work, a method employing a novel derivatization technique was used to target phenolic compounds present in lignin, addressing the lack of selectivity observed in previous studies. This approach features simplified sample preparation and utilizes widely available RPLC coupled with a QqQ, chosen for its enhanced sensitivity compared to QToF instruments. The specific derivatization chemistry employed in this method enables the selective detection and enhanced analysis of lignin components.

2.3 Derivatization in LC-MS/MS

In quadrupole MS, an ion mass scan applies direct current (DC) and alternating current (AC) potentials to four hyperbolic rods positioned symmetrically along one axis. The DC potentials on adjacent rods are oppositely oriented, creating time-varying electric fields. The combined DC and AC potentials generate electromagnetic field, allowing ions of a specific mass to pass through the quadrupole for detection. The quadrupole can function as a mass filter which can scan over a *m/z* range by adjusting the potential. In MS/MS, the first quadrupole (MS 1) acts as a mass filter for ion scanning or selection, the second quadrupole acts as a collision cell, and the third quadrupole (MS 2) scans the resulting ions. Various scanning modes, such as precursor ion scan, product ion scan, neutral loss scan, and selected ion monitoring (SIM), enhance sensitivity by minimizing background noise, as shown in Figure 2 (Lin et al., 2014).

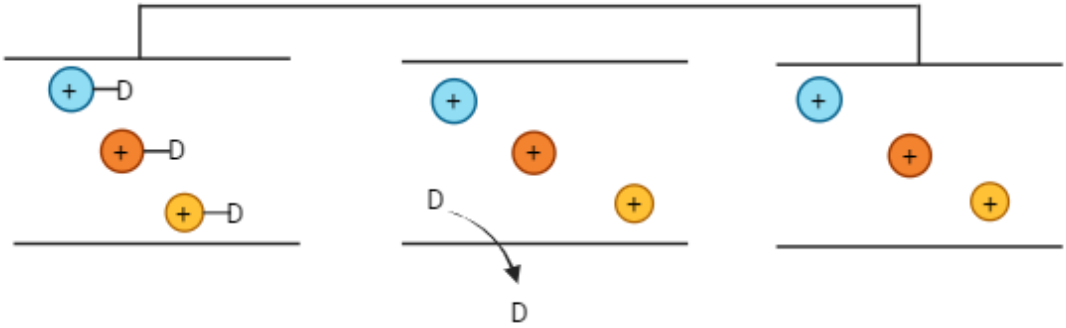
(1) Precursor ion scan mode



MS 1: Scan all *m/z* | Collision cell | MS 2: selected only fragment

(2) Neutral loss scan mode

MS 1 and MS 2 scan simultaneously



MS 1: Scans | Collision cell | MS 2: Selected only fragments that lose neutral molecules

Figure 2. Scan modes of MS/MS. (1) Precursor ion scan mode scans all m/z in MS 1 and selects only fragments in MS 2, (2) Neutral loss scan mode MS 1 and MS 2 are synchronized, MS 1 scans and MS 2 scans at lower m/z than MS 1 by the mass of fragment D.

Not all substances can be studied effectively using RPLC-MS/MS. Some substances have very low ionization efficiencies, making detecting them with the required sensitivity challenging. A substance must be ionizable in the ESI source. First, compounds that exist in their ionic form in a solution are usually efficiently ionized, but charge may also be acquired through gas phase reactions. Second, the compounds should have a hydrophobic properties that can be separated from salts and other potentially interfering substances. Non-polar ions tend to congregate at the surface of droplets, increasing the probability of moving into the gas phase and producing a higher response. Third, it is preferable if the substance degrades rapidly during collision-induced dissociation (CID), producing intense product ions for sensitive MS/MS detection. Chemical modifications are a common way to improve the detection of compounds using HPLC-ESI-MS/MS. This is done by adding components that readily acquire a charge (Santa et al., 2007).

Derivatization changes the structure of the analyte and, therefore, its physical and chemical properties, resulting in higher ionization efficiency. This, in turn, increases the sensitivity of detection, especially when coupled with powerful tools QqQ. Additionally, derivatization modifies the chromatographic retention of the analyte (Gao et al., 2005). In analytical chemistry, combining advanced RPLC-MS with derivatization techniques is crucial for overcoming limitations in analyzing compounds like phenolics. Phenolic compounds have a non-polar nature and lack ionizable groups. Derivatization solves this issue by enhancing the ionization efficiency of the phenolic compounds (Beaudry et al., 2005).

The derivatization reagents that have been reported for use in the analysis of the alcohols and phenols group include dansyl chloride and picolinic acid (Santa et al., 2007), benzoyl chloride (Gao et al., 2003; Malec et al., 2017), S-pentafluorophenyl tris(2,4,6-trimethoxyphenyl)phosphonium acetate bromide (TMPP-AcPFP) (Barry et al., 2003).

Acyl chlorides and sulfonyl chlorides are similar derivative reagents that readily react with nucleophiles like alcohol, thiol, phenol, and amine to form corresponding esters and amides (Xu et al., 2011). The reagents, categorized based on differences in the organic radical group attached to the acyl and sulfonyl, can be further divided into benzoyl chloride and dansyl chloride. Besides impacting retention time, these reagents, with distinct modification groups, offer a unique approach to enhancing MS response. For instance, incorporating an

electron-affinitive group tag (e.g., benzoyl chloride) can boost the positive ion atmospheric pressure chemical ionization (APCI) mode response (Xu et al., 2011).

Dansyl chloride is a valuable tool for improving the detection in mass spectrometry. Its basic secondary amine group helps form ions in the acidic mobile phase, making it easier to detect in positive-ion ESI mode. Additionally, dansyl chloride derivatives produce characteristic fragment ions at *m/z* 171 and 156, which aid in compound identification and increase sensitivity (Xu et al., 2011). Dansyl chloride derivatization, using LC-MS/MS, offers sensitivity and a wide dynamic range, making it a valuable tool for quantifying ethinylestradiol in plasma samples (Anari et al., 2002). A similar approach, utilizing dansyl chloride derivatization, enhances propofol detection limit and signal stability in plasma. This analytical method provides precise measurements. (Beaudry et al., 2005).

The benzoyl chloride derivatization combined with LC-MS/MS potently identifies amine and phenol metabolites in wine. This technique offers higher sensitivity and selectivity than liquid chromatography with UV detection (LC/UV), allowing for the successful quantification of numerous metabolites in wine samples (Malec et al., 2017). Moreover, derivatization methods are highly effective in biological samples of propylene glycol. Using benzoyl chloride derivatization can improve the sensitivity and selectivity of LC-MS/MS, making it suitable for quantifying propylene glycol in plasma and tissue samples. (Gao et al., 2003)

Drawing inspiration from a prior study on amine compounds, the utilization of neutral loss scan mode in LC-MS/MS was explored for the targeted analysis of diethyl ethoxymethylenemalonate (DEEMM)-derivatized amino compounds. This targeted approach decreases the number of compounds detected within a sample since it focuses only on a specific functional group, thereby simplifying analysis and facilitating the identification and characterization of both known and unknown amine derivatives by selectively monitoring the characteristic neutral fragment loss associated with DEEMM derivatization during LC-MS/MS analysis (Maciel et al., 2021).

The review of the literature provides a foundation for the derivatization of phenolic compounds, a technique applied and expanded upon in this work. Derivatization effectively addresses the inherent challenge of poor ionization efficiency in phenolic compounds, enabling their detection and analysis. This work further applies the derivatization-targeted concept, a selective approach that enhances the sensitivity and accuracy of LC-MS analysis by focusing on the modified phenolic compounds and minimizing interference from other sample

components. This is achieved by derivatizing phenolic compounds and employing precursor ion scan or neutral loss scan mode for their specific detection.

2.4 Aim of the study

This project aims to develop a derivatization-based LC-MS method to detect phenolic compounds for lignin structure elucidation. The study seeks to selectively detect phenolic compounds by applying precursor ion scan mode or neutral loss scan mode during LC-MS analysis of derivatized phenolic compounds, shedding light on their correlation with lignin.

3. EXPERIMENTAL

3.1 Chemicals and Materials

All solvents and chemicals were analytical grade. The standards of 2,6-dimethoxyphenol (DMP), 4-methylcatechol (4MC), syringic acid (SA), ferulic acid (FA) (Sigma-Aldrich), 3-methoxycatechol (3MC) (Alfa Aesar), isovanillin (IV) and vanillic acid (VA) (Fluka), dansyl chloride, benzoyl chloride, sodium carbonate, acetone, methanol was purchased from Sigma-Aldrich, sodium bicarbonate (Merck), sodium hydroxide, formic acid, acetonitrile, ethanol (Honeywell), water was prepared using a Millipore Mili-Q Advantage A10 and 0.22-µm regenerated cellulose syringe filter (Sartorius). The properties of the chemical are shown in Annex 1.

3.2 Preparation of eluent, solvent, and standard solution

0.1 M sodium hydroxide

Weighed 0.2 g of sodium hydroxide and transferred it into a 50-mL tube and made it to volume with MiliQ water.

1 M sodium hydroxide

Weighed 2 g of sodium hydroxide and transferred it into a 50-mL tube and made it to volume with MiliQ water.

0.1 M sodium bicarbonate buffer pH 10.6

Weighed 0.0525 g of sodium bicarbonate and 0.4637 g of sodium carbonate anhydrous into a 50-mL tube. Adjusted the volume with MiliQ water and measured pH.

12.5 µL/mL of benzoyl chloride solution

Pipetted 125 µL of benzoyl chloride and transferred it into a 15-mL glass bottle. Added 10 mL of ethanol into the bottle and mixed.

1 mg/mL of dansyl chloride solution

Weighed 10 mg of dansyl chloride and transferred it into a 15-mL glass bottle. Added 10 mL of acetone into the bottle and mixed.

0.1% formic acid solution

Prepared 1100 mL of MiliQ in the mobile phase glass bottle. Added 1.1 mL of formic acid into the same bottle and mixed. Filtered the mobile phase using a 0.22µm pore size polyvinylidene difluoride (PVDF) filter (Millipore).

Standard stock solution (2 mg/mL of each standard)

Weighed the phenolic compounds shown in Table 1. and transferred it into a 15-mL glass bottle. Added 10 mL of methanol into the bottle and mixed.

Table 1. The weight of phenolic compound used for standard stock solution.

Name	Weight (mg)
2,6-dimethoxyphenol	22.69
3-methoxycatechol	20.85
4-methylcatechol	23.13
isovanillin	22.95
syringic acid	20.39
ferulic acid	22.03
vanillic acid	21.08

3.3 Derivatization procedures for phenolic compounds.

Standard solution 1 used for experiment 1

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.1 M sodium hydroxide and 300 µL of 1 mg/mL of dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

Standard solution 2 used for experiment 2

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.1 M sodium hydroxide and 300 µL of 12.5 µL/mL of benzoyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

Standard solution 3 used for experiment 3

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 1 M sodium hydroxide and 300 µL of 1 mg/mL dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

Standard solution 4 used for experiment 4

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 1 M sodium hydroxide and 300 µL of 12.5 µL/mL of benzoyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

Standard solution 5 used for experiment 5

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.1 M sodium bicarbonate buffer pH 10.6 and 300 µL of 12.5 µL/mL of benzoyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

Standard solution 6 used for experiment 6

Pipetted 50 µL of standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.1 M sodium bicarbonate buffer pH 10.6 and 300 µL of 1 mg/mL dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

3.4 The LC-MS/MS condition

The analysis was performed on an Agilent 1290 Infinity II LC system equipped with a pump, autosampler, and heated column compartment. The autosampler was maintained at 22 °C, and the column temperature was set to 40 °C.

Chromatographic separation of the phenolic compounds was achieved using an ACQUITY UPLC BEH C18 1.7 µm 2.1 x 50-mm column with an eluent flow rate of 0.3 mL/min and an injection volume of 1 µL. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). A gradient elution program was employed, as detailed in Table 2.

Table 2. Initial HPLC gradient for the analysis of seven phenolic compounds (A - 0.1 formic acid and B - acetonitrile)

Time (min)	**0**	**2**	**6**	**8**	**10**
A (%)	90	90	2	2	95
B (%)	10	10	98	98	5

Table 3. Final HPLC gradient for the analysis of seven phenolic compounds (A - 0.1 formic acid and B - acetonitrile)

Time (min)	**0**	**2**	**6**	**8**	**10**	**12**
A (%)	60	60	45	45	60	60
B (%)	40	40	55	55	40	40

Table 4. HPLC gradient for the analysis of seven amine compounds (A - 0.1 formic acid and B - acetonitrile)

Time (min)	**0**	**2**	**6**	**8**	**10**
A (%)	90	90	2	2	95
B (%)	10	10	98	98	5

For the initial study, MS was performed in the product ion scan mode for both positive and negative ions, with a scan range of 100 to 1000 *m/z* and a fragmentor voltage of 90 V. From section 3.5, MS was performed in the precursor ion scan mode targeting *m/z* 171 for positive ions, with the scan range of 50 – 500 *m/z*, a fragmentor voltage of 90 V, and a collision energy of 30 eV. Additional MS parameters included: drying gas temperature (290 °C), gas flow (13 L/min), nebulizer pressure (40 psi), sheath gas temperature (380 °C), sheath gas flow (12 L/min), capillary voltage (3000 V positive and negative), and nozzle voltage (0 – 500 eV).

3.5 The solution for optimization of gradient elution system.

Mixed standard stock solution (0.3 mg/mL of DMP, 3MC, 4MC, IV, SA, FA and VA)

Pipetted 500 µL of each standard stock solution, transferred it into a 15 mL glass bottle, and mixed.

Mixed standard solution

Pipetted 50 µL of mixed standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.1 M sodium bicarbonate buffer pH 10.6 and 300 µL of 1 mg/mL of dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial.

3.6 The solution for optimization of the derivatization reaction for phenolic compounds analysis.

Mix standard solution

Prepared the same as topic 3.5.

0.05 M sodium bicarbonate buffer pH 10.6

Weighed 0.0262 g of sodium bicarbonate and 0.2319 g of sodium carbonate anhydrous into a 50-mL tube. Adjusted the volume with MiliQ water and measured pH.

0.125 M sodium bicarbonate buffer pH 10.6

Weighed 0.0656 g of sodium bicarbonate and 0.5796 g of sodium carbonate anhydrous into a 50-mL tube. Adjusted the volume with MiliQ water and measured pH.

0.2 M sodium bicarbonate buffer pH 10.6

Weighed 0.1050 g of sodium bicarbonate and 0.9274 g of sodium carbonate anhydrous into a 50-mL tube. Adjusted the volume with MiliQ water and measured pH.

1 mg/mL dansyl chloride solution

Prepared the same as topic 3.2.

2 mg/mL dansyl chloride solution

Weighed 10 mg of dansyl chloride and transferred it into a 15-mL glass bottle. Added 5 mL of acetone into the bottle and mixed.

3 mg/mL dansyl chloride solution

Weighed 15 mg of dansyl chloride and transferred it into a 15-mL glass bottle. Added 5 mL of acetone into the bottle and mixed.

The optimization of the derivatization reaction for lignin analysis is shown in Table 5.

Table 5. The optimization of the dansyl chloride derivatization reaction.

Solution	B (M)	Ti (min)	T (°C)	D (mg/mL)
1	0.05	10	25	1
2	0.05	10	25	3
3	0.05	10	60	1
4	0.05	10	60	3
5	0.05	60	25	1
6	0.05	60	25	3
7	0.05	60	60	1
8	0.05	60	60	3
9	0.2	10	25	1
10	0.2	10	25	3
11	0.2	10	60	1
12	0.2	10	60	3
13	0.2	60	25	1
14	0.2	60	25	3
15	0.2	60	60	1
16	0.2	60	60	3
17	0.125	35	42.5	2
18	0.125	35	42.5	2
19	0.125	35	42.5	2

B = concentration of sodium bicarbonate buffer pH 10.6, Ti = time, T = temperature, D = concentration of dansyl chloride.

Mixed standard solution for optimization of the derivatization reaction

Pipetted 50 µL of mixed standard stock solution, transferred into a 2-mL Eppendorf tube and combined with the condition in Table 5. The sample was filtered into an autosampler vial.

3.7 The solution for evaluating the impact of dansyl chloride concentration

Mix standard solution for the impact of dansyl chloride on peak area

Pipetted 50 µL of Mixed standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.125 M sodium bicarbonate buffer pH 10.6 and 300 µL of 3 different concentrations of dansyl chloride (2, 2.5, and 3 mg/mL) into the same tube. The sample was filtered into an autosampler vial. The reaction proceeded at the temperature of 25°C for 35 minutes.

2 mg/mL of dansyl chloride and 3 mg/mL of dansyl chloride

Prepared the same as topic 3.6.

2.5 mg/mL of dansyl chloride

Weighed 12.5 mg of dansyl chloride and transferred it into a 15-mL glass bottle. Added 5 mL of acetone into the bottle and mixed.

3.8 The solution for evaluation of dansyl chloride derivatization and LC-MS/MS detection for amine analysis

30 % methanol in 0.1 M hydrochloric acid

Pipetted 1.67 mL of 6 M hydrochloric acid into a 100-mL volumetric flask that contains Mili-Q water and added 30 mL of methanol. Diluted to volume with Mili-Q water and mixed.

Amine standard stock solution (2 mg/mL of each standard)

Weighed the amine compound, as shown in Table 6, and transferred it into a 15-mL glass bottle. Added the solvent, as shown in Table 6, of 30 % methanol in 0.1 M hydrochloric acid into the bottle and mixed.

Table 6. The weight of amine compound and solvent used for amine standard stock solution.

Name	Weight of amine compound (mg)	Weight of solvent (g)
Methylamine	32.67	4.76
Phenylalanine	21.80	7.54
Ethylamine	42.42	9.46
Valine	19.21	6.85
Kynurenine	11.60	4.56
Tryptophan	13.27	4.78
Histamine	23.40	4.70

Amine standard solution

Pipetted 50 µL of Amine standard stock solution and transferred it into a 2-mL Eppendorf tube. Added 250 µL of 0.125 M sodium bicarbonate buffer pH 10.6 and 300 µL of 2 mg/mL of dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial, under the temperature of 25°C and 35 minutes.

3.9 The solution for kraft lignin analysis

50 % (v/v) methanol

Pipetted 5 mL of methanol and transferred it into a 15-mL bottle. 5 mL of MiliQ water was added to the same bottle and mixed well.

Kraft lignin stock solution

Weighed 5 mg of kraft lignin and transferred it into a 2-mL Eppendorf tube. Added 2 mL of 50% methanol. Mixed it.

Kraft lignin solution

Pipetted 50 µL of Kraft lignin stock solution and transferred it into a 2-mL Eppendorf tube. Add 250 µL of 0.125 M sodium bicarbonate buffer pH 10.6 and 300 µL of 2 mg/mL of dansyl chloride solution into the same tube. The sample was filtered into an autosampler vial, under the temperature of 25 °C and 35 minutes.

3.10 Software

All the chemical structures were drawn in ACD/ChemSketch Freeware 2022.2.3. Agilent Quanlitative Analysis Version B.08.00/ Build 8.0.8208.0 was used for chromatogram treatment. All the designs of the experiment (DoE), results analysis, and plots (Lenth, 2009)were created with RStudio software (Posit Software, 2023). All the schemes were created by Biorender.com.

4. RESULTS AND DISCUSSION

4.1 Method development

4.1.1 Evaluation of derivatization reagents for phenolic compounds.

This study investigated the influence of derivatization reagents and bases on detecting phenolic compounds using MS in full scan mode. Six experiments employed a standard mixture of seven phenolic compounds: DMP, 3MC, 4MC, IV, SA, FA, and VA. The structure of the phenolic compounds is shown in Figure 3.

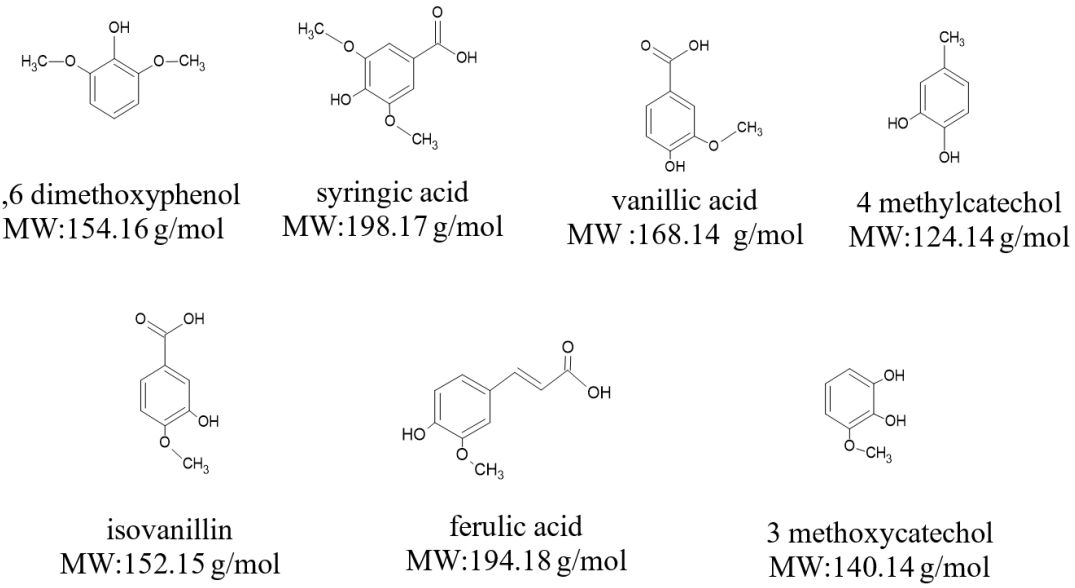


Figure 3. The structures of seven phenolic compounds.

Different combinations of 1 mg/mL dansyl chloride or 12.5 µL/mL benzoyl chloride as derivatizing reagents and 0.1 M sodium hydroxide, 1 M sodium hydroxide, or 0.1 M sodium bicarbonate buffer pH 10.6 were evaluated. The derivatization reaction of phenolic compounds with dansyl chloride and benzoyl chloride is shown in Figures 4 and 5, respectively.

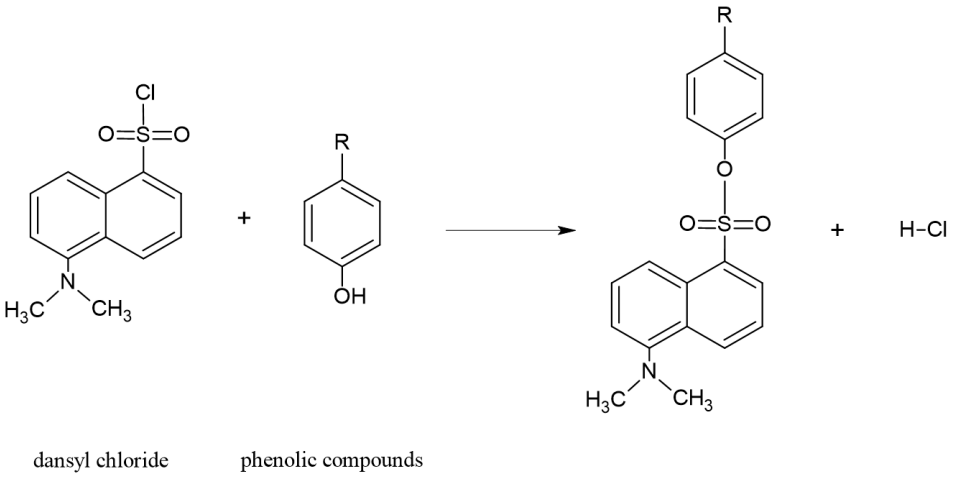
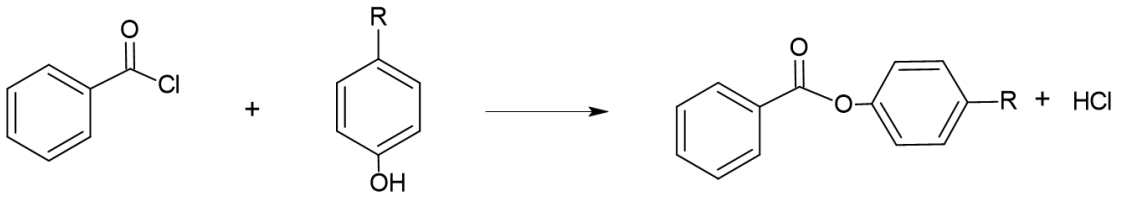


Figure 4. Derivatization reaction of phenolic compounds with dansyl chloride.



benzoyl chloride phenolic compounds

Figure 5. Derivatization reaction of phenolic compounds with benzoyl chloride.

The first experiment utilized 1 mg/mL dansyl chloride with 0.1 M sodium hydroxide, detecting only DMP, 3MC, and 4MC. Subsequently, benzoyl chloride was employed instead of dansyl chloride, maintaining the 0.1 M sodium hydroxide as a base. This modification led to the detection of only DMP and 3MC. Therefore, 1 M sodium hydroxide was implemented paired with either dansyl chloride or benzoyl chloride. The experiment using 1 mg/mL dansyl chloride and 1 M sodium hydroxide detected DMP, 3MC, and 4MC. In contrast, under these conditions, no compounds were detected when 12.5 µL/mL benzoyl chloride was employed.

The last experiment employed 0.1 M sodium bicarbonate buffer pH 10.6 instead of sodium hydroxide alongside both derivatization reagents. While benzoyl chloride exhibited improved reactivity compared to previous experiments, detecting a larger number of phenolic compounds, it did not achieve complete detection. Conversely, dansyl chloride successfully reacted with all seven phenolic compounds when combined with the sodium bicarbonate buffer. Further investigation using the final experiment conditions (dansyl chloride and 0.1 M sodium bicarbonate buffer pH 10.6) explored the optimal MS mode for sensitive detection of the derivatized phenolic compounds by submitting the derivatives to fragmentation.

The fragmentation was studied using product ion scan mode at different collision energies, from 0 to 70 eV. The selected collision energy was 30 eV because the fragments *m/z* 156 and 171, specific to the dansyl-phenol derivatives, are present at the highest intensity (Figure 6). The mass spectra of the dansyl-phenol derivatives are shown in Annex 2.

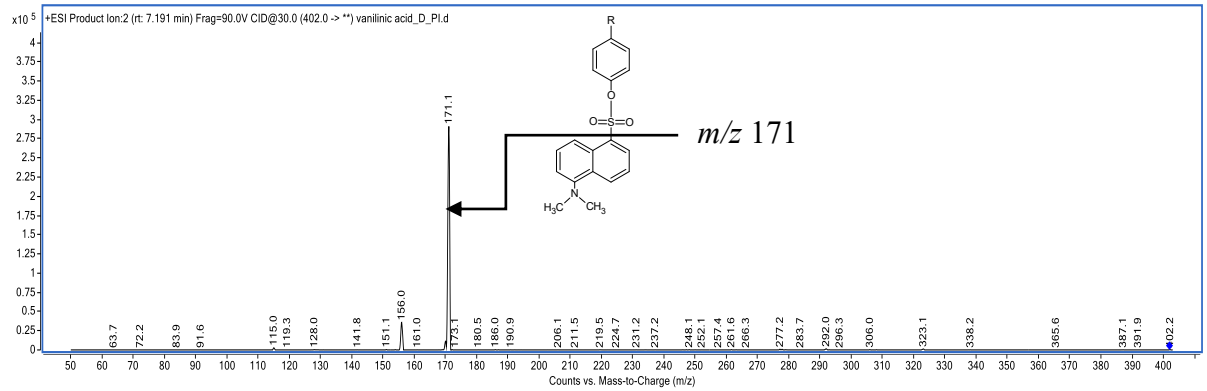
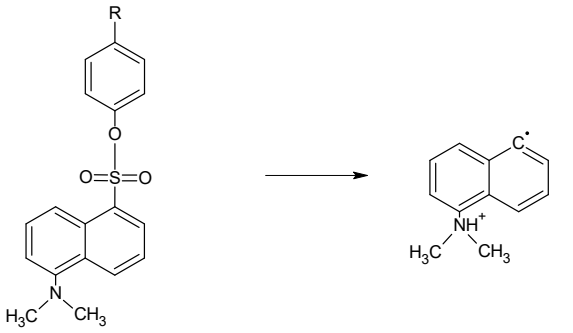
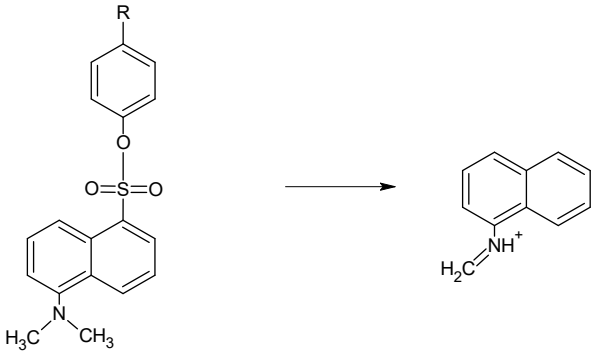


Figure 6. Fragmentation mass spectrum of dansyl-vanillic acid derivative (m/z 402) at collision energy 30 eV.

The formation of the dansyl-phenol derivative involves a nucleophilic substitution reaction, where the hydroxyl group (-OH) of the phenol replaces the chloride atom in dansyl chloride. During mass spectrometric analysis, fragmentation of these derivatives, as shown in Figure 7, can lead to the formation of the *m/z* 171 ion. This ion results from the cleavage of the C-S bond connecting the sulfonyl group to the dimethyl aminonaphthalene moiety, and the resulting positive charge is stabilized by resonance within the aromatic system (Santa et al., 2007; Xu et al., 2011).



$[M + H]^+$ *m/z* 171



$[M + H]^+$ *m/z* 156

Figure 7. Fragmentation pattern of dansyl phenol derivatives in product ion scan mode.

This study revealed that different derivatization reagents and bases significantly impact the detection of derivatives of phenolic compounds. Dansyl chloride demonstrated superior performance compared to benzoyl chloride across various base conditions. Employing a 0.1 M sodium bicarbonate buffer pH 10.6 with 1 mg/mL dansyl chloride yielded the most comprehensive detection of the targeted phenolic compounds. Moreover, the precursor ion scan mode targeting *m/z* 171 can be used to further enhance the selectively for detecting dansyl chloride derivatives and, therefore, phenolic compounds. These findings suggest that this combination represents a promising approach for derivatizing and analyzing phenolic compounds using precursor ion scan mode in LC-MS/MS.

4.1.2 Optimization of the derivatization reaction for phenolic compounds analysis.

Design of experiments (DoE) was employed to explore the impact of four parameters on the sum of peak areas of 7 phenolic compounds and enhance the reaction yield, in terms of a full-factorial design with central points. The four parameters were the concentration of dansyl chloride (testing levels: 0.5, 1.25, and 2 mg/mL), the concentration of sodium bicarbonate buffer solution pH 10.6 (0.05, 0.125, and 0.2 M), the reaction time (10, 35, and 60 minutes), and the temperature (25, 42.5, and 60 °C). The prepared samples were subjected to the designated reaction conditions according to the design (Table 5, section 3.6). The sum of all peak areas in precursor ion scan mode targeted *m/z* 171 was measured for each sample, serving as the response variable (Y).

The data was evaluated using the RSM package in R software with each factor and interaction term in the model. The statistical analysis results of the optimization of the derivatization reaction for phenolic compounds are shown in Table 7. The analysis showed that only two factors, the concentration of dansyl chloride and temperature, had a statistically significant impact ($p < 0.05$) on the sum of peak areas. This suggests that these two factors influence the derivatization efficiency the most. A high R-squared value (0.9715) indicates that the model explains a large proportion of the variation in the data. A highly significant *p*-value for the model (1.722×10^{-6}) suggests strong evidence that the observed effects are not due to chance. A high lack of fit value (0.9601) implies that the model fits the data well. The complete data is shown in Annex 3.

Table 7. Statistical analysis results of the model for the optimization of the derivatization reaction.

Name	*p*-value
xT	0.0014**
xTi	0.4214
xB	0.0122*
xD	9.67×10^{-9}***
xT:xTi	0.0445*
xT:xB	0.1381
xT:xD	0.2514
xTi:xB	0.5408
xTi:xD	0.2416
xB:xD	0.1578
Model	1.72×10^{-6}
Lack of fit	0.9601

The *** *p*-value is <0.001, ** *p*-value is 0.01, * *p*-value is 0.05

A response surface plot was generated using R, depicting the relationship between the two significant factors (dansyl chloride concentration and temperature) and the sum of all peak areas (Y). The plot suggests that the higher the dansyl chloride and the lower the temperature, the higher the sum of the peak areas, as shown in Figure 8. A random residual plot, as shown in Figure 9, further supports the model's validity by indicating that the errors are independent and normally distributed.

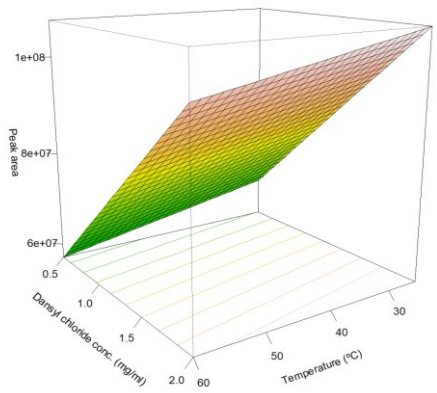


Figure 8. The response surface plot of dansyl chloride concentration and temperature as a function of the sum of the peak areas.

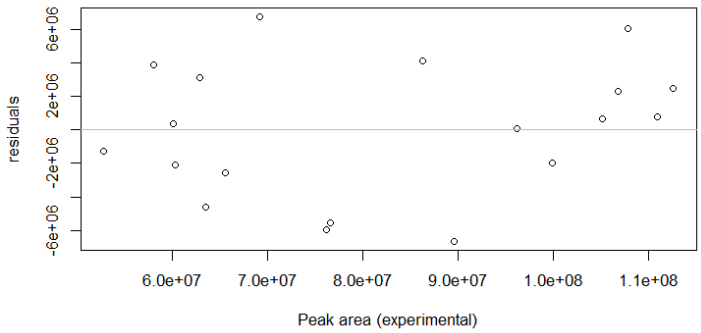


Figure 9. The plot of residuals against the sum of peak area.

4.1.3 Optimization of the dansyl chloride concentration

From section 4.1.2, the higher the concentration of dansyl chloride, the higher the peak area, and, therefore, the derivatization efficiency for phenolic compound analysis, so the effect of dansyl chloride concentration was further investigated. Three concentrations were evaluated: 2 mg/mL, 2.5 mg/mL, and 3 mg/mL. The data is shown in Annex 4. The results are comparable, given the error bars as shown in Figure 10.

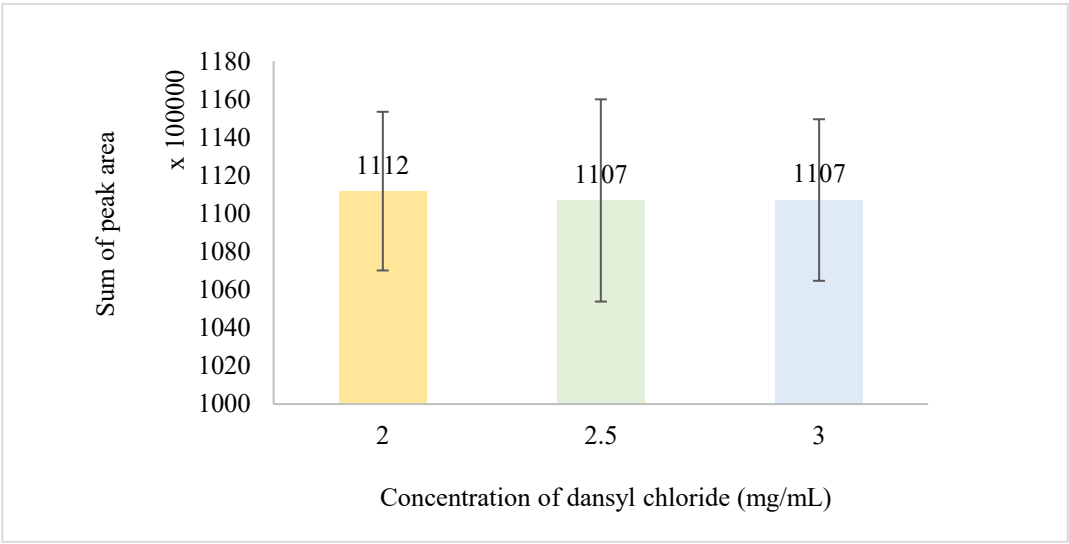


Figure 10. The relationship between the concentration of dansyl chloride (mg/mL) and the sum of peak areas (n = 3; error bars represent standard deviation).

While increasing the concentration to 2.5 mg/mL and 3 mg/mL resulted in lower peak areas, these increases were not statistically significant. The *p*-value is 0.99 ($p > 0.05$, one-way ANOVA). Therefore, considering both analytical sensitivity and economic efficiency, 2 mg/mL was determined to be the optimal concentration of dansyl chloride for this application. The final parameters employed for the detection of phenolic compounds employing dansyl

chloride derivatization were 2 mg/mL of dansyl chloride, 0.125 M of sodium bicarbonate buffer pH 10.6, a temperature of 25 °C, and a reaction time of 35 minutes.

4.1.4 Optimization of the HPLC gradient for phenolic compounds analysis.

This section focuses on optimizing the gradient elution system for HPLC separation of the targeted phenolic compounds. The primary aim was to achieve a separation among the seven compounds, thereby minimizing peak overlap and enabling the detection of potentially more phenolic compounds.

An initial analysis of the standard was conducted using a specific gradient elution program as shown in Table 2, section 3.4. However, upon examination of the obtained precursor ion scan chromatogram (Figure 11), it became evident that all seven phenolic compound peaks retention times were close, resulting in inadequate resolution. Adjustments to the mobile phase composition, which was 0.1% formic acid and acetonitrile, were necessary to address this challenge and achieve optimal separation of all individual compounds.

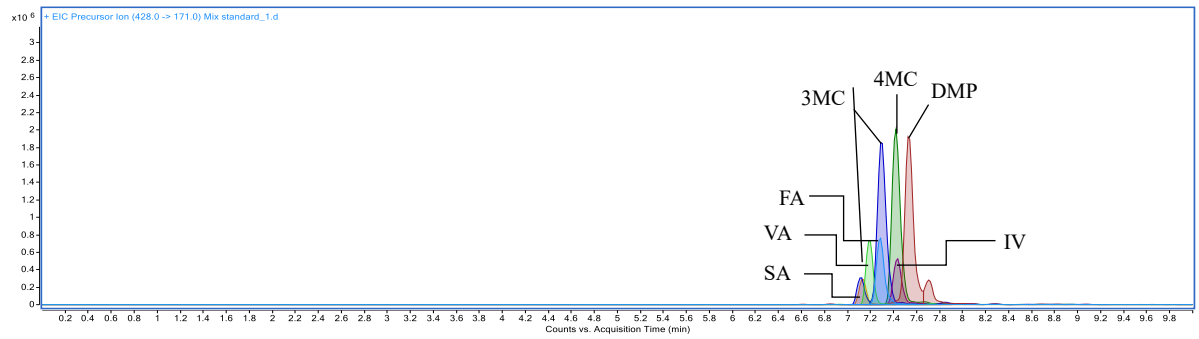


Figure 11. A chromatogram of the seven derivatized phenolic compounds was obtained using an initial gradient program. Detection: precursor ion scan mode

The optimization process involved carefully adjusting the mobile phase composition until a satisfactory level of resolution was attained. This ensured significantly improved separation of each of the seven phenolic compounds compared to the initial chromatogram. This was achieved by starting the gradient program with a higher percentage (90%) of the aqueous mobile phase and decreasing it to 60%. The final optimized gradient elution conditions are presented in Table 3, section 3.4, while the corresponding chromatogram depicting the successful separation is shown in Figure 12. The presence of two peaks for 3-methoxycatechol (3MC) can be attributed to its two hydroxyl groups. During derivatization, the reaction can occur at either hydroxyl group, resulting in two different derivatives with distinct retention times, leading to the observed two peaks.

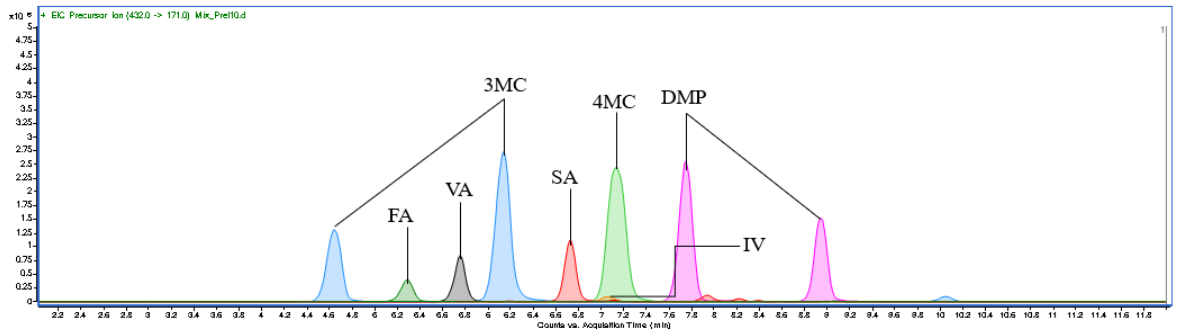


Figure 12. The final gradient program shows a chromatogram in precursor ion scan of seven phenolic compound standard solutions.

This study effectively resolved the previously observed overlapping peaks by successfully optimizing the gradient elution system. This enhanced resolution is crucial for accurately detecting a larger number of phenolic compounds using LC-MS/MS analysis.

4.1.5 Evaluation of dansyl chloride derivatization and LC-MS/MS detection for amine analysis.

The ability to distinguish between amines and phenolic compounds is crucial for accurate analysis, especially when using dansyl chloride for derivatization. Since dansyl chloride reacts with both phenolic and amine compounds, differentiating between them is essential to avoid misinterpretation of results. When analyzing lignin samples, the detection of amino compounds is a possibility, which could lead to inaccurate identification of phenolic compounds if not properly addressed. Therefore, demonstrating the selectivity of this method for phenolic compounds in the presence of amines is essential for ensuring the reliability of the results and its applicability in lignin analysis. A selection of amines for this analysis are methylamine, phenylalanine, ethylamine, valine, kynurenine, tryptophan, and histamine. All samples were derivatized with dansyl chloride using established protocols. To be able to detect dansyl-derivatives of amines, the gradient program employed was the one in Table 4, section 3.4.

Product ion scan mode in the MS detector was employed to investigate the fragmentation patterns of the derivatized amino compounds. The analysis revealed distinct fragmentation patterns for amine and phenolic compounds. The amines presented characteristic fragment ions at *m/z* 170. The observed fragment ions at *m/z* 170 for the derivatized amines are consistent with previous reports in the literature (Zheng & Li, 2012). In contrast, the derivatized phenolic compounds exhibited fragment ions at *m/z* 156 and 171. The mass spectra of dansyl amine derivatives in LC-MS/MS Analysis (*m/z* 170) are shown in Figure 13 and Annex 5. These observations suggest that differentiation between amine and phenolic

compounds can be achieved based on the fragmentation patterns observed in the product ion scan mode of LC-MS/MS analysis following dansyl chloride derivatization. Nonetheless, more amino compound derivatives of dansyl chloride should be submitted to fragmentation to confirm that these are indeed ubiquitous fragments.

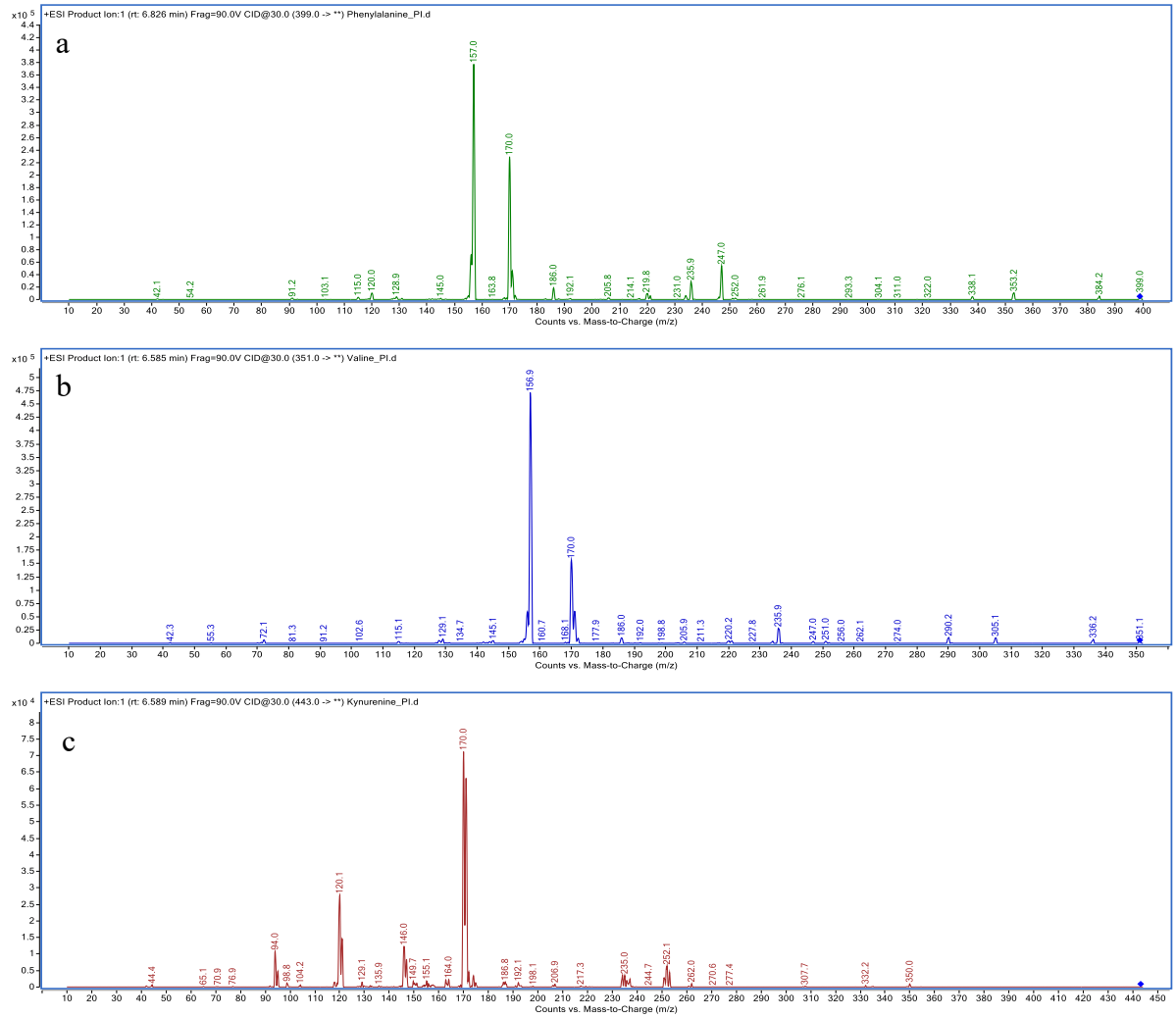


Figure 13: Mass spectra of dansyl amine derivatives in LC-MS/MS Analysis (m/z 170). a. dansyl-phenylalanine, b. dansyl-valine and c. dansyl-kynurenine mass spectrum at collision energy 30 eV.

4.2 Kraft lignin analysis

The developed method was applied to a kraft lignin sample for its characterization based on the presence of compounds with a phenolic group (Figure 14). The analysis found six peaks compared with blank within the 50-500 *m/z* range and putatively annotated phenolic compounds: desaspidinol (5.04 min, 210 g/mol), vanillic acid (5.88 min, 168 g/mol), syringaldehyde (6.19 min, 182 g/mol), apocyanin (8.10 min, 166 g/mol), guaiacol (8.73 min,

124 g/mol), and phenol (9.32 min, 94 g/mol) as shown in Figure 15. The mass spectra of each peak can be found in Figure 16 and Annex 6.

The putative identification was made by comparing the measured molecular weights with the literature (Liang & Wan, 2017). These results show that the analytical method is effective in lignin characterization, suggesting its potential value for lignin valorization efforts where the compositional analysis of lignin is crucial for developing value-added products. Notably, the gradient employed in this method, while optimized for phenolic compounds, does not allow for the detection of amino compounds because the amine compounds elute faster than the phenolic compounds.

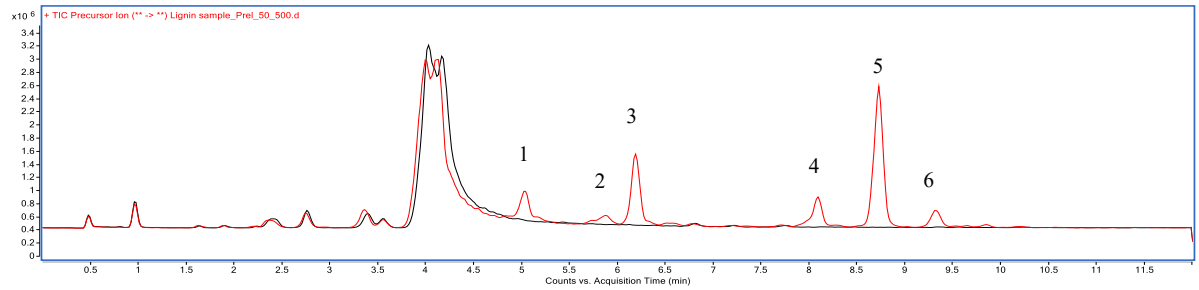


Figure 14. The chromatogram of the kraft lignin sample (red line) compared with blank (black line) on the mass range of 50 – 500 m/z. The peak number indicates the compounds as follows: 1. desaspidinol, 2. vanillic acid, 3. syringaldehyde, 4. apocyanin, 5. guaiacol, and 6. phenol.

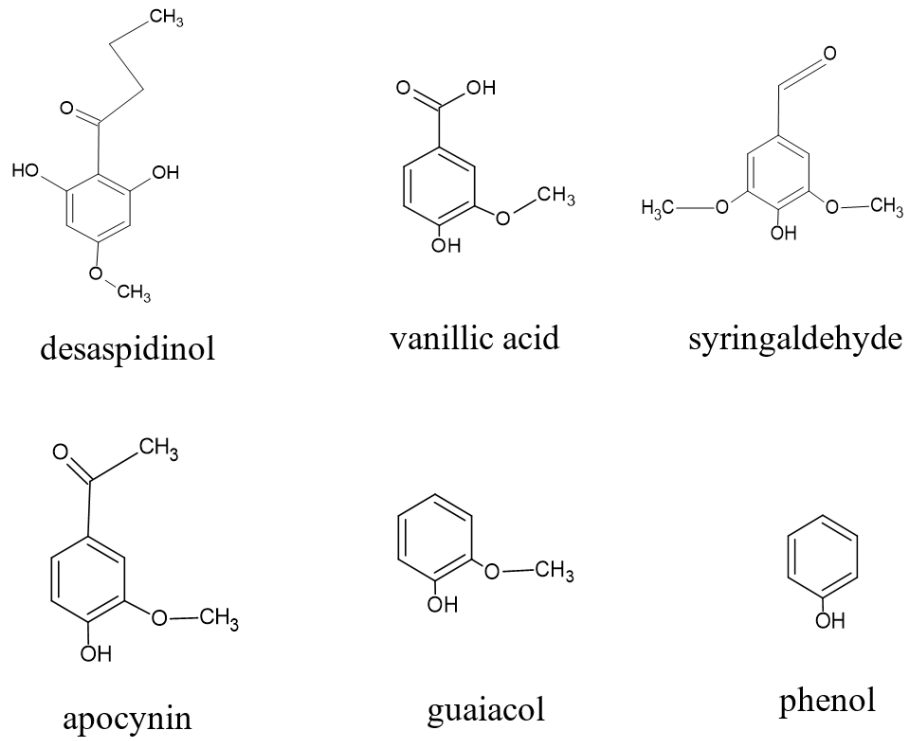


Figure 15. the structure of the phenolic compounds in kraft lignin sample.

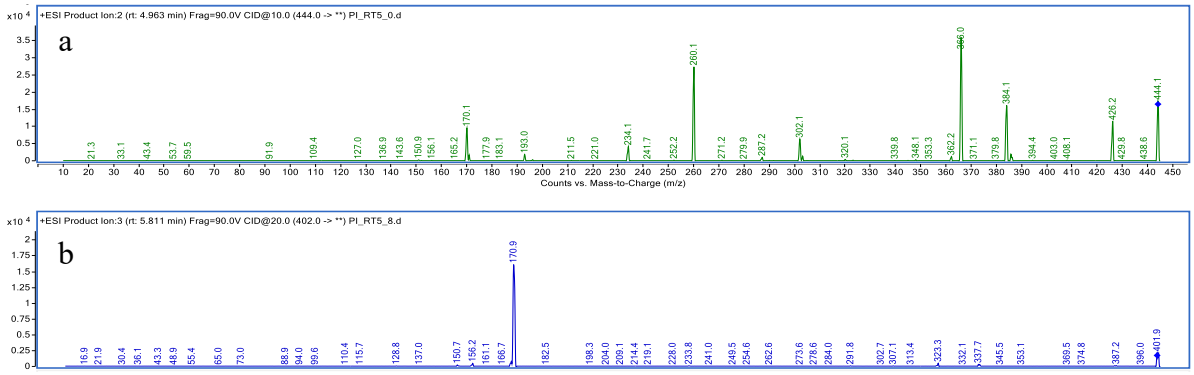


Figure 16. The MS spectra of a. desaspidinol and b. vanillic acid.

5. SUMMARY

In conclusion, this study has established an analytical method for detecting phenolic groups for the characterization of lignin. The study found that suitable conditions for phenolic compound analysis were 2 mg/mL of dansyl chloride as a derivatization reagent, combined with 0.125 M of sodium bicarbonate buffer pH 10.6, a reaction time of 35 minutes, and a temperature of 25 °C. Precursor ion scan mode targeting *m/z* 171 in MS offers selectivity for detecting dansyl-phenol derivative compounds. This method can distinguish between phenolic and amine compounds due to their different fragmentation patterns to ensure the accuracy and reliability of analytical results. The successful identification of phenolic compounds in kraft lignin plays a role in lignin valorization efforts. Future studies will focus on confirming the identities of phenolic compounds within kraft lignin samples. The method will be extended to dimer and trimer structures, as current findings indicate the method is primarily effective for monomer detection. Additionally, the method's applicability will be tested on lignin obtained from other processes. Moreover, rigorous method validation, such as the limit of detection, accuracy, and precision would be a valuable next step if quantitative analysis is of interest.

6. ACKNOWLEDGMENTS

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To my family – my parents and Nattapat – thank you for your endless love and emotional support and for helping me pay for this. Your belief in me means the world.

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ANNEXES

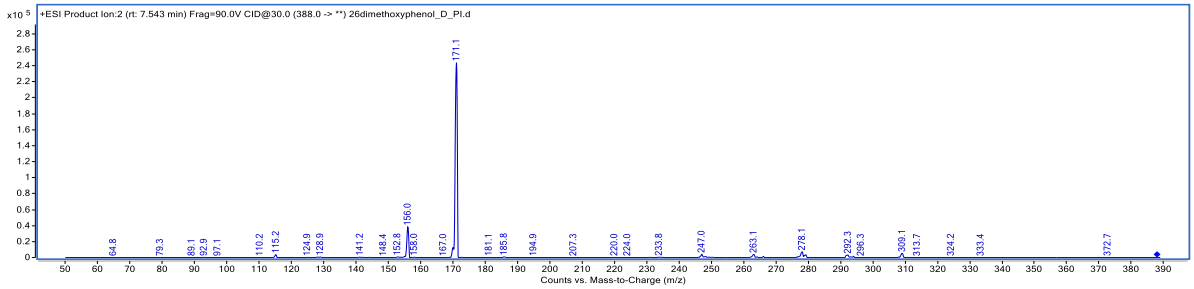
Annex 1

Chemical/Reagent	Producer	Purity, %	MW (g/mol)	Melting point (°C)	Boling point (°C)	CAS No.
2,6-dimethoxyphenol	Sigma-Aldrich	99.0	154.16	50 - 57	261	91-10-1
4-methylcatechol	Sigma-Aldrich	95.0	124.14	67 - 69	251	452-86-8
syringic acid	Sigma-Aldrich	95.0	198.17	205 - 209	440	530-57-4
ferulic acid	Sigma-Aldrich	99.0	194.18	168 - 172	372.3	537-98-4
3-methoxycatechol	Alfa Aesar	98.0	140.14	40 – 43	146 – 147	934-00-9
isovanillin	Fluka	99.5	152.15	81 - 83	112.6 – 116.4	621-59-0
vanillic acid	Fluka	97.0	168.15	208 - 210	257.07	121-34-6
dansyl chloride	Sigma-Aldrich	99.0	269.75	72 - 74	371.3	605-65-2
benzoyl chloride	Sigma-Aldrich	99.0	140.57	-1	209 - 215	98-88-4
sodium carbonate	Sigma-Aldrich	99.0	105.98	851	1600	497-19-8
acetone	Sigma-Aldrich	99.7	58.08	-95	56.05	67-64-1
methanol	Sigma-Aldrich	99.9	32.04	-98	64 - 65	67-56-1
sodium bicarbonate	Merck	99.5	84.01	270	851	144-55-8
sodium hydroxide	Honeywell	98.0	40.00	318	1388	1310-73-2
formic acid	Honeywell	97.5	46.03	8.4	100.8	64-18-6
acetonitrile	Honeywell	99.9	41.05	-45.7	81.6	75-05-8
ethanol	Honeywell	99.8	46.00	-114.1	78.37	67-63-0

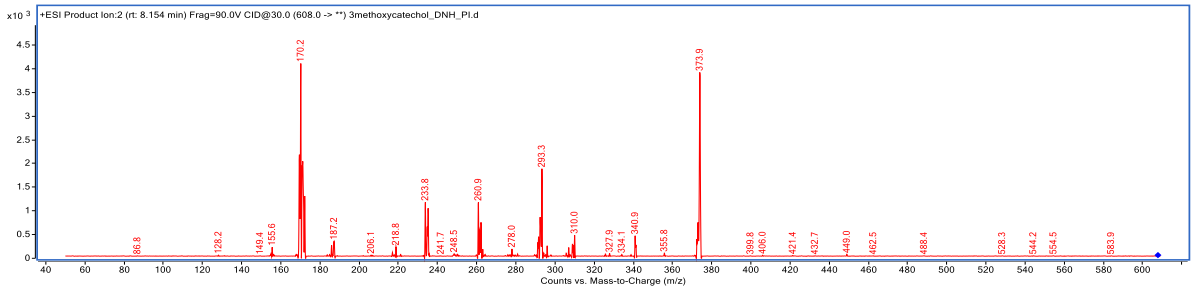
Annex 2

The mass spectra of the dansyl-phenol derivative are as follows,

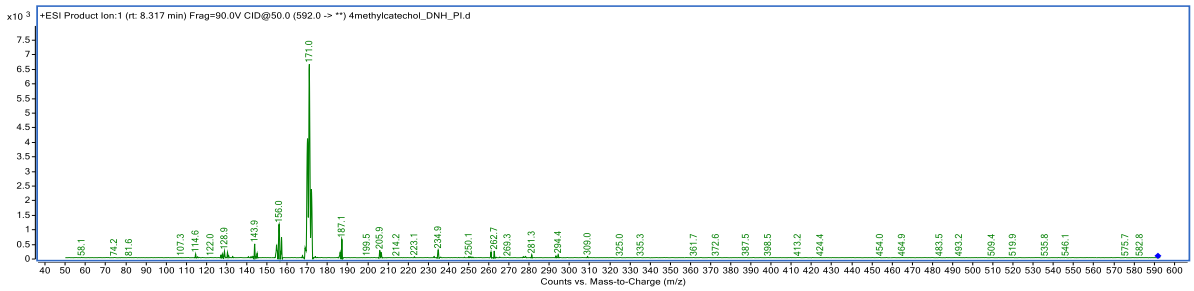
Mass spectrum of the dansyl-2,6 dimethoxyphenol derivative at collision energy 30 eV.



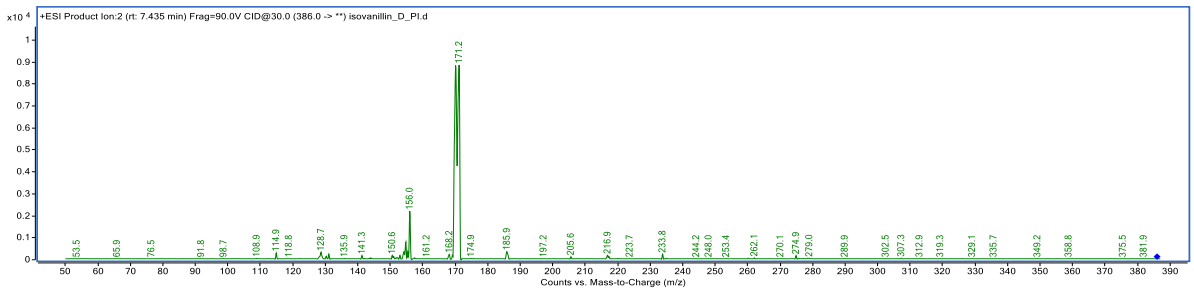
Mass spectrum of the dansyl-3 methoxycatechol derivative at collision energy 30 eV.



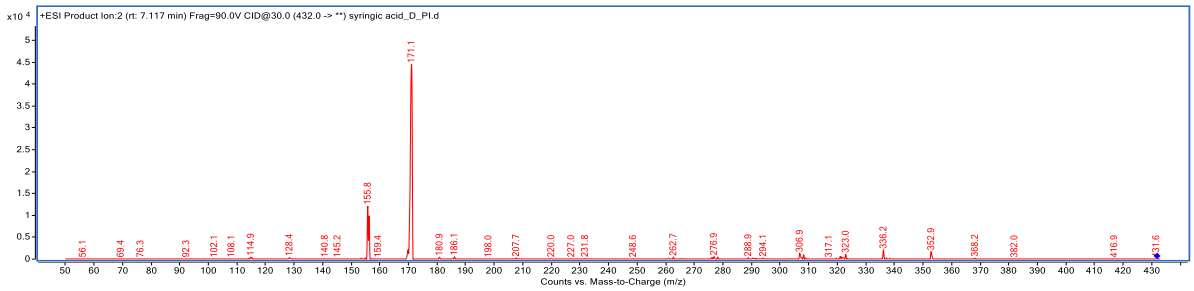
Mass spectrum of the dansyl-4 methylcatechol derivative at collision energy 30 eV.



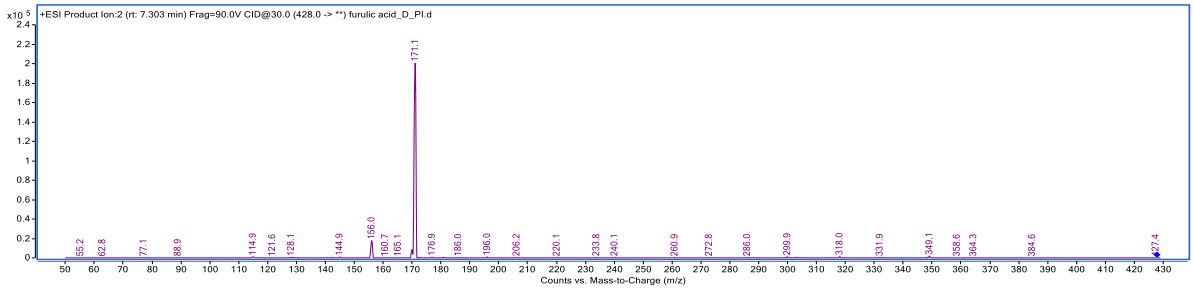
Mass spectrum of the dansyl-isovanillin derivative at collision energy 30 eV.



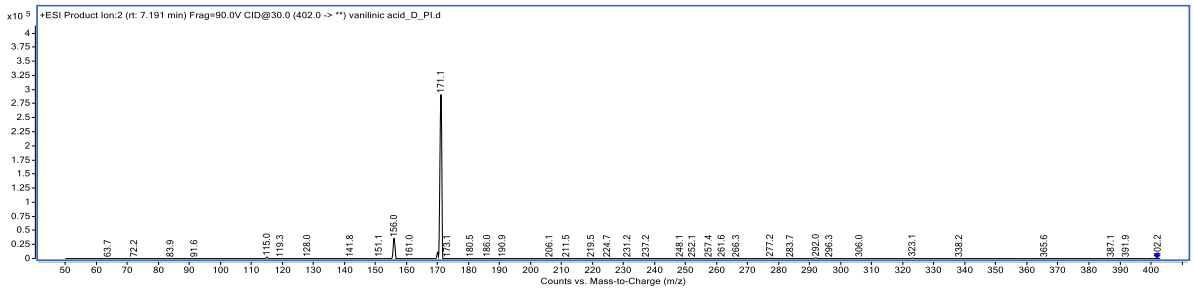
Mass spectrum of the dansyl-syringic acid derivative at collision energy 30 eV.



Mass spectrum of the dansyl-ferulic acid derivative at collision energy 30 eV.



Mass spectrum of the dansyl-vanillic acid derivative at collision energy 30 eV.



Annex 3

Solution	B (M)	Ti (min)	T (°C)	D (mg/mL)	Y
1	0.05	10	25	0.5	60395370
2	0.05	10	25	2	105195777
3	0.05	10	60	0.5	58035292
4	0.05	10	60	2	96245660
5	0.05	60	25	0.5	69183614
6	0.05	60	25	2	106801424
7	0.05	60	60	0.5	52867967
8	0.05	60	60	2	89557339
9	0.2	10	25	0.5	63512114
10	0.2	10	25	2	112628651
11	0.2	10	60	0.5	62917505
12	0.2	10	60	2	107892588
13	0.2	60	25	0.5	65559846
14	0.2	60	25	2	110930213
15	0.2	60	60	0.5	60172818
16	0.2	60	60	2	99872022
17	0.125	35	42.5	1.25	86277669
18	0.125	35	42.5	1.25	76233233
19	0.125	35	42.5	1.25	76634585

B = concentration of sodium bicarbonate buffer pH 10.6, Ti = time, T = temperature, D = concentration of dansyl chloride, Y = sum of all peak area

Annex 4

Solution	B (M)	Ti (min)	T (°C)	D (mg/mL)	Y	Average Y	SD
1	0.125	35	25	2.0	108142943		
2	0.125	35	25	2.0	109470882	111181604	4166332.91
3	0.125	35	25	2.0	115930986		
4	0.125	35	25	2.5	116230803		
5	0.125	35	25	2.5	105646335	110692151	5309416.79
6	0.125	35	25	2.5	110199314		
7	0.125	35	25	3.0	115370058		
8	0.125	35	25	3.0	107066890	110715154	4242127.27
9	0.125	35	25	3.0	109708513		

B = concentration of sodium bicarbonate buffer pH 10.6, Ti = time, T = temperature, D = concentration of dansyl chloride, Y = sum of all peak area

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
2	3	333544811	111181604	17358329908064
2.5	3	332076452	110692151	28189906695764
3	3	332145461	110715154	17995643782896

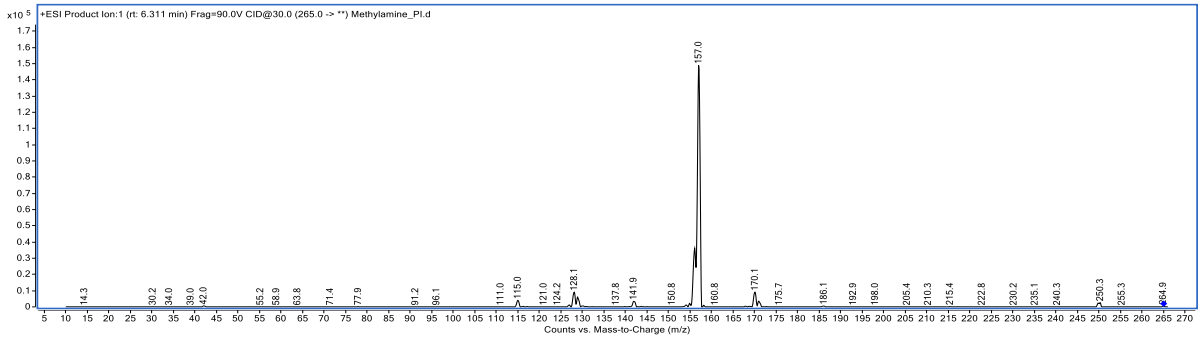
ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	457668979718	2	228834489859	0.01	0.99	5.14
Within Groups	127087760773450	6	21181293462242			
Total	127545429753168	8				

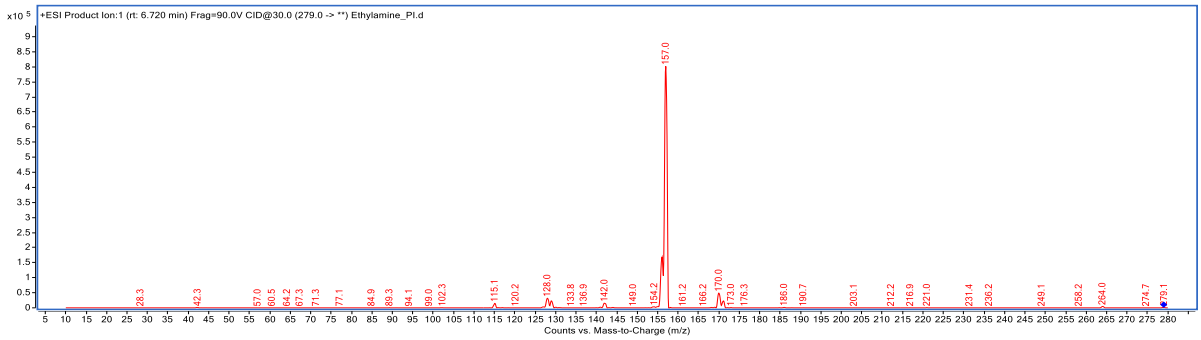
Annex 5

The fragmentation of the dansyl-amine derivative is as follows,

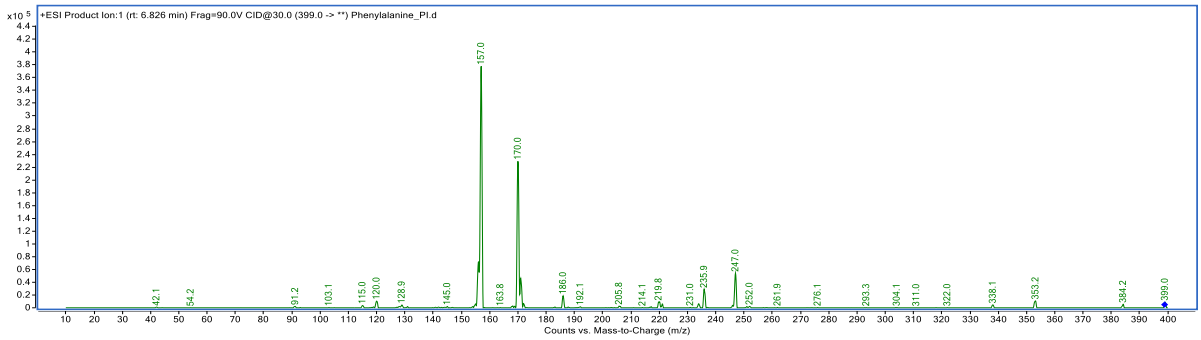
Mass spectrum of the dansyl-methylamine derivative at collision energy 30 eV.



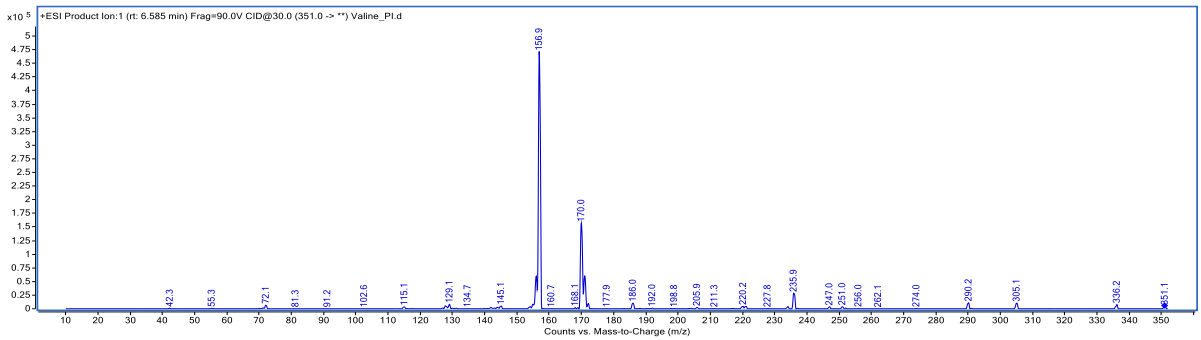
Mass spectrum of the dansyl-ethylamine derivative at collision energy 30 eV.



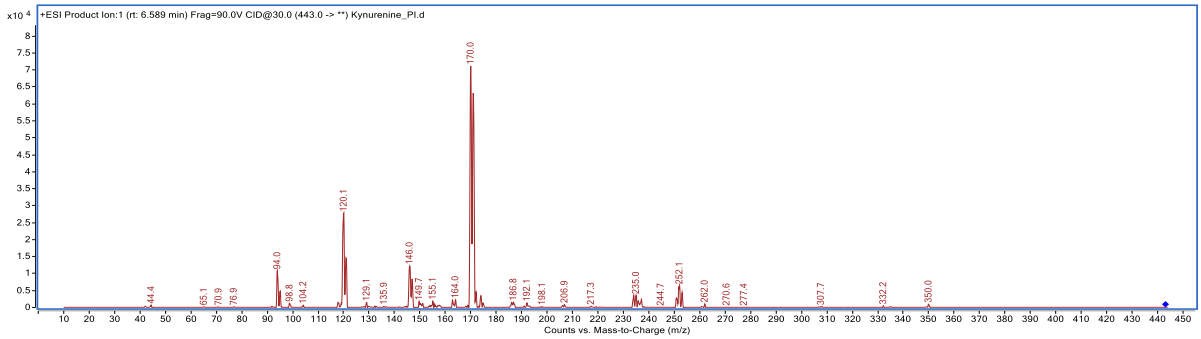
Mass spectrum of the dansyl-phenylalanine derivative at collision energy 30 eV.



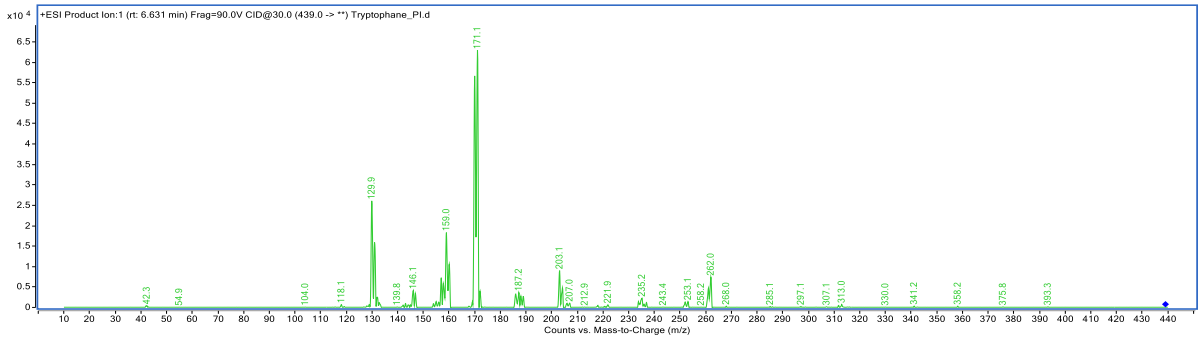
Mass spectrum of the dansyl-valine derivative at collision energy 30 eV.



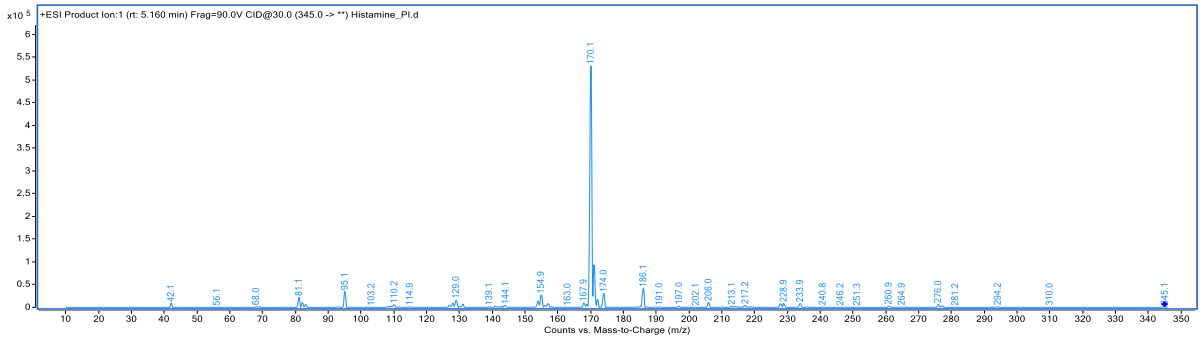
Mass spectrum of the dansyl-kynurenine derivative at collision energy 30 eV.



Mass spectrum of the dansyl-tryptophan derivative at collision energy 30 eV.



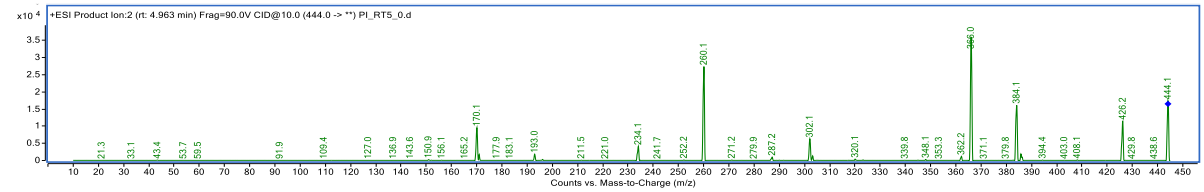
Mass spectrum of the dansyl-histamine derivative at collision energy 30 eV.



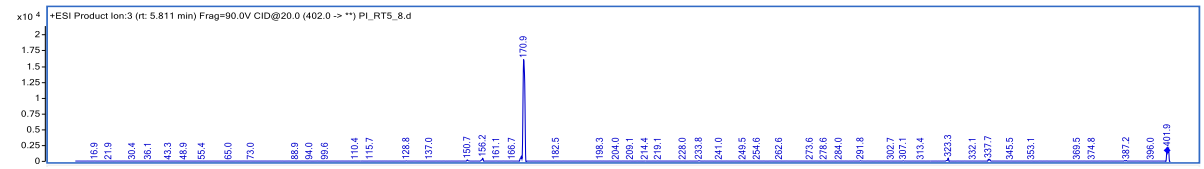
Annex 6

The fragmentation of the dansyl-phenol derivative from the kraft lignin sample is as follows,

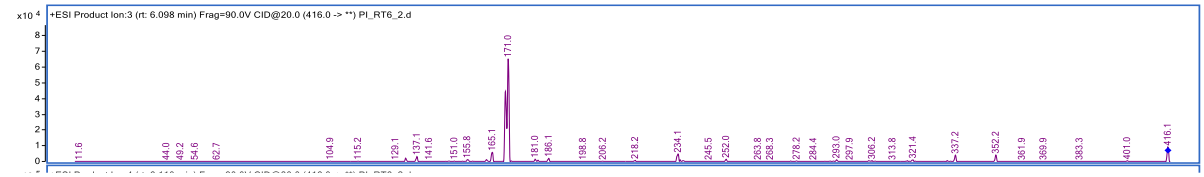
Mass spectrum of the dansyl- desaspidinol derivative at collision energy 30 eV.



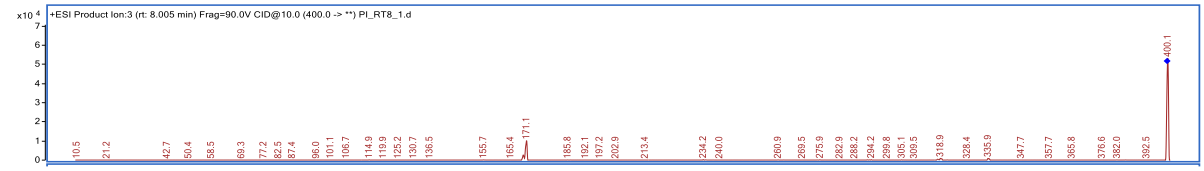
Mass spectrum of the dansyl- vanillic acid derivative at collision energy 30 eV.



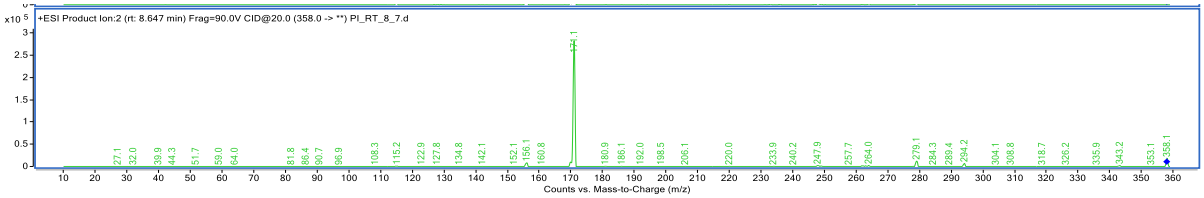
Mass spectrum of the dansyl- syringaldehyde acid derivative at collision energy 30 eV.



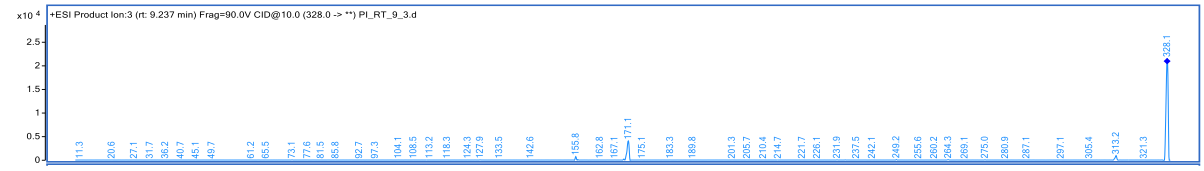
Mass spectrum of the dansyl- apocynin derivative at collision energy 30 eV.



Mass spectrum of the dansyl- guaiacol derivative at collision energy 30 eV.



Mass spectrum of the dansyl- phenol derivative at collision energy 30 eV.



Information sheet

Development of derivatization-based LC-MS method for analysis of lignin components

Abstract: Lignin, a biopolymer found in plant cell walls, is a byproduct of various industries and holds potential as a renewable resource for valuable chemicals. This study focuses on developing an efficient method for analyzing phenolic compounds derived from lignin using high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS). The method involves derivating phenolic compounds with dansyl chloride, and, therefore, enhancing their detection sensitivity and selectivity. The research investigates the optimal conditions for derivatization, including the choice of base, derivatization reagent, and reaction parameters. The developed method can differentiate between phenolic and amine compounds by their distinct fragments *m/z* upon collision, ensuring accurate analysis in complex samples. The developed method is applied to a kraft lignin sample, putatively identifying several phenolic compounds. The findings contribute to lignin valorization efforts by providing a valuable tool for characterizing its phenolic composition.

Keywords: Lignin, phenolic compounds, derivatization, dansyl chloride, high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS), kraft lignin.

CERCS code: P300 analytical chemistry

Derivatiseerimisega LC-MS meetodil baseeruva analüüsimetoodika arendamine ligniini komponentide analüüsiks

Kokkuvõte: Ligniin, taimeraku seinas leiduv biopolümeer, on mitmete tööstusharude kõrvalsaadus ning omab potentsiaali taastuva ressursina väärtuslike kemikaalide tootmiseks. Käesolev uuring keskendub tõhusa meetodi väljatöötamisele ligniinist saadud fenoolsete ühendite analüüsimiseks, kasutades kõrgefektiivset vedelikkromatograafiat/tandem massispektromeetriat (LC-MS/MS). Meetod hõlmab fenoolsete ühendite derivatiseerimist dansüülkloriidiga, mis suurendab nende tuvastamise tundlikkust ja selektiivsust. Töös optimeeriti derivatiseerimise tingimused, sealhulgas aluse, derivatiseeriva reagendi ja reaktsiooni parameetrite valik. Arendatud metoodika võimaldab eristada fenoolseid ja aminoühendeid nende *m/z* järgi, tagades täpse analüüsi keerukates proovides. Väljatöötatud meetodit rakendati krafti ligniini analüüsiks ja identifitseeriti mitmeid fenoolseid ühendeid. Tulemused toetavad ligniini väärindamise jõupingutusi, pakkudes väärtuslikku tööriista selle fenoolse koostise iseloomustamiseks.

Märksõnad: Ligniin, fenoolsed ühendid, derivatiseerimine, dansüülkloriid, kõrgefektiivne vedelikkromatograafia/tandem-massispektromeetria (HPLC-MS/MS), krafti ligniin.

CERCS kood: P300 analüütiline keemia

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