11th يوم البحث العلمي بكلية الصيدلة
College of Pharmacy Research Day

جامعة الملك سعود
King Saud University
كلية الصيدلة

Quantification and Molecular Docking Studies of the Active Principles Isolated from the Edible Plant Sisymbrium irio L.

Presented by:

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Supervisied by:

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Outlines

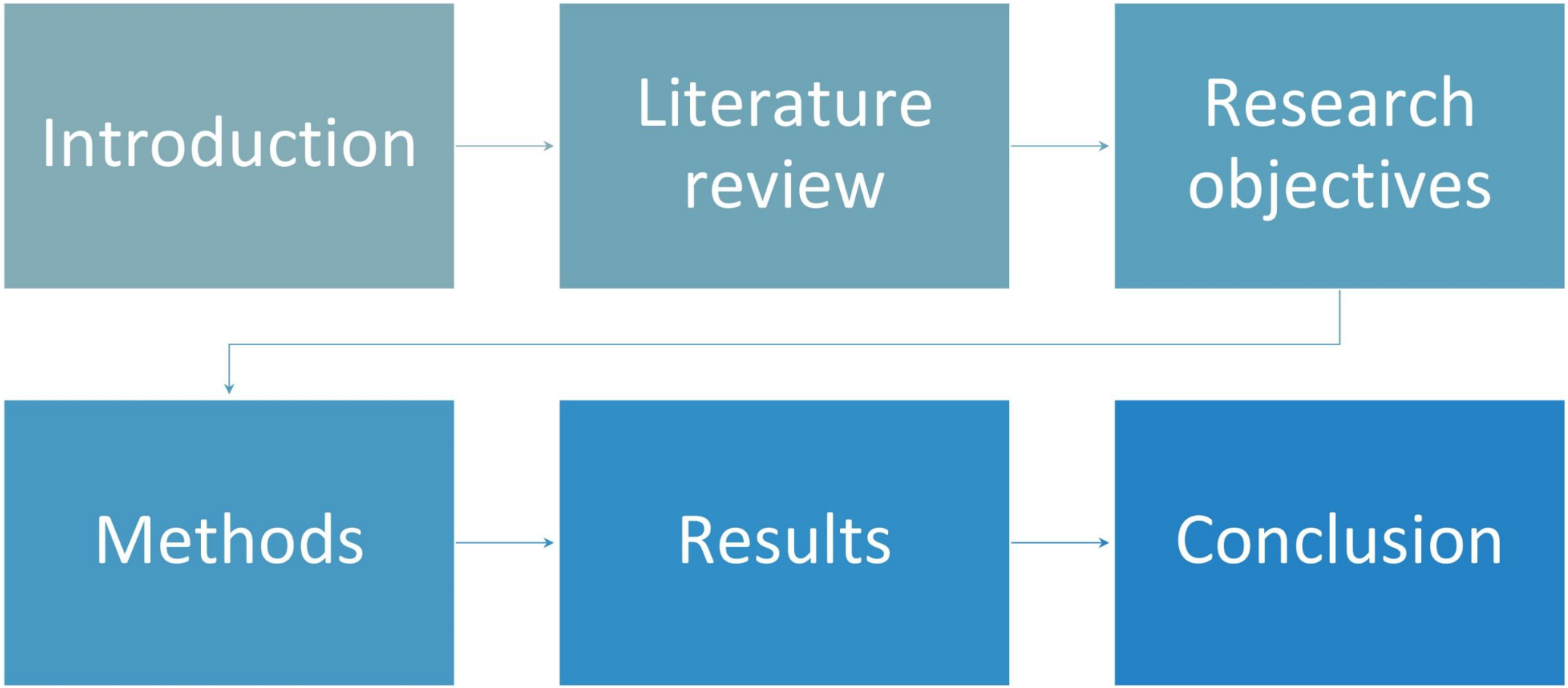
Introduction
Literature review
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Table of abbreviations

abbreviation	Explanation
S. irio	*Sisymbrium irio L.*
PPAR-γ	Peroxisome proliferator- activated receptor gamma
5-HT_1A	Serotonin subtype 1A receptor
5-HT_2A	Serotonin subtype 2A receptor
HPTLC	High Performance Thin Layer Chromatography
TLC	Thin layer chromatography
ALA	α-linolenic acid
RA	Roughanic acid
NMR	Nuclear magnetic resonance
HSQC	Heteronuclear Single-Quantum Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
COSY	Correlation Spectroscopy

Introduction

Sisymbrium irio L. is a member of Brassicaceae family, grows widely in different areas in Saudi Arabia and contains important secondary metabolites.

Members of genus ***sisymbrium*** are used traditionally to treat bronchitis, stomach ailments, voice disorders, sore throats and used as poisons antidote.

Mustard family

broccoli

mustard

radish

cabbage

arugula

cauliflower

kale

[1] Al-Massarani, S., El Gamal, A., Alam, P., Al-Sheddi, E., Al-Oqail, M. and Farshori, N., 2016. *Isolation, biological evaluation and validated HPTLC-quantification of the marker constituent of the edible Saudi plant Sisymbrium irio L.*. Saudi Pharmaceutical Journal.

[2] Mowafy, H., Alanazi, F. and El Maghraby, G., 2012. Development and validation of an HPLC–UV method for the quantification of carbamazepine in rabbit plasma. Saudi Pharmaceutical Journal, 20(1), pp.29-34.

[3] Al-Jaber, N., 2011. Phytochemical and biological studies of Sisymbrium irio L. Growing in Saudi Arabia. *Journal of Saudi Chemical Society*, 15(4), pp.345-350.

[4] Al-Qudah, M. and Abu Zarga, M., 2010. Chemical constituents of Sisymbrium irio L. from Jordan. *Natural Product Research*, 24(5), pp.448-456.

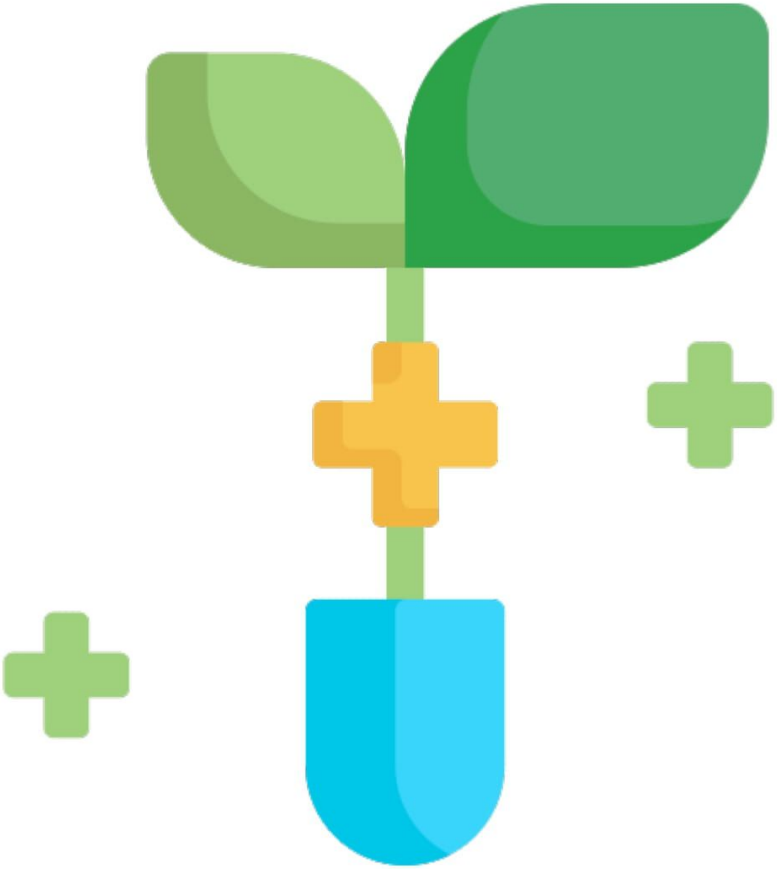
[5] Asilbekova, D., Ozek, G., Ozek, T., Bobakulov, K., Baser, K. and Sagdullaev, S., 2019. Essential Oil and Lipids from Leaves of Ferula kuhistanica. Chemistry of Natural Compounds, 55(6), pp.993-998.

Research Objectives

I. To isolate and then identify pure secondary metabolites from the plant extract and fractions using different chromatographic and spectroscopic techniques.

II. To predict the binding affinity of the different isolates to macromolecular targets, chosen according to their reported pharmacological activities through molecular docking methods.

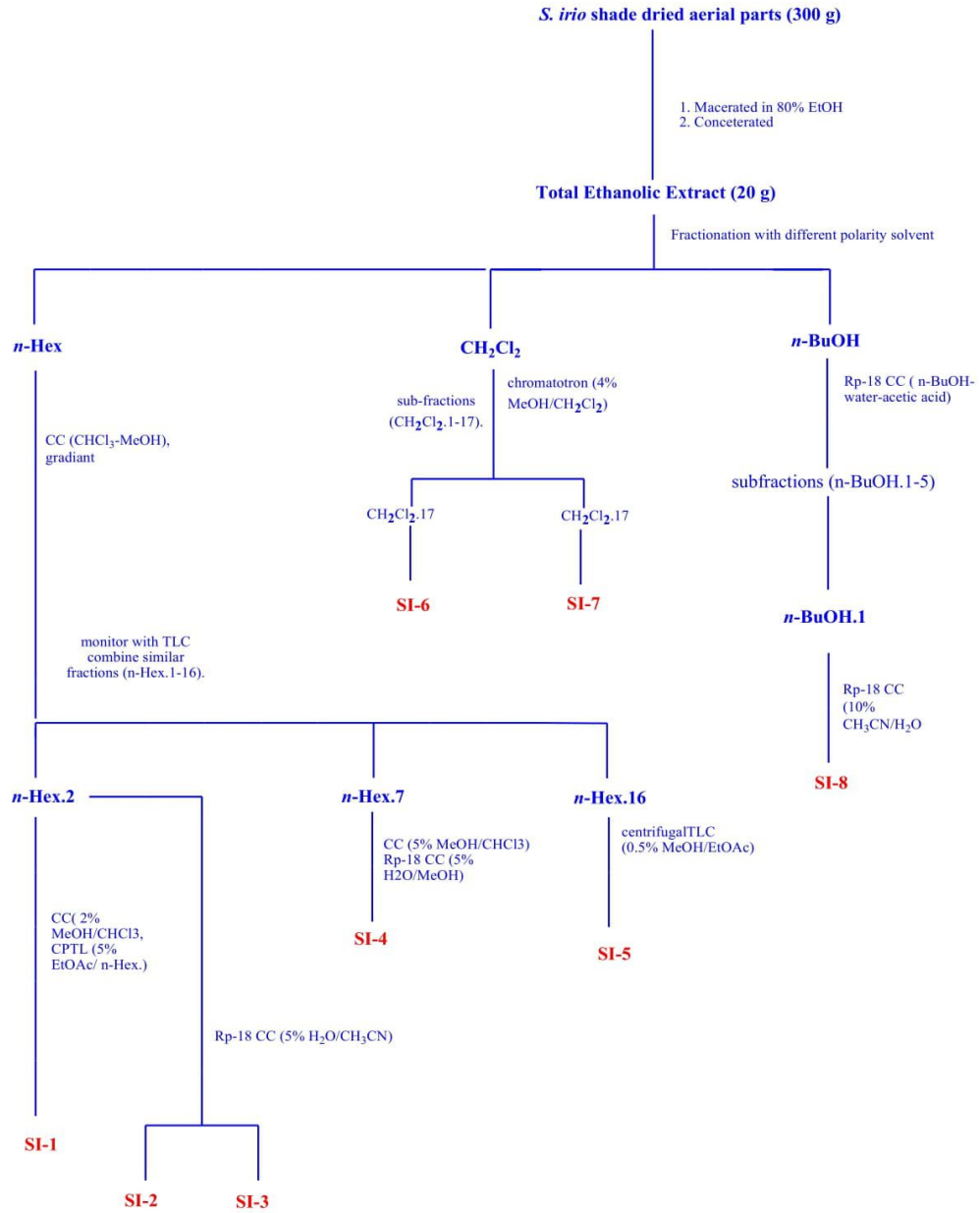
III. To develop a TLC-densitometry (HPTLC) method for quantification of selected biomarker, isolated from the plant.



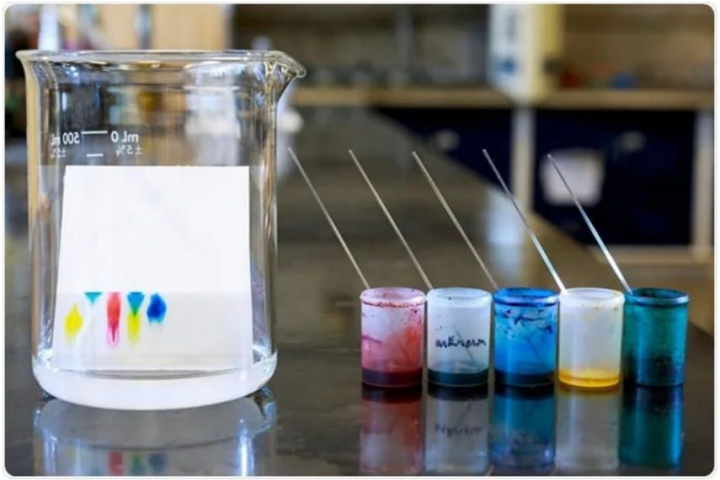
Method

Plant extraction and compounds isolation

S.Irio extraction, fractionation and Isolation scheme



Devices

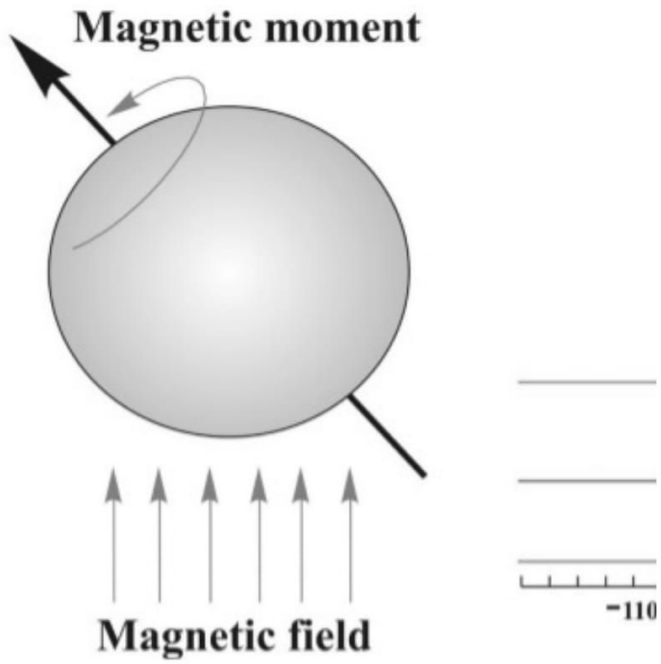


TLC

CycloGraph centrifugal chromatography

Open column chromatography

Nuclear Magnetic Resonance Spectroscopy (NMR)



Molecular docking, targets and ligands preparation

Research Collaboratory for Structural Bioinformatics Protein Data Bank

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5U5L

X-ray Crystal Structure of the PPARgamma Ligand Binding D
Rivoglitazone

DOI: 10.2210/pdb5U5L/pdb

Classification: TRANSCRIPTION/INHIBITOR
Organism(s): Homo sapiens
Expression System: Escherichia coli BL21(DE3)
Mutation(s): No

FASTA Sequence

PDB Format
PDB Format (gz)

PDBx/mmCIF Format
PDBx/mmCIF Format (gz)

PDBML/XML Format (gz)

Pubchem

alpha linolenic acid

Treating this as a text search.

Compounds (102) | Substances (185) | Pathways (44) | BioAssays (41) | Literature (5,472) | Patents (435)

Searching chemical names and synonyms including IUPAC names and InChIKeys across the compound collection. Note that annotations text from compound summary pages is not searched. Read More...

102 results | Filters | SORT BY Relevance

Linolenic Acid; Alpha-Linolenic Acid; 463-40-1; Linolenate; (9Z,12Z,15Z)-Octadeca-9,12,15-Trienoic Acid; ...

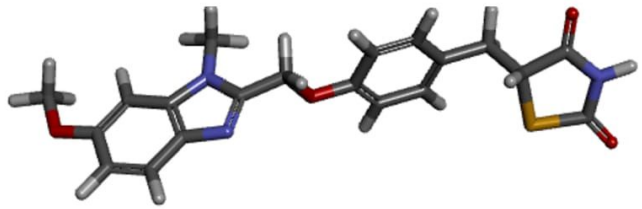
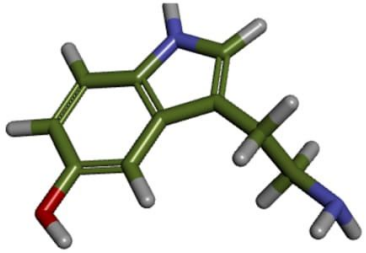
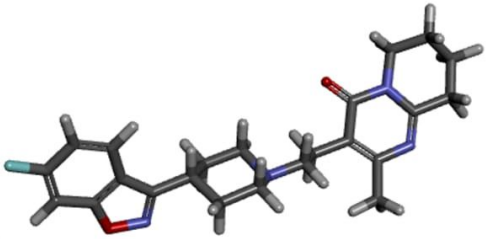
Compound CID: 5280934
MF: $C_{18}H_{30}O_2$ MW: 278.4g/mol
IUPAC Name: (9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid

DOWNLOAD

Summary (Search Results)

CSV | JSON | XML

COMPRESSION:
None | GZip

Programs	**Biovia Discovery studio, AutoDock 4.2.**		
Receptors	**PPAR-γ**	**5-HT_1A**	**5-HT_2A**
Controls	**Rivoglitazone** 	**Serotonin** 	**Risperidone** 
Compounds	**SI-2, SI-3, SI-5, SI-6**	**SI-1, SI-4, SI-7, SI-8**	**SI-1, SI-4, SI-7, SI-8**

Quantification Method

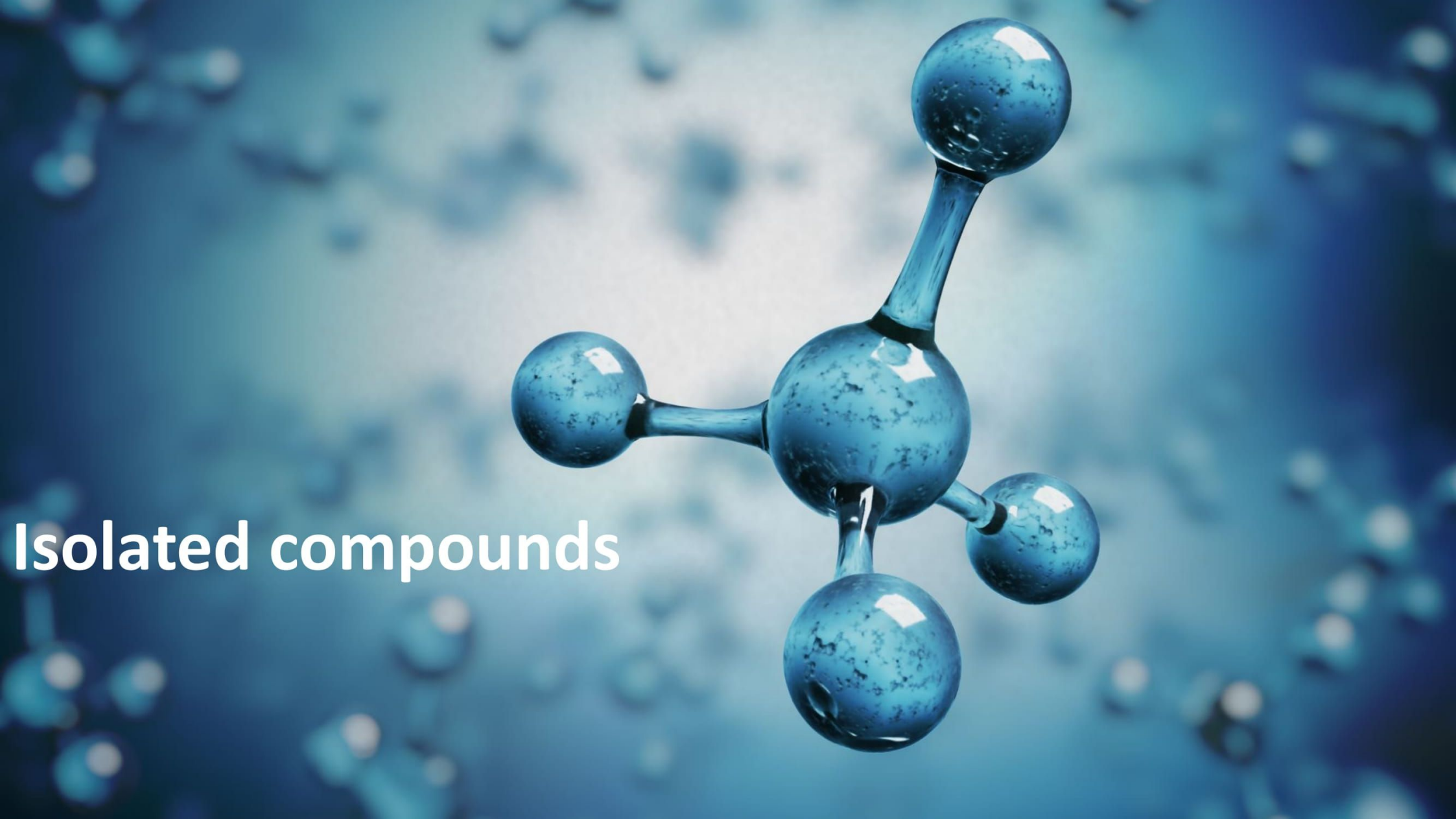
using

High Performance Thin Layer Chromatography (HPTLC)

The developed HPTLC method was validated for

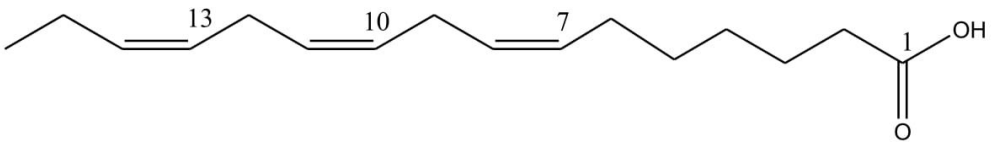
- ACCURACY
- PRECISION
- ROBUSTNESS
- LIMIT OF DETECTION
- LIMIT OF QUANTIFICATION

Results



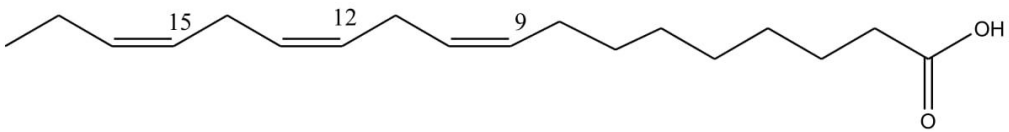
Isolated compounds

Unsaturated Fatty Acids



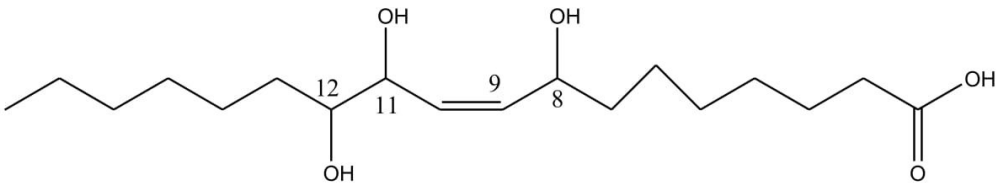
(7Z,10Z,13Z)-hexadecatrienoic acid

SI-2



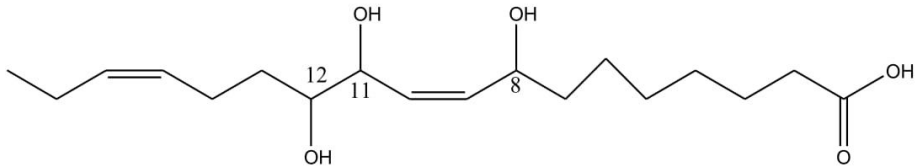
(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid (α-linolenic acid)

SI-3



8,11,12-trihydroxy-9Z-octadecanoic acid

SI-5

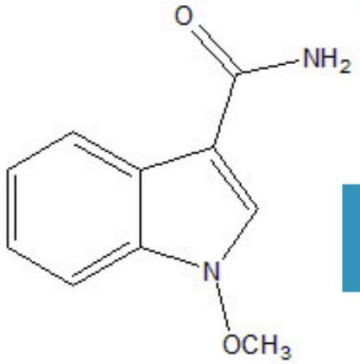


8,11,12-trihydroxy- 9Z,15Z-octadecadienoic acid

SI-6

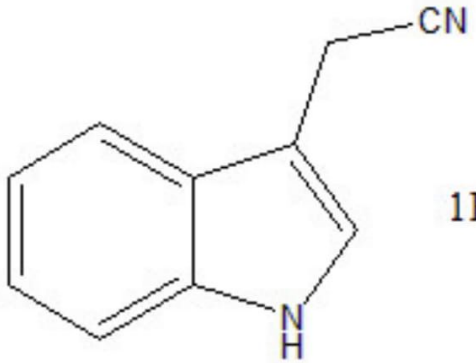
[5] Asilbekova, D., Ozek, G., Ozek, T., Bobakulov, K., Baser, K. and Sagdullaev, S., 2019. Essential Oil and Lipids from Leaves of Ferula kuhistanica. Chemistry of Natural Compounds, 55(6), pp.993-998.
[6] Alexandri, E., Ahmed, R., Siddiqui, H., Choudhary, M., Tsiafoulis, C. and Gerothanassis, I., 2017. High Resolution NMR Spectroscopy as a Structural and Analytical Tool for Unsaturated Lipids in Solution. Molecules, 22(10), p.1663.

Indole alkaloids



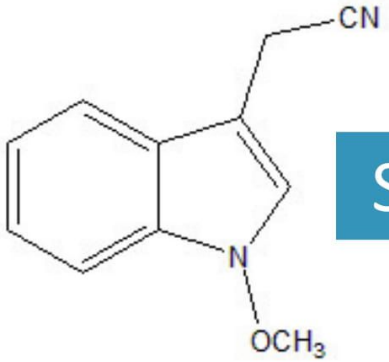
SI-4

1-methoxy-1*H*-indole-3-carboxamide



1H-indole-3-acetonitrile

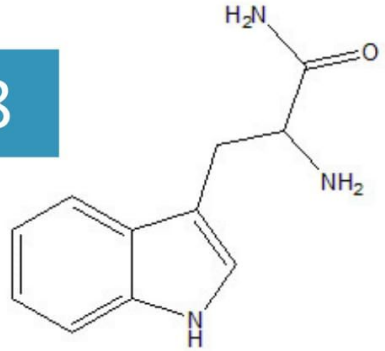
SI-7



SI-1

N-methoxyindole 3-acetonitrile

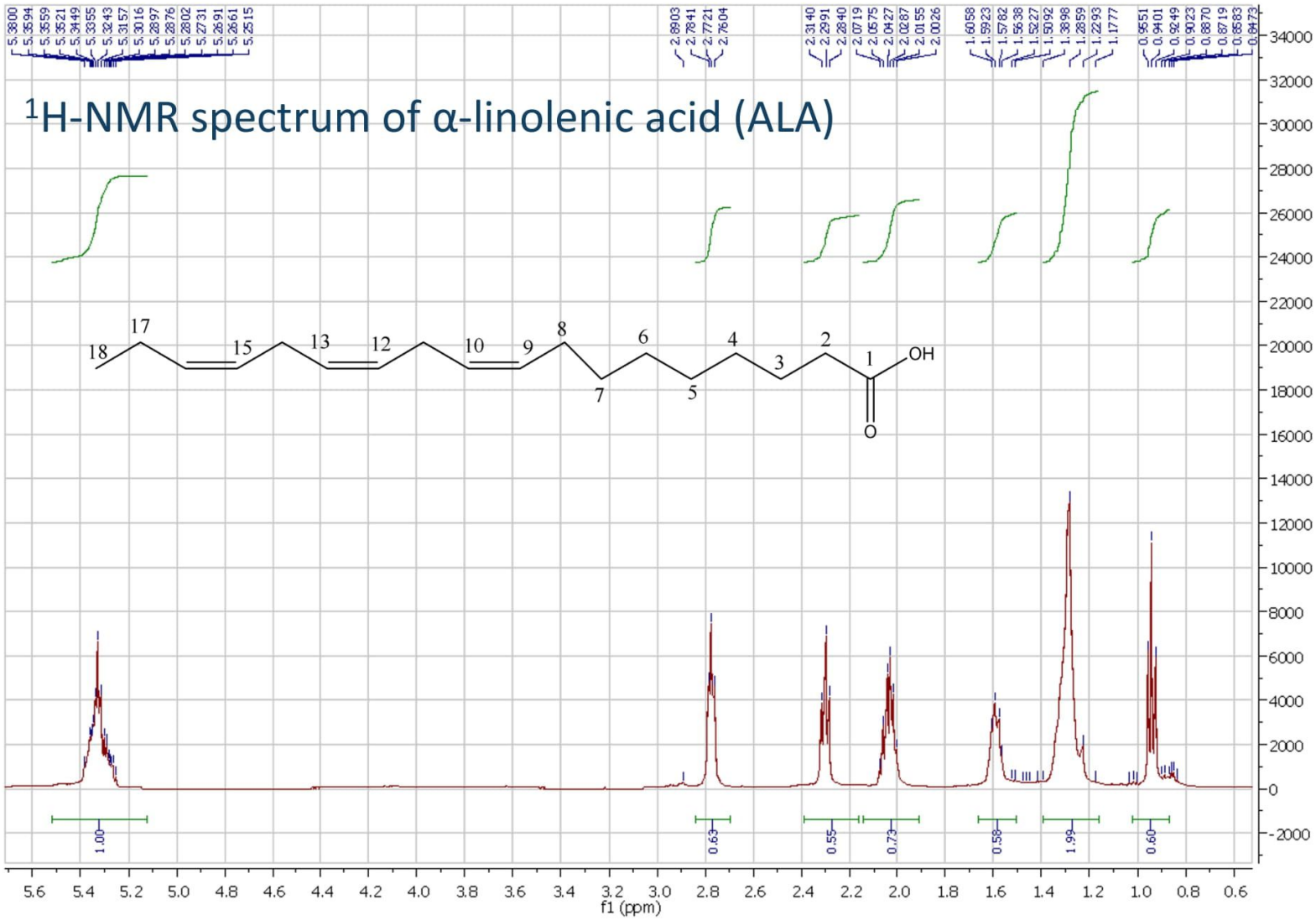
SI-8



1H-Indole-3-propanamide
(α-amino-L-Tryptophanamide)

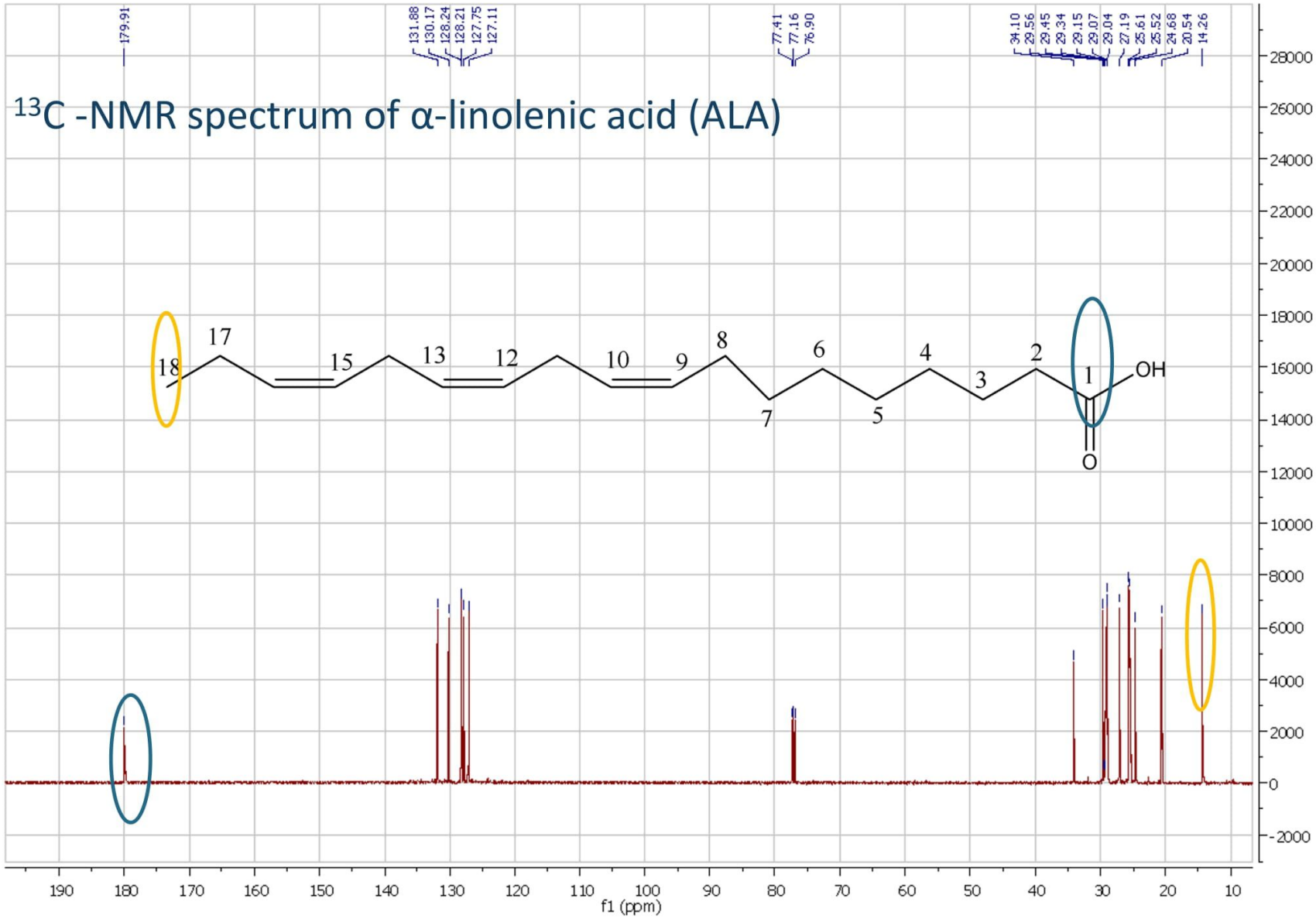
[7] Alhadrami, H.A.; Thissera, B.; Hassan, M.H.A.; Behery, F.A.; Ngwa, C.J.; Hassan, H.M.; Pradel, G.; Abdelmohsen, U.R.; Rateb, M.E. Bio-Guided Isolation of Antimalarial Metabolites from the Coculture of Two Red Sea Sponge-Derived Actinokineospora and Rhodococcus spp. Mar. Drugs 2021, 19, 109.
[8] Prajapati, R., Seong, SH., Kim, HR., Jung, HA., Choi, JS. Isolation and identification of bioactive compounds from the tuber of Brassica oleracea var. gongylodes, Natural Product Sciences, 26(3), 214-220 (2020)

[9] Acheson, K., Schutz, Y., Bessard, T., Ravussin, E., Jequier, E. and Flatt, J., 1984. Nutritional influences on lipogenesis and thermogenesis after a carbohydrate meal. American Journal of Physiology-Endocrinology and Metabolism, 246(1), pp.E62-E70.

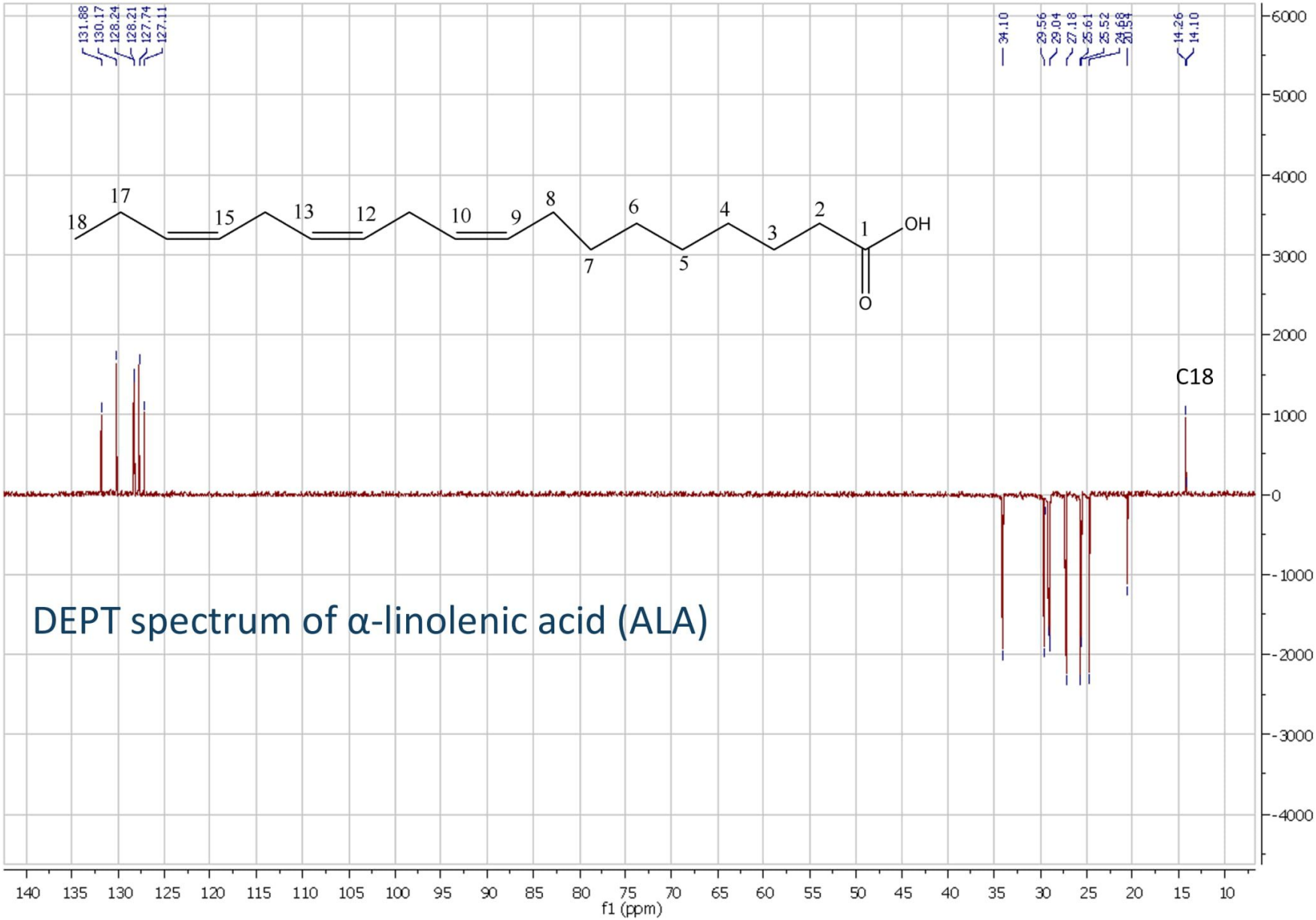


position	δ_H
1	---
2	2.33, 9 t, j 7.6 Hz)
3	1.61, *m*
4	1.35-1.37, *m*
5	1.35-1.37, *m*
6	1.35-1.37, *m*
7	1.35-1.37, *m*
8	2.04-2.08, *m*
9	2.31-5.40, *m*
10	5.31-5.40, *m*
11	2.78, *m*
12	5.31-5.40, *m*
13	5.31-5.40, *m*
14	2.78, *m*

^{13}C -NMR spectrum of α-linolenic acid (ALA)



position	δ_c
1	179.9
2	34.1
3	24.7
4	29.0
5	29.1
6	29.2
7	29.6
8	27.2
9	130.2
10	127.8
11	25.6
12	128.21
13	128.24
14	25.5
15	127.1
16	131.9
17	20.5
18	14.3



DEPT spectrum of α-linolenic acid (ALA)