Original Article

Preformulation Studies Of S-Equol

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Abstract

S-Equol is an intestinal bacterial metabolite of the soybean isoflavone daidzein which has potent Antioxidant, and Anti-inflammatory properties in humans. Evaluation of solubility, Molecular weight, Melting point, Compatibility, and Stability of the Drug by Preformulation Studies should be determined. Preformulation is the Study of the Physicochemical properties of the drug before the formulation process. This can support the necessity for molecular change or offer crucial information for formulation design. This feature offers the basis for medication combinations with medicinal components in dosage form production. The goal of Preformulation research is to create an elegant, stable, effective, and safe dosage form by creating a kinetic rate profile, compatibility with other components, and establishing physicochemical parameters of novel therapeutic compounds. Among these features, drug solubility, and stability are crucial in Preformulation research. Polymorphism with crystal and amorphous forms of the drug molecule results in different chemical, physical, and therapeutic descriptions. This article describes several qualities and strategies for evaluating medication Preformulation Studies of S-Equol.

Keywords: S-Equol, Preformulation Studies, Molecular weight, Melting point, Stability

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INTRODUCTION

Preformulation checking out is step one in the rational improvement of dosage styles of a drug substance. Preformulation research have been advanced in the 1950 & early 1960. It may be described as research of physical and chemical residences of a drug substance by myself and whilst mixed with excipients. Preformulation investigations are designed to deliver all vital facts especially Physicochemical, Physicomechanical, and Biopharmaceutical residences of drug substances, excipients, and packaging materials. The standard goal of Preformulation testing is to generate facts beneficial to the formulator in growing strong and bioavailable dosage paperwork that may be mass-produced1.

Soy foods have commonly been consumed for centuries in northeast Asia of which observational studies show a significant inverse association of dietary intake of soy foods with coronary heart disease (CHD)2. Akira Sekikawa observed that dietary intake of soy isoflavones had a significant inverse association with coronary heart disease (CHD) in Asia. According to evidence from observational studies and short-term RCTs, S-equol is anti-atherogenic, improves arterial stiffness, and may prevent CHD and cognitive impairment/ dementia. Long-term (2-year) RCTs that are well-designed should be pursued. Potentially, soy isoflavones, especially S-equol, are protective against cognitive decline/dementia3.

Premenstrual syndrome (PMS) affects many women's quality of life by causing a variety of psychological, behavioral, and physical symptoms. So, in this study, they estimated the natural S-equol for premenstrual syndrome by enrolling 124 women (aged 20–45 years) with PMS symptoms who are non-equol producers in a double-blind, parallel, randomized, placebo-controlled trial in which they will receive either a natural S-equol supplement (equol 10 mg twice daily) or placebo orally twice daily for three menstrual cycles. During the intervention cycles, the primary outcome measure will be examined. The mean differences in the Daily Record of Severity of Problems total score between the two groups will be computed to compare the primary outcomes between the S-equol and placebo groups. The student's t-test will be used to calculate the p values, with a significance threshold of 5%. (two-sided)4.

Material And Methods

Materials and instruments

List of Chemicals

Table 1: List of Chemicals

S.No	Chemical & API	Manufacturer/ Suppliers
1	S-Equol	Liaoning Kuke Biotechnology Co., Ltd, China
2	Cremophor EL (Kolliphor EL)	Sigma Aldrich Pvt. Ltd. (Bangalore, India)
3	Capryol 90, Isopropyl Myristate, Labrafil	Gattefosse, St-Priest, France
4	Ethanol, Coconut oil, olive oil, Ground nut oil, Soybean oil, sunflower Oil, Castor oil, Oleic acid	Vijaya Enterprises, Hyderabad
5	Methanol(Industrial solvent), DMSO	A.R chemicals, Hyderabad

All the chemicals and excipients were used when received.

Instruments

Table 2: List of Instruments

S.No.	Name of instruments	Manufacturer
1.	Electronic balance	Sartorius, Bangalore, India
2.	UV/Visible spectrophotometer	T60 UV series, LAB INDIA Analytics, Hyderabad
3.	Differential scanning calorimetry	DSC 204 F1 PHOENIX instrument
4.	ATR-FTIR	Bruker EQUINOX 55 FTIR spectrophotometer
5.	XRD	X-ray Diffractometer JDX-3532
6.	NMR spectroscopy	Bruker Av-111 400 Spectrometer(Germany)
7.	Mass spectroscopy	Xevo G2-XS QT of Quadrupole Time-of-Flight mass Spectroscopy(USA)

Preformulation Studies

Preformulation research is to create an elegant, stable, effective, and safe dosage form by creating a kinetic rate profile, compatibility with other components, and establishing physicochemical parameters of novel therapeutic compounds.

Solubility

It is possible to define qualitative solubility as when the two phases are combined to form a homogeneous mixture. According to the introduction of combinatorial chemistry, then the properties of the newly developed active compound will get shift towards higher molecular weight and the lipophilicity of the compounds will get increase and resulting in a decrease in the aqueous solubility of the drug.

Table 3: Solubility criteria

Descriptive	Part of solvent required per part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	10,000 and over

Development of standard calibration curve:

A prepared stock solution of S-equol was made by mixing 10 mg of S-equol in 10ml of Ethanol to give a stock solution of a concentration of 1000µg/ml. From the stock solution,1ml of a solution is taken and makeup to 10ml by a solvent which is 100 µl/ml. From the second stock solution different aliquots of 1ml, 2 ml, 3 ml, 4 ml, and 5 ml were taken in a series of 10ml volumetric flasks, and volumes were made up using Ethanol to get concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml. Thereafter absorbance of each sample was measured within the range of 200 to 400 nm by a UV spectrophotometer. Finally, by taking the concentration on the X-axis and absorbance on the Y-axis, a standard calibration curve was plotted.

Compatibility Studies

Fourier transform infrared spectroscopy (FTIR)

Bruker EQUINOX 55 FTIR Spectrophotometer is used to analyze FT-IR and the spectra were recorded in the region of 4,000 to 400 cm−1 from Osmania university, Hyderabad. The procedure consisted of dispersing a sample (Pure drug and compatibility with excipients) in KBr (200-400 mg) and compressing it into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and a spectrum was obtained.

Differential scanning calorimetry (DSC)5

DSC-60 is used to perform DSC from Osmania university, Hyderabad. DSC is a thermal analysis apparatus measuring how the physical properties of a sample change, along with temperature against time. The pure drug was placed in aluminum pans, crimped, and then heated under a nitrogen flow (30 mL/min) at a scanning rate of 5oC/ min from 25oC to 200oC. Aluminum pans containing the same quantity of indium were used as references. The heat flow as a function of temperature was measured for the drug.

Powder X-ray Diffraction (P-XRD)6

Powder X-ray diffraction analysis was performed to verify the new solid-state formation. P-XRD analysis was conducted for unprocessed NOR and processed NOR (NOR-5) using X-ray Diffractometer JDX-3532 (Osmania University, Hyderabad). Cu Kα radiation in a scanning range of 2θ = 5◦–50◦ was used with tube current 30 mA, operated voltage of 40 kV, step time of 1.0 sec, step size of 0.05◦, divergence slit of 1 degree, scattering slit of 1.0 degree, and receiving slit of 0.2 mm for measurement.

Nuclear Magnetic Resonance (NMR) Spectroscopy7

Nuclear magnetic resonance (NMR) is a key spectroscopic method in analytical and bioanalytical chemistry. The nature and kinds of covalently bound nuclei (hydrogen or carbon) within a molecule can be determined via NMR. It estimates the number of such nuclei under observation using peak area integrations. Recently, several researchers have used NMR to describe chemical compounds, including quantitative 1H, 31P, and 13C-NMR and semi-quantitative HSQC-NMR. The operating frequency of current NMR spectrometers has risen to over 1 GHz.

Mass Spectroscopy8

Mass spectrometry is used to accurately measure the mass of the various molecules within a sample. The four stages of mass spectrometry are – ionization, acceleration, deflection, and detection. The sample is vaporized before being passed into an ionization chamber where it is bombarded by a stream of electrons emitted by an electrically heated metal coil. Because ions are very reactive and have a limited lifetime, they must be formed and manipulated in a vacuum. The atmospheric pressure is around 760 torr (mm of mercury). Ions may be handled at pressures ranging from 10-5 to 10-8 torr (less than a billionth of an atmosphere).MS(ESI) Calculated for C15H14O3:242.274g/mol; Found: 244.604 (M+ +2).

Results And Discussion

Preformulation Studies

The Preformulation studies were performed based on calibration cure in the present study

Physical Properties:

Colour and Appearance: White.

Odour: No Odour.

Melting point: 181ºC -189ºC.

Molecular Weight: 242.27.

Wavelength: 275nm.

Molecular wt: 242.27 g/mol

Structure of S-Equol:

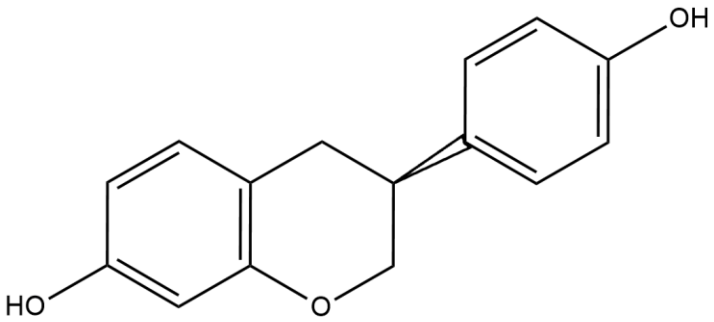


Figure-1:S-Equol Structure

Solubility Study

Solubility studies of S-equol are done in various Oils, Solvents, and Distilled water (Figure-1). The oils used are castor oil, Labrafil, Sunflower oil, Soybean oil, Capryol 90, Isopropyl myristate, Ground nut oil, Olive oil, Coconut oil, Oleic acid, DMSO, Methanol, and Ethanol was determined by adding an excess amount of drug to 1 mL of selected oils and distilled water separately in 5-mL Ria vials and mixing using a vortex mixer. The vials were then kept at 25 ± 1.0-C in an isothermal shaker (JSS College of Pharmacy, Ooty, India) for 72 hours to reach equilibrium. The vials were observed for solubility of the Drug and noted.

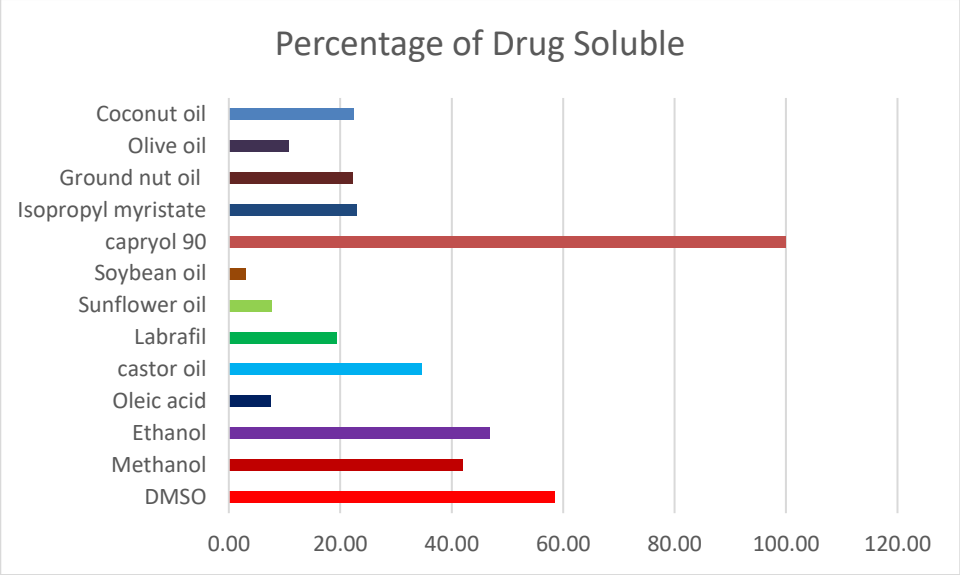


Figure 2: Solubility Profile

Standard Calibration Curve

The maximum wavelength of the Drug observed by UV spectroscopy is 275nm. The drug's calibration curve was produced and it was found that a perfect correlation was observed. The complete linearity between the concentration and absorbance was seen when the concentration ranged from 10µg/ml to 50µg/ml, based on the regression value (R2= 0.9944). Table 4 contains information on the relationship between absorbance and concentration. Figure 3 depicts the Calibration curve.

Table 4: Concentartion and Absorbance.

Concentration	Absorbance
0	0
10	0.252
20	0.585
30	0.901
40	1.166
50	1.363

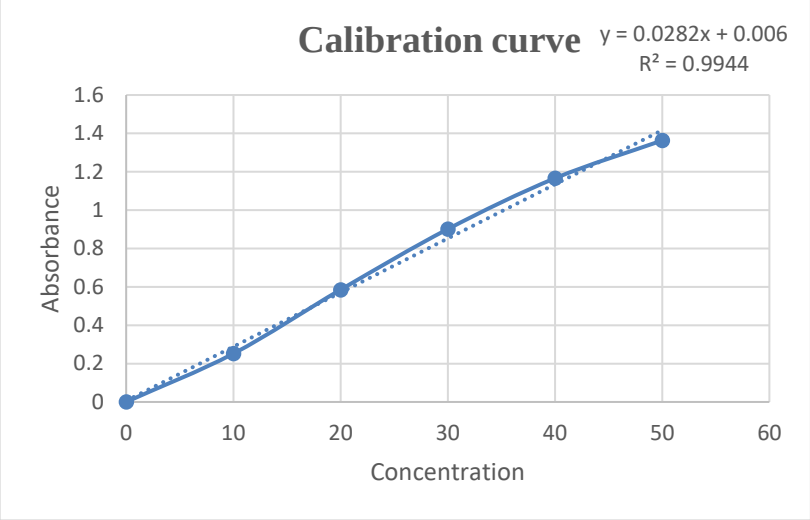


Figure 3: Standard calibration curve

FT-IR

FT-IR of S-Equol

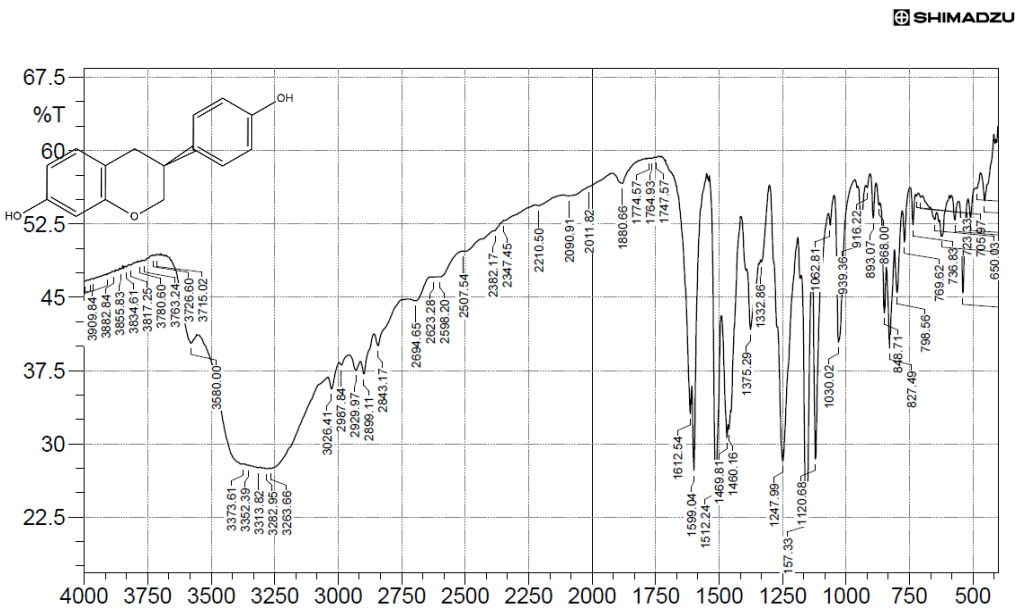
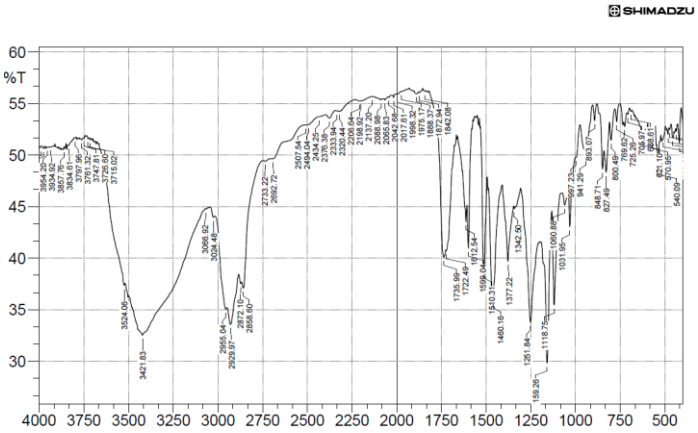


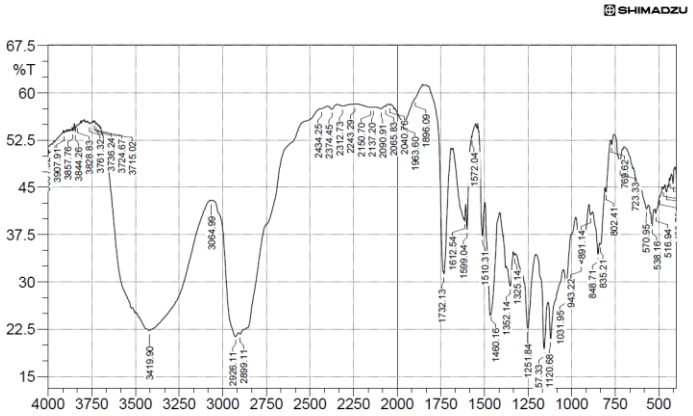
Figure 4:IR Spectroscopy of S-Equol

3580.00 cm-1	O-H Stretching in a hydroxyl group
3026.41 cm-1	-C=C- Stretching
2843.17 cm-1	-C-H- Stretching
1030.62 cm-1	-C-O-C- Bending
1460.16 cm-1	=CH2 Stretching



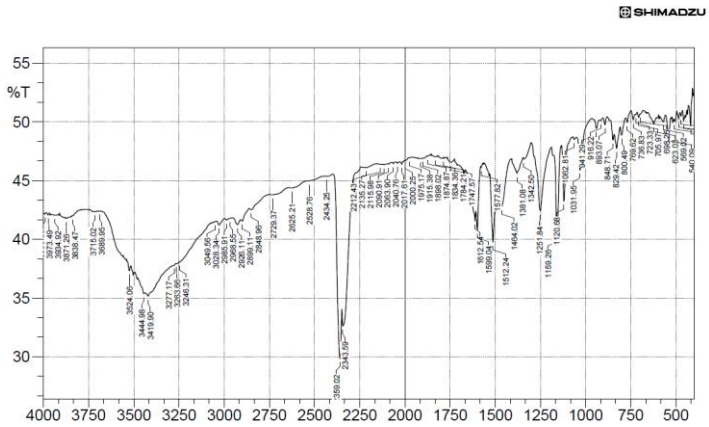
3524.06 cm-1	O-H Stretching in a hydroxyl group
3024.48 cm-1	-C=C- Stretching
2858.60 cm-1	-C-H- Stretching
1031.95 cm-1	-C-O-C- Bending
1460.16 cm-1	=CH2 Stretching

Figure 5:FT-IR of S-Equol with Capryol 90



3419.90 cm-1	O-H Stretching in a hydroxyl group
3064.99 cm-1	-C=C- Stretching
2899.11 cm-1	-C-H- Stretching
1031.95 cm-1	-C-O-C- Bending
1460.16 cm-1	=CH2 Stretching

Figure 6:FT-IR of S-Equol with Cremophor



3524.06 cm-1	O-H Stretching in a hydroxyl group
3028.34 cm-1	-C=C- Stretching
2899.11 cm-1	-C-H- Stretching
1031.95 cm-1	-C-O-C- Bending
1464.62 cm-1	=CH2 Stretching

Figure 7:FT-IR of S-Equol with Ethanol

Differential scanning calorimetry (DSC)

In this study, DSC was used to investigate the interactions and thermal stability of the drug (S-Equol). In the present study, DSC experiments involved the study of thermal behavioral for pure drugs. The DSC thermogram of S-Equol exhibited a sharp endothermic peak at 181.44°C.

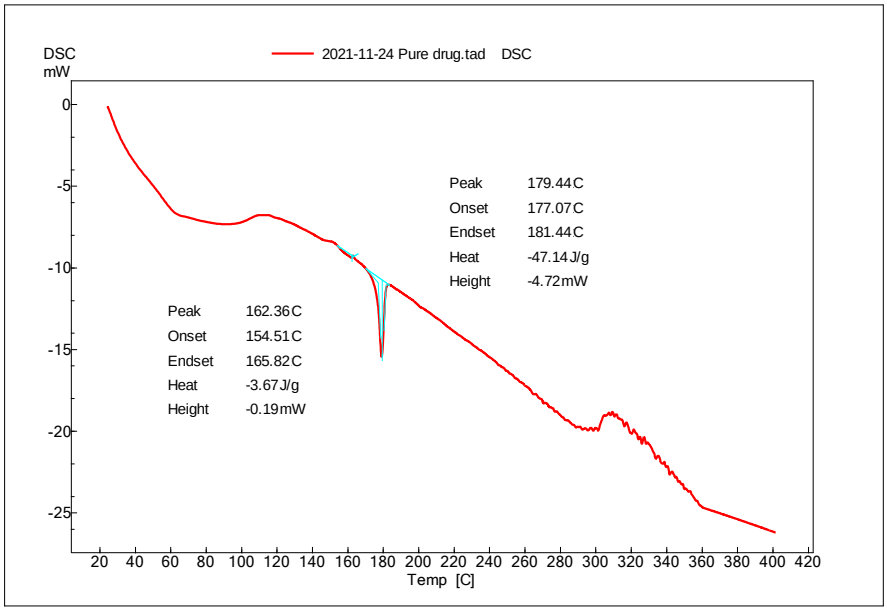


Figure 8: Differential scanning calorimetry of S-Equol

Powder X-ray diffraction

Powder X-ray diffraction was performed to determine the crystallinity of the fabricated drugs. The unprocessed formulation showed sharp peaks indicating its anhydrous crystal nature, while for processed S-Equol, some of these peaks were diffused indicating its conversion to Crystalline form.

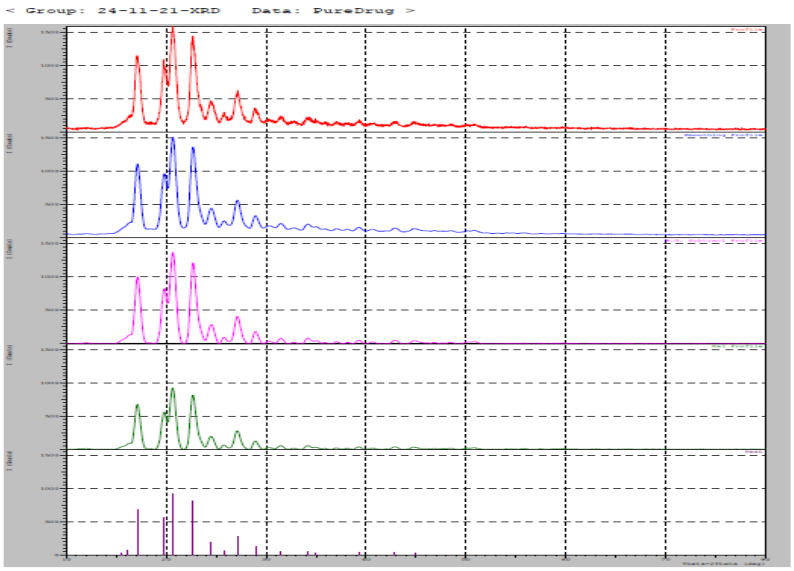


Figure 9: XRD Study of S-Equol

Nuclear Magnetic Resonance (NMR) Spectroscopy

1H NMR Spectrum of Pure Drug(400 MHz, DMSO-d6) δ (PPM): 2.8 (s, 2OH), 6.1, 6.4, 6.8, 6.8, 6.9, 7.1 (s, 7H), 3(1-RC3-H), 3.8-4.2(2-R-CH2-R)

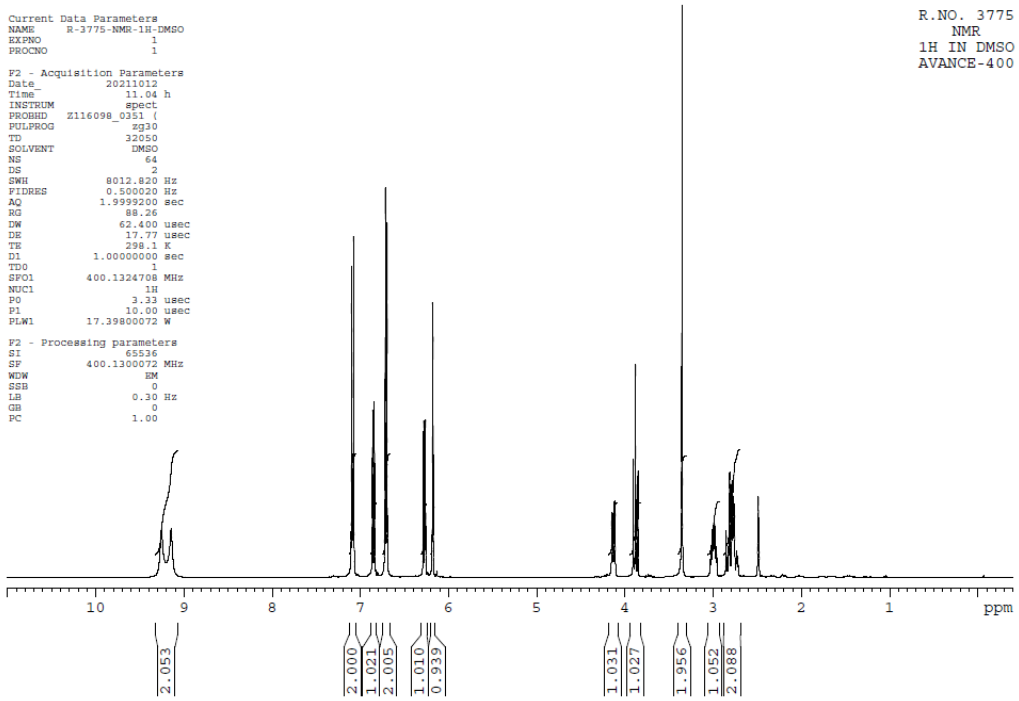


Figure 10: 1H NMR Spectrum of S-Equol

13C NMR Spectrum of Pure Drug(400 MHz, DMSO) δ (PPM): 156.52, 156.17, 154.54, 131.68,130.11,128.34,115.29,112.60,102.52,70.29,40.12,39.91, 39.70,39.49,31.33.

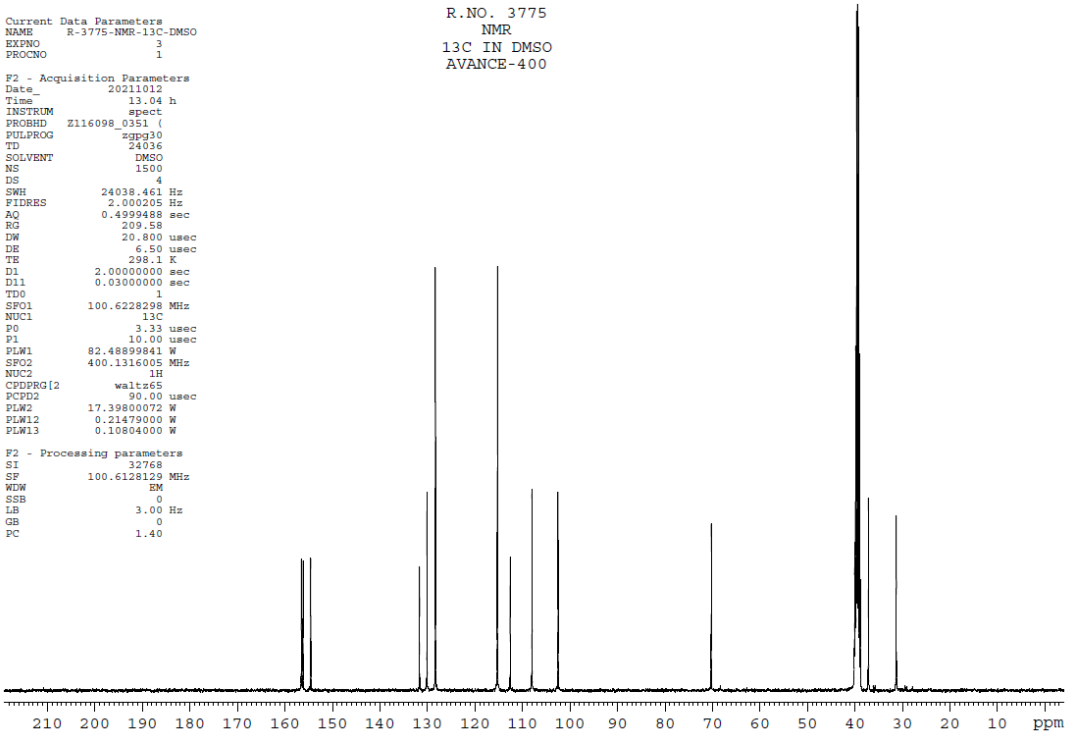


Figure 11: 13C NMR Spectrum of S-Equol

Mass spectroscopy: MS(ESI) Calculated for C15H14O3:242.274g/mol; Found: 244.604 (M+ +2).

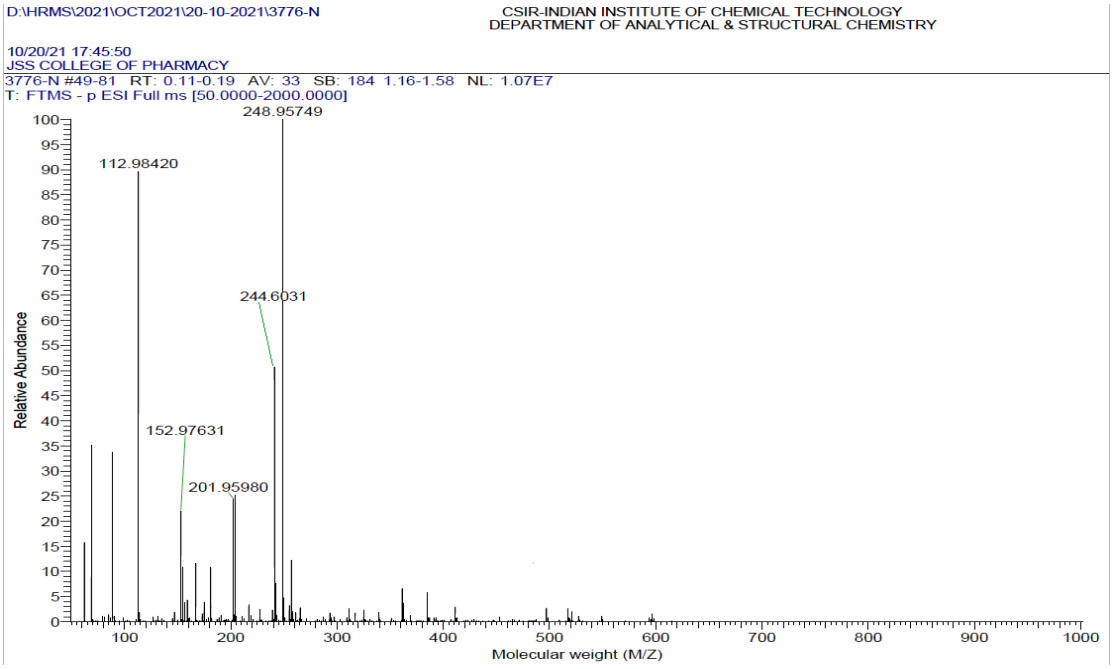


Figure 12: Mass Spectrum of S-Equol

Conclusion

Preformulation research has an extensive part to play in looking ahead to system issues and figuring out logical paths in each liquid and stable dosage shape Technology. By analyzing the physicochemical features of each drug candidate within a therapeutic group, the Preformulation scientist may help the synthetic chemist choose the best molecule and give the biologist appropriate vehicles for eliciting a pharmacological response. In parallel solid-state stability was performed by DSC and XRD. The purity of the compound was identified with NMR, Mass Spectroscopy, and Melting Point. Hence the purity is 98% the drug is future used for Preformulation studies like solubility. The compatibility studies have been done by FT-IR with the Excipients. The Results Show that there is no interaction of the Drug with the Excipients which can then proceed to formulation and optimization. This research article gives details of the above studies concerning any Nanodroplet forms that can be developed with Preformulation studies.

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