**Extraction of Sulforaphane From Broccoli Seeds, its determination by Gas Chromatography/Mass Spectroscopy and its interaction with HDAC**

*A Major Project dissertation submitted*

*in partial fulfilment of the requirement for the degree of*

**Master of Technology**

**In**

**Bio Medical Egineering**

*Submitted by*

**Triyambika Goswami**

**(2K15/BME/12)**
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*Under the supervision of*

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**CERTIFICATE**

This is to certify that the M. Tech. dissertation entitled **“”Extraction of Sulforaphane From Broccoli Seeds, its determination by Gas Chromatography/Mass Spectroscopy and its interaction with HDAC**, submitted by **Triyambika Goswami (2K15/BME/12)** in partial fulfilment of the requirement for the award of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate’s own work carried out by him/her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

**Date:**

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(Project Mentor)
Department of Bio-Technology
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**ACKNOWLEDGEMENT**

First and foremost , my sincere thanks to Dr. D. Kumar, Head, Department of Biotechnology, Delhi Technological University, for giving me an opportunity to study and work in this prestigious institute.

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TRIYAMBIKA GOSWAMI
2K15/BME/12

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## LIST OF ABBREVIATIONS

SFN- Sulforaphane
ROS- Rich Oxygen Species
NRF2- Nuclear Factor Erythroid 2- related factor 2
HAT- Histone Acetyl Transferase
HDAC- Histone Deacetylase Transferase
ALL- Acute Leukocyte
ARE- Antioxident Response Element
CoQ10-Coenzyme Q10
SAHA-Suberoylanilide Hydroxamic Acid
GSH- Glutathione
QR- Quinone Reductase
GC/MS- Gas Chromatography/Mass Spectroscopy

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## ABSTRACT

Cancer is the reason for approximately 13 % of death. Other health issues like cardiovascular disorders, lung inflammation, diabetes, kidney failure etc also affecting people's daily life. People are making their interest for fruits and vegetables rather than medicine because fruits and vegetables contain secondary metabolites which are necessary for good health. In this project I have focused on broccoli extract that is sulforaphane (isothiocyanate). Sulforaphane behaves like antioxidant, antidiabetic, anticarcinogen, anti inflammatory substance etc. I extracted sulforaphane from Broccoli seeds by using liquid liquid phase extraction method which follows crude preparation and it is determined by GC/MS technique.GC/MS does Sulforaphane's 2D and 3D structure is formed by ACD/3D Chem sketch software. In this project interaction of Sulforaphane and HDAC1 is shown by Docking. By Docking amino acids which are interacting with sulforaphane are characterized and free energies which are reqired its interaction is calculated.

## INTRODUCTION

Daily consumption of fruits and vegetables are important to fight against cancer such as lung, oral cavity, larynx ,oesophagus, stomach, pancreas, bladder , prostate, cervix malignancies, ovary, endometrium and breast cancer (Anand, P et al,2008) . Some observations and demonstrations have shown that Cruciferous vegetables like broccoli, cabbage, cauliflower, broccoli sprouts, Kale, watercress can decrease cancer risk and numerous disease.( van Poppel, G., et al. ,1999; Kohlmeier, L.,1997). Broccoli contains sulforaphane( anticancer agent), antioxidants such as vitamin C, quercetin and kaempferol (Zhang, Y et al., 2007,Vallejo, F et al.,**2002**, Koh, E et al.. **2009**) . Among these, sulforaphane [l-isothiocyanato-4-(methylsulfinyl)-butane] is obtained by hydrolysis of glucoraphanin in the presence of myrosinase enzyme (Kore, A. M, 1993).When broccoli is chewed or cut then myrosinase enzyme is activated(A. Yanaka,2007). Sulforaphane has HDAC inhibition activity. It inhibits the activity of class 1 and class 2 HDAC (Myzak MC, et al. 2004).There are other various HDAC Inhibitor like vorinostat, SAHA, Valproic acid which can be used in the combination with sulforaphane to treat many disease.

Sulforaphane reduces ROS Species by inducing( NRF2 transcription factor) via ARE-NRF2 signal Pathway and acts as anticarcinogen( sporn , M.B et al., 2012). Oxidative stress is involved in cardiovascular disorders which is responsible for congestive heart failure, myocardial ischemia, atherosclerosis and chemical induced cardiac tonicity (Molavi, B. et al, 2004). Sulforaphane has shown lower production of ROS and high viability and it reduce oxidative stress (Wu, L.Y., et al,2004).SFN can reduce inflammation of heart, kidney, artery and CNS (Noyan-Ashraf,2005). SFN with its antioxidant function treats retinitis pigmentosa in which photoreceptor cells of eyes are killed and caused blindness. SFN upregulate the retinal levels of TRX, trxR and NrF2 and protect photoreceptor cells (Kong, L., et al., 2007).40 µg/kg SFN prevented streptozotocin (STZ)- induced pancreatic islets apoptosis and diabetes SFN also protects Cell apoptosis and dysfunction which are mediated via NrF2 activation and suppression of transcriptional factor NF-KB pathway (Song, M.Y., et al.,2009).Sulforaphane upregulate aortic Nrf2 expression and transcription. SFN reduces oxidative stress and inflammation which are major cause of diabetic peripheral neuropathy (Giacco, F. et al.,2010). SFN provides neuroprotective effect by activating NrF2 and inhibiting NF-KB signaling. ischemia-induced renal damage is treated by sulforaphane via induction of Nrf2 (D. H. Shin;2010).

Sulforaphane is extracted from Broccoli seesds by the method of liquid liquid phase extraction. Organic solvents like ethyl Acetate, Hexane are used for this method (Kore.A.M.,1993). Gas Chromatograph/Mass Spectroscopy is used to determine sulforaphane in Broccoli Extract (VanEtten et al., 1976; Cole,1976). GC/MS method is reliable and more efficient method to determine sulforaphane. Sulforaphane is effective medicine for many disease. Sulforaphane attracts many researchers because it is a chemopreventive agent. It also has higher healing capacity. There are various bio informatics tools are available (online and offline) which can characterize the sulforaphane.

## REVIEW OF LITERATURE

Sulforaphane (4 – methylsulfinylbutyl isothio cyanate) is an isothiocyanate which is found in cruiferous vegetables ex. Broccoli seeds, broccoli sprouts, Kale seeds, cauliflower etc. Criciferous vegetables contain glucoraphanin, when these vegetables ground or chopped myrosinase enzyme (thio-gluco-side glucohydrolase) converts this glucraphanin into sulforaphane (Fahey, J, W, et al.2001)

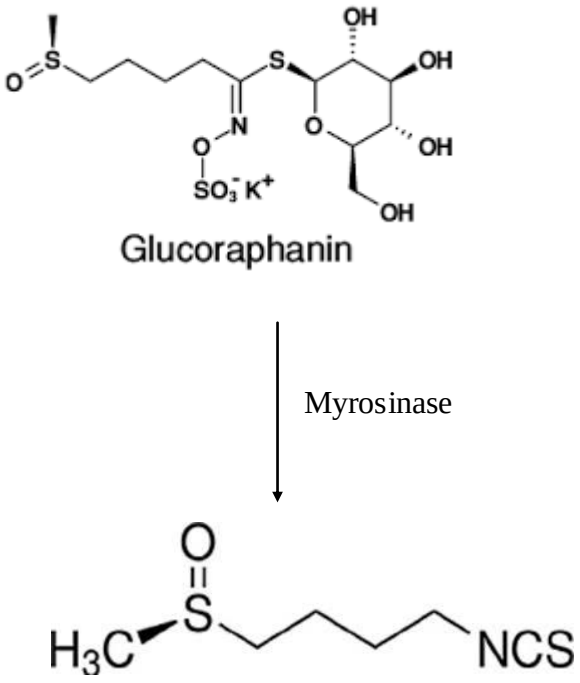


Figure 1 : Formation of Sulforaphane

Sulforaphane is principal inducer of phase II enzymes which are having anticarcinogenic activity (Shang, et al, 1992). Natural Sulforaphane is mainly extracted from broccoli seeds because it has large amount of oil content in broccoli seeds. There is traditional extraction method which includes liquid liquid exhibition (kore, A.M et al. 1993). Various biological effects of sulforaphane are seen in animals and clinical trails. For purification liquid liquid phase extraction is performed (Kore, A.M et al. 1993)

**Functions of Sulforaphane**

SFN can reach high intracellular and plasma concentration due to its good bio availability (Myzak, M.C.et al. 2004). Sulforaphane has anti cancerous, ant diabetic, antioxidant activity it is also useful to treat cancer, Cardiovascular diseases, Acute and chronic lung inflammation, diabetes and other kidney problems.

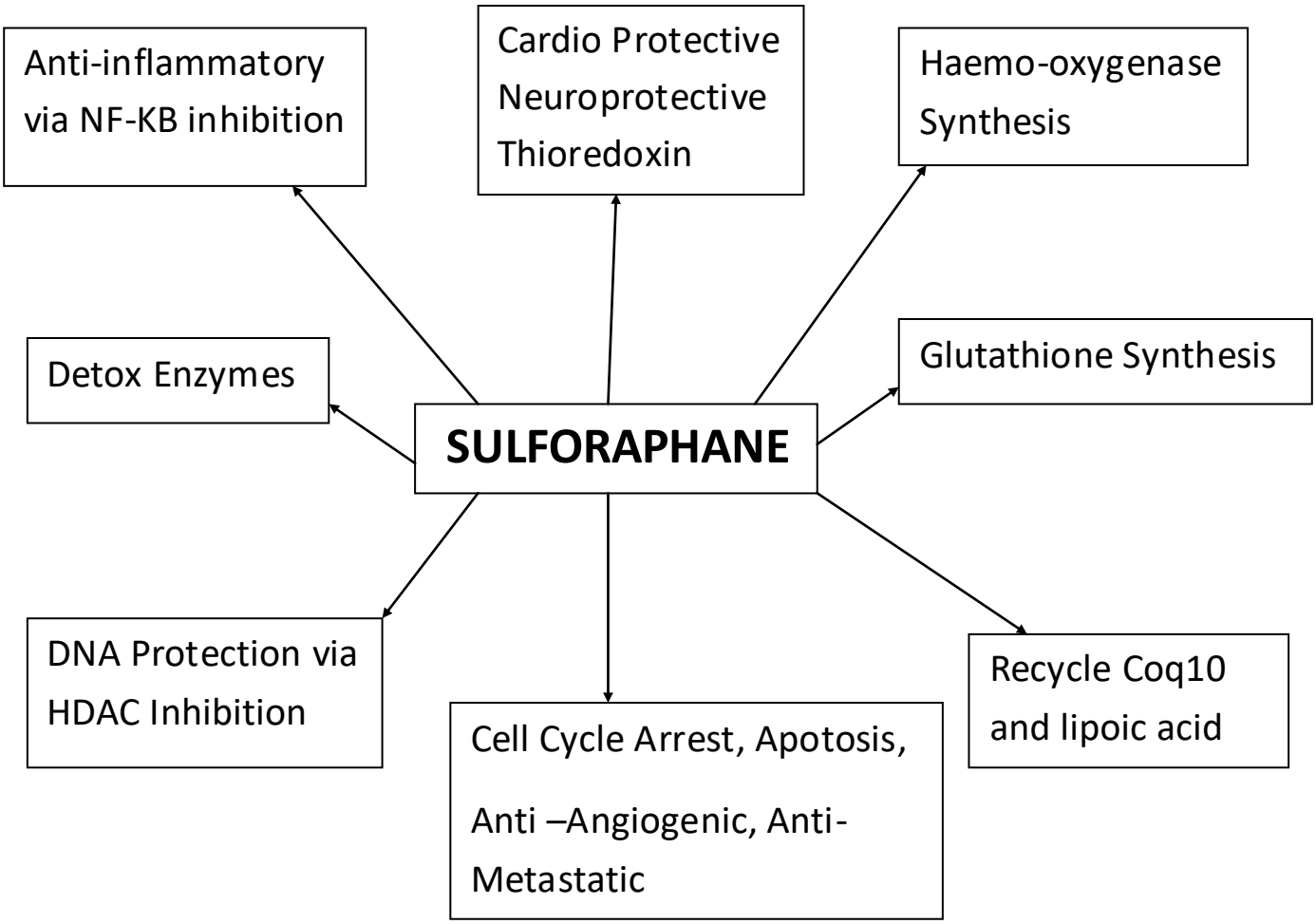


Figure 2 : Function of Sulforaphane

1. Anticancer activity:-

There are various mechanism by which sulforaphane can treat cancer

(1) Cancer cells have increased aerobic glycolysis due to oxidative stress (ROS is accumulated) So when ROS is increased, NRF2 (transcrption factor) Stimulates antistress signaling and suppress oxidative and electrophilic toxicants and inhibit carcinogenesis (sporn , M.B et al., 2012, Lee J.S. et al. 2005), Surforaphane induces phase-II carcinogen detoxification enzymes via ARE –NRF2-Pathway like glutathione trasnferase, NAD(P)H, UDP-glucucronyltransferase and HO-1, which eleminates or decreases electrophilic and oxidative toxicant. (Dinkova-Kostova, A.T.et al 2002). Sulforaphane induces NRF2, ROS depletion is done via NRF2 and depletion of ROS reduces cancirogens (Manda, G;et al 2009)

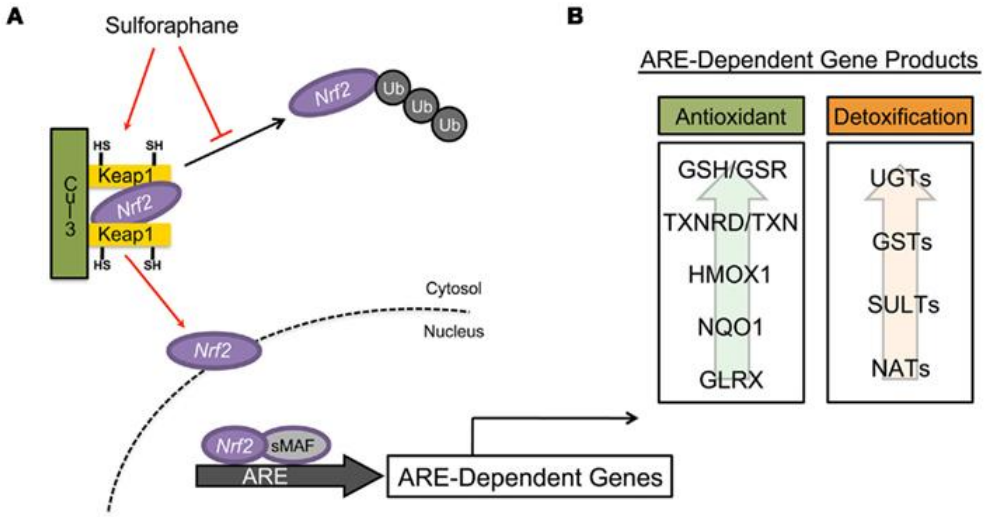


Figure 3: NRF2 mediated anticancerous activity of Sulforaphane

(2) Sulforaphane is well known as HDAC inhibitor. Histone acetylation results chromatin in open form, in this state gene regulation is occurred and histone deacetylation results chromation in closed form (gene-regulation not done). Basset, S.A;et al. 2014) so histone aceylation mechanism depends on HATs and HDACs Struhl, K.et al 1998). There are various HDAC inhibitors known eg. SAHA, Valproic aeed, depsipeptide, sodium butyrate etc.) which are effective against cancer cell line and xenograft models (Fronsdal, K;et al 2005). Sulfroaphane reduce the HDAC activity and down regulation of HDAC proteins which increase histone H3 accetylation at P21 promoter and tubulin and promote cell death (Clerka, J.D. et al. 2001)

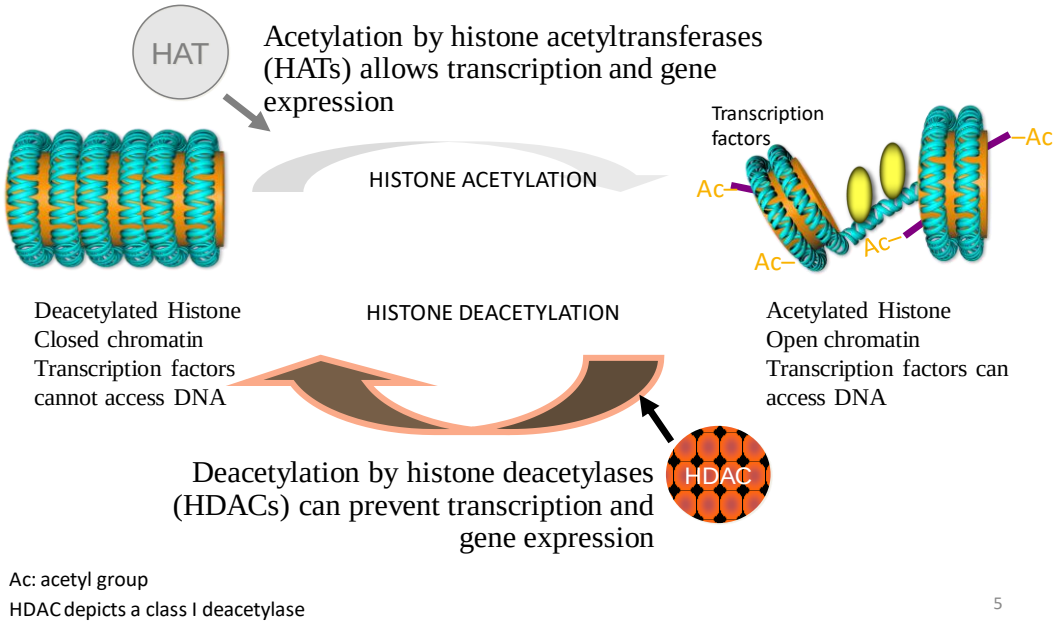


Figure 4 : Histone Acetylation Mechanism

(3) Sulforaphane inhibits cell cycle progression and induces apoptosis in precursor of cancerous cells and tumor cells of different origin. 5-10 µmol/L does not SFN reduces G1 phase cell distribution and induces cancerous cells (HCT/16) apoptosis. 30 µM dose of SFN treatment decreases level of cell cycle regulatory protein cyclin A, cyclin B1 and CDC2 was observed in breast cancer cell (Kanematsu, S. et al. 2010). Treatment of sulforaphane on acute lymphoblastic leukemia (ALL) is dose dependent apoptonis and G2/M cell cycle arrest which is associated with the activation of caspases, p53 independent upregulation of p21, PARP inactivation and inhibition of the Cdc2/Cyclin B1 complex (Zoheny, M.et al. 1982).

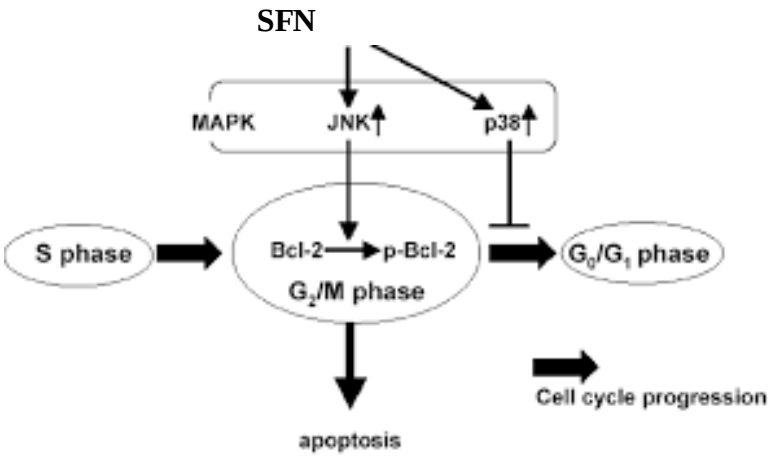


Figure 5: Apoptosis and Cell Cycle Progression by Sulforaphane

(4) Sulforaphane alters cellular signaling and impeding the potential of cancer cells. SFN inhibits multiple oncogenic signaling pathway including NF-KB, Akt; signal transducer and activator of transcription 3 and 5 and many survival pathway proteins(Bhamre, S.et al. 2009).

Health benefits of SFN with its mechanism

| Disease | SFN treatment mechanism |
|---|---|
| (1) Cancer | induction of cell cycle apoptosis cell cycle arrest Induction of NrF2 depletion of ROS |
| (2) Oxidant induced respiratory Disorders | Induction of mucosal phase 2 enzymes, activation of GST and quinine Reduclase |
| (3) Neurodegenerative disorder Cerebral, ischemia, Alzheimer's | Upregulation of anti-oxidant gene acetyl-choline-esterase |
| (4) Diabetes | Oxidative stress reduction |
| (5) Kidney disease | NRF2 induction |
| (6) Ocular disease | NrF2 upregulation of retinal GSH & QR |
| (7) Cardiovascular disease | NrF2 mediated reduction n proinflammatory state |

Table 1 : Sulforaphane's Health Benefit and its Working Mechanism

## METHODOLOGY

MATERIALS AND CHEMICALS REQUIRED

1. 50gm broccoli seeds
2. Glasswares including beaker, conical flask, seperating funnel,stand for seperating funnel, funnel, .22µm filter, whatman filter paper, vaccume filter
3. Ethyl acetate, hexane, anhydrous sodium sulfate, ethanol, methylene chloride, distilled water

METHODOLOGY

CRUDE EXTRACT PREPARATION

1. 50 gm broccoli seeds are taken and homogezed in an analytical grinder
2. 300ml distilled water is added to ground seeds
3. This mixture is kept at 25 degree for 2 hours for spontaneous autolysis
4. Filter this mixture with the help of whatman filter paper
5. Filterate is extracted 3 times with 300ml ethyl acetate
6. Combine all extracts and dried at 35 degree in a rotavapour under vaccume
7. Crude extract is produced
8. For removing impurities and enrichment of sulforaphane, crude extract is treated with liquid liquid phase extraction


Figure 7 : Broccoli seeds in Water after Filteration via whatman filter

Figure 8 : Three times extraction with ethyl acetate

Figure 9 : Mixture of above resultant


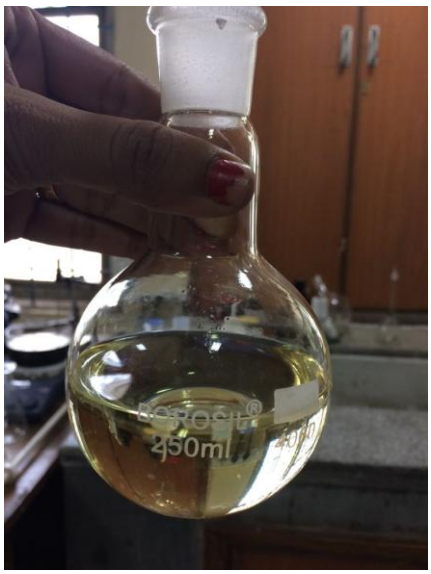


Figure 10 : (a) Mixture in rotavapour (b) Mixture in ethanol

LIQUID LIQUID PHASE EXTRACTION

1. Crude extract is dissolved in 300 ML ethanol (10% (V/V).
2. Wash 3 times with 300 mL of hexane so that non polar contaminant can be removed.
3. Aquous phase will be got
4. Extract 3 lines with 300 ml ethyl acetate.
5. Dry the mixture with 10gm of anhydrous sodium sulfate
6. Filter through a 22 um membrane
7. Dry at $35^0$C under vaccine in a rotary evaporator.
8. Suoforaphane rich extract is got
9. Now it will go for GC-MS for determination of Sulforaphane.

Figure 11 : Three times washing with Hexane

Figure 12 : Three times extraction with Ethyl Acetate

Figure 12 : Sulforaphane rich Extract

Gas Chromatography/Mass Spectroscopy for determination of sulforaphane

1. Concentrated extract is dissolved in 10 ml methylene chloride before inject into GC/MS
2. The split/splitless injector was operated in splitless mode using a 4 mm inside diameter (i.d.) injection liner (Hewlett-Packard, Palo Alto, CA). An HP-5MS fused silica capillary column (Hewlett-Packard, 30 m, 0.25 mm i.d., 0.25 $\mu$m film thickness, cross-linked to 5% phenyl methyl siloxane stationary phase) was used.
3. MS ChemStation software (Hewlett-Packard, version B.02.04 controls the entire mechnism). Temperature of Injector and detector were 250 and 300 °C, respectively. Column oven temperature was initially set at 40 °C for 2 min, then increased to 270 °C (ramp, 10 °C/min), and held for 5 min.
4. To maintain desired carrier gas flow rates for the various experimental conditions EPC is used.
5. For the constant flow conditions, the electronic pressure controller was programmed to maintain a flow rate of 1.0 mL/min throughout the chromatographic separation.
6. For fast initial injection flow conditions, the carrier gas flow rate was programmed for an initial pressure of 25.0 psi (3.0 mL/ min). After 1 min, the pressure was reduced at a rate of 20.0 psi/min to a pressure of 7.1 psi (1.0 mL/min).
7. To maintain a constant 1.0 mL/min flow rate throughout the chromatographic separation, the pressure was increased at a rate of 0.47 psi/ min to a final pressure of 20.3 psi, which was then held for an additional 5 min. Mass spectra were obtained by electron ionization (EI) over a range of 50-550 atomic mass units. Ion source temperature was 177 °C, and the electron multiplier voltage was 1753 eV.

In silico methods for designing structure of sulforaphane and its interaction with histone proteins

1. Download the ACD/3D Chem Sketch Software
2. Draw the elemnt and bonds between chemicals
3. Press the icon for 3D structure
4. Docking is done by using online docking tool
5. HDAC and Sulforaphane interaction is shown in this

## RESULTS

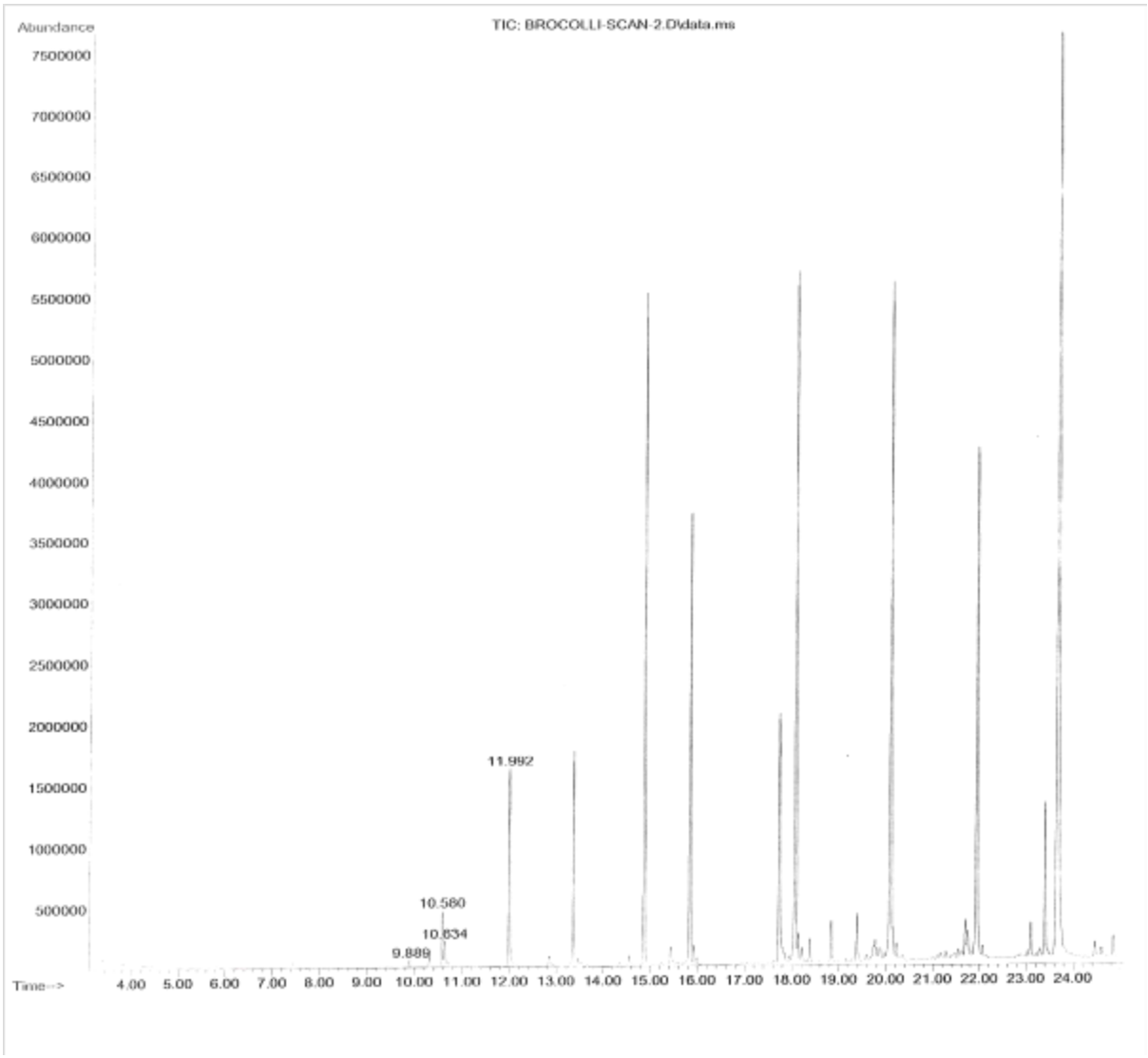


Figure 13 : GC/MS Graph for determination of sulforaphane

Data Path : C:\msdchem\3\DATA\BROCCOLI\
Data File : BROCOLLI-SCAN-2.D
Acq On : 10 Jul 2017 15:15
Operator :
Sample : BROCOLLI-1.D
Misc : SCAN- METHOD
ALS Vial : 16 Sample Multiplier: 1

72, 55, 160

RT – 23.667

Sulforaphane

Integration Parameters: events2.e
Integrator: ChemStation

Method : C:\msdchem\1\METHODS\BROCCOLI.M
Title : PCB

Signal : EIC Ion 72.00 (71.70 to 72.70): BROCOLLI-SCAN-2.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.338 | 712 | 742 | 780 | BV 9 | 1963 | 114986 | 15.50% | 4.397% |
| 2 | 10.312 | 1255 | 1262 | 1274 | VV | 658 | 13829 | 1.86% | 0.529% |
| 3 | 10.581 | 1298 | 1309 | 1324 | BV 2 | 815 | 16454 | 2.22% | 0.629% |
| 4 | 11.991 | 1540 | 1555 | 1567 | BV | 612 | 13083 | 1.76% | 0.500% |
| 5 | 13.358 | 1775 | 1794 | 1805 | BV | 3182 | 61146 | 8.24% | 2.338% |
| 6 | 13.462 | 1805 | 1812 | 1819 | VV | 605 | 9944 | 1.34% | 0.380% |
| 7 | 14.871 | 2039 | 2058 | 2074 | PV | 13706 | 252545 | 34.03% | 9.657% |
| 8 | 15.428 | 2149 | 2156 | 2175 | PV | 658 | 18050 | 2.43% | 0.690% |
| 9 | 15.840 | 2209 | 2228 | 2236 | PV 2 | 7571 | 150200 | 20.24% | 5.744% |
| 10 | 15.918 | 2236 | 2241 | 2249 | VV | 1650 | 26776 | 3.61% | 1.024% |
| 11 | 17.728 | 2550 | 2558 | 2571 | VV | 4284 | 95095 | 12.82% | 3.636% |
| 12 | 18.073 | 2599 | 2618 | 2624 | VV | 13300 | 306404 | 41.29% | 11.717% |
| 13 | 18.131 | 2624 | 2628 | 2634 | VV | 2139 | 36431 | 4.91% | 1.393% |
| 14 | 18.205 | 2634 | 2641 | 2663 | VV | 819 | 22240 | 3.00% | 0.850% |
| 15 | 18.382 | 2663 | 2672 | 2680 | VV 2 | 450 | 9767 | 1.32% | 0.373% |
| 16 | 19.374 | 2840 | 2845 | 2850 | PV | 574 | 8386 | 1.13% | 0.321% |
| 17 | 20.089 | 2953 | 2970 | 2976 | VV | 13889 | 322508 | 43.46% | 12.333% |
| 18 | 20.134 | 2976 | 2978 | 2988 | VV | 2045 | 35775 | 4.82% | 1.368% |
| 19 | 20.215 | 2988 | 2992 | 3000 | VV | 690 | 13860 | 1.87% | 0.530% |
| 20 | 21.150 | 3150 | 3156 | 3161 | VV 2 | 531 | 10603 | 1.43% | 0.405% |
| 21 | 21.926 | 3278 | 3291 | 3297 | VV 2 | 10526 | 230982 | 31.13% | 8.833% |
| 22 | 21.967 | 3297 | 3299 | 3308 | VV 2 | 1490 | 25741 | 3.47% | 0.984% |
| 23 | 22.051 | 3308 | 3313 | 3320 | VV | 674 | 11715 | 1.58% | 0.448% |
| 24 | 23.329 | 3527 | 3537 | 3545 | PV 5 | 459 | 11580 | 1.56% | 0.443% |
| 25 | 23.667 | 3577 | 3596 | 3606 | PV | 25836 | 742029 | 100.00% | 28.375% |
| 26 | 23.743 | 3606 | 3609 | 3619 | VV | 1036 | 23690 | 3.19% | 0.906% |
| 27 | 24.449 | 3723 | 3732 | 3745 | PV | 1030 | 22120 | 2.98% | 0.846% |
| 28 | 24.831 | 3786 | 3799 | 3807 | PV | 450 | 9165 | 1.24% | 0.350% |

Signal : EIC Ion 55.00 (54.70 to 55.70): BROCOLLI-SCAN-2.D\da

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 7.340 | 722 | 742 | 748 | BV 4 | 504 | 14753 | 0.10% | 0.022% |
| 2 | 7.457 | 748 | 763 | 790 | VV 9 | 4798 | 254935 | 1.72% | 0.374% |
| 3 | 9.889 | 1177 | 1188 | 1198 | PV | 2684 | 52678 | 0.36% | 0.077% |
| 4 | 10.313 | 1242 | 1262 | 1281 | PV | 5513 | 125456 | 0.85% | 0.184% |
| 5 | 10.454 | 1281 | 1287 | 1292 | VV 7 | 559 | 10947 | 0.07% | 0.016% |
| 121 | 23.427 | 3552 | 3554 | 3556 | VV 3 | 4925 | 67135 | 0.45% | 0.099% |
| 122 | 23.459 | 3556 | 3559 | 3569 | VV | 5299 | 138237 | 0.93% | 0.203% |
| 123 | 23.542 | 3569 | 3574 | 3576 | VV 2 | 3011 | 58897 | 0.40% | 0.086% |
| 124 | 23.667 | 3576 | 3596 | 3638 | VV | 423420 | 14810390 | 100.00% | 21.739% |
| 125 | 23.926 | 3638 | 3641 | 3655 | VV 10 | 3392 | 147511 | 1.00% | 0.217% |
| 126 | 24.026 | 3655 | 3658 | 3666 | VV 7 | 2260 | 72587 | 0.49% | 0.107% |
| 127 | 24.114 | 3666 | 3674 | 3680 | VV 9 | 1965 | 77068 | 0.52% | 0.113% |
| 128 | 24.258 | 3690 | 3699 | 3706 | VV 10 | 1434 | 65401 | 0.44% | 0.096% |
| 129 | 24.392 | 3715 | 3722 | 3727 | VV 5 | 1540 | 50465 | 0.34% | 0.074% |
| 130 | 24.453 | 3727 | 3733 | 3745 | VV | 7841 | 238472 | 1.61% | 0.350% |
| 131 | 24.537 | 3745 | 3748 | 3751 | VV 3 | 1981 | 35139 | 0.24% | 0.052% |
| 132 | 24.587 | 3751 | 3757 | 3760 | VV | 2687 | 67399 | 0.46% | 0.099% |
| 133 | 24.621 | 3760 | 3762 | 3769 | VV 5 | 1746 | 38092 | 0.26% | 0.056% |
| 134 | 24.712 | 3775 | 3778 | 3788 | VV 7 | 968 | 21915 | 0.15% | 0.032% |
| 135 | 24.833 | 3788 | 3799 | 3814 | PV | 7504 | 241088 | 1.63% | 0.354% |
| 136 | 24.940 | 3814 | 3818 | 3823 | VV 6 | 929 | 22301 | 0.15% | 0.033% |
| 137 | 25.017 | 3823 | 3832 | 3834 | VV 6 | 1895 | 56822 | 0.38% | 0.083% |
| 138 | 25.041 | 3834 | 3836 | 3842 | VV 5 | 1899 | 31766 | 0.21% | 0.047% |

Signal : EIC Ion 160.00 (159.70 to 160.70): BROCOLLI-SCAN-2.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 12.856 | 1695 | 1706 | 1722 | BV | 421 | 10809 | 2.85% | 1.801% |
| 2 | 14.872 | 2049 | 2059 | 2076 | BB | 4062 | 73312 | 19.35% | 12.212% |
| 3 | 17.730 | 2548 | 2558 | 2569 | BV | 2164 | 42523 | 11.23% | 7.083% |
| 4 | 19.374 | 2837 | 2845 | 2855 | BV 2 | 933 | 18058 | 4.77% | 3.008% |
| 5 | 19.767 | 2896 | 2914 | 2921 | BV 2 | 400 | 7998 | 2.11% | 1.332% |
| 6 | 23.089 | 3488 | 3495 | 3517 | VV | 1038 | 21200 | 5.60% | 3.531% |
| 7 | 23.389 | 3540 | 3547 | 3554 | BV | 2779 | 47634 | 12.57% | 7.934% |
| 8 | 23.669 | 3584 | 3596 | 3610 | PV | 16479 | 378813 | 100.00% | 63.099% |

Figure 14 : Mass Spectroscopy results of Broccoli which has shown presence of SFN

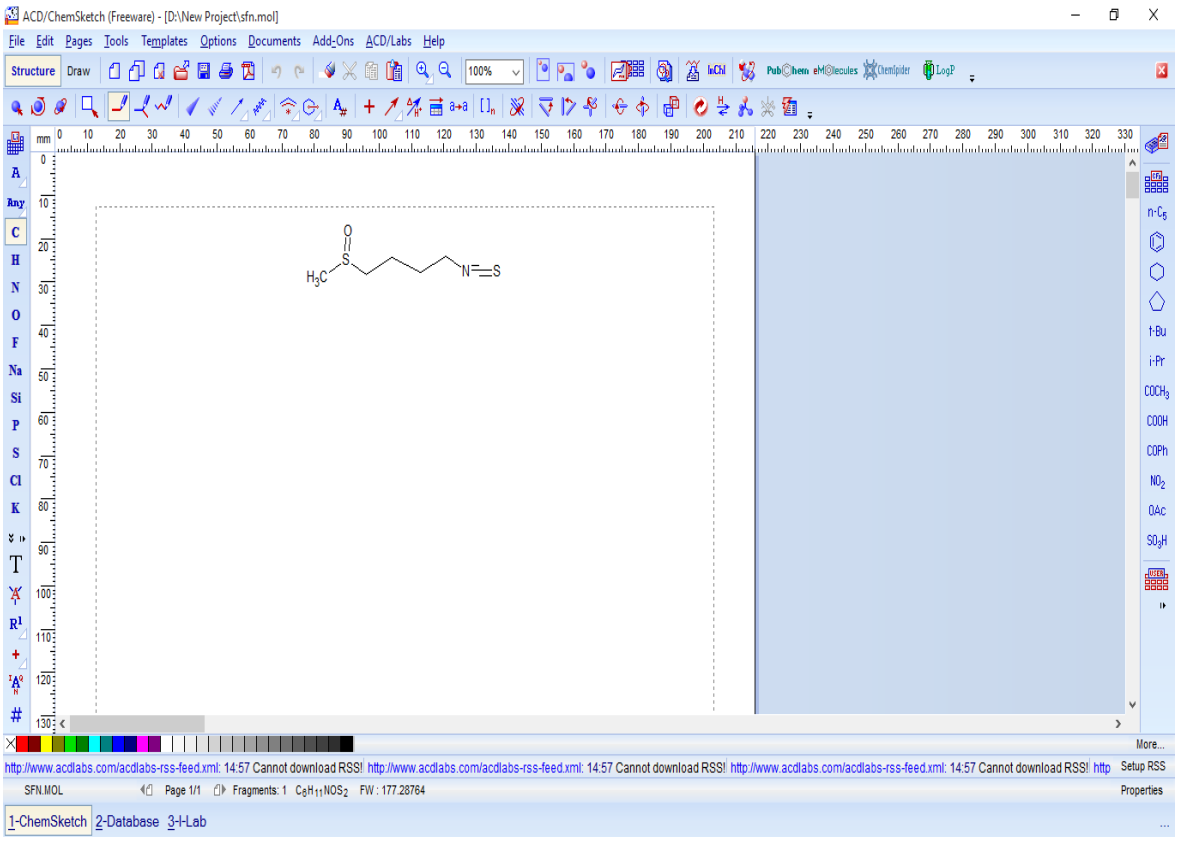


**Figure 15: 2D Structure of sulforaphane**

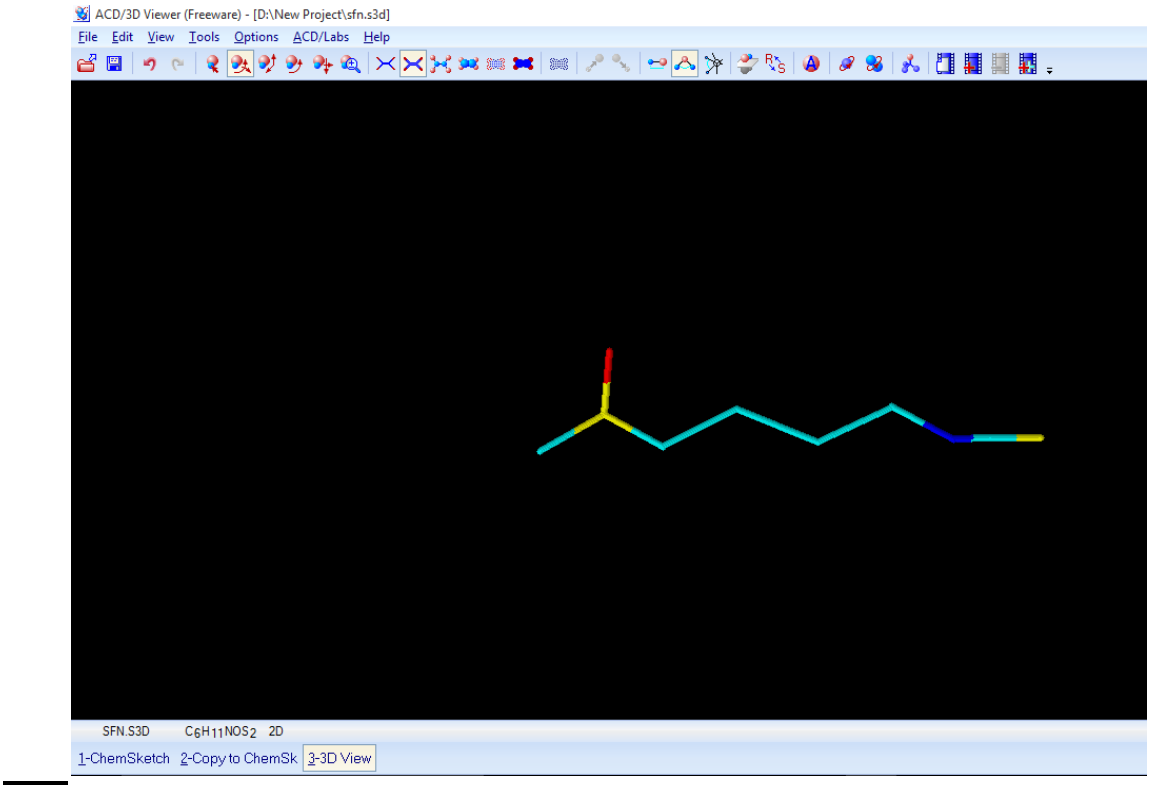


Figure 16 : 3D Structure of Sulforaphane

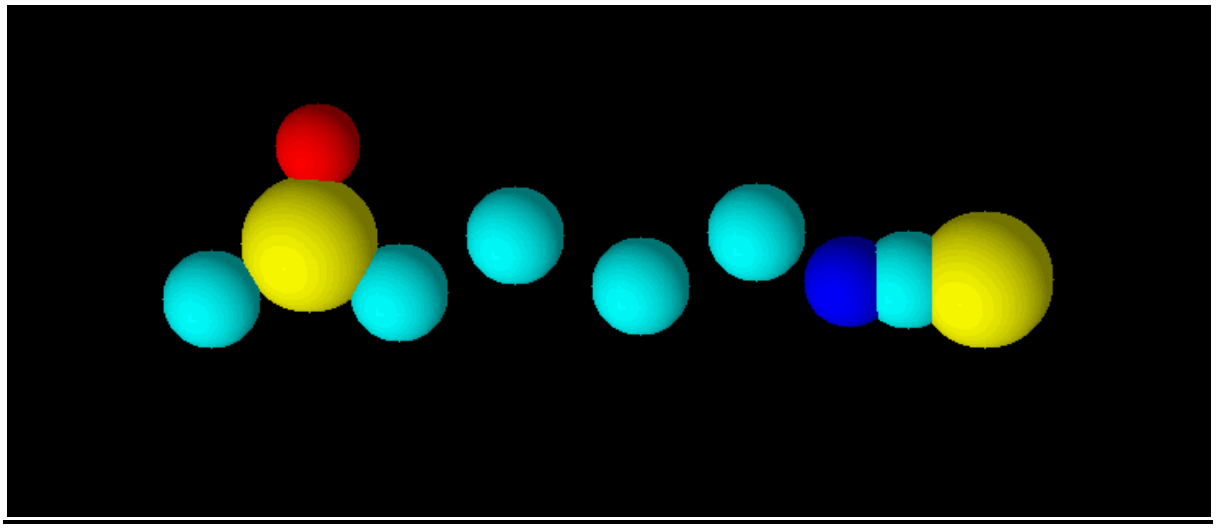

Figure 17 : 3D Structure of Sulforaphane

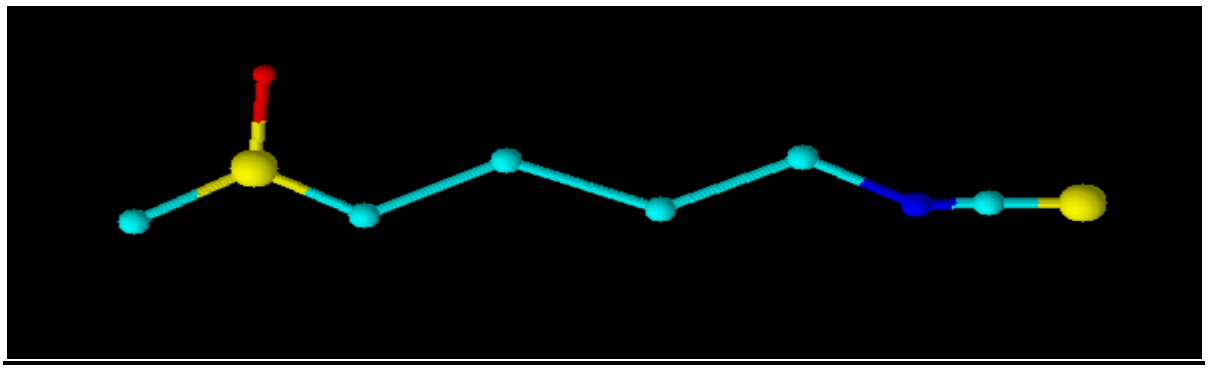

Figure 18: 3D Structure of Sulforaphane

L-Sulforaphane to 3ezp - HYDROLASE

Geometry | Energy | 2D plot | Interaction Table | HBPlot | Methods | Gallery | Parameters

Selected result: 1.

Results Table

| Rank | Est. Free Energy of Binding | Est. Inhibition Constant, Ki | vdW + Hbond + desolv Energy | Electrostatic Energy | Total Intermolec. Energy | Frequency | Interact. Surface | Download |
|---|---|---|---|---|---|---|---|---|
| 1. | -4.07 kcal/mol | 1.05 mM | -5.75 kcal/mol | +0.02 kcal/mol | -5.73 kcal/mol | 40% | 462.826 | download |

Figure 19 – Interaction of Sulforaohane with HDAC 1

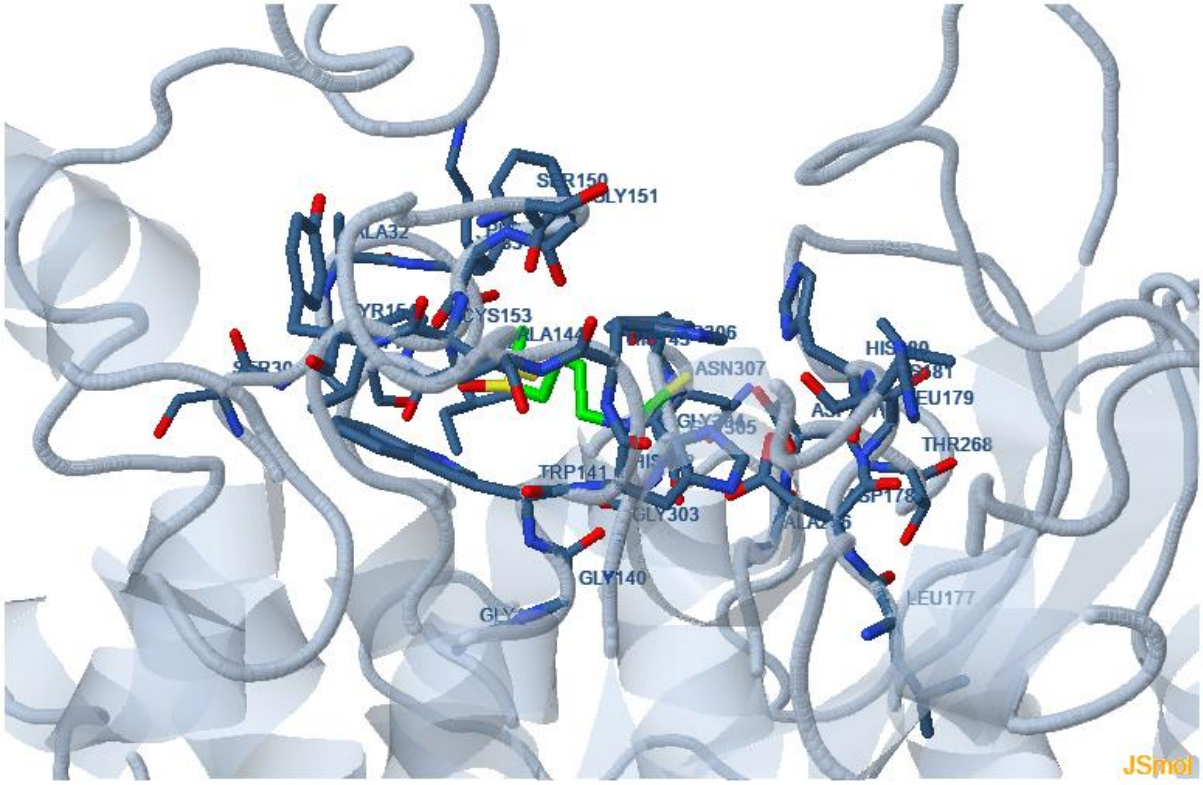


Figure 20 : Interaction of Sulforaphane with HDAC 1

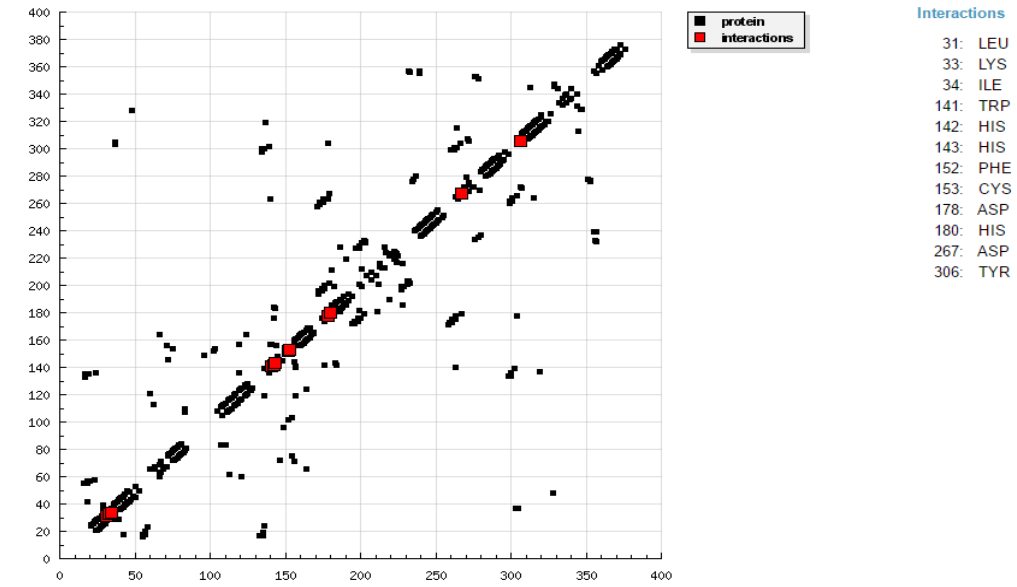


Figure 21 : Interacting Molecule of HDAC 1 and Sulforaphane

## DISCUSSION AND FUTURE PERSPECTIVE

Sulforaphane rich extract is got by liquid liquid phase extraction from broccoli seeds. The extract is in the suspended in 10 % ethanol. Hexane is used to remove non polar contaminant. Anhydrous sodium Sulfate is used to remove water molecule from the extract so that chromatography is done easily. Sulforaphane rich extract goes to GC/MS for determination of SFN. In GC/MS report sulforaphane's ions 72, 55, 160 are present at 23.667 min in 28.375 %, 21.739% and 63.099% respectively. So by the presence of these ions sulforaphane is determined. 2D and 3D structure of sulforaphne is formed by ACD/3D Chem Sketch software. By the Docking HDAC1 and Sulforaphane interaction is shown in this interaction leucine, lysine, Isoleucine, Tryptophan, Histidene, Phenylalanine, cystene, are interacting with sulforaphane's molecule.

Sulforaphane can be used to generate ipsc. Sulforaphane induces Nanog and OCT4 and inhibits HDAC of p53 protein which is a anticancer protein. So in future Sulforaphane can generate induced pluripotent stem cell that will be useful to treat many disease.

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