Supplementary material

Syntheses, biological evaluation and photophysical studies of novel 1,2,3-triazole linked azo dyes.

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Antibacterial activity

The antibacterial activity of all compounds was evaluated by the agar well diffusion method.[1] All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 $\times 10^6$ cfu/mL. 20 mL of Mueller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 2.0 mg/mL of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37^0C for 24 h. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

Determination of Minimum Inhibitory Concentration (MIC) of chemical compounds

MIC of the compounds against bacterial strains was tested through a modified agar well diffusion method.[1] In this method, a two fold serial dilution of each chemically synthesized compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1µg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37^oC for 24 h and observed for the inhibition zones. MIC, taken as the lowest concentration of the chemical compound that completely inhibited the growth of the

microbe, showed by a clear zone of inhibition, was recorded for each test organism. Ciprofloxacin was used as the positive control.

Antifungal activity

The antifungal activity of all compounds was evaluated by poisoned food technique.[2] The molds were grown on sabouraud dextrose agar (SDA) at 25^0C for 7 days and used as inocula. The 15 mL of molten SDA (45^0C) was poisoned by the addition of 100 µL volume of each compound having concentration of 2.0 mg/mL reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25^0C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition.

Percent inhibition of myelial growth = (dc-dt) / dc × 100

dc = average diameter of fungal colony in negative control sets; dt = average diameter fungal colony in experimental sets.

Antioxidant activity

DPPH free radical scavenging assay

Methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a reagent for the spectrophotometric assay.[3] Solutions of five different concentration of all compounds were prepared using methanol. 1 mL of 0.1 mM methanolic solution of DPPH was added to 3 mL solution of the compounds and the mixture was shaken vigorously using vortex mixer. Absorbance was read against a blank at 517 nm after incubation of the reaction mixtures for 60 min in dark at room temperature. Butylated hydroxyl toluene (BHT) was used as a reference

compound. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Radical Scavenging activity (%) = $[(A_0 – A_1)/A_0) \times 100]$

Where A_0 is absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. IC_{50} values were calculated using Microsoft excel programme.

Nitric oxide free radical scavenging assay

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by Griess reaction.[4,5] The reaction mixture (3 mL) containing sodium nitroprusside (1.5 mL, 10 Mm) in phosphate buffer salaine and the solution (1.5 mL) of compound in methanol at different concentrations was incubated at room temperature for 150 min. After incubation 1.5 mL of the reaction mixture was removed and 1.5mL of the Griss reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was recorded at 546 nm. Butylated hydroxyl toluene (BHT) was used as a reference compound. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Nitric oxide radical Scavenging activity (%) = $[(A_0 – A_1)/A_0) \times 100]$

Where A_0 is absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound. IC_{50} values were calculated using Microsoft excel programme.

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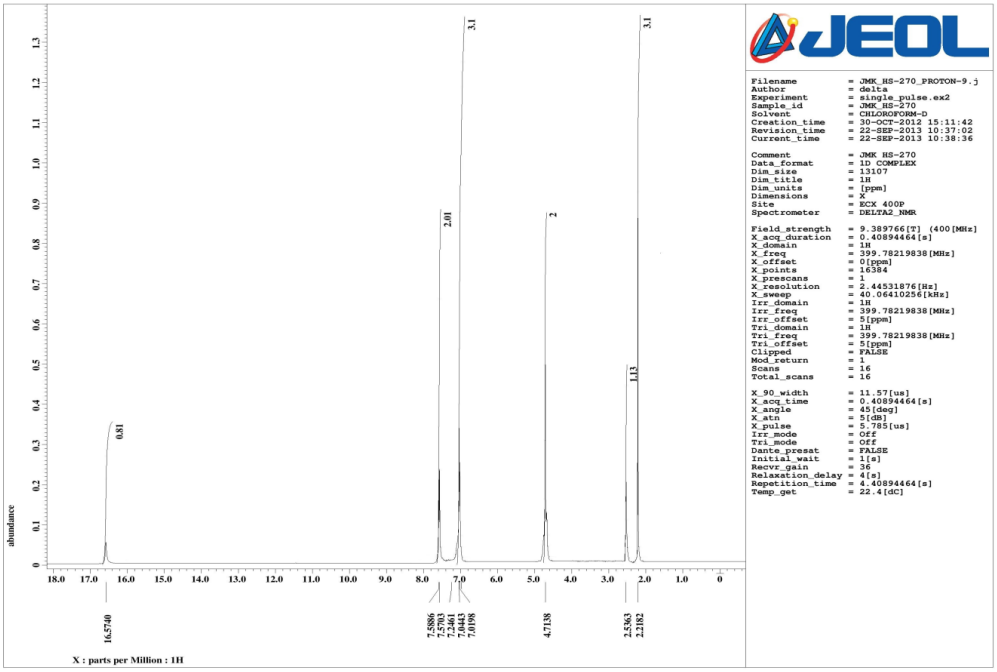

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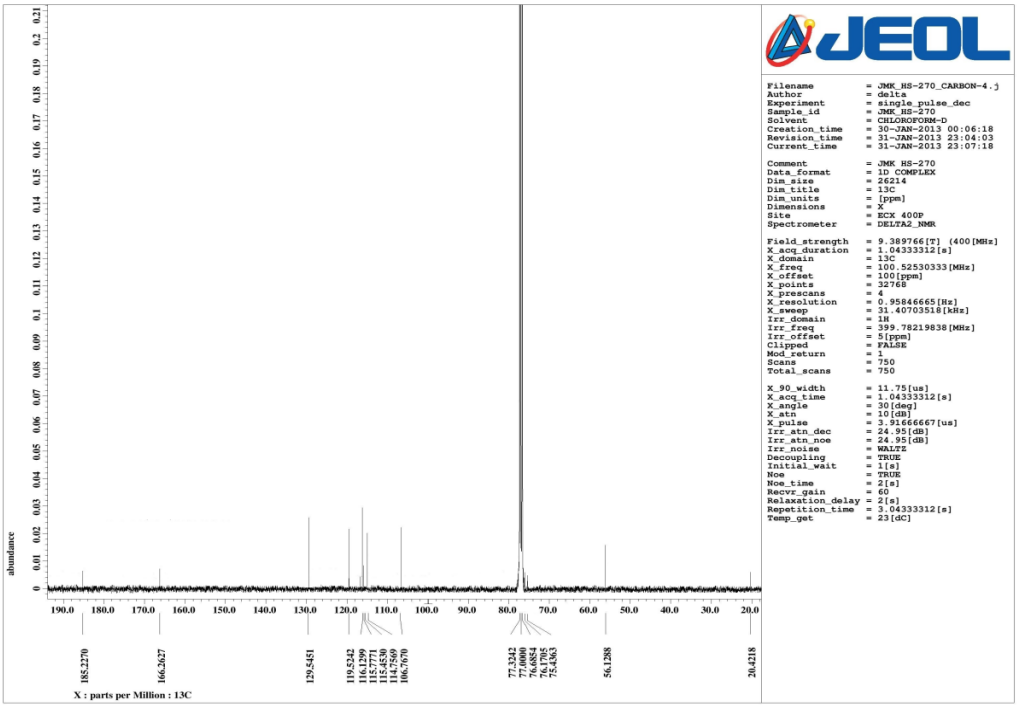

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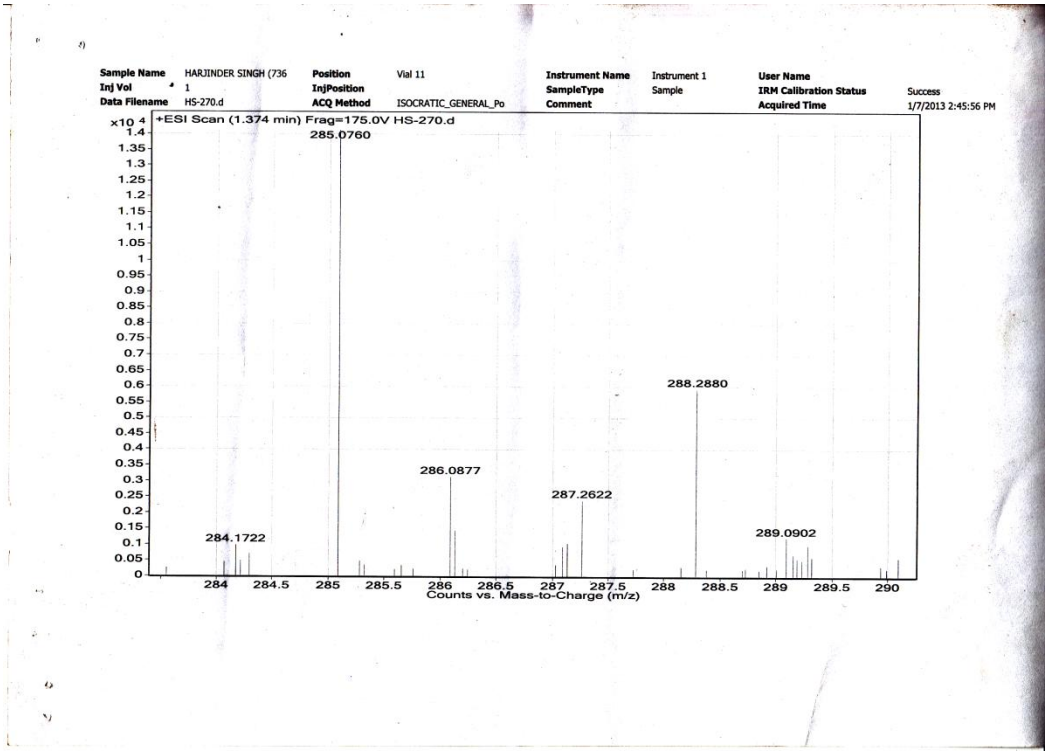

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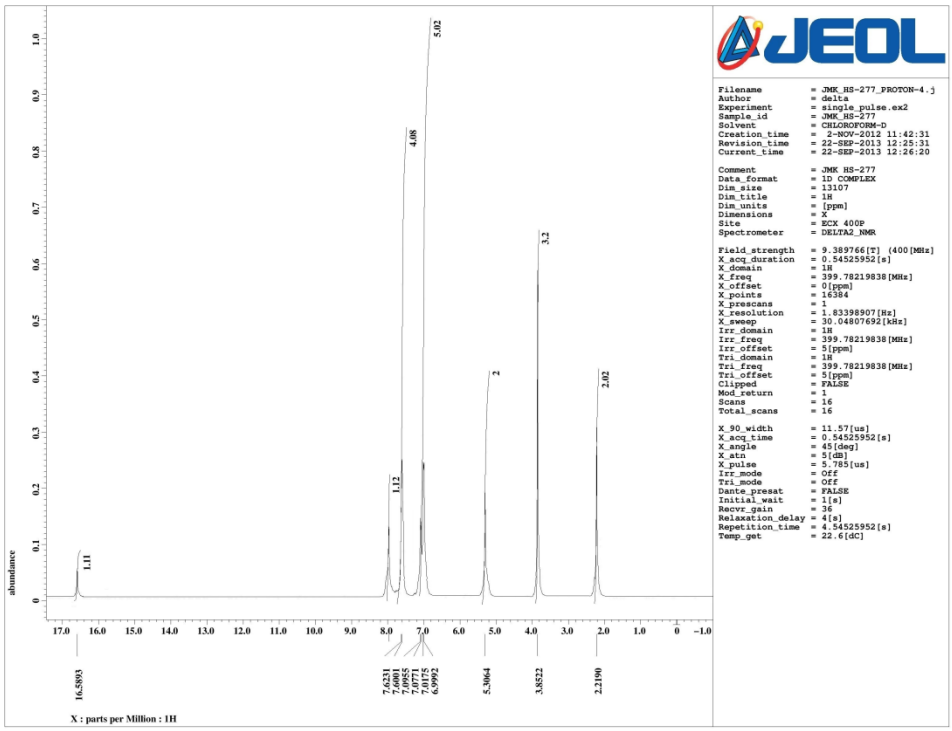

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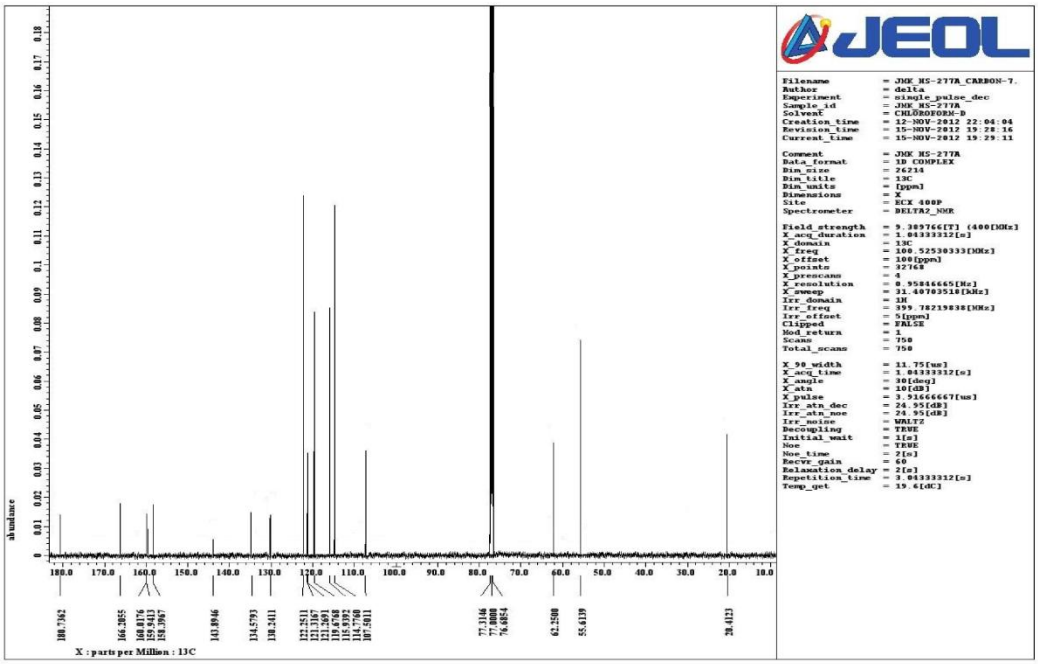

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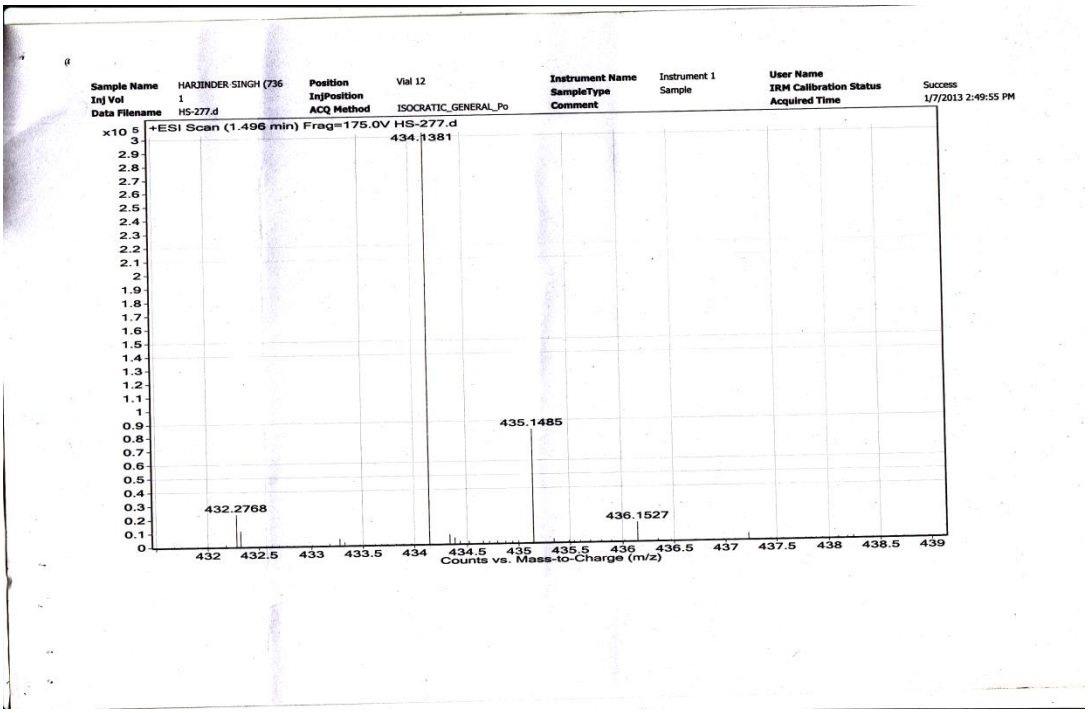

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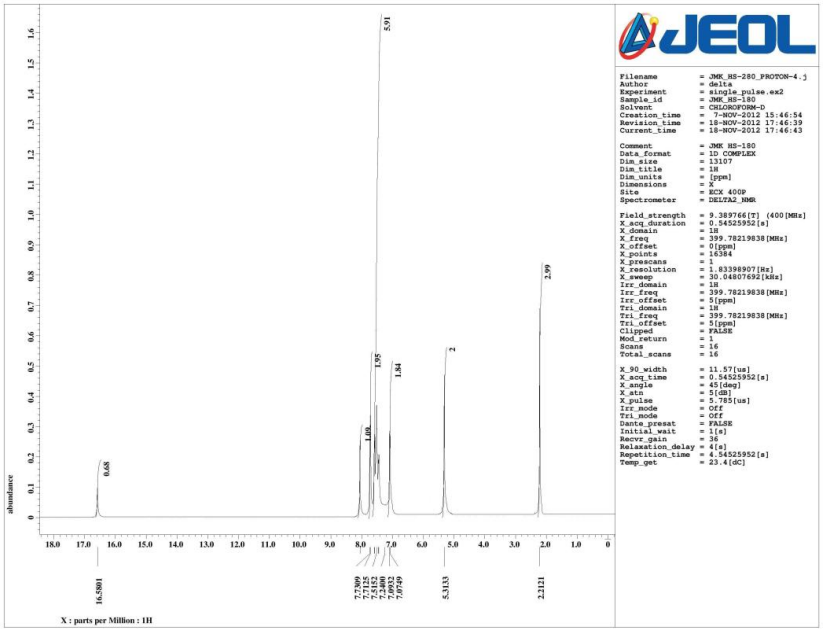

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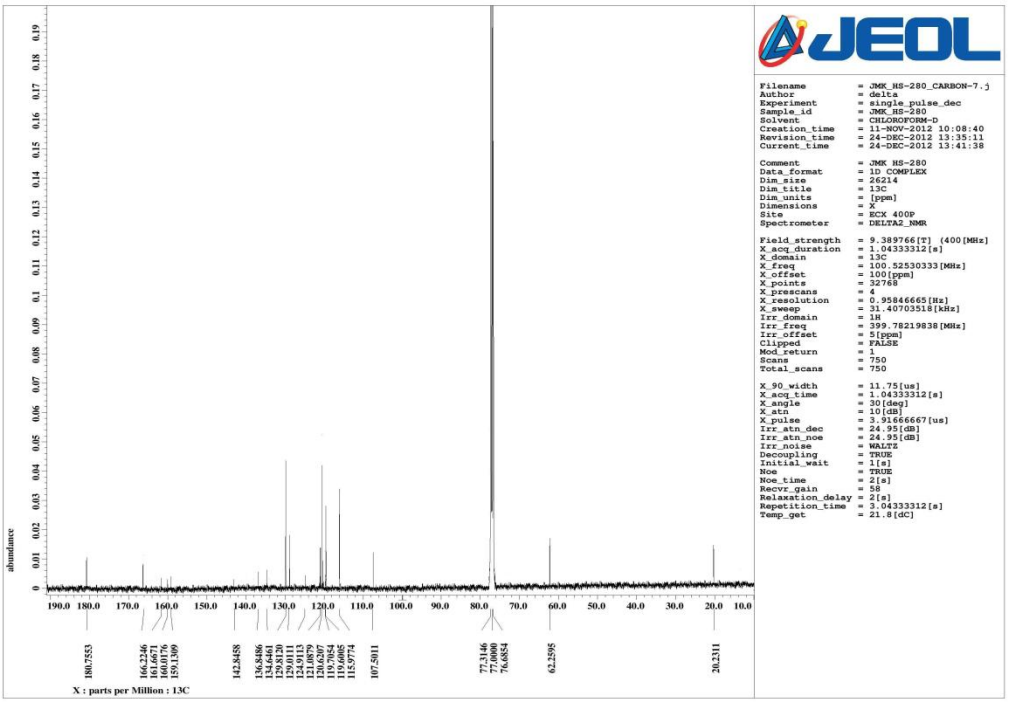

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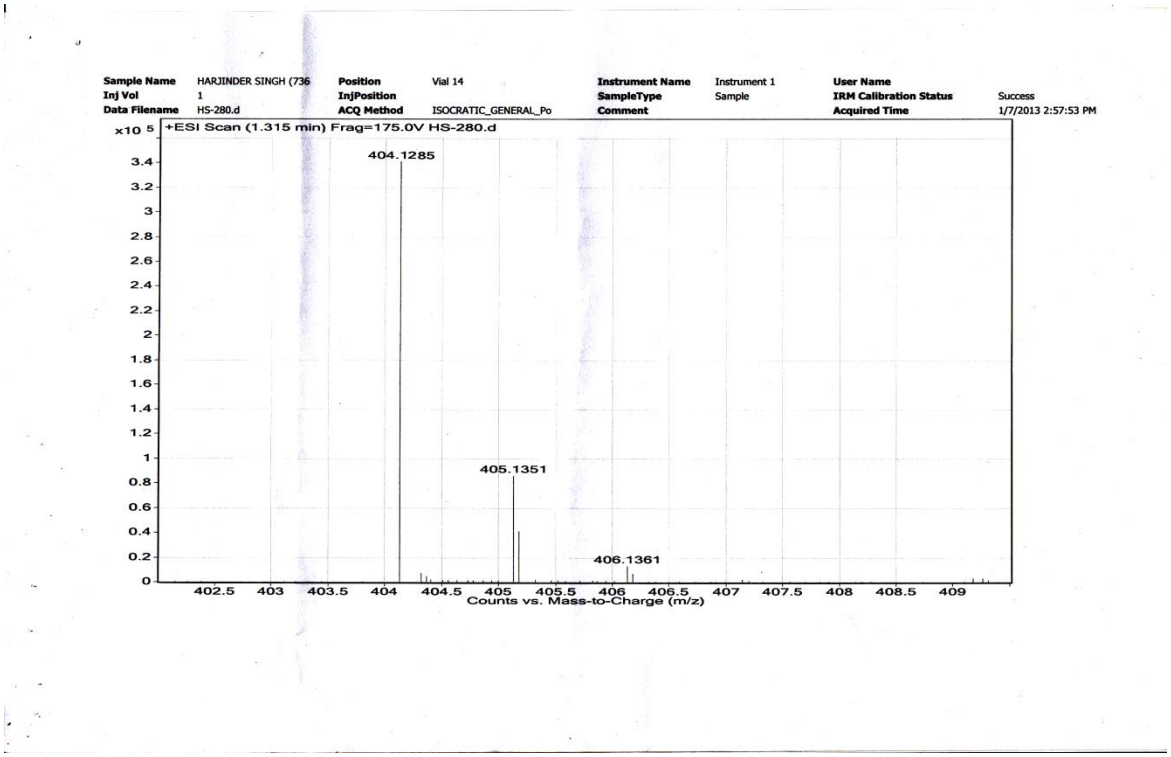

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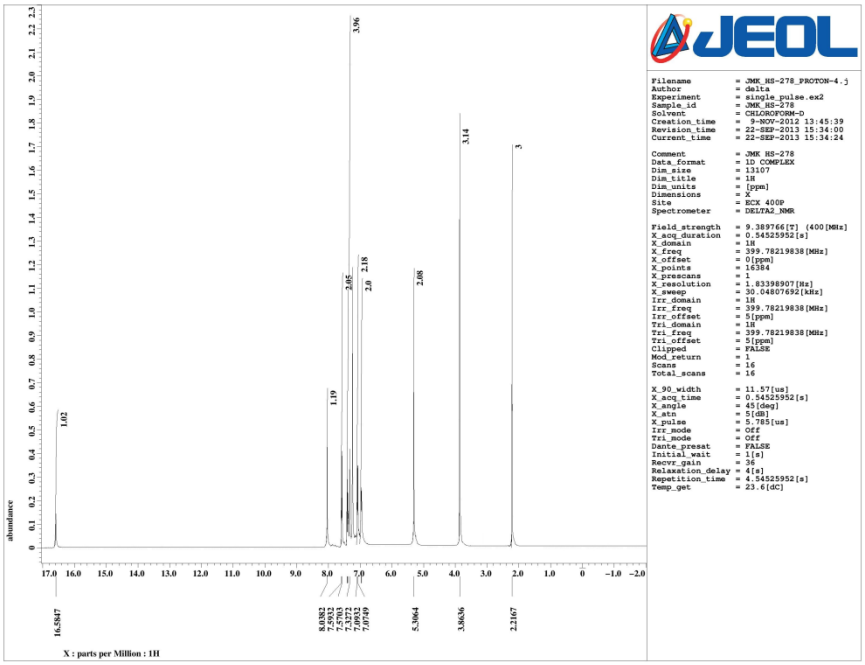

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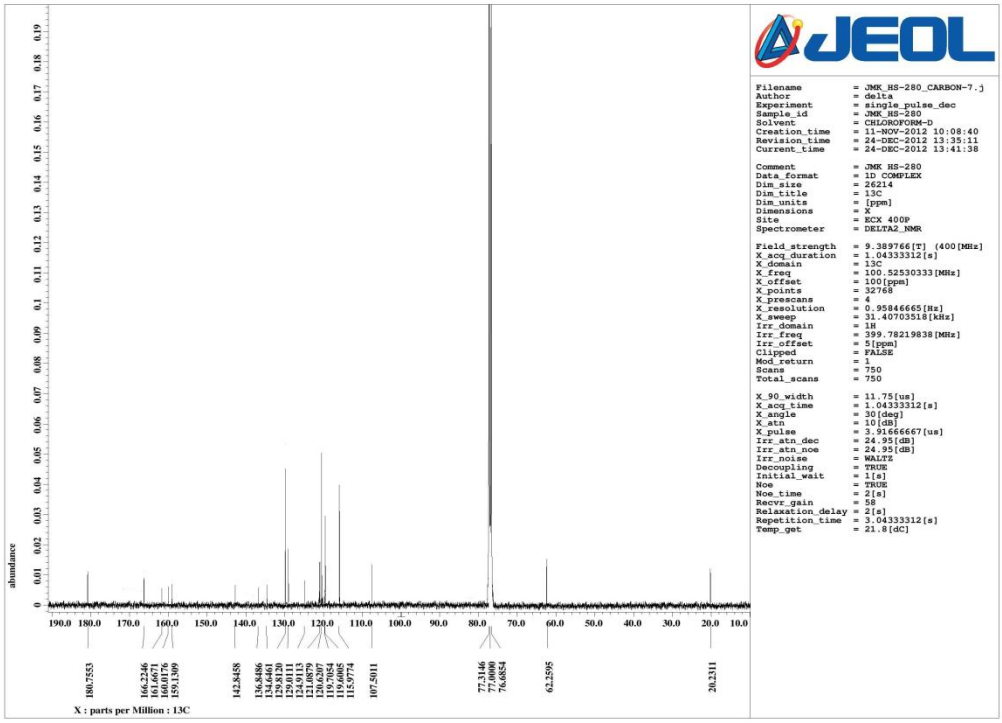

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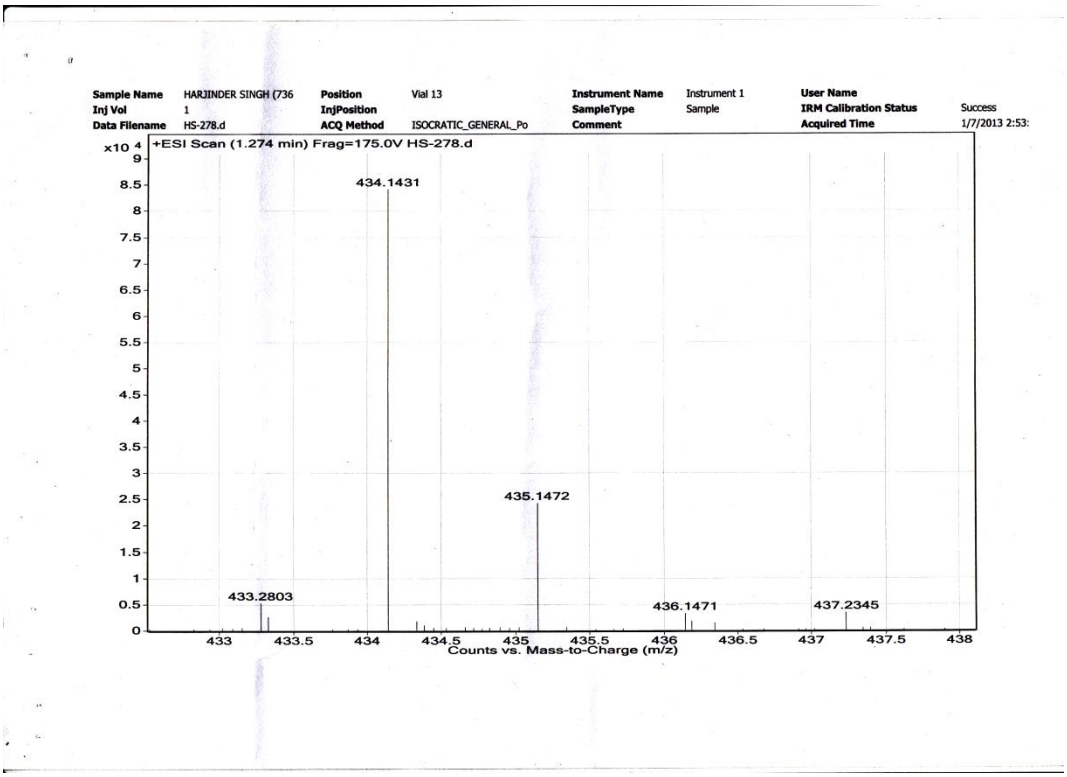

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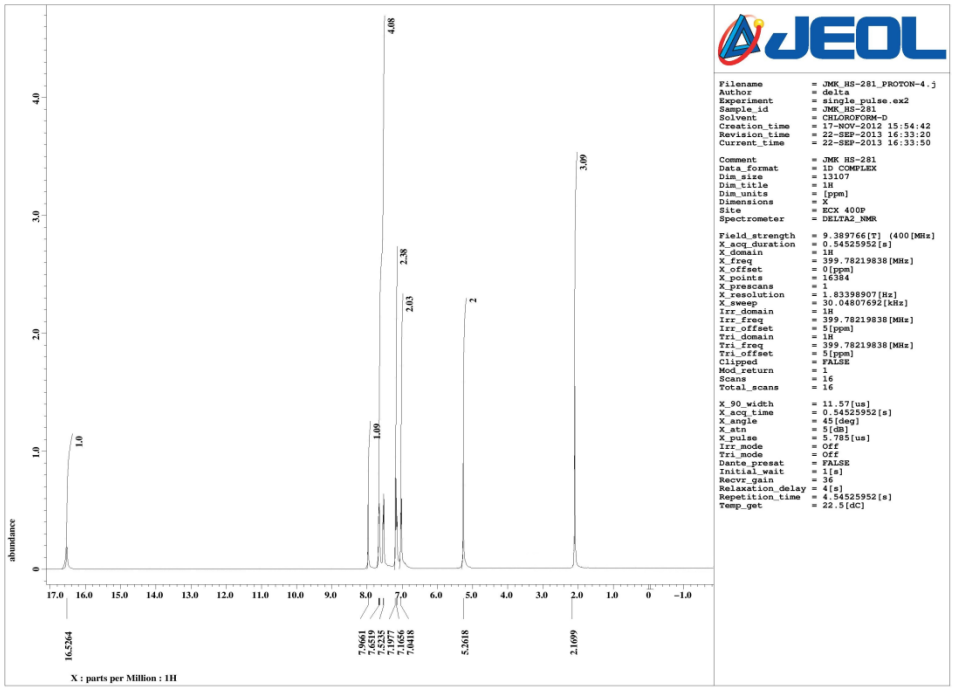

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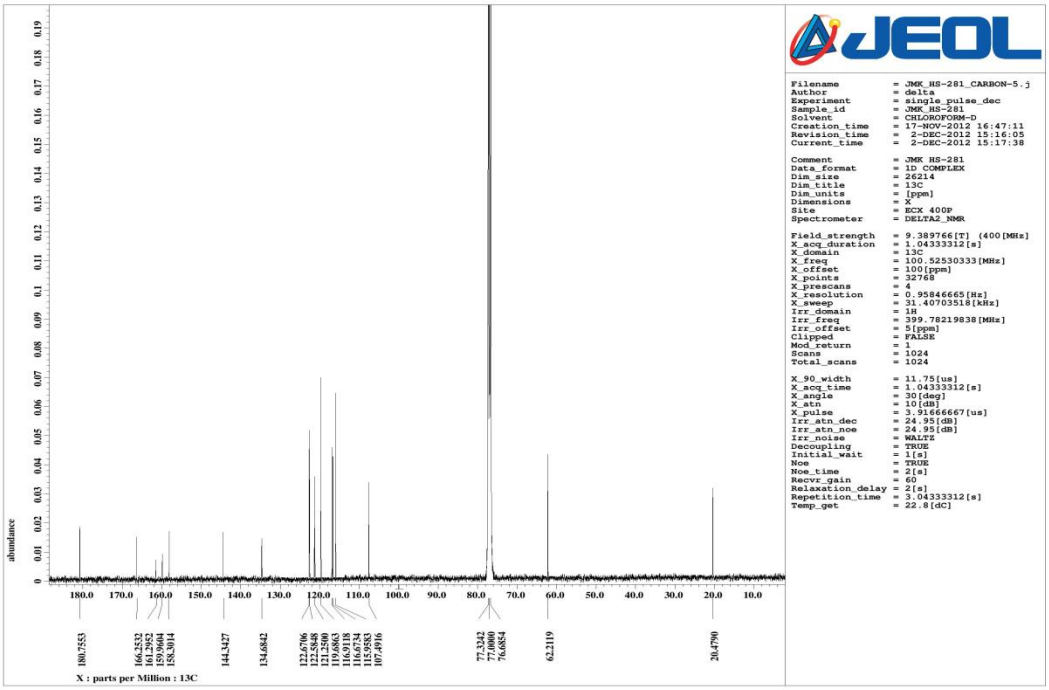

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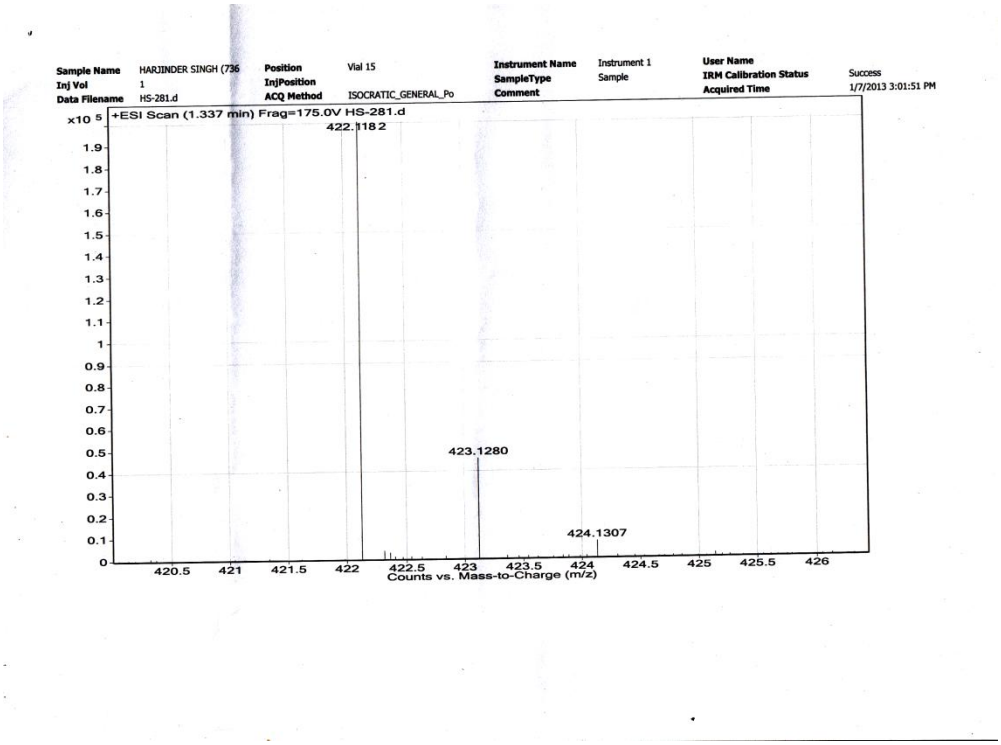

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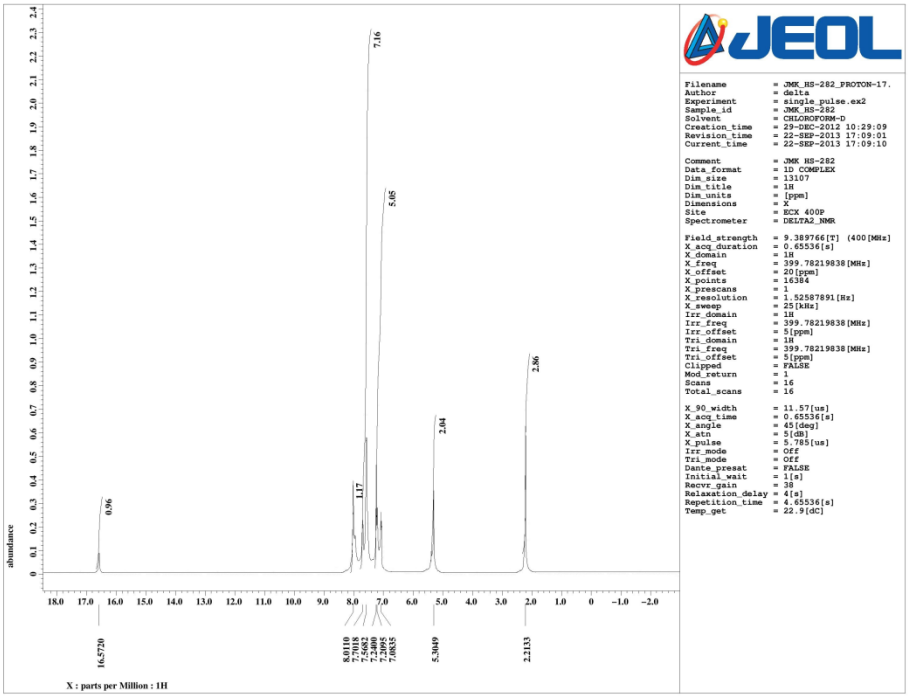

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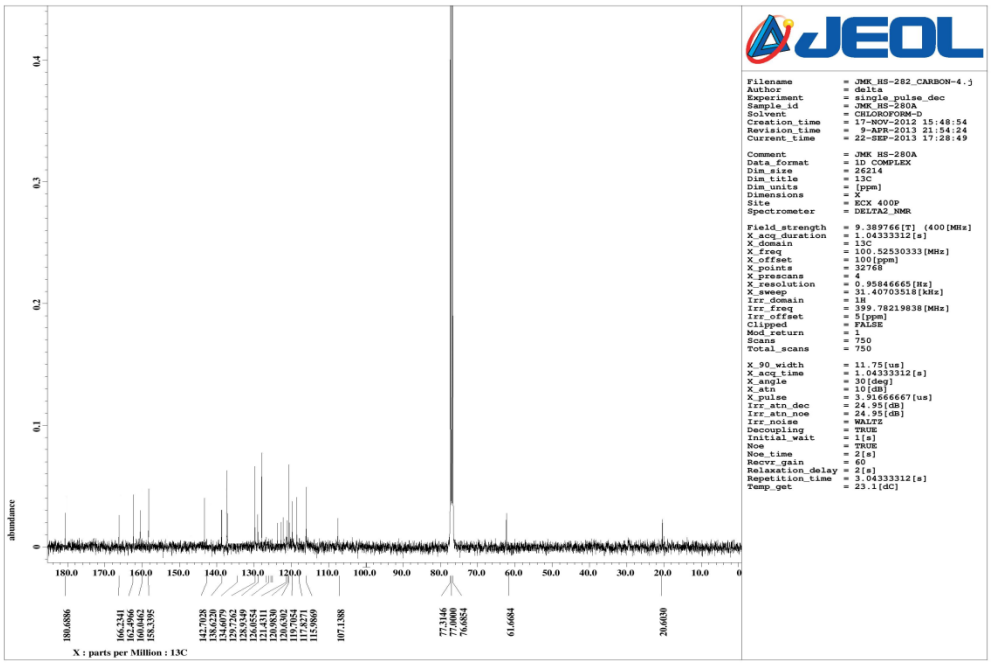

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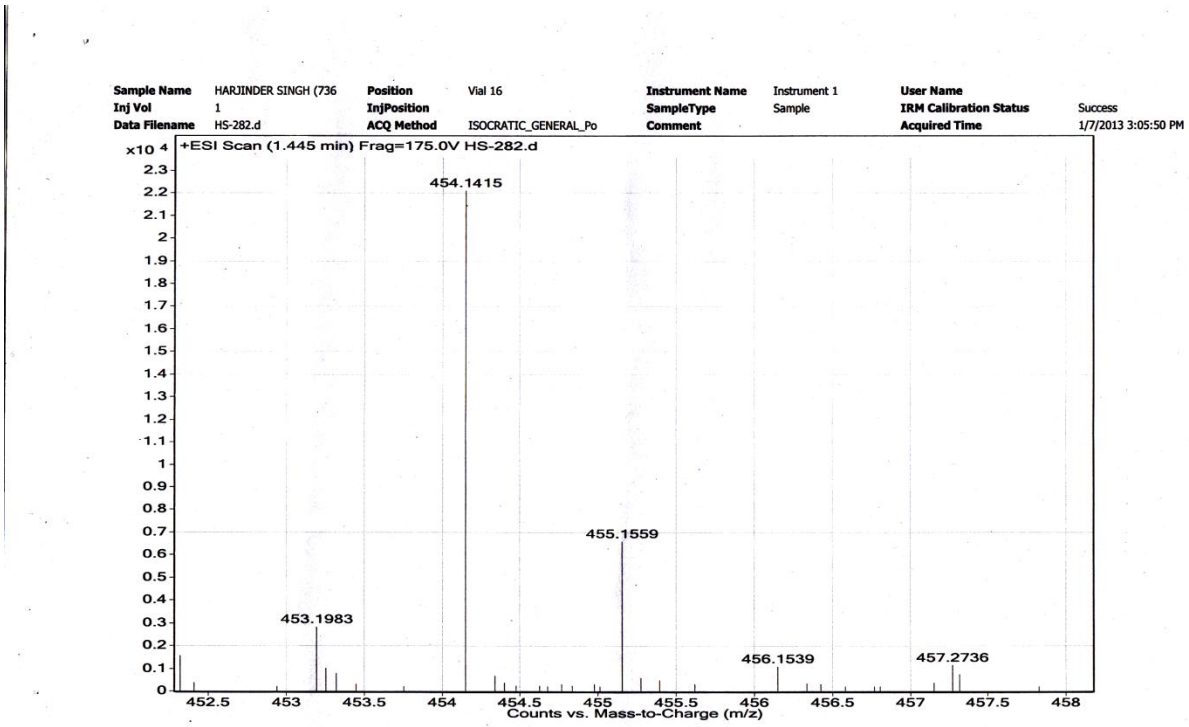

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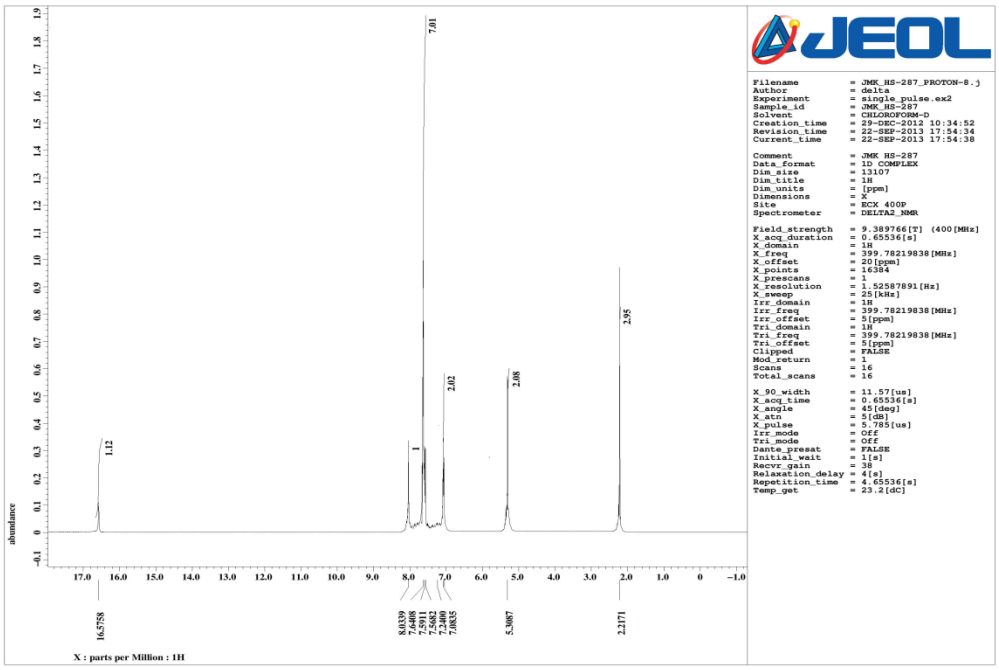

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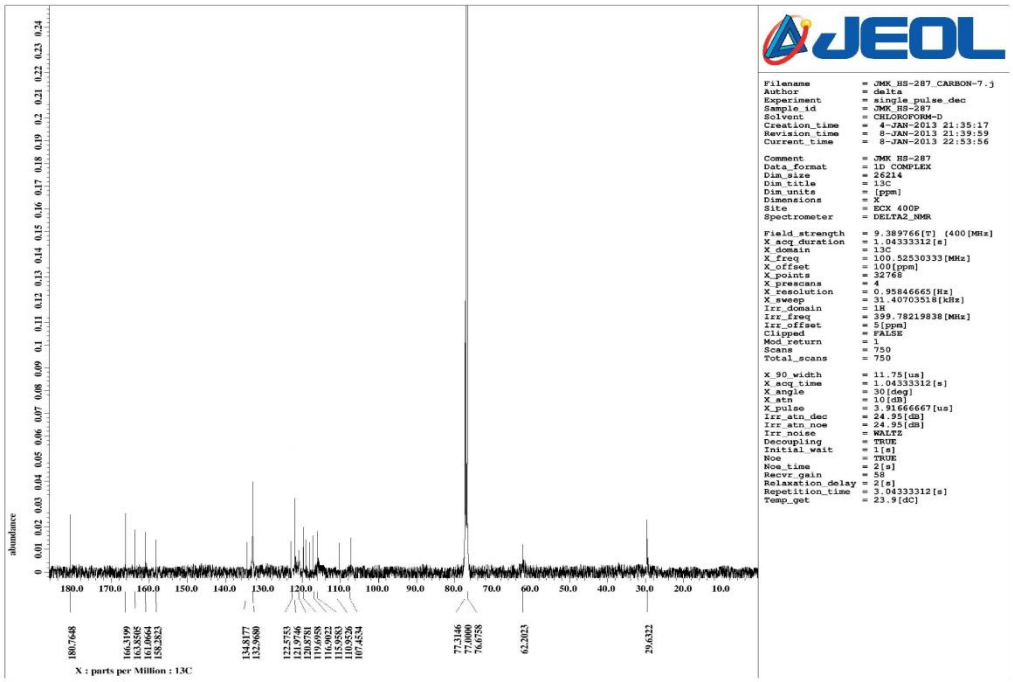

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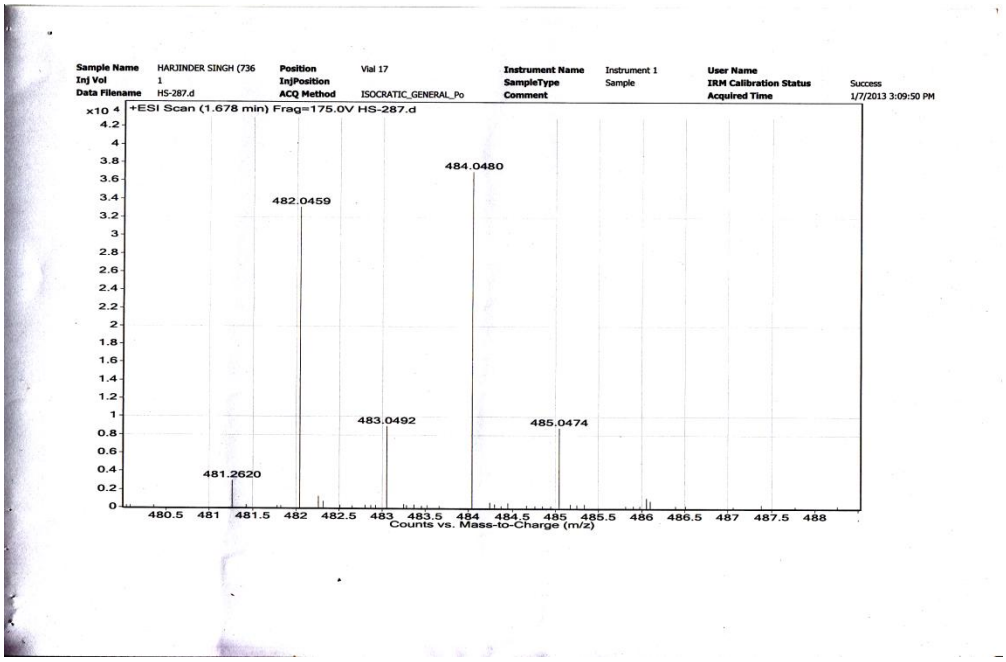

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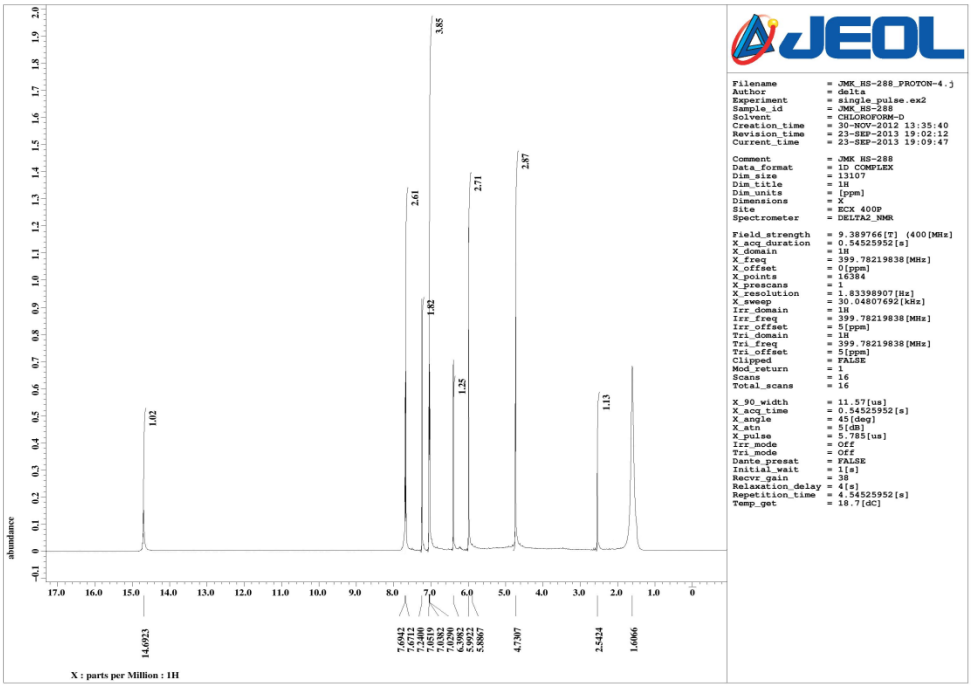

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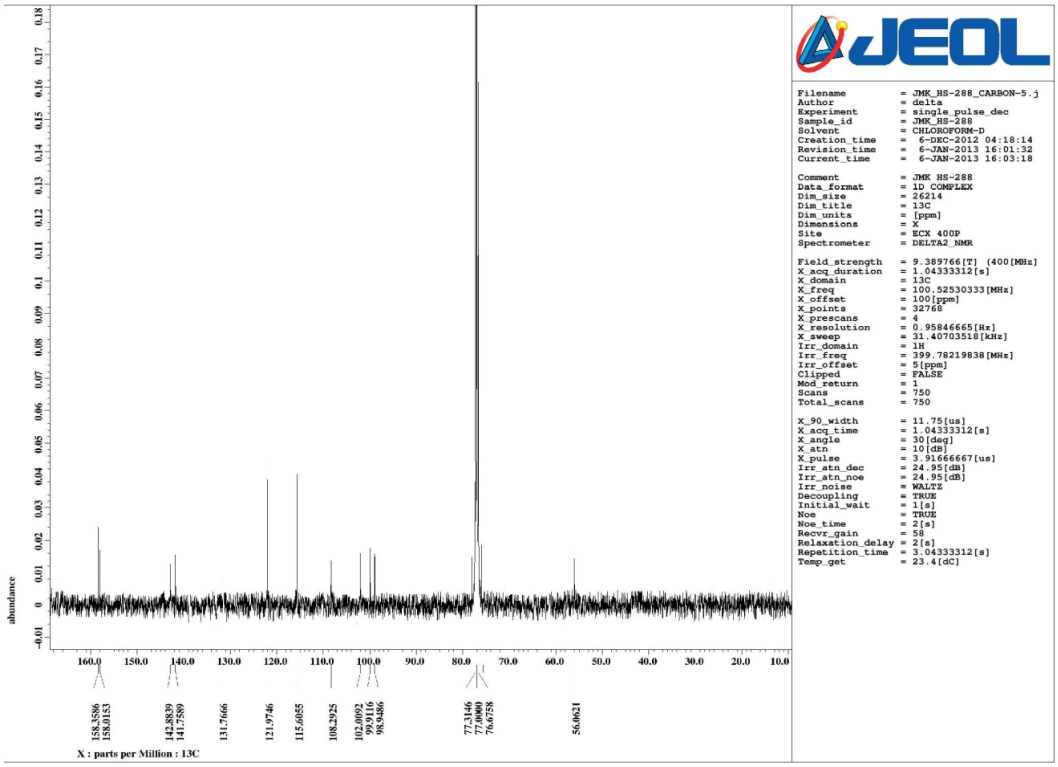

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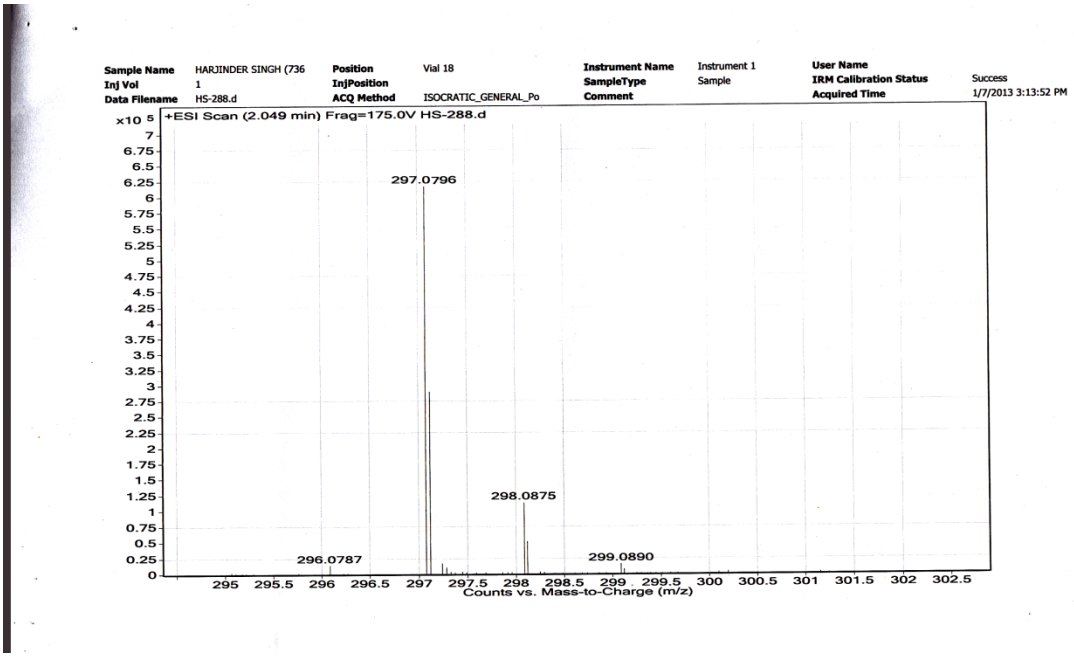

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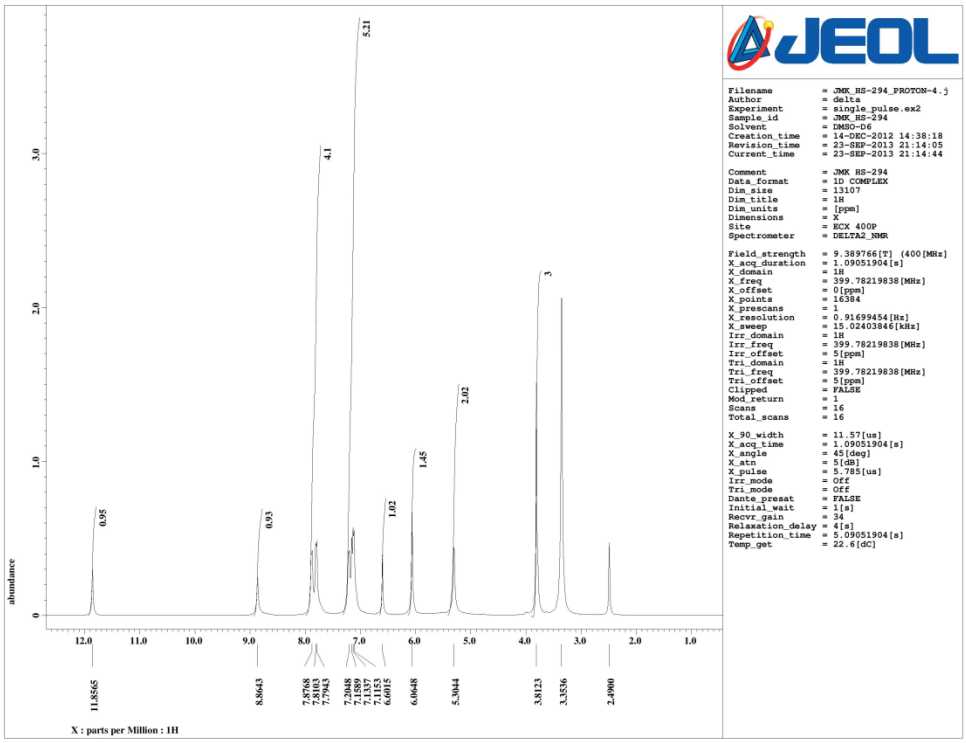

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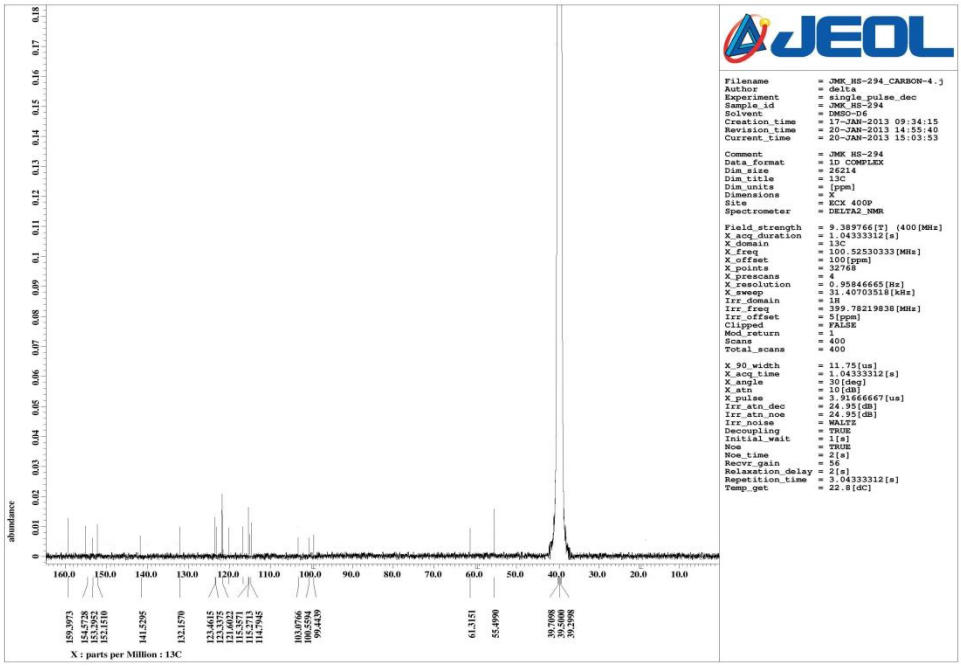

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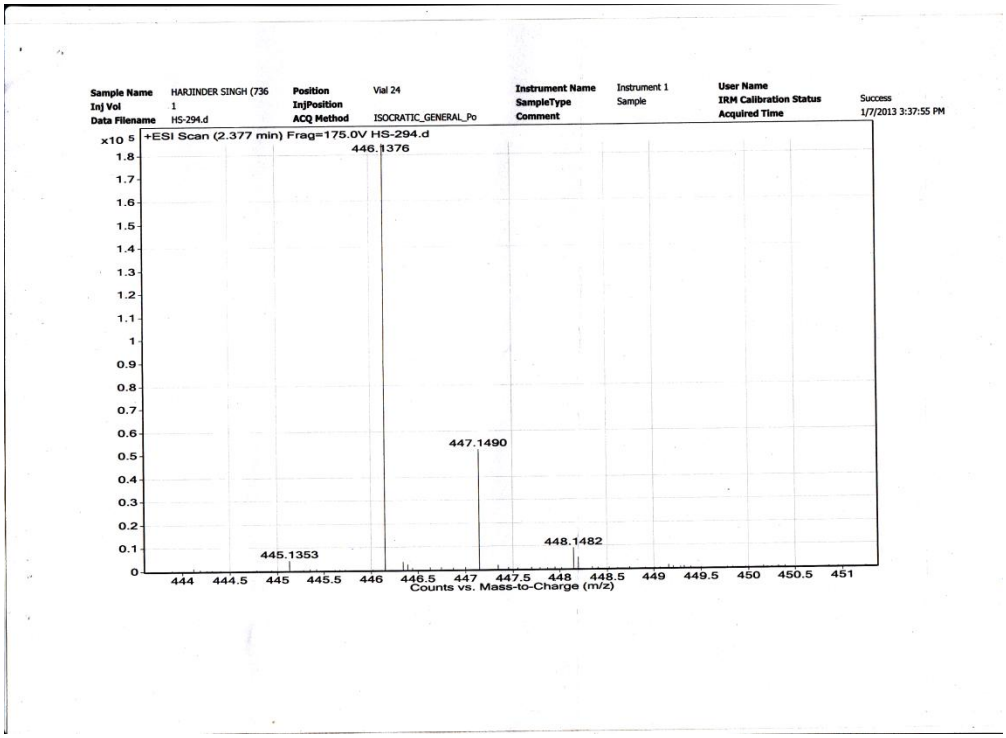

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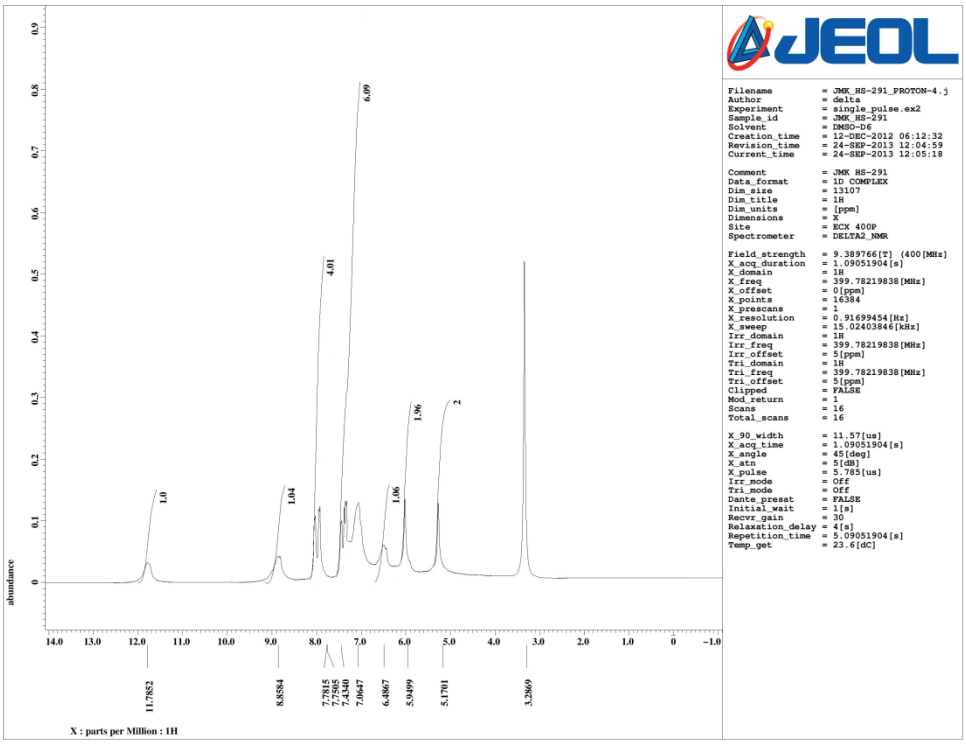

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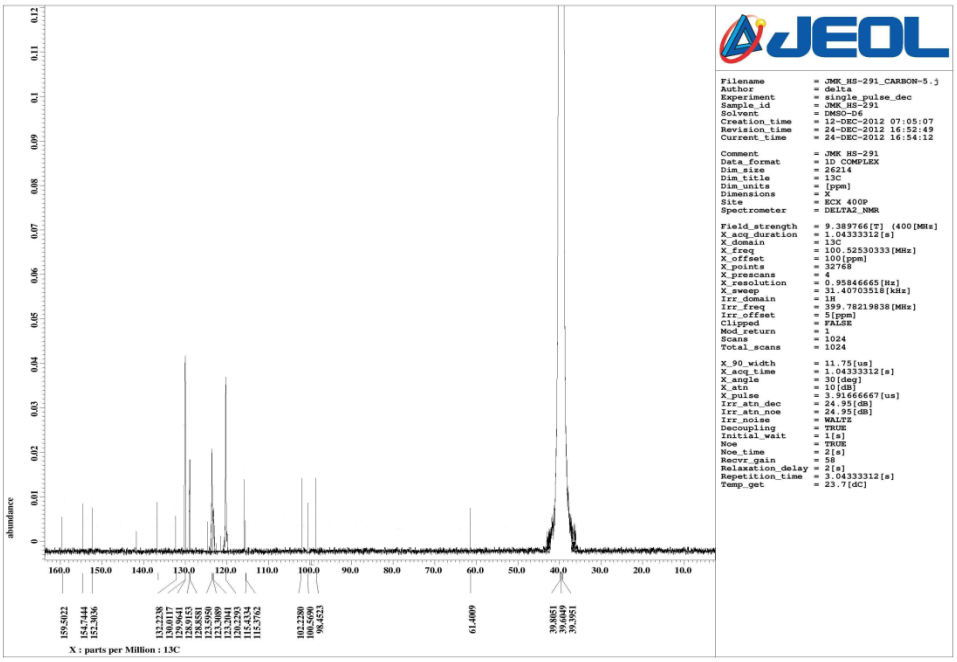

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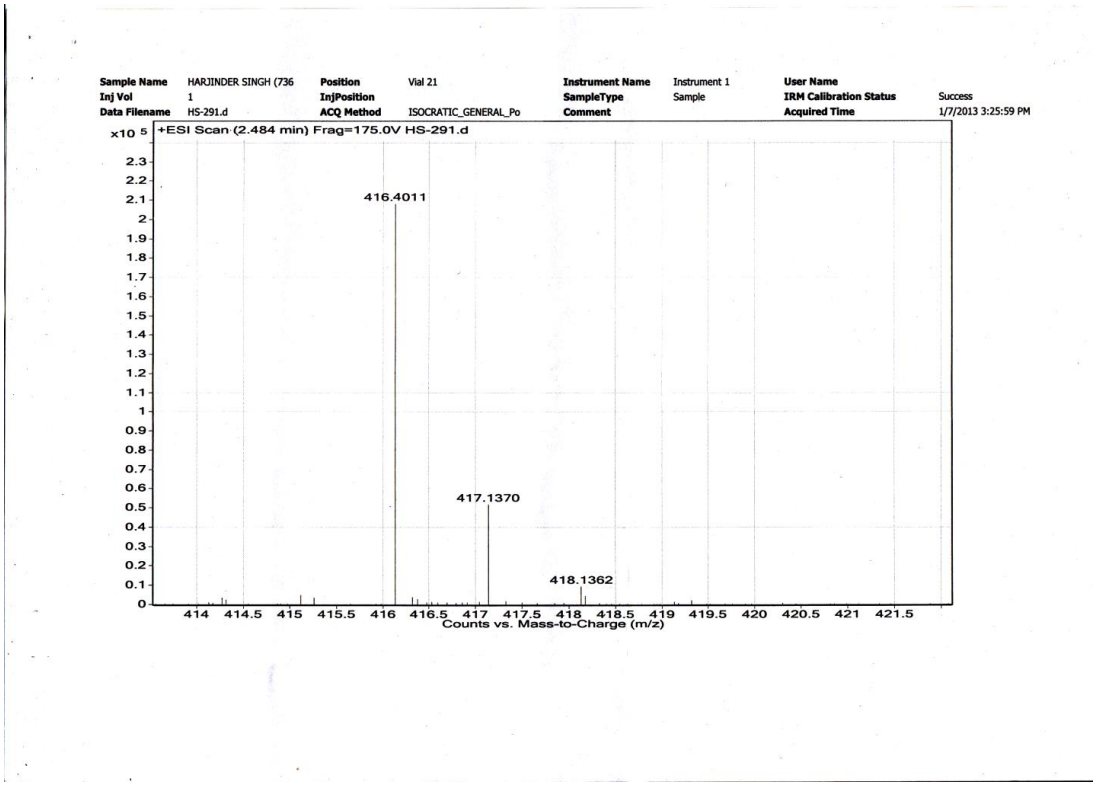

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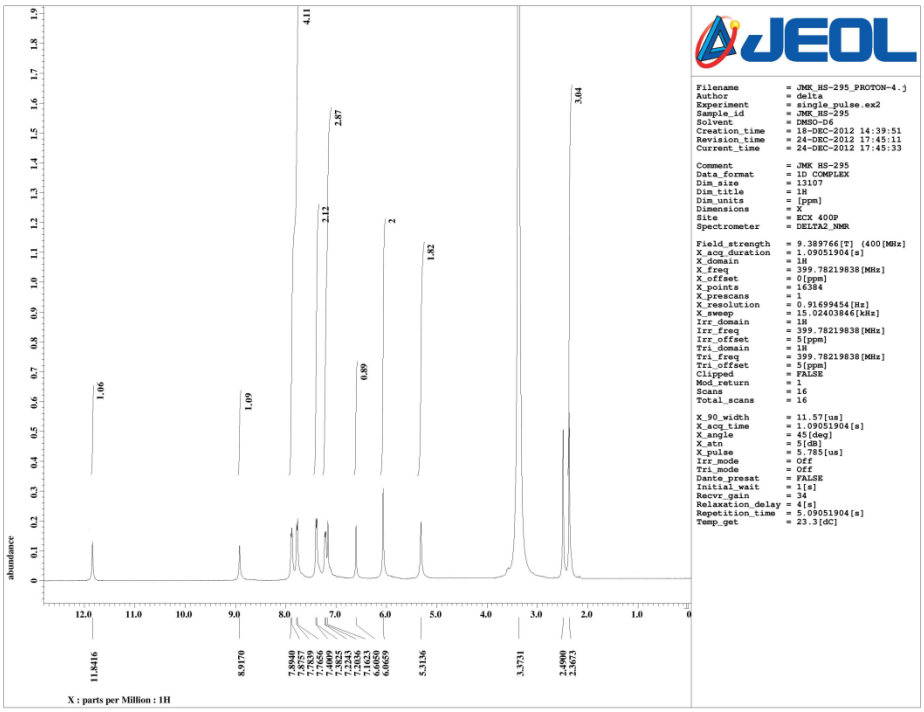

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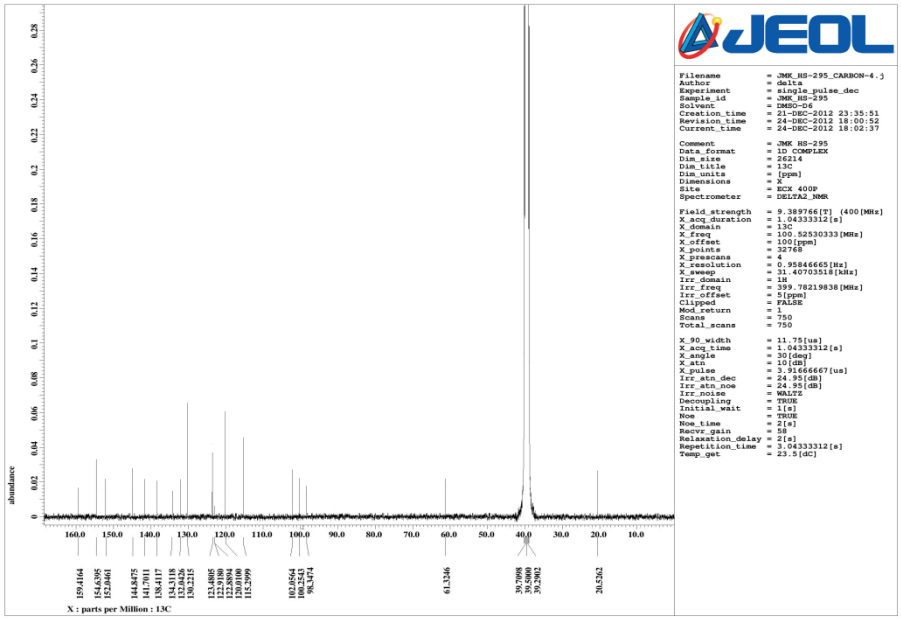

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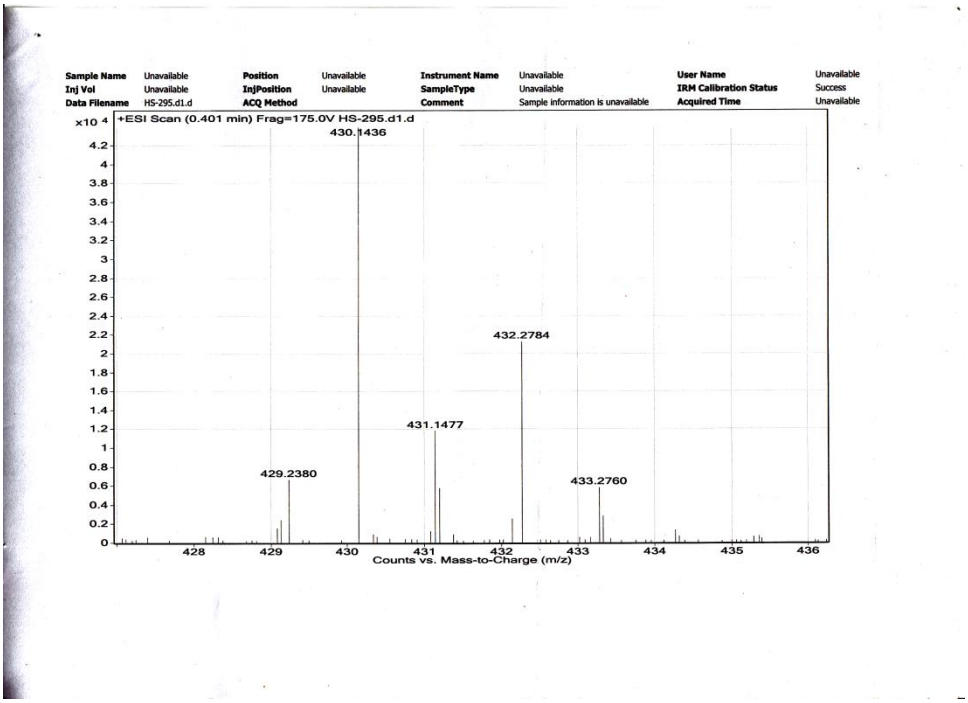

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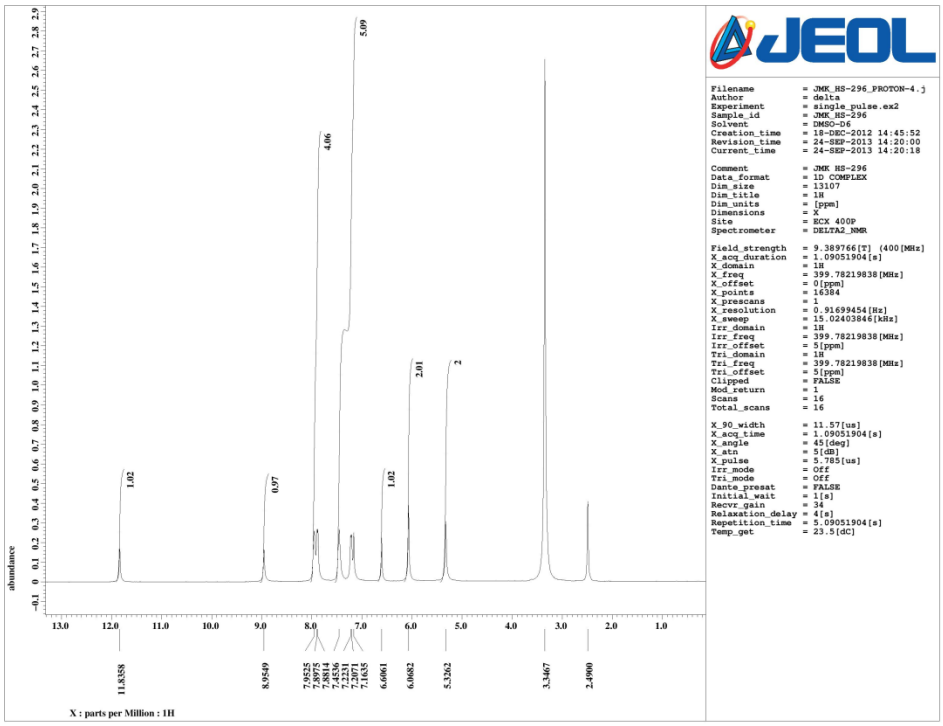

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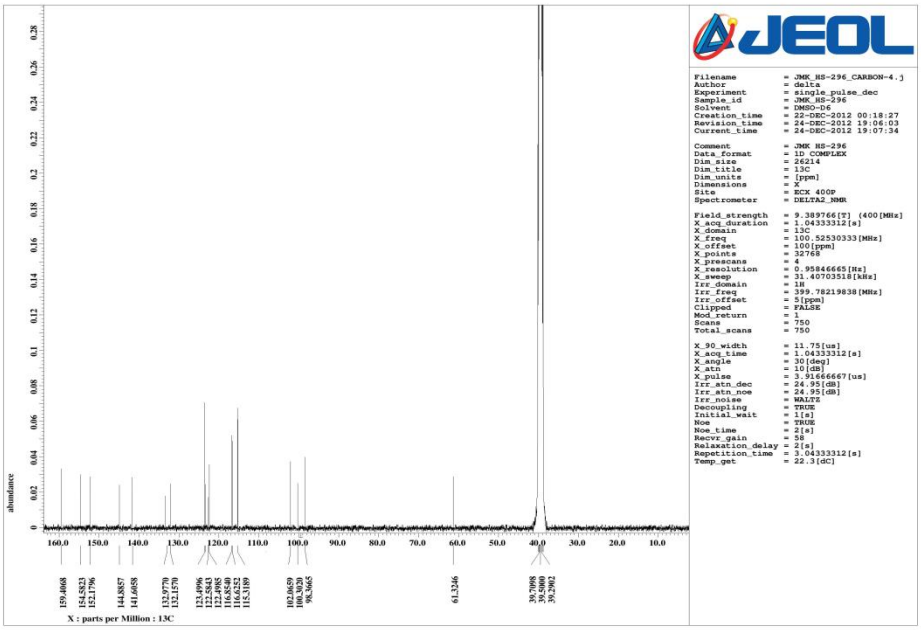

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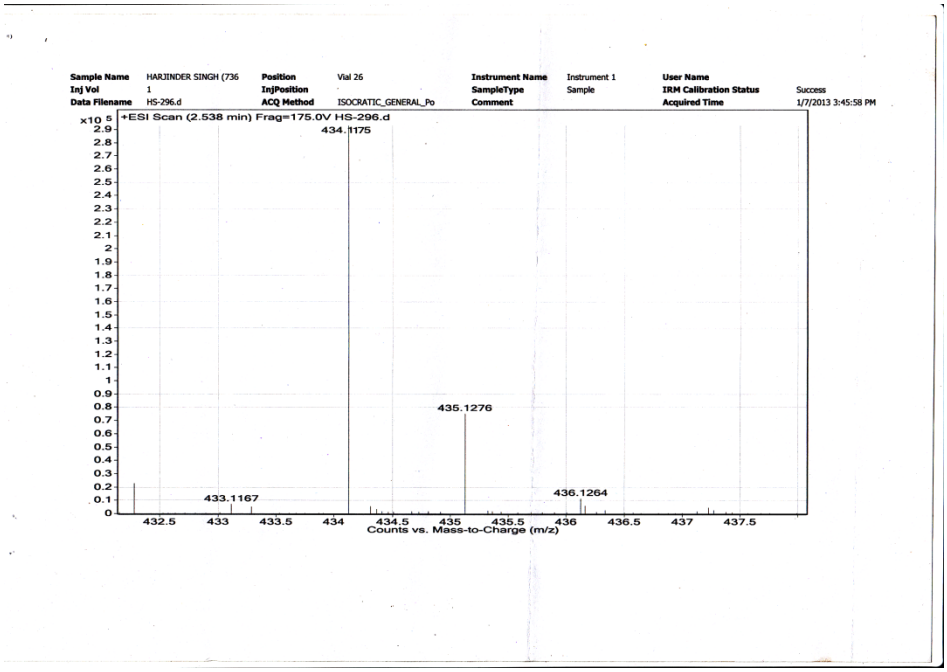

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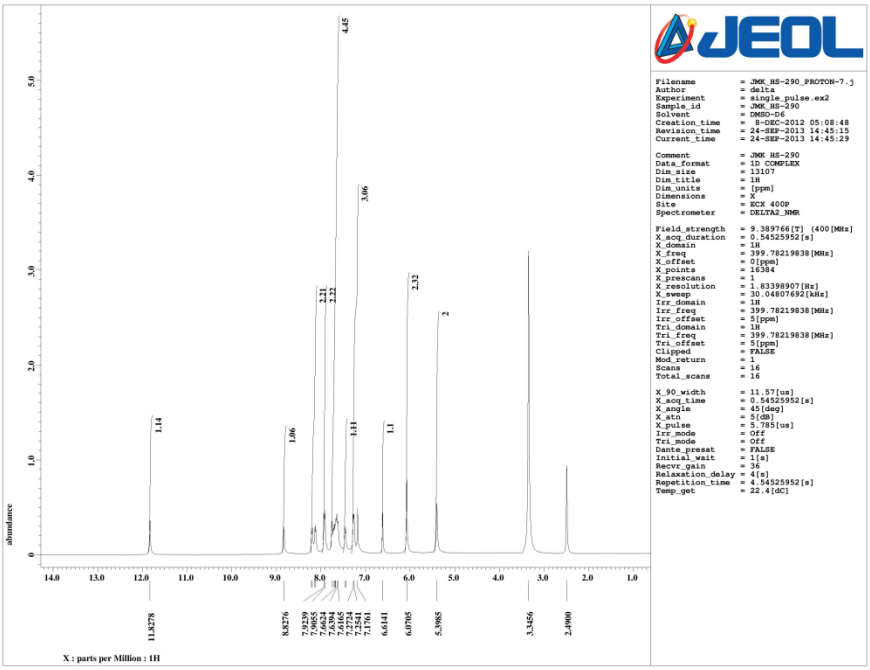

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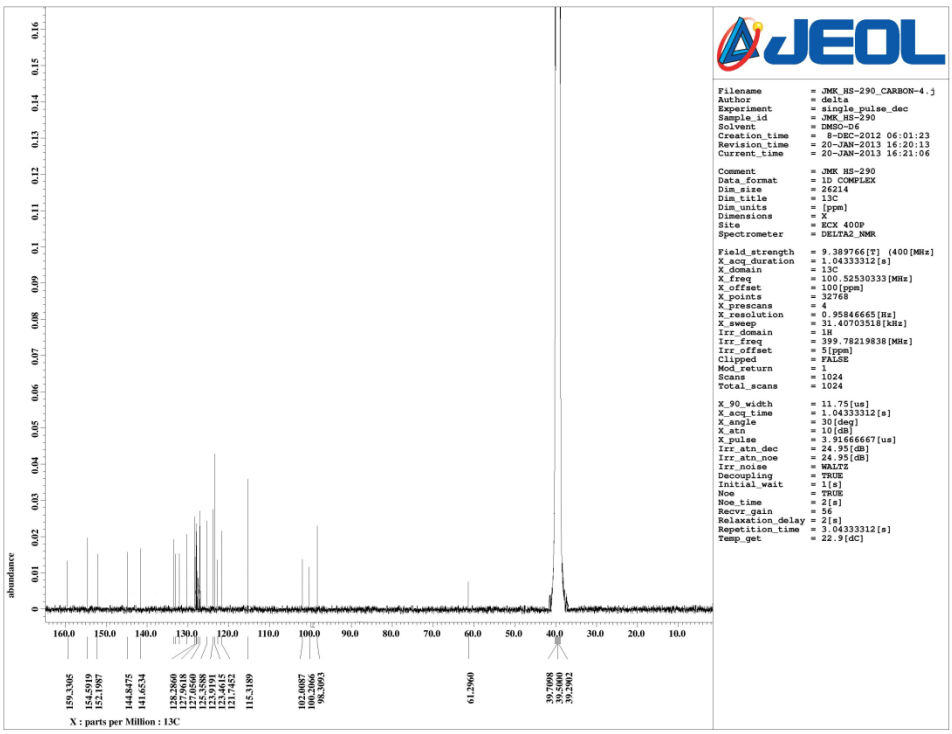

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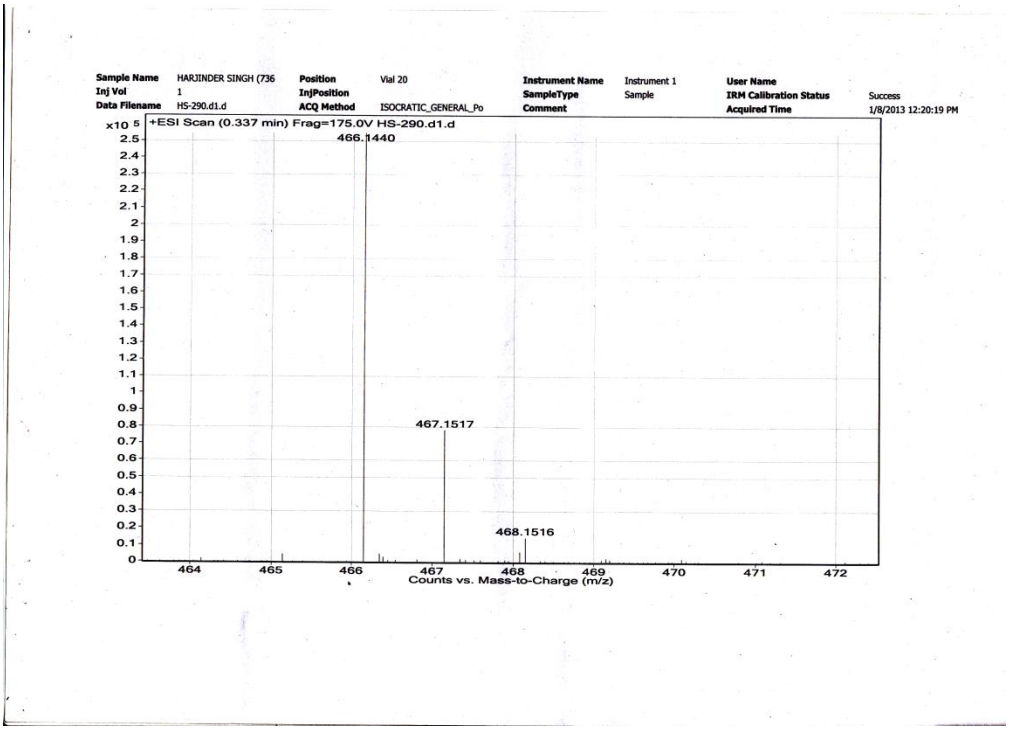

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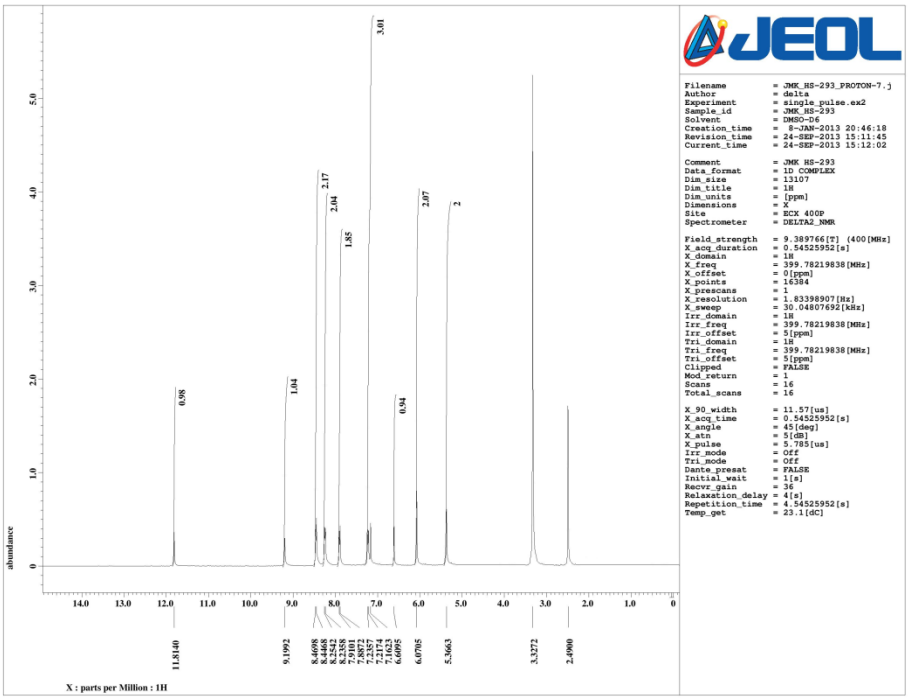

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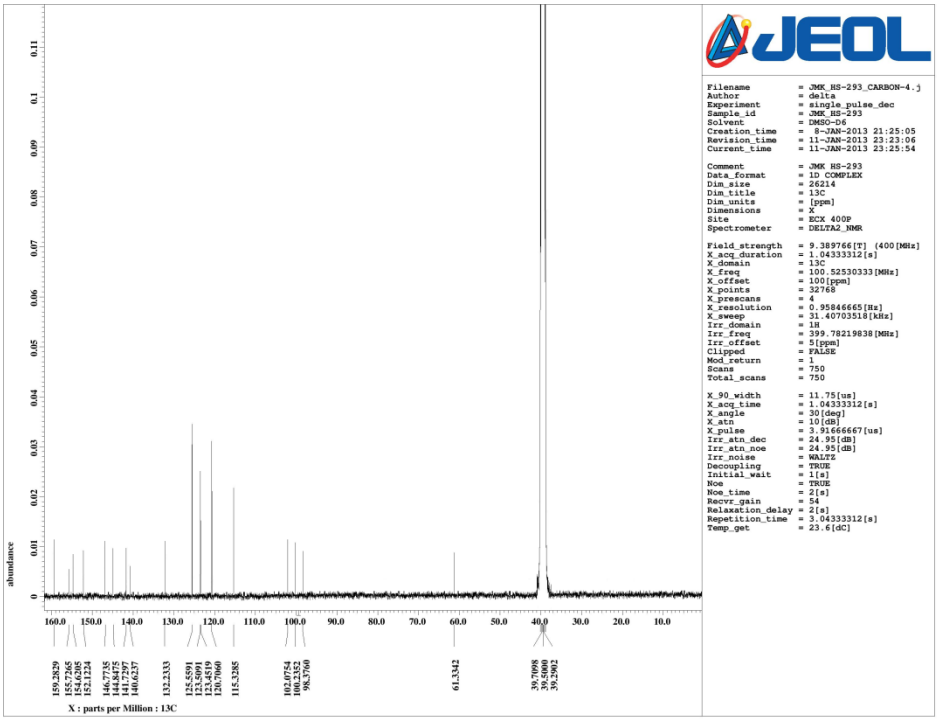

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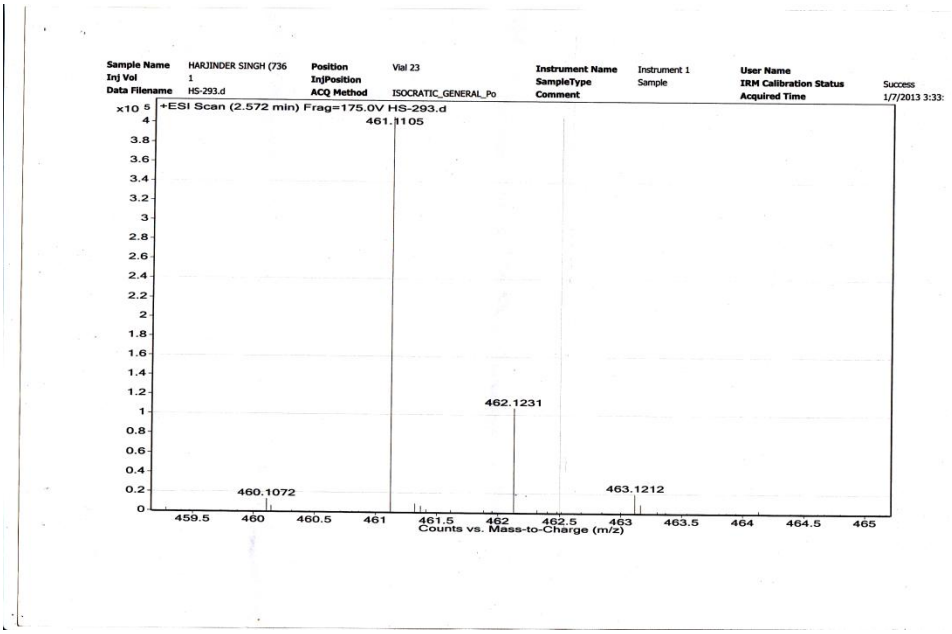

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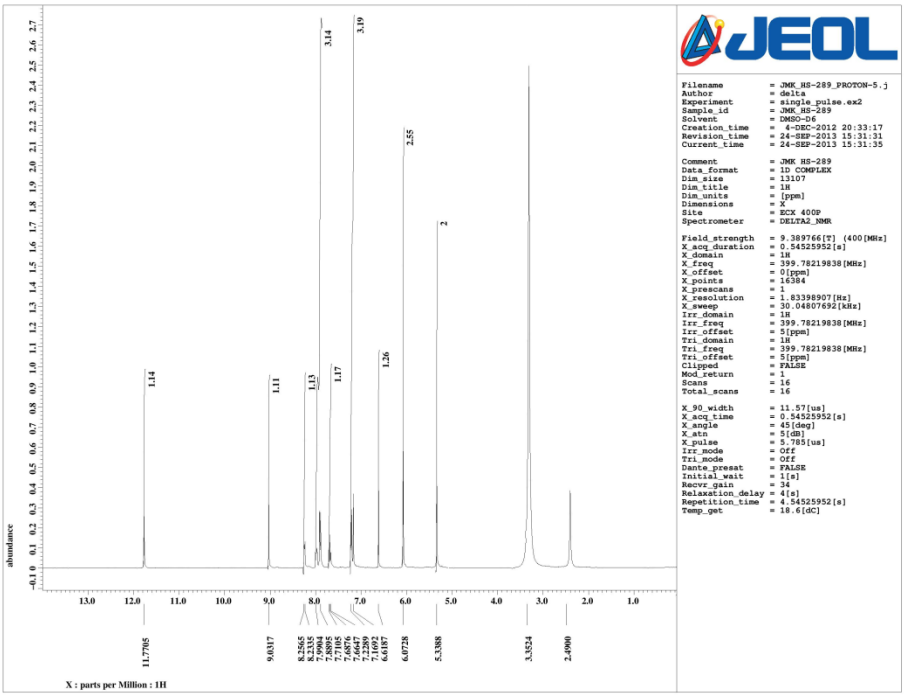

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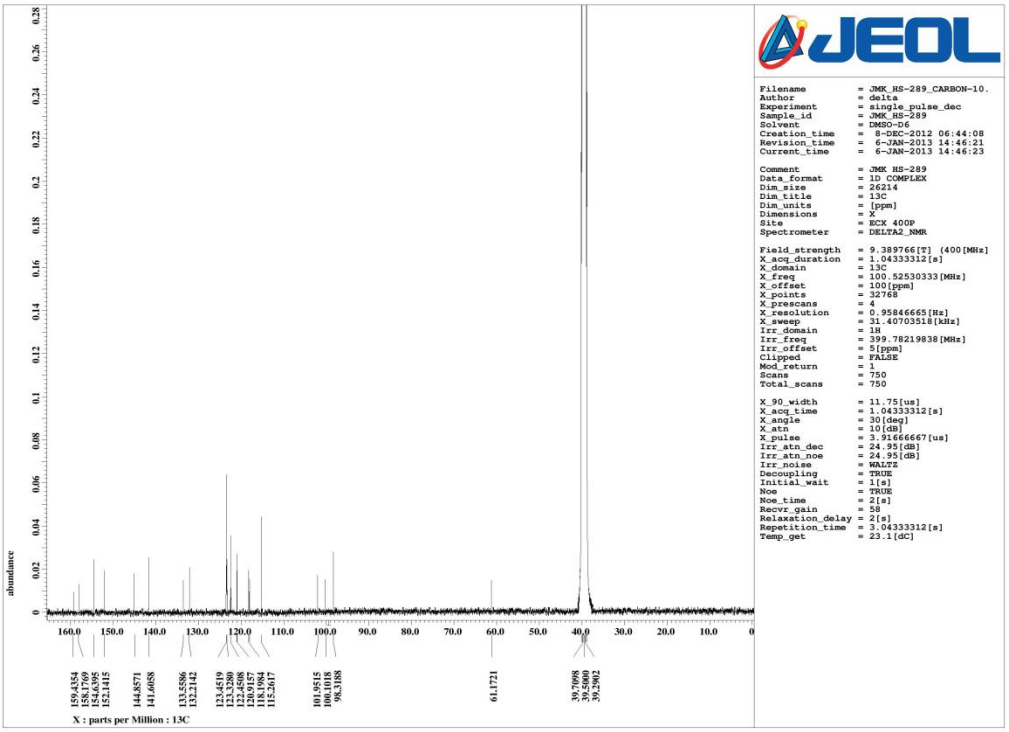

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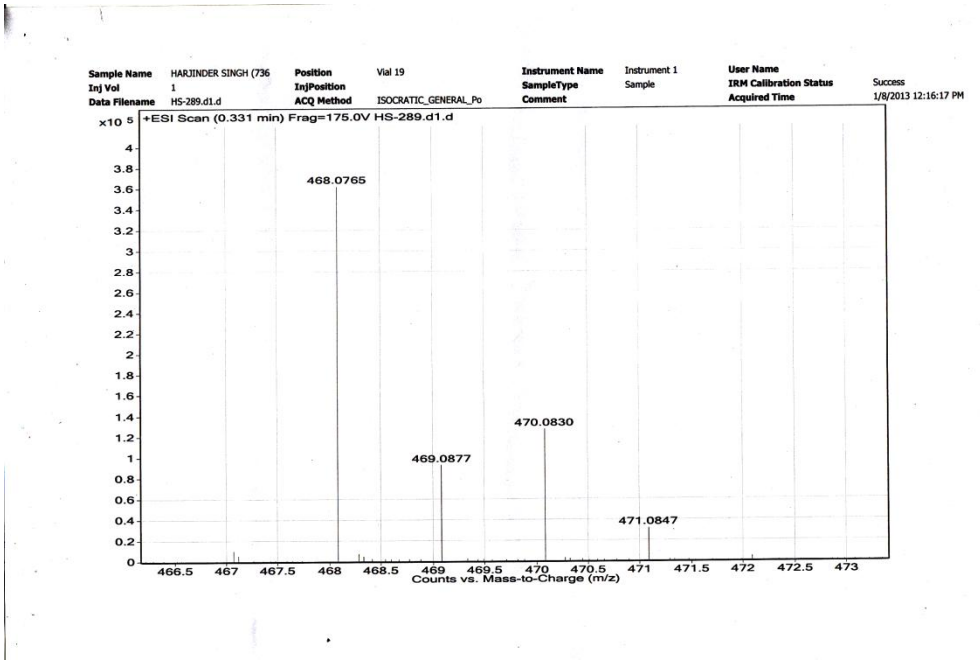

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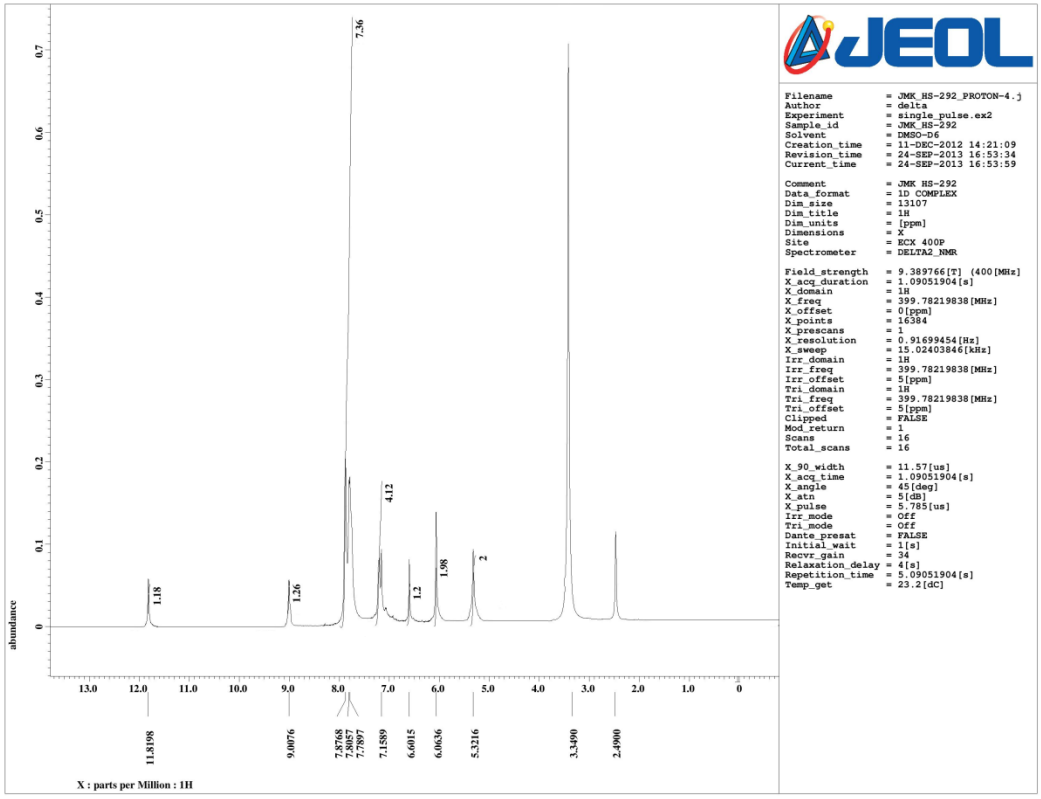

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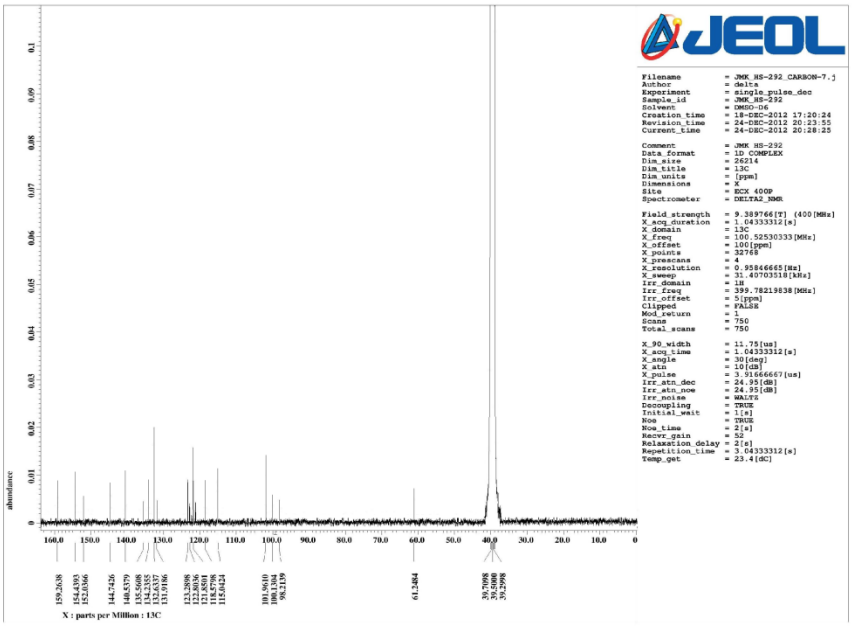
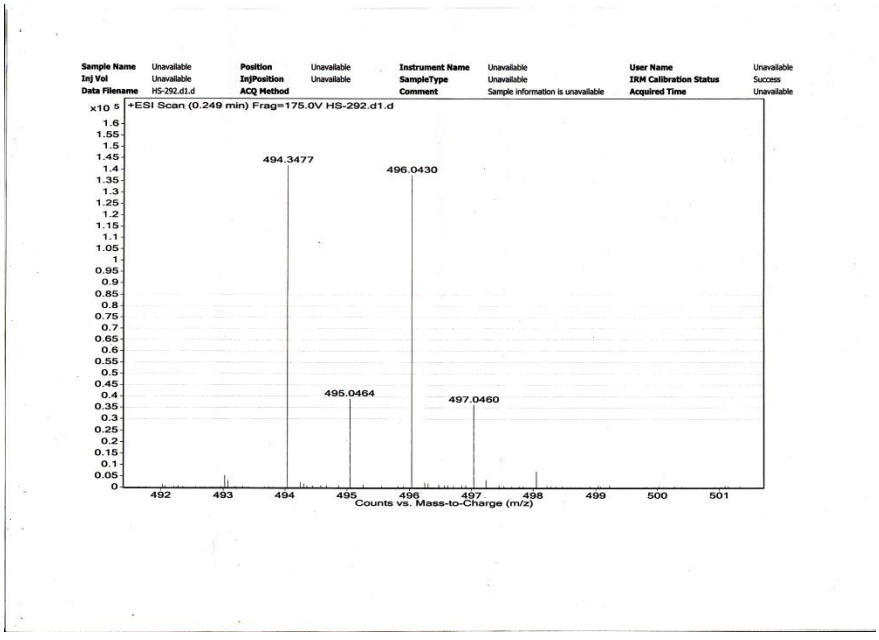
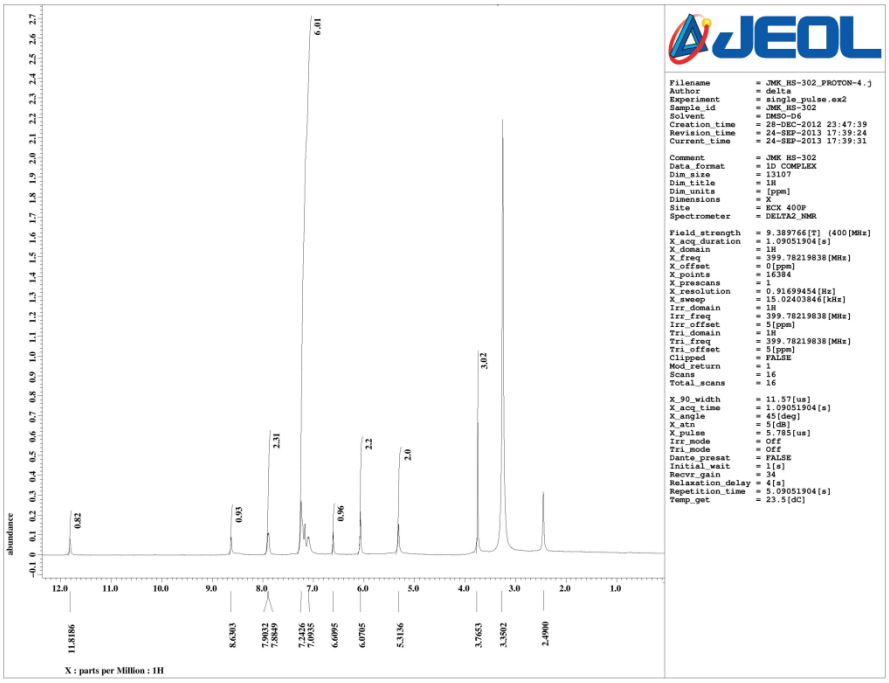


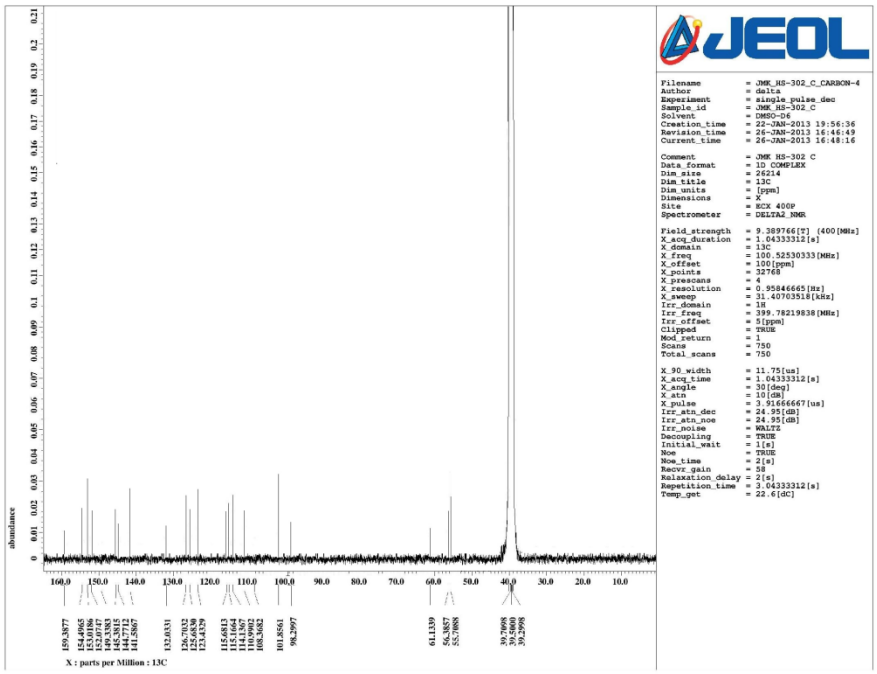
HRMS spectra of compound 6h



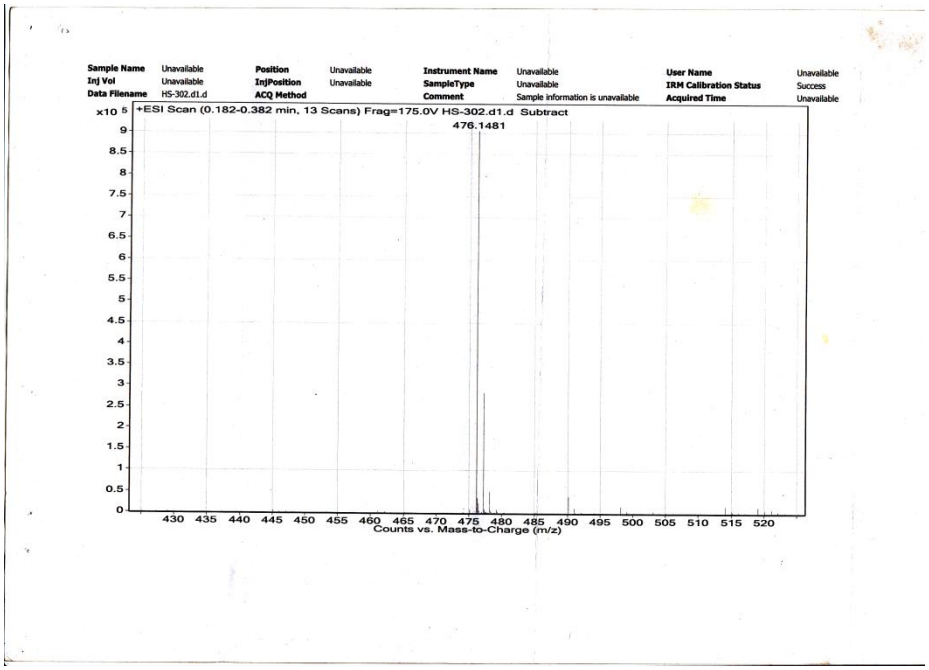
^{1}H spectra of compound 6i



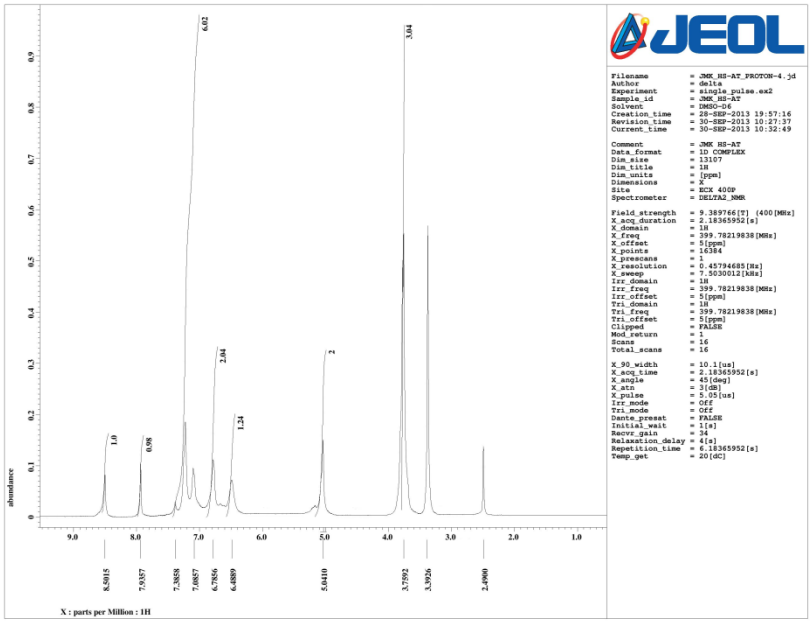
^{13}C spectra of compound 6i



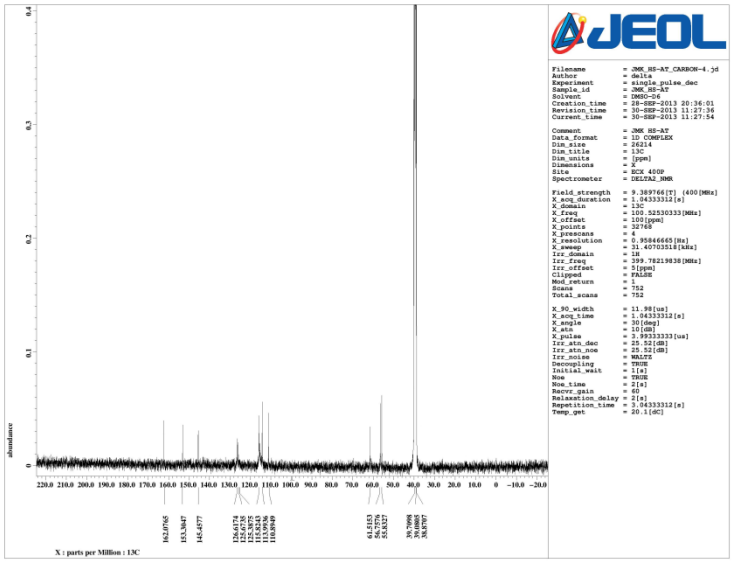
HRMS spectra of compound 6i



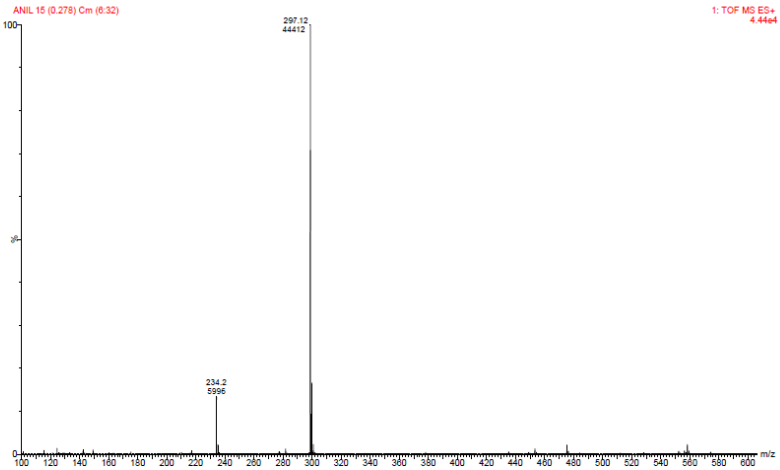
^{1}H spectra of 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)aniline



^{13}C spectra of 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)aniline



MS spectra of 4-((1-(4-methoxyphenyl)-1*H*-1,2,3-triazol-4-yl)methoxy)aniline



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