

Study of Anticonvulsant Clobazam Drug by Sophisticated Instrumentation

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ABSTRACT

Clobazam (7-Chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4(3*H*)-dione) an anticonvulsant drug used since 1984, with molecular formula $C_{16}H_{13}ClN_2O_2$ and molecular mass 300, was characterized with sophisticated instruments like FTIR, UV-NIR, GC-MS, TGA-DTA-DSC and Nano particle size analyzer with zeta potential. The compound was confirmed with the help of GC-MS and the mass of sample was found aggregate with the standard values and mass spectra compare with NIST and WELEY library data. The UV-NIR analysis showed that the λ_{max} was 231 nm with methanol as a diluent. FTIR analysis of the Clobazam gave the characteristics peaks of cyclic amide. HPTLC was used to determine the purity of the drug. Thermal behavior was studied with TGA-DTA-DSC analysis. The particle size of clobazam was measured with the Nano particle size analyzer and Zeta potential of drug found -77.0 mV. The purity of drug check with Preparative HPLC system.

Keywords: clobazam; anticonvulsant drug; cyclic amide; benzodiazepine

1. INTRODUCTION

As of 2005, clobazam (Frisium) is approved in Canada for adjunctive use in tonic-clonic, complex partial, and myoclonic seizures [1]. Clobazam (Urbanyl [2]) is approved for adjunctive therapy in complex partial seizures [3] certain types of status epilepticus, specifically the myoclonic, myoclonic-absent, simple partial, complex partial, and tonic varieties [4], and non-status absence seizures. It is also approved for treatment of anxiety. In India, clobazam (Frisium, Aventis Pharma India, Ltd.) is approved for use as an adjunctive therapy in epilepsy and in acute and chronic anxiety [5]. In Japan, clobazam (Mystan [6]) is approved for adjunctive therapy in treatment-resistant epilepsy featuring complex partial seizures [7]. In New Zealand, clobazam is marketed as Frisium [8]. In the United Kingdom clobazam (Frisium) is approved for short-term (2-4 weeks) relief of acute anxiety in patients who have not responded to other drugs, with or without insomnia and without uncontrolled clinical depression [9]. It was not approved in the US until October 25, 2011, when it was approved for the treatment of seizures associated with Lennox-Gastaut Syndrome [10].

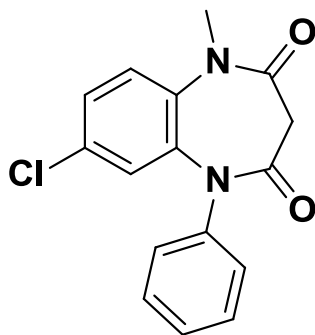


Figure 1. 7-Chloro-1-methyl-5-phenyl-1,5-benzodiazepine-2,4(3H)-dione.

2. OBJECTIVE

Main objective of this study was to become familiar with sophisticated instruments and characterization of the API with the different instruments.

3. MATERIALS, METHOD AND DISCUSSION

3. 1. UPLC/MS/MS

The liquid chromatographic system of Waters Acquity UPLC with PDA (224 nm) and TQD with Mass Link data processing system was used for this entire study and chromatographic separation was achieved by using Waters Acquity BEH C18, 2.4 x 50 mm, 1.7 μ column as stationary phase with binary gradient mode. Sample preparation carried out by 5 mg Clobazam drug dissolved in 25 ml of methanol as diluents. Mobile phase ratio was Ammonium Acetate: Acetonitrile (90:10)

3. 2. Nano particle size analyzer with zeta potential

Analysis carried out with Nano particle Analyzer 52-100, Horiba and sample preparation carried out by 5 mg Clobazam drug dissolved in 25 ml of methanol as diluents. Method for the analysis was scattering angle was 90°, analysis temperature set at 25.0 deg. C and count rate was 1487 kcps.

3. 3. High performance thin layer chromatography (HPTLC)

HPTLC analysis was carried out with Desaga AS 30, Desaga Pro-quant system and sample preparation carried out by 5 mg Clobazam drug dissolved in 25 ml of methanol as diluents. Method for the analysis was Start co. X: 50 mm, Start co. Y: 48 mm, End co. Y: 52 mm, No. of lanes: 10, Mode: Transmission, Evolution mode: Fluorescence, Slit width: 8.0 mm, Slit height: 1.0 mm, Wavelength: 580 nm and Lamp: Deu/tungsten.

3. 4. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis carried out with Shimadzu IRAffinity-1 CE system with IR Solution software and solid sample directly analyzed with ATR (Attenuated Total Reflection) system. Method for the analysis was Measurement mode: % Transmittance, Apodization: Happ-Genel, Number of scans: 20, Resolution: 4.0, Range (cm^{-1}): Min: 650 and Max: 4000.

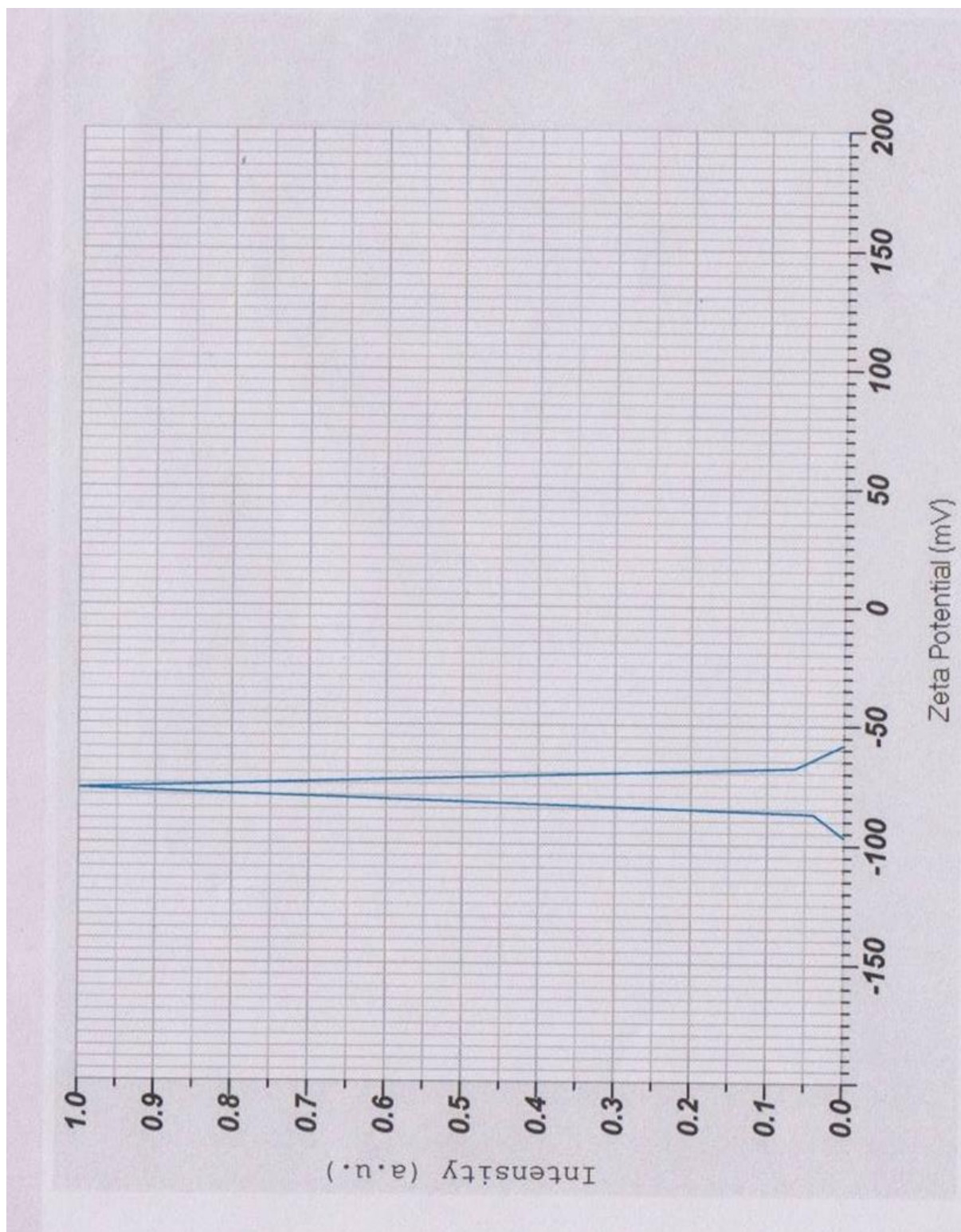


Figure 2. Nano particle size analyzer with zeta potential.

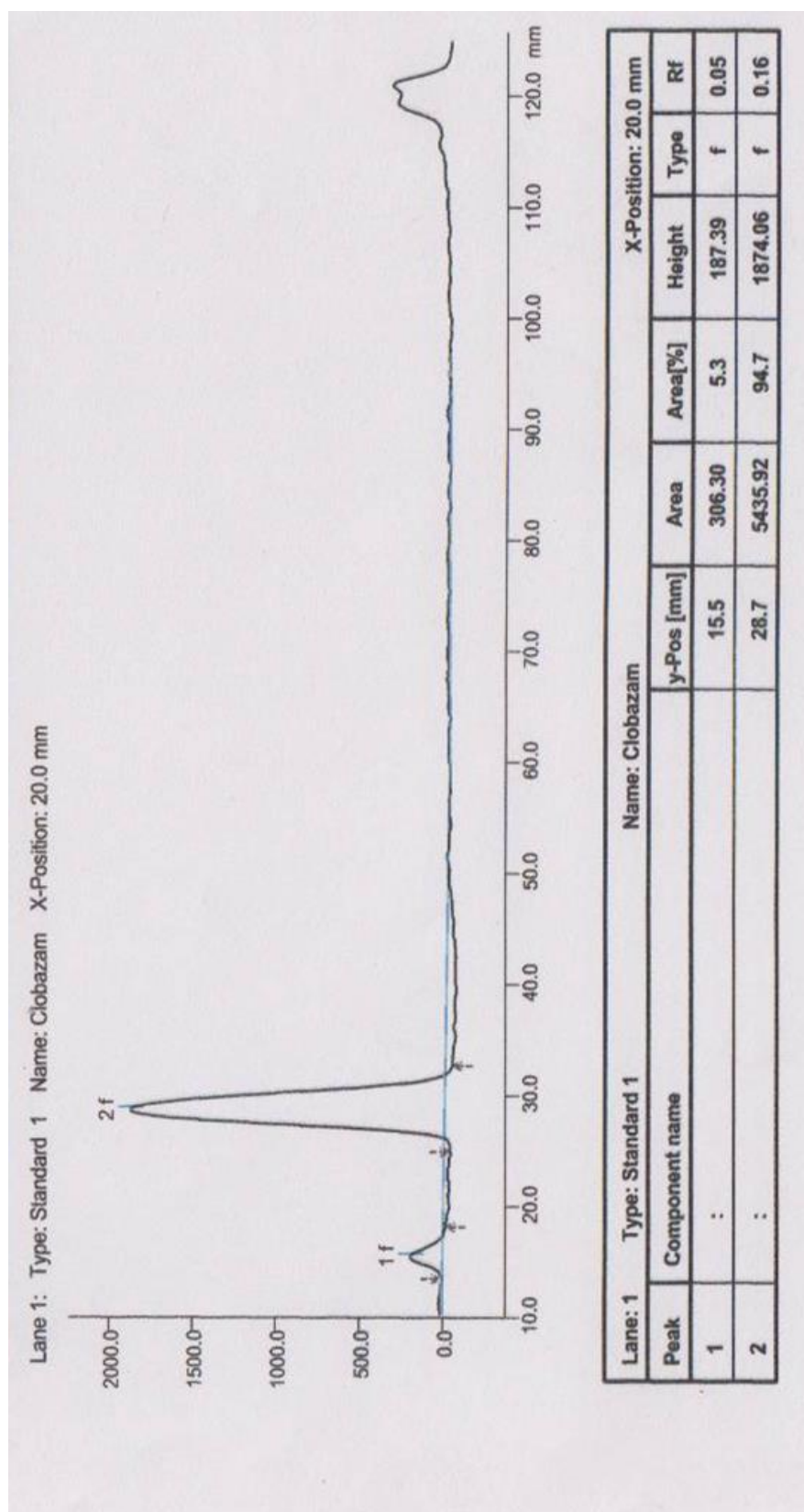


Figure 3. High performance thin layer chromatography (HPTLC).

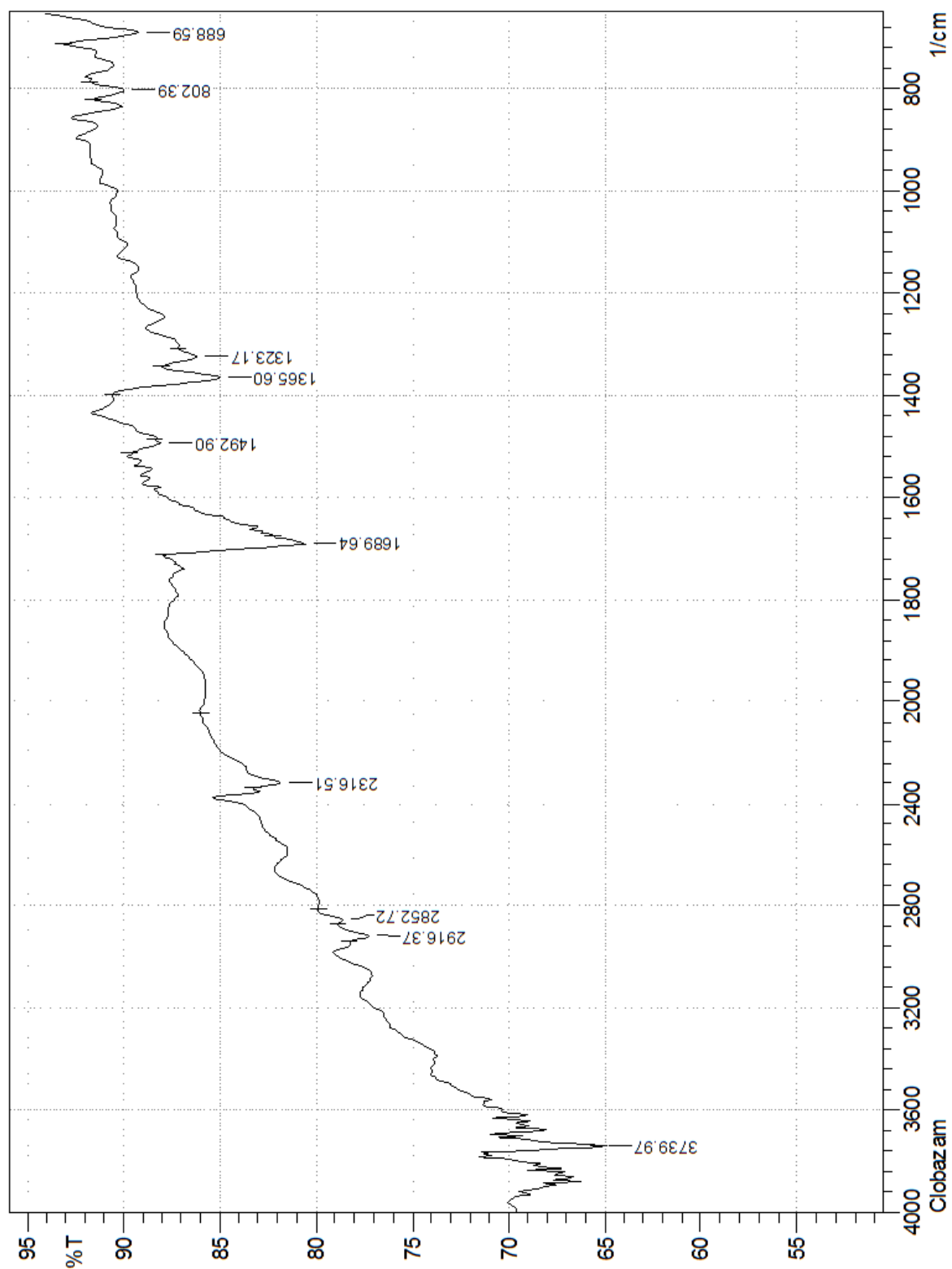


Figure 4. Fourier Transform Infrared Spectroscopy (FTIR).

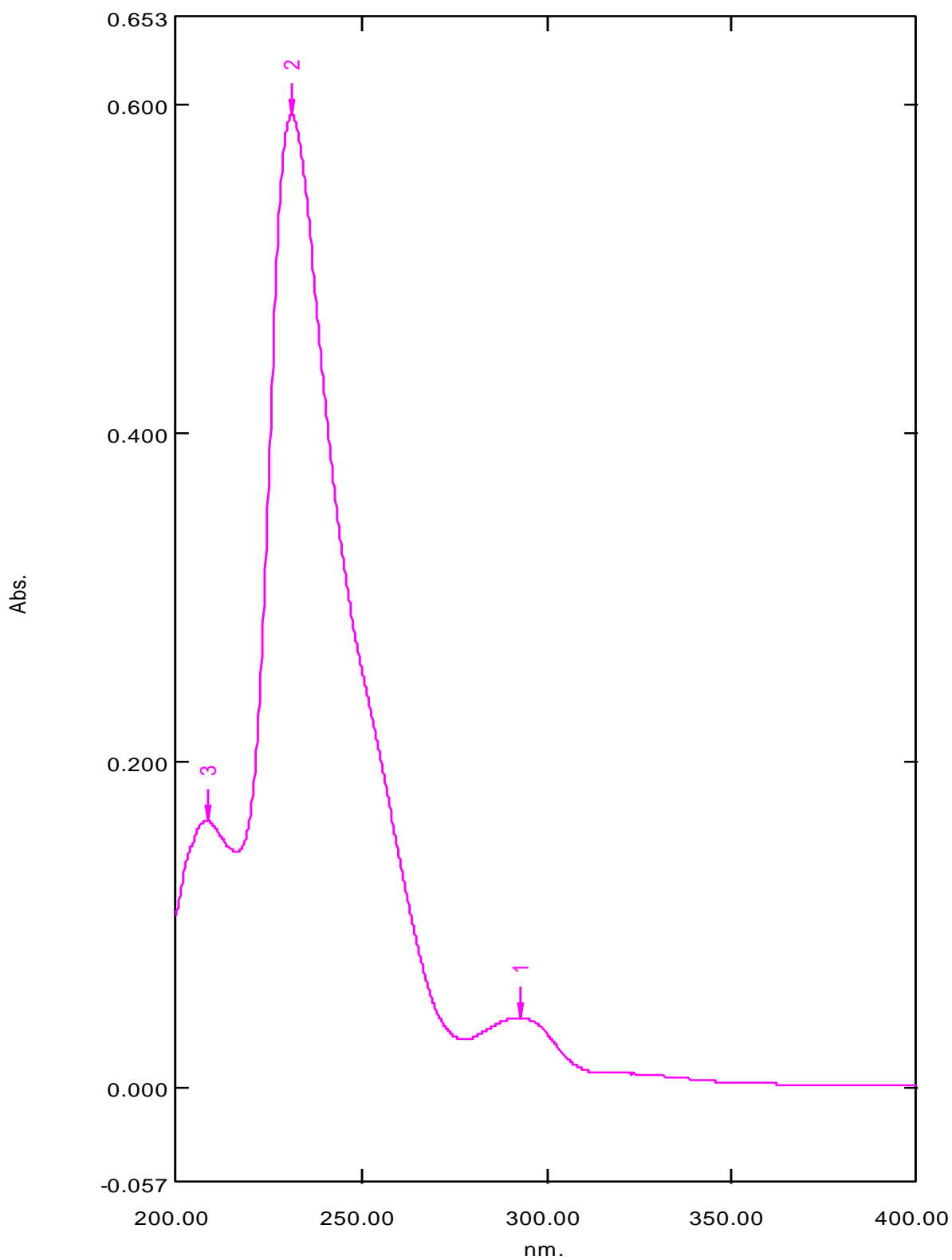


Figure 5. UV-VIS-NIR spectroscopy: (UV-NIR).

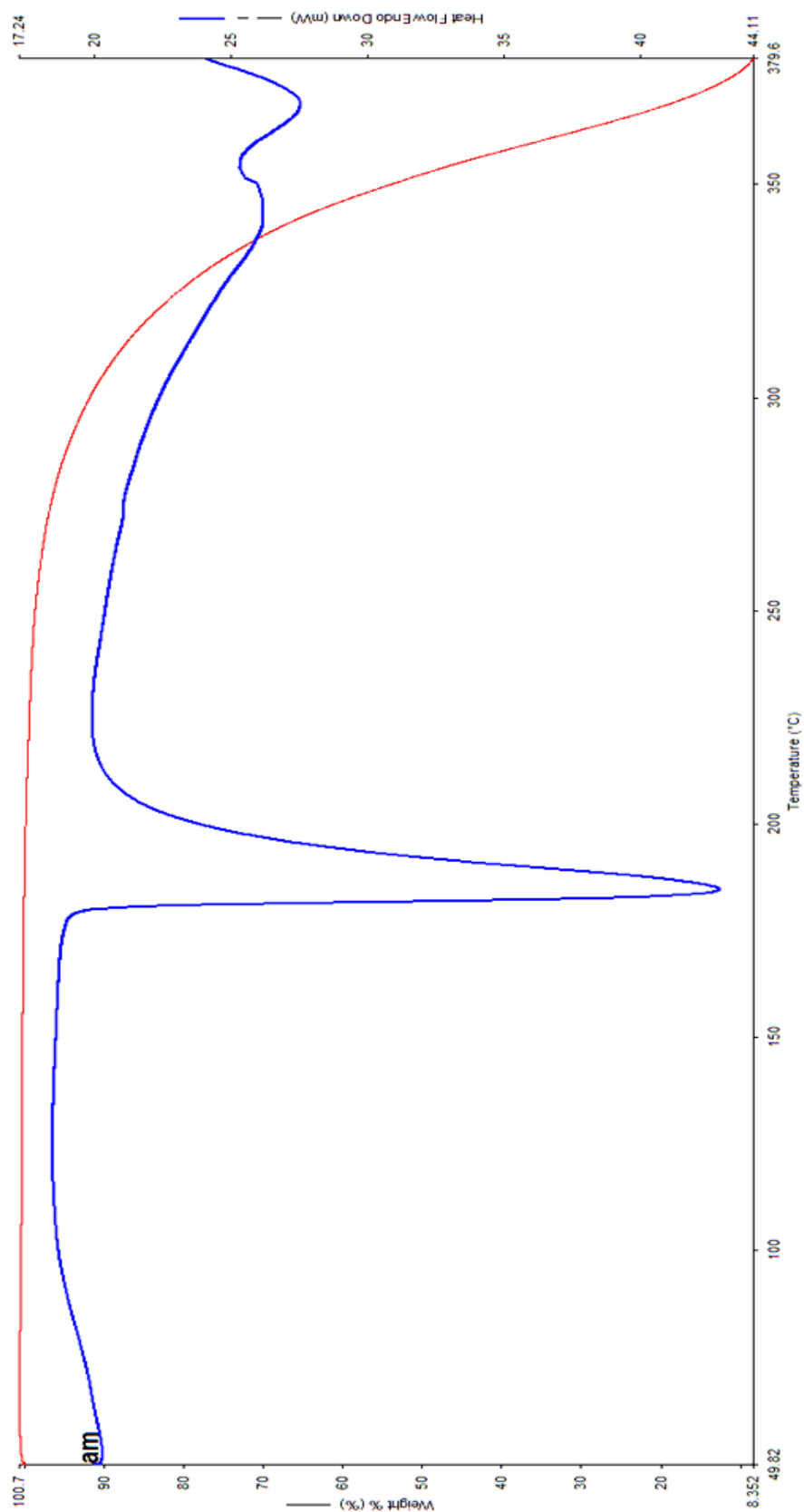


Figure 6. Thermal analysis (TGA-DTA-DSC).

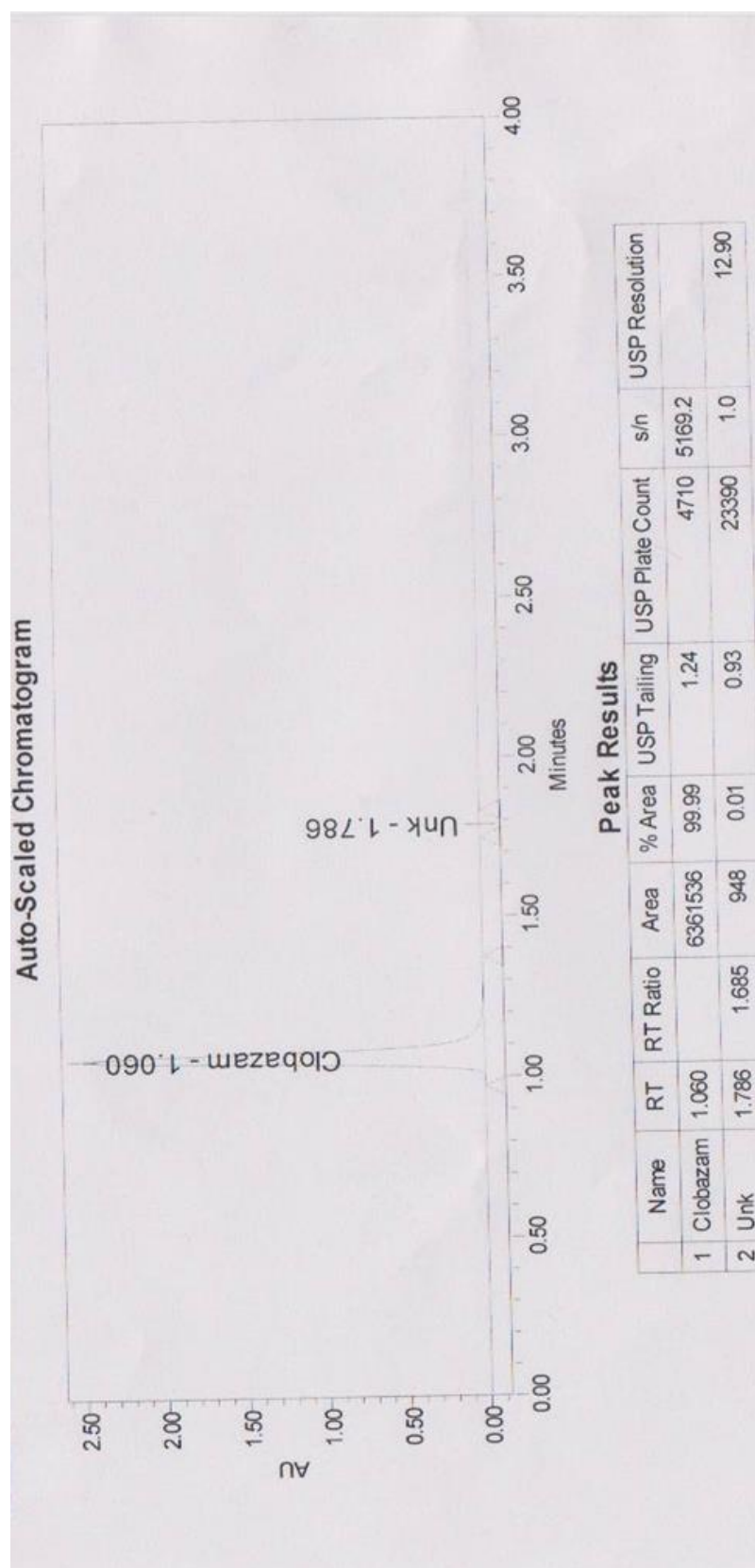


Figure 7. Prep. HPLC.

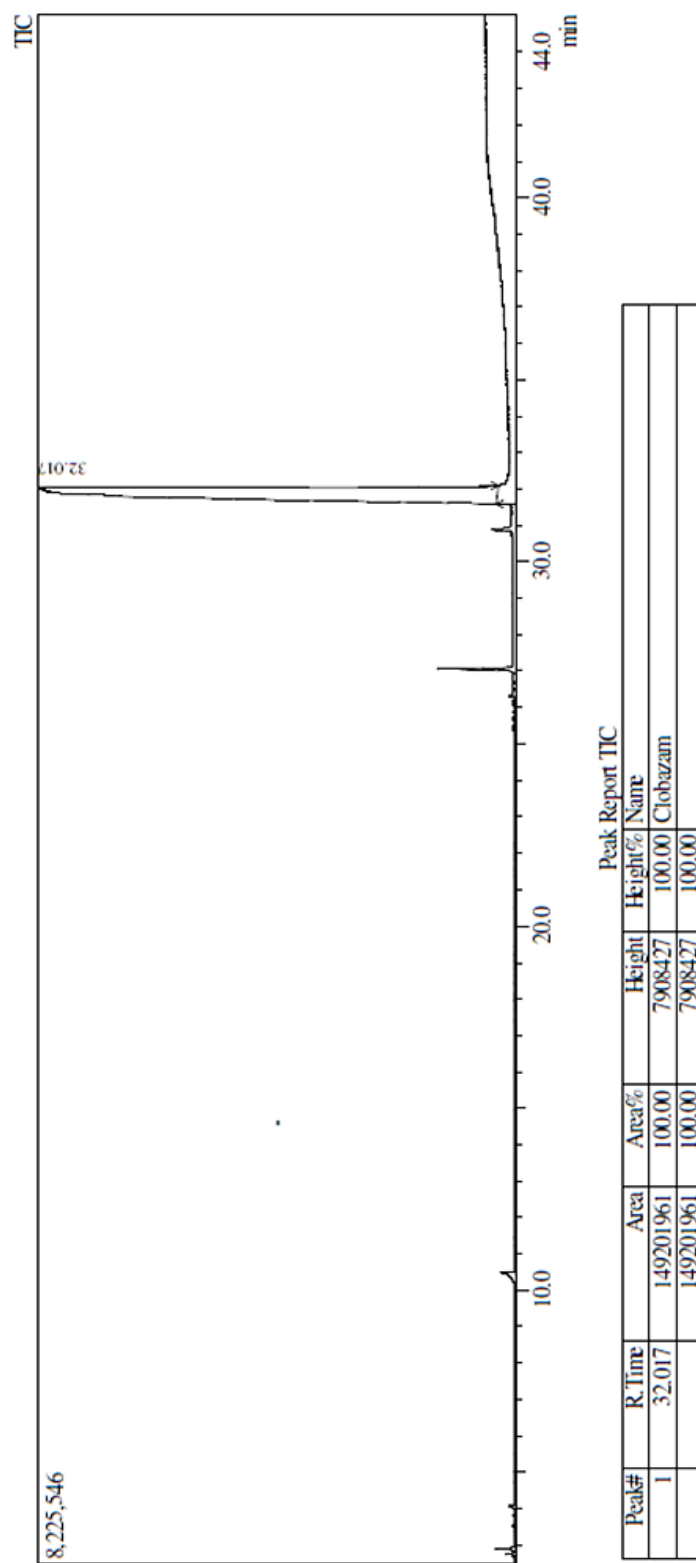


Figure 8. GC-MS analysis.

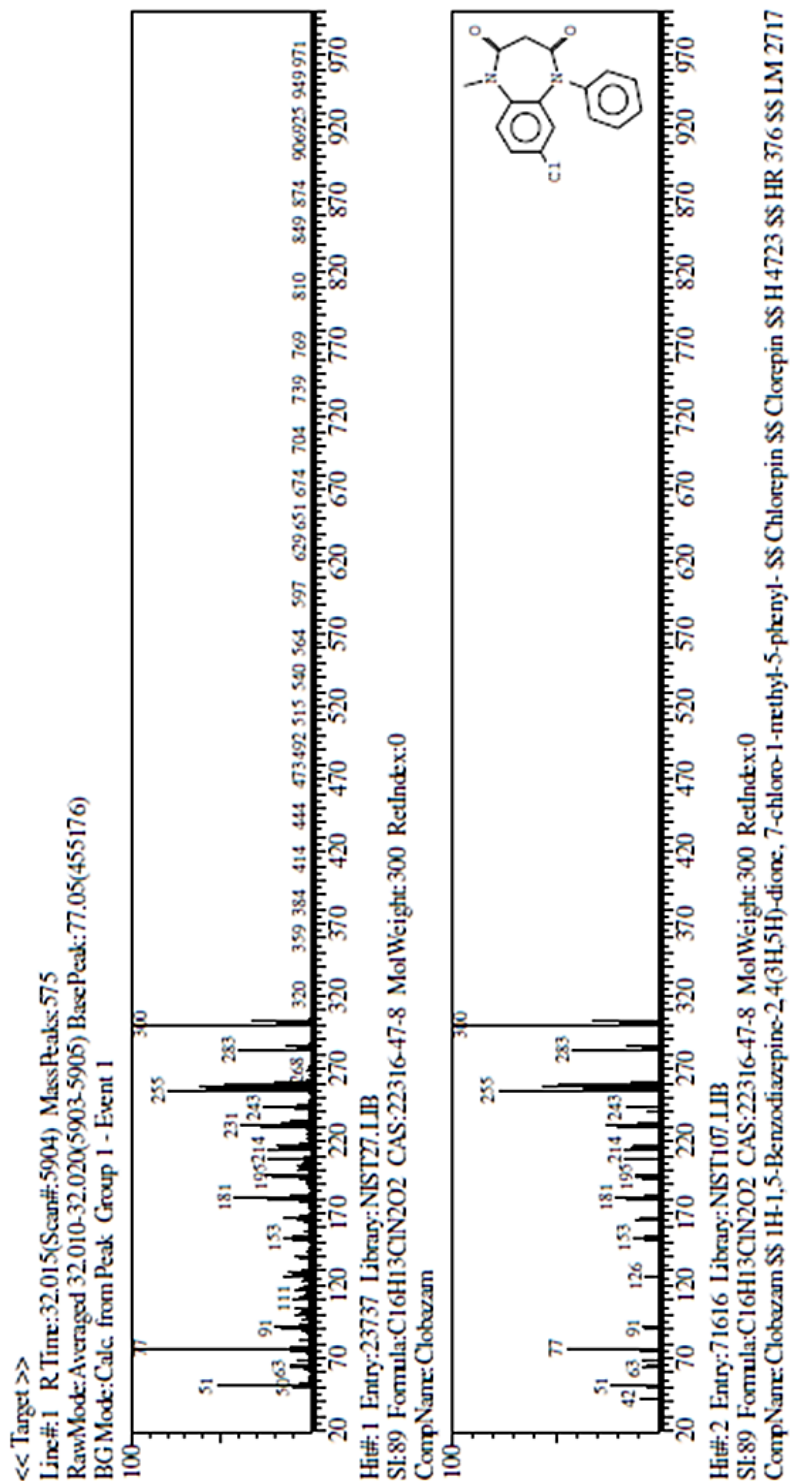


Figure 8(continue). GC-MS analysis.

3. 5. UV-VIS-NIR spectroscopy: (UV-NIR)

UV-NIR analysis was carried out with Shimadzu UV-3600 system with UV probe software and sample preparation carried out by 5 mg Clobazam drug dissolved in 25 ml of methanol as diluents. Further 1 ml of this stock solution was taken and diluted up to 50 ml with diluents. Method for the analysis was wavelength for scan mode start at 200 nm and end at 400 nm, speed for the analysis was set medium.

3. 6. Thermal analysis (TGA-DTA-DSC)

Thermal (TGA-DTA-DSC) analysis was carried out with Perkin-Elmer STA-8000 system with Pyris software and sample preparation carried out by sample take 11.092 mg API powder and directly injected into the system. Method for the analysis was original sample weight: 11.092 mg, Initial temperature: 50 to 400 °C at 20 °C/minute and sample rate set as standard.

3. 7. GC-MS analysis

GC-MS analysis analysis was carried out with Shimadzu make GC-MS QP2010 system with GCMS real time analyzer software in EI mode equipped with a split/split less injector (2500 C), at a split ratio of 1/10, using a SGE make BPX5WCOT (Wall coated open tubular) capillary column (30 × 0.25 mm i.d., 0.25 m film thickness). The oven temperature was 50 °C to 300 °C, at 20 °C/min. Helium was used as a carrier gas at a flow rate of 2.5 ml/min. The injection volume of sample was 1 µl and sample preparation was carried out by 5 mg Clobazam drug dissolved in 25 ml of methanol as diluents. Mass spectrum compare with reported data of NIST and WELEY library.

4. CONCLUSION

The structure was confirmed with the GC MS and UPLC/MS/MS analysis. The positive and negative mass peaks gave the indication of formation of positive and negative fragments. From the thermal analysis it was found that clobazam is highly stable drug. The other spectra were in accordance with the structure of the compound.

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