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Research Article

# SYNTHESIS, ISOLATION, AND CHARACTERIZATION OF HYDROCHLOROTHIAZIDE DIMER IMPURITY

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**ABSTRACT**

Objective: The objective of this study is to find out the unwanted impurity from the Hydrochlorothiazide API (Active pharmaceutical Ingredient).

Method: Impurities ranging from 0.05% to 0.2% of Hydrochlorothiazide were detected by using a simple gradient reversed phase high performance liquid chromatography (RP-HPLC). These impurities were isolated by using Preparative high performance liquid chromatography.

Results: The known impurity of Hydrochlorothiazide that is dimer impurity (Imp-C) was synthesised by chemical synthesis and isolated of purity above 95%. The pure impurity was characterized by using IR, NMR, and MS spectral data and confirm its molecular structure of IUPAC name is N-[[7-(Aminosulfonyl)-6-chloro-2,3-dihydro-1,1-dioxo-4H-1,2,4-benzothiadiazin-4-yl]methyl]-6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-Dioxode.

Conclusion: The Hydrochlorothiazide dimer impurity i.e. Imp-C was properly synthesised and characterized by using modern machinaries.It is the work for the benefit of the human beings, because impurity in the drugs affect the human body.

**Keywords:** Hydrochlorothiazide, Impurities, Spectroscopy, Identification, Characterization and Synthesis

## INTRODUCTION

One of the most common diseases in the developed world is Arterial hypertension. This is the major cardiovascular risk factors for development of coronary heart disease, heart failure, stroke, and chronic kidney disease. In 2000, 970 million people worldwide developed blood pressure and it may increased by 60% in 2025.[1] World Health Organisation gives the result of around 7.1 million deaths per year by Arterial hypertension.[2]

Hydrochlorothiazide is abbreviated as HCTZ.Hydrochlorothiazide is a common diuretic used for hypertension.[3] HCTZ affects the distal renal tubular mechanism of electrolyte reabsorption for diuretic efficiency and increases excretion of sodium and chlorine in approximately the same amounts. The mechanism of the above anti-hypertension drug is not known but may be related to the excretion and distribution of body sodium. The production of HCTZ is with the reaction of 5-chloro-2,4- disulfamylaniline(DSA) and formaldehyde in alkaline solution.[4] The impurity and degradation profiles of a drug substances are critical to its safety assessment and the optimization of the manufacturing process.

This drug belongs to thiazide class of diuretics. It helps to reduce the blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The main site of action in the nephron appears on an electro neutral $Na^+$ $Cl^-$ co transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide includes a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport.[5] On other mechanisms HCTZ is believed to lower peripheral vascular resistance.[6]

This drug sometime used for hypercalciuria, Dent's disease and Meniere's disease. For diabetes insipidus, the effect of thiazide diuretics is presumably mediated by a hypovolemia induced increase in proximal sodium and water reabsorption, thereby diminishing water delivery to the ADH-sensitive sites in the collecting tubules and reducing the urine output.

Thiazides are also used in the treatment of osteoporosis. Thiazides decrease mineral bone loss by promoting calcium retention in the kidney and by directly stimulating osteoblast differentiation and bone mineral formation.[7]

Hydrochlorothiazide, it's IUPAC name is 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzotiadiazine-7-sulfonamide. It is white crystalline solid of melting point of 273$^0$c.

Hydrochlorothiazide is stable and incompatible with strong oxidizing agents and it is insoluble in water. It's molecular formula is $C_7H_8ClN_3O_4S_2$ and the molecular weight is 297.74, and it's monatomic mass is 296.7. The molecular structure is as below in figure no-1

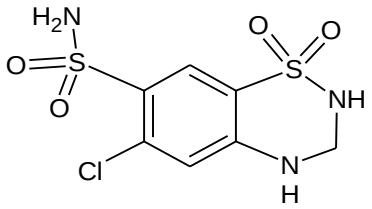


**Fig. 1:**

A literature search revealed that only analytical techniques are available but has less reported synthesis, isolation, and characterization of impurity in the purified form, from hydrochlorothiazide API. The present communication involves the isolation, preparation of impurity and characterization by chromatographic and spectroscopic technique.

## MATERIALS AND METHODS

### Materials and reagents

The raw material of Hydrochlorothiazide dimmer impurity was received from Sitec labs Mumbai, India. The HPLC grade acetonitrile and methanol solvents were obtained from Merck co, Mumbai, India. The HPLC grade Formic acid was obtained from Sigma Aldrich, Mumbai, India.

### High performance liquid chromatography (HPLC Analytical)

An Agilent HPLC system equipped with 1100 series low pressure quaternary gradient pump along with pulse dampener, Photo diode array detector with auto liquid sampler handling system has been used for the analysis of the sample. An X-tera RP-18 , 4.6mm x 5cm x3.5µ column was employed for the testing of reaction mass of Hydrochlorothiazide impurity. The column eluent was monitored at

detection wavelength 275nm. The mobile phase was anhydrous Formic acid 5 ml in 1000 ml hplc grade water and degas it properly. Acetonitrile and Methanol were mixed in 3:1 ratio and degas properly with sonication.The gradient procedure was 00 to 05 min= 97:03, 05 to 14 min= 64 :36, 14 to 18 min = 97:03, 18 to 20 min = 97:03. Chromatography was performed at 35 $^0$c with the flow rate of 1.0 ml/min. Data was recorded by using Chem. station software.

**High performance liquid chromatography (Preparative HPLC)**

Preparative HPLC is the technique of choice for compound isolation and purification within the pharmaceutical and life science industries. Agilent technologies purification solution from nano gram to gram sample quantities. Agilent 1200 Series purification system with low delay volumes optimized for high recovery and purity, with PDA detector and flow rate is 0.001 to 100 ml/min with max. Pressure 400 bar. A ODS-C18 250mm x 21.2mm x 10µ reverse phase silica column was employed for the separation of open ring impurity of Hydrochlorothiazide dimer impurity. Solvent used for the separation was Water (0.1% formic acid): ACN with flow rate of 20 ml/min, with the detection of 275nm.and chromatography was performed at room temperature. The gradient procedure was 00 to 10 min = 80:20, 10 to 30min = 10:90, 30 to 32 min = 10:90, 34 to 35 min = 80:20.

**Microwave**

The use of microwave irradiation in organic synthesis has become increasingly popular within the pharmaceutical and academic arenas, because it is a new enabling technology for drug discovery and development. For the synthesis of the dimer impurity of Hydrochlorothiazide used CEM Discover microwave system. Its a system of ISO 9001-2000 approved. This system perform atmospheric (up to 70ml working volume ) and pressurized (up to 50 ml working volume) reaction. Use a wide range of vessels as well as standard condensers, addition funnels and stirring options with refluxing capability. CEM reaction tubes are pressure rated to 500 psi and use septa that tolerate multiple piercing for reagent addition or sample withdrawal. Microwave system gave faster reactions with increased yields, improved selectivity, and superior reproducibility. Optimization of the reaction very quickly and in fewer steps gave more time to use creativity to explore the available chemical diversity. The instrument specification, overall dimension is (36.2 cm x 43.7 cm x 22.1 cm), weight = 30lbs, Magnetic frequency= 2450 MHz, Power output= 300 Watts, temperature = -90$^0$C to 300$^0$C.

**Mass spectrometry (LC-MS/MS)**

The LC–mass spectrometry (MS) and MS-MS studies were carried out on an Ion trap 6320 Series electron spray ion trap spectrometer (Agilent Technologies).The source voltage was kept at3.0 kV. Parameters: nebulizer gas = 30psi; dry gas = 3 L/min; dry temperature= 150 °C; capillary voltage =24500 to 21500 V. Nitrogen was used as both a sheath and auxiliary gas. Mass range was kept at *m/z* 50–600.The chromatography conditions and mobile phase are column = X-Tera RP(5 cmx4.6 mm x3.5 µ), temperature= 35 $^0$C, Wavelength = 275nm, Mobile phase = Water(10 mm AmmoniumFormate) : (ACN+Meoh) = (3 : 1).The flow rate was maintained at 1.0 mL/min.

**Nuclear magnetic resonance**

The 1H, nuclear magnetic resonance (NMR) spectroscopy experiment of the impurity was carried out at a frequency of 500 MHz at 25 °C on an NMR spectrometer (Varian, Palo Alto, California). 1H chemical shifts are reported on the δ scale in ppm relative to tetra methyl silane 0.00 and$CDCl_3$ (δ77.00 ppm) and DMSO, D6 (δ=39.50) respectively. $^1$H experiments were run using a mixing time of 1000ns.

**FT-IR spectroscopy**

The IR spectra were recorded in the solid state as KBr dispersion medium using Perkin Elmer spectrum 100 FT-IR spectrophotometer.

**Synthesis of impurity**

In a Microwave glass tube, Hydrochlorothiazide API (2gm) was charged with Acetonitrile solvent. In that mixture added para Formaldehyde (0.5gm) of very slowly at 25 $^0$C – 30 $^0$C and stirred for 15-20 mins. After that expose the reaction mass at 120 $^0$C at 40-to-50 watt power in the CEM Discover Microwave for 15 mins interval. After the completion of the reaction, neutralized the mass with aqueous alkaline solution. Reaction mass checked in HPLC and got the required impurity of 10% purity, and checked the reaction mass in LCMS for the confirmation of the required impurity mass. To improve the percentage of the required impurity used preparative chromatography, for the characterization of the impurity.

**RESULTS AND DISCUSSION**

**Detection of impurity by HPLC**

Typical analytical HPLC chromatogram of hydrochlorothiazide bulk drug and it's dimer impurity obtained by using the HPLC method discussed under the heading "High performance Liquid Chromatography (analytical)". The targeted impurity under study is marked as dimmer impurity(Imp-C) eluted at retention time of about 8.966 mins. It was given in figure no-2.

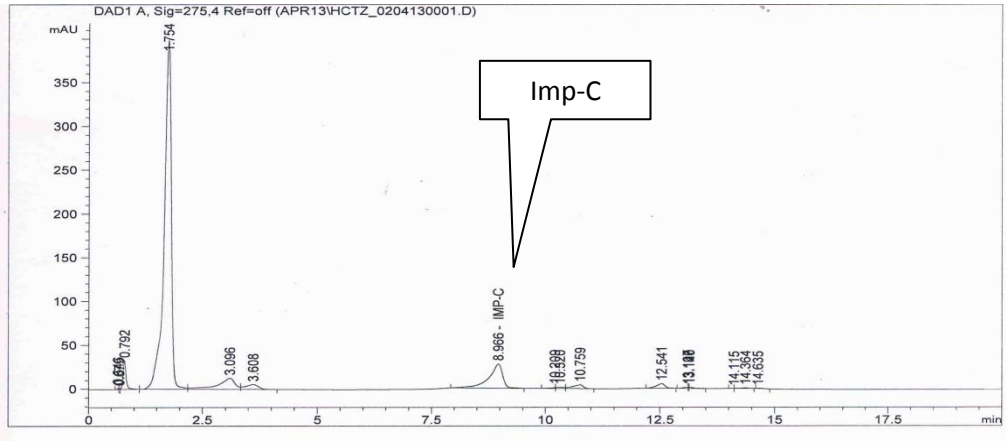


**Fig. 2: Chromatograph of Reaction Mass**

**Isolation of the impurity by PREP-HPLC**

A simple reverse phase chromatographic system, discussed under the heading, "High performance Liquid Chromatography (preparative) was used for isolation of the impurity. In this chromatographic system, the dimmer impurity (Imp-c) eluted at about 16.178 mins. The hydrochlorothiazide dimmer impurity (imp-c) fraction was collected between 14.6min to 17.2min, and it is exhibited in figure no-3 The impurity fraction was concentrated at room temperature under high vacuum on a Buchii Rotavapour

Model R124, the residue was checked after rota, it was degraded. Hence the collected volume from the preparative put directly in the lyopholization for getting solid dimmer impurity (Imp-c). Purity of the impurity was tested in analytical method discussed under the heading, "High Performance Liquid Chromatography" (HPLC). The purity was found to be 97.895 % and it is exhibited in figure no.4, before carrying out spectroscopic experiments.

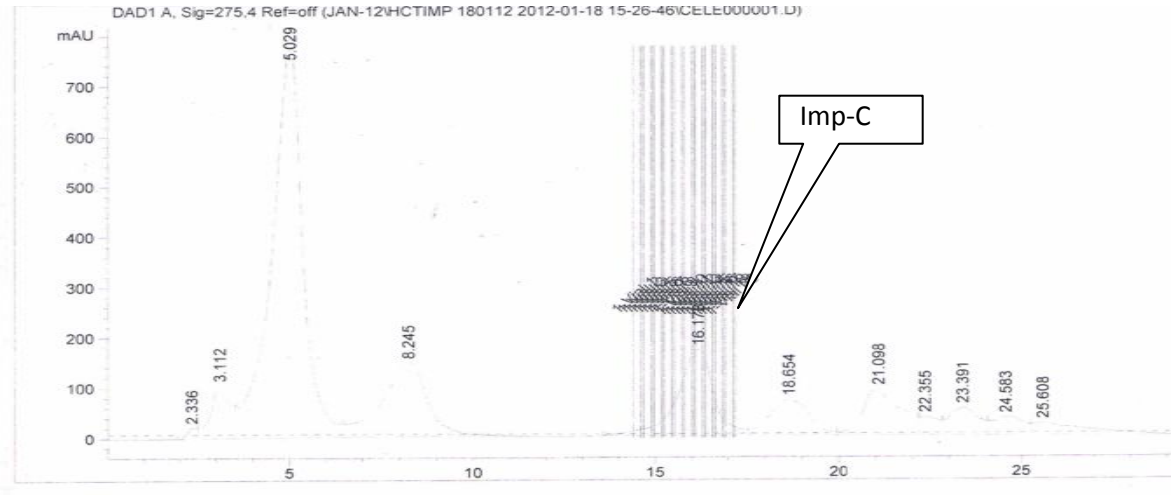


**Fig. 3: Chromatograph of Preparative HPLC.**

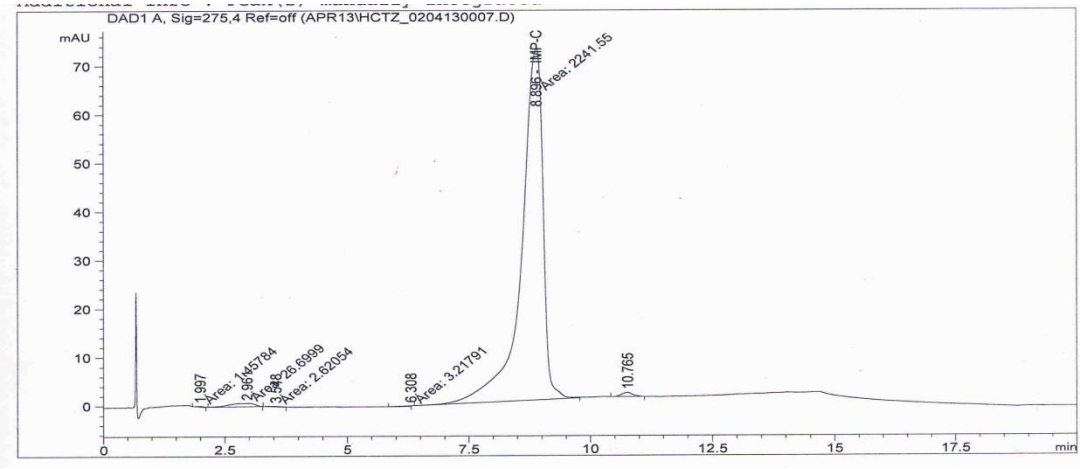


**Fig. 4: Chromatograph of pure Dimer impurity.**

**LC-MS/MS analysis**

LC-MS/MS analysis of hydrochlorothiazide bulk drug sample and dimer impurity of hydrochlorothiazide(Imp-C) was performed using the chromatographic system as described under the heading "Mass Spectrometry (LC-MS/MS)".Result of LC-MS/MS analysis revealed that impurity exhibited molecular ion at m/z(M-1)=604.7amu. and it's MS/MS shown 307.6,217.6 amu.Hydrochlorothiazide API exhibited molecular ion at m/z(M-1)= 295.9amu, and it's MS/MS shown 271.4 amu.

**Structure elucidation**

The Molecular Structure of dimer impurity of hydrochlorothiazide (Imp-C) is as Figure-5. The Molecular Formula is $C_{15}H_{16}Cl_2N_6O_8S_4$ and it's Molecular weight is 607.49 amu and it's monatomic mass is 605.7 amu.

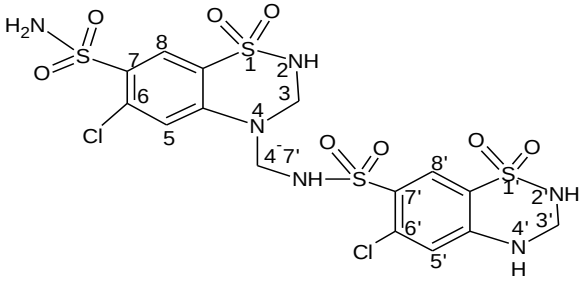

**Fig. 5: Molecular Structure of HCT dimer**.

The IR spectra recorded in the solid state as KBr dispersion. FT-IR data of hydrochlorothiazide and its dimer impurity (Imp-C) exhibited in the figure no-6 and figure no-7. And the mass spectral data exhibited in the Table no-1.

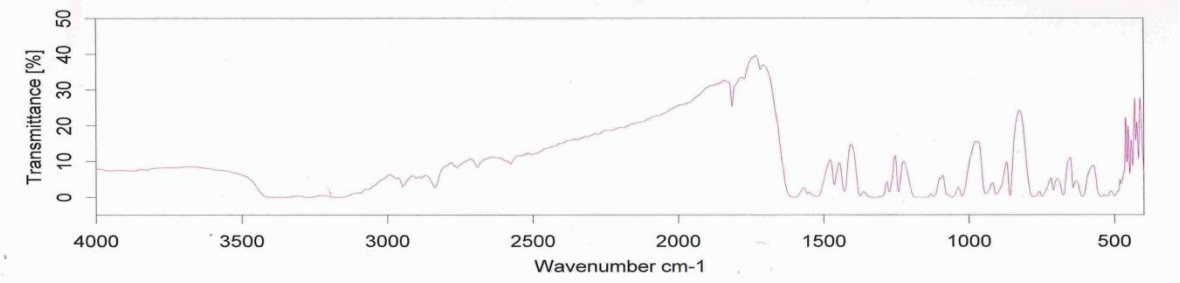


**Fig. 6: IR of HCT API.**

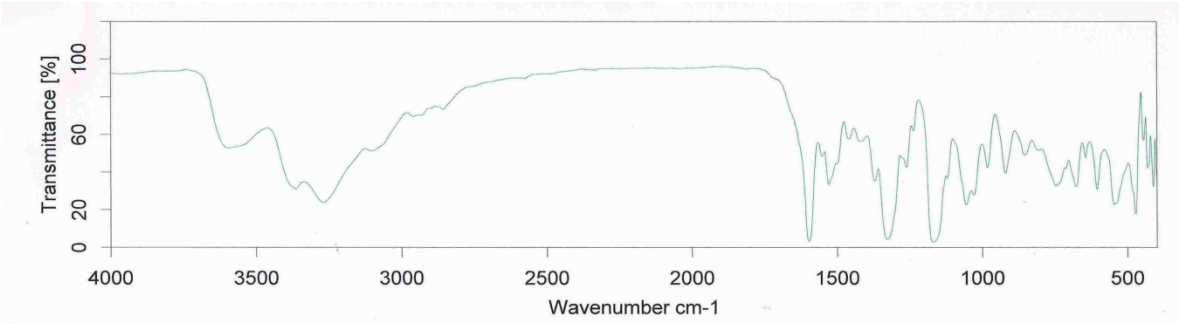


**Fig. 7: IR of HCT dimmer Impurity.**

**Table 1: MS/ MS-MS spectral data of Hydrochlorothiazide and it's dimer impurity (Imp-C).**

| S. No. | Compound | MS/ MS-MS Data |
|---|---|---|
| 1. | Hydrochlorothiazide | m/z 295.9 295.5, 271.4. |
| 2. | Dimmer Impurity (Imp-C) | m/z 604.7 307.6, 217.6. |

Dimer impurity of hydrochlorothiazide (Imp-C), 1H NMR, structure prediction and detailed assignment shown in table-2.

**Table 2: 1H NMR of Hydrochlorothiazide (Impurity-C).**

| Chemical shift (PPM) | Number of protons | Multiplicity | Assignment |
|---|---|---|---|
| 2.483 | | | DMSO |
| 3.294 | | | Water |
| 4.724 | 4 | Doublet | C3'andC4-7' |
| 4.802 | 2 | Doublet | C3 |
| 6.949 | 1 | Singlet | C5' |
| 7.311 | 1 | Singlet | C5 |
| 7.560 | 2 | Singlet | C7-SO2-NH2 |
| 7.929 | 1 | Triplet | N2' |
| 8.029 | 1 | Singlet | C8' |
| 8.129 | 1 | Singlet | N4' |
| 8.326 | 1H | Triplet | N2 |
| 8.816 | 1H | Triplet | C7'-SO2-NH- |

## CONCLUSION

This research paper describes the synthesis, isolation and structure elucidation of process related HCTZ dimmer impurity of Hydrochlorothiazide i.e. Imp-C as reported in USP. The impurity was separated by reverse phase chromatographic technique, by using High performance liquid chromatography (prep-HPLC). The isolated impurity was characterized by using IR, 1H NMR, and LC-MS/MS spectroscopic technique. The synthesis of impurity was also discussed in brief.

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