

SYNTHESIS, ISOLATION, CHARACTERISATION AND QUANTIFICATION OF PROCESS RELATED IMPURITY OF ZIDOVUDINE

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ABSTRACT

Zidovudine is a nucleoside analogue reverse-transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV/AIDS infection. Impurity can be developed in formulation or upon aging of API'S and formulated API'S in medicine. During the processing of Zidovudine, unknown impurities present in the laboratory batches in the range of 0.05–1.0% in HPLC analysis. The process related impurity of Zidovudine, pyrimidine 2, 4(1H, 3H)-dione in bulk and formulations was synthesized, characterized and the RP-HPLC method was developed according to ICH Q₂B guidelines for quantitation of impurity in bulk and formulations. The synthesis of intermediate was carried out by using Propionyl chloride, urea in presence of ethanol as catalyst. Characterisation was done by various sophisticated techniques like IR, NMR- ¹H, ¹³C, MS. The amount of impurity present in bulk drug and formulation was found to be 0.057% and 0.083% respectively which is in limit as per ICH guideline.

Keywords: Impurity, Zidovudine, HPLC, validation.

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INTRODUCTION

Zidovudine chemically known as 1-(3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methylpyrimidine-2,4(1H,3H)-dione, which is inhibitor of enzyme reverse transcriptase. This enzyme is used by HIV (human immune deficiency virus) to synthesise DNA. Zidovudine by inhibiting this enzyme prevents viral DNA from forming.¹ The present study aimed to identify, isolate, characterise and quantify process related impurity associated with Zidovudine bulk and its formulation.²

Regulatory authorities like ICH, USFDA and Canadian drug health agency have stringent condition about purity requirements and identification of impurities in API'S. ICH defines impurity as 'A description of the identified and unidentified impurities present in new drug substance.'³ As drug substance mature through development from early phases in chemical process, concomitant maturing of process related impurity understanding and control is required.⁴ The presence of impurities or its related compounds in a drug substance can have a significant impact on the quality and safety of the drug product. The presence of these unwanted chemicals, even in small amount, may influence the efficacy of the pharmaceutical products.⁵

Qualification of impurity present in bulk drug and formulation is becoming essential by considering safety of patient. According to ICH guidelines, qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity. Thus it suggests need of impurity profiling of drug in pharmaceutical analysis. As per ICH guidelines, the maximum daily dose qualification threshold is, ≤ 2g/day 0.1% or 1 mg per day intake (whichever is lower) ≥ 2g/day 0.05%.⁶ The synthesised compound Pyrimidine 2,4(1H,3H)dione (ZI) is process related impurity of Zidovudine (ZI).⁷⁻¹⁰

The structure of ZI is as follows-

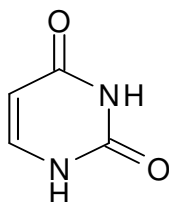


Fig.-1: Pyrimidine 2,4(1H,3H)-dione

EXPERIMENTAL

Chemicals

Propionyl chloride, Urea, KBr (AR grade) (SD fine chemicals), Solvents -Acetonitrile, Methanol, Water (HPLC grade), Ethanol (AR grade) were purchased from Merck Chemicals, India.

Instruments

UV: UV-Vis Spectrophotometer (UV-1650 PC) SHIMADZU INC.

FT-IR: Fourier Transform Infrared Spectrophotometer Model No. (8400S) SHIMADZU.

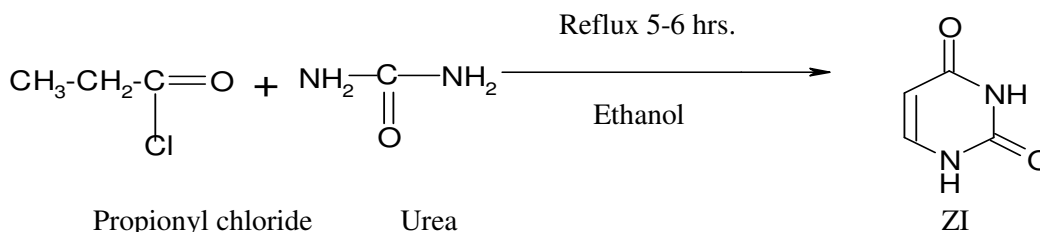
NMR: Characterization of impurities was achieved by using Varian NMR Mercury 300 MHz spectrometer, using DMSO-d₆ as a solvent and TMS as an internal reference standard for the proton experiment. All experiments were conducted at 25°C, and no shift relaxation agents were employed. The ¹H and ¹³C NMR chemical shift values were reported on the δ scale in ppm.

LC-MS: The Q-TOF Micro mass (YA-105) spectrometer capable of recording High Resolution Mass Spectrum (HRMS), Electron spray Ionization (ESI) was used for characterisation of synthesized impurity.

HPLC: The HPLC method was developed by using LC20AD Prominence Liquid Chromatography SPD2 0-A Shimadzu, Japan. The UV-Vis detector and C18 column with dimension on 25 x 0.6 cm was used for the method development with flow rate 1.0ml/min at wavelength 257 nm. The water: methanol: acetonitrile in proportion of (40v:40v:20v) as a mobile phase was selected for method validation of ZI and various parameters according to ICH guidelines (Q₂B) were studied.

Synthesis of Zidovudine impurity

0.1 mole of Propionyl chloride, 0.1 mole of Urea were added in round bottom flask. To it add 10ml of ethanol, stir vigorously. Refluxed for 5-6 hours and pour the solution in cold water and residue of ZI was obtained. Precipitate obtained after filtration of residue and recrystallized it from ethanol.



Scheme-1: Synthesis of Zidovudine impurity

Chromatographic condition

Preparation of stock solution

The stock solution of 100 ug/mL was prepared by dissolving 10mg Zidovudine in 100 ml mobile phase. The dilutions were prepared in concentration range of 200-1000 ng using stock solution and volume was adjusted till the mark with mobile phase.

Preparation of sample solution

The sample solution of Zidovudine formulation was prepared as 100ug/ml stock solution for quantification of these intermediates in Zidovudine formulation. Further dilution was carried out in mobile phase.

RESULTS AND DISCUSSION

Physicochemical properties

The process related Pyrimidine-2,4(1H,3H)dione (ZI) was synthesised and identified by various physicochemical parameters. Percentage yield of ZI was comparatively high (Table-1).

Table-1: Physicochemical properties of ZI

Molecular Formula	Molecular weight	M.P. ^o C	RF	% Yield
C ₄ H ₄ N ₂ O ₂	112	300-302	0.70	80

Characterisation of ZI

UV- Spectrum

The λ_{max} of ZI was recorded in 99% ethanol and found to be 257nm confirming Pyrimidine structure.

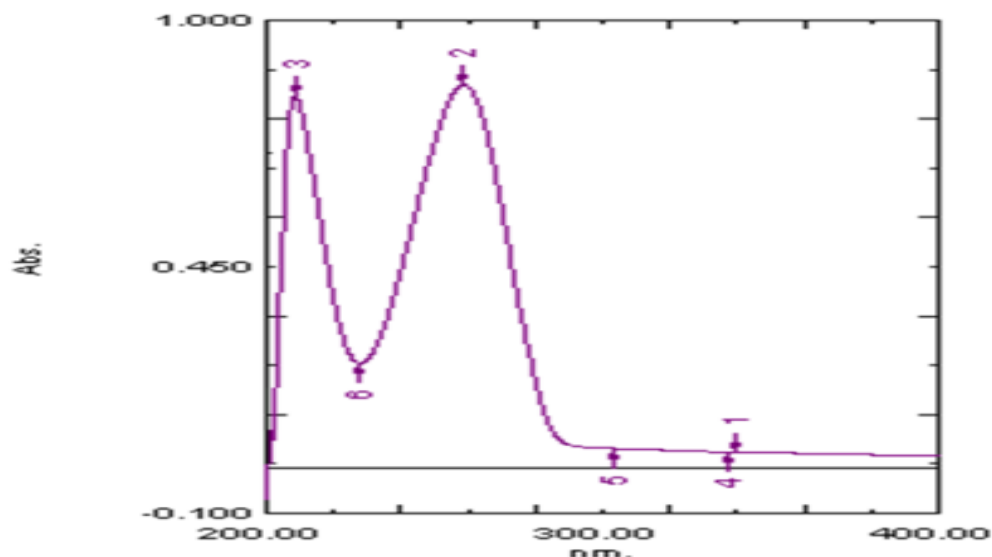


Fig.-2 : UV Spectrum of ZI

IR Data

The functional groups peaks in IR spectrum are as follows. IR (KBr) $\nu^{\text{cm}^{-1}}$: 3400-3200 (N-H stretching), 3150-3000 C-H stretching), 1690-1630 (C=O stretching), 1640-1550 (NH-Bending), 1600-1475 (C=C stretching), 900-600 (oop). This data supports Pyrimidine structure.

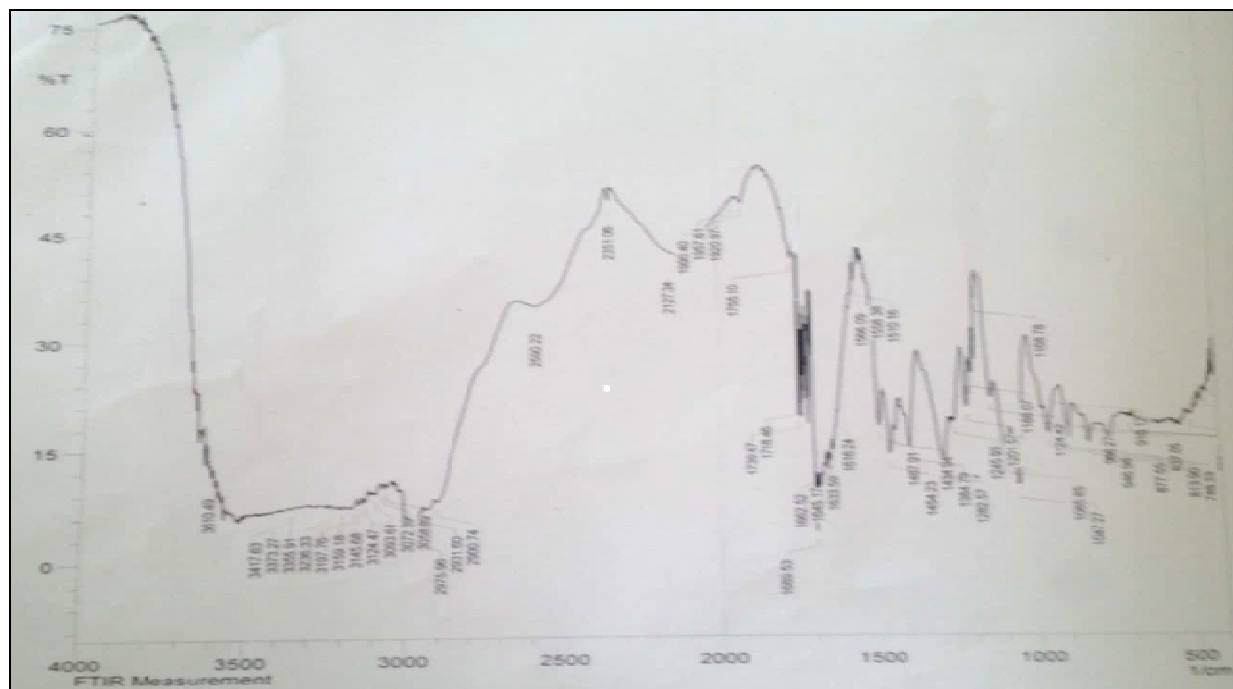


Fig.-3 : IR Spectra of ZI

¹H NMR

The ¹H NMR show peaks occupying different positions confirm pyrimidine ring.

δ = 5.48, 5.46, d, (CH-C=O), 7.412, 7.398, 7.394, 7.380, q, (CH-N-H), 10.79, 10.99, d, (NH-CO-NH)

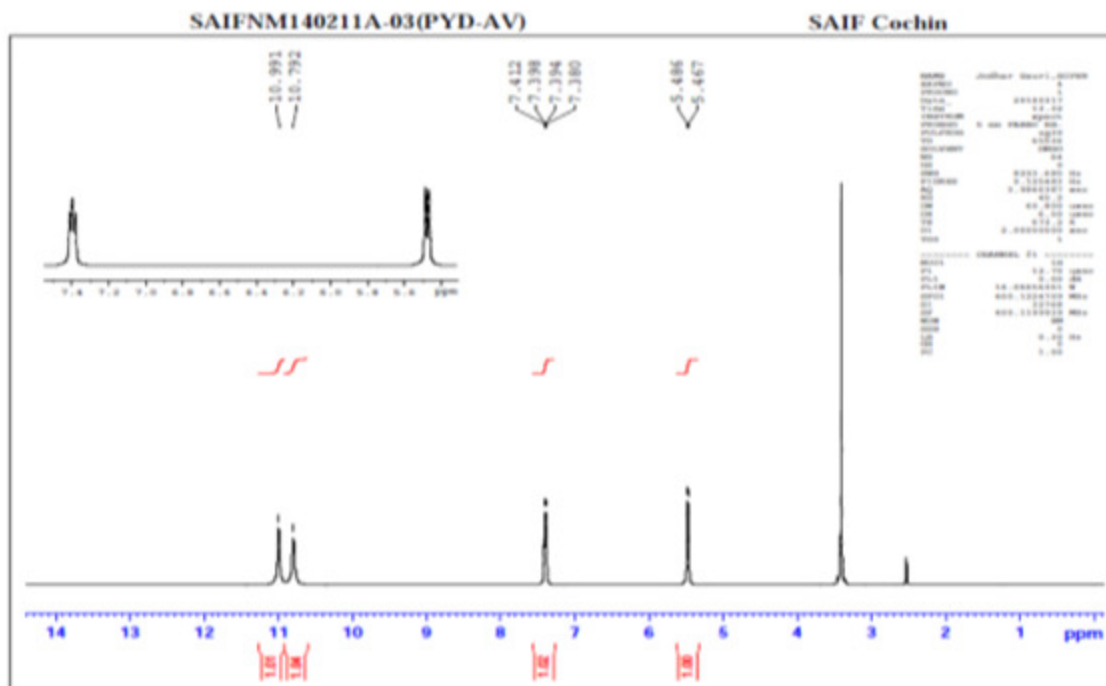


Fig.-4: ¹H NMR Spectra of ZI

¹³C NMR

The presence of carbonyl amide carbon are further confirmed by ¹³ C NMR. Data- δ =151.98 (NH-CO-NH), 164.83(NH- C=O) , 142.63(CH-C=O), 100(CH-NH)

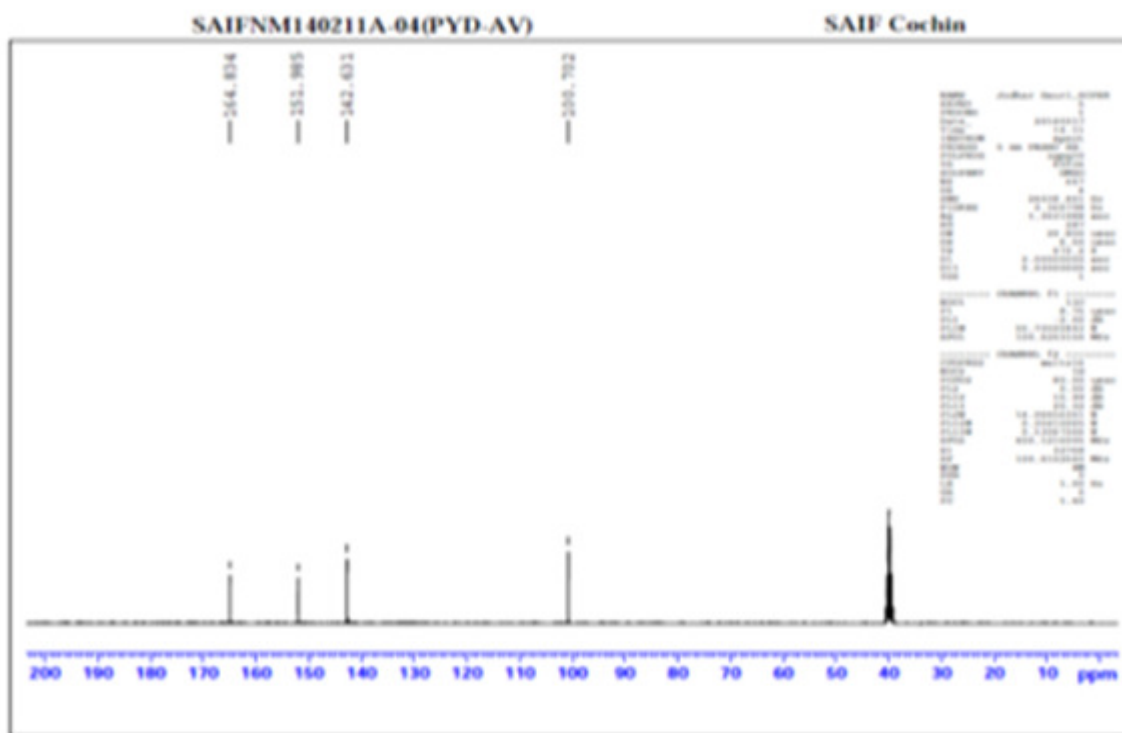
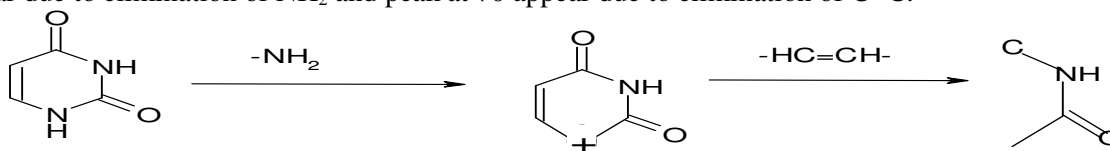


Fig.-5: ¹³C NMR Spectra of ZI

The presence of carbonyl amide carbon are further confirmed by ¹³ C NMR.

LC-MS Data

Peak appear at 112 indicates presence of molecular ion peak. Two major peaks appears at m/e 96 with 100% abundance whereas at m/e 70 the second most abundant peak in the mass spectrum. Peak at 96 appear due to elimination of NH₂ and peak at 70 appear due to elimination of C=C.



Scheme-2: Mass fragmentation pattern of ZI

Mass fragmentation pattern confirm ZI as pyrimidine 2,4 (1H,3H) dione.

Method development by HPLC

The HPLC method was developed for quantification of ZI in bulk and formulation. The method was validated as per ICHQ₂B guidelines .

1. **System Suitability Parameters:** The area of respective concentrations, theoretical plates, number of theoretical plates per cm height and the peak symmetry was recorded.

2. **Linearity** : Dilution of standard impurity in the range of 200-1000ng/ml were prepared by taking suitable aliquots of working standard stock solution in different 10 ml volumetric flasks and diluting upto the mark with mobile phase. 20 μ l was injected from it each time into the column at a flow rate of 1 ml/min. The detector was set at 257 nm and corresponding chromatogram were obtained. A plot of peak area over concentration was constructed. Regression of the plot was computed by least square regression method.

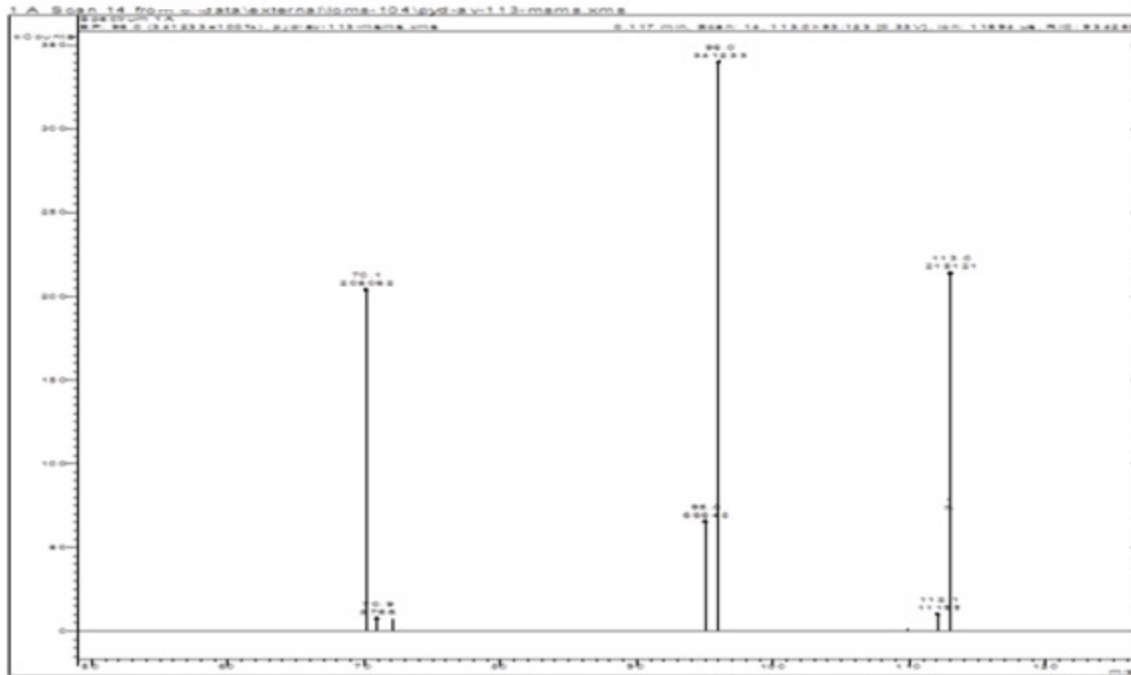
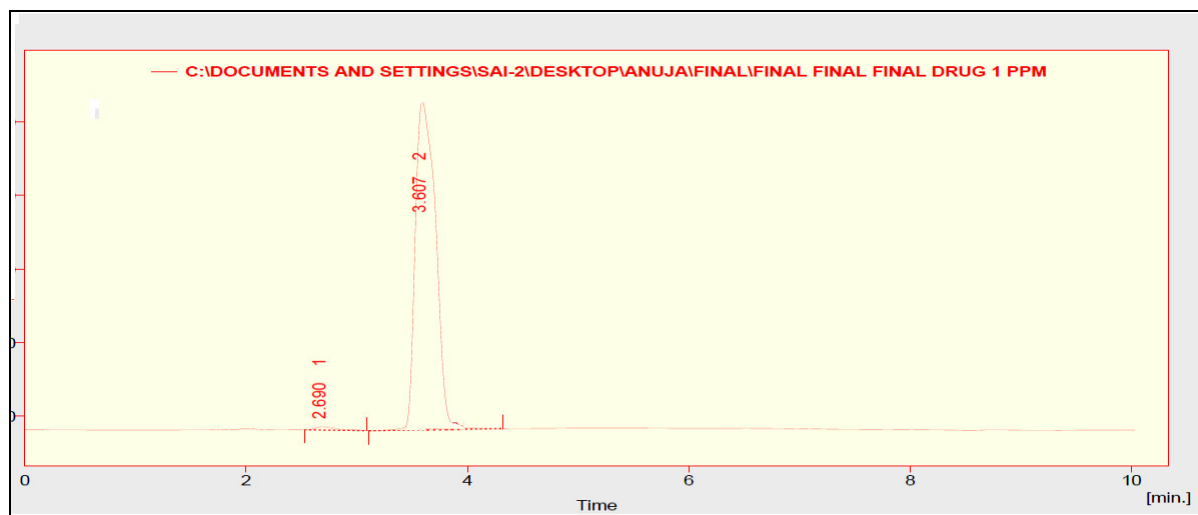


Fig.-6: Mass spectrum of ZI

3. **Precision**: Precision of analytical method was studied by multiple injections of homogenous samples. 6 replicate of 600 ng solution were prepared and injected for precision at the same flow rate of 1ml/min. The intra-day and inter-day precision was used to study the variability of the method. SD and RSD were calculated.
4. **Accuracy**: Accuracy of the method was studied using the method of standard addition. Standard ZI solutions were added to the unknown bulk and tablet formulation of Zidovudine. The percent recovery was determined at three different levels (50%, 75% and 100%). Impurity content was determined and the percent recovery was calculated.
5. **Robustness**: Robustness was studied by changing parameters like change in flow rate. The SD and RSD between the change parameter were calculated.
6. **Ruggedness**: Ruggedness was studied was carried out by using different analysts. The SD and RSD were calculated.
7. **LOD and LOQ** : Limit of detection and limit of quantitation of the method was calculated by formula $LOD = 3.3 \times SD / \text{Slope}$, $LOQ = 10 \times SD / \text{Slope}$
8. **Quantitation of Impurity**: The total amount of ZI present in Zidovudine bulk and formulations was calculated for the synthesized compound and the result was compared to ICH limit. For impurities in new drug substance the limit is 0.1%.

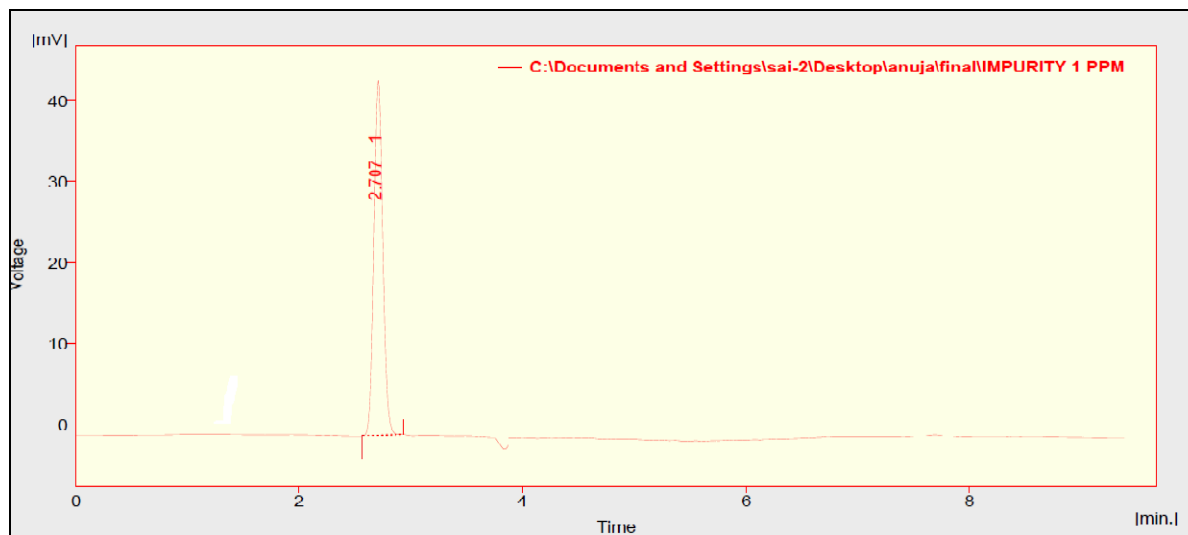
HPLC Chromatogram



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\SAI-2\DESKTOP\ANUJA\FINAL\FINAL FINAL FINAL DRUG 1 PPM)

	Reten.Time [min]	Area [m V.s]	Height [m V]	Area [%]	Height [%]	W05 [min]
1	2.690	12.333	0.953	1.0	1.0	0.18
2	3.617	121.325	94.049	99.0	99.0	0.23
	Total	133.658	95.002	100.0	100.0	

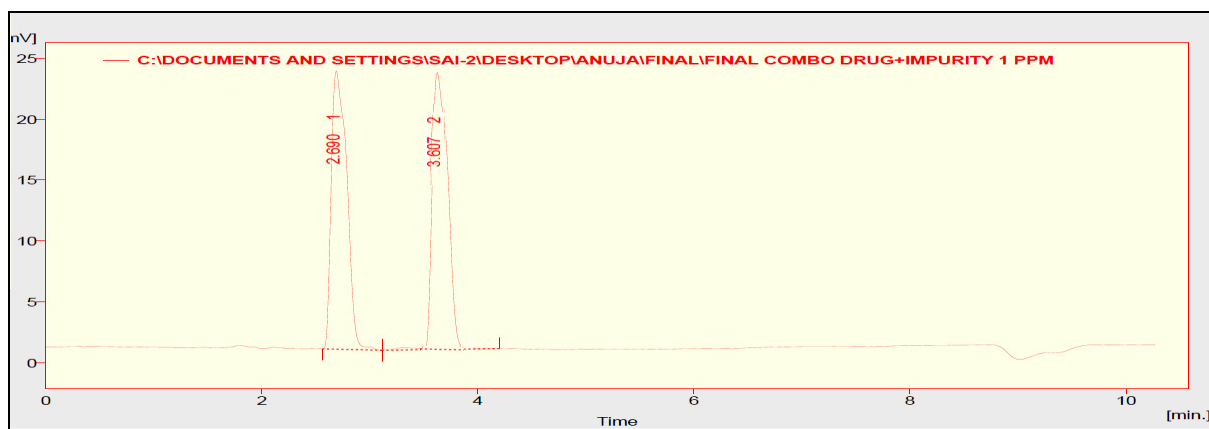
Fig.- 7: Chromatogram of bulk drug



Result Table (Uncal - C:\Documents and Settings\sai-2\Desktop\anuja\final\IMPURITY 1 PPM)

	Reten.Time [min]	Area [m V.s]	Height [m V]	Area [%]	Height [%]	W05 [min]
1	2.707	226.716	43.647	100.0	100.0	0.09
	Total	226.716	43.647	100.0	100.0	

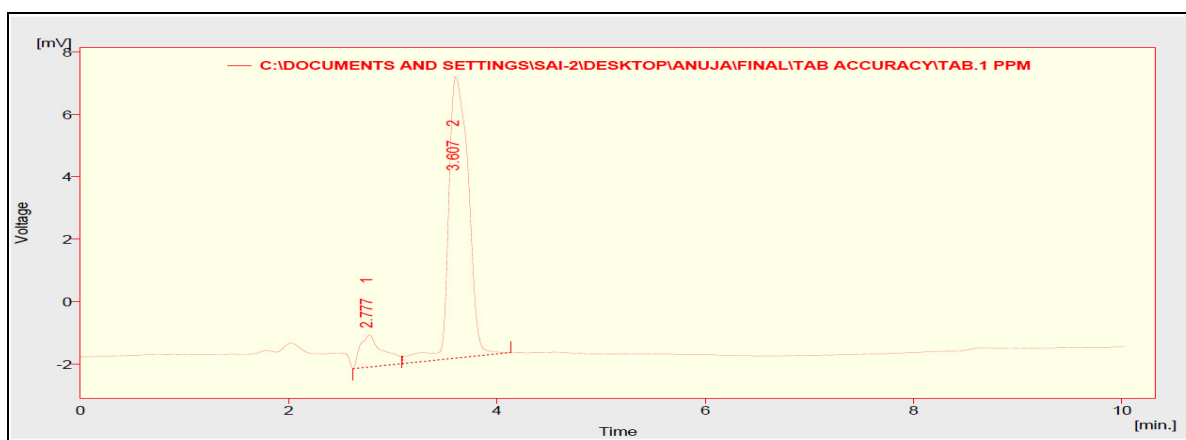
Fig.-8: Chromatogram of ZI



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\SAI-2\DESKTOP\ANUJA\FINAL\FINAL COMBO DRUG+IMPURITY 1 PPM)

	Reten.Time [min]	Area [m V.s]	Height [m V]	Area [%]	Height [%]	W05 [min]
1	2.690	226.845	22.815	57.7	65.0	0.17
2	3.617	166.836	12.283	42.3	35.0	0.23
	Total	394.680	35.098	100.0	100.0	

Fig.-9: Chromatogram of bulk drug & ZI mixture



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\SAI-2\DESKTOP\ANUJA\FINAL\TAB ACCURACY\TAB.1 PPM)

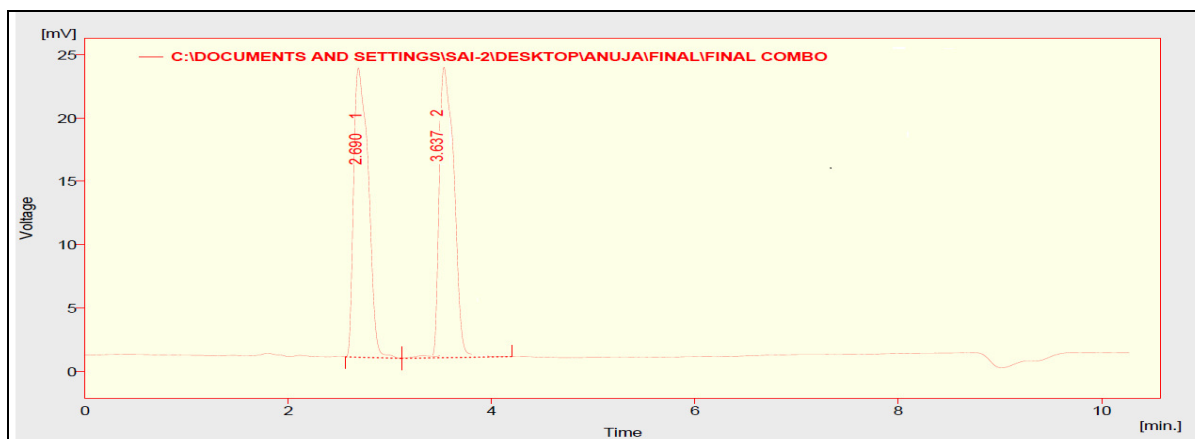
	Reten.Time [min]	Area [m V.s]	Height [m V]	Area [%]	Height [%]	W05 [min]
1	2.777	15.456	1.026	11.3	10.2	0.25
2	3.607	120.832	9.026	88.7	89.8	0.22
	Total	136.289	10.052	100.0	100.0	

Fig.- 10: Chromatograph of Zidovudine tablet

CONCLUSION

The process related impurity in Zidovudine drug substance was synthesised, purified, isolated identified, and characterized by using UV, HPLC(analytical and preparative),Mass, IR and NMR(¹H and ¹³C NMR) techniques. On basis of spectral data, characterisation of impurity was carried out as Pyrimidine-2,4(1H,3H)-dione(ZI).An isocratic reversed-phase high-performance liquid chromatography method was developed, optimized and validated as per ICH guideline. Developed method was found to be efficient, specific, linear, sensitive, precise and accurate and passes as per ICH guideline. Stated method can be

used in routine analysis efficiently for 1-(3-azido-2,3-dideoxy- β -D-erythro-pentofuranosyl)-5-methylpyrimidine-2,4(1H,3H)-dione, in marketed Zidovudine formulations. In future several other impurities for Zidovudine bulk and formulations were identified and proposed analytical methods will be useful for the estimation of these impurities.



Result Table (Uncal - C:\DOCUMENTS AND SETTINGS\SAI-2\DESKTOP\ANUJA\FINAL\FINAL COMBO)

	Reten.Time [min]	Area [m V.s]	Height [m V]	Area [%]	Height [%]	W05 [min]
1	2.690	227.905	22.815	57.7	65.0	0.17
2	3.637	196.836	12.283	42.3	35.0	0.23
	Total	424.741	35.098	100.0	100.0	

Fig.-11: Chromatograph of Zidovudine tablet & ZI mixture

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