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Development of Analytical Method for Simultaneous Estimation By RP-HPLC in Combination Dosage Form of Tinidazole and Nitrofurantoin in Pharmaceutical Formulation

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ABSTRACT:

The RP-HPLC method is simple in the present study A HPLC for Nitrofurantoin and Tinidazole was developed in combined tablet dosage form as pre ICH Guide lines UV Detector and Agilent C 18 (250x4.6mm) 5µ column, injection of 20µl is injected and eluted with the mobile phase of Potassium dihydrogen phosphate buffer with pH3.5: (Acetonitrile & Tetrahydrofuran 64:1 v/v) in the ratio 35:64:1 which was pumped at a flow rate of 1.0ml at 300nm. The peak of Nitrofurantoin and Tinidazole was found well separated within 6 min. both the peaks are well resolved from each other. The developed method will be used for the simultaneous estimation of both the drugs after validate for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustness

Keywords: Tinidazole, Nitrofurantoin, combined tablet dosage, suitability, specificity, linearity, precision, RP-HPLC method

1. INTRODUCTION

Analysis plays a major role today, and it can be considered as an interdisciplinary subject. Pharmaceutical Analysis derives its principles from various branches like chemistry, physics, microbiology etc., pharmaceutical analytical techniques are applied mainly in two areas, via quantitative analysis and qualitative analysis, although there are several other applications. Today, more than 80% of all analytical chromatographic separations are performed using reversed-phase adsorbents which have shown higher versatility compared to normal-phase chromatography adsorbents. Reversed-phase adsorbents have found their use in a wide range of applications such as process purification, isolation of active bio molecules, analytical separation of drugs and metabolites as well as extraction of various contaminants in environmental samples However, Reversed-Phase Chromatography includes a large number of different phases that differ significantly in both chemical and physical properties which will have a significant impact on their chromatographic behaviors. Thus, one chromatographer will have to consider all of the following stationary phase properties while gathering information on the sample to be analyzed or purified

2. MATERIALS AND METHODS:

Nitrofurantoin, Tinidazole, Potassium dihydrogen phosphate obtained from yarrow chem. Products Mumbai AR grade, Acetonitrile, Ortho phosphoric acid ,Water milli-Q grade obtained from loba chem. Pvt ltd

PHARMACEUTICALS Trial – 1:

The trial 1 was performed using mobile phase Acetonitrile and buffer in the ratio 50 : 50 Agilent C18 (250 x 4.6 mm) 5µm packing with flow rate 1.0 ml/min. less separation between Tinidazole and Nitrofurantoin peaks.

Trial – 2:

The trial 2 was performed using mobile phase acetonitrile and buffer in the ratio 80 : 20 Agilent C18 (250 x 4.6 mm) 5 µm packing with flow rate 1.0 ml/min less separation between Tinidazole and Nitrofurantoin peaks

Trial – 3:

The trial 3 was performed using mobile phase sodium acetate and buffer in the ratio 55 : 45 agilent C18 (250 x 4.6mm) 5 µm packing with flow rate 1.0 ml/min less separation between Tinidazole and Nitrofurantoin peaks.

Trial – 4:

The trial 4 was performed using mobile phase ortho phosphoric acid and buffer in the ratio 20 : 80 v/v agilent C18 (250 x 4.6mm) 5 µm packing with flow rate 1.0 ml/min improper peak shape and less separation between Tinidazole and Nitrofurantoin peaks.

Trial – 5:

The trial 6 was performed using mobile phase buffer (pH = 3.5) , acetonitrile and tetra hydro furan in the ratio 35 : 64:1 agilent C18 (250 x 4.6mm) 5 5 µm packing with flow rate 1.0 ml/min proper peak shape and good separation between Tinidazole and Nitrofurantoin peaks found good resolution

2. METHOD DEVELOPMENT

Optimization of mobile phase:

Separation of both the drugs was tried using the following combination of mobile phases. The table gives the details of the same

Table No. 3 Method development trails

Serial no.	Mobile phase	Ratio (v/v)	Elution of peak
1.	Buffer : Acetonitrile	50 : 50	Not properseparation
2.	Buffer : Acetonitrile	80: 20	Not properseparation
3	Buffer (Sodium Acetate) : Acetonitrile	55:45	Separation of peaks
4.	Buffer : [Phosphate) Acetonitrile	20 : 80	improper peak shape.
5.	Buffer: [Acetonitrile: Tetrahydrofuran 35:64:1 v/v] Adjust pH with Ortho phosphoric acid in ratio of 35:64:1	35:64:1	Proper peak shape and good separation between Tinidazole and Nitrofurantoin peaks found good resolution

Out of 5 trials the 5th trial was selected for further studies because when compared to other trails 5th trial was found less in retention time due to the ratio or organic solvent in mobile phase.

Selection of Wavelength

Solution of Nitrofurantoin and Tinidazole were scanned in the UV region and spectrum was recorded (200-400nm). The solvent used was Buffer: [Acetonitrile: Tetrahydrofuran 35:64:1 v/v] Adjust pH with Ortho phosphoric acid in ratio of 35:64:1. It was seen that 300nm both compounds have very good absorbance, which can be used for the estimation of compounds by HPLC.

Selection of Chromatographic Method

Proper selection of the method depends on the nature of the sample (ionic or ionisable or neutral molecules), its molecular weight, pKa value and stability. The drugs selected in the present study are polar and so reversed phase or ion exchange chromatography can be used. The reversed phase HPLC was selected for the initial separation because of its simplicity and suitability.

From the literature survey and with the knowledge of properties of the selected drugs, Aligent C-18 (250x4.6mm) 5µ column was chosen as stationary phase and mobile phase with different compositions such as Potassium dihydrogen phosphate buffer and Acetonitrile was used From all the data observed, obtained, available the initial separation conditions were set to work around.

Effect of Ratio of Mobile Phase

Under the chromatographic conditions mentioned above, the different ratios of mobile phase were tried. The chromatograms were observed for each of the trails, outof which Buffer: (Acetonitrile & Sodium acetate & methanol - 55:45 v/v) in the ratio of 50:50 was selected as the separation was achieved in minimum retention time.

Effect of pH of Mobile Phase

Several trials were made using different Buffer solutions of different pH range. The best separation was achieved with Potassium dihydrogen phosphate adjustthe pH to 3.5 ± 0.1 with dil. Orthophosphoric acid.

Effect of flow rate on separation

The mobile phase consisting of Buffer : [Acetonitrile : Tetrahydrofuron 35:64:1 v/v] and the chromatograms were recorded at flow rates of 0.5ml to 2ml. The sharp peaks were obtained with 1 ml flow rate.

Effect of column (Stationary phase) on separation

At the chromatographic conditions of mixed solutions, combinations of Nitrofurantoin and Tinidazole were injected and chromatograms were obtained using C- 18 column, so C-18 was preferred for further studies.

Reference Standards

Keeping the all above fixed conditions External standard was used.

Optimized Conditions

The following optimized parameters were used as a final method for the simultaneous estimation of Nitrofurantoin and Tinidazole.

Instrument	**:**	**SHIMADZU – LC 2010**
Column	:	Agilent C18 (250x4.6mm) 5µ
Column Oven Temperature	:	30º C
Wave length	:	300nm
Flow rate	:	1.0ml/min
Injection Volume	:	20µl
Runtime	:	8 minutes
Mode of Operation	:	Reverse Phase

Mobile Phase

Solvent A	:	(Potassium dihydrogen phosphate buffer (0.05M): (pH 3.5 ± 0.1)
Solvent B	:	(Acetonitrile and Tetrahydrofuran64:1v/v))
Solvent ratio	:	(Potassium dihydrogen phosphate buffer

(0.05M): (Acetonitrile and Tetrahydrofuran 64:1v/v) pH 3.5 ± 0.1 the ratio of 35:64:1 v/v.

3. RESULTS AND DISCUSSION

The working condition for the HPLC established for Nitrofurantoin and Tinidazole and then was applied on pharmaceutical dosage forms (a combination of Nitrofurantoinand Tinidazole tablet was used) . A simple reverse phase High Performance Liquid Chromatography has been developed. The separation method was carried out by using a mobile phase consisting of Potassium dihydrogen phosphate buffer (0.05M): (Acetonitrile and Tetrahydrofuran 64:1v/v) pH 3.5 ± 0.1 the ratio of 35:64:1 v/v. The deduction was carried out by using UV detector at 300nm. The column was Agilent C 18 (250X4.6mm) 5µ. The flow rate was selected as 1.0ml/min The retention time of Nitrofurantoin and Tinidazole was found to be 2.95 & 4.02. The number of theoretical plates of Nitrofurantoin and Tinidazole was found to be 6679 & 6720 which indicates the efficient performance of the column. These parameters represent the specificity of the method.

Fig. 2 Chromatogram of Trial 1-Blank

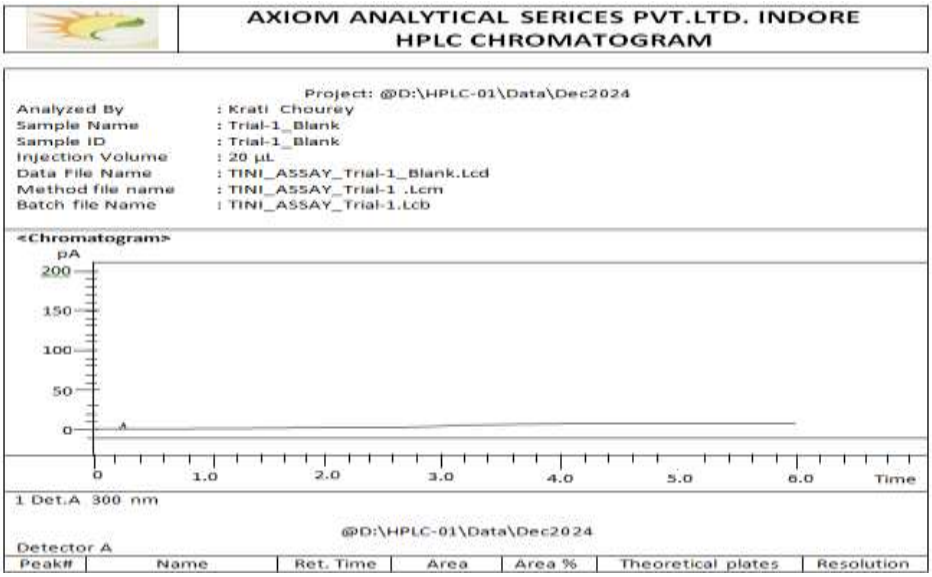


Fig. 4 Chromatogram of Trial 2-Blank

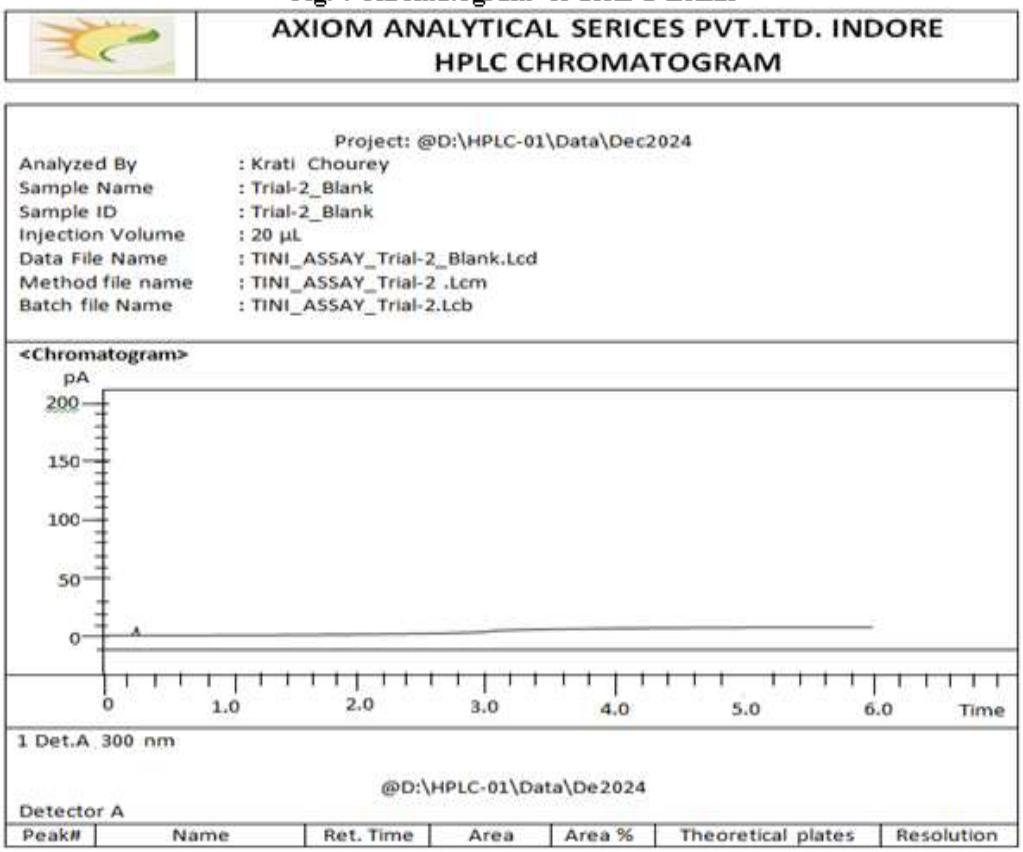
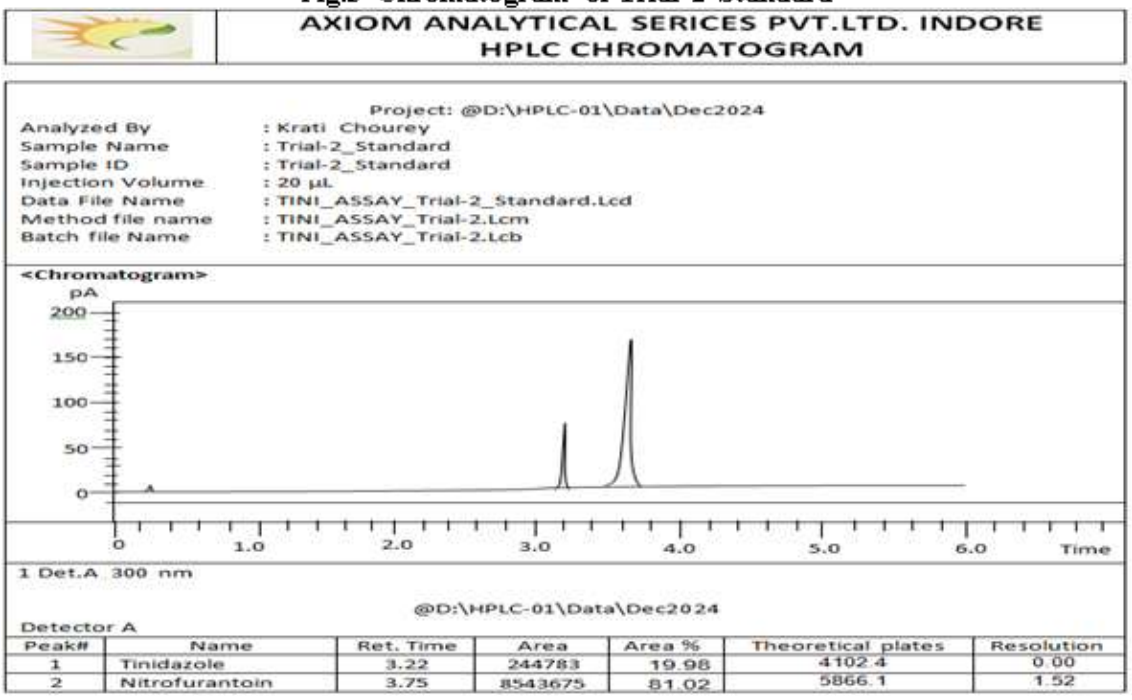


Fig.5 Chromatogram of Trial 2-Standard



Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Tinidazole	3.22	244783	19.98	4102.4	0.00
2	Nitrofurantoin	3.75	8543675	81.02	5866.1	1.52

Fig.6 Chromatogram of Trial 3-Blank

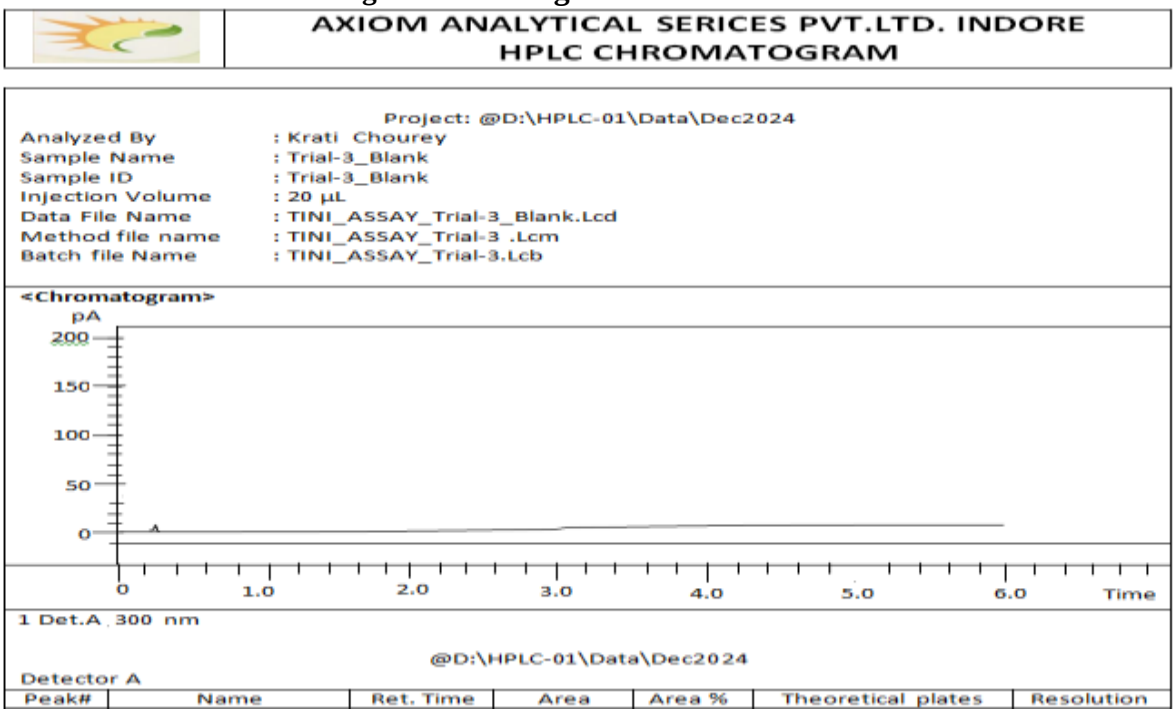
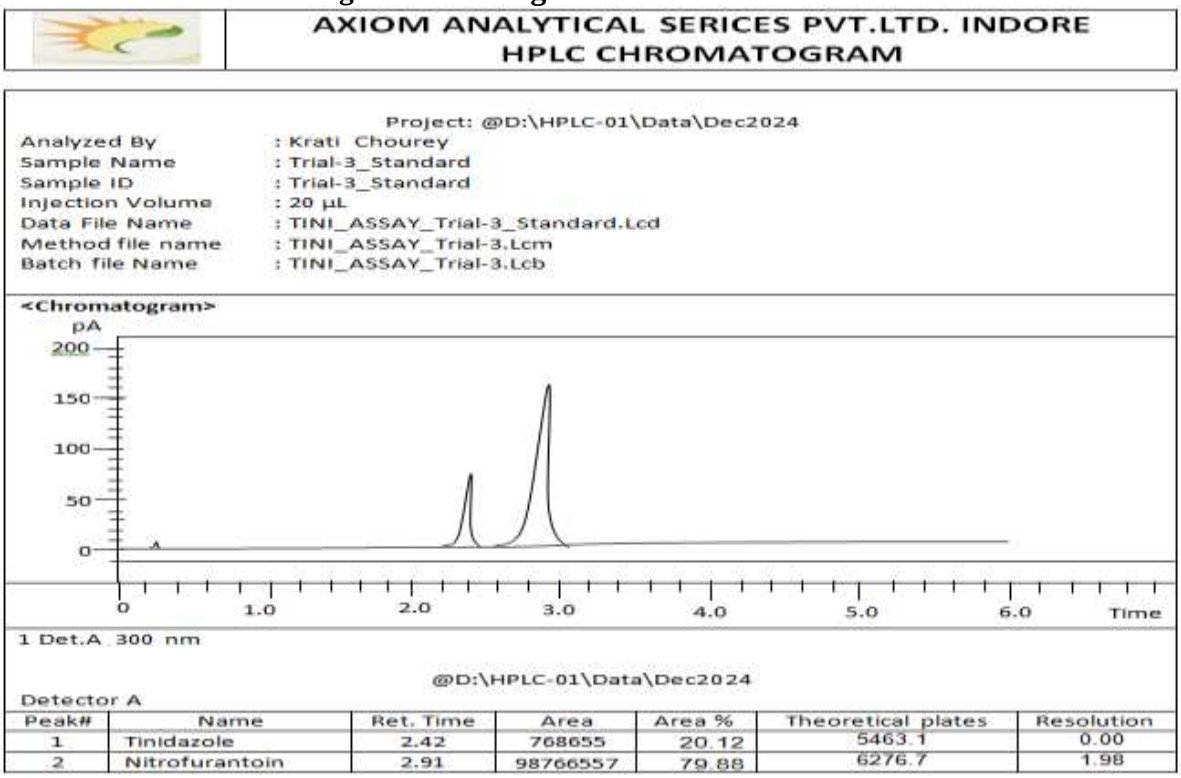


Fig.7 Chromatogram of Trial 3-Standard



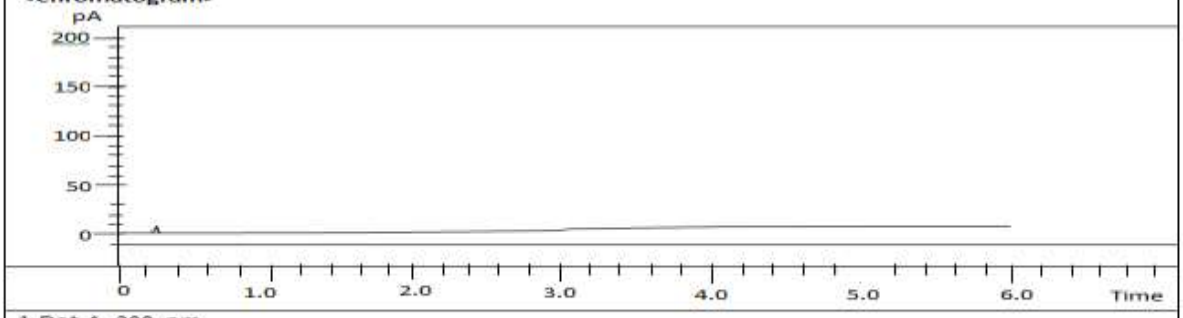
Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Tinidazole	2.42	768655	20.12	5463.1	0.00
2	Nitrofurantoin	2.91	98766557	79.88	6276.7	1.98

Fig. 8 Chromatogram of Trial 4-Blank

AXIOM ANALYTICAL SERICES PVT.LTD. INDORE
HPLC CHROMATOGRAM

Project: @D:\HPLC-01\Data\Dec2024

Analyzed By	: Krati Chourey
Sample Name	: Trial-4_Blank
Sample ID	: Trial-4_Blank
Injection Volume	: 20 µL
Data File Name	: TINI_ASSAY_Trial-4_Blank.Lcd
Method file name	: TINI_ASSAY_Trial-4.Lcm
Batch file Name	: TINI_ASSAY_Trial-4.Lcb



1 Det.A 300 nm

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Detector A

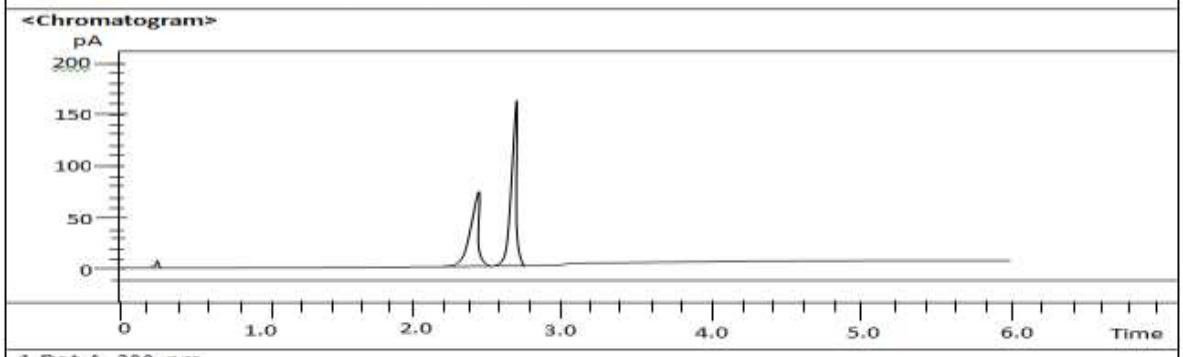
Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution

Fig.9 Chromatogram of Trial 4-Standard

AXIOM ANALYTICAL SERICES PVT.LTD. INDORE
HPLC CHROMATOGRAM

Project: @D:\HPLC-01\Data\Dec2024

Analyzed By	: Krati Chourey
Sample Name	: Trial-4_Standard
Sample ID	: Trial-4_Standard
Injection Volume	: 20 µL
Data File Name	: TINI_ASSAY_Trial-4_Standard.Lcd
Method file name	: TINI_ASSAY_Trial-4.Lcm
Batch file Name	: TINI_ASSAY_Trial-4.Lcb



1 Det.A 300 nm

@D:\HPLC-01\Data\Dec2024

Detector A

Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Tinidazole	2.53	678556	20.45	4563.4	0.00
2	Nitrofurantoin	2.85	67899887	79.55	6599.8	1.02

Fig.10 Chromatogram of Trial 5-Blank

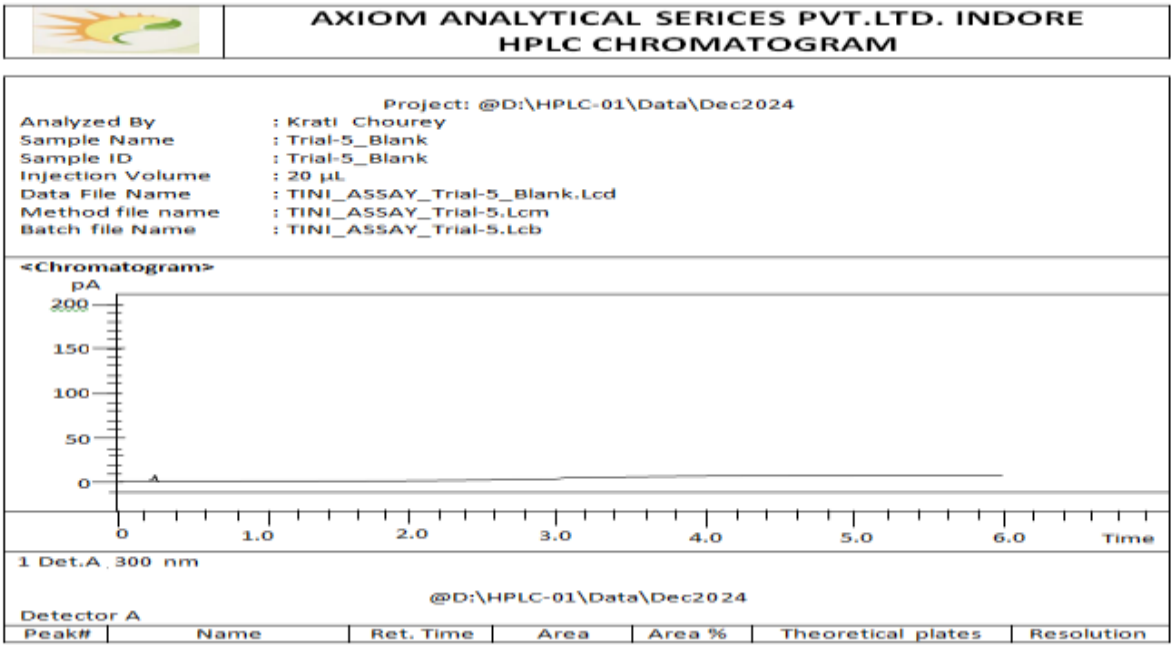
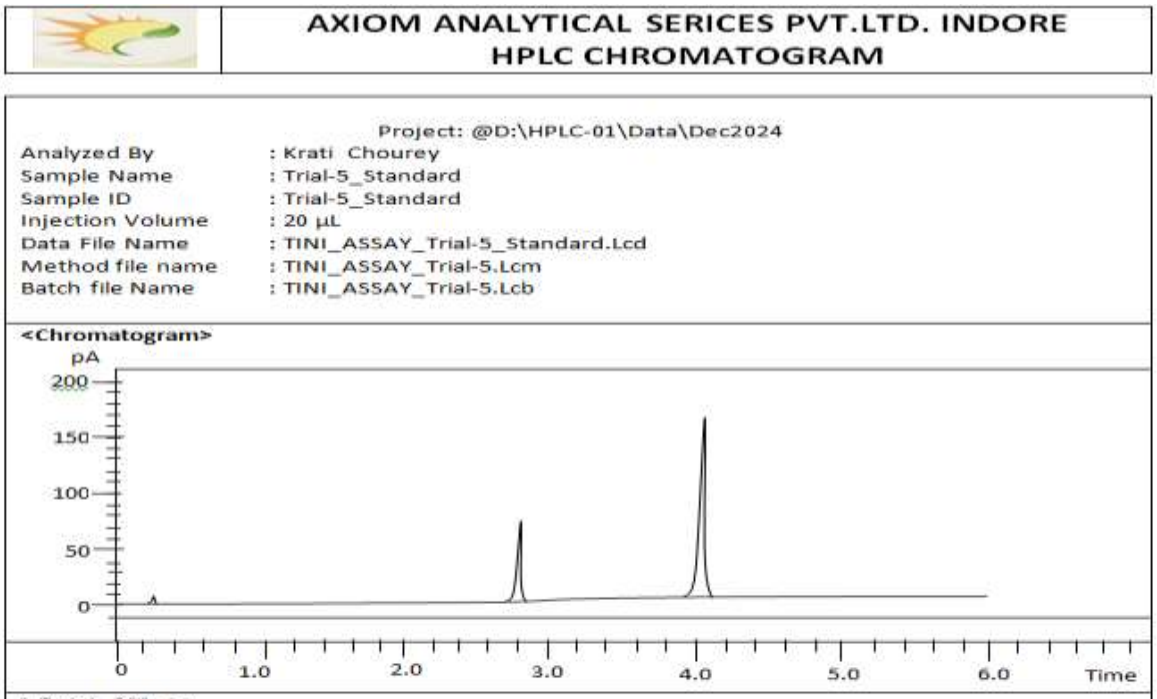


Fig.11 Chromatogram of Trial 5-Standard



Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Tinidazole	2.95	289768	20.88	6879.0	0.00
2	Nitrofurantoin	4.05	89984709	79.12	8722.0	8.55

4. SUMMARY AND CONCLUSION:

A HPLC for Nitrofurantoin and Tinidazole was developed in combined tablet dosage form as pre ICH Guide lines UV Detector and Agilent C 18 (250x4.6mm) 5µ column, injection of 20µl is injected and eluted with the mobile phase of Potassium dihydrogen phosphate buffer with pH3.5: (Acetonitrile & Tetrahydrofuran 64:1 v/v) in the ratio 35:64:1 which was pumped at a flow rate of 1.0ml at 300nm. The peak of Nitrofurantoin and Tinidazole was found well separated within 6 min. both the peaks are well resolved from each other. The developed method will be used for the

simultaneous estimation of both the drugs after validate for various parameters as per ICH guidelines like system suitability, specificity, linearity, system precision, method precision, accuracy, ruggedness and robustnes

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