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Analytical Method Development and Validation for Methylphenidate Hydrochloride and its Impurities by Using RP-HPLC Method

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A B S T R A C T:

A method utilizing Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) has been devised and validated to analyze Methylphenidate Hydrochloride (MPH) and its accompanying impurities in tablet form. The chromatographic conditions were optimized using an Inertsil Symmetry C18 column (100x40 i.d., 3.0 µm particle size) with a mobile phase comprising Acetonitrile: Buffer (30:70v/v) and adjusted to pH 4.0 using acetic acid, flowing at a rate of 1.5 ml/min. Detection and monitoring of eluted compounds occurred at 215 nm for Methylphenidate Hydrochloride (MPH) assay and at 203 nm for related substances (RS) using a PDA detector. Methylphenidate Hydrochloride (MPH), Impurity-A, and Impurity-B were eluted with respective retention times. The RP-HPLC method developed was validated, demonstrating linearity, precision, accuracy, ruggedness, and reliability. Calibration curve plots exhibited linearity. Limits of detection (LOD) for MPH, Imp-A, and Imp-B were 0.03, 0.04, and 0.04 µg/ml, with limits of quantification (LOQ) at 0.1 µg/mL for all. Statistical analysis confirmed the method's suitability for analyzing MPH, Imp-A, and Imp-B in bulk and tablet forms without interference from excipients. Clear resolution among impurities and the main peak was achieved. The validated method adhered to the guidelines set forth by the International Conference on Harmonization. This method is applicable for Methylphenidate HCl containing pharmaceutical formulations for routine quality control and stability studies ensuring safety and efficacy of the product.

Keywords: Reverse phase High Performance Liquid Chromatography, Impurities, Safety, Method validation.

INTRODUCTION:

Chromatography encompasses processes designed for the separation of different species within a mixture, relying on their distribution between a stationary phase and a mobile phase.

Modes of Chromatography: Modes of chromatography are primarily categorized based on the interactions between the solute and the stationary phase, arising from hydrogen bonding, van der Waals forces, electrostatic forces, or hydrophobic forces, or determined by the particle size. Various modes include:

- Normal phase chromatography
- Reversed-phase chromatography
- Reversed phase-ion pair chromatography
- Ion-exchange chromatography
- Affinity chromatography
- Size Exclusion chromatography

Methylphenidate hydrochloride, chemically methyl α-phenyl-2-piperidineacetate hydrochloride, presents as a white, odourless, fine crystalline powder. The infrared spectrum of a mineral oil suspension of methylphenidate hydrochloride and its spectral assignments have been established. The nuclear magnetic resonance (NMR) spectrum has been determined using a Perkin–Elmer R-24B 60 MHz spectrometer at ambient temperature. Despite having two asymmetric carbon atoms, the drug is not optically active as it is a racemic mixture. The diastereoisomer of the drug is also termed as an "erythro isomer." Methylphenidate hydrochloride exhibits a melting point between 224°C and 226°C. RITALIN is available in 10mg or 20mg strengths for oral administration at a concentration of 10mg/mL

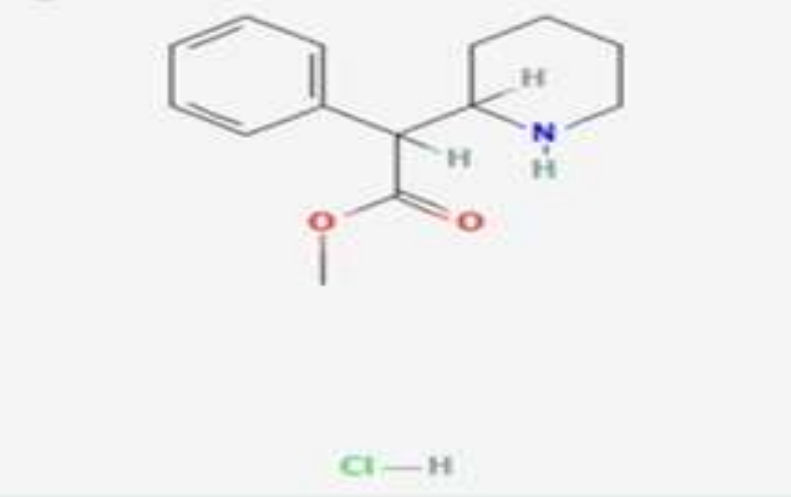


Figure.1 structure of Methylphenidate Hydrochloride

MATERIALS AND METHODOLOGY:

Equipment Used: The analysis was conducted utilizing Shimadzu's Model Alliance High-Performance Liquid Chromatography with a Waters auto sampler–PDA detector 996. Lab Solution software version 3 facilitated data handling. Other apparatus included an analytical balance (Sartorius, SECURA200-10IN), UV/Visible-detector (Standard cell), data handling system (Autochrome-3000), pH meter (Lab India), and a Sonicator. The column employed was an Intertsil ODS-3, with dimensions of 100×4.6mm and 3.0 µm particle size.

Materials and Chemicals: Acetonitrile (HPLC Grade), Octane sulfonic acid sodium salt monohydrate (AR Grade), Triethylamine (AR Grade), and pure samples of Methylphenidate Hydrochloride (MPH), Impurity-A: (2(RS)-Phenyl(2RS)piperidine2-yl)acetic acid, and Impurity-B: (methyl(2SR)-piperidine-2yl) acetate were acquired as gift samples from Pharmaceuticals Hyderabad.

UV-Spectroscopy Wavelength Selection: MPH and Impurity-A, Impurity-B exhibited maximum absorbance at 210 nm and 215 nm, respectively, as observed from UV-Visible spectrophotometer results. The PDA detector was utilized for eluent monitoring in HPLC.

Preparation of Mobile Phase:

- **Mobile Phase A:** 2.158g of octane sulfonic acid sodium salt monohydrate was dissolved in 1000ml water, mixed well, followed by the addition of 1ml triethylamine. The pH was adjusted to 3.50 with dilute ortho phosphoric acid, and the solution was filtered.
- **Mobile Phase B:** Acetonitrile was used.
- **Diluent Preparation:** A mixture of mobile phase-A and mobile phase-B in an 80:20 ratio was prepared.

Sample Preparation:

- **Placebo Solution:** Placebo pellets powder equivalent to about 100mg of Methylphenidate Hydrochloride was dissolved in a 100ml volumetric flask with approximately 70ml of diluent. The solution was sonicated and filtered through a 0.45 nylon filter.
- **Standard Stock Solution:** 20mg of methylphenidate HCl working standard was dissolved in a 200ml volumetric flask with 30ml of diluent, sonicated, and diluted to volume with diluent.
- **Standard Solution:** 2ml of the standard stock solution was transferred into a 100ml volumetric flask and diluted to volume with diluent.
- **Sample Solution:** Pellets powder equivalent to about 100mg of Methylphenidate HCl was dissolved in a 100ml volumetric flask with approximately 70ml of diluent. The solution was sonicated and diluted to volume with diluent.

Procedure:

Sequential injections included a blank (20µl of diluent), a single injection of the placebo solution, a single injection of the sensitivity solution, a single injection of the system suitability solution, six replicate injections of the standard solution, and a single injection of the sample solutions into the chromatograph. Chromatograms were recorded, and the responses were measured accordingly.

- **Preparation of System Suitability solution:**

Accurately weigh and transfer 5mg of USP Methylphenidate HCl related impurity mixture into a 5ml of volume flask ,diluent added and sonicated to dissolve and using diluent make up to mark and dissolve well.

System suitability & System Performance Results for Specificity Study

PARAMETER	RESULT	ACCEPTANCE CRITERIA
S/N ratio for sensitivity solution	13	Not less than 10
Theoretical plates	53782	Not less than 2000
Tailing factor	1.05	Not more than 2.0
%RSD	2.9	Not more than 10.0
Resolution between Imp-B & Methylphenidate HCl	10.25	Not less than 6
System performance	3.2	Not more than 10%

Results and Discussion:

Method development:

Optimization Method

Optimized method Chromatographic conditions

Column	Intertsil ODS-3,100×4.6mm,3.0µm
Mobile *phase*	Octane buffer and Acetonitriel(70:30)
Flow rate	1.3ml
Injection volume	20
Detector	UV 215nm
Column temperature	40˚c
Run time	25 minutes

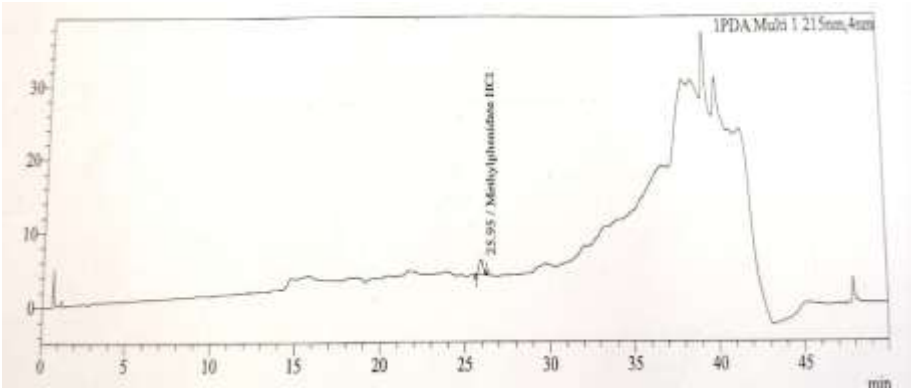


Figure.2 Chromatogram for Methylphenidate HCl

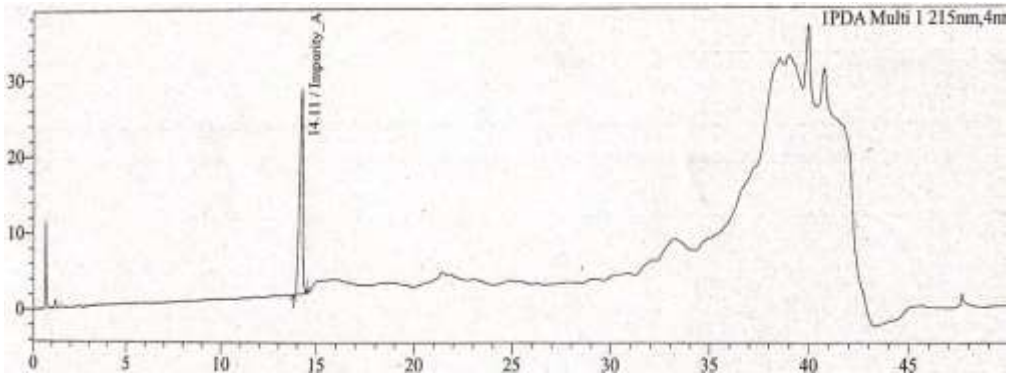


Figure.3 Chromatogram for Impurity A

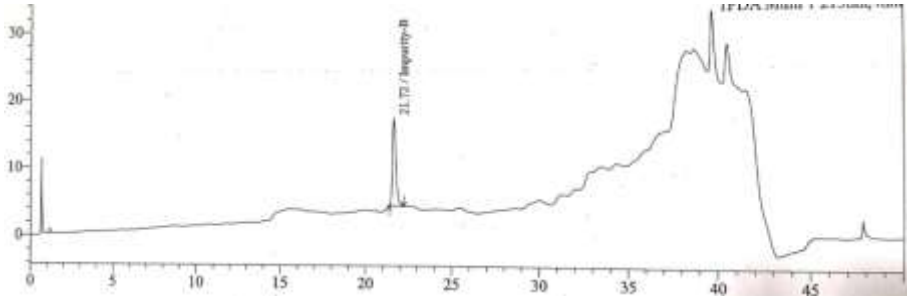


Figure.4 Chromatogram for Impurity B

A. Specificity: An imperative facet, specificity defines the method's capability to discern the analyte's response in the presence of potential impurities. To ascertain selectivity and the method's stability specification, forced degradation studies were conducted as part of the proposed RP-HPLC method. Notably, no interference was observed at the retention time (RT) of MPH in the presence of blank, placebo, or impurities, affirming the method's specificity and its ability to maintain stability even under forced degradation conditions.

Specificty Data for MPH And its Impurities

S.NO	NAME	RT	AREA	RRT
1	Methylphenidate HCl	28.46	16986882	1.00
2	Impurity A	15.36	27617	0.54
3	Impurity B	24.56	1839	0.86

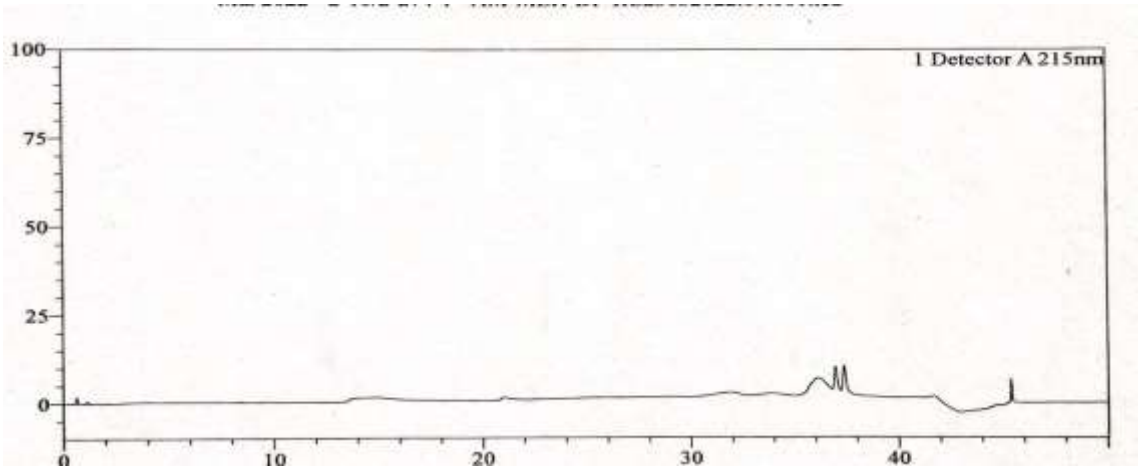


Figure.5 Chromatogram for Blank

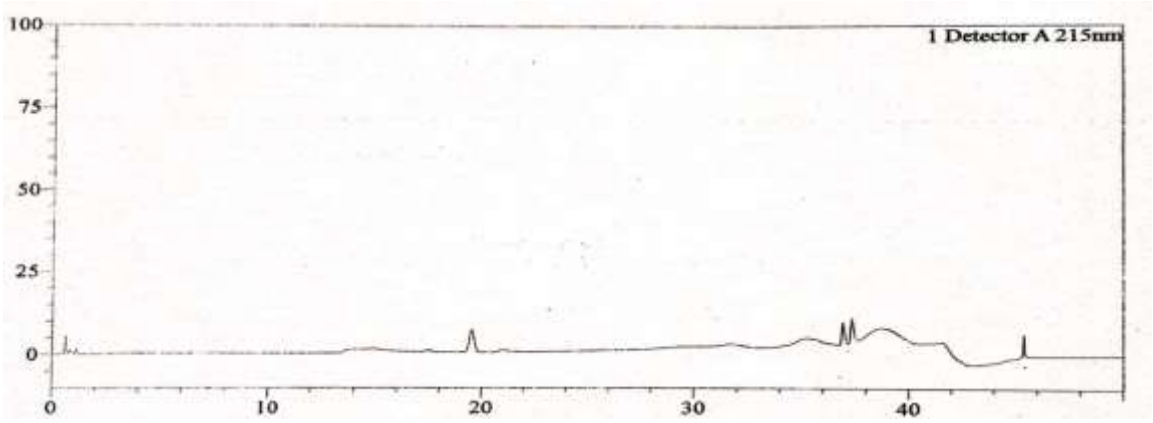


Figure.6 Chromatogram for Placebo

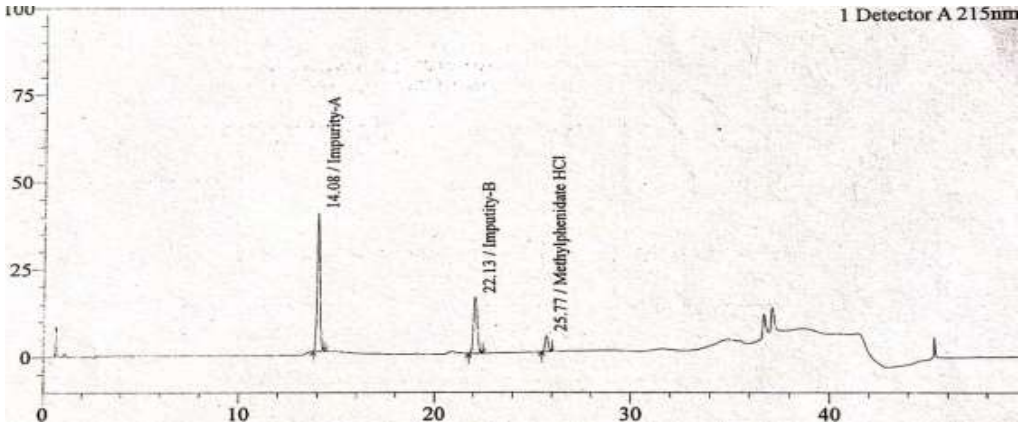


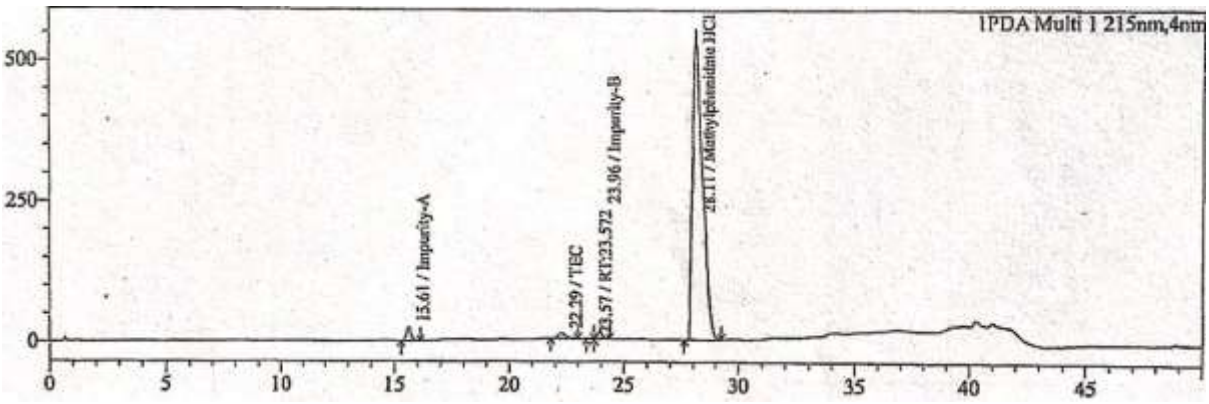
Figure.6 Typical Chromatogram

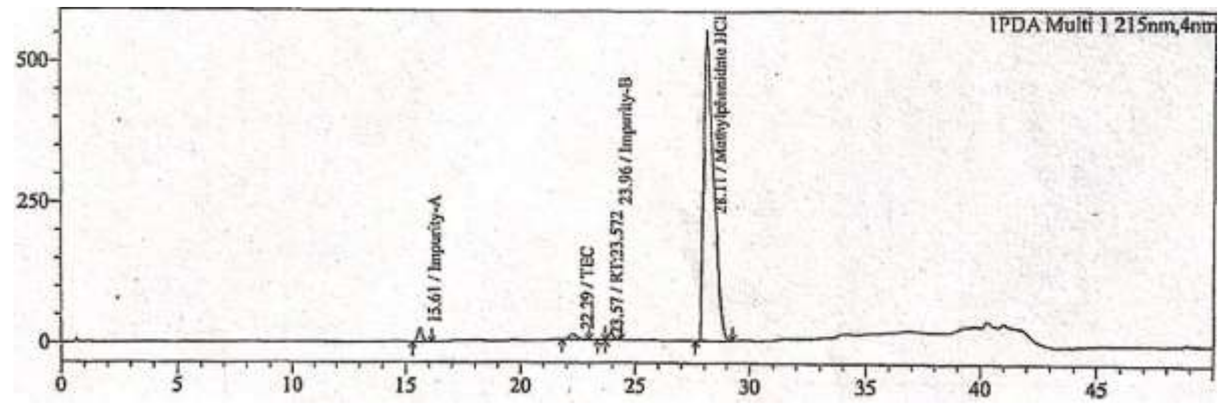
Observation: No interferences were found in the chromatogram of balnk ,Placebo at the retention time of MPH

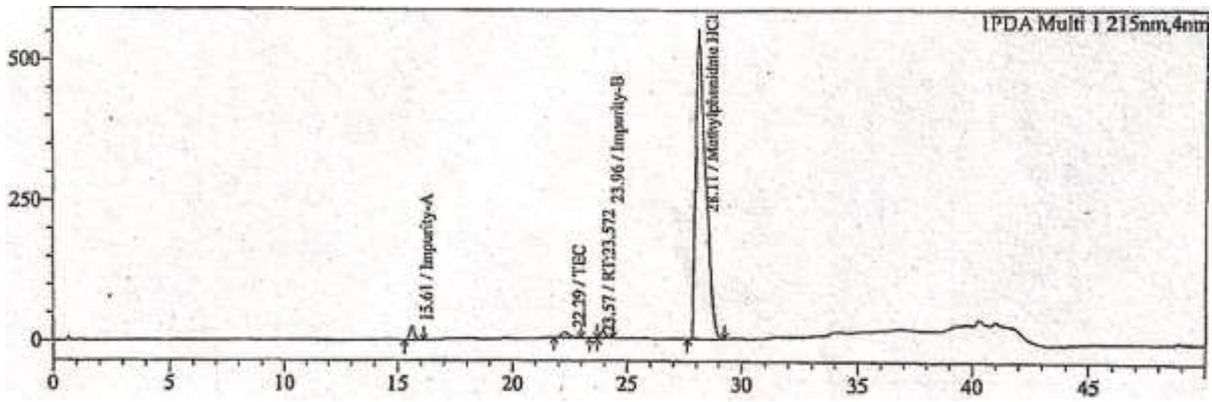
C. Precision: Precision analysis encompassed repeatability and reproducibility, evaluating six identical samples using the same formulation and analytical method. Additionally, intermediate precision was examined by a different analyst, employing varied columns, diverse HPLC systems, and executed on different days.

System precision for Methylphenidate HCl

INJECTION	METHYLPHENIDATE HCL
1	33162
2	34564
3	33472
4	34489
5	35931
6	34870
7	33306
8	35426
9	33124
10	34802
Average	34315
%RSD	2.9




1PDA Multi 1 215nm,4nm
15.61 / Impurity-A
22.29 / TEC
23.57 / RT:23.572
23.96 / Impurity-B
28.11 / Methylphenidate HCl
0
250
500
0 5 10 15 20 25 30 35 40 45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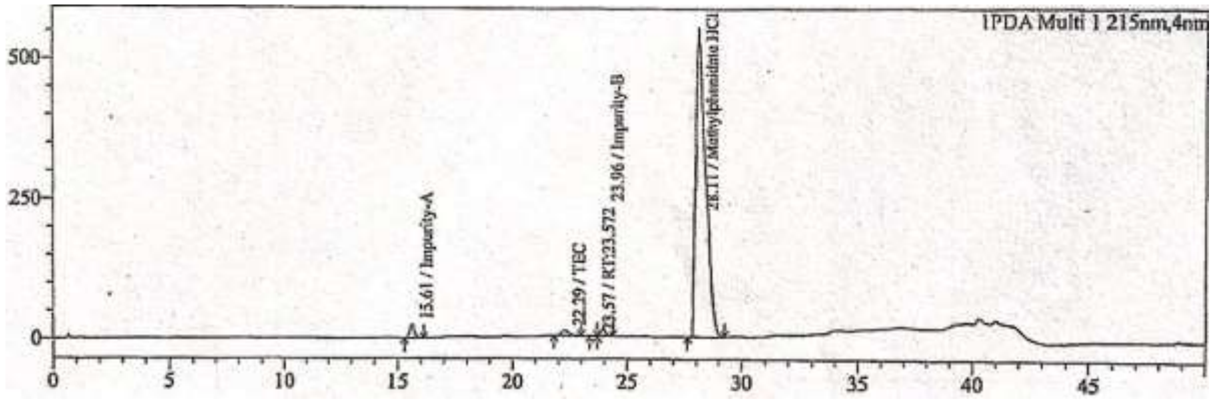
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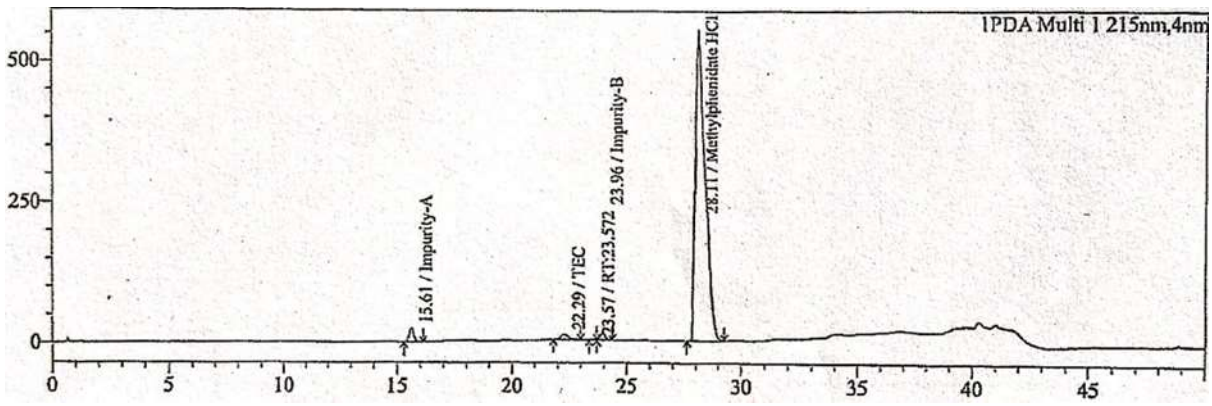
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28.11 / Methylphenidate HCl
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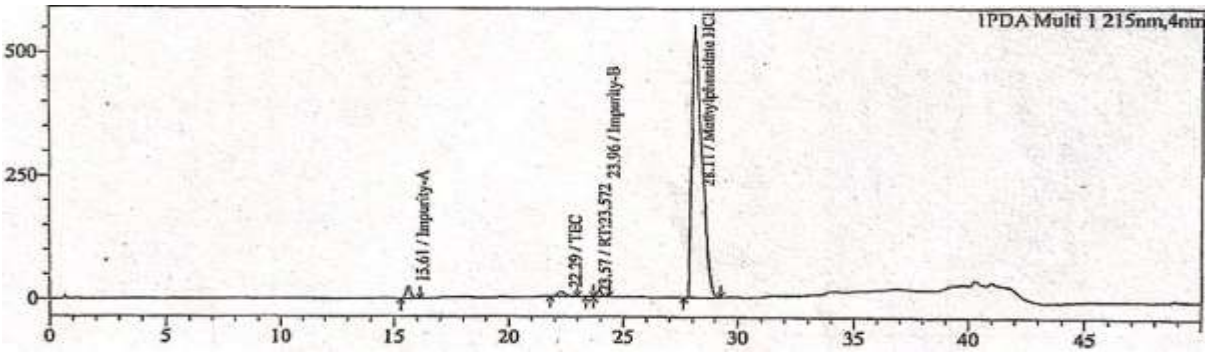


Figure.7 Precision Chromatogram

Limit: Relative standard Deviation shoubl be Less than 2%

D. Linearity: To establish the crucial aspect of linearity, rigorous steps were undertaken. Commencing with the preparation of standard stock solutions of MPH, successive dilutions were meticulously executed, generating a spectrum of solutions at varied concentrations (25%, 50%, 75%, 100%, 125%, 150%). This meticulous procedure facilitated the construction of a comprehensive and well-calibrated curve, delineating the linear relationship between concentration and analytic response, pivotal in ascertaining the method's sensitivity and accuracy across a range of concentrations**...**

System suitability & System Performance Results for Linearity

PARAMETER	RESULT	ACCEPTANCE CRITERIA
S/N ratio for sensitivity solution	122	NLT 10
Theoretical plates	151854	NLT 2000
Tailing factor	1.01	NMT 2.0
%RSD	0.2	NMT 10.0
Resolution between Imp-B & Methylphenidate HCl	12.38	NLT 6
System performance	1.2	NMT 10%

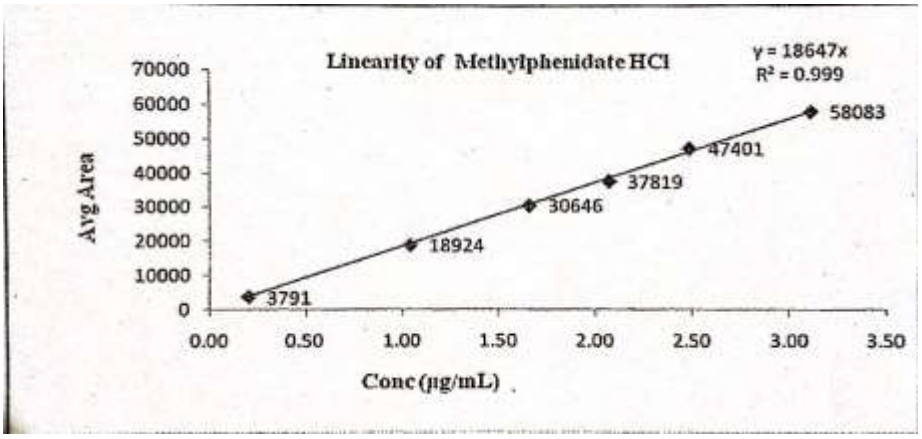


Figure.8 Calibration curve for Methylphenidate HCl

Linearity table for Methylphenidate HCl

LEVEL%	CONCµ/Ml	AREA
10%	0.21	3791
50%	1.04	18924
80%	1.66	30646
100%	2.07	37819
120%	2.49	47401
150%	3.11	58083

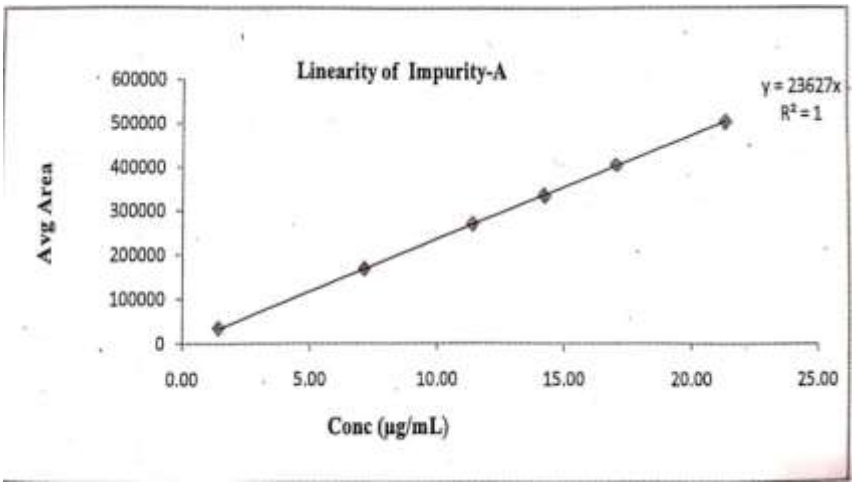


Figure.9 Calibration curve for Impurity

Linearity table for Impurity A

LEVEL%	CONCµ/mL	AREA
10%	1.43	34644
50%	7.13	169097
80%	11.14	270468
100%	14.27	335601
120%	17.12	405482
150%	21.40	505084

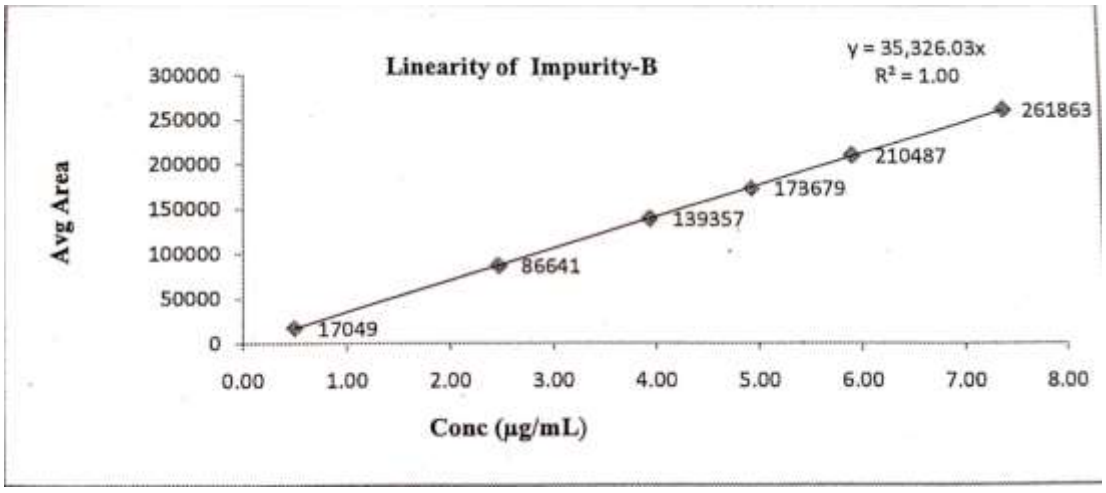


Figure.10 Calibration curve for Impurity B

Linearity table for Impurity B

LEVEL%	CONCµ/mL	AREA
10%	0.49	17049
50%	2.47	86641
80%	3.95	139357
100%	4.94	173679
120%	5.93	210487
150%	7.41	261863

Limit: Correlation coeeficient should not be more than 2

E. **Limit of detection (LOD) and limit of quantification (LOQ):** Determination of the Limit of Detection (LOD) and Limit of Quantification (LOQ) represented a meticulous process, delving into the lower limits of analyte detectability and quantification with precision and accuracy.

This intricate assessment was derived from the calibration curve of linearity, meticulously deciphering the lowest concentration at which the analyte could be reliably detected.

LOQ & LOD table for Methylphenidate HCl

MOLECULE	LOD	LOQ
Methylphenidate HCl	0.05	0.16
Impurity A	0.04	0.08
Impurity B	0.03	0.04

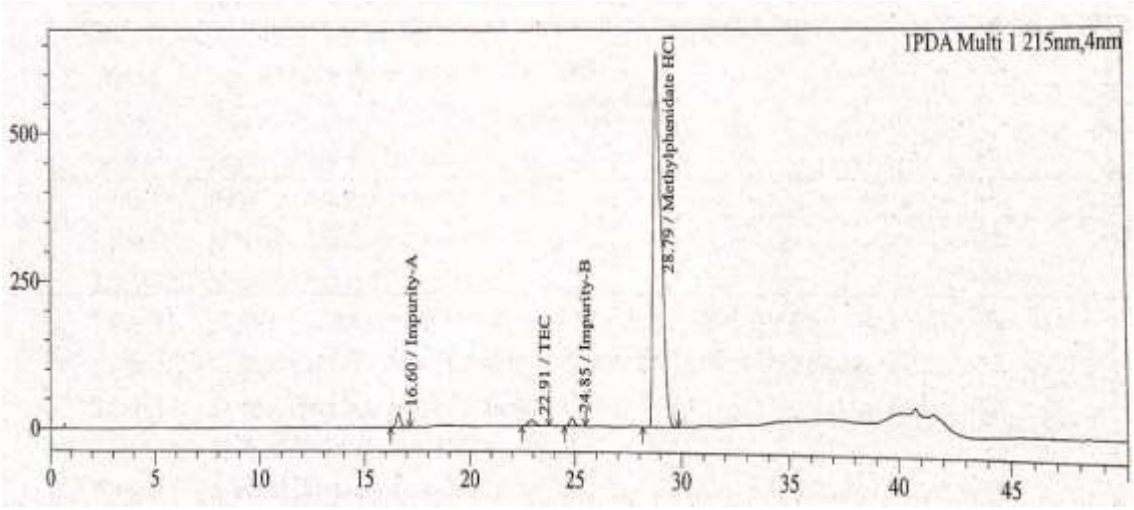


Figure.11 LOD Chromatogram

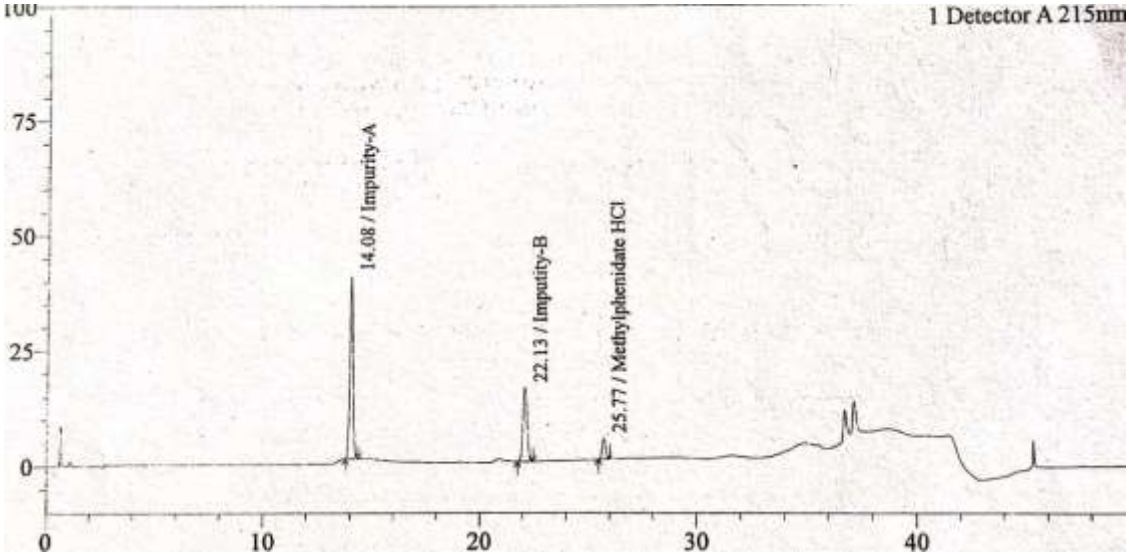


Figure.12 LOQ Chromatogram

Limit: should ne NMT 2

Accuracy: The assessment of Accuracy (Recovery) was meticulously carried out through a methodical process involving the preparation of spiked solutions. This comprehensive procedure entailed blending sample stock solutions with standard stock solutions at distinct levels spanning 50%, 100%, and 150%. To ensure the veracity and reliability of the results, stringent acceptance criteria mandated a % Recovery range falling within the bracket of 98.0 to 102.0 at each level of analysis, reinforcing the precision and fidelity of the method under scrutiny

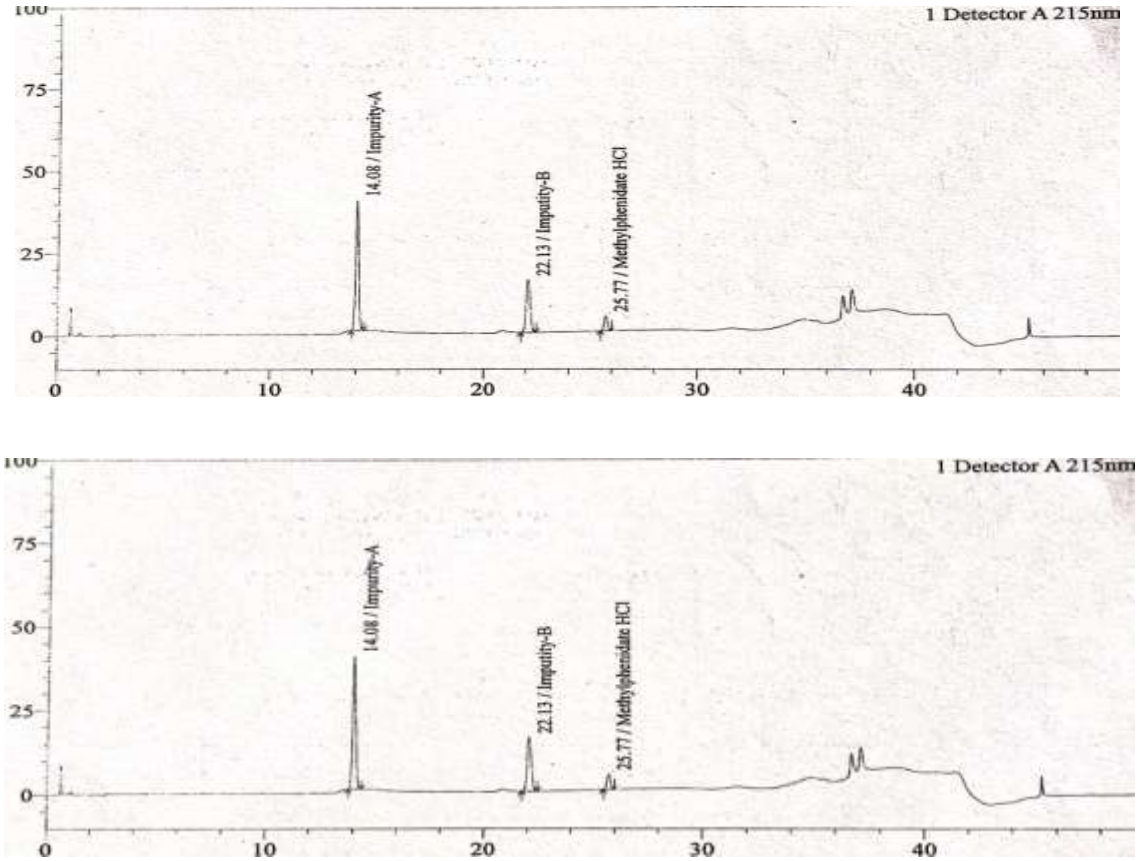
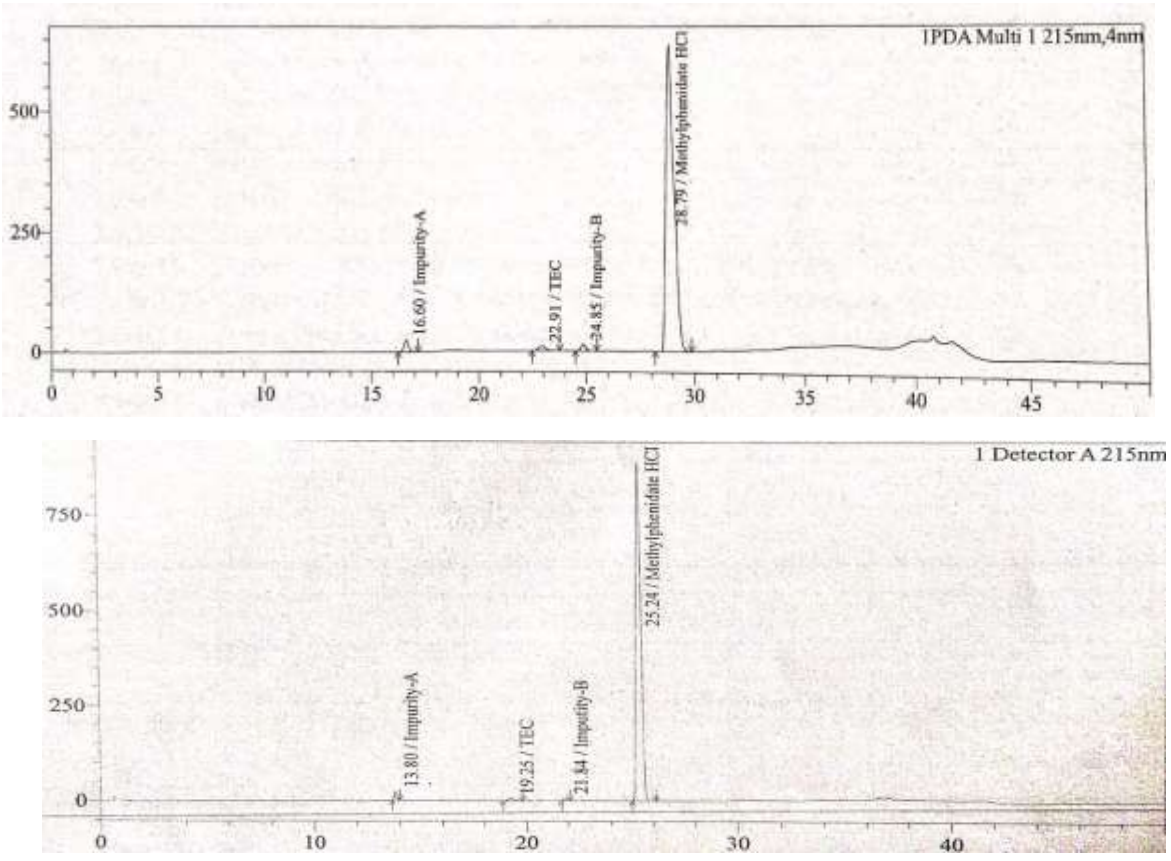


Figure.13 Chromatogram for 50% Acuracy



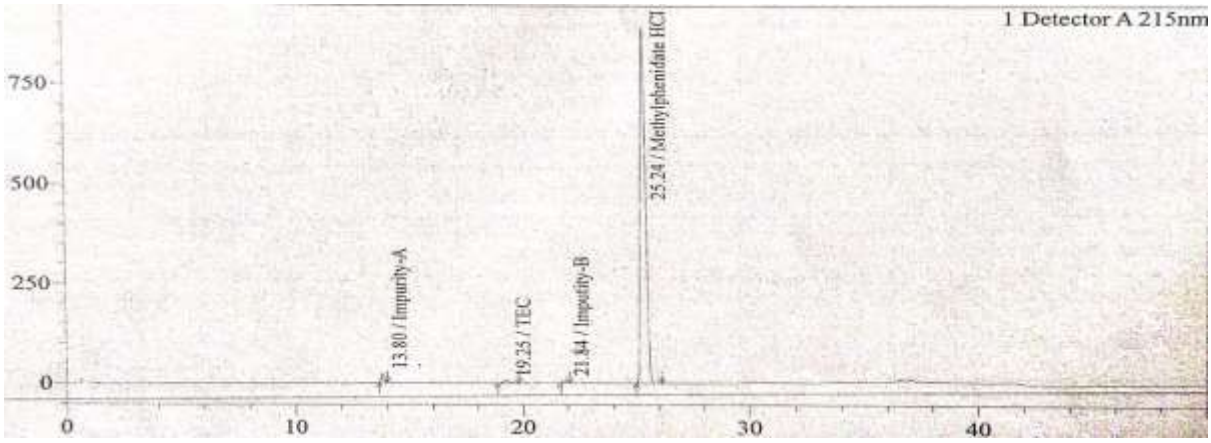
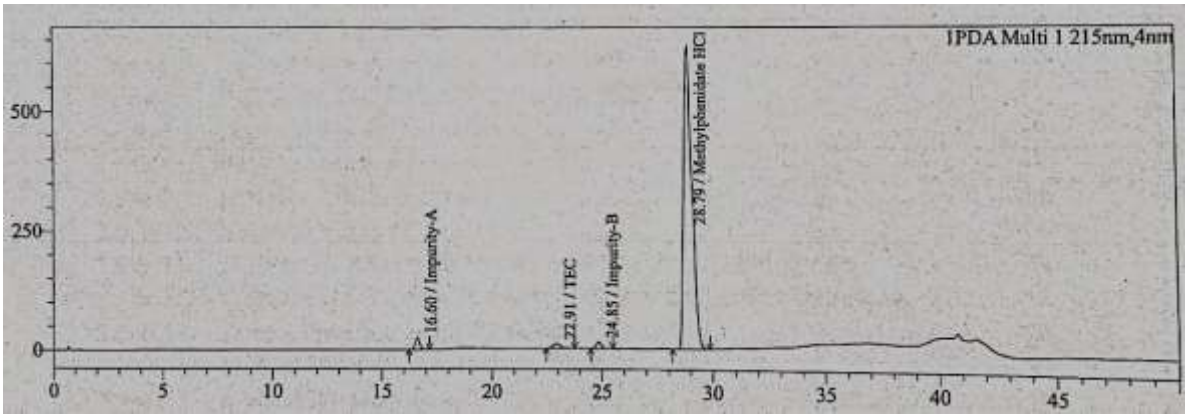
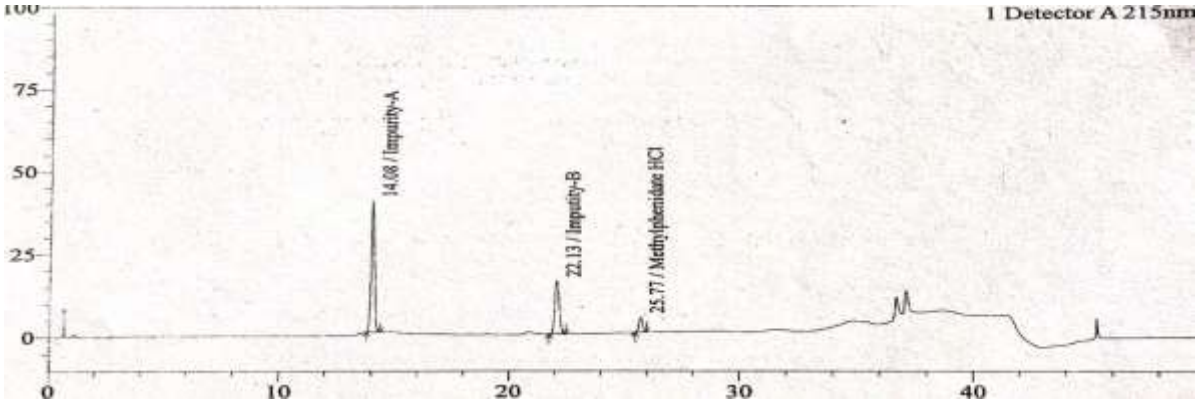
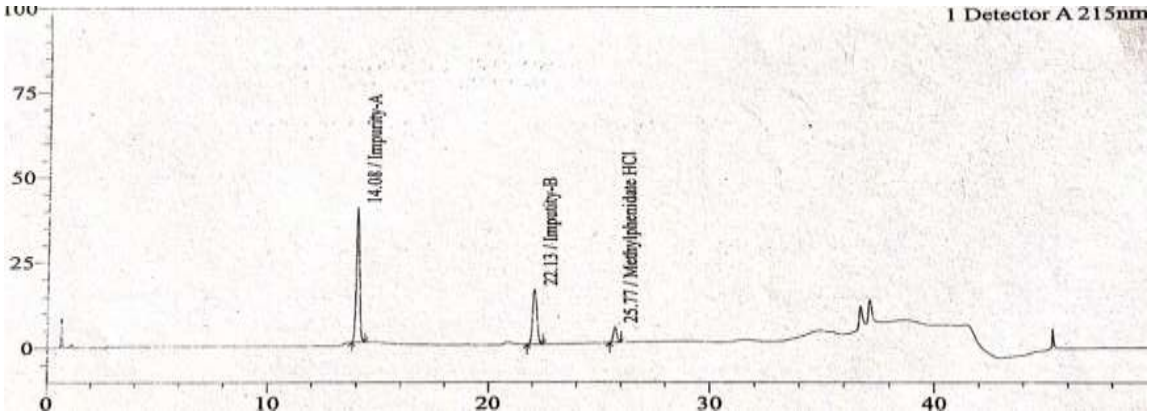


Figure.14 Chromatogram for 100% Accuracy







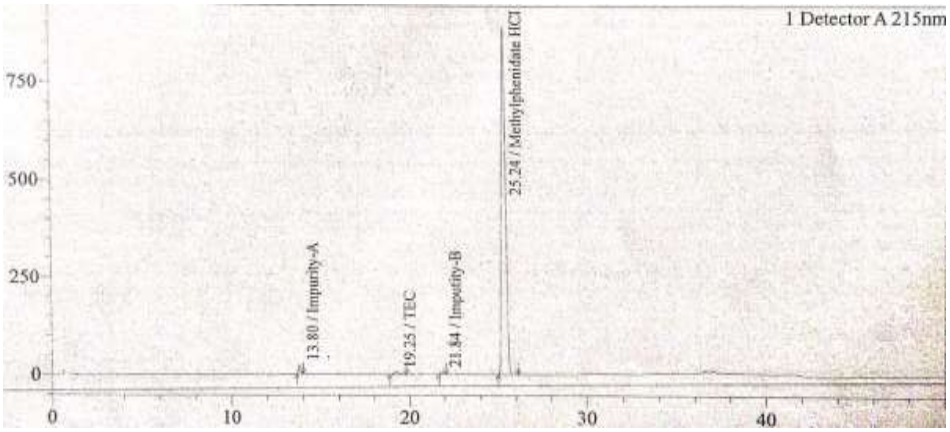


Figure.14 Chromatogram for 150% Accuracy

Accuracy table for Methylphenidate HCl

% Level	Amount spiked(µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	20	19.8	112.7	
	20	20.01	110.8	
	20	20.0	114.5	
100%	40	39.5	111,12	
	40	39.2	109.08	111.24%
	40	39.7	110.05	
150%	60	59.2	108.02	
	60	59.2	112.08	
	60	59.9	110.24	

Limit: Not less than 95% and Not more than 105%

F. Solution Stability: : A comprehensive evaluation of solution stability was conducted, encompassing both sample and standard solutions. These solutions underwent rigorous stability tests, being stored at ambient temperature for a stipulated duration of 24 hours. Subsequently, at specific intervals, re-analysis was undertaken to ascertain the percentage Relative Standard Deviation (% RSD), meticulously comparing the obtained results with those of fresh samples.

Solution Stabilty table

SAMPLE SOLUTION			
TIME POINT	**IMPURITY A**	**IMPURITY B**	**%RSD**
Initial	0.13	0.01	0.01
12th hr	0.14	0.01	0.01
Difference	0.01	NA	NA
24th hr	0.17	0.01	0.01
Difference	0.04	NA	NA
48th hr	0.18	0.01	0.01

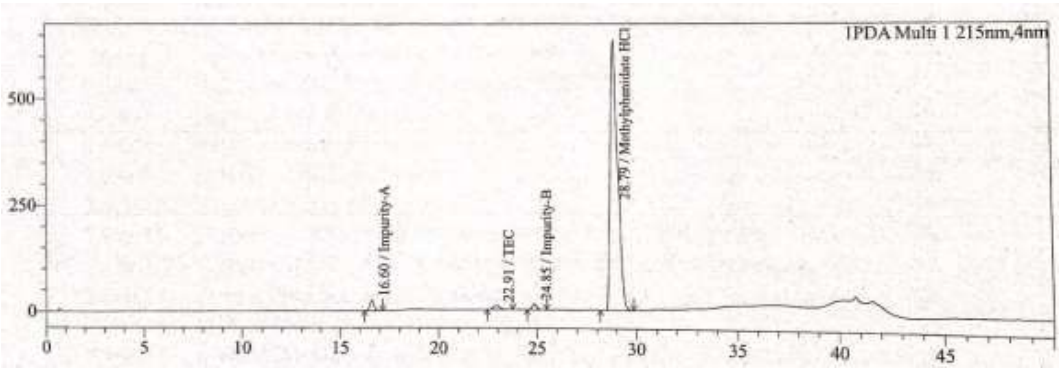


Figure.14 Chromatogram for Solution stability

CONCLUSION:

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC), renowned for its exceptional sensitivity, stands out as a distinctive analytical method renowned for its capacity to efficiently handle multi-component mixtures. For routine analysis, the establishment of methods capable of swiftly and accurately analyzing a vast number of samples within tight timeframes is imperative. The evolution of analytical methods assumes a pivotal role across the spectrum of pharmaceutical endeavors, encompassing discovery, development, and manufacturing processes. By significantly reducing retention times and overall run duration, the method's development achieved a remarkable level of accuracy and simplicity, rendering it suitable for seamless integration into routine quality control procedures within industrial settings.

Acknowledgement:

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Dr. M. AJITHA, HOD, Centre for Pharmaceutical Sciences, UCEST, JNTUH, Hyderabad

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