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Research Article

Development and Validation of a Reverse Phase High-Performance Liquid Chromatographic Method for Quantitative Estimation of Methenamine Hippurate in Tablet Dosage Form

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Abstract

Methenamine Hippurate (MEH) is a urinary antiseptic widely prescribed for the prophylaxis and treatment of recurrent urinary tract infections. The drug acts by releasing formaldehyde in acidic urine, thereby exerting antibacterial activity against a broad spectrum of urinary pathogens. Reliable analytical methods are essential to ensure the quality, safety, and efficacy of pharmaceutical formulations containing Methenamine Hippurate. Although several analytical techniques including spectrophotometry, fluorimetry, gas chromatography, and LC-MS methods have been reported, only limited RP-HPLC methods are available for routine quantitative estimation of Methenamine Hippurate in tablet dosage forms. The present study aimed to develop and validate a simple, rapid, accurate, precise, and economical reverse-phase high-performance liquid chromatographic (RP-HPLC) method for quantitative estimation of Methenamine Hippurate in tablet dosage formulations. Chromatographic separation was achieved using a Phenomenex Kinetex C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of water containing 0.1% orthophosphoric acid and methanol (70:30, v/v) under isocratic conditions. The flow rate was maintained at 1.0 mL/min and detection was performed at 230 nm using a PDA detector. The developed method was validated according to ICH Q2(R1) guidelines for system suitability, linearity, accuracy, precision, ruggedness, robustness, and specificity. Methenamine Hippurate exhibited a sharp and symmetrical chromatographic peak with a retention time of approximately 3.83 min. The developed method demonstrated excellent linearity over the concentration range of 500–1500 µg/mL with a correlation coefficient (R^2) of 0.999. Accuracy studies showed recovery values ranging from 99.70% to 99.87%. Precision and ruggedness studies yielded %RSD values below 2%. The assay of the marketed tablet formulation showed drug content of 99.70% of the labeled claim. Robustness studies confirmed the reliability of the method under deliberate variations of chromatographic conditions.The developed RP-HPLC method was found to be simple, accurate, precise, robust, and economical. The method is suitable for routine quality control analysis, dissolution studies, and stability testing of Methenamine Hippurate tablet formulations.

Keywords: Methenamine Hippurate, RP-HPLC, Method Validation, Tablet Formulation, Urinary Antiseptic, ICH Guidelines, Pharmaceutical Analysis.

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1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections affecting individuals worldwide. Recurrent urinary tract infections pose a significant healthcare burden and often require long-term prophylactic treatment. Methenamine Hippurate (MEH), also known as hexamine hippurate, is a urinary antiseptic extensively used for the prevention and management of chronic and recurrent urinary tract infections. The therapeutic efficacy of Methenamine Hippurate is attributed to its ability to hydrolyze in acidic urine, releasing formaldehyde, which exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative microorganisms without promoting bacterial resistance [1].

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Methenamine Hippurate is chemically designated as 2-benzamidoacetic acid; 1,3,5,7-tetrazatricyclo[3.3.1.$1^{3,7}$]decane and possesses a molecular formula of $C_{15}H_{21}N_5O_3$ with a molecular weight of 319.36 g/mol. The drug is generally administered orally in tablet formulations and is widely employed in long-term prophylactic therapy for recurrent urinary tract infections [2]. Owing to its therapeutic importance, reliable analytical methods are essential for ensuring the quality, safety, efficacy, and regulatory compliance of Methenamine Hippurate pharmaceutical products.

Analytical method development and validation constitute critical components of pharmaceutical quality assurance. High-performance liquid chromatography (HPLC) has become one of the most widely accepted analytical techniques due to its excellent sensitivity, specificity, reproducibility, and suitability for quantitative analysis of pharmaceutical compounds in complex matrices [3,4].

Several analytical methods have been reported for the determination of Methenamine Hippurate and related compounds. Pavitrapok and Williams developed an ion-exchange HPLC method for determination of methenamine in pharmaceutical formulations using a Zorbax SCX-300 strong cation exchange column with UV detection at 212 nm. The method demonstrated excellent linearity and assay values ranging between 98% and 102%, confirming its suitability for pharmaceutical analysis [5].

Raghu Raj Naik et al. reported four validated UV spectrophotometric methods for estimation of Methenamine Hippurate in tablet formulations using different buffer systems. The methods demonstrated linearity within the concentration range of 10–80 µg/mL and were validated according to ICH guidelines, highlighting their applicability for routine quality control analysis [6].Patel et al. developed a green UV spectrophotometric method for estimation of hippuric acid, a degradation and metabolic marker of Methenamine Hippurate, using distilled water as solvent. The method demonstrated excellent sensitivity with correlation coefficient close to 0.999 and low limits of detection and quantification [7].

More recently, Kolli Venkata Durga et al. established an RP-HPLC method for determination of Methenamine Hippurate and its related substances in active pharmaceutical ingredients using an X-Bridge C18 column with gradient elution. Their method demonstrated satisfactory precision, linearity, and compliance with ICH validation requirements [8].

Balasubramanian and Nair developed spectrophotometric and fluorimetric methods based on derivatization of Methenamine and Methenamine Hippurate through Hantzsch condensation reactions. The reported methods exhibited high precision and selectivity for pharmaceutical formulations [9].

Earlier analytical investigations by Strom employed gas-liquid chromatography for determination of Methenamine in pharmaceutical tablets, demonstrating improved sensitivity compared with pharmacopoeial methods available at that time [10]. Similarly, Nieminen et al. reported a gas chromatographic method for determination of Methenamine in biological fluids and successfully applied it to pharmacokinetic investigations [11].

Advanced bioanalytical approaches have also been explored. Kim et al. developed a sensitive LC-MS/MS method for determination of hexamethylenetetramine

(Methenamine) in rat plasma and applied the method to toxicokinetic and bioavailability studies, demonstrating excellent selectivity and sensitivity [12].

Despite these reported analytical methods, literature reveals limited availability of simple and validated RP-HPLC methods specifically developed for routine quantitative estimation of Methenamine Hippurate in tablet dosage forms. Furthermore, many reported methods require specialized instrumentation, complex sample preparation, derivatization procedures, or are intended for biological matrices rather than finished pharmaceutical products.

Therefore, the present investigation was undertaken to develop and validate a simple, economical, accurate, and reproducible RP-HPLC method for quantitative estimation of Methenamine Hippurate in tablet dosage forms. The developed method was validated according to International Council for Harmonisation (ICH) Q2(R1) guidelines with respect to specificity, linearity, accuracy, precision, ruggedness, robustness, and system suitability parameters. The validated method was subsequently applied for routine analysis of a marketed tablet formulation containing Methenamine Hippurate.

2. Materials And Methods

2.1. Materials

Methenamine Hippurate (MEH) reference standard (purity 99.9% w/w) was obtained from Arrow Chem, Mumbai, India. Marketed tablet formulation containing Methenamine Hippurate 1000 mg (MethaClear® Tablets) was procured from a local pharmacy and used for assay studies. The purity of the reference standard as stated by the supplier was accepted for analytical calculations.

All solvents and reagents used during the study were of analytical reagent (AR) or HPLC grade. Methanol (HPLC grade), orthophosphoric acid (OPA), and purified water were obtained from reputed commercial suppliers and used without further purification.

2.2. Instrumentation

Chromatographic analysis was performed using a Waters 600 HPLC system equipped with a photodiode array (PDA) detector and Empower chromatography software. Separation was achieved using a Phenomenex Kintex C18 column (4.6 × 150 mm, 5 µm particle size).

2.3. Selection of Wavelength

A standard solution of Methenamine Hippurate was prepared and scanned in the UV region between 200 and 400 nm using methanol as solvent. The drug exhibited maximum absorbance at 230 nm. Therefore, 230 nm was selected as the analytical wavelength for chromatographic detection**(Figure 1)**.

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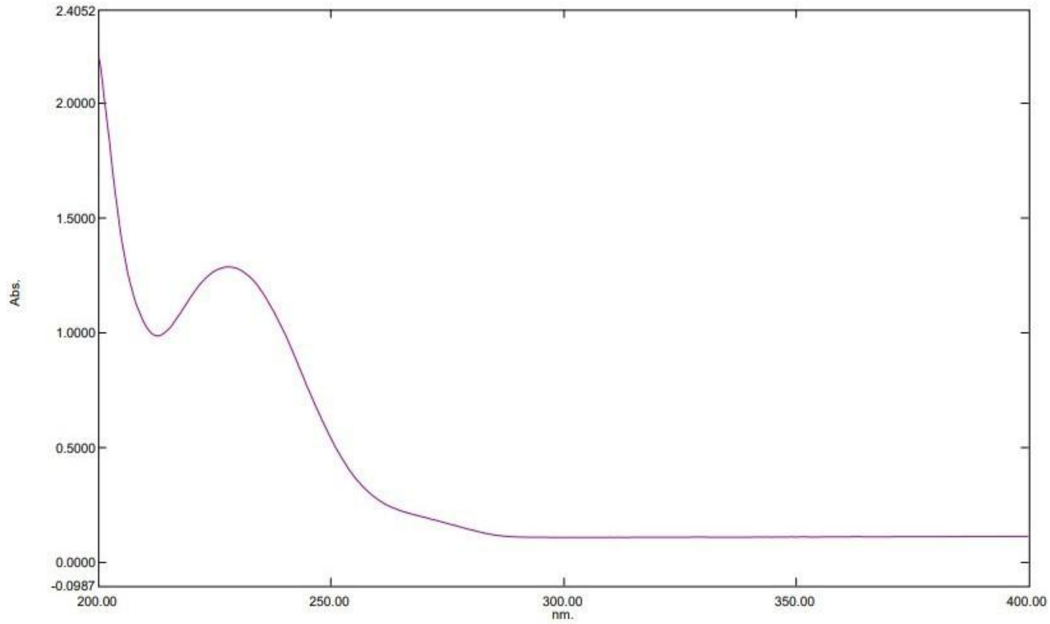

Figure 1. UV Spectrum of Methenamine Hippurate showing λmax at 230 nm.

2.4. HPLC Method Development

2.4.1. Selection of Mobile Phase

Several solvent systems consisting of water, methanol, and their combinations were investigated to obtain satisfactory chromatographic performance. After systematic optimization, a mobile phase consisting of water containing 0.1% orthophosphoric acid and methanol in the ratio of 70:30 (v/v) produced a sharp and symmetrical chromatographic peak with acceptable retention characteristics.

2.4.2. Preparation of Mobile Phase

One milliliter of orthophosphoric acid was transferred into a 1000 mL volumetric flask and diluted to volume with HPLC-grade water to obtain 0.1% OPA solution. The mobile phase was prepared by mixing water (0.1% OPA) and methanol in the ratio of 70:30 (v/v). The mixture was filtered through a 0.45 µm membrane filter and degassed by sonication before use.

Table 1. Optimized Chromatographic Conditions

Parameter	Condition
Column	Phenomenex Kintex C18 (4.6 × 150 mm, 5 µm)
Mobile Phase	Water (0.1% OPA): Methanol (70:30, v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	230 nm
Injection Volume	20 µL
Column Temperature	Ambient
Run Time	15 min

2.5. Preparation of Standard Solution

Accurately weighed Methenamine Hippurate reference standard equivalent to 1000 mg was transferred into a volumetric flask and dissolved in methanol. The solution was sonicated to ensure complete dissolution and diluted appropriately with mobile phase to obtain the desired working concentration.

2.6. Preparation of Sample Solution

Twenty tablets were weighed and finely powdered. An accurately weighed quantity of powder equivalent to 1000 mg of Methenamine Hippurate was transferred into a volumetric flask containing methanol. The mixture was sonicated for complete extraction of the drug, filtered through a membrane filter, and diluted appropriately using mobile phase to obtain the required analytical concentration.

2.7. Assay of Marketed Tablet Formulation

Twenty microliters each of standard and sample solutions were injected separately into the HPLC system after equilibration of the chromatographic column. The chromatograms were recorded and peak areas were measured. Drug content in the marketed formulation was calculated by comparing sample peak area with standard peak area using the validated assay formula.

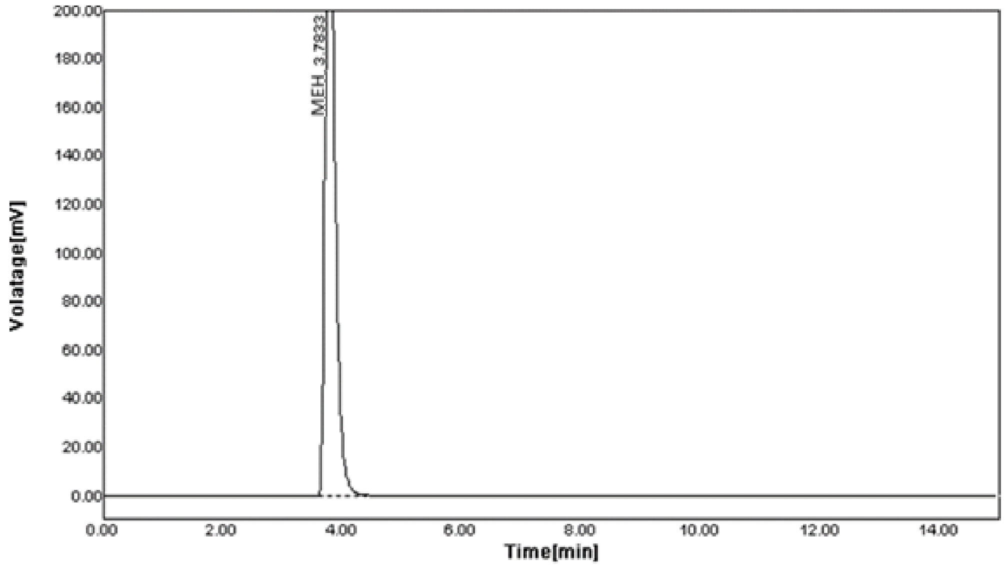

Figure 2. Representative chromatogram of Methenamine Hippurate standard solution.

2.8. Method Validation

Validation of the developed RP-HPLC method was performed according to ICH Q2(R1) guidelines.

2.8.1. System Suitability

System suitability was evaluated using five replicate injections of standard solution. Parameters including retention time, peak area, symmetry factor, and theoretical plate count were recorded.

2.8.2. Linearity

Linearity was assessed over the concentration range corresponding to 80–150% of the target analytical concentration. Calibration curves were constructed by plotting concentration against peak area response.

2.8.3. Accuracy

Accuracy was determined using the standard addition technique at 80%, 100%, and 120% concentration levels. Percentage recovery and %RSD values were calculated.

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2.8.4. Precision

Precision studies included system precision and method precision (repeatability). The results were expressed as percentage relative standard deviation (%RSD).

2.8.5. Robustness

Robustness was evaluated by deliberate variation of chromatographic conditions including Flow rate (±10%), Organic phase composition (±10%) and Detection wavelength (±2 nm)

2.8.6. Specificity

Specificity was evaluated by placebo interference studies to confirm the absence of interfering peaks at the retention time of Methenamine Hippurate.

2.9. Statistical Analysis

Results obtained during validation studies were expressed as mean, standard deviation (SD), and percentage relative standard deviation (%RSD). Acceptance criteria were evaluated according to ICH Q2(R1) recommendations.

3. Results And Discussion

3.1. Method Development and Optimization

The primary objective of the present study was to develop a simple, accurate, precise, and economical RP-HPLC method for quantitative estimation of Methenamine Hippurate in tablet dosage forms. Several chromatographic conditions were investigated to obtain optimum chromatographic performance. Among the various mobile phase systems evaluated, water containing 0.1% orthophosphoric acid and methanol (70:30, v/v) produced a sharp, symmetrical peak with acceptable retention characteristics and satisfactory system suitability parameters. Methenamine Hippurate exhibited a retention time of approximately 3.83 min under optimized chromatographic conditions. The chromatogram showed excellent peak symmetry and chromatographic efficiency**(Figure 2)**.

3.2. System Suitability Studies

System suitability testing was performed prior to sample analysis to ensure adequate chromatographic performance. The low % RSD value demonstrates excellent repeatability of chromatographic response. Theoretical plate count and symmetry factor were within acceptable limits indicating efficient chromatographic separation.

Table 2. System Suitability Parameters

Parameter	Mean Value
Peak Area	409400
Retention Time (min)	3.83
Symmetry Factor	0.84
Theoretical Plates	7127
%RSD of Peak Area	0.65

3.3. Assay of Marketed Tablet Formulation

The developed RP-HPLC method was successfully applied for estimation of Methenamine Hippurate in marketed tablet formulation (MethaClear® Tablets).The assay result demonstrated an average drug content of 99.70% of the labeled claim, indicating excellent formulation quality and content uniformity**(Table 3)**.

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Table 3. Assay Results of Marketed Tablet Formulation

Replicate	Assay (mg)	% Purity
1	1000.0	100.00
2	999.8	99.98
3	999.9	99.99
4	993.3	99.33
5	993.3	99.33
Average	999.7	99.70
SD	0.005	0.36
%RSD	0.36	0.36

The assay results were within acceptable pharmaceutical limits and confirmed suitability of the method for routine quality control applications.

3.4. Precision Studies

The low %RSD value indicates excellent repeatability of the chromatographic system. The % RSD values were 0.42, Theoretical plates were 7037 and mean tailing factor was 1.33. For method precision the recovery assay was 99.53%, % RSD value was 0.38. The method demonstrated satisfactory repeatability with % RSD values below 2%.

3.5. Linearity and Range

Linearity was established over the concentration range corresponding to 80–150% of the target analytical concentration. The calibration plot exhibited excellent correlation between concentration and peak area response with a correlation coefficient (R^2) of 0.999. These results confirm excellent linearity of the developed analytical method.

3.6. Accuracy Studies

Accuracy was evaluated by the standard addition method. Recovery values were within the acceptable range of 98–102%. The recovery for 80, 100 and 120 % addition was 99.79, 99.77 and 99.78 % respectively confirming the accuracy of the developed method.

3.7. Robustness Studies

Robustness studies were performed by deliberate variation of chromatographic parameters including flow rate, mobile phase composition, and detection wavelength. No significant changes were observed in retention time, peak shape, or system suitability parameters following deliberate modifications. The method remained unaffected by small variations in chromatographic conditions, confirming its robustness.

3.8. Specificity

Specificity studies demonstrated the absence of interference from formulation excipients at the retention time corresponding to Methenamine Hippurate **(Table 4)**. No interfering peaks were detected, confirming specificity and selectivity of the developed RP-HPLC method.

Table 4. Placebo Interference Study

Sample	Observation
Placebo	No interference
Standard	Distinct peak observed
Test Sample	Distinct peak observed

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4. Discussion

The present investigation successfully developed and validated a simple, rapid, accurate, precise, and economical RP-HPLC method for quantitative estimation of Methenamine Hippurate (MEH) in tablet dosage forms. The developed analytical method demonstrated excellent chromatographic performance and fulfilled all validation requirements specified by ICH Q2(R1) guidelines.

Methenamine Hippurate remains an important urinary antiseptic for the prevention and management of recurrent urinary tract infections. Due to its widespread clinical use and prolonged administration in prophylactic therapy, reliable analytical methods are essential for quality control, formulation development, stability assessment, and regulatory compliance. Although several analytical techniques have been reported for determination of Methenamine Hippurate and related compounds, many of these methods involve complex instrumentation, derivatization procedures, or are primarily intended for biological samples rather than finished pharmaceutical dosage forms [1,2].

The developed RP-HPLC method employed a Phenomenex Kintex C18 column with a simple mobile phase consisting of water containing 0.1% orthophosphoric acid and methanol (70:30, v/v). Under optimized chromatographic conditions, Methenamine Hippurate produced a sharp and symmetrical chromatographic peak with a retention time of approximately 3.83 min. The relatively short retention time offers significant practical advantages including reduced solvent consumption, decreased analysis time, and increased sample throughput, which are desirable features in routine pharmaceutical quality control laboratories.

System suitability studies demonstrated excellent chromatographic performance. The average retention time was 3.83 min, theoretical plate count exceeded 7000, and the symmetry factor remained within acceptable limits. Furthermore, the %RSD of peak area was found to be only 0.65%, indicating excellent reproducibility of the chromatographic system. Similar observations have been reported by Pavitrapok and Williams, who developed an ion-exchange HPLC method for Methenamine determination and reported satisfactory precision and reproducibility for pharmaceutical analysis [5].

The assay of the marketed tablet formulation demonstrated an average drug content of 99.70% of the labeled claim. These findings confirm the suitability of the developed method for routine quality control analysis and indicate that the marketed product complies with pharmaceutical quality requirements. Similar assay values have been reported in previous analytical investigations involving Methenamine-containing formulations [5,6].

Accuracy studies performed using the standard addition method demonstrated recovery values ranging from 99.70% to 99.87%. These recovery values fall well within the acceptable range of 98–102% recommended by ICH guidelines and confirm the absence of interference from tablet excipients. The recovery results obtained in the present study are comparable with those reported by Naik et al., who developed UV spectrophotometric methods for Methenamine Hippurate and observed recovery values close to 100% [6].

Precision studies revealed excellent repeatability and reproducibility of the analytical procedure. System precision studies produced %RSD values below 1%, while method precision yielded a %RSD of 0.38%. These values are significantly lower than the maximum acceptable limit of 2%, confirming the reliability of the developed method.

Similar precision characteristics have been reported for validated RP-HPLC methods used in pharmaceutical analysis of urinary antiseptics and related compounds [3,7].

Linearity studies demonstrated a direct proportional relationship between concentration and chromatographic response throughout the selected analytical range. The correlation coefficient ($R^2 = 0.999$) indicates excellent linearity and confirms the suitability of the method for quantitative analysis. Comparable linearity values have been reported in RP-HPLC and spectrophotometric methods developed for Methenamine Hippurate and related pharmaceutical compounds [6,8].

Robustness studies were performed by deliberate variation of chromatographic parameters including flow rate, mobile phase composition, and detection wavelength. No significant changes in chromatographic behavior were observed following these modifications. The ability of the method to maintain analytical performance under small experimental variations demonstrates its robustness and reliability for routine laboratory applications. Similar observations have been reported by Durga et al. during validation of RP-HPLC methods for Methenamine Hippurate and related substances [8].

Specificity studies confirmed that no interference from formulation excipients was observed at the retention time corresponding to Methenamine Hippurate. This finding is particularly important for pharmaceutical quality control because excipient interference can significantly affect assay accuracy and method reliability. The specificity demonstrated by the developed method confirms its suitability for routine analysis of tablet formulations.

A comparison of the present method with previously reported analytical procedures reveals several advantages. Spectrophotometric methods reported by Naik et al. and Patel et al. are economical and simple but may lack the selectivity and sensitivity associated with chromatographic techniques [6,7]. Gas chromatographic methods developed by Strom and Nieminen et al. provide adequate sensitivity but require specialized instrumentation and extensive sample preparation [10,11]. LC-MS/MS methods reported by Kim et al. offer superior sensitivity but are expensive and less practical for routine quality control applications [12].

Compared with these analytical approaches, the present RP-HPLC method combines simplicity, economy, selectivity, and reproducibility. The use of a readily available C18 column, simple mobile phase composition, short analysis time, and excellent validation characteristics make the developed method highly suitable for routine pharmaceutical quality control laboratories.

Overall, the developed RP-HPLC method demonstrated excellent specificity, accuracy, precision, linearity, robustness, and system suitability. The method fully complies with ICH Q2(R1) validation requirements and may be successfully employed for routine quality control analysis, formulation development studies, dissolution testing, and stability investigations involving Methenamine Hippurate tablet formulations.

5. Conclusion

A simple, rapid, accurate, precise, robust, and economical RP-HPLC method was successfully developed and validated for quantitative estimation of Methenamine Hippurate in tablet dosage forms.

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Chromatographic separation was achieved using a Phenomenex Kintex C18 column and a mobile phase consisting of water containing 0.1% orthophosphoric acid and methanol (70:30, v/v). The developed method produced a well-resolved chromatographic peak with a retention time of approximately 3.83 min and demonstrated excellent chromatographic performance.

Validation studies confirmed that the method satisfies all ICH Q2(R1) requirements with respect to specificity, linearity, accuracy, precision, robustness, and system suitability. The assay of the marketed tablet formulation demonstrated drug content of 99.70% of the labeled claim, confirming satisfactory product quality and content uniformity.The developed method offers several practical advantages including simple mobile phase preparation, short chromatographic run time, low solvent consumption, excellent reproducibility, high analytical accuracy, cost-effective operation, ease of implementation in quality control laboratories.

The recovery values ranging from 99.70% to 99.87%, correlation coefficient of 0.999, and precision values below 2% further establish the reliability of the developed analytical procedure.

To the best of our knowledge, only a limited number of validated RP-HPLC methods have been reported specifically for routine estimation of Methenamine Hippurate in tablet dosage forms. Therefore, the present method provides a valuable analytical tool for pharmaceutical industries, academic research laboratories, and regulatory quality control agencies involved in the analysis of Methenamine Hippurate formulations.

Future investigations may focus on the development of stability-indicating RP-HPLC methods capable of separating degradation products, bioanalytical applications in biological matrices, and simultaneous estimation of Methenamine Hippurate with other urinary anti-infective agents in combination dosage forms.

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