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METHOD DEVELOPMENT AND VALIDATION OF METHENAMINE HIPPURATE AND ITS RELATED SUBSTANCES IN ACTIVE PHARMACEUTICAL INGREDIENTS BY HPLC

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ABSTRACT

A simple reverse phase HPLC method was developed and validated for the estimation of Methenamine Hippurate and its related Substances in Active Pharmaceutical Ingredient. The analysis was carried out on EMPOWER HPLC model equipped with X-Bridge C_{18} (250 mm× 4.6 mm, 5µm) column and UV-Detector. Buffer: Solvent mixture (95:5) v/v as Mobile Phase-A and Buffer: Solvent mixture (30:70) v/v as Mobile Phase-B (1.36g Potassium hydrogen phosphate and 1.74 g di potassium hydrogen phosphate into 1000 mL volumetric flask containing 1000 mL water, dissolve and adjust pH 7.0 with potassium hydroxide solution (1g in 10ml water) is Buffer) (Acetonitrile and methanol in the ratio of 70:30 %v/v is solvent mixture) at a flow rate of 0.6ml/min was employed for elution and the compounds were detected at 210nm at 35^0C. The method was validated as per the ICH guidelines. Linearity was observed in the concentration range of 50 to 150% with correlation coefficient 1.00. The percent of relative standard deviation of six replicate measurements was found to be 1.6 which indicates that the proposed method was precise. Therefore the developed method was simple, precise and accurate and can be successfully applied for the estimation of Methenamine Hippurate and its related substances in Active Pharmaceutical Ingredient.

KEYWORDS: Methenamine Hippurate, Anti-bacterial, RP-HPLC, Validation, Impurity profile.

INTRODUCTION

Methenamine or Hexamethylenetetramine or Hexamine or Urotropin (Figure 1) is a white crystalline compound is highly soluble in water and polar organic solvents. Methenamine a heterocyclic organic compound with a cage-like structure similar to adamantane. Methenamine hippurate is available as Methenamine hippurate and Methenamine mandelate. Methenamine hippurate is used for the treatment of urinary tract infection. Methenamine hippurate hydrolyzes to ammonia and formaldehyde (nonspecific antibacterial) in acidic urine. Mandelic acid or hippuric acid acidify the urine. Methenamine is chemically 1,3,5,7-tetraazatricyclo [3.3.1.1[3,7]] decane. Methenamine hippurate has molecular formula $C_{15}H_{21}N_5O_3$ and molecular weight 319.37 gm and is also used for the production of plastic materials, explosives, phenolic resins, antibacterial pharmaceutics, disinfecting materials, in the rubber industry as an additive, and so forth. Methenamine was previously determined by ion-exchange HPLC,[2] capillary gas chromatography,[3] spectrophotometry,[4-6] gas chromatography,[7] solid phase spectrophotometry,[8] fluorimetry[9] and in biological samples such as urine by Proton NMR[10] and ion pair extraction,[11] in edible animal tissues[12] by HPLC-MS/MS and in dairy products using GC-MS techniques.[13] In the present study the authors have proposed new zero order and first order derivative spectrophotometric methods for the determination of Methenamine hippurate using four different buffers and all the methods were validated as per ICH guidelines.

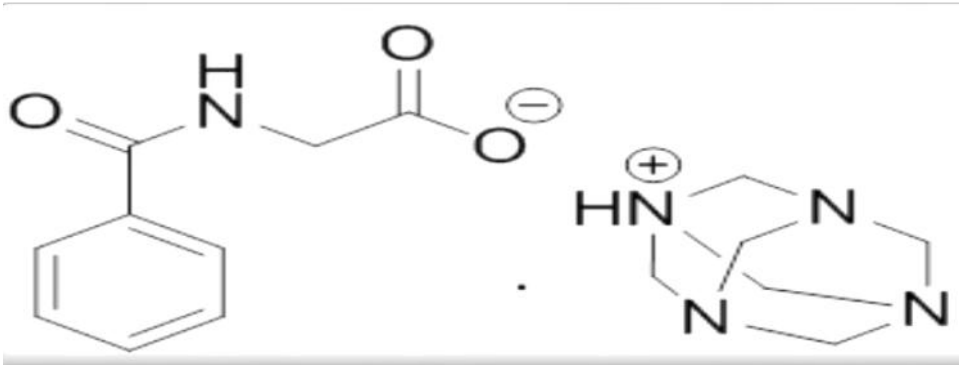

Figure no. 1: Chemical structure of methenamine hippurate.

MATERIALS AND METHODS

Methenamine Hippurate Standard of known Potency, Impurity-A standard (Benzoic Acid) Impurity-B standard, Impurity-Cstandard Impurity-Dstandard Impurity-Estandard Hippuric acid standard.

Preparation of solutions

Preparation of methenamine stock solution: Accurately weight and transfer about 25.0 mg of Methenamine standard into a 25 mL volumetric flask, dissolve and make up to the mark with diluent.

Preparation of Impurities A, B, C, D, E and Hippuric acid stock solution: Accurately weight and transfer about 25.0 mg of each Impurity-A, Impurity-B, Impurity-C, Impurity-D, Impurity-E and Hippuric acid standard into a 25 ml volumetric flask. Add 10-15 mL of Methanol and Sonicate to dissolve. Make up to the mark with Methanol and mix well.

Preparation of system suitability solution: Transfer each 5.0 ml of above stock solutions into a 100 ml volumetric flask and make up to the mark with diluent. Further transfer 3.0 ml of this solution into a 20 ml volumetric flask and dilute up to the mark with diluent and mix well.

Preparation of sample solution: Accurately weigh and transfer about 150.0 mg of sample into a 10 mL volumetric flask, dissolve and make up to the mark with diluent and mix well. Prepare in duplicate.

Method optimization consideration

As per the literature survey there was a need for the short time of analytical method having the good outcome with the lowest concentration range which has to be covered in the range of the sample after cleaning the surface areas used in the manufacturing. During the review of the methods developed it was identified that there was no HPLC method for this drug combination. In the lower concentration there was no method developed for the cleaning samples. Hence this method was developed and validated and applied as per the requirement.

Optimization of chromatographic conditions

After series of trials, the Methenamine Hippurate and its related Substances in Active Pharmaceutical Ingredient. The analysis was carried out on EMPOWER HPLC model equipped with X-Bridge C_{18} (250 mm× 4.6 mm, 5µm) column and UV-Detector. Buffer: Solvent mixture (95:5) v/v as Mobile Phase-A and Buffer: Solvent mixture (30:70) v/v as Mobile Phase-B (1.36g Potassium hydrogen phosphate and 1.74 g di potassium hydrogen phosphate into 1000 mL volumetric flask containing 1000 mL water, dissolve and adjust pH 7.0 with potassium hydroxide solution (1g in 10ml water) is Buffer) (Acetonitrile and methanol in the ratio of 70:30 %v/v is solvent mixture) at a flow rate of 0.6ml/min was employed for elution and the compounds were detected at 210nm at 35^0C.

Validation analytical method

Selectivity and Specificity

To demonstrate the developed method is specific and selective injected the diluent as blank injection then injected the standards individually 10µL in triplicate, In the generated chromatograms there was no interference and the main peaks were identified with the good response and shape.

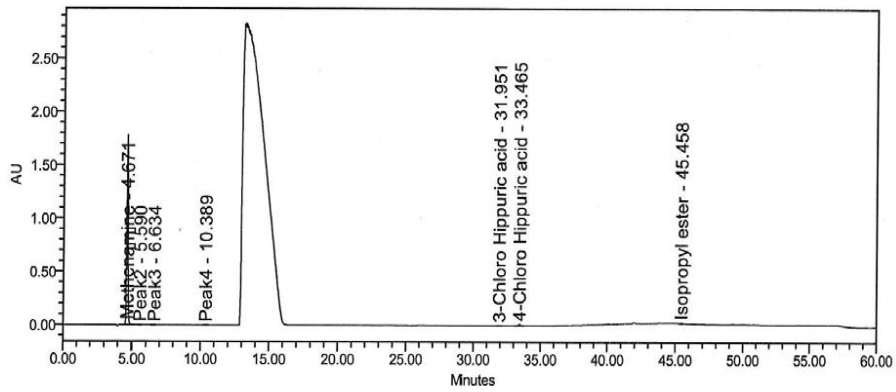

Fig. no. 2: Methenamine chromatogram.

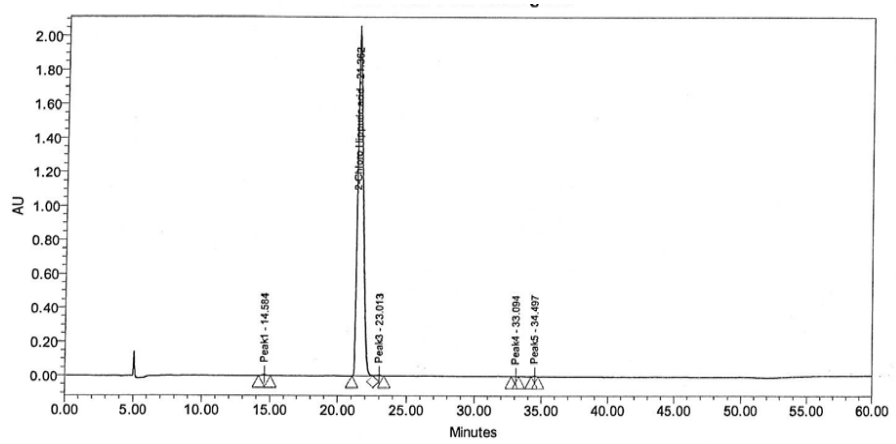

Fig. 3: Chloro hippuric acid chromatogram.

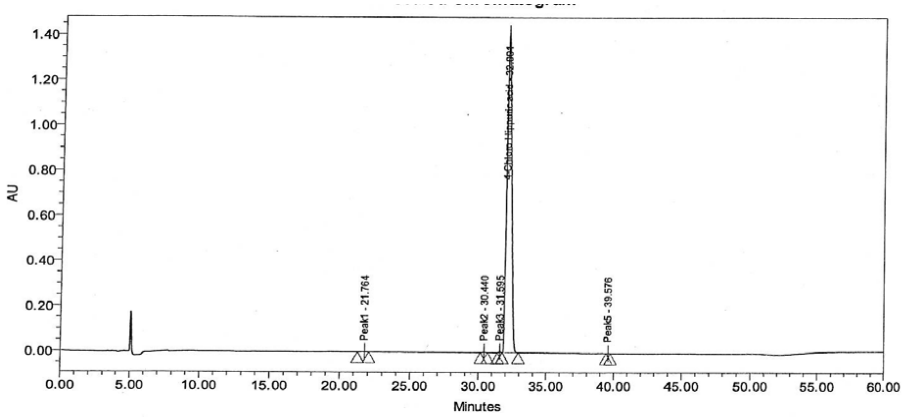

Fig. 6: Isopropyl ester chromatogram.

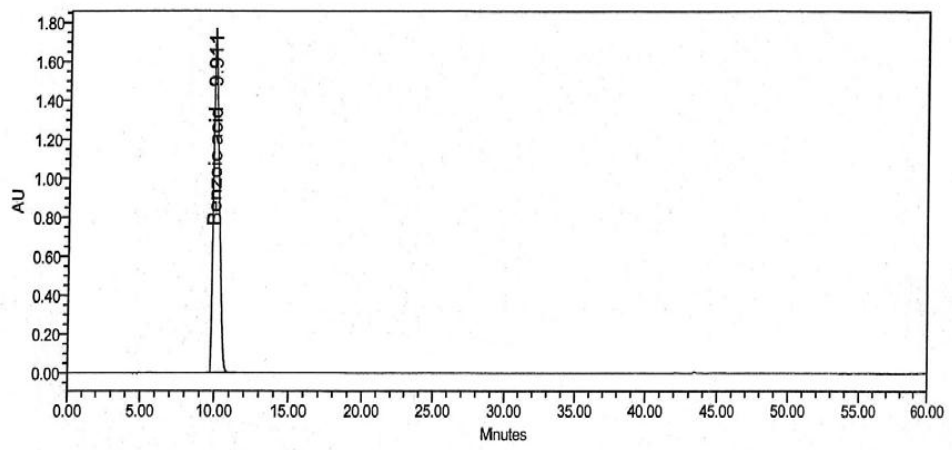

Fig. 7: Benzoic acid chromatogram.

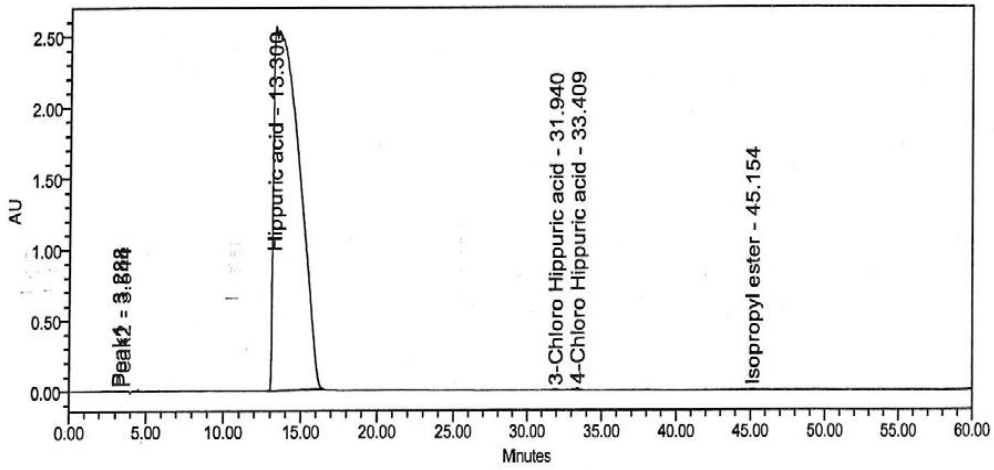

Fig. 8: Hippuric acid chromatogram.

System suitability

For the verification of instrument suitability and execution of sample identification and quantification, Injected the standard preparation six times with the concentration of 200ng/ml and 300 ng/ml combination with the aim of %RSD of area NMT 2.0% and theoretical plates NLT 2000 plates and the tailing factor NMT 1.5 and resolution NLT 4.

Name of the Reference solution	Methenamine	Imp-A	Hippuric acid	Imp-B	Imp-C	Imp-D	Imp-E
Injection-1	11485	336509	294507	457726	576008	310495	214146
Injection-2	11145	326158	285240	447302	563549	303502	209105
Injection-3	11260	335703	293332	456952	574814	310061	213692
Injection-4	11286	337487	295409	462807	583095	313385	215942
Injection-5	10940	335790	294516	459867	579278	312381	215004
Injection-6	11242	337262	293777	457996	574945	310843	213127
Mean	11226	334818	292797	457108	575282	310111	213503

STDEV	179.14	4305.34	3769.79	5242.96	6565.58	3470.93	2372.12
% RSD	1.60	1.29	1.29	1.15	1.14	1.12	1.11
USP Resolution	NA	27.69	14.65	7.11	34.09	3.97	40.92

Precision

After method optimization the method was subjected for the method precision to prove the closeness between the series of measurements from the homogeneous sample with the limit of % RSD for the area NMT 2%. The intermediate precision was verified on the next day with the same limit as % RSD for the area NMT 2%.

Table 2: Method precision solution results.

Name of the Reference solution	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	SMU imp	% Total unknown imps
Precision sol-1	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Precision sol-2	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Precision sol-3	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Precision sol-4	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Precision sol-5	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Precision sol-6	0.05	0.05	0.05	0.08	0.06	0.01	0.32
Mean	0.05	0.05	0.05	0.08	0.06	0.01	0.32
STDEV	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
% RSD	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Accuracy and Recovery

Quality control standards were prepared in the four levels as 80%, 100% and 120% in the combined standard solution with the limit of % Recovery of NLT98% to prove the good accuracy.

Linearity

Linearity is the ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity of Methenamine and impurities are established by injecting different concentrations in the range of 50 % to 150 %.

Table 3: Linearity results.

Name of the solution	Methenamine		Imp-A		Imp-B	
	Conc. (%)	Area	Conc. (%)	Area	Conc. (%)	Area
LOQ Level	0.013	3617	0.0013	8089	0.0013	10427
50% Level	0.0254	6134	0.0253	167051	0.0254	228794
75% Level	0.0381	9163	0.0379	247047	0.0381	336896
100% Level	0.0508	12337	0.0505	334108	0.0507	455280
125% Level	0.0635	15299	0.0632	418357	0.0634	567512
150% Level	0.0762	18168	0.0758	489022	0.0761	666630
Correlation coefficient	1.00	1.00	1.00			
Y-Intercept	396.2	1528.1	22221.9			
% Y-intercept	3.2	0.46	0.5			
Slope	233481.36	6519789.6	8832176.5			

Robustness

The developed method was verified with the minor alterations in the mobile phase composition and column temperature and flow and buffer P^H.

Lower level of quantification

Six LOQ concentrations were verified by considering as S/N ratio is NLT 10 for the LOQ and S/N ratio 3:1 for the LOD. After nominal concentration of first dilution taken 1 ml from 500 ml volumetric flask into 100 ml volumetric flask a to get the concentration 20 ng/ml for Cefixime.For Erdosteine 30 ng/ml .

RESULTS AND DISCUSSION

Method Development and Optimization

After series of trials, the Methenamine Hippurate and its related Substances in Active Pharmaceutical Ingredient. The analysis was carried out on EMPOWER HPLC model equipped with X-Bridge C18 (250 mm× 4.6 mm, 5µm) column and UV-Detector. Buffer: Solvent mixture (95:5) v/v as Mobile Phase-A and Buffer: Solvent mixture (30:70) v/v as Mobile Phase-B (1.36g Potassium hydrogen phosphate and 1.74 g di potassium hydrogen phosphate into 1000 mL volumetric flask containing 1000 mL water, dissolve and adjust pH 7.0 with potassium hydroxide solution (1g in 10ml water) is Buffer) (Acetonitrile and

methanol in the ratio of 70:30 %v/v is solvent mixture) at a flow rate of 0.6ml/min was employed for elution and the compounds were detected at 210nm at 350C.

Method validation

In the developed method, No interference should be observed at the retention time of all known impurities and Methenamine peaks from blank and each other. Peak purity of Methenamine and known impurity peaks should pass for the individual solutions and spiked solution i.e. Purity threshold should be more than purity angle by photo-diode array detector. The %RSD for the six replicate injections of reference solution was less than 10.0. The % RSD for known impurities, single maximum unknown impurity and total impurities results found less than 10.0 from six preparations of precision solutions. A solution containing 0.005% (w.r.t. test concentration) of impurities and Methenamine has been prepared and injected in duplicate to demonstrate that Methenamine peak is detectable at 0.0045% level. The % RSD values obtained for the peak area of Methenamine Hippurate and its known impurities at LOQ level were in the range of 2.3 to 8.9. The % RSD found not more than 15.0 for each analyte from the LOQ solution. The correlation coefficient is more than 0.99 for impurity-A, impurity-B, impurity-C impurity-D and impurity-E. The % y-intercept to average response of 100% standard below the limit (±5%) for each analyte. Hence the method is linear for related substances in Methenamine hippurate. System met the system suitability criteria. The % recovery for impurity-A, impurity-B, impurity-C, impurity-D and impurity-E are within the limit between 70 and 130% for LOQ level and between 80 and 120% for 50 to 150% level. Hence the method is accurate for the quantification of related substances in Methenamine hippurate. The cumulative percent relative standard deviation for the results of each known impurity and total impurities is less than 10 when compared initial to 24 hrs and 48 hrs results together, Hence solution is stable up to 48hrs.

CONCLUSION

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Buffer and Solvent Mixture with 0.6 ml/min flow rate is quite robust. The optimum wavelength for detection was 210 nm at which better detector response for drug and its impurities was obtained. The average retention time for methenamine was found to be 4.66. The linearity

was observed in the range of 50-150% for Methenamine and its related substances with a correlation coefficient of 1.00 respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 80-120 %. Sample to sample precision and accuracy were evaluated using, three samples of five and three different concentrations respectively, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of three days. These results show the accuracy and reproducibility of the assay. Hence, the chromatographic method developed for estimation of Methenamine Hippurate and its Related Substances is said to be rapid, simple, specific, sensitive, precise, accurate and reliable that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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Conflict of interest

Authors assuring that, there is no conflict of interest.

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