RESEARCH ARTICLE

Analytical Method Development and Validation for the Spectrophotometric Estimation of Hipuuric Acid Prodrug (Methenamine Hippurate)

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ABSTRACT

Hippuric acid, pronounced as n-benzoyl glycine, is utilized in the study of nutrition, small bioactive molecules, amino acid derivatives, peptide synthesis, chemical synthesis, cell biology, and chemical biology. It can be found in urine and is produced when glycine and benzoic acid combine. Treatment of urinary tract infections (UTIs) is challenging due to the development of resistant bacterial strains brought on by repeated use of antibiotics and chemotherapy. Methenamine hippurate as a pharmaceutical dosage form hasn't been the subject of any published research, hence a UV-visible spectrophotometric analytical method development has to be created. Hippuric acid (10 g/mL) solution was made, and when it was scanned at 200–400 nm with distilled water as the green solvent, it was discovered that the maximum absorbance was at 228 nm. The linearity concentration range was found with an R^2 = 0.998 correlation coefficient. The recovery experiment, which was conducted at three levels of 80, 100, and 120%, was used to evaluate the procedure's accuracy. The recovery rate was discovered to be between 90 and 120%. The % RSD < 2 it proves that the method is Precise. The results confirmed the approach's high capacity to detect Hippuric acid and were consistent with industry standards. LoD and LoQ were discovered to be 0.169 and 0.048, respectively. This approach results in hipuuric acid in formulations.

Keywords: Hippuric acid, accuracy, linearity, green solvent, prodrug, validation,

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INTRODUCTION

Hippuric acid is mostly utilized in the synthesis processes for diagnosis and study in the fields of cell biology, chemical biology, and small bioactive molecule and amino acid derivatives. Hippuric acid has facilitated the metabolism and excretion of procyanidins in urine. It is a chemical compound that is derived from the carboxylic acid n-benzoyl glycine. It is found in urine and is produced when glycine and benzoic acid react.[1] German scientist Justus Freiher von Liebig initially discovered this in 1829. He first established that it was separate from benzoic acid before going on to pinpoint its components in 1832. Later, the chemical was found in urine[2]. When phenolic compounds, such as those present in fruit juice, wine, and black tea, are taken, hippuric acid is more common in urine. A rhombic prism is created by heating hippuric acid because it is soluble in hot water and a precursor to phenols, hippuric acid (benzoic acid).

Hippuric acid is used to determine the liver's health and function when pyridine, benzene sulphonyl chloride, and distilled water produce a vivid red colour.[3, 4] Therefore, hippuric acid is frequently employed as a marker to identify glue sniffers. Hippuric acid, which the body makes, is used to assess the performance of the liver. Glycine and benzoic acid combine to form hippuric acid and then eliminated from urine (Figure 1).[5-7]

Toluene can physically enter the body through the skin, lungs, or bloodstream, and hippuric acid is a toluene metabolite. A mixed oxidizing enzyme system converts more than 80% of the ingested toluene into benzoic acid and hippuric acid, among other metabolites. For a very long time, hippuric acid was utilized as a biological sign of methylbenzene exposure. It can be found in urine and is produced when glycine and benzoic acid combine. Hippuric acid production by the body is a measure of liver health (Figure 2).[8,9]

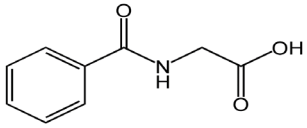

Figure 1: Chemical structure of hippuric acid

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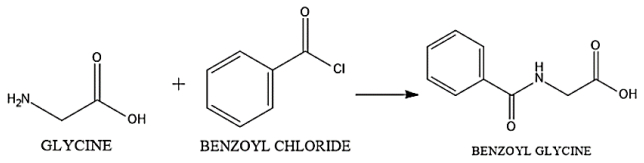

Figure 2: Chemical reaction of hipuuric acid

Reaction

Antibacterial Effect (UTIs Infection)

UTIs are complicated to treat with antibiotics and chemotherapy because they produce resistant bacterial strains when used for lengthy periods of time. Hippuric acid helps to bring urine pH down to a desirable level of 5.5 or lower. The pH of the urine can be lowered by administering ascorbic acid in amounts of 2–4 g per day.[10] Methenamine has been licensed by the USFDA for the prophylaxis of infected UTIs in individuals 6 years of age and older since studies have expressed that it is an efficient antimicrobial-sparing choice in this patient population. Hippuric acid helps to bring urine pH down to a desirable level of 5.5 or lower.

The pH of the urine can be lowered by administering ascorbic acid in amounts of 2–4 g/day. Methenamine and its salts as topical antibacterial agents were proven. The methylenedioxy methamphetamine hippurate, methylenedioxy methamphetamine, and methylenedioxy methamphetamine Mandelate lowest inhibitory dose study in vitro against Staphylococcus aureus, Escherichia coli, and pseudomonas aeruginosa.[11,12]

Parkinson's Disease

Hippuric acid can be used to categorize or identify people who have different neuropsychiatric conditions, including Parkinsonism, epilepsy, etc. Hippuric acid production has been shown to be deficient or impaired in people with the disorders mentioned above.[13]

IR Spectra Characterisation

IR, NMR, and mass spectroscopy were used to determine the structure of compound. Compounds had a significant C=O stretching band at around 1728 cm^{-1} and a strong –NH stretching band at O-H stretch of the hydroxyl group around 3342 cm^{-1}, according to IR spectral data. IR studies revealed a strong C=O stretching band of about 1745 cm^{-1}, -C-H stretch at 2937 cm^{-1} (Figure 3).

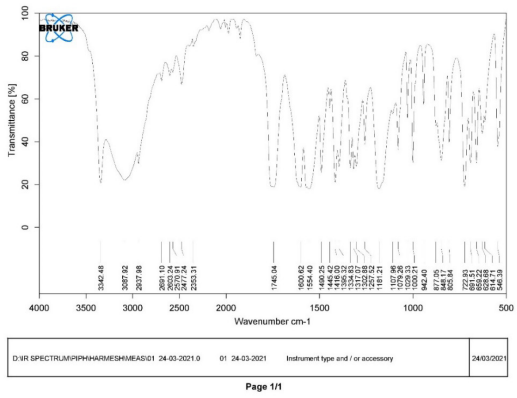

Figure 3: FTIR spectra characterisation of hipuuric acid

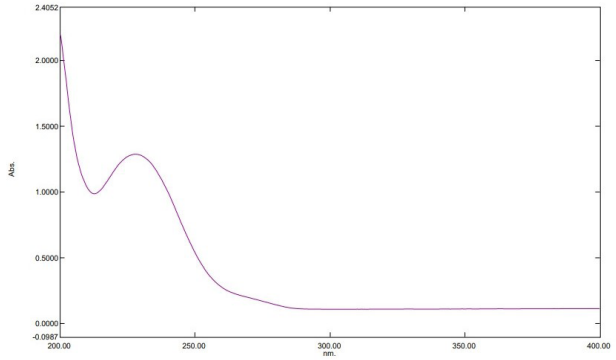

Figure 4: Absorption maxima of standard hippuric acid

MATERIALS AND METHODS

Materials

The Krishna Chemical Industry in Vadodara, Gujarat, provided the Hippuric standard powder.

Instrument

Spectra were measured using a UV visible 1601 Shimadzu twin-beam Spectrophotometer. Two 1-cm long quartz cells that are similar. Digital electronic balance (Mettler Toledo ME204 model).

Selection of Solvent

In addition to being sparingly soluble in $CHCl_3$, Hippuric acid was easily soluble in distilled water, Methanol, and Ethanol. So, for the development and validation of the procedure, distilled Water was selected for solution Preparation.

Working Standard Stock Solution Preparation

Weigh accurately 100 mg of Synthesize Hippuric acid powder added in a 100 mL volume flask, and add distilled water (1000 µg/mL) (Figure 4). Pipette out 1-mL of the aforementioned stock solution into a flask with a 10 mL capacity. Add 100 µg/mL of Distilled water to fill the remaining space. Pipette out 1-mL of the solution into a flask with a 10 mL capacity, then top out the flask with water (10 µg/mL). In a UV-visible spectrophotometer, this solution's absorbance was measured at 228 nm while being scanned between 200 and 400 nm.

Selection of Wavelength

Pipette 100 µg/mL 1 mL of the solution into a flask with a 10 mL capacity, then top out the flask with water (10 g/mL). A UV-visible spectrophotometer was used to scan this solution between 200–400 nm, and with distilled water as a reference, the absorbance brought at 228 nm was calculated.

Dilution

Pipette out 1-mL of the solution into a flask with a 10 mL capacity, volume make up to the mark with distilled water (10 µg/mL). A UV-visible spectrophotometer was used to scan

this solution between 200 and 400 nm, and using pure water as a reference, the absorbance at 228 nm was calculated.

Method Validation

The suggested method was approved by the ICH. The primary aspects that were looked at included linearity, accuracy, precision, specificity, robustness, LoD, and LoQ.

Linearity

Standard calibration curve linearity is evaluated using five concentrations and calibration curves with a range of 2 to 6 µg/mL (Table 2). To create calibration curves, concentration was plotted against absorbance (n = 3). Analysed with linear regression with least squares. A linear regression line with a high correlation coefficient (R2>0.9988) might be created. The regression equation for active is shown in Figures 5 and 6.

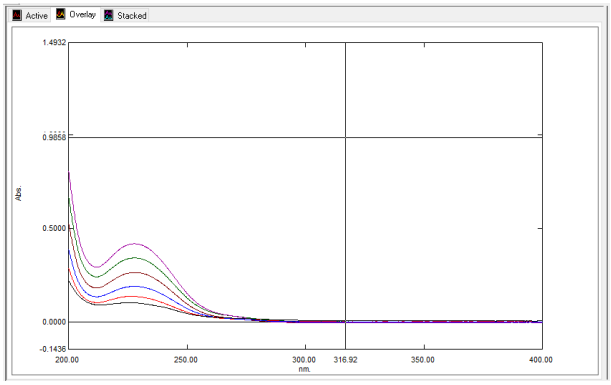

Figure 5: Overlay spectra of standard hippuric acid

Table 1: Linearity and range for standard hippuric acid data at 228 nm

Sr. No	*Calibration curve standard*	*Concentrations (µg/mL)*	*Absorbance*
1	STD1	1	0.0906
2	STD2	2	0.1526
3	STD3	3	0.2122
4	STD4	4	0.2749
5	STD5	5	0.3446
6	STD6	6	0.4173

Accuracy

This study was performed by adding the solution in 3 different concentrations with 9 determinations (2, 4, and 6 µg/mL) to the initial 100 µg/mL solution. The linearity plots were used to calculate the additional medication's % recovery. The results are presented in tables and graphs. The percentage of recovery of known amounts of Hippuric acid was used to measure the accuracy of the method. Spike concentrations of 80%, 100%, and 120%, it is carried out. Each sample was taken three times, with results ranging from 98.61 to 100.61. The procedure offers a high level of accuracy, according to data that was compiled in high recovery. The observed data are all within the permissible recovery thresholds of 90 to 120%.

Precision

It also quantifies repeatability or interday and Intraday precision. It was determined using RSD (relative standard deviation). The percentage RSD results for the triplicates of each concentration were used to calculate the intraday and Interday precision. To determine the percent relative S deviation, the mean value (n = 3) absorbance of each solution was compared to that of the second run on the same day (intraday) and to that of the following day (Interday). There was no noticeable difference between intraday and Interday precision (Table 3 and 4).

LoD, LoQ QUANTIFICATIONS

Different methods are available for LoD and LoQ estimation, and Here calibration standard curve method was used to

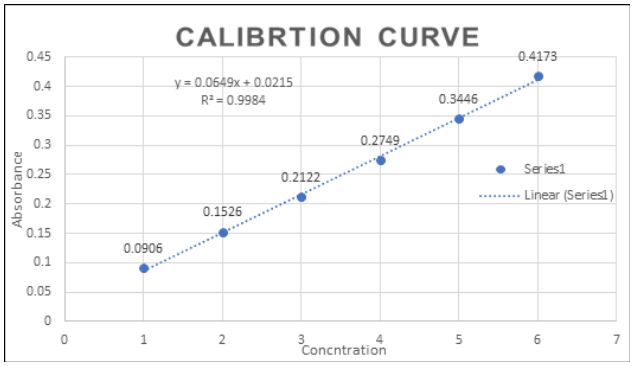

Figure 6: Calibration curve of standard Hippuric acid

Table 2: Standard hippuric acid accuracy study at 228 nm

Level	*Sample conc. µg/mL*	*Drug added (µg/mL)*	*Absorbance*	*Total concn. (µg/mL)*	*Drug recovered*	*Recovery%*	*Mean Std.*	*%RSD*
	3	1.2	0.512					
80%	3	1.2	0.513	4.2	4.26	98.7017	1.074971	1.72
	3	1.2	0.508					
	3	2	0.520					
100%	3	2	0.521	5.0	4.3502	98.8701	1.948638	1.97
	3	2	0.519					
	3	2.6	0.537					
120%	3	2.6	0.532	5.6	4.4	100.2	2.019571	2.00
	3	2.6	0.535					

Table 3: Standard hippuric acid interday precision study at 228 nm

Conc. (µg/mL)	*Abs*	*Mean*	*SD*	*%RSD*
	0.121			
3	0.123	0.123	0.002	1.60
	0.125			
	0.388			
4	0.376	0.381	0.005	1.46
	0.380			
	0.428			
5	0.433	0.434	0.006	1.51
	0.411			

Table 4: Standard hippuric acid intraday precision study at 228 nm

Conc. (µg/mL)	Abs	Mean	SD	%RSD
	0.120			
3	0.119	0.119	0.001	0.840
	0.118			
	0.377			
4	0.384	0.381	0.003	0.999
	0.383			
	0.439			
5	0.429	0.435	0.005	1.32
	0.439			

Table 5: LoD and LoQ quantify

Drug	*LoD (µg/mL)*	*LoQ (µg/mL)*
Hippuric acid (228 nm)	0.048	0.146

determine the LoD and LoQ independently. The LoD and LoQ were calculated using the standard deviation of regression line y-intercepts. Using the response's average slope and standard deviation, and calculated (Intercept).

The LoD is the lowest concentration of analyte in the sample that can be detected but not always quantitated. The lowest amount of analyte in the sample that can be quantitatively measured with the appropriate standard is known as the quantitation limit. LoD and LoQ value quantifies by using this equation (Table 5).

$$LoD = 3.3\sigma/s$$

$$LoQ = 10\sigma/s$$

Where S is the slope of the calibration curve, σ response's standard deviation (Y-intercept).

Robustness

This parameter gives strong and accurate results under a variety of circumstances. The most important parameters were altered, while the others were left unchanged to test the method's durability. Chromatographic parameter changes like that were wavelength shift. The robustness study findings, which are shown in Table 6, showed that the outcomes of the clomiphene citrate determination given in Table 6 were not significantly affected by the slight change in the conditions.

Table 6: Robustness (Wavelength shift) for 5 µg/mL at 227 and 226 nm

Conc.	Abs at 227	Abs at 226	Mean	SD	%RSD
5 µg/mL	0.5523	0.5524	0.5523	0.000141	0.0255
5 µg/mL	0.5527	0.5520	0.5523	0.000495	0.089
5 µg/mL	0.5521	0.5519	0.5528	0.000141	0.025
5 µg/mL	0.5531	0.5534	0.5532	0.000212	0.0383
5 µg/mL	0.5544	0.5548	0.5546	0.000422	0.0744
5 µg/mL	0.5541	0.5547	0.5544	0.000424	0.0764

RESULTS AND DISCUSSION

The method was verified in accordance with ICH standards for various validation parameters performed for Hippuric acid in a concentration range of 2–6 µg/mL. The calibration curve for the approaches was linear. The computed coefficients (r2) were 0.9988 and 0.9988, respectively. The repeatability of the procedures and intermediate day RSD values, both less than 2%, were determined to be evidence of precision. The percentage recovery ranges were discovered to be 90–120%. All measured values fall within the permitted range, indicating a high recovery rate. Therefore, robustness suggested that the decision made by the CC was not significantly affected by the situation's small change. The results showed that the LoD and LoQ were, respectively, 0.048 and 0.146 µg/mL.

CONCLUSION

A simple, precise, green analytical method was developed for the estimation of hipuuric acid prodrug treatment of urinary tract infections is challenging due to the development of resistant bacterial strains brought on by repeated use of antibiotics and chemotherapy. Methenamine hippurate as a pharmaceutical dosage form hasn't been the subject of any published research, hence a UV-visible spectrophotometric analytical method development has to be created. The method has been validated using ICH guideline. The validation method was found to be linear, precise, accurate, specific, and robust. This method is utilized in Pharmaceutical Industry in routine analysis for quality control purposes. Determining hippuric acid using a quick, precise, and affordable UV spectroscopic technique is now possible. The presented method works well for drug estimation in any formulation.

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