Research

Development and Validation of a Novel RP-HPLC Method for Quantification of Metformin Hydrochloride in Floating Tablet Dosage Form

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Abstract:

A simple, accurate and economical method for the estimation of metformin hydrochloride from tablets dosage form and formulated floating tablets. The developed HPLC method is a reverse phase HPLC chromatographic method using C18 column and acetonitrile : phosphate buffer ph adjusted to 6.8-7.4. The linearity was observed in concentration range of 5-40 µg/ml were evaluated. Linear absorbance versus concentration gives regression equation; Y=0.0217x-0.002, with a correlation coefficient (r2) of more than 0.99 in 0.1N HCl. The LOD value in 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions was found to be 0.130, 0.069, 0.02 and 0.07 respectively. The LOQ value in 0.1N HCl, pH 4.6 acetate buffer, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions was found to be 0.140, 0.880, 0.060 and 0.230 respectively. There was absence of any significant change in nature of drug or any interaction between drug and polymers. Stability studies indicated absence of degradation on storage.

KEYWORDS: HPLC, metformin hydrochloride, linearity, phosphate buffer, calibration

INTRODUCTION:

Metformin hydrochloride chemically, N,N-dimethylimidodicarbonimidic diamide hydrochloride[1] is an antidiabetic agent[2]. Metformin, on the other hand can directly and indirectly improve skeletal muscle sensitivity towards insulin. Metformin is an effective biguanide antidiabetic agent that has been used to control blood glucose level of type II diabetic patients for decades and has been considered the first line treatment according to international guidelines.[3]

MATERIAL AND METHODS:

Materials

Metformin HCl was used as the active ingredient. HPMC, was used as polymer. Sodium carbonate was used as an effervescent agent. A Millipore Milli-Q water ultra-pure water system (Millipore, Australia) was used to obtain distilled water. The other ingredients used were stearic acid, magnesium stearate, talc, and k-caragenan. All reagent s were used of analytical grade.

Methodology

UV Spectrophotometric analysis

Preparation of stock solution

- 0.1N HCl solution: 8.3 ml of 37.5% concentrated HCl solution was dissolved in 1000 ml of distilled water.[4]
- pH 6.8 phosphate buffer solution: 50ml of 0.2M Potassium dihydrogen phosphate and 22.4ml of 0.2MNaOH were Prepared and mixed together, volume was made upto 200ml with distilled water (Indian Pharmacopoeia).[5]
- pH 7.4 phosphate buffer solution: 50 ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2M NaOH were prepared and mixed together, volume was made up to 200 ml with distilled water (Indian Pharmacopoeia)..
- Determination of absorption maxima (λmax): A 10 µg/ml solution of mesalamine was prepared in 0.1N HCl buffer solution and it was scanned in UV spectrum. Similar procedure was applied for water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solution[6]

Method Development

Preparation of Calibration Curve:

1) Calibration curve in 0.1N HCl solution - 8.3 ml of 37.5% concentrated HCl solution was dissolved in 1000ml of distilled water.100mg of drug was weighed and dissolved in 100 ml of solution to get the final concentration upto 1000 µg/ml. From this solution the dilutions were prepared in the range of1-14µg/ml in buffer solution.[7]

Absorbance of solution was measured at observed maxima using UV-Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and average absorbance was calculated. The regression line equation was then generated.

2. Calibration curve in Water- 100 mg of drug was weighed and dissolved in 100 ml of water to get the final concentration upto 1000 µg/ml. From this solution the dilutions were prepared in the range of 1-14 µg/ml in buffer solution. Absorbance of solution was measured at observed maxima using UV-Visible spectrophotometer by putting reference standard of medium.[8] The experiment was performed in triplicate and average absorbance was calculated. The regression line equation was then generated.

3) Calibration curve in pH 6.8 phosphate buffer solution – 28.80 gm of disodium hydrogen phosphate and 11.45 gm of potassium dihydrogen phosphate were dissolved in distilled water and volume was made up to 1000 ml with distilled water. 100 mg of drug was weighed and dissolved in 100 ml of buffer solution to get the final concentration upto 1000µg/ml. From this solution the dilutions were prepared in the range of 1-14 µg/ml in buffer solution.[9] Absorbance of solution was measured at observed maxima using UV-Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and average absorbance was calculated. The regression line equation was then generated.

4) Calibration curve in 7.4 phosphate buffer solution - 50 ml of 0.2M potassium dihydrogen phosphate and 39.1ml of 0.2 M NaOH were prepared and mixed together, volume was made up to 200 ml with distilled water. 100 mg of drug was weighed and dissolved in 100 ml of buffer solution to get the final concentration upto 1000 µg/ml. From this solution the dilutions were prepared in the range of 1-14 µg/ml in buffer solution.[10] Absorbance of solution was measured at observed maxima using UV Visible spectrophotometer by putting reference standard of medium. The experiment was performed in triplicate and average absorbance was calculated. The regression line equation was then generated**.**

METHOD VALIDATION

Linearity: For linearity study of developed method, solutions at different concentration (5-50µg/ml) were prepared from the stock solution. The calibration graph was plotted against the concentration and absorbance and the data was treated by linear regression analysis.[11]

Limit of detection (LOD): It is the lowest concentration of the analyte that can be detected. It was calculated by preparing the solution of different concentration from 1-5 µg/ml. LOD was calculated by using the formula:[12]

$$LOD = (3.3*\text{Std. Deviation})/\text{Slope}$$

Limit of quantification (LOQ): It is the minimum quantifiable concentration. It was calculated by preparing the solution of different concentrations ranging from 10-50µg/ml. LOQ was calculated by using the form[13]

LOQ=(10*Std. Deviation)/Slope

Molar Absorptivity:

Molar absorptivity was determined by analysing the sample in UV visible spectrophotometer to determine the amount of light absorbed by the analyte

Ruggedness & Robustness: Ruggedness was determine by analyzing the drug using different analyte at similar condition and absorbance were noted. Robustness was determine by estimating the drug at different level.[14]

RESULT

Method Development
Preparation of Calibration Curve:

Calibration Curve in 0.1 N HCl Solution - The calibration curve of metformin was prepared in 0.1N HCl solution at 211 nm using UV spectrophotometer. Beer's range was found between 0-14 µg/ml with R2 0.990.[15]

Calibration curve in water- The calibration curve of metformin was prepared in Water at 232 nm using UV spectrophotometer. Beer's range was found between 0-14 µg/ml and R2 was 0996.

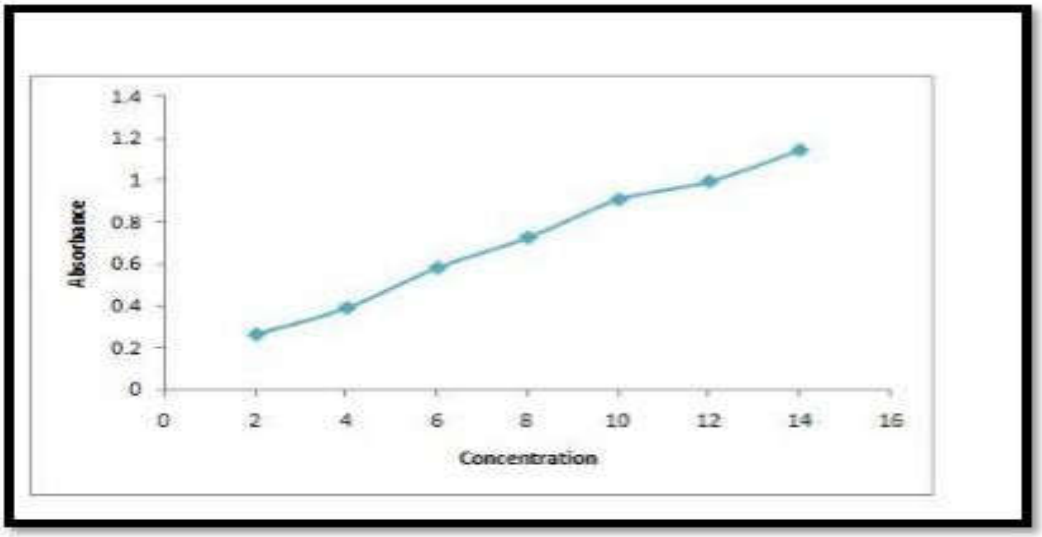
Calibration curve in pH 6.8 phosphate buffer solution –The calibration curve of metformin was prepared in pH 6.8 phosphate buffer solution at 232 nm using UV spectrophotometer. Beer's range was found between 0-14 µg/ml and R2 was 0.998.

Calibration curve in pH 7.4 phosphate buffer solution –The calibration curve of metformin was prepared in pH 7.4 phosphate buffer solution at 233nm using UV spectrophotometer. Beer's range was found between 0-14µg/ml and R2 was 0.990. [16]

S.No	Concentration (µg/ml)	Absorbance in 0.1 N HCl	Absorbance in Water	Absorbance in pH 6.8 phosphate buffer	Absorbance in pH 7.4 phosphate buffer
1.	0	0	0	0	0
2.	1	0 .137	0.099	0.094	0.052
3.	2	0.265	0.160	0.200	0.143
4.	4	0.391	0.315	0.358	0.302
5.	6	0.583	0.476	0.541	0.435
6.	8	0.726	0.590	0.710	0.592
7.	10	0.908	0.738	0.910	0.780
8.	12	0.994	0.903	1.102	0.974
9.	14	1.143	0.983	1.323	1.242

Table 1: Absorbance of drug in various dissolution media

Figure1:Calibration curve of drug in 0.1N HCl solution



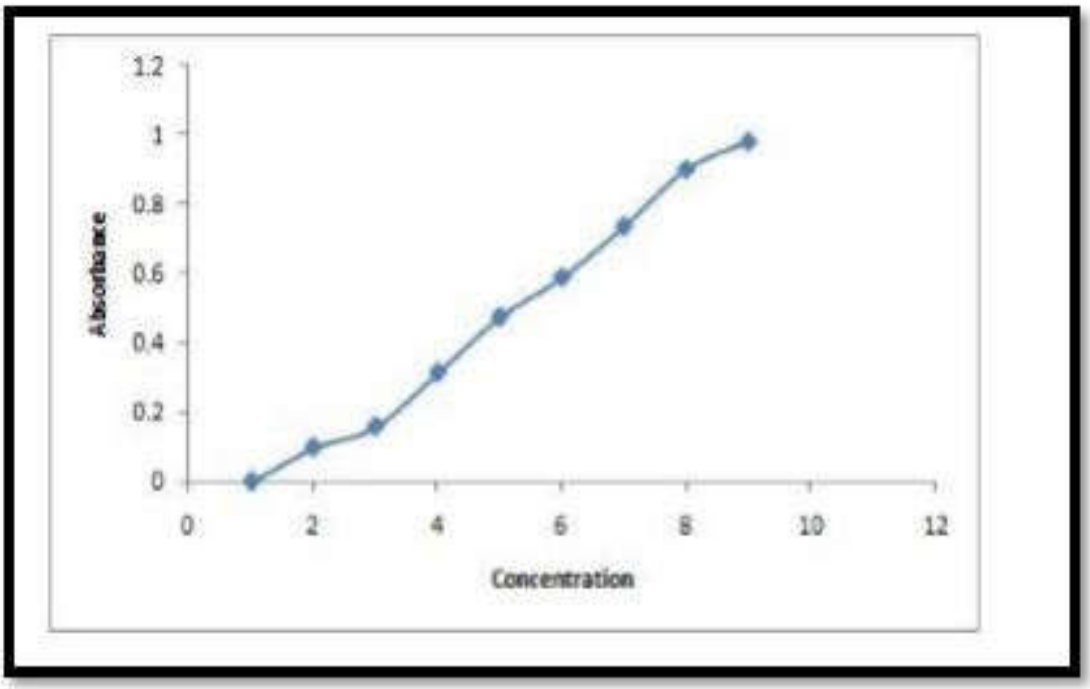


Figure 2: Calibration curve of drug in Water solution

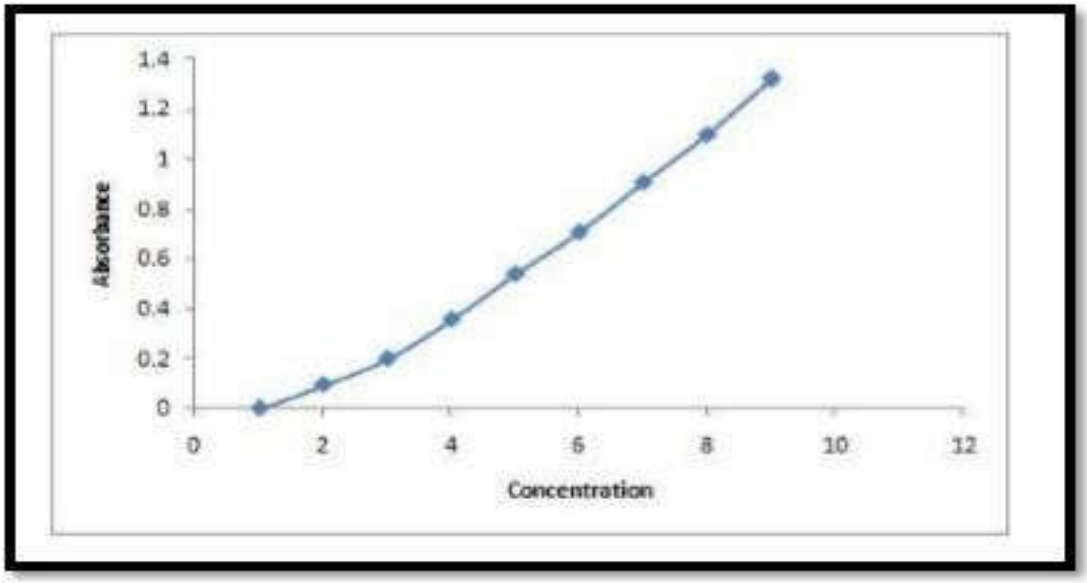


Figure 3: Calibration curve of drug in pH 6.8 phosphate buffer solution

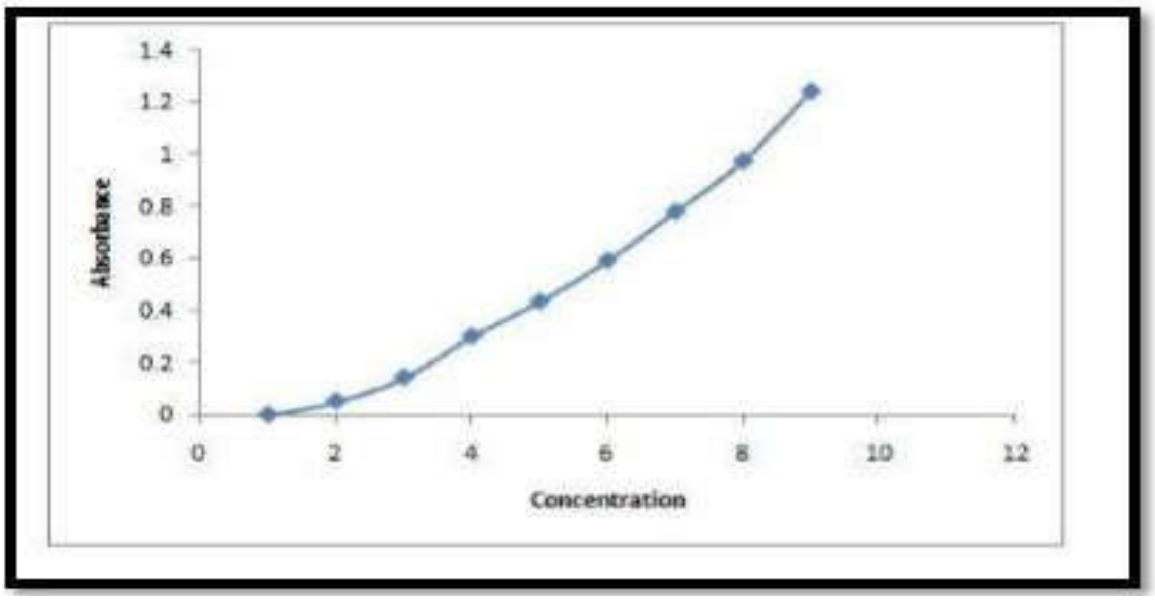


Fig4: Calibration curve of drug in pH7.4 phosphate buffer solution

Method Validation

The method validation was performed with respect to the linearity and range, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ), molar absorptivity, sandell's sensitivity, ruggedness and robustness. For linearity and range the samples of 1-10µg/ml concentrations were scanned for the absorbance value at λmax value 211 nm, 232 nm, 232 nm and 333 nm for 0.1N HCl, pH 6.8 phosphate buffer, Water, pH 7.4 phosphate buffer solutions respectively.[17] These solutions obeyed the Beer-Lambert's law in 0-14µg/ml concentrations with regression coefficient of 0.990, 0.990, 0.998 and 0.996 in 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions respectively.

For LOD, the concentrations from 1-10 µg/ml were scanned for the absorbance value at λmax value 211 nm, 232 nm, 232 nm and 232 nm for 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions respectively. These concentrations proved to be the lowest concentration of drug which can be detected. The LOD value in 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions was found to be 0.130, 0.069, 0.02and 0.07 respectively.[18]

For LOQ, the concentrations from 0-10 µg/ml were scanned for the absorbance value at λmax value211 nm, 232 nm, 232 nm and 233 nm for 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions respectively. These concentrations proved to be the minimum quantifiable concentration. The LOQ value in 0.1N HCl, pH 4.6 acetate buffer, pH 6.8 phosphate buffer and pH 7.4

phosphate buffer solutions was found to be 0.140, 0.880, 0.060 and 0.230 respectively.

Accuracy and precision were analyzed by evaluating 3 concentrations of drug (i.e. 1,6 and 10 µg) in triplicates on same day (Intra-day precision and accuracy) and (Inter- day precision and accuracy). The values observed for accuracy are mentioned in Table 2.

Interday and intraday variation obtained from the drug sample is mentioned in the table 2. Sandell's sensitivity of metformin was found 0.00012, 0.000062, 0.00009 and 0.000052 in 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions respectively.[19]

Table 2: Various method validation parameters of drug in different buffer solutions

S.No	Parameter	0.1N HCl	Water	pH 6.8 phosphate buffer	pH 7.4 phosphate buffer
1.	λ_{max} (nm)	211	232	232	233
2.	Range (µg/ml)	0-14	0-14	0-14	0-14
3.	Slope(m)	0.079	0.071	0.092	0.085
4.	Intercept (c)	0.066	0.021	0.004	0.040
5.	R^2 Value	0.990	0.996	0.998	0.990
6.	LOD(µg/ml)	0.130	0.069	0.02	0.07
7.	LOQ(µg/ml)	0.140	0.880	0.060	0.230
8.	Molar Absorptivity (L.mol-1 cm-1)	20040	10268	15071	8612
9.	Sandell's Sensitivity (µg.cm2/ 0.001 abs	0.00012	0.000062	0.000052	0.00009
10.	Interday Variation(%)	58	11.56	4.7	20
11.	Intraday variation(%)	23.9	10.42	4.5	18.4

Particle Size Analysis of Metformin HCl:

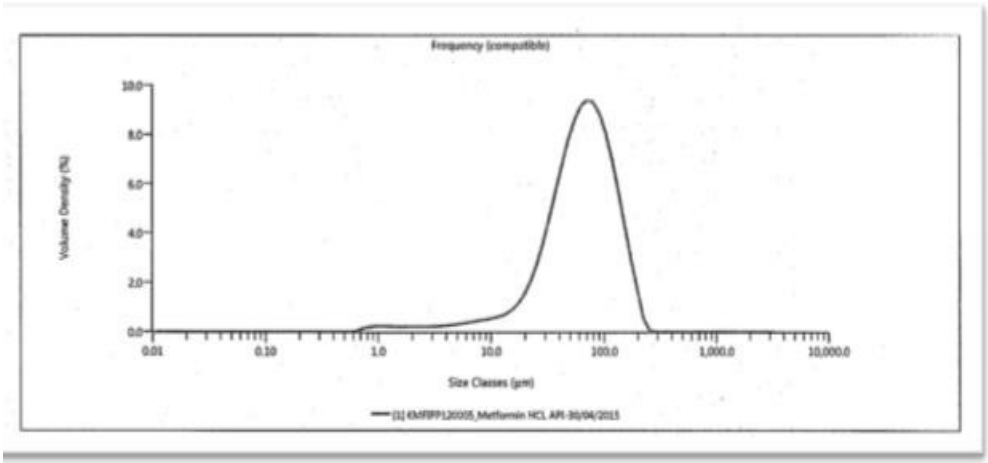


Fig 5: Particle size distribution analysis

The flow properties of powders are dependent upon the particle size distribution as well as particle

shape. Asymmetric particles have poor flow characteristics and hence granulation techniques are used to convert blends of drug and other additives into particles of uniform size having good flow properties.

Preparation of Standard curve of Metformin HCl

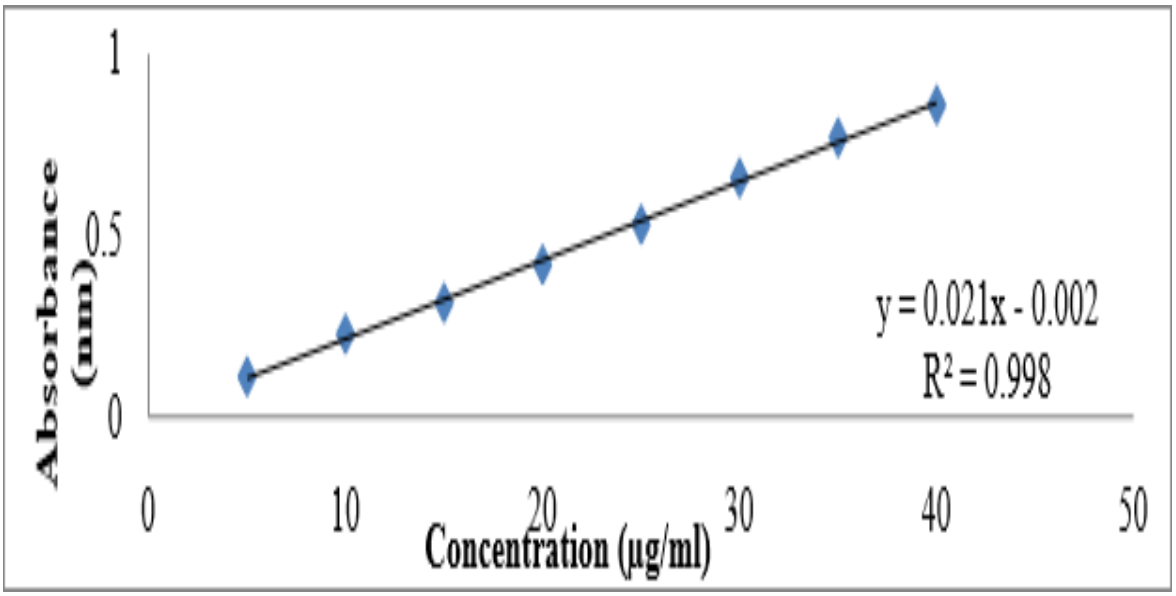


Figure 6: Preparation of Standard Curve of Metformin HCl

Linearity plot of Metformin HCl in the concentration range of 5-40 µg/ml were evaluated. Linear absorbance versus concentration gives regression equation; Y=0.0217x-0.002, with a correlation coefficient (r2) of more than 0.99 in 0.1N HCl.

CONCLUSION:

The linearity was observed in concentration range of 5-50µg/ml is 0.14. The LOD value in 0.1N HCl, Water, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions was found to be 0.130, 0.069, 0.02 and 0.07 respectively. The LOQ value in 0.1N HCl, pH 4.6 acetate buffer, pH 6.8 phosphate buffer and pH 7.4 phosphate buffer solutions was found to be 0.140, 0.880, 0.060 and 0.230 respectively. The present developed and validated method is accurate, precise, economical and simple for the determination of metformin hydrochloride floating tablets. It can be used for the routine analysis of finding the robustness, linearity etc.

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