# Simultaneous Estimation of Metformin and Gliclazide in Tablets using Reverse Phase High Performance Liquid Chromatography

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**A simple, efficient and reproducible method for the simultaneous determination of metformin and gliclazide from tablets has been developed using reversed phase high performance liquid chromatography. The separation was done using a mobile phase consisting of 0.025 M disodium hydrogen phosphate and acetonitrile (25:75%v/v) with pH adjusted to 3.2 with dilute ortho phosphoric acid. Column used was Shimpack CLC $C_8$ (250x4 mm i.d.) 5 $\mu$ with flow rate of 1 ml/min with detection at 240 nm. The external standard calibration method was employed for quantitation. An elution order was metformin (2.8 min) and gliclazide (4.3 min). The linear dynamic range was 5-500 $\mu$g/ml and 10-100 $\mu$g/ml for metformin and gliclazide, respectively. Analytical parameters were calculated and a full statistical evaluation included.**

Metformin[1] is an antidiabetic, which chemically is 1,1-dimethyl biguanide. It is official in IP, BP and USP. Gliclazide[2] is also an antidiabetic but is a benzene sulfonamide derivative. Tablets containing 500 mg of metformin and 80 mg of gliclazide is available in the market as a combined dosage form. The reported analytical methods for the estimation of these two drugs employ either HPLC or spectroscopy[3-8]. The present method aims at developing an isocratic RP-HPLC method for the simultaneous determination of both drugs from tablets.

The apparatus used was a Shimadzu HPLC SPD10-A chromatograph equipped with fixed wavelength UV detector and model 7725i Rheodyne injector with 20 $\mu$l external loop. Column used was Shimpack CLC $C_8$ (250x4 mm i.d.) 5 $\mu$, operating at room temperature. The elution was carried out isocratically at the flow rate of 1 ml/min using disodium hydrogen phosphate (0.025 M) at a pH 3.2 and acetonitrile in 25:75 ratio as mobile phase. The detector was set at 240 nm. Response of peak areas recorded and integrated using software.

Metformin and gliclazide were obtained from Bal Pharmaceuticals, Bangalore with certificate of analysis. HPLC grade acetonitrile and AR grade disodium hydrogen phosphate were obtained from S. D. Fine Chemicals Ltd, Mumbai.

Standard stock solutions of the drugs were prepared by dissolving 600 mg of metformin and 100 mg of gliclazide in 100 ml of mobile phase consisting of disodium hydrogen phosphate (0.025 M at pH 3.2) and acetonitrile in the ratio of 25:75 %v/v. The buffer was prepared by dissolving 3.53 g of disodium hydrogen phosphate in distilled water and diluting to 1000 ml in a volumetric flask. The mobile phase was filtered through 0.45 $\mu$ membrane filter paper and degassed before use.

For linearity studies, five different concentrations in the range of 60-300 $\mu$g/ml of metformin and 10-50 $\mu$g/ml of gliclazide were prepared using mobile phase. The chromatograms of these standard solutions were obtained by injecting 20 $\mu$l of each standard solution and standard curve were obtained by plotting the drug concentrations ($\mu$g/ml) Vs peak areas. The linear regression equation of metformin and gliclazide was, Y=0.0437xconcentration+ 0.0400 and Y= 0.2330xconcentration+(-0.1300), respectively. The correlation coefficient values were found to be 0.9998 and 0.9994 for metformin and gliclazide, respectively.

The external calibration method was employed for

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TABLE 1: SYSTEM SUITABILITY PARAMETERS.

| Parameters | Metformin | Gliclazide |
|---|---|---|
| Resolution | 5 | - |
| Capacity factor | 1.01 | 2.07 |
| Asymmetry factor | 0.99 | 1.00 |
| No. of theoretical plates | 12,268 | 12,956 |
| LOD (ng/ml) | 2 | 6 |
| LOQ (ng/ml) | 10 | 30 |

The results obtained by this method are precise and reproducible for the two drugs, metformin and gliclazide. Reproducibility of the method was done on six samples of metformin and gliclazide and the % RSD was found to be 0.98 and 0.32, respectively. The robustness of the method was confirmed by varying the concentration of the organic phase and buffer in the mobile phase and flow rate. The method was found to be robust in the conditions specified. The system suitability parameters were calculated to confirm the specificity of the developed method and shown in Table 1. The high percentage recovery and low standard deviation data (Table 2) were satisfactory and confirms the accuracy, precision and reliability of the method. Further this method eliminates complicated extraction of individual drugs for quantitation. Both drugs estimated with in 5 min. hence the present method is cost effective and faster, can be used for the routine analysis of these drugs from tablets.

TABLE 2: ESTIMATION OF METFORMIN AND GLICLAZIDE IN FORMULATION.

| Drug | Amount of drug (mg/tablet) | | % label claim | % Recovery |
|---|---|---|---|---|
| | Labeled | Found* | | |
| Metformin | 500 | 499±0.05 | 99.8±0.23 | 99.0±0.08 |
| Gliclazide | 80 | 81.2±0.01 | 102±0.28 | 101± 0.25 |

*Mean±SD of 6 observations

quantitation. The response factor (RF) or sensitivity factor was calculated for both the drugs and used for analyzing formulation. The RF values of metformin and gliclazide were found to be 3738.8 and 2369.2 based on peak area calculation. Based on peak height calculation the RF values were 442.4 and 230.7 for metformin and gliclazide, respectively.

Not less than twenty tablets were weighed and powdered. A quantity of powder equivalent to 10 mg of gliclazide was extracted with 50 ml of methanol. The solution was filtered and 1 ml of the aliquot was diluted to 10 ml with mobile phase.

The formulation solution (20 $\mu$l) was injected in to chromatograph under the condition specified. The peak area obtained was related to slope and intercept values from the calibration data to calculate concentration of both drugs. The mean metformin and gliclazide content obtained from five series of five experiments was 499.1 mg against 500 mg labeled amount of metformin and mean gliclazide content was 81.2 mg against 80 mg labeled amount. The elution order of the drugs is metformin (2.8 min) and gliclazide (4.3 min). Chromatographic figures of merit for the chromatogram of these drugs were calculated. Commonly used excipients like starch, microcrystalline cellulose, talc, lactose and HPMC did not interfere. Analytical recovery studies were carried out from a series of spiked concentrations added to the preanalysed dosage forms.


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