

## Development and Validation of High Performance LCMS Methods for Estimation of Silodosin and Silodosin $\beta$ -D-Glucuronide in Human Plasma

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### Abstract

The aim of present study is to development and validation of analytical methods for estimation of Silodosin and Silodosin  $\beta$ -D-Glucuronide in human plasma in API, method in LCMS. Silodosin is a prescription medication that acts as an alpha-adrenergic blockers used in the treatment for men with benign prostatic hyperplasia, presently commercially available and marketed as Rapaflo in USA and Rapilif, Silodal in other countries worldwide. Drug showed linearity in the concentration range of 0.502 ng/ml to 207.376 ng/ml for Silodosin and 4.121 ng/ml to 302.836 ng/ml for Silodosin  $\beta$ -D-Glucuronide with correlation coefficient consistently greater than 0.99 for Silodosin and Silodosin  $\beta$ -D-Glucuronide. Mass parameters, 496.2/261.0 and 672.2/479.3/261.2 were chosen for analysis by Solid phase extraction method. Different validation parameters to be considered are Accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantitation Limit, and Linearity Range. The results were found to be acceptable as per the guidelines of International Conference on Harmonization (ICH). Both the methods are found to be novel, rapid, linear, precise, accurate, robust and rugged and can be successfully applied for the routine analysis of Silodosin and Silodosin  $\beta$ -D-Glucuronide. The methods are also found to be useful and economical.

**Keywords:** Silodosin; Silodosin  $\beta$ -D-Glucuronide; LCMS; Validation; Solid phase extraction; ICH

### Introduction

This research paper relates to development and validation of LCMS methods for estimation of Silodosin and Silodosin  $\beta$ -D-Glucuronide in API. The IUPAC name is 1-(3-hydroxypropyl)-5-[(2R)-2-[2-[2-(2,2,2-trifluoroethoxy) phenoxy] ethylamino] propyl]-2,3-dihydroindole-7-carboxamide. It is a prescription medication that acts as an  $\alpha$ 1-adrenoceptor antagonist with high uroselectivity used in the treatment of benign prostatic hyperplasia. Presently commercially available and marketed as Rapaflo in USA and Rapilif, Silodal in other countries worldwide. Silodosin is generic and available globally under many brands. Its molecular formula is  $C_{25}H_{32}F_3N_3O_4$  with average molecular weight of 495.534 g/mol and available in white powder form. Solubility in organic solvents such as ethanol, DMSO, and dimethyl formamide, which should be purged with an inert gas. The solubility of silodosin in these solvents is approximately 25 mg/ml, with melting point 105-109°C [1-3]. Chemical structure of Silodosin is given in Figure 1.

Silodosin is used for the symptomatic treatment of benign prostatic hyperplasia. It acts as an  $\alpha$ 1-adrenoceptor antagonist with high uroselectivity. Silodosin is in a class of medications called  $\alpha$ 1A adrenoceptor antagonists. It acts by binding of norepinephrine and epinephrine induces phospholipase C activation, leading to generation of second messengers, including inositol triphosphate and diacylglycerol. Finally, these induce an increase in intracellular calcium levels and smooth muscle contraction. Consequently, blockage of  $\alpha$ 1A-AR induces prostatic and urethral smooth muscle relaxation, and may improve voiding symptoms [2]. However, silodosin also seems to target afferent nerves in the bladder, and thereby acts on bladder over activity and storage symptoms [1,2,4].

Literature survey reveals that determination of Silodosin and Silodosin  $\beta$ -D-Glucuronide in human plasma by LCMS/MS methods unavailable [6-9]. However, as to our best knowledge, none of the methods LCMS has been validated in all the parameters for determination of this drug with a simultaneous method of determining

drug and metabolite. Hence, the aim of present investigations is to develop and validate simple, rapid, accurate, economical and convenient bio analytical methods for determination of Silodosin and Silodosin  $\beta$ -D-Glucuronide.

Validation can be defined as establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. Data obtained from method validation can be used to evaluate the quality, consistency and reliability of analytical method. It is an indispensable part of any good analytical practice [4,6,9].

### The necessity for analytical methods to be validated

The main objective of method validation is to demonstrate the reliability of a particular method for the determination of an analyte concentration in a specific biological matrix, such as blood, serum, plasma, urine, or saliva. Moreover, if an anticoagulant is used, validation should be performed using the same anticoagulant as for the study samples. Generally a full validation should be performed for each species and matrix concerned. Full validation of bioanalytical methods is important: 1) during development and implementation of a novel bioanalytical method. 2) For analysis of a new drug entity. 3) For revisions to an existing method that add metabolite quantification.

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## Advantages of bioanalytical method validation

It results in level of confidence to the developer as well as to the user. The validation process might be a costly and time consuming exercise but results are found to be inexpensive and lead to better time management in the end. It also eliminates annoying repetitions. Typical validation parameters to be considered are Accuracy, Precision, Repeatability, Intermediate Precision, Specificity, Detection Limit, Quantitation Limit, and Linearity Range [4,6,9].

## Materials and Methods

### Materials

The materials used were Silodosin, Silodosin  $\beta$ -D-Glucuronide and Silodosin D6 dihydrochloride (IS) and Methanol. The LCMS instrument used here was AB Sciex, API 3200.

### Sample preparation

**Preparation of stock and working standard solutions for pure drug silodosin:** Transfer accurately weighed (about 10 mg) Silodosin into a 10 ml volumetric flask and dissolve in Methanol. Make up the volume with same and vortex. Concentration of the resultant solution will be about 1000  $\mu$ g/ml.

**Silodosin  $\beta$ -D-Glucuronide:** Transfer accurately weighed (about 10 mg) Silodosin  $\beta$ -D-Glucuronide into a 10 ml volumetric flask and dissolve in Milli-Q water. Make up the volume using Milli-Q water and vortex. Concentration of the resultant solution will be about 1000  $\mu$ g/ml. This solution was further diluted with methanol to get working standard solution of 10  $\mu$ g/ml of drug.

### Preparation of sample solutions

500 ml of Methanol (Methanol:Water::50:50 v/v), 500 ml of Milli-Q water into a 1000 ml of reagent bottle, mix and sonicated.

**Mobile phase:** 800 ml of Acetonitrile (Buffer:Acetonitrile::20:80 v/v) and 200 ml of buffer into a 1000 ml of reagent bottle, mix well and sonicated. Preparation of Buffer (5 mM Ammonium Acetate) approximately weighed 0.385 g of ammonium acetate dissolve in 1000 ml of Milli-Q-water and adjusted solution pH to 9.0 with Ammonia and filtered. Preparation of dilution solvent (methanol:water 50:50% v/v) approximately 250 ml of Milli-Q-water into a 500 ml reagent bottle to it add 250 ml of Methanol and sonicated well.

**Preparation of internal standard stock solution:** Silodosin D6: Transfer accurately weighed (about 10 mg) Silodosin D6 into a 10 ml volumetric flask and dissolve in Methanol and make up the volume using same and vortex. Concentration of the resultant solution will be about 1000  $\mu$ g/ml.

**Preparation of internal standard working solution:** Dilute 5  $\mu$ l of internal standard stock solution to 10 ml using dilution solvent to get IS dilution about 500 ng/ml.

**Preparation of sample solutions:** Sample solutions of different conc. from 10.3688 to 0.0251  $\mu$ g/ml and 15.1418 to 0.2060 for Silodosin and Silodosin  $\beta$ -D-Glucuronide were prepared from above stock solution and diluted with mobile phase.

**Instrumentation:** Quantitation was achieved with LCMS-MS detection using an AB Sciex API-3200 mass spectrometer. Analyst software™ Analyst 1.4.1 was used for processing the analysis data. Estimation was done by mass spectrometric method and chromatographed using a ZORBAX SB-C8, 100 mm  $\times$  4.6 mm, 3.5  $\mu$ m

column. The flow rate was 0.800 ml/min, with splitter under ambient temperature. The auto sampler injection volume was 10  $\mu$ l. The run time was 3.5 minutes.

## Parameters of Validation

### Specificity and selectivity

Selectivity is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. Plasma selectivity was evaluated by analyzing six lots of blank K<sub>2</sub>EDTA human plasma obtained from independent sources.

### Linearity and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of an analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

### Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

### Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

### Reproducibility

Inter-day precision (different days) and intra-day precision (different times in a day) studies were carried out.

### Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The determination was done at three different levels. Three samples of each level were prepared and total 9 determinations done as per ICH conditions. The samples were analyzed and their area measured and results indicated as %RSD.

### Robustness/Ruggedness

The robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

### Detection limit/Quantitation limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used



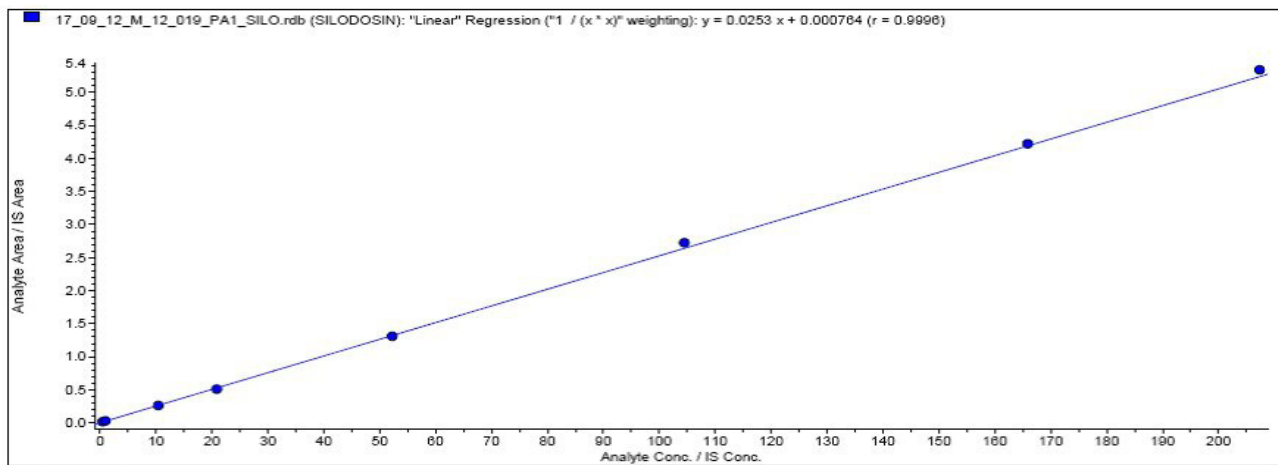


Figure 2: Representative calibration curve for silodosin in human plasma ( $K_2EDTA$ ).

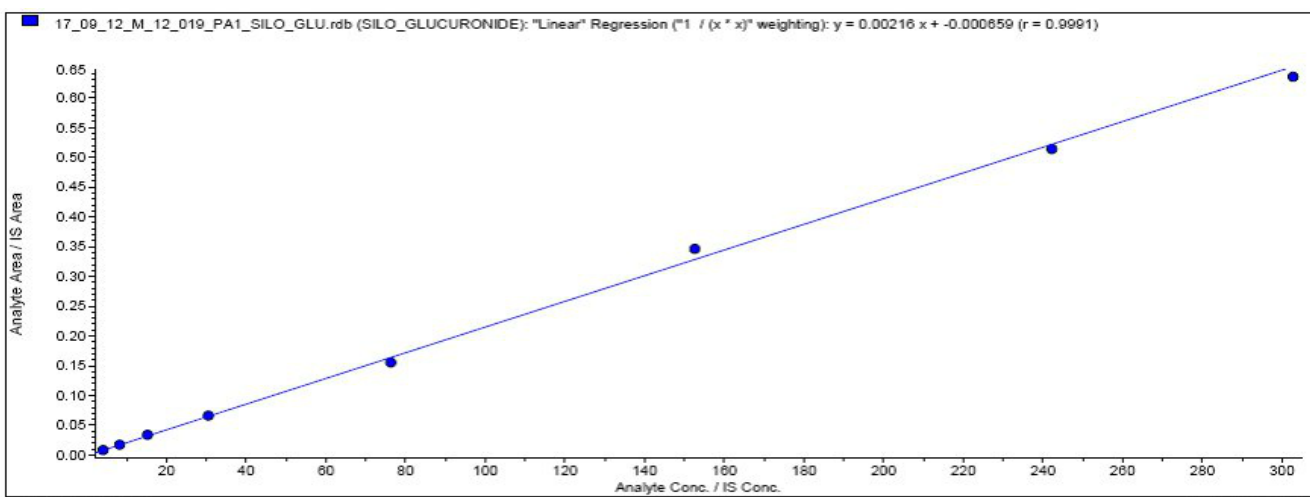


Figure 3: Representative calibration curve for silodosin  $\beta$ -D-Glucuronide in human plasma ( $K_2EDTA$ ).

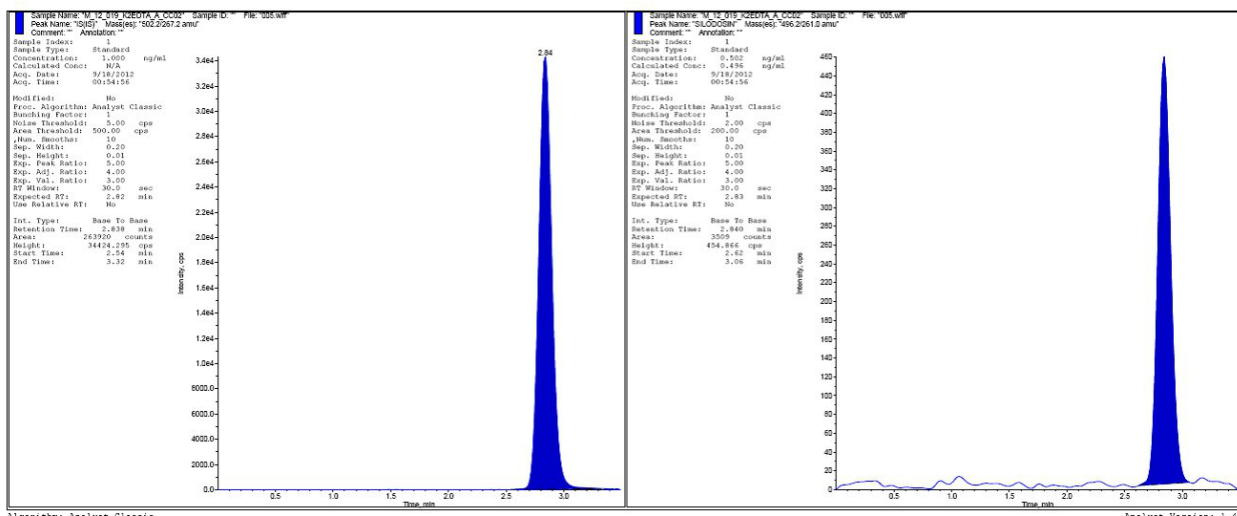


Figure 4: Chromatogram of the LLOQ calibration curve standard for silodosin with internal standard (Silodosin D6).

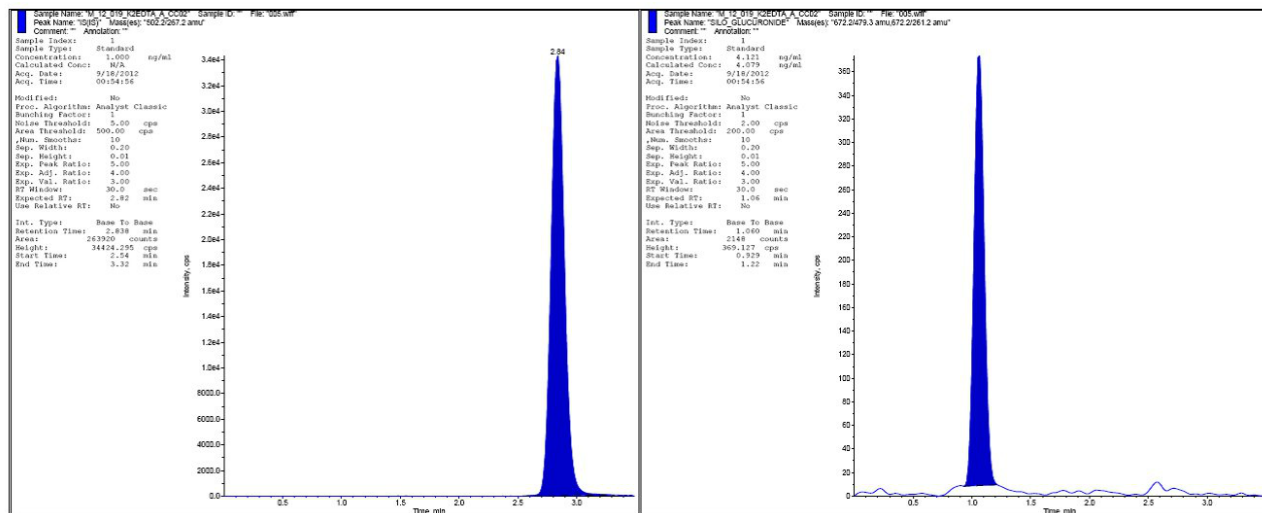


Figure 5: Chromatogram of the LLOQ calibration curve standard for silodosin  $\beta$ -D-Glucuronide with internal standard (Silodosin D6).

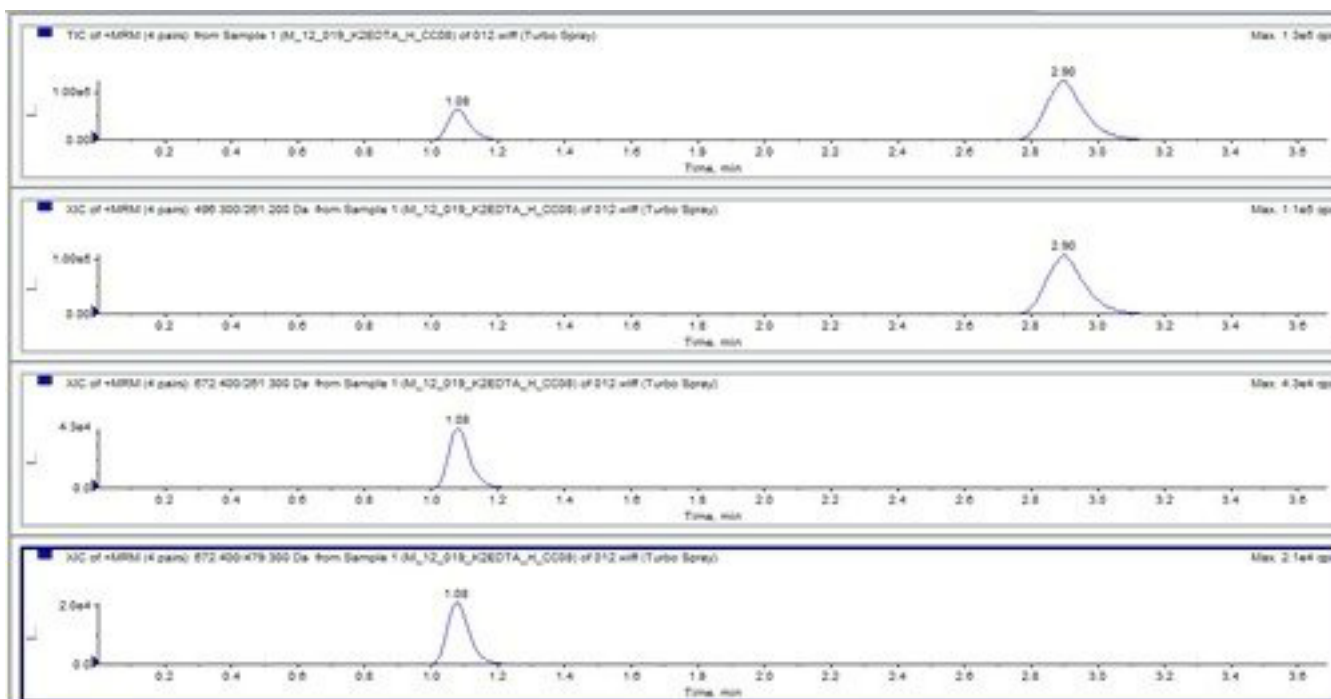


Figure 6: Chromatographic separation of Silodosin, Silodosin  $\beta$ -D-Glucuronide and Silodosin D6 (Internal Standard).

### Ruggedness

To assess ruggedness one batch with injection of standard blank, zero standard blank and 8 non-zero calibration standard with duplication of LLOQ and ULOQ injection was performed by a different analyst using a different column. The variation observed in the results were negligible, hence the method meets ruggedness criteria.

### Conclusions

A sensitive and selective LC-MS/MS method to quantitate Silodosin and Silodosin  $\beta$ -D-Glucuronide in  $K_2EDTA$  in Human plasma over the

concentration range 0.502 to 207.376 ng/ml for Silodosin and 4.121 to 302.836 ng/ml for Silodosin  $\beta$ -D-Glucuronide was successfully validated. This method is suitable for subject sample analysis and incurred sample analysis to support bioequivalence/ bioavailability and/or pharmacokinetic studies involving formulations of Silodosin and Silodosin  $\beta$ -D-Glucuronide.

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