Simultaneous estimation of silodosin and silodosin β-D-glucuronide in human plasma using LC-MS/MS for a pharmacokinetic study

Siva Sankara Rao Yadlapalli a,b, Naresh Kumar Katari a, Surendra Babu Manabolu a and Vasu Babu Ravi a,b

aDepartment of Chemistry, GITAM University, Hyderabad, India; bAnaCipher Clinical Research Organization, Ramanthapur, Hyderabad, India

ABSTRACT
A rapid, simple, sensitive and selective LC-MS/MS method has been developed and validated for simultaneous quantification of silodosin and silodosin β-D-glucuronide human plasma using stable labelled isotopes as internal standards. Solid phase extraction technique (SPE) was used for the extraction and the method validated over a range of 0.20 ng/mL to 100.56 ng/mL for silidisin and 0.20 ng/mL to 101.63 ng/mL for silodosin β-D-glucuronide. The chromatographic separation was achieved on Cosmosil Adze C18 (4.6 × 100 mm, 3 µm) column using a mobile phase consisting of acetonitrile, methanol and 10 mM ammonium acetate buffer 50:20:30 (v/v/v) at a flow rate of 1.000 mL/min with run time of 4 min. The API-4500 LC-MS/MS was operated under the multiple-reaction monitoring mode using electrospray ionization. The developed assay method was successfully applied to a pharmacokinetic study in humans.

Definitions

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained under the prescribed conditions. Precision is defined as the ratio of standard deviation/mean (%).

Accuracy: The accuracy of an analytical procedure expresses the closeness of the determined value to the value which is accepted either as a conventional true value or an accepted reference value. Accuracy is defined as (determined value/true value) × 100%.

1. Introduction
Silodosin, an α1-adrenoceptor antagonist with high uroselectivity. 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 (Figure 1). Consequently, blockade of α1A-AR induces prostatic and urethral smooth muscle relaxation, and may improve voiding symptoms. However, silodosin also seems to target afferent nerves in the bladder, and thereby acts on bladder over activity and storage symptoms [1–5].

As per the literature, few LC-MS methods have been reported for the quantification of silodosin alone [6] and with its active metabolite silodosin β-D-glucuronide [7,8] in human plasma. The reported method utilizes solid phase extraction (SPE) [7] and liquid–liquid extraction [6,8] for sample preparation. Nair et al. [7] present simultaneous determination of SLD and KMD-3213G in human plasma with linear ranges of 0.50–207.38 and 4.12–302.84 ng/mL, respectively. However, no sample preparation details were discussed in the paper and also the method was used only for spiked samples not applied for real sample analysis. Silodosin alone was determined by Zhao et al. [6] with the range of 0.50–50.0 ng/mL. But this method is only suitable for silodosin may not suitable for simultaneous analysis of its metabolite. In 2017, another LC-MS method was developed by Shah et al. [8] for silodosin and KMD-3213G in the range of 0.10–80.0 ng/mL for both analytes. However, the runtime of > 6 min, which is very high and are not suitable for routine high-throughput bioanalysis of silodosin and its metabolite.

The objective of present study is to develop and validate the analytical method for estimation of silodosin and silodosin β-D-glucuronide in human plasma in API method using LC-MS/MS. The present LC–MS/MS method is carried over a range of 0.20–100.56 ng/mL of silodosin and 0.20–101.63 ng/mL after direct elution from SPE cartridges and loaded into vials without evaporation, drying and reconstitution steps, thereby, lessening time makes the method more suitable for commercial purposes. Also, the method utilized low plasma volume (100 µL) compared with earlier reports and achieved greater sensitivity than earlier reports.
After validation, method was successfully applied to a clinical pharmacokinetic study in healthy male volunteers following single oral administration of silodosin 4 mg capsule. Also, the results were authenticated by incurred sample reanalysis (ISR). [6–8].

2. Experimental

2.1. Chemicals and reagents

The reference samples of silodosin and silodosin β-D-glucuronide, internal standards silodosin-d4 and β-D-glucuronide-d4 were procured from Daicel Chiral Technologies, (Hyderabad, India). Water used for the LC–MS/MS analysis was prepared from Milli-Q-water purification system procured from Millipore (Bangalore, India). Acetonitrile and methanol were of HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). Analytical grade ammonium acetate and formic acid was purchased from Merck Ltd., (Mumbai, India). The control human plasma sample was procured from Deccan’s Pathological Labs (Hyderabad, India).

2.2. Instrumentation and chromatographic conditions

A Shimadzu LC-20 AD Series HPLC system (Shimadzu Corporation, Kyoto, Japan) was used to inject 20-μL aliquots of the processed samples on Cosmosil Adze C18 (4.6 × 100 mm, 3 μm Genius Technologies, USA) column, which was kept at ambient temperature (20 ± 5°C). The isocratic mobile phase, mobile phase consisting of acetonitrile, methanol and 10 mM Ammonium acetate buffer 50:20:30 (v/v/v) as the mobile phase delivered at a flow rate of 1.0 mL/min into the mass spectrometer electrospray ionization chamber.

Quantitation was achieved with MS/MS detection in positive ion mode for the analytes and IS using AB Sciei API-4500 mass spectrometer (Foster City, CA, USA) equipped with Turboionspray™ interface at 500°C. The ion spray voltage was set at 5500 V. The source parameters viz., the nebulizer gas, curtain gas, auxiliary gas and collision gas were set at 45, 35, 45 and 10 psi, respectively. The compound parameters viz. the declustering potential (DP), collision energy, entrance potential and collision cell exit potential were 90, 35, 10, 9 V for silodosin and Silodosin-d4, 20, 48, 10, 8 V for silodosin β-D-glucuronide and silodosin β-D-glucuronide-d4, respectively. Detection of the ions was carried out in the multiple-reaction monitoring mode (MRM) by monitoring the transition pairs of silodosin m/z – 496.50 (parent) and 261.10 (product) and silodosin β-D-glucuronide m/z – 672.50 (parent) and 244.10 (product) internal standard silodosin-d4 m/z – 500.50 (parent) and 261.10 (product) and silodosin β-D-glucuronide-d4 m/z – 676.50 (parent) and 244.10 (product) in positive ion mode. The analysis data obtained were processed by Analyst software™ (version 1.6.3).

2.3. Preparation of standard solutions

Weighed about 2.02 mg of silodosin, 2.12 mg of silodosin β-D-glucuronide, 2.19 mg of silodosin-d4, 2.03 mg of silodosin β-D-glucuronide-d4 working standards and transferred to a 2-mL clean glass volumetric flasks separately, dissolved in methanol and made up the volume with the same to produce a solution of 1 mg/mL. For the preparation of calibration curve standards and quality control samples two separate stock solutions were prepared and used.

2.4. Preparation of calibration curve standards and quality control samples

Calibration curve standard consisting of a set of 10 non-zero concentrations ranging from 0.20 to 100.56 ng/mL of silodosin and 0.20–101.63 ng/mL of silodosin β-D-glucuronide were prepared. Prepared quality control samples consisted of concentrations of 0.21 ng/mL (LLOQ QC), 0.61 ng/mL (LQC), 15.17 ng/mL (MQC1), 50.55 ng/mL (MQC2) and 75.79 ng/mL (HQC) for silodosin and 0.21 ng/mL (LLOQ QC), 0.61 ng/mL (LQC), 15.29 ng/mL (MQC1), 50.96 ng/mL (MQC2) and 76.40 ng/mL (HQC) for silodosin β-D-glucuronide. These samples were stored at −70°C until use.

2.5. Sample processing

The samples were thawed at room temperature and vortexed to ensure complete mixing of the contents. 200 μL of the plasma sample was pipetted into pre-labelled Radio Immuno Assay vial tubes, 20 μL of internal standard dilution (108.56 ng/mL silodosin-d4, 504.35 ng/mL of silodosin β-D-glucuronide-d4 was added to it and vortexed, except in blank plasma samples where 20-μL diluent was added and vortexed. Also processed 200 μL of each sample with addition of 20 μL of internal standards dilution and vortexed. Then added 625 μL of 1% formic acid buffer and vortexed. The sample mixture was loaded onto Phenomenex Starata X-33 μM polymeric sorbent cartridges (30 mg/1CC) that were pre-conditioned with 1.0 mL of HPLC grade methanol followed by 1.0 mL Milli Q grade water. After applying the maximum pressure wash the
extraction cartridge washed with 1.0 mL of 1% formic acid buffer followed by 1.0 mL of Milli-Q water and 1.0 mL of washing solution. The sample was eluted 1.0 mL of mobile phase and the eluted samples were transferred into loading vials and loaded into the autosampler.

### 2.6. Pharmacokinetic study design

A pharmacokinetic study was performed in healthy male subjects \((n = 12)\). The ethics committee approved the protocol and the volunteers provided with informed written consent. Twenty four (24) blood samples \((1 \times 6 \text{ mL})\) were collected in pre-labelled K2 EDTA \((\text{BD, Franklin, NJ, USA})\) vacutainers. Single venous blood sample were withdrawn at pre-dose \((0.00)\) and 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 8.00, 12.00, 16.00, 20.00, 24.00, 36.00, 48.00 and 72.00 hours post-dose. The tubes were centrifuged at 3200 rpm for 10 min and the plasma was collected. The collected plasma samples were stored at \(-70^\circ\text{C}\) until their use. Plasma samples were spiked with the IS and processed as per the extraction procedure described earlier. Along with the clinical samples, the QC samples of each six replicates at low, middle 1, middle 2 and high concentration levels were also assayed. Plasma concentration-time profile of silodosin and silodosin \(\beta\)-D-glucuronide was analysed by non-compartmental method using WinNonlin Version 5.1. An incurred sample re-analysis (ISR) was also conducted by selecting the 24 subject samples \((2 \text{ samples from each subject})\) near \(C_{\text{max}}\) and the elimination phase. The variability \%(difference\) between original value and ISR values were within \(\pm 20\%\).

### 3. Results and discussion

### 3.1. Method development

Mass parameters were optimized in both positive and negative ionization modes for the analytes and IS. Good response was found in positive ionization mode. Data of the multiple-reaction monitoring were considered to get better selectivity. To develop a rapid, sensitive and simple assay method for the extraction and quantification of silodosin and silodosin \(\beta\)-D-glucuronide during method development different options were tried to optimize chromatography parameters. The selectivity of MS/MS detection was also expected to be beneficial in developing a selective and sensitive method. LC–MRM is a very powerful tool for pharmacokinetic studies since it provides sensitivity and selectivity requirements for analytical methods. Thus, the MRM technique was chosen for the assay development. The MRM state file parameters were optimized at a concentration of 50 ng/mL to maximize the response for the analyte. The chromatographic conditions, especially the composition of mobile phase, were optimized through several trials to achieve good resolution and symmetric peak shapes for the analytes as well as a short run time. Separation was attempted using various combinations of methanol, acetonitrile and buffer with varying contents of each component on different columns like C8 and C18 of different makes like Chromolith, Hypersil, Hypurity advance, Zorbax, Kromasil and Intertsil. It was found that a mixture of methanol, acetonitrile and 10 mM ammonium acetate 50:20:30 \((\text{v/v/v})\) could achieve this purpose and was finally adopted as the mobile phase. Cosmicsil Adze C18 \((4.6 \times 100 \text{ mm}, 3 \mu\text{m})\) column gave a good peak shape and response even at LLOQ level for both the analytes and internal standards. The retention time of silodosin, silodosin-d4 and silodosin \(\beta\)-D-glucuronide, silodosin \(\beta\)-D-glucuronide-d4 was low enough \((3.04\) and 1.01 min\) allowing a small run time of 4.0 min.

### 3.1.1. Optimization of chromatography

Protein precipitation was tried initially using acetonitrile and methanol as precipitating agents but the response was inconsistent especially at the LLOQ level moreover, moderate drug protein binding of about 27–45\% was reported. Thus, the simple SPE technique was employed for the sample preparation in this work and provided high recoveries of the drugs. The use of stable labelled isotopes of the analyte as internal standard is recommended for bioanalytical assays to increase assay precision and limit variable recovery between analyte and the IS. For an LC-MS/MS analysis, utilization of stable isotope-labelled drugs as IS proves to be helpful when a significant matrix effect is possible. At the initial stages of this work, several compounds were investigated to find a suitable internal standard and finally silodosin and silodosin \(\beta\)-D-glucuronide stable labelled isotopes silodosin-d4 and silodosin \(\beta\)-D-glucuronide-d4 were found to be best for the present method.

### 3.2. Method validation

The validation of the method was carried out as per Europe, Middle East, and Africa (EMEA) and US Food and Drug Administration (FDA) guidelines. The parameters determined were selectivity, specificity, matrix effect, method ruggedness, linearity, precision, accuracy, recovery, stability, run size evaluation and dilution integrity.

### 3.2.1. Selectivity and specificity

The degree of interference by endogenous plasma constituents with the analytes and IS was assessed by inspection of chromatograms derived from processed blank plasma sample. As shown in Figure 2. No significant direct interference in the blank plasma traces was observed from endogenous substances in drug-free plasma at the retention time of the analytes and internal standards.
No interference was observed at retention time of silodosin, silodosin β-D-glucuronide, silodosin β-D-glucuronide-d4 when working concentration of silodosin-d4 injected. Similarly no interference was observed at retention time of silodosin, silodosin-d4, silodosin β-D-glucuronide when working concentration of silodosin β-D-glucuronide-d4 injected as shown in Figure 3.

3.2.2. Sensitivity
The lowest limit of reliable quantification for silodosin in human plasma was set at the concentration of the LLOQ 0.20 ng/mL and silodosin β-D-glucuronide in human plasma was set at the concentration of the LLOQ 0.20 ng/mL. The precision and accuracy for silodosin at this concentration was found to be 1.96% and 108.62%, and silodosin β-D-glucuronide at this concentration was found to be 7.62% and 111.58%, respectively was shown in Figure 4.

3.2.3. Matrix effect
No significant matrix effect was observed in eight batches including lipemic and Hemolysed plasma for silodosin and silodosin β-D-glucuronide at low (LQC) and high (HQC) concentrations. For silodosin, the precision for IS normalized matrix factor at LQC and HQC level was found to be 3.34% and 1.00% respectively and IS normalized factor was 1.02 for LQC and 1.00 for HQC.

3.2.4. Linearity
A regression equation with a weighting factor of 1/(concentration ratio) 2 of drug to IS concentration was judged to produce the best fit for the concentration-detector response relationship for silodosin and silodosin β-D-glucuronide in human plasma. Correlation coefficient (r) was greater than 0.99 in the concentration range of 0.20–100.56 ng/mL for silodosin and the concentration range of 0.20–101.63 ng/mL for silodosin β-D-glucuronide.

3.2.5. Precision and accuracy
See Table 1.

3.2.6. Recovery
The mean overall recovery of silodosin was 84.92% with a precision range of 0.75–3.62% and silodosin β-D-glucuronide was 83.83% with a precision range of 0.74–3.10%. The mean recovery of internal standard silodosin-d4 was 85.54% with a precision from 1.77% to 2.30% and silodosin β-D-glucuronide-d4 was 84.88% with a precision ranging from 1.46% to 1.88%.

3.2.7. Dilution integrity
Dilution integrity samples were prepared by spiking 1.71 times and 3.42 times of highest standard concentration. Silodosin precision and accuracy, for a concentration (171.78 ng/mL) was 0.76% and 105.78% and

![Figure 2](image-url). Typical MRM chromatograms of blank in human plasma silodosin (A) and silodosin β-D-glucuronide (B).
silodosin $\beta$-D-glucuronide precision and accuracy, for a concentration (173.18 ng/mL) was 1.02% and 104.79%, respectively. Similarly, silodosin precision and accuracy, concentration (343.56 ng/mL) was 0.87% and 106.62% and silodosin $\beta$-D-glucuronide precision and accuracy, concentration (346.35 ng/mL) was 0.61% and 105.12%, respectively.

3.2.8 Run size evaluation
Forty sets of each of LQC, MQC1, MQC2 and HQC samples were processed and analysed for run size evaluation. One hundred and sixty QCs out of 160 QCs of run size evaluation were within 15% of their respective nominal values.

Within-batch precision and accuracy for silodosin at LQC, MQC1, MQC2 and HQC was 1.96%, 0.96%, 0.71% and 0.99%, respectively, and the accuracy was 104.49%, 108.90%, 104.16% and 104.61%, respectively.

Within-batch precision and accuracy silodosin $\beta$-D-glucuronide at LQC, MQC1, MQC2 and HQC was 3.27%, 1.43%, 1.18% and 1.03%, respectively and accuracy was 102.58%, 107.71%, 104.67% and 105.75%, respectively.
Table 1. Intra-day/inter-day precision and accuracy of the method for determining silodosin and silodosin β-D-glucuronide in plasma samples.

| Analyte                      | Concentration added (ng/mL) | Concentration found (mean ± SD; ng/mL) | Precision (%) | Accuracy (%) |
|------------------------------|-----------------------------|----------------------------------------|---------------|--------------|
|                              |                             | Intra-day precision and accuracy (n = 12; 6 from each batch) | Inter-day precision and accuracy (n = 30; 6 from each batch) |
|                              |                             | (mean ± SD; ng/mL)                     | Precision (%) | Accuracy (%) |
| Silodosin                    | 0.21                        | 0.23 ± 0.01                            | 4.43          | 111.41       |
|                              | 0.61                        | 0.64 ± 0.02                            | 3.03          | 105.08       |
|                              | 15.17                       | 16.09 ± 0.19                           | 1.20          | 106.13       |
|                              | 50.55                       | 52.69 ± 1.22                           | 2.31          | 104.24       |
|                              | 75.79                       | 81.16 ± 2.24                           | 2.76          | 107.09       |
| Silodosin β-D-glucuronide    | 0.21                        | 0.21 ± 0.03                            | 12.07         | 98.92        |
|                              | 0.61                        | 0.61 ± 0.031                           | 5.06          | 99.02        |
|                              | 15.29                       | 16.09 ± 0.31                           | 1.90          | 105.24       |
|                              | 50.96                       | 52.88 ± 1.08                           | 2.05          | 103.76       |
|                              | 76.40                       | 81.15 ± 2.91                           | 3.58          | 106.22       |

Note: SD: standard deviation; n: number of replicates.

Table 2. Stability data of silodosin and silodosin β-D-glucuronide under different conditions (n = 6).

| Stability test                  | Silodosin | Silodosin β-D-glucuronide |
|---------------------------------|-----------|---------------------------|
|                                | QC nominal concentration (ng/mL) | Mean ± SD | Stability (%) | Precision (%) |
|                                |           | (ng/mL)               |              |              |
| Auto-sampler stability (at 10°C 79 h) | 0.61       | 0.67 ± 0.02           | 109.72       | 3.01         |
|                                 | 75.79      | 78.79 ± 0.90          | 103.96       | 1.14         |
| Wet-extract stability (at room temperature 72 h) | 0.61       | 0.66 ± 0.02           | 108.2        | 3.27         |
|                                 | 75.79      | 79.39 ± 0.79          | 104.75       | 0.99         |
| Bench-top stability (20 h)      | 0.61       | 0.65 ± 0.00           | 107.77       | 0.67         |
|                                 | 75.79      | 79.02 ± 0.56          | 104.27       | 0.71         |
| Freeze-thaw stability (five cycles) | 0.61      | 0.65 ± 0.01           | 107.58       | 1.61         |
|                                 | 75.79      | 79.16 ± 0.36          | 104.46       | 0.46         |
| Re injection stability (47 h)   | 0.61       | 0.66 ± 0.02           | 108.07       | 2.74         |
|                                 | 75.79      | 80.06 ± 0.91          | 105.64       | 1.14         |
| Long-term Stability (at –70°C for 30 days) | 0.61      | 0.60 ± 0.01           | 98.57        | 1.56         |
|                                 | 75.79      | 72.77 ± 1.27          | 96.03        | 1.75         |

Note: SD: standard deviation, n: number of replicates.

Figure 5. Mean plasma concentration-time profile of silodosin in human plasma following oral dosing of silodosin 4 mg capsule to healthy volunteers (n = 12).
Figure 6. Mean plasma concentration-time profile of silodosin $\beta$-D-glucuronide in human plasma following oral dosing of silodosin 4 mg capsule to healthy volunteers ($n = 12$).

Table 3. Pharmacokinetic parameter for silodosin ($n = 12$, Mean ± SD).

| PK parameter | Silodosin |
|--------------|-----------|
| $t_{\text{max}}$ (h) | 2.04 ± 1.64 |
| $C_{\text{max}}$ (ng/mL) | 52.48 ± 24.78 |
| $\text{AUC}_{0-t}$ (ng h/mL) | 211.08 ± 75.48 |
| $\text{AUC}_{0}\text{-inf}$ (ng h/mL) | 215.00 ± 75.58 |
| $t_{1/2}$ (h) | 8.29 ± 2.64 |
| Kel (h$^{-1}$) | 0.09 ± 0.04 |

Table 4. Pharmacokinetic parameter for $\beta$-D-glucuronide ($n = 12$, Mean ± SD).

| PK parameter | Silodosin $\beta$-D-glucuronide |
|--------------|---------------------------------|
| $t_{\text{max}}$ (h) | 5.92 ± 2.40 |
| $C_{\text{max}}$ (ng/mL) | 17.15 ± 4.59 |
| $\text{AUC}_{0-t}$ (ng h/mL) | 491.61 ± 183.57 |
| $\text{AUC}_{0}\text{-inf}$ (ng h/mL) | 531.66 ± 223.06 |
| $t_{1/2}$ (h) | 17.04 ± 3.66 |
| Kel (h$^{-1}$) | 0.04 ± 0.01 |

3.2.9. Stabilities
See Table 2.

4. Pharmacokinetic study results
In order to verify the sensitivity and selectivity of this method in a real-time situation, the present method was used to test for silodosin and silodosin $\beta$-D-glucuronide in human plasma samples collected from healthy male volunteers ($n = 12$). The mean plasma concentrations against time profile of silodosin and Silodosin $\beta$-D-glucuronide are depicted in Figures 5 and 6. The pharmacokinetic parameters estimated are shown in Tables 3 and 4.

5. Conclusion
The LC-MS/MS assay reported in this paper is rapid, simple, specific and sensitive for simultaneous quantification of silodosin and silodosin $\beta$-D-glucuronide in human plasma and is fully validated according to commonly acceptable FDA and EMEA guidelines. The method showed suitability for pharmacokinetic studies in humans. The cost-effectiveness, simplicity of the assay and usage of solid phase extraction, and sample turnover rate of less than 4.0 min per sample, make it an attractive procedure in high-throughput bioanalysis of silodosin and silodosin $\beta$-D-glucuronide. From the results of all the validation parameters, we can conclude that the developed method can be useful for BA/BE studies and routine therapeutic drug monitoring with the desired precision and accuracy.

Acknowledgements
The authors gratefully acknowledge AnaCipher Clinical Research Organization, Hyderabad, India for providing necessary facilities for carrying out this study.

Disclosure statement
No potential conflict of interest was reported by the authors.

ORCID
Siva Sankara Rao Yadlapalli http://orcid.org/0000-0003-0114-9686
Naresh Kumar Katari http://orcid.org/0000-0002-5737-8528
Vasu Babu Ravi http://orcid.org/0000-0002-6446-3042

References
[1] EMA: Science medicine Health. Silodyx, INN-silodosin, annex I: summary of product characteristics. European Medicines Agency, 30 Churchill Place, Canary Wharf, London E14 5EU, United Kingdom, 2015.
[2] FDA. Guidance for industry. bioanalytical method validation. US department of health and human services, USA, 2015.

[3] Xia Z, Yu Wang L, Junyu X, et al. Determination of silodosin in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B. 2009;877:3724–3728.

[4] Cui Y. Determination of silodosin in human plasma by liquid chromatography–tandem mass spectrometry. J Chromatogr B. 2009;877(29):3724–3728.

[5] Yoshida M. New clinical evidence of silodosin, an α1A selective adrenoceptorantagonist, in the treatment for lower urinary tract symptoms. Int J Urol. 2012;19(4):1–11.

[6] Zhao X, Liu Y, Xu J, et al. Determination of silodosin in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B. 2009;877(29):3724–3728.

[7] Nair SM, Ravi Kumar P, Sharma M, et al. Development and validation of high performance LCMS methods for estimation of silodosin and silodosin β-D-glucuronide in human plasma. Pharm Anal Chem. 2016;3:1.

[8] Shah PA, Shrivastav PS. Determination of silodosin and its active glucuronide metabolite KMD-3213G in human plasma by LC-MS/MS for a bioequivalence study. Biomed Chromatogr. 2017. doi:10.1002/bmc.4041.