Novel LC–MS/MS method for the quantification of silodosin and its metabolites in human plasma

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Benign prostatic hyperplasia (BPH) is a medical condition caused by the enlargement of the prostate gland as a result of hyperplasia of both the glandular epithelial and stromal cell compartments [1]. BPH has become a major cause for the increasing number of lower urinary tract symptoms (LUTS) in men, such as frequent urination, a weak urine stream and dribbling of urine, which can all worsen a patient's quality of life. This disease generally appears at the age of 50 or above in males and is comorbid with hypertension and erectile dysfunction. Moreover, the demographics of the population across the world indicate that aging is one of the most prominent causes of such health issues. To study the symptoms associated with BPH and bladder outlet obstruction, it is essential to have innovative strategies that can withstand the test of changing times. In response to this need, several safe and effective medical treatment options have been introduced to alleviate the symptoms at an early stage to lower the disease progression rate.

Over the last two decades, α -adrenoceptor (α -AR) antagonists have become the first-line treatment therapy for men with LUTS, suggestive of bladder outlet obstruction caused by BPH. Initially, phenoxybenzamine, a nonselective first generation α_1 - and α_2 -AR antagonist was used to treat LUTS. However, it received limited success because of its associated orthostatic and dizziness-related side effects on account of its poor tolerability. In October 2008, the US FDA approved silodosin as the second, third-generation α_{1A} selective antagonist after tamsulosin for the management of BPH. It has demonstrated significant effectiveness in improving the storage/irritating and voiding/obstructive symptoms compared with a placebo [2]. Furthermore, in a urodynamic study, silodosin assisted in improving detrusor overactivity and in reducing the grade of obstruction [3]. Silodosin is highly selective for the α_{1A} -AR, with an affinity that is 583- and 55.5-fold higher than that for the α_{1B} - and α_{1D} -ARs, respectively [3,4]. Compared to the currently available α_1 -AR antagonists, silodosin is 1400-times more specific in terms of α_{1A} -AR versus α_{1B} -AR binding than doxazosin and terazosin and 40-times greater compared with tamsulosin. Such increased selectivity for α_{1A} -AR is envisaged to provide additional relief from LUTS in patients with BPH without increasing the rate of serious adverse cardiovascular events [5]. In this editorial, we evaluate the capabilities and scope of the existing bioanalytical methods and propose a novel fit-for-purpose liquid chromatography-tandem mass spectrometry (LC–MS/MS) method in human plasma that can meet the current requirements for the analysis of silodosin and its metabolites.

Bioanalytical platforms for silodosin: past, present & future

Since its approval in 2008, there have been very few bioanalytical methods developed for the quantitation of silodosin [6–8]. LC–MS/MS so far has been the preferred technique for analysis; however, a complete account of bioanalytical method development incorporating sample extraction, chromatographic separation and mass spectrometric detection is currently limited. The validated LC–MS/MS method reported by Zhao *et al.* [6] allowed

the quantification of silodosin in human plasma. Here, the LLOQ of silodosin was 0.50 ng/ml using 500 μ l plasma sample. After sample preparation from liquid–liquid extraction with methyl *tert*-butyl ether, silodosin was analyzed on Zorbax C8 column. Electrospray ionization was performed in the positive mode due to the basic nature of silodosin, and the developed method was further applied to a pharmacokinetic study in two healthy subjects. Metabolism of silodosin via the glucuronidation pathway generates the major silodosin β -D-glucuronide metabolite designated as KMD-3213G, which has demonstrated significant pharmacological activity. Although the method developed here is sensitive and rapid enough to quantify silodosin in human plasma, it does not allow the analysis of KMD-3213G. The other two methods have essentially used the same approach to study the safety and pharmacokinetics of silodosin [7] and the effect of genetic polymorphisms on silodosin pharmacokinetics in healthy Chinese subjects [8].

Only two reports discuss simultaneous determination of silodosin and KMD-3213G in human plasma [9,10] based on LC–MS/MS analysis. The method reported by Nair *et al.* [9] was developed and validated in spiked plasma samples using solid-phase extraction. Based on the optimized conditions, the recovery of silodosin was close to 60% and that of KMD-3213G around 90%. The linear concentration range for silodosin and KMD-3213G was 0.502–207.376 and 4.121–302.836 ng/ml, respectively. However, the method was presented with no discussion on method development and application thereof. In one very recent research study, Shah *et al.* [10] critically evaluated each step of analysis (e.g., including extraction and chromatography) that directly impacts the method performance for the quantification of silodosin and KMD-3213G. The important feature of the method was complete baseline separation of the analytes and the selectivity achieved in lipemic and hemolyzed plasma samples, which are critical for reliable estimations in plasma samples. Though the analysis time was 6 min on Symmetry C18 column under gradient elution, the method was fivefold-times more sensitive (0.10 ng/ml) compared with all other methods. Moreover, the method required just 300 μ l plasma sample for processing. Further, the method was successfully applied for a bioequivalence study in healthy subjects and the reliability in the measurement of subject samples was demonstrated by reanalysis of selected incurred samples.

Although this area holds great promise for fine-tuning therapy and reducing the side effects of nonsubtype α_1 -AR antagonists, bioanalytical developments for silodosin seem to be minuscule since its approval. Thus, there is tremendous scope for the development of novel LC–MS/MS-based methods that integrate the following aspects:

- Analysis of the inactive metabolite, KMD-3293 along with the parent drug and its active glucuronide metabolite;
- Simultaneous analysis for a possible combination therapy involving tamsulosin or dutasteride with silodosin for BPH-associated LUTS;
- Implementation of design of experiment (DOE) approach for method development;
- Use of high-resolution MS (HRMS) for high-throughput analysis and automation.

The measurement of plasma concentration of drugs and its major active and inactive metabolites is of immense interest in various types of investigations, including possible drug–drug interactions, pharmacokinetic studies, associated side effects and toxicity data of metabolites. According to Watson Pharmaceuticals NDA document [11], the combined systemic exposure of both the metabolites was 60–90% higher than the parent drug in a pharmacokinetic study. This suggests the importance of evaluating the plasma concentration of the inactive silodosin metabolite, KMD-3293. Glucuronidation is always a major metabolic detoxification pathway, which results in the formation of inactive water-soluble species or metabolites that are more active than the parent drug. The bioanalytical assay should be adequately robust under different conditions to generate reliable results. Further, interconversion of the drug–metabolite pair is also important at every step of the assay. During mass detection, glucuronide fragmentation should always be evaluated to avoid overestimation of the parent drug concentration. Additionally, the chromatographic separation of the metabolite from the parent drug is an equally important aspect of method development.

The other facet that could be explored is the suitability of the method to analyze fixed dose combination formulations, as silodosin monotherapy associated with a minor adverse event such as ejaculation failure in some cases [12]. The assessment of combination therapy comprising of silodosin with other uroselective drugs that have a complimentary mode of action can help to prevent such adverse events. This will further push the development of fixed-dose combination therapy in the market and allow for better compliance with minimal side effects. Furthermore, to develop an efficient bioanalytical methodology, the DOE approach is essential to prove method robustness. Traditional method development is realized by optimizing one parameter at a time

(e.g., mobile composition, pH, temperature etc.), while DOE can assist to account for the most probable interactions between various factors that affect the bioanalytical procedure. This leads to precise estimations with minimum experimental workup. To find the most influential operating parameter, different DOE methods, such as Box–Behnken design, Fractional Factorial design, Central Composite design and Plackett–Burman design, can be screened. For the selection of the optimal values of important bioanalytical parameters, response surface designs can then be evaluated [13,14].

Over the past two decades, LC–MS/MS using triple quadrupole mass spectrometers has evolved into a mainstream approach for the quantitative bioanalysis of drugs and metabolites. In a routine sense, the determination of drugs in biological samples is considered as a multistep procedure involving calibration, sample pretreatment, chromatographic separation and detection by MS. The new generation of high-resolution mass spectrometry systems offer high-throughput quantitative analyses in combination with information-rich qualitative data with high sensitivity, scan-to-scan reproducibility, accuracy, dynamic range and resolution. This approach can lead to short analytical run times (typically 0.5–1.5 min) for targeted quantitative analysis of silodosin, its metabolites and other uroselective agents in a fixed-dose combination therapy. With the ever-growing role of automation in bioanalytical approaches, especially in the area of sample processing and online tandem chromatographic techniques (such as multidimensional development or ultra-performance convergence chromatography), there can be considerable saving in operation time with increased separation selectivity. This can further enhance the productivity of the method with superior results.

Conclusion

So far, LC–MS/MS has been the indispensable tool for quantitative bioanalysis of silodosin, a promising candidate to treat BPH-associated LUTS. New advanced bioanalytical methods with higher sensitivity, speed, selectivity and robustness are required to increase the domain of analysis for silodosin and its metabolites. With the existing LC–MS/MS methods, it is difficult to unravel new tasks of dealing with combination therapy. More novel and fit-for-purpose bioanalytical methods are essential to meet this requirement. As it stands today, bioanalytical developments for silodosin and its metabolites are at a minuscule level, thus, there lies a tremendous scope for further development of novel bioanalytical platform using LC–MS/MS to extend the domain of analysis.

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