Editorial

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Bioanalysis

Should commonly prescribed drugs be avoided as internal standard choices in new assays for clinical samples?

"If one goes back in time to a few decades earlier, the technology advancement seen in the present day scenario was limited..."

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Nuggehally R Srinivas
Suramus Bio, J.P. Nagar I Phase, Bangalore 560078, Karnataka, India
Tel.: +91 8042085501
srini.suramus@yahoo.com

Dependence on internal standard

The use of internal standard in chromatography-based bioanalytical methods employing HPLC, ultra-performance LC (UPLC) or LC with triple quadruple (LC–MS/MS) has been well accepted and is often considered critical for the performance of the assay in terms of accuracy and precision points of view. If one goes back in time to a few decades earlier, the technology advancement seen in the present day scenario was limited for the various components of bioanalysis such as extraction, chromatography and detection. The dependence on the liquid–liquid extraction process including back-extraction steps, the derivatization process to improve the sensitivity of the quantitation by introducing a new chemical tag on the analyte (especially applicable for drug racemates and for drugs that had a poor UV absorbance potential for HPLC detection) and inconsistent column-to-column performance, all in totality necessitated the use of an internal standard that compensated for the associated variability in the three critical steps of quantitation. Today, the technology advancement has grown leaps and bounds as observed in: newer column technologies with variety of stationary phases; innovative solid-phase extraction systems; interesting column switching devices to promote online extraction and detection; and most importantly the continuous nuances in the mass spectral detection options. Since most of the LC–MS/MS rely upon an efficient protein precipitation step for the extraction process and with the current column technology rendering consistent chromatography, one would question if there is a need of internal standard at all for the quantitation. However, the introduction of mass spectral detection brings in a new challenge of matrix effect, which may affect the ionization of the analyte and cause variability in the quantitation [1]. Therefore, suitable internal standard may be required that behave in an identical fashion with that of the analyte, not only during extraction and chromatography but also during the ionization/detection process.

Internal standard: pharmaceutical industry

From a pharmaceutical industry perspective, the internal standard story begins, when the focus on bioanalysis starts to emerge in the drug-discovery process upon the identification of a new hit chemical series leading to the creation of lead candidates that would need high throughput and efficient bioanalysis. Initially, some discovery related *in vitro* ADME screens and *in vivo* PK studies that rely on a single time point (i.e., 1 h concentration) may be performed with or without the use of an internal standard [2]. However, when there is a certainty on a few lead candidates to be considered for further preclinical profiling, it is important to consider the incorporation of an internal standard in the bioanalysis. The changing needs and

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increased challenges during the discovery, preclinical and clinical development of novel chemical entities (NCEs) have been well documented [3]. In the initial stages of development of a bioanalytical method, it is customary to use a close analog of the NCE to serve as the internal standard because it is expected to provide ideal characteristics to compensate for the experimental variability. As the NCE advances to the next milestone of candidate nomination and further development of NCE occurs, many innovator companies prefer to use a stabel label isotope of the NCE as the internal standard, especially since the assays are LC–MS/MS based. The use of stable labeled isotope of the NCE addresses the various issues during extraction, chromatography and detection, and enables unambiguous quantitation of the NCE. When the NCE enters clinical phase of development, the same stable labeled isotope of the NCE will continue to serve as internal standard for the bioanalysis of human samples from various Phase I studies and other clinical pharmacology studies [3].

Explosion of newer assays

After NCE (i.e., drug) approval, other research communities (e.g., generic companies, specialty companies and academic researchers, to name a few) become actively involved in generic formulation development, specialty product development and performing various preclinical/clinical pharmacology work, as the case may be, on the marketed drug. This is the time, when numerous bioanalytical methods are developed for the analysis of the drug based on the need of the researcher; these methods may be tailor made to impart more sensitivity, improve throughput, make it more suitable for therapeutic drug monitoring or incorporate the analysis of certain key metabolite(s) and/or other drug(s). A review of the literature suggests the availability of plethora of assays for several well-established drugs in various therapeutic/disease segments such as immunosuppressants (mycophenolic acid) [4], congestive heart failure (digoxin) [5], antiplatelet drug (clopidogrel) [6], oncology (irinotecan) [7], antibiotics (azithromycin) [8], lipid lowering drug (niacin) [9] and so on.

> "...many innovator companies prefer to use a stabel label isotope of the novel chemical entity as the internal standard, especially since the assays are LC–MS/MS based."

In spite of the availability of plenty of assays for established drug(s), newer assays for the same drug(s) are continuously being reported in the literature. The intent of this editorial is not to question the need or relevance of the newer assays, but provide some introspective thoughts on the choice of internal standard when newer assays are being developed and reported.

Common drugs as internal standards: issues

In my view and based on discussions that I had with several experienced colleagues in bioanalysis, while enough scrutiny and scientific diligence is exercised in developing a method with the selected internal standard, the choice of the internal standard in some of the reported assays may still not be an ideal one; simply because the chosen internal standard appears to be a commonly prescribed drug and therefore it may restrict a wider applicability of the newly published assay especially in the patient population. Several examples are presented from the published assays to deliver this key message.

Wang *et al.* published a LC–MS/MS for the simultaneous quantitation of salicylic acid and dipyridamole that used two internal standards, namely diazepam and rosiglitazone [10]. One would ask the question as to why structurally dissimilar drugs were chosen as internal standards? However, the choice of the two internal standards was made from a drug ionizability point of view, owing to the use of both positive ionization (dipyridamole) and negative ionization (salicylic acid) in the quantitation of the respective drugs [10]. Because of the increased use of rosiglitazone in diabetic patients, the application of such an assay that incorporates rosiglitazone as the internal standard may be limited for all clinical samples.

Shafi *et al.* used telmisartan as the internal standard for the quantitation of linagliptin using LC–MS/MS [11]. Although no specifics were discussed on the selection of telmisartan as an internal standard, such an assay would pose challenges in clinical samples of such antihypertensive patients that may be prescribed telmisartan.

Elbarbry and Shoker reported a simple HPLC assay for the quantitation of mycophenolic acid and employed naproxen as the internal standard [12]. Likewise, Benech *et al.* used ketoprofen for the analysis of mycophenolic acid, in human peripheral blood mononuclear cells [13]. Because of the increased use of naproxen/ketoprofen as a pain reliever and anti-inflammatory drugs, the applicability of such assays especially in organ transplant patients who have consumed commonly prescribed drugs such as naproxen or ketoprofen may become a challenge.

Mannemale and Nagarajan described a LC–MS/MS for the simultaneous quantitation of amlodipine and aliskiren using hydrochlorothiazide as the internal standard [14]. While the method supported analysis of amlodipine and aliskiren plasma levels when given in new fixed dose combinations, the choice of hydrochlo-

rothiazide may largely limit the use of such novel assay in patient population because hydrochlorothiazide is commonly added to antihypertensive regimen. To underscore this point, recently a UPLC–MS/MS assay was published, which measured amlodipine, aliskiren and hydrochlorothiazide commonly used as a triple agent in a single analytical run [15].

Gopinath *et al.* reported a LC–MS/MS for the simultaneous quantitation of fluoxetine and olanzapine using duloxetine as the internal standard. While the method was used for the assessment of samples in a bioavailability/bioequivalence (BA/BE) study in healthy subjects [16], the applicability of such an assay for patient samples may be problematic because duloxetine with its unique mechanism may also be coprescribed with either of the two drugs.

From the above, it is evident that although internal standards serve the purpose of an unambiguous quantitation of the reference drugs [11–16], the applicability of such assays outside of the designed PK study appeared to be dictated by other factors such as polypharmacy, self-medication and/or type of patient population. It may be possible in certain cases that the choice of internal standard may be made to aid in the ground work for a separate assay development for the internal standard at a later time merely by reversing the reference drug to be the internal standard in the developed assay. Such a strategy, although innovative in nature, may be applicable perhaps to support BA/BE studies in healthy subjects. Therefore, it may be important for authors to comment on the feasibility of the newly developed assays in its application for a wider patient pool with any cautionary notes for due consideration.

In my opinion, if new assays are developed and validated to serve the purpose of supporting a single or a few clinical pharmacology studies including BA/BE studies only in healthy subjects, then there is no concern on the choice of internal standard. However, if new assays are intended to be applied for large pool of patient samples or for the purpose of therapeutic drug monitoring, it may be prudent to scrutinize the selection of internal standards to ensure that certain commonly prescribed drugs and/or self-medication drugs (e.g., antipyretics, pain relievers and anti-inflammatory drugs) are avoided in the internal standard selection process.

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