Monitoring Internal Standard Response Difference between Known and Unknown Samples with LC-MS/MS Based Method for Detecting Matrix-Induced Systematic Error

Yansheng Liu, Yu-Hui Fu, Marsha Luna, Victor Green, Moo-Young Kim – KCAS, Shawnee, Kansas

INTRODUCTION

A new approach for additional quality control through monitoring internal standard (IS) variability with LC-MS/MS based methods has been established in our laboratory. The goal was to detect potential random and systematic errors that might be induced by the matrix difference between known samples (standards and QCs) and unknown (incurred) samples. The quantitation error may be caused by unique clinical or preclinical sample matrix and may not be detected during normal review of the results of known samples or even by results of incurred sample repeats (ISR) as indicated by the data in this presentation.

The data generated in 14 different clinical and preclinical studies have been examined with a unique LC-MS/MS method for each study. One study, completed prior to new established SOP for monitoring IS variability was revisited. Although all of the examined methods had passed matrix effect evaluation very well using different lots of commercial matrices during method development and validation, three studies failed the IS variability criteria including the revisited study.

All other examined studies passed the criteria very well with majority of examined batches within ±5% in terms of the difference of mean or CV. Due to limit of space, the data from three studies that failed the IS response variability criteria are shown and discussed in this presentation. The discussion will be limited to IS response variability associated with potential systematical error induced by matrix difference between known and unknown samples. In results and discussion, %Diff. of Mean or %CV represents %difference of mean or %CV of IS responses between standards and unknown samples.

METHOD

The IS responses observed for known and unknown samples respectively in the batch were separated into two groups for statistical calculations. When the difference between two groups in the mean and/or CV% of IS responses was over 15%, an investigation was triggered to evaluate potential systematic and random error induced by the different IS behaviors. The results of incurred sample reanalysis (ISR) obtained using either the modified or original procedure compared to the original results for supporting the investigation conclusion.

General information for LC-MS/MS systems:

HPLC : Shimadzu LC-10ADvp or LC-20AD
Autosampler : Perkin Elmer 200 Series or Leap Technologies HTP PAL.
Mass Spec : AB Sciex API 3000 or API 4000 LC-MS/MS
Typical MP A : Ammonium Formate buffer or acidic solution in low content of organic solvent or deionized water
MP B : MeOH or Acetonitrile based solution

RESULTS AND DISCUSSION

Case 1: An old study for determination of pseudoepherine in human plasma using epherine-d_3 as internal standard was revisited and investigated. The method involved liquid-liquid extraction followed by chromatography on a Betasil Phenyl (100x2.1 mm) column and detection using AB Sciex API 3000 LC-MS/MS system. A high variability of IS response was found for each batch in the study. The overall precision and accuracy for all QC samples at three concentration levels in a total of 68 batches are 3.2 to 4.8 (CV%) and -2.5 to -1.5 (bias%) respectively. The mean determined for 82 pooled incurred samples in 41 batches is 202 ng/mL with precision at 6.6 (CV%). The results indicate that the internal standard tracked the analyte very well in controlling the random error, but the internal standard is not always effective in controlling matrix-induced systematic error.

Table 1. IS response difference and pooled ISR results

Run #	B002	B009	B207
% Diff. of Mean	39.4	-32.7	-6.1
% Diff. of %CV	2.4	22.4	11.1
Results for Pooled ISR	200 / 214	225 / 214	213 / 216

The unknown samples analyzed with and without dilution were employed to evaluate the potential matrix-induced systematic error. The known sample matrix was used for dilution of unknown samples. The comparison results shown in table 2 indicate that no systemic error can be found for the samples from B002 in which the IS responses for unknown samples were significantly higher than the IS responses observed for standards. A significant systematic error was observed for B009 in which the % difference of mean IS responses was negative and significant as shown in Tables 1 and 3.

Table 2. Comparison between the diluted and undiluted with enhanced IS responses for incurred samples.

	Determined Concentration / Dilution factor			
Original in B002	198 / 1x	118 / 1x	209 / 1x	19.4 / 1x
Repeat in B207	193 / 2x	117 / 2x	205 / 2x	17.5 / 2x
Difference	2.6%	0.85%	1.9%	10.3%

Table 3. Comparison between the diluted and undiluted with suppressed IS responses for incurred samples.

	Determined Concentration / Dilution factor				
Original in B009	234 / 1x	34.8 / 1x	66.9 / 1x	182 / 1x	319 / 1x
Repeat in B207	145 / 2x	19.8 / 2x	38.8 / 2x	101 / 2x	188 / 2x
Difference	47.0 %	54.9%	53.1%	57.2%	51.7%

Case 2: Analysis of a client's drug in dog plasma was conducted using deuterated IS on an API 4000 LC-MS/MS following liquid-liquid extraction. The %difference of mean IS responses between unknown samples and standards was significant when a batch was chromatographed on single HPLC column. The batch was re-injected for the purpose of investigation on the same day using a modified method on two HPLC columns with a valve switching system for further on-line cleanup as shown in Figure 1.

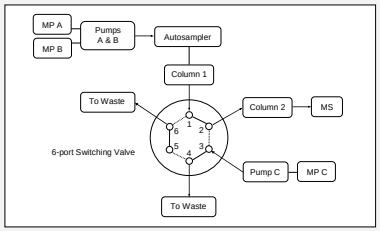

Figure 1. Valve-switching setup for on-line cleanup

The IS response difference between unknown samples and standards was significantly reduced with valve switching on-line cleanup as shown in Table 4. The determined results with two different methods are shown in Figure 2. No significant systematic error was observed when the IS responses between unknown and standards were significant with single column method. The mean of difference between two measurements for unknown samples above LLOQ was 7.5%. It indicated that the deuterated IS could track drug well and limit the matrix induced systematic error. Although the original method was acceptable, the method has been modified and validated later with on-line cleanup procedure for reliable performance.

Table 4. IS response difference with two methods

	Single column	Valve switching
%diff of Mean	43.0	-7.0
Diff of %CV	2.0	6.4

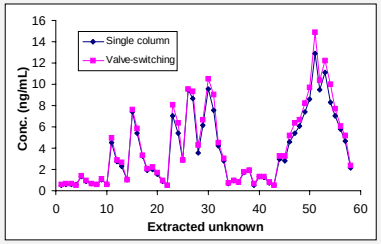

Figure 2. Comparison of two measurements

Case 3: Hydromorphone in clinical plasma samples was extracted with added deuterated IS using SPE, chromatographed on a Betasil silica 50x 2.1 mm column and detected using API 4000 LC/MS/MS System. Two batches for a clinical study were analyzed initially and significant IS response difference between standards and unknown samples was detected as shown in Table 5.

Table 5. IS response difference

	B001	B002
%diff of Mean	49.4	58.7
Diff of %CV	15.1	-5.9

An investigation was conducted by reanalyzing selected unknown samples with dilution using known sample matrix. The determined concentrations (pg/mL) are shown in Table 6. Inconsistent results were found between diluted and undiluted samples. The root cause responsible for systematic error was found to be the interference peak co-eluted with deuterated IS through further investigation. The different chromatographic methods tested could not resolve the issue and a different IS was used for modified method to fix the problem.

Table 6. Comparison of determined results with/without dilution

	Non-diluted	x5 dilution/%diff	x5 dilution/%diff
Unknown# 1	1430	1920 / 34.3	1830 / 28.0
Unknown# 2	1290	1650 / 27.9	1520 / 17.8
Unknown# 3	866	1290 / 49.0	1220 / 40.9

CONCLUSION

- The matrix-induced systematic error can not always be effectively detected by QC and ISR results during preclinical and clinical studies.
- Monitoring IS variability, especially the IS response difference between known and unknown samples is necessary and the significant matrix difference between known and unknown samples can be effectively detected.
- A selected IS may track an analyte very well in controlling random error such as induced during analytical process, but may not be able to correct matrix-induced systematical error.
- Our results confirmed general opinion: the IS response variability data can not be used as criteria for accepting batch or rejecting batch, but is valuable for triggering investigation.
- It will be a concern when an LC-MS/MS based method is lacking IS or shows significant retention difference between IS and analyte.

ACKNOWLEDGEMENTS

The entire KCAS LC/MS operation team has contributed to the data examined and presented by authors in this presentation.