Clinica Chimica Acta 486 (2018) 205–208

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Investigation of transition ion ratio variation for liquid chromatography-tandem mass spectrometry: A case study approach☆

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ARTICLE INFO

Keywords:
Transition ion ratio
Liquid chromatography
Mass spectrometry

ABSTRACT

Background: A transition ion ratio (TIR) is the ratio of one fragment over another from the same precursor and is frequently monitored in liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays for analyte identification. The Clinical and Laboratory Standards Institute (CLSI) C50-A guidelines give a static percent allowable TIR deviation based on the TIR level. Anecdotally, we observed failures of these rules for some of our LC-MS/MS assays. We determined what parameters may affect TIRs in a clinical setting and whether TIR variations may be analyte, matrix, instrument service, and/or concentration dependent.

Methods: Data was collected from the validation and selected periods after implementation for urine benzodiazepines (7 analytes) and plasma azole antifungals (6 analytes). TIRs for the calibrators and quality control materials on a Thermo TSQ™ Quantum Ultra from July 2016 to February 2017 for benzodiazepines in urine and Thermo TSQ™ Vantage from May 2016 to Oct 2016 for azoles in serum were monitored.

Results: The statistically significant day-to-day TIR shift ranged from 5.7 to 27.0% of the days studies for benzodiazepines and from 5.6 to 27.8% of the days studied for azoles excluding shifts caused by instrument services. Instrument service had significant impact on all benzodiazepines except oxazepam with p-values ranging from 1.79×10^{-6} to 1.53×10^{-39} and 4 of the 6 azoles (fluconazole, isavuconazole, voriconazole, and itraconazole) with (p from 7.89×10^{-3} to 1.98×10^{-12}). Lorazepam, α-hydroxyalprazolam, and hydroxyitraconazole showed significant concentration dependent TIR variations.

Conclusions: TIR variations may be affected by instrument services, and can be concentration and analyte dependent. Instead of using a static percent deviation rule, establishment of TIR variation criteria for each analyte during test development and validation may provide a more useful tool for analyte identification.

1. Introduction

Mass spectrometry (MS) is increasingly used in the clinical laboratory [1–6]. The most common type of MS in the clinical laboratory for targeted quantitation is triple quadrupole MS operating in selected reaction monitoring (SRM) mode. One precursor-to-product ion is termed a transition or product ion. Transition ions are thought to be a physical property of a compound of interest (COI). If one maintains the same MS settings, a MS should produce consistent ion fragmentation. By measuring multiple transition ions, transition ion ratios (TIR) can be produced that reflect the COI. If used correctly, monitoring the TIR improves the specificity of a liquid chromatography/tandem mass spectrometry (LC- MS/MS) assay. These TIRs allow mass spectroscopists to determine if a peak is truly a COI or an analytical interferent [7]. However, for isobaric compounds with very similar structures these TIRs may not provide sufficient specificity.

TIR guidelines in clinical laboratories are available through Clinical and Laboratory Standards Institute (CLSI) C50-A guidelines [8]. These guidelines give a static percent allowable TIR deviation based on the TIR level [8]. As an example, a TIR of 10–20% has a static allowable

Abbreviations: UAMCLZ, 7-aminoclonazepam; UOHALP, α-hydroxyalprazolam; UOHTR, α-hydroxytriazolam; AMU, atomic mass unit; CLSI, Clinical and Laboratory Standards Institute; COI, compound of interest; FLU, fluconazole; OHITRA, hydroxyitraconazole; ISA, isavuconazole; ITRA, itraconazole; ULORZP, lorazepam; UNDIAZ, nordiazepam; UOXAZP, oxazepam; POSA, posaconazole; SAD, static allowable deviation; SRM, selected reaction monitoring; UTEMZ, temazepam; TIR, transition ion ratio; VOR, voriconazole

☆ A portion this study was presented at the 69th AACC Annual Meeting in 2017.

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https://doi.org/10.1016/j.cca.2018.08.009
Received 4 May 2018; Received in revised form 12 June 2018; Accepted 7 August 2018
Available online 09 August 2018
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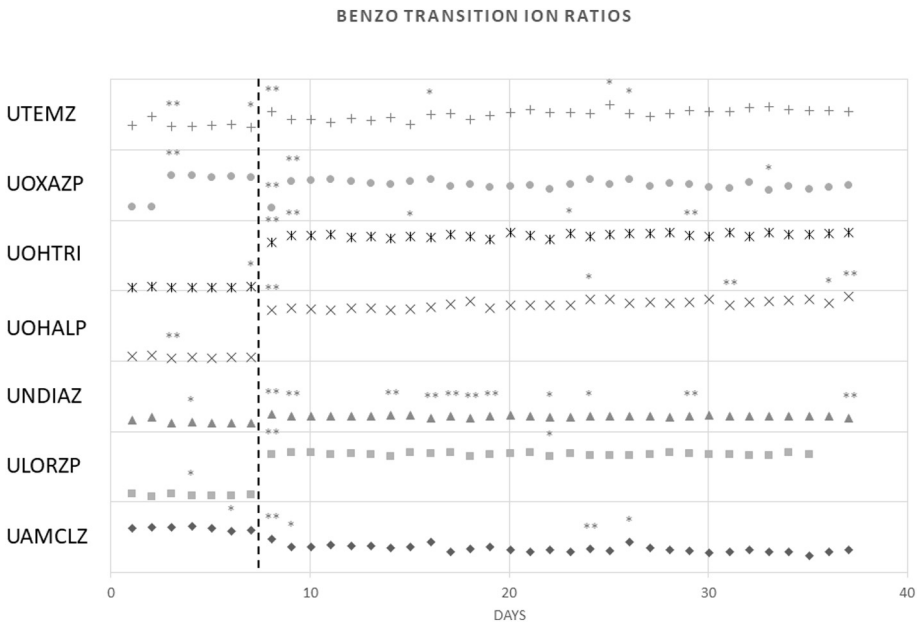

Fig. 1. Benzodiazepine Normalized TIRs. Average daily TIR were calculated then a min-max normalization was performed. Min-max normalization sets the min to zero and the max to 1. Each COI is stacked on the y-axis and the x-axis is day number. Statistical significance was identified using two tailed student's *t*-test. Dashed line indicates instrument service performed to the instrument. * = p-value < 0.05; ** = p-value < 0.01.

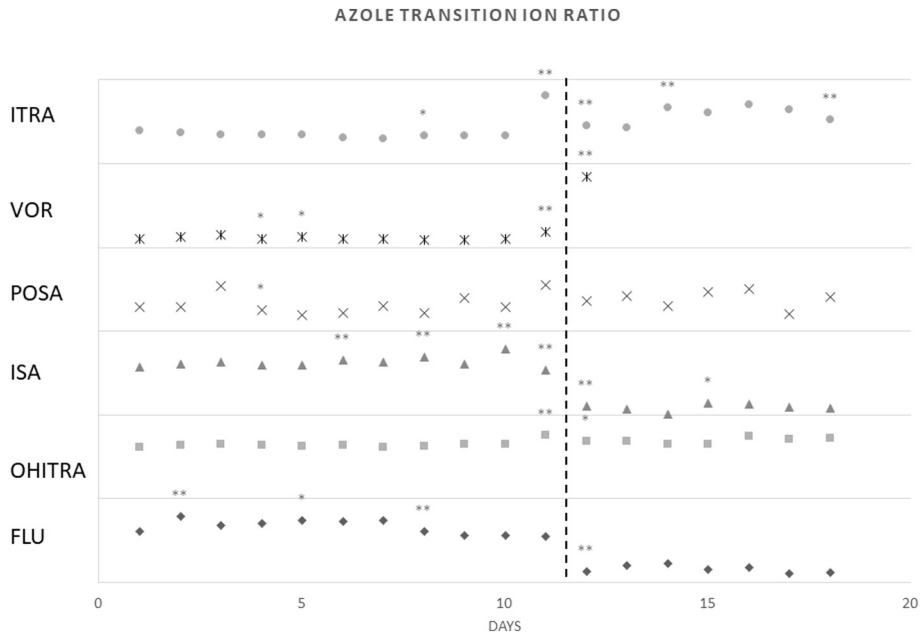

Fig. 2. Azole Normalized TIRs. Average daily TIR were calculated then a min-max normalization was performed. Statistical significance was identified using two tailed student's t-test. Dashed line indicates instrument service performed to the instrument. * = p-value < 0.05; ** = p-value < 0.01.

deviation (SAD) of 30%. There could be circumstances where these SADs are too large for some COIs and too small for others; hence, the SAD may lead to improper interpretation of the COI or interference. These levels are in place under the assumption that on a specific instrument and for a certain COI these levels do not change. Previously, a study showed that TIRs vary based on ion intensity which was used as a proxy for concentration [9]. Anecdotally, we observed failures of these rules for some of our LC-MS/MS assays and began an investigation into the factors that have impacts on TIR variations.

2. Methods

TIRs were collected from assay validation and selected periods after assay implementation. Data sets were non-contiguous, single day preparations. A benzodiazepine data set that contained multiple lots of spiked human urine with known levels (calibrators and quality controls [QC]) of 7-aminoclonazepam (UAMCLZ; precursor ion: 286 *m/z*→ ;product ion 1: 121 *m/z*; product ion 2: 222 *m/z*), lorazepam (ULORZP; 321→229;194), nordiazepam (UNDIAZ; 271→140;208), α-hydroxyalprazolam (UOHALP; 325→279;243), α-hydroxytriazolam (UOHTRI; 359→176;242), oxazepam (UOXAZP; 287→241;104), and temazepam (UTEMZ; 301→177;255) and was analyzed by LC-MS/MS with patient and validation samples. Briefly, internal standard solution and urine sample were mixed and enzymatically hydrolyzed at 60 °C with red abalone β-glucuronidase. Then the mixture was diluted with methanol and water prior to direct injection. Calibrator levels were 50, 100, 200, 500, 1000, 5000 ng/ml and QC values were 200 and 1000 ng/ml. Data analyzed contained TIRs for the calibrators and QCs on a Thermo TSQ™ Quantum Ultra from July 2016 to February 2017. Azole antifungal analysis [10], the second data set, measured fluconazole