Comment on: Determination of serum levels of imatinib mesylate in patients with chronic myeloid leukemia: validation and application of a new analytical method to monitor treatment compliance

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Whether therapeutic drug monitoring is beneficial for imatinib mesylate used in the treatment of chronic myeloid leukemia is currently under investigation[1], but most previous studies have indicated a significant association between trough serum concentrations greater than 1000 ng/mL and positive clinical response[2]. In this issue, a mass spectrometry (MS)-based method for determination of imatinib concentrations in human serum is presented[3]. While MS is often regarded as a difficult and expensive technique, requiring highly skilled personnel, the authors have chosen a robust and economical single-quadrupole instrument that is appropriate for the clinical lab setting. However, although compatible methods have been reported[4], most MS-based procedures, including the earliest reported assays[5,6], have required tandem mass spectrometry (MS/MS)[2], a technique for which single-quadrupole mass spectrometers are not well-suited. Therefore, new methods for imatinib quantitation compatible with low-cost single-quadrupole instruments are clearly valuable, especially in light of the expected increase in demand if therapeutic drug monitoring does become standard practice.

Selectivity is often advanced to justify using MS for therapeutic drug monitoring. Improved selectivity, by enabling analyte quantitation in more complex matrices, allows sample preparation procedures to be simplified, which can reduce bias and total analysis time. Only protein removal by methanol precipitation was required in the presented method. MS is most powerful when combined with an appropriate separation. While gas chromatography is important for volatile molecules, liquid chromatography (LC) has wider applicability. Electrospray ionization (ESI) is the LC-MS interface of choice since the predominance of molecular ions simplifies data analysis. Sensitivity is enhanced by techniques like selected ion monitoring (SIM), where the instrument acts as a filter allowing only a single ion to reach the detector. SIM can be extended, by switching rapidly between targeted ions, to allow small numbers of components to be simultaneously monitored. Calibration curves must be established for the matrix in question to allow absolute quantitation. The presented method follows this SIM approach, targeting imatinib, the imatinib metabolite CGP 74588, and an internal standard. While not required in the presented method, the instrument used is capable of concurrent SIM of more than three analytes. Furthermore, simultaneous analysis is often unnecessary because an ion need only be targeted around the expected elution time, increasing the number of compounds that can be monitored.

A further advantage of MS is that isotopically labeled analogs of targeted analytes can be used as internal standards. Deuterated analogs behave nearly identically to targets, while using heavier isotopes (e.g., ^{13}C, ^{15}N, ^{18}O) results in standards that are essentially indistinguishable until a mass spectrum is recorded. However, deuterated analogs are usually the more economical choice (imatinib-D_8 was used in the presented method). In contrast to most other methods, where similar compounds must be used, the use of isotopically labeled standards gives greater confidence in assessments of sample preparation losses and biases. While LC-MS with SIM can often be sufficient, as it was in the presented method, interfering species having similar mass-to-charge (*m/z*) ratios and elution times to targets will sometimes be encountered. If modifications to the chromatographic method are not successful in separating target from interfering molecules, selected reaction monitoring (SRM) can be used. SRM relies on MS/MS to distinguish ions having the same or similar *m/z* ratios. In this technique, characteristic fragment ions are monitored in addition to the molecular ion. By following a number of fragments (or transitions) for each analyte, exceptional selectivity can be achieved. However, as mentioned above, single-quadrupole instruments, such as that used in the presented method, are not appropriate for this approach (in-source fragmentation MS/MS is possible in single-quadrupole mass spectrometers, but targeted ions cannot be isolated prior to fragmentation).

As referred to previously, the chief drawbacks of LC-MS approaches are often held to be cost and the requirement for highly skilled personnel. Furthermore, sample preparation protocols designed for other detection methods can sometimes be difficult to adapt for MS (e.g., ESI is incompatible with detergents). While current single-quadrupole instruments are among the

Conflict-of-interest disclosure:
The author declares no competing financial interest

Submitted: 1/4/2013
Accepted: 2/6/2013

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DOI: 10.5581/1516-8484.20130024

most robust and accessible mass spectrometers available, training requirements are certainly more demanding than for techniques like LC with absorbance detection. However, well-designed software can somewhat alleviate such concerns. In the future, cost-control may be enhanced through innovative solutions where, for example, multiple chromatographic systems share a single mass spectrometer[7]. Runs on each LC system are staggered, with eluate only directed toward the MS interface when targeted peaks are expected. Since elution windows for key peaks are often only a small fraction of the total operating cycle time, sample throughput can be greatly increased without requiring additional MS instrumentation.

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