A REVIEW: USE OF DEUTERATED INTERNAL STANDARDS IN MASS SPECTOMETRY TECHNIQUES

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Immunosuppressive drugs are used to prevent transplant rejections in organ-transplanted patients but there has to be effective therapeutic drug monitoring because an inadequate or incorrect dosage could lead to intoxication or transplant rejection. There have been many designed protocols for the quantification of these drugs and most of them involve the use of high performance liquid chromatography but Armin et al in their research work chose to design a protocol following the mass spectrometry method which is an upcoming method due to its specificity and sensitivity which was observed to be notably higher. Five immunosuppressive drugs which included Cyclosporine A, Tacrolimus, Sirolimus, Everolimus and Mycophenolic acid were evaluated in whole blood and plasma using deuterated internal standards.

After observing that previous methods using high performance liquid chromatography lacked appropriate internal standards / mycophenolic acid to complete the analytic spectrum, the authors decided to use mass spectrometry method incorporating deuterated standards. Cyclosporine A, Tacrolimus, Sirolimus, Everolimus were measured in whole blood while mycophenolic acid was measured in plasma. In order to minimize imprecision, elaborate sample preparation was required to separate the molecules of interest from patients' blood matrix molecules.

Generally, the sample preparation consists of precipitation with a mixture of zinc sulphate and organic solvents. The authors made sure that the protocols used were according to internationally accepted guidelines. They also discovered that adding water before sample precipitation prevented sample clotting and improved extraction efficiency but this was observed to also increase the dilution which makes the need for a highly mass spectrometer almost inevitable. An intra-assay precision test was carried out in which each concentration was extracted five times and measured in series.

During the erythrocyte lysis stage of the protocol, the authors discovered that the use of water alone for lysis left some erythrocytes undamaged when examined microscopically, they then introduced treatment which yielded complete erythrocyte lysis which is a mandatory criteria for good reproducibility. The data obtained from the mass spectrometry analysis showed that all

drugs revealed linear behavior up to the highest concentration of calibrator Assay sensitivity as well as data forprecision and accuracy exceeded clinical requirements. Retention of analytes and deuterated standards were concordant in time, whereas the common standards showed a slight difference in retention time.

The authors were able to overcome the challenge of accuracy and precision which is usually the case when internal standards are used by using deuterated standards and this served as a plus especially in the quantification of cyclosporine A. Furthermore, it was noted that the use of this deuterated standards compensated for most of the measurement errors that could have resulted from either ion suppression or enhancement since deuterated standards co-elutes with the analytes.

Surprisingly, the authors did not fail to state some of the challenges encountered in the course of their research such as identification of most appropriate sample dilution, matrix composition of material used and asserting the purity of the reagents used. It is worthy to note that the results of their findings were also compared with those of other laboratories and from this they suggested that it is not only the immunoassays that need to be standardized but also the physical methods.

From the above results and discoveries, the mass spectrometry was seen to have better specificity, higher sensitivity and was considered to be “economic”. Although the use of the term economic by the authors was not very clear because in stating the likely demerits of the mass spectrometry method they included high acquisition costs alongside the need for academic expertise, complete validation process and back-up system.

Commendation goes to the authors for maintenance and cost reduction wits as they stated that rinsing with methanol for one minute during separation in the analytical column lifetime to at least three months or 4500 analyses. Overall, the mass spectrometry should be subjected to thorough validation process as the authors already suggested in their work, and also mass spectrometry standards should be established and made available so that results from different laboratories can be reliably compared. More people should also be trained on the mass spectrometry techniques in order to include the available technique since it will help overcome one of the challenges associated with setting up the mass spectrometry method.

The authors have done a good job by writing the paper with an enticing flow and choice of words that carries along the reader to the very end.

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