Metabolomic Analysis using the Absolute*IDQ*™ p150 Kit in combination with the AB Sciex 5500 QTrap® Mass Spectrometer

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Introduction

The Absolute*IDQ*™ p150 Kit is a commercially available product for targeted metabolomics which can simultaneously identify and quantify a large number of endogenous metabolites in small sample amounts. The Kit enables the quantification of 163 metabolites from 4 different compound classes (amino acids, acylcarnitines, glycerophospho-/sphingolipids and hexoses) in a single assay. The assay is performed using a flow injection analysis method (FIA-MS/MS). The Kit was developed and validated on the AB Sciex API 4000™ and 4000 QTrap® triple quadrupole mass spectrometers. However, the new AB Sciex 5500 QTrap® instrument is also widely distributed, and there is a demand for running the Absolute*IDQ* Kit on this instrument. The main difference between the two instruments is the higher sensitivity of the 5500 system. We tested and evaluated the analytical performance of the Absolute*IDQ* Kit on the 5500 QTrap. In this application note, we report on its performance results and the observed differences compared to the 4000 QTrap instrument.

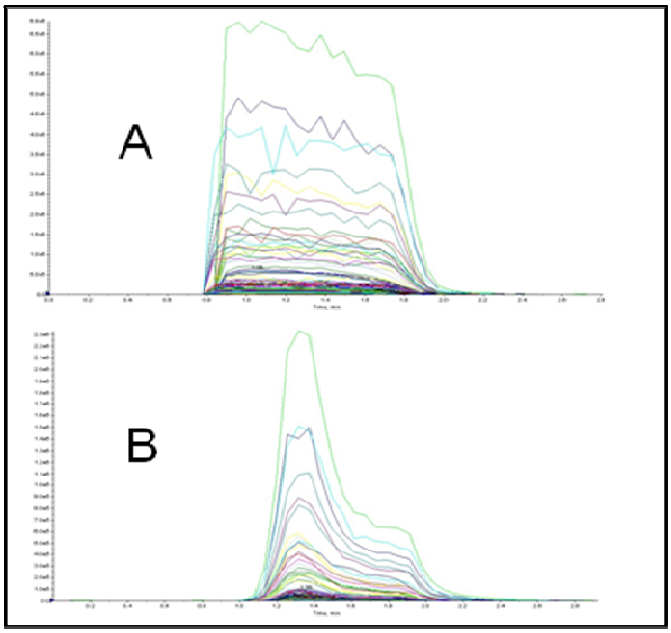

Figure 1: Examples for extracted ion chromatograms (XIC) obtained with 5500 QTrap (A) vs. 4000 QTrap (B). *Identical samples (Biocrates Quality Control samples) were used. The XICs obtained in positive ion mode are shown.*

Methods

The Absolute*IDQ* Kit was prepared as described in detail in the User Manual. We analyzed three quality control samples (QC1, QC2 and QC3) that are provided with each Kit. The QCs represent lyophilized human plasma samples containing metabolites in different concentration levels for respectively low (unspiked), medium (spiked) and high (spiked) QCs. Each QC was employed in five replicates using the standard sample volume of 10 µL. We also applied a series of 5 µL sample volume in addition to the standard volume. After completing the plate preparation and metabolite extraction, the samples were filtered by centrifugation. The obtained filtered extracts were finally diluted with the specified FIA running buffer. Because of the high sensitivity of the 5500 instrument, we generated dilutions of 1:100, 1:75, 1:50 and 1:25 (for the 4000 instrument, a 1:3 dilution is used as standard practice). Therefore, a small amount of the extracts was transferred into another empty deep well plate and the running solvent was added. The acquisition methods for the 5500 QTrap instrument were adapted from the 4000 system, but not modified. The declustering potential values (DP) that were expected to be higher in the 5500 QTrap system were maintained, since we observed impairment when increasing the DPs. The curtain gas value was not changed either, and was kept at a value of 20. Increasing the curtain gas value had no significant impact on noise improvement.
To assess potential instrument-related differences, the same samples were analyzed on three different 5500 QTrap instruments (Novartis, DSM and Biocrates) and compared with measurements on a 4000 QTrap instrument (Biocrates). The standard flow injection method of the Absolute*IDQ* Kit, comprising two subsequent 20 µL injections (one for positive and one for negative ion mode), was applied for all measurements. Multiple reaction monitoring (MRM) detection was used for quantification.

Results and Discussion

Figure 1 illustrates the FIA signals that were obtained using the two instrument types. The filtrate dilution (1:3) established for the 4000 QTrap is a good compromise between acceptable ion suppression and intensity level. Increasing the dilution causes a loss of sensitivity, whereas decreasing amplifies suppression effects.

However, due to the higher diluted extracts that were injected into the 5500 QTrap, suppression effects were not observed over all tested dilution ratios. Thus, the aim is to make use of the high instrument sensitivity by selecting an adequate extract dilution.
In general, the data obtained compare well with each other and provide good results on reproducibility and accuracy. The coefficient of variation (CV) was compared separately for the different metabolite classes measured by the Absolute*IDQ* Kit. The CVs of all replicate series (using 5 µL and 10 µL sample volume) and of all extract dilution series, respectively, were calculated. They are exemplified in Figure 2 by means of two metabolite representatives. The determined CVs show excellent values throughout and are well below 15%. The results, however, clearly show increased CVs when a sample volume of 5 µL was applied.

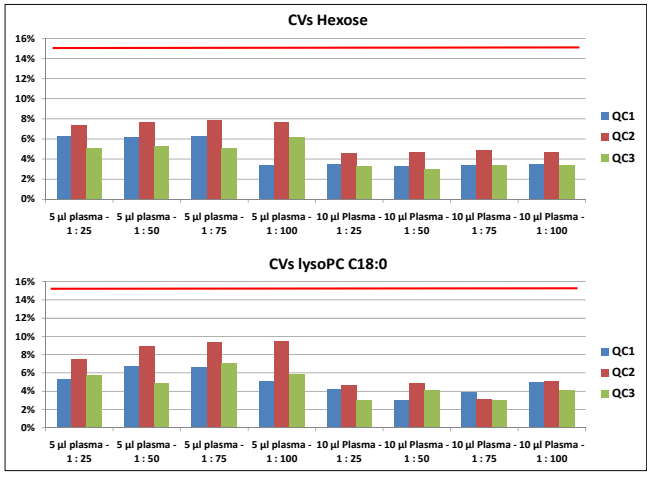

Figure 2: Comparison of CV values
Three quality control plasma samples (n = 5) were analyzed with different sample volumes and different extract dilutions. Mean values of the coefficient of variation (CV) were calculated.

In addition, also the accuracy of quantitation was slightly impaired when using a sample volume of 5 µL, as illustrated by two other metabolite representatives in Figure 3. For this reason, we do not recommend to pipette 5 µL sample volume to the Kit plate, but to maintain the standard volume of 10 µL.

After this finding, we focused on comparing the different intensity levels obtained by the different extract dilutions that were tested. We found that the 1:75 dilution delivers intensities close to those obtained by the 1:3 dilution on the 4000 QTrap system. Higher concentrated extracts cause no suppression effects on the 5500 QTrap instrument (as described above) and can be used with certainty to achieve more sensitivity. The 1:100 dilution leads to a low intensity level and is not recommendable.

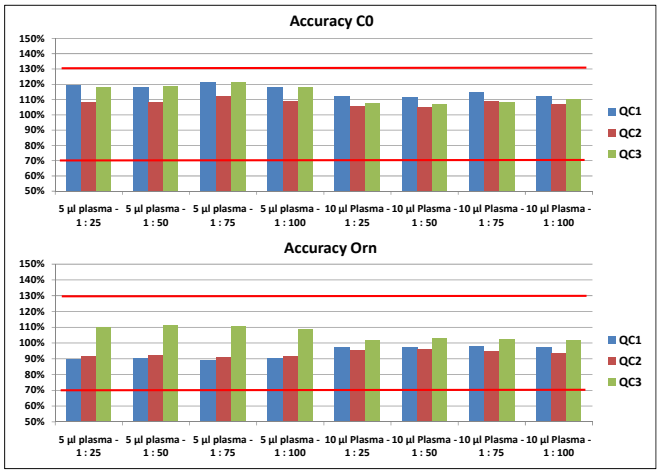

Figure 3: Comparison of accuracy values
Three quality control plasma samples (n = 5) were analyzed with different sample volumes and different extract dilutions. Mean values of the accuracy were calculated.

We found that the 1:50 extract dilution is a well-suited choice, since a high intensity level is obtained that is otherwise still well below the saturation range of the detector. In this regard, there is a higher risk when using a 1:25 dilution. We observed that the sensitivity can differ comparing several 5500 QTrap instruments and that each instrument behaves somewhat differently. A 1:25 extract dilution can also be applied, but should be done after consultation with the Biocrates customer support. Finally, the CV values obtained at the different test sites for the 1:50 dilution were compared and are shown in Figure 4.

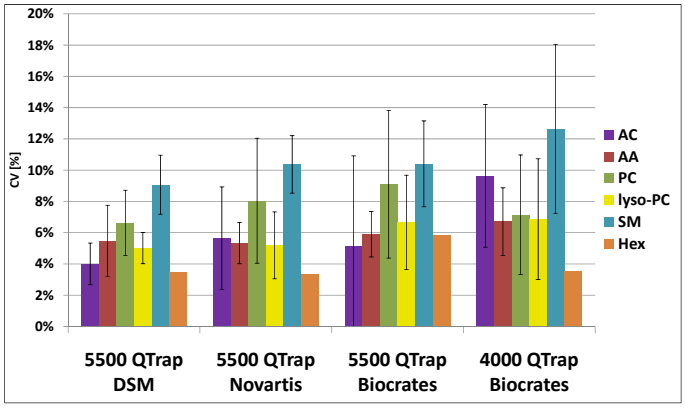

Figure 4: Comparison of CV values (three test sites)
The coefficients of variation (CVs) obtained by analyzing the unspiked quality control sample QC1 (n = 5, measured on four different instruments) are shown. 10 µL sample volume was pipetted and 20 µL of a 1:50 extract dilution was injected (1:3 dilution for the 4000 QTrap). Mean values of the CV and standard deviations were calculated for the different metabolites classes. Only analytes with values above the limit of detection (LOD) were considered.

Due to the high sensitivity of the 5500 QTrap, the noise level is also slightly increased, having an impact on the limit of detection (LOD) too. Therefore, for many analytes, the LOD was higher when using the 5500 QTrap instrument. The LOD was defined as 3 times signal to noise level, which was calculated by using zero samples with identical processing but without adding any plasma sample (10 µL PBS).

Met*IQ*™ Software

The proprietary Met*IQ* Software is an integral part of the Absolute*IDQ* Kit. In the MetVal module of the software, an automated quality assessment of the data is performed. One function of MetVal is to determine if the intensities for the blank and the internal standards are within the ranges set in the method operation procedure (OP). Since the Absolute*IDQ* Kit has been validated for the 4000 instrument, the values in its OP are only valid for this type of instrument. However, to enable customers with a 5500 instrument to use the MetVal module, these values have been adjusted in the Met*IQ* Software. Therefore, the higher intensities observed with the 5500 instrument were taken into account. However, a full validation of the method was not performed. A separate OP with the 5500 QTrap is available as a software patch for Absolute*IDQ* Kit users.

Conclusion

The data presented in this application note clearly reveal that the Absolute*IDQ* Kit can also be used in combination with the 5500 QTrap mass spectrometer. The Kit shows an excellent performance with this instrument with regard to reproducibility and accuracy. It is recommended to use a sample volume of 10 µL and an extract dilution of 1:50 for running the Kit assay on the 5500 QTrap. A specific Met*IQ* Software patch will automatically apply the increased intensity level caused by the higher sensitivity of the 5500 QTrap system.

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