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Metabolomic Analysis using the Absolute*IDQ*™ Kit in combination with the 3200 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 from different metabolite classes. The kit can detect and quantify 163 endogenous metabolites in small amounts (10 µL) of plasma samples. The kit was developed and validated on the API 4000™ and 4000 QTrap® triple quadrupole mass spectrometers. However, the 3200 QTrap® instrument is also widely distributed, and there is also demand for running the Absolute*IDQ* kit on this instrument. The main difference between the two instruments is the lower sensitivity of the 3200 series. In this application note, we tested and evaluated the performance of the Absolute*IDQ* kit on the 3200 QTrap instrument and report on the observed differences compared to 4000 QTrap instruments.

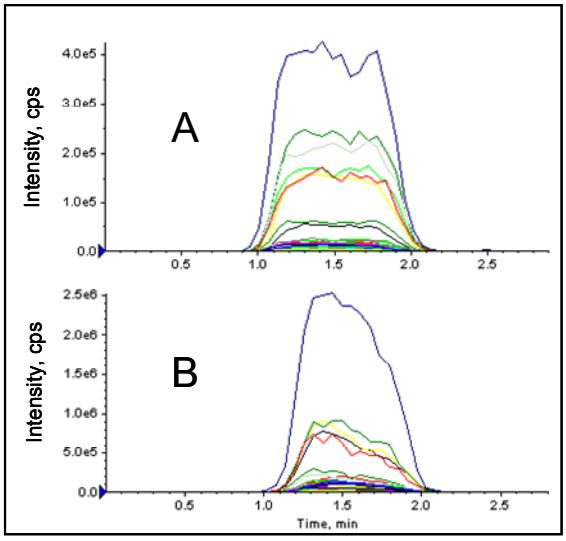

Figure 1: Examples for extracted ion chromatograms (XIC) obtained with 3200 QTrap (A) vs. 4000 QTrap (B). *Identical samples (Biocrates Standard) were used. The XICs obtained in positive mode are shown.*

Methods

The Absolute*IDQ* kit was prepared as described in detail in the User Manual. Acquisition methods for the 4000 QTrap instrument were converted using the *"Convert Method"* script in the Analyst® Software. The conversion affected the values for declustering potential (DP), which are lower in the 3200 QTrap system. The other MS parameter (CE and CXP) were kept identical, whereas the 3200-specific mass dependant values (CEP) were taken from default calibration curves.
To assess potential instrument-related differences, the same samples were analyzed on two different 3200 QTrap instruments and compared with two 4000 QTrap instruments. In addition to Standards and Quality Control samples that are provided with each kit, a pooled human plasma sample was analyzed in five replicates. The standard flow injection method of the Absolute*IDQ* kit comprising two subsequent 20 µL injections (one for positive and one for negative detection mode) was applied for all measurements. Multiple reaction monitoring (MRM) detection was used for quantification.

Results and Discussion

Figure 1 shows the intensities obtained using the two instrument types. Intensities (counts per second, cps) are about 5-10 times lower using the 3200 QTrap instrument. Furthermore, the obtained data are comparable, and the same MRM pairs exhibited the highest relative intensity in both cases.
Additionally, the coefficient of variation (CV) was compared separately for the different metabolite classes measured by the Absolute*IDQ* kit. Identical plasma samples were processed and analyzed with four different instruments. The results showed that the mean of the CV values using the 3200 QTRAP was below 15 % for all metabolites classes and even below 10 % for some classes (Figure 2). Furthermore, it was clear that each instrument behaves somewhat differently. In general, the CV values were slightly lower using the 4000 QTrap instrument. For many analytes the limit of detection (LOD) was, as expected, somewhat worse using the 3200 QTrap instrument. The LOD was defined as 3 times signal to noise level, which was calculated by using a zero sample with identical processing but

without adding any plasma sample (10 µL water or buffer).

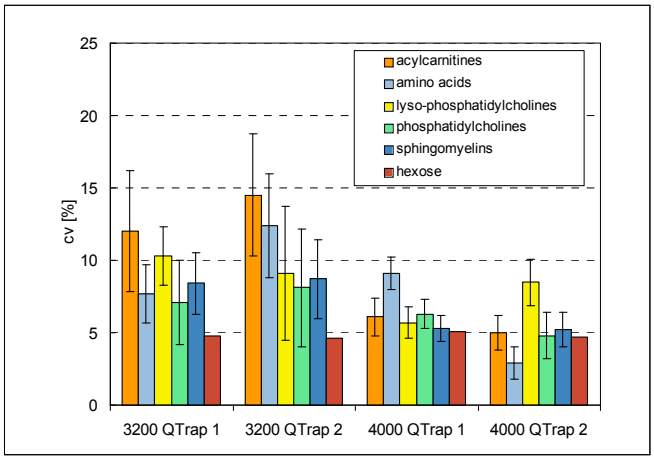

Figure 2: Comparison of CV values
Pooled human plasma samples (n=5) were analyzed with four different instruments. Mean values of the coefficient of variation (CV) and standard deviations were calculated for the different metabolites classes. Only analytes with values above LOD were used.

Met*IQ*™ Software

The proprietary Met*IQ* software is a central 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 SOP. Since the Absolute*IDQ* kit has been validated for the 4000 instrument, the values in its SOP are only valid for this type of instrument. However, to enable customers with a 3200 instrument to use the MetVal module, these values have been adjusted in the Met*IQ* software. By doing this the lower intensities that have been observed with the 3200 instrument were taken into account, however a full validation of the method was not performed. A separate operating procedure (OP) with the 3200 QTrap is available as software patch for Absolute*IDQ* kit users.

Comparison of two plasma datasets

For further comparison 82 human plasma samples from four different groups were independently prepared on two different Absolute*IDQ* kit plates and measured with both a 3200 QTrap (Hannover Medical School) and a 4000 QTrap instrument (Biocrates). The data were analyzed using statistical tools integrated in the latest version of the Met*IQ* software. In general metabolites that revealed significant differences (p-values ≤ 0.0001, Kruskal-Wallis test) clearly exhibited a similar behaviour across all groups, independent of the assay preparation and the instruments used. An example of this is shown for two kit metabolites in figure 3. Thus, the variations due to sample preparation or use of different instruments are significantly lower than the observed changes in the metabolite concentrations for the four patients groups. The concentration of one amino acid was clearly higher in the healthy control group, whereas a phosphatidyl-choline (PC) was increased after treatment with drugs, even more pronounced with drug 1.

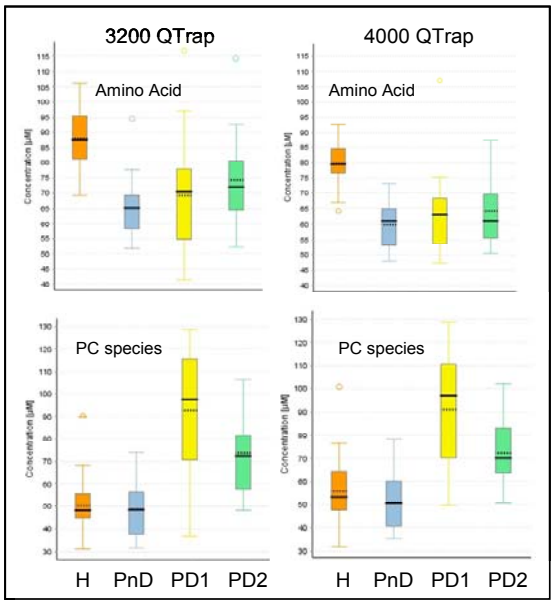

Figure 3: Box plots for selected metabolites.
The four groups consisted of 17-22 samples each of healthy control (H), patients without treatment (PnD), patients treated with drug 1 (PD1) and patients treated with drug 2 (PD2)

Conclusion

The data presented in this application note clearly reveal that the Absolute*IDQ* kit can also be used in combination with the 3200 QTrap mass spectrometer. A specific Met*IQ* software patch will automatically apply changes to account for the lower sensitivity of the 3200 series.

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