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Detection of Metabolites in Different Animal Plasma Using the Absolute*IDQ*™ p150 Kit

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Introduction

The Absolute*IDQ* kit is based on a targeted metabolomics approach. This approach aims to simultaneously identify and quantify a large number of endogenous metabolites from different metabolite classes. The recently launched Absolute*IDQ* p150 kit allows the quantification of 163 metabolites from different compound classes (Table 1). It has been validated for human plasma samples according to the *FDA Guidance for Industry „Bioanalytical Method Validation".*
Analysis of plasma samples from different animal species is of high interest in a variety of research areas, for example in pharmaceutical and toxicological research. In this application note, we have tested and evaluated the AbsoluteIDQ kit with different animal plasma. Pooled murine, rat, canine, porcine and bovine plasma have been used. Below are the observed results.

Table 1: Metabolites in the AbsoluteIDQ™ p150 Kit

Metabolite Class	Analytes
acylcarnitines (AC)	41
amino acids (AA)	14
hexose (H1)	hexose pool
lyso-phosphatidylcholines (LPC)	15
phosphatidylcholines (PC)	77
sphingomyelins (SM)	15

Methods

The plasma samples listed in Table 2 were analyzed and compared with human plasma. The Absolute*IDQ* kit was prepared as described in detail in the User Manual. In brief, 10 µL of plasma was added to the center of the filter on the upper 96-well kit plate, and the plasma samples were dried using a nitrogen evaporator. Subsequently, 20 µL of a 5 % solution of phenyl-isothiocyanate was added for derivatization of the amino acids. After incubation, the filter spots were dried again using a nitrogen evaporator. The metabolites were extracted using 300 µL of a 5 mM ammonium acetate solution in methanol. The extract was obtained by centrifugation into the lower 96-deep well plate.

The extracts were diluted with 600 µL of the MS running solvent and were analyzed using a 4000 QTrap® instrument (Applied Biosystems/MDS Sciex). The standard flow injection method of the Absolute*IDQ* kit using two subsequent 20 µL injections (one for positive and one for negative mode analysis) was applied for all measurements. Multiple reaction monitoring (MRM) detection was used for quantification.

Table 2: Characteristics of different animal plasma

Plasma	Species	Gender	Age	Anti-coagulant
murine	B57BL6	mixed	8-11 weeks	EDTA K3
rat	Wistar	mixed	8-11 weeks	EDTA K3
canine	Beagle	mixed	> 1 year	EDTA K3
porcine	not specified	mixed	not specified	EDTA K3
bovine	F2 Charolais × deuts. Holstein	mixed	> 6 months	EDTA K3

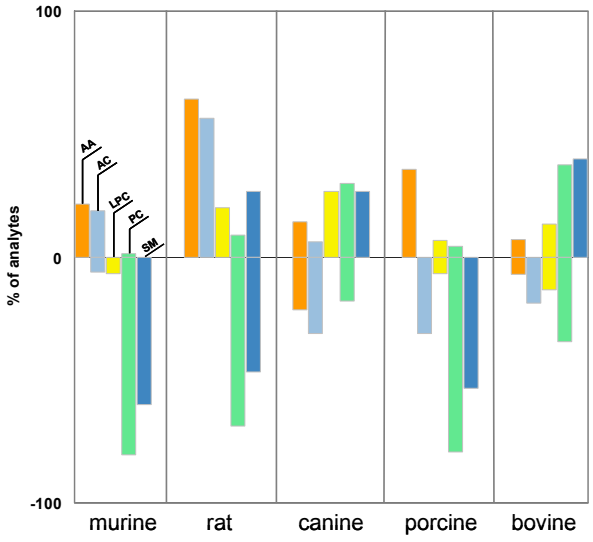

***Figure 1: Comparison of median metabolite concentrations in animal plasma with the Absolute**IDQ* **kit.** *The percentage of metabolites within a given class that exhibited significantly higher (150 %) or lower (50 %) concentration values compared to human plasma is given. For the figure, only analytes with values greater than LOD were used, i.e. 14 amino acids (AA), 16 acylcarnitines (AC), 15 lyso-phosphatidylcholines (LPC), 67 phosphatidyl-cholines (PC), and 15 sphingomyelins (SM).*

Results and Discussion

A full validation of the kit method for the different animal plasma was outside the scope of this application note. However, several important parameters were evaluated with the aim to give confidence of using the Absolute*IDQ* kit with different animal plasma.
In Figure 1, a class-wise comparison of the metabolite concentrations in the different animal plasma is shown. This figure provides an overview of the percentage of metabolites in the different classes in which the concentration ranges differ more than ± 50 % when compared to human plasma.

Internal Standards

The kit's internal standards (IS) are essential for quantification. Therefore the signal intensity of the MRM pairs of the IS was evaluated in relation to the values obtained for human plasma. This supplies information about ion suppression in the different plasma. Furthermore, the unusual increase in intensity for certain MRM transitions would indicate the presence of an interfering substance in the tested animal plasma.
In general, lower intensities (increase of ion suppression) were found for the IS of several amino acids in murine, rat and canine plasma and for some acylcarnitines in rat plasma. However, the intensities were high enough to still allow good quantification. There was no indication of interfering substances in any of the animal plasma.

Coefficient of variation (CV)

For all tested animal plasma the intraday CVs of metabolite concentrations within the evaluated quantification range (defined in the Analytical Specifications p150, Absolute*IDQ* kit) were below 15 % and in many cases, even below 10 %. There were only 5 phosphatidylcholines, namely PC aa C42:0, PC aa C42:1, PC ae C42:2, PC ae C44:3, PC ae C44:4, that exhibited CV values of 20-25 % in some animal plasma.

On-board stability

The on-board stability is defined as the time frame in which the kit plate can be processed after the sample material has been added (Kit plate stored at 4°C in the dark). For bovine plasma the on-board stability was 6 hours. Canine, rat, murine, and porcine plasma were less stable, hence a direct processing of the kit plate after sample addition is necessary. Especially lyso-phosphatidylcholines exhibited increased concentration values after 6 hours storage. This could be explained by intrinsic lipase activity that seems to differ significantly in the investigated animal samples.

In addition to these general findings, information is given below regarding metabolite concentrations significantly differing in comparison to human plasma.

Acylcarnitines

In a typical pool of human plasma (from healthy people) there are on average 23 of 41 measured acylcarnitines below the limit of detection (LOD). Canine, porcine and bovine plasma exhibited an even higher number of analytes that were below LOD (32, 36 and 35, respectively). To a small extent, this was also the case for rat and murine plasma, although, in comparison to human plasma, the concentrations of C14, C16:1, C18:1-OH and C6 (C4:1-DC) in rat plasma were measurable (above LOD). Remarkably, in rat plasma, the concentration of 9 different acylcarnitines exceeded the human ones by more than 2-fold.

Hexose

As shown in figure 2 murine and rat plasma exhibited dramatically higher hexose (H1) concentrations than human plasma. This can be explained by the significantly higher energy metabolism in rodents.

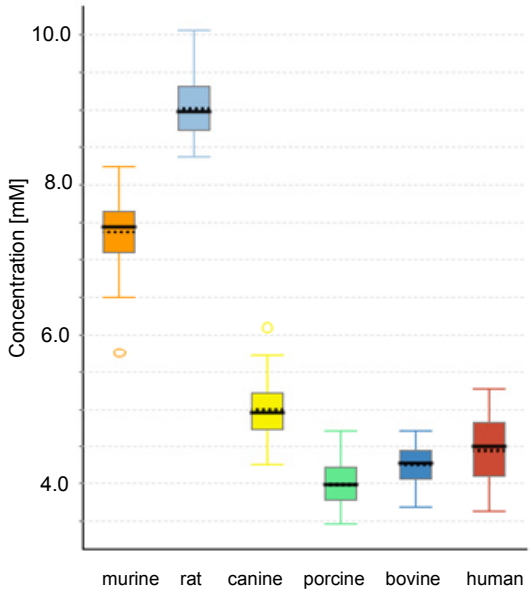

Figure 2: Median hexose (H1) concentration in the different plasma samples

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Amino acids

In comparison to human plasma the concentration values in canine plasma of the amino acids glycine, ornithine and tyrosine plasma were less than 50 %; in bovine plasma this was true for the proline concentration. Most of the amino acid concentrations were between 50 to 150 % of the human values. Extremely increased concentrations were measured for rat plasma: 9 amino acids (out of 14) values were higher than 150 % of the human level (Gln, Gly, Met, Orn, Phe, Ser, Thr, Try, xLeu).
This was also partly the case for murine (Met, Phe, Orn), canine (Gln, Thr), porcine (Gly, Met, Orn, Thr, xLeu), and bovine (Thr) plasma.

Lyso-phosphatidylcholines

The concentrations of 5 lyso-phosphatidylcholines (lysoPC) were below LOD in pooled human plasma. There was one additional analyte below the LOD in both rat and murine plasma: lysoPC a C28:1. However, the concentration of lysoPC C14:0 was high enough in rat, porcine and bovine plasma that it exceeded LOD in contrast to human plasma. All animal plasma also exhibited levels of certain lysoPCs that were not detectable human material: lysoPC C24:0 (in all 5 species) and lysoPC C26:0 (only in bovine).

Phosphatidylcholines

In murine and rat plasma one phosphatidylcholine was detectable which was not detectable in human: PC ae C42:0. In general, for mouse, rat and porcine plasma, the majority of PC concentrations were significantly below 50 % of the human plasma levels though they were still in the detection range. In contrast, a majority of canine and bovine PC concentrations exceeded 150 % of the human levels and exhibited a species-specific pattern.

Sphingomyelins

The sphingomyelin concentrations of all analyzed plasma lay in the detection range. Qualitatively, canine and bovine plasma exhibited primarily elevated levels of sphingomyelins in comparison to human plasma. A more than 10-fold higher concentration of SM C26:1 in bovine plasma is a remarkable example of this. On the contrary, in murine (9 out of 15) and porcine plasma (8 out of 15), a majority of sphingolipid concentrations were measured at values below 50 % of human. Rat plasma was the only plasma with more varied pattern of concentration levels: While analytes with long side chains were higher, e.g. SM (OH) C24:1, SM C26:0, analytes with side chain lengths below C20 were lower in concentration (clearly less than 50 %) in comparison to human plasma values.

Conclusion

The data presented in this application note clearly reveal that the Absolute*IDQ* kit, normally specified for human plasma, can be used for analysis of murine, rat, canine, porcine and bovine plasma samples. Additional information on the 163 analyzed metabolites in the different animal plasma is provided as supplementary information for Absolute*IDQ* kit users upon request.

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