The present LC/MS method was developed for the monitoring of dog plasmatic concentration of
glucosamine (GLcN). In this scope, relatively low plasmatic concentrations of GLcN were expected,
ranging from 50 ng/mL to 1000 ng/mL. As this method must be the most sensible, liquid chromatography coupled to simple quadrupole mass spectrometry detection (LC/MS) was selected
thanks to its efficiency and usefulness for this objective. Additionally, a solid phase extraction (SPE)
step was performed to avoid matrix effect as well as ion suppression.
Due to the ionisable character of the compound of interest, a mixed-mode strong cation exchange
(Plexa PCX) disposable extraction cartridge (DEC) was selected. 350 µL of the plasma sample was
first acidified with 35 µL of trichloroacetic acid (10%). 25 µL of miconazole, the internal standard (IS),
at 1000 ng/mL were then added. 325 µL of resulting sample were treated. The cartridges were
successively conditioned with 1 mL of acetonitrile and 500 µL of a 20 mM formic buffer at pH=3.0. The
washing step was realized with 500 µL of acetonitrile. The elution was led using 1 mL of a mixture of
acetonitrile and 5% ammonia (70/30, v/v). Following this extraction, the solution was evaporated to
dryness and the residue was recovered in 150 µL of a 5 mM ammonium hydrogen carbonate buffer at
pH=7.5. Finally, 20 µL of this last solution were injected into the chromatographic system.
The separation was carried out on an Agilent Zorbax SB-CN column (250 x 4.6, 5 µm) using a mobile
phase consisting in a mixture of methanol and 5 mM ammonium hydrogen carbonate buffer at pH=7.5
(95/5, v/v). The detection was led at a m/z ratio 180.0 and 417.0 for GLcN and IS respectively.
Reliability of the results was demonstrated through the validation of the method using an approach
based on the accuracy profile [1-2], allowing to manage the risk associated to the use of these methods
in routine analysis [3]; it is thus guaranteed that each future results will fall in the +/-30% acceptance
limits with a probability of at least 95%.
This study was sponsored by KitoZyme sa (Belgium).

[2] Ph. Hubert et al, Harmonization of strategies for the validation of quantitative analytical