Determination of stress-related hormones in horse hair and sheep wool by means of liquid chromatography coupled to hybrid high resolution mass spectrometry: validation and application to real samples

A. Manucci[1], R. Rosato[1], M. Ricci[1], F. Castellani[1], F. Cerasoli[1], M. Podaliri[1], G. Centorotola[1], L. Iannetti[1], E. Dalla Costa[2], E. Atallah[2] and G. Saluti*[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100, Teramo

[2]Università degli Studi di Milano – Dipartimento di Medicina Veterinaria e Scienze Animali, Via dell'Università, 6, 26900, Lodi

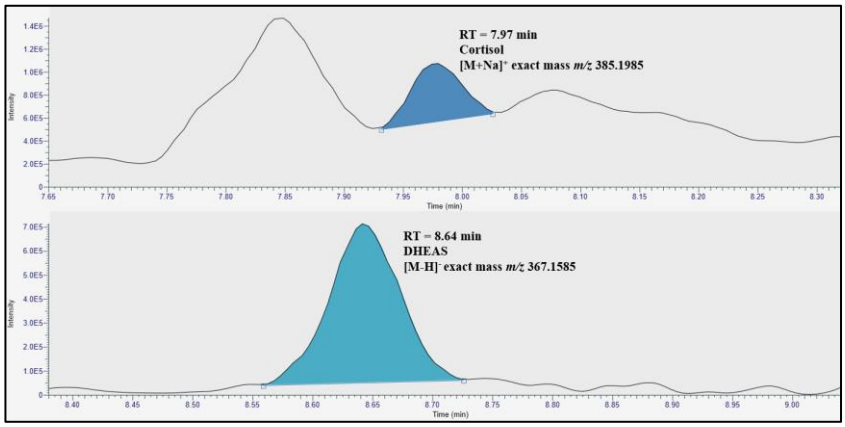

Figure 1 – Chromatogram of cortisol and DHEAS in a wool sheep sample at 13 and 8 pg mg^{-1}, respectively.

Concentration levels of molecules linked with emotional state can be an animal health and welfare assessment tool. Cortisol, its metabolite cortisone, dehydroepiandrosterone (DHEA) and its sulfate metabolite (DHEAS) have been taken into account as stress-related hormones [1, 2]. The development/validation of a quantitative method for endogenous compounds is challenging because of the difficulty for obtaining analyte-free authentic matrices as quality control samples, so surrogate analytes (i.e., cortisol-$^{13}C_3$, cortisone-$^{13}C_3$, DHEA-$^{13}C_3$, DHEAS-$^{13}C_3$) have been used to circumvent the issue, thanks to their similar physico-chemical properties. After the optimization of LC-HRMS/MS conditions and preliminary experiments using the previously published sample preparation protocol, the method was validated on sheep wool and horse mane hair sample according to ICH guideline [3 - 5]. During the validation study, 40 experiments (five replicates for each spiking level repeated on two different days) were carried out at four different concentrations (i. e. 5, 10, 20 and 100 pg mg^{-1}). The estimated performance characteristics (selectivity, linearity, accuracy, lower limit of detection) were satisfactory. By way of example, apparent recoveries obtained by isotopic dilution, ranged between 85 % and 109 % and the coefficients of variation were lower than 15 % (within-run precision and between-run precision) for both of the investigated matrices.

Afterthat, the method has been applied to several samples of animals living in marginal areas and Figure 1 shows the chromatogram of cortisol and DHEAS in a wool sheep sample.

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