

PROJECT REVIEW

"Development of a two-dimensional HPLC method for the GC/C/IRMS analysis of corticosteroids and low concentrated urinary metabolites"

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The project that we propose implies the development of a novel two-dimensional HPLC purification method for the carbon isotopic analysis of two groups of molecules, corticoids and low concentrated steroids. Prednisolone is a direct metabolite of the synthetic corticoid prednisone but it can also be derived from the dehydrogenase bacterial enzymatic activity of cortisol. In the same manner cortisone, the parent molecule of cortisol, could produce considerable quantities of prednisone as well, yielding false positive cases. Additionally, numerous methods for the purification and ^{13}C analysis of boldenone and nandrolone metabolites have been developed in the past years; however, to our knowledge, none yet has proven to produce measurements as precise and accurate as the testosterone metabolites, being unquestionably more concentrated.

Furthermore two HPLC purifications are commonly required for their purification, from which testosterone metabolites are left aside due to the complexity of the separation even if the latter come necessary in the detection of multi-positives cases. To ascertain the exogenous origin of the corticoids, difficult to chromatographically purify due to their higher polarity, and to efficiently purify boldenone or nandrolone metabolites including testosterone metabolites, the development of a novel two dimensional HPLC method through the heart-cutting technique is sought for a rapid purification of those compounds from the urine matrix without any derivatization. The rationale behind this method development is (i) to save instrumental time for the purification of the compounds, (ii) obtain the same purification power as with two subsequent HPLC cleansing runs and (iii) to gain intensity, precision and accuracy owed to fewer manipulations and chemical reactions. Finally, we wish to validate the methods by confirming the absence of isotopic fractionation through the analysis of all the tested metabolites using the developed HPLC technique and to publish the methods used for routine $\delta^{13}\text{C}$ measurements.