# Plackett-Burman design as a tool to develop a generic extraction for U-HPLC-Orbitrap-MS analysis of glucocorticoids in livestock urine.

Nathalie De Clercq[1], Julie Vanden Bussche[1], Siska Croubels[2], Philippe Delahaut[3] and Lynn Vanhaecke[1]

[1]Ghent University, Faculty of Veterinary Medicine, Research group of Veterinary Public Health and Zoonoses, Laboratory of Chemical Analysis, Salisburylaan 133, B-9820 Merelbeke, Belgium.
[2]Ghent University, Faculty of Veterinary Medicine, Department of Pharmacology, Toxicology and Biochemistry, Salisburylaan 133, B-9820 Merelbeke, Belgium.
[3]CER Groupe, Département Santé, Rue du Point du Jour 8, B-6900 Marloie, Belgium.

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The natural glucocorticoids, cortisol and cortisone, are steroid hormones secreted by the adrenal cortex. Their anti-inflammatory properties have led to the development of synthetic glucocorticoids exerting even higher inflammatory activities, including prednisolone and methylprednisolone, nowadays commonly used as therapeutic drugs. Besides, corticosteroids are known to display growth-promoting effects, which has led to their illegal abuse in livestock. In order to protect the consumer against potential harmful residues present in the derived animal products, their use for growth-promoting purposes in cattle has been prohibited within the European Union (EC Council directive 96/22). Recent developments within the field of analytical chemistry and the resulting lowering limits in detection, have resulted in the regular detection of prednisolone-positive bovine urine samples. In order to determine the origin of this prednisolone, a general extraction protocol was developed. The sample preparation was based on a two-step liquid-liquid extraction with *tert*-butyl methylether and was developed by using a Plackett-Burman design. This experimental design identifies the most significant independent factors in a minimal number of experiments. The time saving approach allows estimating all main effects clear of any confounded two-factor interactions and does not describe interaction between factors. Analysis was executed on an ultra-high performance liquid chromatograph coupled to an Orbitrap mass spectrometer and successfully validated accordingly 2002/657/EC. The limit of quantification (LOQ) for dihydrocortisone, cortisol and cortisone ranged from 0.3-0.8 µg $L^{-1}$. $CC_{\beta}$s for prednisolone, prednisone and methylprednisolone were respectively 0.4 µg $L^{-1}$, 0.3 µg $L^{-1}$ and 0.8 µg $L^{-1}$. Recovery ranged from 85%-105%, repeatability (RSD) and within-laboratory reproducibility (RSD) were lower than 13.9% and 16.4% respectively. This method allowed the simultaneous and accurate analysis of the endogenous (cortisol, cortisone) and exogenous (prednisone, prednisolone, methylprednisolone) glucocorticoids and potential metabolites or derivates thereof in urine and demonstrates the usefulness of a Plackett-Burman experimental design in generic extraction development.