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Development and Validation of Mitotane Determination in Plasma Samples by LC-DAD

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Introduction: Mitotane (o,p'-DDD) is the drug of choice for adrenocortical carcinomas (ACC) and its measurement in plasma is essential to control drug administration.

Objective: To develop and validate a simple, reliable and straightforward method for Mitotane determination in plasma samples.

Method: Drug free plasma samples were collected in potassium-ethylenediamine tetraacetate (K-EDTA) tubes and spiked with 1.0, 2.5, 10.0, 25.0 and 50.0 µg/mL of DDD. p,p'-DDD was used as internal standard and was added at 25.0 µg /mL to each sample, standards and controls. Samples were submitted to protein precipitation with acetonitrile and then centrifuged and 50 uL sobrenadant was injecting into a 1260 Infinity II liquid chromatography system (Agilent Technologies) and DDD and IS were detected at 230 nm by diodo array detector (DAD) in a 12 minutes isocratic mode with a solvent mixture of 60% acetonitrile and 40% formic acid in water 0.1% pump mixed, at 0.6 mL/min flow rate. Chromatography column Waters Acquity HSS T3 was kept at 28°C. We evaluated sensitivity, precision, presence of carry-over, recovery, linearity and the accuracy of this method.

Results: Our method resulted in a symmetrical peak shape and good baseline resolution for DDD (mitotane) and 4,4'-DDD (internal standard) with retention times of 6.0min, 6.4min, respectively, with resolution higher than 1.5. Endogenous plasma compounds did not interfere with

the evaluated peaks when blank plasma and spiked plasma with standards were compared. Linearity was assessed over the range of 1.0 to 50.0 $\mu\text{g/mL}$ for DDD ($R^2 > 0.9987$ and a 97.8% - 105.5% extraction efficiency). Analytical sensitivity was 0.98 $\mu\text{g/mL}$, functional sensitivity was 1.00 $\mu\text{g/mL}$, intra-assay and inter-assay coefficient of variation were less than 9.98%, carry-over was not detected, recovery ranged from 98% to 117% and high accuracy was observed (89.4% to 105.9%) with no matrix effects for mitotane measurements. Patient's samples results were compared with previous measurement by GC-MS method with a high correlation ($r=0.88$ and bias=-10.2%) showed that our method is useful for routine mitotane measurement.

Conclusion: DDD determination in plasma samples by our developed and validated method is simple, robust, efficient and sensitive for therapeutic drug monitoring and dose management in order to achieve therapeutic index of mitotane in patients with adrenocortical cancer.

Presentation: Saturday, June 11, 2022 1:00 p.m. - 3:00 p.m.