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(Poster)

DETERMINATION OF MITOTANE AND PRINCIPAL METABOLITE BY A SIMPLE HPLC-UV METHOD AND ITS VALIDATION IN HUMAN PLASMA SAMPLE

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Introduction

Mitotane (dichlorodifenyldichloroethane, DDD) it is a polychlorinated compound derivative of dichlorodiphenyltrichloroethane (DDT) that is prescribed to in inoperable adrenocortical renal carcinoma and Cushing's syndrome (1,2). DDD and its principal metabolite, dichlorodiphenylethene (DDE), can accumulate in fat tissues (3) and their plasma concentrations are more related with clinical improvement, than those of dichlorodiphenylacetate (DDA), another metabolite of DDD (4). Therapeutic monitoring of plasma concentrations is thus required to combine good therapeutic efficacy with acceptable toxicity. HPLC methods constitute a valid alternative to gas chromatography, and plasma concentrations of 14–20 mg/L are considered therapeutic concentrations.

Materials and Methods

Chromatographic conditions: stationary phase was represented by a Higgins Analytical C18 5 μm column (250mmx4.6 mm), maintained at 35 oC. Mobile phase was made by H2O/acetonitrile (10/90, v/v) and pumped at flow of 1.0 ml/min. Peaks of interest were monitored at wavelenght of 226 nm.

Human plasma sample preparation: 200 μl of plasma sample was added with aldrin (as Internal Standard) and 200 μl of acetonitrile for protein precipitation. Samples were vortexed for 30 sec and centrifuged at 12000 rpm x 10 minutes. Clear supernatants (50 μl) were injected within the HPLC apparatus.

Results

The average recovery of analytes was 95%, and the method was linear (r2=0.9988 and 0.9964 for DDD and DDE, respectively) within the range 1-40 mg/L. The values of limit of quantitation and detection were 0.2 mg/L and 0.3 mg/L for DDD and 0.066 mg/L and 0.099 mg/L for DDE, respectively. It is worth noting that sample preparation and run time are short enough to allow the analysis of at least 4 samples per hour (15 min total run). Indeed, the retention time (RT) of DDD and DDE are 7.06 min and 9.42 min, respectively, while the RT of the internal standard is 12.67 min. Finally, the validation process returned inter- and intra-day mean accuracies (1.30% and 1.60%, respectively) and precision (6.45% and 7.70%, respectively) within the limit of FDA guidelines. Therefore, the method has been adopted at the TDM Lab of Clinical Pharmacology Unit, University Hospital, Pisa, for routine monitoring of mitotane plasma concentrations in patients affected by adrenocortical renal carcinoma.

References:

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