

Analysis of amitriptyline and its metabolites

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AIMS

Amitriptyline, a tricyclic antidepressant, has been renowned for over half a century for its efficacy, pharmaco-economic benefits and potential adverse effects. Most analytical procedures utilised for recommended therapeutic drug monitoring only account for amitriptyline as the parent drug and nortriptyline as the active demethylated metabolite. This project aimed to determine the hydroxylated metabolites which may prove expedient due to possible clinical effects, particularly cardiovascular toxicity.

METHODS

Solutions of 100 µg/mL amitriptyline, nortriptyline, cis- and trans- hydroxy-amitriptyline, cis- and trans- hydroxy-nortriptyline, and clomipramine were prepared in HPLC-grade water and mixed together by transferring 0.5 mL of each solution into amber-coloured vials which were stored at 4 °C until analysed. Reversed-phase HPLC triplicate runs were carried out using Agilent 1260 Infinity Series[®] II liquid chromatography system with UV detection. A Kinetex[®] C18 LC Column at a temperature of 27 °C was used as stationary phase and acetonitrile and a phosphate buffer as mobile phase. Flow rate, pH and percentage of organic modifier were adjusted to optimise separation of the six tricyclic compounds and clomipramine as internal standard.

A simple chromatographic procedure was developed, for simultaneous assay of amitriptyline, nortriptyline and the isomeric hydroxy-metabolites. By attuning the critical parameters, adequate separation of these compounds, which are similar in structure, was attained.

CONCLUSION

The proposed method, including relevant sample preparation procedures, is apposite for validation in the analysis of pharmaceutical impurities and pharmacokinetic studies. Genetic and environmental factors determine enzyme activity which may be linked to variations in metabolite-to-parent drug concentration ratios and clinical events.

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RESULTS

Good chromatographic outcomes were achieved with isocratic conditions comprising of 31% acetonitrile and 69% phosphate buffer at pH5.6 as mobile phase, using a flow rate of 0.5 mL/min and a detection wavelength set at 210 nm. These parameters resulted in the separation of trans-10-hydroxy nortriptyline, trans-10-hydroxy amitriptyline, cis-10-hydroxy nortriptyline, cis-10-hydroxy amitriptyline, nortriptyline and amitriptyline, eluting at 4.3, 4.7, 5.4, 6.0, 15.9 and 19.9 minutes, respectively; Figure 1.

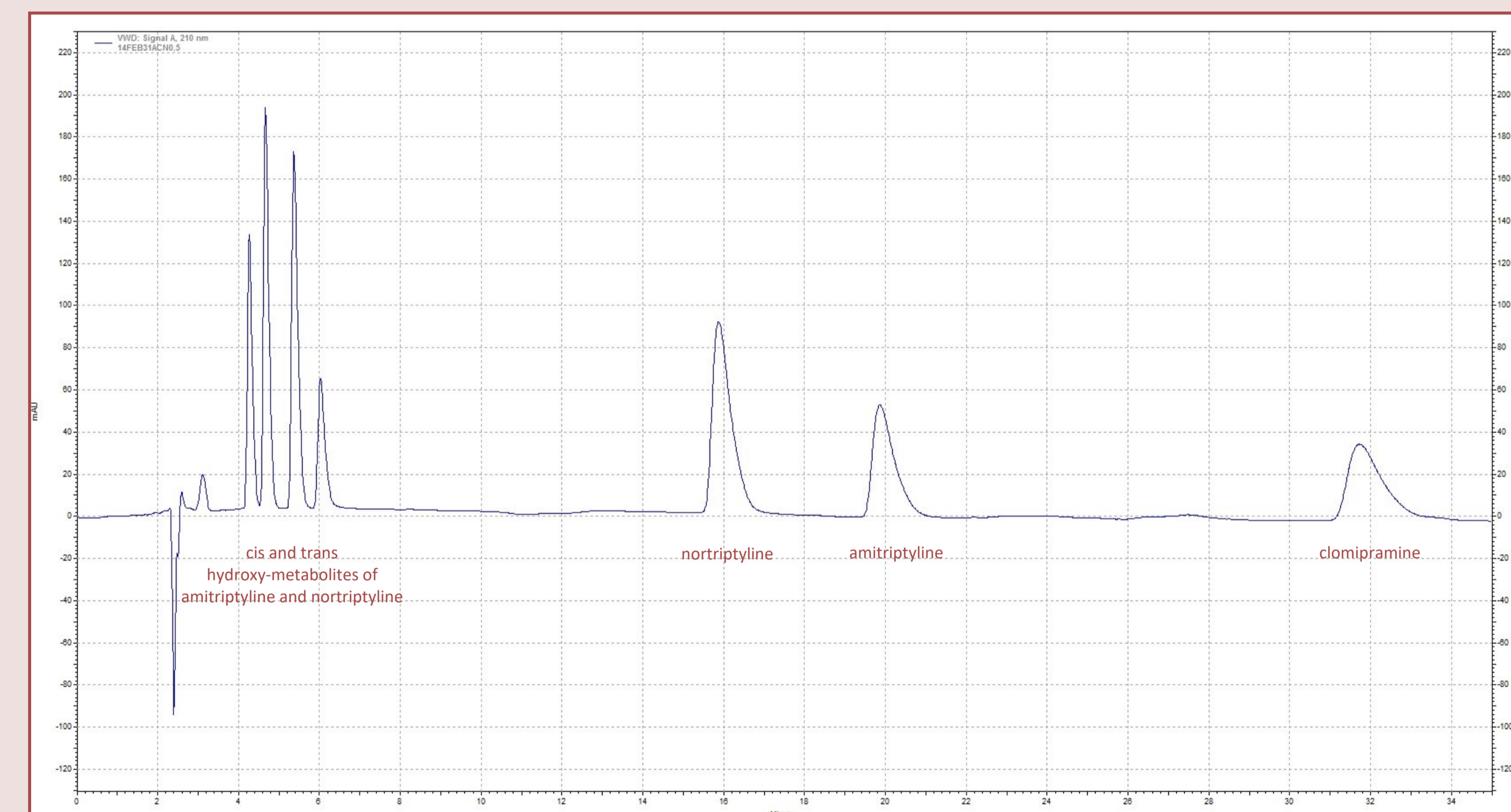


Figure 1: Chromatogram showing separation of amitriptyline, nortriptyline and the hydroxy-metabolites, with clomipramine as internal standard