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Technical note

Improved clean-up procedure for the high-performance liquid chromatographic assay of clomipramine and its demethylated metabolite in human plasma

I. Altieri, S. Pichini, R. Pacifici, P. Zuccaro*

Clinical Biochemistry Department, Istituto Superiore di Sanita', Roma, Italy

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Abstract

A rapid and selective assay of clomipramine and its metabolite desmethylclomipramine in human plasma, based on high-performance liquid chromatography with UV detection has been developed. The compounds were subjected to solid-phase extraction, using Extrelut 1 cartridges. Recoveries ranged between 88–95% for clomipramine, and 75–80% for desmethylclomipramine. This method has been used for therapeutic monitoring of clomipramine and its metabolite in individuals treated with this drug.

During the last five years, the method of Godbillion and Gauron [1] for high-performance liquid chromatographic determination of clomipramine and desmethylclomipramine has been used in our laboratory to monitor plasma concentration of these substances in individuals treated with the antidepressant drug. The disadvantage of HPLC method reported above is a laborious three-step liquid–liquid extraction procedure, which does not provide very clean plasma extracts and makes the analysis too long for therapeutic drug monitoring of a large number of samples. Furthermore, an investigation on the possible interferences with other drugs administered as a comedication, is needed especially for the new drugs commercialized in the last few years. Here we wish to report the improvements we developed using a solid-phase extraction procedure for plasma samples and a slightly modified mobile phase for chromatographic analysis. Extrelut-1 glass columns (Merck, Bracco, Milan, Italy) used for the extraction procedure are pre-packed columns filled with 700 mg diatomaceus earth. A 500-μl aliquot of plasma, with 100 μl of imipramine (0.5 μg/ml ethanolic solution) added as an internal standard, was mixed with 500 μl of 0.5 *M* NaOH and transferred to an Extrelut-1 glass column. After 10 min, analytes were eluted under gravity with 5 ml *n*-hexane–isoamylic alcohol (98:2). The organic phase was evaporated to dryness under nitrogen and redissolved in 100 μl of mobile phase. The absolute recovery of the extraction ranged between 88–95% for clomipramine, and 75–80% for desmethylclomipramine. The chromatography was performed on a Merck–Hitachi L-6200 intelligent pump equipped with an Merck–Hitachi L-4200 UV–Vis detector set to 214 nm and an Merck–Hitachi D 2000 computing integrator. A Partisphere silica column (5 μm

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particle size, 12.5 cm × 4.6 mm I.D., Whatman, Clifton, NJ, USA) was used with a mobile phase consisting of hexane–ethanol–dichloromethane–diethylamine ($77:18:5:3\cdot10^{-3}$). The flow-rate was 1.3 ml/min. A typical chromatogram of clomipramine and its metabolite desmethylclomipramine, from plasma sample of an individual monitored for these substances, is shown in Fig. 1 and compared to a blank plasma. Drugs checked for potential interference with clomipramine and its metabolites were: fluoxetine, desipramine, amitriptiline and doxepin. They were extracted with a good recovery using our procedure but they showed retention times different from those of our analytes, so that they did not interfere and could be monitored using same methodology. The within-day and between-day coefficients of variation observed with this method were 2.8 and 3.8% for 100 ng/ml clomipramine and 3.2 and 5% for 100 ng/ml desmethylclomipramine. In conclusion, the modifications brought to the method of Godbillon and Gauran enabled quick and reliable monitoring of clomipramine and its metabolite in patients and investigation of the steady-state pharmacokinetics when the drug was used alone or in combination.

References

[1] J. Godbillion and S. Gauron, J. Chromatogr., 204 (1981) 303–311.

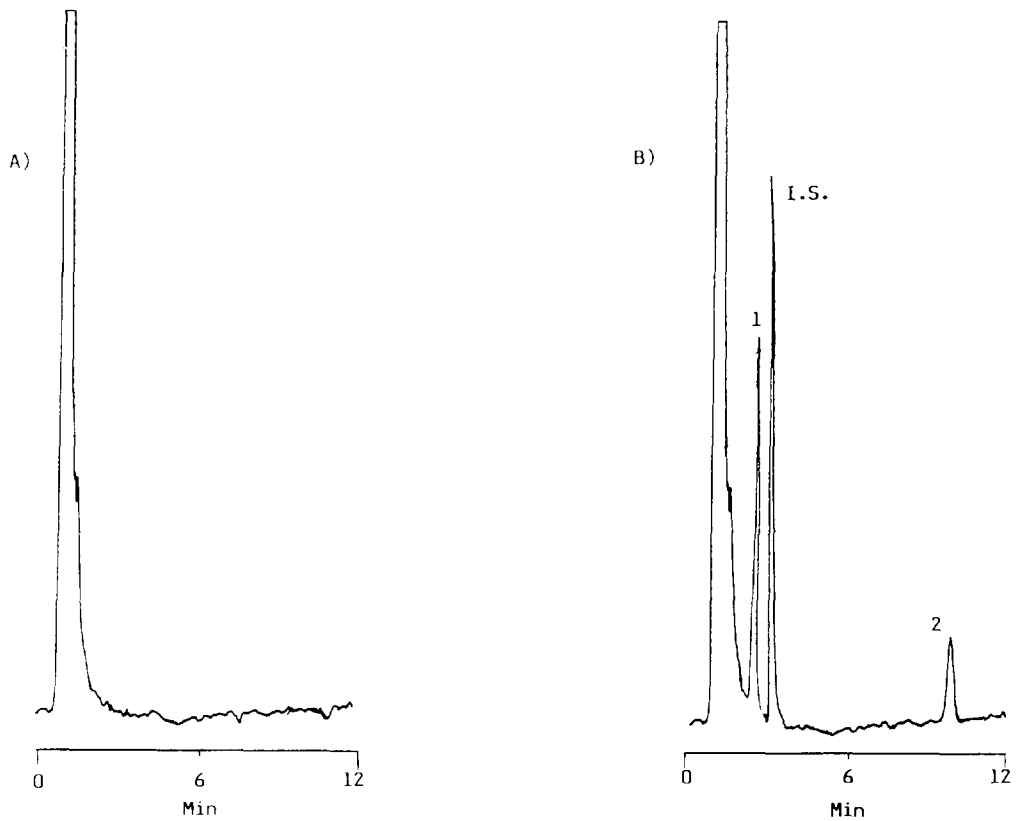

Fig. 1. Chromatograms of (A) extract of 0.5 ml blank plasma sample, (B) extract of 0.5 ml plasma sample of a patient containing 42 ng/ml clomipramine (1), 53 ng/ml desmethylclomipramine (2) and imipramine as an internal standard (I.S.).