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Note

Improved procedure for the determination of niridazole in biological fluids by high-performance liquid chromatography

J. J. MILLER, B. M. JONES, P. R. MASSEY and J. R. SALAMAN
K.R.U.F. Institute of Renal Disease, Cardiff Royal Infirmary, Cardiff (Great Britain)
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Niridazole, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone, is marketed by Ciba under the trade-name Ambilhar. Although this drug has been used for many years for the treatment of schistosomiasis[1] it is only recently that niridazole has been found to be immunosuppressive[2–6]. A previous gas–liquid chromatographic method[7] for the determination of niridazole in urine and serum was sensitive only to 250 ng/ml of sample and had a retention time for niridazole of 27.75 min. The procedure described here allows for the determination down to 50 ng/ml of sample and a much improved time period for assay (7.5 min).

MATERIALS AND METHODS

Analar-grade reagents were used throughout. Methanol was redistilled before use.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was carried out using an Anachem 4153 chromatograph (Anachem, Luton, Great Britain) fitted with an Altex 153 analytical UV detector with an 8-μl flow cell. The detector was fitted with a 365-nm filter. The column was 250 mm × 4.6 mm I.D. packed with Spherisorb ODS, mesh size 5 μm, and injection of samples on to this column was achieved via a loop injector. The isobaric flow-rate was 0.5 ml/min using methanol–water (4:1) as solvent. Under these conditions it was found that niridazole had a retention time of 7.5 min.

Extraction procedure

A 1-ml sample of either urine or serum was acidified with 1 ml of 1 *M* HCl and extracted by shaking with 5 ml of 1,2-dichloroethane. The whole was allowed to stand for 5–10 min before the lower organic phase was removed and concentrated by rotary evaporation. The sample was redissolved in the methanol–water eluent (1 ml) and aliquots were subsequently injected into the high-performance liquid chromatograph.

DISCUSSION

There was found to be a good linear response for HPLC of a known concentration of niridazole (100 μg–50 ng) which were dissolved in the eluent. Further, under the conditions employed all urine and serum samples tested to date by HPLC have been free from interfering peaks.

The use of a wavelength filter at 365 nm to detect the 5-nitro group present in niridazole (absorption maximum 380 nm) results in the greatest sensitivity for the detection of the parent compound. However, it has been found possible to use a 254-nm filter for the detection of niridazole in biological fluids, with an obvious resultant fall in the sensitivity of the assay. Using this filter system it was only possible to detect levels of 100 ng/ml of sample.

RESULTS

As has previously been reported niridazole has been used for many years for the treatment of schistosomiasis but not as an immunosuppressant[1,3]. We are currently involved in a clinical trial comparing niridazole + azathioprine + prednisolone with the more standard immunosuppressive regime of azathioprine + prednisolone in kidney transplant recipients, a study which requires an improved, more sensitive assay for niridazole. These requirements have now been attained by the use of HPLC.

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