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Note

An unusual column effect during the analysis of 5-fluorocytosine by high-performance liquid chromatography

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5-Fluorocytosine (Alcobon; flucytosine; 5FC) is an orally active drug used in the treatment of systemic mycoses[1]. Measurement of serum levels can be important to ensure that they are sufficient to prevent developed resistance and that they are not excessively high to cause dose-dependent leucopenia and thrombocytopenia. 5-Fluorocytosine is excreted unchanged by the kidneys and so levels are especially important in renal failure.

Previous reports[2–4] on the analysis of 5-fluorocytosine by high-performance liquid chromatography have largely employed precipitation with trichloroacetic acid followed by direct injection onto reversed-phase columns. This method of analysis was employed by our laboratory. However, the use of one type of column led to an unusual chromatographic event.

EXPERIMENTAL

Instrumental conditions

The column used was a 10 cm × 4.8 mm I.D. Waters Novapak C_{18} (4 μm particle size). The mobile phase was 0.1 *M* phosphate buffer, pH 7, at a flow-rate of 2 ml/min. UV detection was at 280 nm, 0.04 a.u.f.s., chart speed 0.5 cm/min. The internal standard was 5-methylcytosine (Sigma).

Method

The method is based on that of Miners *et al.*[2] except that a Waters Novapak C_{18} column was substituted for a Waters μBondapak C_{18} column.

RESULTS AND DISCUSSION

5-Fluorocytosine is a polar compound and unless an ion-pairing agent is used[4], the mobile phase must be largely[3] or wholly[2] aqueous depending on the column used. In our system, a radially compressed Waters Novapak C_{18} column was selected which normally gave acceptable chromatograms (Fig. 1a) using an aqueous phosphate buffer at pH 7. However, on occasions the chromatogram changed abruptly causing a drop in retention of both 5-fluorocytosine and the internal standard (Fig. 1b). This effect never occurred during continuous analyses but always took place immediately

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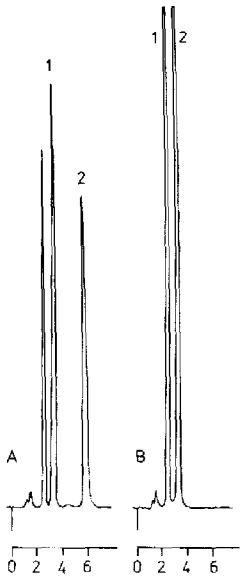

Fig. 1. Chromatograms of a serum 5-fluorocytosine extract obtained (A) with normal chromatographic operation or (B) following the described chromatographic change. Peaks: 1 = 5-fluorocytosine; 2 = 5-methylcytosine (internal standard). Conditions: column: Waters Novapak C_{18} 4 μm 10 cm × 4.8 mm I.D.; mobile phase: 0.1 *M* phosphate buffer, pH 7; detection: UV at 280 nm, 0.04 a.u.f.s.

after the mobile phase flow was stopped for a period of time (normally about 1–2 h). The original chromatogram could be restored by simply injecting 100 μl of methanol onto the column. A similar effect could not be reproduced with our Waters μBondapak C_{18} column.

The cause of this column behaviour is not immediately clear, but the fact that it has not been observed with mixed aqueous organic mobile phases and that it is reversible by the injection of methanol points to a solvation effect on the stationary phase. In normal operation, the octadecyl chains in the phase bonded monolayer are solvated and project perpendicularly into the mobile phase. It is suggested that after a period of operation in wholly aqueous conditions, the solvation of the monolayer is disrupted presumably by complete loss of the organic solvent molecules. The configuration of the monolayer then changes (possibly flattens) by the action of hydrophobic forces between its carbon chains resulting in a loss of column performance. The establishment of strong hydrophobic interactions is prevented by the presence of an organic modifier.

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