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Note

Separation of *E*- and *Z*-isomers of clomiphene citrate by high-performance liquid chromatography using methenamine as mobile phase modifier

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Clomiphene citrate is used in the treatment of infertility. The commercial product is an isomeric mixture (Fig. 1). The British Pharmacopeia (B.P.) and United States Pharmacopeia (U.S.P.) specify between 30 and 50% of the *Z*-isomer and as the two isomers (*E* and *Z*) exhibit different pharmacological activities[1] it is essential to control accurately the isomer ratio. The B.P. method relies on differences in the infra-red spectra of the two isomers but this method is more in the nature of a limit test, lacking in accuracy and inapplicable to the finished dosage form[2]. The U.S.P. method uses high-performance liquid chromatography (HPLC) on a 2-m alumina column and a very complex eluent[3]. This method has proven impossible to validate. More recently, an HPLC method based on silica and using triethanolamine as a mobile phase modifier has been described[4].

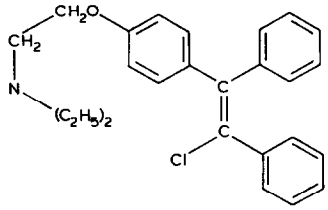

Fig. 1. Structure of clomiphene.

We report a method using methenamine as a modifier on silica and dichloromethane–acetonitrile as a mobile phase. This system gives excellent resolution and very rapid column equilibration as opposed to the B.P. method.

EXPERIMENTAL

Separations were carried out on 25 × 0.46 cm stainless-steel columns packed with either 10-μm LiChrosorb Si 60 (Merck, Darmstadt) or Spherisorb 10-μm silica (Phase Separations, Clwyd, U.K.). The mobile phase was 20% acetonitrile in dichloromethane (HPLC grade solvents, Biolabs, Jerusalem) containing 0.1% (w/v) methenamine (B.D.H., Poole, U.K.). Analyses were carried out using a Model 5030

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chromatograph (Varian, Walnut Creek, CA, U.S.A.) with a Varian UV50 detector set at 254 nm and a Rheodyne 7120 valve loop injector equipped with a 10-μl loop. Peak areas were determined using a Varian 110L CDS integrator.

DISCUSSION

Methenamine is available as a pure crystalline solid or may easily be recrystallized, whereas more usual base modifiers such as diethylamine, triethanolamine and other aliphatic amines commercially available are impure and difficult to purify. The lengthy equilibration time required for the B.P. HPLC method in order to obtain a stable baseline may be, at least in part, due to the presence of impurities in the triethanolamine modifier (equilibration times upwards of 3 h are normal). The methenamine modifier gives a stable baseline and reproducible retention times for the two isomers within 30 min.

Separate solutions of the pure *E*- and *Z*-isomers of clomiphene citrate were used to demonstrate linearity of response (Fig. 2), and the reproducibility of the isomer ratio was determined giving a coefficient of variation of 1.16%. The isomers were well resolved with a resolution factor of 1.3 (Fig. 3a). Using 5-μm silica, resolution factors greater than 1.6 could be achieved. The method is also applicable to

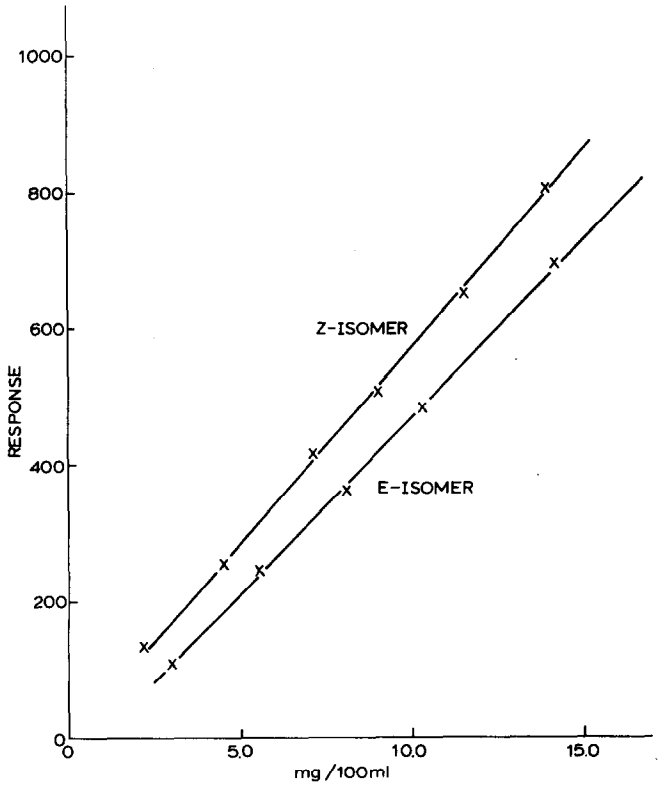

Fig. 2. Curves of peak area *vs.* concentration of the *Z*- and *E*-isomers of clomiphene citrate.

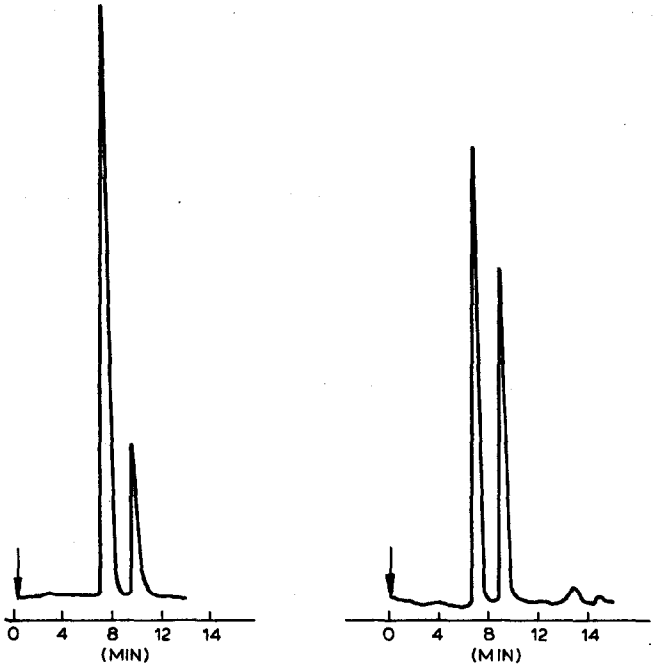

Fig. 3. Separation of the *E*- and *Z*-isomers of clomiphene before (left) and after (right) exposure to sunlight.

the finished dosage form using essentially the same procedure as described in the B.P. 1980 for sample preparation but with the substitution of dichloromethane for alcohol-free chloroform.

Solutions of clomiphene citrate are known to be photochemically unstable and the specificity of the HPLC system was demonstrated by analysis of a solution after exposure to UV light (Fig. 3b). The additional peaks observed are presumably the phenanthrene derivatives that would be produced by such irradiation.

Methenamine is a very weak base and columns in continuous use in our laboratories for over a year have shown no sign of loss of efficiency.

ACKNOWLEDGEMENTS

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4 *British Pharmacopoeia 1980*, H.M.S.O, London, 1980.