Journal of Chromatography, 319 (1985) 467–470
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,435

Note

Mitotane (*o,p'*-DDD) emulsion and tablet analysis by high-performance liquid chromatography

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(Received November 28th, 1984)

Mitotane, 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD), is the drug of choice for the treatment of adrenocortical carcinoma[1]. Mitotane has also been used in the treatment of metastatic Leydig cell carcinoma[2] and in selected patients with pituitary ACTH-dependent Cushing's syndrome[3,4]. In its recommended large doses, mitotane has severe side effects which can limit its use[5]. Moolenaar *et al.*[6] have recommended that mitotane be administered in presence of a lipoid medium such as chocolate, milk or an oil emulsion to improve absorption of the drug. It is the purpose of this paper to formulate such an emulsion and to develop a method for its analysis. One of the high-performance liquid chromatographic (HPLC) procedures developed for this analysis was also adapted for a content uniformity assay of mitotane tablets[7].

EXPERIMENTAL

Apparatus

The chromatographic system consisted of Altex (Berkeley, CA, U.S.A.) Models 110A pump and 153 fixed-wavelength (254 nm) detector equipped with a Rheodyne Model 7125 20-μl loop injector (Berkeley, CA, U.S.A.). Whatman (Clifton, NJ, U.S.A.) 250 × 4.6 mm I.D. columns, Partisil PxS 10/25 ODS-3 or Partisil PxS 5/25 were employed. These columns were preceded with guard columns (100 × 4.6 mm I.D.) packed with Whatman Co:Pell ODS or HC Pellosil 30–38 μm pellicular material respectively.

Materials

Mitotane and the internal standard, 1,1-bis-(*p*-chlorophenyl)-2,2-dichloroethylene (*p,p'*-DDE) (Aldrich, Milwaukee, WI, U.S.A.) as well as corn oil (Mazola, Best Foods, Englewood Cliffs, NJ, U.S.A.) and tragacanth (MCB, Norwood, OH, U.S.A.) were used as received. Apple-cherry syrup is formulated by the University of Michigan Hospital Pharmacy from sweetened apple-cherry juice (Miesel, Detroit, MI, U.S.A.). Glass distilled solvents cyclohexane, isooctane and methanol (MCB) were used for HPLC.

Mitotane emulsion consisted of 10 g mitotane, 50 ml corn oil, 0.2 g tragacanth, 20 ml apple-cherry syrup and 6 ml ethanol. Purified water was added to give a volume of 100 ml.

0021-9673/85/$03.30

The mitotane was dissolved in the corn oil with mild heat on a steam bath and mixed in a dry mortar with the tragacanth. Water (25 ml) was added and emulsified. The ethanol and apple-cherry syrup were combined, added to the primary emulsion in three portions and emulsified after each addition. Water was added to make the product measure 100 ml and mixed.

Analysis of mitotane emulsion

p,p'-DDE (10 mg) was added as an internal standard to 5 ml of the emulsion and extracted for 15 min with 40 ml of isooctane–ethanol (98:2) in a separatory funnel. The isooctane layer was brought up to 50 ml with isooctane and 1.0 ml of this mixture was diluted with isooctane to 10 ml to give the desired concentration of 1 mg/ml of mitotane and 0.02 mg/ml *p,p'*-DDE for HPLC analysis.

Two HPLC systems were developed in which the first system employed the C_{18} column with methanol–water (85:15) at a flow-rate of 1.0 ml/min and changing to methanol–methylene chloride (50:50) at 2.0 ml/min to remove the corn oil. The second system used the silica column with isooctane–cyclohexane (80:20) at 1.0 ml/min and changing to isooctane–ethanol (98:2) at 2.0 ml/min to remove the corn oil.

Mitotane tablets

An HPLC content uniformity assay for mitotane tablets (Lysodren, Bristol Labs., Syracuse, NY, U.S.A.) involved reducing a single tablet to a fine powder in a mortar and pestle, adding 10 mg of *p,p'*-DDE as internal standard and extracting with isooctane (4 × 10 ml). Each extract was filtered into a 50-ml volumetric flask and isooctane added to the mark. An aliquot of this extract was diluted 1 to 10 to give a solution of approximately 1 mg/ml of mitotane and 0.02 mg/ml of the internal standard. This solution was used for HPLC analysis with the silica isooctane–cyclohexane system employed for the analysis of the mitotane emulsion.

RESULTS AND DISCUSSION

The initial attempts to formulate a mitotane emulsion were taken from the USP XX Formulation for Mineral Oil Emulsion[7] with a solution of mitotane in corn oil replacing the mineral oil.However, tragacanth proved to be a more efficient emulsifying agent than acacia and a fruit syrup flavor such as the apple-cherry syrup used in our formulation proved to be more effective in masking the corn oil taste than the vanillin suggested in the USP Mineral Oil Emulsion. The solubility of mitotane in corn oil was about 30% at room temperature. However, in the final formulation this was reduced to 20% so as not to risk mitotane crystallization upon exposure to lower temperatures.

Isooctane–ethanol (98:2) proved to be a satisfactory water-immiscible system to extract the mitotane from the more polar substituents in the emulsion. A single extraction of 15 min with the use of an internal standard proved to be a consistent method of making this separation prior to HPLC.

HPLC conditions were developed to separate mitotane from the internal standard, *p,p'*-DDE on both a C_{18} reversed-phase column and on a silica column. The separation using methanol–water (85:15) at a flow-rate of 1.0 ml/min is shown in Fig.

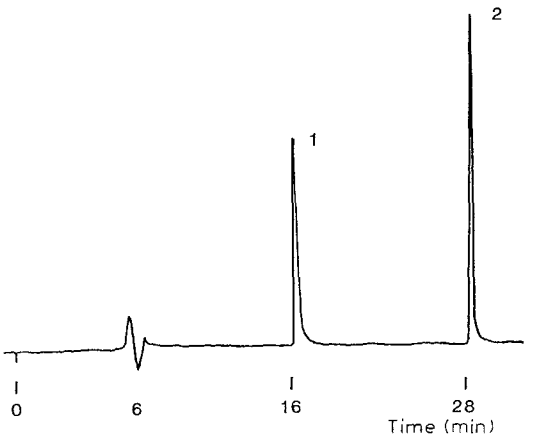

Fig. 1. HPLC analysis on a C_{18} column of mitotane (1) and internal standard (2).

1 and Fig. 2A illustrates this separation on a silica column using isooctane–cyclohexane (80:20). In both cases the solvent system required for these separations resulted in the retention on the column of the corn oil used in the emulsion. The other substituents in the emulsion did not interfere with these separations. The silica column was used in preference to the C_{18} column for the analysis of the emulsion because (1) the corn oil could be removed in less time (2) the silica column could be re-equilibrated more quickly and (3) the water-immiscible solvent system necessary to extract mitotane and internal standard from the emulsion is more compatable with the eluting solvent system than it would be with the reversed-phase system.

With the silica column, isooctane–ethanol (98:2) at 2.0 ml/min for 15 min was required to remove the corn oil. This had to be followed by re-equilibration of the

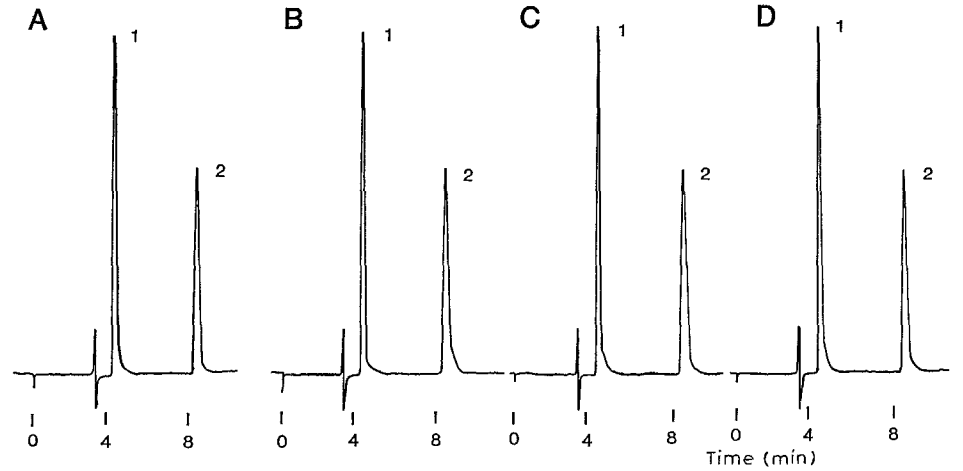

Fig. 2. HPLC analysis on a silica column of internal standard (1) and mitotane (2). (A) Standards on re-equilbrated column. (B) Emulsion extract on re-equilibrated column. (C) Standards after two prior emulsion injections. (D) Emulsion extract after two prior emulsion injections.

column with at least 120 ml of the isooctane–cyclohexane eluting system to obtain consistent mitotane to internal standard peak height ratios. Corn oil retained on the column also changed this ratio. Fig. 2 compares the slight change in peak height ratios after repeated injection of these compounds extracted from the emulsion without removal of the corn oil and re-equilibration of the column. It was found that at least three mitotane emulsion injections could be performed without removal of the corn oil if peak height ratios were compared to a known standard mixture injected just prior to the emulsion extract injection to be measured. The results of ten measurements for three extractions done in this manner resulted in a recovery of 100.1 ± 0.02%.

When the silica column isooctane–cyclohexane HPLC system was applied to isooctane extractions of individual mitotane 500-mg tablets the recovery from a single tablet with five chromatographic measurements of the extract was 512 ± 4 mg. The results of fourteen measurements of four individual tablets was 508 ± 7 mg.

ACKNOWLEDGEMENTS

We gratefully acknowledge the recognition and support of the APhA Academy of Pharmaceutical Sciences Pharmaceutical Analysis and Control Section through their 1984 Undergraduate Award in Pharmaceutical Analysis to S.P.M. as well as support to J.E.S. from Grant ROICA 37794 of the National Cancer Institute, DHHS.

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