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## Simultaneous Determination of Amitriptyline Hydrochloride and Chlordiazepoxide from Tablets by HPTLC

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A simple, precise and rapid HPTLC method is developed for the simultaneous determination of amitriptyline hydrochloride and chlordiazepoxide from tablets using chloroform: methanol: ammonia (92:8:0.2 v/v) as a mobile phase. Metronidazole is used as an internal standard. RF values obtained were 0.31, 0.40, 0.53 for metronidazole, chlordiazepoxide and amitriptyline hydrochloride respectively.

**A**MITRIPTYLINE hydrochloride, chlordiazepoxide and their pharmaceutical dosage forms are official in U.S.P.<sup>1</sup>, I.P.<sup>2</sup> and Extra Pharmacopoeia.<sup>3</sup> Literature survey shows volumetric,<sup>4</sup> spectrophotometric<sup>5</sup> and HPLC<sup>6-7</sup> methods for the determination of amitriptyline hydrochloride in single pharmaceutical dosage forms or in combination with some other drugs. For chlordiazepoxide, spectrophotometric<sup>8-11</sup>, HPLC<sup>12-14</sup> and TLC<sup>15</sup> methods have been reported. In this communication we propose a simple, precise and accurate HPTLC method for the simultaneous determination of amitriptyline hydrochloride and chlordiazepoxide from a tablet formulation. The proposed method is faster and economic when compared to U.S.P. official HPLC method.

### EXPERIMENTAL

The following instruments and material have been used throughout the study Camag Linomat IV sample applicator, Camag TLC scanner II, CATS 3.15 v software, silicagel 60F<sub>254</sub> HPTLC glass plates and Camag twin through chamber, Amitriptyline hydrochloride and chlordiazepoxide were procured from Merind (I) Ltd. Chromatographic grade chloroform, methanol, ammonia (E. Merck, India) were used. Standard stock solutions were prepared by the following procedure. Fifty mg of amitriptyline hy-

drochloride and 20 mg of chlordiazepoxide were taken in a 100 ml volumetric flask, 50 ml of methanol was added and ultrasonicated for 5 minutes and finally diluted upto the mark with methanol.

### Chromatography

Ten microlitres of each of the solutions (5-20 mcg/ml and 2-8 mcg/ml for amitriptyline hydrochloride and chlordiazepoxide respectively each containing 8 mcg/ml of metronidazole as an internal standard) were applied to 10 X 10 cm silica gel 60F<sub>254</sub> HPTLC glass plate in narrow bands of 6 mm width in the increasing concentration range using a sample applicator. The plate was developed to a distance of 60 mm in a twin trough chamber using chloroform: methanol: ammonia (92:8:0.2 v/v) as a mobile phase. Time required for the development was 20 minutes. Densitometric evaluation was done by a TLC scanner II controlled by Cats 3.15 v software and absorbance was measured at 248 nm.

### Determination of amitriptyline hydrochloride and chlordiazepoxide from tablets.

From a requisite quantity of 20 powdered tablets, test solution was prepared in methanol containing 4 and 10 mcg/ml of amitriptyline hydrochloride and chlordiazepoxide respectively and 8 mcg/ml of met-

ronidazole as an internal standard. Ten microlitres of the above solution was applied, developed, dried and scanned as per the above described.

## RESULTS AND DISCUSSION

Various mobile phases were tried for the separation but a mixture of chloroform: methanol: ammonia (92:8:0.2 v/v) gave good resolution of amitriptyline hydrochloride, chlordiazepoxide and metronidazole (I.S). RF values obtained were 0.31, 0.40 and 0.53 for metronidazole, chlorodiazepoxide and amitriptyline hydrochloride respectively. A linear relationship was obtained within the concentration range of 50-200 ng for amitriptyline hydrochloride and 20-80 ng for chlordiazepoxide.

The calibration curves could be represented by the linear regression equations which are as follows:

$$Y(\text{Amitriptyline hydrochloride}) = 0.008615 X + 0.0207 \quad (r=0.998)$$
$$Y(\text{Chlordiazepoxide}) = 0.02626 X - 0.1438 \quad (r=0.989)$$

These equations were used for direct evaluation of the drugs from tablets. The average content obtained from replicate analysis (n=7) for amitriptyline hydrochloride was 24.66 mg/tab (98.64%, RSD=1.725) and for chlordiazepoxide was 9.92 mg/tab (99.2%, RSD=1.36 %). Average % recovery obtained for amitriptyline hydrochloride and chlordiazepoxide were 99.55% and 99.54% respectively.

The proposed method is simple, precise, rapid and economical and involves a single step sample preparation and hence can be used for routine analysis in quality control laboratories.

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