Studies on A New Antifilarial Agent, 2,2'- Dicarbomethoxy amino 5,5'-Dibenzimidazolyl Ketone*

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High performance liquid chromatographic and TLC densitometric assay methods for 2,2'-dicarbomethoxy amino 5,5'-dibenzimidazolyl ketone (C.D.R.I. Code No. 82/437) a new antifilarial compound, are described. The sensitivity of the HPLC method was found to be 0.5 mcg/ml, and that of the TLC densitometric method 0.5 mcg.

2,2'-DICARBOMETHOXY amino 5,5'-dibenzimidazolyl ketone (compound 82/437) (I)[1,2] is a new antifilarial agent[3] developed at C.D.R.I., Lucknow. It is now in phase I clinical trials. To check the identity and purity of this compound and for the quality control of bulk samples and formulations, two estimation methods, one an HPLC and method a second TLC Densitometric method were developed.

Compound 82/437 is a brown powder with a molecular formula $C_{19}H_{16}N_6O_5$, molecular weight 408 with a melting point more than 300°. It is soluble in formic acid, pyridine and DMSO and practically insoluble in hexane, chloroform, methanol and water. Standard solution of the drug was prepared by dissolving 5.0 mg of compound 82/437 in 2 ml warm DMSO and making the volume up to 25 ml by methanol. Working standards were prepared in mobile phase from stock solution in the range of 1.0 - 50.0 ug/ml by sequential dilution. The contents of 20 powdered tablets or capsules of 82/437 were mixed and amount equivalent to 5.0 mg of compound 82/437 was weighed and vortexed with 2 ml of warm DMSO for 2 min and then diluted to 25 ml with methanol to get sample solution.

The HPLC system consisted of a Perkin Elmer 250 solvent delivery pump, Perkin Elmer LC 235 diode array detector, Rheodyne 7125 injector fitted with a 20 ul loop, a C_{18} column Lichrospher 100 RP-18, 5 um, 250x4 mm [E. Merck] and G.P. 100 printer plotter. The mobile phase was a mixture of 20 volumes of acetonitrile and 80 volumes of 0.05 M aqueous solution of potassium dihydrogen phosphate adjusted to pH6 with sodium hydroxide. The chromatographic procedure was carried out using a flow rate of 1.0 ml per minute and a detection wavelength of 305 nm.

For the TLC demitometric method a dual wavelength TLC (Shimadzu model CS-910) fitted with a Shimadzu U-235 data recorder, precoated silica gel plates 60F254 with a layer thickness 0.25 mm (E. Merck) and microsyringe (25 uL, Top) was used. Chromatography was carried out in a glass TLC tank saturated with the solvent system, chloroform : methanol 17:3 and the chromatogram were developed to a height of at least 15 cm. Plates were removed, air dried and the spots were scanned at 315 nm using Dual wavelength transmission mode with back ground subtraction and using a light beam of 1x10 mm. Chromatograms were recorded, peak areas measured and plotted against concentration to get a calibration curve.

Twenty five uL each of standard 82/437 solution, test solution and blank (methanol) were applied on a TLC plate. Plates were developed, dried, scanned,

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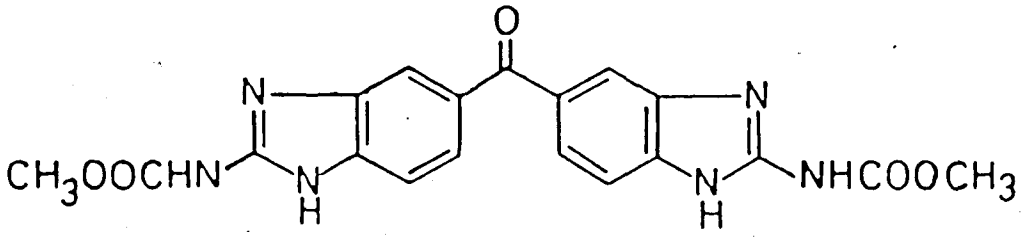

82/437 (I)

Fig. 1

Table 1
Analysis of different formulations of 2,2'-dicarbomethoxy 5,5'-dibenzimidazolyl ketone (CDRI Compound No. 82/437)

(A) By HPLC estimation method

Formulation No.	Ingredients	Amount of Drug Incorporated (mg)	Amount of Drug recovered		% Recovery of added compound	
			HPLC	TLC	HPLC	TLC
1.	Microcrystalline cellulose	50	50.0 mg	50.85 mg	100	101.7
2.	Lactose	50	51.78 mg	48.17 mg	103.6	96.3
3.	Starch	50	48.22 mg	49.15 mg	96.4	98.3

peak area calculated and content of 82/437 determined with reference to standard run side by side.

Known amounts of reference standard (R.S.) 82/437 were added to the mixed contents of preanalysed tablets and total amount of 82/437 was reanalyzed by interpolation on the corresponding calibration graphs. The accuracy of the method was calculated on the basis of the difference in the contents before and after adding R.S. and the precision was obtained by calculating the inter-day relative standard deviations (R.S.D).

A C 18 column was used to separate compound 82/437.0.05 M potassium dihydrogen phosphate buffer adjusted to pH 6 with sodium hydroxide : acetonitrile 80:20 was found to be the optimum mobile phase for the detection and effective resolution of 82/437 from the impurities in bulk drug samples and from the other contents in the formulations. The retention time of 82/437 was 10 min. Under these conditions and no interfering peaks were detected. The lower detection limit was 0.5 ug/ml.

External standardization of peak height was used for the determination of I. The calibration graph was linear over the range 1-5 ug/ml with a correlation coefficient (r) of 0.9991 and from 10 to 50 ug/ml with a correlation coefficient (r) of 0.9979.

The calibration curve for 82/437 was linear in the range of 0.5 to 10 ug in TLC densitometric method. The correlation coefficient and lower detection limits were 0.9921 and 0.5 ug respectively.

Assay data of bulk drug samples, tablets and capsules analyzed by the present methods are presented in Table 1. Further, the presence of excipients

in tablets and capsules also did not interfere in the estimation of 82/437 by the proposed methods.

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Chemical Constituents and Bioactivity Studies of *Hibiscus micranthus* Linn.

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Petroleum ether and benzene extract of leaves and stem of *Hibiscus micranthus* afforded long chain alkanes, alcohols, an acid, a ketone and β-sitosterol and the ether extract of aerial parts furnished phenolic acids. The ethanolic extract of aerial parts of roots demonstrated significant antifungal and anticancer activity.

ALTHOUGH a number of plants of the genus Hibiscus (Fam. Malvaceae) have been widely studied[1-4], a survey of literature revealed no reports on the chemical investigation of *H. micranthus*. Hence, a systematic chemical and pharmacological examination of this plant was undertaken. Herein, we report the isolation of some alkanes, alcohols, an acid, a ketone, β-sitosterol and some phenolic acids from pet. ether and benzene extract of leaves and stem separately and from ether extract of the aerial parts alongwith the antimicrobial, antiviral and antitumor efficacy of ethanolic extract of aerial parts and roots.

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The plant material was collected from the campus of the University of Rajasthan, Jaipur and identified from the Herbarium, Department of Botany. The dried ethanolic extract of leaves and stem after reextraction with pet. ether and benzene separately and the dried ethanolic extract of the aerial part after reextraction with ether were taken up for phytochemical studies.

The TLC behaviour of the pet. ether and benzene extract of leaves were same, so these were mixed together and subjected to column chromatography over silica gel. Elution of the column with pet. ether afforded pentacosane[5] (m.p. 55-57°), nonadecanone-10 (m.p. 64-65°) and docosyl alcohol[5]