HPLC Estimation Method of a New Spermicidal and Anti HIV Compound 1-(4-methoxy phenyl),5-piperidino Penta-1,4-diene-3-one in its Dosage Form#

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HPLC method for the estimation of a new spermicidal and anti HIV agent, 1-(4-methoxy phenyl), 5-piperidino penta-1,4-diene-3-one. tartarate [CDRI compound 87/132] in bulk samples and formulations is described. The calibration curve was linear in the range of 50-500 µg/ml. This method was used for the estimation of this compound in its formulations.

1-(4-methoxy phenyl), 5-piperidino penta-1,4-diene-3-one tartarate[1,2] [I, Figure 1] was synthesised as a spermicidal agent. The spermicidal activity of this compound was found to be twenty times the activity of nonoxynol-9, the most commonly used spermicidal agent. This compound was devoid of vaginal irritation and was found to be a potent anti HIV agent. Standard compound 87/132 is a brownish yellow powder with molecular weight 285. It was obtained from this institute.

Methanol and chloroform used were of AR grade. 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 µl loop, a C_{18} column Lichrospher 100 RP-18, 5 µm, 250x4 mm (E. Merck) and GP 100 printer plotter (Perkin Elmer).

Compound 87/132 (5 mg) was dissolved in 10 ml methanol to get a standard solution with concentration of 0.5 mg/ml. Stability of 87/132 in this solution was also checked. It was observed that not more than 5% of 87/132 decomposed when kept in its solution form for 24 h at room temperature. Formulation[3] equivalent to 5 mg of compound or 5 mg of bulk drug sample was dissolved in water, treated with sodium bicarbonate (5% solution) to pH 7.2, extracted with chloroform (3x3 ml). The chloroform layer was separated, dried and concentrated to dryness. The viscous mass obtained was dissolved in methanol and volume was made to 10 ml.

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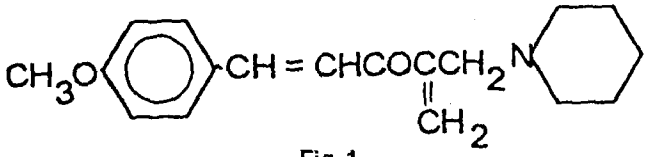

Fig. 1

The mobile phase for HLPC consisted of a mixture of acetonitrile and 0.05 M KH_2PO_4 adjusted to pH 4.0 (30:70). The compound eluted out at about 9 min using a flow rate of 1.5 ml per minute and a detection wave-length of 340 nm. Six calibration standards were prepared by serial dilution from the stock solution to obtain concentrations of 10, 50, 100, 200, 350 and 500 µg/ml.

Compound (I) was added to formulation (n=3) at three concentration levels (50, 200 and 500 µg/ml and processed as described above. Concentrations were calculated from the standard curve. The accuracy of the method was calculated based on the difference between the mean calculated and added concentrations (%DFA) while precision was determined by calculating the inter-day and intra-day coefficient of variation (%CV).

The HPLC method described herein provides good separation of compound 87/132 from the other constituents. Fig. 2 shows a chromatogram of a) mobile phase, b) standard 87/132 c) crude 87/132 in the reaction mixture. Under the chromatographic conditions used the retention time of I was about 9.0 min, other constituents extracted did not interfere since they eluted either before or after the peak of interest. The lower limit of quantification of (I) was 50 µg/ml.

Table I - Inter and intra assay variations

	Intra assay variation			Inter assay variation		
Conc. Inj. (μg/ml)	Conc. Found (Mean±S.D.) (μg/ml)	%C.V.	%DFA	Conc. Found (Mean+S.D.) (μg/ml)	% C.V.	%DFA
10	22.10±0.138	0.624	121	21.698±0.631	2.908	116.98
50	45.65±1.378	3.018	-8.7	45.25±0.689	1.522	-9.5
100	101.18±1.649	1.629	1.18	101.16±3.302	3.264	1.16
200	190.36±4.012	2.108	-4.82	187.716±8.02	4.272	-6.142
350	346.54±4.373	1.262	-0.99	350.636±8.68	2.475	0.182
500	498.34±10.84	2.175	-0.33	490.412±17.72	3.615	-1.972

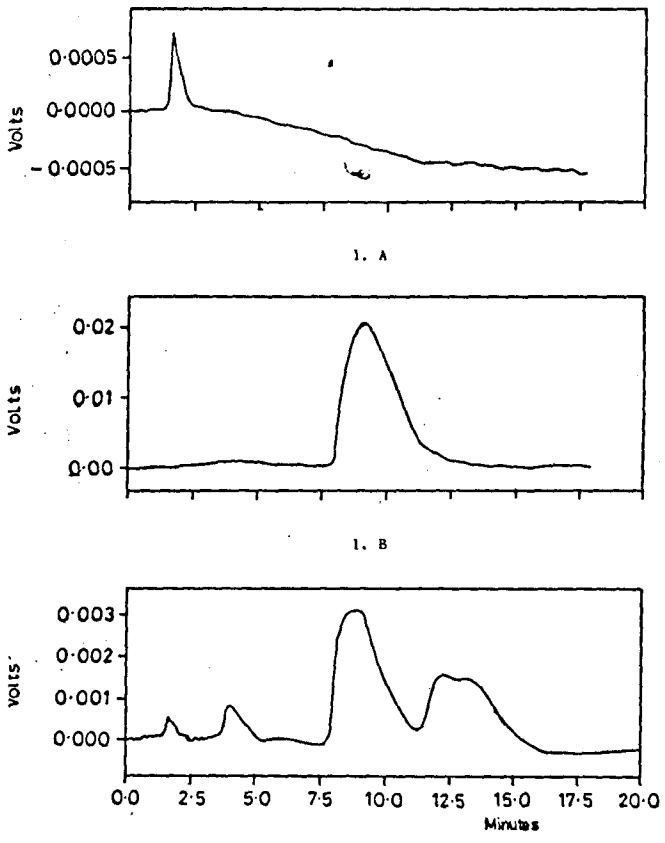

Fig. 2 : Chromatogram of a) mobile phase, b) standard 87/132 and c) crude 87/132 in the reaction mixture

The photodiode array detector gave a peak purity index, which indicated a pure peak without any interference from other ingradients of formulations. The peak was identified by its retention time and absorption maxima at 340 nm. Linear least square regression analysis of the calibration graph demonstrated linearity in the range 50-500 μg/ml. A typical standard curve (R=0.9983) could be described by the equation

Unknown conc. = 347.735 x peak area-16.213.

The reproducibility and accuracy of the method is calculated by inter and intra assay precision (%RSD) of concentration found and by calculating % of mean deviation from actual concentration respectively. Both of them were found to be well within the acceptable limits[4] (table-I) except in the case of 10 μg/ml. Thus the minimum detection limits is 10 μg/ml while the minimum quantitation limit is 50 μg/ml.

The present method provides sensitive assay method with proper resolution of 87/132. No interference from the

Table II - Analysis of different samples and formulation of C.D.R.I. compound No. 87/132

Batch No.	Drug incorporated (mg)	Drug found (mg)%
Formulation 1	25	23.905
Formulation 2	25	25.186
Sample 1		98.23
Sample 2		98.74

other constituents of formulations were observed. Table I shows the inter and intra assay variations results. Different formulations and bulk drug samples of 87/132 were analysed by this method and the results are given in table No. 2.

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Synthesis, Pharmacological Evaluation and QSAR Studies of 4,5-Dihydro-4-[(substituted Phenyl) Methylene]-5-oxo-2-Phenyl/methyl-1H-Imidazole-1-Acetic Acids

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A series of (Z)-4,5-dihydro-4-[(substituted phenyl)methylene]-5-oxo-2-phenyl-1H-imidazole-1-acetic acids (1-11) and a few of (Z)-4,5-dihydro-4-[(substituted phenyl)methylene]-2-methyl-5-oxo-1H-imidazole-1-acetic acids (12-14) were synthesized and evaluated for antiinflammatory activity. Ten compounds showed significant antiinflammatory activity. Compound 2 exhibited activity comparable to phenylbutazone. It also showed significant antiarthritic activity and was less ulcerogenic than phenylbutazone. Five compounds exhibited significant analgesic activity. Several compounds showed good activity in scavenging the stable free radical DPPH. QSAR studies suggested that none of the physicochemical parameters studied showed good correlation to the antiinflammatory activity.

We have previously reported the antiinflammatory activity of a number of compounds containing styryl carbonyl moiety namely, phenylbutenones[1], chalcones[2], cinnamic acids[3], 3-(benzylideneamino) coumarins[4], styryl sydnones[5] and so on. The present study describes the synthesis and antiinflammatory, antiarthritic and analgesic activities, ability to scavenge DPPH free radical and gastric ulcerogenicity of the title compounds. QSAR studies were carried out on the antiinflammatory activity of the compounds. The title compounds **(1-14)** were synthesized by the reaction of substituted oxazolones with glycine in fused sodium acetate and glacial acetic acid[6]. The intermediate (Z)-4-(substituted benzylidene)-2-phenyl/methyl-oxazol-5(4H)-ones were synthesized by condensing ring substituted aromatic aldehydes with benzoylglycine or acetylglycine respectively, in presence of acetic anhydride and anhydrous sodium acetate[7]. The synthesis and antiinflammatory activity of compound **12** was reported earlier[6].

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