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Determination of Nicoumalone by Isocratic RP-HPLC Method

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A simple, rapid and reproducible high performance reversed phase liquid chromatographic method has been developed for the estimation of nicoumalone in bulk drug samples and pharmaceutical dosage forms using kromosil-C_{18} column. The mobile phase consists of acetonitrile and trifluroacetic acid in water in the ratio of 20:80 v/v, respectively and was pumped at rate of 1.0 mL/min at 30 °C. The detection was carried out at 254 nm and the calibration curve was linear in the range of 5 to 25 µg/mL. The method was statistically validated for its linearity, precision and accuracy. The intra-day and inter-day variation was found to be less than 1 % showing high precision of the assay method. The proposed RP-HPLC method used for determining nicoumalone in bulk drug sample or in pharmaceutical formulation.

Key Words: Nicoumalone, RP-HPLC.

INTRODUCTION

Nicoumalone[1-3] is also known as acenocoumrol which is chemically 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2*H*-1-benzopyran-2-one. Nicoumalone is a coumarin derivatives used as an oral anticoagulants with actions similar to warfarin. It is used in the management of thromboembolic disorders. Literature survey reveals, that few spectrophotometric methods have been reported *i.e.* spectrophotometric methods for the determination of drug in presence of iodine and wool fast blue, also by using *p*-N-N-dimethyl phenylenediamine dihydrochloride[4,5]. Determination of drug in human plasma for pharmacokinetic interaction studies with antacids and adrenoreceptor antagonists (tamsulosin) by HPLC method have been reported[6,7]. Therefore there is a need for study of fast, low cost and selective method is obvious, especially for routine quality control analysis of pharmaceutical formulation. The aim of this study is to develop a simple, rapid, precise and accurate reverse phase HPLC method for the determination of nicoumalone in bulk drug samples or in pharmaceutical dosage forms.

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EXPERIMENTAL

Quantitative HPLC was performed on shimadzu prominence isocratic HPLC system with LC 20 AT pump, SPD-20A detector, spinchrom CFR software column (kromosil C_{18}, 250 × 4.6 mm; 5 μm).

Pure sample of nicoumalone were obtained as gift sample from Nicholas pirmal, India limited. Acetonitrile, methanol, water (HPLC grade Qualigens), trifluroacetic acid (AR grade). The commercial available nicoumalone tablet claimed to contain 4 mg of drug were procured from local market.

Procedure

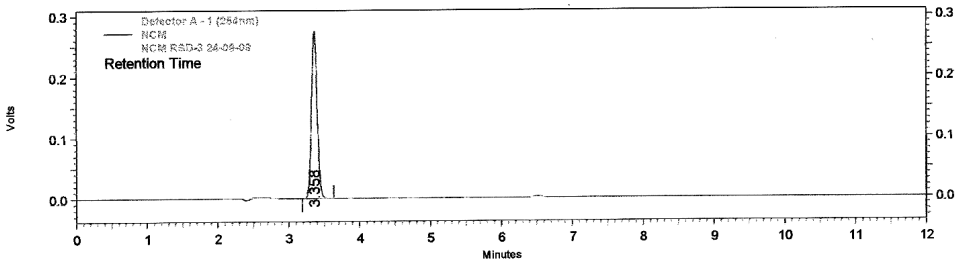
Chromatographic condition: The content of the mobile phase was acetonitrile and 0.1 % trifluro acetic acid were prepared in the ratio of 20:80. The content of the mobile phase were filtered before use through 0.45 μm membrane filter, degassed with helium purge for 15 min and pumped from the respective solvent reservoirs to the column at the flow rate of 1.0 mL/min which yielded a column back pressure 138-141 kg/cm^2. The run time was set for 12 min and the column temperature was maintained at 30 °C. The volume of injection loop was 10 μL. Prior to the injection of drug solution, the column was equilibrated for at least 0.5 h with mobile phase flowing through the system. The elements were monitored at 305 nm and the data were acquired, stored and analyzed with 'spinchrom CFR software (Shimadzu)'.

Standard solution: Weighed accurately about 50 mg of nicoumalone standard into a 100 mL volumetric flask, dissolved it and make upto the mark with methanol. Subsequent dilution of this solution were made with mobile phase to get concentration of 2.5 to 25 μg/mL of nicoumalone. The standard solution prepared as above were injected 5 times into a column at a flow rate of 1.0 mL/min. The peak area of drug concentration were calculated. The regression of the drug concentration over the peak areas was obtained. This regression equation was used to estimate the amount of nicoumalone in tablet dosage form. Nicoumalone solution containing 10 and 40 μg/mL were subjected to the proposed HPLC analysis for finding out intra and interday variations. The recovery studies were carried out by adding known amount of nicoumalone to pre-analyzed and subjecting to proposed HPLC method.

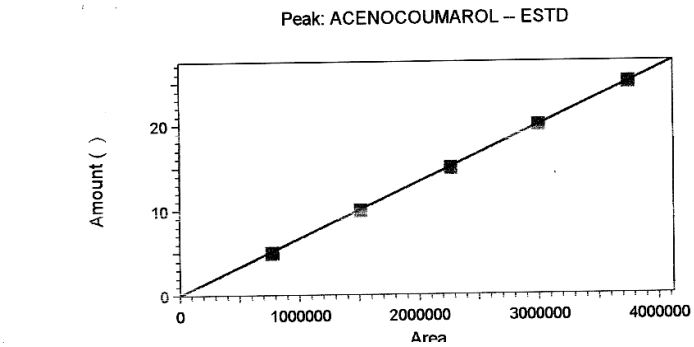
Estimation of nicoumalone: Twenty tablets each containing 4 mg were weighed and powdered. An accurately weighed portion of the powder equivalent to 50 mg of nicoumalone was transferred to a 50 mL volumetric flask containing 20 mL of methanol. The contents of the flask were sonicated for 15 min to dissolve nicoumalone and made upto volume with methanol and the resulting mixture was filtered through 0.45 μm filter. Subsequent dilution of this solution were made with mobile phase to get concentration of 0.2 to 25 μg/mL. This solution (10 μL) was injected 6 times into the column. The mean values of peak areas for six such determinations were calculated and the drug content in the tablet was quantified using the regression equation obtained above. The same procedure was followed for the estimation of nicoumalone in other commercial available tablet dosage forms.

R.S.D. of nicoumalone

S.No.	mg/mL	Area	Retentation	
1	10	1529398	3.358	
2	10	1527760	3.359	
3	10	1527873	3.358	S.D = 1808.7747
4	10	1525029	3.358	Mean = 1526988.667
5	10	1524687	3.360	R.S.D. = 0.1184
6	10	1527185	3.358	



Chromatogram of nicoumalone



	Level 1	Level 2	Level 3	Level 4	Level 5
Area	771308	1512387	2263830	3000960	3751372
Amount	5	10	15	20	25
RF	6.48249e-006	6.61206e-006	6.62594e-006	6.66453e-006	6.66423e-006
Last Area					
Rep StDev					
Rep %RSD					
Rep Area 1	771308	1512387	2263830	3000960	3751372

Linearity of nicoumalone

RESULTS AND DISCUSSION

The present study was carried out to develope a sensitive, precise and accurate RP-HPLC method for the analysis of nicoumalone in bulk sample or pharmaceutical dosage forms. The column pressure varied from 138-141 kg/cm^2 the retention time for nicoumalone was 3.358 min for a run period of 12 min. Each of samples was injected six times and same retention time were observed in all cases. The peak

area of different concentration set up as above were caculated and average value for 6 such determinations are shown in (Table-1). The peak area for drug solution was reproducible as indicated by low coefficient of variation (0.118). A good linear relationship (0.9998) was observed between the concentration of nicoumalone and the respective peak areas. The calibration graph was found to be Y = 149101.88 where Y is the peak area and C is the concentration of nicoumalone in the range of 2.5 to 25 µg/mL when the nicoumalone solution containing 10 and 40 µg/mL were analyzed by the proposed RP-HPLC method for the finding out the intra and inter day variation, a low co-efficient of variation was observed (Table-2). This shows that the present HPLC method was highly precise. The amount of drug was shown in (Table-3). About 99.19 % nicoumalone could be recovered from the pre-analyzed sample indicating the high accuracy of the proposed RP-HPLC method. The drug content in the tablet was quantified using the proposed analytical method. The mean content of nicoumalone in two different brands of tablet dosage forms is shown in (Table-4). The absence of additional peaks indicates no interference of the excipents used in the tablet. The tablets were found to contain 98.0 to 99.15 % of the labeled amount. The less than 1 % C.V. indicates the reproducibility of the assay of nicoumalone in the tablets dosage form. The proposed RP-HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

TABLE-1
CALIBRATION OF THE RP-HPLC METHOD FOR THE ESTIMATION OF NICOUMALONE

Concentration of nicoumalone (µg/mL)	Peak area (n=6)	Concentration of nicoumalone (µg/mL)	Peak area (n=6)
2.5	385654	20.0	3000960
5.0	771308	22.5	3376080
7.5	1156962	25.0	3751372
10.0	1512387	Regression equation (Y*)	
12.5	1890483	Slope (b)	149101.68
15.0	2263830	Intercept (a)	24868.86
17.5	2641135	Correlation coefficient (r)	0.9998

*y = bC+ a; Y is peak area and C is the concentration of nicoumalone in the range of 2.5 to 25 µg/mL.

TABLE-2
INTER- AND INTRA-DAY PRECISION FOR NICOUMALONE ASSAY IN PHARMACEUTICAL DOSAGE FORMS BY THE PROPOSED RP-HPLC METHOD

Concentration of nicoumalone (µg/mL)	Observed concentration of nicoumalone			
	Intra-day		Inter-day	
	Measured conc. (µg/mL) ± SD	% C.V.	Measured conc. (µg/mL) ± SD	% C.V.
10	1097.2512	0.1186	473.764	0.0510
40	330.5384	0.0259	2413.90	0.1892

TABLE-3
EXPERIMENTAL VALUES OBTAINED IN RECOVERY TEST FOR NICOUMALONE TABLETS BY PROPOSED HPLC METHOD

Amount of drug added (µg) to drug solution /powder tablet formulation	Recovery from drug solution		Recovery from powdered tablet formulations	
	Mean Amount (µg) Found (n=6)	Mean % recovery (n = 6)	Mean Amount (µg) Found (n=6)	Mean % recovery (n = 6)
20	19.85	99.25	19.62	99.05
40	39.83	99.58	39.86	99.65

TABLE-4
MEAN (±SD) AMOUNT OF NICOUMALONE IN TBLET DOSAGE FORMS BY THE PROPOSED HPLC METHOD

Brand of tablet	Labelled amount of drug (mg)	Mean (±SD) amount found (mg) by the proposed method (n=6)	Mean (±SD)% labeled amount (n=6)
T_1	4.0	3.96	99.19
T_2	4.0	3.94	98.50

T_1 = Acitrom 4.0 (Nicholas piramal); T_2 = Nicoz 4.0 mg (Ajanta pharma)

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REFERENCES

1. www.wikipedia.com
2. M.J. O'Neil, The Merck Index-An Encyclopedia of Chemicals, Drugs & Biologicals. Merck & Co. Inc. White House N.J., edn. 13, p. 32 (1955).
3. S.C. Sweetman, Martindale-The Complete Drug Reference, Pharmaceutical Press, London (UK), edn. 33, p. 940 (2006).
4. C.S.P. Sastry, G.S. Rao, Y.P. Naidu and K.R. Srinivas, *Talanta*, **45**, 1227 (1998).
5. C.S.P. Sastry, T.T. Rao and A. Sailaja, *Int. J. Pharmaceut.*, **93**, 13 (1993).
6. A.S. Khalit, F. Viviare, A. Nagger, A. Iman and I. Assem, *Int. J. Pharmceut.*, **19**, 307 (1984).
7. P. Rolan, J. Terpstra, C. Clarke, F. Mullins and J.N. Visser, *Br. J. Clin. Pharmacol.*, **55**, 341 (2003).

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