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## A Simple and Sensitive HPTLC Method for the Determination of Content Uniformity of Nicorandil Tablets

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A simple, sensitive HPTLC method was developed for the analysis of nicorandil in its commercial single component tablet formulations (5 mg). The stationary phase was precoated silica gel 60 F<sub>254</sub>. The mobile phase used was a mixture of chloroform:methanol:ammonia, (9:1:0.1 v/v). Detection of the spots was carried out at 254 nm. The method was validated in terms of linearity (200-600 ng/spot), precision (intra-day variation:0.503 to 1.960%, inter-day variation:0.333 to 3.070%), accuracy (97.69 to 100.26%) and specificity. The limit of detection and limit of quantification for nicorandil were found to be 40 ng/spot and 200 ng/spot, respectively. The proposed method was successfully applied to determine nicorandil content of 10 individual tablet units of two market formulations, after extracting nicorandil with methanol. Both the formulations complied with the USP specifications (RSD less than or equal to 6%) of the content uniformity test. The proposed HPTLC method can analyse ten or more formulation units simultaneously on a single plate and provides a faster and cost-effective quality control tool for routine analysis of nicorandil tablet formulations.

Nicorandil, N-(2-hydroxyethyl)nicotinamide nitro ester, is a potassium channel opener employed in the treatment of cardiovascular disorders<sup>1-3</sup>. Various analytical methods like HPLC (coupled with solid-phase extraction, liquid extraction, UV detection)<sup>4-6</sup> and GC-MS<sup>9</sup> have been reported for the determination of nicorandil in biological fluids. HPLC methods have also been reported for stability studies and pharmacokinetic studies<sup>10</sup>.

Recently various formulations (5 and 10 mg tablets) of nicorandil have been introduced in the market. As it is established that content uniformity requirements may be applied where the product to be tested contains 50 mg or less of active ingredient which comprises 50% or less by the weight of dosage form unit<sup>11,12</sup>, it is essential to determine content uniformity for nicorandil formulations.

The present study describes development and valida-

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tion of a simple, specific, sensitive, accurate and precise HPTLC method for the determination of nicorandil in tablets and its application in estimating the content uniformity of nicorandil tablets.

### MATERIALS AND METHODS

Nicorandil working standard was procured as a gift sample from Torrent Pharmaceuticals Ltd., Ahmedabad. Silica gel 60 F<sub>254</sub> TLC plates (20x20 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single component uncoated tablet formulations of nicorandil (5 mg) (Formulation 1 and 2) were purchased from market. Chloroform, ammonia (30% v/v) and methanol (A.R., Ranbaxy Ltd., New Delhi) were used for mobile phase preparation and as solvents.

A Camag HPTLC system (Switzerland) comprising of Camag Linomat IV semiautomatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag CATS 4 software covering single level content uniformity test

option, Camag twin-trough chamber (20x10 cm) and sonicator (Model FS-4, Frontline Ind., Mumbai) were used during the study.

#### Preparation of standard nicorandil solution and samples:

Nicorandil (10 mg) was weighed accurately and transferred to 10 ml volumetric flask. It was dissolved in 5 ml of methanol and adjusted to the mark with methanol. It was further diluted with methanol to obtain the final concentration 20 µg/ml of nicorandil. Ten uncoated tablets (each containing 5 mg nicorandil) were taken and each tablet was finely powdered. Powder of each tablet was dissolved in 5 ml of methanol. The solution was sonicated for 10 min. The solution was filtered through Whatman filter paper No. 41 and the residue was washed with methanol and the volume was adjusted to 10 ml with the same solvent. One millilitre of the solution was further diluted to 10 ml to have concentration of nicorandil equivalent to 50 µg/ml.

#### HPTLC method and chromatographic conditions:

TLC plate was pre-washed with methanol. The plate was activated by drying it in oven at 50° for 5 min. The plate was allowed to cool at room temperature. The chromatographic estimations were performed using following conditions; stationary phase, precoated silica gel 60 F<sub>254</sub> aluminum sheets (20x10 cm) (pre-washed with methanol and activated for 5 min at 50°); mobile phase, chloroform:methanol:ammonia (9:1:0.1 v/v); chamber saturation time, 1 h; temperature, 25±1°; migration distance, 45 mm; wavelength of scanning, 254 nm; slit dimensions, 4x0.1 mm; scanning speed, 5 mm/s. Following spotting parameters were used - band width, 5 mm; space between two bands, 3 mm and spraying rate, 15 s/µl.

#### Chromatographic separation:

Twenty five microlitres of standard solution of nicorandil (20 µg/ml) was applied on the pre-washed and activated plate under nitrogen stream using semiautomatic spotter. It was developed at constant temperature in a Camag twin-trough chamber previously saturated for 1 h with 10 ml mixture of chloroform:methanol:ammonia (9:1:0.1 v/v) as the mobile phase up to 45 mm. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 254 nm in reflectance mode with Camag TLC Scanner 3 using CATS 4 software incorporating the track optimization option.

#### Preparation of calibration curve:

Aliquots of 10, 15, 20, 25 and 30 µl of standard solution

of nicorandil (20 µg/ml) were applied on the TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was dried, developed and photometrically analysed as described earlier.

#### Validation of method:

The method was validated in terms of linearity, accuracy, specificity, inter-day and intra-day precision, repeatability of measurement of peak area as well as repeatability of sample application. The limit of quantification and limit of detection for nicorandil were also determined.

#### Determination of nicorandil in formulation:

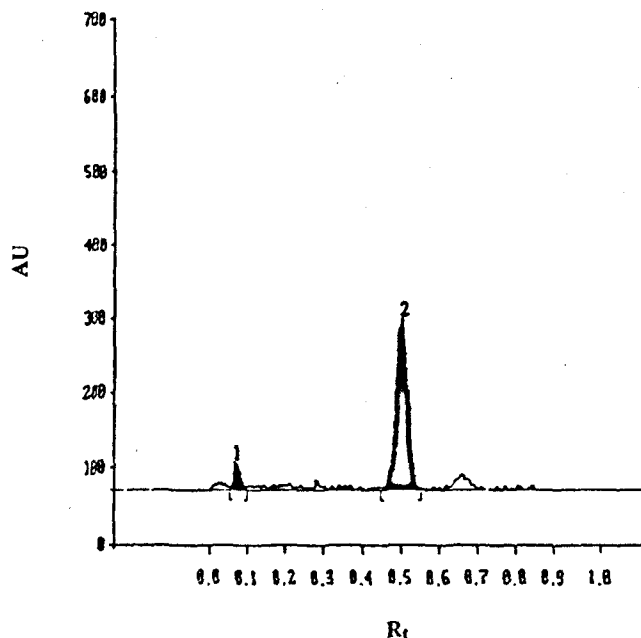
Ten microliters of sample solution for formulation 1 and 2 (50 µg/ml) were applied on TLC plates, developed and scanned as described earlier. Amount of nicorandil present in the sample solution was determined by using single level content uniformity test option in Camag CATS 4 software.

## RESULTS AND DISCUSSION

Literature survey indicated that various methods have been reported for analysis of nicorandil in biological fluids<sup>4-9</sup>. Most of them are HPLC and GC-MS which are sophisticated, costly and time consuming. These methods may not be employed for routine analysis purposes like assay and content uniformity determination for formulation, which are the routine quality control requirements. Therefore it was thought to utilize HPTLC, a versatile, speedy and cost effective technique. Keeping physicochemical properties of nicorandil in mind, a HPTLC method was developed to determine nicorandil content of the tablets.

Since nicorandil is freely soluble in methanol, tablet powder was extracted with methanol. Sonication for 10 min helped to extract completely nicorandil from tablet matrix. Various solvent systems like mixtures of chloroform:methanol, chloroform:methanol:triethylamine, chloroform:2-propanol were tried to separate and resolve spot of nicorandil from the spots of impurities and other excipients of formulations. The mixture of chloroform:methanol:ammonia (9:1:0.1 v/v) could resolve nicorandil spot with better peak shape.

Combination of chloroform and methanol offered optimum migration ( $R_f = 0.52 \pm 0.04$ ) and resolution of nicorandil from other components of formulation matrix (fig. 1). On the other hand, ammonia solution helped in sharpening of the peak. Even presaturation of TLC chamber with mobile phase for 1 h assured better reproducibility in migration of nicorandil and better resolution.



**Fig. 1: Chromatogram of nicorandil from formulation.**  
Chromatogram showing resolution of nicorandil (peak 2) from components of formulation matrix.

The linearity of response for nicorandil was determined by analysing corresponding standards for each concentration in the range of 200 to 600 ng/spot in 5 replicates. It was observed that the responses for various concentrations of standard nicorandil were linear in the range of 200 to 600 ng/spot, with a correlation coefficient of 0.9987. The average linear regression equation was represented as  $y = 0.1663x - 91.2718$ , where  $x$  = concentration of nicorandil and  $y$  = peak area. The limit of detection and limit of quantification for nicorandil were found to be 40 ng/spot and 200 ng/spot, respectively.

Accuracy of analysis, in terms of systemic error involved, was determined by calculating recovery of nicorandil by standard addition method at 5 levels of the calibration curve ( $n=3$ ). The results indicated that the recovery of added nicorandil ranged between 97.69 to 100.26% ensuring that the method is accurate.

The intra-day precision was determined by analyzing standard nicorandil solutions in the concentration range of 200-600 ng/spot for three times on the same day while inter-day precision was determined by analysing corresponding standards daily for 3 d over a period of one week. The intra-day and inter-day coefficients of variation were found to be in the range of 0.503% to 1.960% and 0.333% to

3.070%, respectively. The smaller values of intra- day and inter-day variations in the analysis indicate that the method is precise.

Repeatability of measurement of peak area was determined by spotting 20  $\mu$ l of nicorandil standard solution on a TLC plate and developing the plate. The separated spot of nicorandil was scanned seven times without changing position of the plate and relative standard deviation (RSD or % C.V.) for measurement of peak area was calculated and was found to be 0.30%.

Repeatability of sample application was assessed by spotting 20  $\mu$ l of nicorandil standard solution seven times on a TLC plate by semiautomatic spotter, followed by development of plate and recording the peak areas for seven spots. The RSD for the peak area values was calculated and was found to be 2.30%. Both the RSD values, for measurement of peak area and sample application, were well below the instrumental specifications (1% and 3%, respectively), ensuring proper functioning of HPTLC system.

To confirm the specificity of the proposed method, nicorandil tablet solution was spotted on the TLC plate, developed and scanned as described earlier. It was observed that excipients present in formulation did not interfere with peak of nicorandil ( $R_f = 0.52 \pm 0.04$ ) (fig. 1). The purity of the nicorandil peak was determined by comparing the spectra at three different levels i.e. at peak start (S), peak apex (M) and peak end (E). Good correlation between these three spectra indicated the purity of nicorandil peak (correlation,  $r(S,M) = 0.9997$ ,  $r(M,E) = 0.9920$ ). The spectrum of nicorandil extracted from tablet was also compared with spectrum of standard nicorandil, which showed good correlation ( $r=0.9928$ ).

Different validation parameters for the proposed HPTLC method for determining nicorandil content are summarized in Table 1. The proposed HPTLC method was found to be rapid, simple, specific, sensitive, precise and accurate.

This method was applied to determine the content of nicorandil in two different market samples of single component nicorandil tablets. The nicorandil content of individual tablet unit (10 replicates) for both the formulations was calculated by comparing the area of nicorandil from formulation unit with that of standard nicorandil. The corresponding data along with RSD in the content for each formulation are given in Table 2. The diagrammatic representations generated using Camag CATS 4 software single level content uniformity option for 10 units of the tablet formulations, Formu-

TABLE 1: SUMMARY OF VALIDATION PARAMETERS

Parameter	Results
Linearity range	200-600 ng/spot
Correlation coefficient	0.9987
Limit of detection	40 ng/spot
Limit of quantification	200 ng/spot
Accuracy (n=3)	97.69-100.26%
Precision (%C.V.)	
Repeatability of application (n=7)	2.30
Repeatability of measurement (n=7)	0.30
Inter-day (n=3)	0.333-3.070
Intra-day (n=3)	0.503-1.960
Specificity	Specific

Different validation parameters of the proposed HPTLC method for determination of nicorandil in formulation.

TABLE 2: ANALYSIS OF NICORANDIL TABLET DOSAGE FORMS FOR CONTENT UNIFORMITY

Amount of nicorandil found			
Formulation 1 <sup>a</sup>		Formulation 2 <sup>a</sup>	
(mg)	% Content	(mg)	% Content
4.32	86.39	4.73	94.67
4.85	97.08	5.27	105.48
4.90	98.06	4.78	95.58
4.93	98.59	5.49	109.85
5.07	101.32	4.98	99.55
5.10	102.06	5.09	101.76
4.70	94.02	5.34	106.72
4.70	94.02	4.79	95.79
5.10	101.98	5.00	100.09
5.16	103.27	5.03	100.55
%C.V. <sup>b</sup> 5.26%		5.02%	

Note: 1) Amount of nicorandil according to label claim = 5mg; and 2) USP specifications for content uniformity: <sup>a</sup>Range for drug content 85 to 115%; <sup>b</sup>% C.V. for drug content ≤ 3%.

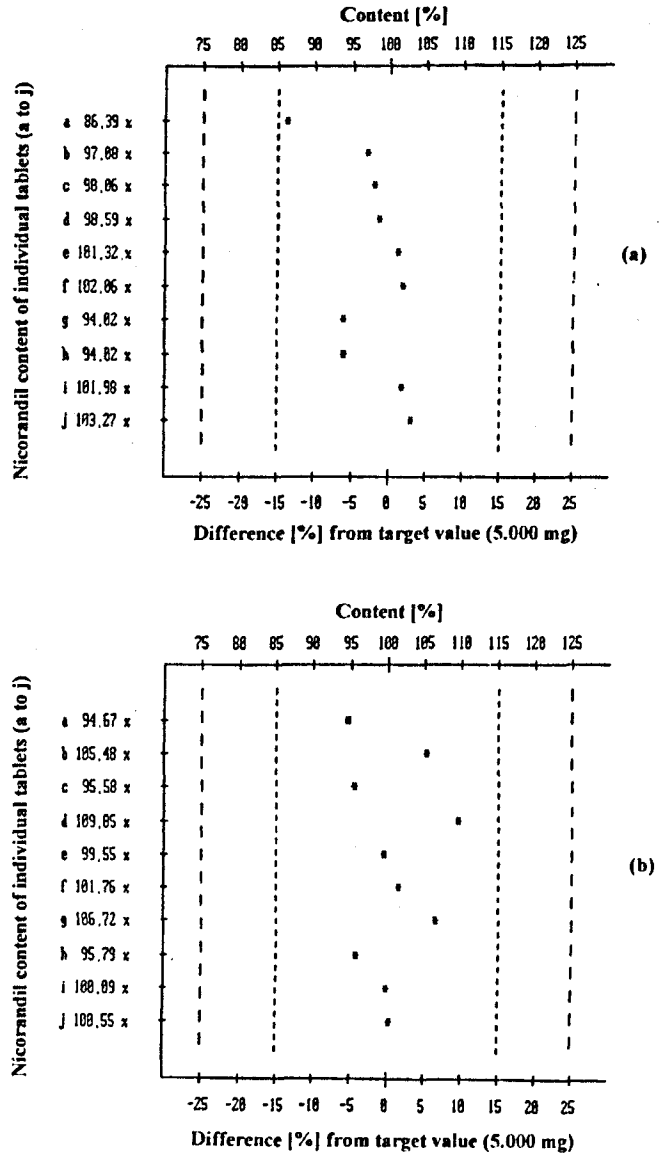


Fig. 2: Statements of content uniformity.

Diagrammatic representative (statement) of percentage content in ten individual tablet units of a) Formulation 1 and b) Formulation 2 (USP limit for drug content = 85 to 115%).

Formulation 1 and 2 are shown in fig. 2a and 2b, respectively.

According to USP if the average of the limits specified in the potency definitions in the individual monograph is 100% or less unless otherwise specified in the individual monograph, the requirement for dose uniformity are met if the amount of an active ingredient in each of 10 dosage units as determined from weight variation or content uniformity

mity method is within the range of 85 to 115% of the label claim and RSD is less than or equal to 6%<sup>11,12</sup>. Thus, since the content of individual tablet unit and the RSD between the content of 10 tablet units fall within the permissible limits according to USP, both Formulation 1 and 2 comply with the content uniformity test of USP.

Since, to perform content uniformity test employing other methods, like HPLC or UV/Vis spectrophotometry, one has to analyse the specified number of dosage form units serially, which proves to be time consuming. On the other hand, using proposed HPTLC method one can analyze 10 or more dosage form units on a single plate simultaneously. Thus proposed method proves to be very fast and cost-effective and can be employed in pharmaceutical industry for determination of content uniformity of single component uncoated nicorandil tablet dosage forms on routine basis.

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