Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Suppl 4, 2012 ISSN - 0974-2441

Research Article

A VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF NEBIVOLOL AND INDAPAMIDE IN SOLID DOSAGE FORM

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Received: 23 September 2012, Revised and Accepted: 21 October 2012

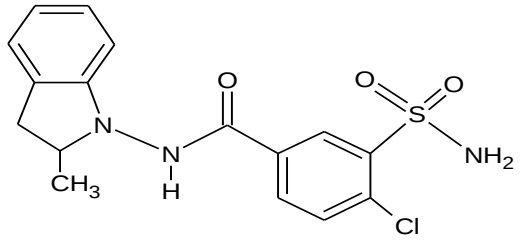
ABSTRACT

A simple, rapid, accurate and reliable HPTLC method has been established for simultaneous estimation of Nebivolol and Indapamide in pharmaceutical dosage form. Method was developed on silica gel 60 F_{254} TLC plates using ethyl acetate: methanol: dil. ammonia, (8.5: 0.8: 1.0 v/v/v), as mobile phase. This system resolved compact bands for Nebivolol (R_f~0.43) and Indapamide (R_f~0.64). The developed HPTLC method was validated in accordance with ICH guideline in terms of linearity and range, accuracy, precision, specificity, sensitivity and robustness. The validated linearity ranges found were 500–4000 ng /spot (r^2= 0.9994) and 300–1050 ng/spot (r^2= 0.9989) for Nebivolol and Indapamide, respectively. The method was found sensitive, precise, accurate and specific and be used for quantitative estimation of both the analytes in commercial pharmaceutical dosage form.

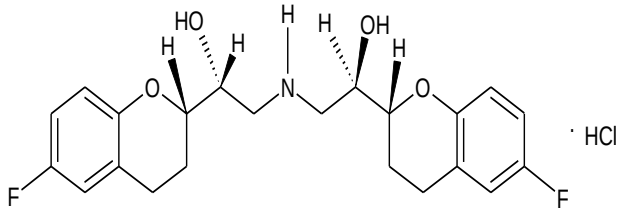
Keywords: HPTLC, Nebivolol, Indapamide, Validation

INTRODUCTION

Nebivolol chemically, 1-(6-fluorochroman-2-yl) - { [2 - (6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino} ethanol, is a third-generation vasodilating cardio selective β-blocking agent. Its molecular formula is $C_{22}H_{25}F_2NO_4$•HCl and it has a molecular weight of 405.435 g/mol[1,2]. Indapamide chemically, 4-chloro-N-(2-methyl-2, 3-dihydroindol-1-yl)- 3-sulfamoyl-benzamide, is a non-thiazide sulphonamide diuretic. Its molecular formula is $C_{16}H_{16}ClN_3O_3S$ and it has a molecular weight of 365.84 g/mol[3]. The combination is used for the treatment of hypertension.



Indapamide



Nebivolol

A recent literature survey revealed that few methods are available for the determination of Nebivolol hydrochloride in pure, pharmaceutical dosage forms and/or biological fluids, it includes high-performance liquid chromatography (HPLC)[4-7], high-performance thin-layer chromatographic (HPTLC)[8-10], UV spectrophotometric[11-12], Spectrofluorometric[13] method. Numerous different analytical methods have been developed for quantitative determination of Indapamide in pure, pharmaceutical dosage forms and/or biological fluids. These methods include high performance liquid chromatography[14-15], high-performance thin layer chromatography[16-17] and Spectrophotometry[18] method. However, there is no method for the simultaneous determination of these two drugs by high performance thin-layer chromatography (HPTLC).

The intended purpose of this investigation was to develop and validate a sensitive, precise, accurate and specific HPTLC method for the simultaneous estimation of Nebivolol and Indapamide in pharmaceutical dosage form.

EXPERIMENTAL

Materials and Reagent

Indapamide was gifted by Dishman pharmaceuticals and chemicals Ltd, Ahmedabad. All chemicals were of analytical reagent grade (Merck, India). Formulation, NEBULA-D tablets, was procured from local market.

Preparation of Standard and Sample solutions

Accurately weighed Nebivolol (50 mg) and Indapamide (15 mg) were dissolved in and diluted with methanol up to 100 ml to obtain a standard stock solution of Nebivolol (500 µg/ml) and Indapamide (150 µg/ml).

To determine the content of Nebivolol and Indapamide simultaneously in tablets (label claim: 5mg Nebivolol and 1.5 mg Indapamide per tablet), twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 5 mg Nebivolol and 1.5 mg Indapamide was weighed. Then equivalent weight of the drug was transferred into a 10 ml volumetric flask containing 5 ml methanol, sonicated for 10 min and diluted to 10 ml with methanol to obtain solution of Nebivolol (500 µg/ml) and Indapamide (150 µg/ml). The mixture was filtered using whatmann filter.

Chromatographic conditions

A CAMAG HPTLC system equipped with Linomat V applicator, TLC Scanner IV, and an integrated software winCATS was used for the analysis. HPTLC was performed on 20 cm × 10 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck, India). Standard and sample solutions were applied to the plates, as 6 mm bands, distance between tracks 10 mm, under a stream of nitrogen, by means of a Camag Linomat V sample applicator fitted with a 100-µl Hamilton syringe. Plates were then developed, at room temperature, with ethyl acetate: methanol: dil. ammonia 8.5:0.8:1 (*v/v/v*) as mobile phase, in a 20 cm × 10 cm Camag twin-trough chamber previously saturated for 20 min. The development distance was 80 mm. After development the plates were removed from the chamber, dried in air, and densitometric scanning was performed at 274 nm (Figure 1) with a Camag TLC Scanner-4 with winCATS software at a slit width of 4.00 × 0.30 mm, scanning speed of 20 mm/s, and data resolution of 100 µm/step.

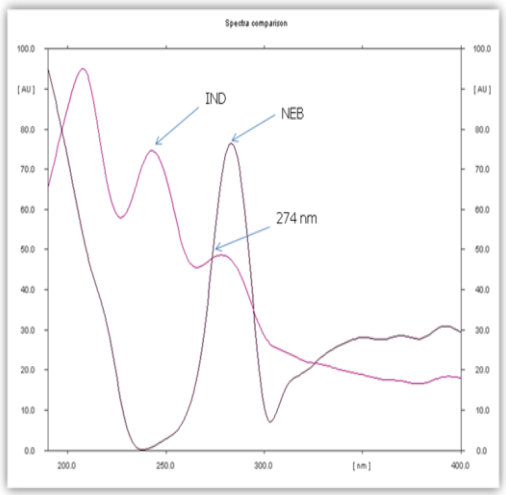


Fig 1: Overlaid Spectra of Nebivolol and Indapamide

RESULTS AND DISCUSSION

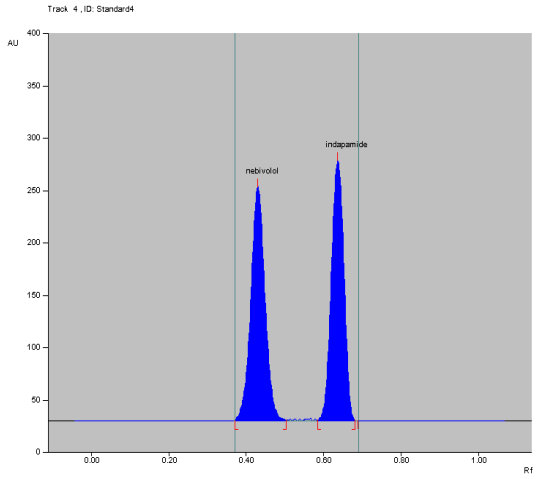


Fig 2: Chromatogram obtained from HPTLC of NEB and IND standard drug solution (NEB R_f ~ 0.43 and IND R_f ~ 0.64)

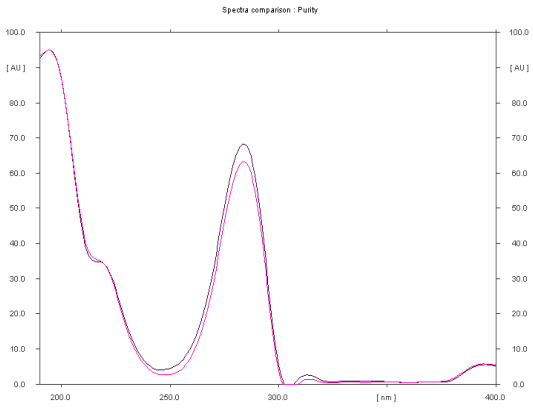


Fig 3: Peak purity spectra of Nebivolol

Optimization of Chromatographic Conditions

Several mobile phases were tried to achieve good separation of Nebivolol and Indapamide. Finally mobile phase consisting of ethyl acetate: methanol: dil. ammonia (8.5:0.8:1, v/v/v) gave good resolution with R_f values of 0.43 and 0.64 for Nebivolol and Indapamide respectively.

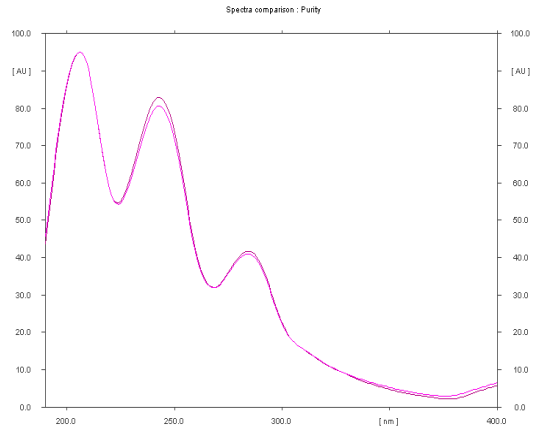


Fig 4: Peak purity spectra of Indapamide

Method validation

The method was validated in accordance with ICH guideline19.

Linearity

Linearity is generally evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. For determining linearity, calibration curves were plotted over a concentration range of 500-4000 ng/spot and 300-1050 ng/spot for Nebivolol and Indapamide, respectively. Accurately prepared standard solutions of Nebivolol and Indapamide (1,2,3,4,5,6,7,8 µl) were applied to the plate. Calibration plots were constructed by plotting peak area against the corresponding amount of each drug. Each reading was the average of 6 determinations (Table 1).

Table 1: System suitability parameter

PARAMETER	NEBIVOLOL	INDAPAMIDE
Rf value	0.43	0.64
Linearity range	500-4000ng/spot	300-1050ng/spot
Slope ± SD	2.382667 ± 0.03	7.7475 ± 0.08
Intercept± SD	914.8803 ± 17.05	782.278 ±105.90
Correlation coefficient	0.99938	0.9989

n=6, SD= standard deviation

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was determined by performing recovery studies at three different levels of standard additions. Accuracy was checked by adding 80, 100 and 120 % amount of Nebivolol and Indapamide to pre-analyzed sample (1500 ng of Nebivolol and 450 ng of Indapamide) and subjected to the proposed HPTLC method (Table 2).

Table 2: Results from recovery studies (n = 3)

Components	Initial amount (ng/spot)	Amount added (ng/spot) (n=3)	Amount recovered (ng/spot) (n=3)	% Recovery	% RSD
Nebivolol	1500	1200	2958.09	100.87	1.51
	1500	1500	2983.24	98.55	1.37
	1500	1800	3080.94	101.82	1.47
Indapamide	450	360	945.21	101.32	1.33
	450	450	969.76	101.76	0.26
	450	540	971.22	101.89	0.78

RSD=Relative standard deviation

Precision study (Intra-day and Inter-day)

The precision of an analytical method is the closeness of agreement between series of measurements obtained from multiple sampling of the same sample. Precision was measured by analysis of sample solutions at three different concentrations. The intraday precision was determined by analyzing three different concentrations of

Nebivolol (500, 2000 and 4000 ng/spot) and Indapamide (300, 600 and 1050 ng/spot) six times in a day. Interday precision was determined by analyzing three different concentrations of Nebivolol (500, 2000 and 4000 ng/spot) and Indapamide (300, 600 and 1050 ng/spot) six times on six consecutive days. The results are shown in Table 3.

Table 3: Results of Precision (Intra-day and Inter-day)

Drug	Concentration (ng/spot)	Intraday Precision % RSD (n=6)	Interday Precision % RSD (n=6)
Nebivolol	500	0.31	1.28
	2000	0.40	1.59
	4000	0.27	0.93
Indapamide	300	1.14	1.16
	600	0.60	1.21
	1050	0.41	1.47

RSD=Relative standard deviation

LOD and LOQ

The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined and the limit of detection (LOD) of an individual analytical procedure is the lowest amount of an analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOQ and LOD value represent the sensitivity of the proposed analytical method. LOD and LOQ of the drugs were calculated using the following equations.

$LOD = 3.3 \times [\sigma]/S$

$LOQ = 10 \times [\sigma]/S$

Where σ = the standard deviation of the response and S = the standard deviation of y intercept of regression lines (Table 4).

Table 4:LOD and LOQ for Nebivolol and Indapamide

Parameter	Nebivolol	Indapamide
LOD (ng/spot)	23.61	45.11
LOQ (ng/spot)	71.56	136.68

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present in the sample matrix. The specificity of the method was ascertained by analyzing standard drug and sample. The peak purity for Nebivolol and Indapamide was tested by correlation of spectra acquired at the peak start (s), peak apex (m), and peak end (e) positions. Correlation between these spectra confirmed the purity of the Nebivolol peak (correlation r (s,m) = 0.999995, r (m,e) = 0.999996) and for Indapamide peak (correlation r (s,m) = 0.999996, r (m,e) = 0.999998) respectively. Thus it can be concluded that the excipients did not interfere with the peaks from standard drug solutions.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters. The robustness of the method was checked by introducing small deliberate changes in various method parameters like mobile phase composition, saturation time, migration distance, time between spotting to chromatographic development, and time between chromatographic development to scanning, and the results are examined. Study was carried out at 450 ng/spot and 1500 ng/spot concentration of Indapamide and Nebivolol respectively (Table 5). The %RSD obtained after a small change in the

parameters was used as an indicator of the robustness of the method. The RSD values less than 2 indicate the robustness of the method.

Table 5: Results from robustness study

Robust condition		% RSD Nebivolol	% RSD Indapamide
Mobile phase composition	Ethyl acetate(8.3 ml)	0.87	0.72
	Ethyl acetate(8.7 ml)	1.13	1.90
	Methanol(0.6 ml)	0.94	1.66
	Methanol(1.0 ml)	1.59	0.19
	Dil.ammonia(0.8 ml)	0.46	1.83
	Dil.ammonia(1.2 ml)	0.51	1.83
Run distance	9 cm	0.88	1.07
	7 cm	1.30	1.54
Saturation time	0 min	0.30	1.61
	15 min	0.96	1.26
	25 min	0.96	0.47
After 15 min of application plate was developed		1.09	0.81
After 20 min of application plate was developed		0.32	1.34
After 15 min of development plate was scanned		1.00	0.92
After 20 min of development plate was scanned		0.86	0.88

RSD =Relative standard deviation

Solution stability study

Table 6 shows the results obtain in the solution stability study at different time intervals for test preparation. It was concluded that the test preparation solution was found stable up to 48 hour at ambient temperature with the consideration of < 2.0 % in % assay value difference against interval value.

Table 6: Results of solution stability

Interval	% Assay (n=3) Nebivolol	Indapamide
Initial	99.50839 ± 0.41	100.0876 ± 0.20
24 hr	98.94461 ± 0.85	99.95902 ± 0.85
48 hr	98.55042 ± 0.43	99.40723 ± 0.64

Assay of the marketed formulation

Assay provides the exact value of the amount of an analyte present in the formulation against label claim or stated amount. From the test solution 3 µl (containing 1500 ng of NEB and 450 ng of IND) was applied to the plate for assay. The concentration was determined by regression analysis. Results are shown in table 7.

Table 7: Results of assay

Component	Conc. applied (ng/spot)	Conc. Found (ng/spot)	Label claimed (mg)	Amount found (mg)	% Assay	%RSD
Nebivolol	1500	1520.09	5.0	5.07	101.4	0.20
Indapamide	450	447.52	1.5	1.49	99.6	0.77

n=3, RSD=Relative standard deviation

Conclusion

The developed HPTLC method is suitable for simultaneous estimation of Nebivolol and Indapamide in pharmaceutical dosage form is accurate, precise, specific, robust, and rapid. Thus it can be conveniently adopted for quality-control analysis.

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