Asian Journal of Chemistry Vol. 22, No. 8 (2010), 5951-5955

Development of High Performance Thin Layer Chromatographic Method for Simultaneous Estimation of Atenolol and Nefidipine in Combined Dosage Form

MADHURI RAMTEKE*, AVINASH KASTURE† and NARENDRA DIGHADE†
Adv. V.R.M. Institute of Diploma in Medical Laboratory Technology, R-2, Electronic Zone, MIDC, Hingna Road, Nagpur-441 110, India
E-mail: shende_madhuri@rediff.com

An accurate and precise high pressure thin layer chromatographic method for simultaneous estimation of atenolol and nefidipine in their combined dosage form has been developed. The study employs kieselghur 60, GF254 on aluminium foil and a mobile phase comprising cyclohexane:methanol:ethyl acetate:ammonia (5:1.5:3:0.5 v/v). The detection was carried out at 230 nm. The linear detector response for atenolol was observed between 5.7-18.9 µg/mL while for nifedipine 2.3-7.0 µg/mL. The recovery study was carried out by standard addition method. The results of recovery were 99.76 ± 0.216, 100.72 ± 0.216 for atenolol and 100.04 ± 1.069, 99.89 ± 1.058 for nifedipine.

Key Words: Atenolol, Nefidipine, HPTLC, Validation.

INTRODUCTION

Atenolol[1] is an antihypertensive, antianginal and antiarrhythmic drug and chemically is 4-(2-hydroxy-3-isopropyl aminopropoxy)-phenylacetamide. Nifedipine[2] is antianginal and antihypertensive and chemically is dimethyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylate. The Indian Pharmacopoeia describes non-aqueous titration method for the assay of atenolol and nefidipine. Gas liquid chromatography[3], reversed phase high performance liquid chromatography[4], ultra violet spectrophotometry[5-8], colorimetric estimation[9], miscellar electrokinetic chromatography[10], HPLC[11], HPTLC[12] are few methods reported in literature for the analysis of atenolol and nefidipine from their respective formulations. HPLC[13] and RP-HPLC[14] method is also reported for simultaneous estimation of atenolol and nefidipine in combined dosage form.

EXPERIMENTAL

All chemical and reagents were of AR/HPLC grade. The instruments used in the present study was Camag-HPTLC system comprising of Camag Linimat IV automatic sample applicator, Camag TLC Scanner III with CATS 4 software, Camag twin trough glass chamber were used.

†Department of Pharmaceutical Sciences, Mahatma Jyotiba Fuley Shaikshanik Parisar, Amravati Road, Nagpur-440 033, India.

Chromatographic conditions

Stationary phase: The stationary phase used was of kieselghur60, GF254 TLC precoated aluminium foiled plates and the mobile phase selected was cyclohexane: methanol:ethyl acetate:ammonia (5:1.5:3:0.5 v/v). The saturation time was 15 min, thickness of plate was 200 µm, sample application: 6 mm band, separation technique: ascending, temperature: 20 ± 5 ºC, relative humidity: 50-60 %, migration distance: 70 mm, scanning mode: absorbance, detection wavelength: 230 nm. The detection wavelength was selected from overlain spectra of both the drugs in methanol.

Selection of wavelength: The separated bands on HPTLC plates were scanned over the wavelength of 200-400 nm.

Calibration curve response: Standard solution ranging from 3-21 µL was applied on TLC plates by microlitre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 230 nm. Peak height and areas were recorded for each concentration of drugs and the calibration curves were constructed.

Preparation of standard and sample solutions

Standard solutions: An accurately weighed quantity of about 100 mg each of atenolol and nifedipine were transferred to two separate 100 mL volumetric flasks. Both drugs were dissolved in about 50 mL of methanol and then volume was made up to the mark with the same solvent (concentration-1 mg/mL of atenolol and nefidipine, respectively).

Mixed standard solution: Accurately weighed quantities of 50 mg atenolol and 20 mg of nifedipine were transferred to a 50 mL volumetric flask. The drugs were dissolved in 30 mL of methanol and finally the volume was made up to the mark with methanol [concentration 1 mg/mL (1 µg/µL) concentration of atenolol and 0.4 mg/mL (0.4 µg/µL) of nifedipine, respectively].

Analysis of laboratory mixture

Preparation of standard laboratory mixture: Accurately weighed quantities of 50 mg of atenolol and 20 mg of nifedipine was transferred to 50.0 mL volumetric flask and dissolved in methanol and volume was made up to mark.

Preparation of sample mixtures: Three laboratory mixtures of atenolol and nifedipine were prepared by appropriately weighing the quantities of drug samples so as to get the concentration in the range of 1 and 0.4 mg/mL of atenolol and nifedipine, respectively.

On the HPTLC plates one spot of standard and six spots of sample were applied, developed and scanned densitometrically at 230 nm. The per cent estimates of both the drugs were calculated using the formula:

$$\% \text{ Estimated} = \text{Amount estimated/Amount applied} \times 100$$

The results are given in Table-1.

TABLE-1
PER CENT ESTIMATION OF DRUG FROM LABORATORY MIXTURE, MARKETED FORMULATION AND THEIR RECOVERY STUDIES

Sample	Statistics	Estimate of labeled claim (%)				Recovery (%)			
		ATN		NFD		ATN		NFD	
		By height	By area	By height	By area	By height	By area	By height	By area
Laboratory mixture	Mean	100.71	99.99	100.72	100.30	99.83	99.69	99.05	99.84
	SD	0.221	0.529	0.265	0.233	0.245	0.475	0.730	0.316
	CV	0.219	0.529	0.263	0.232	0.245	0.476	0.737	0.316
Marketed preparation	Mean	100.44	99.48	99.98	100.11	99.76	100.72	100.04	99.89
	SD	1.538	2.790	0.678	1.811	0.216	0.216	1.069	1.058
	CV	1.531	1.809	0.678	0.391	0.216	0.214	1.069	1.059

Each % labeled claim is the mean of five readings.

Estimation of drugs in marketed preparations: Twenty tablets were weighted and finely powdered. An accurately weighed quantity of tablet powder equivalent to 50 mg of atenolol was transferred in 50 mL volumetric flask and 25 mL methanol was added to it. The content was shaken for about 10 min and then the volume was adjusted up to the mark with methanol. The solution was filtered using Whatmann paper 1. The filtrate was diluted further to get the final concentration of standard solution as 1.0 and 0.4 mg/mL of atenolol and nifedipine, respectively. The per cent labeled claim of drug estimated in the marketed formulation was calculated by using the formula:

% Labeled claim = Amount estimated/Amount applied (on labeled claim basis) × 100

The results are shown in Table-1.

Validation of proposed method: The proposed method was validated by considering the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out the recovery studies by standard addition method. The recovery study was performed to determine the possible interference due to the excipients present in the marketed formulation. The method was found to be accurate on the basis of the results shown in Table-1 indicating no interference of excipients in the recovery of both the drugs.

Precision: Standard deviation and relative standard deviation of the observation were determined and results shown in Table-2 were found to be within the prescribed standard limits. The results are shown in Table-2.

RESULTS AND DISCUSSION

Various pure solvents of varying polarity like methanol, ethyl acetate, chloroform, toluene, cyclohexane, *etc.* and their mixtures in different proportions were tried as mobile phase for development of chromatogram. The mobile phase found to be most appropriate was cyclohexane:methanol:ethyl acetate:ammonia (5:1.5:3.0:

TABLE-2
RESULTS OF THE PRECISION STUDY BY PROPOSED METHOD

Weight of tablet powder (mg)	Drug estimated (%)			
	Atenolol		Nefidipine	
	Peak height	Peak area	Peak height	Peak area
162.69	100.78	102.06	99.20	101.80
164.00	98.76	99.88	100.35	98.20
164.22	101.78	96.52	100.40	100.35
Mean	100.44	99.48	99.98	100.11
SD	1.538	2.790	0.678	1.811
CV (%)	1.531	2.805	0.678	1.809

Each % estimation is the mean of five observations.

0.5 v/v). It gave the good resolution of two compounds with R_f values as 0.07 for atenolol and 0.69 for nefidipine. The densitometric evaluation of the chromatogram was done on 230 nm as both the drugs have sufficient absorbance and better sensitivity at the specified wavelength. The linearity response was observed in the concentration range of 5.7-18.9 µg/mL for atenolol and 2.3-7.6 µg/mL for nefidipine. The per cent estimate of drugs in laboratory mixture were found to be 100.71 ± 0.221, 99.99 ± 0.529, 100.72 ± 0.265 and 100.30 ± 0.233 by peak height and peak area for atenolol and nefidipine, respectively. The per cent estimates of drugs in marketed formulation were 100.44 ± 1.538, 99.48 ± 2.790, 99.98 ± 0.678 and 100.11 ±1.811 for both the drugs. The accuracy of the method was determined by recovery studies using standard addition method. Results of the estimation by recovery studies of both the drugs were *ca.* 99-100 % indicating the non interference of excipients.

The replicate estimation of atenolol and nefidipine in the same batch of tablet as analyzed by the proposed method gave concurrent results indicating the reliability of the method. The values of SD and RSD and coefficient of correlation were within the prescribed limit of < 2 %, showing high precision of the method.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Torrent Pharmaceuticals Ltd., Indrad, for providing gift samples of atenolol and nifedipine and to the Head of Department of Pharmaceutical Sciences, Nagpur for providing the necessary facilities to carry out the research work.

REFERENCES

1. The Indian Pharmacopoeia, The Controller of Publications, New Delhi, India, p. 72 (1996).
2. The Indian Pharmacopoeia, The Controller of Publications, New Delhi, India, p. 511 (1996).
3. G.S. Sadana and A.B. Ghogare, *Indian Drugs*, **28**, 142 (1990).
4. S.V. Erram and H.P. Tipnis, *Indian Drugs*, **29**, 436 (1992).
5. D.M. Shingbal and S.R. Bhangle, *Indian Drugs*, **24**, 490 (1987).
6. M.S Bhatia, S.G. Kaskhedikar and S.C. Chaturvedi, *Indian Drugs*, **34**, 576 (1997).
7. S. Singh and R. Jain, *Indian Drugs*, **34**, 678 (1997).

8. S. Gangwal and P. Trivedi, *Chem. Abstr.*, **130**, 357251r (1999).
9. J. Emmanual and U.S. Quenim, *The Eastern Pharmacist*, **151**, 34 (1991).
10. A.P. Argekar and J.G. Sawant, *Chem. Abstr.*, **131**, 9726x (1999).
11. A.P. Argekar and J.G. Sawant, *Chem. Abstr.*, **132**, 142078z (2000).
12. A.P. Argekar and J.G. Sawant, *J. Liq. Chromatogr. Rel. Technol.,* **22***,* 1571 (1999).
13. H.L. Rau, A.R. Aroor and P.G. Rao, *Indian Drugs*, **28**, 283 (1991).
14. S.V. Erram and H.P. Tipnis, *Indian Drugs*, **30**, 462 (1993).

(*Received*: 7 September 2009; *Accepted*: 30 April 2010) AJC-8645

THE 5TH IUPAC INTERNATIONAL SYMPOSIUM ON MACRO- AND SUPRAMOLECULAR ARCHITECTURES AND MATERIALS (MAM-10): NEW SCIENCE AND TECHNOLOGIES FOR THE IMPROVEM

15 — 20 AUGUST, 2010

MONTEGO BAY, JAMAICA

Contact:
E-mail: mam-10@uwimona.edu.jm
Website: http://www.mona.uwi.edu/mam-10