A VALIDATED DENSITOMETRIC METHOD FOR ANALYSIS OF TELMISARTAN AND ATORVASTATIN CALCIUM IN FIXED DOSE COMBINATION

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ABSTRACT

A simple, specific, accurate and precise high-performance thin-layer chromatographic method for analysis of Telmisartan and Atorvastatin calcium in fixed dose combination has been developed. The method uses aluminium plates coated with silica gel 60 F₂₅₄ as stationary phase and toluene: methanol (7: 3, v/v) as mobile phase. Densitometric evaluation of the separated bands was performed at 280 nm. The two drugs were satisfactorily resolved with R_f values 0.50 ± 0.01 and 0.29 ± 0.00 for Telmisartan and Atorvastatin calcium, respectively. The respective calibration plots were found to be linear over the range 200–1000 and 200–700 ng/band for Telmisartan and Atorvastatin calcium, respectively. This method has been successfully validated and applied for the analysis of drugs in pharmaceutical formulation.

Keywords: High-performance thin-layer chromatography, telmisartan, atorvastatin calcium

INTRODUCTION

Atorvastatin calcium (ATV), (βR,dR)-2-(4-fluorophenyl)-β,d-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt, is a synthetic cholesterol-lowering agent¹ and is official in *Indian Pharmacopoeia*. Official assay method is by HPLC². Literature survey reveals spectroscopic method for determination of ATV in combination with amlodipine besylate³. HPTLC methods have been reported for estimation of ATV in biological sample as a single drug⁴ and in pharmaceutical sample in combination with ezetimibe⁵. Several HPLC methods are reported for determination of ATV in bulk drugs and tablets⁶⁻⁷. Stability indicating HPLC methods are also reported for estimation of ATV in combination with other drugs⁸⁻¹⁰.

Telmisartan (TEL) is described chemically as 4[(1, 4-dimethyl-2-propyl (2, 6-bi-1H-benzimidazol-1-yl) methyl] [1, 1-biphenyl]-2-carboxylic acid¹¹. TEL is useful in the treatment of mild to moderate hypertension¹². Few spectrophotometric methods have been reported for estimation of TEL in pharmaceutical dosage form as a single drug or in combination with other drugs¹²⁻¹³. HPTLC methods are also reported for the determination of TEL in formulation¹⁴⁻¹⁵.

Extensive literature survey reveals that no HPTLC method is available for determination of Atorvastatin Calcium and Telmisartan in combined tablet dosage form. Aim of present work was to develop a simple, precise, accurate and economical HPTLC method for determination of binary drug formulation.

EXPERIMENTAL

Reagents and Chemicals

Methanol, toluene (all AR grade) were obtained from Thomas Baker (chemicals) Pvt. Ltd. (Mumbai, India). Standard bulk drug samples of TEL (99.65 % pure) and ATV (99.83 % pure) were provided as gift sample by Glenmark Pharmaceuticals Ltd. (Nasik, India) and Hetero Drugs Ltd. (HP, India), respectively. The pharmaceutical dosage form used in this study was Tele Act ST 20 film coated tablets (Hetero Drugs Ltd. HP, India) labeled to contain telmisartan 20 mg and atorvastatin calcium IP equivalent to atorvastatin 10 mg per tablet.

Instrumentation and Chromatographic Conditions

Chromatography was performed on 20 cm X 10 cm aluminum plates precoated with silica gel 60 F₂₅₄ (E. Merck, Darmstadt, Germany). Before use the plates were prewashed with methanol and activated at 110 °C for 5 min. Samples were applied to the plates as bands 6 mm wide and 6.8 mm apart by means of a Camag (Switzerland) Linomat 5 sample applicator equipped with a 100 µL syringe (Hamilton, Bonaduz, Switzerland). Linear ascending development was performed in a 20 cm X 10 cm twin trough glass chamber (Camag) using toluene: methanol (7: 3, v/v) as mobile phase after saturation

of the chamber with mobile phase vapor for 30 min. The development distance was 9 cm and the development time approximately 30 min. After chromatography the plates were dried in a current of air by use of a hair dryer. Densitometric scanning was performed with a Camag TLC Scanner 3 at 280 nm for all measurements. The scanner was operated by Wincats software Version 1.4.2. The source of radiation was a deuterium lamp emitting a continuous UV spectrum between 200 and 400 nm. The slit dimensions were 5 mm X 0.45 mm and the scanning speed was 20 mm/s.

Preparation of Standard Stock Solutions

A standard stock solution of TEL and ATV were prepared by dissolving 10 mg drug in 10 mL methanol to furnish a concentration of 1 mg/mL. From this stock solution 1 mL was further diluted to 10 mL with methanol to get working standard solution of 100 µg/mL of each drug.

VALIDATION

The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines¹⁶.

Linearity

The working standard solutions of TEL (100 µg/mL) and ATV (100 µg/mL) were applied by overspotting on a TLC plate, in the range 2–10 µL, by use of the Linomat 5 sample applicator and 100 µL syringe. The plate was developed and scanned under the conditions described above. Each amount was analyzed five times and peak areas were recorded. Calibration plots of peak area against respective amount were established separately for TEL and ATV.

LOD and LOQ

LOD and LOQ were calculated, in accordance with ICH guidelines, as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Precision

To study intra-day variation, solutions of TEL and ATV were prepared and applied to the TLC plates in linearity range (3 concentrations × 3 replicates). All the solutions were analyzed on the same day to record any intra day variation in the results. To study inter-day variation, analysis of three standard solutions in triplicate in linearity range was performed on different days.

Robustness

The effect of small, deliberate variation of the analytical conditions on the peak areas of the drugs was examined. Time from application to development (0, 10, 20, 30 min), from development to scanning (0, 30, 60, 90 min), volume of mobile phase (± 10 %) and development distance (± 10 %) were varied. One factor at a time was changed to study the effect.

Accuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three

different levels 80, 100, and 120 % to pre-analyzed sample solution. Three replicates were analyzed for each level.

RESULTS AND DISCUSSION

Selection of Detection Wavelength

After chromatographic development, bands were scanned over the range 200–400 nm (spectrum scan speed 100 nm/s) and the spectra were overlain. At 280 nm, TEL as well as ATV has considerable absorbance so this wavelength was selected as detection wavelength (Fig. 1).

Fig. 1: Overlay spectrum of ATV and TEL recorded in-situ on plate from 200-400 nm.

Method Development

Different mobile phases containing toluene, methanol, chloroform, ethyl acetate, and triethylamine in different proportions were examined (results not shown). Toluene: methanol (7:3, v/v) was finally selected because it resulted in acceptable resolution of the bands with R_f values of 0.50 ± 0.01 for TEL and 0.29 ± 0.00 for ATV. The densitogram obtained from an overspotting solution of TEL and ATV is shown in Fig. 2.

Fig. 2: Densitogram obtained from a mixed standard solution of ATV (300 ng/band; $R_f 0.29 \pm 0.00$) and TEL (300 ng/band; $R_f 0.50 \pm 0.01$) at 280 nm.

Analysis of Tablet Formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg ATV was weighed and transferred to a 10 mL volumetric flask containing approximately 5 mL methanol. The mixture was ultrasonicated for 5 min and diluted to volume with methanol. The solution was filtered using Whatman no. 41 paper. From stock solution 1 mL was taken and diluted to 10 mL with methanol. 3 μ L of this stock solution was applied to a TLC plate. After chromatographic development the peak areas of the bands were measured at 280 nm and the amount of each drug in sample was determined from the respective calibration plots. The analytical procedure was repeated six times for the homogenous powdered sample. The results of commercial formulation analysis are shown in Table 1.

Table 1: Results of commercial formulation analysis.

Label Claim (mg/TAB)	% Label Claim Estimated (Mean $\pm$ S.D) (n = 6)	% R. S. D.
TEL-20	99.76 $\pm$ 1.22	1.22
ATV-10	99.59 $\pm$ 1.28	1.28

The calibration plots were found to be linear over the ranges 200–1000 and 200–700 ng/band for TEL and ATV, respectively. Results of validation parameters are shown in Table 2.

Table 2: Validation Parameters.

PARAMETERS	ATV	TEL
Linearity range (ng/band)	200-700	200-1000
Regression equation*	$y = 5.5354x - 254.61$	$y = 4.6025x + 1672.1$
r^2 *	0.9994	0.9943
Precision (% RSD)		
Intraday	0.87	0.56
Interday	1.21	1.52
LOD (ng/band)	43.54	50.14
LOQ (ng/band)	143.68	165.48

*average of five determinations

The results of recovery studies are shown in Table 3.

Table 3: Recovery studies of TEL and ATV.

	Amount Taken (ng / band)	Amount added (ng / band)	Total Amount found (ng / band)	% Recovery (Mean $\pm$ S.D)
TEL	300	240	541.78	100.33 $\pm$ 0.79
	300	300	597.96	99.66 $\pm$ 1.02
	300	360	656.63	99.49 $\pm$ 0.69
	300	240	537.62	99.56 $\pm$ 1.11
ATV	300	300	603.00	100.5 $\pm$ 0.82
	300	360	652.87	98.92 $\pm$ 0.59

CONCLUSION

The validated HPTLC method employed here proved to be simple, rapid, accurate, precise, sensitive and robust and can thus be used for routine analysis of TEL and ATV in a combined tablet dosage form.

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REFERENCES

1. The Merck index, 13th Ed., Merck and Co. Inc, White House Station. N J (1997) pp.868.
2. Indian Pharmacopoeia Vol II, New Delhi: The controller of Publication: (2007) pp.749.
3. R. Sahu, V. B. Patel, *Indian J. Pharm Sci.*, **69**, 110, (2007).
4. A. Jamshidi, A. R. Nateghi, *Chromatographia*, **65**, 763, (2007).
5. B. G. Chaudhari, N. M. Patel, P. B. Shah, K. P. Modi, *Indian J. Pharm. Sci.*, **68**, 793, (2006).
6. X. S. Miao, C. D. Metcalfe, *J. Chromatogr. A*, **998**, 133, (2003).
7. S. Ertürk, E. S. Akta, L. Ersoy, S. Fiçiciolu, *J. Pharm. Biomed. Anal.*, **33**, 1017, (2003).
8. A. Mohammadi, N. Rezanour, M. Ansari Dogaheh, F. Ghorbani Bidkorbeh, M. Hashem, R. B. Walker, *J. Chromatogr. B*, **846**, 215, (2007).
9. B. G. Chaudhari, N. M. Patel, P. B. Shah, *Chem. Pharm. Bull.*, **55**, 241, (2007).
10. B. G. Chaudhari, N. M. Patel, P. B. Shah, L. J. Patel, V. P. Patel, *J. Am. Oil Chem. Soc.*, **90**, 1539, (2007).
11. The Merck Index, 13th Ed., Merck and Co. Inc, White House Station. N J (2001) pp.1628.
12. M. S. Palled, M. Chatter, P. M. N. Rajesh, A. R. Bhat, *Indian J. Pharm. Sci.*, **68**, 685, (2006).
13. S. Bankey, G. G. Tapadiya, S. S. Saboo, S. Bindaiya, D. Jain, S. S. Khadbadi, *International Journal of Chem Tech Research* 1,183 (2009).
14. N. J. Shah, B. N. Suhagia, R. R. Shah, P. B. Shah, *Indian J. Pharm. Sci.*, **69**, 202, (2007).
15. C. Prabhu, G. S. Subramanian, A. Karthik, S. Kini, M. S. Rajan, N. Udupa, *J. Planar Chromatogr.*, **20**, 477, (2007).
16. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Complementary Guideline on Methodology dated 06 November 1996, incorporated in November 2005, London.