Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2013, 5(3):202-207
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Simultaneous estimation of atorvastatin calcium and pioglitazone hydrochloride in tablet dosage form by reverse phase liquid chromatography and high performance thin layer chromatography

Hiral J. Panchal[1*] and Bhanubhai N. Suhagia[2]

[1]*Arihant School of Pharmacy and Bio-Research Institute, Adalaj, Gandhinagar, Gujarat, India*
[2]*Faculty of Pharmacy, D. D. University, Nadiad, Gujarat, India*

ABSTRACT

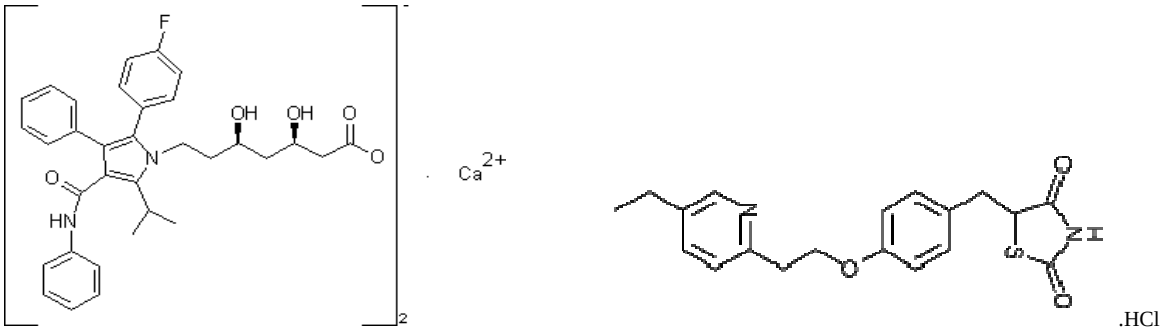
Two simple and sensitive methods to determine atorvastatin calcium (ATO) and pioglitazone hydrochloride (PIO), in tablet dosage form, were developed and validated using reverse phase liquid chromatography (LC) and high performance thin layer chromatography (TLC). The LC separation was achieved on a Phenomenex Luna C_{18} column (250mm, 4.6mm i.d., 5µm), in the isocratic mode using 0.65M ammonium acetate buffer: acetonitrile (50: 50 v/v), pH 6.9 ±0.05, as the mobile phase at a flow rate of 1.2 mL/min. The retention times were about 4.64 and 6.39 min for ATO and PIO, respectively. Quantification was carried out at 258 nm over the concentration range of 0.5-3 µg/mL for each drug. The TLC separation was achieved on silica gel 60 F_{254} HPTLC plates using with acetone: benzene: acetic acid glacial (2.6 + 7.36 + 0.04 v/v/v), as the mobile phase. The Rf values were about 0.28 and 0.61 for ATO and PIO, respectively. Quantification was performed with ultraviolet (UV) detection at 258 nm over the concentration range of 50-300 ng/spot for each. Both methods were validated, and the results were compared statistically. They were found to be simple, specific, accurate and precise. The methods were successfully applied for the determination of ATO and PIO in tablet dosage form without any interference from common excipients.

Key words: Atorvastatin calcium, Pioglitazone hydrochloride, Tablet dosage form, Reverse Phase Liquid Chromatography and High Performance Thin Layer Chromatography

---

INTRODUCTION

Atorvastatin calcium (ATO), [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate (Figure 1), is a fully synthetic statin used as the calcium salt in the treatment of hyperlipidemia [1]. ATO is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyses the conversion of HMG-CoA to mevalonate, an early and rate limiting step in cholesterol biosynthesis [2].



(a) (b)

FIGURE 1: Chemical structure of (a) ATO and (b) PIO

Pioglitazone (PIO), 5-((4-(2-(5-ethyl-2-pyridinyl)ethoxy)phenyl)methyl)-,(+-)-2,4-thiazolidinedione (Figure 1), is an antihyperglycaemic agent which increases hepatic and peripheral insulin sensitivity, thereby inhibiting hepatic gluconeogenesis, and also effective in reducing some measures of cardiovascular risk and arteriosclerosis [3].

Literature survey revealed that different analytical method for estimation of ATO have been reported, which include spectrophotometric methods **[**4-6], high performance thin layer chromatography [7, 8] determination in plasma by HPTLC [9, 10], high performance liquid chromatography (HPLC) with UV detection [11-13], stability indicating HPLC with UV detection [14, 15], stability indicating HPLC with fluorescence detection [16], determination in human plasma by HPLC with UV detection [17], LC-tandem mass spectrometry (LC-MS/MS) for determination in human plasma [18, 19], LC-ESI-MS determination in human plasma [20].

Literature survey revealed that different analytical method for determination of PIO have been reported, which include spectrophotometric methods [21, 22], high performance thin layer chromatography with UV detection [23-26], high performance liquid chromatography with UV detection [27, 28], stability indicating HPLC [29, 30], HPLC determination in human plasma with UV detection [31], determination of PIO and its metabolites in human serum using LC and solid phase extraction [32], determination by HPLC and MEKC method [33].

The present investigation describes a simple, accurate, sensitive and precise Reverse phase liquid chromatography and High performance thin layer chromatographic methods for the simultaneous estimation of ATO and PIO in pharmaceutical formulation.

MATERIALS AND MEHTODS

Reagents and materials

ATO and PIO powder with 99.95 and 99.97 % purity, respectively. Tablet dosage form: PIAT; Atorvastatin calcium 10 mg and Pioglitazone hydrochloride 10 mg (Cadila Pharma Ltd., Ahmedabad) were purchased from local market. LC grade methanol, acetonitrile and ammonium acetate were from Finar Chemicals Pvt. Ltd. (Ahmedabad, India). The water for LC was prepared by triple glass distillation and filtered through nylon 0.45µm-47mm membrane filter (Millipore, Bedford, MA). For TLC, aluminium backed silica gel 60 F_{254} 20 x 20 cm HPTLC plates (E. Merck KGaA, Darmstadt, Germany) with 0.2 mm layer thickness. AR grade methanol, acetone, benzene and glacial acetic acid (99.5%) were from Finar Chemicals Pvt. Ltd. (Ahmedabad, India).

Apparatus and chromatographic conditions

LC

A Shimadzu (Kyoto, Japan) LC system (LC-2010CHT) equipped with autosampler, photodiode array (PDA) detector and Phenomenex (Torrence, CA) Luna C_{18} column (250 mm, 4.6mm i.d., 5µm) was used. A Sartorius CP224S (Gottingen, Germany) analytical balance, and an ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used. The LC system was operated isocratically at 25 ± 2°C using mobile phase comprised of 0.65M ammonium acetate buffer: acetonitrile (50: 50 v/v), pH 6.9 ± 0.05, at a flow rate of 1.2 mL/min. The mobile phase was filtered through nylon 0.45µm-47mm membrane filter and was degassed before use. The determination was performed at 258 nm using LC solution (v 1.2; Shimadzu) software. The injection volume was 20 µL and the total run time was 10 min.

TLC

A Camag (Muttenz, Switzerland) TLC system equipped with Linomat V auto sprayer, Scanner-III, flat bottom twin trough developing chamber (20 x 10 cm) and UV cabinet with dual wavelength (254 nm and 366 nm) UV lamp. Before use the plates were washed with methanol and activated at 110°C for 5 min. The mobile phase was comprised of Acetone: benzene: acetic acid glacial (2.6 + 7.36 + 0.04, v/v/v). Samples were applied to the plates as 6 mm bands, with a Hamilton (Reno, Nevada, USA) HPTLC syringe (100µl), keeping distance (6 mm) between bands, distance (15 mm) from the plate side edge and distance (10 mm) from the bottom of the plate. A sample application rate of 10µL/s was used. The chamber saturation time was 30 min at temperature 25 ± 2°C. The development distance was 7 cm. Plates were removed from chamber, dried by means of hot air. The densitometric scanning was performed at 258 nm in absorbance-reflectance mode with winCATS software (v 1.3.3; Camag). The slit dimensions were 6.00 × 0.45 mm and the scanning speed was 100nm/s. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 360 nm.

Preparation of ATO and PIO Standard solutions

Accurately weighed ATO (10 mg) and PIO (10 mg) standards were transferred to a 50 ml volumetric flask, dissolved in and diluted to the mark with methanol to obtain standard stock solution for ATO (200 µg/ml) and PIO (200 µg/ml). Aliquot of the solution (2.5 ml) was transferred to a 50 ml volumetric flask, and diluted to the mark with mobile phase for HPLC, while with methanol for HPTLC to obtain working standard solution for ATO (10 µg/ml) and PIO (10 µg/ml).

Preparation of Sample solution

Twenty tablets were weighed and average weight was calculated. Accurately weighed powder equivalent to 10 mg ATO and 10 mg of PIO was transferred in a 50 mL volumetric flask and methanol (30 mL) was added. The solution was sonicated for 15 min. The flask was allowed to cool down to room temperature, and the volume was diluted to the mark with methanol to obtain the sample stock solution for ATO (200 µg/mL) and PIO (200 µg/mL). The solution was filtered through 0.45µm-47mm membrane filter. An aliquot (2.5 mL) was transferred to a 50 mL volumetric flask, and diluted to the mark with mobile phase for LC, and with methanol for TLC to obtain working sample solution for ATO (10 µg/mL) and PIO (10 µg/mL). For LC, An aliquot (1 mL) of the working test solution was transferred to a 10 mL volumetric flask, and diluted to the mark with mobile phase to obtain the

sample solution for ATO (1 µg/mL) and PIO (1 µg/mL). For TLC, An aliquot (15 µL) of the working sample solution was applied on plates, to obtain the sample concentration for ATO (150 ng/spot) and PIO (150 ng/spot).

Method validation

The methods were validated for the following parameters following the International Conference on Harmonization (ICH) guidelines (34).

Specificity

The specificity of the methods was established by comparing the chromatograms and measuring the peak purities of ATO and PIO from standard and sample solutions of the tablet dosage forms. For LC, the peak purity spectra of ATO and PIO were recorded using PDA detector. For TLC, the peak purity of ATO and PIO were assessed by comparing spectra acquired at the peak start (S), peak apex (M), and peak end (E) of a spot. Correlation between ATO and PIO spectra from standard and sample was also established.

Linearity (Calibration curve)

LC

Mixed working standard solutions (0.5, 1, 1.5, 2, 2.5 and 3 mL equivalent to 0.5, 1, 1.5, 2, 2.5 and 3 µg/mL for ATO and PIO, each) were transferred in a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. An aliquot (20 µL) of each solution were injected under the operating chromatographic conditions as described earlier. Chromatograms were recorded. Calibration curves were constructed by plotting peak areas versus concentrations, and the regression equations were calculated. Each response was average of three determinations.

TLC

Mixed working standard solutions (5, 10, 15, 20, 25 and 30 µL equivalent to 50, 100, 150, 200, 250 and 300 ng/spot for ATO and PIO, each) were applied to the plate. The plate was developed and analyzed. The chromatograms were recorded and the peak areas were noted. Calibration curves were constructed by plotting peak area versus concentration, and the regression equations were calculated. Each response was average of three determinations.

Accuracy (% Recovery)

The accuracy of the methods was determined by calculating recoveries of ATO and PIO by the standard addition method. Known amount of standard solutions of ATO (0, 0.5, 1 and 1.5 µg/mL) and PIO (0, 0.5, 1 and 1.5 µg/mL), and ATO (0, 50, 100 and 150 ng/spot) and PIO (0, 50, 100 and 150 ng/spot) for TLC were added to a prequantified sample solutions of ATO and PIO (1 and 1 µg/mL) for LC and (150 and 150 ng/spot) for TLC. Each solution was injected in triplicate and the percentage recovery was calculated by measuring the peak areas and fitting these values into the regression equations of the calibration curves.

Precision

The intraday and interday precisions of the proposed methods were determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of ATO (1, 2 and 3 µg/mL) and PIO (1, 2 and 3 µg/mL) for LC, and ATO (100, 200 and 300 ng/spot) and PIO (100, 200 and 300 ng/spot) for TLC. The results are reported in terms of relative standard deviation.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the ATO and PIO, for both LC and TLC, were calculated using the standard deviation of responses and slopes using signal-to-noise ratio.

Robustness

LC

Robustness of the method was studied by changing the extraction time of ATO and PIO from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.2 mL/min and column oven temperature by ± 2 ^{0}C.

TLC

Robustness of the method was studied by changing the extraction time of ATO and PIO from tablet dosage forms by ± 2 min, composition of mobile phase by ± 0.2 mL of organic solvent, development distance by ± 1 cm, and temperature.

System-Suitability Test

System suitability tests are used to verify that the resolution and repeatability of the system were adequate for the analysis intended. The parameters used in this test were asymmetry of the chromatographic peak, peak resolution and repeatability, as RSD of peak area for replicate injections. The precision of the instruments was checked by repeatedly injecting (n = 6) solution of ATO and PIO (1 µg/mL, each) for LC; while ATO and PIO (150 ng/spot, each) for TLC.

For TLC, the repeatability of sample application was checked by measuring area of six bands having same concentration of ATO (150 ng/spot) and PIO (150 ng/spot) applied on the same plate, while the repeatability of measurement of peak area was checked by repeatedly measuring (n = 6) area of one band of ATO (150 ng/spot) and PIO (150 ng/spot) applied on the same plate without changing the position of plate. The results are reported in terms of relative standard deviation.

Estimation of ATO and PIO in tablet dosage forms

The responses of sample solutions were measured at 258 nm for quantitation of ATO and PIO by the proposed methods. The amount of ATO and PIO present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for ATO and PIO, respectively.

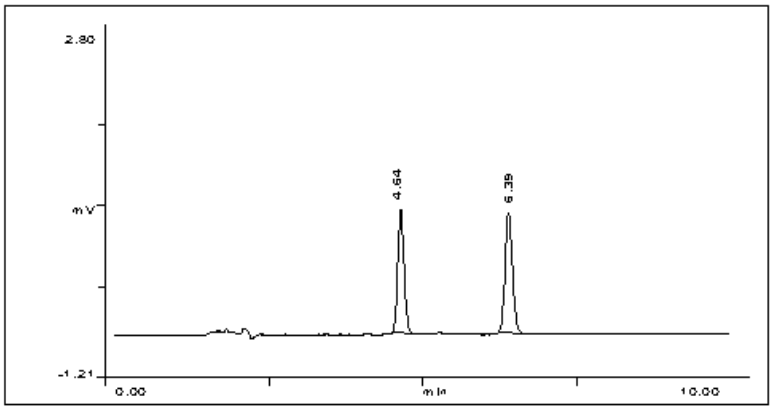
RESULTS AND DISCUSSION

LC

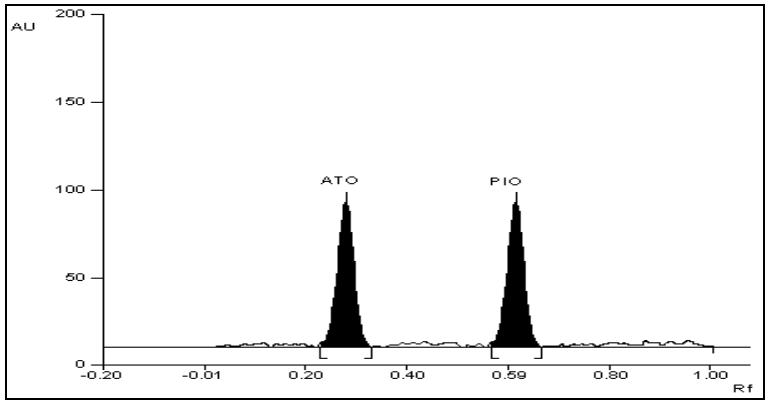
The mobile phase consisting of 0.65M ammonium acetate buffer: acetonitrile (50: 50, v/v), pH 6.9 ± 0.05, at a flow rate of 1.2 mL/min, was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for ATO and PIO. Quantification was achieved at 258 nm based on peak area. The retention times were about 4.64 and 6.39 min for ATO and PIO, respectively (Figure 2).

TLC

The mobile phase consisting of acetone: benzene: acetic acid glacial (26.0: 73.6: 0.4, v/v/v) was found to be satisfactory to obtain good peak symmetry, better reproducibility and repeatability for ATO and PIO. Quantification was achieved with ultraviolet detection at 258 nm based on peak area. The Rf values were about 0.40 and 0.20 for ATO and PIO, respectively (Figure 2).



(a)



(b)

FIGURE 2: (a) Liquid chromatogram of ATO (1 µg/mL) and PIO (1 µg/mL) from tablet dosage form, at 258 nm, with retention time of 4.64 and 6.39 min, respectively; (b) Thin Layer chromatogram of ATO (150 ng/spot) and PIO (150 ng/spot) from tablet dosage form, at 258 nm, with Rf of 0.28 and 0.61, respectively

Method validation

Specificity

Both methods were found to be specific as no significant change in the responses of ATO and PIO was observed after 24 h. The excipients present in tablet dosage forms didn't interfere with the chromatographic responses of ATO and PIO, as the peak purities of ATO and PIO from sample solution were >0.99 for LC. For TLC, the peak purity r(S, M) = 0.9998 and r(M, E) = 0.9991 for ATO and r(S, M) = 0.9999 and r(M, E) = 0.9991 for PIO were found. Also good correlation (r = 0.9998) for ATO and (r = 0.9998) for PIO were obtained between standard and sample spectra. Peak purity > 0.99 for both LC and TLC indicates the methods specificity.

Linearity (Calibration curve)

Linear correlation was obtained between peak area and concentration for ATO and PIO each, in the range of 0.5-3 µg/mL for LC, and 50-300 ng/spot for TLC. The linearity of the calibration curves were validated by the value of correlation coefficient of the regression (*r*). The regression analysis of the calibration curves is shown in Table 1.

TABLE 1: Regression analysis of the calibration curves for ATO and PIO by the proposed LC and TLC-Densitometric methods

Parameter	LC		TLC	
	ATO	PIO	ATO	PIO
Linearity range	0.5-3 µg/mL	0.5-3 µg/mL	50-300 ng/spot	50-300 ng/spot
Slope	235164.8065	100091.3782	10.4397	8.1836
Standard deviation of slope	25.2963	0.1549	0.0161	0.0219
Intercept	- 174.7312	- 8883.4003	23.1987	27.9920
Standard deviation of intercept	127.6127	7.2648	5.4235	4.5921
Correlation coefficient, *r*	0.9991	0.9990	0.9990	0.9991

Accuracy (% Recovery)

The recovery study was carried out by the standard addition method. The percent mean recoveries obtained for ATO and PIO were 99.51 ± 0.21 % and 99.61 ± 0.27 % for LC, while 99.86 ± 0.39 % and 99.87 ± 0.43 % for TLC, which were satisfactory (Table 2).

TABLE 2: Results of recovery studies for ATO and PIO by the proposed LC and TLC-Densitometric methods (n=3)

Method	Drug	Amount taken	Amount added	Amount found	Recovery ± SD, %	% RSD
LC	ATO (µg/mL)	1	0	1.01	100.09 ± 0.26	0.26
		1	0.5	1.49	99.51 ± 0.33	0.33
		1	1	1.97	98.60 ± 0.17	0.17
		1	1.5	2.48	99.83 ± 0.09	0.09
	PIO (µg/mL)	1	0	1.01	100.10 ± 0.11	0.11
		1	0.5	1.51	100.23 ± 0.40	0.40
		1	1	1.99	99.89 ± 0.20	0.20
		1	1.5	2.49	99.96 ± 0.38	0.38
TLC	ATO (ng/spot)	150	0	149.40	99.60 ± 0.29	0.29
		150	50	200.20	100.10 ± 0.41	0.41
		150	100	249.53	99.81 ± 0.54	0.54
		150	150	299.97	99.99 ± 0.35	0.35
	PIO (ng/spot)	150	0	149.85	99.90 ± 0.30	0.30
		150	50	200.14	100.07 ± 0.52	0.52
		150	100	250.28	100.11 ± 0.49	0.49
		150	150	298.23	99.41 ± 0.42	0.42

Precision

For LC, % RSD for repeatability was found to be 0.48 and 0.10 for ATO and PIO, respectively. For TLC, % RSD for the repeatability of sample application were found to be 0.60 and 0.85; while for the repeatability of measurement of peak area were found to be 0.10 and 0.19 for ATO and PIO, respectively.

The value of % RSD for intraday and interday variations were found to be in range of 0.10-0.20 and 0.17-0.94 for ATO, and 0.14-0.40 and 0.23-0.84 for PIO, respectively for LC; while 0.84-1.07 and 1.09-1.13 for ATO, and 0.73-1.09 and 1.05-1.28 for PIO, respectively for TLC. The % RSD values indicate the proposed methods are precise.

LOD and LOQ

The LOD and LOQ were found to be 0.0046 and 0.0139 µg/mL for ATO, 0.0065 and 0.0198 µg/mL for PIO, respectively for LC; while 1.9687 and 5.9657 ng/spot for ATO, and 2.6751 and 8.1065 ng/spot for PIO, respectively for TLC.

Robustness

The methods are found to be robust as the results were not significantly affected by slight variation in the chromatographic conditions for both LC and TLC.

System-Suitability Test

The results are reported in Table 3.

TABLE 3: System suitability test parameters for ATO and PIO by the proposed LC method

Parameter	ATO ± % RSD	PIO ± % RSD
Retention time, min	4.64 ± 0.11	6.40 ± 0.08
Tailing factor	1.13 ± 0.46	1.10 ± 0.50
Asymmetry	1.15 ± 0.48	1.12 ± 0.49
Theoretical plates	10051.67 ± 0.36	14243.00 ± 0.58

Estimation of ATO and PIO in tablet dosage forms

The proposed Liquid chromatography and Thin Layer chromatography were successfully applied for determination of ATO and PIO in tablet dosage forms. The results obtained for ATO and PIO were comparable with the corresponding labeled claim percentage (Table 4).

TABLE 4: Analysis results for ATO and PIO tablet dosage form by the proposed LC and TLC-Densitometric methods

Formulation	ATO ± SD (n=5), %		PIO ± SD (n=5), %	
	LC	TLC	LC	TLC
PIAT	100.09 ± 0.26	99.60 ± 0.29	100.10 ± 0.11	99.90 ± 0.30

$t_{calculated} < t_{table}$ (P = 0.05)

COMPARISON OF THE PROPOSED METHODS

The assay results for ATO and PIO, in tablet dosage forms, obtained using Liquid chromatography and Densitometric-thin Layer chromatography were compared statistically by applying the paired *t*-test. The calculated *t*-value for ATO (0.36) and PIO (0.29) for LC and TLC are less than the tabulated *t*-value for ATO and PIO (2.78) at the 95% confidence interval. Therefore, no significant difference is found in the content of ATO and PIO determined by the proposed methods.

CONCLUSION

Two methods were developed for determination of ATO and PIO based on different analytical techniques. Both methods were validated and found to be simple, sensitive, specific, accurate, precise and robust. Statistical comparison of the assay results for ATO and PIO in tablet dosage forms by both methods indicated no significant difference. Hence, both methods can be used successfully for the routine analysis of pharmaceutical dosage forms of ATO and PIO.

REFERENCES

[1] H. S. Malhotra, K. L. Goa, *Drugs*, **2001**, 61, 1835-1881.
[2] J. M. Mary, J. H. Richard, C. C. Pamela; Lippincott's illustrated Reviews-Pharmacology, Lippincott Williams & Wilkins, London, **2002**, 2, 207-216.
[3] J. Waugh, G. M. Keating, G. L. Plosker, S. Easthope, D. M. Robinson, *Drugs*, **2006**, 66(1), 85-109.
[4] N. Erk, *Anal. lett*., **2003**, 36(12), 2699-2711.
[5] V. Nagaraj, V. Kalamkar, R. Mashru, *Anal. Sci*., **2007**, 23, 445-451.
[6] R. Sahu, V. B. Patel, *J. Pharm. Sci*., **2007**, 69(1), 110-111.
[7] B. G. Chaudhari, N. M. Patel, P. B. Shah, K. P. Modi, *J. Pharm. Sci*., **2006**, 68(6), 793-796.
[8] S.S. Yadav, D. V. Mhaske, A. B. Kakad, B. D. Patil, S. S. Kadam, S. R. Dhaneshwar, *J. Pharm. Sci*., **2005**, 67(2), 182-188.
[9] A. Jamshidi, A. R. Nateghi, *Chromatographia*, **2007**, 65, 763-766.
[10] G. Bahrami, B. Mohammadi, S. Mirzaeei, A. Kiani, *J. Chromatogr. B*, **2005**, 826, 41-45.
[11] S. Erturk, A. E. Sevinc, L. Ersoy, S. Ficicioglu, *J. Pharm. Biomed. Anal*., **2003**, 33(5), 1017-1023.
[12] D. A. Shah, K. K. Bhatt, M. B. Shankar, R. S. Mehta, T. R. Gandhi, S. L. Baldania, *J. Pharm. Sci*., **2006**, 68(6), 796-799.
[13] K. R. Rajeswari, G. G. Sankar, A. L. Rao, J. V. Seshagirirao, *J. Pharm. Sci*., **2006**, 68(2), 275-277.
[14] B. G. Chaudhari, N. M. Patel, P. B. Shah, *Chem. Pharm. Bull*., **2007**, 55(2), 241-246.
[15] A. Mohammadi, N. Rezanour, D. M. Ansari, B. F. Ghorbani, M. Hashem, R. B. Walker, *J. Chromatogr. B*, **2007**, 846(1-2), 215-221.
[16] B. G. Chaudhari, N. M. Patel, P. B. Shah, L. J. Patel, V. P. Patel, *J. AOAC*, **2007**, 90(6), 1539-1546.
[17] A. Kher, *J. AOAC*, **2007**, 90(6), 1547-1553.
[18] B. B. Gholamreza, M. Bahareh, M. Shahla, K. Amir, *J. Chromatogr. B*, **2005**, 826(1-2), 41-45.
[19] B. V. Dohalsky, J. Huclova, B. Barrett, B. Nemec, I. Ulc, D. I. Jelinek, *Anal. Bioanal. Chem*., **2006**, 386(2), 275-285.
[20] M. Hermann, H. Christensen, J. L. E. Reubsaet, *Anal. Bioanal. Chem*., **2005**, 382(5), 1242-1249.
[21] L. Ma, J. Dong, X. J. Chen, G. J. Wang, *Chromatographia*, **2007**, 65(11-12), 737-741.
[22] A. Goyal, I. Singhvi, *Ind. Pharm.*, **2007**, 6(55), 91-93.
[23] M. B. Shankar, V. D. Modi, D. A. Shah, K. K. Bhatt, R. S. Mehta, G. Madhira, B. J. Patel, *J. AOAC*, **2005**, 88(4), 1167-1172.
[24] R. Bhushan, D. Gupta, A. Jain, *J. Planar Chromatogr*, **2006**, 19(110), 288-296.
[25] K. Manoj, P. Muthusamy, S. Anbazhagan, *Indian Drugs*, **2004**, 41(6), 354-357.
[26] G. Anna, H. Hanna, B. Anna, *J. Liq. Chromatogr*., **2005**, 27(13), 2057-2070.
[27] R. T. Sane, S. N. Menon, S. Inamdar, M. Mote, A. Menezes, *J. Planar Chromatogr*., **2004**, 17(2), 154-156.
[28] R. T. Sane, S. N. Menon, S. Inamdar, M. Mote, G. Gundi, *Chromatographia*, **2004,** 59(7-8), 451-453.
[29] A. Jedlicka, J. Klimes, T. Grafnetterova, *Pharmazie*, **2004**, 59(3), 178-182.
[30] D. B. Wanjari, N. J. Gaikwad, *J. Pharm. Sci*., **2005**, 67(2), 256-258.
[31] S. Pattana, N. Penporn, and S. Aurasorn, *J. Chromatogr. B*, **2006**, 843(2), 164-169.
[32] Z. Z. Williams, M. G. Williams, *J. Pharm. Biomed. Anal*., **1996**, 14(4), 465-473.
[33] T. Radhakrishnaa, D. R. Sreenivas, G. O. Reddy, *J. Pharm. Biomed. Anal*., **2002**, 29(4), 593-607.
[34] ICH Steering Committee. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use; Validation of Analytical Procedure-Methodology, Geneva, Switzerland, **2005**.