Int J Pharm Bio Sci 2012 Oct; 3(4): (P) 645 - 655

Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

USE OF MICELLAR MOBILE PHASE FOR THE CHROMATOGRAPHIC SIMULTANEOUS DETERMINATION OF ATENOLOL AND INDAPAMIDE IN PHARMACEUTICAL DOSAGE FORM

SAVITA S YADAV AND JANHAVI R RAO*

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy Erandwane, Pune -411038, India

ABSTRACT

Rapid liquid chromatographic procedure has been developed for the simultaneous determination of Atenolol and Indapamide in a bulk drug and pharmaceutical formulation. The method uses C18 stationary phases and micellar mobile phases of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15% (v/v) 1- propanol as organic modifier and ultraviolet detection at 229 nm are used for the determination. Under these conditions, the studied indapamide and atenolol elute between 5.59 ± 0.10 and 8.058 ± 0.034 min at a 1.5 mL/min flow rate. Limits of detection are well below the concentrations of the drugs found in the commercial pharmaceutical preparation analyzed. The recoveries of the analytes in the pharmaceutical preparations are in the range 99.59 to 99.96% for atenolol and 99.03 to 99.14% for indapamide, respectively.

KEYWORDS: **RP-HPLC, atenolol, indapamide, MLC, simultaneous determination, validation**

JANHAVI R RAO
Department of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy Erandwane, Pune -411038, India

INTRODUCTION

Atenolol, 4-(2-hydroxy-3- [(1-methylethyl) amino] propoxy] benzeneacetamide, is an antihypertensive, antianginal, and antiarrhythmic[1]. Indapamide is a non-thiazide sulphonamide diuretic drug, generally used in the treatment of hypertension, as well as decompensate cardiac failure. Chemically indapamide is 3-(amino sulfonyl) -2methyl- 4-imdol-1-yl) benzamide. Atenolol and Indapamide are official in the IP, BP and USP[2-4]. Different procedures have been developed in order to determine atenolol and indapamide either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using several analytical techniques such as Spectrophotometric techniques[5-8]. Several methods based on separation techniques, including HPTLC[9-11], LC–MS[12] and mainly HPLC[13-17] in reverse phase mode (RP-HPLC) have been also proposed. Because in conventional RP-HPLC are required high concentrations of organic solvents, buffers and gradient elution are required. Micellar liquid chromatography (MLC) is an alternative mode to the conventional reversed-phase liquid chromatography, in which an aqueous solution of a surfactant above its critical micellar concentration is used as mobile phase. So the mobile phase is composed by surfactant micelles and monomers and the stationary phase remains constantly and reproducibly modified by the adsorption of surfactant monomers[18]. The technique is an interesting alternative because of the lower cost and toxicity, the often improved selectivity, and the separation of compound mixtures of diverse polarity without requiring gradient elution. The aim of this work was to develop simple and rapid method for the analysis of pharmaceutical preparation containing atenolol and indapamide. In order to adjust the eluent strength of the micellar mobile phase and reduce the analysis time sodium dodecyl sulfate (SDS) and a small amount of 1-propanol was used[19].

EXPERIMENTAL

Reagents and standards

The surfactant was used to prepare the different mobile phases assayed: sodium dodecyl sulphate (SDS, 99%, Merck Chemicals, Mumbai, India) anionic surfactant. Surfactants were dissolved in 0.01M aqueous solutions of phosphate buffer pH 3, prepared with disodium hydrogen phosphate and citric acid (analytical reagent, Merck Chemicals, Mumbai, India) to adjust the pH of the micellar eluent. After that, an adequate amount of 1-propanol (HPLC grade, Merck Chemicals, Mumbai, India) was added to the micellar eluent to obtain the working concentration (v/v). Atenolol and indapamide were supplied, as a gift, by Emcure Pharmaceuticals Ltd., Pune, India. Aten-D tablet containing 50 mg atenolol and 2.5 mg indapamide (Batch No. ZHK 3280) were obtained commercially within their shelf life. Stock standard solutions of atenolol and indapamide were prepared by dissolving the compound in 0.07M SDS solution. Working solutions were prepared by dilution of the stock standard solutions in the mobile phase solution used. The solutions were stored in the refrigerator at 4 ◦C and they were stable at least for 15 days. Double distilled water was used throughout. The mobile phases and the solutions injected into the chromatograph were vacuum-filtered through 0.45 µm nylon membrane filter (Pall India Pvt. Ltd.).

Instrumental and measurement

The HPLC system consisted of Intelligent HPLC pump model (Jasco PU 2080 Plus). The solutions were injected into the chromatograph through a Rheodyne valve, with a 20 µL loop. The detector consisted of a UV/ VIS (Jasco UV 2075 Plus). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. ODS Hypersil C18 (250 mm, 4.6 mm, 5 µm) column was used. The mobile phase flow rate

was 1.5 mL/min. an ultrasonic bath was used to remove the air from the mobile phases.

Sample preparation

Twenty tablets of the pharmaceutical formulation Aten-D (containing 50 mg atenolol and 2.5 mg indapamide) were assayed. They were crushed to a fine powder and an amount of the powder corresponding to approximately 50 mg atenolol and 2.5 mg indapamide was weighed in a 25 mL volumetric flask. The powder obtained was dissolved in methanol. After that, an adequate volume of aliquot was taken and diluted with 0.07M SDS solution and sonication (for 30 min) the solution was diluted to volume with 0.07 M SDS solution and filtered through 0.45 µm nylon membrane filter (Pall India Pvt. Ltd). Finally, an aliquot of the clean solution was injected into the chromatograph.

METHOD VALIDATION

Sample Analysis

From the filtered sample solution 80 µg/mL for atenolol and 4 µg/mL for indapamide were injected into the chromatogrph. The analysis was repeated five times

Precision

The intra-day precision (RSD, %) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Inter- day precision (RSD %) was assessed by analyzing drug solutions within the calibration range on three different days over a period of a week.

Limits of Detection and Quantitation

To determine the limits of detection (LOD) and quantitation (LOQ), solutions of concentration in the lower part of the linear range of the calibration plot were used. LOD and LOQ were calculated using the equations LOD = 3.3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and S is the slope of the corresponding calibration plot.

Specificity

The specificity of the method was ascertained by analysis of drug standards and samples. The mobile phase resolved both the drugs very efficiently, as shown in Fig. 1a & 1b. The identities for atenolol and indapamide were confirmed by comparing their t_R with those of standards.

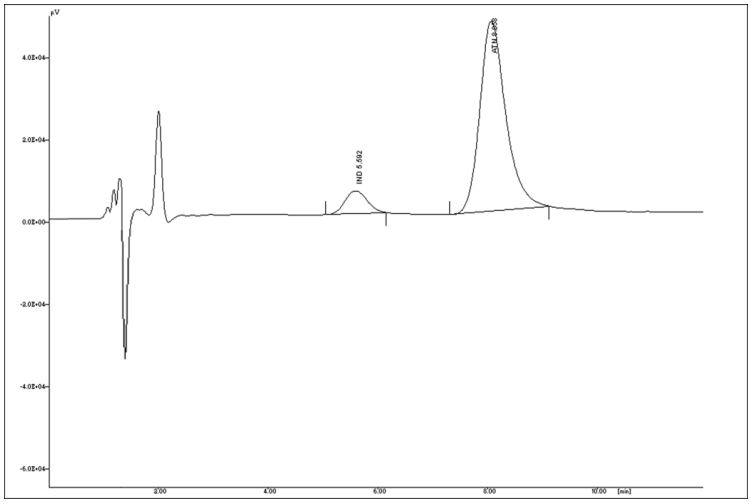


Figure 1a

Chromatogram of atenolol (t_R = 8.058 min) and indapamide (t_R = 5.59 min) from standard

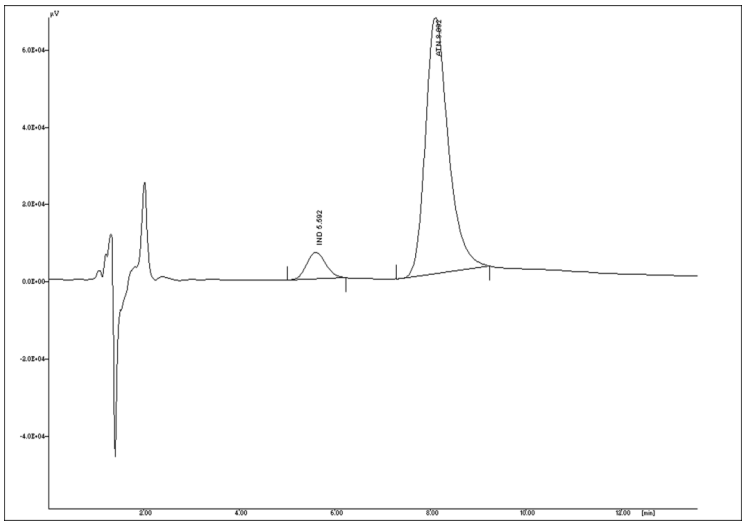


Figure 1b
Chromatogram of atenolol (t_R = 8.092 min) and indapamide (t_R = 5.592 min) from tablet sample

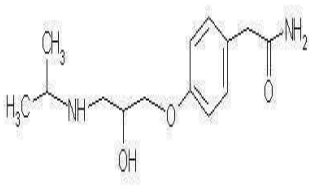
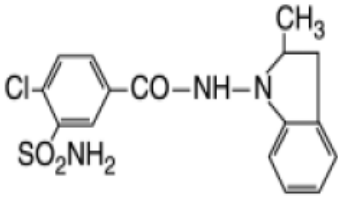
Accuracy
Analyzed samples were over applied with an extra 80, 100, and 120% of the drugs from standard solutions of atenolol and indapamide and the mixtures were reanalyzed by use of the method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug at different levels in the formulation.

Robustness
Robustness was assessed by deliberately changing the chromatographic conditions and studying the effects on the results obtained.

RESULTS AND DISCUSSION

Chromatographic efficiencies
The cationic nature of beta blocker and diuretic produces broad asymmetrical peaks in RPLC with aqueous-organic mobile phases and conventional C18 columns, due to the ionic interaction of the charged solutes with the free silanol groups on the alkyl-bonded reversed-phase packings[20]. The use of low pH and the addition of salts or other blocking agents able to bind silanol groups, such as tertiary and quaternary amines, is a common practice to decrease peak tailing of basic drugs. Another alternative is the use of special stationary phases where the silanol groups are base-deactivated [21]. The dissociation constants (pKa) of indapamide and atenolol in water are in the range 8.8- 9.6 (Table 1).

Table 1
Structures, dissociation constants and (o/w) partition Coefficients of the Atenolol and Indapamide

Compound	Structure	pKa	Log P (o/w)
Atenolol		9.6	0.23
Indapamide		8.8	2.7

Although the pH of the mobile phase does not affect their retention, the efficiencies of chromatographic peaks increases and the asymmetries decrease when the pH decreases[22]. For this reason, the experimental work was carried out at low pH (pH 3). Peak efficiency (expressed as theoretical plates, *N*) was estimated at 10% of peak height according to Foley and Dorsey[23]. Asymmetry factors were calculated as the ratio (*B*/*A*) of the distance between the center and the tailing and leading edge of the chromatographic peak, measured also at 10% of peak height. All simulations and optimizations were performed with the software Borwin. Tables 2 list the mean values of N and B/A for the atenolol and indapamide eluted from the ODS Hypersil C18 column using micellar SDS–propanol mobile phases. The low efficiencies and highly symmetrical peaks obtained in these conditions are indicative of the presence of free silanol groups in the column, which interact with both the drugs. It should be noted that with the micellar-organic mobile phases, the efficiencies decreased at increasing concentration of the surfactant, and increased with the volume fraction of propanol, which is the expected behaviour. Moreover, both the drugs yielded nearly Gaussian peaks with the SDS–propanol mobile phases.

Table 2
Efficiencies and asymmetry factors for Atenolol and Indapamide eluted with mobile phases of SDS–propanol[a]

Compound	0.07 M SDS		0.1 M SDS		0.15 M SDS	
	N	B/A	N	B/A	N	B/A
Atenolol	4334.82	1.125	3654.71	1.350	2400.06	1.392
Indapamide	2024.47	1.14	2021.11	1.251	1508.61	1.253

[a] ***Mean values for 15% propanol.***

Elution strength

The elution strength was measured for the different micellar-organic system. For MLC, the experimental design consisted of three mobile phases which covered a domain from 0.07 to 0.15M SDS and from 5 to 15% (v/v) propanol. The elution strength (i.e. sensitivity of the retention of solutes) of the surfactant depends on the concentration of organic modifier, and vice versa. The elution strength of propanol changed only slightly for concentrations of SDS between 0.07 and 0.15 M. It decreased at increasing concentration of the surfactant for the most polar drugs. The elution strength of the surfactant usually increased with the concentration of propanol.

ANALYTICAL DATA

Validation of method Linearity

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 20–120 µg/mL for atenolol and 1–6 µg/mL for indapamide. The linear regression equations were Y = 19832X + 47847 (r^2 = 0.9998) for atenolol and Y = 49773X - 7942 (r^2 = 0.9993) for indapamide. The plots obtained from linear regression and residuals analysis are given in Fig 2a and 2b for Atenolol and 3a and 3b for Indapamide.

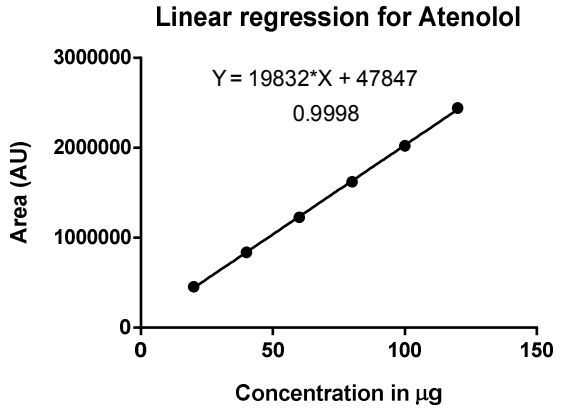


Figure 2a
Plot obtained from linear regression analysis for atenolol.

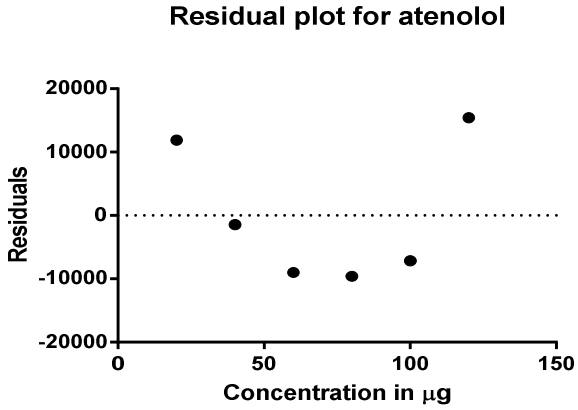


Figure 2b
Residuals plot obtained from linear regression analysis for atenolol.

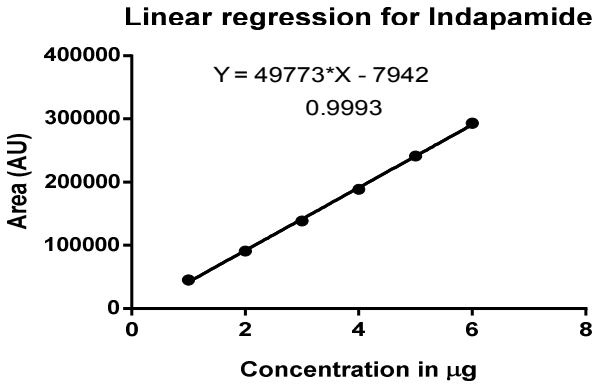


Figure 3a
Plot obtained from linear regression analysis for indapamide.

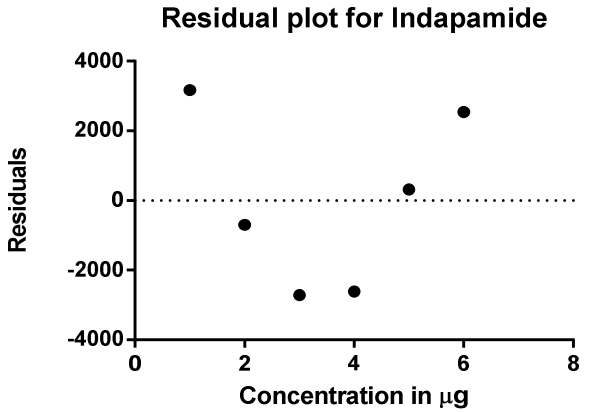


Figure 3b
Residuals plot obtained from linear regression analysis for indapamide

Precision: The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table 3 reveal the high precision of the method.

Table 3
Precision Study

Concentration (µg/mL)	Intra-day precision			Inter-day precision		
	Measured Conc. ± SD	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery[a] (%)
Atenolol						
40	39.96 ± 0.12	0.119	99.99	39.98 ± 0.175	0.175	99.95
80	79.79 ± 0.39	0.393	99.74	79.88 ± 0.562	0.564	99.85
120	119.61 ± 0.305	0.304	99.68	120.33 ± 0.76	0.75	100.3
Indapamide						
2	1.98 ± 0.112	0.11	99.00	1.99 ± 0.39	0.39	99.5
4	3.97 ± 0.85	0.84	99.25	3.99 ± 0.87	0.88	99.75
6	6.03 ± 0.34	0.33	100.5	5.98 ± 0.19	0.20	99.66

[a]***Mean from three estimates***

Limit of Detection and Quantitation

The limit of detection and quantitation calculated were found 2, 5 µg/mL, respectively for atenolol and 0.1, 0.6 µg/mL for indapamide. This indicates the method is sufficiently sensitive.

Accuracy

When the method was used for extraction and subsequent analysis of both drugs from the pharmaceutical dosage forms, and the extract was over applied with 80, 100, and 120% of additional drug, the recovery was listed in Table 4.

Table 4
Recovery studies

Drug	Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	Recovery[a] (%)
Atenolol	50	80	90	89.63	99.59
		100	100	99.96	99.96
		120	110	119.87	99.89
Indapamide	2.5	80	4.5	4.46	99.14
		100	5.0	4.95	99.06
		150	5.5	5.45	99.03

[a]***Mean from five estimates***

Robustness

The relative standard deviation of peak areas was less than 2%. The RSD shown in Table 5 indicate the robustness of the method.

Table 5
Robustness of the method[a]

Chromatographic factors	Level	Chromatographic changes in t_R	
		Atenolol	Indapamide
A: Flow rate mL/min.			
1.4	-0.1	8.098	5.608
1.5	0.0	8.092	5.592
1.6	+0.1	8.058	5.575
Mean ± SD		8.083 ± 0.022	5.592 ± 0.017
B: % of propanol in the mobile phase			
% 14	-1.0	8.083	5.600
% 15	0.0	8.092	5.592
% 16	+1.0	8.042	5.582
Mean ± SD		8.072 ± 0.027	5.591 ± 0.0090

[a]***Mean from three estimates***

Sample Analysis

When the Aten-D tablets were analyzed, sharp and well defined peaks for indapamide and atenolol were obtained at t_R 5.59 and 8.09 min, respectively, when scanned at 229 nm. The amount of the label claim measured were 99.79 ± 0.20 % for atenolol and 99.36 ± 0.11 % for indapamide.

CONCLUSION

In this paper, a new, simple, rapid micellar liquid chromatographic procedure for the simultaneous determination of atenolol and indapamide in pharmaceutical preparation is proposed. The LOD and % R.S.D. values are sufficiently good for the applicability of this method in the quality control of this pharmaceutical formulation. Due to the versatility of the interactions in micellar liquid chromatography, it is possible to determine a great variety of compounds including those with high hydrophobicity in adequate times of analysis. The relatively low amount of organic solvent required in MLC is very attractive. This reduces the toxicity, flammability, environmental impact and cost of the mobile phases. Propanol is less toxic than methanol and acetonitrile, and is highly retained in SDS micellar solutions, which reduces the risk of evaporation. Micellar mobile phases can, therefore, be stable for long periods of time.

ACKNOWLEDGEMENT

The authors would like to thank, Emcure Pharmaceutical Ltd. Pune, India for providing a gift sample of standard atenolol and indapamide. The authors would like to thank, Dr K R Mahadik, Principal, Poona College of Pharmacy, Pune, India for providing necessary facilities to carry out the work.

REFERENCES

1. O'Neil M J, The Merck Index, 14th ed, Merck Research Laboratories, Whitehouse Station, New Jersey: 142, (2006).
2. Indian Pharmacopoeia, Vol 1. Government of India, Ministry of Health and Family Welfare, Controller of Publications, Delhi: 72–73, (1996).
3. British Pharmacopoeia, Vol 1. The stationary office, London: 179–181, (2005).
4. United States Pharmacopoeia, 28th ed, Vol 1. Rockville, MD: The United States Pharmacopeial Convention: 193, (2005).
5. Kasture A V, Ramteke M, Simultaneous UV-spectrophotometric method for the

estimation of atenolol and amlodipine besylate in combined dosage form. Indian J Pharm Sci., 68(3): 394-396, (2006).
6. Al-Ghannam S M, A simple spectrophotometric method for the determination of β-blockers in dosage forms. J Pharm Biomed Anal., 40(1): 151-156, (2006).
7. Singhvi, Goyal A, 2007, Visible spectrophotometric estimation of aceclofenac and indapamide from tablets using folin-ciocalteu reagent. Indian J Pharm Sci. 69(1):164-165.
8. Ribeiro D S M, Prior J A V, Santos J L M, Lopes J A, Lima J L F C, Exploiting the oxidative coupling reaction of MBTH for indapamide determination. Talanta, 79(4): 1161-1168, (2009).
9. Argekar A P, Powar S G, Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography. J Pharm Biomed Anal., 21(6): 1137-1142, (2000).
10. Sathe S R, Bari S B, Simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in tablets by high-performance thin-layer chromatography with UV absorption densitometry. Acta Chromatogr., 19: 270-278, (2007).
11. Dewani M G, Bothara K G, Madgulkar A R, Damle M C, Simultaneous Estimation of Perindopril Erbumine and Indapamide in Bulk Drug and Tablet Dosage Form by HPTLC. International Journal of Comprehensive Pharmacy, 2(1): 1-4, (2011).
12. Jain D S, Subbaih G, Sanyal M, Pande U C, Shivastav P, Liquid chromatography-tandem mass spectrometry validated method for the estimation of indapamide in human whole blood. J Chromatogr B Anal Technol Biomed Life Sci, 834 (1-2): 149-154, (2006).
13. Sivakumar T, Venkatesan P, Manavalan R, Valliappan K, Development of a HPLC method for the simultaneous determination of losartan potassium and atenolol in tablets. Indian J Pharm Sci., 69(1):154-157, (2007).
14. Barman R K, Islam M A U, Ahmed M et al., Simultaneous high-performance liquid chromatographic determination of Atenolol and Amlodipine in pharmaceutical-dosage form, Pak J Pharm Sci., 20(4): 274-279, (2007).
15. Naveen Kumar, Verma N, Omveer Singh, Joshi N, Kanwar G S, Estimation of Atenolol by Reverse Phase High Performance Liquid Chromatography. E J Chem., 7(3): 962-966, (2010).
16. Pai J B, Shetty A S, Gopinath B, Gangaprasad Chenna, Development and Validation of RP-HPLC Method for Quantitative estimation of Indapamide in Bulk and Pharmaceutical dosage forms, Int.J. PharmTech Res., 3(3):1482-1487, (2011).
17. Patel A R, Chandrul K K, Method Development and Validation of Simultaneous Estimation Telmisartan and Indapamide by Reverse Phase-High Performance Liquid Chromatography in Pharmaceutical Dosage Forms. Asian Journal of Biochemical and Pharmaceutical Research, 1(1): 39-49, (2011).
18. Martınez-Algaba C., Escuder-Gilabert L., Sagrado S., R.M. Villanueva-Camanas R. M, Medina-Hernandez M. J., Comparison between sodium dodecyl sulphate and cetyl trimethyl ammonium bromide as mobile phases in the micellar liquid chromatography determination of non-steroidal anti-inflammatory drugs in pharmaceuticals, Journal of Pharmaceutical and Biomedical Analysis, 36: 393–399, (2004).
19. Martınez-Algaba C., Bermudez-Saldana J. M., Villanueva-Camanas R. M, Sagrado S., Medina-Hernandez M. J., Analysis of pharmaceutical preparations containing antihistamine drugs by micellar liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 40: 312–321, (2006).

20. Basci N. E., Temizer A., Bozkurt A., Isimer A., Optimization of mobile phase in the separation of beta-blockers by HPLC, J. Pharm. Biomed. Anal. 18 (4-5): 745-50, (1998).
21. Saarinen M. T., Siren H., Riekkola M. L., Screening and determination of beta-blockers, narcotic analgesics and stimulants in urine by high-performance liquid chromatography with column switching, J Chromatogr B Biomed Appl, 664(2): 341-6, (1995).
22. Rapado-Martınez I, Villanueva-Camanas R. M. Garcıa-Alvarez-Coq.ue M. C, Micellar Liquid Chromatography: A Worthy Technique for the Determination of β-Antagonists in Urine Samples. Anal. Chem, 71(2): 319-326, (1999).
23. Foley J.P., Dorsey J.G., Equations for calculation of chromatographic figures of merit for ideal and skewed peaks. Anal. Chem., 55: 730-737, (1983).