Research Article

Micellar Liquid Chromatographic Method for Simultaneous Determination of Atenolol and Aspirin in Bulk and Pharmaceutical Dosage Form

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Accepted on: 06-03-2016; **Finalized on:** 31-03-2016.

ABSTRACT

Micellar liquid chromatographic method has been developed for the simultaneous determination of Atenolol and Aspirin in a bulk drug and pharmaceutical formulation. Chromatographic separation achieved isocratically on ODS hypersil C18 column (5 µm, 250 mm × 4.6 mm) and micellar mobile phase of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15% (v/v) 1- propanol as organic modifier in the ratio 70: 30 v/v and ultraviolet detection at 225 nm are used for the determination. Under these conditions, the studied Aspirin and Atenolol elute between 4.033 ± 0.02 and 6.042 ± 0.031 min at a 1 mL/min flow rate. Parameters such as linearity, precision, accuracy, specificity and robustness are studied as reported in the ICH guidelines. Linearity for Atenolol and Aspirin was in the range of 2-12 µg/mL and 4-24 µg/mL respectively. The mean recoveries obtained for Atenolol and Aspirin were 99.55 and 99.61 % respectively. Developed method was found to be accurate, precise, selective and rapid for simultaneous estimation of Aspirin and Atenolol.

Keywords: Atenolol, Aspirin, MLC, simultaneous determination, validation.

INTRODUCTION

Micellar liquid chromatography (MLC) is an efficient alternative to conventional reversed-phase liquid chromatography with hydro-organic mobile phases. Most MLC procedures uses hybrid micellar mobile phases containing a surfactant above the critical micellar concentration and a relatively small amount of organic solvent to increase the elution strength and improve the efficiencies. The anionic sodium dodecyl sulphate (SDS) is the most usual surfactant in MLC, but it also requires the addition of an organic solvent to decrease the retention times and increase the efficiency. In particular, positively charged basic compounds are strongly retained by the stationary phase modified by adsorption of SDS monomers and require the addition of a strong solvent, such as propanol or pentanol[1-3].

Atenolol (ATN), 4-(2-hydroxy-3- [(1-methylethyl) amino] propoxy] benzeneacetamide, is an antihypertensive, antianginal, and antiarrhythmic[4]. Aspirin (ASP) is chemically, 2-(acetyloxy) benzoic acid and exerts its anti-inflammatory, analgesic and antipyretic actions. Aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) inhibit the activity of the enzyme called cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever[5].

Literature survey revealed that there are several methods available to determine ATN and ASP either alone or in combination with other drugs in pharmaceutical formulations and biological fluids using various analytical techniques such as spectrophotometric techniques[6-8], several methods based on separation techniques, including HPTLC[9-10], LC–MS[11-12] and HPLC[13-16] have been also reported. There is no MLC method for the simultaneous determination of these drugs in combined dosage form. Hence a new MLC method has been developed for the estimation of ATN and ASP in combined dosage form. The developed method is simple, precise, selective, and rapid and can be used for routine analysis. The structures of the drugs are shown in Figure 1.

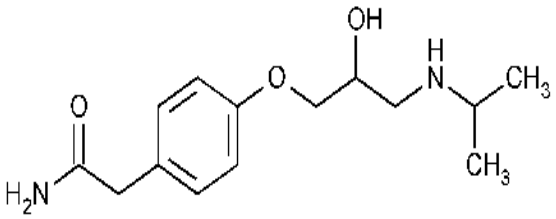

Figure 1a: Chemical structure of ATN

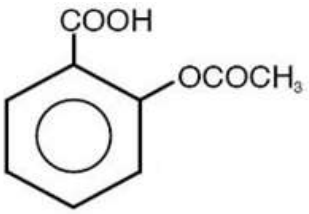

Figure 1b: Chemical structure of ASP

MATERIALS AND METHODS

Chemicals and Reagents

Aspirin and Atenolol were kindly supplied by Wockhardt Pharmaceuticals Ltd., Aurangabad and Emcure Pharmaceuticals Ltd, Pune, India. ATO-PRIN tablet containing 50 mg Atenolol and 100 mg Aspirin were

obtained commercially within their shelf life. For MLC work double distilled water was prepared in the laboratory. 1-propanol, Sodium dodecyl sulphate, disodium hydrogen phosphate and citric acid used were of HPLC grade and analytical grade reagent and were purchased from Merck Chemicals, Mumbai, India.

MLC instrument and chromatographic conditions

Chromatographic separation was achieved using MLC System consisted of Intelligent HPLC pump model (Jasco PU 2080 plus), an auto sampler and UV/VIS (Jasco UV 2075 plus) detector. The output signal was monitored and processed using Jasco Borwin version 1.5, LC-Net II/ADC software.

ODS Hypersil C18 (250 mm, 4.6 mm id, 5 µm particle size) was used as the stationary phase. Mobile phase consisting of 0.07M sodium dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15 % (v/v) 1-propanol as organic modifier in the ratio 70:30 v/v was delivered at a flow rate of 1 mL/min. The detector was set at the wavelength of 225 nm. Injection volume was kept 20 µL.

Preparation of standard and sample solutions

A standard mixed stock solution of atenolol and aspirin was prepared by accurately weighing atenolol (50 mg) and aspirin (100 mg) into a 25 mL volumetric flask. The drugs were dissolved in methanol and the solution was diluted to volume.

The stock solution was further diluted with mobile phase to obtain a solution of ATN (2 µg/mL) and ASP (4 µg/mL), respectively.

Twenty tablets of the pharmaceutical formulation ATO-PRIN (containing 50 mg atenolol and 100 mg aspirin) were assayed. They were crushed to a fine powder and an amount of the powder corresponding to approximately 50 mg ATN and 100 mg ASP 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.

System suitability

From the filtered sample solution 2 µg/mL for ATN and 4 µg/mL for ASP were injected into the chromatograph. The analysis was repeated six times to test the system suitability for their retention time, resolution (Rs), theoretical plates number (N) and tailing factors (T).

Method Validation

The developed MLC method was validated as per International Conference on Harmonization (ICH) guidelines for the parameters like specificity, linearity and range, LOD and LOQ, precision (intraday and interday precision), accuracy and robustness[17].

Sample analysis

20 µL of working standard solution and sample solutions were injected into the liquid chromatograph and the chromatograms were recorded. From the peak area of ASP and peak area of ATN the amount of the drugs in the sample were calculated.

Linearity and range

For determining linearity, calibration curves were plotted over a concentration range of 2-12 µg/mL for ATN and 4-24 µg/mL for ASP, respectively. A 20 µL of sample solution was injected into the chromatographic system using fixed volume loop injector. Chromatograms were recorded. Calibration plots were constructed by plotting peak area against the corresponding amount of each drug.

Limit of detection and limit of quantitation

The LOD and LOQ were calculated according to the 3.3 σ/s and 10 σ/s criteria, respectively; where σ is the standard deviation of the peak area and s is the slope of the corresponding calibration curve.

Precision

Precision studies were carried out to establish the intraday and interday precision of proposed method. 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.

Accuracy

To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. The experiment was conducted in triplicate. Percentage recovery and relative standard deviation were calculated.

Robustness

To check the robustness of proposed method, small, deliberate changes were made to the chromatographic condition. A study was performed by changing the flow rate and % of propanol in the mobile phase (± 1%). Standard solution prepared as per test method and injected into the MLC system. Flow rate change was done by varying flow rate at from 0.9 mL/min to 1.1 mL/min.

RESULTS AND DISCUSSION

Method development

The MLC procedure was optimized for simultaneous determination of ATN and ASP. Good resolution of both the components was obtained with 0.07M sodium

dodecyl sulfate (SDS) pH 3 adjusted with phosphate buffer and 15 % (v/v) 1-propanol as organic modifier in the ratio 70:30 v/v.

The flow rate of 1 mL/min was optimum. UV detection was made at 225 nm. At this wavelength ATN and ASP can be quantified.

Hence, 225 nm determined empirically has been found to be optimum. The average retention times for ASP and ATN was found to be 4.033 and 6.042 min, respectively (Figure 2).

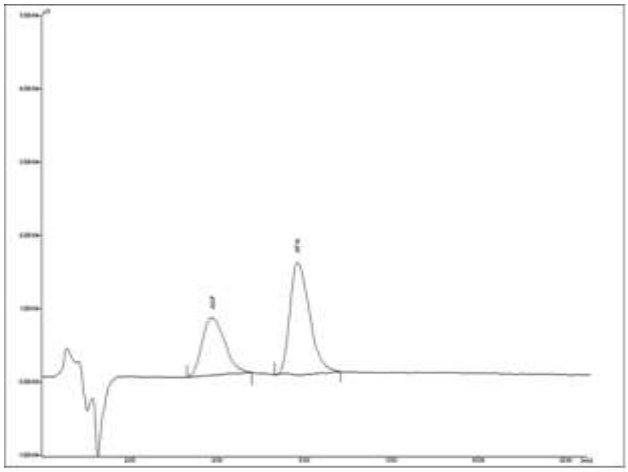

Figure 2: Chromatogram of ASP and ATN

System suitability

To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 1.

Table 1: System suitability parameters (n=6)

Parameters	ASP	ATN
Retention Time in min	4.033	6.042
Resolution (Rs)	0.00	4.729
Theoretical plates number (N)	2959	3785
Tailing Factor	1.351	1.214

Linearity

Linearity was accessed by visualizing the calibration graph and plot of the residuals.

The points distributed equally above and below the trend line showed linearity.

The linear regression equations were Y = 26481X + 108761 (r^2 = 0.9995) for ATN and Y = 13211X + 113353 (r^2 = 0.9996) for ASP.

The plots obtained from linear regression are given in Figures 3 and 4 for ATN and ASP, respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limits of detection and quantitation were found to be 0.8 µg/mL and 2 µg/mL for ATN and 2 µg/mL and 4 µg/mL for ASP, respectively. This indicates the method is sufficiently sensitive.

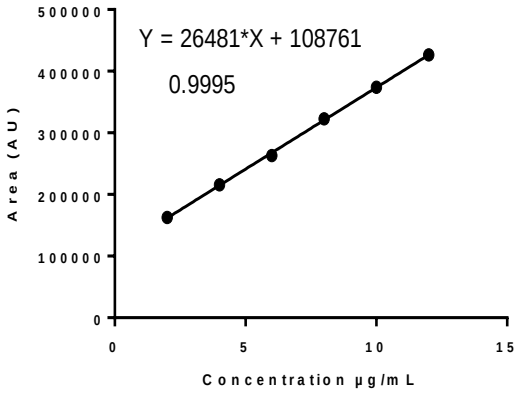

Figure 3: Calibration curve for ATN

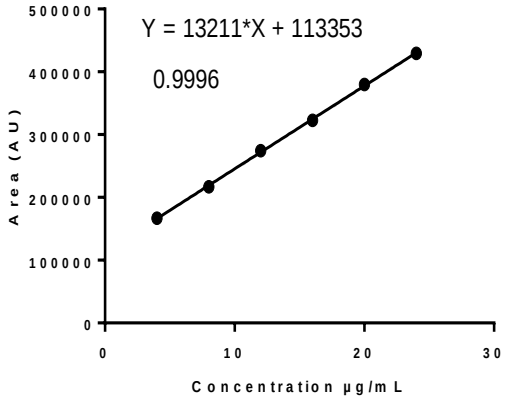

Figure 4: Calibration curve for ASP

Precision

The precision of the method was expressed as relative standard deviation (RSD, %). Results calculated as % RSD values for intraday and interday precision studies are shown in Table 2 and found to be satisfactory.

Accuracy

Accuracy was determined at three levels 80%, 100% and 120% of the target concentration in triplicate and the percentage recovery for the amount added was calculated. The results are presented in Table 3.

Robustness

Robustness of the method was determined by performing same analysis at slightly different parameters from the optimized conditions of selected method. There were no significant changes in the retention times of ASP and ATN when the flow rate (± 0.1 mL/min.) and % of propanol in the mobile phase (± 1%) were changed. The low values of the % RSD indicate the robustness of the method, as shown in Table 4.

Table 2: Precision studies

Conc. (µg/mL)	Intra-day precision (n=3)			Inter-day precision (n=3)		
	Measured Conc. ± SD	(%) RSD	Recovery (%)	Measured Conc. ±SD	(%) RSD	Recovery (%)
Atenolol						
4	3.975 ± 0.031	0.78	99.38	3.973 ± 0.041	1.04	99.33
8	7.96 ± 0.072	0.91	99.50	7.97 ± 0.090	1.13	99.63
12	11.92 ± 0.13	1.09	99.33	11.91 ± 0.144	1.21	99.25
Aspirin						
8	7.97 ± 0.089	1.12	99.63	7.95 ± 0.083	1.05	99.38
16	15.96 ± 0.171	1.07	99.75	15.92 ± 0.175	1.10	99.50
24	23.89 ± 0.244	1.02	99.54	23.83 ± 0.27	1.14	99.29

Table 3: Recovery studies

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount recovered (mg)	(%) Recovery	Mean (%) Recovery (± SD)
ATN 50	80	90	89.70	99.67	99.55 ± 0.182
	100	100	99.34	99.34	
	120	110	109.60	99.64	
ASP 100	80	180	179.44	99.69	99.61 ± 0.161
	100	200	198.83	99.42	
	120	220	219.37	99.71	

Table 4: Robustness evaluation of ATN and ASP

Conditions	Level	ATN		ASP	
		Rt (min.)	% RSD of peak area	Rt (min.)	% RSD of peak area
A: Flow rate mL/min.					
0.9	-0.1	6.095	1.02	4.092	1.11
1.0	0.0	6.042	1.10	4.033	1.05
1.1	+0.1	6.000	1.13	4.010	1.14
Mean ± SD		6.046 ± 0.048	1.083 ± 0.0569	4.045 ± 0.042	1.10 ± 0.0458
B: % of propanol in the mobile phase (± 1%)					
% 14	-1.0	6.092	1.07	4.095	1.03
% 15	0.0	6.042	1.14	4.033	1.11
% 16	+1.0	6.004	1.19	4.012	1.18
Mean ± SD		6.046 ± 0.044	1.133 ± 0.0603	4.047 ± 0.043	1.107 ± 0.075

Analysis of marketed formulation

The chromatogram of the sample extracted from conventional tablets showed peaks of ASP (Rt 4.033 min.) and ATN (Rt -6.042 min) well resolved from other tablet excipients. The percent contents of ASP and ATN per tablet by proposed method was found to be 99.67 % and 99.60 %, respectively.

CONCLUSION

An accurate, sensitive and precise micellar liquid chromatographic method has been developed and fully validated for quality control analysis of atenolol and aspirin in bulk and Pharmaceutical dosage form. The developed method was found to be simple and have certain advantages associated with this method such as high selectivity, sensitivity and less hazardous. Moreover, the lower solvent consumption along with the short analytical run time leads to a cost effective and eco-friendly chromatographic procedure.

Acknowledgement: The authors would like to thank, Emcure Pharmaceutical Ltd. Pune and Wockhardt Pharmaceuticals Ltd., Aurangabad, India for providing a gift sample of standard atenolol and Aspirin. 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.

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Source of Support: Nil, **Conflict of Interest:** None.