ISSN : 0974-7419 Volume 16 Issue 4
Analytical CHEMISTRY
An Indian Journal
Full Paper
ACAIJ, 16(4) 2016 [147-155]

New stability indicating RP – HPLC and Spectrophotometric methods for the determination of amoxapine in tablet dosage form

Uma Maheswar Korrapati, Rambabu Chintala*
Department of chemistry, Acharya Nagarjuna University, Guntur, A.P., (INDIA)
E-mail: rbchintala@gmail.com

ABSTRACT

A simple, selective, linear, precise and accurate and validated stability indicating RP-HPLC method [M 1] and two spectrophotometric methods [M 2 & M 3] were developed for rapid assay of Amoxapine in tablet dosage form. In Method 1, a chromatographic system was employed with a Zodiac C18 column, a mobile phase of methanol(MeOH): acetonitrile(ACN): water in a proportion of 10:80:10 (v/v/v) and UV7000 Techcomp detector was used at a detection wavelength of 254nm. The forced degradation studies are carried as per ICH guidelines and a complete separation of the degradation peak with the drug peak was observed and hence is specific for the estimation of Amoxapine in the presence of its degradation products. The chromogenic reagents like 1,10 Phenanthroline (*o*-PHEN) (M 2) and Naphthaquinine sulfate (NQS) (M 3) were used in colour development for the estimation of amoxapine in pure and dosage forms. The methods were validated as per the ICH guidelines and adopted for the assay of Amoxapine in the bulk drug and formulations. © 2016 Trade Science Inc. - INDIA

KEYWORDS

Amoxapine;
RP-HPLC method;
1,10 Phenanthrolene, NQS;
Validation;
Assay.

INTRODUCTION

Amoxapine (amoxapine tablets) is an antidepressant of the dibenzoxazepine class, chemically distinct from the family dibenzazepines, dibenzocycloheptenes and dibenzoxepines, though it is often classified as a secondary amine tricyclic antidepressant. It is the N-demethylated metabolite of loxapine. It is designated chemically as 2-Chloro-11-(1-piperazinyl) dibenz [b, f][1,4] oxazepine. Amoxapine is used in the treatment of depression, anxiety disorders, panic disorder, and bipolar disorder. It also has properties similar to those of atypical antipsychotics[1,2] and may be used in the treatment of schizophrenic psychosis off-label.

It works by increasing the amounts of certain natural substances in the brain that are needed to maintain mental balance. Common side effects of

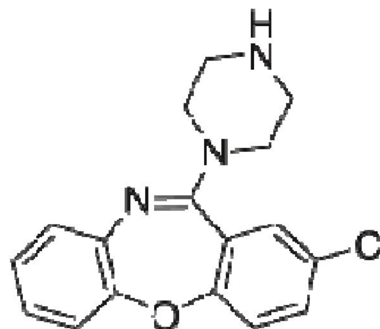

Figure 1 : Structure of amoxapine

amoxapine include hypotension, drowsiness, dry mouth, constipation, blurred vision, fatigue and vertigo[3]. Additionally, due to the drug's and its metabolite 7-hyroxy amoxapine's potent blockade of dopamine receptors, it can cause neuroleptic malignant syndrome as well as acute extra pyramidal symptoms and tardive dyskinesia. Cardiovascular and anticholinergic side effects are much reduced compared to other tri- and tetra cyclic antidepressants. Very few determination methods like LCMS[4], HPLC[5], Bioanalytical[6,7], simultaneous determination[8,9] and a spectrophotometric[10] method are so far reported. We now repot, three new methods one stability indicating RP-HPLC and two spectrophotometric for its estimation.

EXPERIMENTAL

Materials

Amoxapine reference standard was provided by RV Labs Pvt. Ltd., and the formulation tablets were purchased from a local pharmacy. HPLC grade methanol (MeOH), acetonitrile (ACN), Water were purchased from Merck Specialities Pvt. Ltd., Mumbai. All other chemicals and reagents such as Naphtha Quinine sulfate, Sodium Hydroxide, ferric chloride and 1, 10 phenanthroline were of AR grade and were purchased from Qualigens Fine Chemicals, Mumbai.

Instrumentation

In M 1, the PEAK chromatographic system with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop and variable wavelength programmable UV detector UV7000 Techcomp was used. The output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. In Method-2 and 3, a UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Denver electronic analytical balance (SI-234) and Systronics digital pH meter were used for weighing and to adjust pH of the mobile phase respectively.

Preparation of solutions

In Method 1, an amount of the pure drug equivalent to 10mg of Amoxapine was accurately weighed and transferred into a 10ml volumetric flask, dissolved with 10ml of mobile phase, sonicated for five minutes and made up to the mark with mobile phase. The solution was filtered through Ultipor N66 Nylon 6, 6 membrane sample filter paper. To prepare sample solution, the average weight of five tablets of the drug (Amolife – 50mg) was determined and powdered them with mortar. An amount of powder equivalent to 10mg of Amoxapine was weighed accurately and transferred into a clean 10 ml volumetric flask and dissolved in mobile phase solution. Then the solution was made up to the volume with mobile phase. The solution was sonicated for 5min and filtered through 0.45 µm membrane filter. Then about 5.0ml of the stock solution of standard and sample separately transferred into two 100ml volumetric flasks and diluted up to the mark with mobile phase. Appropriate volumes of these solutions were further diluted with mobile phase to prepare solutions of required concentration. An amount of pure drug equivalent to10mg was accurately weighed and dissolved in 10ml of double distilled water in a standard flask. About 1.0ml and 3.0ml of this stock solution was measured and made up to 10.0ml to get a concentration of 100 µg/ml and 300 µg/ml and was used as stock solution in M 2 and M 3 respectively. The sample solution was prepared by dissolving an amount of the powdered tablet (Amolife – 50mg) equivalent to 10mg of Amoxapine in a clean 10 ml volumetric flask and made up to the mark with distilled water.

Preparation of reagents

0.25% $FeCl_3$ solution was prepared by dissolving 250mg of ferric chloride in double distilled water and made up to 100ml. 0.2% 1,10 phenanthroline and 0.5% NQS solutions were prepared by dissolving 200mg or 500mg of 1,10 phenanthroline or NQS in water and made up to 100ml. About 8.7ml of concentrated HCl was transferred into a clean 100ml volumetric flask and diluted up to the mark with double distilled water to make the final concentration of the resulting solution 1N.

Method Development

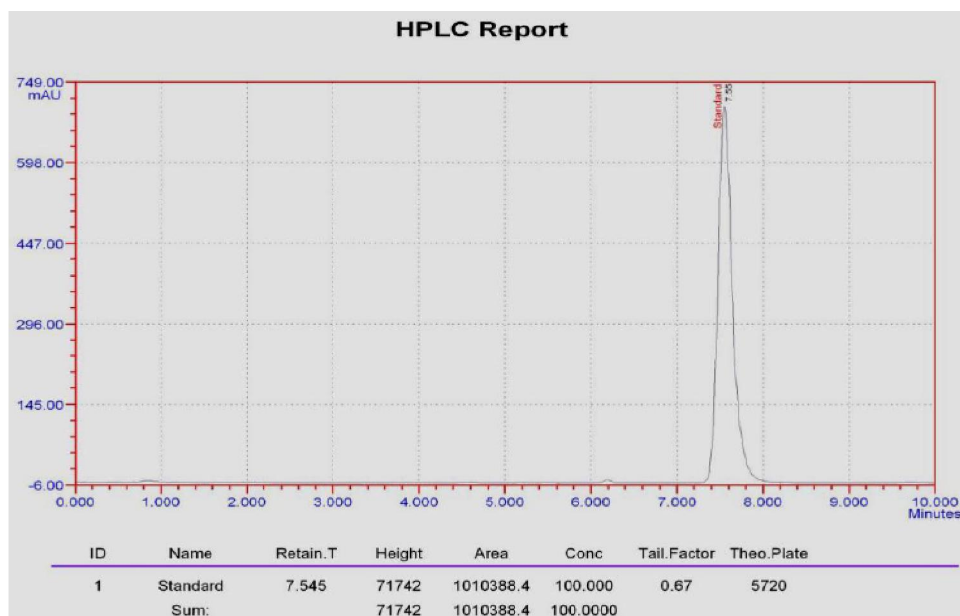

Figure 2 : Standard chromatogram of amoxapine

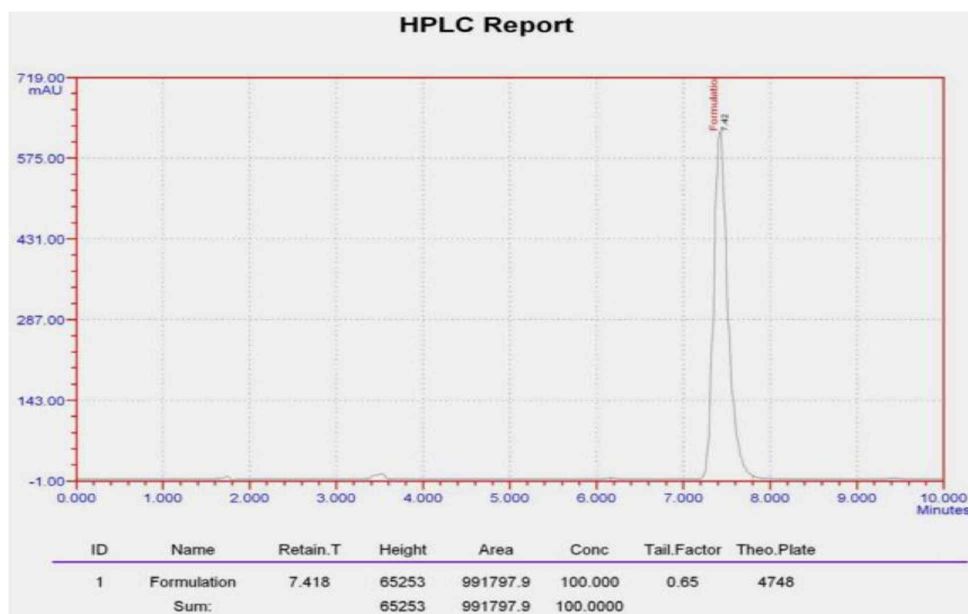

Figure 3 : Typical chromatogram of amoxapine formulation

M 1 (RP-HPLC): While developing the RP-HPLC method, different trails for the systematic study of the effect of various factors like detection wavelength, composition and *p*H of the mobile phase and flow rate were undertaken by varying one parameter at a time and keeping all other conditions constant. The optimized chromatographic conditions were found to be Zodiac, C18 (250mmx4.6mm, 5µ particle size) as column, mixture of MeOH: ACN:Water in the ratio 10: 80: 10 (v/v) as mobile phase with a pH of 4.6 at a flow rate of 0.8ml/min, a run time of 10 minutes and detection wavelength at 254 nm. Typical RP-HPLC chromatograms of Amoxapine standard (Rt=7.545min) and sample (Rt=7.418min) were recorded by injecting 20µl of standard or sample and represented in Figure-2 & Figure-3 respectively. The system suitable parameters were presented in TABLE-1.

Stress degradation studies

Amoxapine was exposed to different stress conditions and the degradation products were well sepa-

Full Paper

TABLE 1 : System suitability parameters of amoxapine

Parameters	Values
λ max (nm)	254
Beer's law limit	60-240 ppm
Correlation coefficient	0.999
Retention time	7.54
Theoretical plates	5720
Tailing factor	0.67
Limit of detection	1.25 ppm
Limit of quantification	4.0ppm

phase), working standard and sample solutions were injected separately into the HPLC system and chromatograms were recorded under the optimized chromatographic conditions. In case of spectrophotometric analysis, absorbance was measured for the colored products of the standard and sample in Method-2 and Method-3. The experimental results were given in TABLE-4.

Precision

The precision of the proposed methods was ex-

TABLE 2 : Stress degradation studies

Degradation Condition	Degradation Time	Retention Time	% Degradation	% Recovery	Tailing Factor	Theoritical Plates
Acid	48 Hrs	7.402	19.5	80.5	1.35	10953
Base	48 Hrs	7.715	18.0	82.0	0.86	5565
Photolytic	48 Hrs	7.085	30.9	69.1	1.28	9524
UV light	48 Hrs	7.402	20.8	79.2	1.08	10893
Thermal	48 Hrs	7.547	20.7	79.3	1.52	8271
Peroxide	48 Hrs	7.417	30.1	69.9	1.60	6363
Aqueous	48 Hrs	7.787	24.1	75.9	1.11	12475

TABLE 3 : Optical parameters of the proposed methods

Sl.No.	Parameter	Method – 2 [o-PHEN]	Method – 3 [NQS]
1.	Wavelength Maxima	535nm	540nm
2.	Molar Absorptivity	0.243×10^3	4.117×10^3
3.	Sandell's sensitivity	0.0202µg/cm	0.00598 µg/cm
4.	Linearity Range	2.5 – 15.0 µg/ml	10 – 60 µg/ml

rated with greater resolution. The conditions and extent of degradation were shown in TABLE 2.

Visible spectrophotometric methods

In developing visible spectrophotometric methods, optimized conditions were identified by making different trails by varying one of the parameters such as concentration of standard, volume of the reagents, order of addition of the reagents, temperature and time for color development and keeping other as constant, and the optimized procedures were presented below. The suitable optical parameters of the developed methods are given in TABLE 3.

Method-2 : About 1.0ml of stock solution (100 µg/ml) was accurately transferred into 10 ml graduated test tube, about 0.5 ml of 0.25% $FeCl_3$ solution and 2.0 ml of 0.2% 1,10 phenanthroline were added. The tube was heated in water bath up to 30 min. after cooling to room temperature, about 2.0 ml of 1N HCl was added and made to 10 ml with distilled water. The absorbance of the colored solution was scanned over a range of wavelength 400-800nm against a reagent blank and the wavelength of maximum absorbance was found to be 535nm. The absorption spectrum was presented in Figure-4. This method was based on the formation of ferrous tris-o-phenanthroline complex with 1, 10 -phenanthroline at pH ~ 3±0.2.

Method-3: In this method, in a 10ml graduated test tube an aliquot of standard drug, 1.0 ml of 0.01M NaOH solution and 1.0ml of NQS solution were taken and the tube was stoppard immediately and shaken well until the appearance of colour. The absorption spectrum was scanned from 400-800nm against a reagent blank and 540nm was found to be wavelength of maximum absorbance. The absorp-

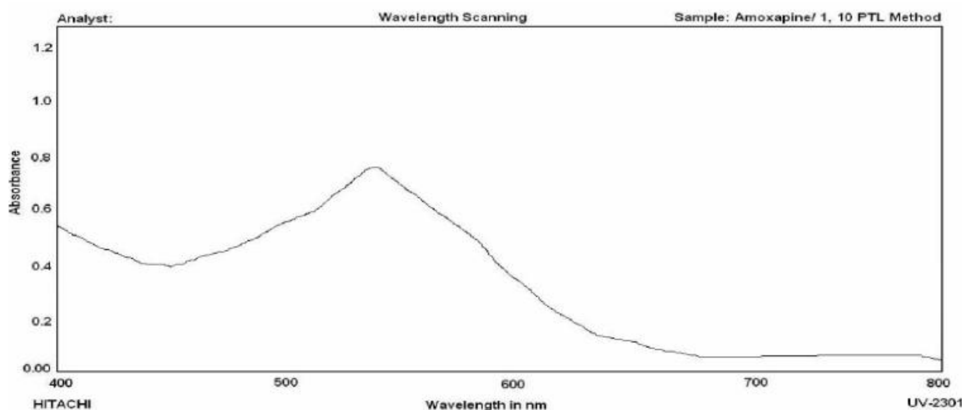

Figure 4 : Wavelength scanning of 1, 10 phenanthrolene method [M2]

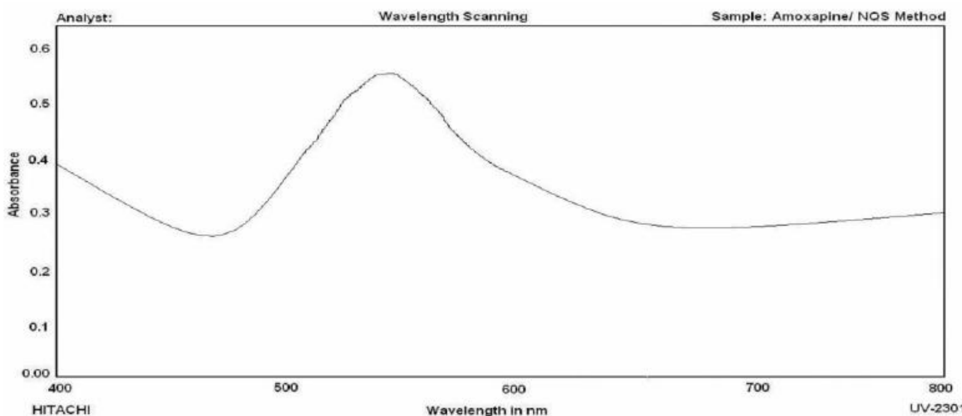

Figure 5 : Scanning spectra of NQS method [M3]

TABLE 4 : Specificity of the proposed methods

	Method 1	Method 2	Method 3
Solution	Peak Area	Absorbance	Absorbance
Standard	1010384.4	0.468	0.428
Sample	991797.9	0.466	0.426

TABLE 5 : Intraday precision results

Sl. No.	Method – 1		Method – 2		Method – 3	
	Concentration	Peak Area	Concentration	Absorbance	Concentration	Absorbance
1.	150	1010388	7.5	0.467	30	0.428
2.	150	1008626	7.5	0.469	30	0.425
3.	150	1006051	7.5	0.471	30	0.427
4.	150	1002845	7.5	0.466	30	0.424
5.	150	1004497	7.5	0.465	30	0.423
6.	150	1007444	7.5	0.464	30	0.426
Mean	1006642		0.467		0.426	
SD	2756		0.002608		0.001949	
RSD	0.002738		0.005585		0.004575	
CV	0.2738		0.5585		0.4575	

tion spectrum was given in Figure-5.

Method validation

Specificity: In the proposed RP-HPLC method, to find out specificity, about 20µL of blank (mobile

TABLE 6 : Interday precision results

Sl. No.	Method – 1		Method – 2		Method – 3	
	Concentration In µg/ml	Peak Area	Concentration In µg/ml	Absorbance	Concentration µg/ml	Absorbance
1.	150	1015607	7.5	0.459	30	0.416
2.	150	1012650	7.5	0.457	30	0.413
3.	150	1007384	7.5	0.458	30	0.415
4.	150	1010860	7.5	0.456	30	0.414
5.	150	1013680	7.5	0.454	30	0.412
6.	150	1014174	7.5	0.455	30	0.411
Mean	1012393		0.457		0.414	
SD	2810		0.001949		0.001949	
RSD	0.002776		0.004265		0.004265	
CV	0.2776		0.4265		0.4265	

TABLE 7 : Accuracy results of the proposed methods

Method	Spike Level%	Target Concentration µg/ml	Spiked Concentration µg/ml	Total Concentration µg/ml	Concentration obtained * µg/ml	% Recovey
Method 1	50	60	30	90	89.94	99.93
	100	60	60	120	119.15	99.29
	150	60	90	150	150.08	100.05
Method 2	50	05	2.5	7.5	7.505	100.06
	100	05	5.0	10.0	10.10	101.56
	150	05	7.5	12.5	12.63	101.06
Method 3	50	20	10	30	30.13	100.46
	100	20	20	40	40.47	101.14
	150	20	30	50	50.46	100.92

*mean of five determinations

pressed in terms of intra-day and inter-day precision. The intraday and inter day precision were determined by measuring the response of the instrument (peak area in Method-1 and absorbance in Method-2 &3) six times within two different days by freshly preparing working standard solution and measuring the signal of the instrument following the standard procedures for the Method 1,2 and 3. The precision was expressed in terms of standard deviation, relative standard deviation (RSD) and coefficient of variance *i.e.*, percent of Relative standard deviation (%RSD). The results of intraday and inter day precision of the proposed method were presented in TABLE-5 and TABLE-6 respectively.

Accuracy

Accuracy of the proposed methods was determined by calculating percent of recovery of Amoxapine by the method of standard addition. A known amount of Amoxapine standard was added to a pre analyzed sample and the amount of Amoxapine was estimated by measuring the response of the instrument (peak area in Method-1 and absorbance in Method-2 &3) three times over the specified concentration range (50%, 100% and 150% with respect to target concentration) and amount of Amoxapine was estimated by following the standard procedures for these three methods. The accuracy results were given in TABLE-7.

Linearity

In method -1, appropriate aliquots of standard Amoxapine stock solution were taken in a series of volumetric flasks and made up to the mark. The response of the instrument (peak area) was measured thrice at each concentration, and calibration curve

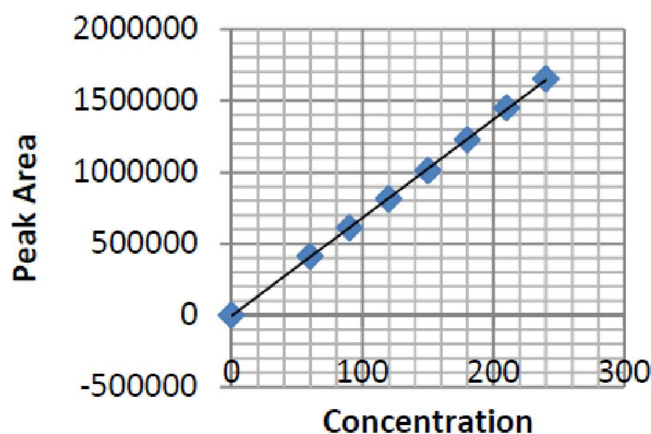

Figure 6 : HPLC method [M1]

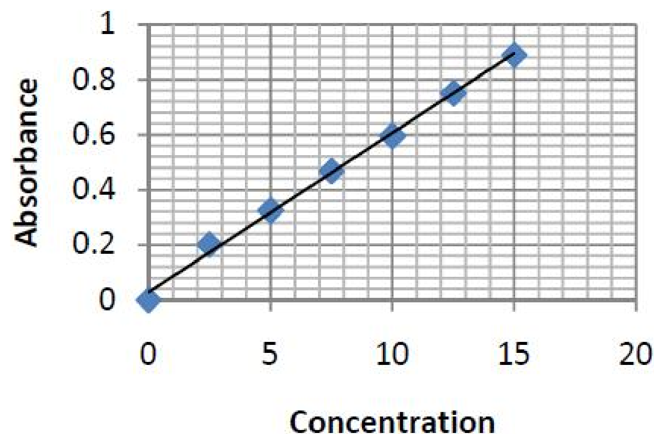

Figure 7 : 1,10-Phenanthrolene method [M2]

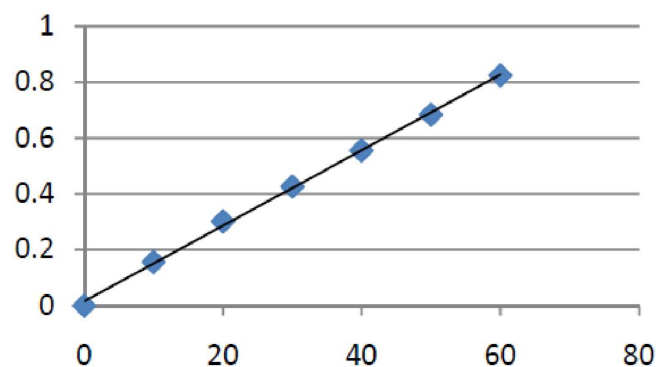

Figure 8: NQS Method [M3]

was constructed by plotting the mean peak area versus concentration of Amoxapine. The linearity plot was presented in Figure-6. In Method – 2 and 3, the absorbance of the colour developed was measured after 5min at 535nm and 540nm respectively against a reagent blank and a linear plot was drawn with absorbance against concentration and represented in Figure – 7 & 8. The results of linearity in these methods were presented in TABLE-8.

Sensitivity

The sensitivity of the proposed methods are presented in terms of limit of detection (L.O.D.) and limit of quantification (L.O.Q.) and these were calculated from standard deviation of response and slope of the calibration curve(s) by using the formulae LOD = 3Sa / b and LOQ = 10Sa / b respectively and given in TABLE-9.

Formulation analysis

The prepared sample solution was analyzed by adopting the standard procedures and percent of assay was determined, and the assay results were given in TABLE-10.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions. Ruggedness was tested by changing the operator in two different days at 60μg/ml concentration and the %RSD was 0.67 and was found to be under the acceptance criteria.

Robustness

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate variations in method parameters. In the present investigation, robustness was tested by ±5% change in the mobile phase ratio, wavelength of the detector and the pH of the detector at a concentration of 50μg/ml solution. It was found that there is no remarkable change in the results with all the changed conditions.

RESULTS AND DISCUSSION

The main objective of the present investigation was to develop and validate a stability indicating liquid chromatographic and two visible spectrophotometric methods using 1, 10 Phenanthroline and NQS as complexing agents for color development for the determination of Amoxapine in pure and formulations.

The drug Amoxapine being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. The concentration

Full Paper

TABLE 8 : Linearity 22of the methods

Sl. No.	Method – 1		Method – 2		Method – 3	
	Concentration In µg/ml	Peak Area	Concentration In µg/ml	Absorbance	Concentration In µg/ml	Absorbance
1.	60	410865	2.5	0.201	10	0.156
2.	90	612186	5.0	0.325	20	0.301
3.	120	813338	7.5	0.468	30	0.426
4.	150	1010388	10.5	0.596	40	0.556
5.	180	1226055	12.5	0.751	50	0.684
6.	210	1447868	15.0	0.889	60	0.824
7.	240	1650402	-	-	-	-
Slope	6873.6		0.057		0.013	
Intercept	5772.8		0.028		0.015	
CC	0.9998		0.998		0.998	

TABLE 9 : Sensitivity of the methods in terms of LOD and LOQ

Sl.No.	LOD	LOQ
Method – 1	1.25µg/ml	4.00µg/ml
Method – 2	0.18 µg/ml	0.62 µg/ml
Method – 3	0.75µg/ml	2.5µg/ml

TABLE 10 : Formulation analysis results

Method	Brand Name	Dosage Form	Concentration prepared (µg/ml)	Amount found in µg/ml	% Assay
Method – 1	Amolife 50	Tablet	150	147.24	98.2
Method – 2	Amolife 50	Tablet	7.5	7.45	99.3
Method – 3	Amolife 50	Tablet	30	29.87	99.5

of the methanol and acetonitrile were optimized to give symmetric peak with short run time based on asymmetric factor and peak area obtained. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and precise. The absence of additional peaks indicates no interference of the excipients. The high percentages of recovery (99.29 to 101.56) indicate that the proposed methods are highly accurate. The studies of linearity between the response and concentration of the drug indicate that these methods are linear within the best suitable rage (60 – 240, 2.5 – 15.0 and 10 – 60 µg/ml for Method-1, 2 and 3 respectively) and correlation coefficient was found to be not less than 0.9980.

CONCLUSION

The proposed methods for the assay of Amoxapine are found to be simple, sensitive, precise, accurate, robust, rugged and linear over a suitable range. The proposed RP-HPLC method is specific for the determination of Amoxapine even in the presence of its degradation products with an assay in acceptable limit and hence can be employed as a stability indicating one. The proposed Spectrophotometric methods are of wide applicability due to the longer stability of the formed coloured complexes. The tablet dosage forms were analyzed successfully and the assay was simple, rapid and found to be within the preferred limits. Hence, may be applied for the quality analysis of Amoxapine.

REFERENCES

[1] B.M.Cohen, P.Q.Harris, R.I.Altesman, J.O.Cole; The American Journal of Psychiatry, **139(9)**, 1165–1167 **(1982)**.

[2] R.Apiquian, A.Fresan, R.E.Ulloa; Neuropsychopharmacology, **30(12)**, 2236–44

(2005).

[3] J.L.Kinney, R.L.Evans; Clinical Pharmacy, **1(5)**, 417–24 **(1982)**.

[4] J.S.Zimmer, S.R.Needham, C.D.Christianson, C.M.Piekarski, C.N.Sheaff, K.Huie, A.R.Reed, L.Takahashi; Bioanalysis, **2(12)**, 1989-2000 **(2010)**.

[5] M.Scott, Johnson, Gloria Nygard, S.K.Wahba Khalil; Journal of Pharmaceutical Sciences, **73(5)**, 696–699 **(1984)**.

[6] Hisako takeuchi, Shinichi yokota, Shigehiko shimada, Yoshio ohtani, Sadanori miura, Hiroaki kubo; Current therapeutic research, **53(4)**, **(1993)**.

[7] Einosuke Tanaka, Masaru Terada, Takako Nakamura,, Shogo Misawa, Choei Wakasugi; Journal of Chromatography B, Biomedical Sciences and Applications, **692(29)**, 405–412 **(1997)**.

[8] Hue, Bernadette, Palomba, Bertrand, Giacardy-Paty, Marie, Bottaï, Thierry, Alric, Robert, Petit, Pierre; Therapeutic Drug Monitoring, **20(3)**, 335-339 **(1998)**.

[9] M1.Gupta, A.Jain, K.K.Verma; J Sep.Sci., **33(23-24)**, 3774-80 **(2010)**.

[10] Patel S.Samir, C.Vijayakrishna, Aradhya, Satishkumar A.Shetty, Manzoor Ahmed; International Journal of Universal Pharmacy and Bio Sciences, **4(1)**, 29-35 **(2015)**.