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Development and validation of a rapid resolution liquid chromatographic (RRLC) method for the determination of Atomoxetine HCl in its pharmaceutical dosage form

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Abstract

The present work described the development of a rapid resolution liquid chromatographic (RRLC) method for atomoxetine in its pharmaceutical dosage form Strattera ®. The analysis was achieved on Agilent Eclipse XDB C18 column (50 mm x 4.6 mm i.d, 1.8 µm particle size) using mixture of aqueous 0.04 M Glacial acetic acid and 0.03M triethylamine (TEA), pH 4.6 - Acetonitril (42 : 58, v/v) as mobile phase at flow rate of 0.27 ml/min and UV detection at 220 nm. The developed method was linear over the concentration range of 4-40 µg /ml (r = 0.99969) with a limit of detection and quantitation 0.44 µg /ml and 1.32 µg /ml for atomoxetine. The developed RRLC method was validated with respect to specificity, linearity, accuracy, and precision, limit of detection and limit of quantitation. The statistical analysis proved that the developed method for quantification of atomoxetine as bulk drug and from pharmaceutical preparation is reproducible and selective. The proposed RRLC method can be used fo the quality control of formulated products containing atomoxetine.

Rapid resolution liquid chromatography RRLC has become an increasingly useful approach to achieve higher throughput, improve sensitivity and reduce costs. The agilent 1200 rapid resolution LC system enables faster analysis (theoretically up to 20x) than with conventional high performance liquid chromatography (HPLC) while maintaining equivalent resolution. This is achieved by using sub-2 micron column particle chemistry.

Keywords: Developed (RRLC); (HPLC); Atomoxetine HCl; Pharmaceutical preparation

1. Introduction

1.1. Atomoxetine HCL

Atomoxetine is the first non-stimulant drug approved for the treatment of an attention –deficit hyperactivity disorder (ADHD). It is sold in the form of Hydrochloride salt of Atomoxetine. It is a selective nor-adrenaline inhibitor. The formal chemical name (IUPAC) is (R)-N-methyl-3-phenyl-3-(o-tolyloxy) propan-1-amine (Figure 1)[1].

Atomoxetine is classified as a norepinephrine reuptake inhibitor, and is approved for use in children, adolescents, and adults. However, its efficacy has not been studied in children under six years old. Its advantage over stimulants for the treatment of ADHD is that it has less abuse potential than stimulants, is not scheduled as a controlled substance and has proven in clinical trials to offer 24 hour coverage of symptoms associated with ADHD in adults and children [1].

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Strattera was originally intended to be a new antidepressant drug; however, in clinical trials, no such benefits could be proven. Since norepinephrine is believed to play a role in ADHD, Strattera was tested and subsequently approved as an ADHD treatment. Clinical experiments are currently being undertaken to test Atomoxetine use in weight loss programs with obese people or those with a binge eating disorder [2].

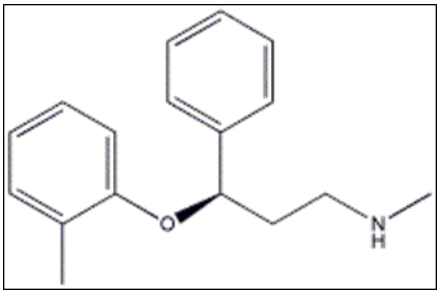


Figure 1 Structures of Atimoxetine HCL

Atomoxetine was analyzed by HPLC method in United States pharmacopoeia which has a very tedious, time – consuming, and complicated mobile phase using gradient flow technique [3].

Literature survey reveals that several methods have been reported like UV, HPLC, HPLC-MS, HPTLC for the assay of atomoxetine HCl [4-19]. the proposed method is supposed to be time and chemical - saving (it saves the chemicals used in mobile phase, solvent and the analyzed drug itself), accurate and simple to achieve good estimation of the concentrations of atomoxetine HCL in bulk and pharmaceutical dosage form.

2. Material and methods

2.1. Experimental

2.1.1. Apparatus

- Agilent RRLC system
- Cole Parmer pH meter

2.1.2. Materials and reagents

- Acetonitril was of HPLC grade, glacial acetic acid and triethylamine were analytical grade.
- **Authentic:** atomoxetine HCl, kindly supplied by Lilly
- **Pharmaceutical formulation:** Strattera ®

2.1.3. RRLC Conditions

The RRLC separation and quantitation were achieved on

- **Column:** Agilent Eclipse XDB C18 column (50 mm x 4.6 mm i.d, 1.8 μm particle size)
- **Mobile Phase: aqueous** 0.04 M Glacial acetic acid and 0.03M triethylamine (TEA), (pH 4.6) - Acetonitril (42: 58, v/v)
- **Flow rate:** 0.27 ml/min
- **Injection volume:** 0.8 μl
- **Temperature:** ambient temperature
- **Detector:** 220 nm.

2.1.4. Standard solution

Stock solution of atomoxetine (1 mg/ml) was prepared by dissolving a weight of atomoxetine HCL which is equivalent to 100 mg of atomoxetine base in 100 ml of mobile phase.

2.1.5. Preparation of calibration curve

The working solution was prepared by further dilution of the stock standard solution with the mobile phase to reach the concentration range of 4-40 μg /ml for atomoxetine. Triplicate 0.8 μl injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The peak area values were plotted against corresponding concentrations, linear relationship was obtained.

2.1.6. Pharmaceutical formulation preparation

The content of ten capsules of Strattera® was weighed. A portion of the powder equivalent to 40 mg of atomoxetine was accurately weighed, transferred separately to 100 ml volumetric flask, and dissolved in 100 ml mobile phase using ultrasonic bath (15 min) and then filtered through 0.45 μm membrane filters (Millipore, Milford, MA). Further dilution was carried out with mobile phase to reach calibration range.

2.2. System suitability

The system suitability parameters including capacity factor (k'), selectivity (α), resolution (R_s), tailing factor (T), and theoretical plate (N) listed in Table 1. All parameters were satisfactory with good specificity for the stability assessment of atomoxetine.

Table 1 System suitability parameters of atomoxetine

Compound	Rt	k′	α	Rs	(T)	(N)
Atomoxetine	2.33	0.27	-	-	1.358	3475

2.2.1. Application to pharmaceutical formulation

Table 2 Determination of atomoxetine in Strattera ® capsules by the proposed RRLC method

Claimed μg /ml	Found μg /ml	Recovery %
4	4.04	101
8	8.2	102.5
16	15.99	99.9
24	24.4	101.7
32	31.63	98.8
40	39.82	99.6
Mean		100.583
S.D		1.393

Table 3 Statistical comparison between the proposed RRLC method and the reported method for the determination of atomoxetine in Strattera ® capsules

Commercial product	Proposed method	Reference method
Recovery a ± SD	100.583 ± 1.393	99.293 ± 1.54
t	1.52	2.571b
F	2.68	5.05b

a: Mean of six determinations; b : The theoretical values of t and F at P = 0.05

The proposed method was successfully applied to determine atomoxetine in its dosage form (Strattera ®) capsules. Six replicates determination were made. Satisfactory results were obtained for atomoxetine in good agreement with label claims (Table 2). The results obtained were compared statistically by Student's t - test (for accuracy), and variance ratio

F - test (for precision) with the reported method. [20]. The results in (Table 3) showed that the t and F values were smaller than the tabulated values indicating that there was no significant difference between the proposed and reported methods.

3. Results and discussion

After the trials of various mobile phase systems such as 0.1% phosphoric acid solution: Acetonitrile (35:65 v/v), acetonitrile : sodium hexanesulfonate pH4 (70:30 v/v), methanol : phosphate buffer pH3.5 : THF (30:63:7 v/v/v), the proposed mobile phase which is a solution of teta —n-butylammonium hydroxide + triethylamine (adjust pH to 3.5 with phosphoric acid) : Acetonitril (375 : 625, v/v) was found to be satisfactory giving good resolution peaks. Also several columns and pHs were tested to achieve the best resolution. Figure 2

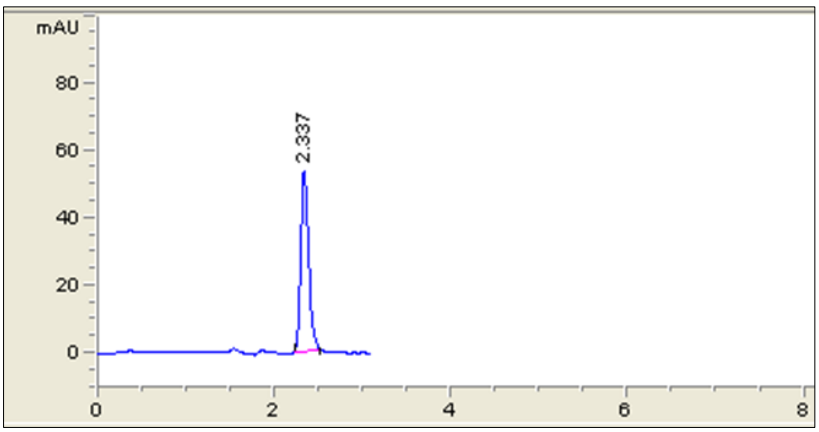
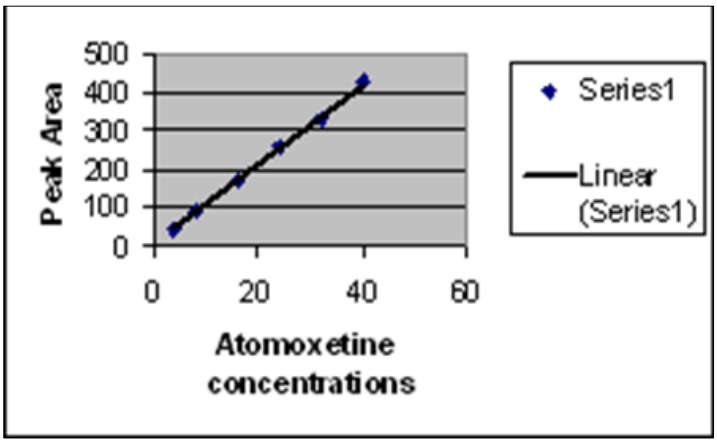


Figure 2 Chromatogram of Atomoxetine HCl



Y=10.54395X+1.684101 R2=0.99969

Figure 3 Calibration curve of atomoxetine HCl was prepared by plotting graph of concentration v/s area and equations for straight line was obtained.(x-axes (Atomoxetine concentration ,y-axes (Peak Area)

3.1. Validation of the method

3.1.1. Linearity

The linearity of the proposed method for determination of atomoxetine was validated by analyzing different concentrations of the drug. According to the international conference on harmonization ICH [21], at least five concentrations must be used. In this study six concentrations were chosen, ranging between 4-40 µg /ml of atomoxetine. Each concentration was repeated three times. The high value of correlation coefficient and the intercept value was not statistically ($p< 0.05$) different from zero (Table 4) validate the linearity of the calibration graphs. Typically the regression equation was Y= 1.684101 + 10.54395 C (r = 0.99969).

Table 4 Assay parameters and regression characteristic of atomoxetine determined by the proposed RRLC method

Parameters	**Atomoxetine**
Linearity range μg /ml	4-40
Detection limit μg /ml	0.44
Quantitation limit μg /ml	1.32
Regression equation(y*) n	6
Slope (b)	10.54395
Standard deviation of slope	0.130608
Relative standard deviation of slope	1.24
Confidence limit of slope at 95% confidence limit	10.18132 - 10.90657
Intercept (a)	1.684101
Standard deviation of intercept	3.170664
Confidence limit of intercept at 95% confidence limit	-7.11907- 10.48728
Correlation coefficient (r)	0.99969
Standard error of regression	4.074746

Y = a + b C where C is the concentration of compound in μg /ml and Y is the peak area

3.1.2. Precision

In order to prove the validity and applicability of the proposed method and reproducibility of the results mentioned, three concentrations of the cited drug were carried out. (Table 5) show the values of Intra-day and Inter-day relative standard deviation (RSD %) for different concentrations.

Table 5 Evaluation of precision for the determination of atomoxetine by the proposed RRLC method

Compound	**Theoretical Concentration μg /ml**	**Intra-day Concentration Meana**	**RSD%**	**Inter-day Concentration mean b**	**RSD%**
Atomoxetine	8	8.34	0.85	8.23	0.4
	16	15.52	0.76	15.47	1.05
	32	31.68	0.82	31.84	1.1

a Mean of three determinations; b Mean of three different days

3.1.3. Range

The calibration range was established through consideration of the practical range necessary, according to the drug concentration present in the pharmaceutical product, to give accurate, precise, and linear results. The calibration range of the proposed method is given in (Table 4).

3.1.4. Limit of detection and quantitation

According to ICH recommendation [21] the approach based on the S.D of the response and the slope was used for determination of the detection (**DL**) and quantitation limit (**QL**) by means of the following equations:

$$DL = \frac{3.3\sigma}{s}$$

Where **σ** = the standard deviation of the response
S = the slope of the calibration curve

$$QL=\frac{10\sigma}{s}$$

Where **σ** = the standard deviation of the response
S = the slope of the calibration curve

3.1.5. Specificity

Peak purity was examined using photodiode array detector and indicates specificity of the method.

3.1.6. Accuracy

This study was performed by adding known amounts of the studied drug compound to known concentration of the commercial pharmaceutical capsule (standard addition method). The resulting mixtures were analyzed and the results obtained were compared with the expected results (Table 6) suggested the good accuracy of the proposed methods.

Table 6 The application of Standard addition technique to the analysis of atomoxtine by the proposed RRLC method

Claimed μg /ml	Add μg /ml	Found μg /ml	Recovery %
4	4	3.996	99.9
4	8	8.03	100.3
4	16	15.99	99.9
4	24	24.31	101.3
4	32	32.38	101.19
Mean S.D			100.52, 0.68

3.1.7. Robustness

Robustness is the measure of capacity of analytical methods to remain unaffected by small but deliberating variations of the operation parameters. Variation of the pH of (aqueous 0.04 M Glacial acetic acid and 0.03M triethylamine) of the mobile phase by ± 0.2, organic solvent strength of the mobile phase by ± 2 %, and detector wavelength by ± 2 nm did not have significant effect on chromatographic resolution of the RRLC method.

3.1.8. Solution stability

Solution of studied drug in mobile phase exhibit no absorbance or chromatographic change for 2weeks when kept at room temperature, and for 3 months when stored in the refrigerator at 4 °C

4. Conclusion

The proposed RRLC method provide simple, accurate, and reproducible quantitative analysis for determination of atomoxetine in bulk and in pharmaceutical dosage form without any interference from the excipients. The method was completely validated. Owing to its sensitivity, simplicity and short analysis time, it is suitable for routine analysis in quality control laboratories to assay the drug.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicts of interest.

References

[1] http://en.wikipedia.org/wiki/Atomoxetine

[2] Atomoxetine@3DChem.com

[3] Authorized USP pending monographs guideline http://www.usp.org.

[4] Validated RP-HPLC and UV spectrophotometry method for the estimation of atomoxetine hydrochloride in pharmaceutical dosage forms C.Patel, M.Patel, S.Rani, J.Chrom.B, 2007, 850,356-60.

[5] Hetal R. Prajapati, Paras N. Raveshiya, Bhavesh B. Jadav, Divyesh M. Mahakal Development and Validation of High Performance Thin-Layer Chromatographic Method for Determination of Atomoxetine Hydrochloride in Pharmaceutical Dosage Forms Der Pharma Chemica, 2012, 4(1):194-201.

[6] Comparison of different ligand densities in immunoaffinity chromatography of the plantibody HB-01 coupled to Sepharose CL-4B to purify the rHBsAgG.Wei,L.Wenbiao, G.Guixin, Z.Jun, J.Chrom.B,2007,854,128-34.

[7] A quality by design approach to impurity method development for atomoxetine hydrochloride (LY139603) F.Peter,A.bernard, J.Pharm. Biomed.Anal. 2008, 46,431-41.

[8] Simultaneous quantification of atomoxetine as well as its primary oxidative and O-glucuronide metabolites in human plasma and urine using liquid chromatography tandem mass spectrometry (LC/MS/MS)H.John,L.Richard,D.George,J. Pharm.Biomed.Anal.,2005,38,720-33.

[9] Formulation and Evaluation of Sustained Release Tablets Containing Atomoxetine Hydrochloride P.N.Raveshiya, H.R.Prajapati, J.Pharm.Res., 2011, 4(6),1720-1722.

[10] Determination of atomoxetine in human plasma by a high performance liquid chromatographic method with ultraviolet detection using liquid-liquid extraction,Guo W1, Li W, Guo G, Zhang J, Zhou B, Zhai Y, Wang C J-Chromatogr,-B:-Anal- Technol-Biomed-Life-Sci. 2007, 846(1)351-354

[11] Simultaneous quantification of atomoxetine as well as its primary oxidative and O-glucuronide metabolites in human plasma and urine using liquid chromatography tandem mass spectrometry (LC/MS/MS) John H.MullenaRichard L.ShugertaGeorge D.PonsleraQiminLia1BhaskarSundarambHeather L.CoalesbJoseph E.Yakupkovicb2Richard M.LeLacheurbWilliam J.WheeleraFrank J.BelasaJohn-MichaelSauera3 J-pharm-biomed-Anal. 2005, 38(4):720-733

[12] Determination of the enantiomer and positional isomer impurities in atomoxetine hydrochloride with liquid chromatography using polysaccharide chiral stationary phases Jane A.SellersBernard A.OlsenPaul K.OwensPeter F.Gavin J-pharm-Biomed. Anal. 2006, 41(4):1088-1094.

[13] A new high performance liquid chromatographic method for quantification of atomoxetine in human plasma and its application for pharmacokinetic study Chaula Patel, Minal Patel, Shubha Rani, Manish Nivsarkar, Harish Padh, Journal of Chromatography B, 2007 , 850, Issues 1–2, , 356-360.

[14] Electrochemical behavior of atomoxetine and its voltammetric determination in capsules M. Pérez-Ortiz, C. Muñoz, C. Zapata-Urzúa, A. Álvarez-Luej. Talanta, 2010, 82, Issue 1, 30, 398-403

[15] Development and Validation of High Performance Thin-LayerChromatographic Method for Determination of Atomoxetine Hydrochloride in Pharmaceutical Dosage FormsHetal R. Prajapati*, Paras N. Raveshiya, Bhavesh B. Jadav, Divyesh M. Mahakal Der Pharma Chemica, 2012, 4 (1):194-201

[16] Development and validation of a stability-indicating RP-HPLC method for determination of atomoxetine hydrochloride in tablets, Sejal K Patel 1, Natvarlal J Patel, J AOAC Int. 2010 , 93(4):1207-14.

[17] Development and Validation of a Rapid HPLC Assay for the Simultaneous Determination of Three Psychoanaleptic Drugs in Pharmaceutical Formulations .V. Nagaraju & A. M. Crouch Journal -of- liquid-chromatography -and -related- technologies , 2008, 31(5-8):722-732

[18] RP-LC Determination of Atomoxetine HCl from its Pharmaceutical Dosage Form ,Sudhir Sadanand Kamat ,Vishal B. Choudhari ,Vinayak Vele and Swarup Prabhune ,chromatographia Journal ,2008,67(1-2): 143-146 .

[19] Determination of atomoxetine in human plasma by a high performance liquid chromatographic method with ultraviolet detection using liquid-liquid extraction. Guo W1, Li W, Guo G, Zhang J, Zhou B, Zhai Y, Wang C ,Journal-of- chromatography,-B:- Analytical technologies -in -the- Biomedical- and -life-s ciences , 2007, 854(1-2):128-134

[20] International Conference on Harmonization (ICH).

[21] Straterra® registeration file provided by Lilly.