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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ATOMOXETINE HYDROCHLORIDE AND SODIUM BENZOATE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, precise, and stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of Atomoxetine Hydrochloride and Sodium Benzoate in pharmaceutical oral liquid dosage form. Chromatographic separation was achieved on an Intersil ODS-3 column (150 x 4.6 mm, 5 um) using a gradient elution programme with Mobile Phase A (phosphate buffer, pH 3.0 +/- 0.05) and Mobile Phase B (buffer: methanol, 20: 80 v/v) at a flow rate of 1.0 mL/minute, with detection at 270 nm and a total run time of 25 minutes. Atomoxetine Hydrochloride and Sodium Benzoate eluted at approximately 12.4 minutes and 5.3 minutes, respectively. The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness. The method showed excellent linearity over the range of 575-1725 ug/mL for Atomoxetine Hydrochloride (r = 0.999) and 100-300 ug/mL for Sodium Benzoate (r = 0.9999). Percentage recovery for both analytes was within 98.0-102.0%, and the relative standard deviation (%RSD) for precision studies was below 2.0% at every level. As no official pharmacopoeial method currently exists for the simultaneous estimation of Atomoxetine Hydrochloride with Sodium Benzoate preservative, the proposed method is recommended for routine quality control analysis of this combination in pharmaceutical dosage forms.

KEYWORDS: Atomoxetine Hydrochloride; Sodium Benzoate; RP-HPLC; Method Validation; ICH Guidelines; Stability-indicating.

1. INTRODUCTION

Attention-Deficit Hyperactivity Disorder (ADHD) is a chronic neurodevelopmental condition characterised by inattention, hyperactivity, and impulsivity that affects children and frequently persists into adulthood. Atomoxetine Hydrochloride, marketed under the brand name Strattera, is a selective norepinephrine reuptake inhibitor (SNRI) approved for the treatment of ADHD in patients six years of age and older. Unlike stimulant medications, Atomoxetine does not produce a significant increase of dopamine in the nucleus accumbens and therefore carries a low potential for abuse. Chemically, Atomoxetine Hydrochloride is (3R)-N-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine hydrochloride, with a molecular formula of C17H21NO·HCl and a molecular weight of 255.36 g/mol.

Sodium Benzoate is a widely used pharmaceutical preservative that inhibits microbial growth in liquid formulations by disrupting anaerobic fermentation within the microbial cell at low intracellular pH. In oral liquid formulations, Atomoxetine Hydrochloride is commonly co-formulated with Sodium Benzoate as a preservative; however, no single method is described in any

pharmacopoeia for the simultaneous estimation of Atomoxetine Hydrochloride together with Sodium Benzoate. Several individual RP-HPLC, UV-spectrophotometric, HPTLC, and LC-MS/MS methods have been reported in the literature for the estimation of Atomoxetine Hydrochloride alone in bulk drug, plasma, and dosage forms, but a validated method for the drug-preservative combination has not been established.

High-Performance Liquid Chromatography (HPLC) remains the most widely applied technique for quantitative and qualitative analysis of pharmaceutical products because of its high resolution, sensitivity, reproducibility, and suitability for a broad range of analytes. In reversed-phase HPLC (RP-HPLC), a non-polar stationary phase (commonly C8 or C18 bonded silica) is used with a polar or moderately polar mobile phase, and separation is governed by hydrophobic interactions between the analyte and the stationary phase.

The aim of the present study was, therefore, to develop and validate a simple, accurate, precise, and stability-indicating RP-HPLC method, in accordance with ICH Q2(R1) guidelines, for the simultaneous estimation of Atomoxetine Hydrochloride and Sodium Benzoate in pharmaceutical oral dosage form.

2. DRUG PROFILE

2.1 Atomoxetine Hydrochloride

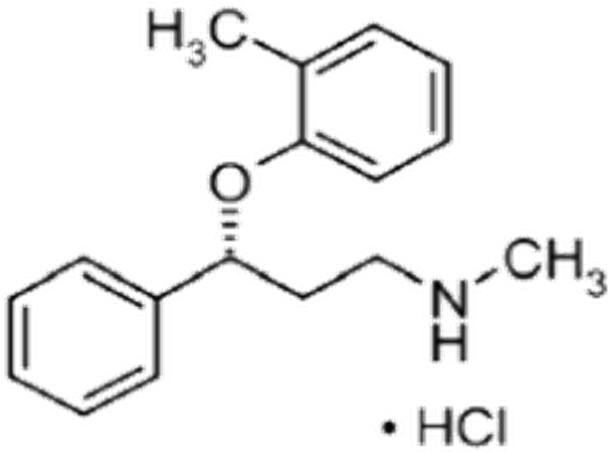

Figure 1: Structure of Atomoxetine Hydrochloride.

Property	Description
IUPAC name	(3R)-N-Methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine hydrochloride
Molecular formula	C17H21NO . HCl
Molecular weight	255.36 g/mol (free base)
Category	Selective Norepinephrine Reuptake Inhibitor (SNRI)
Appearance	White, granular powder
Melting point	161-165 degC
LogP / pKa	3.95 / 9.8

2.2 Sodium Benzoate

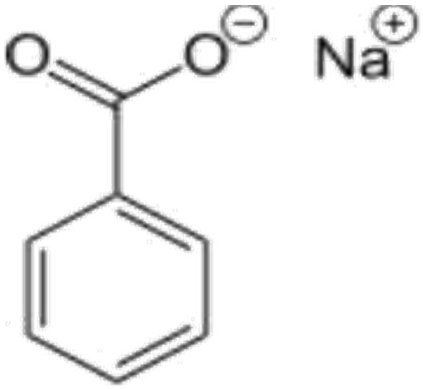

Figure 2: Structure of Sodium Benzoate.

Property	Description
Molecular formula	C6H5COONa
Molecular weight	144.10 g/mol
Category	Pharmaceutical preservative
Appearance	White crystalline powder
Mechanism of action	Inhibits anaerobic fermentation of glucose at low intracellular pH, suppressing microbial growth

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Atomoxetine Hydrochloride working standard was procured from ZCL Chemicals Ltd., and Sodium Benzoate reference standard was procured from Avantor. HPLC-grade methanol (Rankem), sodium dihydrogen phosphate anhydrous (Spectrochem), 1-hexane sulphonic acid sodium monohydrate salt (Merck Lichropur), and orthophosphoric acid 88% (Merck Emparta) were used. Milli-Q or equivalent water was used throughout the study.

3.2 Instrumentation

Chromatographic separation was carried out on a Waters Alliance 2695 HPLC system equipped with a UV and photodiode array (PDA) detector, controlled using Empower 3 software. An analytical balance (Mettler Toledo XP2U), pH meter (Metrohm 780), ultrasonicator (Leelasonic-100), FT-IR spectrophotometer (ABB MB 3000), and melting range apparatus (Labindia MK2/40H) were also used for drug identification and solution preparation.

3.3 Chromatographic Conditions

Parameter	Condition
Column	Intersil ODS-3, 150 × 4.6 mm, 5 µm
Mode	Gradient elution
Mobile Phase A	Phosphate buffer, pH 3.00 ± 0.05
Mobile Phase B	Buffer : Methanol (20: 80 v/v)
Flow rate	1.0 mL/minute
Wavelength	270 nm
Injection volume	10 µL
Column/sample temperature	5°C (sample cooler)
Run time	25 minutes

Gradient Programme

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	44	56
18.0	6	94
18.1	44	56
25.0	44	56

3.4 Preparation of Buffer and Mobile Phase

The buffer was prepared by dissolving 6.0 g of sodium dihydrogen phosphate anhydrous and 4.12 g of 1-hexane sulphonic acid sodium monohydrate salt in 1000 mL of water, adjusting the pH to 3.00 ± 0.05 with diluted orthophosphoric acid, and filtering through a 0.45 µm PVDF membrane filter. Mobile Phase B was prepared by mixing buffer and methanol in a 20: 80 v/v ratio and degassing by sonication. The diluent consisted of water and methanol in a 60: 40 v/v ratio.

3.5 Preparation of Standard and Sample Solutions

A standard stock solution containing Atomoxetine Hydrochloride (1150 µg/mL) and Sodium Benzoate (200 µg/mL) was prepared by dissolving 115 mg of Atomoxetine Hydrochloride and 20 mg of Sodium Benzoate reference standard in 70 mL of diluent in a 100 mL volumetric flask, sonicating to dissolve, and diluting to volume with diluent. The sample solution (label claim: Atomoxetine Hydrochloride 4.6 mg/mL and Sodium Benzoate 0.8 mg/mL) was prepared by transferring 5 mL of sample into a 20 mL volumetric flask, diluting with diluent, and filtering through a 0.45 µm PVDF Millex-HV filter prior to injection.

3.6 Method Validation

The developed method was validated as per ICH Q2(R1) guidelines for the following parameters: system suitability, specificity, linearity and range, accuracy, precision (system, method, and intermediate precision), limit of detection (LOD), limit of quantification (LOQ), and robustness.

System suitability was assessed from five replicate injections of standard solution, evaluating theoretical plates, tailing factor, and %RSD of peak area. Specificity was established by injecting blank, placebo, standard, and sample solutions and confirming the absence of interference at the retention times of the analyte peaks. Linearity was evaluated at five concentration levels (50-

150% of the target concentration): 575-1725 µg/mL for Atomoxetine Hydrochloride and 100-300 µg/mL for Sodium Benzoate. Accuracy was determined by the standard addition method at 50%, 100%, and 150% levels, in triplicate. Precision was assessed through system precision (n = 5), method precision (n = 6), and intermediate precision (n = 6, different analyst/day/instrument/column lot). LOD and LOQ were calculated from the standard deviation of the response and the slope of the calibration curve (LOD = 3.3σ/S; LOQ = 10σ/S). Robustness was evaluated by deliberately varying flow rate (±0.1 mL/min), wavelength (±2 nm), and mobile phase pH (±0.2).

4. RESULTS AND DISCUSSION

4.1 Method Development

Several mobile phase combinations and stationary phases were evaluated to achieve adequate resolution and peak shape for Atomoxetine Hydrochloride and Sodium Benzoate. Isocratic systems using phosphate buffer/acetonitrile and phosphate buffer/methanol combinations produced either poor resolution of the Sodium Benzoate peak or unacceptable tailing of the Atomoxetine peak. A gradient elution programme using an Intersil ODS-3 column (150 × 4.6 mm, 5 µm) with Mobile Phase A (phosphate buffer, pH 3.0) and Mobile Phase B (buffer: methanol, 20: 80 v/v) produced well-resolved, symmetrical peaks for both analytes and was therefore selected as the final chromatographic condition. Under these conditions, Sodium Benzoate eluted at approximately 5.3 minutes and Atomoxetine Hydrochloride at approximately 12.4 minutes. The detection wavelength of 270 nm was selected based on PDA scanning (200-400 nm) of the standard solution, at which both analytes showed adequate absorbance.

4.2 System Suitability

Parameter	Atomoxetine HCl	Sodium Benzoate	Acceptance Criteria
Mean retention time (min)	12.38	5.26	-
Theoretical plates	>37,000	>10,400	NLT 2000
Tailing factor	1.20	1.03	NMT 2.0
% RSD (n=5)	0.23	0.11	NMT 2.0%

All system suitability parameters were within acceptance criteria, confirming the suitability of the chromatographic system for the intended analysis.

4.3 Specificity

No interference from the blank or placebo solution was observed at the retention times corresponding to Atomoxetine Hydrochloride (~12.4 min) and Sodium Benzoate (~5.3 min). Peak purity angle values were lower than the peak purity threshold for both analytes in standard and sample solutions, confirming that the developed method is specific and stability-indicating.

4.4 Linearity and Range

Parameter	Atomoxetine Hydrochloride	Sodium Benzoate
Range (µg/mL)	575 - 1725	100 - 300
Slope	2702.6	3649.5
Intercept	54224	757.44
Correlation coefficient (r)	0.999	0.99995

Both analytes exhibited excellent linearity across the tested concentration ranges, with correlation coefficients exceeding the acceptance limit of 0.999 (NLT 0.999).

4.5 Limit of Detection and Limit of Quantification.

Analyte	LOD (ppm)	LOQ (ppm)
Atomoxetine Hydrochloride	10.86	32.93
Sodium Benzoate	2.35	7.12

4.6 Accuracy

Level	Mean % Recovery (Atomoxetine HCl)	% RSD	Mean % Recovery (Sodium Benzoate)	% RSD
50%	101.30	0.60	100.89	0.76
100%	100.85	0.50	99.66	0.51
150%	100.40	0.58	100.11	0.81

Recovery for both analytes was found within the acceptance criterion of 98.0-102.0% at all three concentration levels, with %RSD values below 2.0%, confirming the accuracy of the proposed method.

4.7 Precision

Precision Type	%RSD (Atomoxetine HCl)	%RSD (Sodium Benzoate)
System precision (n=5)	0.57	0.53
Method precision (n=6)	0.67	0.58
Intermediate precision (n=6)	0.63	0.57

All precision studies yielded %RSD values well below the acceptance limit of 2.0%, demonstrating that the method is repeatable and rugged across analysts, instruments, and column lots.

4.8 Robustness

Deliberate variation of flow rate (±0.1 mL/min), detection wavelength (±2 nm), and mobile phase pH (±0.2) produced %RSD values of the peak area not exceeding 0.9% for either analyte at any condition tested, confirming that the method is robust to small, deliberate variations in operating parameters.

4.9 Assay

Assay of the marketed formulation, performed in six replicates, gave a mean assay value of 100.85% (%RSD 0.50) for Atomoxetine Hydrochloride and 99.94% (%RSD 0.44) for Sodium Benzoate, both within the acceptable assay range.

4.10 Stability of Analytical Solutions

Solution stability studies showed that the standard solutions of Atomoxetine Hydrochloride and Sodium Benzoate remained stable up to 72 hours and 60 hours, respectively, while the sample solutions were stable up to 74 hours for Atomoxetine Hydrochloride and 68 hours for Sodium Benzoate, with recovery/assay values remaining within the acceptance range of 98.0-102.0% throughout the stability period.

5. CONCLUSION

A simple, specific, accurate, precise, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Atomoxetine Hydrochloride and Sodium Benzoate in pharmaceutical oral dosage form, in accordance with ICH Q2(R1) guidelines. The method demonstrated excellent linearity, accuracy, and precision, with all validation parameters meeting acceptance criteria. Since no pharmacopoeial method currently exists for the simultaneous estimation of this drug-preservative combination, the proposed RP-HPLC method can be reliably employed for routine quality control and stability testing of Atomoxetine Hydrochloride formulations containing Sodium Benzoate as a preservative.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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