DEVELOPMENT OF ULTRAVIOLET-SPECTROPHOTOMETRIC METHOD FOR ANALYSIS OF AMOXAPINE IN PHARMACEUTICAL DOSAGE FORM

SHWETA RANGARI, SHRADDHA PATIL, RUTUJA MAHAJAN, ABRAR AHMAD, NISHIKANT A RAUT*
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur – 440 033, Maharashtra, India.
Email: nishikantraut29@gmail.com

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ABSTRACT

Objective: Knowing the exact amount of active pharmaceutical ingredient (API) in pharmaceutical dosage form is of utmost importance to meet regulatory requirements and to ensure patient safety. Spectrophotometric analysis provides a simple, efficient, and economic approach for estimation of API in pharmaceutical dosage form. In the present work, we have developed simple, sensitive, and highly economic ultraviolet (UV) spectrophotometric method for the estimation of amoxapine in a pharmaceutical formulation.

Methods: Amoxapine shows maximum absorbance of light at wavelength 297 nm in water. The linearity study revealed that it obeys Beer-Lambert’s law over the range of 2–20 µg/mL. Absorptivity value of amoxapine was found to be 206.6±1.341.

Result: The tablet formulation was successfully analyzed by developed UV spectrophotometric method. The developed method was validated as per International Conference on Harmonization guidelines with respect to accuracy, precision, specificity robustness. The limit of detection and limit of quantitation was found to be 1.98 and 60.50 ng/mL, respectively.

Conclusion: The developed method is simple, precise, accurate, and cost-effective and can be used for routine analysis of amoxapine.

Keywords: Amoxapine, Ultraviolet-spectrophotometry, Method development, Validation.

INTRODUCTION

The pharmaceutical analysis is a branch of pharmaceutical sciences having immense importance in the drug development and good manufacturing practices. Knowing the exact amount of active pharmaceutical ingredient (API) in pharmaceutical dosage form is of utmost importance to meet regulatory requirements and to ensure patient safety. Spectrophotometric analysis provides a simple, efficient, and economic estimation of API in pharmaceutical dosage form [1,2].

Amoxapine (Fig. 1) is a tricyclic antidepressant, acts by inhibiting reuptake of neuronal transmitter and help in controlling agitation and anxiety along with depression [3,4]. Chemically, amoxapine is 2-chloro-11-(piperazine-1-yl) dibenzo-[b,f] [1,4] oxazepine having molecular weight 313.7 g/mol and molecular formula C\textsubscript{16}H\textsubscript{16}ClN\textsubscript{3}O [5].

There are numerous methods available for analysis of amoxapine, such as high-performance liquid chromatography [6-9], high-performance liquid chromatography-MS/MS [10], and spectrofluorimetric method [11]. The spectrophotometric method using chromogenic reagents such as phenanthroline and naphthoquinone sulfate for color development and first-order spectrophotometric method using ethanol as a solvent are also reported [12,13]. All the methods available are either costly or time-consuming. Hence, the aim of proposed research work was to develop simple, accurate, precise, and economic ultraviolet (UV)-spectrophotometric method for estimation of amoxapine in pharmaceutical dosage form.

METHODS

Instruments

UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan), with UV probe 2.52 software and 10 mm rectangular quartz cell was employed to develop UV-spectrophotometric method. AR2130 balance (Adventurer, New Jersey, USA) was used for all weighing procedures.

Standard, tablet formulation, chemicals, and reagents

Amoxapine (purity 99.2%) was obtained as a gift sample from Mehta Pharmaceutical Industries, Mumbai (India). Amoxapine tablet formulation (Amolife 50 mg, La Pharma, Ahmedabad, Gujarat) was purchased from regional pharmacy shop. All other chemicals and reagents (Sisco Research Laboratories Pvt., Ltd. (Mumbai, India) used were of analytical grade, procured from local vendor and used without further purification. Double-distilled water was used throughout the experiment.

EXPERIMENTAL AND RESULT

Standard stock and working solution preparation

The standard stock solution was prepared by dissolving an accurately weighed quantity of amoxapine (10 mg) in aliquot portion of methanol and volume was made up to 10 ml with water. The working solution was prepared by diluting the 0.1 ml of standard stock solution with water so as to get a concentration of 10 µg/ml.

Determination of absorbance maxima (λ\textsubscript{max})

The amoxapine solution in water (10 µg/ml) was freshly prepared from stock solution and scanned in the UV range of 400–200 nm (Fig. 2) against blank. The well-defined spectrum was observed with absorbance maxima at 297 nm.

Linearity and range

The standard stock solution was serially diluted with water to different concentrations such as 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28 and 30 µg/ml. The absorbances of these solutions were recorded at wavelength 297 nm. The plot of calibration curve was plotted for absorbance versus concentration (Fig. 3). The calibration curve shows the linearity over the concentration range from 2 µg/ml to 20 µg/ml.
Determination of absorptivity value
Absorptivity value was determined by weighing five different weights and preparing five dilutions for precise result. For this, the standard stock solution of amoxapine for each weight was diluted in five different volumetric flasks with water to obtain 10 µg/ml of concentrations. The absorbance of each solution was recorded at 297 nm using 10 mm quartz cell. The absorptivity values for five different weights were calculated using formula mentioned below. The results obtained from the absorbance of each solution were used for calculation of absorptivity value, and results are depicted in Table 1.

Absorptivity value = \[
\frac{\text{Absorbance}}{\text{Concentration of analyte (g/100 ml)}}
\]

Application of proposed method for analysis of tablet formulation
For analyzing the tablet formulation of amoxapine, 20 tablets were weighed to determine average weight and powdered. Accurately weighed quantities of tablet powder equivalent to 10 mg amoxapine were transferred in 10 ml of volumetric flask. The content of flask was dissolved in aliquot part of methanol by vigorous shaking, and the volume was made with water. The final concentration of 10 µg/ml was obtained by diluting the aliquot portion of these stock solutions with water. The absorbance of these solutions was measured at 297 nm wavelength, and the amount of drug was estimated. The data of estimation are depicted in Table 2.

Validation
International conference on harmonization (ICH) Q2 (R1) guidelines were referred to validate the proposed method with respect to accuracy, precision, linearity, range, specificity and ruggedness [14].

Accuracy
The recovery of amoxapine in tablet formulation was ascertained by standard addition method. The preanalyzed tablet powder was spiked with pure drug at three different levels, 80%, 100%, and 120% of the pre-analyzed tablet powder. The three replicates of each concentration were studied, and percentage of recovery was calculated. The results of accuracy are shown in Table 3.

Precision
The repeatability and intermediate precision of proposed method was analyzed on same day, different day and by different analyst. The results of precision studies are depicted in Table 4.

Specificity
The deliberated degradation of tablet sample was carried out in different condition such as acidic, basic, oxidative, thermal, and UV. The results are shown in Table 5.

Limit of detection (LOD) and limit of quantitation (LOQ)
The LOD and LOQ were calculated using formulae for LOD (LOD=3.3×σ/S) and LOQ (LOQ=10×σ/S). Where σ stands for standard deviation (SD) of the response and S stands for slope of calibration curve of the analyte. The results of LOD and LOQ are shown in Table 6.

Robustness
The robustness of proposed method was examined by doing small deliberate change in method parameters for instance, change in wavelength. The results are depicted in Table 7.

### Table 1: Absorptivity value of amoxapine

| Weights (mg) | Absorptivity value* | Mean ±SD | % RSD |
|--------------|---------------------|----------|-------|
| 10.1         | 206.46              | 206.80   | 0.606 |
| 9.9          | 205.06              | ±1.253   |       |
| 10.1         | 206.46              |          |       |
| 10.3         | 208.02              |          |       |
| 10.2         | 208.04              |          |       |

*Each value is mean of five observations. RSD: Relative standard deviation

### Table 2: Application of proposed method for the analysis of amoxapine in tablet dosage form

| Weight of tablet powder (mg) | Absorbance* | % Estimation* | % RSD |
|-----------------------------|-------------|---------------|-------|
| 28.84                       | 0.209       | 100.32±0.931  | 0.928 |

*Value is a mean of six observations. SD: Standard deviation, RSD: Relative standard deviation
Table 3: Results of recovery study

| Weight of tablet powder (mg) | Absorbance | Amount recovered (µg/mL) | % Recovery* mean±SD | % RSD |
|-----------------------------|------------|--------------------------|---------------------|-------|
| 28.47                       | 0.354      | 18.37                    | 102.0±0.724         | 0.709 |
| 27.74                       | 0.380      | 19.68                    | 98.44±0.897         | 0.911 |
| 26.05                       | 0.425      | 22.02                    | 100.9±0.942         | 0.941 |

*Value is mean of three determinations. RSD: Relative standard deviation, SD: Standard deviation

Table 4: Results of analysis of amoxapine on intraday, interday, and by different analyst

| Amolife (Avg. wt. 137.71 mg for 50 mg of amoxapine) | Intraday | Interday | Different analyst |
|-----------------------------------------------------|----------|----------|-------------------|
| Mean*±SD                                            | 99.63±0.751 | 99.80±0.510 | 100.5±0.609 |
| % RSD                                               | 0.753     | 0.511     | 0.686             |

*Value is mean of six observations. RSD: Relative standard deviation, SD: Standard deviation

Table 5: Results of specificity studies

| Conditions     | Weight of tablet powder (mg) | Percentage of amoxapine* mean±SD | % RSD |
|----------------|------------------------------|----------------------------------|-------|
| 0.1 N HCl      | 29.22                        | 99.95±0.746                      | 0.746 |
| 0.1 N NaOH     | 30.14                        | 99.27±0.985                      | 0.992 |
| 3% H2O2        | 29.60                        | 99.3±0.482                      | 0.482 |
| Thermal (60°C) | 28.19                        | 100.9±0.773                      | 0.766 |
| UV             | 28.97                        | 99.3±0.752                      | 0.757 |

*Each value is mean of three observations. RSD: Relative standard deviation, UV: Ultraviolet, SD: Standard deviation

Table 6: Regression analysis

| Parameters       | Values          |
|------------------|-----------------|
| Linearity range  | 2–20 µg/mL      |
| Correlation coefficient (r) | 0.995         |
| Slope            | 0.019           |
| LOD              | 19.8            |
| LOQ              | 60.50           |

LOD: Limit of detection, LOQ: Limit of quantitation

Table 7: Results of robustness study

| Parameter       | Amolife | Mean*±SD | % RSD |
|-----------------|---------|----------|-------|
| Wavelength      | 295 nm  | 100.75   | 0.927  |
|                 | 299 nm  | 99.51    | 0.795  | 0.763 |

*Value is mean of three determinations. RSD: Relative standard deviation, SD: Standard deviation

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AUTHORs CONTRIBUTION
First author SR conceptualized and designed the study, SP, RM and AH have assisted SR in the experiment, SR wrote the manuscript and NAR optimized study design, supervised complete experimental work and completed final drafting of the manuscript. All correspondence of the manuscript were managed by NAR.

CONFLICTS OF INTERESTS
Authors declare no conflicts of interest.

REFERENCES
1. Lotfy HM, Saleh SS. Recent development in ultraviolet spectrophotometry through the last decade (2006-2016): A review. Int J Pharm Pharm Sci 2016;8:40-56.
2. Zadubke N, Shahi S, JadHAV A, Borde S. Development and validation of spectrophotometric method for estimation of carbamazepine in bulk and tablet dosage form. Int J Pharm Pharm Sci 2016;8.234-8.
3. Jee S, Dawson G, Brodgen, R. Amoxapine: A review of its pharmacology and efficacy in depressant state. Drug Eval 1982;24:1-23.
4. Apiquian K, Ullao R, Fesam A, LozyzaC, Nicolin H, Kapor S. Amoxapine shows atypical antipsychotic effect in patients with schizophrenia: Results from a prospective open-label study. Schizophr Res 2002;59:35-39.
5. Tassel J, Hassan F. Liquid chromatographic determination of amoxapine and 8-hydroamoxapine in human serum. Clin Chem 1982;28:2514-62.
6. Selinger K, Lebel G, Hill H, Anslow J. A high performance liquid chromatographic method for the analysis of amoxapine in human plasma. J Pharm Biomed Anal 1989;8:1001-7.
7. Vijaykrishna AC, Samir SP, Ahmed M, Shetty AS, Kuppast IJ, Anilkumar SM, et al. RP-HPLC method development and validation for estimation of amoxapine in tablet dosage form. World J Pharm Pharm Sci 2015;4:11113-9.
8. Gupta M, Jain A, Verna K. Determination of Amoxapine and nortriptylline liquid-liquid micro extraction and high performance liquid chromatography. J Sep Sci 2010;33:3774-80.
9. Steven M, Jain A, Verna K. Determination of antidepressant, maprotiline and amoxapine and their metabolite in plasma by liquid chromatography. Clin Chem 1983;29:314-8.
10. Zimmer JS, Needham SR, Christianson CD, Piekarski CM, Sheaff CN, Huie K, et al. Validation of HPLC-MS/MS methods for analysis of loxapine, amoxapine,7-OH-loxapine, 8-OH-loxapine and N-oxide in human plasma. Bioanalysis 2010;2:1989-2006.
11. Karasakal A, Ulu S. Development and validation of a sensitive spectrophluorimetric method for the determination of amoxapine in human plasma and urine. J Biol Chem 2013;29:2984-7.
12. Korrupati U, Chintala R. New stability indicating RP-HPLC and spectrophotometric methods for the determination of amoxapine in tablet dosage form. Anal Chem 2016;16:147-56.
13. Patel S, Vijaykrishna C, Shetty S, Ahmad M. Development and validation of first order derivative spectrophotometric method for estimation of amoxapine in bulk and tablet dosage form. Int J Pharm Biomed Sci 2015;4:29-35.
14. International Conference of Harmonization: Q2 (R1) Validation of Analytical Procedure: Methodology. Geneva: 1996.