A Comprehensive Review of the Analytical Development and Validation of Alprazolam in Bulk and Pharmaceutical Dosage Form

Gaurav M. Prajapati1, Devang R. Ghuge2

1SGMSPM’s Sharadchandra College of Pharmacy, Otur, Pune, Maharashtra, India
2Kasturi Shikshan Sanstha College of Pharmacy, Shikrapur, Pune, Maharashtra, India

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*Corresponding author: Gaurav M. Prajapati
SGMSPM’s Sharadchandra College of Pharmacy. Otur, Pune, Maharashtra, India

Abstract

Alprazolam is approved in 2003 by US FDA. Alprazolam is available as an extended-release tablet, a mouth dissolving tablet (a tablet that dissolves rapidly in the mouth), as well as a concentrated solution (liquid). For the treatment of severe anxiety and panic disorder, alprazolam is one of the most commonly prescribed benzodiazepines. IPUAC name of Alprazolam is 8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a] [1, 4] benzodiazepine. The molecular formula and molecular Weight is C17H15ClFN4 and 326.75 g/mol. These articles may serve as an overview of Alprazolam with their drug profile, pharmacology, pharmacokinetics, and analytical HPLC procedures that are commonly employed in determining common provision issues. These reviews cover topics such as mobile phase, mobile phase ratio, column, retention time, flow rate, UV detector wavelength, run time, and more. Linearity, percent recovery, detection limit, and quantification limit are all validating parameters. The pharmaceutical analysis serves as an internal control for pure and pharmaceutical dosage forms, which is critical for quality assurance. The development of analytical methods has emerged as a crucial study activity.

Keywords: Drug profile, Pharmacology, Pharmacokinetic, Analytical HPLC Method, retention time, flow rate.

INTRODUCTION

Psychotropic medications have a variety of medicinal and scientific purposes, but they frequently cause users to experience positive changes, pushing them to take them on a regular basis. As a result, these psychoactive chemicals are overused, that is, they are taken frequently despite the risks and bad repercussions to one’s health [1, 2]. Alprazolam (ALP) is a new generation 1, 4-benzodiazepine synthesised from 8-chloro-1-methyl-6-phenyl-4H-[1, 2, 4] triazolo [4, 3-]-[1, 4]. It's a benzodiazepine that's mostly used as an anxiolytic in humans and might help with depression and panic attacks. It's important to remember that alprazolam, like any other benzodiazepine, is ineffective in alleviating anxiety and strain generated by daily stress. ALP is also used to treat panic disorders, both with and without agoraphobia [3, 4]. It affects the brain's neurotransmitters, which are imbalanced and unstable during anxiety. It works by increasing the effects of a natural substance in the body called GABA. It's used to treat anxiety disorders, panic attacks, and anxiety induced by stress or sadness. To avoid withdrawal symptoms, benzodiazepine therapy should be terminated gradually by decreasing a patient's dose. The drowsiness that alprazolam can produce is one of its most common side effects. As a drug of abuse, alprazolam has been coupled with alcohol to increase the sedative effects of the substance, which can lead to coma and death [5]. On October 16, 1981, the FDA approved alprazolam [6].
Table-1: Drug profile of Alprazolam

<table>
<thead>
<tr>
<th>Sr.no</th>
<th>Parameter</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alprazolam</td>
<td><img src="image" alt="Chemical Structure of Alprazolam" /></td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Physical State</td>
<td>White Crystalline Powder</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>IUPAC</td>
<td>8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine</td>
<td>[7]</td>
</tr>
<tr>
<td>4</td>
<td>Molecular formula</td>
<td>C₁₇H₁₂ClFN₄</td>
<td>[8]</td>
</tr>
<tr>
<td>5</td>
<td>Molecular Weight</td>
<td>326.75 g/mol</td>
<td>[9]</td>
</tr>
<tr>
<td>6</td>
<td>Melting Point</td>
<td>228-228.50°C</td>
<td>[10]</td>
</tr>
<tr>
<td>7</td>
<td>Trade Names</td>
<td>Xanax, Niravam, Xanax XR</td>
<td>[11]</td>
</tr>
<tr>
<td>8</td>
<td>BCS Class</td>
<td>Class II (High Permeability and Low Solubility)</td>
<td>[12]</td>
</tr>
<tr>
<td>9</td>
<td>Onset of action</td>
<td>20-60 minute</td>
<td>[13]</td>
</tr>
</tbody>
</table>
| 10    | Elimination half-life | Full release: 11~13 hours  
Extended release: 11~16 hours                  | [14]      |
| 11    | Solubility         | It is slightly soluble in chloroform, soluble in alcohol, slightly soluble in acetone and insoluble in water | [15]      |
| 12    | Uses               | Treat anxiety disorders and panic disorder                             | [16]      |

Pharmacology

The gamma-aminobutyric acid (GABA) type A receptor is a positive allosteric modulator of alprazolam. GABA’s actions are amplified when it binds to the receptor, causing neurons in the brain to be inhibited. This has anticonvulsant, antidepressant, and muscle relaxant properties. The central nervous system action of alprazolam varies depending on the dosage [17].

Mechanism of Action

![Molecular Structure of Alprazolam](image)

Alprazolam is a benzodiazepine, a type of psychotropic medicine. The GABA-A receptor is occupied by benzodiazepines. This receptor has five subunits, such as alpha, beta, gamma, delta, epsilon, and rho. Two alpha-1 subunits, two beta-2 subunits, and one gamma-2 subunit make up a GABA-A receptor in the CNS. Between the alpha-1 and gamma-2 subunits is where the benzodiazepine binding site is located. Studies in mice reveal that the alpha-1 subunit mediates benzodiazepine sedation, amnesia, and ataxia, whereas the alpha-2 and alpha-3 subunits mediate anxiolytic and muscle-relaxing actions. Additionally, evidence reveals...
that BNZ-1 receptors are involved in sedation and anti-anxiety, whereas BNZ-2 receptors are involved in muscle relaxation, anticonvulsant action, memory, and motor coordination. GABA-A receptors appear to be coupled to benzodiazepine binding sites, improving the effects of GABA by increasing GABA affinity at the GABA-A receptor. The calming or inhibitory effects of alprazolam on the human neurological system are mediated via the primary inhibitory neurotransmitter GABA when coupled to the GABA-A receptor [18, 19].

Pharmacokinetics

After oral treatment, alprazolam is rapidly absorbed, with a peak plasma concentration of 1 to 2 hours. Oral alprazolam has a bioavailability of 80 to 100 percent. Alprazolam binds to serum protein, primarily albumin, to the tune of 80%. Alprazolam is converted to 4-hydroxyalprazolam and alpha-hydroxyalprazolam metabolites in the liver by cytochrome P450 3A4 (CYP3A4). The kidneys filter alprazolam and its metabolites, which are then eliminated in the urine. In healthy people, alprazolam has a plasma half-life of roughly 11.2 hours [20].

Side Effects

Anterograde amnesia and concentration problems [21], Urinary retention (infrequent) [22], Skin rash, respiratory depression, constipation, drowsiness, dizziness, lightheadedness, fatigue, unsteadiness, and impaired coordination, vertigo [23], Jaundice (very rare) [24], Ataxia, slurred speech [25], Disinhibition [26]. Some other paradoxical reactions such as mania, agitation, hyperactivity, restlessness [27-29], aggression [30], Twitches, and tremor [31]. There are few food and interaction such as, when alprazolam is used with the herb kava, it might lead to a semi-comatose state [32]. Hypericum plants, on the other hand, can diminish alprazolam plasma levels and so reduce its therapeutic impact [33-35] ketoconazole, fluvoxamine, nefazodone, cimetidine, erythromycin, norfluroxetine, itraconazole, propoxyphene, and ritonavir are all CYP3A4 inhibitors that delay alprazolam's hepatic clearance, potentially resulting in its buildup [36]. Alprazolam usage during pregnancy has been linked to congenital defects [37]. Drug usage throughout the third trimester can lead to fetal drug dependency and symptoms of withdrawal in the postpartum period, as well as newborn flaccidity and respiratory issues. However, rapid stopping of benzodiazepines owing to teratogenesis concerns has a significant risk of triggering severe symptoms and a significant rebound impact of an underlying mental problem in long-term users. Abrupt cessation of psychiatric medicines, such as benzodiazepines, can potentially cause spontaneous miscarriages [38-40].

Analytical chemistry is a discipline of science that uses cutting-edge technology to determine the composition of substances using analytical techniques. Both qualitative and quantitative outcomes are possible. Analytical tools serve a critical role in obtaining high-quality and consistent analytical data. As a result, everyone in the analytical laboratory must be concerned about equipment quality assurance. Spectral, chromatography, electrochemical, hyphenated, or other analytical procedures are used. The process of choosing an effective assay procedure to identify the components of a formulation is known as analytical method development [41-43].

The validation study's findings are detailed in the validation report. Its objective is to offer information about the features that were examined during the study, the findings that were achieved, and how those results were interpreted. A validation report typically contains the following information:

1. Validation protocol.
2. Analytical method
3. The validation parameters
4. The results
5. Interpretation of the results
6. Relevant validation information
7. Details of the reference materials
8. Details of batch number
9. Details of the equipment used for the study
10. References to the laboratory details

Typical validation parameters recommended by FDA, USP, and ICH is as follows:

1. Specificity
2. Linearity and Range
3. Precision
   (A) Method precision (Repeatability)
   (B) Intermediate precision (Ruggedness)
4. Accuracy
5. Solution stability
6. Limit of Detection (LOD)
7. Limit of Quantification (LOQ)
8. Robustness

Method validation is a broad topic that encompasses a variety of validation factors and procedures for various levels of criteria dependent on the analytical method's intended usage. During routine use, the validated method clarifies unexpected or unknown problems. The verified approach has a low confidence level. Following the construction of a technique, it must be verified to ensure that it meets the requirements for its intended usage.
Table-2: Reported method development and validation

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Mobile phase</th>
<th>Flow rate</th>
<th>Retention time</th>
<th>Detection</th>
<th>Column</th>
<th>Linearity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>phosphate buffer: acetonitrile (40:60 v/v)</td>
<td>1ml/min</td>
<td>2.342 min</td>
<td>225 nm</td>
<td>Symmetry C18 (4.6 x 250mm, 5μm)</td>
<td>5-25 μg/ml</td>
<td>[44]</td>
</tr>
<tr>
<td>2.</td>
<td>ACN:0.05M Phosphate Buffer (55:45) Ph 7.2 with TEA (Triethanolamine)</td>
<td>1.5ml/min</td>
<td>3.7 min</td>
<td>264 nm</td>
<td>C18 JNJ Analytical (4.6×25×5μ)</td>
<td>5-15 μg/ml</td>
<td>[45]</td>
</tr>
<tr>
<td>3.</td>
<td>Acetonitrile and phosphate buffer (50:50 v/v) pH 4.5</td>
<td>1ml/min</td>
<td>5.178 min</td>
<td>225 nm</td>
<td>C18 column (4.6 x 250 mm, 5 μm)</td>
<td>0.5-1.50 μg/ml</td>
<td>[46]</td>
</tr>
<tr>
<td>4.</td>
<td>acetonitrile and potassium phosphate buffer (pH6.0±0.1) (40:60 v/v)</td>
<td>1.5 ml/min</td>
<td>3.01 min</td>
<td>254 nm</td>
<td>Cosmosil C-8 column</td>
<td>50-150 μg/ml</td>
<td>[47]</td>
</tr>
<tr>
<td>5.</td>
<td>Water (ph 6): Methanol:Triethylamine (70:30:0.1 % v/v/v)</td>
<td>1ml/min</td>
<td>3.181 min</td>
<td>216 nm</td>
<td>ODS-BP Hyperchrome C18 column</td>
<td>50 - 150 μg/ml</td>
<td>[48]</td>
</tr>
<tr>
<td>6.</td>
<td>Acetonitrile and phosphate buffer (50:50 v/v)</td>
<td>1ml/min</td>
<td>1.89 min</td>
<td>225 nm</td>
<td>C18 column (4.6 x 250 mm, 5 μm)</td>
<td>0.5-2.0μg/ml</td>
<td>[49]</td>
</tr>
<tr>
<td>7.</td>
<td>Acetonitrile: Water 75:25 (pH adjusted to 2.75 with 0.1% orthophosphoric acid)</td>
<td>1.1 ml/min</td>
<td>3.14 min</td>
<td>224 nm</td>
<td>C18 column, Phenomenex</td>
<td>120-600μg/ml</td>
<td>[50]</td>
</tr>
<tr>
<td>8.</td>
<td>Acetonitrile : phosphate buffer pH 5.5 (45: 55 v/v)</td>
<td>1ml/min</td>
<td>4.51 min</td>
<td>230 nm</td>
<td>Nucleosil C8 column</td>
<td>4–14 mg/mL</td>
<td>[51]</td>
</tr>
<tr>
<td>9.</td>
<td>acetonitrile and 0.02M KH2PO4 buffer (65:35 v/v) and 0.1% Tri Fluoro Acetic acid (TFA)</td>
<td>1ml/min</td>
<td>5.182 min</td>
<td>230 nm</td>
<td>Hypersil BDS C18 (250 mm x 4.6 mm x 5 μm)</td>
<td>5.0–75.0 mg/mL</td>
<td>[52]</td>
</tr>
<tr>
<td>10.</td>
<td>acetonitrile and water (80:20 % v/v)</td>
<td>1ml/min</td>
<td>6.2 min</td>
<td>236 nm</td>
<td>RP Cell Pack C18 column (250 mm x 4.6 mm x 5 μm)</td>
<td>0.25-1.5 μg/mL</td>
<td>[53]</td>
</tr>
<tr>
<td>11.</td>
<td>acetonitrile/water 40:60 v/v</td>
<td>1ml/min</td>
<td>3.34 min</td>
<td>230nm</td>
<td>Lichrospher RP-18 column (25cm x 5 μm)</td>
<td>2.5 to 40 μg/mL</td>
<td>[54]</td>
</tr>
<tr>
<td>12.</td>
<td>methanol: phosphate buffer pH 7 (70:30)</td>
<td>1ml/min</td>
<td>3.8 min</td>
<td>221nm</td>
<td>Phenomenex C18 (150mm x 4.6mm x 5μm)</td>
<td>0.8-1.2 μg/ml</td>
<td>[55]</td>
</tr>
<tr>
<td>13.</td>
<td>Phosphate buffer: Acetonitrile (30:70 v/v pH:4.0)</td>
<td>1ml/min</td>
<td>2.15 min</td>
<td>229 nm</td>
<td>ZORBAX C8 (150mm x 4.6mm x 5μm)</td>
<td>0.6-1.4μg/ml</td>
<td>[56]</td>
</tr>
</tbody>
</table>

CONCLUSION
Antidepressant medication alprazolam is used to treat anxiety and panic attacks. The methodologies for studying and analysing Alprazolam in pure form and pharmaceutical dosage form are described in the previous work. According to a review of the literature, many approaches for developing and validating procedures for diverse medications have been documented. The current study shows how HPLC in pure form and pharmaceutical dosage form was used to evaluate Alprazolam. These approaches are described for the creation of Alprazolam drug methods and their validation parameters. Following formulation, drug analysis is critical for determining the drug's identity and metabolites.

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REFERENCES


