WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Review on Preparation and Evaluation of Alprazolam

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ABSTRACT

The treatment of anxiety disorders or the temporary symptomatic relief of symptoms associated with excessive anxiety are the intended uses of ALPRAZOLAM. Anxiolytic therapy is typically not necessary for anxiety or tension brought on by daily stress. The medication ALPRAZOLAM is prescribed to treat generalized anxiety disorder (GAD). The hallmarks of Generalized Anxiety Disorder (GAD) include excessive or unrealistic worry and anxiety (apprehensive expectations) about two or more life circumstances, for a duration of six months or more, during which the individual has experienced these concerns on most days. ALPRAZOLAM is also recommended for the treatment of panic disorder, whether or not it is accompanied by agoraphobia. Recurrent panic attacks are a hallmark of panic disorder. Patients who have a history of known hypersensitivity to alprazolam, any ingredient in the product's formulation, or other benzodiazepines should not take ALPRAZOLAM. An alternative high-performance thin-layer chromatographic technique is created to separate and estimate the drug substance alprazolam's starting material and synthesis-related intermediates. With regard to test concentration, the HPTLC method can identify impurities at a level of 0.05 percent.

Keywords: Alprazolam, GAD, Anxiety, HPTLC, Hypersensitivity.

Introduction

A well-known class of substances with a variety of effects on the central nervous system are benzodiazepines. Benzodiazepines exhibited a variety of biological activities, including anticonvulsive and antitumoral effects. One common sedative-hypnotic that is prescribed to treat insomnia is benzophenone. Alprazolam, in contrast to traditional benzodiazepines (BZDs), binds to the GABAA receptor's BZ site, which is located at the a1/c2 subunit interface. It has a relatively low affinity for GABAA that contains the a2/a3 subunits and no discernible affinity for the 5 subtypes.

Generalized anxiety disorder (GAD) can be treated with ALPRAZOLAM (alprazolam). The hallmarks of Generalized Anxiety Disorder (GAD) include excessive or unrealistic worry and anxiety (apprehensive expectation) about two or more life circumstances, for a duration of six months or more, during which the individual has experienced these concerns on more days than not. In these patients, at least six of the following eighteen symptoms are frequently present: Autonomic Hyperactivity (shortness of breath or smothering sensations; palpitations or accelerated heart rate; sweating, or cold, clammy hands; dry mouth; dizziness or lightheadedness; nausea, diarrhea, or other abdominal distress; flushes or chills; frequent urination; trouble swallowing or "lump in the throat"); Motor Tension (trembling, twitching, or feeling shaky); muscle tension, aches, or soreness; restlessness; easy fatigability); Alertness and Scanning (trouble falling or staying asleep; irritability; feeling tense or on edge; exaggerated startle response); difficulty concentrating or "mind going blank" due to anxiety). These symptoms cannot be brought on by an organic factor or be a side effect of another mental illness. Moreover, ALPRAZOLAM (alprazolam) is recommended for the treatment of panic disorder, whether or not agoraphobia is present. Recurrent panic attacks are a hallmark of panic disorder. At least four of the following symptoms must be present during a panic attack, which are isolated episodes of extreme fear or discomfort: Dyspnea, vertigo, shakiness, sweating, choking, nausea, abdominal distress, depersonalization, derealization, paresthesias, flushes, chills, chest pain or discomfort, fear of dying, fear of going insane, or fear of acting irrationally are some symptoms of this condition. Chloroform, acetone, ethyl acetate, and methanol (50:50:50:5) are used as the mobile phase in the TLC method as described in USP3,4. The test concentration is 40 mg/mL, and the sample is evaluated against 0.1, 0.3, and 0.5% standard solutions (alprazolam) under short-wavelength UV light (254 nm).

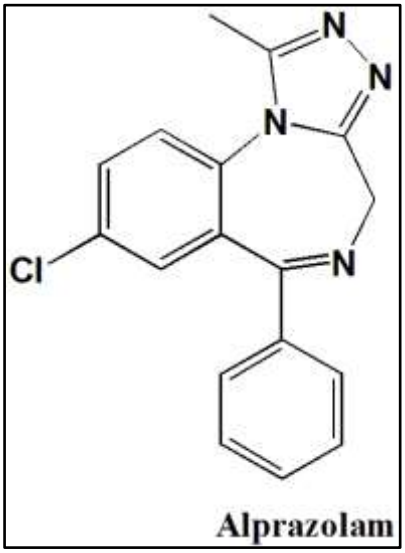

Figure 1: Structure of Alprazolam

Synthesis of Alprazolam

i. 6-chloro-2-hydrazino-4-phenylquinoline (3) is produced when 2,6,-dichloro-4-phenylquinoline (1) reacts with hydrazine (2).

ii. Triethyl orthoacetate (4) and boiling (3) in xylene cause heterocyclization, which results in the formation of a triazole derivative (5).

iii. (5) oxidatively cleaves to form 2-[4-(3′-methyl-1,2,4-triazolo)] by employing sodium periodate (6) and ruthenium dioxide (7) in an acetone-water system.5-chlorobenzophthalenone (8).

iv. 2-[4-(3′-methyl-5′-bromomethyl-1,2,4-triazolo)] is obtained by oxymethylating the (8) with formaldehyde (9), then substituting the hydroxyl group with phosphorous tribromide (10)(11) 5-chlorobenzophenone.

v. Alprazolam is produced when an amino group (such as ammonia) is substituted for the bromine atom, leading to spontaneous heterocyclization. [2]

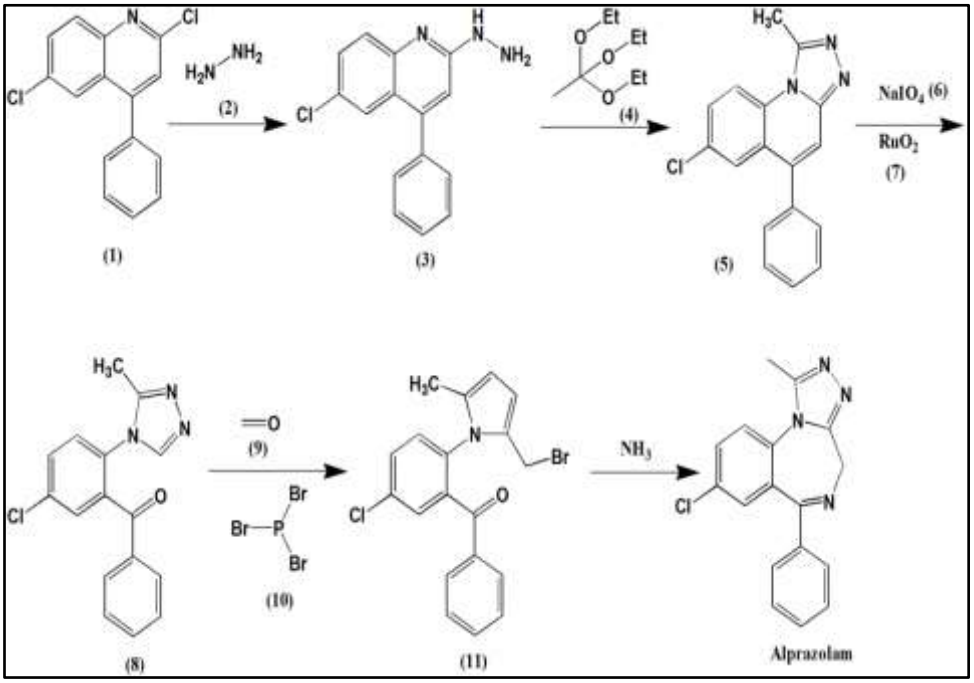

Figure 2: Synthesis process of Alprazolam

Mechanical of Action

i. Alprazolam affects the BNZ1 and BNZ2 benzodiazepine receptors.

ii. Alprazolam breaks down into 4-hydroxylprazolam and α-hydroxylprazolam, which are two active metabolites. The potency of the former metabolite is 0.20 times that of alprazolam, whereas the latter metabolite is 0.66 times more potent.

iii. While BNZ2 action affects memory, coordination, muscle relaxation, and anticonvulsive activities, BNZ1 action results in sedation and anti-anxiety effects.

iv. The medication also has a calming effect when it binds to GABAA receptors, increasing GABA binding to the receptors and inhibiting the nervous system.

Structure Active Relationship (SAR)

- • To bind with derivatives of 5-phenyl-1,4-benzodiazepin-2-one, Ring A needs to have an aromatic or heteroaromatic ring.
- • The functional anxiolytic activity is increased by an electronegative group located at ring A's 7th position.
- • The functional anxiolytic activity will be reduced by substitutions with the electronegative group on ring A at positions 6, 8, or 9.
- • When the drug's ring A is a heterocycle, its pharmacological activity is low.
- • Ring B requires a proton-accepting group in order to bind with GABAA.
- • Maximum activity is noted when the proton accepting group is present on ring B's 2-position and in coplanar spatial orientation with ring A.
- • The selectivity for binding with GABA BZR subpopulations is altered when sulfur is substituted for oxygen in ring B, but the anxiolytic properties are maintained.
- • The agonist activity remains unaffected, however substitution of the ring B's imine nitrogen or methylene 3-position results in a decrease in antagonist activity.
- • Rapid excretion occurs for derivatives containing the 3-hydroxy moiety.
- • The tert-butyl group and other sterically large substituents on ring B lower the receptor's affinity and in vivo activity.
- • Nitrogen at position 4 and the 4,5 double bond are not necessary for anxiolytic activity.
- • If the C=N bond is switched out for a C-N bond, the BZR affinity is reduced.

• The binding with BZR does not require the presence of 5-phenyl ring C.

• The agonist activity of the drug is reduced when substitution occurs at the para position of ring C.

• When substitution occurs at the ortho position, the drug's agonist property remains unchanged.

• The affinity of the BZR increases when 1,2-bond f the ring C is annelated with another ring that is rich in electrons, like imidazole.

Physiochemical Properties

SL No.	Physiochemical Properties	
1	Molecular weight	308.8 g/mol
2	Physical appearance	Solid; crystals from ethyl acetate
3	Melting point	228-229°C
4	Water partition coefficient	2.12
5	Solubility	13.1 mg/ml in water
6	Presence of ring	Diazepine, benzene, triazole
7	Number of chiral centers	Not present

Table 1: Physiochemical properties

Alprazolam Warning

When alprazolam is taken with other medications, there is an increased risk of sedation, coma, and severe or life-threatening breathing issues. Inform your physician about any opiate medications you are taking or intend to take for cough, such as codeine (in Triacin-C, in Tuzistra XR) or hydrocodone

(in Anexsia, in Norco, in Zyfrel); or for pain, such as fentanyl (Actiq, Duragesic, Subsys, etc.), hydromorphone (Dilaudid, Exalgo), meperidine (Demerol), methadone (Dolophine, Methadose), morphine (Astramorph, Duramorph PF, Kadian), oxycodone (in Oxycet, in Percocet, in Roxicet, others), and tramadol (Conzip, Ultram, in Ultracet). Your doctor will closely monitor you and may need to adjust the dosages of your medicationsIf you take alprazolam along with any of these drugs and experience any of the following side effects, contact your doctor right away or go to the emergency room right away: unusual feelings of lightheadedness, drowsiness, breathing difficulties or sluggishness, or lack of responsiveness. If you are unable to seek treatment for yourself, make sure your family members or caregiver know which symptoms are serious enough to call for emergency medical attention or a visit to the doctor.

Alprazolam could lead to addiction. Never take a higher dose, take it more frequently, or take it for longer than your doctor prescribes. Inform your physician if you have ever overused prescription medications, used street drugs, or consumed large amounts of alcohol. Avoid using illicit drugs or alcohol while receiving treatment. During your alprazolam treatment, alcohol consumption and drug use on the streets increase the likelihood that you will encounter these severe, potentially fatal side effects. Additionally, disclose to your physician any history of depression or other mental health conditions.

Particularly if you take alprazolam for a few days to a few weeks, it may result in physical dependence, a condition where unpleasant physical symptoms arise if a medication is abruptly stopped or taken in smaller doses. Consult your doctor before reducing the dosage or stopping this medication. Abruptly stopping alprazolam can exacerbate your health and result in withdrawal symptoms that can linger for more than a year. Most likely, your doctor will gradually reduce the amount of alprazolam you take. If you encounter any of the following symptoms, contact your physician or seek emergency care: unusual movements; ringing in your ears; anxiety; memory problems; difficulty concentrating; sleep problems; seizures; shaking; muscle twitching; changes in mental health; depression; burning or prickling feeling in hands, arms, legs or feet; seeing or hearing things that others do not see or hear; thoughts of harming or killing yourself or others; overexcitement; or losing touch with reality.

Side Effects

The following symptoms may be experienced: drowsiness, lightheadedness, headache, fatigue, dizziness, irritability, talkativeness, difficulty concentrating, dry mouth, increased salivation, changes in sex drive or ability, nausea, constipation, changes in appetite, weight changes, difficulty urinating, and joint pain.

Contraindications

Patients who have a history of known hypersensitivity to alprazolam, any ingredient in the product's formulation, or other benzodiazepines should not take ALPRAZOLAM (alprazolam). Additionally, patients with myasthenia gravis, acute narrow-angle glaucoma, severe respiratory insufficiency, severe hepatic insufficiency, or sleep apnea syndrome should not take ALPRAZOLAM. Alprazolam, however, is safe to use in open-angle glaucoma patients who are getting the right care.

Because ketoconazole and itraconazole greatly impair alprazolam's metabolism by CYP3A4, co-administration of alprazolam with these medications is contraindicated (see DRUG INTERACTIONS, Drug-Drug Interactions).

Experimental

M/S Natco Pharma Limited (Hyderabad, India) was the manufacturer of the drug substance alprazolam as well as all the related substances mentioned above. Merck provided analytical grade ethyl acetate, methanol, and methylene chloride. We used Merck's pre-coated Silica Gel 60F254 TLC plates. High-performance thin-layer chromatograph equipment (CAMAG, Germany) is used in this developmental study.

Diluent: A mixture of 4: 1 chloroform and methanol.

System suitability solution: A mixture having 0.08 mg/mL each of impurity-1, impurity-2, impurity-3, impurity-4, and alprazolam (0.2 % with respect to test concentration) in diluent.

Reference solution (a): A mixture having 0.04 mg/mL each of impurity- 1, impurity-2, impurity-3, impurity-4, and alprazolam (0.1 % with respect to test concentration) in diluent.

Reference solution (b): A mixture having 0.08 mg/mL each of impurity- 1, impurity-2, impurity-3, impurity-4, and alprazolam (0.2 % with respect to test concentration) in diluent.

Reference solution (c): A mixture having 0.12 mg/mL each of impurity- 1, impurity-2, impurity-3, impurity-4, and alprazolam (0.3 % with respect to test concentration) in diluent.

Test solution: 400 mg of alprazolam in 10 mL of methanol (40 mg/ mL).

Mobile phase: Mix 8 mL of methylene chloride, 0.5 mL of methanol, and 0.25 mL of ethyl acetate.

Application volume: 5 µL.

Elution: In order to pre-saturate the TLC plate with the vapors of the mobile phase, it must be developed in a closed chamber with walls lined with filter paper. The TLC plate must be allowed to air dry after elution before being seen under a UV lamp set at 254 nm. A Camag Linomat-IV scanner will be used to perform densitometric scanning on the TLC plate.**Scan parameters:** The typical densitometric scan parameters are as below.

Distance between tracks	14.0 mm
Bandwidth	20 nm
Lamp	Mercury
Wavelength	254 nm
Slit dimension	8.0 × 0.9 mm
Data step resolution	50 µm
Measurement mode	Absorption/Reflection

Table 2: Scan Parameters

If the system's suitability solution revealed five fully separated spots that corresponded to impurities-1, impurity-2, impurity-3, impurity-4, and alprazolam, the HPTLC system was judged appropriate for use. A system suitability solution will be used to identify the components that are being studied. For estimating the corresponding impurity level in the test application, Reference solutions (a), (b), and (c) must be utilized.

Results

System suitability

When five completely separated spots are obtained after applying a system suitability solution containing alprazolam and all related impurities 1, 2, 3, and 4 at approximately 0.2% (0.08 mg/mL) in relation to the test concentration of 40 mg/mL, the HPTLC chromatographic system is considered suitable. Five clearly separated spots led to the system suitability solution, indicating that the acceptance criteria were satisfied. As a result, the HPTLC method's system suitability is determined.

Power of the method-specificity or resolution

By figuring out each component's Rf value, the developed HPTLC analytical method's ability to separate the components is determined. With regard to the test concentration of 40 mg/mL, each of the components under investigation is applied separately at a concentration of 0.8% (0.08 mg/mL). each component was identified using its retardation factor (Rf). The documented method was followed in the analysis of the alprazolam drug substance (40 mg/mL) spiked with each of the impurities at 0.2 % (0.08 mg/mL). The corresponding Rf values show that each of the study's components was well resolved.

Component	**Rf value**	**Spot. No.**
2-Chloro acetamide-5-chloro benzophenone (Impurity-1)	0.83	5
Nordiazepam (Impurity-2)	0.45	3
Thionordiazepam (Impurity-3)	0.77	4
2-(2-Aceto hydrazinyl)-7-chloro-5-phenyl-3H-1,4-benzodiazapine (Impurity-4)	0.16	1
Alprazolam	0.25	2

Table 3: R_f Value.

It is evident from the chromatogram and the Rf values that the procedure can separate all of the components with adequate resolution. As a result, this HPTLC technique is unique and tailored to the given situation. Six replicate applications of an alprazolam sample spiked with the relevant impurities at 0.2% (0.08 mg/mL) relative to the test concentration are used to calculate the precision. On an HPTLC, the developed TLC plate is scanned. The answers are noted and the RSD (%) is computed. The RSD (%) values for each of the five compounds show how accurate the procedure is. Regarding the test concentration (40 mg/mL), the linearity of the method is examined over a range of 0.05% (0.02 mg/mL) to 0.50 % (0.2 mg/mL). Applications using an alprazolam mixture sample spiked with impurities at concentration levels of 0.5% (0.02), 0.1% (0.04), 0.15 % (0.06 mg/mL), 0.2% % (0.08 mg/mL), 0.30 % (0.12 mg/mL), and 0.50 % (0.2 mg/mL) were conducted in relation to a test concentration of 40 mg/mL.

S. No.	Impurity-1	Impurity-2	Impurity-3	Impurity-4	Alprazolam
1	7896.1	5296.8	6477.7	8489.1	82389.6
2	8042.9	5017.6	6562.7	8495.0	86177.0
3	7906.2	5066.6	6668.8	8347.2	79591.4
4	7872.6	5068.7	6713.0	8422.2	76848.5
5	8058.1	5255.7	6747.3	8576.1	80984.8
6	8104.0	5213.5	6934.5	8300.4	85199.0
Average	7980.0	5153.2	6684.0	8438.3	81865.1
RSD (%)	1.25	2.26	2.37	1.21	4.27

Table 4

The TLC plate eluted is air-dried and is densitometrically scanned on Camag TLC Scanner. The correlation coefficient for the concentration taken and area response obtained on HPTLC

Impurity-1		Impurity-2		Impurity-3		Impurity-4	
Conc. (%)	Area response	Conc. (%)	Area response	Conc. (%)	Area response	Conc. (%)	Area response
0.05	3797.1	0.05	2363.4	0.05	1022.6	0.05	4335.9
0.10	5060.0	0.10	3928.4	0.10	2992.1	0.10	6371.7
0.15	7312.9	0.15	5591.7	0.15	4975.4	0.15	7328.0
0.20	8434.5	0.20	6224.6	0.20	6041.5	0.20	8067.4
0.30	10707.7	0.30	8376.8	0.30	8620.2	0.30	9537.8
0.50	13804.9	0.50	13259.6	0.50	12159.1	0.50	13526.9
CC	0.9820	CC	0.9970	CC	0.9854	CC	0.9909

Linear regression equation : Y = 22143.09X + 3388.514 for Impurity-1
Y = 23446.63X + 1543.981 for Impurity-2
Y = 22143.09X + 3388.514 for Impurity-3
Y = 19038.25X + 4069.663 for Impurity-4

Table 5

By adding spikes to the alprazolam sample at three different concentrations—0.1% (0.04 mg/mL), 0.2% (0.08 mg/mL), and 0.3% (0.12 mg/mL)—the recoveries of impurities were evaluated. The recovery percentage varies between 89.1% and 101.5% across all impurities.

Application of each component at a level of 0.025 percent (0.01 mg/mL) with respect to test concentration (40 mg/mL) allows for the study of the detection level (L.O.D.) for each component. Every component was identified. At a test concentration of 40 mg/mL, the presence of contaminants in the alprazolam sample matrix is also confirmed.

Description	0.1 % Level	0.2 % Level	0.3 Level
	Impurity-1		
Conc. obtained	0.102	0.205	0.270
Conc. taken	0.101	0.202	0.303
Recovery (%)	101.000	101.500	89.100
	Impurity-2		
Conc. obtained	0.098	0.184	0.281
Conc. taken	0.100	0.200	0.300
Recovery (%)	98.000	92.000	93.700
	Impurity-3		
Conc. obtained	0.090	0.194	0.286
Conc. taken	0.101	0.202	0.303
Recovery (%)	89.100	93.000	94.400
	Impurity-4		
Conc. obtained	0.099	0.200	0.278
Conc. taken	0.100	0.200	0.300
Recovery (%)	99.000	100.000	92.700

Table 6

Conclusion

Based on the aforementioned, it can be concluded that the HPTLC method developed is specific enough to identify, separate, and estimate any potential impurities in the drug substance alprazolam.

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