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

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Abstract **Review Article**

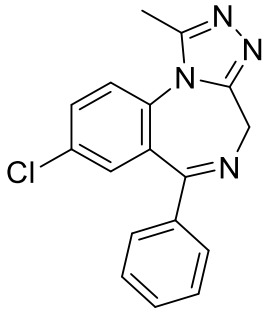
Alprazolam is approved in 2003 by US FDA. Alprazolam is available as an extended-release tablet, a mouth dissolving tablet (a tablet that dissolves rapidly in the mouth), as well as a concentrated solution (liquid). For the treatment of severe anxiety and panic disorder, alprazolam is one of the most 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 $C_{17}H_{12}ClFN_4$ 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 agarophobia [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

Sr.no	Parameter	Result	Reference
1	Alprazolam		-
2	Physical State	White Crystalline Powder	-
3	IUPAC	8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a] [1,4]benzodiazepine	[7]
4	Molecular formula	$C_{17}H_{12}ClFN_4$	[8]
5	Molecular Weight	326.75 g/mol	[9]
6	Melting Point	228-228.50C	[10]
7	Trade Names	Xanax, Niravam, Xanax XR	[11]
8	BCS Class	Class II (High Permeability and Low Solubility)	[12]
9	Onset of action	20~60 minute	[13]
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

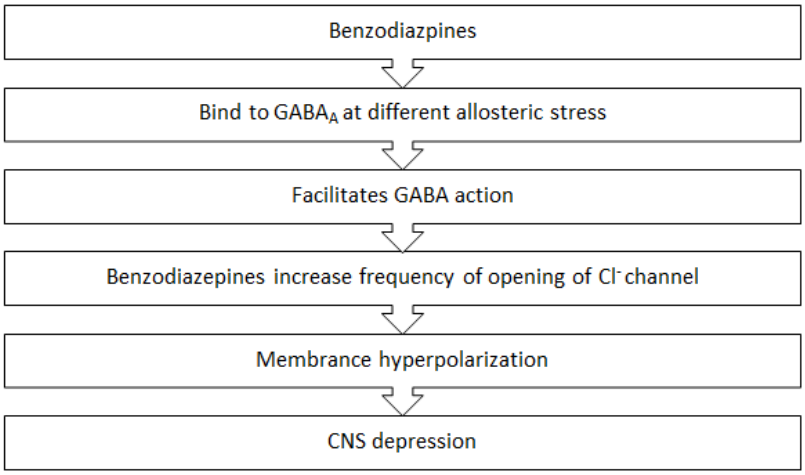


Fig-1: Mechanism of action

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, norfluoxetine, 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

Sr.No.	Mobile phase	Flow rate	Retention time	Detection	Column	Linearity	References
1.	phosphate buffer: acetonitrile (40:60v/v)	1ml/min	2.342 min	225 nm	Symmetry C18 (4.6 x 250mm, 5µm)	5-25 µg/ml	[44]
2.	ACN:0.05M Phosphate Buffer (55:45) Ph 7.2 with TEA (Triethanolamine)	1.5ml/min	3.7 min	264 nm	C18 JNJ Analytical (4.6×25×5µ)	5-15 µg/ml	[45]
3.	Acetonitrile and phosphate buffer (50:50 v/v) pH 4.5	1ml/min	5.178 min	225 nm	C18 column (4.6 × 250 mm, 5 µm).	0.5-1.50 µg/ml	[46]
4.	acetonitrile and potassium phosphate buffer (pH6.0±0.1) (40:60 v/v)	1.5 ml/min	3.01 min	254 nm	Cosmosil C-8 column	50-150 µg/ ml	[47]
5.	Water (ph 6): Methanol:Triethylamine (70:30:0.1 % v/v/v)	1ml/min	3.181 min	216 nm	ODS-BP Hyperchrome C18column (250 mm, 4.6 mm, 5 µm)	50 - 150 µg/ml	[48]
6.	Acetonitrile and phosphate buffer (50:50 v/v)	1ml/min	1.89 min	225 nm	C18 column (4.6 × 250 mm, 5 µm).	0.5-2.0µg/ml	[49]
7.	Acetonitrile: Water 75:25 (pH adjusted to 2.75 with 0.1% orthophosphoric acid)	1.1 ml/min	3.14 min	224 nm	C18 column, Phenomenex (250mm x 4.60mm x 5 µm)	120-600µg/ml	[50]
8.	Acetonitrile : phosphate buffer pH 5.5 (45: 55 v/v)	1ml/min	4.51 min	230 nm	Nucleosil C8 column (150 mm x 4.6 mmx 5 µm)	4–14 mg/mL	[51]
9.	acetonitrile and 0.02M KH2PO4 buffer (65:35 v/v) and 0.1% Tri Fluoro Acetic acid (TFA)	1ml/min	5.182 min	230 nm	Hypersil BDS C18 (250 mm×4.6 mm, 5 µm)	5.0–75.0 mg/mL	[52]
10.	acetonitrile and water (80:20 % v/v)	1ml/min	6.2 min	236 nm	RP Cell Pack C18 column (250 mm × 4.6 mm x 5 µm)	0.25-1.5 µg/mL	[53]
11.	acetonitrile/water 40:60 v/v	1ml/min	3.34 min	230nm	Lichrospher RP-18 column (25cm x 5 µm)	2.5 to 40 µg/mL	[54]
12.	methanol: phosphate buffer pH 7 (70:30)	1ml/min	3.8 min	221nm	Phenomenex C18 (150mm x 4.6mm x 5µm)	0.8-1.2 µg/ml	[55]
13.	Phosphate buffer: Acetonitrile (30:70 v/v pH:4.0)	1ml/min	2.15 min	229nm	ZORBAX C8 (150mm x 4.6mm x 5µm)	0.6-1.4µg/ml	[56]

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

1. Griffin, C. E., Kaye, A. M., Bueno, F. R., & Kaye, A. D. (2013). Benzodiazepine pharmacology and central nervous system–mediated effects. *Ochsner Journal*, *13*(2), 214-223.
2. Ait-Daoud, N., Hamby, A. S., Sharma, S., & Blevins, D. (2018). A review of alprazolam use,

misuse, and withdrawal. *Journal of addiction medicine*, *12*(1), 4.
3. Dawson, G. W., Jue, S. G., & Brogden R. N. (1984). Alprazolam. *Drugs*, 27(2), 132-147.
4. Fawcett, J. A., & Kravitz, H. M. (1982). Alprazolam: pharmacokinetics, clinical efficacy, and mechanism of action. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *2*(5), 243-253.
5. Verster, J. C., & Volkerts, E. R. (2004). Clinical pharmacology, clinical efficacy, and behavioral toxicity of alprazolam: a review of the literature. *CNS drug reviews*, *10*(1), 45-76.
6. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=018276
7. https://en.wikipedia.org/wiki/Alprazolam
8. https://pubchem.ncbi.nlm.nih.gov/compound/Alprazolam#section=InChI-Key
9. Ntoupa, P. S. A., Papoutsis, I. I., Dona, A. A., Spiliopoulou, C. A., & Athanaselis, S. A. (2021). A fluorine turns a medicinal benzodiazepine into NPS: the case of flualprazolam. *Forensic Toxicology*, *39*(2), 368-376.
10. https://www.chemsrc.com/en/cas/28981-97-7_946819.html
11. https://www.rxlist.com/consumer_alprazolam_xanax/drugs-condition.htm
12. Roul, L. K., Manna, N. K., Parhi, R. N., Sahoo, S., & Suresh, P. (2012). Dissolution rate enhancement of alprazolam by solid dispersion. *Indian J Pharm Educ Res*, *46*(1), 38-44.
13. Sealock, K., Lilley, L. L., Collins, S. R., & Snyder, J. S. (2016). *Pharmacology for Canadian health care practice*. Elsevier Health Sciences.
14. American Society of Health-System Pharmacists (13 November 2017). "Alprazolam Monograph for Professionals". Drugs.com. Retrieved 25 October 2018.
15. Index, M. (1989). An encyclopedia of chemicals, drugs, and biologicals. Merck & Co. Inc., Whitehouse Station, NJ, USA. 1130.
16. Ait-Daoud, N., Hamby, A. S., Sharma, S., & Blevins, D. (2018). A review of alprazolam use, misuse, and withdrawal. *Journal of addiction medicine*, *12*(1), 4.
17. Huybrechts, I. (1991). The pharmacology of alprazolam: a review. *Clinical therapeutics*, *13*(1), 100-117.
18. Masiulis, S., Desai, R., Uchański, T., Serna Martin, I., Laverty, D., Karia, D., ... & Aricescu, A. R. (2019). GABAA receptor signalling mechanisms revealed by structural pharmacology. *Nature*, *565*(7740), 454-459.
19. Ibáñez, J., González de la Aleja, J., Gallego, J. A., Romero, J. P., Saíz-Díaz, R. A., Benito-León, J., & Rocon, E. (2014). Effects of alprazolam on cortical activity and tremors in patients with essential tremor. *PLoS One*, *9*(3), e93159.
20. Greenblatt, D. J., & Wright, C. E. (1993). Clinical pharmacokinetics of alprazolam. *Clinical pharmacokinetics*, *24*(6), 453-471.
21. Barbee, J. G. (1993). Memory, benzodiazepines, and anxiety: integration of theoretical and clinical perspectives. *The Journal of clinical psychiatry*.
22. Dodds, T. J. (2017). Prescribed benzodiazepines and suicide risk: a review of the literature. *The primary care companion for CNS disorders*, *19*(2), 22746.
23. Rawson, N. S., & Rawson, M. J. (1999). Acute adverse event signalling scheme using the Saskatchewan Administrative health care utilization datafiles: results for two benzodiazepines. *The Canadian Journal of Clinical Pharmacology= Journal Canadien de Pharmacologie Clinique*, *6*(3), 159-166.
24. Noyes, R., DuPont, R. L., Pecknold, J. C., Rifkin, A., Rubin, R. T., Swinson, R. P., ... & Burrows, G. D. (1988). Alprazolam in panic disorder and agoraphobia: Results from a multicenter trial: II. Patient acceptance, side effects, and safety. *Archives of general psychiatry*, *45*(5), 423-428.
25. Cassano, G. B., Toni, C., Petracca, A., Deltito, J., Benkert, O., Curtis, G., ... & Klerman, G. (1994). Adverse effects associated with the short-term treatment of panic disorder with imipramine, alprazolam or placebo. *European Neuropsychopharmacology*, *4*(1), 47-53.
26. Michel, L., & Lang, J. P. (2003). Benzodiazepines and forensic aspects. *L'encephale*, *29*(6), 479-485.
27. Arana, G. W., Pearlman, C., & Shader, R. I. (1985). Alprazolam-induced mania: two clinical cases. *The American journal of psychiatry*.
28. Winston, A. (1985). Three case reports of acute paroxysmal excitement associated with alprazolam treatment. *Am J Psychiatry*, *142*, 859-861.
29. Khanna, S., Anand, U., & Banerjee, A. (1996). Alprazolam-induced hypomania. *Australian and New Zealand journal of psychiatry*, *30*(4), 550-552.
30. Rapaport, M., & Braff, D. L. (1985). Alprazolam and hostility. *The American Journal of Psychiatry*, *142*(1), 146-146.
31. Béchir, M., Schwegler, K., Chenevard, R., Binggeli, C., Caduff, C., Büchi, S., ... & Noll, G. (2007). Anxiolytic therapy with alprazolam increases muscle sympathetic activity in patients with panic disorders. *Autonomic Neuroscience*, *134*(1-2), 69-73.
32. Izzo, A. A., & Ernst, E. (2009). Interactions between herbal medicines and prescribed drugs. *Drugs*, *69*(13), 1777-1798.
33. Izzo, A. A. (2004). Drug interactions with St. John's Wort (Hypericum perforatum): a review of the clinical evidence. *International journal of clinical pharmacology and therapeutics*, *42*(3), 139-148.
34. Madabushi, R., Frank, B., Drewelow, B., Derendorf, H., & Butterweck, V. (2006).

Hyperforin in St. John's wort drug interactions. *European journal of clinical pharmacology*, *62*(3), 225-233.
35. Izzo, A. A., & Ernst, E. (2001). Interactions between herbal medicines and prescribed drugs. *Drugs*, *61*(15), 2163-2175.
36. Dresser, G. K., Spence, J. D., & Bailey, D. G. (2000). Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. *Clinical pharmacokinetics*, *38*(1), 41-57.
37. Oo, C. Y., Kuhn, R. J., Desai, N., Wright, C. E., & McNamara, P. J. (1995). Pharmacokinetics in lactating women: prediction of alprazolam transfer into milk. *British journal of clinical pharmacology*, *40*(3), 231-236.
38. Iqbal, M. M., Sobhan, T., & Ryals, T. (2002). Effects of commonly used benzodiazepines on the fetus, the neonate, and the nursing infant. *Psychiatric Services*, *53*(1), 39-49.
39. García-Algar, Ó., López-Vílchez, M. Á., Martín, I., Mur, A., Pellegrini, M., Pacifici, R., ... & Pichini, S. (2007). Confirmation of gestational exposure to alprazolam by analysis of biological matrices in a newborn with neonatal sepsis. *Clinical Toxicology*, *45*(3), 295-298.
40. Einarson, A., Selby, P., & Koren, G. (2001). Abrupt discontinuation of psychotropic drugs during pregnancy: fear of teratogenic risk and impact of counselling. *Journal of Psychiatry and Neuroscience*, *26*(1), 44.
41. Gouveia, B. G., Rijo, P., Gonçalo, T. S., & Reis, C. P. (2015). Good manufacturing practices for medicinal products for human use. *Journal of Pharmacy & Bioallied Sciences*, *7*(2), 87.
42. Food and Drug Administration. Current good manufacturing practices for finished pharmaceuticals (21 CFR, Part 211). Food and Drug Administration, Rockville, MD. (Online.) http://www. accessdata. fda. gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch. cfm. 2008.
43. McDowall, R. D. (2005). Effective and practical risk management options for computerised system validation. *The Quality Assurance Journal: The Quality Assurance Journal for Pharmaceutical, Health and Environmental Professionals*, *9*(3), 196-227.
44. GV, C. (2015). RP-HPLC method development and validation for simultaneous estimation of sertraline HCL and alprazolam in tablet dosage form.
45. Bais, S., Bhavsar, M., Singhvi, I., & Chandewar, A. (2014). Analytical method de velopment and validation for the estimation of alprazolam and sertraline hydrochloride by HPLC. *Pharma Res*, *11*(1).
46. Kawade, D. P., Shah, S. K., & Asnani, A. J. Development Of Rp-Hplc Method For Simultaneous Estimation Of Alprazolam And Fluoxetine Hydrochloride In Pharmaceuticaltablet Dosage Form.
47. Sharma, A., Gaurav, K., & Srivastava, R. (2021). A Comparative Estimation of Alprazolam in Pharmaceutical Formulations by Validated HPLC and HPTLC Techniques. *Analytical Chemistry Letters*, *11*(2), 187-197.
48. Shah, S. K. (2016). Payal P. Chauhan, Divya Y. Patel. *Eurasian Journal of Analytical Chemistry*, *11*(2), 101-113.
49. Shah, S. K., Dangre, S. C., & Charbe, N. B. (2012). Development of RP-HPLC Method for Simultaneous Estimation of Alprazolam and Fluoxetine Hydrochloride in Pharmaceutical Tablet Dosage Form. *Research Journal of Pharmacy and Technology*, *5*(7), 938-941.
50. Shukla, S., Kumar, P., Srivastava, R. S., & Trivedi, P. (2010). RP-HPLC method development and its validation for simultaneous estimation of Alprazolam and Fluoxetine hydrochloride in pharmaceutical dosage form. *Eurasian Journal of Analytical Chemistry*, *5*(3), 239-245.
51. Patel, R. B., Patel, M. R., Shankar, M. B., & Bhatt, K. K. (2009). Simultaneous determination of alprazolam and fluoxetine hydrochloride in tablet formulations by high-performance column liquid chromatography and high-performance thin-layer chromatography. *Journal of AOAC International*, *92*(4), 1082-1088.
52. Rao, H., Ahmad, S., Madni, A., Ahmad, I., & Shahzad, M. N. (2020). Single-step extraction for simultaneous quantification of desvenlafaxine and alprazolam in human spiked plasma by RP-HPLC. *Plos one*, *15*(9), e0238954.
53. Malothu, N., Senagashetty, D. M., & Katamaneni, P. RP-HPLC Method Development and Validation For Simultaneous Estimation of Paracetamol and Alprazolam in Bulk and Pharmaceutical Dosage Forms.
54. Atta-Politou, J., Parissi-Poulou, M., Dona, A., & Koutselinis, A. (1991). A simple and rapid reversed phase high performance liquid chromatographic method for quantification of alprazolam and α-hydroxyalprazolam in plasma. *Journal of liquid chromatography*, *14*(19), 3531-3546.
55. http://globalresearchonline.net/journalcontents/v56-2/07.pdf
56. Putchakavala, P. R. (2014). *International Journal of Research in Pharmaceutical and Nano Sciences*, 3(5), 473-481.