



Extraction Study of Alprazolam from Human Serum

Vamsi Puri*, *Brilliant College of Pharmacy, Abdullapurmet(M), Rangareddy (Dist),
Hyderabad, Telangana,India

ABSTRACT

An open access  journal

Alprazolam may be a benzodiazepine drug containing Triazole rings. it's short half-life and used for the treatment of anxiety and anxiety disorder, depression etc. at the side of medicine, this is often extremely abused drug in the Asian nation also as everywhere the world thanks to its high efficiency. This drug is additionally employed in varied sorts of criminal activities like stealing, robbery, stupefying activities, physical assault and crime against girls. This drug comes below the class of Drug expedited sex crime (DFSA). The analysis work was aimed to extract and determine the benzodiazepine in a blood sample. The benzodiazepine was extracted by deproteination and sonication as per standard operating procedure. The identification of alprazolam was done by color tests and analyzed by GC-MS. Ammonium- n-Vanadate test gave a play of color was discovered when the specific interval of your time within the case of alprazolam drug. Bratton Marshall check showed purple color, that was modified to fade color when jiffy. The GC-MS study showed the retention time of benzodiazepine in the extracted sample was 21.85 min that matches with a retention time of operating normal resolution (21.83 min). the color changes discovered in extracted sample conjointly matches to the changes discovered in normal operating resolution. The GC-MS methodology was found to be easy and appropriate, for extraction and determination, alprazolam from the various suspected biological samples comes in Forensic Science Laboratories. The GC-MS knowledge confirmed that the strategy of extraction used is reliable for benzodiazepine analysis from the blood sample.

Supporting Information:

Received: 25 June 2018
Accepted: 30 June 2018
Published: 04 July 2018

Competing Interests:
The authors have declared
that no competing interests
exist.

Corresponding author address

Vamsi Puri*,
*Brilliant College of Pharmacy,
Abdullapurmet(M),
Rangareddy (Dist),
Hyderabad, Telangana,India

Copyright: © 2018
Www.ijaps.net
Published under a
Creative Commons
Attribution 4.0

Keywords: Alprazolam, Metabolite, Psychotropic, Identification

Introductions

The alprazolam could be a benzodiazepine group of drug. it's one in every of the foremost potent medicine of benzodiazepine group. alprazolam is employed as antidepressant to counter depression, additionally accustomed treat psychological disorder, panic disorder still as in treatment of sleep disorder, nausea because of therapy induced nausea and vomiting. This drug is generally administered by body process in type of pills and as blood vessel and intra muscular injection in liquid dose. whereas the most route of elimination is from urine . [1-2].

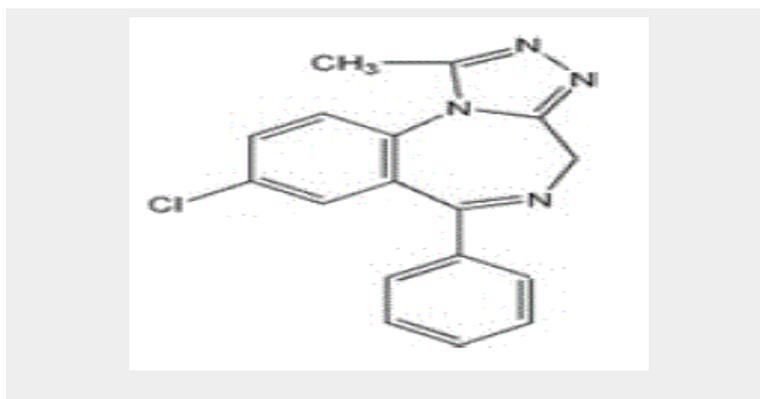
Chemical Properties

Alprazolam that could be a triazolo analog of the 1,4 benzodiazepine category of central nervous system-active compounds. The chemical name of alprazolam is 8-chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3- α] [1,4] benzodiazepine. The formula is $C_{17}H_{13}ClN_4$ which corresponds to a mass of 308.76.

The molecular formula is portrayed below:

$C_{17}H_{13}ClN_4$ which corresponds to a molecular weight of 308.76.

The structural formula is represented below:



Alprazolam is a white crystalline powder, which is soluble in methanol or ethanol but which has no appreciable solubility in water at physiological pH.

These drugs are wide use in criminal activities rather than its therapeutic use. In rave parties and bars, alprazolam is by choice mixed in consumable products like soft-drinks, alcoholic beverages, food stuff and used for crime against ladies, thus this drug is also called assault drug, and alternative criminal activities like theft, robbery, homicide, suicide and stupefying activities. alprazolam is an addictive drug, even at recommended dosages for long term use of this turn out serious health deteriorating result like development of addiction, tolerance and physical dependence. unlike barbiturates, large doses of benzodiazepines are seldom fatal unless combined with alternative medication/alcohol to boost the result of alternative drugs like heroin or cocaine, addicts typically use benzodiazepines and alternative depressants to boost their “high” or alter the facet effects of drug absorb giant dose. long term users of alprazolam shows several withdrawal symptoms like increased anxiety, nausea, aching in limbs, sleeplessness, blurred vision, diarrhoea, muscle cramps, sweating, seizure, agitation, sensitivity to light and sound [6,7]. alprazolam also has some side effects, that terribly among patients looking on quantity of doses and for the period. Common side effects are drowsiness, light-headedness, dizziness, irritability, concentration loss, enlarged secretion, reduce drive, changes in appetite, issue urinating, depression, confusion, issues with speech, mood swings, dangerous thoughts, loss with coordination or balance

Material and Methods:

Chemicals: Ammonia, Ammonium-n-Vanadate Benzene, Chloroform (AR & HPLC grade), Ethyl acetate, Methanol, Hexane, Bismuth Subnitrate, α -Naphthyl ethylene-diamine, Acetone, Isopropanol, Formic acid, HPLC grade chloroform, Iodine, Hydrochloric Acid, Sodium Nitrite, Potassium Iodide, Sulphuric Acid and Sulphanilic Acid were procured from Merck India. Standard: Standard Alprazolam of percentage purity 99.7 % w/w was procured from Indian Pharmacopoeia Commission, Ghaziabad, U.P., India.

Preparation of Sample: About 15 ml of human blood was taken into tarson tube, and five mg of crushed finely divide powder of alprazolam pill was another to that. it absolutely was agitated for

15 min, so tarson tube was placed within the incubator for 72 h at 37°C. After 72 h, tarson tube was taken out from the brooder and it absolutely was unbroken at temperature for cooling. The blood within the tarson tube fashioned a lump

Sl No	Exhibits	Colour(AVT)	Colour (BMT)	Result
1	Blank	Yellow	No color	Negative
2	Alprazolam Standard	Parrot green	Purple color	Positive
3	Extracted sample	Parrot green	Purple color	Positive

AVT – Ammonium Vanadate test and BMT - Bratton- Marshall test.

Preparation of ordinary stock solution: regarding five mg of ordinary benzodiazepine drug was weighed on the balance and poured into a capped tubing containing 5 ml of HPLC Grade chloroform. Then cap of test tube was closed and shaken well for correct dissolution. This 5 ml resolution of ordinary alprazolam (5 mg) had strength a thousand ppm.



Fig 2. Color observed (a) Ammonium- n-Vanadate Test, (b) Bratton-Marshell Test.

Preparation working standard solution:

The 5 ml (Strength 1000 ppm) of standard stock solution was diluted to 50 ml, to prepared working standard solution of strength 100 ppm solution.

Preparation working standard solution:

The 5 ml (Strength 1000 ppm) of standard stock solution was diluted to 50 ml, to prepared working standard solution of strength 100 ppm solution.

Table 2. Observed Peaks of GC-MS for sample and standard of Aprazolam.

Exhibit	CD	Spectrogram Data		
No/type	RT (min)	SPA (Library)	CPE (Library)	QSA (%)
1/SA	21.83	308.0, 279.0, 245.0, 224.0, 204.0, 177.0, 155.0, 137.0, 102.0, 77.0, 51.0	308.1, 279.1, 245.1, 204.1, 176.9, 137.0, 101.9, 77.0	99
2/ES	21.85	308.0, 279.0, 245.0, 224.0, 204.0, 177.0, 155.0,137.0,102.0, 77.0, 51.0	308.0, 279.1, 204.1, 177.0, 154.1, 136.9, 102.0, 77.0, 50.9	99

SA – Standard alprazolam, ES – Extracted sample, RT – Retention time, CD – Chromatographic data, SPA – Standard Peaks of Alprazolam, CPE - Comparable Peaks on Exhibits and QSA - Quantity Matches with standard Alprazolam.

Solid-phase Extraction of the drug from blood sample: the entire blood was transferred to grinding equipment and ground terribly finely. The blood was deprotonated by keeping it underneath sonication at 60o for thirty-five min. the method was recurrent three times by taking a break of 5 min between each cycle for cooling the sample. The phosphate buffer of pH scale half dozen was extra to the entire blood and Vortex for 30 s. the entire blood was transferred to the capped tube for centrifuge at 3000 revs for 5min. The supernatant layer was collected from the tube and rest was discarded. The SPE Column (C18) was washed with II Samuel acid (in water), followed by laundry with the 50 yule wood spirit (in water). after the laundry, the

supernatant layer was passed from SPE column. The drug was absorbed on the SPE bead. The absorbed drug from SPE column was eluted by passing 5 a solution of Ammonia in wood spirit. The wash was collected in a beaker. The wash was further gaseous within the china dish and reconstituted in HPLC grade chloroform [10,11]. Identification of Alprazolam: Screening/ color test: Ammonium- n-Vanadate Test: regarding two drops of extracted sample, operating customary answer furthermore as blank (Water) were taken on spot tile and dried at temperature. when complete drying, one to two drops of plant product was extra followed by one cubic centimeter of chemical agent one (100 mg of Ammonium- n-Vanadate dissolved in ten cubic centimeters of conc. H₂SO₄) [10,11]. Bratton Marshall Test: regarding two drops of extracted sample, operating customary answer furthermore as blank (Water) were taken on spot tile and dried at temperature. Then three drops of chemical agent two (10 yuletide H₂SO₄), was added. regarding three drops of chemical agent three (0.1 % NaNO₃), three drops of chemical agent four (0.5 yule Sulphanilic acid) and one drop of chemical agent five (0.1% α -Naphthyl ethylenediamine) were extra and mixed [10,11]. Gas Chromatography-Mass spectroscopy: The extracted sample and dealing customary answer was filtered through twenty-two μ m syringe filter and stuffed into five cubic centimeter vials. The vials were placed in automobile sample receptacle. The machine sampler was taken out the sample with the assistance of syringe and directly injected into the injection port. operative Condition of gigacycle per second-MS (Agilent 6890 N Network GC system & 5975 inserts XL Mass Selective MSD detector of column SLB-5MS of dimension thirty m \times zero.25 mm) and injection temperature of 280 °C, mode of Split mode (1/20), oven and detector temperature was 300 and 150 °C, carrier gas was He with flow of one ml/min, the runtime was twenty-five min [10,11].

Results and Discussions:

Ammonium- n-Vanadate test gave a play of color was observed when a specific interval of your time that are green within the case of alprazolam drug. Bratton Marshall check showed purple color, that was modified to fade color when few minutes as color detail given in Table one. within the projected methodology, the Table two unconcealed that the retention time of alprazolam in the extracted sample was 21.85 min that matches with the retention time of

operating customary answer (21.83 min). the color changes ascertained in extracted sample additionally matches to the changes ascertained in a customary operating answer. each the on top of data confirms that the strategy of extraction used is reliable for alprazolam analysis from a blood sample.

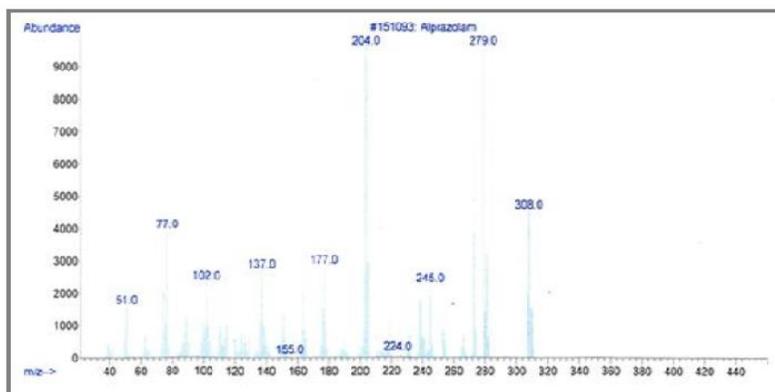


Fig 3. GC- MS chromatogram of Alprazolam (as per library).

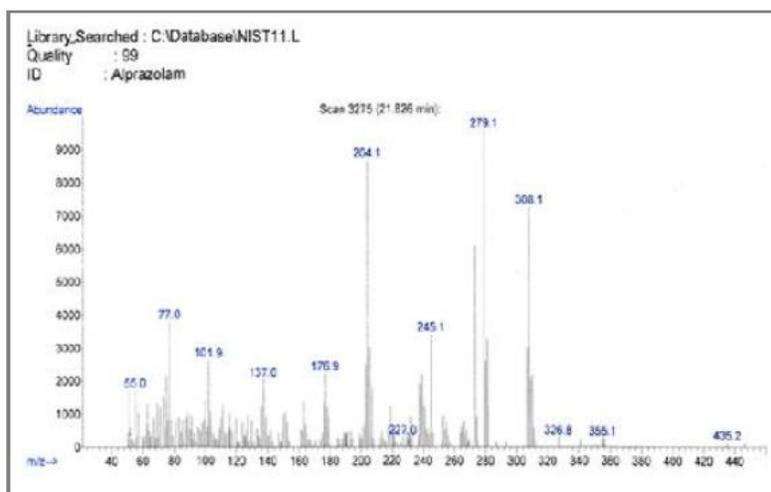


Fig 4. GC- MS chromatogram of working standard solution.

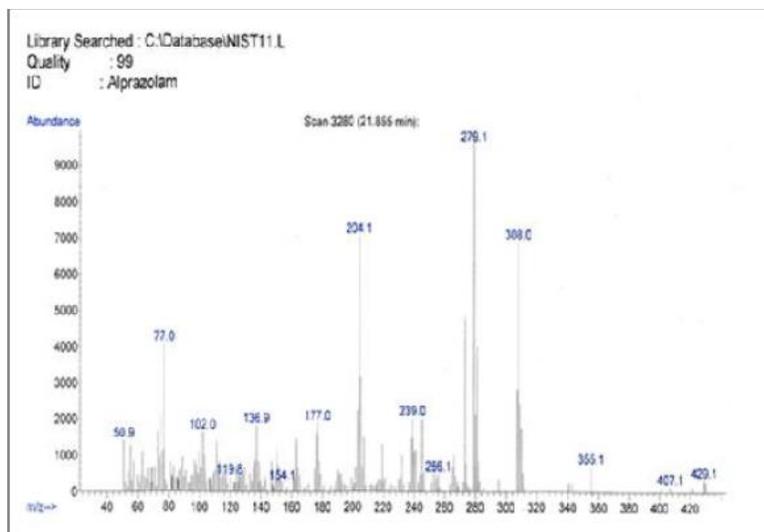


Fig 5. GC- MS chromatogram of extracted sample.

Conclusion:

In the present work, a simple and sensitive GC-MS methodology has been developed for the determination of the alprazolam within the human blood. The extraction method during this paper was very easy and no important matrix interference had seen by the endogenous compounds. This method is extremely simple and appropriate, for extraction and determination, alprazolam from the different suspected biological samples comes in Forensic Science Laboratories. The alprazolam is most often encountered drug among sizable amount alternative downer category medication. This methodology is sensitive, specific and reliable, any additional development and validation of this method allow the analysis of the broad range of benzodiazepine drugs like chlordiazepoxide, Quazepam, Estazolam, and triazolam while not purchasing new equipment and material.

References

1. Charles LW, Wagdy WW, Charles LWJ. Drug and Chemical Blood-Level Data 2001. Forensic Sci Int, 2001; 122; 107-123.
2. Kakkar A, Kumar S. Alprazolam Poisoning. J Indian Acad Forensic Med, 2014; 36(4): 432-433.
3. Frank M, Ulrike MS, Burkhard M. Molecular Pathology in Forensic Medicine – Introduction. Forensic Sci Int, 2010; 203(1-3): 3-14.

4. Substance use Disorders: Manual for paramedical staff. New Delhi: National Drug Dependence treatment Centre, AIIMS; 2010. pp. 13-15.
5. Moffat AC, Davivd O. Clarke's Analysis of Drugs and Poisons in pharmaceuticals, body fluids and post- mortem material. 14th ed. London: London Pharmaceutical; 2011.
6. Detection of Drugs of Abuse in Body Fluids: A manual for laboratory Personnel. New Delhi: Drug Dependence Treatment Centre, AIIMS; 2010. pp. 28- 82.
7. Heather CA. History of Benzodiazepines: What the Textbooks May Not Tell You. Presentation at the 3rd Annual Benzodiazepine Conference. Bangor, Maine, 2005.
8. Dariusz B, Marta BS, Ewa T, Bogdan S. Application of gas chromatography/ mass spectrometry (GC/MS) to the analysis of benzodiazepines. Problems Forensic Sci, 2004; 49: 5–37.
9. Huidobro AL, Ruperez FJ, Barbas C. Pharmaceutical applications Isolation, identification and determination of the major degradation product in alprazolam tablets during their stability assay. J Pharm Biomed Anal, 2007; 44: 404–413.
10. Winek CL. Role for Hospital Pharmacist in Toxicology and blood level information. Clin Toxicol, 1970; 3(4): 543-551. 11. Winek CL. Tabulation of therapeutic, toxic and lethal concentration of drugs and chemicals in blood. Clin Chem, 1976; 22(6): 832-836.