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Extraction and identification of Alprazolam from human blood

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ABSTRACT: Background: Alprazolam is a benzodiazepine drug containing Triazole rings. It has short half-life and used for the treatment of anxiety and panic disorder, depression etc. Along with prescription drug, this is highly abused drug in India as well as all over the globe due to its high potency. This drug is also used in various types of criminal activities like theft, robbery, stupefying activities, physical assault and crime against women. This drug comes under the category of Drug Facilitated Sexual Assault (DFSA). **Aim:** The research work was aimed to extract and identify the Alprazolam in blood sample. **Method:** The alprazolam was extracted by deproteinationa and sonication as per standard procedure. The identification of alprazolam was done by color tests and analysed by GC-MS. **Results:** Ammonium- n-Vanadate test gave a play of color was observed after specific interval of time in the case of Alprazolam drug. Bratton Marshell test showed purple colour, which was changed to fade colour after few minutes. The GC-MS study showed the retention time of Alprazolam in extracted sample was 21.85 min which matches with retention time of working standard solution (21.83 min). The colour changes observed in extracted sample also matches to the changes observed in standard working solution. **Conclusion:** The GC-MS method was found to be simple and suitable, for extraction and determination Alprazolam from the different suspected biological samples comes in Forensic Science Laboratories. The GC-MS data confirmed that the method of extraction used is reliable for Alprazolam analysis from blood sample.

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INTRODUCTIONS:

The Alprazolam is a benzodiazepine group of drug. It is one of the most potent drugs of benzodiazepine group. Alprazolam is used as antidepressant to counter depression, also used to treat anxiety disorder, panic disorder as well as in treatment of Insomnia, nausea due to chemotherapy induced nausea and vomiting. This drug is normally administered by ingestion in form of pills and as intravenous and intra muscular injection in

liquid dose. While the main route of elimination is from urine [1-2].

Common Name : Alprazolam, IUPAC Name: 8-chloro-1-methyl-6-phenyl-4H-triazolo[4,3-α]1,4-benzodiaze pine, Trade name: Alprax, Novo-Alprazol, Alpram, Xanax XR, Alpronax, Bestrol, Street name: Bars, Benzo, Dogbones, Forgetful Pills, Gold Bars, Xannies, Molecular formula:$C_{17}H_{13}ClN_4$, Molecular mass : 308.8 g/Mole, Boiling point: 508.9 °C, Melting point: 228.8 °C, Half Life: 6.3 to 26.9 h, Physical form: White crystalline powder, Polymorphism: Present in different forms, Therapeutic dose: 0.25 to 3 mg and LD_{50} (mouse; orally): 1020 mg/kg.

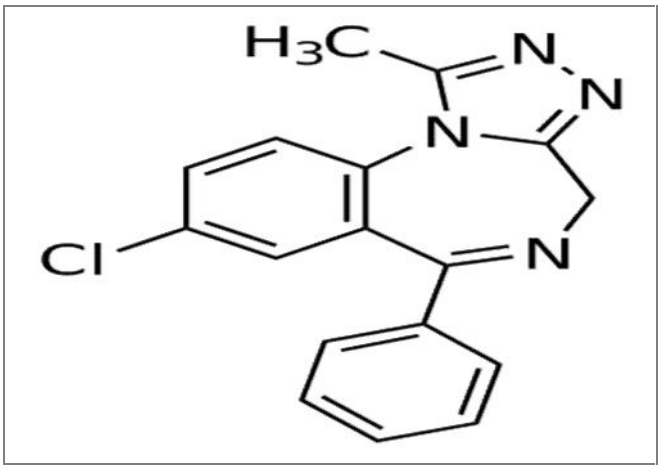


Fig 1. Chemical Structure of Alprazolam.

These drugs are widely use in criminal activities rather than its therapeutic use. In rave parties and bars, Alprazolam is intentionally mixed in consumable products like soft-drinks, alcoholic beverages, food stuff and used for crime against women, so this drug is also called Date rape drug, and other criminal activities like theft, robbery, homicide, suicide and stupefying activities. The only cause in increasing misuse of pharmaceutical preparation like Alprazolam is the easy availability of these preparations. Alprazolam is a highly potent psychoactive drug mentioned in Serial no. 30 of clause (xxiii) under Section 2 of NDPS Act.1985 as a "Controlled Substance or Psychotropic Substance: drug which mentioned in schedule - 'H' at serial no. 15 of Drug[3-5].

Alprazolam is an addictive drug, even at recommended dosages for long term use of this produce serious health deteriorating effect like development of addiction, tolerance and physical dependence. Unlike barbiturates, large doses of benzodiazepines are rarely fatal unless combined with other drugs/alcohol to enhance the effect of other drugs like heroin or cocaine, addicts generally use benzodiazepines and other depressants to enhance their "high" or alter the side effects of drug take in large dose. Long term users of alprazolam shows many 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, which very among patients depending on amount of doses and for the time period. Common Side effects are Drowsiness, light-headedness, dizziness, irritability, concentration loss, increased salivation, reduce sex drive, changes in appetite, difficulty urinating, depression, confusion, problems with speech, mood swings, suicidal thoughts, loss with coordination or balance [8].

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 5 mg of crushed finely divide powder of Alprazolam tablet was added to it. It was shaken for 15 min, and then tarson tube was placed in the incubator for 72 h at 37ºC. After 72 h, tarson tube was taken out from the incubator and it was kept at room temperature for cooling. The blood in the tarson tube formed a lump.

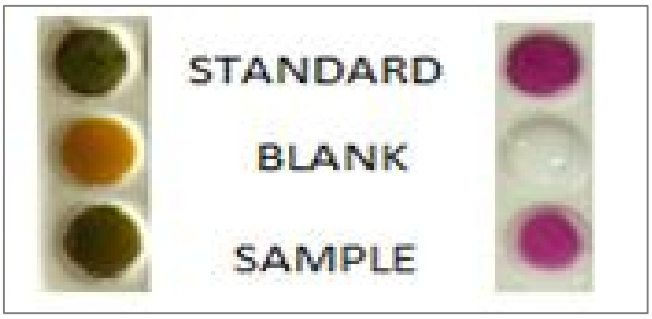
Table 1. Various observations of color tests.

Sl. No	Exhibits	Color (AVT)	Color (BMT)	Result
1	Blank	Yellow	No color	Negative
2	Alpra-zolam Standard	Parrot green	Purple color	Positive
3	Extracted sample	Parrot green	Purple color	Positive

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

Preparation of Standard stock solution:

About 5 mg of Standard Alprazolam drug was weighed on the weighing balance and poured into a capped test tube containing 5 ml of HPLC Grade chloroform. Then cap of test tube was closed and shaked well for proper dissolution. This 5 ml solution of Standard Alprazolam (5 mg) had strength 1000 ppm.



(a) (b)

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.

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 drug from blood sample:

The whole blood was transferred to grinding apparatus and grinded very finely. The blood was deprotonated by keeping it under sonication at 60^{o} for 35 min. The process was repeated 3 times by taking break of 5 min between each cycle for cooling the sample. The phosphate buffer of pH 6 was added to the whole blood and Vortex for 30 s. The whole blood was transferred to the capped test tube for centrifuge at 3000 rpm for 5 min. The supernatant layer was collected from the test tube and rest was discarded. The SPE Column (C18) was washed with 2 % formic acid (in water), followed by washing with the 50 % Methanol (in water). After the washing, 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 % solution of Ammonia in Methanol. The elute was collected in beaker. The elute was further evaporated in the china dish and reconstituted in HPLC grade chloroform [10,11].

Identification of Aprazolam:

Screening/ Colour test:

Ammonium- n-Vanadate Test: About 2 drops of extracted sample, working standard solution as well as blank (Water) were taken on spot tile and dried at room temperature. After complete drying, 1 to 2 drops of ethanol was added followed by 1 ml of reagent 1 (100 mg of Ammonium- n-Vanadate dissolved in 10 ml of conc. H_2SO_4) [10,11].

Bratton Marshell Test: About 2 drops of extracted sample, working standard solution as well as blank (Water) were taken on spot tile and dried at room temperature. Then 3 drops of reagent 2 (10 % H_2SO_4), was added. About 3 drops of reagent 3 (0.1 % $NaNO_3$), 3 drops of reagent 4 (0.5 % Sulphanilic acid) and 1 drop of reagent 5 (0.1% α-Naphthyl ethylene diamine) were added and mixed [10,11].

Gas Chromatography-Mass spectroscopy:

The extracted sample and working standard solution was filtered through 22 µm syringe filter and filled into a 5 ml vials. The vials were placed in auto sample tray. The auto sampler was taken out the sample with the help of syringe, and directly injected into the injection port. Operating Condition of GC-MS (Agilent 6890 N Network GC system & 5975 insert XL Mass Selective MSD detector of column SLB-5MS of dimension 30 m × 0.25 mm) are 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 rate of 1 ml/min, the run time was 25 min [10,11].

RESULTS AND DISCUSSIONS:

Ammonium- n-Vanadate test gave a play of color was observed after specific interval of time that are orange → Brown → chocolate brown → grayish-green → olive green → parrot green in the case of Alprazolam drug. Bratton Marshell test showed purple colour, which was changed to fade colour after few minutes as colour detail

given in Table 1. In the proposed method, the Table 2 revealed that the retention time of Alprazolam in extracted sample was 21.85 min which matches with retention time of working standard solution (21.83 min). The colour changes observed in extracted sample also matches to the changes observed in standard working solution. Both the above data confirms that the method of extraction used is reliable for Alprazolam analysis from blood sample.

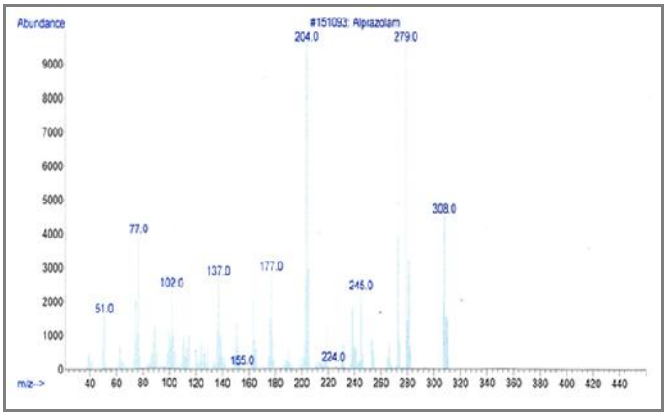


Fig 3. GC- MS chromatogram of Alprazolam (as per library).

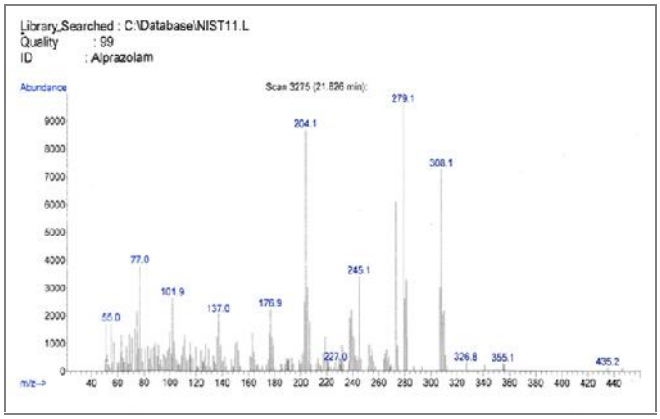


Fig 4. GC- MS chromatogram of working standard solution.

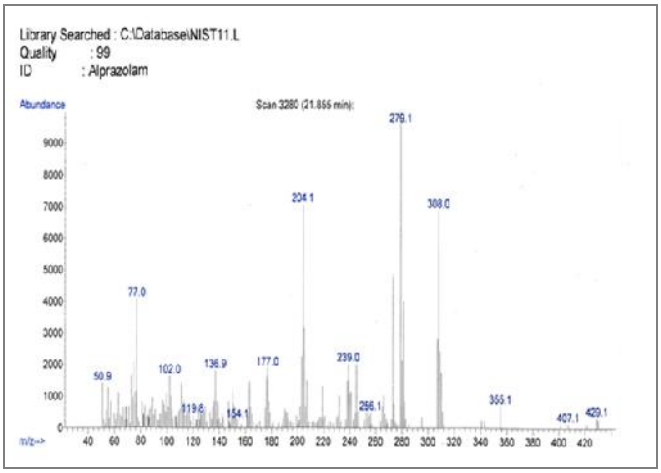


Fig 5. GC- MS chromatogram of extracted sample.

CONCLUSION:

In the present work, a simple and sensitive GC-MS method has been developed for the determination of the Alprazolam in the human blood. The extraction process in this paper was very simple and no significant matrix interference had seen by the endogenous compounds. This method is very simple and suitable, for extraction and determination Alprazolam from the different suspected biological samples comes in Forensic Science Laboratories. The Alprazolam is most frequently encountered drug among large number other benzodiazepine class drugs. This method is sensitive, specific and reliable, further more development and validation of this method allows the analysis of the broad range of benzodiazepine drugs like Chlordiazepoxide, Quazepam, Estazolam, and Triazolam without purchasing new equipment and material.

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