

New Color Test and Solvent Systems for the Identification of Alprazolam Drug in Food and Biological Matrices by Thin Layer Chromatography

Mohammad Imran*, P. Sharma**, Risha Jasmine Nathan Singh*

Authors affiliation: *M.Sc.Forensic Science, ** Senior Scientific Assistant/ Faculty Chemistry, LNJN National Institute of Criminology & Forensic Science, Ministry of Home Affairs, Govt. of India, Rohini, Delhi 110085.

Reprints requests: Mohammad Imran, M.Sc. Forensic Science, LNJN National Institute of Criminology & Forensic Science, Ministry of Home Affairs, Govt. of India, Rohini, Delhi 110085.

E-mail: forensic.imran@gmail.com

Abstract

Alprazolam is as a high-potency benzodiazepine drug that is often encountered in medico-legal cases of drug facilitated sexual assaults, robberies and stupefying activities. Its detection by traditional color tests and thin layer chromatography is routinely done in forensic science laboratories using food and biological samples. In this study we have modified a color test and have tested various new solvent systems for TLC method of analysis of Alprazolam and have found that these can be successfully employed in routine analyses in FSLs so as to produce more effective results.

Keywords: Alprazolam; Drug Facilitated Sexual Assault (DFSA); Color test; Solvent System; TLC; UV.

Introduction

Alprazolam is a benzodiazepine group drug which acts as sedative/ depressant and often misused in criminal activities like date-rapes, robberies, assaults and other crimes by spiking in soft-drinks, alcoholic beverages, food-stuffs etc [1][2]. It is one of the most potent drugs of its group and shows synergetic effects when used with other drugs or alcohol [3][4]. The symptoms include severe sedation, behavioural changes and intoxication and higher doses may prove fatal [4][5]. It is also covered in NDPS Act 1985 as a Psychotropic Substance [6].

Literature abounds with information about various methods of detection of Alprazolam in biological and non-biological matrices [7]. Color tests and Thin Layer Chromatography (TLC) are the oldest methods and have been improvised over decades. In the present study, we have validated a color test that has been used for acidic drugs belonging to benzodiazepine class and successfully performed

it on Alprazolam which is basic in nature [7] after slight modification. Additionally, we have developed new solvent systems for TLC method which, to the best of our knowledge, has not been mentioned in literature.

Materials Used

Preparative TLC Plates were purchased from Merck. Ammonium-n-vandate was purchased from Thermo. Acetic Acid; Acetone; Ammonia; Ammonium Sulphate; Ammonium Chloride; Benzene; Bismuth Subnitrate; Chloroform; Carbon tetrachloride; Diethyl-ether; Absolute Ethanol (Merck); Ethyl acetate; Isopropanol; Phosphoric Acid; Potassium Iodide; Methanol; Hexane; Hydrochloric Acid; Nitric Acid; Sodium Sulphate anhydrous; Sodium Tungstate; Sulphuric Acid; Sodium Nitrite were purchased from Merck.

All the chemicals were of HPLC grade.

Methodology

Standard Preparation

Alprazolam (Alprax) tablets (0.5mg) were purchased from Torrent Pharmaceutical Ltd. and dissolved in chloroform to prepare 0.4 mg/ml solution.

Sampling

Five categories of samples were collected: A-Cream Biscuit (Sunfeast Bounce Choco Twist Cream Biscuits); B-Cold-drink (Thums up 600ml pack); C-Alcoholic Beverage (Magic Moment VODKA with apple green flavor); D-Animal Viscera (Goat Liver); E- Post-mortem human blood. All the samples were tested negative for the presence of Alprazolam by TLC before sample preparation.

Sample Preparation

Powdered sample A (~17.0788 g); samples B & C (~50 ml); homogenised sample D (~100g) and sample E (~20 ml) were taken separately and powdered Alprax tablets (~2mg) were added to each of the above samples and mixed thoroughly. They were kept at room temperature (27°C) for not more than 6 hours before extraction (mean plasma elimination half-life range of alprazolam = 6.3-26.9 hours) [8].

Extraction

Food samples A, B & C were extracted using Acid-Base Extraction technique [7] as follows: The samples

were made acidic (pH=3) and extracted with diethyl ether thrice and then passed through anhydrous sodium sulphate. This organic layer was separated and evaporated to dryness and kept aside as acidic fraction for further analysis. The aqueous layer was made basic (pH=8) and extracted with chloroform: diethyl ether (3:1) and passed through anhydrous sodium sulphate. This organic layer was separated and evaporated to dryness and kept aside as basic fraction for further analysis. Sample D was first digested using ammonium sulphate method and further extracted using the same Acid-Base Extraction technique as mentioned above. The acidic and basic fractions were separately collected for analysis. Sample E was processed by "Sodium-Tungstate Digestion Method" and extracted by the same technique as done for other samples. The acidic and basic fractions were separately collected for analysis.

Color Test

Mandelin Test [9] was modified in our study by using 20% ammonium-n-vandate in concentrated sulphuric acid instead of dilute acid as mentioned in the literature.

Thin Layer chromatography: Acidic & basic fractions of all the samples were reconstituted individually by chloroform and TLC was performed. Different solvent systems were tested and the results compared (Table 1). The spots were visualized under UV light (254nm) and then after spraying with Dragendorff's reagent [10].

Table 1: Solvent Systems Tested for Alprazolam

S.No	Solvent System	Ratio
I	Ethyl acetate	100 %
II	CHCl ₃ : Acetone	90 : 10 80 : 20 70 : 30 60 : 40
III	CHCl ₃ : MeOH	97 : 03 90:10** 60 : 40
IV	Ethyl acetate : MeOH : NH ₃	85:10:5 (No spot) 85:10:25 50:40:10 40:45:15 45:45:10*
V	CHCl ₃ : Acetone: Ethyl acetate : MeOH	50:50:50:5 50:50:50:10
VI	Benzene : Acetone : NH ₃	50:50:2 50 : 50 :10*
VII	Hexane : CHCl ₃ : MeOH	50:50:10 50:40:10 10:40:50*
VIII	C CCl ₄ : MeOH	70 : 30 75 : 25 60:40*
IX	Acetone : CHCl ₃ : isopropanol	90:05:05 80 :10 :10 75:20:05

*Best Solvent Systems found in our study

**Good Solvent system mentioned in literature [7]

UV-Visible Spectrophotometry [11]

TLC spots for Alprazolam standard and samples were eluted separately on different TLC plates and visualized under UV light (254nm). The spots were scraped and dissolved in 0.1N HCl and analysed [9] by double beam UV-Visible Spectrophotometer (ECL UV5704SS).

Result and Discussion

Mandelin Test has been mentioned in the literature for acidic drugs but it was modified in our study to be applicable for Alprazolam (a basic benzodiazepine drug) to give satisfactory results. Positive results were obtained as detected by a play of colours (orange-> brown-> chocolate-brown-> greyish- green -> olive- green->parrot green) with an interval of 1-2 minutes between two consecutive colors for Alprazolam in Standard and prepared samples when compared with blank solutions.

TLC is one of the most popular techniques used in forensic science laboratories for screening of drugs in biological & non-biological samples. In our study, we have discovered the best solvent systems that can be used for detection of Alprazolam drug, which to the best of our knowledge have not been mentioned in the literature; (Ethyl Acetate: Methanol: Ammonia::45:45:10; Rf=0.90); (Benzene: Acetone: Ammonia:: 50:50:10; Rf=0.46); (Hexane: chloroform : methanol:: 10:40:50; Rf=0.85); (Carbon tetrachloride: methanol::60:40; Rf=0.79). The color of the spots as observed under UV light (254nm) was dark bluish black with no fluorescence and after spraying with Dragendorff's reagent was orange, as expected from this drug of benzodiazepine class.

The UV-Visible Spectrograph pattern and I_{max} of food and beverage samples A,B&C (213.5nm, 215.0nm & 216.5nm respectively) was found to show hypsochromic shift from that of the standard drug (218.0 nm) upon analysis. The spectra from viscera and blood samples D & E (219.0nm & 219.0nm) respectively were found to be comparable with that of the standard drug (218.0 nm) with an error ± 1 .

Fig. 1: Standard Alprazolam

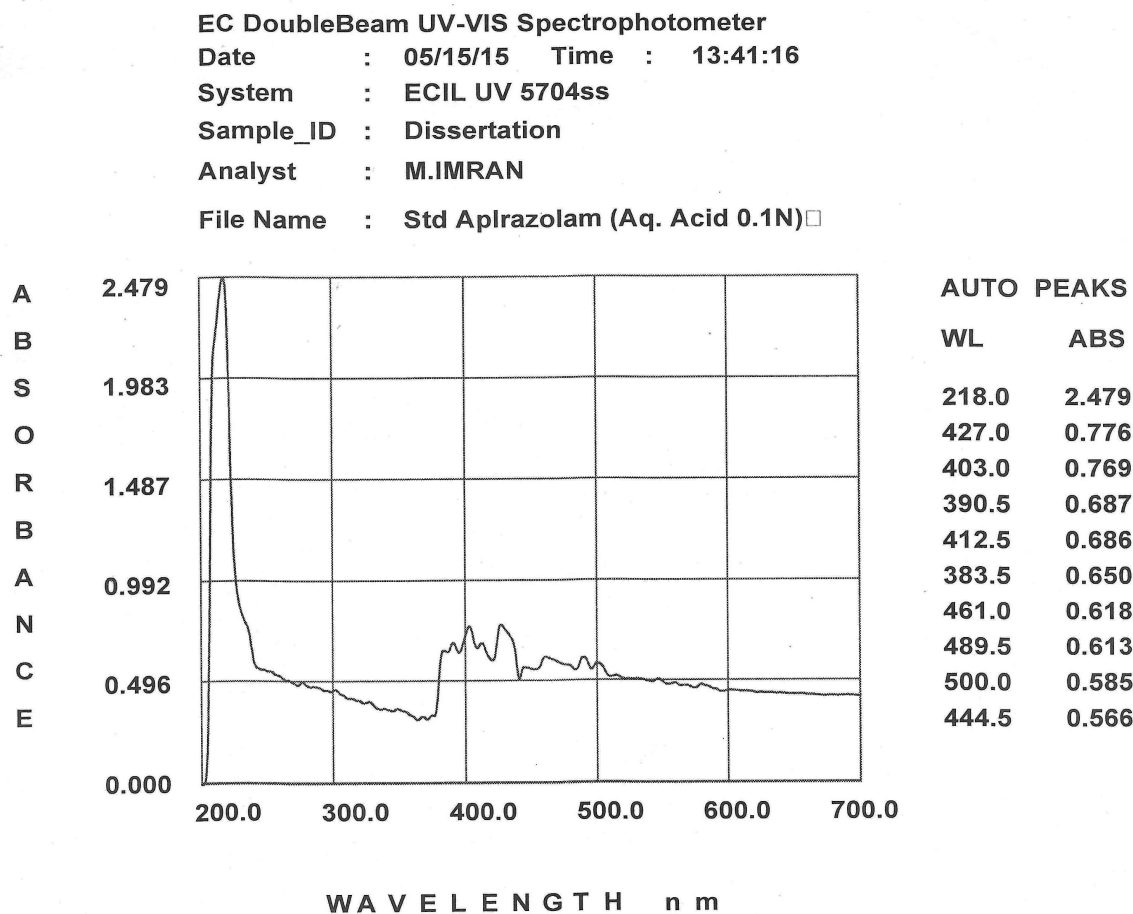


Fig. 2: Sample A

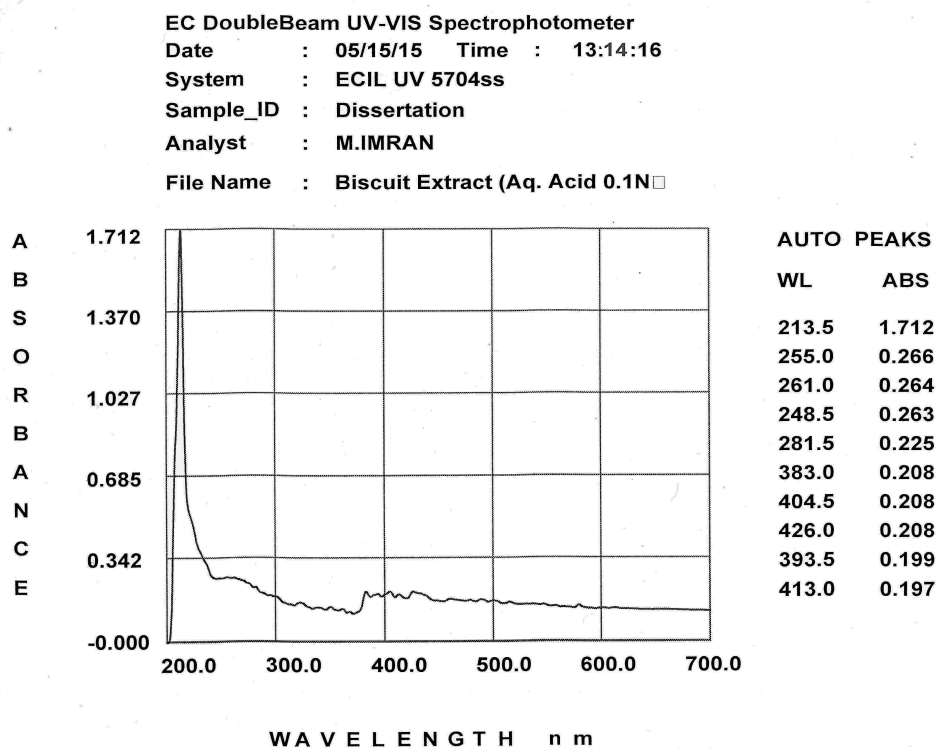


Fig. 3: Sample B

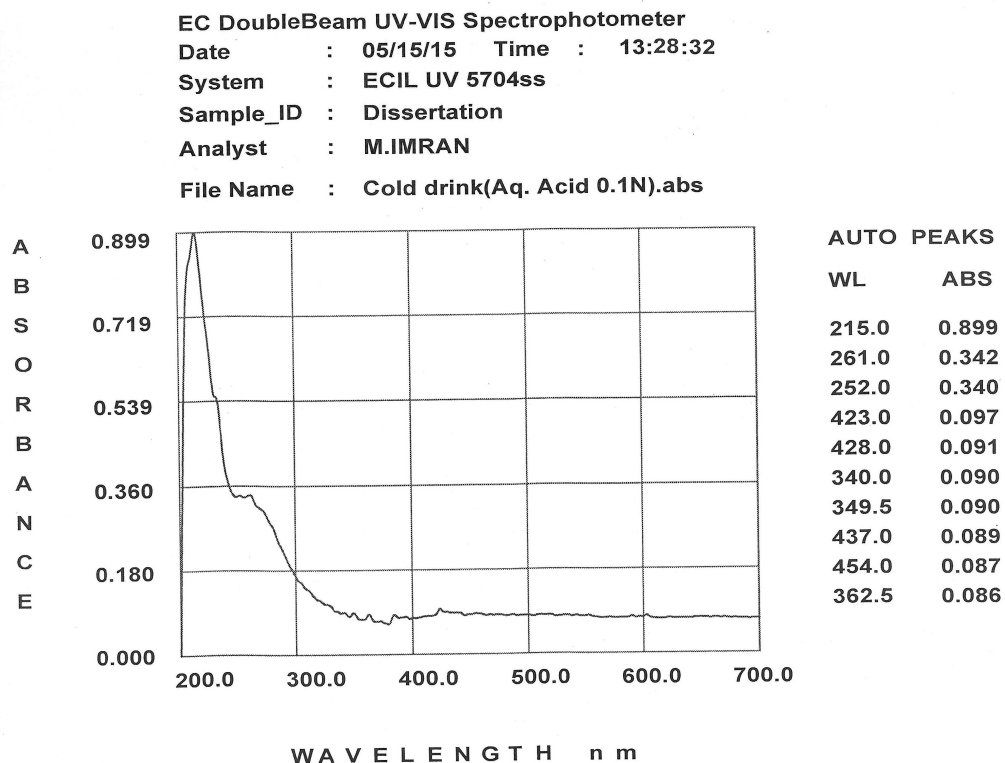


Fig. 4: Sample C

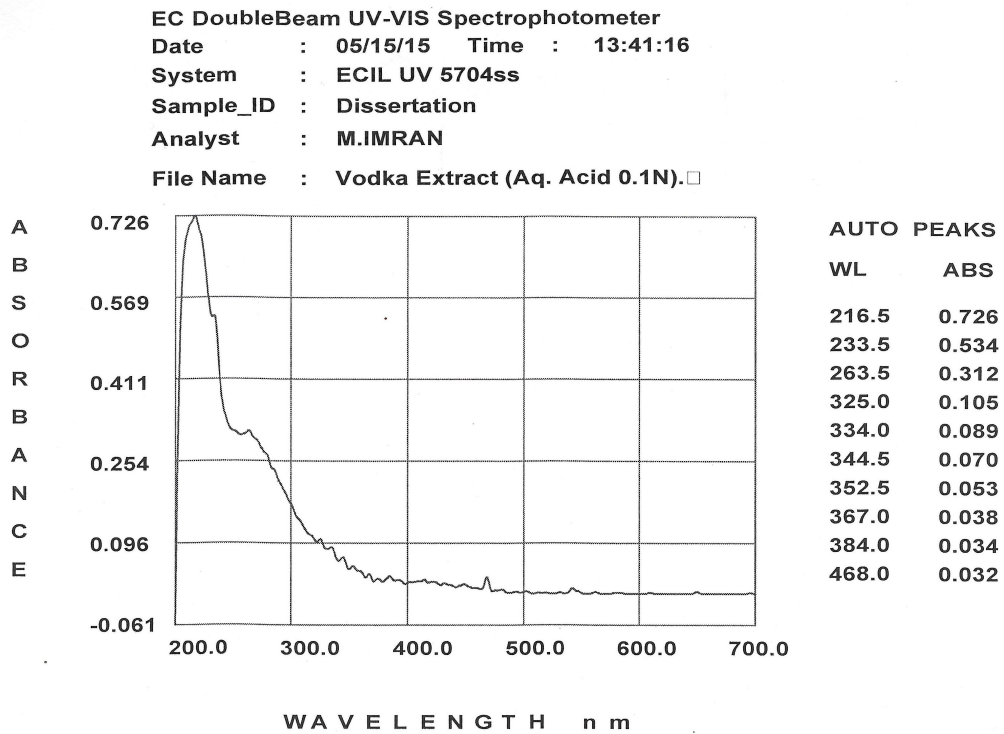


Fig. 5: Sample D

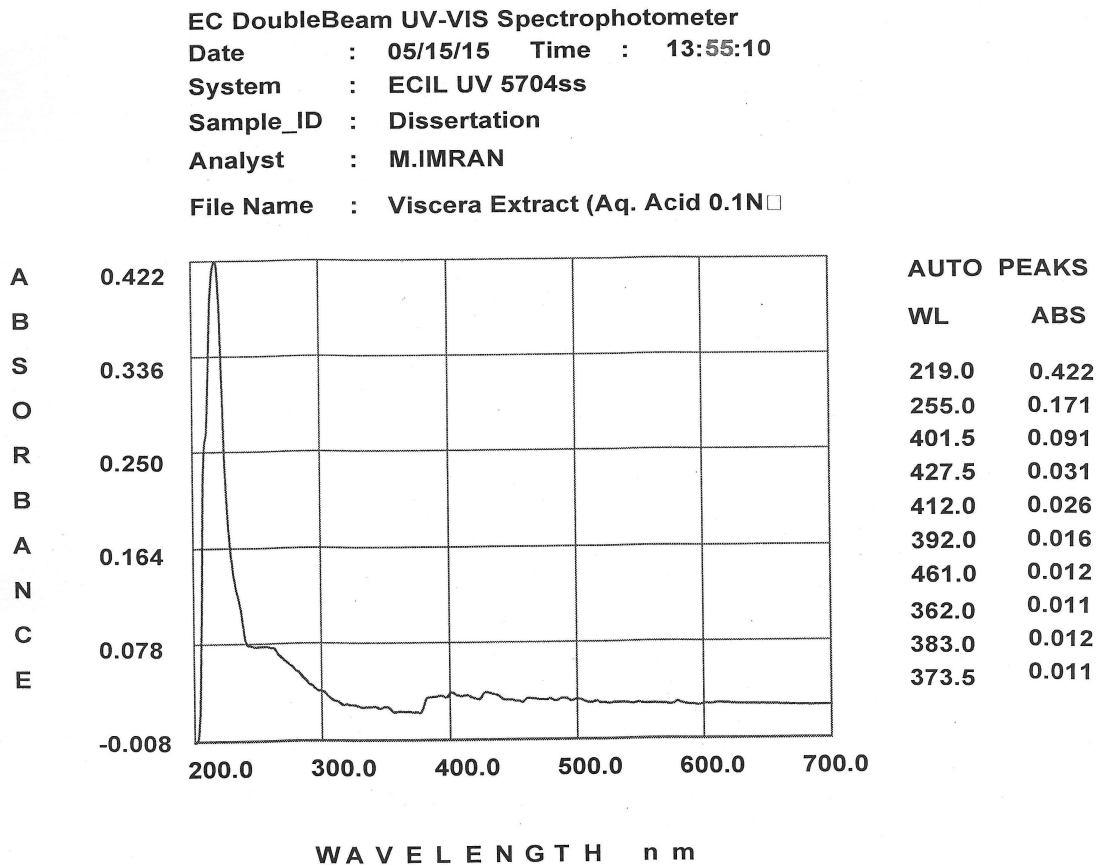
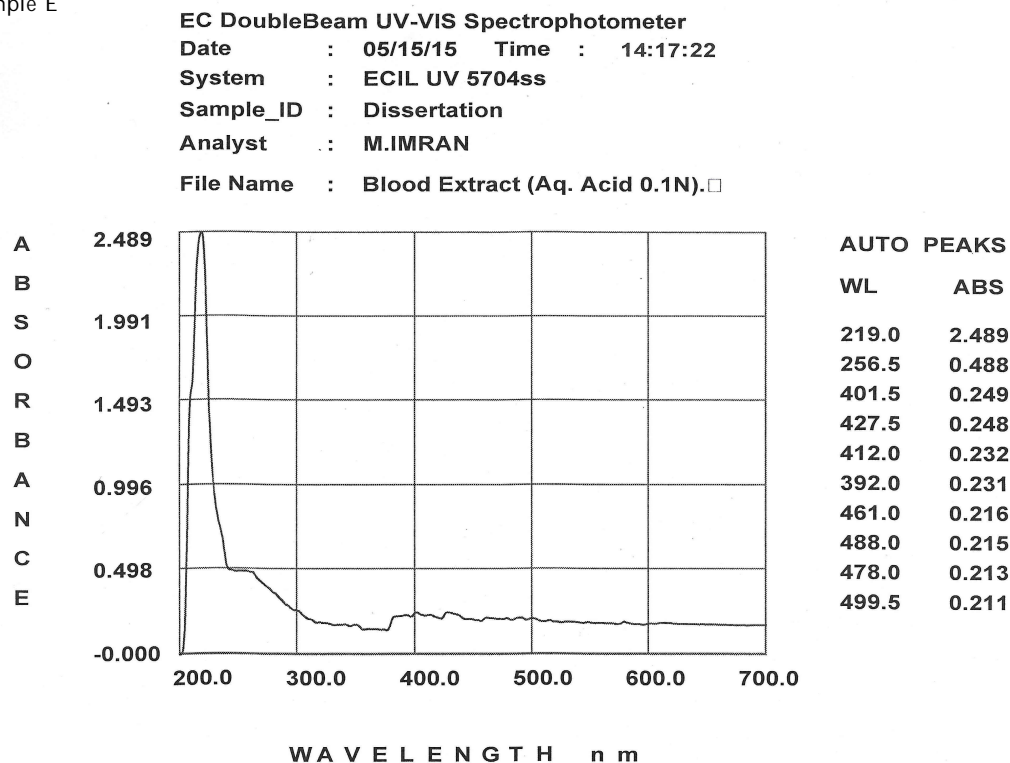


Fig. 6: Sample E



Conclusion

The Mandelin color with a slight modification as found in our study test can be successfully employed for the testing of Alprazolam in routine forensic analyses. Our study also suggests that TLC method can be improvised by changing the solvent systems for detection and screening of this drug in forensic science laboratories. This drug can be tested in both food and biological samples.

Acknowledgement

The authors are thankful to The Director Mrs. Rina Mitra (IPS) and Dy. Inspector General of Police Mr. Sandeep Mittal (IPS), LNJN National Institute of Criminology & Forensic Science, Ministry of Home Affairs, Govt. of India, Delhi for encouragement and laboratory support.

References

- Ender Senol, Ahsen Kaya, Aytac Kocak, Ekin O. Aktas. Watch Out for Nuts in Your Travels: An Unusual Case of Drug-Facilitated Robbery. *J of Travel Med*, 16(6): 431-432, 2009.
- Benny Monheit. Focus Street Drug Prescription Drug Misuse. Reprinted from *Australian Family Physician*, 39(8): 540-546, 2010.
- Fazili Rifat, Rashid Arsalaan F. Alprazolam as a suicidal drug-a new trend. *J of Punjab Acad of Forensic Med & Toxic*, 12(2): 79-81, 2012.
- Clarke's Analytical Forensic Toxicology, Edited by Sue Jickells, Adam Negrusz. The Pharmaceutical Press UK, ISBN 978 0 85369 705 3, 2008.
- Ankita Kakkar, Sushil Kumar. Alprazolam Poisoning. *J Indian Acad of Forensic Med*, 36(4), 2014.
- The Narcotic Drugs and Psychotropic Substances Act. 1985, amended 2003, Universal Law Publishing Co. Pvt. Ltd.
- Toxicology Manual 2005, Directorate of Forensic Science (Ministry of Home Affairs, Govt. of India), Selective Scientific Publication.
- J. C. Fleishaker, J. P. Phillips, M. G. Eller PhD and R. B. Smith. Pharmacokinetics and Pharmacodynamics of Alprazolam Following Single and Multiple Oral Doses of a Sustained-Release Formulation. *The J of Clin Pharmac*, 29(6): 543-549, 1989.
- Vogel's Textbook of Practical Organic Chemistry 5th Ed. Furnis B.S. Et All, Longman, 1989.
- G. Hancu, Eniký Fülöp, Aura Rusu, Eleonora Mircia and Á. Gyéresi. Thin Layer Chromatographic Separation of Benzodiazepine Derivates.

Analele Universităţii din Bucureşti – Chimie
(serie nouă), 20(2):181 – 188, 2011.

Spectrophotometric Method for the estimation of
Alprazolam in Tablet Dosage Form, 3(1):161-164,
2011.

11. Ata. Kiran Kumar, A. Mohanakrishna, M.
Sudheer, K.Sai Rajesh and P. Ramalingam. UV
-

Red Flower Publication Pvt. Ltd.

Presents its Book Publications for sale

- | | |
|--|---------------------|
| 1. Breast Cancer: Biology, Prevention and Treatment | Rs.395/\$100 |
| 2. Child Intelligence | Rs.150/\$50 |
| 3. Pediatric Companion | Rs.250/\$50 |

Order from

Red Flower Publication Pvt. Ltd.

48/41-42, DSIDC, Pocket-II, Mayur Vihar, Phase-I

Delhi - 110 091 (India)

Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205

E-mail: redflowerpppl@gmail.org, redflowerpppl@vsnl.net

Website: www.rfpppl.co.in