

## COMPARISON OF BIOAVAILABILITY AND BIOEQUIVALENCE OF HERBAL ANXIOLYTIC DRUGS WITH MARKETING DRUG ALPRAZOLAM

Meera Sumanth\*, Swathy Nedunuri

Department of Pharmacology, Viveswarapura Institute of Pharmaceutical sciences, 22<sup>nd</sup>  
main, 24<sup>th</sup> cross, BSK II Stage, Bangalore -560070. Karnataka, India.

Article Received on  
04 Oct 2014,

Revised on 30 Oct 2014,  
Accepted on 25 Nov 2014

### \*Correspondence for

#### Author

Meera Sumanth

Department of  
Pharmacology,  
Viveswarapura Institute  
of Pharmaceutical  
sciences, 22<sup>nd</sup> main, 24<sup>th</sup>  
cross, BSK II Stage,  
Bangalore -560070.  
Karnataka, India.

### ABSTRACT

If the herbal remedies have to prove as an alternate to the allopathic drug treatment, it is essential to understand its bioequivalence with allopathic drugs. In this study bioavailability and bioequivalence of *Withania somnifera* (Ashwagandha), *Bacopa monnerie* (Brahmi) is compared with Alprazolam. A single oral dose of 1mg/kg, 0.42gm/kg/day, 0.42 gm/kg/day of Alprazolam, Ashwagandha, Brahmi respectively, were administered to overnight fasted albino Rabbits (1.5-1.8 kg, either sex, n=6). Immediately after drug administration, 0.5 ml of blood was withdrawn at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours from marginal ear vein, and plasma was extracted by liquid-liquid extraction. About 20µl was injected into HPLC system. All the drugs were well absorbed with peak blood concentrations occurring at 1-2 hr. A significant decrease was found in C<sub>max</sub> and AUC of *Withania somnifera* and *Bacopa monnerie*. The relative bioavailability of

Ashwagandha (60-70%) was found to be more significant than Brahmi when compared with Alprazolam. The bioequivalence of Ashwagandha with Brahmi and Alprazolam is Ashwagandha (14.25%) = Brahmi (18.42%) = Alprazolam (23.15%). Brahmi is more bioequivalent with Alprazolam as compared to Ashwagandha. The analysis using HPLC was found to be rapid, selective, accurate, precise, dependable and easy to perform.

**KEYWORDS:** Bioavailability, Bioequivalence, HPLC, *Withania somnifera*, *Bacopa monnerie*, Alprazolam.

## INTRODUCTION

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral components. Benzodiazepines are most commonly used drugs for anxiety. Alprazolam is short-acting benzodiazepine which possess anxiolytic, sedative, hypnotic, skeletal muscle relaxant, anticonvulsant, and amnesic properties. <sup>[1,2]</sup> When taken orally, it is well absorbed in gastro intestinal tract. Apart from allopathic drugs, herbal remedies are an excellent alternative to prescribed medications for anxiety. Many of these medications are successful in the treatment of anxiety and have fewer side effects. <sup>[3]</sup> There are many medicinal plants with Anxiolytic activity-*Bacopa monnerie* (Brahmi), *Withania somnifera* (Ashwagandha), *Lactuca virosa*, *Rhodiola rosea*, *Hypericum perforatum*, *Matricaria recutita*, *Mitragyna speciosa*, *Piper methysticum* etc. *Withania somnifera* (Ashwagandha) is a potential herbal drug for treatment of stress, anxiety, psychomatic disorders, anti-oxidant, blood pressure, memory enhancement. <sup>[4,5]</sup> *Bacopa monnerie*(Brahmi) is beneficial in treatment of anxiety, neurosis and mental fatigue. <sup>[6]</sup>

*Bioavailability* is defined as rate and extent of absorption of unchanged drug from its dosage form, or amount of drug that reaches systemic circulation. Bioavailability actually describes absorption and half-life of drugs. <sup>[7]</sup> For dietary supplements, herbs and other nutrients in which the route of administration is always oral, bioavailability designates simply the quantity or fraction of the ingested dose that is absorbed. *Bioequivalence* is defined as when two or more drugs or dosage forms enter into systemic circulation at same rate and extent in a unchanged form i.e., their plasma concentration time profiles will be identical without significant statistical differences, which is determined, based on the relative bioavailability of the innovator medicine versus the generic medicine. <sup>[8]</sup> Evaluation of bioavailability and bioequivalence of a drug and its formulations gives us an indication of the absorption, potency,  $t_{1/2}$  and other kinetic parameters of drug. *Withania somnifera* and *Bacopa monnerie* had been proved for its Anxiolytic activity. <sup>[9,10]</sup> If, the herbal remedies have to prove as an alternate to the allopathic drug treatment, it is essential to understand its bioequivalence with allopathic drugs. Hence in the present study, an attempt is made to compare the bioavailability and bioequivalence of *Withania somnifera*, *Bacopa monnerie* extracts with marketed drug Alprazolam.

## METHODOLOGY

### *Drugs, Reagents and Chemicals*

Alprazolam was obtained as a gift sample from Unimax labs. Ashwagandha and Brahmi were obtained as a gift sample from Green Chem Bengaluru. The reagents Acetonitrile and Methanol, HPLC grade, were from SD Fine Chemicals, (Mumbai, India). HPLC grade water was obtained from Spectrochem Pvt Ltd, (Mumbai, India). All the solvents and solutions for HPLC analysis were filtered through a millipore filter of pore size 0.45  $\mu\text{m}$  and degassed by ultrasonication.

### *Animals*

Eighteen healthy New Zealand Albino Rabbits, 1.5- 1.8 kg, of either sex were procured, from Pet Planet, Bangalore for experimental purpose. Rabbits were housed at our Institute's animal house facilities until they gained significant weight suitable for the present investigation. All the animals were acclimatized for 2 weeks prior to study under standard husbandry conditions, i.e. room temperature of  $27^{\circ} \pm 5^{\circ} \text{C}$ , relative humidity 45-55% and natural day and night cycle. The animals had free access to standard rabbit pellet (Pranav Agro Industry, Bangalore), with water supplied *ad libitum* under strict hygienic conditions.

All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration no.152/99/ CPCSEA. The protocol was approved by "Institutional Animal Ethical Committee" (IAEC) of Visveswarapura Institute of Pharmaceutical sciences.

### *HPLC System*

The liquid chromatographic system consisted of the following components: Shimadzu HPLC model (VP series) containing LC-20AT (VP series) pump, variable wavelength programmable UV/Vis detector SPD-10AVP and rheodyne injector (7725i) with 20  $\mu\text{l}$  fixed loop. Chromatographic analysis was performed using spinchrom software on a phenomenex luna C18 column with 250 $\times$ 4.6 mm i.d. and 5  $\mu\text{m}$  particle size. The composition of mobile phase is critical factor for separation. Mobile phase used was Acetonitrile : water (75:25) for Alprazolam, Ashwagandha and Brahmi. Prior to use, the mobile phase was filtered with a 0.45  $\mu\text{m}$  membrane filter and degassed under reduced pressure for 10 min. The column was maintained at 30 $^{\circ}\text{C}$ . The compounds were eluted isocratically at a flow rate of 1.0 ml/min. Analysis was carried out by Reverse phase High performance liquid chromatography (Rp

HPLC).<sup>[11,12]</sup> Detection was performed by UV absorption at 221 nm for Alprazolam<sup>[13]</sup> 225nm for Ashwagandha<sup>[14]</sup> and 205nm for Brahmi.<sup>[15]</sup>

### ***Preparation of Stock and Standard Solutions***

The Stock solution of Alprazolam (1mg/ml) was prepared in HPLC grade Acetonitrile(ACN), which is stable for 3 months at 4°C. Working standard solutions (100, 150, 250, 500, 1000 ng/ml) were prepared from stock solution. Standard calibration samples were prepared by spiking 0.5ml of drug free rabbit plasma to achieve final concentrations of 100-1000 ng/ ml. Internal Standard (Midazolam) (10mg/ml) was prepared in HPLC grade ACN and stored at 4°C.<sup>[16,17]</sup>

### ***Bioavailability and Bioequivalence Studies***

Overnight fasted albino rabbits were divided into 3 groups, of 6 animals each. Animals of group 1, 2 and 3 were administered standard drug Alprazolam (1.0mg/kg p.o.) *Withania somnifera* (0.42gm/kg/day p.o.) *Bacopa monnerie*(0.42 gm/kg/day p.o) respectively. Animals of group 1, 2 and 3 were further divided into subgroups 1A,1B, 2A, 2B and 3A, 3B (n=3 each)for generic and marketed(brand) drugs administration respectively, in the same doses as mentioned earlier. Based on their human dose-1000 mg/day, doses of *Withania somnifera*, *Bacopa monnerie* were calculated, using conversion factor based on body surface area of Rabbits.<sup>[18]</sup>

$$\text{Rabbit dose} = \text{human dose} \times 0.07 \dots (\text{For 1.5 Kg})$$

Human therapeutic dose is considered as low dose and double the therapeutic dose is considered as higher dose. Dose of Alprazolam was selected based on earlier studies.<sup>[14]</sup> The rabbits were also fasted up to 3 h after drug administration, so as to avoid drug-food interaction. Immediately after drug administration, 0.5 ml of blood was withdrawn at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 hours by marginal ear vein of rabbit, collected in EDTA tubes and centrifuged at 5000 rpm for 10-15 min. Plasma samples were stored at -20°C until the time of analysis.<sup>[11, 19]</sup> Plasma was extracted by Liquid -liquid extraction,(To 0.5 ml plasma, 25µl of internal standard (Midazolam) was added and agitated, extracted with 1ml of ACN, agitated for 1min and centrifuged at 5000 rpm for 10 min. Upper layer (about 1ml) was collected in EDTA tubes, reconstituted with 1 ml CAN) and about 20 µl is injected into HPLC system.

### Pharmacokinetic Determination

The data was represented in a plasma level-time curve. The area under time curve ( $AUC_{0-24}$ ) was calculated using Trapezoid rule. The maximum concentration ( $C_{max}$ ) and maximum time ( $T_{max}$ ) were obtained directly from generated data. Half-life ( $T_{1/2}$ ) was determined from the semi-log plot of the data. Total AUC was calculated by the formula,  $AUC_{total} = AUC_{0-24} + C_{24}/K_e$ .

### Statistical Analysis

Data was expressed as mean  $\pm$  SEM. Statistical comparison between different groups were done using One-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test,  $P < 0.05$  was considered significant. Statistical analysis was carried out using Graph Pad Prism version 3.0 (GraphPad Software Inc., San Diego, Calif., USA).

## RESULTS

A significant increase in  $C_{max}$  of generic Alprazolam was found when compared with marketed Alprazolam. (Fig no.1) A significant increase in  $C_{max}$  of Brahmi (from Green chem., Bangalore) was found when compared with marketed Brahmi. (Fig no.2) A significant increase in  $C_{max}$  of Ashwagandha (from Green chem., Bangalore) was found when compared with marketed Ashwagandha. (Fig no.3) The HPLC assessment of Pharmacokinetics of Alprazolam, Ashwagandha, Brahmi are listed in table no 1. It shows decrease in  $C_{max}$  and AUC of *Withania somnifera* and *Bacopa monnerie* as compared to marketed drug Alprazolam.

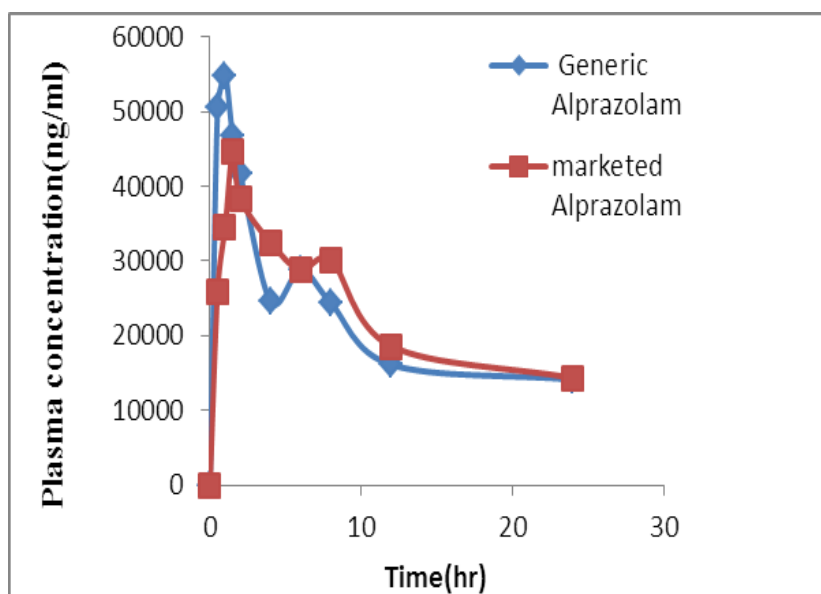


Fig no.1: Plasma concentration- time profile of Alprazolam

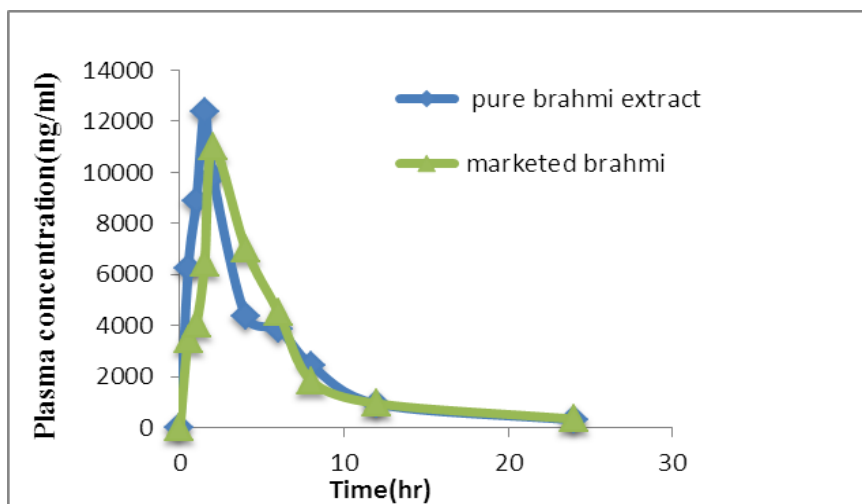


Fig no.2: Plasma concentration- time profile of *Brahmi*

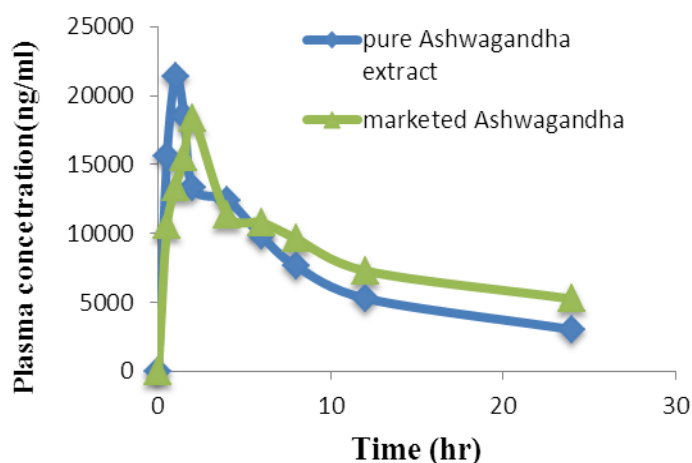


Fig no. 3: Plasma concentration- time profile of *Ashwagandha*.

Table 1:HPLC Assessment of Pharmacokinetic parameters for Alprazolam, Ashwagandha and Brahmi.

Sl. no	Parameter	Alprazolam pure	Alprazolam marketed	<i>Withania somnifera</i> extract	<i>Withania somnifera</i> marketed	<i>Bacopa monnerie</i> Extract	<i>Bacopa monnerie</i> marketed
1	C <sub>max</sub> (ng/ml)	54845.5	44532	21360.7	18317.8	12397.4	9990.1
2	T <sub>max</sub> (hr)	1	1.5	1	2	1.5	2
3	AUC (ng/ml/hr)	521453.3	378091.2	339927.9	195826.3	166071.6	110914.78
4	t <sub>1/2</sub> (hr)	24.19	15.49	27.69	18.29	35.07	12.386

C<sub>max</sub>- Peak plasma drug concentration, T<sub>max</sub>- Time to reach peak plasma concentration, AUC- Area under plasma level –time curve, t<sub>1/2</sub>- Biological half-life.

Values expressed as mean ± SEM, n=6, one way ANOVA, followed by Tukey Kramer post hoc test.

## DISCUSSION

HPLC is popular method for analysis of herbal medicines because it is easy and use is not limited by volatility and stability of the sample compound. HPLC is used to analyze almost all the compounds in herbal medicines.<sup>[19]</sup> In this study, rabbits were administered a single oral dose 1mg/kg, 0.42gm/kg/day, 0.42gm/kg/day of Alprazolam, Ashwagandha, Brahmi respectively. It was found that the drugs were well absorbed with peak blood concentrations occurring at 1-2 hr. A significant decrease in  $C_{max}$  and AUC of *Withania somnifera* and *Bacopa monnerie* were found. The relative bioavailability of Ashwagandha (60-70%) was found significant. The variation in bioavailability of Alprazolam, a triazolobenzodiazepine derivative, is due to presence of benzodiazepine moiety, which gets absorbed more readily than herbal drugs. The variation in bioavailability of *Withania somnifera* is due to presence of alkaloid group, which gets absorbed more readily than saponin glycosides present in *Bacopa monneri*.<sup>[20]</sup>

The HPLC method of analysis was found to be simple, rapid, selective, accurate, precise, dependable and easy to perform. The principle advantages of this method were found to be use of simple liquid-liquid extraction as a part of chromatographic procedure and better sensitivity. This isocratic HPLC-UV method for analysis of plasma samples may be recommended for pharmacokinetic studies as well as for therapeutic drug monitoring.

## CONCLUSION

The relative bioavailability of Ashwagandha (60-70%) is more significant than Brahmi when compared with Alprazolam. The bioequivalence of Ashwagandha with Brahmi and Alprazolam is Ashwagandha (14.25%) = Brahmi (18.42%) = Alprazolam (23.15%). Brahmi is more bioequivalent with Alprazolam as compared to Ashwagandha. The analysis using HPLC was found to be rapid, selective, accurate, precise, dependable and easy to perform.

## REFERENCES

1. Baldessarini RJ, Goodman and Gilman's The Advanced Pharmacological Basis of Therapeutics, 11<sup>th</sup>ed, Editors Hardman GJ, Limbird LE, McGraw Hill Companies, USA, 2006; 450-55.
2. Giovanni AF, Silvana G, Piera B, Gianni SAR, Sandra C, Francesco M. Benzodiazepines and anxiety sensitivity in panic disorder, Prog. Neuropsychopharmacol. Biol. Psychiatry, 1994; 18: 1163-8.

3. Mukherjee PK. Quality control of herbal drugs, 2<sup>nd</sup> ed. Business Publication, New Delhi, 2008; 45-65.
4. Kulkarni SK and Ashish Dhir. *Withania somnifera*. An Indian ginseng, Prog. Neuropsychopharmacol. Biol. Psychiatry, 2008; 32: 1093-1105.
5. Dale Kiefer. Ashwagandha Stress Reduction, Neural Protection, and a Lot More from an Ancient Herb. (Online) 2006.  
AvailablefromURL:[http://www.lef.org/magazine/mag2006/jun2006\\_report\\_ashwa\\_01](http://www.lef.org/magazine/mag2006/jun2006_report_ashwa_01).
6. Deepak R, Gitika B, Gautam P, Raghwendra P, Satyawar S, Hemant KS. Adaptogenic effect of *Bacopa monnieri* (Brahmi) Pharmacol. Biochem. Behav, 2003; 75: 823-30.
7. Shargel L, Susanna WU, Andrew Yu. In: Applied Biopharmaceutics and Pharmacokinetics, 5<sup>th</sup> ed, McGraw-Hill, New York, 1999; 452-7.
8. Heaney RP. Factors influencing the measurement of bioavailability, taking calcium as a model. J. Nutri, 2000; 131: 1344S-8S.
9. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. Phytomedicine, 2000; 7(6): 463-9.
10. Bhattacharya SK, Ghosal S. Anxiolytic activity of a standardized extract of *Bacopa monnieri*: an experimental study. Phytomedicine, 2007; 5(2): 77-82.
11. Venkatesh AB, Ulrike G, Claudia K, Markus V, Hartman D. Pharmacokinetics and Bioavailability of herbal medicinal products, Phytomedicine, 2002; 9: 1-3.
12. Bhandari P, Kumar N, Singh B, Singh V, Kaur I. Silica-based monolithic column with evaporative light scattering detector for HPLC analysis of bacosides and apigenin in *Bacopa monnieri*. J. Sep.Sci., 2009; 32(15-16): 2812-8.
13. Shukla S, Pankaj K, Hari NM, Sushant KS, Trivedi P, Radhey SS. RP-HPLC method development and its validation for simultaneous estimation of Alprazolam and Fluoxetine hydrochloride in Pharmaceutical dosage form, Eur. J. Ana. Chem., 2010; 5(3): 239-45.
14. Narayan DC, Girish CP, Laxminarain M, Neelam S, Rakesh T, Rajender S. Analysis of withanolides in root and leaf of *Withania somnifera* by HPLC with photodiode array and evaporative light scattering detection,. Phytochem. Ana, 2008; 19(2): 148-54.
15. Mohammadreza R, Yalda HA, Hakemi L, Maryam M, Gheise B. Simultaneous determination of Clobazam and its major metabolite in human plasma by a rapid HPLC method. J. Chromat.B, 2005; 823: 167-71.

16. Mohammadreza R, Yalda HA, Kambiz AM, Solatani F. An improved HPLC method for rapid quantitation of diazepam and its major metabolites in human plasma, *Talanta*, 2008; 75: 671-6.
17. Bhattacharya SK, Muruganandam AV. Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol.Biochem.Behav*, 2003; 75: 547-55.
18. Ghosh MN. Fundamentals of experimental Pharmacology, 4<sup>th</sup> ed, Hilton and company, Kolkata, 2008; 178.
19. Yi-Zeng L, Pishan X, Kelvin C. Quality control of herbal drugs. *J. Chrom. A*, 2004; 812: 53-70.
20. Ganzera M, Gampenrieder J, Pawar RS, Khan IA, Stuppner H. Separation of the major triterpenoid saponins in *Bacopa monnieri* by high-performance liquid chromatography, *Analytica Chimica Acta*, 2004; 516: 149-154.