Research Article
ISSN: 0974-6943

V. Dilip Kumar et al. / Journal of Pharmacy Research 2011,4(1),50-52

Available online through
http://jprsolutions.info

Simultaneous estimation of citalopram hydrobromide and dothiepin hydrochloride in human plasma by HPLC method

V. Dilip Kumar[1], Mahesh N. M[1], B.M. Gurupadayya[2], V. Ravi[3]
[1]*Department of Pharmacology, JSS College of Pharmacy, JSS University, Shivarathreeshwara Nagar, Mysore-570 015.*
[2]*Department of Pharmaceutical Chemistry, JSS College of Pharmacy, JSS University Shivarathreeshwara Nagar, Mysore-570 015.*
[3]*Department of Pharmaceutics, JSS College of Pharmacy, JSS University Shivarathreeshwara Nagar, Mysore-570 015.*

Received on: 15-09-2010; Revised on: 18-11-2010; Accepted on:13-12-2010

ABSTRACT

This was a study to develop a rapid, precise, accurate and sensitive high performance liquid chromatographic method for the simultaneous estimation of citalopram hydrobromide and dothiepin hydrochloride in spiked human plasma. The chromatographic method was standardized using a Phenomenex C-18 column (250 x 4.6 mm i.d., 5 µm particle size) with UV detection at 234 nm and flow rate of 1 ml/min. Salbutamol was spiked in plasma as internal standard (IS). The mobile phase consisting of acetonitrile:water (35:65 v/v) containing of 0.01% orthophosphoric acid and 0.01% sodium chloride. The regression equation for citalopram hydrobromide and dothiepin hydrochloride were Y = 0.0282X +0.0750 and Y= 0.0312 X + 0.1187 respectively. LOD and LOQ were found to be 7.978 and 26.595 ng/mlrespectively for citalopram and 11.413 and 38.044 ng/ml respectively for dothiepin. The method was validated and produced accurate and precise results for estimation of the two drugs in human plasma.

Key words: Citalopram, Dothiepin, HPLC, Plasma, Estimation

INTRODUCTION

Citalopram HBr, 1-(3-(dimethylamino) propyl-1-(p-fluorophenyl)-5-phthalancarbonitrile hydrobromide is a selective serotonin reuptake inhibitor and dothiepin HCl, 3-(dibenzo [*b,e*]thiepin-11(6*H*)-ylidene)-*N,N*-dimethylpropan-1-amine hydrochloride. is a tricyclic antidepressant both of which are used in treating depression. Citalopram produces its pharmacological action by inhibiting the serotonin (5 HT, 5-Hydroxytryptamine) and whereby it increases the serotonin levels in the synaptic clefts (1,2). Dothiepin is a tricyclic antidepressant which acts by inhibiting the uptake of both serotonin and nor adrenaline levels in the synaptic clefts by inhibiting the respective amine transporters (3). A large proportion of patients do not get an ideal result from the first antidepressant that they are given, and it is frequently necessary to consider alternatives which may involve combinations of drugs. Clinicians were found to prescribe 5% of the combination of SSRI and TCA in Australia for treating severe depression (4). Various cases of pharmacokinetic and pharmacodynamic interactions resulted between these two classes of drugs and were reported, the severity of which varies from simple dizziness to even death (5). The severity of interaction depends on the relative plasma drug concentration. Citalopram is metabolised by CYP2D6, 2C19 and 3A4 and dothiepin by CYP2D6 (6). Citalopram dose dependently inhibits CYP2C19 and 2D6 and to a mild extent 3A4 (7, 8). Various CYP isoenzymes are involved in metabolism of dothiepin. Dothiepin in recent studies was found to inhibit CYP2C19 (9,10). Due to pharmacokinetic interaction the plasma levels of drugs were expected to rise. In order to study the interaction potential of these two drugs, the plasma levels of the drugs should be estimated. Various analytical methods have been developed for analysis of citalopram and dothiepin individuallyin both pharmaceutical and biological fluids (11-20), but there is no method for simultaneous estimation method of citalopram and dothiepin in plasma.

Hence, we developed and optimized an analytical method which is easy, simple, sensitive, accurate, and precise and with good extraction recoveries for the estimation of citalopram and dothiepin simultaneously in plasma. The following high performance liquid chromatographic method provides opportunity for the simultaneous estimation of citalopram and dothiepin in human plasma.

***Corresponding author.**
Dr. B.M. Gurupadayya,
Department of Pharmaceutical Chemistry,
JSS College of Pharmacy, JSS University,
Shivarathreeshwara Nagar, Mysore-570 015
Fax Number: +91 0821-2548359
***Tel.:** +91 9242886136*
***E-mail:**bm_guru2004@yahoo.co.in*

MATERIALS AND METHODS

Chemicals

Citalopram hydrobromide was kindly provided by Torrent Pharmaceuticals, Ahmadabad. Dothiepin hydrochloride was obtained as a gift sample from Hetero chemicals, Hyderabad. Salbutamol sulphate was supplied by Cipla, Mumbai. The HPLC grade acetonitrile was purchased from Merck Specialities Private Limited, Mumbai, India. HPLC grade water and analytical grade sodium chloride, sodium hydroxide, hydrochloric acid, orthophosphoric acid, hexane and iso-amylalcohol were purchased from Ranbaxy Fine Chemicals Limited, New Delhi, India.

Instrumentation

The HPLC system consists of SHIMADZU LC-2010AHT with auto sampler. Chromatographic separations were achieved by using a Phenomenex C_{18} column (250 x 4.6 mm i.d., 5 µm particle sizes). The column was maintained at room temperature. Injections of sample were made by auto injector. The mobile phase was 35% acetonitrile in water consisting of 0.01% sodium chloride and 0.01% ortho phosphoricacid. The flow is isocratic with a flow rate of 1 ml/min. The detector is UV with a detection wavelength of 234 nm. Chromatogram was recorded and peaks were quantified by means of PC based LC Solution Software. Cooling centrifuge (REMI, Vasai, India), Vibra Cell Sonicator (Sonics and Materials, Inc., Newtown, USA), Refrigerator (REMI, Vasai, India).

Stock and Standard solutions

Stock solutions of citalopram hydrobromide and dothiepin hydrochloride at 1000 µg ml-1were prepared by dissolving each 100 mg of each in 100 ml of HPLC water and stored at 4 °C. The stock solutions of each drug were further diluted with HPLC water to give working solutions of 10 µg ml^{-1}. Drug free plasma aliquots of 1 ml each were spiked with various concentrations of standard solutions obtained by suitably diluting working solution to give a series of concentration with least plasma concentration of 50 ng/mlof both citalopram and dothiepin.

Sample Preparation

To each of the above plasma aliquots in centrifuge tubes, 10 ml of standard solution of salbutamol sulphate as internal standard (IS) was added to give a final concentration of 100 µg ml^{-1}. Contents of the centrifuge tubes were made alkaline with 0.2 ml of 1M sodium hydroxide. An aliquot of 10 ml hexane containing 1% v/v isoamyl alcohol was added and mixture was sonicated for 5 min using a sonicator. After centrifugation at 1500 g for 10 min, the aqueous layers were frozen by immersing the tubes into a cooling bath consisting of dry ice and ethanol. The hexane layers were decanted into another centrifuge tubes and 0.2 ml of 0.05 M hydrochloric acid was added. The mixture was shaken centrifuged and frozen as mentioned above, and the organic layer was discarded and 50 ml of acid extract was injected into the chromatograph.

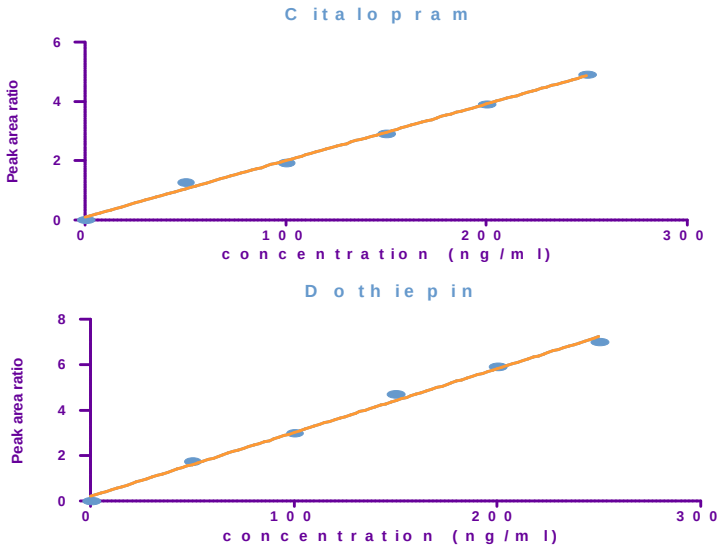

Figure 1: (A) Calibration curve of citalopram hydrobromide between plasma concentrations of 0 to 250 ng/mland (B) Calibration curve of dothiepin hydrochloride between plasma concentrations 0 to 250 ng/ml.

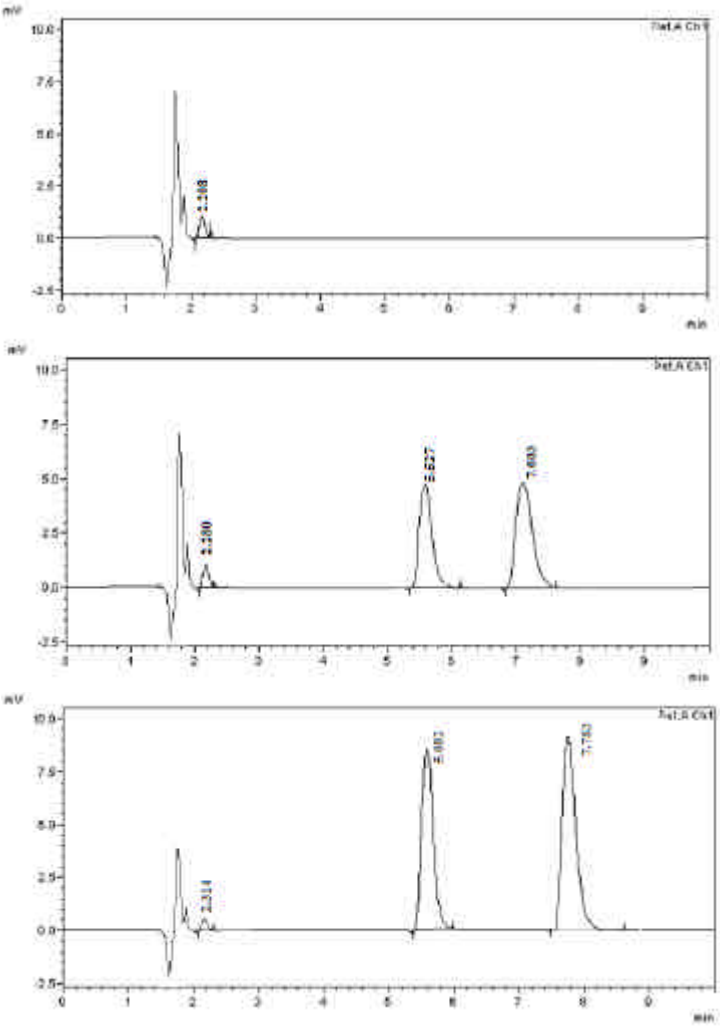

Figure 2: Typical HPLC chromatograms after extraction of (A) Blank plasma spiked with only IS. (B) Blank plasma spiked with each citalopram and dothiepin to make final concentration of 250 ng/ml. (C) Plasma obtained from rat after 4 hours of oral administration of citalopram and dothiepin each with 10 mg/kg.

Table 1. Accuracy and Precision

Drug	Nominal concentration (ng/ml)	Intraday (n=6) Accuracy (%)	Precision (%)	Interday (n=6) Accuracy (%)	Precision (%)
Citalopram	100	98.6	2.2	99.6	2.8
	250	96.0	2.7	94	5.4
	500	96.0	1.5	96.2	2.1
Dothiepin	100	98.5	1.5	103.6	3.9
	250	97	1.2	97.2	1.8
	500	101.5	2.4	94.7	2.3

Calibration Curves, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Calibration curves for both citalopram and dothiepin were plotted individually by taking the peak areas of drug/Internal standard versus concentration. The slopes of the plots for citalopram and dothiepin were determined by the method of least square regression analysis. The LOD and LOQ for citalopram and dothiepin by the proposed method were determined using calibration curves. LOD and LOQ were calculated as 3.3 s/S and 10 s/S, respectively, where S is the slope of the calibration curve and s is the standard deviation of y-intercept of regression equation (n=6).

Accuracy and Precision

Intraday reproducibility precision and accuracy were assessed for three different concentrations of 100, 250 and 500 ng/ml of citalopram and dothiepin with replicates of 6 were analyzed on the same day and the concentrations were calculated by extrapolating peak area ratios to standard curves obtained on that day. The procedure was repeated on three separate days to allow the determination of interday precision and accuracy. Accuracy and precision were determined by comparing the calculated concentrations of the extracted standard plasma analytes with different known concentrations of analytes. Accuracy was expressed as the mean % error and precision was expressed as the coefficient of variance (%).

Extraction recovery

The extraction efficiencies of citalopram and dothiepin were determined by taking replicates of 100 and 300 ng/ml. The plasma samples of one milliliter were spiked with both the drugs with above concentrations and internal standard and were extracted. One milliliter of 0.05N HCl containing both the drugs at same concentrations were used for reference. The recoveries were studied by comparing the peak area ratio of both the drugs in plasma extracts and in acid solution.

Stability

Stabilities of the drugs in plasma samples for short term, freeze thaw and processed samples of citalopram and dothiepin were assessed to confirm the stability of both the drugs through the procedure. Aliquots of plasma containing both the drugs with concentrations each of 100 and 300ng/ml were analyzed immediately and after storage. Short-term stability was assessed after 5 hours standing at room temperature then samples were subjected to two freeze–thaw cycles to assess drug stability. Finally, stability of the processed samples stored for 24 hours at room temperature in the auto sampler was determined.

Application of Method

After obtaining approval from Institutional Animal Ethics Committee, Wistar rats were used for the applicability of the study *in vivo.* Two groups of rats (n=6), one for control and another for sample were used. To control group normal saline solution was given orally and to sample group therapeutic doses of citalopram hydrobromide (10 mg/kg) (21) and dothiepin hydrochloride (10 mg/kg) (22) were given orally as a solution. After 4 hours blood was collected by retro-orbital puncture using a micro haematocrit tube and collected in a heparinized centrifuge tubes. Centrifuge the tubes at 2000 rpm for 5 min at 4 ^{0}C. Separate the plasma and drugs were extracted and estimated using this HPLC method and were expressed as mean ± SEM.

RESULTS

The chromatograms of the two drugs, citalopram and dothiepin were as shown in figure 2. The retention times of citalopram, dothiepin and internal standard were found to be 5.52, 7.60 and 2.28 minutes respectively for the given set of chromatographic conditions.

Linearity, LOD and LOQ

The calibration curve for citalopram was found to be linear between the concentration range of 50 to 250 ng with mean average correlation coefficient, r^2 = 0.9958. The calibration curve for dothiepin was found to be linear between the

concentration range of 50 to 250 ng with $r^2 = 0.9943$. Both the calibration curves pass through the origin, which justifies the use of single point calibration. The regression equation for citalopram hydrobromide and dothiepin hydrochloride were Y = 0.0282 X + 0.0750 and Y= 0.0312 X + 0.1187 (Y = peak area drug/IS peak area ratio and X = concentration) respectively. LOD and LOQ for citalopram and dothiepin were calculated from the respective regression equations. LOD and LOQ were found to be 7.978 and 26.595 ng/mlrespectively for citalopram and 11.413 and 38.044 ng/mlrespectively for dothiepin.

Accuracy and Precision

The accuracy and precision data for both citalopram and dothiepin at three different concentrations obtained from the above method are shown in table 1. The average intraday accuracies and precisions ranged from 96% to 98.6% and 1.5% to 2.7% respectively for 100, 250, 500 ng/mlsolutions of citalopram hydrobromide. For dothiepin the average intraday accuracies and precisions were ranged from 97% to 101.5% and 1.2% to 2.4% respectively for three sets of dothiepin hydrochloride solution. The average interday accuracies and precisions ranged from 94% to 99.6% and 2.1% to 5.4% respectively for three sets of citalopram hydrobromide concentration. For dothiepin the average interday accuracies and precisions were ranged from 94.7% to 103.6% and 1.8% to 3.9% respectively for three sets of dothiepin hydrochloride solution.

Extraction Recovery

The mean recoveries of citalopram in spiked plasma (n=6) at concentrations of 100 and 300 ng/mlwere 88% ± 1.2 and 92% ± 2.4 respectively and for dothiepin 92% ± 1.5 and 95% ± 1.8 at 100 and 300ng/mlL respectively.

Stability

The plasma concentrations of the samples spiked with citalopram and dothiepin before 5 hours did not show any significant ($P<0.05$) change from that of freshly spiked plasma. The accuracies were ranging from 96.3% to 98.4% and 99.5% to 103% for citalopram and dothiepin respectively. The plasma concentrations of both analytes in spiked plasma samples subjected to freeze–thaw cycles did not vary significantly ($P<0.05$) when compared to fresh samples. Accuracy ranged from 98% to 102% for citalopram and from 97% to 101% for dothiepin. and after storage of reconstituted extracts in the auto sampler varied from 99% to 102% for citalopram and 97% to 102% for dothiepin.

Application of Method

The plasma samples from Wistar rats of both control and sample group were extracted and analysed using HPLC and chromatograms were obtained. The chromatograms did not show any additional peaks other than that of subject drugs and the internal standard. That is there is no interaction between the peaks of drugs with the peaks of their metabolites. The mean plasma concentrations of citalopram and dothiepin were 12.65 ± 1.63 µg $ml^{-1}$and 18.73 ± 1.28 µg ml^{-1} respectively.

DISCUSSION

The estimation of plasma concentrations of citalopram and dothiepin can help in finding out the interaction potential of two drugs. This estimation of changes in plasma concentrations of citalopram and dothiepin allows physicians and psychiatrists to adjust the doses of treatment regimen. For this an analytical method which was rapid, accurate, simple and good extraction recovery for simultaneous quantitative analysis of citalopram and dothiepin was needed which does not exist. In this study, such an analytical method was developed and validated.

In the initial studies, the wavelength for UV detection was determined by scanning a UV overlay spectra in the wavelength range of 600 to 200 nanometers for the solutions of citalopram hydrobromide, dothiepin hydrochloride and salbutamol sulphate in 0.05M HCl. Maximum absorption of all the three solutions was found at 234 nanometers and was chosen as detection wavelength.

A mobile phase consisted of acetonitrile (78 : 28) and ammonium formate (45 mmol/L, pH 4.0) was used according to the method developed by Mendoza et al(11) using C_{18} column with a flow rate of 0.8 ml/min and was found that the retentions for dothiepin is crossing over 20 minutes. Further to decrease the run time and for rapid elution the mobile phase of 40% v/v acetonitrile containing 0.01% v/v orthophosphoric acid and 0.01% sodium chloride was used which was similar to that used by Jonathan et al (12) used for estimation of citalopram in breast milk. All the three drugs eluted rapidly in 7 minutes, but, there is no resolution of citalopram and dothiepin peaks. So, in order to resolve the peaks, the acetonitrile content were further changed to 35% which was the same used by Ilett KF et al (13) for estimation of dothiepin in breast milk. This mobile phase eluted all the three drugs with short retention times and eluted all the three drugs in 8.3 minutes which was rapid.

With the above changes in the detection wavelength, mobile phase content and extraction recovery procedures, a simple, rapid, accurate and precise and sensitive HPLC method for simultaneous estimation of citalopram and dothiepin in plasma was developed which can be applied clinically for the interaction study.

CONCLUSION

The HPLC method mention above for the simultameous determination of citalopram and dothiepin in human plasma is rapid, sensitive, reproducible, and can be applicable for pharmacokinetic studies of subject drugs in combination.

ACKNOWLEDGEMENTS

The authors express their gratitude to Torrent Pharmaceuticals, Ahmedabad; Hetero Chemicals, Hyderabad; Cipla, Mumbai for providing gift samples of citalopram hydrobromide, dothiepin hydrochloride and salbutamol sulphate Respectively. Thanks are also extended to Dr. H.G. Shivakumar, Principal, JSS College of pharmacy, Mysore for providing necessary facilities and his constant support.

REFERENCES

[1] Plaznik A, Stefanski R, Kostowski W, Restraint stress-induced changes in saccharin preference: the effect of antidepressive treatment and diazepam. Pharmacol Biochem Behav 1989; 33: 755-759.
[2] Winslow JT, Insel TR, Serotonergic and catecholaminergic reuptake inhibitors have opposite effects on the ultrasonic isolation calls of rat pups. Neuropsychopharmacology 1990; 3: 51-59.
[3] Bourin M, John P, Martine H, Glen B, Marie C. A schematic representation of the psychopharmacological profile of antidepressants. Prog. Neuro- psychophatmacol. & Biol. Psychiat 1996; 20(8): 1389-1402.
[4] McManus P, Andrea M, Philip M,. Don B, John D. Co-prescribing of SSRIs and TCAs in Australia: how often does it occur and who is doing it ?. Br. J. of Clinical Pharmacol. 2001; 51(1): 93-98.
[5] Sheldon P, Steve W. Detrimental Antidepressant Drug–Drug Interactions: Are They Clinically Relevant? Neuro psychopharmacology. 2006; 31:1605–1612.
[6] Kukoyi O, Argo TR, Carnaham RM. Exacerbation of panic disorder with rifampin therapy in a patient receiving citalopram. Pharmacotherapy 2005; 25: 435-437.
[7] Jeppesen U, Gram LF, Vistisen K, Loft S, Poulsen HE, Brosen K. Dose- dependent inhibition of CYP1A2, CYP2C19 and CYP2D6 by citalopram, fluoxetine, fluvoxamine and paroxetine. Eur. J. Clin. Pharmacol. 1996; 51(1): 73-78.
[8] Karin H, Norio Y, Gunnel T, Jolanta W, Lars LG, Leif B. Metabolism of citalopram enantiomers in CYP2C19/CYP2D6 phenotyped panels of healthy Swedes. Br. J. Clin. Pharmacol. 2003; 56: 415-421.
[9] Rees JA. Clinical interpretation of pharmacokinetic data on dothiepin hydrochloride (Dosulepin Prothiaden). J. Int. Med. Res 1988; 9:98–102.
[10] Whyte IM, Dawson AH, Buckley NA. Relative toxicity of venlafaxine and selective serotonin reuptake inhibitors in overdose compared to tricyclic antidepressants. Quarterly Journal of Medicine. 2003; 96(5): 369-374.
[11] Mendoza L, Hajduch M, Kekulova H, Svobodova X, Mihal V, and Svoboda M. Bioequivalence of two brands of citalopram 40 mg tablets after single oral administration to healthy volunteers. Biomed. Papers. 2005; 149(1):169–72.
[12] Jonathan R, Judith HK, Peter LH, Micheal P, Rolland K, Kenneth FI. Citalopram and desmethylcitalopram in human milk; distribution, excretion and effects in breast feed infants. Br. J. Clin Pharmacol 2000; 50:263-68.
[13] Ilett KF, Lebedevs TH, Wojnar-horton RE, Yapp P, Roberts MJ, Dusci J et al. The excretion of dothiepin and its primary metabolites in breast milk. Br. J. Clin. Pharmac. 1993; 33: 635-639.
[14] Rop PP, Viala A, Durand A, Conquy T, Determination of Citalopram, amitriptyline and clomipramine in plasma by reversed-phase high-performance liquid chromatography. J Chromatogr 1985; 338(1):171-178.
[15] Eva N. Citalopram in newborns: A case report. The international association of forensic toxicologists.[Online].2006 [cited 2009 Aug 10]; Avaliable from: URL:http://www.TIAFT.org/proceedings/ CT-p-07_full.pdf.
[16] Ole VO, Kristian L. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites. Journal of Chromatography B 1996; 675:83-88.
[17] Overo KF. Preliminary studies of the kinetics of citalopram in man. Europ. J. clin. Pharmacol. 1978; 14:69-73.
[18] Oyehaug E, Ostensen ET, Salvesen B. High-performance liquid chromatographic determination of citalopram and four of its metabolites in plasma and urine samples from psychiatric patients. J Chromatogr. 1984; 308:199-208.
[19] Akerman KK, Jolkkonen J, Huttunen H, Penttilä I. High-performance liquid chromatography method for analyzing citalopram and desmethylcitalopram from human serum. Ther. Drug Monit 1998; 20(1):25-29.
[20] Raggi MA, Pucci V, Mandrioli R, Sabbioni C, Fanali S. Determination of Recent Antidepressant Citalopram in Human Plasma by Liquid Chromatography- Fluorescence Detection. Chromatographia 2003; 57 : 273-278.
[21] Vallo M, Lembit A. Acute and Chronic Citalopram Treatment Differently Modulates Rat Exploratory Behavior in the Exploration Box Test: No Evidence for Increased Anxiety or Changes in the [3H]Raclopride Binding. Pharmacology. 1999; 58:59–69.
[22] Krishnamurthy M, DeSouza C, Selvaraj V, Venkataraman BV, Nagarani MA, Joseph T. Effects of ECT-dothiepin combination on learning in rats. Indian J Exp Biol. 1993; 31(10): 831-833.

Source of support: Nil, Conflict of interest: None Declared