International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.3, No.4, pp 1909-1915, Oct-Dec 2011

Isocratic Liquid Chromatographic Method for Analysis of Amisulpride in Pharmaceutical Preparations.

Madhurjya Neog, Rikesh Patel and Sangita Sharma*

Department Of Chemistry, Hemchandracharya North Gujarat University, Patan-384265, Gujarat, India

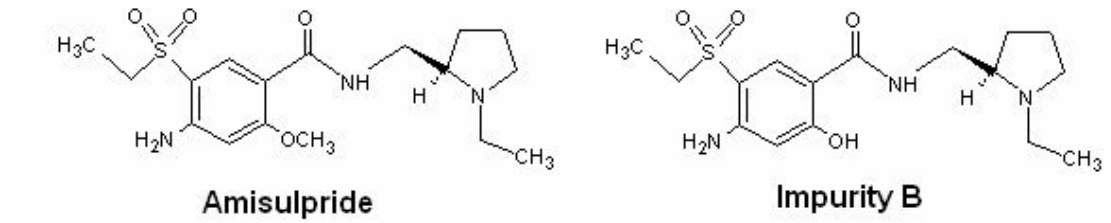
Corres.athor: researchsharma@yahoo.com

Abstract: An Isocratic liquid chromatography method for rapid estimation of Amisulpride in solid dosage forms is investigated. The separation is performed by using a reversed-phase C18 column (250 × 4.6 mm, 5µm). The retention times of Amisulpride and its Impurity (Imp-B) are 28.49 min and 32.45 min respectively with sharp peak shapes and resolution greater than 3.0. Results of the analysis are validated statistically and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.
Key words: Amisulpride, Ion Pair Chromatography, Reverse phased.

INTRODUCTION

Amisulpride[1] is chemically 4-Amino-*N*-[[(2*RS*)-1-ethylpyrrolidin-2-yl] methyl]-5-(ethylsulphonyl)-2-methoxybenzamide (Molecular mass 369.48 $gmol^{-1}$). Amisulpride is a selective dopamine antagonist and is used in the treatment of schizophrenia.. It has a high affinity for D_2 (Ki 2.8 nM) and D_3 (Ki 3.2 nM) dopaminergic. Amisulpride is not approved by the Food and Drug Administration for use in the United States, but it is used in Europe (France, Germany, Italy, Switzerland, Russia, United Kingdom) and Australia to treat psychoses and schizophrenia[2-3]. Literature survey reveals the estimation of Amisulpride in pharmaceutical formulations by various spectrophotometric[4], HPLC[4-11], HPTLC[11], Capillary zone electrophoresis[4, 12], Voltammetric[13] methods. The present work deals with the development of ion pair liquid chromatographic method for the quantitative estimation of Amisulpride in bulk and pharmaceutical Preparations. The specificity of the method was performed and no degradation product was obtained in the stress conditions (hydrolysis, oxidations, photolysis and thermal stress). As per ICH suggestion[14, 15] probable Impurity of Amisulpride (Impurity B as per the British Pharmacopoeia) 4-amino-N-[[(2RS)-1-ethylpyrrolidin-2-yl]methyl]-5-(ethylsulphonyl)-2-hydroxybenzamide (Figure 1) was separated from the drug. The main advantage of the developed method is the usefulness for routine analysis in quality control labs due to short run time and no need of gradient program. The developed method can be used for assessing the stability of Amisulpride in bulk drugs and pharmaceutical dosage form The developed method was validated with respective linearity, accuracy, precision, LOD, LOQ and robustness.

Figure 1: Chemical Structures of Amisulpride and its Impurity (Imp-B)



EXPERIMENTAL

Material and reagents

Amisulpride bulk drug and its impurity Imp-B were obtained form Molecule Analytical Lab. Sulpitac-200 (Sun Pharmaceutical Ind. Ltd., Silvassa) and Soltus 50 (LC Orbit Life science Pvt Ltd. Puducherry) tablets are obtained from market. Potassium dihydrogen phosphate and Methanol were obtained from E. Merck. O-phosphoric acid and octane sulphonate were obtained from Spectrochem. All chemicals and reagents used have an analytical or LC grade. Milli-QWater was used throughout the experiment

Chromatographic Condition

LC system used was an isocratic Shimadzu system with LC-20 AT double reciprocating pump and SPD-20 A UV detector. The out-put signal was monitored and processed using Spinchrom software. The chromatographic column Inertsil ODS (250 × 4.6 mm, 5µm) reverse phased C18 was used. The column was maintained at 40°C. The instrumental setting was at a flow of 1 ml min^{-1}. A Rheodyne injector with a 10 µl loop was used for the injection of sample. The detection wavelength was 225 nm.

Mobile Phase

The mobile phase consists of buffer containing 700 mg L^{-1} of sodium octanesulphonate as ion pair reagent and Methanol in the ratio of (30:70 v/v) and. The buffer used in the mobile phase contained 50 mM of Potassium dihydrogen phosphate in double-distilled water (pH-2.0 by o-phosphoric acid). The mobile phase was premixed and filtered through a 0.45 micron membrane filter and degassed.

Preparation of standard stock solution

All solutions were prepared on a weight basis and solution concentrations were also measured on a weight basis to avoid the use of an internal standard. Standard solution of Amisulpride was prepared by dissolving the drugs in the diluent and diluting them to the desired concentration. Methanol was used as diluent. 50 mg of Amisulpride was accurately weighed, transferred to a 50 ml volumetric flask, dissolved and diluted to 50 ml with diluent. From this stock solution 5 ml were transferred into a 50 ml volumetric flask and diluted to volume with diluent. This final solution contained 100 µg ml^{-1} of Amisulpride.

Sample Solutions (Tablets)

Twenty tablets of Amisulpride from each batch were finely ground using agate mortar and pestle. The ground material, equivalent to 100 mg of the active pharmaceutical ingredient, was extracted into diluent in a 100 ml volumetric flask by vortex mixing followed by ultra sonication and made up to volume by diluent. The solution was filtered through a 0.45-micron filter and an appropriate concentration of sample (100 µg ml^{-1} assay concentration) was prepared in diluent at the time of analysis.

Specificity/Selectivity

Specificity is the ability of the method to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include degradation products, matrix, etc. The specificity of the developed LC method for Amisulpride was carried out in the presence of its degradation products. Stress studies were performed for Amisulpride bulk drug to provide an indication of the stability-indicating property and specificity of the proposed method. Intentional degradation was attempted to stress condition exposing it with acid (5 M hydrochloric acid), alkali (5 M sodium hydroxide), hydrogen peroxide (30%), heat (110°C) and UV light (254 nm wavelength) to evaluate the ability of the proposed method to separate Amisulpride from its degradation products. For heat and light studies, the study period was 24 h whereas for acid, base and oxidative degradation the study period was 2 h in 60°C. Peak purity of the test was carried out for Amisulpride by using a PDA detector in

stress samples. Assay studies were carried out for stress samples against Amisulpride reference standard and the mass balance (% assay + % sum of all impurities + % sum of all degradants) was calculated. The most common excipients which may present in Amisulpride commercial tablets are Lactose, Starch, methyl cellulose, colloidal silica and Magnesium stearate.[16] These excipients were injected in a mixture in the optimized conditions to show the specificity of the method in formulation of Amisulpride.

Procedure for force degradation studies for Amisulpride

Acidic Degradation

5.0 ml of 5 M hydrochloric acid was added to 10.0 ml of drug solution (1000 µg ml^{-1}). This solution was allowed to stand for 2h at 90°C in water bath, the solution was allowed to attend ambient temperature then the solution was neutralized by 5 M sodium hydroxide to pH 7 and the volume made up to 100 ml with diluent.

Alkali Degradation

5.0 ml of 5 M sodium hydroxide was added to 10.0 ml of drug solution (1000µg ml^{-1}). This solution was allowed to stand for 2h at 90°C in water bath, the solution was allowed to attend ambient temperature then the solution was neutralized by 5 M hydrochloric acid to pH 7 and the volume made up to 100 ml with diluent.

Oxidative Degradation

5.0ml of 30% hydrogen peroxide was added to 10 ml of drug solution (1000 µg ml^{-1}). This solution was allowed to stand for 2h at 90°C in water bath, the solution was allowed to attend ambient temperature. The volume made up to 100 m1 with diluent.

Thermal Degradation

About 50 mg of drug substance were kept at 110°C for 24 h then the solution was prepared to achieve a final concentration of 100 µg ml^{-1}.

UV Degradations

About 50 mg of drug substance were exposed to UV short wavelength 254 nm light for 24 h. Then the solution was prepared to achieve a final concentration of 100 µg ml^{-1}.

RESULT AND DISCUSSION

Optimization of chromatographic condition

Imp-B (as per the British Pharmacopoeia) was the most likely impurity present in bulk samples. The main difficulty of the chromatographic method was to get the separation of Imp-B from the Amisulpride peak in ODS column. Attempts were made by using different C18 and C8 stationary phases. The chromatographic conditions were optimized with respect to specificity, resolution and time of analysis. Effects of pH (1.5 – 7) and ionic strength (10 - 80 mM L^{-1}) were investigated using phosphate buffer. It was found that the retention time of Amisulpride did not significantly alter at pH 2–4 and ionic strength between 30 and 60 mM L^{-1}. The optimum conditions are given in ‘‘Experimental’’. The tailing factor obtained was less than 2 and retention time was also about 28 min for the main peak and 32 min for Imp-B. The total run time is short and ultimately increases productivity thus reducing the cost of analysis per sample. The two peaks were well separated with a resolution of greater than 3.0 (Figure 3. B) and the number of theoretical plates for the Amisulpride peak was 12366.

Results of Force Degradation Experiments

Degradation was not observed in Amisulpride bulk sample during stress conditions like UV light, heating to 110°C, acid and base hydrolysis (Figure 2. A-E). In oxidative degradation non polar impurities were generated and were well separated from the peak of Amisulpride. Peak purity test results confirmed that the Amisulpride peak was homogeneous and pure in all the analyzed stress samples. The mass balance of stressed samples was close to 100%. The assay of Amisulpride is unaffected in the presence of Imp-B

Figure 2: Typical LC chromatograms of stressed test samples of Amisulpride.

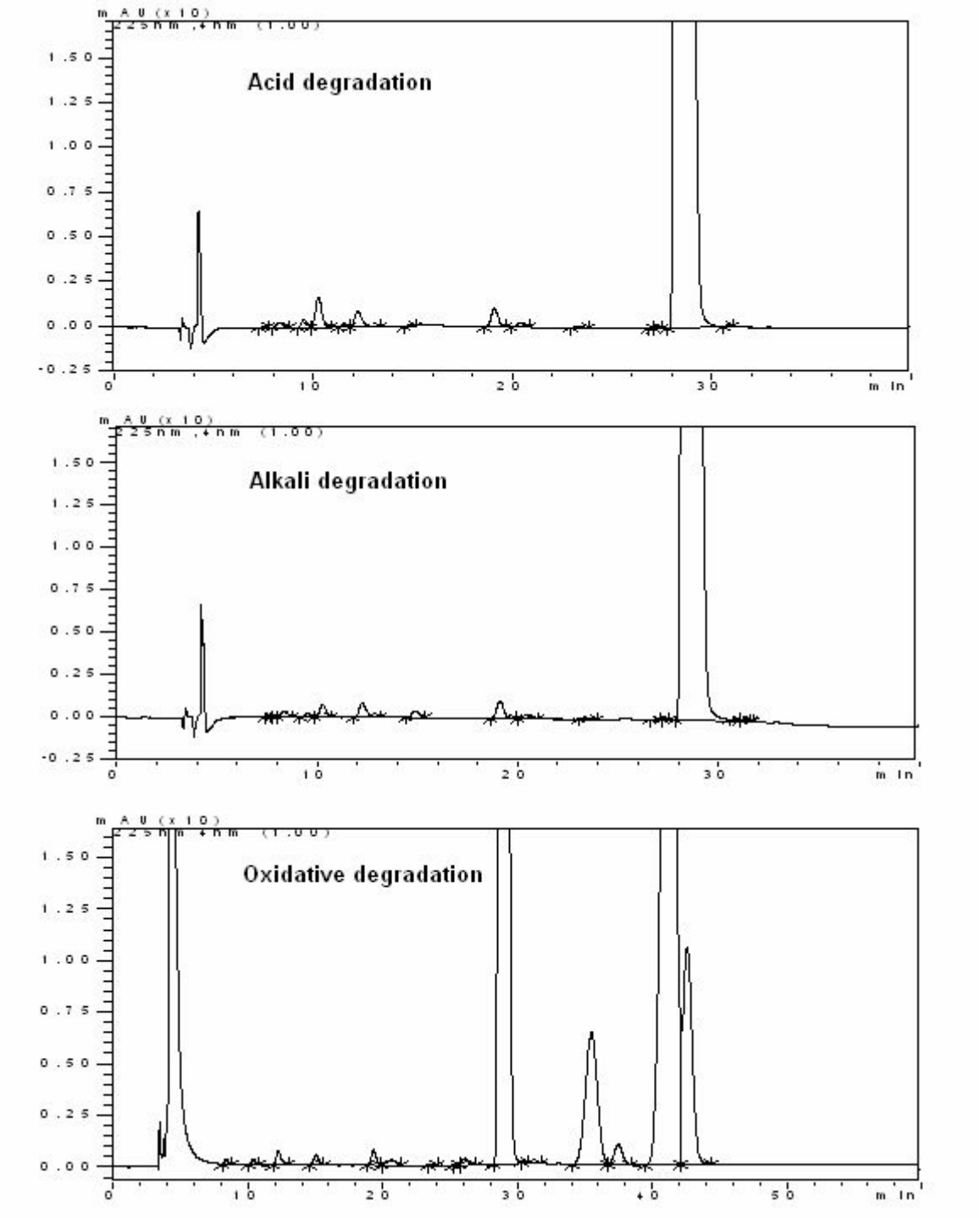
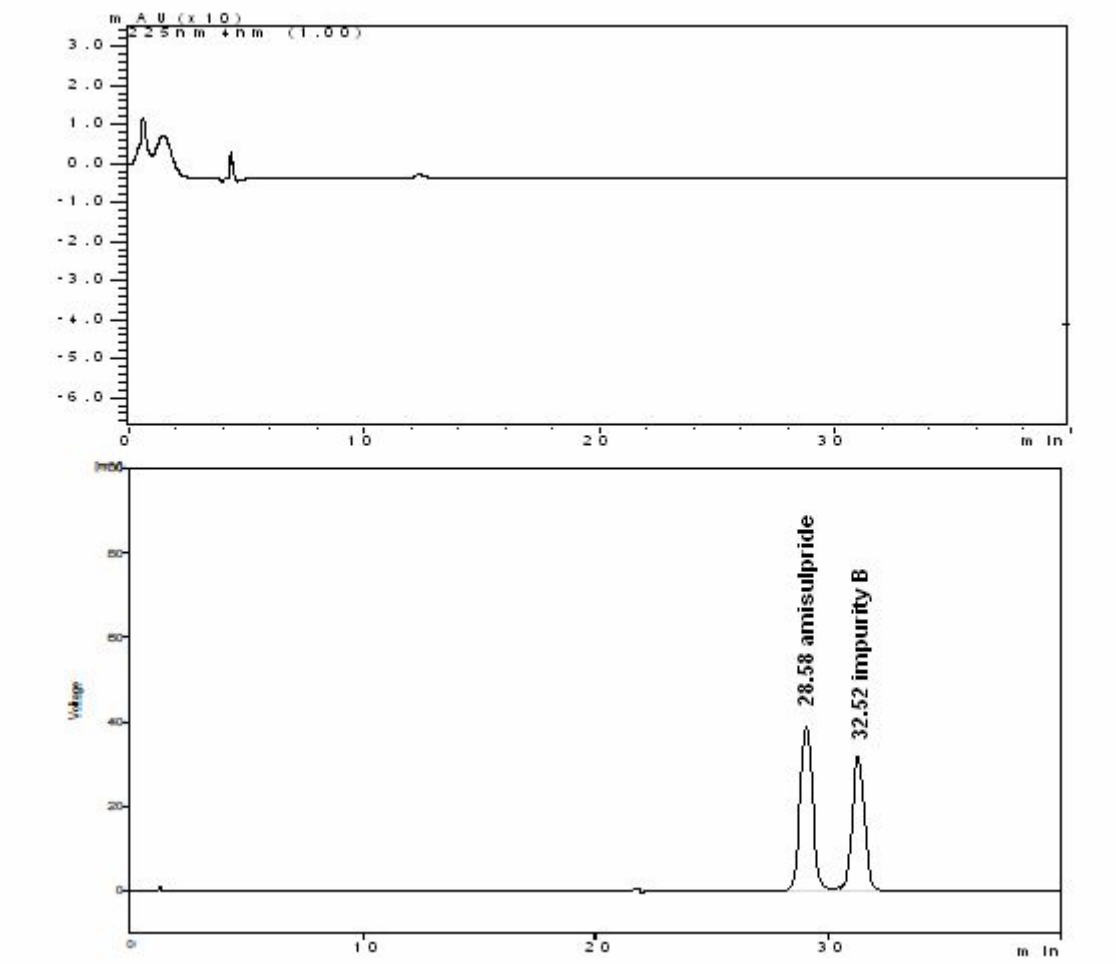


Figure 3: Typical LC chromatograms of blank and spiked samples (Imp-B)



Method Validation

Precision

Assay of method precision (intra-day precision) was evaluated by carrying out six independent assays of test samples of Amisulpride against reference standard. The intermediate precision (inter-day precision) of the method was also evaluated using two different analysts and different days in the same laboratory. The percentage of RSD and mean of six assay values obtained by two analysts were 0.25, 99.98 % and 0.56, 99.89 % respectively.

Accuracy (Recovery Test)

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the pre analysed samples. The recovery was performed at three levels, 80, 100 and 120 % of the assay concentration (100 µg ml^{-1}). Three samples were prepared for each recovery level. The recovery values for Amisulpride ranged from 98.59 to 100.13 % and the % RSD for nine determinations was 0.53.

Linearity

The linearity of the response of the drug was verified at six concentration levels, ranging from 10 to 200 % of the targeted level (100 µg ml^{-1}), of the assay concentration. Standard solutions containing 80–120 µg ml^{-1} of Amisulpride in each linearity level were prepared.

Linearity solutions were injected in duplicate. The equation of the calibration curve for Amisulpride obtained y = 59.0075x – 21.2804 where, y is the response in mV and x is concentration in µg ml^{-1}. The calibration graphs were found to be linear in the aforementioned concentrations. The coefficient of determination was 0.999. The Y-intercept bias was found 0.36 for Amisulpride.

Limit of Detection and Limit of Quantification
The limit of detection (LOD) and limit of quantification (LOQ) was found 0.5 and 1.1 µg ml^{-1} respectively. LOD and LOQ values were calculated based on signal-to-noise ratio (S/N). For LOD and LOQ, signal-to-noise ratio were 4 and 12 respectively.

Robustness:
To determine the robustness of the developed method experimental conditions were purposely altered and the percentage relative standard deviation of the area of the analyte peak were evaluated. The resolution between Amisulpride and Imp-B was not less than 2 in all conditions. The flow rate of the mobile phase was 1.0 ml/min. To study the effect of flow rate on resolution, it was changed by 0.2 units from 0.8 to 1.2 ml/min. The effect of of pH was studied by varying pH 0.2 unit from 1.8 to 2.2. The effect of percent organic strength on resolution was studied by varying methanol from -2 to +2%. The effect of column temperature on resolution was studied at 35°C and 45°C instead of 40°C, while the other components were held constant in chromatographic condition. The percentage relative standard deviation of the area of the peak due to Amisulpride was found less than 1.00. The difference between retention time obtained in optimized method and in robustness test conditions is less then 2 min for the drug.

Stability of Analytical Solution
The stability of the standard solutions and the sample solutions were tested at intervals of 6 h for 48 h. The stability of solutions was determined by comparing results of the assay of the freshly prepared standard solutions. The percent difference in area for the assay preparation and standard preparation determined up to 48 h for Amisulpride were less than 2.0 %. The results indicate that the solutions were stable for 48 h at ambient temperature.

Determination of Active Ingredients in Tablets
The validated LC method was applied to the determination of Amisulpride in tablets. Two batches of the tablets were assayed and the results are shown in Table 1 indicating that the amount of drug in tablet samples met with requirements (90 – 110 % of the label claim).

Table 1: Assay result for Amisulpride in the formulation product:

Batch	Label Value	Found (mg)	% Label Claim
1	200	199.854	99.93
2	50	49.793	99.59

CONCLUSION

The developed LC method was specific, selective robust, rugged and precise and can be used for routine quality control analysis of Amisulpride in bulk drugs and pharmaceutical dosage form. It can be conveniently used for assessing assay, related substances and dissolution of tablets of the pharmaceutical dosage form containing Amisulpride in quality control laboratories as previously reported.

ACKNOWLEDGEMENTS

The authors are grateful to Molecule Analytical Laboratories Ahmedabad for gift samples (Amisulpride and Imp-B) and for providing laboratory facilities for this research work.

REFERENCES

1. British Pharmacopoeia. Her Majesty's Stationary Office British Pharmacopoeia Commission. London; Vol. I, 2008; pp: 133.
2. Lecrubier Y, Azorin M, Bottai T, Dalery J, Garreau G, Lempérière T, Lisoprawski A, Petitjean F, Vanelle J.M. "Consensus on the practical use of amisulpride, an atypical antipsychotic, in the treatment of schizophrenia", Neuropsychobiology, 2001; 44: 41.
3. Kaplan O and Arline O. "Psychotropic medications around the world" Psychiatric Times., 2004; 21: 5.
4. Robert S, Lukasz K, Hanna H, Izabela S. "Comparative validation of amisulpride determination in pharmaceuticals by several chromatographic, electrophoretic and spectrophotometric methods", Anal. Chim. Acta., 2007; 590: 195.
5. Ascalone V, Ripamonti M, Malavasi B. "Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. Application to pharmacokinetics", J. chromatogr. B Biomed. Appli., 1996; 676: 95.

6. Péhourcq F, Ouariki S, Bégaud B. "Rapid high-performance liquid chromatographic measurement of amisulpride in human plasma: application to manage acute intoxication", J. Chromatogr. B Analyt. Technol. Biomed. Life. Sci., 2003; 789: 101.
7. Bohbot M, Doare L, Diquet B. "Determination of a new benzamide, amisulpride, in human plasma by reversed-phase ion-pair high-performance liquid chromatography", J. Chromatogr., 1987; 416: 414.
8. Nirogi R, Bhyrapuneni G, Kandikere V, Mudigonda K, Ajjala D, Suraneni R, Mukkanti K. "Liquid chromatography tandem mass spectrometry method for the quantification of amisulpride with LLOQ of 100 pg/mL using 100 µL of plasma", Biomed. Chromatogr., 2008; 22: 1424.
9. Moulin A, Truffer D, Rauch-Desanti C, Istin M, Grognet JM, Dufour A. "Comparison of HPLC and RIA methods applied to the quantification of amisulpride in human plasma", PubliCEA., 1991; 507.
10. Mokrim R, Brunet C, Cazin M, Gressier B, Luyckx M, Dine T, Robert H, Cazin JC. "Amisulpride evaluation by radioreceptor assay comparison with HPLC procedure after single administration in the rabbit", Methods Find. Exp. Clin. Pharmacol., 1993; 15: 41.
11. Julia Sachse, Sebastian Härtter, Harald Weigmann, Christoph Hiemke. "Automated determination of amisulpride by liquid chromatography with column switching and spectrophotometric detection", J. Chromatogr. B., 2003; 784: 405.
12. Musenga A, Mandrioli R, Morganti E, Fanali S, Raggi MA. "Enantioselective analysis of amisulpride in pharmaceutical formulations by means of capillary electrophoresis", J. Pharm. Biomed. Anal., 2008; 46: 966
13. Özkan SA, Bengi Uslu, Zühre Sentürk. "Electroanalytical characteristics of amisulpride and voltammetric determination of the drug in pharmaceuticals and biological media", Electroanalysis, 2004; 16: 231.
14. Stability Testing of New Drug Substances and Products (Q1AR2), ICH Harmonised Tripartite Guideline, 2003.
15. Validation of Analytical Procedures: Methodology (Q2R1), ICH Harmonized Tripartite Guidelines, 2005
16. Public Assessment reports. Amisulpride Tablets. 50, 100, 200, 400 mg.Focus Pharmaceutical Ltd. Medicine and Healthcare products Regulatory Agency: London, 2006.
Website:
http://www.mhra.gov.uk/Publications/PublicAssessmentReports

*****