Turk J Pharm Sci 10 (3), 367-376, 2013

Original article

STABILITY INDICATING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY AND CONTENT UNIFORMITY STUDY OF AMISULPRIDE IN PHARMACEUTICAL DOSAGE FORM

Batuk DABHI[1], Kalpen CHAVDA[1], Anand JINDANI[1], Vishwa DHINOJA[1], Madhavi PATEL[1], Hetal JEBALIYA[1], Yashwantsinh JADEJA[1], Denish KARIA[2], Anamik SHAH*[1]

[1] Saurashtra University, Department of Chemistry, Rajkot-360 005, Gujarat, INDIA
[2]Arts Commerce and Science College, Borsad, Anand, Gujarat, INDIA

Abstract

A reliable and sensitive isocratic stability indicating RP-UPLC method has been developed and validated for quantitative analysis and content uniformity study of Amisulpride in tablets. An isocratic method for analysis of Amisulpride was achieved on C18 (100x2.1) mm particle size 1.7 µ columns at of 0.20 mL/min flow rate within shorter runtime of 3 min. Photodiode array detector was used to monitor the eluate at 280 nm. The mobile phase consisted of Buffer-ACN (50:50 v/v), (Buffer: 2 mL Ortho phosphoric acid in 1L water). The drug was subjected to oxidation, hydrolysis, photolysis and thermal degradation. Response was a linear function of drug concentration in the range of 20-80 µg/ml (r^2= 0.999) with a limit of detection and quantification of 0.1 and 0.3 µg/mL respectively. Accuracy (recovery) was between 99 to 101 %. Degradation products resulting from the stress studies did not interfere with the detection of Amisulpride the assay is stability-indicating.

Key words: *Method validation, Amisulpride, Content uniformity, UPLC.*

Farmasötik Dozaj Formlarındaki Amisülpiridin İçerik Uygunluğu Analizi İçin Stabilite Göstergeli Bir Ultra Performans Sıvı Kromatografik Yöntem

Amisülprid'in kantitatif analizi ve tabletlerde içerik uygunluğu analizi için güvenilir ve duyarlı bir stabilite gösterici izokratik RP-UPLC metodu geliştirilmiş ve validasyonu yapılmıştır. Amisülprid analizi, bu izokratik metot ile C18 (100×2.1)mm ve partikül büyüklüğü 17 µ olan kolon üzerinde 0.20 mL/dk akış hızında ve 3 dk'dan daha kısa süre içerisinde gerçekleştirilmiştir. Eluat kontrolü için 280 nm'de fotodiyot dizi dedektörü kullanılmıştır. Hareketli faz tampon-ACN (50:50 h/h) bileşiminden oluşmaktadır (Tampon: 1L su içerisinde 2 mL ortofosforik asit). İlaç; oksitlenme, hidroliz, fotoliz ve termal bozunmaya tabi tutulmuştur. Sırasıyla 0.1 ve 0.3 µg/mL gözlenebilme ve tayin sınırları içerisinde 20-80 µg/mL (r^2=0.999) konsantrasyon aralığında amisülprid için doğrusal bir cevap alınmıştır. Doğruluk (geri kazanım) % 99 – 101 arasındadır. Stres çalışmaları sonucunda meydana gelen bozunma ürünleri amisülprid'in tayinine girişim yapmamıştır ve çalışma stabilite göstericidir.

Anahtar kelimeler: *Yöntem validasyonu, Amisulprid, İçerik uygunluğu, UPLC.*

***Correspondence:** E-mail: anamik_shah@hotmail.com; Tel: 9825215656

INTRODUCTION

Amisulpride (Fig 1) is chemically, 4-Amino-N-[(1-ethyl-2-pyrrolidinyl) methyl]-5-ethylsulfonyl)-2-benzamide; 4-amino-n-((1-ethyl-2-pyrrolidinyl) methyl)-5-(ethylsulfonyl)-2-methoxybenzamide. It belongs to a class of drugs called antipsychotic used to treat psychosis in schizophrenia and episodes of mania in bipolar disorder. In small doses it is also used to treat depression.

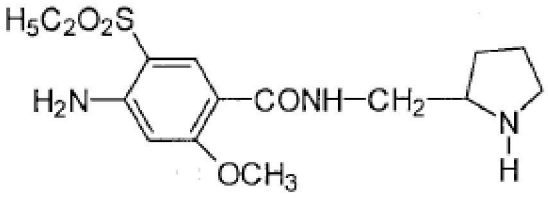

Figure 1. Amisulpride.

A detailed survey of analytical literature for Amisulpride revealed few methods based on a variety of techniques such as UV-Spectrophotometry, High performance thin layer chromatography(HPTLC),HPLC and UPLC. Since a UPLC method has many advantages over that of a HPTLC & HPLC method. UPLC is often the first choice for developing an analytical method as compare to HPTLC and HPLC. Quantitative analysis of Amisulpride by HPLC(1) , by spectrometric(2) and HPLC method in human plasma(3-6) ,quantitative analysis by mass analysis(7-11), and some HPLC(12, 13) methods of analysis as good separation technique and comparison of different methods are reported in literature. None of the reported analytical procedure describes UPLC method for quantitative analysis of Amisulpride.

The objective of this work was to develop a simple, precise, reliable and rapid stability indicating liquid chromatographic method for assay of Amisulpride and for determination of the content uniformity of a tablet formulation.

MATERIALS AND METHODS

Amisulpride reference standard (label claim 99.24 % pure) was provided by sun pharmaceutical industries Ltd. HPLC grade acetonitrile and orthophosphoric acids were obtained from Merck India Limited, Mumbai, India. Analytical grade hydrochloric acid sodium hydroxide pellets and hydrogen peroxide solution 30 %(v/v) were obtained from Ranbaxy and 0.45 filters was obtained from Ranbaxy. High quality deionised water was obtained from a Milli-Q (Millipore, Milford, MA, USP) purification system.

Chromatography

The chromatography system used to perform development and validation of this assay method consisted of Waters UPLC with PDA detector connect to a multiple instrument data acquisition and data processing system (Empower).Chromatographic analysis was performed on acquity BEH phenyl (100x2.1 mm id,1.7 µm particle size) column. Seperation was achieved using a mobile phase consist of actonitrile-OPA (Ortho Phosphoric Acid) buffer (50:50,v/v) solution at flow rate of 0.2 mL/min. The eluent was monitored using PDA detector at a wavelength 280 nm. The Column was maintained at 30°C temperature and injection volume 1 µL was used. Mobile phase was filtered through 0.45 and 0.22 µm filter prior to use.

Preparation of buffer solution

2 mL HPLC grade ortho phosphoric acid in 1 L milli-Q water.

Preparation of standard solutions

Stock solution (500 µg/mL) of Amisulpride standard was prepared by transferring accurately weighed 25 mg of amisulpride standard into a 50 ml volumetric flask and adding 35 ml acetonitrile. Mixture was sonicated for 2 min to dissolve the amisupride and the solution was then diluted to volume with the same solvent.

Standard stock solution (50 µg/mL) was prepared by diluting 5 mL standard stock solution to 50 mL, in a volumetric flask, with the same solvent mixture.

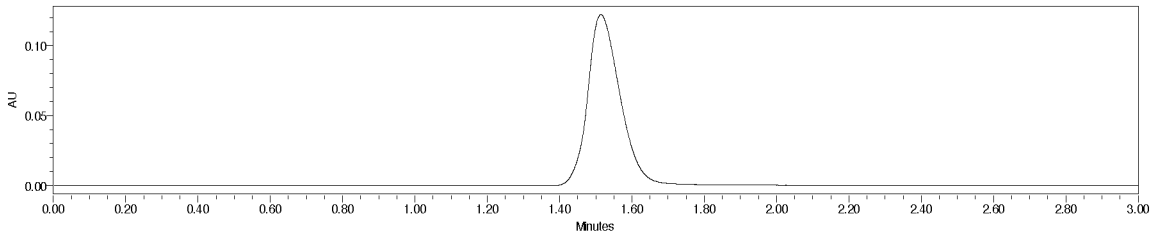

Figure 2. Chromatogram of standard amisulpride.

Preparation of test solutions

To prepare stock solution (500 µg/mL) for assay, 20 tablets were weighed and mixed. An aliquot of power equivalent to the weight of 5 tablets was accurately weighed and transferred to 100 mL volumetric flask. Acetonitrile, 70 ml was added to the flask and the mixture was sonicated for 2 min with normal hand shaking. The contents of flask were then left to return to room temperature and diluted to volume with the same solvent. This solution (10 mL) was filtered through a 0.45 and 0.22 µm nylon syringe filters.

To prepare test solution (50 µg/mL) for assay 5 ml test stock solution was transferred to 50 mL volumetric falsk and diluted to volumetric flask and dilute to volume with Acetonitrile and shakewell.

Method validation

Method was developed using same concentration of analyte for both, assay and determination of content uniformity study. Method could be validated simultaneously except determination of precision for content uniformity study. The specificity of the method was evaluated and there was no interference from placebo components (prepare in solution) or from products resulting from forced degradation.

Forced degradation studies

To perform the forced degradation study 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and photolytic conditions. For acidic degradation the drug was heated under reflux with 1M HCl at 80 °C for 2 hr and the mixture was neutralized. For alkaline degradation the drug was treated with 0.1M NaOH at 80 °C for 2 hr and the mixture was neutralized. For degradation under oxidizing conditions the drug was heated under reflux with (6 % v/v) H_2O_2 at 80° for 2 hr. For thermal degradation the powered drug was exposed at 70 °C for 48 hr. For photolytic degradation, powdered drug was exposed to sunlight for 48 hr. The placebo was also

subjected to the same stress condition to determine whether any peaks arose from the declared recipients. After completion of the treatments the solutions were left to return to room temperature and diluted with acetonitrile to furnish 50 μg/mL solutions. The purity of the drug peak obtained from the stressed sample was measured using PDA detector.

Precision

System precision was evaluated by analyzing the standard solution five times and method precision (repeatability) was evaluated by assaying six sets of test samples prepared for assay determination and ten sets of samples prepared for determination of content uniformity, all on same day(intra-day precision) system precision and method precision were also determined by performing the same procedures on a different day (inter-day precision) and by another person under the same experimental conditions(intermediate precision).

Linearity

Eight solution was prepared containing 20 μg/mL, 30 μg/mL, 40 μg/mL, 50 μg/mL, 60 μg/mL, 70 μg/mL, 80 μg/mL amisupride concentration which correspond to 40, 60, 80, 100, 120, 140 and 160 % respectively, of the test solution concentration .Each solution was injected in duplicate. Linearity was evaluated by linear-regression analysis.

Accuracy

Accuracy was assessed by determination of the recovery of the method at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration, three sets were prepared and injected in duplicate.

Robustness

The robustness of the method was evaluated by assaying test solution after slight but deliberate changes in the analytical conditions. The factor chosen for this study were the flow rate (±0.1 mL/min),mobile phase composition (Buffer-Acetonitrile, 48:52 and 52:48,v/v) and using different batch of UPLC column.

Solution stability

Stability of solution was evaluated for standard solution and the test preparation. The solution were stored at 5°C and at ambient temprature without protection of light and tested after 12, 24, 36 and 48 hr. The response for the aged solution was evaluated by comparision with freshly prepared solution.

System stability

The stability of the chromatographic system was tested before each stage of validation. Five replicate injection of standard preparation were injected and asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

RESULT AND DISCUSSION

In this work an analytical UPLC method for assay of amisulpride in a tablet formulation was developed and validated. The basic chromatographic condition were designed to be simple and easy to use and reproduce and were selected after testing the different condition that affect UPLC analysis, for example column, aqueous and organic components of mobile phase, proportion of mobile phase components, detection wavelength, diluents and concentration of analyte. Acquity BEH phenyl column (100 x 2.1 mm) particle size 1.7 μ was used because of its advantages of high resolving capacity better reproducibility and low tailing.

For mobile phases of different composition containing water adjusted to acidic pH by addition of Ortho phosphoric acid and methanol resulted in poor peak shape. When methanol was replaced by acetonitrile better peak shape was obtained. The portion of mobile phase components was optimized to reduce retention times and enable good resolution of amisulpride from the degradation products.

Table 1. Linearity study of amisulpride.

Linearity	Level % of Level	Concentration (µg/mL)	Mean area
1	40	20.02	302115
2	60	30.02	457352
3	80	40.03	602114
4	100	50.04	753785
5	120	60.05	903887
6	140	70.06	1049288
7	160	80.06	1204156
		Correlation co-efficient:	0.999
		Slope:	14970
		Intercept:	15441

A detection wavelength of 280 nm was selected after scanning the standard solution over range 190-400 nm by use of the PDA detector. Detection at 280 nm resulted in good response and good linearity.

The drug substance was easily extracted from the pharmaceutical dosage form using water: acetonitrile (50:50,v/v). The tablet dispersed readily in water and the drug substance was freely soluble in acetonitrile. Solutions of standard and test preparations were found to be stable in this solvent mixture, by using same concentration of analyte for assay and for determination of content uniformity both methods could be validated simultaneously except for determination of precision.

After development of the analytical method, it was validated in accordance with ICH (14, 15) and USP guidelines. This furnished evidence the method was determined by checking for interface with the drug from placebo components. The specificity of the method was also evaluated by the forced degradation study. The peak purity angle is smaller than that of peak threshold angle means there was no interface with the analyte peak from degradation products. major degradation up to 9.50 % occurred under photolytic condition. Under thermal condition the drug was degraded by approximately 7.50 %, 7.95 % degraded under Acidic condition, 4.27 % degradation occure under alkali conditions and 1.90 % degradation occure under oxidative condition.

To determine linearity a calibration graph was obtained by plotting amisupride concentration against peak area. Linearity was good in the concentration range 20-80 µg/mL. The regression equation was y = 149706x + 154418 where x is the concentration in µg/mL and y is the peak area in absorbance units; the correlation coefficient was 0.999.

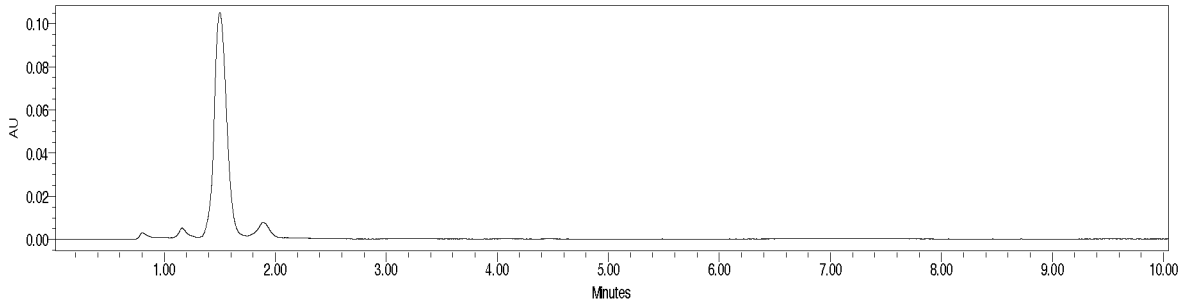

Figure 3. Acid degradation study of amisulpride.

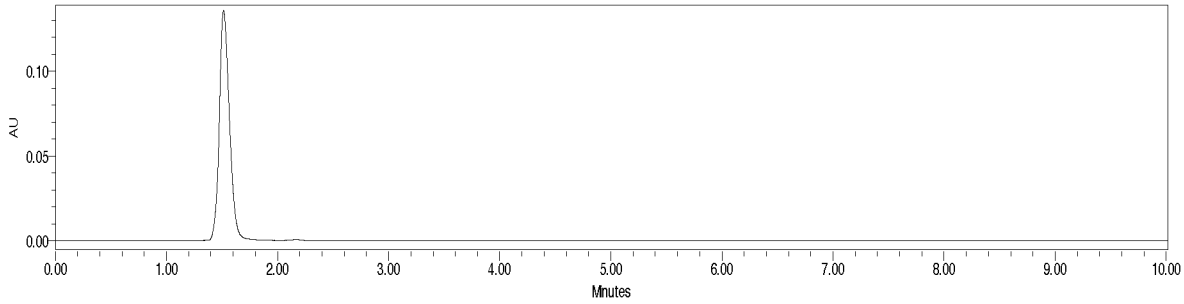

Figure 4. Base degradation study of amisulpride.

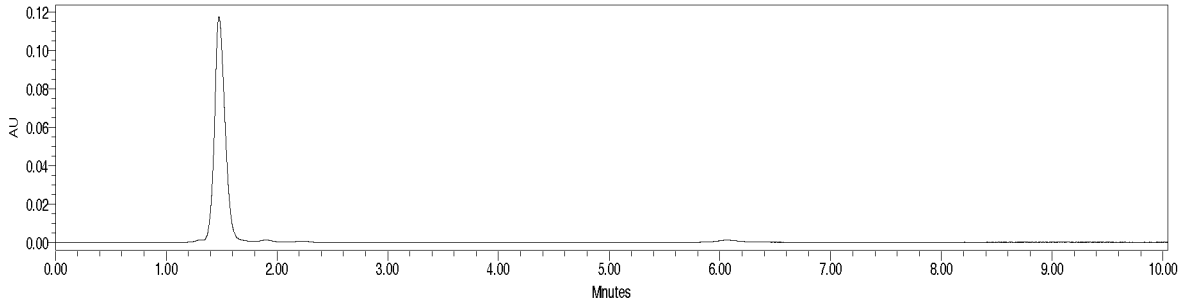

Figure 5. H_2O_2 Degradation study of amisulpride.

For assay (n=6) and determination of content uniformity (n=10). % RSD for the system precision was 0.45 % and 0.37 % respectively, on the same day (intra-day) and 0.41 % and 0.55 % on different day (inter-day).The mean values of method precision (repeatability) were 101.3 %, RSD 0.28 % for assay on the same day(intra-day) and 101.6 %, RSD 0.61 % for assay on the different days (inter-day). Intermediate precision was established by determining the overall (inter-day and inter-day) method precision for assay. For intermediate precision, overall assay value (n=12) was 101.4 and % RSD was 0.37 %.

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method, known amounts of amisupride (25, 50 and 75 µg/mL) were added to a placebo preparation and the amount of amisupride recovered, in the

presence of placebo interference, was calculated. The mean recovery of the amisupride was between 99.6 and 101.7 % which is satisfactory.

Table 2. Degradation study of amisulpride.

Degradation Condition	Total Degradation, %	Major Impurity, %
Acidic	7.95	6.22
Alkali	4.27	0.40
Oxidative	1.90	2.00
Thermal	7.50	5.78
Photolytic	9.50	0.12

The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method, known amounts of amisupride (25, 50 and 75 µg/mL) were added to a placebo preparation and the amount of amisupride recovered, in the presence of placebo interference, was calculated. The mean recovery of the amisupride was between 99.6 and 101.7 % which is satisfactory.

Table 3. Accuracy Study of amisulpride.

Levels %	No	Amount of drug added(µg/mL)	Amount of drug found(µg/mL)	Recovery (%)	Mean recovery (%)	RSD(%)
	1	25.20	25.18	99.92		
50	2	25.43	25.35	99.69	100.01	0.38
	3	25.50	25.61	100.43		
	1	50.39	50.26	99.74		
100	2	50.81	50.89	100.16	99.89	0.23
	3	50.70	50.59	99.78		
	1	74.96	75.15	100.25		
150	2	75.26	75.21	99.93	100.03	0.19
	3	75.42	75.35	99.91		

The robustness of the method was assessed by assaying test solution under different analytical conditions deliberately changed from the original conditions. For each different analytical condition the standard solution and test solution were prepared separately. The result obtained from assay of the test solution was not affected by varying the condition and was in accordance with the true value. System suitability data were also found to be satisfactory during variation of the analytical conditions. The analytical method therefore remained unaffected by slight but deliberate changes in the analytical conditions.

During study of the stability of the stored solution of standards and test preparations for assay determination the solutions were found to be stable for up to 36 h. Assay values obtained after 36 h were statistically identical with the initial value without measurable loss. Before each measurement of validation data, a system suitability test was performed by measurement of general characteristics such as peak asymmetry, number of theoretical plates and RSD (%) of

peak area observed for a standard solution. The values obtained were satisfactory and in accordance with in-house limits.

Table 4. Precission study of amisulpride.

Study	Sr.No.	Assay (%)	Mean Assay (%)	Std. Dev.	% RSD
Method Precision	1	99.15	99.35	0.2868	0.2586
	2	99.50			
	3	99.92			
	4	99.32			
	5	99.20			
	6	99.33			
Intermediate Precision	1	99.29	99.21	0.0891	0.0899
	2	99.26			
	3	99.31			
	4	99.20			
	5	99.13			
	6	99.09			

The intensive approach described in this manuscript was used to develop and validate a liquid chromatographic analytical method that can be used for assay of amisupride in a pharmaceutical dosage form. Degradation products produced as a result of stress did not interfere with detection of amisupride and the assay methods can thus be regarded as stability indicating.

Table 5. Robustness study of amisulpride.

Robust Condition	Assay (%)	% Assay Difference	RT, Minutes	System Suitability Parameters	
				Theoretical Plates	Asymmetry
At 0.19 mL/min flow rate	99.35	0.00	1.59	5215	1.32
At 0.21 m/min flow rate	99.29	0.06	1.44	5421	1.32
Buffer-Acetonitrile (52:48, v/v)	99.36	0.01	1.52	5368	1.34
Buffer-Acetonitrile (48:52, v/v)	99.35	0.00	1.50	5497	1.33
Column (Lot Change)	99.33	0.02	1.52	5342	1.33

This HPLC method for assay of amisupride in a tablet formulation was successfully developed and validated for its intended purpose. The method was shown to specific, linear, precise, accurate and robust. Because the method separates amisupride and all degradation products formed under variety of stress conditions it can be regarded as stability indicating. This

mehtod is recommended to the industry for quality control of drug content in pharmaceutical preparation.

ACKNOWLEDGEMENT

The author are grateful to the Sun Pharmaceuticals, ankleswer, Gujarat, India and the Department of Chemistry. Saurashtra university (UGC-SAP Sponsored and DST-FIST Funded) Rajkot, Gujarat, India, respectively, for the gift samples of amisulpride, standard and for providing instrumental facilities. Special thanks are due to "National Facility for Drug Discovery through New Chemicals Entities (NCE's) Development and Instrumentation Support to Small Manufacturing Pharma Entities" Program under Drug and Pharma Research Support (DPRS) jointly funded by Department of Science & Technology, New Delhi, Goverment of Gujarat Industries Commissionerate and Saurashtra University, Rajkot.

REFERENCES

1. Malavasi B, Locatelli M, Ripamonti M, Ascalone V, Determination of amisulpride, a new benzamide derivative, in human plasma and urine by liquid-liquid extraction or solid-phase extraction in combination with highperformance liquid chromatography and fluorescence detection, Application to pharmacokinetics, J Chromatogr B Biomed Appl 676, 107–115, 1996.
2. Robert S, Łukasz K, Hanna H, Izabela S, Comparative validation of amisulpride determination in pharmaceuticals by several chromatographic, electrophoretic and spectrophotometric methods, Anal Chim Acta, 590(2), 195-202, 2007.
3. Pehourcq F, Ouariki S, Begaud 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, 789, 101–105, 2003.
4. Sachse J, Hartter S, Weigmann H, Hiemke C, Automated determination of amisulpride by liquid chromatography with column switching and spectrophotometric detection, J Chromatogr B Analyt Technol Biomed Life Sci, 784, 405–410, 2003.
5. 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, Eur J Drug Metab Pharmacokinet 3, 507–512, 1991.
6. Kudris IV, Skakun NN, Orlova IN, Libina VV, Kulikov AU, Analysis of amisulpride in human plasma by SPE and LC with fluorescence detection, Chromatographia, 73, 67–74, 2011.
7. 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 microL of plasma, Biomed Chromatogr 22, 1424–1433, 2008.
8. Gschwend MH, Arnold P, Ring J, Martin W, Selective and sensitive determination of amisulpride in human plasma by liquid chromatography-tandem mass spectrometry with positive electrospray ionisation and multiple reaction monitoring, J Chromatogr B Analyt Technol Biomed Life Sci 831, 132–139, 2006.
9. Couchman L, Morgan PE, Flanagan RJ, Basic drug analysis by strong cation-exchange liquid chromatography-tandem mass spectrometry simultaneous analysis of amisulpride and metamfetamine and amphetamine in serum/plasma, Biomed Chromatogr 25, 867–872, 2011.

10. Zou S, Wen Y, Lin Q, Determination of amisulpride in plasma by LC/MS/MS, Zhongguo Xiandai Yingyong Yaoxue 26, 483–486, 2009.
11. Jitsufuchi N, Kudo K, Tokunaga H, Imamura T, Selective determination of sultopride in human plasma using highperformance liquid chromatography with ultraviolet detection and particle beam mass spectrometry, J Chromatogr B Biomed Sci Appl 690, 153–159, 1997.
12. Ghulam A, Tony K, Development and validation of Liquid chromatography method for the determination of methyl salicylate in a medicated cream formulation, Turk J Pharm Sci 8 (2), 117-126, 2011.
13. Maryam H, Application of UV-Spectrophotometry and HPLC for determination of Venlafaxone and its four related substance in pharmaceutical dosage forms, Turk J Pharm Sci 8 (2), 91-104, 2011.
14. ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October 1994, 1-5.
15. ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November 1996, 1-8.

Received: 26.09.2012
Accepted: 02.01.2013