International Journal of Biomedical and Pharmaceutical Sciences ©2013 Global Science Books

Stability-Indicating High Performance Liquid Chromatographic Method for Estimation of 9-Hydroxyrisperidone in Tablet Formulation

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ABSTRACT

A stability-indicating high performance liquid chromatographic (HPLC) method for estimation of 9-hydroxyrisperidone (HRD) was developed and validated. HRD was separated and quantified on a Nucleosil C8 column (150 mm length, 4.6 mm id, 5 µm particle size) using a blend of methanol-ammonium acetate buffer pH 5.0 (95/05 v/v) as a mobile phase and at a flow rate of 1.3 mL/min. Quantification was achieved with a UV detector at 238 nm over a concentration range of 5-30 µg/mL. The applied HPLC method allowed separation and quantification of HRD with good linearity (r^2 – 0.999) in the studied concentration range. The limit of detection and limit of quantification were found to be 1.04 and 3.16 µg/mL, respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. HRD stock solution was subjected to different stress conditions. The degraded product peaks were well resolved from the pure drug peak with significant differences in their retention time values. Stressed samples were assayed using the developed HPLC method. Statistical analysis of the data showed that the method is precise, accurate, reproducible, and selective for the analysis of HRD. The method was successfully applied for the estimation of HRD in tablet dosage form.

Keywords: HPLC, 9-hydroxyrisperidone, stability-indicating method
Abbreviations: **HPLC**, High performance liquid chromatography; **HPTLC**, high performance thin layer chromatography; **HRD**, 9-hydroxyrisperidone; **ICH**, International Conference on Harmonization

INTRODUCTION

9-Hydroxyrisperidone (HRD), (±)-3-{2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)piperidino]ethyl}-6,7,8,9-tetrahydro-9-hydroxy-2-methyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one, is a primary active metabolite of an older antipsychotic drug, resperidone. HRD has been approved by the FDA for the treatment of schizophrenia since 2006 (Tan *et al*. 2009; Patel *et al*. 2010).

A literature survey revealed one high performance thin layer chromatography (HPTLC) (Patel *et al*. 2010) method which was developed and reported from our laboratory for the determination of HRD in microemulsion formulations and for its *in vitro* release. Tan *et al*. (2009) developed an LC-MS-MS method using Gemini 3 µm C18 (50 mm × 4.6 mm) HPLC column with a gradient mobile phase of water/methanol/formic acid/ammonium hydroxide at a flow rate of 1.0 ml/min for the detection and estimation of HRD in human plasma using Sciex API 4000 Liquid Chromatography - Mass Spectrometry - Mass Spectrometry (LC-MS-MS) in ionspray mode. Jadhav *et al*. (2011) developed and reported a Reverse Phase Liquid Chromatography (RP-LC) method using Hypersil BDS C18 (250 mm × 4.6 mm, 5 µm) column, thermostated at 45°C for detection of different process related to impurities in the bulk sample of HRD. Jane *et al.* (2011) reported HPLC, visible spectrophotometric and UV spectrophotometric methods for estimation of HRD in bulk and dosage forms. The LC methods reported for the estimation of HRD in dosage form show longer retention times and consequently generate a large amount of solvent waste, which may adversely affect the environment, therefore they demand expensive disposal processes. In this context, we attempted to develop a stability-indicating LC method to estimate HRD which is simple and rapid.

The present study describes the validation of a stability-indicating RP-HPLC method for determination of HRD in the presence of its degradation products according to International Conference on Harmonization (ICH) guideline. The developed method was applied for routine analysis of HRD in pharmaceutical tablet dosage form.

EXPERIMENTAL

Materials and methods

The pure HRD powder was obtained form Torrent Research Centre (Gandhinagar, India). HPLC grade methanol, acetonitrile, water, triethylamine, tetrahydrofuran were purchased from E. Merck (India) Ltd., Mumbai. Analytical grade sodium hydroxide, hydrochloric acid, potassium dehydrogen phosphate, ammonium acetate, glacial acetic acid were purchased from Allied Chemical Corporation, Baroda.

Apparatus

The method was developed using a PerkinElmer (Waltham, MA) Series 200 HPLC instrument equipped with a Series 200 diode array detector, Series 200 quaternary gradient pump, Series 200 column oven, manual injector (Rheodyne valve) with 20 mL fixed loop, Turbochrom navigator software (Version 6.1.1.0.0:K20), and Nucleosil (SGE, Austin, TX) C8 column (150 × 4.6 mm id, and 5 µm particle size).

Chromatographic condition

The Nucleosil C8 column was used at ambient temperature. The

Received: 12 October, 2012. Accepted: 28 October, 2012.

Original Research Paper

mobile phase consisted of methanol-ammonium acetate buffer (95 + 05, v/v) with the final pH adjusted to 5.0 ± 0.02 and was pumped at flow rate 1.3 mL/min. The mobile phase was filtered through a Nylon 0.45 µm membrane filter and degassed before use. The elution was monitored at 238 nm, and the injection volume was 20 µL.

Preparation of 9-hydroxyrisperidone standard stock solution

A 10 mg of standard HRD accurately weighed and transferred to a 100 ml volumetric flask and dissolved in 30 ml mobile phase. The flask was sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 100 µg/ml HRD. The above solution was filtered through Whatman filter paper (0.45 µm).

Sample preparation for determination of 9-hydroxyrisperidone in tablet dosage form

Twenty tablets were weighed and finely powdered. A mass equivalent to 10 mg HRD was weighed and transferred to a 100 mL volumetric flask, and mobile phase (15 mL) was added. The solution was sonicated for 15 min, and the final volume was diluted to the mark with mobile phase to obtain a solution of HRD (100 mg/mL). An aliquot of this solution was further diluted to 100 mL with mobile phase to obtain a 10 mg/mL solution of HRD, which was used for determination of HRD. The solutions were filtered through a Nylon 0.45 mm membrane filter.

METHOD VALIDATION

Validation of the developed HPLC method was carried out as per the ICH guidelines Q2 (R1).

Specificity

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. Commonly used excipients (starch, microcrystalline cellulose and magnesium stearate) were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined. Specificity was also studied by performing forced degradation study using acid and alkali hydrolysis, chemical oxidation and dry heat degradation studies and interference of the degradation products were investigated. HRD was weighed (100 mg) and transferred to two separate 100 ml volumetric flasks, dissolved in few ml of methanol and diluted up to the mark with methanol. This stock solution was used for forced degradation studies.

1. Alkali hydrolysis

To the separate 25 ml volumetric flask, 1 ml stock solutions of HRD was taken and 5 ml of 3M NaOH was added to perform base hydrolysis. Flasks was heated at 80°C for 2 h and allowed to cool to room temperature. Solution was neutralized with 3M HCl and diluted up to the mark with mobile phase.

2. Acid hydrolysis

To the separate 25 ml volumetric flask, 1 ml stock solutions of HRD was taken and 5 ml of 3M HCl was added to perform acid hydrolysis. Flasks was heated at 80°C for 2 h and allowed to cool to room temperature. Solution was neutralized with 3M NaOH and diluted up to the mark with mobile phase.

3. Oxidative stress degradation

To perform oxidative stress degradation, appropriate aliquots of stock solutions of HRD was taken in separate 25 ml volumetric flasks and 5 ml of 3% hydrogen peroxide was added. Flask was heated in a water bath at 80°C for 2 h. and allowed to cool to room temperature and diluted up to the mark with mobile phase.

4. Dry heat degradation

Analytically pure sample of HRD was exposed in oven at 80°C for 2 h. The solid was allowed to cool and 100 mg of HRD was weighed, transferred to separate 100 ml volumetric flask and dissolved in few ml of methanol. Volume was made up to the mark with the methanol. Solution was further diluted by mobile phase taking appropriate aliquots.

All solutions were passed through a 0.45 µm Whatman filter paper and injected in the liquid chromatographic system and chromatograms were recorded.

Calibration curve (linearity of the HPLC method).

Calibration curves were constructed by plotting peak area vs. concentrations of HRD, and the regression equations were calculated. The calibration curves were plotted over the six different concentration range 5-30 µg/mL of HRD. Aliquots of standard working solution (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL) were transferred to a series of 10 mL volumetric flasks and diluted to the mark with mobile phase. Aliquots (20 µL) of each solutions were injected under the operating chromatographic condition described above (n = 6).

Precision

The intra-day and inter-day precision studies were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for three different concentrations of HRD (10, 15, 20 µg/mL), and the results are reported in terms of relative standard deviation. The instrumental precision studies were carried out by estimating response of 3 different concentrations of HRD (10, 15, 20 µg/mL) six times and results are reported in terms of relative standard deviation.

Detection limit and quantitation limit

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Accuracy (% recovery)

The accuracy of the method was studied at three levels: 80, 100 and 120%. The accuracy of the method was determined by calculating recovery of HRD by the standard addition method. Known amounts of standard solution of HRD (0, 18, 20 and 22 µg/mL) were added to prequantified sample solutions of tablet dosage form. The amount of HRD was estimated by applying values of peak area to the regression equations of the calibration curve.

Robustness

Robustness of method was studied by changing the flow rate (±10%) composition of organic phase (±2%) and the pH (± 0.2%) of mobile phase.

Solution stability

Stability of sample solutions was studied at ambient temperature for 24 h.

Table 1 Degradation of 9-hydroxyrisperidone under different stress conditions.

Stress condition		Name	Standard 9-Hydroxyrisperidone concentration	Retention time (min)	% Area
Acidic	API	HRD	20 µg/ml	2.60	85.20
		Unknown I	20 µg/ml	5.51	14.80
	SAMPLE	HRD	20 µg/ml	2.61	84.29
		Unknown I	20 µg/ml	5.52	15.69
Alkali	API	HRD	20 µg/ml	2.62	82.00
		Unknown I	20 µg/ml	5.52	15.80
		Unknown II	20 µg/ml	6.31	2.20
	SAMPLE	HRD	20 µg/ml	2.62	83.65
		Unknown I	20 µg/ml	5.52	16.34
Oxidative	API	HRD	20 µg/ml	2.60	100
	SAMPLE	HRD	20 µg/ml	2.60	100
Thermal	API	HRD	20 µg/ml	2.60	100
	SAMPLE	HRD	20 µg/ml	2.60	100

System suitability test

The system suitability test was carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicate injections of a reference solution containing 20 µg/mL HRD. The parameters measured were peak area, retention time, theoretical plates, and tailing factor (peak symmetry).

RESULTS AND DISCUSSION

HPLC method development and optimization

Validation of a stability-indicating analytical method should demonstrate the capability of the method for the quantitation of the active pharmaceutical ingredient and the determination of possible degradation products without any interference. To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time. Ammonium acetate buffer resulted in high sensitivity compared with phosphate buffer and phosphoric acid solution. The use of methanol resulted in better sensitivity and short analysis time, improving the peak symmetry (about 1.16). Columns from different sources were evaluated, and the Nucleosil C8 analytical column was selected, as it provided the best chromatographic performance and acceptable peak characteristics, including tailing factor and number of theoretical plates. Moreover, acceptable resolution of HRD and the degradation products was obtained, confirming the stability-indicating capability of the proposed method. For selection of the best wavelength of detection, a PDA detector was used.

A satisfactory separation with good peak symmetry and steady baseline was achieved with Nucleosil C8 column and methanol-ammonium acetate buffer pH 5.0 (95 + 05, v/v) as mobile phase at flow rate of 1.3 mL/min. The quantitation of HRD was achieved at 238 nm. The optimized conditions of the HPLC method were validated for the analysis of HRD in tablet formulations and application for QC. **Fig. 1** shows a typical chromatogram obtained by the proposed RP-HPLC method, demonstrating the resolution of the symmetrical peak corresponding to HRD. The retention time observed (2.60 min) allows a fast determination of the drug, which is suitable for QC laboratories.

Method validation

Forced degradations are performed to provide indications of the stability-indicating properties of an analytical method, particularly when there is no information available about the potential degradation products. The acidic condition caused significant decrease of the HRD peak area, with one additional peak detected. Under basic condition a significant decrease of the HRD peak area was also observed, with two additional peaks detected. **Fig. 2A** represents the chromatogram of acid degraded sample which showed additional peak at 5.51 min while **Fig. 2B** represents the chromatogram of base degraded sample showed additional peak at 5.52 min and 6.31 min. The force degradation study in oxidative and thermal degradation of HRD, resulted in a non-significant decrease of the HRD peak area without any detectable degradation products (**Fig. 2C, 2D**). The peaks of the degraded products were well resolved from HRD peak. **Table 1** outlines the percent degradation shown by HRD at each stress condition. HRD was found to be most susceptible to degradation under basic conditions more than under any other stress conditions as evident from the percentage of intact drug.

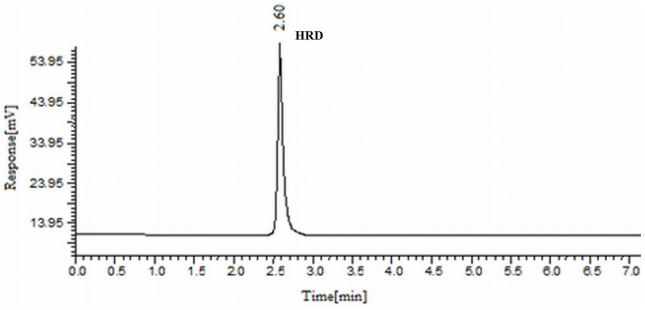

Fig. 1 Chromatogram of standard solution containing 20 µg/mL 9-hydroxyrisperidone.

Specificity is the ability to accurately and specifically measure the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the degree of interference from other active ingredients, excipients, impurities, and degradation products. Specificity in a method ensures that a peak response is due to a single component only. In present study, the ability of the method to separate the drug from its degradation products and the non-interference of the excipients indicate the specificity of the method. Values of peak purity index were higher than 0.9999. These results indicated that the proposed method is specific and stability-indicating, and can be applied for stability studies and QC analysis of entecavir in pharmaceutical products, with advantages when compared to previously published methods.

The linearity of a method is defined as its ability to provide measurement results that are directly proportional to the concentration of the analyte. The linearity of the detector was obtained by diluting the analyte stock solution and measuring the associated responses, while the linearity of the analytical method was determined by making a series of concentrations of the analyte from independent sample preparations (weighing and spiking). The linearity data described in present study demonstrates acceptable linearity for HRD over the range of 80 to 120% of the target concentration. Linear correlation was obtained between peak areas and concentrations of HRD in the range of 5 – 30 µg/ml. The following regression equation was found by plotting the peak area (y) versus the HRD concentration (x) expressed in µg/mL: $y = 25513x + 43915$. The correlation coefficient (r^2 : 0.999) obtained for the regression line

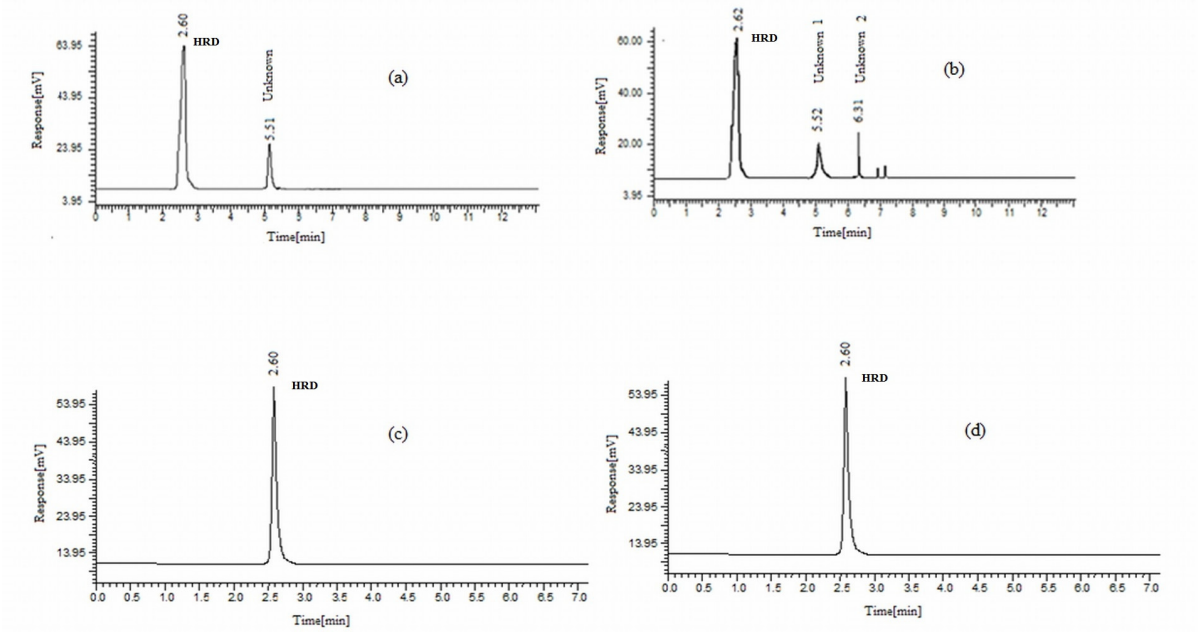

Fig. 2 Chromatogram of 9-hydroxyrisperidone and degradation products. (**A**) Acid degradation; (**B**) Alkali degradation; (**C**) Peroxide degradation; (**D**) Thermal degradation.

Table 2 Regression analysis of calibration graphs for 9-hydroxyrisperidone for the proposed HPLC.

Parameter	9-Hydroxyrisperidone
Concentration range (µg/ml)	5-30
Slope	25513
Standard deviation of the slope	128.92
Intercept	43915
Standard deviation of the intercept	846.1
Correlation coefficient	0.999

demonstrates the excellent relationship between peak area and concentration of HRD. Data of regression analysis were summarized in **Table 2**. Moreover, the relative SE of slope can be used as a parameter with respect to the precision of the regression, as a general acceptance criterion for the linearity performance of the analytical procedure. This parameter should be comparable to the RSD obtained in the evaluation of the precision. The result obtained for the RSD of the slope was 0.13%, which is lower than the mean value 0.50% for the RSD of the precision.

The precision, evaluated as the repeatability of the method, was studied by calculating the RSD for six determinations of the 20 mg/mL sample of HRD performed on the same day and under the same experimental conditions. The obtained RSD value was 0.535%. The RSD values for repeatability study was found to be <1%, which indicates that the proposed method is repeatable. The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on 3 different days (interday); the mean values obtained were 99.51 and 100.13% with RSDs of 0.20 and 0.47%, respectively. The between-analysts precision was determined by calculating the mean values and the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 99.46 and 99.98% with RSDs of 0.22 and 0.47%, respectively. The RSD values for intermediate precision was found to be <2%, which indicates that the proposed methods are reproducible.

The accuracy was assessed from by the standard addition method for three replicate determinations of three different solutions containing 18, 20, and 22 mg/mL HRD. The recoveries were obtained in a range of 99.46 to 100.10% for HRD using proposed HPLC method (**Table 3**). The high values indicate that the proposed HPLC method is accurate.

The LOD and LOQ were determined from slopes of linear regression curves. The limit of detection (LOD) and limit of quantification (LOQ) for HRD were found to be 1.04 and 3.16 µg/mL, respectively. (**Table 3**) LOD and LOQ data show that HRD can be accurately determined in the microgram quantity.

Table 3 Summary of validation parameters for the proposed HPLC methods.

Parameter	HPLC
LOD[a]	1.04 µg/mL
LOQ[b]	3.16 µg/mL
Accuracy, %	99.46-100.10
Repeatability (RSD[c], %, n=6)	0.535
Precision (RSD, %)	
Interday (n=3)	0.207-0.499
Intraday (n=3)	0.214-0.474
Specificity	Specific
Solvent suitability	suitable

[a] LOD = Limit of detection.
[b] LOQ = Limit of quantification.
[c] RSD = Relative standard deviation.

Table 4 Chromatographic conditions and range investigated during robustness study.

Variable	Range	HRD, %	RSD, %	Optimized value
Flow rate (ml/min)	1	99.63	0.32	1.3
	1.2	99.88	0.22	
	1.3	100.13	0.17	
Percentage of methanol	4.0	99.46	0.21	5.0
	5.0	99.96	0.13	
	6.0	99.13	0.28	
pH of mobile phase	4.8	99.89	0.18	5.0
	5.0	100.07	0.14	
	5.2	99.38	0.23	

The results and the experimental range of the selected variables evaluated in the robustness assessment are given in **Table 4**, together with the optimized values. There were no significant changes in the chromatographic pattern when the modifications were made in the experimental conditions, thus showing the method to be robust. Solvent suitability study was carried out to establish the stability of the sample in an analytical solution (diluent) over a period of time during routine analysis. The stability of sample solutions was tested at intervals of 12 h, 24 h, and up to 48 h. The method was found to be rugged as there was no change in

Table 5 System suitability test parameters of 9-hydroxyrisperidone for the proposed HPLC method.

System Suitability Parameters	9-hydroxyrisperidone	RSD %	Status
Retention times (R_T)	2.61	0.32	Pass
Peak area	256445	0.79	Pass
Theoretical plates (N)	7768	0.87	Pass
Tailing factor (A_S)	1.16	0.25	Pass

Table 6 Assay results for the tablet dosage form using the proposed HPLC.

Parameter	PPD ± SD[a] (n[b] = 5), %
	HPLC
Tablet formulation	99.99 ± 0.52

[a] SD = Standard deviation.
[b] n = Number of determination.

area of HRD. Results of this solvent suitability study showing non-significant change (<2%) relative to freshly prepared samples. The RSD values calculated in the system suitability test for the parameters studied were within the acceptable range (RSD <2.0%), as shown in **Table 5**, indicating that the system is suitable for the analysis intended.

Method application

The proposed RP-HPLC method was applied for the determination of HRD in tablet dosage forms, without prior separation of the excipients of the formulation. The results in **Table 6** demonstrate the quality of the analyzed pharmaceutical samples and the applicability of the method for QC analysis.

CONCLUSIONS

This study is a typical example of the development of a stability-indicating assay following ICH guidelines. The results of stress testing undertaken according to the ICH guidelines reveal that the method is specific and stability-indicating. Based on the peak purity results obtained from the analysis of forced degradation samples using the described method, it can be concluded that there is no other coeluting peak with the main peaks, and the method is specific for the determination of HRD in the presence of degradation products.

A simple and rapid isocratic stability-indicating RP-HPLC method has been developed and validated for determination of HRD in tablet dosage form. The results of the validation studies show that the RP-HPLC method is sensitive, accurate, specific, and stability-indicating. It possesses significant linearity (r^2 = 0.9999), precision with a mean RSD of 0.5%, high efficiency and resolution, and no interference from the excipients or degradation products, as was demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of HRD in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

ACKNOWLEDGEMENTS

Authors are grateful to the Torrent research centre for gratis sample of HRD.

REFERENCES

Andhariya JV, Dhoru MM, Shinde RB, Mehta PJ (2009) Development and validation of RP-RRLC method for estimation of paliperidone and its impurities in extended release dosage form. Available online: www.scientificipca.org

ICH Harmonised Tripartite Guideline: Validation of Analytical procedures: Text and Methodology, Q2 (R1) Geneva, 2005, http://www.ich.org.

Jadhav SA, Landge SB, Choudhari PM, Solanki PV, Bembalkar SR, Mathad VT (2011) Stress degradation behavior of paliperidone, an antipsychotic drug, and development of suitable stability-indicating RP-LC method. *Chromatography Research International* **2011**, 1-10

Jane M, Joshi CK, Aghera J (2011) Analytical method for estimation of Paliperodine. *International Journal of Research in Pharmaceutical Sciences* **2 (2)**, 158-161

Patel RB, Patel MR, Bhatt KK, Patel BG (2010) HPTLC method development and validation: Quantification of paliperidone in formulations and *in vitro* release study. *Analytical Methods* **2**, 525-531

Tan MS, Breau A, Bugge JC (2009) Quantitative determination of paliperidone in human plasma by LC-MS-MS. *AAPS Journal* **11**, 3917