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Simultaneous estimation of risperidone, olanzapine and quetiapine and their degradation products by hplc

Deepa Sharma[1], Kona S. Srinivas[2], Pallavi Gupta[2], Dhananjay P. Dwivedi[2], Harish Dureja[3], Manju Nagpal[1], Pankaj Rakha[1], Surinder Goyal[1*]

[1]Department of Pharmacy, Rajendra Institute of Technology and Sciences, 4th Miles Stone, Hisar Road, Sirsa, India.
[2]Analytical Department, New Drug Discovery Research, Ranbaxy Research Laboratories, Gurgaon, India.
[3]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, India.

Abstract

A rapid, specific reversed phase HPLC method has been developed for simultaneous determination of risperidone, olanzapine and quetiapine. Drugs were subjected to stress conditions such as acidic, alkaline and oxidative hydrolysis. Chromatographic separation of these pure drugs was carried out on Luna C18 (250*4.6, 5μm) with a 50:50 (v:v) mixture of 20mM ammonium acetate and acetonitrile as mobile phase. The flow rate was 1.0 mL min^{-1} and the analysis was monitored at 235 nm by UV detection. The system and method precision was found to be less than 1%. The assay results were linear from 35 to 65 μg mL^{-1} for risperidone ($R^2 \geq 0.991$), olanzapine ($R^2 \geq 0.992$) and quetiapine ($R^2 \geq 0.999$). Method validated showed it to be specific, precise, robust and linear over the range of analysis. Separation was complete within 10 min. Degradation studies revealed that degradation products do not interfere with the determination of drugs.

Keywords: Risperidone, Olanzapine, Quetiapine, HPLC, Force degradation

Introduction

Schizophrenia is a life-long illness that affects approximately 1% of the human population (Meltzer 1999a and 1999b, Lewis and Lieberman 2000). The sustained use of combinations of antipsychotic medications i.e. "antipsychotic polypharmacy"—is an increasingly common treatment strategy for the management of schizophrenia (Centorrino et al. 2005). Also the use of atypical antipsychotics is increasing these days than those of traditional neuroleptic antipsychotic (Clark et al. 2002). Over the past several years, atypical antipsychotic drugs have supplanted typical antipsychotic drugs in the treatment of schizophrenia because of superior efficacy and reduced side effects (Nash 1991, Meltzer 1999a and 1999b). Combinations of "atypical" antipsychotic medications are well tolerated and may be effective in the management of schizophrenia disorder (Lerner et al. 2004) V. At present, five atypical antipsychotic drugs have been approved for use in the United States, which includes clozapine, olanzapine, risperidone, ziprasidone, and quetiapine (Borison et al. 1986, Wetzel et al. 1995, Bymaster et al. 1996, Janssen et al. 1988, Daniel et al. 1999). A lot of research work has been reported on method development for simultaneous analysis of one or more than one antipsychotic drugs. For example, simultaneous estimation of five antipsychotic drugs (olanzapine, haloperidol, chlorpromazine, ziprasidone, and risperidone) has been reported (Zhang et al. 2007) and another research work showed simultaneous determination of levomepromazine and clozapine

***Corresponding author:** drgoyal@rediffmail.com

and their main metabolites (Mercolini et al. 2007). But literature survey showed no direct method reported for simultaneous estimation of risperidone, olanzapine and quetiapine in their combined form. Risperidone, olanzapine and quetiapine are chemically known as 3-(2-(4-(6-fluoro-1,2-benzisoxazole-3-yl)-1-piperidinyl) ethyl)-6,7,8,9-tetrahydro-2- methyl-4H-pyrido[1,2-a]pyrimidin-4-one, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1,5]benzodiazepine, 2-[2-(4-dibenzo[b,f][1,4]thiazipin-11-yl-1-piperazinyl)ethoxy]-ethanol fumarate (2:1) respectively.

The aim of the present study is to develop a rapid, specific and validated HPLC method for the determination of risperidone, olanzapine and quetiapine in their combined pure form. The developed method was validated for specificity, precision, linearity and solution stability. Drugs were also subjected to stress conditions such as acidic, alkaline and oxidative hydrolysis (30% v/v H_2O_2).

Material and Method

Apparatus

The HPLC system used was of a 2695 series with photo diode array detector of 2996 series (Alliance – Waters). The system is equipped with an inbuilt solvent degasser, quaternary pump, auto sampler and a reversed phase Luna C18 (250*4.6, 5 µm) column.

Reagents and Materials: Risperidone, olanzapine and quetiapine pure powder were provided by Ranbaxy Research Laboratories, Gurgaon, India. HPLC grade acetonitrile was procured from Rankem fine chemicals, India. Ammonium acetate, acetic acid, sodium hydroxide, ammonia, hydrogen peroxide, hydrochloric acid were obtained from Qualigens' fine chemicals, India and were of analytical grade. Hydrophilic PVDF syringe filter 0.45 µm was procured from Millipore Millex-HV, India. Milli-Q water was used to prepare solutions. A diluent consisting of 50% ammonium acetate buffer and 50% acetonitrile was used to prepare solution.

Chromatographic conditions

Separation was achieved at 40 °C temperature on column using mobile phase at a flow rate of 1 mL/min. The mobile phase consisted of 50% of 20 mM ammonium acetate buffer (adjusted to pH = 6.7±0.5 with ammonia or acetic acid) and 50% acetonitrile. The mobile phase was filtered and degassed before use. The monitoring wavelength was 235 nm and the injection volume was 20 µL.

Preparation of risperdone, olanzapine and quetiapine standard and sample solutions

25 mg each of risperidone, olanzapine and quetiapine was taken in a 50 mL volumetric flask and dissolved in diluent, sonicated for 5 min and volume was adjusted to 50 mL. Then 5 mL of this solution was transferred to 50 mL volumetric flask and volume was adjusted with a diluent to obtain a concentration of 50 ppm.

Method validation

The developed method was validated for specificity, precision, linearity, robustness, solution stability and force degradation. The validation was performed in accordance with ICH guidelines.

System Suitability: System suitability test was performed to confirm the reproducibility of the equipment to be used for the intended validation. The test was performed by injecting 20 µL of the standard solution. System suitability parameters like peak asymmetry and theoretical plates were monitored.

System Precision: Standard solution (50 ppm) was injected in six replicate injections to check the %RSD for finding the precision of the system to be used for validation.

Method Precision: Method precision or Intra-assay precision data were obtained by repeatedly analyzing, in one laboratory on one day, aliquots of homogeneous sample, each of which independently prepared according to method procedure. Five injections of standard solution of 50 ppm were injected to check the system suitability. Then samples

were prepared six times and each of those was injected in duplicate. Mean of all of these values gives rise to the assay value obtained from method precision.

Specificity: The specificity was determined by analyzing the blank. Any interference was analyzed by the peak purity, which was calculated using Empower software. The justification of injecting 20 µL of blank is to compare and identify whether there is appearance of any peak in the blank sample due to contamination. Two injections of sample solution containing 50 ppm of each drug were injected and the peak purity of analyte peaks was monitored.

Linearity: Calibration curves were constructed by plotting peak areas vs concentrations of risperidone, olanzapine and quetiapine respectively, and the regression equations were calculated. The calibration curves were plotted over the concentration range 35 to 65 $\mu g\, mL^{-1}$ for risperidone, olanzapine and quetiapine. From the working standard stock solution (500 ppm), 7, 4, 5, 6, 13 mL were transferred to series of 100, 50, 50, 50, 100 mL of volumetric flask respectively. Aliquots (20 µL) of each solution were injected under the operating chromatographic condition.

Robustness: For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions. Standard solution of 50 ppm was prepared and injected five times, whereas the sample of 50 ppm was prepared three times, injected in duplicate to see the influence of variation in organic phase (±2%), mobile phase (±0.2), flow rate (±10%) and temperature (±5 °C).

Stability: A sample solution of drugs (50 ppm) was prepared and kept at 25°C in the auto sampler till 24 h to indicate the sample stability in analytical solution at 25°C.

Forced Degradation

In the forced degradation studies, drugs were allowed to degrade in acidic, basic and oxidative conditions at room temperature and at high temperature (50°C) for different periods.

Acid hydrolysis: Drugs were allowed to hydrolyze under two different acidic conditions: mild acidic (0.01 N, 1 N, 2 N, 4 N, 5 N HCl) and concentrated acidic (10 N and 12 N HCl). For that 25 mg of each drug was weighed, dissolved in the 5 mL of respective acidic strengths and kept for 2, 8, 24, 36 h at room temperature and at 50°C, samples were withdrawn, neutralized with base sodium hydroxide (NaOH) and the sample solutions were prepared as specified.

Alkaline hydrolysis: Like acid hydrolysis, same procedure was followed for alkaline hydrolysis by taking the different strengths like 0.001 N, 0.002 N, 0.004 N, 0.01 N, 0.1 N, 0.2 N, 0.5 N, and 1 N NaOH to carry out alkaline hydrolysis. However before adding dilute NaOH, a diluent (acting as cosolvent) was added to avoid solubility problem.

Oxidative degradation: The drugs were treated with 0.5 and 1.0 mL of 30% v/v hydrogen peroxide (H_2O_2) at room temperature for 2 h to carry out oxidative hydrolysis and the sample solutions were prepared as specified.

Result and Discussion

Optimization of chromatographic conditions

To optimize the HPLC parameters, several mobile phase were tried. A satisfactory separation and good peak symmetry for Risperidone, Olanzapine and Quetiapine was obtained with a mobile phase consisting of 50 % ammonium acetate buffer (pH 6.7) and 50% acetonitrile to obtain better reproducibility and repeatability. Quantification was achieved with UV detection at 235 nm based on peak area. Complete resolution of the peaks with clear baseline separation was obtained.

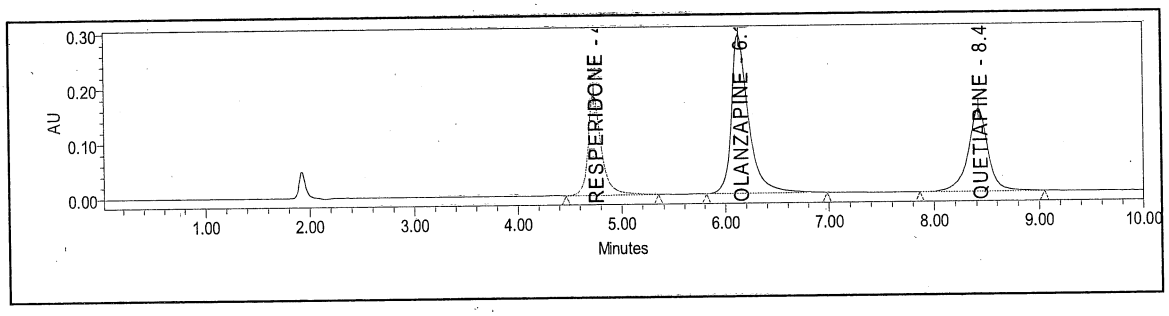
Validation of the proposed method

The validation study allowed the evaluation of the method for its suitability for routine analysis. The retention time obtained for risperidone, olanzapine and quetiapine are 4.7, 6.159 and 8.431 min respectively. System suitability parameters obtained for risperidone, olanzapine and quetiapine, were (1) asymmetry factor 1.34, 1.43 and 0.95, (2) theoretical plates 8341, 8432 and 11742 respectively.

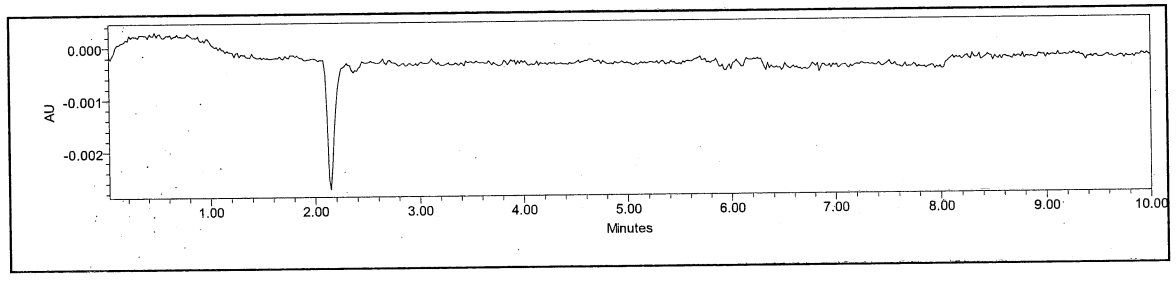
System precision: The low RSD values of risperidone (0.21%), olanzapine (0.27%) and quetiapine (0.27%) reveals that the system in use is precise.

Method precision: The RSD values for risperidone, olanzapine and quetiapine in the combined form were found to be 0.49, 0.42 and 0.16% respectively. The low RSD values indicate that the proposed method is repeatable.

Specificity: The resulting chromatogram obtained from blank showed no interference due to contamination. Two injections of 50 ppm sample solution did not show any peak other than that of risperidone, olanzapine and quetiapine Figures 1a and 1b.



(a)



(b)

Figure 1. (a) A typical chromatogram of specificity, (b) blank

Linearity: Risperidone, olanzapine and quetiapine showed linear response in the range of 35 to 65 μg mL^{-1} (70–130%). The corresponding linear regression equation was $y = 33733\ x - 164281$, $y = 69174\ x + 288557$ and $y = 38716\ x + 31754$ with square of correlation coefficient (R^2) of 0.991, 0.992 and 0.999 for risperidone, olanzapine and quetiapine respectively.

Robustness: The influence of variation in organic phase (±2%), mobile phase (±0.2), flow rate (±10%) and column temperature (±5°C) has been evaluated by %RSD of assay should not be more than 2% (Table 1). A small but deliberate variation in method parameter provides an indication of its reliability during normal usage.

Table 1: Robustness parameters

DRUGS	Flow 1 mL/min		Column Temp. 40°C		Buffer pH 6.7		Organic phase 50%	
	-0.1	+0.1	-5°C	+5°C	-0.2	+0.2	-2%	+2%
	%RSD							
Risperidone	0.98	0.49	1.13	0.94	0.91	0.76	1.45	0.69
Olanzapine	0.62	0.41	1.32	1.56	1.15	0.66	0.43	0.10
Quetiapine	1.16	0.76	1.01	0.96	0.80	0.86	1.46	0.86

Stability: The samples were found to be stable at 25°C for 24 h and were analyzed by calculating the cumulative %RSD which was found to be less than 2%.

Force degradation: Analysis of all stress samples were performed using ammonium acetate buffer (pH 6.7) and acetonitrile (50:50 v:v) as the mobile phase. The stress testing may provide information about the degradation pathway and selectivity of the analytical method.

Acid hydrolysis: Under mild acidic conditions, no reasonable amount of drug was found to be degraded. But under concentrated acidic condition, mild degradation of all the three drugs was observed (Table 2). The degradation of quetiapine was higher in 12 N HCl and its degraded product is shown in Figure 2.

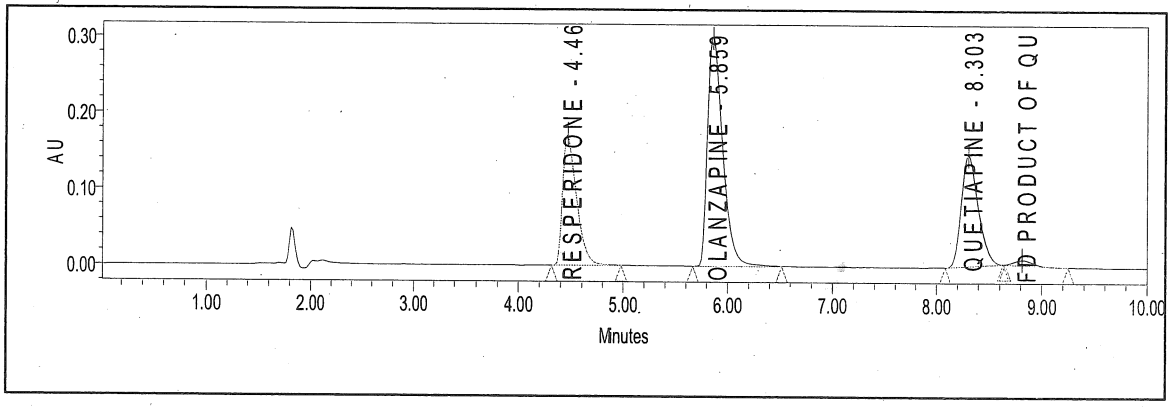


Figure 2. Chromatogram showing degraded product of quetiapine by acidic hydrolysis

Table 2. Degradation studies by acidic hydrolysis

Peak purity data of degradation studies- Acidic hydrolysis			
	Degradation %	Purity Angle	Purity Threshold
Risperidone			
5 mL 12 N HCl 36 h	18	0.115	0.319
Olanzapine			
5 mL 12 N HCl 36 h	17	0.072	0.274
Quetiapine			
5 mL 12 N HCl 36 h	26	0.101	0.309

Alkaline hydrolysis: No significant degradation of resperidone and quetiapine was observed at room temperature and at temperature of 50°C (Table 3). But in case of olanzapine, 18% of the drug was found to degrade in 1 N NaOH at 50°C (Figure 3).

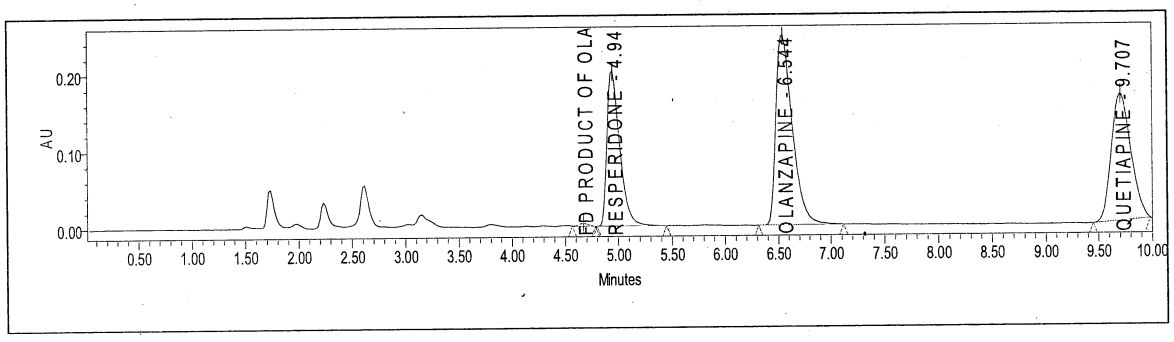


Figure 3. Chromatogram showing degraded product of Olanzapine in alkaline hydrolysis.

Table 3. Degradation studies by alkaline hydrolysis

Peak purity data of degradation studies- Alkali degradation			
	Degradation %	**Purity Angle**	**Purity Threshold**
Risperidone			
5 mL 0.1 N NaOH 24 hours 50 °C	1	0.131	0.33
5 mL 1 N NaOH 24 hours 50 °C	3	0.11	1.102
Olanzapine			
5 mL 0.1 N NaOH 24 hours 50 °C	0	0.071	1.069
5 mL 1 N NaOH 24 hours 50 °C	18	0.078	1.07
Quetiapine			
5 mL 0.1 N NaOH 24 hours 50 °C	2	0.131	0.330
5 mL 1 N NaOH 24 hours 50 °C	6	0.11	1.102

Oxidative hydrolysis: The drugs were allowed to degrade in 0.5 to 1 mL of 30% H_2O_2. 10 to 30% of degradation was observed in 0.5 mL of H_2O_2 at room temperature in all three drugs but in 1 mL of H_2O_2 extensive degradation i.e. 30-90% was observed for all three drugs (Table 4). Various degraded products of risperidone, olanzapine and quetiapine is shown in Figure 4.

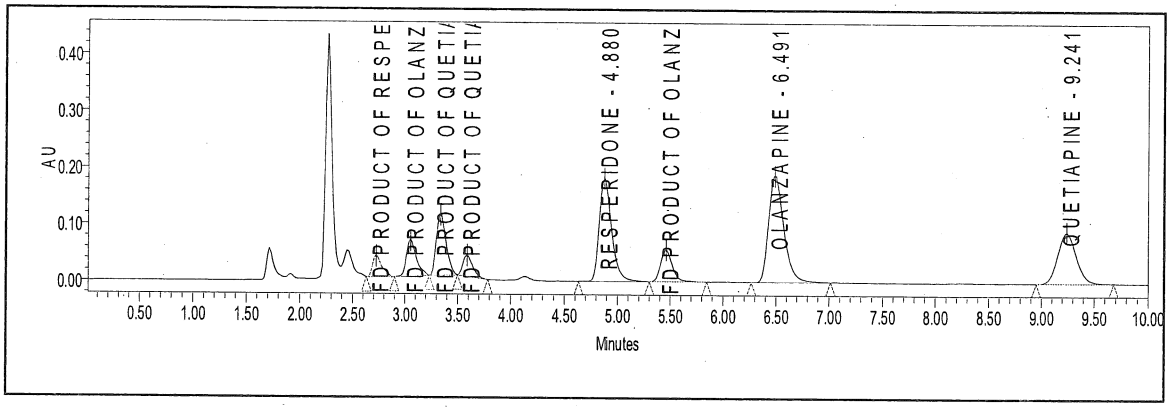


Figure 4. Chromatogram showing degraded product of risperidone, olanzapine and quetiapine by oxidative hydrolysis

Table 4. Degradation studies by oxidative hydrolysis

Peak Purity data of degradation studies- H_2O_2 Degradation			
	Degradation %	**Purity Angle**	**Purity Threshold**
Risperidone			
0.5 mL 30% H_2O_2 2 h	11	0.177	1.159
1.0 mL 30% H_2O_2 2 h	30	0.576	1.187
Olanzapine			
0.5 mL 30% H_2O_2 2 h	40	0.112	1.113
1.0 mL 30% H_2O_2 2 h	79	0.238	1.239
Quetiapine			
0.5mL 30% H_2O_2 2 h	28	0.192	1.213
1.0mL 30% H_2O_2 2 h	87	0.549	1.608

Conclusion

A rapid, specific isocratic HPLC method has been developed for the simultaneous determination of risperidone, olanzapine and quetiapine using a photo diode array (PDA)/UV detector. The method was validated for precision, specificity, linearity, robustness and stability and stability in analytical solution for pure drugs. The study brings forward new and interesting aspects on the decomposition behavior of risperidone, olanzapine and quetiapine. Stress testing of the drug substance can help identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule. Forced degradation studies revealed that possible degradation products do not interfere with the determination of risperidone, olanzapine and quetiapine. The method uses a simple mobile phase composition easy to prepare with little or no variation. The rapid run time of 10 min and the relatively low flow rate (1 mL/min) allows the analysis of a large number of samples with less mobile phase that proves to be cost-effective.

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