A Stability Indicating HPLC Method for the Determination of Electrochemically Controlled Release of Risperidone

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Abstract

A rapid stability indicating reversed-phase high-performance liquid chromatography (HPLC) method is developed for the determination of the electrochemically controlled risperidone release from a novel drug delivery system, based on the intrinsically conducting polymer (ICP), polypyrrole. The chromatographic separation was carried out on a C_{18} column using acetonitrile–potassium dihydrogen phosphate (45:55, v/v, pH 6.5; 0.05 M) as the mobile phase. The isocratic flow is at 1.0 mL/min, with a runtime of 6 min, and the UV detection is at 237 nm. This provided a calibration curve linear over the range of 1–100 µg/mL. Intra-day and inter-day accuracy range between 98.4% and 102.6%, and the RSD for precision is <1.43%. The limit of detection and quantitation were determined to be 0.001 µg/mL and 0.01 µg/mL, respectively. The analytical method confirmed risperidone is stable for the oxidizing electric potential and the acidic environment involved during the manufacture and operation of the novel drug delivery system. The rate of risperidone release from polypyrrole depended on electrical stimulation applied to the polymer. This HPLC method is significantly faster than previously published methods and is the first to investigate the effect of an oxidizing potential on risperidone stability, which is essential for the evaluation of controlled delivery from an ICP-based system.

Introduction

Risperidone is an antipsychotic agent used to treat schizophrenia (1). Risperidone (Figure 1), a benzisoxazole derivative, is chemically known as 3-[2-[4-(6-fluoro-1,2-benzisoxazole-3-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4H-pyrido[1,2-a]pyrimidin-4-one (2). Patients with mental health conditions are known to be poorly adherent to medication regimes, frequently resulting in unsatisfactory treatment outcomes (3). Few controlled release atypical antipsychotic preparations are available on the market. Controlled release formulations offer benefits to patients such as maintaining body drug concentrations at therapeutically desired levels and improving adherence (4).

The novel drug delivery system (NDDS) presented is based on the intrinsically conducting polymer (ICP), polypyrrole (PPy). PPy has found applications in medicine as drug delivery systems, biosensors, and for nerve regeneration (5,6). PPy can be altered between oxidized and reduced states, causing subsequent changes in polymer charge, permeability, and volume. These properties are being exploited to create a drug delivery system where the release rate is responsive to electrical stimulation. Such a system would have multiple applications not only in mental health but across many therapeutic fields.

Forced degradation testing plays an important role in pharmaceutical product development (7,8). Regulatory guidance suggests potential degradation products should be formed through elevated temperature, by using acidic, basic, and oxidative conditions, and through photolysis (9). Several high-performance liquid chromatography (HPLC) methods are reported in the literature for the quantification of risperidone in various media (10–16). However, only two of these deal with the determination of risperidone in the presence of degradation products or impurities (12,16). El-Sherif et al. used HPLC and thin-layer chromatography to quantify risperidone in the presence of impurities. Tomar et al. used acidic, basic, and oxidative conditions to form degradation products. Acid and base treatments led to the formation of 9-hydroxyrisperidone, while oxidative degradation lead to the formation of N-oxide of risperidone. The stability indicating HPLC method presented by Tomar et al. had a runtime of 25 min. As this method used a gradient flow, an additional 4 min would be required between runs to allow for system equilibration. Such a lengthy runtime is a limitation for the routine analysis of risperidone where time and costs must be considered.

In this manuscript, a specific stability indicating analytical method to unequivocally determine risperidone in the presence

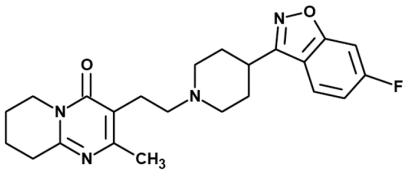

Figure 1. Risperidone structure.

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of degradation products and excipients (17) is presented. During the manufacture of the NDDS, risperidone is exposed to harsh conditions, specifically to an acidic environment and an oxidizing electrical potential. There are no published studies examining the stability of risperidone to an oxidizing electrical potential. The presented method is required to quantify risperidone in the presence of degradation products that may form in response to both chemical and electrical stress. In addition, the method must be capable of quantifying risperidone in the presence of any excipients that may be co-released with the drug. The method should have a reasonably fast run time, making it attractive for the routine analysis of risperidone.

This body of work looks at (*i*) HPLC method development, (*ii*) stress studies of risperidone, (*iii*) HPLC method validation, (*iv*) recovery from spiked plasma samples, and (*v*) application of the HPLC method to risperidone released from the NDDS.

Experimental

Reagents and chemicals

Risperidone was gifted by Douglas Pharmaceuticals (Auckland, New Zealand). *p*-Toluene sulfonic acid (pTS) and pyrrole were purchased from Aldrich (Sydney, Australia). All the reagents and solvents used in the mobile phase preparation for HPLC were of analytical reagent (AR) and HPLC grades, respectively. All other chemicals used in the study were at least reagent grade. Water used in the preparation of the formulation, and buffers was obtained by reverse osmosis (MilliQ unit, Millipore, Billerica, MA) of demineralized water.

Chromatographic conditions

A reversed-phase HPLC method was utilized for the quantification of risperidone. An Agilent series 1200 (Agilent Corporation, Waldbronn, Germany) comprising of a quaternary pump, an autosampler and photodiode array (PDA) detector were used, with data acquisition by Chemstation software (Agilent Corporation). For the routine analysis of solutions containing risperidone, a 5 to 10 min runtime to increase the speed of the sample analysis with a reduction in associated costs was aimed for. Various columns, mobile phases, mobile phase combinations and flow rates were trialed to achieve optimum performance and peak separation.

Stress studies of risperidone

Risperidone is practically insoluble in water (more than 10 mL of solvent required per 1 mg of solute) (2). Due to this, acetonitrile was used as a cosolvent to prepare all degradation solutions, except the basic NaOH solution (7). In the basic solution, acetonitrile was replaced with methanol.

The oxidative degradation of risperidone was forced by mixing risperidone in a solution and hydrogen peroxide (H_2O_2) in an aqueous solution, providing risperidone at 1 mg/mL and peroxide at 0.15%, v/v. Degradation occurring in acid conditions was tested by mixing risperidone in solution with an HCl aqueous solution, providing risperidone at 1 mg/mL and HCl at 1 M. To test degradation under basic conditions, risperidone in solution was mixed with a NaOH aqueous solution, providing risperidone at 1 mg/mL and NaOH at 1 M. Degradation at elevated temperatures was examined with risperidone in both solution and solid states. To test the solution state, risperidone in the solution was mixed with water, giving a final risperidone concentration of 1 mg/mL; this was stored at 55°C. In addition, risperidone powder was stored at 55°C in an open glass container until the time of analysis when ~ 1 mg of powder was mixed with 10 mL of acetonitrile. The photolytic degradation of risperidone was tested by storing drug powder in a clear glass container at 40°C, in a Binder Incubator KBF240 series (Binder, Germany), providing illumination levels compliant with ICH guidelines (18). At the time of analysis, ~ 1 mg of powder was mixed with 10 mL of acetonitrile.

Exposure to manufacture conditions

Acidic solution. Mimicking the conditions used in the manufacture of the NDDS, risperidone (0.09 M) was dissolved in a solution of methanol–water (7:3, v/v) containing pTS (0.1 M) and pyrrole (0.2 M). The final solution pH was 3.90. The solution was stored at 20°C and analyzed after 24 h.

Oxidizing potential. Risperidone (0.9 M) and pTS (0.1 M) dissolved in methanol–water (7:3, v/v) was exposed to a potential of 2 V for 1 h at 20°C in a three-electrode cell comprising a stainless steel working electrode, a platinum counter electrode, and a Ag/AgCl reference electrode.

Unless otherwise stated, all stressed samples were stored at 55°C in a Binder Incubator BD240 series (Binder, Germany). The study aimed to generate between 5% and 20% degradation of risperidone (7). Degradation was estimated as the area of the risperidone peak compared to the area of the evolved degradation peaks.

Method validation

The developed HPLC method was validated for the linearity, the range, the limit of detection (LOD) and quantitation (LOQ), and the accuracy and precision. To achieve this, a primary stock solution of risperidone 1 mg/mL was prepared in acetonitrile. Working solutions (0.1 mg/mL and 0.01 mg/mL) were prepared by diluting the stock solution with acetonitrile. From the working standards, 6 calibration standards in the range of 1–100 µg/mL were prepared with 5 replicates by diluting with acetonitrile. The solutions were prepared fresh daily. The LOD and LOQ were determined by injecting serially lower concentrations of risperidone. The LOD was taken to be the lowest risperidone concentration, showing a peak with a signal-to-noise ratio of at least 3, while the LOQ required a signal-to-noise ratio of at least 10.

The intra-day accuracy and precision were determined by the analysis of 5 replicates of 3, 10, 30, and 100 µg/mL. The inter-day accuracy and precision were determined by the analysis of the same concentrations with 5 replicates over 3 days, giving a total of 15 measurements. The precision was determined as %RSD, while the accuracy was determined by comparing the measured concentrations against the true concentrations.

Risperidone stability in release media

Acetonitrile–potassium dihydrogen phosphate (pH 6.5, 0.05 M) (45:55, v/v) was used as the media to test risperidone release from the NDDS. Risperidone, dissolved in this media at a concentration of 87.14 µg/mL, was stored either at –20°C, 4°C, or

25°C. The concentration of risperidone remaining was tested at 3 h, 8.5 h, 24 h, 48 h, 7 days, and 14 days. Triplicate samples were tested at each time point for each condition.

Recovery from plasma samples

Blank plasma samples were spiked with known amounts of risperidone dissolved in methanol (1 mg/mL) to concentrations of 20, 50, or 100 µg/mL, with 5 replicates at each concentration. Risperidone was extracted from the samples by taking 25 µL of plasma and adding 25 µL of water and 100 µL of acetonitrile. After thorough mixing, the samples were left to stand for 10 minutes before being centrifuged at 10000 rpm for 5 min. The supernant was injected onto the HPLC system.

Application of the analytical method to risperidone release from the novel drug delivery system

PPy films containing risperidone were prepared as previously described (19). The excipients used in this formulation are pyrrole and pTS. For the chromatographic method to be applicable to drug release studies, it must be able to resolve the risperidone peak from exicipent peaks and any degradation products that may form during the manufacture process.

Following preparation, in-vitro controlled release of risperidone from the drug delivery system was tested into a stirred dissolution vessel containing acetonitrile–potassium dihydrogen phosphate (pH 6.5, 0.05 M) (45:55, v/v). The release of risperidone was measured under three different release conditions. A control group was created by analyzing the release without any stimulation. In the other two groups, electrical stimulation was used to alter the redox state of the polymer. The release from the oxidized or reduced PPy was tested by applying a constant potential of either +0.6 V or –0.6 V (versus an Ag/AgCl reference electrode), respectively. Electrical stimulation was applied using a CH Instruments Model 440 electrochemical workstation (Austin, Texas). For each of the protocol tested, 5 replicate films were used. Following controlled release studies samples were stored at 4°C and analyzed within 7 days.

Results and Discussion

Chromatographic conditions

Based on previously published reports, several RP-HPLC columns were tested. The optimum system performance and peak separation was achieved using a Gemini analytical column (250 × 4.6 mm, particle size 5 µm) (Phenomenex, Auckland, New Zealand) and a C18 precolumn (12.5 × 4.6 mm) of the same packing. After testing differing mobile phases in various combinations, an isocratic flow rate of 1 mL/min was used, with the mobile phase consisting of acetonitrile–potassium dihydrogen phosphate (pH 6.5, 0.05 M) (45:55, v/v). The UV detection was achieved at 237 nm. Figure 2 shows risperidone eluting as a narrow peak around 4.5 min.

Stress studies of risperidone

Chromatograms are presented for the different degradation conditions. The degradation experiments were stopped when 5–20% degradation had been achieved or after 3 weeks, whichever occurred first. The purity of the risperidone peaks were tested using a PDA detector and Chemstation software by examining 5 UV spectra across the risperidone peak. Two spectra were taken before the apex of the peak, 1 spectrum at the apex and 2 after the peak apex; the correct and matching spectra indicated the peak corresponded to risperidone and was pure. The UV scans have been inset into the corresponding chromatograms. The risperidone peak can be seen eluting around 4.5 min.

Under oxidative conditions, a degradation peak of risperidone was visible at 3.0 min (Figure 3). The peak at 2.5 min in Figure 3 can be attributed to H_2O_2, as an identical peak was observed when an injection of 0.15% H_2O_2 without risperidone was made.

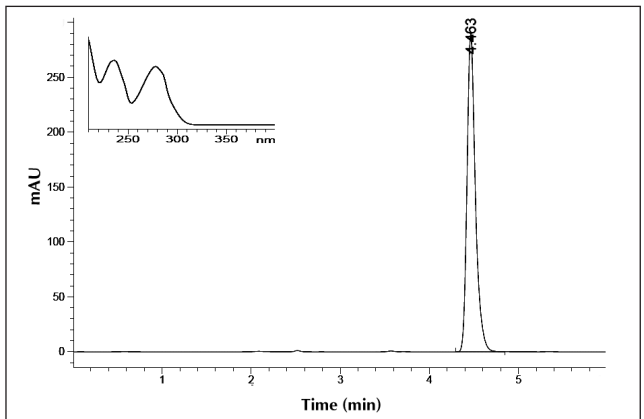

Figure 2. Risperidone standard injection (1 mg/mL).

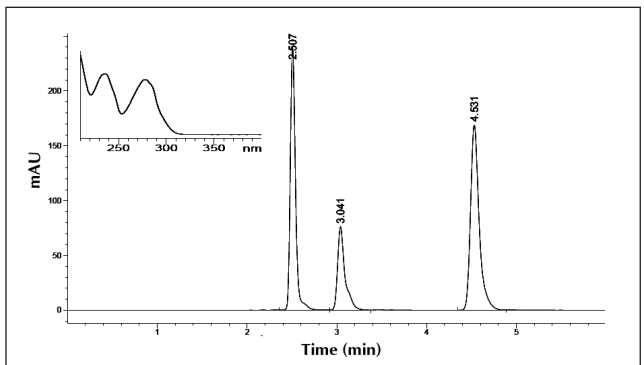

Figure 3. Risperidone (1 mg/mL), in H_2O_2 (0.15% v/v) after 30 min.

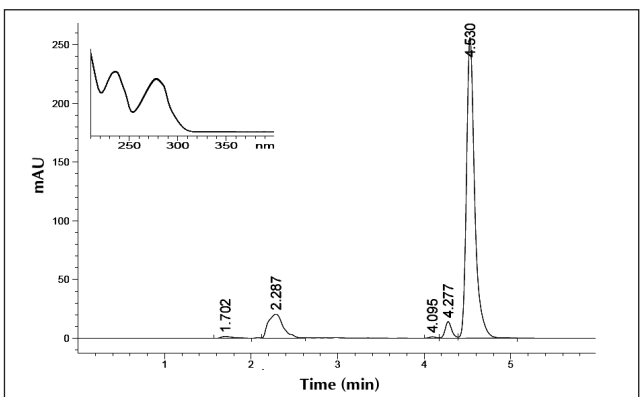

Figure 4. Risperidone (1 mg/mL) in 1 M HCl after 3 weeks.

The main degradation peaks formed under acidic conditions can be seen eluting at 2.3 and 4.3 min (Figure 4). The resolution between the risperidone peak and the degradation peak at 4.3 min was 1.74, providing adequate resolution (2). Basic degradation resulted in new peaks eluting at 2.7 and 4.1 min (Figure 5). UV spectra of these degradation peaks showed them to be different species to those formed by acidic degradation. This is in contrast with Tomar et al., who report the products of an acid and base degradation to be the same (16). When risperidone dissolved in acetonitrile, and water was kept at an elevated temperature, the main thermal degradation product can be seen eluting around 3 min (Figure 6). However, when risperidone was kept as a powder at the same temperature for an equal length of time, no degradation was observed. As the risperidone powder was stable at 55°C for 3 weeks without any light exposure, any degradation seen in risperidone powder stored at 40°C and exposed to light could be attributed to photodegradation. The sample kept under these conditions demonstrated risperidone is photostable for 3 weeks.

More than a 5% degradation of risperidone was observed when stressed by acid, base, and oxidative conditions and when risperidone was kept in solution for 3 weeks. In all cases the risperidone peak was shown to be pure and adequately resolved from other peaks (resolution > 1.74) (2). With risperidone kept as a dry powder, even after 3 weeks at elevated temperature and with light exposure, no degradation was observed.

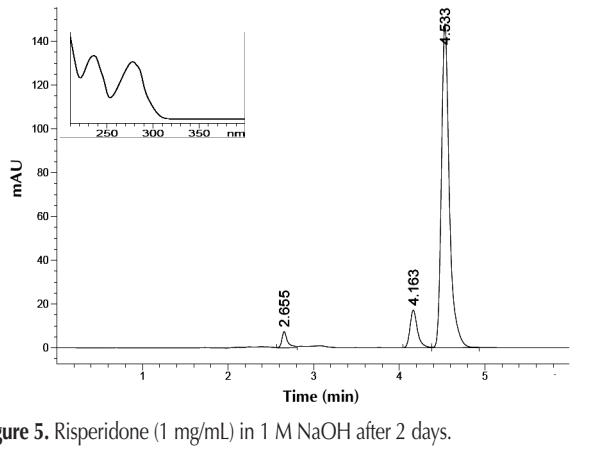

Figure 5. Risperidone (1 mg/mL) in 1 M NaOH after 2 days.

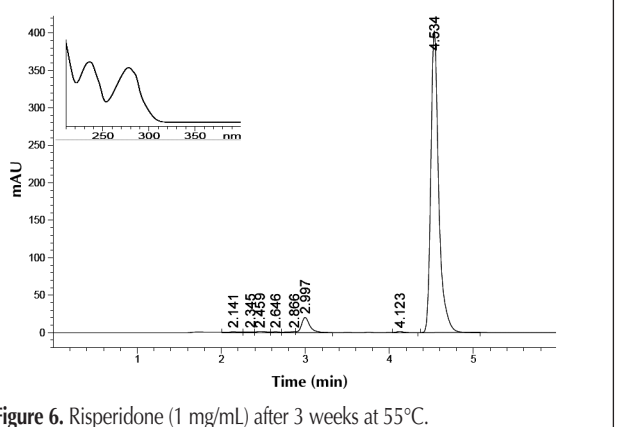

Figure 6. Risperidone (1 mg/mL) after 3 weeks at 55°C.

Exposure to manufacture conditions

Acidic solution. After 24 h of storage, no degradation of risperidone was observed. No additional peaks were observed on the chromatogram, and the risperidone peak was observed to be pure. During NDDS, manufacture solutions are freshly prepared daily, and the 24 h period tested is beyond the length of time risperidone would usually be exposed to this environment.

Oxidizing potential. After 1 h of exposure to 2 V, no degradation of risperidone was observed. No additional peaks were observed on the chromatogram, and the risperidone peak was observed to be pure. This is in excess of the potential risperidone is exposed to during NDDS manufacture, usually 1.5 V for 16 min.

It is interesting to note that while oxidation with H_2O_2 resulted in risperidone degradation, exposure to 2 V did not. The chemical oxidant has a standard reduction potential of +1.776 V (20), that is to say H_2O_2 has an oxidizing strength equivalent to 1.776 V. The electrical oxidizing potential was set at 2 V; this is the potential difference between the reference and working electrodes. However, there is a potential drop between the electrode surface and the liquid media; therefore, the drug molecules are exposed to a lower potential. Chemical oxidants are preferred in forced degradation studies (7); however, it is difficult to correlate the strength of a chemical oxidant with an actual oxidizing electrical potential. Therefore, it is essential when developing a stability indicating HPLC method for use with an ICP based drug delivery system that forced drug degradation is also explored electrically.

Validation

Calibration curves for risperidone showed good linearity in the 1–100 µg/mL range. The representative linear equation obtained from linear regression analysis was $y = 14.4x - 1.84$, with a correlation coefficient of $r^2 > 0.9999$. The LOD and LOQ levels were 0.001 µg/mL and 0.01 µg/mL respectively. Accuracy and precision data for the presented HPLC method is shown in Table I.

Risperidone stability in release media

Following storage at either –20°C, 4°C, or 25°C risperidone solutions at all time points up to and including 14 days were stable. The average amounts of risperidone remaining in solution remained at 100 ± 1%.

Recovery from plasma samples

Recovery was calculated from an area under the risperidone peak extracted from spiked human plasma compared with those

Table I. Accuracy and Precision Data of the HPLC Method

	Intra-day		Inter-day	
Conc. (µg/mL)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
3	101.7	0.50	102.6	0.96
10	98.4	0.02	99.5	0.29
30	100.3	0.39	100.4	1.43
100	100.1	0.24	100.7	0.96

from standards of risperidone dissolved in acetonitrile using the calibration curve described above. Levels of recovery are presented in Table II. This demonstrates the ability to use the presented HPLC method for analysis of biological samples.

Application of the analytical method to risperidone release from the novel drug delivery system

Figure 7 shows a representative chromatogram following the analysis of the release media. The risperidone, the pTS, and the pyrrole peaks are well resolved from each other with no degradation peaks present. Individual injections of risperidone, pTS, and the pyrrole standards were made to aid in the identification of the different peaks. The risperidone, pTS, and pyrrole peaks can be seen eluting around 4.4, 2.7, and 5.3 min, respectively. The peak at 2.45 min was also present when a blank injection of acetonitrile–potassium dihydrogen phosphate (pH 6.5, 0.05 M) (45:55, v/v) was made. The inset shows 5 UV spectra taken from across the risperidone peak showing this peak to be pure.

Table II. Recovery of Risperidone from Plasma

Conc. (µg/mL)	Recovery (%) (mean ± SD)
20	96.6 ± 4.1
50	99.8 ± 1.4
100	101.2 ± 1.4

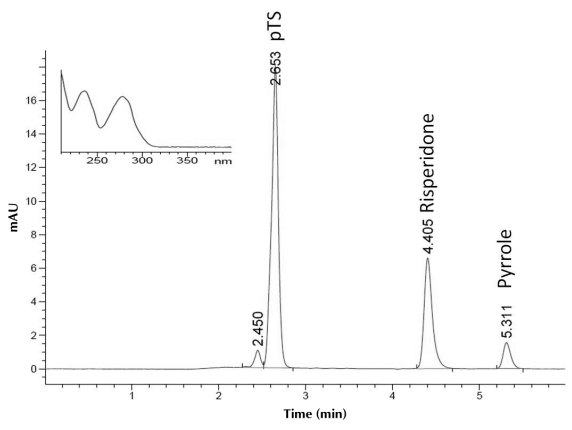

Figure 7. Representative chromatogram of a release sample.

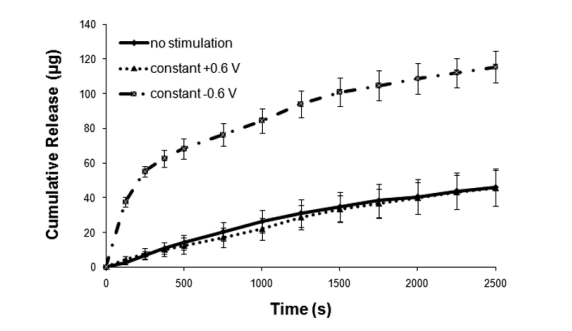

Figure 8. Average risperidone release from PPy films under different release conditions. Error bars represent standard error (n = 5).

The risperidone release was measured from PPy films under different release conditions (Figure 8). The release rate of risperidone varied depending on the electrical stimulation applied to the film. The lowest levels of risperidone were released when either no electrical stimulation or an oxidizing +0.6 V was applied to the films. The similar rates seen in these two conditions would occur as a freshly prepared polymer is in the oxidized state and would be expected to remain in this state if no further stimulation occurs. When a constant –0.6 V was applied to reduce the polymer, the greatest amount of risperidone was released. Based on previous mechanistic studies, this increase in release was due to subsequent changes in polymer charge, permeability, and volume (19). The presented HPLC method has been used to evaluate the stability of a risperidone releasing system based on PPy (21).

Conclusion

A specific stability indicating RP-HPLC method has been developed for the analysis of risperidone release from a novel drug delivery system based on polypyrrole. This method is significantly faster than previously published methods and would be associated with reductions in time requirements and solvent costs. Degradation products of risperidone were generated under forced conditions; these did not interfere with risperidone analysis. For the evaluation of drug release from ICP systems, it is essential that risperidone is exposed to an oxidizing electrical potential, not only a chemical oxidant. The validation work has shown the method to be accurate and precise, with an excellent linearity over the range of 1–100 µg/mL. The HPLC method was applied to risperidone samples extracted from plasma with excellent recovery. The developed stability indicating HPLC method was able to show that risperidone is stable to the manufacture and release processes of the NDDS. Formulation excipients were detected and did not interfere with risperidone quantification. The rate of risperidone release from the PPy based drug delivery system depends on the electrical stimulation applied to the polymer. Such a system offers benefits as the rate of drug release can be optimized according to the requirements of the individual.

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