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Electrochemical and Spectrophotometric Methods to Assay of Risperidone an Antipsychotic Drug in Pharmaceutical and Biological Samples

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The electrochemical behaviour of risperidone and its quantitative determination was investigated using voltammetric and spectrophotometric methods. The diffusion coefficient and number of transferred electrons were calculated by using voltammetric methods. Cyclic voltammetricstudies showed that risperidone shows a characteristic adsorption/desorption behaviour on the hanging mercury drop electrode. Therefore, new, rapid, selective and simple electrochemical methods including differential pulse voltammetry, square-wave voltammetry and simple spectrophotometric methods for the direct determination of risperidone in pharmaceutical dosage forms without time-consuming steps prior to drug assay were developed. In these methods limit of detection (LOD) values were found to be range between 1.3×10^{-8} and 6.4×10^{-8} mol L^{-1}. Proposed methods were applied to determine the content of risperidone in commercial pharmaceutical preparations and biological samples. The methods were found to be highly accurate and precise, having a relative standard deviation of less than 4 %.

Key Words: Risperidone, Differential pulse voltammetry, Square wave voltammetry, Spectrophotometric method, Hanging mercury drop electrode, Assay.

INTRODUCTION

Risperidone (RPN) is an atypical antipsychotic. It was approved in 1993 for the treatment of schizophrenia. In 2007, risperdal (RPL) was approved as the only drug agent available for treatment of schizophrenia in youth ages. Risperidone contains the functional groups of benzisoxazole and piperidine as part of its molecular structure (Fig. 1). It is chemically known as 3[2-[4-[6-fluoro-1,2-benzisoxazol-3-yl]piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido[1,2-a]pyrimidin-4-one. This drug belongs to a class of antipsychotic drugs known as atypical neuroleptics. It is a strong dopamine antagonist. It has high affinity for D2 dopaminergic receptors. It has actions at several 5-HT (serotonin) receptor subtypes[1]. It is effective in the treatment of both positive and negative symptoms of schizophrenia which is a mental illness that causes disturbed or unusual thinking, loss of interest in life and strong or inappropriate emotions. Risperidone has a lower potential to cause extra pyramidal

side effects compared to classical antipsychotics[2]. Recent evidence from open trials suggests that risperidone may be also beneficial in the treatment of schizoaffective and bipolar disorders[3].

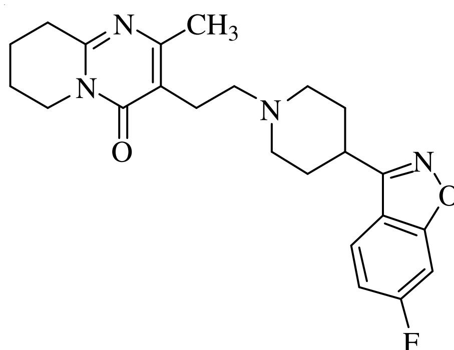

Fig. 1. Structural formula of risperidone (RPN)

In the literature there have been reported high-performance liquid chromatography[4-6], liquid chromatography-mass spectroscopy[7-9], liquid chromatography[10], high-performance liquid chromatography and thin layer densitometry[11] methods for the determination of risperidone in pharmaceuticals or biological fluids. Isolation of degradation products of risperidone[12] and structural studies of impurities of risperidone by hyphenating techniques[13] also have been reported in the literature. In pharmacopoeias[14], methods of infrared absorption spectrophotometry and liquid chromatography were described for the identification of RPN. All these reported methods are either not sufficiently sensitive or tedious and require highly sophisticated instrumentation. Although RPN is electroactive molecule on mercury electrode there is only a few studies in literature which is dealing with electrochemical behaviour and development of electrochemical method to determine the RPN[15,16].

Electrochemical methods have been proved to be very sensitive for the determination of organic molecules including drugs and related molecules in pharmaceutical dosage forms and biological fluids. These methods are faster, easier and cheaper than spectroscopic and chromatographic methods. Spectrophotometric methods on drug analysis are also very simple, fast, economic and reproducible.

The purpose of this study is to establish the experimental conditions for the determination of RPN and to investigate the electrochemical behaviour and to propose possible reduction mechanism of RPN on hanging mercury drop electrode (HMDE) using cyclic voltammetry and controlled potential electrolysis techniques. This study is also purposed to develop a new, rapid, selective and simple electrochemical methods including differential pulse voltammetry (DPV), square wave voltammetry (SWV) and simple spectrophotometric methods for the direct determination of RPN in raw materials, pharmaceutical dosage forms and biological samples without time-consuming extraction, separation and evaporation steps prior to drug assay.

EXPERIMENTAL

All voltammetric measurements such as cyclic voltammetry (CV), controlled potential coulometry, square wave voltammetry (SWV) and differential pulse voltammetry (DPV) were carried out using a CH-instrument electrochemical analyzer (CHI 760). A three electrode cell system incorporating the hanging mercury drop electrode (HMDE) as working electrode, platinum wire auxiliary electrode (BAS MW-1034) and an Ag/AgCl reference electrode (MF-2052 RE-5B) was used in all experiments.

A three electrode combination system for bulk electrolysis was consisted of mercury pool (55.4 cm^2) as working electrode, coiled platinum wire auxiliary electrode (23 cm) (BAS MW-1033) and Ag/AgCl reference electrode (BAS MF-2052 RE-5B).

All pH measurements were made with Thermo Orion Model 720A pH ion meter having an Orion combined glass pH electrode (912600) which had been calibrated with pH 4.13 and 8.20 stock buffer solutions before measurements.

A PG instrument T-80 recording double beam UV-visible spectrophotometer with data processing capacity was used for spectrophotometric analysis. The deionized water was supplied from Human Power I^+, ultra pure water system. All the data were obtained at ambient temperature

Standard sample of RPN (99 %, from Janssen-Cilag) was used to plot the calibration curve. Stock solution of RPN (4×10^{-3} mol L^{-1}) was prepared in 25 mL of ethanol (Merck). Calibration solutions were prepared by appropriate dilution of the stock solution over the range of desired concentrations with Britton-Robinson (BR) buffer.

All chemicals used both in preparation of Britton-Robinson (BR) buffer solution, such as phosphoric acid (Riedel), boric acid (Riedel), acetic acid (Merck), sodium hydroxide (Merck) and in the preparation of NH_3 (Merck) -NH_4Cl (Merck) buffer solution were analytical reagent grade. Double-distilled deionized water was used in preparations of all the solutions.

Risperdal (RPL) (from Eczacibasi) tablets were used as pharmaceutical dosage form which contains 1 mg of RPN and some amount of lactose monohydrate as excipients per tablet. To prepare the solutions of tablets, initially the drug content of ten tablets was weighed, finely powered and mixed. The average mass per tablet was determined. A sample equivalent to one tablet was weighed and transferred in to a 100 mL calibrated flask and completed to the volume with ethanol. The contents of the flask were sonicated for 0.5 h to achieve complete dissolution. After solution step, content of flask was centrifuged 0.5 h at 1500 rpm. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with BR buffer.

Voltammetric procedure: In cyclic voltammetry, 9 mL of RPN solution in BR was placed into the electrochemical cell for each time. The solution was deoxygenated with purified argon (99.99 % purity) for 2 min before running for the first cycle

and 30 s for following cycles. After deareation, a new hanging mercury drop was formed and then the voltammograms were recorded by applying a negative-going scan from -1.20 to -1.70 V.

In controlled-potential electrolysis, 50 mL of 1.2×10^{-4} mol L^{-1} RPN solution in BR was placed into the mercury pool electrode (55.4 cm^2). The solution was deoxygenated for 25 min before running electrolysis. The applied potential was hold constant at -1.75 V and the electrolysis was performed for 5 h with stirring continuously.

RESULTS AND DISCUSSION

Electrochemical behaviours of RPN: The electrochemical behaviour, diffusion and adsorption properties of RPN were studied by using cyclic voltammetry and controlled-potential electrolysis. In cyclic voltammetry, a single well-defined reduction peak was observed at a potential of about -1.58 V. There exist no peaks when an only blank BR buffer solution was scanned and peak intensity increases with increasing concentration of RPN, concluded that this peak is due to the reduction of RPN molecules. There is also an anodic peak indicating the reversible nature of RPN-electrode interaction, but the ratio of anodic peak current to cathodic peak current ($i_{p,a}/i_{p,c}$) is less than unity (Fig. 2).

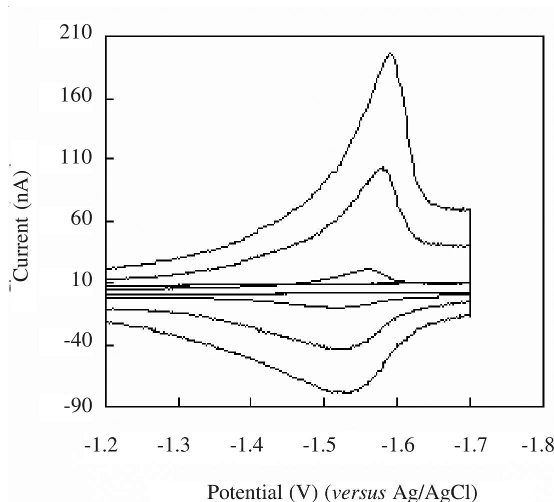

Fig. 2. Cyclic voltammograms of risperidone in BR buffer solution at pH 10.3 scan rate: 0.100 V s^{-1}. (a) Blank BR buffer, (b) 2.3×10^{-5} mol L^{-1}, (c) 6.4×10^{-5} mol L^{-1}, (d) 1.2×10^{-4} mol L^{-1}

In fact for an ideal reversible electrochemical mechanism, ratio of peak currents is unity and potential of peak is not affected by scanning rate[17]. The influences of the potential scan rate on cathodic peak current (i_p) and cathodic peak potential (E_p) were investigated for 1.2×10^{-4} mol L^{-1} RPN in the 0.010-10.000 V s^{-1} scan rate range.

The peak potential shifted to more negative values as scan rate increased (Fig. 3). These investigations showed that electrode reaction is quasi-reversible one.

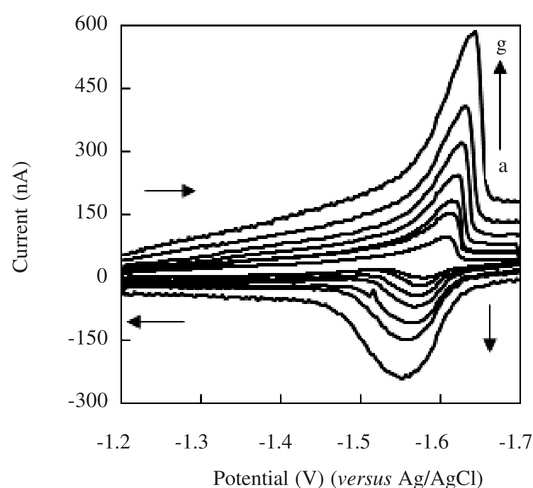

Fig. 3. Cyclic voltammograms of 1.2×10^{-4} mol L^{-1} risperidone in BR buffer solution at pH 10.3 at different scan rates. (a) 0.050 V s^{-1}, (b) 0.075 V s^{-1}, (c) 0.100 V s^{-1}, (d) 0.150 V s^{-1}, (e) 0.225 V s^{-1}, (f) 0.300 V s^{-1}, (g) 0.500 V s^{-1}

The pH of the medium showed that the peak potential shifts to more cathodic region as pH increased. This shows existence of proton in mechanism of electrode reaction.

Linear plots of peak current *versus* square-root of scanning rate (i_p *versus* $\nu^{1/2}$) should be obtained for diffusing electroactive species, whereas species adsorbed on the electrode surface should result in linear plots of i_p *versus* ν. When the scan rate varied from 0.010-0.500 V s^{-1} in 1.2×10^{-4} mol L^{-1} RPN, a linear dependence of the peak intensity i_p (A) upon the scan rate (V s^{-1}) was found as given equation $i_{p,c}$ (A) = $1.06 \times 10^{-6}\nu + 8.03 \times 10^{-8}$ with $R^2 = 0.996$, confirmed an adsorption behaviour. In addition, the peak current decreased with pH values increasing and the peak current increased as the buffer concentration increasing, indicated that the electrode reaction controlled mainly by a surface interaction.

A plot of logarithm of peak current (A) *versus* logarithm of scan rate (V s^{-1}) gave a straight line with a slope of 0.926 for RPN, close to the theoretical value of 1 for adsorbed species, which expressed that electrode process is controlled by adsorption[17].

In controlled-potential electrolysis, the result of bulk electrolysis showed that no significant change in both peak current and peak potential was observed before and after electrolysis. These results supported that deduction on the observed reduction wave as a catalytic adsorptive one. To find the number of electron(s), following relations were used in data of cyclic voltammetry:

$$ip = n^2F^2\Gamma A\nu/4RT \quad (1)$$

and the relation $$Q = nFA\Gamma \quad (2)$$

where i_p is the peak current (A), Q is the charge (C) consumed by the surface process as calculated by the integration of the area under the peak, n is total number of electrons transferred in electrode reaction. G is the surface coverage of adsorbed substance, (mol cm^{-2}), A is the working mercury electrode area (0.0145 cm^2) and F is the Faraday constant (96485 C mol^{-1}), ν is the scanning rate (V s^{-1})[17]. By substitution the Γ term of the equation 2 to the first equation, it is easy to get a new relation for n:

$$n = 4i_pRT/FQ\nu \quad (3)$$

In the potential scanning rate range from 0.010-0.500 V s^{-1} numbers of electron(s) transferred in electrode reaction (n) was calculated by directly using an equation given above for an each scan rate and by using the slope of peak current *versus* scanning rate for the series of the scan rate. As a result of both methods, calculation and graph method, number of electrons was found between 1.89 and 2.15 so number of electron in electrochemical step is 2. The surface coverage of adsorbed substance (Γ) was found as 2.10×10^{-11} mol cm^{-2} when 0.010 V $s^{-1} \leq \nu \leq$ 0.075 V s^{-1} and 1.18×10^{-11} mol cm^{-2} for the scanning rate between 0.100 and 0.500 V s^{-1}. At higher scan rates, shapes of cyclic voltammograms are distorted. Thus it was not aimed to calculate the electrochemical parameters of RPN at a scan rates higher than 0.500 V s^{-1}.

The peak current for an adsorption-desorption couple (at 298.15 K) is given by the equation[18]:

$$i_p = (1.09 \times 10^6)n^2ACD^{1/2}\nu t^{1/2} \quad (4)$$

Diffusion coefficient of RPN was calculated by using the slope of the plot of peak current *versus* potential scan rate (i_p *versus* ν). Diffusion coefficient was found as 1.84×10^{-8} cm^2 s^{-1}.

As a results of electrochemical parameters given above, the mechanism can be proposed as electro reduction of carbonyl group of RPN. Since the structure of RPN molecule has carbonyl group, activated by the neighboring nitrogen and based on the observed transfer of two electrons, it can be postulated that the electrode reaction is due to the reduction of carbonyl group activated by the neighboring nitrogen.

Voltammetric assay of RPN: Because of being an electroactive molecule on the hanging mercury electrode surface, the electrochemical assay of RPN was established various voltammetric techniques including differential pulse voltammetry (DPV) and square-wave voltammetry (SWV). In the present study, initially variables such as supporting electrolyte, pH, RPN concentration and instrumental parameters were optimized as given below:

The peak responses for the studied drug were affected by the type of supporting electrolytes. Two different supporting electrolytes were examined including BR and NH_3/NH_4Cl buffers. The highest peak current and the best peak shape were

obtained in the presence of BR buffer. Peak current increased with increasing buffer concentration in the range between 0.01 and 0.20 mol L^{-1}. The suitable concentration of each component of BR buffer was described as 0.04 mol L^{-1}.

The pH of a solution is a critical factor affecting both the rate and equilibrium state of the accumulation process and the rate of the electrode reaction. The influence of the pH on the DPV and SWV response was studied at HMDE of 1.2×10^{-5} mol L^{-1} RPN between pH values from 7-12. The peak current increased with the increasing pH of the supporting electrolyte till it reached a much enhanced value over the pH range 8-12 (Fig. 4). At pH values higher than 10.3, the peak current decreased gradually together with a poor peak definition up to pH 12 and at pH values lower than 8 peak shapes (symmetry and resolution) were distorted. Therefore, a BR buffer of pH 10.3 was chosen as a supporting electrolyte in the rest of the present quantitation work.

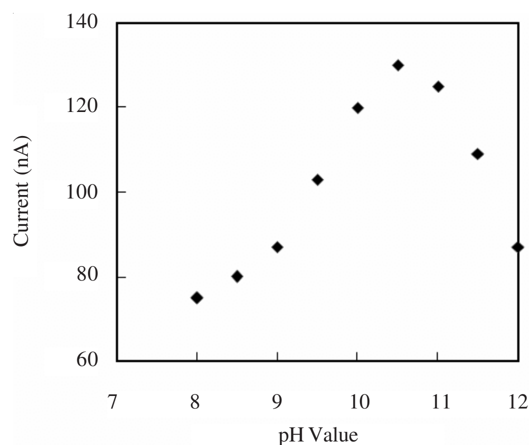

Fig. 4. Influence of pH on the peak current in cyclic voltammogram of 1.2×10^{-5} mol L^{-1} risperidone (scan rate: 0.100 V s^{-1})

The square-wave (SW) response markedly depends on the parameters of the excitement signal. In order to obtain a well-defined square-wave voltammetric peak shape, the optimum instrumental conditions frequency (f), scan increment (ΔEi) and pulse-amplitude (ΔEa) were studied for 5×10^{-7} mol L^{-1} RPN in a BR buffer of pH 10.3 at a HMDE. The optimum instrumental conditions were found as f = 25 Hz, ΔEi = 4 mV and ΔEa = 15 mV.

To establish the linearity range (working range) of RPN in both DPV and SWV, seven different standard solutions were used ranged from 2.3×10^{-7}-2.0×10^{-5} mol L^{-1} (Figs. 5A and 6A). For each concentration five reproducible measurements were taken and mean of these measurements was used to plot the calibration curve. Result of concentration studies showed that an average peak current of reduction wave is change linearly with RPN concentration, in the range from 2.3×10^{-7}-7.5×10^{-6} mol L^{-1} (Figs. 5B and 6B).

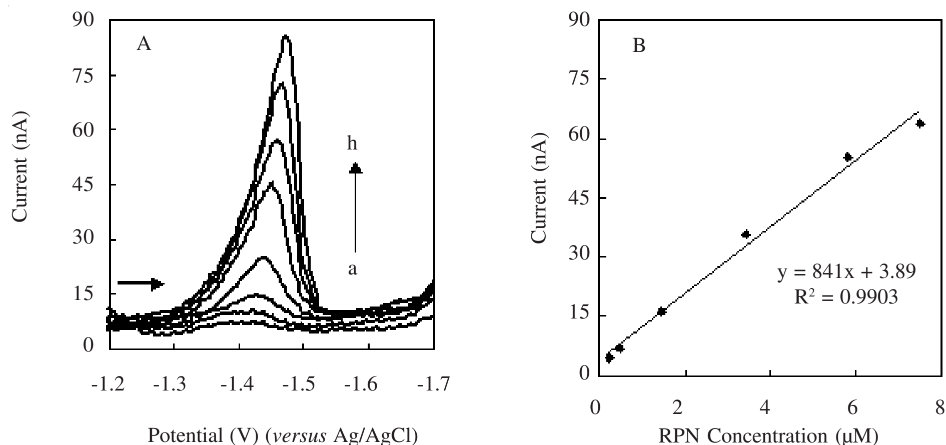

Fig. 5. Results of concentration studies of risperidone by means of DPV (A) effect of RPN concentration on reduction wave intensity in DPV: (a) base line, (b) 2.3×10^{-7} mol L^{-1}, (c) 5.0×10^{-7} mol L^{-1}, (d) 1.4×10^{-6} mol L^{-1}, (e) 3.4×10^{-6} mol L^{-1}, (f) 5.8 $\times 10^{-6}$ mol L^{-1}, (g) 7.5×10^{-6} mol L^{-1}, (h) 2.0×10^{-5} mol L^{-1} (B) calibration curve of RPN in DPV from (b) to (g) given in (A)

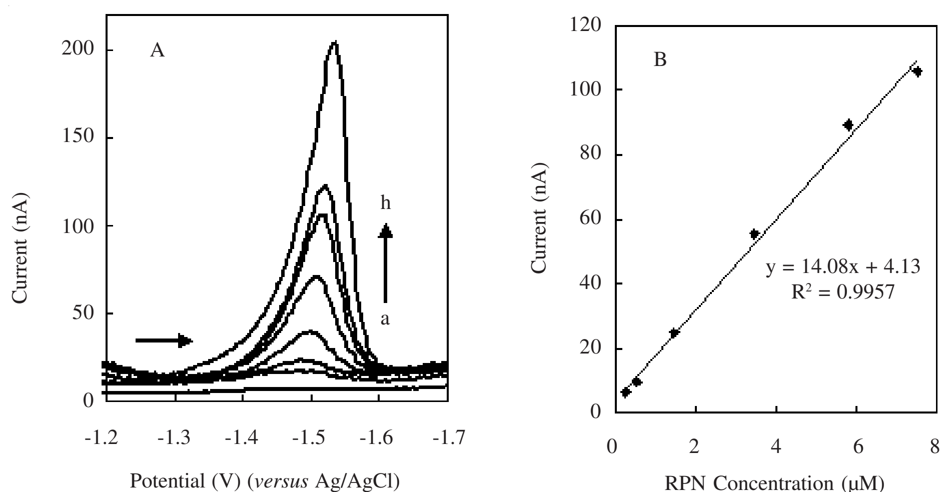

Fig. 6. Results of concentration studies of risperidone by means of SWV (A) effect of RPN concentration on reduction wave intensity in SWV: (a) base line, (b) 2.3×10^{-7} mol L^{-1}, (c) 5.0×10^{-7} mol L^{-1}, (d) 1.4×10^{-6} mol L^{-1}, (e) 3.4×10^{-6} mol L^{-1}, (f) 5.8 $\times 10^{-6}$ mol L^{-1}, (g) 7.5×10^{-6} mol L^{-1}, (h) 2.0×10^{-5} mol L^{-1} (B) calibration curve of RPN in SWV from (b) to (g) given in (A)

Limit of detection (LOD) and limit of quantitation (LOQ) values for RPN were calculated using the relations: LOD = 3 s/m and LOQ = 10 s/m. The abbreviation of s is the standard deviation of the peak currents of blank BR solutions (five runs) and m is the slope of the related calibration curve, LOD values for SWV and DPV

were found to be 1.3×10^{-8} and 2.2×10^{-8} mol L^{-1}, respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

Spectrophotometric assay of RPN: For maximum sensitivity, spectrophotometric absorbance measurements are ordinarily should be made at a wavelength corresponding to absorption maximum because change in absorbance per unit of concentration is greatest at this point. To obtain the wavelength at which RPN has a maximum absorbance, firstly baseline correction of blank BR buffer was scanned and then wavelength scanning from 800-200 nm was performed by using standard RPN solutions at pH 10.3. This scanning was repeated for five different standard solution of RPN. These investigations show that maximum absorption of RPN occurs at 277 and 238 nm (Fig. 7).

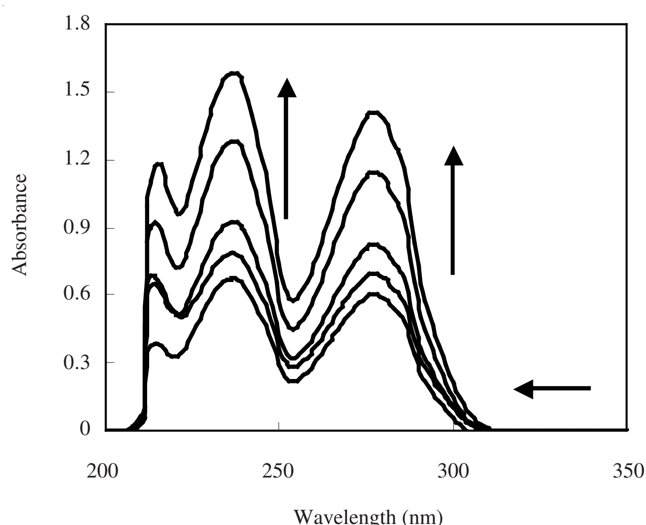

Fig. 7. Results of concentration studies of risperidone by means of UV-vis spectrophotometry after base line correction (at pH 10.3), in BR buffer

After investigation of wavelengths, calibration studies were performed. To determine the linearity range and to plot the calibration curve nine different standard solution were prepared from 2.6×10^{-7}-1.2×10^{-5} mol L^{-1}. Absorbance of RPN is linearly changed with RPN concentration at range from 2.6×10^{-7}-1.2×10^{-5} mol L^{-1} when 277 nm was used and from 2.7×10^{-6}-4.0×10^{-5} mol L^{-1} when 238 nm was used.

Validation of methods: Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limits of detection and quantitation, accuracy, reproducibility, stability, selectivity and robustness[19]. Values of such parameters are given in Tables 1-6.

TABLE-1
PARAMETERS OF CALIBRATION AND VALIDATION FOR PROPOSED METHODS

Method	Electrochemical		Spectrophotometric	
Parameters	DPV	SWV	at 277 nm	at 238 nm
Linearity (working) range[a]	2.3×10^{-7} 7.5×10^{-6}	2.3×10^{-7} 7.5×10^{-6}	2.7×10^{-7} 1.2×10^{-5}	2.8×10^{-6} 4.1×10^{-5}
Slope[b]	0.0084	0.0146	24047.07	43552.32
Intercept[c]	3.9×10^{-9}	3.4×10^{-9}	0.009	-0.144
Regression coefficient (R^2)	0.990	0.999	0.992	0.993
Standard deviation of regression	2.5×10^{-9}	1.4×10^{-9}	0.010	0.054
Standard deviation of slope	3.7×10^{-4}	2.1×10^{-4}	917.46	1448.33
Standard deviation of intercept	1.9×10^{-9}	1.1×10^{-9}	0.005	0.018
Limit of detection (LOD)[a]	2.2×10^{-8}	1.3×10^{-8}	6.1×10^{-8}	3.4×10^{-8}
Limit of quantification (LOQ)[a]	7.1×10^{-8}	4.1×10^{-8}	2.0×10^{-7}	1.1×10^{-8}
Repeatability of current (RSD, %)[d]	1.13	0.88	0.73	1.08
Repeatability of potential (RSD, %)[d]	0.11	0.13	–	–

[a]mol L^{-1}. [b]For voltammetric methods in A L mol^{-1} and for spectrophotometry in L mol^{-1} cm^{-1}. [c]For voltammetric methods in A and for spectrophotometry in absorbance unit. [d]For voltammetry peak current and peak potentials were investigated, whereas in spectrophotometry absorbance values of the same sample were used.

TABLE-2
RESULTS OF SAMPLE ANALYSIS IN DPV

Samples	RPN content (mg)	Found values (mg)	Reported value*	RSD (%)
First day	1.00	1.00, 1.06, 0.99, 1.01, 1.03	1.02 ± 0.032	2.72
Second day	1.45	1.44, 1.46, 1.49, 1.43, 1.42	1.45 ± 0.032	1.92

*Results in average value ($\bar{x} \pm t \cdot s/\sqrt{N}$) at 95 % confidence level.

TABLE-3
RESULTS OF SAMPLE ANALYSIS IN SWV

Samples	RPN content (mg)	Found values (mg)	Reported value*	RSD (%)
First day I	0.50	0.49, 0.50, 0.50, 0.52, 0.48	0.49 ± 0.017	2.98
First day II	1.00	1.00, 1.01, 0. 99, 1.03, 1.06	1.02 ± 0.032	2.73
Second day	1.45	1.49, 1.46, 1.44, 1.42, 1.45	1.45 ± 0.030	1.78

*Results in average value ($\bar{x} \pm t \cdot s/\sqrt{N}$) at 95 % confidence level.

TABLE-4
RESULTS OF SAMPLE ANALYSIS IN UV-VIS SPECTROPHOTOMETRY at 277 nm

Samples	RPN content (mg)	Found values (mg)	Reported value*	RSD (%)
First day	0.75	0.77, 0.74, 0.74, 0.76, 0.75	0.75 ± 0.013	1.73
Second day I	1.00	1.00, 1.02, 1.07, 1.00, 1.08	1.03 ± 0.044	3.72
Second day II	1.25	1.23, 1.27, 1.25, 1.20, 1.26	1.24 ± 0.032	2.23

*Results in average value ($\bar{x} \pm t \cdot s/\sqrt{N}$) at 95 % confidence level.

Assay of RPN in samples: In order to evaluate the applicability of the proposed method to pharmaceutical preparations and biological samples, RPN was determined

in the pharmaceutical preparation (risperdal tablets), spiked human urine and spiked human serum by using direct calibration method. Analytical and statistical parameters obtained in voltammetric studies for standard RPN were used in the assay studies.

TABLE-5
RESULTS OF REAL SAMPLE ANALYSIS IN UV-VIS SPECTROPHOTOMETRY AT 238 nm

Samples	RPN content (mg)	Found values (mg)	Reported value*	RSD (%)
First day	0.75	0.78, 0.76, 0.79, 0.74, 0.73	0.76 ± 0.029	3.35
Second day I	1.00	1.06, 0.98, 1.05, 0.99, 1.00	1.02 ± 0.042	3.59
Second day II	1.25	1.20, 1.22, 1.26, 1.28, 1.32	1.26 ± 0.055	3.80

*Results in average value ($\bar{x}$ ± t·s/√N) at 95 % confidence level.

TABLE-6
RESULTS OF ANALYSIS IN BIOLOGICAL SAMPLES IN BOTH DPV AND SWV

Samples	RPN content (mg)	Found values (mg)	Reported value*	RSD (%)
A (DPV, Serum)	1.00	1.02, 0.99, 1.00, 0.99, 1.00	1.00 ± 0.012	1.04
B (SWV, Serum)	2.22	2.25, 2.23, 2.19, 2.20, 2.22	2.22 ± 0.027	1.08
C (DPV, Urine)	2.43	2.54, 2.47, 2.44, 2.40, 2.45	2.46 ± 0.059	2.09
(SWV, Urine)	1.00	1.06, 0.98, 1.05, 0.99, 1.00	1.02 ± 0.042	3.59

*Results in average value ($\bar{x}$ ± t·s/√N) at 95 % confidence level.

Assay of RPN in tablets: Proposed method was first applied to assay of RPN in risperdal tablets. The results of applications to pharmaceutical preparations found by using proposed method were given in Tables 2 and 3 for DPV and SWV, respectively and 4 and 5 for spectrophotometry at different wavelengths. As seen in Tables, average recoveries are in good agreement with the RSD values less than 3 %, which is a good evidence of validity of method. Thus, the precision is quite satisfactory for the analysis of pharmaceutical preparations as well as bulk samples.

Assay of RPN in biological samples: In order to investigate the applicability of the proposed methods to biological samples, method was applied to spiked human urine and spiked human serum samples. In both of the applications, analytical and statistical parameters found or calculated in studies of standard RPN were used. Analysis studies in biological samples were performed as direct calibration method.

Application to spiked urine: Urine sample from healthy individuals was centrifuged at 4000 rpm. Into a set of 10 mL volumetric flasks, separate aliquots of urine (1 mL) were spiked with varying amounts of RPN. The volumes were adjusted to 10 mL with BR buffer at pH 10.3. A 1 mL aliquot from each solution was diluted to 10 mL with the same buffer and transferred into the measuring vessel. Voltammograms were recorded as under construction of calibration curve. The results of applications to spiked urine found by using proposed method are given in Table-6.

Application to spiked human serum: Serum samples, obtained from healthy individuals were stored frozen until assay. After gentle thawing, an aliquot volume

of sample was fortified with RPN dissolved in ethanol to achieve appropriate concentration. The solution was centrifuged for 15 min at 4000 rpm to remove the precipitated serum proteins and the supernatant was taken carefully. Appropriate volume of supernatant liquor was transferred in the 10 mL volumetric flask. The sample solution obtained above was applied to the proposed method for determination of RPN. The results of applications to spiked human serum found by using proposed method are given in Table-6.

As seen in Table-6 average recoveries are in good agreement with low RSD values less than 4 % for both human serum and human urine, which is a good evidence of validity of method. These results indicate that the content of RPN in the pharmaceuticals and biological samples such as human urine and human serum can be safely determined by using proposed methods without interference from excipients in the preparations and biological liquids. The method can be applied to pharmaceuticals and biological samples after a simple dilution step with direct measurements.

Interference studies: During an application of proposed method to biological samples and tablets, before adding a standard solution of molecule under investigation, voltammetric base line of biological medium was measured by applying the same procedures as applied to calibration studies with standard samples (for blank biological base line). In such an applications there exist no extra voltammetric signal, indicates that there is no significant interferences of various inorganic cations, anions and some organic substances found in pharmaceutical preparations (tablets) and biological mediums (human urine and human serum). Furthermore recovery values of spiked samples are sufficient as pharmaceuticals. Because of these investigations no further interference studies were carried out.

Conclusion

The study of electroactive compound, risperidone (RPN) in BR buffer solution provides new assay methods for electrochemical and spectrophotometric means. The current signal due to the reduction process was a function of the amount of RPN in electrochemical methods and absorbance is a function of concentration of RPN in spectrophotometric method. The proposed methods provide a sensitive and selective method of RPN assay without further purification of compounds found in pharmaceutical dosage forms. The developed methods DPV, SWV and spectroscopy have detection limits 2.2×10^{-8}, 1.3×10^{-8} and 6.1×10^{-8} mol L^{-1}, respectively. These methods can be used to assay of RPN in pharmaceutical preparations and biological samples. These methods are more sensitive to already reported different spectrophotometric, chromatographic and electrochemical methods given in references. The proposed method has distinct advantages over other existing methods regarding sensitivity, time-consuming and lower detectability. In addition no sophisticated instrumentation is required. Consequently, the proposed method has the potential of a good analytical alternative for determining risperidone in pharmaceutical formulations.

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