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Original Article

High-performance liquid chromatographic method for simultaneous determination of iloperidone and idebenone in spiked plasma

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ABSTRACT

Background: Iloperidone (ILP) is a second-generation atypical antipsychotic agent and idebenone (IDB), a synthetic analogue of coenzyme Q10 is an important cell membrane antioxidant. No validated analytical method for simultaneous determination of ILP and IDB in rat plasma has been reported till date.

Objective: To develop and validate a simple reversed phase high performance liquid chromatography method for simultaneous determination of iloperidone and idebenone in rat plasma.

Methods: A liquid–liquid extraction method was used for deproteination of plasma samples using methanol as an extraction solvent. Chromatographic separations were done using isocratic conditions. Mobile phase containing acetonitrile and 0.025 M KH_2PO_4, pH 6 (60:40) at a flow rate of 1 mL/min was utilized for efficient separation. The UV detector was set at 277 nm. Risperidone was used as an internal standard.

Results: The limits of detection (LOD) for iloperidone and idebenone were 10 and 20 ng/ml, while the limits of quantification (LOQ) were 30 and 35 ng/ml, respectively. The standard curves for iloperidone and idebenone in plasma were linear over the range of 0.05–20 μg/ml, with the correlation coefficients of 0.9993 and 0.9985, respectively. All the validation parameters, such as accuracy, intra and inter-day precision were within the required limits. The samples were stable at −80 °C and −20 °C as compared to 4 °C storage temperature when subjected to repeated freeze–thaw cycles.

Conclusion: The proposed method proves to be a sensitive method because of its potential to simultaneously determine iloperidone and idebenone in rat plasma in a single HPLC run.

1. Introduction

Iloperidone (ILP) is a novel atypical antipsychotic agent, chemically known as (1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]propoxy]-3-methoxyphenyl]ethanone) (Fig. 1). It is commercially available in the form of oral tablets for the treatment of adults with schizophrenia.[1]

Idebenone (IDB) is a ubiquinone derivative chemically known as 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-p-benzoquinone (Fig. 1). Idebenone is approved in Canada and

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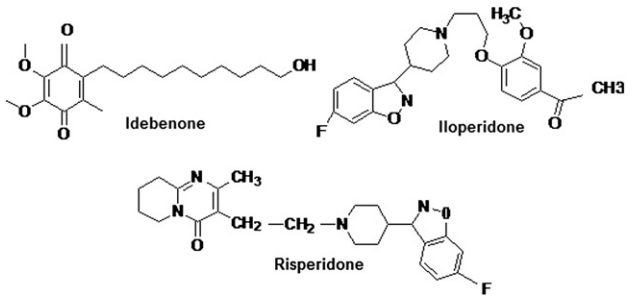

Fig. 1 – Chemical structure of idebenone, iloperidone and risperidone.

Europe in the form of oral tablets for the treatment of Friedreich's Ataxia. It also demonstrates very good antioxidant and neuroprotective properties.[2]

The role of oxidative stress in neurodegenerative diseases has already been established. Idebenone possesses unique ability to cross the blood brain barrier and ability to inhibit lipid peroxidation thereby inducing protection from neuronal damage.[2,3] Hence it was envisaged that the concomitant administration of antipsychotic agent ILP with antioxidant agent IDB may prove beneficial for the effective treatment of psychosis. To demonstrate the efficacy of developed formulation, a simple, fast and reliable method for determination of drug in the plasma is required. There are various reports which disclose different analytical methods for estimation of ILP[4,5] or IDB[6,7] individually from bulk drug or from biological samples.

To the best of our knowledge, no validated analytical method for simultaneous determination of ILP and IDB in spiked rat plasma has been reported till date. Therefore, the objective of this study was to develop a new, simple, rapid, precise, accurate, specific and validated RP-HPLC method for simultaneous determination of ILP and IDB from rat plasma.

2. Materials and methods

Iloperidone and risperidone were kindly provided as gift samples by Symed Labs (Hyderabad, India) and Wockhardt Limited (Aurangabad, India), respectively. Idebenone was purchased from Xi'an Bosheng Biological Technology Co Ltd, China. All other chemicals and reagents used were of HPLC grade and purchased from Merck Chemicals, India. Nylon filter paper of 0.45 μm (Millipore) was purchased from Pall life science, Mumbai, India. For all analysis, double-distilled water filtered through a 0.45 μm membrane filter was used.

2.1. Instrumentation

The HPLC system equipped with an Intelligent LC Pump (model Jasco PU 2080) with an autosampler programmed at 20 μL capacity per injection was used for the analysis. The detector consisted of UV/VIS detector (Jasco UV 2075). Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. A guard column (RP-18, 33 mm Kromasil®) and a Thermo Scientific ODS Hypersil (250 mm × 4.6 mm, 5μ) C-18, column was used for separations.

2.2. Chromatographic conditions

Combinations of different solvents were tested in order to find the suitable mobile phase for efficient elution of the drugs. The optimized mobile phase consisted of a mixture of acetonitrile and 0.025 mM potassium dihydrogen phosphate monohydrate buffer, pH 6.0 (HI 98107, pHep® pH Tester, Hanna Instruments, Mumbai, India) adjusted using concentrated aqueous solution of sodium hydroxide in the ratio of 60:40 (v/v). The flow rate of 1 ml/min was maintained throughout the analysis. The detector was model operated at a wavelength of 277 nm. Risperidone was used as an internal standard (Fig. 1).

2.3. Standard solutions and spiked plasma samples

Standard solutions of ILO, IDB and RSP were prepared separately by dissolving 10 mg of standard drug in 10 ml of methanol to yield the final concentration of 1000 μg/ml. These stock solutions were stable for at least three months when stored at 4 °C. Working standard solutions of ILO, IDB and RSP were prepared daily by diluting the stock solutions to desired concentrations with suitable solvent.

Plasma standards were prepared by spiking blank rat plasma (100 μl) with ILO, IDB and RSP standard solutions, respectively. For calibration in plasma, standards from 0.05 to 20 μg/ml were prepared by spiking blank rat plasma with 50 μl of working stock solutions of ILO and IDB, respectively. Quality control samples were independently prepared at three different concentrations of 0.1 μg/ml (LOQ, low), 8.0 μg/ml (MOQ, medium) and 15.5 μg/ml (HOQ, high) of ILO and IDB in the same way as described above.

2.4. Preparation of plasma samples

Blood samples were collected from retro-orbital plexus of the rat. During collection, blood samples were mixed thoroughly with di-sodium EDTA in order to prevent blood clotting. Samples were centrifuged at 7000 rpm for 20 min at 4 °C. Separated plasma (supernatant) was transferred into prelabelled tubes and stored in a refrigerator until the completion of analysis. Liquid–liquid extraction method was used for the preparation of plasma samples that are to be injected into HPLC system. To 100 μl of blank plasma, 50 μl of internal standard solution (10 μg/ml) was added. To this added 300 μl of methanol as an extraction solvent. This mixture was vortexed for 10 min followed by centrifugation (AllegraTM 64R Centrifuge, Beckman–Coulter India Pvt Ltd) at 7000 rpm for 20 min at 4 °C. The supernatant was collected and a volume of 20 μl was injected into the HPLC system.

2.5. Validation of bioanalytical method

The HPLC method was validated for linearity, specificity, sensitivity, precision, accuracy and stability according to FDA guidelines.[8]

2.6. Linearity

The linearity of the detector response for the test compounds was evaluated by injecting a total of nine calibration (working) standard solutions (0.05–20 μg/ml) covering the working