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Determination of pramipexole (U-98,528) in human plasma by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry

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Abstract

A highly sensitive and selective HPLC–MS–MS method was developed for the determination of pramipexole in human plasma. The analytes, pramipexole and BHT-920 (internal standard), were extracted from plasma at basic pH with methyl *tert.*-butyl ether (MTBE). MTBE was evaporated to dryness and reconstituted in 100 μl of (95:5) methanol–water. Chromatographic separation was achieved on a Zorbax SB-CN column with a mobile phase of (15:5:80) water–0.1 *M* ammonium acetate–methanol. The analytes were detected utilizing HPLC in conjunction with atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (MS–MS). The assay was linear in the concentration ranges of 50 to 5000 pg/ml. The analysis of pooled quality controls (150, 750, and 3000 pg/ml) demonstrated excellent precision with relative standard deviations (R.S.D.) (n=18) of 7.2%, 5.3% and 5.2%, respectively. The method is accurate with all intra-day (n=6) and overall (n=18) mean values being less than 11.7% from theoretical.

Keywords: Pramipexole

1. Introduction

Pramipexole (2-amino-4,5,6,7-tetrahydro-6-propyl-amino-benzthiazole-dihydrochloride) is a dopamine autoreceptor agonist that is in Phase III trials for the treatment of Parkinson's disease, and Phase II for schizophrenia. Therefore, a highly sensitive (50 pg/ml in human plasma) and selective method for the determination of pramipexole in plasma is necessary to support pharmacokinetic evaluation in humans. High-performance liquid chromatography (HPLC) methods for the determination of pramipexole in human plasma and urine with electrochemical detection [1] and UV detection [1] have been developed using solvent extraction [1]. However, the methods require relatively longer (>15 min) HPLC analysis times. This is because of the necessity to separate interference peaks from the peaks of interest.

The combination of HPLC and atmospheric pressure chemical ionization tandem mass spectrometry (APCI–MS–MS) provides many benefits, including analytical ruggedness as well as enhanced sensitivity and selectivity. Particularly, it enables the use of fast

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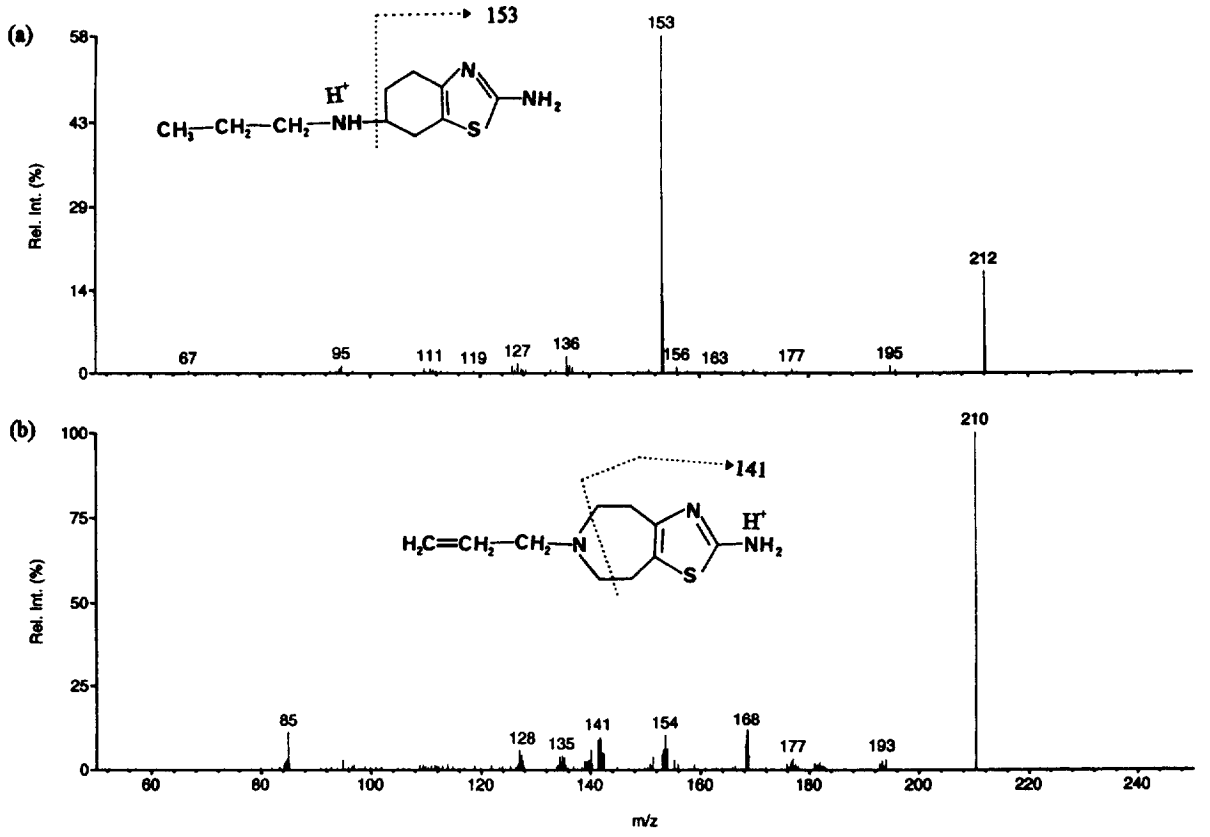

Fig. 1. Molecular structure and mass spectrum of (a) pramipexole and (b) BHT-920, internal standard.

liquid chromatographic separations at a flow-rate of 0.5–1.5 ml/min with very short analysis time. This technique has received a lot of attention and has been used to determine various drugs in biological fluids [2–9].

This paper presents a highly sensitive and selective HPLC method for the quantitation of pramipexole in human plasma, which has an analysis time of less than 5 min. This method utilizes atmospheric pressure chemical ionization (APCI) tandem mass spectrometry (MS–MS) detection.

2. Experimental

2.1. Materials

Pramipexole dihydrochloride (U-98,528E, SND 919 CL2 Y, purity 99.9%) and internal standard (BHT-920, I.S., purity 99.2%) were obtained from Upjohn (Kalamazoo, MI, USA). Heparinized human plasma was purchased from Worldwide Biological. Methanol and methyl *tert.*-butyl ether (MTBE), HPLC-grade, were obtained from Burdick and Jackson (Muskegon, MI, USA). Glacial acetic acid (GR grade) was obtained from EM Science (Gibbstown, NJ, USA). Sodium hydroxide was obtained from Mallinckrodt (Paris, KY, USA) and ammonium acetate (HPLC-grade) was obtained from Fisher (Fairlawn, NJ, USA). Deionized water was processed through a Milli-Q water purification system, (Millipore, Bedford, MA, USA).

2.2. Chromatographic systems

HPLC–MS–MS was performed on a Sciex (Thornhill, Canada) Model API III triple-quadrupole mass spectrometer interfaced via a Sciex heated nebulizer probe to a Hewlett-Packard 1090 L HPLC system with a 250-μl sample loop. The nebulizer probe

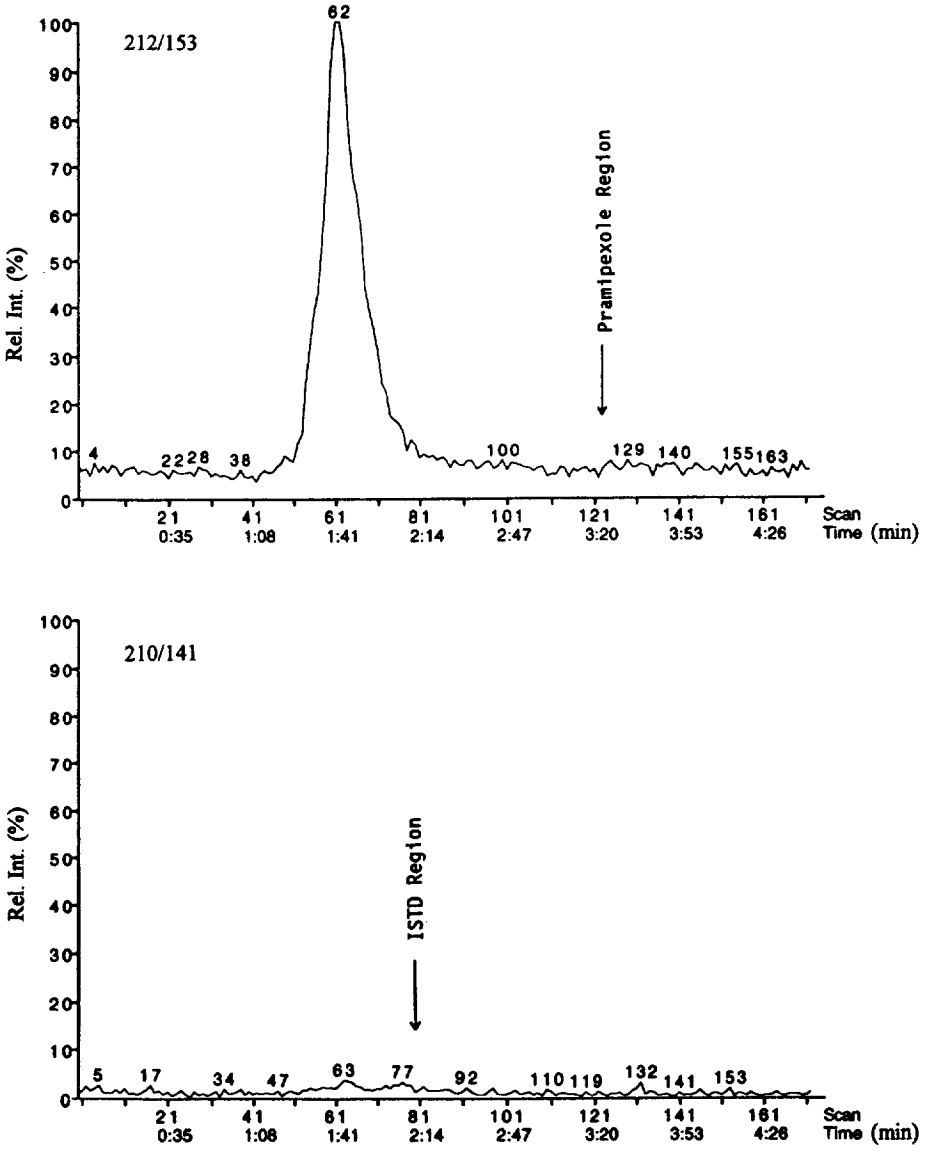

Fig. 2. Chromatogram of blank human plasma.

temperature was set at 470°C. The nebulizing gas pressure and auxiliary flow were set at 550 kPa and 1.5 l/min, respectively. Gas-phase chemical ionization was effected by a Corona discharge needle at 3 μA and positive ions were sampled into the quadrupole mass analyzer via a 0.0045-inch pinhole aperture. The mass spectrometer was set to detect the protonated molecular ions $[M+H]^+$ at m/z 212 (pramipexole) and 210 (internal standard) via the first quadrupole filter (Q1), to produce collision-induced fragmentation in Q2, and to monitor, via Q3, the product ions at m/z 153 and 141 for pramipexole and internal standard, respectively. The orifice potential was set at 45 V and the dwell time was 500 ms. The analytical column was a Zorbax SB-CN (150×4.6 mm I.D., 5 μm particle size, Mac-Mod, Chadds Ford, PA, USA). The mobile phase was water–0.1 *M* ammonium acetate–methanol (15:5:80)

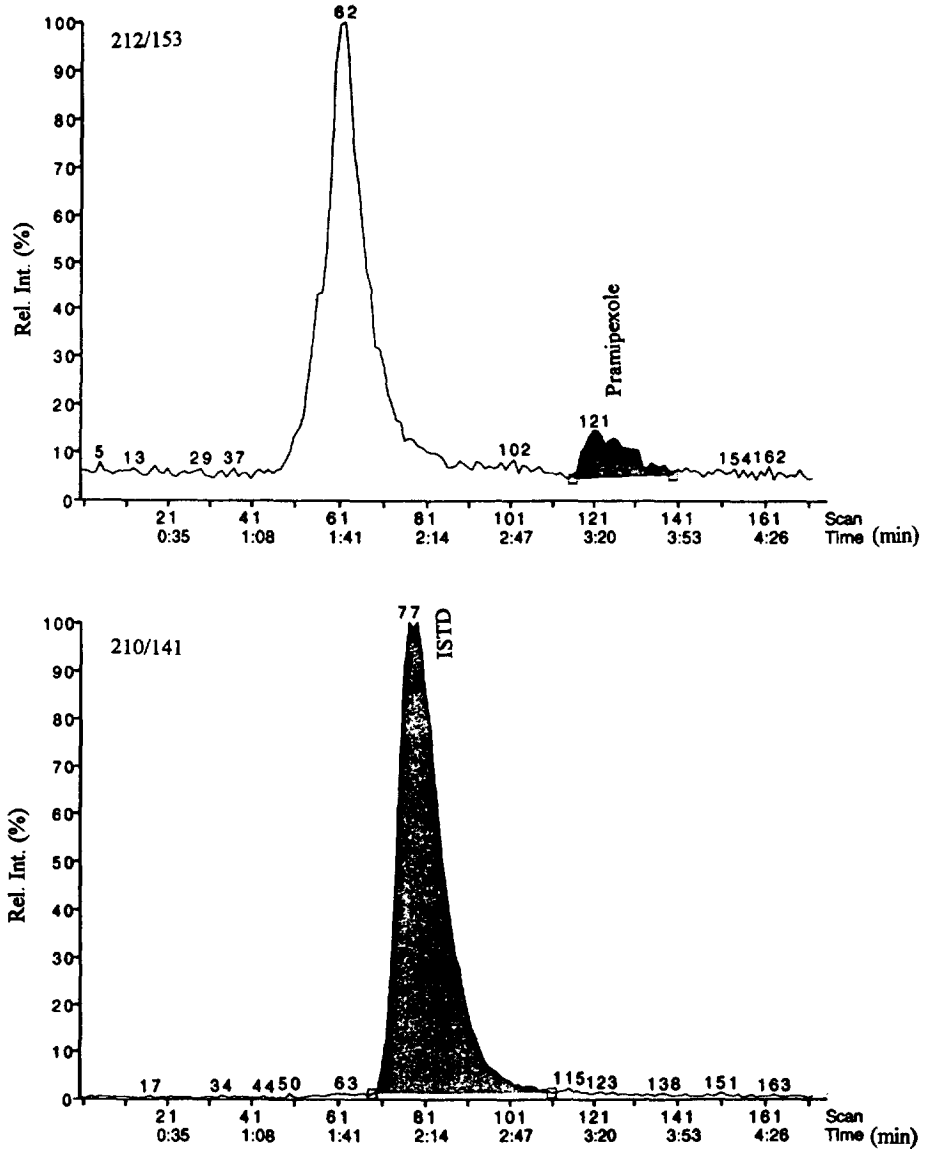

Fig. 3. Chromatogram of a 50 pg/ml calibration standard in human plasma.

at a flow-rate of 1.2 ml/min. Data were collected and processed using Macintosh Quadra 950 and 800 computers, respectively. The software used to process the data was Sciex API v2.4.

2.3. Preparation of standard solutions

A stock solution of pramipexole (100 μg/ml, free base) was prepared by dissolving 7.19 mg of pramipexole dihydrochloride salt in 50 ml water. The factor to convert from the salt to the free base is 1.431 (MW pramipexole dihydrochloride/MW pramipexole=302.28:211.28). A stock solution of internal standard (100 μg/ml) was prepared by dissolving 5 mg of BHT-920 (internal standard) in 50 ml of water. Standard solutions of pramipexole (1 to 100 ng/ml) and internal standard (180 ng/ml) were prepared by diluting the stock solutions with 1% glacial acetic acid. The analytes in these solutions were stable for at least four months when

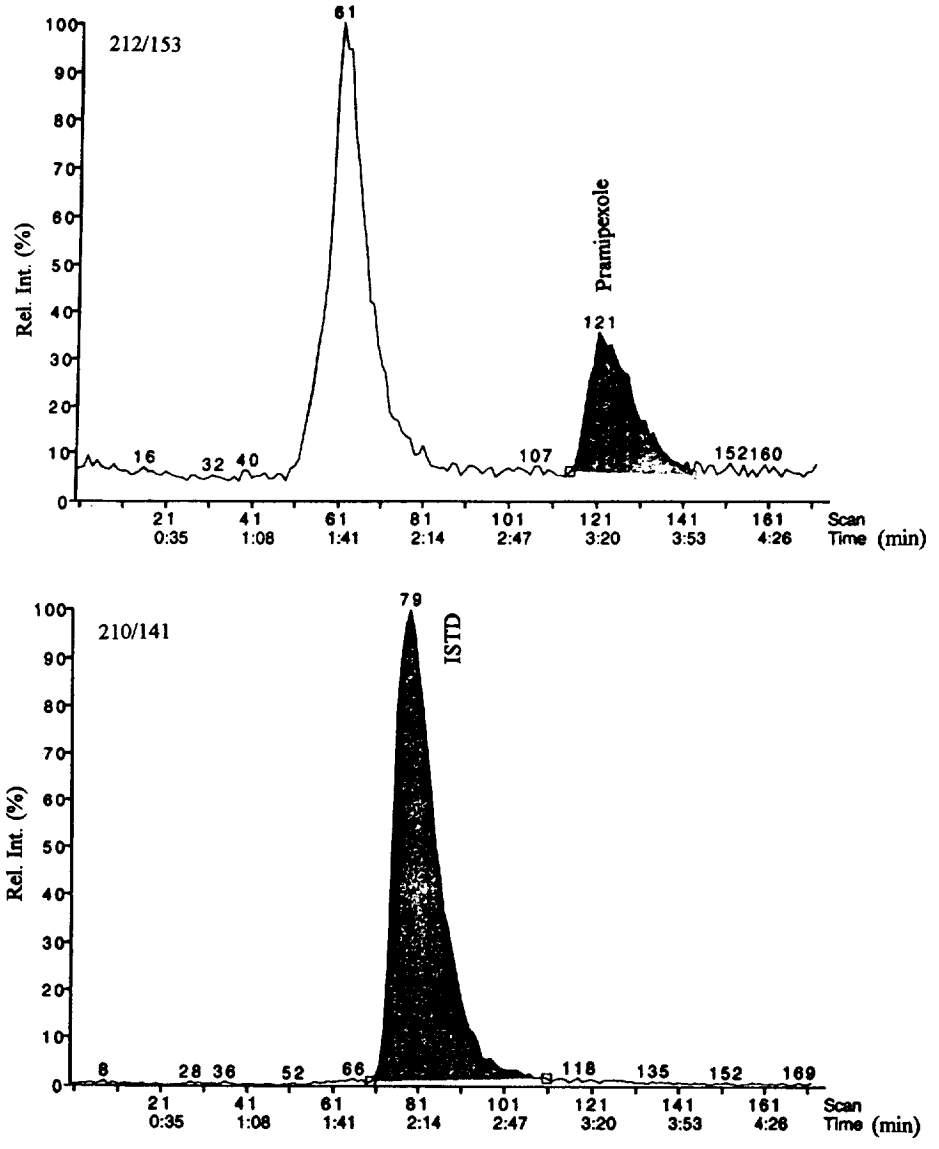

Fig. 4. Chromatogram of a 150 pg/ml quality control in human plasma.

stored in polypropylene bottles at 5°C. Pramipexole and the internal standard were found to adsorb to glass. Therefore, all standards and quality controls were prepared and stored in polypropylene flasks and vials.

2.4. Quality control samples

Pooled quality control samples (quality controls) were prepared to determine the precision and accuracy of the method, and to evaluate the stability of samples. A quality control pool at a concentration above the curve range (over-curve control) was also prepared to evaluate parallelism when analyzed using partial volume.

Plasma quality control pools (150, 750, and 3000 pg/ml pramipexole) were prepared by diluting 150 μl of 200 ng/ml, 750 μl of 200 ng/ml, and 3 ml of 200 ng/ml pramipexole, respectively, to a 200-ml volume of human plasma. The over-curve control

Table 1
Calibration curve data for pramipexole in plasma

Calibration standard concentration (pg/ml)	Calculated concentration (mean±S.D., n=6) (pg/ml)	R.S.D. (%)	Deviation (%)
50	50.3±0.68	1.4	0.6
100	95.7±3.69	3.9	−4.3
200	208±10.2	4.9	4.1
500	487±12.9	2.7	−2.7
1000	960±30.0	3.1	−4.0
2000	2060±51.7	2.5	3.0
5000	5100±117	2.3	2.0

Results of the validation study over a 3-day period.

(9000 pg/ml) was prepared by diluting 0.9 ml of 1 μg/ml to 100 ml with human plasma.

All quality control pools were aliquoted into polypropylene vials and stored at −20°C. The plasma quality control pools were stable for more than nine months.

2.5. Sample preparation

Calibration standards were prepared by adding 50 μl of the appropriate pramipexole standard solutions (1.00 to 100 ng/ml) to 1 ml of blank plasma. Calibration standards, samples, and quality controls were processed by adding 1 ml of plasma, 50 μl of internal standard solution (180 ng/ml), 100 μl of 1 *M* sodium hydroxide, and 6 ml of MTBE into labelled polypropylene centrifuge tubes. The tubes were capped and mixed on a vortex mixer for 5 min, and centrifuged at 630 *g* for 5 min. The aqueous layer was frozen in a dry ice–acetone bath, and the organic layer was transferred to a clean tube. The MTBE was dried down under nitrogen and reconstituted in 100 μl methanol–water (95:5). Aliquots of 70 μl were injected onto the HPLC system. The extracted samples were stable for at least 48 h.

Table 2
Precision and accuracy of the assay for pramipexole in plasma

Quality control concentration (pg/ml)	Calculated conc. (overall mean±S.D., n=18) (pg/ml)	R.S.D. (%)	Deviation (%)
150	144±10.4	7.2	−4.1
750	715±38	5.3	−4.7
3000	2820±146	5.2	−6.0

Results of the validation studies over a 3-day period with six determinations per day.

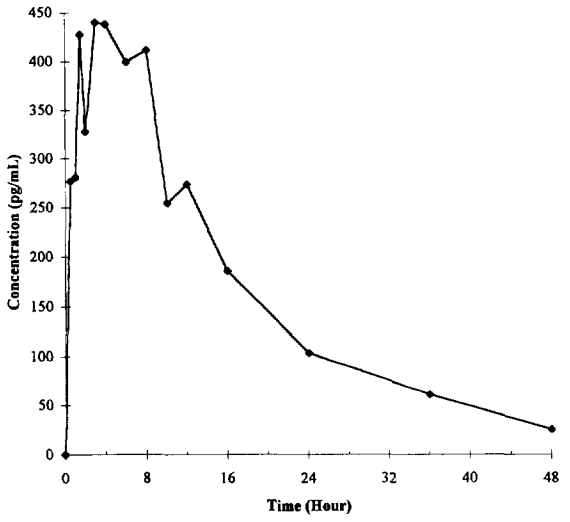

Fig. 5. Plasma pharmacokinetic profile from one individual following a single 0.25 mg oral dose of pramipexole.

2.6. Validation

Duplicate calibration curves were analyzed on each of three days. Triplicate quality controls at each concentration were analyzed with each calibration curve. The calibration curves were obtained by weighted (1/concentration*concentration) least-squares linear regression analysis of the peak-area ratios of pramipexole/internal standard versus the concentration of pramipexole. The equations of the calibration curves were used to calculate the concentration of pramipexole in the samples and quality controls from their peak area ratios.

3. Results and discussion

3.1. Separation and specificity

The molecular structures and mass spectra of pramipexole and BHT-920 (internal standard) are shown in Fig. 1. Representative chromatograms of standards and quality controls are shown in Fig. 2, Fig. 3 and Fig. 4. The mean retention times of pramipexole and the internal standard were 3.4 and

2.2 min, respectively. Blank plasma from ten pools was tested for endogenous interferences. There were no endogenous interferences found in the pramipexole and internal standard regions for all of the lots tested (Fig. 2).

3.2. Linearity, precision and accuracy

Calibration curve data for pramipexole are contained in Table 1. Calibration curves for pramipexole in plasma were linear in the concentration range of 50 to 5000 pg/ml. The correlation coefficients were greater than 0.9978 for all curves.

Data from the spiked quality control samples are shown in Table 2. The within-day precision of the method, as measured by the relative standard deviation (R.S.D.) of the daily mean ($n=6$), was less then 6.9%. The overall precision was 7.2%, 5.3%, and 5.2% R.S.D. ($n=18$) for the 150, 750, and 3000 pg/ml pramipexole quality controls, respectively.

The accuracy of the method was determined by comparing the means of the measured concentrations with the nominal (theoretical) concentrations. All of the daily mean ($n=6$) and overall mean ($n=18$) values for the quality controls were within 11.7% of their expected values.

3.3. Limit of quantitation

The limit of quantitation (LOQ) was set at 50 pg/ml pramipexole in human plasma. Six replicates of the lowest standard (50 pg/ml) were analyzed to evaluate the LOQ. At the LOQ, the R.S.D. ($n=6$) of the peak-area ratios was 6.2%, the R.S.D. ($n=6$) of the measured concentrations was 7.9%, and the deviation of the mean ($n=6$) of the measured concentrations from their nominal value was 4.1%.

3.4. Extraction recovery

Extraction recoveries were determined by comparing the peak area of extracted calibration standards with the peak area of unextracted recovery standard at the same nominal concentration. The recovery for pramipexole and internal standard were 81.6 and 85.4%, respectively.

3.5. Parallelism

A quality control pool containing 9000 pg/ml pramipexole was prepared and analyzed at the partial volumes of 200 and 400 μl (diluted to 1 ml with blank human plasma). The mean ($n=6$) value for each partial volume was within 3.9% of their expected values. The precision was better than 3.9% R.S.D. ($n=6$) at both partial volumes.

In addition, the high quality control pool which contains 3000 pg/ml pramipexole was analyzed at the partial volumes of 200 and 400 μl (diluted to 1 ml with blank human plasma). The mean ($n=6$) value for each partial volume was within 4.7% of their expected values. The precision was better than 5.6% R.S.D. ($n=6$) at both partial volumes.

3.6. Application

The method was applied for the determination of the plasma level of pramipexole in humans. Fig. 5 presents the pramipexole pharmacokinetic profile of one individual from a clinical trial. Plasma concentrations were measured for 48 h following single oral administration of 0.25 mg of pramipexole. Fig. 5 also demonstrates that the sensitivity of this assay is adequate to define the elimination profile of pramipexole in humans.

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