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## Determination of pramipexole (U-98,528) in human plasma and urine by high-performance liquid chromatography with electrochemical and ultraviolet detection

Yau Yi Lau<sup>a,\*</sup>, Glenn D. Hanson<sup>a</sup>, Nita Ichhpurani<sup>b</sup>

<sup>a</sup>Department of Biopharmaceutics, Corning Hazleton Inc., 3301 Kinsman Boulevard, Madison, WI 53704, USA

<sup>b</sup>Bioanalytical Services, The Upjohn Company, Kalamazoo, MI 49001, USA

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### Abstract

A sensitive and selective high-performance liquid chromatographic (HPLC) method was developed for the determination of pramipexole in human plasma and urine. Plasma/urine is made alkaline before pramipexole and BHT-920 (internal standard) are extracted by ethyl ether and back-extracted with a solution that contains heptanesulfonic acid. Separation is achieved by ion-pair chromatography on a Zorbax Rx C<sub>8</sub> column with electrochemical detection at 0.6 V for plasma and ultraviolet detection at 286 nm for urine. The retention times of pramipexole and internal standard are approximately 14.4 and 10.7 min, respectively. The assay is linear in concentration ranges of 50 to 15 000 pg/ml (plasma) and 10 to 10 000 ng/ml (urine). The correlation coefficients are greater than 0.9992 for all curves. For the plasma method, the analysis of pooled quality controls (300, 3000, and 10 000 pg/ml) demonstrates excellent precision with relative standard deviations (R.S.D.) ( $n=18$ ) of 1.1%, 2.3%, and 6.8%, respectively. For the urine method, quality control pools prepared at 30, 300, and 3000 ng/ml had R.S.D. values ( $n=18$ ) of 2.9%, 1.7%, and 3.0%, respectively. The plasma and urine controls were stable for more than nine and three months, respectively. The mean recoveries for pramipexole and internal standard from plasma were 97.7% and 98.2%, respectively. The mean recoveries for pramipexole and internal standard from urine were 89.8% and 95.1%, respectively. The method is accurate with all intra-day ( $n=6$ ) and overall ( $n=18$ ) mean values for the quality control samples being less than 6.4 and 5.8% from theoretical for plasma and urine, respectively.

**Keywords:** Pramipexole

### 1. Introduction

Pramipexole (2-amino-4,5,6,7-tetrahydro-6-propylaminobenzthiazole dihydrochloride) is a dopamine autoreceptor agonist. It is found to decrease the extracellular concentrations of dopamine and its metabolites in the striatum of freely moving rats

through a reversible interaction with the dopamine D2 receptor [1,2]. In mice, pramipexole is also found to reduce exploratory locomotor activity [2]. It is now in Phase III trials for the treatment of Parkinson's disease, and Phase II for schizophrenia. A sensitive and selective method for the determination of pramipexole in plasma is necessary to support pharmacokinetic evaluations in human.

The purpose of the present study is to develop a

\*Corresponding author

sensitive and selective method for the determination of pramipexole in human plasma and urine.

## 2. Experimental

### 2.1. Materials

Pramipexole dihydrochloride (U-98,528E, SND 919 CL2 Y, purity 99.9%) and internal standard (BHT-920, purity 99.2%) were from Upjohn (Kalamazoo, MI, USA). Heparinized human plasma was purchased from Interstate Blood Bank. Human urine was donated by Corinig Hazleton employee. Acetonitrile and ethyl ether, HPLC grade, were from Fisher (Fairlawn, NJ, USA) and Burdick and Jackson (Muskegon, MI, USA), respectively. Glacial acetic acid (GR grade), potassium dihydrogenphosphate anhydrous, sodium acetate trihydrate were from EM Science (Gibbstown, NJ, USA). Sodium hydroxide was from Mallinckrodt (Paris, KY, USA). Heptanesulfonic acid sodium salt monohydrate were from Fluka (Ronkonkoma, NY, USA). Deionized water was processed through a Milli-Q water purification system, Millipore, before use.

### 2.2. Chromatographic system

The HPLC system consisted of an ABI/Kratos Spectroflow 400 pump (Foster City, CA, USA), a Perkin-Elmer ISS-100 autoinjector (Norwalk, CT, USA), and an ESA 5100A electrochemical detector with a 5011 high-sensitivity dual-electrode analytical cell and a 5020 guard cell. Electrode 2 was set at 0.6 V for detection, electrode 1 was set at 0.2 V for impurity screening, and the guard cell potential was 0.65 V for mobile phase conditioning. The analytical column was a Zorbax Rx C<sub>8</sub> (250×4.6 mm I.D., 5 μm particle size, Mac-Mod, Chadds Ford, PA, USA) with a Brownlee RP-8 pre-column (15×3.2 mm I.D., 7 μm particle size, ABI, San Jose, CA, USA). The mobile phase was prepared by dissolving 10.2 g potassium dihydrogenphosphate, 10.2 g sodium acetate, and 4.5 g heptanesulfonic acid sodium salt in 3 l of water and adjust the pH to 3.5 with acetic acid. The solution is filtered through a 0.45-μm membrane, 450 ml filtered acetonitrile added, and degassed. The flow-rate was 1.2 ml/min. Data collec-

tion and calculations were on an HP1000 Model A900 computer with a 3350A laboratory automation system (Hewlett-Packard, Palo Alto, PA, USA).

### 2.3. Preparation of back-extraction solution

Dissolve 1.7 g of potassium dihydrogenphosphate, 1.7 g of sodium acetate, and 0.5 g of heptanesulfonic acid sodium salt in 500 ml of water and adjust the pH to 3.5 with acetic acid. Refrigerate when not in use. The solution is stable for more than six months.

### 2.4. Preparation of standard solutions

A stock solution of pramipexole (1 mg/ml, free base) was prepared by dissolving 14.31 mg of pramipexole dihydrochloride salt (U-98,528E) in 10 ml of 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 10 mg of BHT-920 (internal standard) in 100 ml of water. Working solutions of pramipexole (1 to 300 ng/ml and 0.2 to 100 μg/ml for plasma and urine, respectively) and internal standard (30 ng/ml and 15 μg/ml for plasma and urine, respectively) were prepared by diluting the stock solutions with 1% acetic acid. These solutions were stable for at least six months when stored in polypropylene bottles at 5°C. Pramipexole and the internal standard are found to adsorb to glass. Therefore, all standards and controls were prepared and stored in polypropylene flasks and vials.

### 2.5. Control samples

Pooled quality control samples (controls) were prepared to determine the precision and accuracy of the method, and to evaluate the stability of samples. Over-curve control pools were also prepared to evaluate accuracy and precision when analyze using partial volume.

Plasma control pools (300, 3000, and 10 000 pg/ml pramipexole) were prepared by diluting 100 μl of 300 ng/ml, 1 ml of 300 ng/ml, and 200 μl of 5 μg/ml pramipexole, respectively, to 100 ml of human plasma. An over-curve control (25 000 pg/

ml) was prepared by diluting 0.5 ml of 5  $\mu\text{g/ml}$  pramipexole to 100 ml with human plasma.

Urine control pools (30, 300, and 3000 ng/ml pramipexole) were prepared by diluting 60  $\mu\text{l}$  of 50  $\mu\text{g/ml}$ , 600  $\mu\text{l}$  of 50  $\mu\text{g/ml}$ , and 3 ml of 100  $\mu\text{g/ml}$  pramipexole, respectively, to 100 ml of human urine. An over-curve control (30 000 ng/ml) was prepared by diluting 3 ml of 1 mg/ml pramipexole to 100 ml with human urine.

All control pools were aliquoted into polypropylene vials and stored at  $-20^\circ\text{C}$ .

### 2.6. Sample preparation

Calibration standards were prepared by adding 50  $\mu\text{l}$  of the appropriate pramipexole working solutions (1 to 300 ng/ml for plasma; 0.2 to 100  $\mu\text{g/ml}$  for urine) to 1 ml of blank plasma or urine. Clinical specimens and controls were prepared by aliquoting 1 ml into polypropylene tubes. Calibration standards, clinical specimens and controls were processed by adding 50  $\mu\text{l}$  of internal standard working solution, 100  $\mu\text{l}$  of 1 M sodium hydroxide, 6 ml of diethyl ether, into labeled polypropylene centrifuge tubes. The tubes were capped and mixed on a vortex mixer for 5 min, and centrifuged at 2500 g for 5 min. The aqueous layer was frozen in a dry ice–acetone bath, the organic layer was transferred to a clean tube and back-extracted with 100  $\mu\text{l}$  and 200  $\mu\text{l}$  (plasma and urine, respectively) of back-extraction solvent. The aqueous layer was frozen in a dry ice–acetone bath, the organic layer was discarded. Residual organic phase was removed by briefly blow under nitrogen. The removal of ethyl ether is extremely critical for the urine method because the presence of trace amount of ethyl ether increases the peak height of the internal standard relative to pramipexole. Aliquots (50  $\mu\text{l}$ ) were injected into the HPLC system for both plasma and urine samples.

### 2.7. Validation

Duplicate calibration curves were analyzed on each of three days. One reagent blank (water substituted for plasma or urine), blank plasma or urine, control zero (blank plasma or urine spiked with internal standard) and triplicate controls at each concentration were analyzed with each calibration

curve. The calibration curves were obtained by weighted ( $1/C$ ) least-squares linear regression analysis of the peak-height ratios of pramipexole/internal standard versus the concentration of pramipexole. The equations of the calibration curves were then used to calculate the concentration of pramipexole in the samples and controls from their peak-height ratios.

## 3. Results and discussion

### 3.1. Separation and specificity

The molecular structures of pramipexole (U-98,528) and BHT-920 (internal standard) are shown in Fig. 1. Representative chromatograms of standards and controls demonstrate excellent peak shape and separation between pramipexole and internal standard (Fig. 2 and Fig. 3). The mean retention times of pramipexole, and the internal standard were 14.4, and 10.7 min, respectively. Electrochemical detection was used for plasma analysis because it provided excellent sensitivity and selectivity. UV detection was used for urine analysis because the

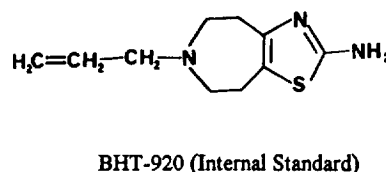
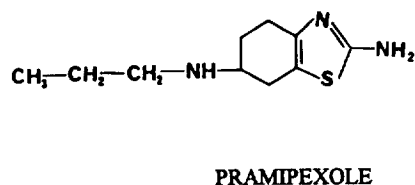


Fig. 1. Molecular structures of pramipexole and BHT-920, internal standard.

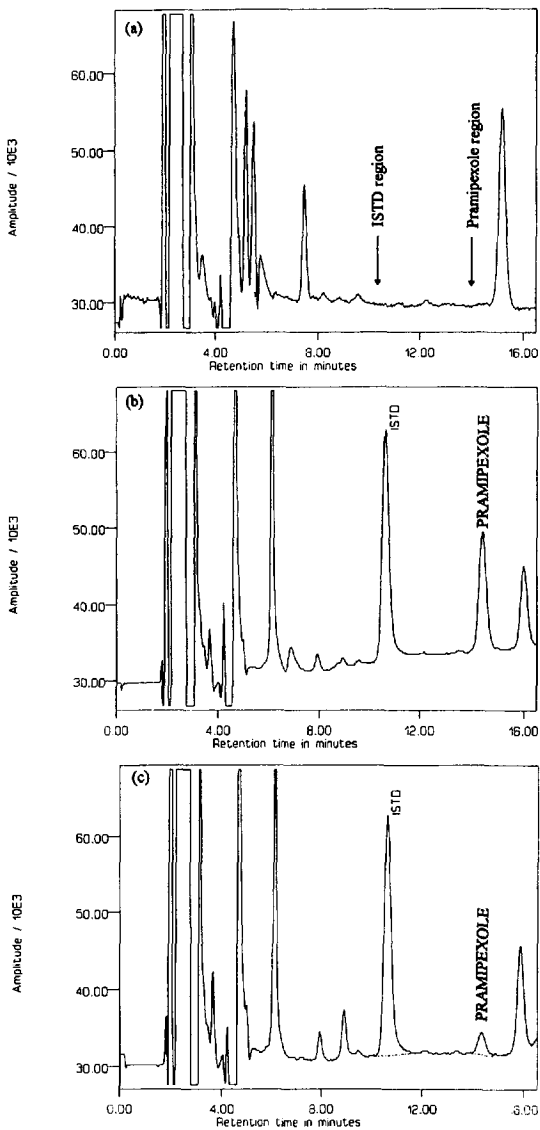


Fig. 2. (a) Chromatogram of blank human plasma; (b) 1000 pg/ml calibration standard in human plasma; (c) plasma from one human subject taken 0.5 h after dosing (orally).

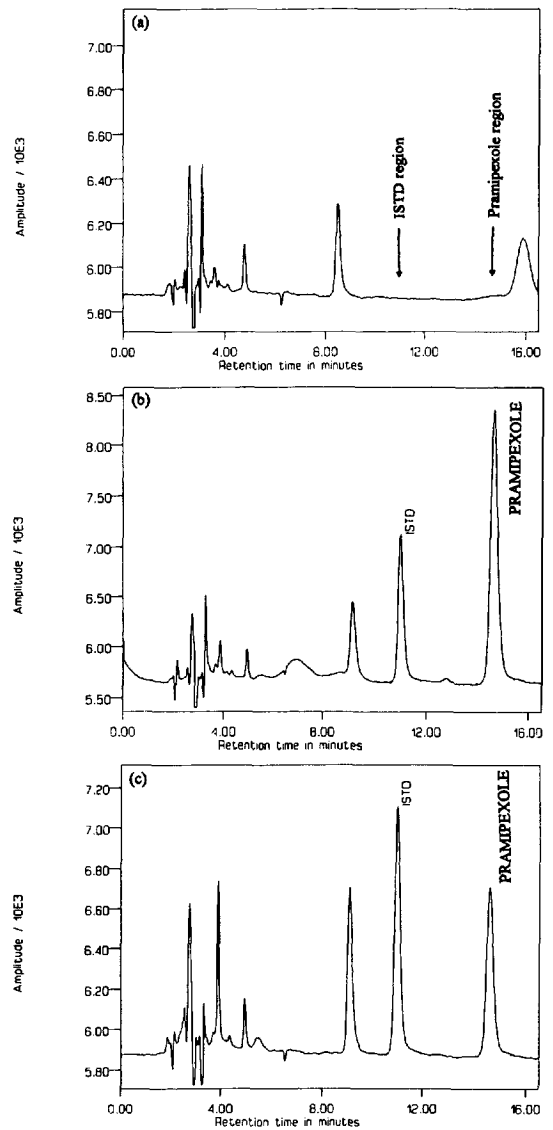


Fig. 3. (a) Chromatogram of blank human urine; (b) 1000 ng/ml calibration standard in human urine; (c) urine from one healthy human subject collected 6–8 h after dosing (orally).

higher concentration of pramipexole in urine made electrochemical detection unnecessary.

Blank plasma and urine from six pools were tested for endogenous interferences. The pramipexole, and internal standard regions were clean for all of the lots tested (Fig. 2 and Fig. 3).

### 3.2. Linearity, precision and accuracy

Calibration curve data and parameters for pramipexole are in Table 1, Table 2 and Table 3. Calibration curves for pramipexole in plasma were linear in the concentration range of 50 to 15 000

Table 1  
Calibration curve parameters for pramipexole in human plasma and urine ( $n=6$ )

Matrix	Slope (mean $\pm$ S.D.)	R.S.D. (%)	Mean intercept	Mean correlation coefficient
Plasma	6.87E-4 $\pm$ 1.57E-5	2.3	-0.0077	0.9998
Urine	1.97E-3 $\pm$ 4.36E-5	2.2	0.00313	0.9997

Results of validation studies over 3-day periods.

Table 2  
Calibration curve data for pramipexole in plasma

Calibration standard concentration (pg/ml)	Calculated concentration (mean $\pm$ S.D., $n=6$ ) (pg/ml)	R.S.D. (%)	Deviation (%)
50	53.1 $\pm$ 1.87	3.5	6.2
100	102.1 $\pm$ 4.5	4.4	2.1
200	197 $\pm$ 10.2	5.2	-1.5
500	494 $\pm$ 4.3	0.9	-1.2
1000	950 $\pm$ 24.1	2.5	-5.0
2000	1990 $\pm$ 36	1.8	-0.5
5000	5050 $\pm$ 91	1.8	1.0
15 000	15 000 $\pm$ 98	0.7	0.0

Results of validation study over a 3-day period.

pg/ml and 10 to 10 000 ng/ml in urine. The correlation coefficients are greater than 0.9992 for all curves.

Data from the spiked control samples are shown in

Table 3  
Calibration curve data for pramipexole in urine

Calibration standard concentration (ng/ml)	Calculated concentration (mean $\pm$ S.D., $n=6$ ) (ng/ml)	R.S.D. (%)	Deviation (%)
10	9.95 $\pm$ 0.78	7.8	-0.5
20	19.8 $\pm$ 1.19	6.0	-1.0
50	49.2 $\pm$ 0.8	1.6	-1.6
100	100.5 $\pm$ 3.8	3.8	0.5
500	500 $\pm$ 20.5	4.1	0.0
1000	1040 $\pm$ 28	2.7	4.0
5000	5130 $\pm$ 107	2.1	2.6
10 000	9800 $\pm$ 100	1.0	-2.0

Results of validation study over a 3-day period.

Table 4  
Precision and accuracy of the assay for pramipexole in plasma

Control concentration (pg/ml)	Calculated concentration (overall mean $\pm$ S.D., $n=18$ ) (pg/ml)	R.S.D. (%)	Deviation (%)
300	318 $\pm$ 3.4	1.1	6.0
3000	2940 $\pm$ 68	2.3	-2.0
10 000	9630 $\pm$ 653	6.8	-3.7

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

Table 4 and Table 5. The within-day precision of the methods as measured by the relative standard deviation (R.S.D.) of the daily mean ( $n=6$ ) was less than 11.4% and 3.8% at the three control concentrations in human plasma and urine, respectively. The overall precision for the plasma method was 1.1%, 2.3%, and 6.8% R.S.D. ( $n=18$ ) for the 300, 3000, and 10 000 pg/ml pramipexole controls, respectively. The overall precision for the urine method was 2.9%, 1.7%, and 3.0% R.S.D. ( $n=18$ ) for the 30, 300, and 3000 ng/ml pramipexole controls, respectively.

The accuracy of the method was determined by comparing the means of the measured concentrations with the nominal (theoretical) concentrations of pramipexole in the plasma and urine controls. All of the daily mean ( $n=6$ ) and overall mean ( $n=18$ ) values for the controls were within 6.4% and 5.8% of their expected values for plasma and urine, respectively.

A control pool containing 25 000 pg/ml pramipexole in plasma was prepared and analyzed at the partial volumes of 200 and 400  $\mu$ l. The mean

Table 5  
Precision and accuracy of the assay for pramipexole in urine

Control concentration (ng/ml)	Calculated concentration (overall mean $\pm$ S.D., $n=18$ ) (ng/ml)	R.S.D. (%)	Deviation (%)
30	28.6 $\pm$ 0.83	2.9	-4.6
300	296 $\pm$ 5.2	1.7	-1.4
3000	3030 $\pm$ 91	3.0	1.0

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

( $n=6$ ) values for both partial volumes were within 3.6% of their expected values. A control pool containing 30 000 ng/ml pramipexole in urine was also prepared and analyzed at the partial volumes of 100 and 200  $\mu$ l. The mean ( $n=6$ ) values for both partial volumes were within 4% of their expected values. The precision was better than 1% and 4.4% R.S.D. ( $n=6$ ) at both partial volumes for the plasma and urine method, respectively. In addition, the high control for the urine method was prepared and analyzed at the same partial volumes. The mean ( $n=6$ ) values for both partial volumes were within 1% of their expected values. The precision was better than 4.8% R.S.D. ( $n=6$ ) at both partial volumes.

### 3.3. Limit of quantitation

The limit of quantitation (LOQ) was set at 50 pg/ml and 10 ng/ml pramipexole in plasma and urine, respectively. At least five replicates of the lowest standard (50 pg/ml or 10 ng/ml) were analyzed to evaluate the LOQ.

For the plasma method, at the LOQ, the R.S.D. ( $n=6$ ) of the peak-height ratios was 7.0%, the R.S.D. ( $n=6$ ) of the measured concentrations was 6.6%, and the deviation of the mean ( $n=6$ ) of the measured concentrations from their nominal value was 6.4%.

For the urine method, at the LOQ, the R.S.D. ( $n=5$ ) of the peak-height ratios was 5.7%, the R.S.D. ( $n=5$ ) of the measured concentrations was 2.4%, and the deviation of the mean ( $n=5$ ) of the measured concentrations from their nominal value was 0.2%.

### 3.4. Absolute recoveries

Absolute recoveries were determined by comparing the peak heights of extracted calibration standards with the peak heights of unextracted recovery standards at the same nominal concentration. Excellent recovery and specificity was achieved in both plasma and urine assay by using a simple solvent extraction-ion pair back extraction. The mean ( $n=16$ ) recoveries for pramipexole and internal standard from plasma were 97.7% and 98.2%, respectively. The mean recoveries for pramipexole and internal standard from urine were 89.8% ( $n=14$ ) and 95.1% ( $n=16$ ), respectively.

### 3.5. Stability

The stability of pramipexole was determined by measuring the concentration changes in the control samples over time. Stock and working solutions of pramipexole in 1% acetic acid were stable for at least four months when stored at 5°C. The plasma controls stored in polypropylene at -20°C were stable for more than nine months while urine controls were stable for more than three months.

Stability was tested by subjecting the plasma and urine controls to two and three freeze-thaw cycles, and storage for 24 h at room temperature. The thawing and refreezing of controls, and the storage of controls at room temperature had little effect on the precision or accuracy of the results. The mean ( $n=3$ ) value was within 9.0% and 7.0% of their expected values for plasma and urine method, respectively.

Process stability was tested by extracting two sets of calibration standards with duplicate controls. One set was stored overnight at room temperature, and the other at 5°C before analyzing. The storage of extracted samples at room temperature or 5°C had little effect on the accuracy of the control results. The mean ( $n=2$ ) value was within 9.2% and 4% of their expected values for plasma and urine method, respectively, at room temperature. The mean ( $n=2$ ) value was within 8.4% and 3% of their expected values for plasma and urine method, respectively, at 5°C.

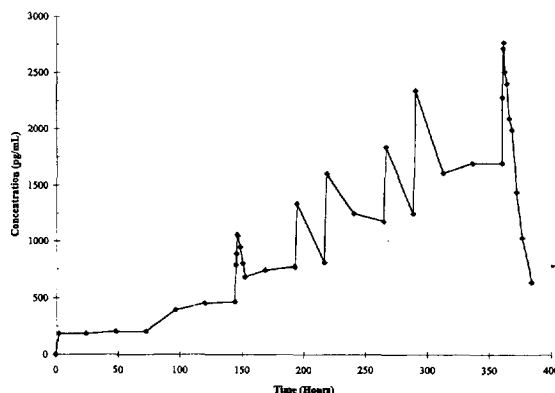


Fig. 4. Mean pramipexole pharmacokinetic profile from an ascending dose clinical study.

### 3.6. Application of the method to biological specimens

This new assay procedure is precise, accurate, selective and sensitive for the quantitation of pramipexole in human plasma and urine. The ruggedness of the assays have been demonstrated by applying the methods to several clinical studies. Fig. 4 presents the mean pramipexole pharmacokinetic profile from an ascending dose clinical study.

### References

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