*Journal of Chromatography*, 568 (1991) 494–500
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5922

## Short Communication

# Determination of a novel steroidal androgen receptor antagonist (Win 49596) in human plasma using solid-phase extraction and high-performance liquid chromatography with ultraviolet detection

DONNA M. SKEE*

*Department of Drug Metabolism and Pharmacokinetics, Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY 12144 (USA)*

JACK A. COOK

*Pharmacokinetics/Drug Metabolism Department, Parke-Davis Research Center, 2800 Plymouth Road, Ann Arbor, MI 48106 (USA)*

and

DAVID P. BENZIGER

*Department of Drug Metabolism and Pharmacokinetics, Sterling Research Group, 81 Columbia Turnpike, Rensselaer, NY 12144 (USA)*

(First received September 18th, 1990; revised manuscript received March 27th, 1991)

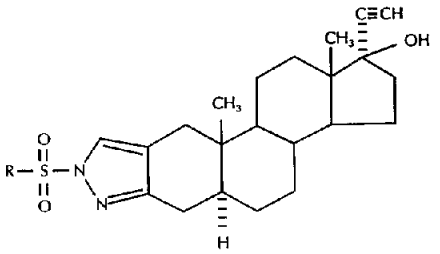
ABSTRACT

A rapid, sensitive and selective method was developed for the determination of a novel steroidal androgen receptor antagonist (Win 49596, I) in human plasma. The procedure involved extraction from plasma using a solid-phase phenyl support and elution directly onto a reversed-phase $C_8$ column using a mobile phase consisting of 0.2 mol/l sodium acetate buffer at pH 7–acetonitrile (45:55, v/v). Drug was monitored by ultraviolet detection at a wavelength of 238 nm. Linear responses were observed for standards over the range 0.01–5.0 μg/ml. The minimum quantifiable level was 0.02 μg/ml, using a 0.5-ml plasma sample. The precision was 5.5% and the accuracy ranged from −9.4% to 0.23%. The analytical method has been used to quantify I in plasma from dogs and rats and is projected for use with human plasma from clinical trials.

## INTRODUCTION

Win 49596 (I) is a novel steroidal methanesulfonyl pyrazole androgen receptor antagonist with the chemical name (5α,17α)-1′-(methylsulfonyl)-1′-*H*-pregn-20-yno[3,2-*c*]pyrazol-17-ol (Fig. 1). It was effective in the treatment of benign pros-

0378-4347/91/$03.50 



| Compound | R | Mol. Wt. |
|---|---|---|
| I | $CH_3$ | 416.6 |
| II (Internal Standard) | $CH_3(CH_2)_3$ | 458.7 |

Fig. 1. Structures of I and II.

tatic hyperplasia (BPH) in animal models [1,2] and is currently undergoing clinical evaluation.

This report describes a method based on high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection using a solid-phase phenyl support for sample preparation.

## EXPERIMENTAL

### *Reagents*

I and the internal standard, Win 51352 (II, Fig. 1), were synthesized at Sterling Research Group (Rensselaer, NY, USA) and used without further purification. Acetonitrile and methanol were HPLC grade from Fisher (Fair Lawn, NJ, USA). Sodium acetate from Mallinkrodt (Paris, KY, USA) was analytical grade. Water was obtained from a Sybron/Barnstead ORGANICpure™ system (Boston, MA, USA) fed with deionized, distilled water. All other chemicals were obtained commercially (reagent grade or better) and used without further purification.

### *Standard solutions*

A stock standard solution was prepared by dissolving I at 100 μg/ml in acetonitrile. This stock standard solution was then diluted in acetonitrile to achieve stocks from which a 50-μl portion was used to prepare calibration curve standards. A 40 μg/ml solution of II in acetonitrile was used as the internal standard stock solution.

A stock solution of 0.2 mol/l sodium acetate buffer was prepared by dissolving 16.4 g of anhydrous sodium acetate in 1 l of water and adjusting to pH 7 with 0.2 mol/l acetic acid.

*Preparation of calibration curve standards*

Aliquots (50 μl) of the appropriate standard solutions were added to conical centrifuge tubes containing 0.5 ml of control human plasma (potassium oxalate anticoagulant) to give calibration curve standard concentrations of 0 (acetonitrile compensate), 0.01, 0.02, 0.05, 0.07, 0.10, 0.20, 0.50, 0.70, 1.0, 2.0 and 5.0 μg/ml.

*Preparation of spiked plasma samples*

Sets of randomized spiked plasma were prepared under single-blind conditions. The set contained triplicate samples at final concentrations of 0 (acetonitrile compensate), 0.084, 0.15, 0.63 and 1.8 μg/ml in plasma. One set was frozen for seven days at −20°C prior to analysis.

*Sample preparation procedure*

To each calibration curve standard or spiked sample (0.5 ml) were added 25 μl of the internal standard solution and 350 μl of acetonitrile. Each tube was vigorously vortex-mixed for 20 s and then centrifuged at approximately 950 *g* for 10–15 min to remove any precipitate. The supernatants were immediately transferred to clean tubes containing 0.5 ml of water. The samples (approximately 0.8 ml) were loaded onto an advanced automated sample processor (AASP) phenyl cassette (Varian, Sugar Land, TX, USA), preconditioned with three volumes (approximately 2 ml each) of methanol and one volume of water, and then washed with one volume of 20% acetonitrile in water. The cassettes were placed in the AASP and the pretreated samples eluted directly onto the chromatographic system.

*Instrumentation and chromatographic conditions*

The HPLC system consisted of a mini-pump (Milton Roy, Riviera Beach, FL, USA), a Kratos 773 variable-wavelength UV detector (ABI Analytical, Ramsey, NJ, USA) and an AASP. The HPLC column was filled with reversed-phase material Partisphere 5 $C_8$ (5 μm, 12.5 cm × 4.6 mm I.D.) (Whatman, Clifton, NJ, USA). The guard column (23 mm × 2.9 mm I.D.) was packed with Bondapak phenyl/Corasil 37–50 μm packing material (Waters Assoc., Milford, MA, USA).

The mobile phase, consisting of 0.2 mol/l sodium acetate buffer at pH 7–acetonitrile (45:55, v/v), was delivered at 2 ml/min. The valve reset time on the AASP, the time that the mobile phase passes through the cassette to elute the sample, was 0.5 min and the cycle time was 13.5 min to accommodate peaks eluting after the internal standard.

The effluent was monitored using a Kratos Model 773 variable-wavelength UV detector at a wavelength of 238 nm, the maximum absorbance for I. The system was operated at room temperature.

*Data processing*

The output from the detector was interfaced with a Hewlett-Packard Model

3357 laboratory automation system for data acquisition and processing. Drug concentrations were determined by inverse prediction from a linear regression of peak-height ratios (I/internal standard) as a function of standard concentrations [3]. The minimum quantifiable level of the assay was defined as the lowest calibration standard having a regression-estimated concentration which was within ± 15% of its nominal value. The observed concentrations for the prepared, spiked samples were expressed as the percentage difference from nominal values, and the range of these percentages was used to define the accuracy of the assay. The overall accuracy was defined as the overall mean of the percentage differences from the nominal concentration. The precision of the assay was estimated as the overall standard deviation of the percentage differences from the nominal concentration.

## RESULTS AND DISCUSSION

### *Chromatography*

Fig. 2 shows a plasma sample taken from a dog 2 h after oral administration of a 5 mg/kg dose of I. The approximate retention times were 2.5 min for I and 4.8 min for II.

Work prior to the validation of this method indicated that a $C_8$ bonded phase gave shorter retention times than $C_{18}$ and better separation from endogenous material than either $C_2$ or phenyl. The spherical particle shape also gave better resolution than irregular silica of other $C_8$ columns. A precolumn was used to protect the analytical column rather than for added separating capability. However, bulk Partisphere 5 $C_8$ material was unavailable for the precolumn and the phenyl bonded phase was therefore chosen because it would have little effect on the separation. I is a neutral compound and so sodium acetate, adjusted to pH 7 with an ionic strength of 0.2 mol/l, was chosen for its effect on resolution and peak shape and our experience that it could be used long term without affecting the HPCL system. Phenyl solid-phase material was chosen for sample prepara-

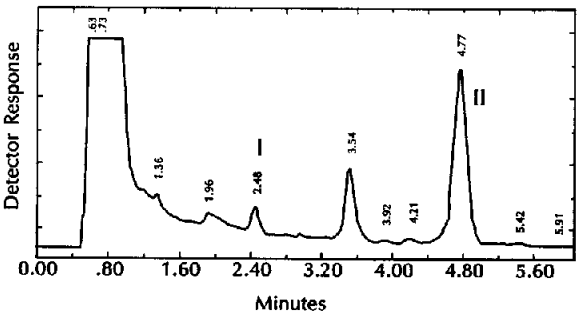



Fig. 2. Computer-generated chromatogram of plasma sample taken from a dog 2 h after oral administration of a 5 mg/kg dose of I; assayed concentration of I was 169 ng/ml.

tion because it retained I and II sufficiently to allow other materials to be washed off, yet weakly enough so that a relatively small volume of mobile phase would be needed to elute them without band broadening.

*Linearity and limit of detection*

A regression analysis of the peak-height ratio *versus* concentration showed linearity over the range 0.01–5.0 μg/ml. The coefficient of determination was 0.99 or greater for each standard curve. The minimum quantifiable level was 0.02 μg/ml.

*Precision and accuracy*

Table I shows the observed concentrations for the prepared plasma validation sample. Numbers presented in the table are rounded for ease of presentation, and

TABLE I

CONCENTRATION DATA FOR I IN PREPARED PLASMA SAMPLES

| Nominal concentration (μg/ml) | Fresh set | | Frozen set | |
|---|---|---|---|---|
| | Concentration found (μg/ml) | Difference (%) | Concentration found (μg/ml) | Difference (%) |
| 0.084 | 0.080 | −5.3 | 0.076 | −9.1 |
| | 0.082 | −2.3 | 0.065 | −22.8 |
| | 0.087 | 3.5 | 0.087 | 3.8 |
| Mean | 0.083 | −1.4 | 0.076 | −9.4 |
| S.D. | 3.8 | | 11.2 | |
| C.V. (%) | 4.5 | | 14.7 | |
| 0.15 | 0.14 | −0.87 | 0.14 | −5.5 |
| | 0.14 | −8.4 | 0.15 | 0.55 |
| | 0.14 | −5.2 | 0.16 | 5.7 |
| Mean | 0.14 | −7.4 | 0.15 | 0.23 |
| S.D. | 2.9 | | 8.4 | |
| C.V. (%) | 2.1 | | 5.6 | |
| 0.63 | 0.60 | −4.6 | 0.61 | −2.9 |
| | 0.57 | −9.2 | 0.69 | −6.3 |
| | 0.60 | −4.2 | 0.61 | −3.4 |
| Mean | 0.59 | −6.0 | 0.60 | −4.2 |
| S.D. | 17.7 | | 11.6 | |
| C.V. (%) | 3.0 | | 1.9 | |
| 1.8 | 1.72 | −4.6 | 1.73 | −3.7 |
| | 1.74 | −3.1 | 1.73 | −4.1 |
| | 1.77 | −1.7 | 1.68 | −6.9 |
| Mean | 1.74 | −3.1 | 1.71 | −4.9 |
| S.D. | 25.6 | | 31.2 | |
| C.V. (%) | 1.5 | | 1.8 | |

accuracy to more than three significant figures should not be inferred. Calculations were performed on unrounded numbers and may differ slightly from the rounded numbers in the table. Precision was 5.5%. Accuracy ranged from −9.4% to 0.23%; the overall mean accuracy was −4.5%.

*Recovery*

The extraction efficiency was 79.7% for I and 68.8% for II. Concentration had no affect on the percentage extracted.

*Effect of freezing on sample integrity*

There was no apparent freezing affect (Table I) as assayed concentrations of samples frozen for seven days at −20°C were not markedly different from nominal concentrations or from samples analyzed immediately after preparation.

*Utility*

The utility of the method has been demonstrated by the analysis of plasma samples from rats and dogs from various studies and is projected for use with human plasma from clinical trials. Approximately 100 samples can be analyzed per day.

The performance characteristics of the analytical method were investigated over a three-week period. The mean (±S.D.) concentration for the frozen quality control samples of I was 0.299 ±0.004 μg/ml (nominal 0.3 μg/ml), indicating no apparent degradation of I in frozen plasma. The coefficient of determination ($r^2$) from each calibration curve was 0.99 or greater. The coefficient of variation (C.V.) for the mean values of the calibration curve slope was 0.032% ($n=5$). The analytical column has performed satisfactorily for approximately 300 injections before routine replacement would occur.

## CONCLUSION

A rapid, sensitive and selective method has been developed for the determination of I in human plasma. The preparation and run time for the method is minimal, and this has proven beneficial considering the large number of samples required for toxicologic, pharmacologic and pharmacokinetic studies.

## ACKNOWLEDGEMENTS

We would like to acknowledge the assistance of Mr. Eric Gower in the preparation of this manuscript and Mr. Paul Erdtmansky for his technical assistance.

REFERENCES

1 P. E. Juniewicz, B. M. Lemp, T. A. Barbolt, T. K. LaBrie, M. McCarthy, J. R. Reel and F. H. Batzold, *Prostate*, 16 (1990) 1–14.
2 P. E. Juniewicz, M. McCarthy, B. M. Lemp, T. A. Barbolt, C. Shaw, D. M. Hollenbaugh, R. C. Winneker, J. R. Reel and F. H. Batzold, *Endocrinology*, 126 (1990) 2625–2634.
3 R. R. Sokal and F. J. Rohlf, *Biometry*, Freeman, San Francisco, CA, 1969, p. 229.