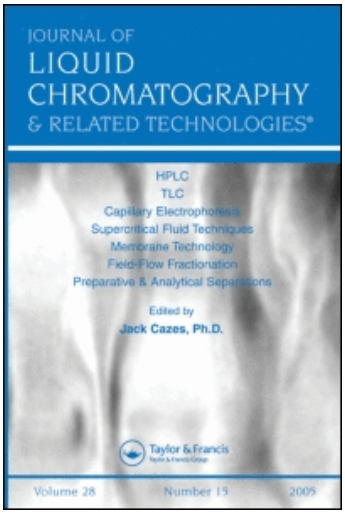


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# DEVELOPMENT OF AN HPLC METHOD FOR THE ANALYSIS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE IN A POTENTIAL ANTIPSYCHOTICACTIVE PHARMACEUTICAL INGREDIENT

J. J. Krikke[a]; G. L. Karrick[a]; S. R. Hagen[a]
[a] Warner-Lambert Parke-Davis, Holland, MI, U.S.A.

Online publication date: 19 September 2000

**To cite this Article** Krikke, J. J. , Karrick, G. L. and Hagen, S. R.(2000) 'DEVELOPMENT OF AN HPLC METHOD FOR THE ANALYSIS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE IN A POTENTIAL ANTIPSYCHOTICACTIVE PHARMACEUTICAL INGREDIENT', Journal of Liquid Chromatography & Related Technologies, 23: 16, 2553 — 2562
**To link to this Article: DOI:** 10.1081/JLC-100100509
**URL:** http://dx.doi.org/10.1081/JLC-100100509

J. LIQ. CHROM. & REL. TECHNOL., 23(16), 2553–2562 (2000)

# DEVELOPMENT OF AN HPLC METHOD FOR THE ANALYSIS OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE IN A POTENTIAL ANTIPSYCHOTICACTIVE PHARMACEUTICAL INGREDIENT

J. J. Krikke,* G. L. Karrick, S. R. Hagen

Warner-Lambert Parke-Davis
Chemical Development
188 Howard Avenue
Holland, MI 49424, USA



## ABSTRACT

An HPLC assay was developed which could detect 2 ppm of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) in CI-1007 maleate bulk pharmaceutical chemical. The method is an ion-pairing HPLC assay which uses a mobile phase containing sodium dodecyl sulfate (SDS) (600 0.083M SDS at pH4: 400 Acetonitrile) and a $C_8$ column (Waters Symmetry $C_8$, 250 x 4.6 mm) to selectively separate MPTP, a neurotoxin that destroys brain dopamine neurons and produces Parkinson-like symptoms when administered intravenously, and HPTP (4-phenyl-1,2,3,6-tetrahydropyridine) from CI-1007 and maleic acid. This method exhibited improved system precision, linearity, limit of quantitation, and accuracy, in comparison to a previously developed HPLC method that used an ammonium acetate buffered mobile phase.

## INTRODUCTION

1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) has been shown to cause symptoms similar to those seen in patients with Parkinson's disease.[1] Symptoms from MPTP were first observed in the early 1980s when illicit drug synthesis inadvertently led to MPTP contamination. Investigation led to the first publication in 1983 that made a connection between MPTP and Parkinson-like symptoms.[2]

Studies of the biochemistry of MPTP followed, and necessitated the development of suitable analytical techniques. Shih and Markey described a gas chromatographic (GC) method for determining MPTP in mouse brain tissue.[3] Jindal and Lutz used GC, and GC coupled with mass spectrometry, to study MPTP and analogs in rat urine.[4] Rollema et al. described a high performance liquid chromatographic (HPLC) assay for MPTP in biological samples using electrochemical detection.[5] Shinka et al. used cation exchange HPLC to determine MPTP in brain tissue, and Naoi et al. described MPTP studies with human brain synaptosomes using reverse phase HPLC with fluorometric detection.[6,7]



Little work has been done in the area of determining MPTP as a potential impurity in bulk drugs. Kramer et al. recently noted that MPTP was a contaminant in a candidate drug intended for use in psychiatric disorders.[8] They described an HPLC method for determining the presence of MPTP in tissue and plasma. However, no mention was made of how MPTP was determined in the drug candidate. A review of the literature provided no information on how MPTP is determined in bulk drug substances using HPLC.

A method for determining MPTP in active pharmaceutical ingredient (API), CI-1007 maleate, is outlined below. CI-1007, R(+) 1,2,3,6-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexene-1-yl)methyl]pyridine is a potential antipsychotic agent.[9] Since the molecule is based on phenyl-1,2,3,6 tetrahydropyridine, it was suspected that MPTP may be a potential impurity.

Originally an ammonium acetate buffered HPLC method was developed for detection of the MPTP impurity in CI-1007 maleate API. This assay was further developed and compared to an ion-pair HPLC method. The original method gave little retention on the column, and it was thought that this could be alleviated by ion pairing the basic amine groups with an acidic ion pairing reagent, leading to increased retention and improved plate count. The development, experiments, and results from each method are described.

## EXPERIMENTAL

Notes: The final mg/mL concentrations of HPTP, MPTP, and CI-1007 listed below refer to the free base forms of these compounds. The analyses were performed using a Waters 700 WISP autosampler, a 600E pump, and a 996 photodiode array detector. The detector output was processed using Waters version 2.1 Millennium software. All sample preparations and HPLC mobile phase wastes were treated with bleach prior to disposal.

### Ammonium Acetate Method

An experimental gradient method was modified after transfer from another Parke-Davis laboratory. The first 15 minutes of the solvent program were changed to an isocratic elution in order to give more consistent retention times and to positively identify MPTP. The acetonitrile (MeCN) content in the sample preparation was increased to prevent crystallization of CI-1007 upon standing and to eliminate clogging of the autosampler tubing.

The detection wavelength was changed to 242 nm from 254 nm, as this was determined to be the absorption maxima of MPTP, and the detector sensitivity was subsequently improved by approximately 40%. The injection volume was decreased from 150 µL to 75 µL to avert peak broadening. Lastly, the $C_{18}$ guard column was removed and the overall gradient program used to clean and regenerate the column was reduced from 96 to 60 minutes without any adverse effects. Limited validation experiments were performed on this modified method.



#### *Instrument Parameters*

Column: Column: Zorbax Rx-$C_8$, 5 µm, 250 x 4.6 mm (30°C); Mobile Phase: Mobile phase A= 835 parts (0.02 M $NH_4OAc$/pH 4): 165 parts MeCN, Mobile phase B= 250 parts (0.02 M $NH_4OAc$/pH 4): 750 parts MeCN; Program: 0-15 min (100% A at 1 mL/min), 25 min (100% B), 26-44 min (100% B at 1.5 mL/min), 45-59 min (100% A at 1.5 mL/min), 60 min (100% A at 1 mL/min); Wavelength: 242; Loop Size: 200 µL (methanol needle wash solution); Injection Volume: 75 µL.

#### *Solutions, Procedure, and Calculations*

Standard Solutions: solutions containing between 2 and 39 ppm of HPTP and MPTP in mobile phase A were prepared for MPTP quantitation and linearity determination.

Sample Solutions: 0.135 g samples of CI-1007 maleate salt were dissolved in 5 mL of 1 DMSO: 4 MeOH and diluted to 10 mL with mobile phase A to give 9.98 mg/mL CI-1007 free base.

Spiked Test Solutions: samples of CI-1007 maleate salt were prepared as stated above, except that MPTP was added from a stock solution to yield a 4 ppm spike.

## Ion-Pair HPLC Method

An alternative method was developed which utilized sodium dodecyl sulfate (SDS) as an ion-pair reagent. Initially, a 4.6 x 250 mm Zorbax $C_8$ (5 µm) column and a mobile phase of one part acetonitrile (MeCN) and one part SDS were used to resolve MPTP from HPTP and maleic acid. CI-1007 was then eluted with a second eluent consisting of 875 parts MeCN and 125 parts SDS (0.01 M at pH4). In order to improve peak shape, a 4.6 x 250 mm Waters Symmetry $C_{18}$ (5 µm) column was used. Next, in order to improve the resolution of MPTP from HPTP, the mobile phase was modified to contain 475 parts water, 125 parts 0.04 M SDS (pH 4) and 400 parts MeCN. Finally, a 4.6 x 250 mm Waters Symmetry $C_8$ (5 µm) was tested and found to provide satisfactory results. This column was, therefore, used in subsequent validation experiments.

### *Instrument Parameters*

Column: Waters Symmetry $C_8$, 5 µm, 250 x 4.6 mm (30°C); Mobile phase: Mobile phase A= 475 parts $H_2O$: 125 parts (0.04 M SDS/pH 4): 400 parts MeCN, Mobile phase B= 125 parts (0.04 M SDS/pH 4): 875 parts MeCN; Program: 0-30 min (100% A at 1 mL/min), 31-44 min (100% B at 1.5 mL/min), 45-59 min (100% A at 1.5 mL/min), 60 min (100% A at 1 mL/min); Wavelength: 242 nm; Loop size: 200 µL (methanol needle wash solution); Injection Vol: 50 µL.

### *Solutions, Procedure, and Calculations*

Standard Solutions: solutions containing between 2 and 98 ppm of HPTP and MPTP in mobile phase A were prepared for MPTP quantitation and linearity determination.

Sample Solutions: 0.135 g samples of CI-1007 maleate salt were dissolved in 5 mL of 1 DMSO: 4 MeOH and diluted to 10 mL with mobile phase A to give 9.98 mg/mL CI-1007 free base.

Spiked Test Solutions: samples of CI-1007 maleate salt were prepared as stated above, except that MPTP was added from a stock solution to yield a 4 ppm spike.

## RESULTS AND DISCUSSION

### Ammonium Acetate Method

System Suitability and Precision: A 2 ppm standard chromatogram displayed HPTP at about 12.0 minutes and MPTP at about 13.0 minutes with a resolution of 2.2. The HPTP gave a tailing factor of 1.0 and a plate count of 12874, while the MPTP afforded a tailing factor of 1.0, a plate count of 12523 and a signal to noise ratio of 4.9:1 (Figure 1).

Six replicate injections of a 4 ppm HPTP and MPTP standard solution were performed. The ppm levels of both impurities were determined using a calibration curve generated from 2 and 6 ppm standard solutions. The relative standard deviations (RSD) for the six determinations were 5.23% for HPTP and 4.94% for MPTP.

Linearity of Response: Four different standard solutions were prepared and injected whose MPTP concentrations varied from 0.0000197 mg/mL to 0.00003939 mg/mL (2-39 ppm MPTP for a 10 mg/mL sample). A linearity plot



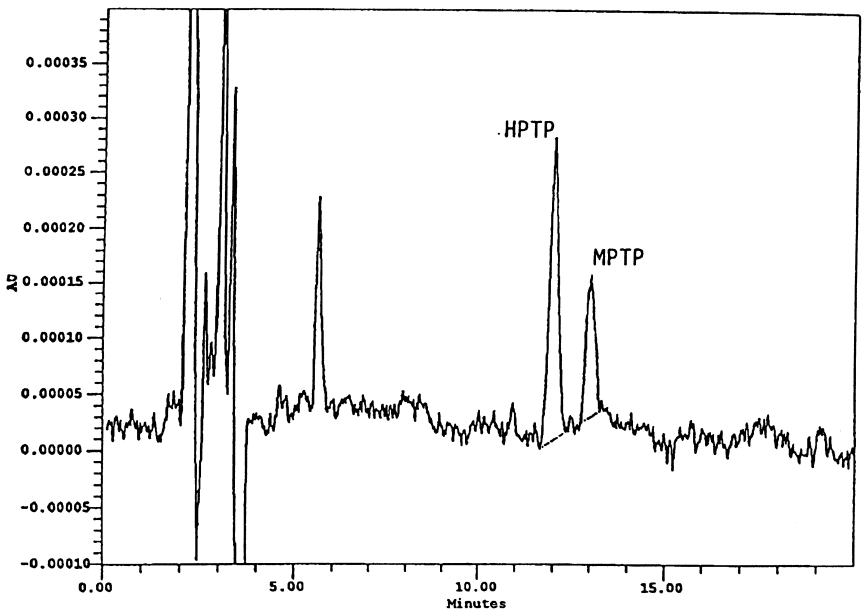


**Figure 1**. Ammonium acetate HPLC method, 2 ppm working standard.

was constructed by plotting the MPTP concentration (x-axis) against the area of MPTP (y-axis). Results of the linear regression afforded a correlation coefficient of 0.99981 with response factors being within 29% of the average system precision response factor.

Limit of Quantitation and Detection: Six replicate injections of a 4-ppm MPTP standard solution were performed. The ppm levels of MPTP were determined for each injection using a calibration curve generated from 2 and 6 ppm standard solutions. The RSD for the six determinations was calculated at 4.94%. This demonstrated that 4 ppm MPTP could be quantitated. A 2 ppm standard solution was analyzed and gave a signal to noise ratio of 5:1 for MPTP, indicating that 2 ppm of MPTP could be detected.

Accuracy: Lots XH381193 (4.2 ppm MPTP), XH381095 (4.7 ppm MPTP), XH171094 (<2 ppm MPTP) and XH020194 (<2 ppm MPTP) were spiked respectively with 4 ppm of MPTP. Subsequent single determinations of these spiked test solutions gave unacceptable recoveries of 165%, 145%, 211%, and 178%, respectively, for MPTP.



**Ion-Pair HPLC Method**

System Suitability and Precision: A 2 ppm standard chromatogram displayed HPTP at about 21.5 minutes and MPTP at about 22.3 minutes with a resolution of 1.46. The HPTP gave a tailing factor of 1.1 and a plate count of 18598. The MPTP afforded a tailing factor of 0.9, a plate count of 23680, and a signal to noise ratio of 3.0:1 (Figure 2).

Six replicate injections of a 5 ppm HPTP and 2.5 ppm MPTP test solution were performed. The ppm levels of both impurities were determined for each injection using a calibration curve. The RSD for the six determinations were computed at 3.12% for HPTP and 2.91% for MPTP.

Method Precision: After constructing a linear regression plot with standard solutions, single determinations were performed on six different weights of a sample that contained 3 ppm of MPTP. The RSD of the calculated values was 16.3%. This showed that the method generated reproducible results at the 3 ppm level.

Linearity of Response: Five different standard solutions were prepared and injected whose MPTP concentrations varied from 0.0000197 mg/mL to 0.0009847 mg/mL (2-98 ppm MPTP for a 10 mg/mL sample). A linearity plot was constructed by plotting the MPTP concentration (x-axis) against the area of MPTP (y-axis). Results of the linear regression afforded a correlation coefficient of 0.99997 with response factors being within 22% of the average system

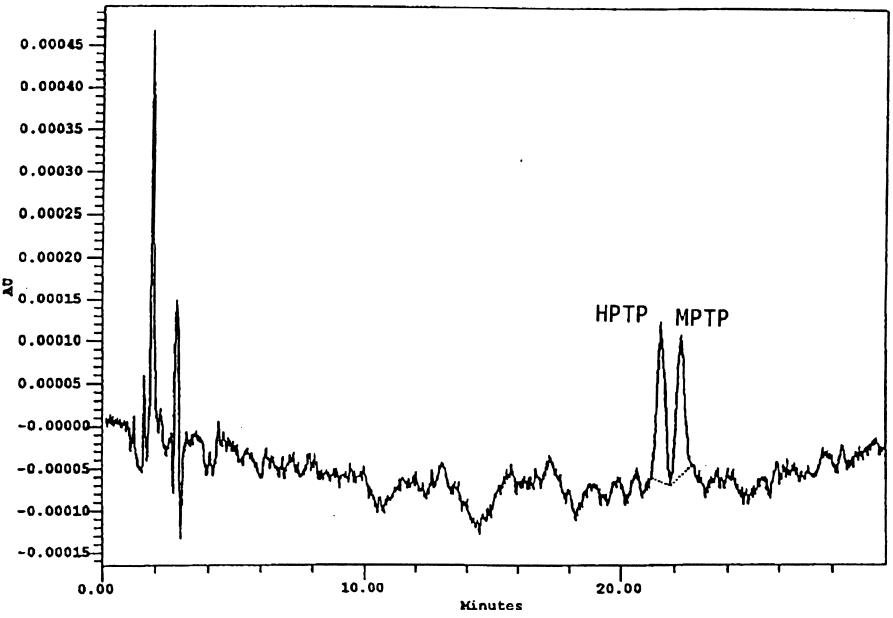


**Figure 2**. Ion-pair HPLC method, 2 ppm working standard.



precision response factor. This data indicated that the detector response was proportional to the level of MPTP from 2-98 ppm.

Limit of Quantitation and Detection: Six replicate injections of a 2.5 ppm MPTP test solution (Lot XH381193) were performed. The ppm levels were determined using a calibration curve generated from standard solutions. The RSD for the six determinations was calculated at 2.91%. This indicated that this method could reproducibly quantitate as little as 2.5 ppm of MPTP. A 2 ppm MPTP standard solution was analyzed and gave a signal to noise ratio of 3:1, indicating that this method could detect 2 ppm of MPTP.

Accuracy: Lots XH381193 (2.67 ppm MPTP), XH381095 (2.68 ppm MPTP), XH171094 (<2 ppm MPTP) and XH020194 (<2 ppm MPTP) were spiked with 4 ppm of MPTP Subsequent determinations of these spiked test solutions gave satisfactory recoveries of 93%, 87%, 93%, and 86% of MPTP, respectively. This experiment demonstrated that this method was accurate for MPTP quantitation.

Stability of Solution: Two different samples of XH381095 were originally analyzed to show 2.31 ppm and 2.30 ppm MPTP. The solutions were then re-assayed eight days later. The subsequent analyses showed 2.48 ppm and 2.45 ppm MPTP, respectively. Since these values deviated only 7.4% and 6.7% from the original assay, this experiment demonstrated that the solutions were stable for at least 8 days.

Lot Test Results: All of the CI-1007 maleate API lots produced to date were analyzed for MPTP by the new ion-pair method and the results are shown in Table 1.

Intermediate Precision: The ion-pair method was transferred to a second analyst in a different laboratory, and eight lots of CI-1007 maleate API were assayed for MPTP. During initial transfer of the method to the second laboratory, it was found that the type of autoinjector used was important. Specifically, a variable volume injector had to be used. For example, the first laboratory used a Waters 700 WISP equipped with a 200 µL loop, while the second used an Alcott 718 which was equipped with a 200 µL loop and a 50 µL syringe.



**Table 1**

**MPTP Content in CI-1007 API Lots, Measured with the Ion-Pair HPLC Method**

| Lot Number | ppm MPTP, Analyst 1 | ppm MPTP, Analyst 2 |
|---|---|---|
| 220693 | < 2 | < 2 |
| 280793 | 143 | 184 |
| 290793 | 145 | 264 |
| 381193 | 3 | 3 |
| 371193 | < 2 | 2 |
| 020194 | < 2 | < 2 |
| 171094 | < 2 | < 2 |
| 010195 | < 2 | -[1] |
| 080295 | < 2 | - |
| 180595 | < 2 | - |
| 210595 | < 2 | - |
| 381095 | 3 | 2 |
| 33704x75 | < 2 | - |

[1] Not assayed by analyst 2.

Both laboratories used methanol as the needle wash solvent for the variable volume injectors. A positive displacement fixed loop injector (Alcott 728) was tried, but did not perform well. It is thought that sample solubility and subsequent sample delivery onto the column was improved when a variable volume injector was used. The results obtained by the second analyst are shown in Table 1, and were comparable to results obtained in the first laboratory, except for lot XH290793 (2nd crop).

## CONCLUSIONS

Despite the finding that each method could quantitate and resolve MPTP from HPTP at the 2-4 ppm level, the study disclosed that the ion-pair procedure exhibited improved system precision, linearity, limit of quantitation, and accuracy. In addition, use of the photodiode array detector indicated that the method was selective for MPTP.



## ACKNOWLEDGMENTS

We would like to thank Dr. Charles Kulier for assistance with the references, and Dr. Donald Butler for informative discussions.

## REFERENCES

1. R. Lewin, Science, **224**, 1083 (1984).

2. J. W. Langston, P. Ballard, J. W. Tetrud, I. Irwin, Science, **219**, 979 (1983).

3. M. C. Shih, S. P. Markey, Biomed, Environ. Mass Spectrom., **13(2)**, 85 (1986).

4. S. P. Jindal, T. Lutz, Res. Commun. Subst. Abuse, **13(2)**, 137 (1992).

5. H. Rollema, D. S. Fries, J. B. DeVries, D. Mastebroek, A. S. Horn, Life Science, **37(17)**, 1633 (1985).

6. T. Shinka, N. Castagnoli, Jr., E. Y. Wu, M. K. P. Hoag, A. J. Trevor, J. Chromatography, **398**, 279 (1987).

7. M. Naoi, T. Takahashi, T. Nagatsu, Anal. Biochem., **162**, 540 (1987).

8. P. J. Kramer, J. Caldwell, A. Hofmann, P. Tempel, G. Weisse, Human & Exp. Toxicology, **17**, 283 (1998).

9. J. L. Wright, B. W. Caprathe, D. M. Downing, S. A. Glase, T. G. Heffner, J. C. Jaen, S. J. Johnson, S. R. Keston, R. G. MacKenzie, L. T. Meltzer, T. A. Pugsley, S. J. Smith, L. D. Wise, D. J. Wustrow, J. Med Chem., **37**, 3523 (1994).

Received December 7, 1999 Author's Revisions May 22, 2000
Accepted December 27, 1999 Manuscript 5224