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Determination of Memantine HCl by UV Spectrophotometry using MPCN as an UV-labelling Reagent

Sun-Sook Jang†, Jung-Kap Choi and Gyurng-Soo Yoo
College of Pharmacy, Chonnam National University, Kwangju 500-757, Korea
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자외부 유도화제인 MPCN을 이용한 memantine HCl의 정량

장선숙† · 최중갑 · 유경수
전남대학교 약학대학
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Abstract : Spectrophotometric assay for memantine HCl was developed by using sodium 1-(4-methoxyphenyl) cinnamonitrile-2-sulfonate (MPCN) as an UV labelling reagent. The optimum derivatizing condition was obtained by incubation for 30 min at pH 5.0 in the presence of 20-fold molar excess of the labelling reagent. The absorbance of reaction product was measured at 324.5 nm and detection limit was 0.43 μg/ml. A linear range was 5.0×10^{-6}~6.5×10^{-5} M of memantine HCl. The linear regression analysis of absorbance at various drug concentration was r=0.9998 (n=6). When applied to memantine HCl ampule and tablet, it gave the mean contents of 100.08±0.72% and 99.75±0.77%, respectively.

요약 : Memantine HCl을 자외부 유도화제인 MPCN과 반응시켜 흡광광도법으로 정량하였다. 유도화반응은 pH 5, 50℃에서 MPCN을 1:20배 몰비로 사용하여 30분간 반응시켰으며 dichloromethane으로 추출한 후 324.5nm에서 흡광도를 측정하였다. 그 결과 memantine HCl의 최종 농도로써 5.0×10^{-6}~6.5×10^{-5}M 사이에서 직선성을 나타냈으며 detection limit는 0.43μg/ml이었다. 본 분석법을 제제에 적용하였을 때 주사제는 100.08±0.72%였고 정제는 99.75±0.77%로, 부형제에 의한 영향 없이 간편하고 신속하며 재현성 있는 결과를 나타내므로 의약품의 품질관리에 효과적으로 응용될 수 있으리라 사료된다.

Key words : Spectrophotometric assay, memantine HCl, sodium 1-(4-methoxyphenyl) cinnamonitrile-2-sulfonate (MPCN), UV labelling reagent

1. Introduction

Memantine HCl (1,3-dimethyl-5-aminoadamantane, DMAA, D145) has been shown to affect the neurotransmitter system. It interferes with the uptake, binding and release of dopamine and 5-hydroxytrypamine (5-HT) in the central nerve system[1] and improves the disturbances of the extrapyramidal system as observed in Parkinson disease which is characterized by deficit of dopamine and 5-HT.[2~4]

Existing assay methods for memantine HCl in-

clude titrimetric chloride determination with 0.1 N of mercuric (Ⅱ) nitrate, potentiometry with 0.1 N of perchloric acid[5], and GC method.[5] However, titrimetric method is considered to be undersirable because it is generally regarded as being prone to make individual errors and as having comparatively low sensitivity. GC method requires further instrumentation and efforts than spectrophotometric method.

We describe here the UV detection method employing MPCN as an UV labelling reagent.[6] It provides a reliable and simple method for the analysis of memantine HCl.

2. Materials and Methods

1) Apparatus

UV absorption spectra were recorded on a Perkin-Elmer Lambda 5 UV/VIS double beam spectrophotometer.

2) Reagents and Chemicals

MPCN was synthesized from 2-formylbenzene sulfonic acid sodium salt (2-FBS), and 4-methoxyphenylacetonitrile (4-MPA) (Aldrich Chemical Co., Milwaukee, WI, USA) according to the method described by Jang *et al.*[6] Reference standard, tablets and ampules of memantine HCl were obtained from the Merz Co., (Frankfurt/M., FR Germany). Acetate buffer solution was prepared by dissolving 8.2 g of sodium acetate in water, adjusting pH to 2.0~8.0 with acetic acid and diluting with water to make 100 ml.

All other chemicals and solvents used were the highest purity of commercially available.

3) Preparation of stock solution

The stock solutions of MPCN and memantine HCl was prepared in pH 5.0 acetate buffer to be 1.0×10^{-2} M and 5.0×10^{-4} M, respectively. The stock solution of MPCN was stable for up to one week at room temperature. While that of memantine HCl shoud be prepared freshly.

4) Assay procedure

To obtain the calibration curve, various concentrations of memantine HCl (5.0×10^{-4}~1.0×10^{-5} M) were prepared by diluting the memantine HCl stock solution with pH 5.0 acetate buffer. Each solution (2.0 ml) was transferred to screw-capped test tubes and 2.0 ml of MPCN stock solution was added.

The solutions were mixed well and incubated in water bath at 50℃ for 30 min. Upon cooling to room temperature, the reaction product was extracted with 5 ml of dichloromethane (DCM) using vortex for a min.

Each solution was then transferred into 60 ml of separating funnels, allowed to stand until two phases were separated completely. This extraction procedure was repeated once. The collected organic phase was made up to 10 ml with DCM, and absorbance was measured at 324.5 nm. A blank was run concurrently.

For the analysis of dosage forms, 20 of memantine HCl tablets were powdered well and the quantity equivalent to 10 mg of memantine HCl was accurately weighed and dissolved in acetate buffer. The solution was filtered into a 100 ml volumetric flask and adjusted to the volume. Memantine HCl solution (2 ml) containing to 60 μg/ml was transferred to a test tube and assayed as described above. Ampules (10 mg/2 ml) were also assayed by the same procedure of tablets.

3. Results and Discussion

1) Effect of pH

MPCN (14.12 mg) and memantine HCl (2.15 mg) were dissolved in 10 ml of acetate buffer (pH 2.0~8.0) to make 4.0×10^{-3} M and 1.0×10^{-3} M, respectively. After diluting memantine HCl solution to 2.

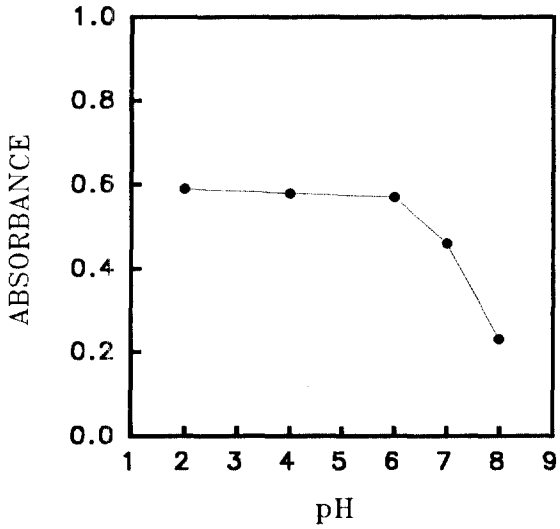

Fig. 1. Effect of pH on the derivatization of MPCN-memantine
MPCN : 4.0×10^{-3} M
Memantine HCl : 2.0×10^{-4} M

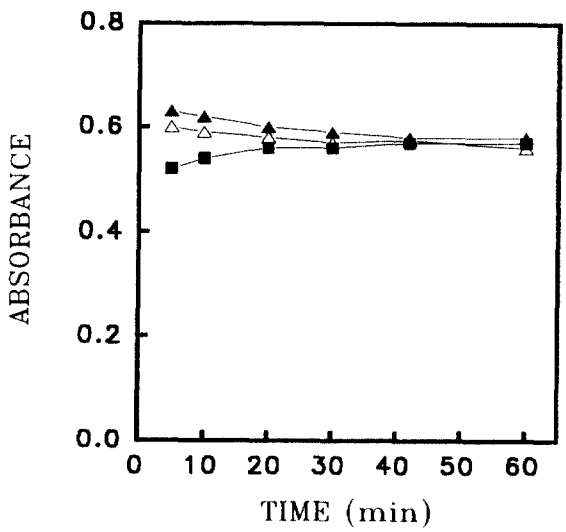

Fig. 2. Effect of reaction time and temperature on the derivatization of MPCN-memantine
MPCN : 4.0×10^{-3} M
Memantine HCl : 2.0×10^{-4} M

0×10^{-4} M, each sample was analyzed by following the assay procedure at different pHs. As shown in *Fig.* 1, absorbance was not affected significantly by the change of pH in the range of 2.0~6.0. At the pH higher than 6.0, however, there was a dramatic decrease in absorbance. Therefore, subsequent experiments were carried out at pH 5.0.

2) Reaction time and temperature

Reaction time was examined from 5 to 60 min the temperature of 20, 40, and 50℃. According to the results given in *Fig.* 2, reaction temperature did not influence significantly on derivatization reaction as far as reaction time was given sufficiently. The stabilized and constant absorbance was obtained by the incubation at 50℃ for 30 min.

3) Reaction molar ratio

Reaction between memantine HCl and MPCN was affected by the molar ratio of two compounds. The concentration of MPCN was varied from 5 to

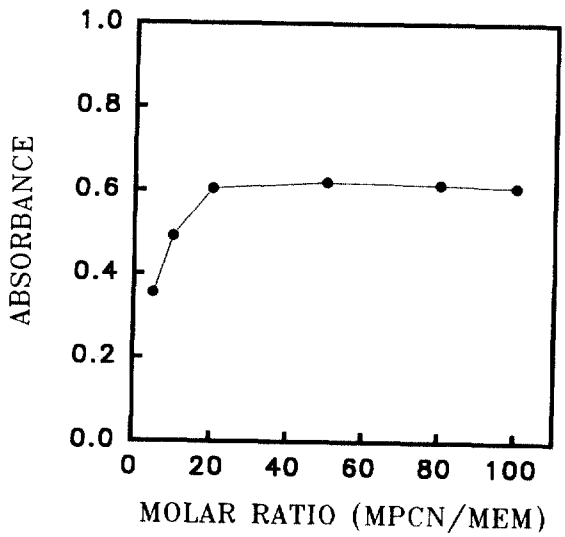

Fig. 3. Effect of reaction molar ratio on the derivatization of MPCN-memantine
Memantine HCl : 2.0×10^{-4} M

100-fold molar excess at the fixed concentration of memantine HCl. As shown in *Fig.* 3, maximum absorbance was obtained with 20-fold or more mo-

lar ratio of MPCN used. The quantities of MPCN beyond the 20-fold excess did not affect absorbance significantly.

4) Extraction efficiency of organic solvents

Reaction product was extracted with various organic solvent such as dichloromethane, chloroform, benzene, and hexane. *Table* 1 shows the extraction efficiency of organic solvents. Nonpolar solvents such as benzene and hexane showed very poor extraction efficiency. Therefore, dichloromethane was chosen as an extraction solvent in this experiment.

Table 1. Extraction efficiency of organic solvents

Solvent	Absorbance
dichloromethane	0.572
chloroform	0.564
benzene	0.149
hexane	–

MPCN : 4.0×10^{-3} M
Memantine HCl: 2.0×10^{-4} M

5) Effect of extraction times

To maximize the recovery of the reaction product of memantine-MPCN, extraction was carried out up to four times with vigorous shaking for a min. Maximum extraction was achieved with twice extraction (data not shown).

6) Stability of memantine-MPCN derivative

Reaction product was stable for up to 8 hours when stored at ambient temperature (data not shown).

7) Calibration curve

The stock solution of memantine HCl (M.W. 215.76) was diluted from 5.0×10^{-4} to 1.0×10^{-5} M. Aliquots of this solution (2 ml) were treated with MPCN following the assay procedure. Linearity

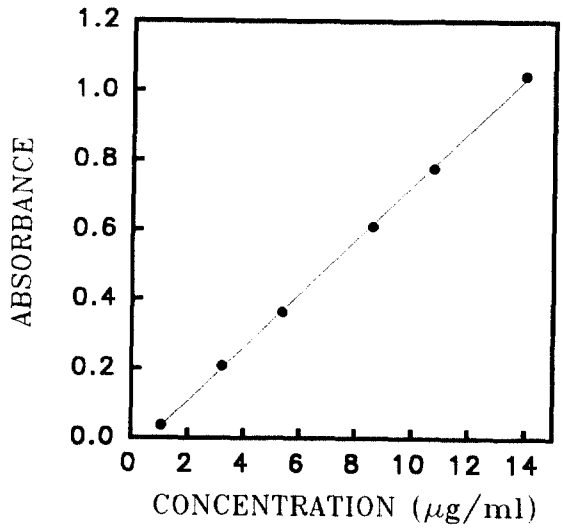

Fig. 4. Calibration curve of MPCN-memantine derivative

was obtained over the range of concentration from 5.0×10^{-6} M to 6.5×10^{-5} M (1.08 μg/ml to 14.02 μg/ml) in final assay solution (*Fig.* 4).

The linear regression analysis of absorbance vs. memantine HCl concentration gave a slope of 0.0775, an intercept of −0.0498, and a correlation coefficient, r, of 0.9998 (n=6).

The minimum detectable amount was found to be 0.43 μg/ml of memantine HCl.

8) Assay of dosage forms

This proposed method was applied to the deter-

Table 2. Determination of memantine HCl in commercial preparations

Samples	Ampule	Tablet
Theoretical amount(μg/ml)	24	24
Found* (μg/ml)	24.02±0.17	23.94±0.19
Recovery %	100.08	99.75
% RSD**	0.72	0.77

*Mean±SD based on triplicate determinations of each sample
**Relative standard deviation

mination of memantine HCl in tablets and ampules. Data revealed that the method provides good recovery and precision (*Table* 2).

These data indicated that none of tablet excipients, such as lactose and aerocil 200, interfered with the assay.

Acknowledgement

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