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Spectrophotometric Determination of Amantadine Sulfate after Ion-Pairing with Methyl Orange

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Abstract □ A convenient spectrophotometric method was examined for the determination of amantadine sulfate (AMTS) which has no UV-VIS chromopohores. AMTS was ion-paired quantitatively with methyl orange (MO) at 70℃ for 30 min. The ion-paired complex was extracted with dichloromethane and the absorbance was measured at 421.5 nm. A linear relationship was observed in the range of 2.5×10^{-7} M to 3.75×10^{-6} M and the correlation coefficient was 0.999 (n=3). This assay method was applied to the quantification of AMTS in commercial tablet form with good recovery and high precision.

Keywords □ Amantadine sulfate, methyl orange, ion-pair, UV-VIS spectrophotometry

Many compounds of pharmaceutical interest are polar and ionizable. Therefore, classical solvent extraction procedures for their concentration often fail. The analysis of such compounds has presented particular difficulties in obtaining specificity and accuracy. The principle of ion-pair extraction is based on the formation of a complex between an analyte ion and a suitable counter-ion. Significant advantages may be obtained by using ion-pair chromatography for the resolution of mixture of ionizable and nonionizable compounds and for improving sensitivity of detection by use of an UV-VIS absorbing counter-ion for a nonabsorbing analyte ion[1].

AMTS, 1-aminoadamantane sulfate, introduced as an antiviral agent for the prophylaxis of A_2 influenza was unexpectedly found to cause symptomatic improvement of patients with parkinsonism[2-4]. This drug is assumed to act by releasing dopamine from intact dopaminergic terminals that remain in the nigrostriatum of patients with parkinson's disease.

A few analytical methods for AMTS have been reported. Indirect procedures carried out after derivative formation include HPLC determinations[5] which are based on the conversion of AMTS into 3-(4,6-difluorotriazinyl) amino-7-methoxycoumarin derivative at high reaction temperature (140℃). Other direct assay methods include capillary isotachophoresis using conductivity detector[6], NMR analysis[7] and GLC methods[8-11]. However, most of these are not adequate for the routine assay of AMTS because either sensitivity is not good or the formation of a suitable derivative is time consuming. In case of NMR method, it is difficult to fix detection conditions due to instrumental variations. Here we present an assay method for AMTS using MO as an ion-pairing agent. It was quite sensitive because of strong absorptivity of MO in the visible range and was found to give satisfactory result when applied to the dosage form.

EXPERIMENTAL SECTION

Apparatus

Perkin-Elmer Lambda 5 UV-VIS spectrophotometer was used for spectrophotometric determination. The Philip Harris constant temperature circulating water bath used for the reaction and vortex mixer was used for the extraction of reaction product. Mass spectrum was recorded on a Jeol JMS-DX 303 model.

Chemicals

MO was a first grade (Junsei Chemical Co., Ltd., Tokyo) and AMTS reference standard and P.K.

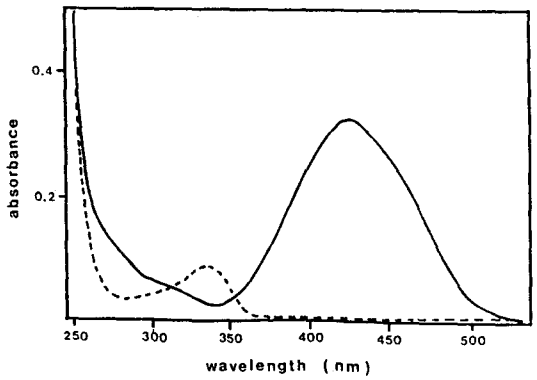


Fig. 1. Absorption spectra of the extracted reaction product (—) in dichloromethane layer and MO (---) in dichloromethane.

Merz tablets were obtained from Han Wha Pharmaceutical Company. All other solvents used for the extraction of reaction product such as dichloromethane, chloroform, hexane and benzene were of reagent grade.

Preparation of stock solutions

AMTS stock solution (1.25×10^{-4} M) was prepared by dissolving the weighed amount (2.5 mg) of AMTS standard in 50.0 m*l* of distilled water. MO stock solution (5.0×10^{-3} M) was prepared by dissolving the weighed amount (81.3 mg) in 50.0 m*l* of distilled water.

Examination of assay conditions

Effect of pH: AMTS and MO stock solutions were mixed in the 0.2 M phosphate buffer of varying pH of 4.0 to 8.0. They were reacted at 70℃ for 1 hr in the constant-temperature circulating water bath. The reaction mixture was cooled in ice water and vortexed for 1 min. The product was extracted with 4.0 m*l* dichloromethane and the absorbance was measured at 421.5 nm 1 hr after the extraction.

Effect of reaction time and temperature: Reaction time and temperature were varied from 10 to 60 min and from 40 to 80℃, respectively. 30 samples were prepared at various time and temperature conditions. Other conditions were the same as mentioned above.

Effect of reaction molar ratio: Reaction molar ratio between AMTS and MO was varied from 1 : 5 to 1 : 300. Samples were heated at 70℃ for 30 min.

Effect of extraction solvent: Polar and non-polar solvents such as dichloromethane, chloroform, hexane, benzene, diethyl ether or ethyl acetate were examined for the extraction of the reaction product.

Stability test: The change in absorbance with time at the ambient temperature was measured at 421.5 nm.

Application to the dosage form

20 tablets of P.K. Merz (100 mg of AMTS/tablet) were well ground and the quantity equivalent to 2.5 mg of AMTS was accurately weighed and dissolved in 50.0 m*l* of distilled water. 200 μ*l* of this solution was transferred to screw-capped test tubes and MO stock solution was added at 250-fold molar ratio. Samples were then treated according to the assay produre, described above.

RESULTS AND DISCUSSION

The UV/VIS spectrum of the reaction product between AMTS and MO was shown in Fig. 1. The reaction product extracted with dichloromethane showed a maximal absorbance at 421.5 nm. As previously reported[12], it was presumed that this reaction between AMTS and MO associated with ion-pairing in consideration of mass spectrum. The peak of ion-paired product was appeared at $m/z=$ 457 as a $(M+H)^+$ by the FAB-MS. Particularly, in the case of the FAB-MS, the peak at $m/z=479$, $(M+Na)^+$ ion, was appeared as a impure peak due to the trace amount of methyl orange Na salt simutaneously extracted with the ion-pair product to the organic phase[13] (Fig. 2). The structure of the ion-pairing product between AMTS and MO was given in Scheme 1.

Optimization of assay conditions

Effect of pH: The reaction product showed a maximum absorbance when reacted in pH 6.0 phosphate buffer solution because AMTS and MO were both present in ionic forms (Fig. 3). However, similar absorbance was obtained when reacted in the distilled water and thus we used distilled water for the reaction.

Effect of reaction time and temperature: The samples reacted at 40 to 60℃ had relatively low absorbances with time in comparison with ones at 70℃ and 80℃ (Fig. 4). On the other hand, the samples reacted at

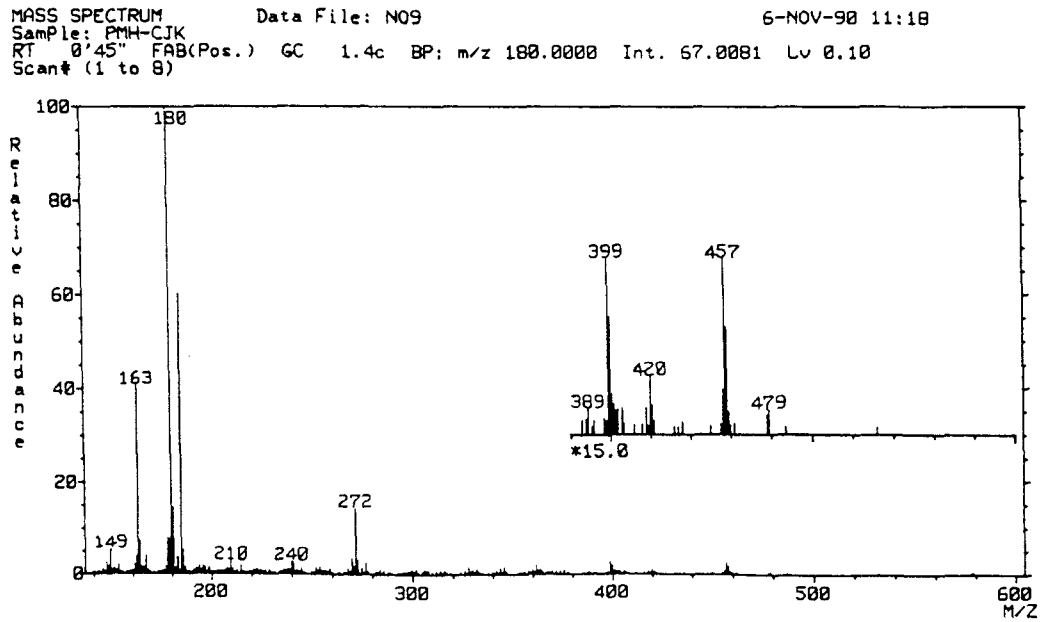


Fig. 2. Mass spectrum of the reaction product.

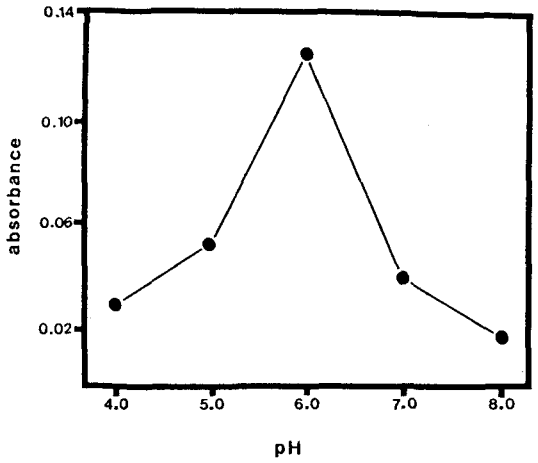
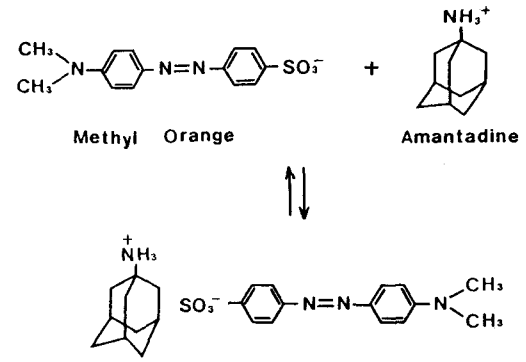


Fig. 3. Effect of pH on the ion-pair formation between AMTS and MO.
Absorbance was measured at 421.5 nm.



Scheme 1. Scheme of ion-pairing reaction between AMTS and MO.
Molecular weights of AMTS & MO are 398. 52 and 327.33, respectively.

70℃ and 80℃ showed similar absorbances, however, a little variation with time was observed in the samples reacted at 80℃. Therefore, heating at 70℃ for 30 min was chosen as the overall optimal reaction time and temperature.

Effect of molar ratio: The absorbance was gradually increased with molar ratio. However, it did not affect the absorbance beyond 250-fold molar ratio (Fig. 5).

Efficiency of extraction solvent: Ethyl acetate and diethyl ether were not suitable for extraction of ion-pairing product because the phase separation was very slow and molar absorptivity of the product was low. Extraction efficiencies of non-polar solvents such as benzene and hexane were poor as shown in Table I. Therefore, dichloromethane was chosen in this experiment because it showed the highest absorbance among the tested organic solvents.

Stability test: The absorbance was decreased during the first 1 hr after reaction, it was assumed that

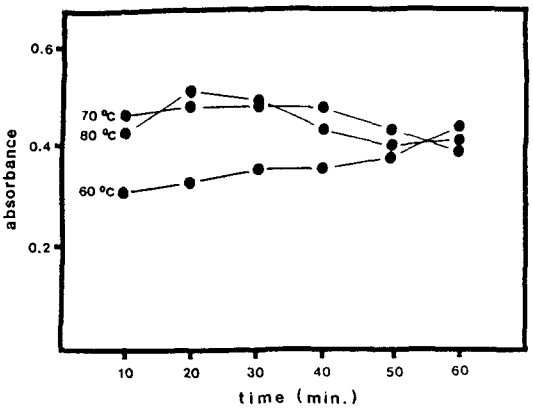


Fig. 4. Effect of reaction time and temperature on the ion-pairing reaction of AMTS and MO.
Absorbance was measured at 421.5 nm.

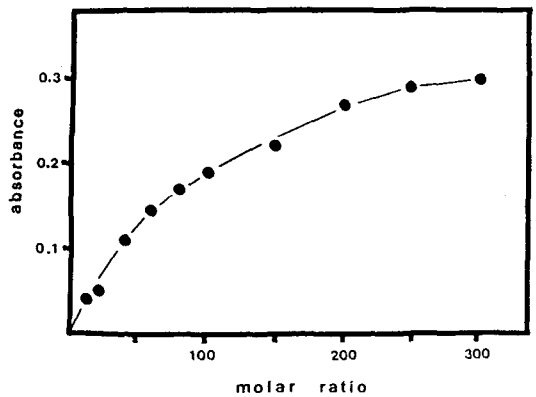


Fig. 5. Effect of reaction molar ratio of AMTS and MO on changes in absorbance.

ion-paired product may need time to be stabilized. It was explainable according to the report of E. Tomlinson that the dissociation of ion-pair product is increased in the organic phase due to the difference of polarity between the two layers when the polar solvent for the extraction of ion-pair product is used[14]. However after that time, it was stable up to 4 hr (Fig. 6).

Calibration curve: Various concentrations (6.3×10^{-8} M-6.3×10^{-6} M) of AMTS were used for plotting a calibration curve. The linearity between AMTS concentrations and the absorbance of AMTS-MO ion-pairing product solution was obtained from 2.5 $\times10^{-7}$ M to 3.75×10^{-6} M and the correlation coefficient was 0.999 (n=3) given in Table II. The detection limit was 6.3×10^{-8}M (S/N ratio=2).

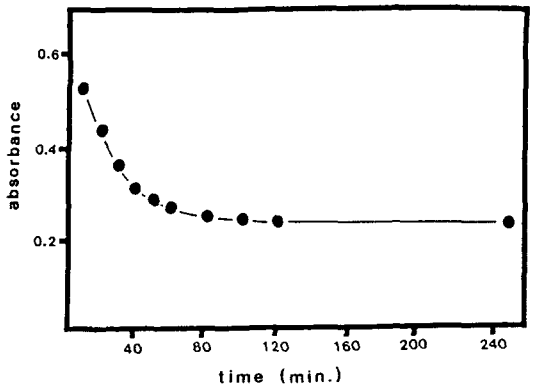


Fig. 6. Stability of the reaction product.
Absorbance was measured at 421.5 nm.

Table I. Extraction efficiency of organic solvents ($*\lambda_{max}$=421.5 nm)

Solvents	Absorbance* (Mean± SD)
Dichloromethane	0.582± 0.002
Chloroform	0.312± 0.001
Benzene	0.084± 0.002
Hexane	0.067± 0.001

*Mean± SD based on triplicate determinations

Table II. Estimated linearity of AMTS by spectrophotometry

Theoretical concn.($\times10^{-6}$ M)	Absorbance	Calculated concn.(X)	Response factor*
0.25	0.015	0.30	1.20
0.50	0.030	0.56	1.12
1.00	0.052	0.95	0.95
1.50	0.077	1.39	0.93
2.00	0.114	2.04	1.02
2.50	0.141	2.51	1.00
3.13	0.177	3.14	1.00
3.75	0.214	3.79	1.01

X=(absorbance−intercept)/slope
r=0.999 (n=3)
Intercept= −0.002
Slope=0.057
*Response factor=calculated concentration/theoretical concentration

Assay of AMTS in dosage form: According to the assay result on AMTS tablets by this method, it showed a good recovery and precision without any interferences by tablet additives such as gelatin, dye, poly-

Table III. Determination of AMTS in commercial preparation

Amount added (μg/ml)	2.42
Amount found (μg/ml)	2.41± 0.04
Recovery(%)	99.6 ± 1.7
% RSD	1.7

*Mean± SD based on triplicate determinations

ethylene glycol 6000, lactose, microcrystalline cellulose and colloidal silicone dioxide (Table III).

CONCLUSION

We established a new spectrophotometric method for AMTS assay using ion-pairing technique. The AMTS stock solution (1.25×10^{-4} M) and MO stock solution (5.0×10^{-3} M) were prepared in distilled water. The optimal reaction molar ratio was 1 : 250. The reaction condition was 70℃ for 30 min. After the reaction, samples were cooled immediately in an ice water bath and extracted three times with 4.0 ml of dichloromethane using vortex mixer for 30 sec. The absorbances were measured at 421.5 nm 1 hr after the extraction. This proposed accurate, convenient and economical method using MO as a ion-pairing agent could be applied to the routine assay of AMTS for quality control.

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