# Blood Concentrations of Amitriptyline and Its Metabolite in Rats after Acute Oral Administration of Amitriptyline

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## Abstract

**Amitriptyline (AMT), a tricyclic antidepressant that is a dibenzocycloheptadine derivative, is frequently used. However, the case reports of AMT-related fatalities are increased, nowadays, due to the low levels of toxic and fatal concentration in blood. So, this study was carried out to determine the concentrations of AMT and its demethylated metabolite, nortriptyline (NTR), after acute single oral administration of AMT in rats. Blood samples were collected five times from the opthalmic venous plexus at 0, 1, 2, 4, and 8 h after acute single oral administration of AMT in toxic doses of 10 (Group I) or 20 mg/kg (Group II), and the concentrations of AMT and NTR and the mean ratios of AMT to NTR (AMT/NTR) in the blood were periodically determined at designated times. The blood concentrations of AMT and NTR were identified and quantitated by gas chromatography with thermionic specific detection and gas chromatography–mass spectrometry after solid-phase extraction with a Clean Screen DAU column. The peak blood concentrations of AMT and NTR in Group I were 0.34 and 0.28 µg/mL, respectively, and those of AMT and NTR in Group II were 0.59 and 0.43 µg/mL, respectively, and were reached at 1 h after single oral administration.**

## Introduction

Amitriptyline (AMT), 3-(10,11-dihydro-5H-dibenzo[a,d]-cycloheptene-5-ylidene)-*N*,*N*-di-methyl-1-propanamine, a tricyclic antidepressant with sedative effects that is a dibenzocycloheptadine derivative, is frequently used especially in the neuropsychiatry for depression, depressive personality disorder and nocturia, and so on. However, the reports of cases of AMT-related fatalities have increased recently because of the low level of toxic and fatal concentrations that cause fatal dysrhythmia, and amitriptyline is the most frequently involved drug in tricyclic overdose, accounting for half or more of the poisonings in some studies (1,2).

Various analytical techniques have been employed for the determination of AMT and nortryptyline (NTR) in blood (3). An early method used thin-layer chromatography, and most methods are based on high-performance liquid chromatography with liquid–liquid extraction for sample preparation (4). In this study, the blood concentrations of AMT and NTR were identified and quantitated by gas chromatography with thermionic specific detection (GC–TSD) and gas chromatography–mass spectrometry (GC–MS) after solid-phase extraction (SPE) with a Clean Screen DAU column.

Fatal AMT intoxications are not rare, and several cases have been reported in the last few years. AMT is mainly metabolized by *N*-demethylation to NTR, but very few publications deal with postmortem concentrations of AMT in the blood (5).

The purpose of this study was to determine the blood concentrations of AMT and its demethylated metabolite, NTR, and the mean ratio of AMT to NTR (AMT/NTR) after acute single oral administration of AMT in rats.

## Experimental

### Reagents

Amitriptyline HCl, nortriptyline HCl, and ethyl *p*-piperidyl-acetylaminobenzoate were obtained from Sigma Chemical Co. All the other reagents were of analytical grade and used without further purification.

### Apparatus

The vacuum manifold and solid-phase column (Clean Screen DAU) were purchased from World Monitoring Corp. (Horsham, PA).

### Instruments and conditions

A Varian model 3400 GC equipped with a thermionic specific detector was used for the quantitation of AMT and NTR in

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blood. Operating GC conditions were as follows: column, a fused-silica capillary DB-1 (15 m × 0.25 mm); column temperature, programmed from 150°C to 260°C at 10°C/min; initial time, 1 min; final time, 10 min; injector temperature, 260°C; detector temperature, 270°C; carrier gas, He; flow rate, 40 mL/min.

A Finnigan MAT GCQ was used to identify AMT and NTR. Conditions were as follows: column, a fused-silica capillary DB-5MS (15 m × 0.25 mm); ionization energy, 70 eV; ion source temperature, 180°C; transferline temperature, 270°C; EM voltage, 1400 V.

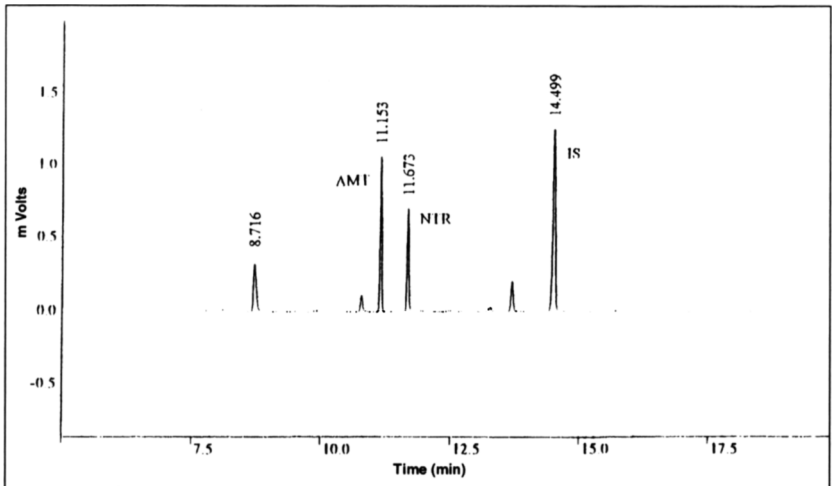


**Figure 1.** The GC chromatograms of AMT and NTR.

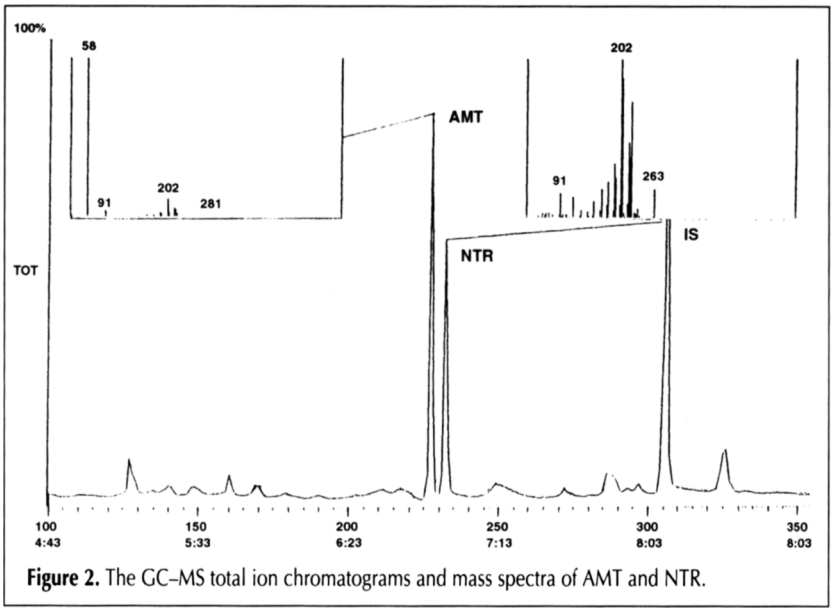


**Figure 2.** The GC–MS total ion chromatograms and mass spectra of AMT and NTR.

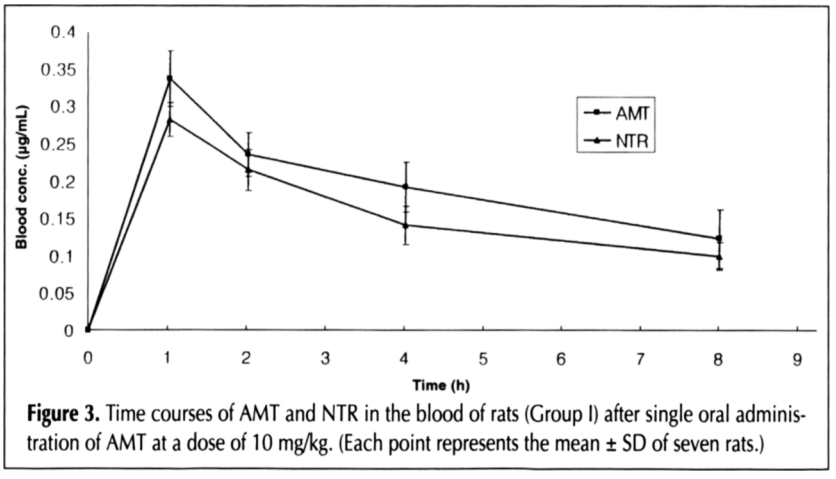


**Figure 3.** Time courses of AMT and NTR in the blood of rats (Group I) after single oral administration of AMT at a dose of 10 mg/kg. (Each point represents the mean ± SD of seven rats.)

### Animal study

Male Sprague-Dawley rats weighing between 250 and 300 g were housed five per cage with free access to food and water under a 12-h light/12-h dark cycle. Rats were divided into three study groups of seven, 21 rats total. In the first group (control group), rats received a single oral administration of distilled water. In the second group (Group I), they received a single oral administration of 10 mg/kg of AMT. The last group (Group II) received a single oral administration of 20 mg/kg of AMT.

Blood samples were collected five times from the opthalmic venous plexus at 0, 1, 2, 4, and 8 h after acute single oral administration of AMT with toxic concentration of 10 (Group I) or 20 mg/kg (Group II) to rats, and the concentrations of AMT and NTR and the AMT/NTR in the blood were periodically determined at designed times.

### SPE method

The extraction was performed with Clean Screen DAU columns, which were installed on a vacuum manifold. The column was preconditioned with 3 mL of methanol, 3 mL of deionized water, and 1 mL of 100mM phosphate buffer (pH 6.0). One milliliter of blood was used for the analysis. Three milliliters of deionized water was added to and mixed with a 1-mL blood sample, then centrifuged for 10 min at 2000 rpm. The pellet was discarded. After 2 mL of 0.1M phosphate buffer (pH 6.0 ± 0.5) was added to a 1-mL blood sample, the sample was vortex mixed for 30 s and loaded onto the column at a flow rate of 1 mL/min.

The column was washed with 3 mL of deionized water and 3 mL of methanol and then dried under vacuum for 5 min. The analytes were eluted from the column with 3 mL of methylene chloride/isopropanol/ammonia water (78:20:2). The eluates were evaporated to dryness under nitrogen, 100 μL of internal standard was added, and then 1 μL was injected onto the GC.

### Preparation of standards

The stock standard solutions of AMT-HCl and NTR-HCl were separately prepared in ethanol to give the concentration of 1 mg/mL.

The working standard solutions were prepared in the concentration range 0.05–1 µg/mL by appropriate dilution with ethanol. Ethyl *p*-piperidyl-acetylaminobenzoate 5 µg/mL in ethanol was used as internal standard solution.

### Determination of calibration curve

The calibration curves for AMT and NTR were determined by adding 0.05, 0.1, 0.5, 1, and 1.5 µg of AMT and NTR to 1 mL of drug-free blood. These solutions were then extracted according to the methods described.

### Measurement of recovery rate

The recovery rates were evaluated from 1-mL blood samples spiked with 0.1, 0.5, and 1 µg of AMT-HCl and NTR-HCl.

## Results and Discussion

The blood concentrations of AMT and NTR and AMT/NTR were measured at 0, 1, 2, 4, and 8 h after the rats were dosed with AMT (Group I with 10 mg/kg; Group II with 20 mg/kg).

Figure 1 shows the GC chromatograms of AMT and NTR, and Figure 2 shows the GC–MS total ion chromatograms and the typical mass spectra of AMT and NTR.

The calibration curves for AMT and NTR were determined by adding 0.05, 0.1, 0.5, 1, and 1.5 µg of AMT and NTR to 1 mL of drug-free blood. The calibration curves of AMT and NTR were linear over the concentration range 0.05 to 1.5 µg with $y = 0.0132x - 0.0221$ ($r = 0.9999$) and $y = 0.0242x - 0.0354$ ($r = 0.9998$), respectively.

The extraction recovery was determined from control drug-free blood at 0.1, 0.5, and 1 µg of concentration levels of AMT-HCl and NTR-HCl. The recovery rates were found to be 89, 92, and 92% for AMT and 92, 90, and 94% for NTR.

The time courses of AMT and NTR in the blood of rats after single oral administration of AMT at a dose of 10 mg/kg (Group I) and 20 mg/kg (Group II) were shown in Figures 3 and 4, respectively.

The concentrations of AMT and NTR and the AMT/NTR in blood of rats after single oral administration of AMT are tabulated in Table I.

In Group I, the blood concentrations varied from 0.12 (± 0.04) to 0.34 (± 0.04) µg/mL for AMT and from 0.10 (± 0.02) to 0.28 (± 0.02) µg/mL for NTR, and the AMT/NTR ranged from 1.09 to 1.45. In Group II, the blood concentrations varied from 0.24 (± 0.03) to 0.59 (± 0.13) µg/mL for AMT and from 0.18 (± 0.04) to 0.43 (± 0.06) µg/mL for NTR, and the AMT/NTR ranged from 1.23 to 1.38.

Maximum blood concentrations ($C_{max}$) of AMT and NTR in Group I were 0.34 and 0.28 µg/mL and those in Group II were 0.59 and 0.43 µg/mL. The time required to achieve the maximum concentration ($t_{max}$) in blood was 1 h for both AMT and NTR. In addition, Miyake et al. (6) reported that the serum levels of NTR after oral administration were high compared with the results obtained after intravenous injection. In addition, they reported that $t_{max}$ in serum was 0.25 and 0.5 h for AMT and NTR, respectively. They also reported that the demethylated metabolite, NTR, could not be detected following intravenous injection of the parent drug AMT. Similar findings have also been reported for imipramine (IMP) (7). The major hydroxylation and demethylation pathways of AMT are similar to those of IMP (8). Miyake et al. (6) reported that AMT and NTR, extremely lipophilic compounds, would distribute immediately to the tissues when administrated systemically, and they insisted that formed NTR, which was present in a smaller amount than AMT, would also be distributed immediately because of its high lipophilicity (6). In general, ratios of the parent drug to its metabolite may be useful in evaluating the "acuteness" of some ingestions, because the ratios decrease with time as the parent drug is converted to metabolite (9,10). In this study, the AMT/NTR in Group I and Group II were 1.09–1.45 and 1.23–1.38, respectively, and the AMT/NTR ratios in Group I and Group II were all more than 1.00. Therefore, they suggested an acute ingestion by Group I and Group II.

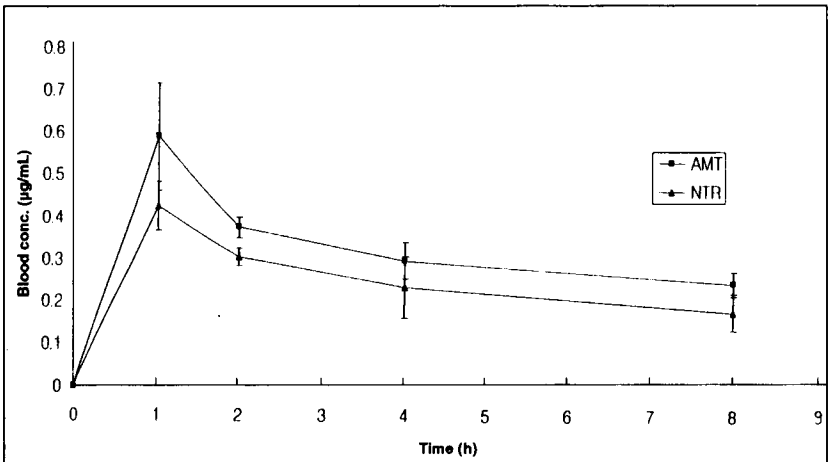


**Figure 4.** Time courses of AMT and NTR in the blood of rats (Group II) after single oral administration of AMT at a dose of 20 mg/kg. (Each point represents the mean ± SD of seven rats.)

**Table I. The Concentrations of AMT and NTR and AMT/NTR in Blood of Rats after Single Oral Administration of AMT**

| | Group 1 concentration (µg/mL) | | | Group 2 concentration (µg/mL) | | |
|---|---|---|---|---|---|---|
| Time (h) | AMT (mean ± SD) | NTR (mean ± SD) | AMT/NTR | AMT (mean ± SD) | NTR (mean ± SD) | AMT/NTR |
| 1 | 0.34 ± 0.04 | 0.28 ± 0.02 | 1.19 | 0.59 ± 0.13 | 0.43 ± 0.06 | 1.38 |
| 2 | 0.24 ± 0.03 | 0.21 ± 0.03 | 1.09 | 0.37 ± 0.03 | 0.30 ± 0.02 | 1.23 |
| 4 | 0.19 ± 0.03 | 0.13 ± 0.02 | 1.45 | 0.30 ± 0.04 | 0.23 ± 0.07 | 1.28 |
| 8 | 0.12 ± 0.04 | 0.10 ± 0.02 | 1.22 | 0.24 ± 0.03 | 0.18 ± 0.04 | 1.37 |

* Abbreviations: AMT, amitriptyline; NTR, nortriptyline; AMT/NTR, the ratio of amitriptyline and nortriptyline.

## Conclusions

The $C_{max}$ of AMT and NTR in Group I were 0.34 and 0.28 µg/mL, and those in Group II were 0.59 and 0.43 µg/mL. The $t_{max}$ in blood was 1 h for both AMT and NTR. The AMT/NTR in Group I and Group II were 1.09–1.45 and 1.23–1.38, respectively. The AMT/NTR in Group I and Group II are all more than 1.00. Therefore, they suggested an acute ingestion and did not differ significantly from one another.

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