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Quantitation of the tacrine analogue octahydroaminoacridine in human plasma by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and sensitive method based on liquid chromatography–tandem mass spectrometry (LC–MS/MS) has been developed for the determination of octahydroaminoacridine in human plasma using tramadol as internal standard (I.S.). Sample preparation involved pH adjustment with sodium carbonate followed by solvent extraction with dichloromethane:ethyl ether (40:60, v/v). Chromatographic separation was achieved on a Venusil MP-C18 column (5 μm, 100 mm × 4.6 mm) using acetonitrile:10 mM ammonium acetate:formic acid (30:70:1, v/v/v) as mobile phase. Detection utilized an API 4000 system operated in the positive ion mode with multiple reaction monitoring of the analyte at m/z 203.1 → 175.1 and of the I.S. at m/z 264.1 → 58.0. The method was linear in the range 0.01–10 ng/ml with a lower limit of quantitation of 0.01 ng/ml. Intra- and inter-day precisions measured as relative standard deviation were <3.15% and <5.01%, respectively. The method was successfully applied to a pharmacokinetic study involving oral administration of a tablet containing 4 mg octahydroaminoacridine succinate to healthy volunteers. Pharmacokinetic parameters for octahydroaminoacridine include C_{max} 1.19 ± 0.53 ng/ml, T_{max} 0.77 ± 0.17 h, AUC_{0-t} 3.42 ± 1.01 ng h/ml and $t_{1/2}$ 2.89 ± 0.56 h.

1. Introduction

Alzheimer's disease (AD) is a multifaceted neurodegenerative disorder affecting millions of elderly people worldwide [1]. It is the most common type of dementia with a prevalence which continues to rise [2]. The cause of the disease is unknown but it appears to involve a cholinergic deficit in the brain. This has led to the use of a number of acetylcholinesterase inhibitors (AChEIs) including tacrine (tetrahydroaminoacridine, THA), galanthamine and donepezil. Unfortunately these drugs have limited efficacy in memory enhancement and provide only palliative treatment since they do not stop the neurodegenerative process [3–5]. Therefore, there remains an urgent need to develop new anti-AD agents with more potent therapeutic efficacy.

Octahydroaminoacridine (1,2,3,4,5,6,7,8-octahydroacridin-9-amine, OHA) (Fig. 1) is a THA analogue currently undergoing phase 1 clinical trials in China. Like rivastigmine, it acts as a dual anticholinesterase, being an effective inhibitor of both acetylcholinesterase and butyrylcholinesterase. Preclinical pharmacodynamic studies suggest that OHA is a more potent anti-AD drug than either rivastigmine or donepezil but it remains to be seen whether its use is associated with lower hepatotoxicity than observed with THA [6].

To date, various methods have been developed for the quantitation of THA and its dimmer, bis(7)-tacrine, in biological samples including high-performance liquid chromatography (HPLC) with fluorescence [7], and tandem mass spectrometric (MS/MS) [8,9] detection. However, to our knowledge, no assay method for the determination of OHA has been reported. This paper describes a rapid and sensitive assay based on liquid chromatography tandem mass spectrometry (LC–MS/MS) capable of monitoring plasma concentrations of OHA after administration of a therapeutic oral dose to healthy volunteers.

2. Experimental

2.1. Reagents and chemicals

OHA succinate (purity >99%) was supplied by Changchun Huayang High-Technology Limited Corporation (Changchun, P.R.

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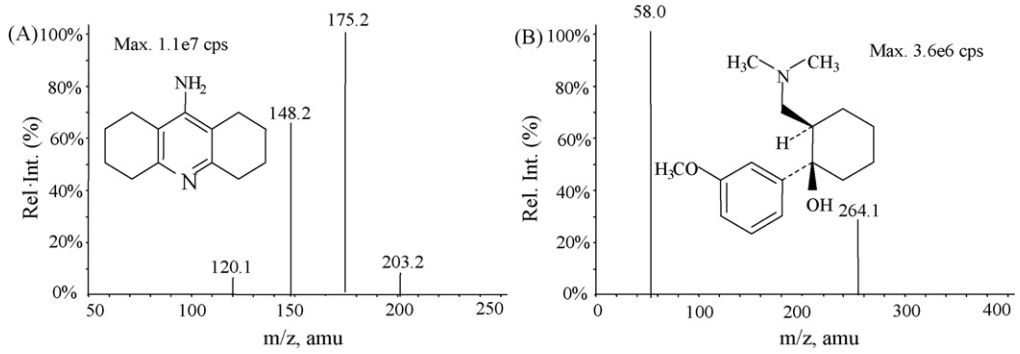

Fig. 1. Full-scan product ion scans of $[M+H]^+$ and structures for (A) octahydroaminoacridine and (B) tramadol.

China). Tramadol (purity >99%) for use as internal standard (I.S.) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). HPLC grade acetonitrile was purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other reagents were of analytical grade and used without further purification. Distilled water, prepared from demineralized water, was used throughout the study. Drug-free human plasma was obtained from the Changchun Blood Donor Service (Changchun, P.R. China).

2.2. Preparation of calibration standards and quality control (QC) samples

A stock solution of OHA succinate (containing OHA 1 mg/ml) in distilled water was diluted with distilled water to give a series of standard solutions with OHA concentrations in the range 0.05–50 ng/ml. A series of calibration standards was then prepared by spiking blank plasma samples (500 μl) with 100 μl aliquots of standard solutions to give OHA concentrations in plasma of 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 ng/ml. Low, medium and high QC samples were prepared by spiking blank plasma samples with QC solutions (0.03, 0.3 and 3 ng/ml) prepared independently in the same way. A stock solution of I.S. (1 mg/ml) in acetonitrile was diluted with acetonitrile:water (50:50, v/v) to produce an I.S. working solution (3 ng/ml).

2.3. Sample preparation

Frozen plasma samples were thawed at room temperature and subjected to liquid–liquid extraction as follows. To 500 μl plasma (or a calibration standard or QC sample) in a 10 ml capped glass tube was added 100 μl I.S. working solution, 100 μl distilled water, 100 μl 1 M Na_2CO_3 solution and 3.5 ml dichloromethane:ethyl ether (40:60, v/v). The mixture was vortexed for 30 s and then mechanically shaken for 15 min. After centrifugation at 3500 × *g* for 5 min, the organic layer was transferred to another glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The sample was reconstituted in 300 μl mobile phase, vortexed, transferred into a plastic autosampler vial and 20 μl injected into the LC–MS system.

2.4. LC–MS/MS

The HPLC system (Agilent 1100 series) consisted of a binary pump, an autosampler, a column oven maintained at 30 °C and a Venusil MP-C18 column (5 μm, 100 mm × 4.6 mm). The mobile phase was 30% acetonitrile 70% 10 mM ammonium acetate buffer (1% formic acid) delivered at a flow rate of 1.0 ml/min. An approximately 1:1 split of the column elute was included prior to entry into the mass spectrometer. Detection was performed on a Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) with an electrospray ionization (ESI) source operated in the positive ion mode. Multiple reaction monitoring (MRM) at unit resolution involved transitions of the protonated forms of OHA at *m/z* 203.1 → 175.1 (quantifier) and 203.1 → 148.1 (qualifier) and of tramadol at *m/z* 264.1 → 58.0. Full-scan product ion mass spectra of OHA and tramadol are shown in Fig. 1. Optimized MS conditions were as follows: curtain gas, gas 1 and gas 2 (all nitrogen) 20, 45 and 45 units, respectively; dwell time 200 ms; ion spray voltage 5000 V; source temperature 550 °C; declustering potentials 100 V for OHA and 29 V for tramadol; collision energies 50 eV (*m/z* 203.1 → 175.1) and 56 eV (*m/z* 203.1 → 148.1) for OHA and 40 eV for tramadol.

2.5. Assay validation

Assay validation was performed according to FDA guidelines [10]. Linearity was assessed by weighted ($1/x^2$) least-squares linear regression of analyte: I.S. peak area ratios for calibration curves prepared in triplicate on three different days. Precision (as relative standard deviation (RSD)) and accuracy (as relative error (RE)) were evaluated based on assay of QC samples on three different days. The lower limit of quantitation (LLOQ) was defined as the lowest concentration of analyte that can be determined with precision and accuracy of ±20%. Specificity was performed by analyzing blank plasma samples from six different subjects. Recovery was determined by comparing peak areas of QC samples with those of post-extraction spiked blank plasma. Matrix effects were evaluated by comparing peak areas of analyte in QC samples with those in standard solutions [11]. Long-term and short-term stability were studied by assaying samples after storage for 1 month at −20 °C and storage at room temperature for 6 h, respectively. Stability during three successive freeze–thaw cycles and in processed samples stored at room temperature (25 °C) for 12 h were also evaluated.

2.6. Pharmacokinetic study

The analytical method was applied to determine plasma concentrations of OHA in healthy volunteers after a single oral dose containing 4 mg OHA succinate. The clinical protocol was approved by the Ethics Committee of Beijing University First Hospital, P.R. China and all volunteers gave informed consent. Volunteers (n = 10; 5 males, 5 females) were non-drinkers of alcohol and did not consume any concomitant medication during the study. Serial blood samples (5 ml at each time point) were collected from the antebrachium vein prior to dosing and at 0.33, 0.67, 1, 2, 4, 6, 8, 10, 12, 16 and 24 h after dosing. Plasma was separated by centrifugation at 3000 × *g* for 10 min and kept frozen at −20 °C until analysis. Plasma concentration–time profiles for OHA in each subject were analyzed by noncompartmental analysis using Topfit 2.0.

Table 1
Precision and accuracy for the determination of octahydroaminoacridine in human plasma (data are based on assay of six replicates on three different days).

Spiked conc. (ng/ml)	Calculated conc. (mean ± SD, ng/ml)	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy RE%
0.03	0.031 ± 0.001	3.15	5.01	4.35
0.30	0.31 ± 0.01	2.61	2.03	4.28
3.00	2.98 ± 0.08	2.73	3.20	−2.80

3. Results and discussion

3.1. Method development

Full-scan product ion spectra of the $[M+H]^+$ ions of OHA and tramadol are shown in Fig. 1. In the mass spectrum of OHA, the product ion at *m*/*z* 175.1 was more abundant than that at *m*/*z* 148.1. Accordingly, the transition *m*/*z* 203.1 → 175.1 was selected as quantifier and the transition *m*/*z* 203.1 → 148.1 as qualifier. The transition *m*/*z* 264.1 → 58.0 was used to monitor tramadol.

Chromatographic conditions were optimized for resolution, peak shapes of analyte and I.S., sensitivity and run time. Three commercially available C18 (5 μm) chromatographic columns (Zorbax Extend, Nucleosil 150 mm × 4.6 mm; Venusil MP 100 mm × 4.6 mm) were evaluated and the Venusil MP-C18 column chosen because it gave the best resolution and peak shapes with minimal matrix effects. Mobile phases containing 10 mM ammonium acetate with either 0.5% or 1.0% formic acid and either methanol or acetonitrile were evaluated and acetonitrile–10 mM ammonium acetate with 1.0% formic acid selected based on it providing the best sensitivity. Using a flow rate of 1.0 ml/min, the retention times for analyte and I.S. were 1.77 and 1.93 min, respectively.

In terms of sample preparation, a one-step protein precipitation method using acetonitrile was found to be unsuitable due to the resulting high percentage of organic solvent left in the samples. Liquid–liquid extraction (LLE) from basified plasma followed by removal of solvent and reconstitution in mobile phase gave the best combination of high recovery of analyte and I.S. with minimal matrix effects.

3.2. Method validation

The assay was found to be highly specific since there were no endogenous peaks in plasma at the retention times of I.S. and OHA. Representative chromatograms of blank plasma, plasma spiked with OHA at the LLOQ (0.01 ng/ml) and a study sample taken 0.67 h after the oral dose of OHA succinate are shown in Fig. 2. The assay was linear in the range 0.01–10 ng/ml ($y = 0.685x + 0.00082$, $r = 0.9997$) with intra- and inter-day precisions of <3.15% and <5.01%,

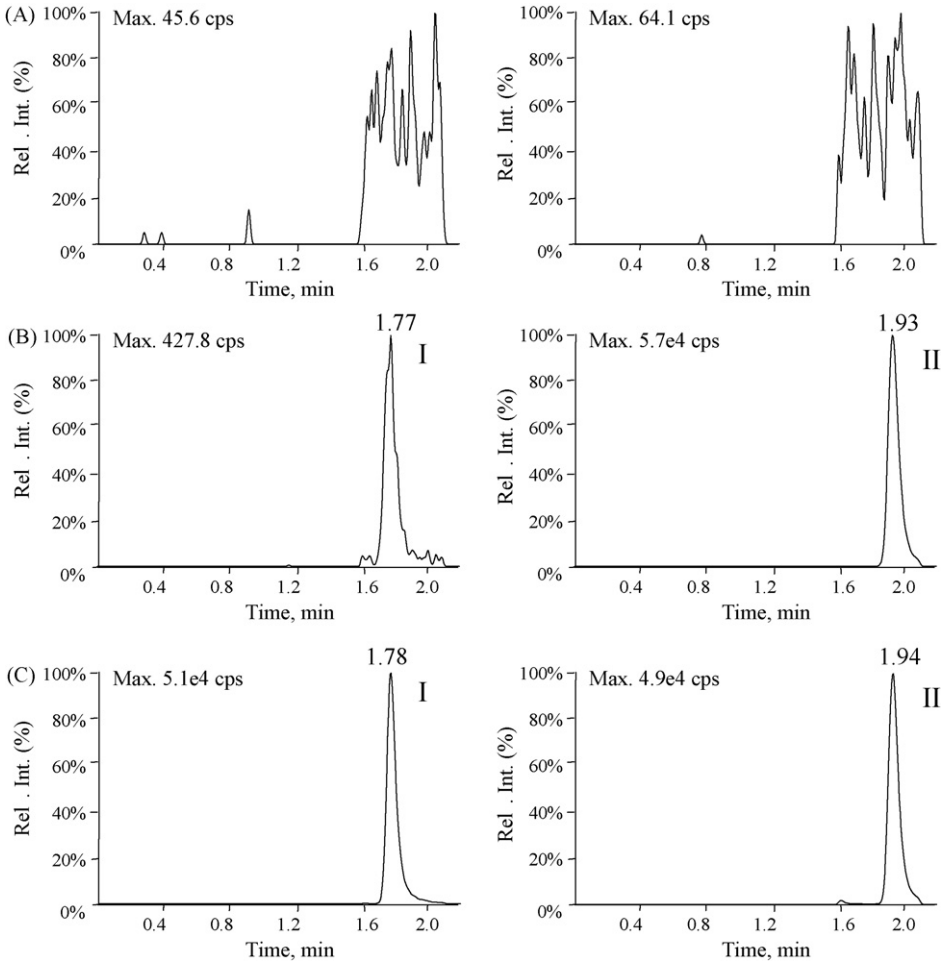

Fig. 2. Representative MRM chromatograms of octahydroaminoacridine and tramadol in human plasma. (A) Blank plasma; (B) blank plasma spiked with 0.01 ng/ml octahydroaminoacridine (I) and tramadol (II); (C) plasma sample from a human volunteer 0.67 h after a single 4 mg oral dose of octahydroaminoacridine succinate.

Table 2
Stability of octahydroaminoacridine under various conditions (data are means ± SD of three replicates).

Storage conditions	Initial conc. (ng/ml)	Final conc. (ng/ml)
Human plasma at room temperature for 6 h	0.03	0.031 ± 0.001
	0.30	0.31 ± 0.01
	3.00	2.92 ± 0.08
Processed samples at room temperature for 12 h	0.03	0.031 ± 0.002
	0.30	0.31 ± 0.02
	3.00	2.86 ± 0.07
Human plasma after three freeze/thaw cycles	0.03	0.03 ± 0.002
	0.30	0.31 ± 0.01
	3.00	2.94 ± 0.11
Human plasma for 1 month at −20 °C	0.03	0.03 ± 0.001
	0.30	0.29 ± 0.01
	3.00	2.84 ± 0.15

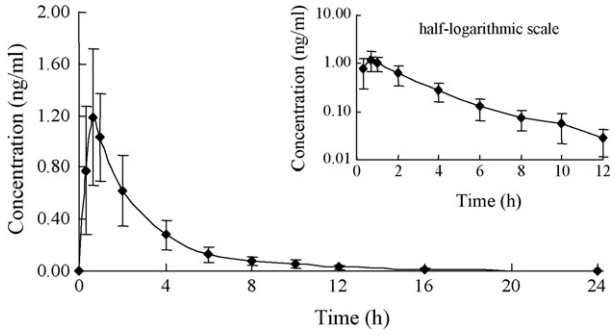

Fig. 3. Plasma concentration–time profile of octahydroaminoacridine after administration of a tablet formulation containing 4 mg octahydroaminoacridine succinate to healthy volunteers. Data are mean ± SD for 10 volunteers (5 males and 5 females).

respectively and accuracy in the range −2.80% to 4.35% (Table 1). Matrix effects were shown to be minimal based on ratios of peak areas of OHA in low, medium and high QC samples with those in corresponding standard solutions of 98.5 ± 5.2%, 105.4 ± 4.7% and 104.1 ± 2.9%, respectively. The ratio for the I.S. was 98.3 ± 3.3%. Recoveries of OHA in low, medium and high QC samples were also satisfactory with concentrations of 95.2 ± 4.0%, 98.4 ± 3.5% and 97.7 ± 2.8% of nominal values, respectively. The recovery of the I.S. was 85.7 ± 3.2%. In terms of stability (Table 2), OHA was shown to be stable under all the conditions examined.

3.3. Pharmacokinetic study

The mean concentration–time profile of OHA after oral administration of a tablet containing 4 mg OHA succinate is shown in Fig. 3. The mean peak plasma concentration (C_{max}) was 1.19 ± 0.53 ng/ml at a time (T_{max}) of 0.77 ± 0.17 h after the dose. The mean area under the plasma concentration–time curve (AUC_{0-t}) was 3.42 ± 1.01 ng h/ml and the mean elimination half-life ($t_{1/2}$) 2.89 ± 0.56 h. The results confirm that the assay is suitable for pharmacokinetic studies of OHA given at a therapeutic dose for the treatment of Alzheimer's disease.

4. Conclusion

A rapid and sensitive LC–MS/MS method for the determination of octahydroaminoacridine in human plasma after a therapeutic oral dose has been developed and validated. The method allows efficient analysis of the large numbers of samples involved in clinical studies.

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