Determination of atomoxetine in human plasma by LC-MS/MS and clinical application to Chinese children with ADHD

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Introduction

- Atomoxetine (ATX) is a selective and potent norepinephrine reuptake inhibitor, which was the first non-stimulant medicine approved in the United States for the treatment of ADHD.
- The CPIC guideline for personalized atomoxetine therapy is based on the CYP2D6 genotype information and the peak plasma concentration of atomoxetine. Therefore, a highly rapid, sensitive and reproducible method is critical for the clinical implementation of the guideline.
- This study utilized LC-MS/MS for the quantification of ATX with simple protein precipitation using only 50.0 µL of human plasma. The problem of inconsistent retention time between the plasma samples and the solution samples of atomoxetine was solved. Linearity was achieved using an extremely wide range, from 0.500 to 2,000 ng/mL in plasma. With a complex needle wash solution, carryover contamination was eliminated successfully.
- This method was successfully implemented on pediatric patients with ADHD and provided valuable information to enable clinicians decide dose selection and titration.

Results

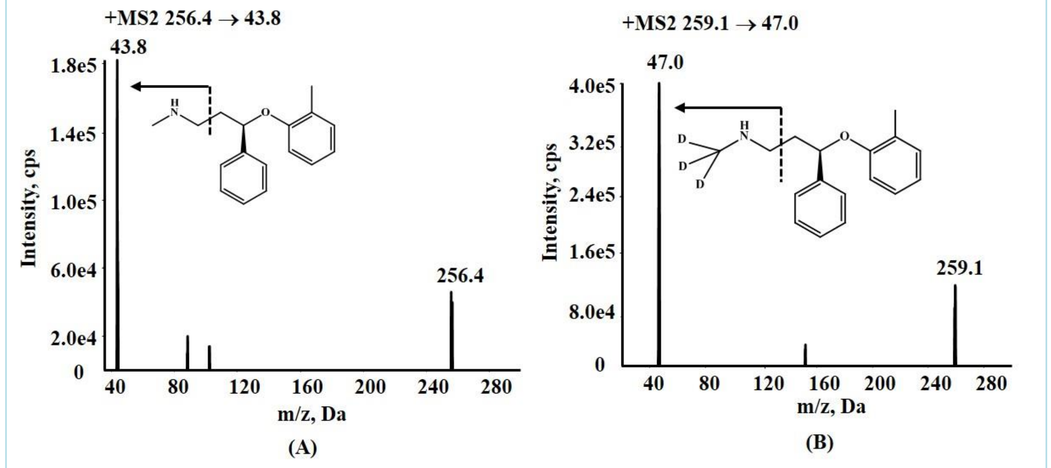

Fig. 1 Product ion mass spectra of ATX (A) and ATX-d3 (IS, B).

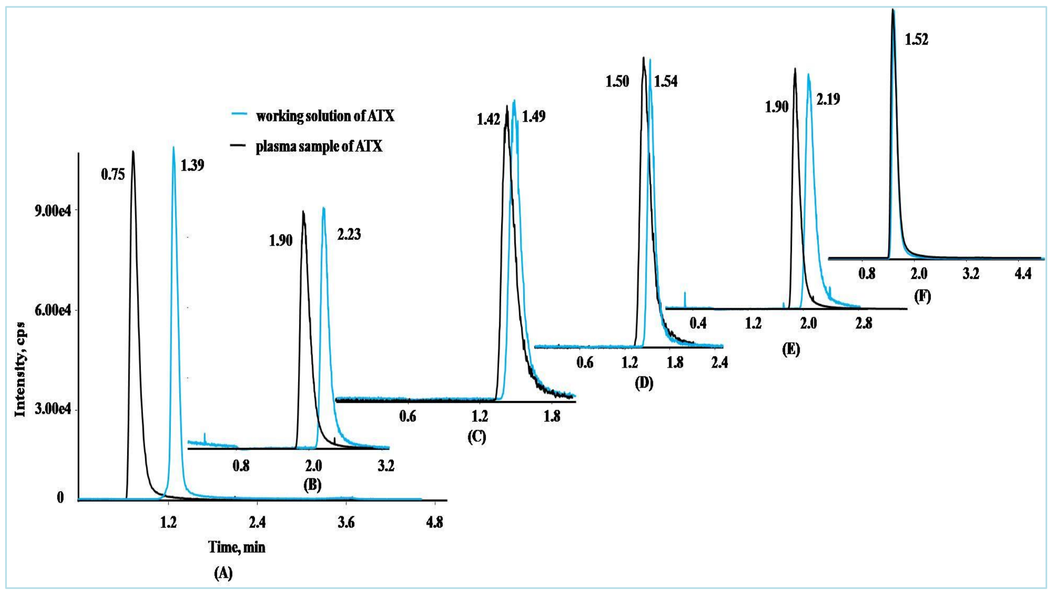

Fig. 2 The effect of ammonium acetate concentration in the mobile phase on the retention time of ATX between plasma samples and working solutions. (A) 0.1 mM formic acid in the mobile phase (pH 4.48); (B) 0.1 mM formic acid and 0.2 mM ammonium acetate in the mobile phase (pH 4.94); (C) 0.1 mM formic acid and 0.5 mM ammonium acetate in the mobile phase (pH 5.35); (D) 0.1 mM formic acid and 1 mM ammonium acetate in the mobile phase (pH 5.60); (E) 0.1 mM formic acid and 2 mM ammonium acetate in the mobile phase (pH 5.87); (F) 0.1 mM formic acid and 5 mM ammonium acetate in the mobile phase (pH 6.26).

Conclusions

- A rapid and sensitive LC-MS/MS method was developed and validated for the quantification of ATX in human plasma.
- The reproducible LC-MS/MS method was applied to pediatric patients with ADHD.
- Given the increasing number of prescriptions of atomoxetine, the determination of ATX levels in plasma can provide significant information in clinical practice, which might facilitate dose selection and titration. Hence, the clinical response can be improved without adverse effects.

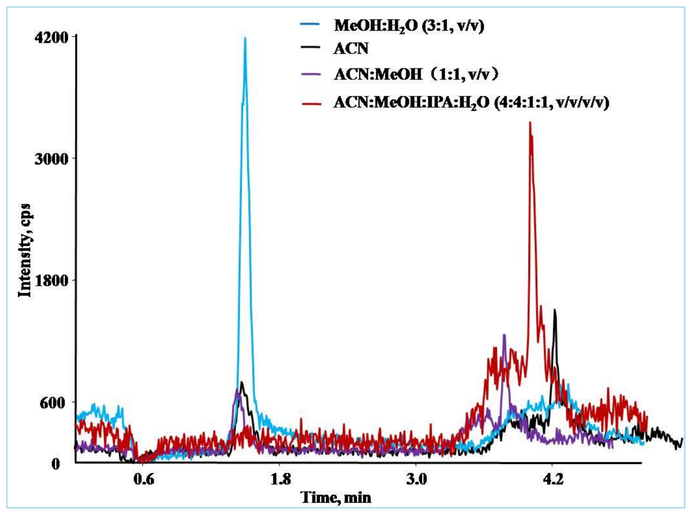

Fig. 3 The effect of carryover using different needle wash solutions.

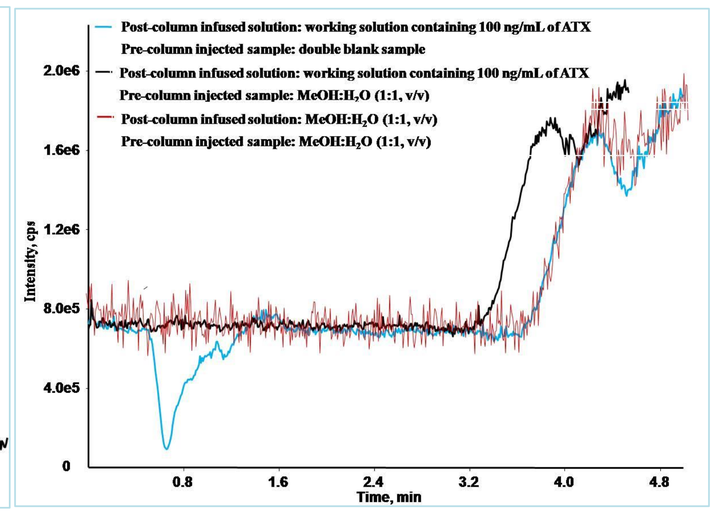

Fig. 4 Chromatograms of different samples with post-column infusion and pre-column infusion.

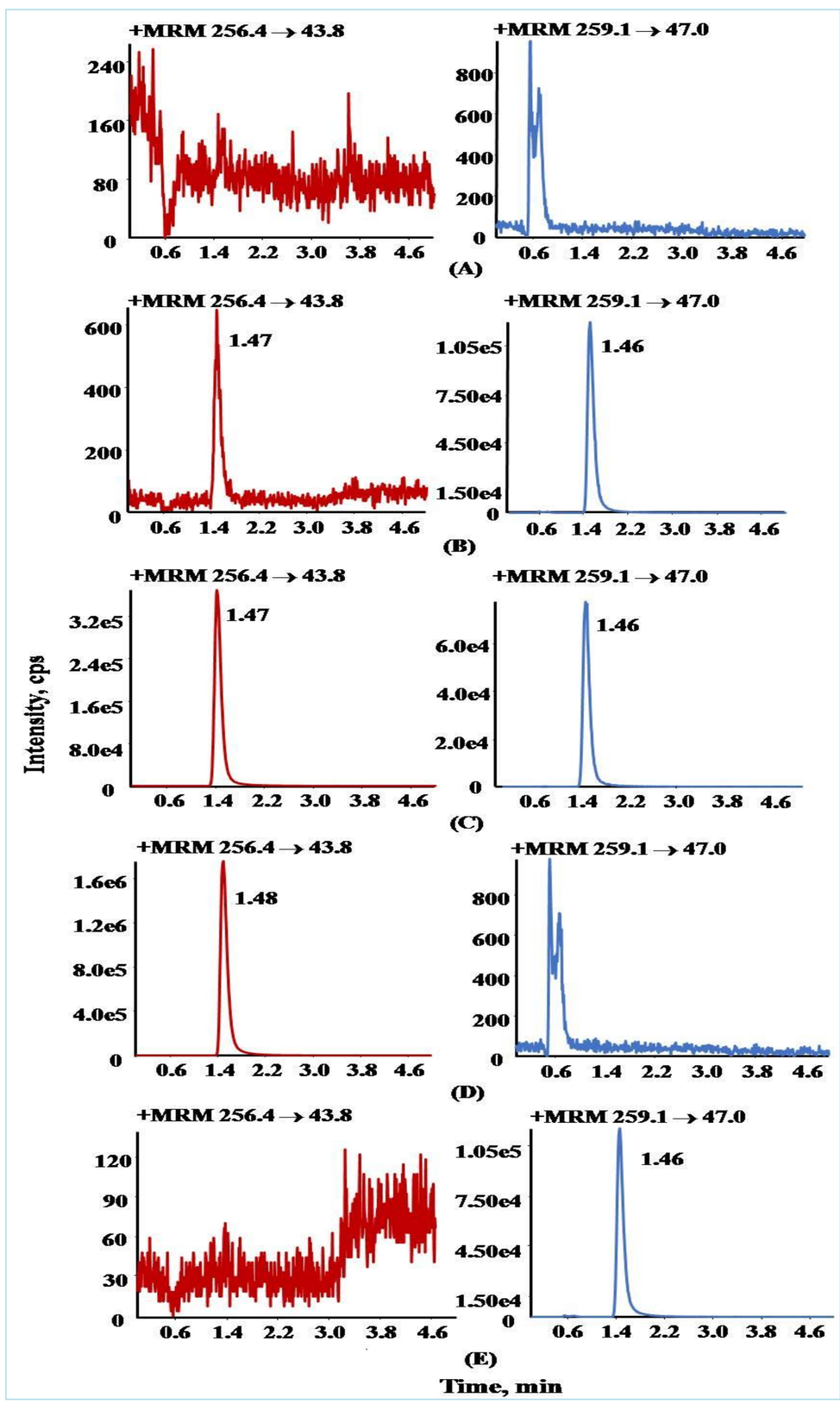

Fig. 5 Typical MRM chromatograms of (A) double blank sample; (B) plasma sample spiked with ATX at LLOQ (0.500 ng/mL) and ATX-d3 (IS) at 15.0 ng/mL; (C) a plasma sample from a subject at 2 h after being administered a dose of 25 mg/day atomoxetine; (D) ULOQ without IS; (E) control blank.

	Subject 1	Subject 2	Subject 3	Subject 4
Age	**9**	**8**	**12**	**11**
Sex	**male**	**male**	**female**	**male**
ATX dose (mg/day)	**25**	**25**	**25**	**10**
ATX dose (mg/kg/day)	**0.63**	**0.77**	**0.50**	**0.34**
Genotype of CYP2D6	***10*10* (IM)	***1*2* (NM)	***1*10* (NM)	***1*10* (NM)
Blood sample collecting after dosing (h)	**2.0**	**1.0**	**1.8**	**2.0**
C_{max} of ATX (ng/mL)	**658**	**272**	**260**	**251**
Therapeutic recommendation	**Concentration is adequate, consider maintaining the dose.**	**If response is inadequate, consider a proportional increase in the dose to approach a 400 ng/mL concentration.**	**If response is inadequate, consider a proportional dose increase to achieve a concentration of ~ 400 ng/mL. If unacceptable side effects are present at any time, consider a reduction in dose or switching to other medications.**	**If response is inadequate, consider a proportional dose increase to achieve a concentration of ~ 400 ng/mL. If unacceptable side effects are present at any time, consider a reduction in dose or switching to other medications.**

Table 1 Peak concentrations and dosing recommendations for ATX based on CYP2D6 genotype for the four pediatric subjects.

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