

In Vivo Metabolic Profiling of Carbamazepine in Brain and CSF Using an Advanced Hybrid Triple Quadrupole-Linear Ion Trap System and Fast Chromatography

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OVERVIEW

- Metabolic Profiling in Brain Homogenate and CSF
- High Sensitivity and Fast Scanning Speed
- IDA Methodology Combined with UPLC Separation

INTRODUCTION

Metabolite profiling in tissues is potentially a very useful tool in drug discovery and development. For example, profiling metabolites in the target organ can yield useful information about the mechanism of action, therapeutically active metabolites and potential toxicity. However, carrying out such experiments is challenging analytically due to the low levels of metabolites often present in tissues and the complexity of the matrix. Furthermore, it is most useful to perform such *in vivo* studies at physiologically relevant dose levels of the compound of interest. Dosing the compound at high levels in order to overcome analytical limitations can yield misleading results since the metabolic profile at high doses may be quite different than that at physiologically relevant concentrations.

Profiling metabolites in the central nervous system (CNS) is of particular interest. Performing qualitative LC-MS/MS analysis on cerebrospinal fluid (CSF) and brain homogenate is challenging due to the high sensitivity requirements, matrix complexity and the low sample volume available for CSF. In this study we evaluate a new high sensitivity fast scanning hybrid triple quadrupole-linear ion trap LC-MS/MS system for metabolite profiling in CSF and brain homogenate. The hybrid system enables the use of precursor ion and constant neutral loss scans to detect metabolites in a complex matrix and obtain the MS/MS product ion data in the same injection. We also evaluate the fast scanning capability of this system to perform such experiments under UPLC separation conditions for short run times and enhanced throughput.

MATERIALS AND METHODS

Carbamazepine (CBZ) was administered by *IV* at 4 mg/Kg to eight male Sprague-Dawley rats. Terminal collection of plasma, CSF and harvesting of the brain was performed at 15 minutes, 1, 3 and 6 hours on two rats each. An additional two rats were used for collection of pre-dose controls.

CSF was prepared for analysis by direct dilution with aqueous mobile phase: 50 μ L of sample was diluted with 75 μ L of mobile phase for an overall dilution factor of 2.5 fold. Brains were homogenized with water at a 4:1 (volume:weight) ratio. 200 μ L of homogenate was protein precipitated with 1 mL of 50/50 methanol/acetonitrile. Following centrifugation, the supernatant was dried down under nitrogen then reconstituted in 100 μ L of aqueous mobile phase. This process gave the same overall dilution factor as the CSF (2.5 fold).

A QTRAP[®] 5500 hybrid triple quadrupole linear ion trap LC/MS/MS system equipped with an Acquity UPLC was used for all analysis. A gradient separation was performed under the following conditions:

Mobile Phase A: Water with 10 mM ammonium formate (pH 3)
Mobile Phase B: 95/5 (v/v) Methanol / Water with 10 mM ammonium formate (pH 3)
LC column: Acquity UPLC BEH C18 2.1x100 mm, 1.7 μ m
Column Temperature: 65 $^{\circ}$ C
Flow Rate: 450 μ L/min
Total Analysis Time: 7 minutes
Injection Volume: 10 μ L

Analysis was performed in Electrospray using a Turbo V ionization source. Information Dependant Acquisition (IDA) was used for all experiments. Survey scans for this study were constant neutral loss (NL) and predictive MRM (pMRM). The NL method consisted of two simultaneous survey scans for the loss of 43 and 80 at a scan speed of 1,000 Da/s. This was followed by two dependent EPI scans at 10,000 Da/s (collision energy 30 eV, collision energy spread 20 eV). The pMRM method consisted of 176 transitions (dwell time 2 ms) and were built automatically using LightSight[®] Software. The dependent EPI scans were the same as described above for NL. Dynamic Background Subtraction (DBS) was activated for all experiments and all MS/MS data was acquired in IDA mode with no additional injections required to acquire further MS/MS data on peaks of interest. Data was processed using LightSight[®] Software Version 2.1 and Analyst[®] Software Version 1.5.

RESULTS

I. In Vivo Metabolites Detected in CSF by Neutral Loss-IDA

Fig 1. Carbamazepine structure and product ion spectrum

An examination of the product ion spectrum of CBZ (Fig 1) shows a prominent fragment at m/z 194 corresponding to the neutral loss of 43 as shown. Therefore a NL of 43 scan is an obvious survey scan choice for detecting metabolism involving the tricyclic ring structure. Conversely, m/z 194 is a suitable fragment for a pMRM study.

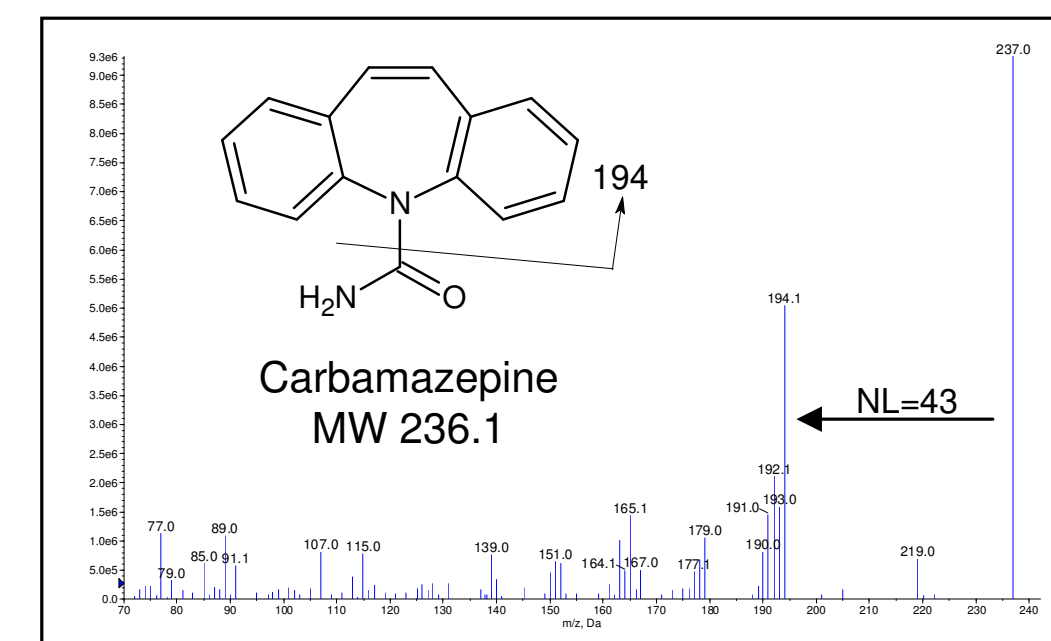
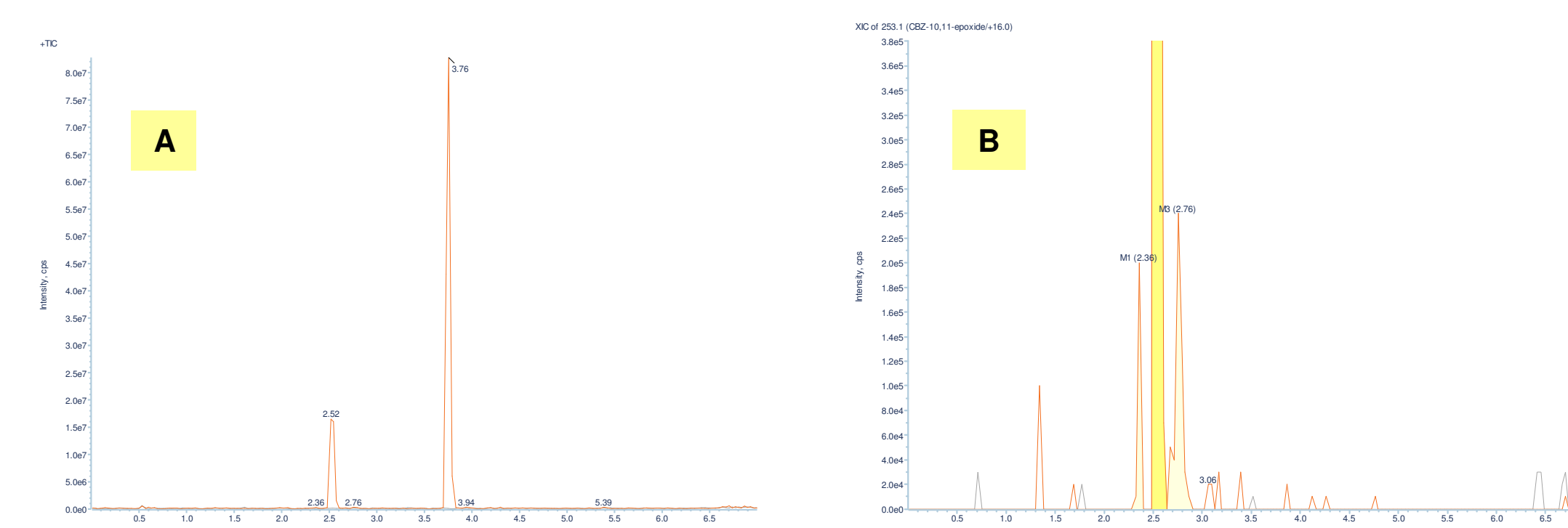
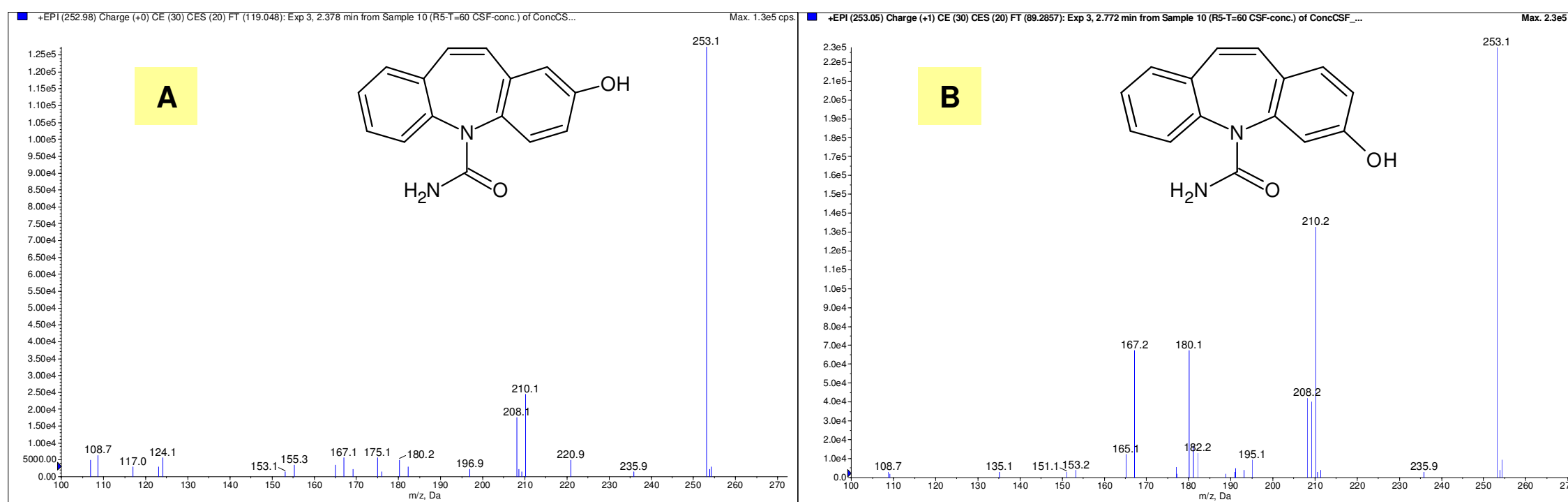


Fig 2: CSF sample collected 1 hour after IV administration. (a) TIC for the NL of 43 and (b) XIC of 253.1



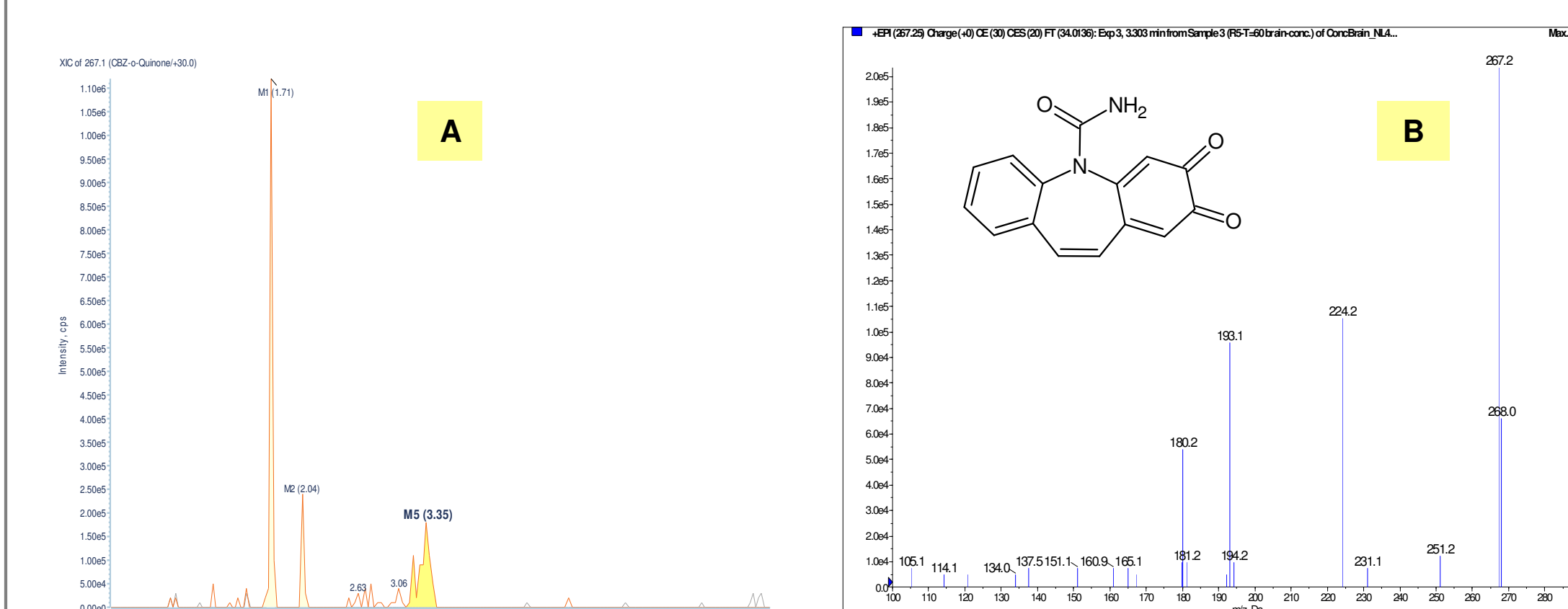
The NL of 43 TIC of the 1 hour CSF sample (Fig 2a) shows two major peaks at 2.52 and 3.76 minutes with m/z of 253 and 237 corresponding to CBZ-epoxide and CBZ, respectively. Closer examination of the XIC for m/z 253 reveals two additional metabolites at 2.36 and 2.76 minutes (Fig 2b, y-axis expanded). The MS/MS data of the two minor metabolites is consistent with mono-hydroxylated CBZ (Fig 3a and 3b) and comparison with synthetic standards confirms the two minor metabolites as 2-hydroxy CBZ (retention time of 2.36 min) and 3-hydroxy CBZ (retention time of 2.76 min).

Fig 3: MS/MS of metabolites detected in CSF. (a) 2-OH CBZ (b) 3-OH CBZ



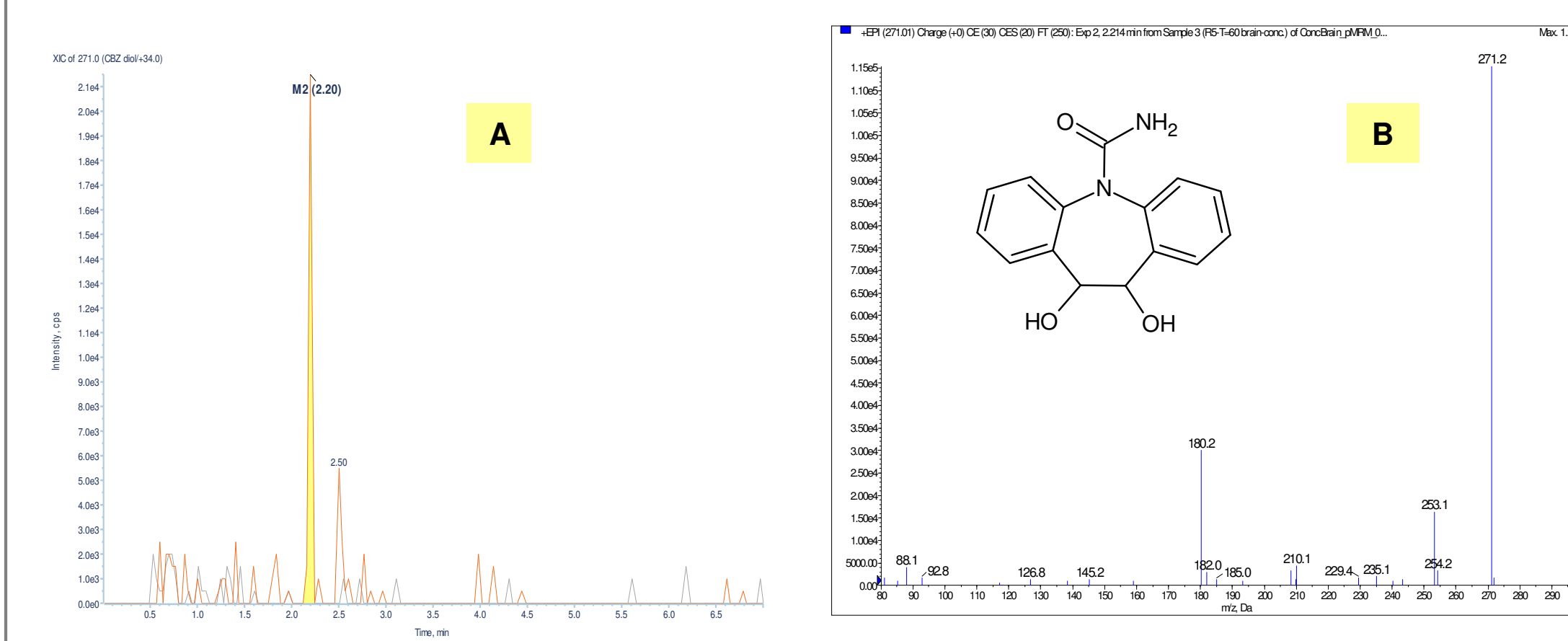
II. In Vivo Metabolites Detected in Brain Homogenate by Neutral Loss and pMRM-IDA

Fig 4: CBZ-o-Quinone detected in brain homogenate at 1 hour post administration by NL of 43. (a) XIC of m/z 267 and (b) MS/MS fragmentation obtained in the same injection



The same survey scan designs (NL and pMRM) were used for profiling metabolites in brain homogenate. NL of 43 detected the epoxide and hydroxyl metabolites reported in CSF. In addition, a peak at 3.35 minutes m/z 267 (gain of 30) was detected (Fig 4). The MS/MS fragmentation is consistent with CBZ-o-Quinone.

Fig 5. Early eluting species consistent with CBZ-10,11 reduced-diol. (a) pMRM detection in brain homogenate and (b) MS/MS fragmentation



pMRM of the brain homogenate detected all of the metabolites reported above. In addition an early eluting peak (RT 2.2 min) was observed in the 271 \rightarrow 180 MRM transition (Fig 5). The MS/MS fragmentation and the polar nature of this species are consistent with CBZ-10,11-diol. This is an interesting finding since the mechanism of formation of this diol is thought to be from the 10,11-epoxide which is detected in abundance in both brain and CSF.

Fig 6. PK profiles of CBZ and CBZ-10,11-epoxide in (a) CSF and (b) brain

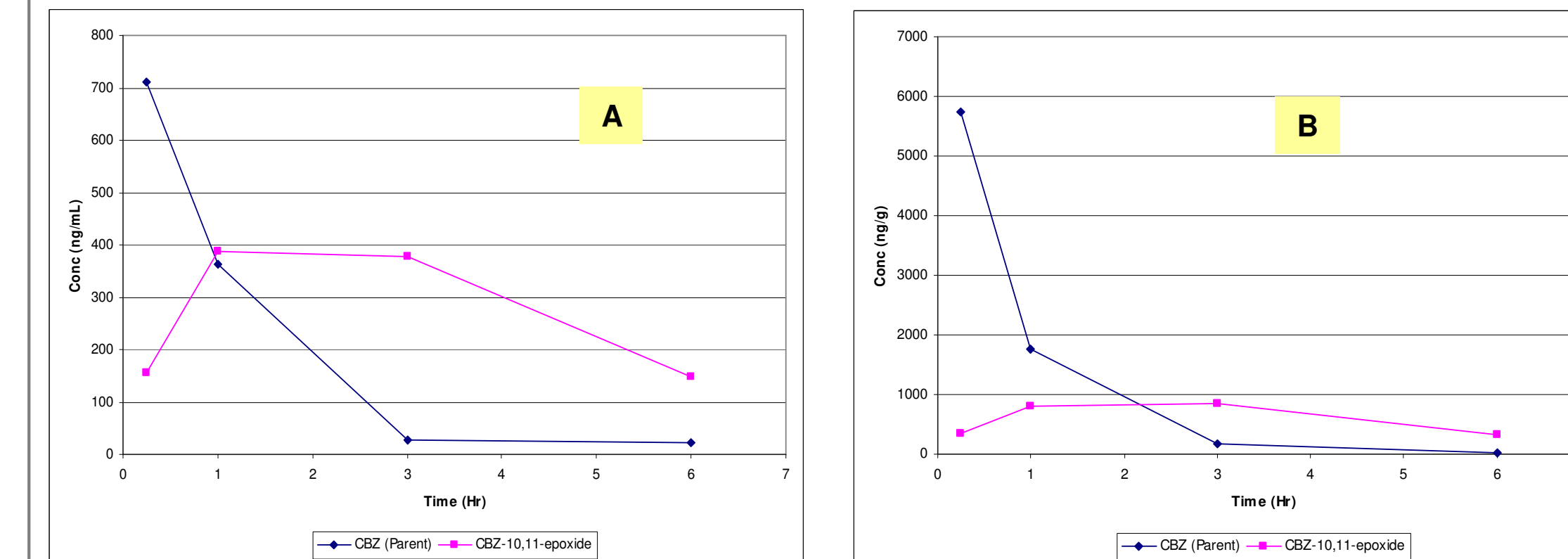


Fig 6 shows the concentrations of CBZ parent and its main metabolite (CBZ-10,11-epoxide) in CSF and brain after a 4 mg/Kg *IV* dose using standard MRM quantification method. CBZ-10,11-epoxide was also detected with abundant sensitivity in the 1 hour samples using neutral loss scanning. Although the hydroxyl metabolites (2-OH and 3-OH CBZ) were not quantified in this study, it is clear that they were present at much lower levels than the epoxide as shown by the NL scan (Fig 2b). The successful detection and confirmation of these low level metabolites, especially in CSF, using this approach demonstrates the feasibility of fast triple quadrupole scanning functions for high sensitivity profiling of low level metabolites in the CNS.

CONCLUSIONS

- *In vivo* metabolites of CBZ were successfully detected and confirmed in the CNS of the rat following administration of a physiologically relevant dose level.
- New hybrid triple quadrupole-ion trap technology with high sensitivity and fast scanning enabled the detection of these metabolites at low levels including in the CSF. Short run times taking advantage of high efficiency UPLC type separations were successfully used owing to the very fast triple quadrupole and ion trap scanning speed capability.

- Selective scanning modes such as neutral loss and pMRM were effective in detecting the metabolites with appropriate coverage and sensitivity. The neutral loss mode demonstrated very high sensitivity for low level metabolites.

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