In Vivo Metabolic Profiling of Carbamazepine at Physiologically Relevant Concentrations Using Hybrid Quadrupole-Linear Ion Trap Technology

TPX 581

Tanya N. Gamble[1], Henrianna Y. Pang[2], Sophie Pan[2], Yingbo Yang[2], William Cui[2], Douglas J. Turk[2], and Hesham Ghobarah[1], [1]Applied Biosystems/MDS Analytical Technologies, Concord, Canada, [2]NoAb Biodiscoveries Inc., Mississauga, Canada

ABSTRACT

•Metabolic profiling of carbamazepine metabolites using fast LC and sensitive MS techniques
•Combining selective quadrupole scans with sensitive trap mode dependent scans facilitate a comprehensive detection of metabolites in two injections

INTRODUCTION

Traditionally, *in vitro* and *in vivo* metabolite profiling has been performed at higher doses due to limitations in sensitivity and selectivity of mass spectrometry detection. These high doses can cause a metabolic profile that is significantly different from what is present at physiologically relevant concentrations. As a result, it is highly desirable to perform such in vivo studies at relevant doses which are often much lower and pose significant analytical challenges for selective and sensitive qualitative analysis.

Carbamazepine, a drug used for the treatment of epilepsy and bipolar disorders, was used in this study to evaluate the use of a highly sensitive hybrid-quadrupole linear ion trap mass spectrometer for metabolic profiling at physiologically relevant concentrations.

MATERIALS AND METHODS

Carbamazepine was adminstrated intravenously to Sprague-Dawley rats at 4 mg/Kg dose. Plasma samples were collected at pre-dose, 15, 60, 180, and 360 min, and stored at -20°C prior to analysis. Each sample was prepared using simple protein precipitation with 50/50 methanol/acetonitrile, centrifuged, dried down and reconstituted in 0.1/5/95 formic acid/acetonitrile/water..

Carbamazepine and its metabolites where chromatographically separated using an Acquity UPLC® system.

Mobile Phase A:	10 mM ammonium formate pH 3
Mobile Phase B:	95% methanol/5% 10 mM ammonium formate pH 3
Weak Solvent Wash:	50/50 methanol/water
Strong Solvent Wash:	methanol
LC column:	Acquity UPLC BEH C18 2.1x100 mm, 1.7µ
Column Temperature:	65°C
Flow Rate:	450 µL/min
Injection Volume:	10 µL
Total Analysis Time:	7 minutes

Time (min)	%A	%B
0	80	20
0.4	80	20
0.5	65	35
2.5	55	45
5.5	35	65
6	5	95
6.5	5	95
6.7	80	20

Table 1: LC gradient

Mass spectrometric detection was performed using a QTRAP® 5500 system in positive mode. The high speed data acquisition facilitated the combination of multiple experiments in a single Information Dependent Acquisition (IDA) method. Combinations of survey scans (predictive MRM, precursor ion, neutral loss) were used to profile the samples. LightSight® software was used to create and submit acquisition methods, as well as interpret the data. Structure elucidation was performed using ACD Labs version 12 software.

Parameter	Setting
Ionization Mode	TurboIonSpray
Polarity	Positive
IonSpray Voltage	4500
Temperature	550
Gas 1 (Nebulizer)	50
Gas 2 (Heater)	50
Curtain Gas	25
Declustering Potential	50
Collision Energy	30
Collision Energy Spread	20
Quad Resolution	Q1/Q3 unit

Table 2: General MS parameters

Figure 1: AB SCIEX QTRAP® 5500 system

RESULTS

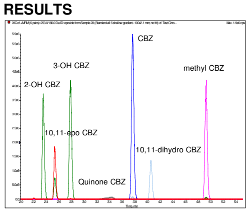

Figure 2: Metabolite standards were used to optimize chromatography and confirm identity of potential metabolites

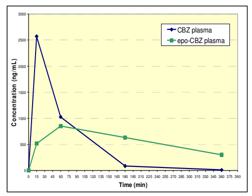

Figure 3. Profile of parent and 10,11-epoxide metabolite. Each data point represents the average concentration (ng/mL) of two rats.

The increased scan speed (up to 2000 Da/s in quad mode and 20 kDa/s in LIT mode) of the AB SCIEX QTRAP® 5500 system opens up new possibilities for combining different scans modes (quadrupole and linear ion trap) to detect metabolites. During this study, multiple methods were investigated to determine the metabolites of carbamazepine.

A predictive MRM (pMRM) approach was used first pass to detect potential metabolites. Four additional experiments were performed for comparison, including a combined neutral loss approach of NL 80 for sulfate conjugates and NL 176 for glucuronide conjugates.

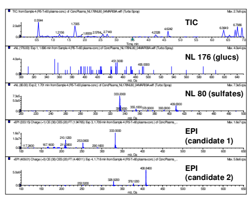

Figure 4. Combined NL of 176 & 80 to detect glucuronide and sulfate conjugates

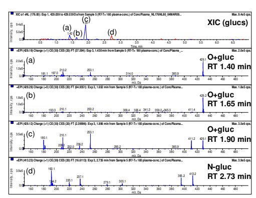

Figure 5. EPI spectra of glucuronide metabolites.

Figure 4 illustrates the dual-survey IDA method to detect sulfate and glucuronide conjugates. The total cycle time (if 2 candidate ions meet the idea criteria) is 1.1s. In this acquisition, at RT 1.7 min, the NL 80 survey scan produced two peaks above the IDA threshold. Two information rich Enhanced Product Ion (EPI) spectra were acquired. Based on the fragmentation, m/z 333 is proposed to be a result of oxidation plus sulfonation. The identity of the m/z 409 peak is unknown, and believed not to be related to carbamazepine. Figure 5 shows the MS/MS spectra for the glucuronide species. The strong m/z 180 peak found in O+gluc CBZ at RT 1.90 min suggests that the 10,11 position has been modified in forming this metabolite

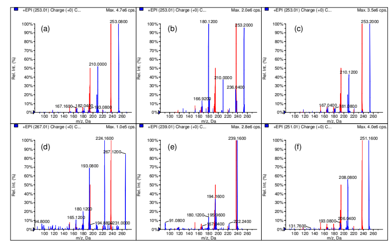

Figure 6: Representative MS2 spectra for potential metabolites: (a) 2OH CBZ, (b) 10,11-epo CBZ, (c) 3OH CBZ, (d) o-quinone CBZ, (e) dihydro CBZ, (f) methyl CBZ. The MS2 spectrum for parent drug has been overlaid in red.

Table 5 summarizes the potential metabolites detected in the rat plasma. Based on sample/control comparison, and the presence of characteristic fragment ions or neutral losses, twenty potential metabolites have been identified. Proposed structures can be found in Table 6. Four of the potential metabolites require further investigation. Six of the metabolites were confirmed (2-OH CBZ; 10,11-epoxide CBZ; 3-OH CBZ; o-quinone CBZ; 10,11-dihydro CBZ and 2-methyl CBZ) by the analysis of standards prepared from synthetic materials.

Table 5. Comparison of the potential metabolites found using the six experiments explored during this study

Peak ID	Biotransformation	Mass Shift	R.T. (min)	pMRM	NL80 NL176	Prec180	Prec194 NL43	Prec194 Prec210 NL43
M1	Oxidation + OH+H (2,3-trans diol CBZ)	34	1.33	✓		✓		✓
M2	Oxidation + Glucuronidation	192	1.4	✓	✓			
M3	Oxidation + Sulfonation	96	1.6	✓	✓		✓	✓
M4	Oxidation + Glucuronidation	192	1.64	✓	✓			
M5	Oxidation + Sulfonation	96	1.73	✓	✓	✓	✓	✓
M6	unknown m/z 283	46	1.76	✓				✓
M7	Oxidation + Glucuronidation	192	1.91	✓	✓	✓		✓
M8	Oxidation + OH+H (10,11-trans diol CBZ)	34	2.2	✓		✓		✓
M9	unknown m/z 290.1	53	2.2		✓			
M10	Oxidation (2-OH CBZ)	16	2.35	✓			✓	✓
M11	Oxidation (10,11-epo CBZ)	16	2.54	✓		✓	✓	✓
M12	unknown m/z 283	46	2.57	✓		✓	✓	✓
M13	Glucuronidation	176	2.72	✓	✓		✓	✓
M14	unknown m/z 283	46	2.72	✓			✓	✓
M15	Oxidation (3-OH CBZ)	16	2.75	✓		✓	✓	✓
M16	unknown m/z 255	18	3.17	✓		✓	✓	
M17	Keto (Ox-2H) + Oxidation (o-quinone CBZ)	30	3.41	✓		✓	✓	
M18	Parent (CBZ)	0	3.7	✓			✓	✓
M19	Hydrogenation (10,11-dihydro CBZ)	2	4.02	✓				
M20	Methylation (2-methyl CBZ)	14	4.93					✓

All time points were diluted 10x in mobile phase A and subjected to the pMRM approach. Subsequent analysis was performed on the concentrated samples for rat 5 (t=60 min) and rat 7 (t=120 min). The pMRM approach detected and performed full scan MS/MS for 19/20 potential metabolites. Although other scan combinations were explored, the two-injection strategy of pMRM followed by a dual NL for phase 2 conjugates proved to be successful in identifying the potential metabolites of carbamazepine.

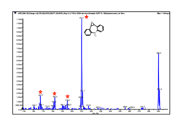

Figure 7: EPI spectra of unknown metabolite m/z 290. Highlighted peaks are common to other CBZ metabolites

Table 6: Summary of potential metabolites detected

Peak ID	Proposed Structure	m/z	t=15 min RO3	t=15 min RO4	t=60 min RO5	t=60 min RO6	t=120 min RO7	t=120 min RO8	t=360 min RO9	t=360 min R10
M1		271	✓	✓	✓	✓	✓	✓	✓	✓
M2		429		✓	✓	✓	✓	✓	✓	✓
M3		333			✓	✓	✓			✓
M4		429		✓	✓	✓				
M5		333	✓	✓	✓	✓	✓	✓	✓	✓
M6		283			✓					
M7		429		✓	✓	✓	✓	✓	✓	✓
M8		271	✓	✓	✓	✓	✓	✓	✓	✓
M9		290			✓		✓			
M10		253	✓	✓	✓	✓	✓	✓	✓	✓
M11		253	✓	✓	✓	✓	✓	✓	✓	✓
M12		283	✓	✓	✓	✓	✓	✓	✓	✓
M13		413	✓	✓	✓	✓		✓		
M14		283	✓	✓	✓	✓	✓			
M15		253	✓	✓	✓	✓	✓	✓	✓	✓
M16		255				✓	✓		✓	
M17		267	✓	✓	✓	✓	✓	✓	✓	✓
M18		237	✓	✓	✓	✓	✓	✓	✓	✓
M19		239	✓	✓	✓	✓				
M20		251	✓	✓	✓					

CONCLUSIONS

•Twenty potential metabolites were detected in rat plasma
•The increased speed and sensitivity of the QTRAP® 5500 system allows for profiling of metabolites at physiologically relevant metabolites
•The highly selective quadrupole scan modes combined with sensitive linear ion trap modes of operation facilitate the detection of metabolites at low levels.
•Combining multiple survey scans in a single IDA experiment detects more potential metabolites in a single injection

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