Increasing Throughput in Metabolite Identification Studies with Ultra Performance Liquid Chromatography / Tandem Mass Spectrometry

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OVERVIEW

Utility of the UPLC™/tandem MS approach in the rapid detection and characterization of metabolites of structurally diverse drugs with a broad spectrum of physiochemical attributes (acidic, basic and neutral compounds) is examined.

INTRODUCTION

Characterization of major biotransformation pathways in a given chemical series prone to high metabolic instability allows medicinal chemists to devise appropriate chemical intervention strategies to abrogate the issue and in addition, such studies have also proven useful in probing the potential of lead compound(s) in a chemical series to undergo bioactivation to reactive electrophilic intermediates. However, there may be cases where the need to detect more than just the major metabolite within a given series is crucial. In such cases, bioanalytical methodology involving LC-MS/MS has mostly relied upon longer chromatographic separations to resolve/identify multiple metabolites in a given mixture [1,2]. Ultra performance liquid chromatography (UPLC™) is a new regime of separation science that maintains the benefits of traditional HPLC separation while increasing the parameters of speed, sensitivity and resolution. These improvements are achieved utilizing new LC columns, which incorporate sub-2µm packing materials and thus provide great chromatographic performance with the ability to operate at higher backpressures due to higher mobile phase linear velocities.

METHODS

Three test compounds, buspirone, indomethacin, and a neutral amide derivative of indomethacin were chosen to represent basic, acidic, and neutral drugs, respectively. Metabolic profiles of these test compounds in NAPDH-supplemented human liver microsomes were then analyzed with a UPLC™ system coupled to a triple quadrupole mass spectrometer operating in the electrospray ionization positive mode. Precursor and product ion scanning modes were used to evaluate metabolite formation and characterization.

Materials: Buspirone, indomethacin, indomethacin amide, and NADPH were purchased from Sigma-Aldrich (St. Louis, MO). Human liver microsomes pooled from 53 individual donors was purchased from BD Gentest (Woburn, MA).

Microsomal Incubations: Stock solutions of test compounds were prepared in methanol. The final concentration of methanol in the incubation media was 0.2% (vol/vol). Incubations were carried out at 37°C for 60 min in a shaking water bath. The incubation volume was 1 mL and consisted of the following: 0.1 M potassium phosphate buffer (pH 7.4), human liver microsomes (P450 concentration = 0.5 mM), NADPH (1.2 mM) and test substrates (20 mM). The reaction mixture was prewarmed at 37°C for 2 min before adding NADPH and incubations were terminated by the addition of ice-cold acetonitrile (1 mL). The solutions were centrifuged (3000 rpm for 10 min) and the supernatants were dried under a steady nitrogen stream. The residue was reconstituted with mobile phase and analyzed for metabolite formation.

LC/MS/MS Conditions:

Waters® Micromass® Quattro Premier™ mass spectrometer
Desolvation Gas Flow: 700 L/hr
Source Temperature: 120° C
Desolvation Temperature: 350° C
Collision Cell Pressure: $2.59 \times 10^{(-3)}$ mbar

UPLC™: Waters ACQUITY UPLC™ System
LC columns: ACQUITY UPLC™ BEH C18 (indomethacin and amide) or BEH Shield RP18 (buspirone), 2.1 x 50 mm 1.7 µm
Mobile Phase: A1: 10 mM ammonium formate, B1: acetonitrile
A2: 0.1% formic acid ,B2: 0.1% formic acid in acetonitrile
LC gradient: Flow Rate 0.4-0.6 mL/min
Run Time: 5.2-7.0 min
Gradients: 98% A1, hold 0.5min, to 40% B1 at 3 min (buspirone)
90% A2 to 100% B2 over 4 min (indomethacin and amide)
Injection Volume: 5 µL

RESULTS AND DISCUSSION

Fig 1. Representative UPLC™/MS/MS extracted product ion chromatograms of Buspirone (base) and its metabolites obtained by CID of the MH+ ion in human liver microsomal incubations.

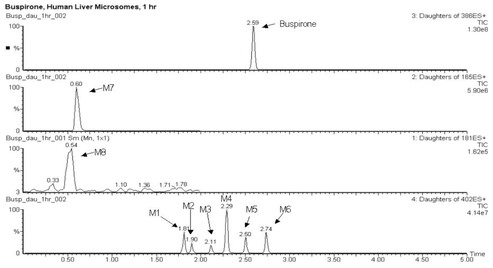


Fig 2. Representative UPLC™/MS/MS precursor ion chromatograms of Buspirone and its metabolites in human liver microsomal incubations.

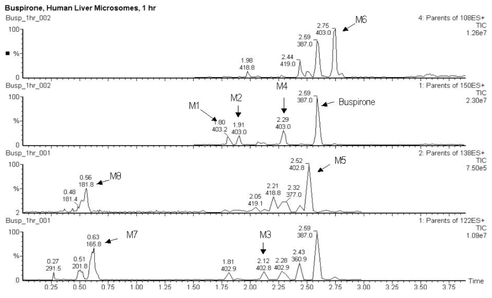


Fig 3. Representative UPLC™/MS/MS extracted product ion chromatograms of Indomethacin (acid) and its metabolites obtained by CID of the MH+ ion in human liver microsomal incubations.

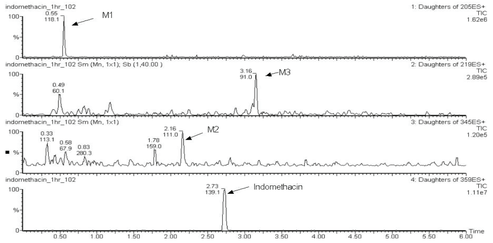


Fig 4. Representative UPLC™/MS/MS precursor ion chromatograms of Indomethacin and its metabolites in human liver microsomal incubations.

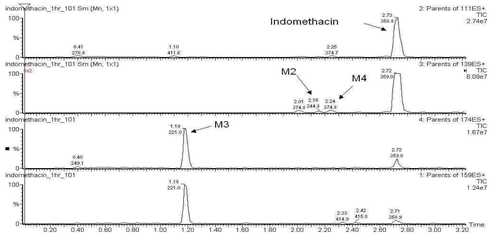


Fig 5. Representative UPLC™/MS/MS extracted product ion chromatograms of Indomethacin amide 1 (neutral) and its metabolites obtained by CID of the MH+ ion in human liver microsomal incubations.

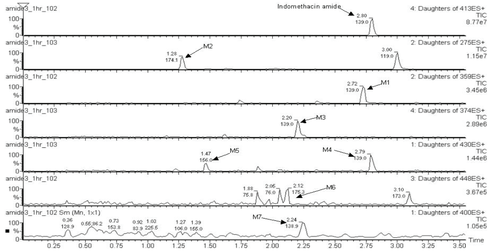


Fig 6. Representative UPLC™/MS/MS precursor ion chromatograms of the Indomethacin amide 1 and its metabolites in human liver microsomal incubations.

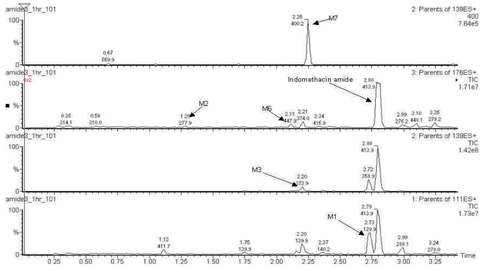
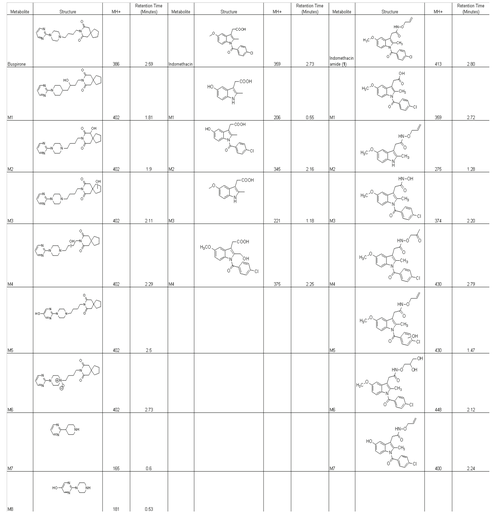


Table 1. Major metabolites of Buspirone, Indomethacin, and Indomethacin amide 1 following an *in vitro* incubations in human liver microsomes.



CONCLUSIONS

- UPLC™/tandem MS is a powerful tool in the rapid detection and characterization of metabolites of structurally diverse drugs irrespective of physiochemical attributes (acidic, basic and neutral compounds).
- Compared with traditional HPLC/MS/MS, UPLC™/MS/MS demonstrates significant advantages such as faster separations, sharper peaks, and increased sensitivity with minimal sample preparation and without changing the detection system.
  - -The UPLC™ methods presented here have resulted in a decrease in analysis time from 45 minutes to 5-6 min utes, resulting in approximately a 7-9-fold increase in productivity.
  - -The increased sensitivity of UPLC™ has enabled us to reduce our injection volume by 4-fold, from 20 µL to 5 µL of sample.
- An extensive metabolite ID profile was generated in 2 analysis runs or less with the described methods. It has previously required a minimum of six scan functions per run in precursor or product ion mode to generate the data necessary for preliminary metabolite identification.
- This UPLC™/Quattro Premier™ combination facilitates the acquisition of a large number of scan functions from highly resolved peaks in one run while maintaining adequate peak characterization.

REFERENCES

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2. Kalgutkar AS, Henne KR, Lame ME, Vaz AD, Collin C, Soglia JR, Zhao SX, Hop CE. Metabolic activation of the nontricyclic antidepressant trazodone to electrophilic quinone-imine and epoxide intermediates in human liver microsomes and recombinant P4503A4. *Chem Biol Interact.* ***2005*** *Jun 30;155(1-2):10-20.*

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