Measurement of Dextromethorphan and Dextrorphan in Human Urine Using a Novel Isocratic Liquid Chromatography – Tandem Mass Spectrometry Method

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BACKGROUND

A large number of drugs are metabolised by the highly polymorphic cytochrome P450 (CYP) enzymes. The principal five enzymes involved in biotransformation processes are CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4.[1] The interaction of drugs with these enzymes, which can be present in induced, inhibited or polymorphic forms, may affect the metabolism, toxicity and efficacy of the drug. A number of genotypes can exist for a particular CYP enzyme, which may or may not express a phenotypic change in drug metabolism.[2] The enzyme CYP2D6 has variant genotypes that express different phenotypes related to the rate a drug is metabolised. Determination of these describes whether the individual is a poor, extensive or ultra rapid metaboliser.[3]

Dextromethorphan is recognised for its therapeutic properties as an antitussive drug and is widely available without prescription. Its availability as an orally administered drug enables its exploitation as a probe drug for oxidative phenotyping.[4-5] This poster focuses on the detection of dextromethorphan (DXM) and dextrorphan (DR) using a novel isocratic liquid chromatography-tandem mass spectrometry (LC/MS/MS) method. More than one CYP enzyme is involved in the metabolism of DXM to its metabolites; DR, 3-methoxymorphinan and 3-hydroxymorphinan (Figure 1). DXM is metabolised to the *0*-demetheylated metabolite, DR, via the CYP2D6 enzyme pathway. The ratio of DXM to DR reflects the CYP2D6 enzyme activity and the subsequent classification of metabolic rate.[4]

AIM

To develop a LC/MS/MS method for the measurement of DXM and DR in human urine. This method was to be used in a clinical study investigating oxidative phenotyping. Many researchers have measured DXM and DR using high performance liquid chromatography (HPLC) with fluorescence, UV or mass spectrometric detection. Published methods commonly use C8 columns, either isocratically or with a gradient system, in an attempt to reduce the difference in elution times between these two compounds. Gradient systems are preferred, but this often results in longer equilibration times and more solvent waste. Here we report a LC/MS/MS method using a novel isocratic system, which elutes DXM, DR and the internal standard (ethylmorphine) in less than 4 minutes.

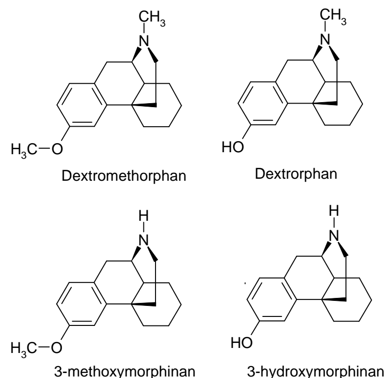

Figure 1: Structures of dextromethorphan and its metabolites

EXPERIMENTAL

Materials
Dextromethorphan hydrobromide, dextrorphan tartrate and β-glucuronidase were obtained from Sigma-Aldrich (Poole, Dorset, England). HPLC grade acetonitrile and methyl-tert-butyl-ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Analar grade formic acid and sodium hydroxide (40% solution) were obtained from BDH (Lutterworth, Leicestershire, England). De-ionised water was prepared on site (ELGA Limited).

Extraction
Internal standard (ethylmorphine, 2.5mg/L, 100μL), sodium hydroxide (1M, 100μL) and MTBE (2mL) were added to each urine calibrator, control or human sample (100μL). The tubes were capped, mixed (approx. 5 minutes) and then centrifuged (3500rpm, 5 minutes). The upper organic layer from each was transferred to a tube containing 0.1% formic acid (500μL). After mixing (approx. 5 minutes) and centrifugation (3500rpm, 5 minutes) the top solvent layer was removed and discarded. The aqueous extracts were then transferred to autosampler vials ready for analysis. The human urine samples were pre-incubated with β-glucuronidase for a minimum of 12 hours prior to extraction.

HPLC Conditions and MS Parameters
The HPLC equipment consisted of a Perkin Elmer PE200 series autosampler (injection volume, 10μL) and pump. A silica column (Supelcosil LC-Si, 10cm x 4.6mm, 5μm) was maintained at 50°C in a Schimadzu CTO-10A column oven. The mobile phase, consisting of acetonitrile/de-ionised water/formic acid (50/50/0.2) was pumped at 1mL/min and split 10:1 before entering the mass spectrometer.

A Sciex API2000 triple quadrupole mass spectrometer equipped with a turbo-ion spray interface maintained at 500°C was used for detection. The method was run in positive ionisation mode and set to detect the precursor and product ions of dextromethorphan (m/z: 272.04/212.98), dextrorphan (m/z: 257.99/198.80 and ethylmorphine (m/z: 314.01/165.08). The total run time was 5.0 minutes.

CALIBRATION CURVES

Figure 2 and Figure 3 show calibration curves for both DXM and DR urine calibrators respectively. The assay range for DXM was 0 to 5060ng/mL and 0 to 4950ng/mL for DR.

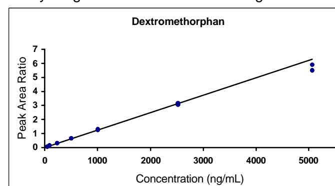

Figure 2: Dextromethorphan, regression linear through zero, weighting (1/(x*x)), y = 0.00125x, r = 0.9989.

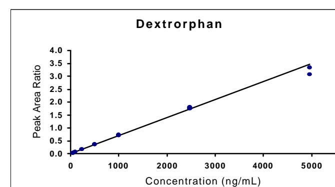

Figure 3: Dextrorphan, regression linear through zero, weighting (1/x*x)), y = 0.000701x, r = 0.9984.

REPRESENTATIVE CHROMATOGRAMS

The ratio of DXM to DR allows classification of metabolic rate; ultra-rapid metaboliser (ratio < 0.003), extensive metaboliser (ratio 0.003 to 0.03), intermediate metaboliser (ratio 0.03 to 0.3) and a poor metaboliser (ratio > 0.3). Figure 4 and Figure 5 show chromatograms for a poor and an extensive metaboliser respectively.

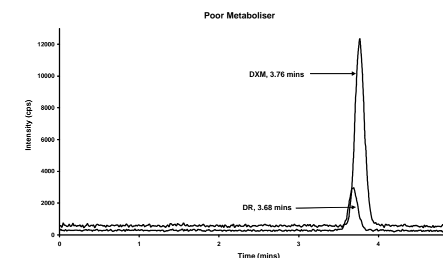

Figure 4: A chromatogram illustrating the DXM (1149 ng/mL) to DR (355 ng/mL) ratio (3.237) for a poor metaboliser.

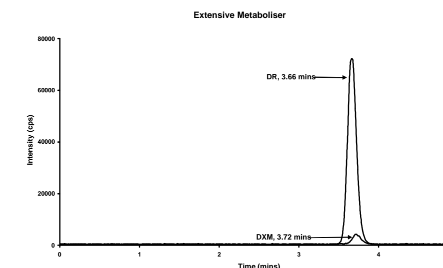

Figure 5: A chromatogram illustrating the DXM (336 ng/mL) to DR (12814 ng/mL) ratio (0.026) for an extensive metaboliser.

SUMMARY

The aim to develop a method for the measurement of dextromethorphan and dextrorphan in human urine using LC/MS/MS was achieved. A novel isocratic method using a silica column gave good retention of the drug, metabolite and internal standard. An isocratic method has advantages over using a gradient method as it does not use as much mobile phase and avoids long equilibration times, allowing a much more efficient method for detection. This method will be used in our clinical toxicology laboratory for clinical studies and research investigations of oxidative phenotyping.

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