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

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INTRODUCTION

The analysis of dextromethorphan and its metabolites; dextrorphan, 3-methoxymorphinan and 3-hydroxymorphinan (Figure 1), has been encountered in both clinical and forensic settings. However this poster will focus on dextromethorphan and dextrorphan.

Dextromethorphan has therapeutic properties as an antitussive drug and is available without prescription as a component of cough remedies. The drug can be orally administered and is widely available, allowing it to be exploited in research as a probe drug for oxidative phenotyping.[1-2] A large number of drugs are metabolised in the body by the Cytochrome P450 (CYP) enzymes, which are highly polymorphic. CYP3A4, CYP2C19 and CYP2D6 are a few examples of these enzymes involved in the biotransformations.[3] Dextromethorphan is metabolised to the *0*-demethylated metabolite, dextrorphan, via CYP2D6 enzyme pathway. The ratio of dextromethorphan to dextrorphan reflects the CYP2D6 enzyme activity and can be used to classify an individual as either a poor or extensive metaboliser.[1]

Dextromethorphan and dextrorphan can be routinely detected and quantitated by gas chromatography-mass spectrometry (GC/MS) in our forensic toxicology service, and has been present in several post mortem cases. This type of abuse is not unexpected as a desperate individual will try to use any legal or illegal drug that is readily available to them. The recreational abuse of dextromethorphan (DXM) is also increasing. Its use is characterised in two ways; firstly as a drug knowingly being consumed and secondly being sold as ecstasy. Countries reporting use in these contexts include America, Korea[4] and Spain[5]. Its increased use is believed to be related to cost, legal access and wide availability.

AIM

To develop a liquid chromatography-tandem mass spectrometry method (LC/MS/MS) for the measurement of dextromethorphan and dextrorphan in human urine. This method was to be used in a clinical study investigating oxidative phenotyping. Many researchers have measured dextromethorphan and dextrorphan 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 dextromethorphan, dextrorphan and the internal standard, ethylmorphine in less than 6 minutes.

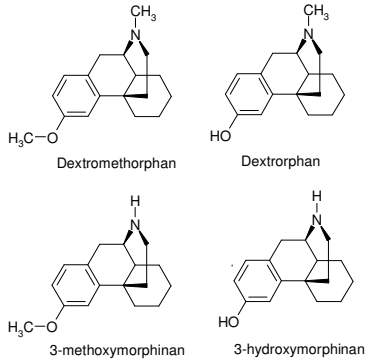

Figure 1: Structures of Dextromethorphan and its metabolites

EXPERIMENTAL

Extraction

Internal standard (ethylmorphine, 2.5mg/L, 100µL), sodium hydroxide (100µL, 1M) and methyl tertiary butyl ether (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, 5minutes) 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). Total run time of method was 6.0mins.

CALIBRATION CURVES

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

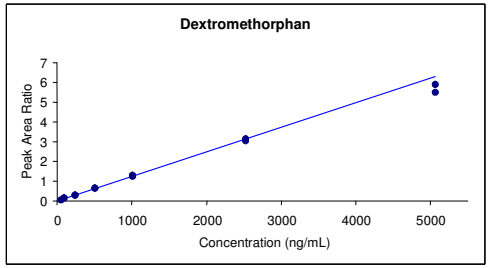

Figure 2: Dextromethorphan calibration curve. Regression linear through zero, weighting (1/(x*x)), y = 0.00125x, r = 0.9989.

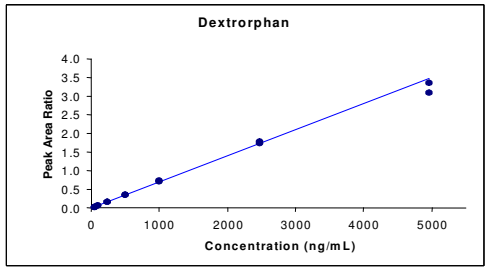

Figure 3: Dextrorphan calibration curve. Regression linear through zero, weighting (1/x*x)), y = 0.000701x, r = 0.9984.

REPRESENTATIVE CHROMATOGRAMS

Figure 4 represents a typical chromatogram for a urine calibrator. The retention times of dextromethorphan, dextrorphan and ethylmorphine are approximately 4.4, 4.3 and 4.7 minutes respectively.

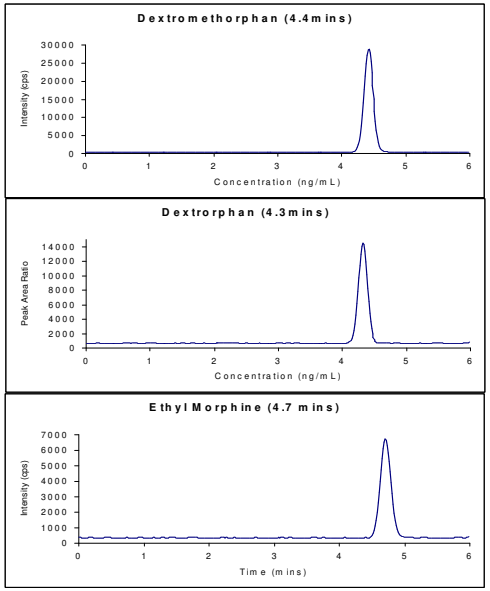

Figure 4: Representative chromatogram of a urine calibrator; dextromethorphan (4.4min), dextrorphan (4.3min) and ethylmorphine (4.7min).

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 because 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 investigating oxidative phenotyping. The method can also be used for any assays that require the measurement of these drugs and can be used for different sample matrices such as blood. In the forensic toxicology laboratory we currently use GC/MS for the qualitative and quantitative analysis of dextromethorphan and dextrorphan. However, the LC/MS/MS method has the advantage that it uses less sample volume for detection. This will be advantageous when there is limited sample which is often the case with post-mortem samples.

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