METABOQUAN-R

UPLC/MS/MS ANALYSIS OF 9 METABOLITES FOR CYTOCHROME P450 INHIBITION STUDIES IN A SINGLE ANALYTICAL RUN

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METHODS

MetaboQuan-R is a high-throughput, generic UPLC MS/MS platform designed to be applicable to as many different compound classes as possible. By changing only the chromatographic gradient and maintaining the same mobile and stationary phase, MetaboQuan-R can be used in the field of biomedical research for targeted metabolomics, lipidomics and proteomics applications consecutively (1).

Here we apply this platform to the discovery DMPK arena. CYP Inhibition studies require the analysis of various metabolites that have varying physio-chemical properties. Due to this variety, these metabolites are often run on separate analytical platforms. The generic nature of the MetaboQuan-R set-up allows the analysis of these metabolites to be combined into a single injection. In this study we demonstrate the analysis of 9 metabolites covering the CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 isoforms. The data acquired was then compared to that obtained with established separate methods

LC/MS Conditions

LC System: ACQUITY I-Class FL
Column: CORTECS T3, 2.1 x 30mm, 2.7µm

Mobile Phase A: 0.01% Formic Acid (aq)
+ 0.2mM Ammonium Formate
Mobile Phase B: 0.01% Formic Acid in 50/50 ACN/IPA
+ 0.2mM Ammonium Formate
Column Temp (°C): 60

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	1	90	10	Initial
0.2	1	90	10	6
0.8	1	2	98	7
1.7	1	2	98	6
1.71	1	90	10	6

Table 1: LC Gradient

MS Parameters

MS System: Xevo TQ-S Micro

Metabolite	Precursor ion (m/z)	Product ion (m/z)	Cone Voltage (V)	Collision Energy (eV)
Acetaminophen	152.1	110.1	75	13
6-hydroxybupropion	238.1	167.1	45	20
6-hydroxypaclitaxel	892.2	308.1	60	27
4-hydroxydiclofenac	312.1	230.1	10	27
4-Hydroxytolbutamide	287.1	107.1	35	10
4-hydroxymephenytoin	235.1	150.1	45	22
Dextrorphan	258.1	157.1	45	33
1-hydroxymidazolam	342.1	203.1	35	25
6β-hydroxytestosterone	305.1	269.1	55	14
Metoprolol (IS)	268.1	116.1	30	20

Table 2: MRM Parameters

A Single, Rapid and Generic UPLC/MS/MS set-up for Discovery DMPK Studies

CYP Inhibition assay for 9 isoforms reduced from 3 injections to a single analytical run

SCAN ME

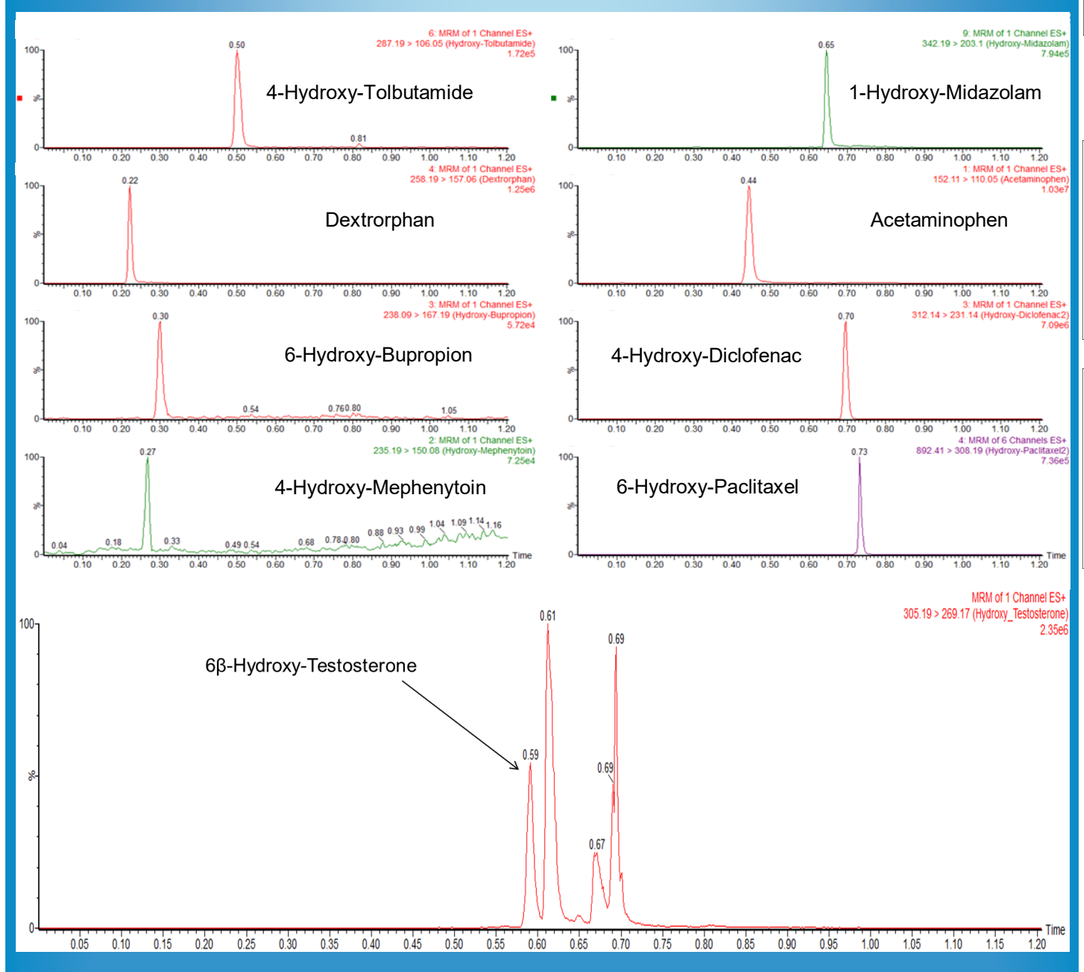
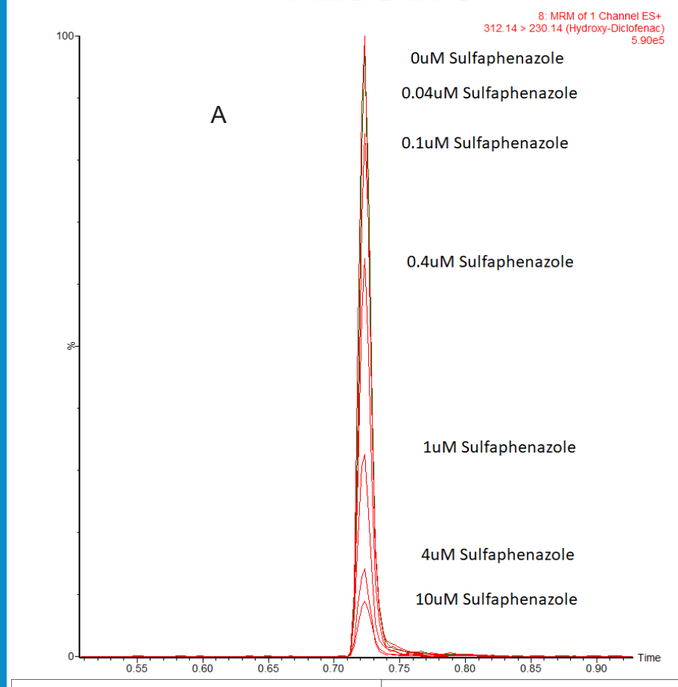


Figure 1. Chromatograms of the 9 metabolites analysed for the CYP Inhibition of the 9 isoforms shown in table 2

RESULTS



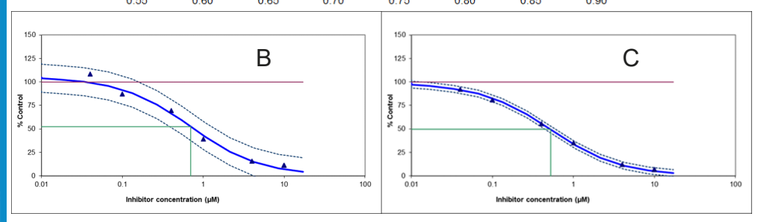
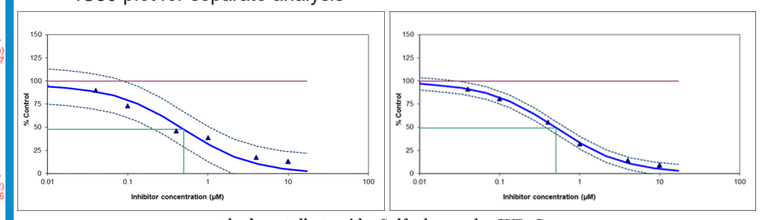
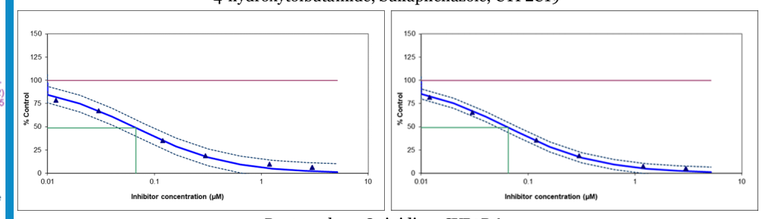


Figure 2: A - Chromatograms of 4-hydroxy-diclofenac showing the different levels of Inhibition of CYP2C9 at various concentrations of Sulfaphanazole. **B** - IC50 plot for combined 9 in 1 experiment **C** - IC50 plot for separate analysis



4-hydroxytolbutamide; Sulfaphenazole; CYP2C19



Dextrorphan; Quinidine; CYP2D6

Figure 3: - Example IC50 plots demonstrating the agreement between the 9 in 1 combined method (left) and the established separate method (right)

DISCUSSION

A chromatographic method was successfully developed on the MetaboQuan-R platform for the analysis of the 9 metabolites listed in table 2. This methodology successfully separated 6β-Hydroxy-Testosterone from its isobaric interferences (Fig 1). When this method was applied to a CYP Inhibition assay in which all 9 metabolites were combined together, the IC50 values agreed with the separate assay for all except three metabolites. Two of these (hydroxy -paclitaxel and hydroxy-mephenytoin) failed the comparison due to poor reproducibility. These most probably failed due to issues with the cassetting of the samples together prior to analysis. The third metabolite that failed the comparison was Acetaminophen. It is suspected that this is due to an interference from the thermal degradation of Phenacetin in the samples. Both of these issues are to be further investigated in future studies.

(1) - www.waters.com/targetedomics