CAPSULES notes and applications from BASi
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Drug Interaction / Phenotyping Assay

Method Profile

Identify drug candidates with unacceptable drug interaction liability sooner rather than later.
Avoid expense of failed clinical trials due to statistical anomalies.
Rapid turn-around of samples by experts in chromatographic and mass spectral separations.

Variations in the metabolism of a drug can lead to unpredictable blood concentrations, increasing the incidence of toxic side effects, decreasing efficacy and/or poor activation of prodrugs. These variations can be caused by human polymorphism, which has been studied across geographic and racial boundaries, and by interference of concomitantly administered drugs. With worldwide distribution of pharmaceuticals and increased use of multi-drug treatment regimens, tools are needed to evaluate these risks.

Phenotyping Assay

Use of multi-drug "cocktails" to evaluate these risks has become more frequent. The technique is simple. If a drug candidate is found *in vitro* to be a substrate for one or more of the Cytochrome P-450 enzyme systems, that candidate can be further tested in humans. To this end, it becomes necessary to phenotype the study subjects. This is accomplished by dosing subjects with a probe compound(s) that has been shown to be metabolized to a product exclusively by the P-450 enzyme system(s) in question. Plasma and urine samples are collected over an eight-hour period and the concentration of probe compounds and their metabolites determined. The ratio of substrate/ metabolite is used to measure a clinical subject's metabolic capacity for the specific P-450 in question, and compared to literature values.

In one scenario, one weekend is required to determine a subject's phenotype for specific enzyme systems. The screen is conducted prior to a clinical study, to determine whether a study panel is representative of the population at large, or to set up a panel which is representative. This reduces the potential for artifacts due to a non-representative distribution of metabolic capabilities among subjects. Such artifacts could adversely impact decisions based on early clinical studies with small numbers of test subjects.

Drug Interactions

The same technique can also be utilized to evaluate directly the interaction potential of a drug candidate. In this case, the study outlined above would be repeated after a suitable washout period, with the candidate drug administered with the probe compounds. The study design includes a suitable randomization to reduce period effects. As in the case above, the concentration of the probe compound(s) and its metabolite(s) is determined and compared to the previously determined control values. If the results are judged significant, more specific interaction studies could be conducted.

Seven probe drug/metabolite pairs in various combinations can be used to characterize the activity of six major metabolic isozymes (see ***T1***). As much as 90% of known human drug metabolism involves one or more of these enzymes. The drugs were chosen not only for the enzymatic specificity of the monitored biotransformation products, but also for their safety and lack of bioanalytic, pharmacokinetic, and pharmacodynamic interaction. The literature is rich with data on their use. BASi has developed several LC/MS/MS methods to measure combinations of these drugs and metabolites.

For Example

The multiple reaction monitoring (MRM) LC/MS plots (***F1***) show data gathered during development of this method. Plots of spiked urine and plasma represent results that might be expected for normal, fast metabolizers. Blanks were run to confirm that background interference is not a problem. The bottom right plot shows a typical result that might be seen for a slow metabolizer for mephenytoin. It can be seen in this case that the metabolite* of mephenytoin is present at about 5% of the level in the normal subject.

*T1. Screening drug/metabolite pairs and the enzyme systems they model. (*methods under development)*

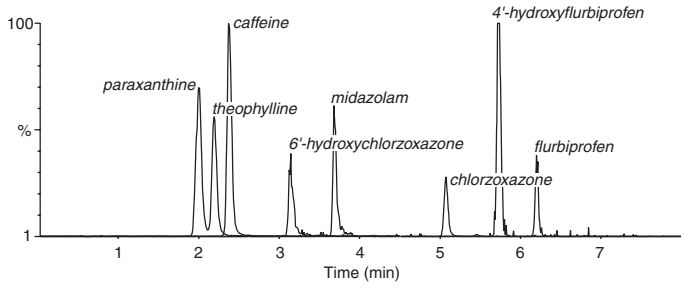
Test Drug/Metabolite	Enzyme System	Typical Dose (mg)	Matrix
Mephenytoin/4'-hydroxymephenytoin	CYP-2C19	100	urine
Dextromethorphan/dextrorphan	CYP-2D6	30	urine
Flurbiprofen/4'-hydroxyflurbiprofen	CYP-2C9	100	urine/plasma
Midazolam	CYP-3A	10	plasma
Dapsone/acetyldapsone	N-acetyltransferase	100	plasma
Caffeine/paraxanthine	CYP-1A2	100	plasma
Chlorzoxazone/6'-hydroxychlorzoxazone	CYP-2E1	250	plasma
Omeprazole/5'-hydroxyomeprazole	CYP-2C19	*	plasma
Diclofenac/4'-hydroxydiclofenac	CYP-2C9	*	plasma

BASi Links

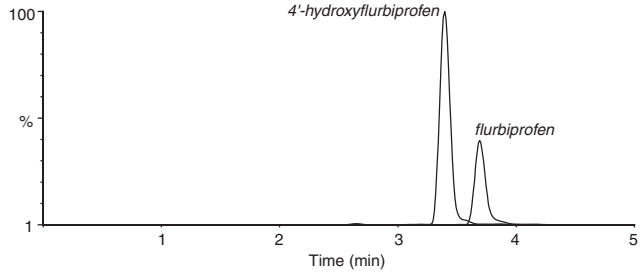
1. *www.currentseparations.com/issues/19-1/19-1e.pdf Drug Metabolism and Pharmacokinetics in Drug Discovery: A Primer for Bioanalytical Chemists, Part I*
2. *www.currentseparations.com/issues/19-3/19-3e.pdf Drug Metabolism and Pharmacokinetics in Drug Discovery: A Primer for Bioanalytical Chemists, Part II*
3. *www.bioanalytical.com/info/poster/pdf/Chan-09.pdf Investigation of* InVivo *Metabolism of Silibinin in Liver Microsomes and Cytochrome P450 Isozyme Identification*
4. *www.bioanalytical.com/info/poster/pdf/Zhu-08.pdf Rapid Quantitative Determination of Multiple Cytochrome P450 Probe Substrates by Gradient Liquid Chromatography/Electrospray Ionization-Ion Trap Mass Spectrometry*
5. *J Chromatography B, 750: 371-379, 2002. Rapid and Quantitative Determination of Metabolites from Multiple Cytochrome P450 Probe Substrates by Gradient Liquid Chromatography/Electrospray Ionization-Ion Trap Mass Spectrometry*

F1. Example MRM chromatograms.

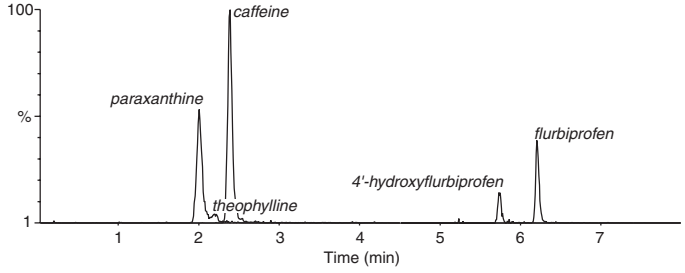
Standard mix in plasma



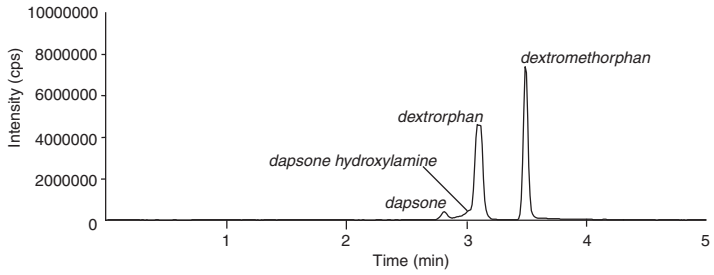
Urine: CYP-2C9 probe drug flurbiprofen



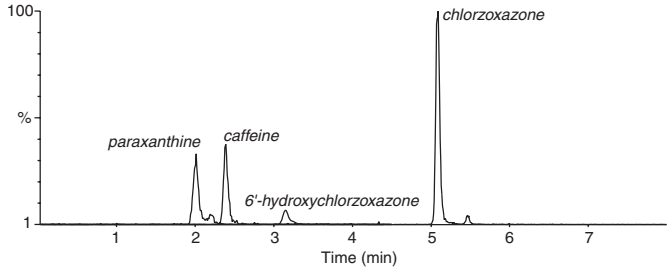
Patient plasma sample: caffeine/flurbiprofen



Urine: CYP-2D6 probe dextromethorphan



Patient plasma sample: caffeine/chlorzoxazone



Urine: CYP-2C19 probe mephenytoin