HOW DO YOU USE STEREO-SELECTIVE METABOLISM STUDIES TO IMPROVE YOUR DRUG DISCOVERY AND DEVELOPMENT PROCESS?

WHITE PAPER

The Importance of Stereo-Selective Metabolism in Drug Discovery and Development

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INTRODUCTION

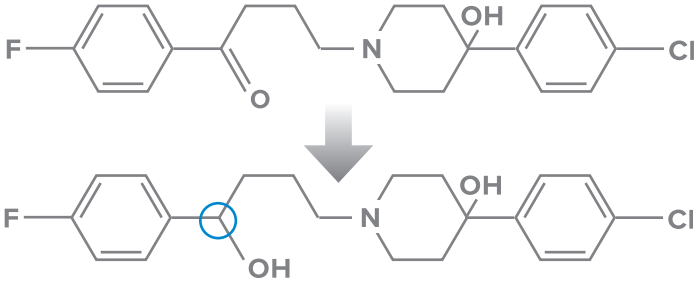
"In order to understand the actions of drugs it is an absolute necessity to have knowledge of the transformations they undergo in the body...we must not judge drugs according to the form and amount administered, but rather according to the form and amount which actually is eliciting the action."[1]

This statement was written more than 150 years ago—in 1859—by Rudolph Buchheim (1820–79), a pioneer in experimental pharmacology. It lays out as elegantly as any modern statement or regulatory guidance the very mission and importance of drug metabolism, pharmacokinetics ("amount"), and pharmacology ("action"), and—especially—the role of metabolite profiling and identification ("transformations" and "form").

There are few aspects of drug metabolism and metabolite profiling more essential—and more interesting - than exploring stereo-selective (also known as enantio-selective or chiral-selective) metabolism. For the better part of a century, the consideration of chirality in drug metabolism was limited to academic study and/or to natural products, owing in no small part to the limits of separations chemistry. However, chirality has been earning ever-greater importance in drug discovery and development, such that most of the new molecular entities reaching the market in the first decades of the 21st century are single enantiomers, rather than the racemic mixtures (or achiral drugs) that dominated the latter half of the 20th century. Stereo-selective metabolism has important impacts on scientific aspects such as pharmacokinetics, pharmacodynamics, drug safety, and bio-analytical chemistry; but also on regulatory, intellectual property, business and product development, and other important considerations.[2-4]

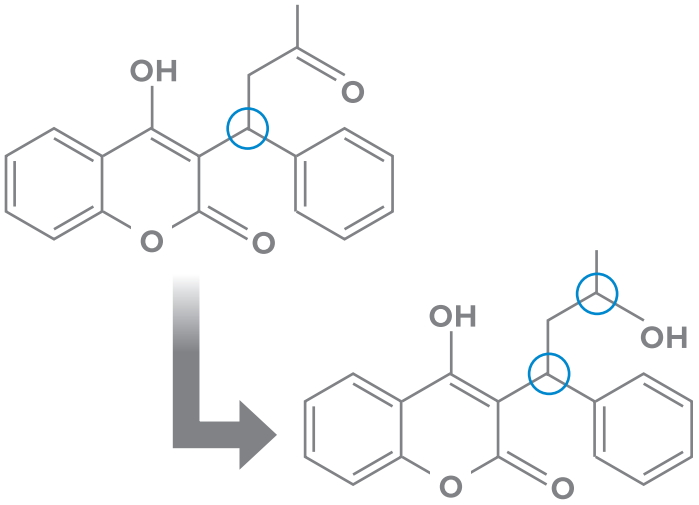
TYPICAL REACTIONS

While direct enantio-selective effects on the physical–chemical aspects of adsorption, distribution, or elimination are minimal or are only now being studied, their effects on metabolism are the rule rather than the exception. This applies to the drugs as substrates or products and applies across multiple enzyme systems (P450, Phase II, etc.). Examples include:



The **prochiral/achiral** metabolism (carbonyl reduction) of haloperidol (in humans, S-hydroxy-haloperidol is preferred) to a chiral product;

- The **chiral to chiral** transformation of (S)-warfarin to (S)-7- or (S)-6-hydroxywarfarin;
- **Chiral to achiral** transformations, such as those involving 1,4-dihydropyridine calcium antagonists to their pyridine analogs;
- **Chiral to diastereomer** transformations such as the keto-reduction of warfarin, resulting in two pairs of diastereomeric alcohols;



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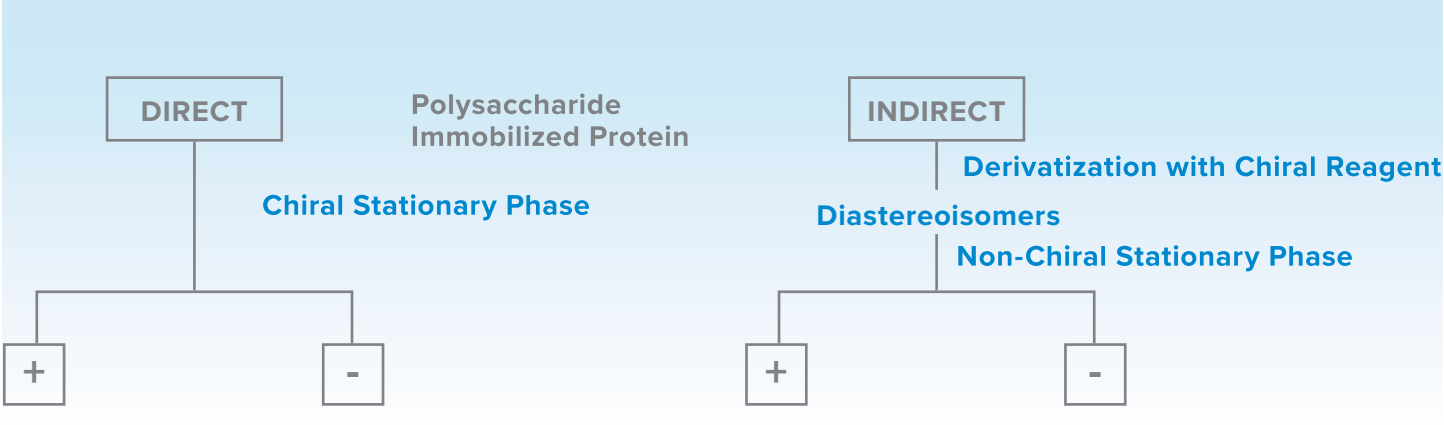


Bio-Analytical Chemistry Approaches[6]

- **Chiral inversions** (enzymatic and non-enzymatic) such as those that occur with 2-arylpropionic acid NSAIDS.

POTENTIAL DRUG-DRUG INTERACTIONS

The administration of racemic drugs is polypharmacy, by definition; add in yet another drug, and the effects on drug-drug interactions (DDIs), pharmacology, or other drug safety considerations can be significant when the induction or inhibition of one drug influences the PK behavior of the other(s), and reinforces the importance of considering the exploration of chirality in metabolite profiling exercises.

Examples include the interaction of warfarin with cimetidine [decreases the clearance of (R)-warfarin] or with sulfinpyrazone [decreases the clearance of (S)-warfarin]. Likewise, racemic mixtures can exert effects on pharmacodynamics and/or safety:

- The efficacy may be in a single enantiomer, the antipode being biologically inert;
- The pharmacological activity of each of the enantiomers may be different, such that each can be developed on its own merits;
- The enantiomers may have opposite effects at the same target;
- The pharmacological activity lies in both enantiomers, but adverse safety is only associated with one.

BIO-ANALYTICAL CHEMISTRY APPROACHES

As mentioned earlier, a driving factor in the increased appreciation of chiral-driven drug metabolism, PK, and pharmacodynamics has been an improvement in column chemistries to achieve the necessary separations. Indeed, it has been stated that the "development of complex PK models and plasma–concentration–effect relationships based on 'total' drug concentrations following administration of a racemate are of limited value and potentially useless."[5]

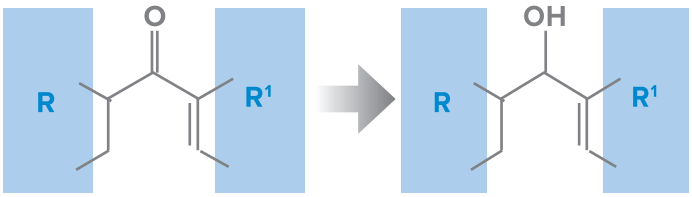
As with all quantitative analytical chemistry, the methods should aim for accuracy and reproducibility, with minimal sample preparation, and the employment of sample-friendly mobile phases or pH. However, special diligence in method development and validation is required for chiral separations. The preferred method is direct separation on a chiral stationary phase (e.g., polysaccharide or immobilized protein). An alternative is an indirect method in which the enantiomers are derivatized with a chiral reagent to produce diastereoisomers, which can then often be separated on conventional column chemistries.

AN EXAMPLE OF STEREO-SELECTIVE METABOLISM

The current movement towards single enantiomers as drug candidates, noted above, should result in mitigation of the DDIs that are potentially associated with racemic mixtures. This does not alleviate the concern or challenges that are associated with achiral-to-chiral transformations and/or chiral-to-chiral or chiral-to-diastereomer transformations.

A hypothetical case study (based on actual data, but anonymized) of stereo-selective metabolism and metabolite profiling, is described below.[7,8]

Consider a small molecule drug candidate that demonstrates good efficacy in a mouse pharmacology model and exhibits acceptable safety and PK profiles in preclinical studies. Early metabolite profiling efforts in mouse indicated that the most significant metabolite pathway was keto-reduction to form the corresponding alcohol (similar to the example of haloperidol described above), now with a chiral center:



Based on these findings, attention should be given to possible stereo-selective metabolism to the chiral alcohol, with several important questions to consider:

- What is the in vitro metabolism of the compound in other preclinical species and in human?

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- Can an initial bio-analytical method of the enantiomers be achieved with minimal method development/sample prep?
- Can the bio-analytical method be optimized and made suitable for validation?
- Is there stereo-selective (i.e., to the R- or S-form) metabolism to the alcohol?
- Do the in vivo results reflect the in vitro results?
- What enzyme is responsible for the metabolism?
- Are there regulatory/clinical/intellectual property/business development implications?

In vitro experiments (liver S9) in multiple species indicated that metabolism to the S-enantiomer of the keto-reduced metabolite was preferred (>90% in rat, dog, and human; >80% in mouse). However, the keto-reduced alcohol was not observed in monkeys: this would have significant implications for selection of species for toxicological studies. Pharmacokinetic experiments in mouse, rat, and dog were all consistent with the companion in vitro experiments: that is, the S-enantiomer was preferred over the R-enantiomer, and consistent with the ratios observed in the in vitro experiments. Furthermore, plasma exposure of the keto-reduced metabolite in other species was several-fold higher than the parent compound (which, in turn, raises the typical questions for a "disproportionate metabolite"). Finally, the S-enantiomer of the keto-reduced metabolite was also found to have significant efficacy against the target.

From a bio-analytical perspective, initial analyses employed an immobilized protein column at hand that offered very good separation of the enantiomers of the keto-reduced metabolite, but required long (>60 minutes) run times, yielded suboptimal peak shapes (and corresponding suboptimal sensitivity), and was not robust enough to accommodate repeated injections of biological samples. Subsequent investigations resulted in the successful employment of a different chiral column chemistry that allowed for shorter run times and improved sensitivity so that the metabolite could be monitored in clinical studies in a cost-effective manner.

This hypothetical case study provides a good example of the need to consider possible enantio-selective metabolism and its impact on PK, pharmacodynamics, toxicology, and bio-analytical chemistry; not to mention regulatory, intellectual property, business and product development, and other important considerations.[8]

STEREO-SELECTIVE METABOLISM AT EAG LABORATORIES: WE KNOW HOW

EAG has more than three decades of experience in the conduct of ADME (adsorption, distribution, metabolism, and excretion) studies with preclinical species to support drug discovery and development.

Our study directors design and oversee the in-life stages at partner sites with AAALAC-accredited facilities that are vetted and qualified by EAG. Dose preparation, mass balance determinations, sample preparation, extractions, metabolite profiling, and unknown metabolite identification are performed by highly experienced scientists in EAG's metabolism groups. Our scientists can also facilitate related services such as quantitative whole body autoradiography.

Specific capabilities include:

- Experts with significant publication records in drug metabolism—We Know How
- Radiolabeled and non-radiolabeled ADME studies—preclinical species or human clinical trial support
- Sample preparation and extraction method development
- Chromatographic method development
- Radiochromatographic profiling
- LC-MS/MS identification (including high resolution mass spectrometry)
- Proposal of metabolic pathways
- Radio-labeled, stable-labeled or non-radiolabeled synthesis of drug and metabolites

ABOUT THE AUTHOR

James Schmidt, EAG Life Sciences Senior Scientific Advisor for Trace Analysis and Structural Chemistry, Custom Synthesis, and Product Chemistry, brings more than three decades of experience in xenobiotic metabolism, bio-analytical chemistry, and structural elucidation with private, government, and industrial laboratories. His special interests include small molecule metabolic stability, metabolite profiling and identification, chiral separations, and pharmacologically active metabolites. He is the author or co-author of several peer-reviewed posters and papers, as well as scientific reports to satisfy regulatory requirements of both the EPA and FDA. Most recently, he contributed the chapter on "Metabolite Profiling" to the book New Horizons in Predictive Drug Metabolism and Pharmacokinetics (Alan G. E Wilson, ed., RSC Publishing, 2015). James is based at our Columbia, Missouri (formerly ABC Laboratories) location.

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1. A. Conti and M. H. Bickel, Drug Metab. Rev., 1977, 6, 1–50.
2. V. Campo, L. Bernardes and I. Carvalho, Curr. Drug Metab., 2009, 10, 188–205.
3. D . Brocks, Biopharm. Drug Dispos., 2006, 27, 387–406.
4. Chirality in Drug Design and Development, ed. I. Reddy and R. Mehvar, CRC Press, New York, 2004.
5. A . Hutt, Metab. Drug Interact., 2007, 22, 79–112.
6. Adapted from Campo, et al. (ref. 2, above)

Hypothetical Case Study: *The author has had the privilege of working on several interesting examples of stereo-selective metabolism in his career. The hypothetical case study presented in this white paper is based on one of those examples; additional data on the example is reported here:*

7. J. Schmidt, A. Nouraldeen, L. Moran, L. Li and A. Wilson, Enantio-selective and Species-Dependent Carbonyl Reductase Metabolism of LX6171, 12th Annual Conference on Drug Metabolism and Applied Pharmacokinetics, 2009.
8. L. Li, W. Heydorn, J. Kramer, A. Nouraldeen, J. Schmidt, J. Jiang, L. Moran and A. Wilson, Metabolism Mediated CYP2B Induction by LX6171 (3′-chlorobiphenyl-4-yl)-1-(pyrimidine-2-yl) piperidin-4-yl methanone in the Rat, International Society for the Study of Xenobiotics (ISSX) Annual Meeting, 2009.

Other recent case studies are reported here:

9. J. Schmidt, “Metabolite Profiling,” in A.G.E. Wilson (ed.), New Horizons in Predictive Drug Metabolism and Pharmacokinetics, RSC Publishing, Cambridge, UK, 2015.

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