The Wonder of MS Finger Printing – A M9 Story from the Development of GSK A

Cathy Chen, Ernest Schubert, Janine Rogers, Igor Goljer, Steve Castellino
Structure ID Group, Drug Metabolism and Pharmacokinetics, PTS, GlaxoSmithKline, King of Prussia, PA 19406, USA

An abundance of oxidative metabolites on GSK-A's cyclohexyl moieties were observed from *in vivo* preclinical and human metabolism. These isobaric metabolites have similar retention times as well as identical empirical formulae and MS/MS spectra. Consequently, a strategy was needed to consistently identify each isobaric metabolite across a variety of species and matrices which did not solely rely on retention time. An MS^n finger print approach was developed to achieve this goal and was applied throughout the course of the study to characterize these isobaric species.

The MS^3 spectra contained 6-8 isobaric fragments of different intensities to form a unique pattern for each metabolite which constituted the basis of our "finger print" method. These "MS-finger prints" were reproducible across species and biological matrix thereby providing unambiguous identification of each metabolite. Consequently, our study to estimate the quantities of circulating drug related material in human could be reliably conducted.

A discrepancy was observed in the MS^n finger prints between a synthetic standard for metabolite M9 and that detected in human. This observation led to the discovery of a new metabolite, M22. Careful comparison of the MS^n finger prints of M2-M6 and examination of the current metabolic scheme led to the proposed structure of M22 (later confirmed by NMR). The story proved the finger print tool is robust, reliable, and powerful.