Pharmaceutical MANUFACTURING

STABLE ISOTOPES PROVIDE A NEW PAT TOOL

USED TO AUTHENTICATE PRODUCTS, STABLE-ISOTOPIC ANALYSIS CAN ALSO BE USED TO IMPROVE PROCESS UNDERSTANDING AND CONTROL

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Scientists have used stable isotope analysis for decades, employing isotope-ratio mass spectrometry to trace the "isotopic fingerprints" of natural materials. Within the past few years, pharmaceutical manufacturers and regulators have also begun to test the technique, which is relatively inexpensive and extremely precise, to authenticate pharmaceutical ingredients and products (Refs. 1-5).

In addition, isotopic analysis can also provide information about the manufacturing process, during which raw materials are manufactured into intermediates, active pharmaceutical ingredients (APIs) and, finally, into drug products. Stable-isotopic values are affected by the "fractionation" that occurs during the manufacturing process (Box, p. 29). The measurement of variables such as reaction type and rate, and separation processes such as recrystallization can be used to monitor reactions in various steps of Process Analytical Chemistry (PAC).

Recently, Molecular Isotope Technologies LLC (Niantic, Conn.) conducted joint studies with FDA's Division of Pharmaceutical Analysis using isotopic analysis to study the API, Naproxen [6,7]. Now, Molecular Isotope Technologies and Johnson & Johnson (J&J) are exploring the technique's potential in helping identify counterfeit products based on their processes, or to provide information in process patent infringement cases [8].

In this article, we discuss how isotopic analysis can be used to track the isotopic fractionation that occurs in synthetic pharmaceutical manufacturing processes. We also present and discuss preliminary results of J&J studies using isotopic analysis to evaluate the antiepileptic drug, Topiramate. So far, these results suggest that this analytical tool could be a practical, cost-effective way to analyze and control pharmaceutical manufacturing processes.

FUNDAMENTALS OF ISOTOPIC ANALYSIS

To very briefly review the basics, stable isotopes exist naturally as mass variants of chemical elements. Carbon-13, for instance, consisting of seven neutrons and six protons, accounts for approximately 1.1 percent of all carbon in nature; carbon-12 accounts for approximately 98.9 percent. The variation in the $^{13}C/^{12}C$ ratios—and other isotopic ratios of pharmaceutical components—can be used to trace the "isotopic provenance" or "fingerprint" of APIs or drug products via isotope-ratio mass spectrometry. Figure 1 is a schematic diagram of an Elemental Analyzer/Mass Spectrometer. In an EAMS, samples are dropped from a carousel to a high-temperature oven where they are combusted to small molecules such as CO_2 or N_2, whose isotope ratios are subsequently measured on an isotope-ratio mass spectrometer.

When both the sources of starting materials and of the manufacturing

process are fixed, stable-isotopic values for the products will be predictably constant and provide an identifiable and reproducible fingerprint. Over the last six years, authentication of pharmaceutical APIs and drug products, based on their isotopic compositions, has been shown to be a highly specific means of identifying the manufacturers and individual batches of products [1-5]. Both pharmaceutical manufacturers and regulatory agencies are interested in the technology as a forensic layer of product security.

However, FDA-DPA's Naproxen studies show that natural stable-isotopic ratios can also be measured to identify pharmaceutical material sources and determine the processes by which APIs are made (Figure 2). In 2002, J&J decided to test the technique's effectiveness in distinguishing between 53 samples of Topiramate that were made by three different synthetic pathways [6,7]. The initial goal was to determine whether the technique could be used as a tool in process patent infringement cases.

The observed isotopic results for the Topiramate samples can be grouped into a relatively small number of clusters consistent with synthetic fractionation. Three major isotopic-fractionating processes include:

- Synthetic reaction pathways (reaction mechanisms determining batch-to-batch isotopic variations via synthetic fractionation)
- Fractional crystallization
- Size fractionation (sieving, particle sorting).

FRACTIONATION EXAMINED

Isotopic fractionation between light and heavy isotopes occurs when chemical reactions do not proceed to completion, or when multiple products are formed, and those isotopes are unevenly distributed among the reactants and products. In principle, the isotopic compositions of chemical products can be predicted from the isotopic compositions of the starting materials together with knowledge of the fractionations. However, fractionations can be quantitatively predicted only when complete mass balances are available and when the kinetic and equilibrium isotope effects associated with all relevant chemical reactions are accurately known [9].

Synthetic isotope fractionations are potential quantitative process monitors that could be used to integrate specific reaction variables that contribute to the isotopic composition of the synthetic product. In a given process for which the isotopic compositions of the reactants are known and the synthetic-isotopic fractionation has previously been determined, the isotopic composition of the product has a predictable isotopic value. If the observed value is not as predicted, then something in the synthetic process has varied. That may have been the reaction

IMPORTANT ISOTOPIC LAWS AND PRINCIPLES

1. Stable-isotopic composition of materials is a function of the isotopic composition of raw materials or reagents and processes of synthetic isotope fractionation, which is equivalent to saying that it is a function of thermodynamic and kinetic properties.

2. To account for the mass and isotopic composition of pharmaceutical materials, these laws are fundamental:

   - Mass balance: $M_a + M_b = M_T$; and,
   - Isotope mass balance: $M_a\delta_a + M_b\delta_b = M_T\delta_T$

   Where, M_a, M_b, and M_T are the masses of component a, b, and their total T. δ_a, δ_b, and δ_T are the stable isotopic compositions of sample a, sample b, and their total T. So, in a simple system (e.g., Reactant A → Product B), where all components can be accounted for as "reactants" or "products," then the isotopic composition is rigorously defined by the preceding laws.

3. Generally, heavy isotopes preferentially partition to the more condensed phase (i.e., solid > liquid > gas)

STABLE-ISOTOPIC FRACTIONATION

Figure 4 is a simple schematic diagram showing the general concept of isotopic fractionation between reactants (δ_R; viz., δ_A, δ_B) and a product (δ_P; viz., δ_C). Commonly, the isotopic difference or fractionation is denoted $\Delta\delta$ (= $\delta_P - \delta_R$), where δ_P = isotopic compositions (in ‰) of the product (δ_P) and reactant (δ_R). More precisely and formally,

the isotopic difference is denoted $\epsilon_P = [(\delta_R + 1000)/(\delta_P + 1000) - 1]10^3$. Typically, the most important variable in synthetic-isotopic fractionation is the rate of the potentially-fractionating reaction. The forcing function for reaction rate may be factors such as temperature, pressure, or availability of reactants.

A straightforward application of synthetic-isotope fractionation in manufacturing consistency, currently underway for Topiramate synthesis, is monitoring a simple two-reagent (A + B) reaction that produces one product C. In this reaction, where reagent A is in excess, reagent B is limiting, the overall fractionation of the reaction is given by ΔA:

A + B → C (ΔA, A in excess, B limiting)

where, ΔA for a given reaction is a function of the isotopic compositions (δ) of components A, B, and C.

Note that (i) measuring ΔA (calculated from measurements of δ_A, δ_B, δ_C) periodically would give a quantitative index of the consistency of drug component synthesis, and (ii) with tabulation of the isotopic compositions of the product C (δ_C) and documentation of its synthetic pathways at the manufacturer, the details of the synthetic pathway of the products can be accessed via subsequently-measured batch-specific isotopic composition of products from either the supply chain or from the marketplace.

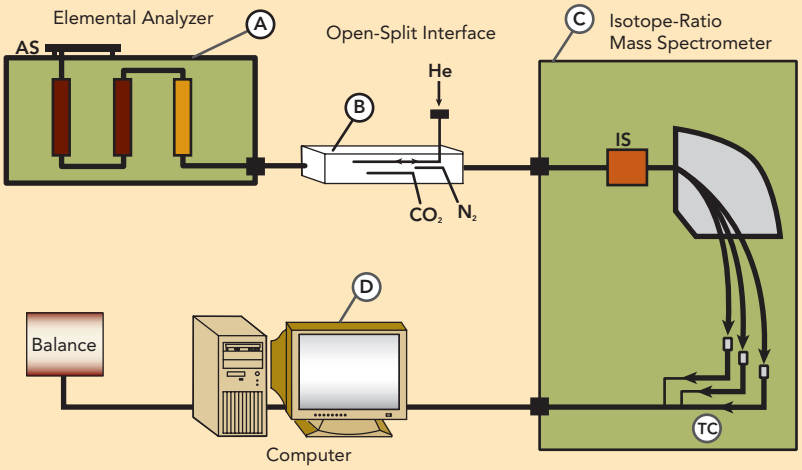

Figure 1. A schematic drawing of an Elemental Analyzer/Isotope-Ratio Mass Spectrometer (EA/IRMS)

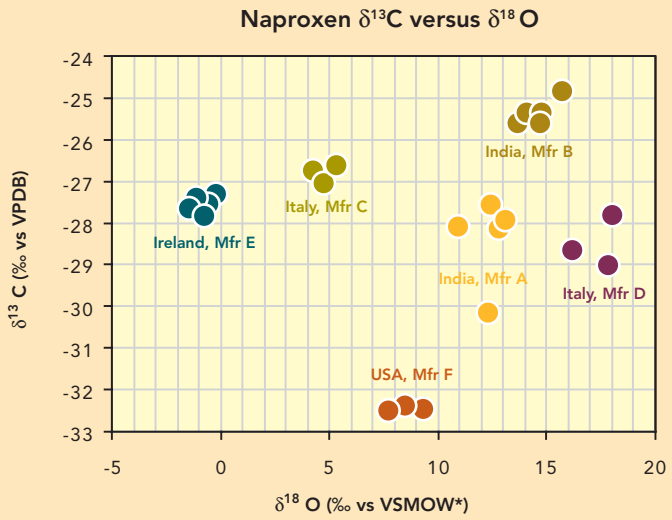

Figure 2. A bivariate isotope ($\delta^{13}C$ vs. $\delta^{18}O$) graph of Naproxen data from the FDA-DPA study [8]. The clustering indicates the identity (isotopic provenance) of manufacturers of the API.

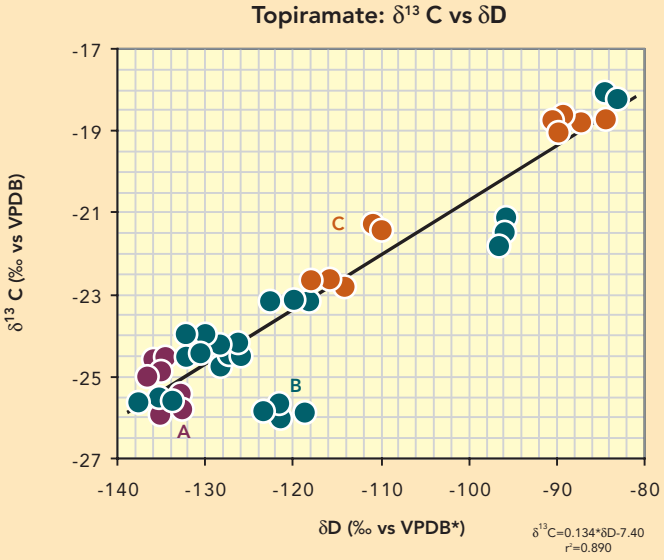

Figure 3. A bivariate isotope ($\delta^{13}C$ vs. δD) graph of Topiramate data from the Johnson & Johnson study [6-7]. The clustering identifies samples manufactured through different synthetic pathways at a variety of plants throughout the world.

**VSMOW, or "Vienna Standard Mean Ocean Water," and VPDB, "Vienna Pee Dee Belemnite," are International Atomic Energy Agency standard materials used in reporting isotopic data.*

rate as modulated by factors such as pressure, temperature or reagent abundance. Isotopic composition integrates such reaction variables and therefore can be used to monitor them.

Preliminary studies seek to differentiate the effects of raw materials from synthetic pathways in the synthesis of pharmaceutical intermediates and, ultimately, the synthetic fractionation of either partial or total synthetic pathways. When those factors are quantified, they should be reproducible, so that they present a means by which to monitor the consistency of synthetic processes. We have just begun this endeavor, but decades of isotopic experience in the earth sciences provide a solid background for such research.

ENCOURAGING EARLY RESULTS

Although the isotopic compositions of the starting materials used to produce the archived Topiramate samples were not controlled, the isotopic results were graphically grouped or "clustered" depending on the synthetic pathway used. The clustering of the data in Figure 3 into a small number of groups (rather than a random distribution) is consistent with either or both a limited number of synthetic pathways and/or a limited number of raw material sources. In ongoing research, we are controlling—and thus, developing a quantitative understanding of—the specific isotopic contributions of each of the starting materials and the synthetic processes to the products' observed isotopic compositions.

While the observed clustering may be related to only the different synthetic pathways used, effects due to the varying isotopic compositions of the reagents and starting materials may also have contributed. Because the samples had been produced in a variety of different laboratories and manufacturing plants throughout the world, it was not possible to trace the suppliers of the various raw materials, reagents, and exact reaction conditions used during manufacture.

At the present stage of development, stable-isotopic analyses are being performed offline at a separate analytical facility. However, we envision

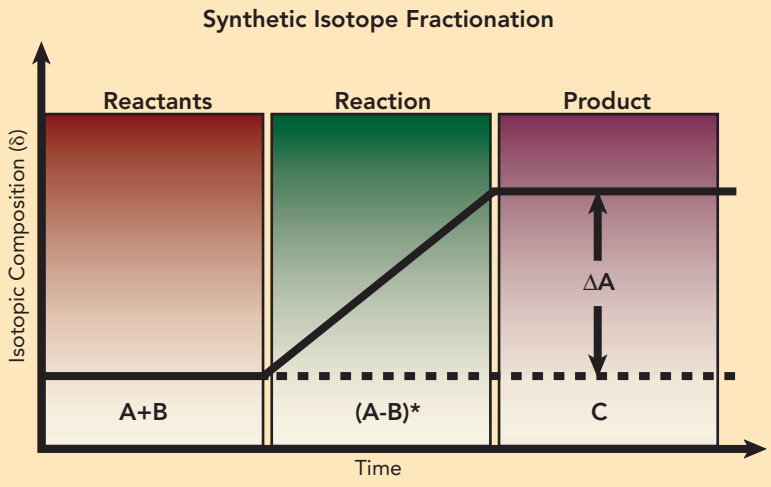

Figure 4. The effect on a two-component reaction (A + B) forming the product C, accompanied by a synthetic isotope fractionation (ΔA) of reagent A during the reaction.

at-line isotopic analyses to become the preferred mode of application for synthetic-isotope analyses. At-line mass-spectroscopic analyses are already underway in the pharmaceutical industry [10]. In fact, in addition to mass spectrometer manufacturers that produce laboratory instruments (Thermo Electron, San Jose, Calif., and GV Instruments, Manchester, U.K.), at least one firm, Monitor Instruments (Cheswick, Pa.), has already developed compact, on-line cycloid isotope-ratio mass spectrometry for the biochemical and geochemical industries.

At-line isotopic measurements can easily be performed on samples removed from the reaction stream. To our knowledge, there are presently no on-line methods for isotope-ratio measurements for solids such as pharmaceutical materials, although there are some for gases. However, given the existing multi-sample carousel systems for isotope analysis, only an at-line sampling system would be necessary to acquire nearly real-time samples and analyses.

PROSPECTUS

The prospect for stable-isotopic analysis in PAT is generally promising, since the ideas of synthetic-isotope fractionation described here have been developed and well accepted in the earth-science isotope community for decades. The dynamic range (= observed range/1σ precision) of isotope-ratio mass spectrometric measurements for pharmaceutical materials is typically high (~50-100), indicating a marked utility for quantifying authentication of both products and processes.

Since 1999, pharmaceutical-isotope analysis has progressed from stable-isotopic authentication, largely observational work, of pharmaceutical products to the early stages of stable-isotopic analysis in PAC. The basic scientific principles for understanding stable-isotopic fractionation as a natural process monitor have been in place for decades. We are just now beginning to evaluate them for industrial applications. The FDA PAT guidance [11] and the cGMP initiative [12] encourage the implementation of new technologies. Assuming that we find adequate dynamic ranges in process measurements of interest, it is reasonable to expect that stable-isotope mass spectrometry may become one of the analytical technologies used in PAT.

Acknowledgements. The authors thank John M. Hayes for important contributions to this paper on synthetic-isotope fractionation.

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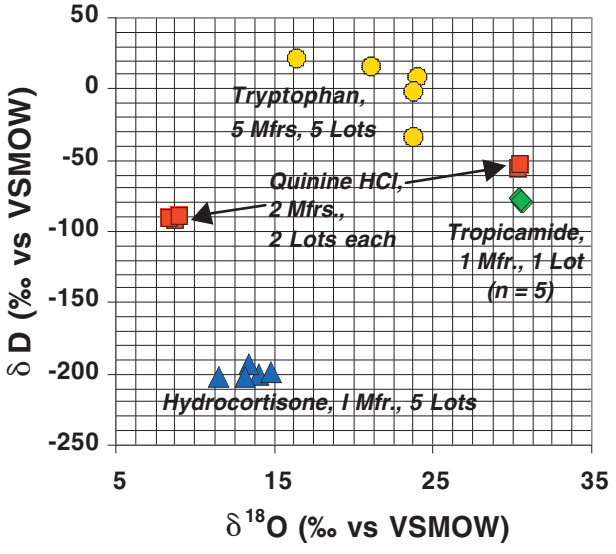
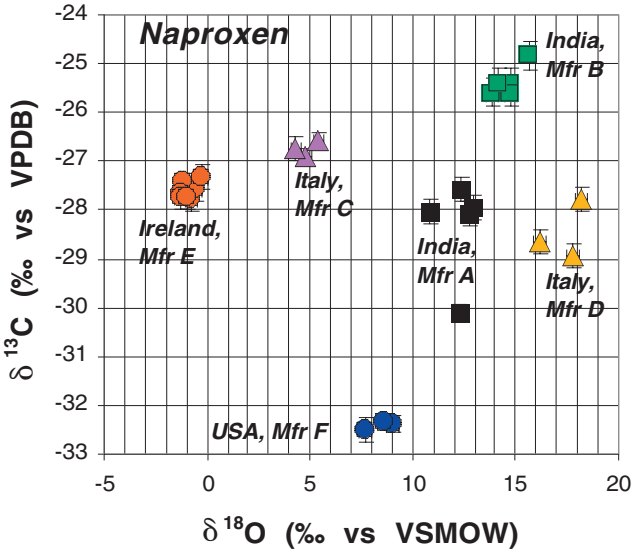
We can differentiate sources of drugs by their natural "fingerprints."

Four APIs: Mixed Sources



Individual batches and manufacturers of Active Pharmaceutical Ingredients (APIs) are easily distinguished using our MSIA technology. (Jasper *et al., J. Pharm. Biomed. Anal.,* 2004, 35(1):21-30).

One API: Six Sources



Individual manufacturers of a single API are also distinguished by our MSIA technology. (FDA Anticounterfeiting Mtg., Oct. 15, 2003)

Isotopic Analysis of Batch Products: A Clear Difference

All chemical compounds have distinctive ratios of natural stable isotopes, thus every batch of pharmaceutical materials has its own highly-specific isotopic fingerprint. Multiple Stable Isotopic Analysis (MSIA) can therefore be used to identify, track and classify batches, and thus to mitigate counterfeiting, diversion, theft, patent infringement and liability issues.

With this highly-specific stable isotope approach, Molecular Isotope Technologies provides a novel technology for pharmaceutical and regulatory clients to protect their products.

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