Using stable isotopes to authenticate pharmaceutical materials

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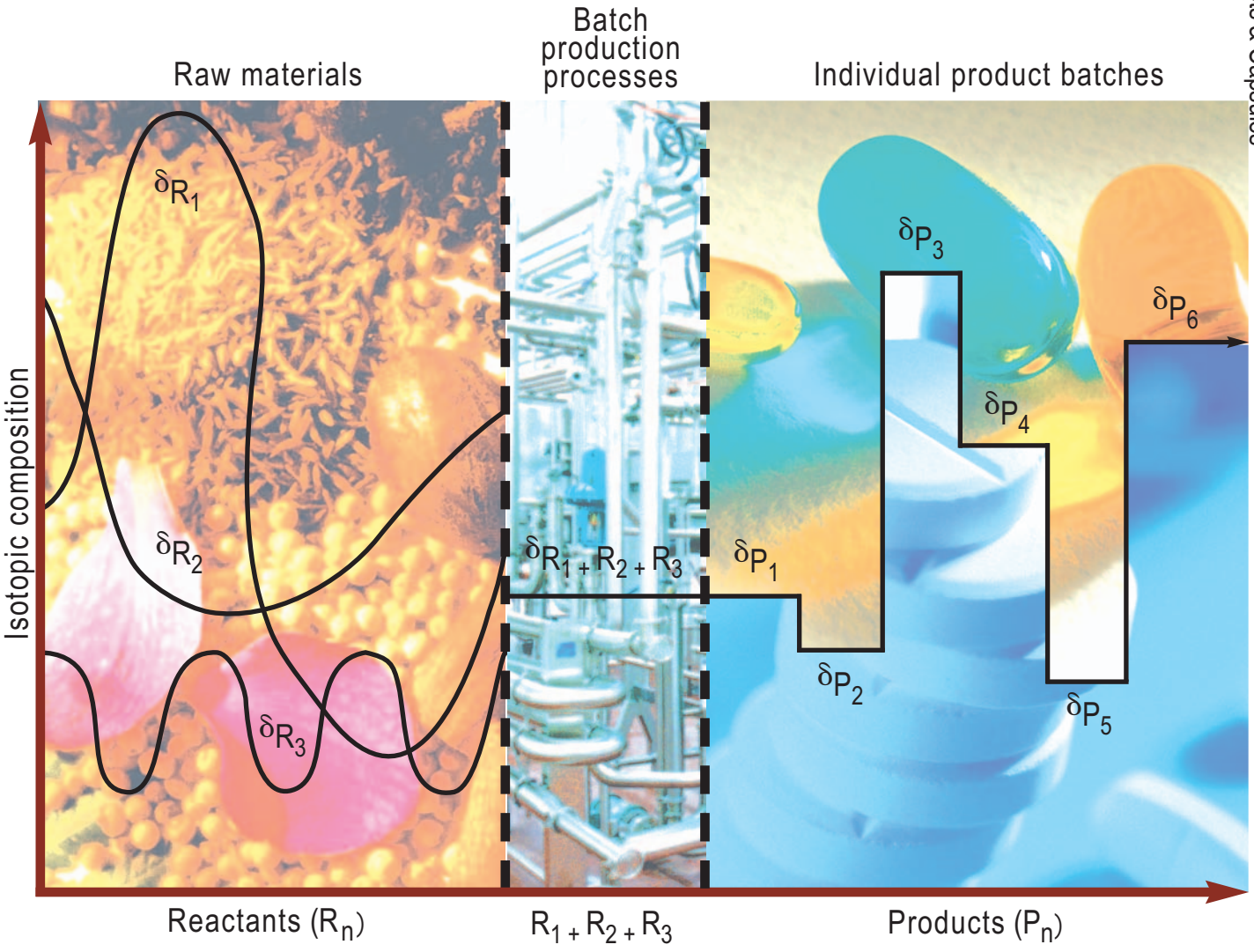

Manufacturers and US regulatory authorities are confronting pharmaceutical security using overt, covert, and forensic techniques. Many overt techniques are already in place. Covert techniques, such as radio-frequency identification (RFID) and secure business practices, have recently gained prominence. Forensic techniques, however, are only now receiving particular attention. This article reviews the application of one forensic technique, multiple stable isotopic analysis (MSIA), as a means to authenticate pharmaceuticals.

Purposeful misidentification of pharmaceutical materials threatens the efficacy of end-products and intermediates, consumer confidence, and the economic well-being of pharmaceutical manufacturers. Thus pharmaceutical manufactures and regulatory agencies have a strong interest in ensuring product authenticity and security [1]. The main areas of concern associated with purposeful misidentification are counterfeiting, diversion (also known as countertrading), vicarious liability, theft, and patent infringement.

In addition to these concerns, the sale of diverted and counterfeited pharmaceutical materials has supported international terrorism in the USA and aboard. To defend against these threats, the FDA recently delineated three classes of methods to bolster pharmaceutical security. They are overt, covert, and forensic approaches [2]. This article reviews some forensic approaches, with a particular focus on multiple stable isotopic analysis (MSIA). Figure 1 lists the salient features of MSIA.

FIGURE 1

Salient features of multiple stable isotopic analysis

- Natural, non-radioactive isotopes
- Conducted on natural or ambient concentrations (nothing added)
- Internationally standardized (International Atomic Energy Agency)
- Green chemistry
- Statistically defined
- Digital (numerical) data
- Compatible with RFID

The FDA's three classes of pharmaceutical security are

Overt techniques. These protective measures are visible to the naked eye and include holograms, color-shifting dyes, and watermarks.

Covert techniques. These protective measures are invisible to the naked eye, and special equipment is often needed to detect them. They include watermarks, inks, and dyes that fluoresce or absorb ultraviolet (UV) light, invisible bar codes, radio-frequency identification (RFID), and secure business practices.

Forensic techniques. These protective measures use sophisticated analytical equipment to identify chemical markers, taggants, or other unique chemical properties of a substance. [**Author's note:** *I prefer the term "analytical" instead of "forensic." Forensic implies the commission of a crime, whereas analytical has no such implication. Therefore, I have substituted, as practicable, "analytical" for "forensic" in the remainder of this article.*]

At the moment, industry and governmental agencies are focused on the covert techniques of RFID and secure business practices [3]. RFID uses microchips to store data on the provenance of a given product. Inserting a microchip into pharmaceutical packaging enables one to track and trace the pharmaceutical product through electronic scanning. Secure business practices are methods that have the cumulative effect of continuously tracking the pathways of the materials to ensure that they are not diverted or altered.

There are several analytical approaches that characterize, or "fingerprint," pharmaceutical materials to authenticate them. They include, but are not limited to,

- analysis of organic impurities by mass spectroscopy;
- analysis of crystalline polymorphs by nuclear magnetic resonance;
- analysis of electromagnetic spectra by infrared (IR) or UV spectroscopic characterization;
- analysis of trace metals by inductively coupled plasma/mass spectrometry (ICP-MS);
- analysis of product-reacted, chemically sensitive dyes by spectroscopy; and
- analysis of natural stable isotopes by isotope-ratio mass spectrometry (IRMS).

This article focuses on IRMS analysis of naturally existing, low-molecular-weight, stable isotopes, such as those of carbon, nitrogen, hydrogen, and oxygen as they occur in their "natural" or ambient concentrations. These isotopes are intrinsic to virtually all pharmaceutical components, including active pharmaceutical ingredients (APIs), excipients, and medicines in final dosage form.

Stable isotopes

To understand the utility of stable isotopic ratio analysis via IRMS, one needs to understand stable isotopes. Stable isotopes were formed at the origin of the universe. They are simple mass variants of chemical elements whose masses are determined by the number of protons and neutrons in a given element's nucleus. For example, carbon-12 (^{12}C) is composed of six neutrons and six protons. For reference, ^{12}C accounts for about 98.89 percent of all natural carbon. By contrast, ^{13}C is composed of seven neutrons and six protons, and accounts for about 1.11 percent of carbon.

Other common stable isotope pairs include nitrogen (^{14}N and ^{15}N), oxygen (^{16}O and ^{18}O), hydrogen (^{2}H and ^{1}H), and so on. Some elements also have radioactive isotopes, but in practice these are only measurable in a few elements. Radioactive ^{14}C, for example, represents only about 1 part per trillion of all natural carbon. (By contrast, stable isotopes are non-radioactive and exist naturally in pharmaceuticals and in virtually all other common materials.) Therefore, nothing needs to be added to pharmaceutical products to isotopically trace them. The stable isotopes themselves become a batch-specific tracer of APIs and other substances.

Scientists have used stable-isotope ratios to trace the source, or "isotopic provenance," of natural materials since the 1950s, when isotope-ratio mass spectrometers first became available. Among the 112 elements, 62 are known to have at least 252 stable isotopes, yielding the potential for many different isotopic ratios that can serve as tracers. By choosing just three or four ratios of various stable isotopes in a certain product, an estimated upper limit of specificity that reaches one-to-millions, even one-to-billions, can be achieved.

Examples of the utility of stable isotopes include tracing different photosynthetic pathways that impart distinctive isotopic compositions to plant organic materials. These organic materials, as well as fossil fuel sources and inorganic materials, are the raw materials of all pharmaceutical components.

Answers to frequently asked questions about MSIA

What about "quality" matters? As mentioned in the main article, two factors determine a substance's "isotopic fingerprint:" raw materials and synthetic processes. For practical purposes, the isotopic variations observed in individual drug batches do not relate to drug quality, such as the drug's efficacy, trace contamination, etc. Instead, MSIA gives the overall manufacturing "fingerprint" or "isotopic provenance" of the pharmaceutical substance.

What about efficacy? Natural isotopic variations are so small that their effect on efficacy is immeasurably small. By their instrumental nature, stable isotopic measurements are much more precise than any means used to measure efficacy.

Is there radioactivity? No. By definition, stable isotopes are nonradioactive.

Why was the technique not recognized earlier? There was only one isotope-ratio mass spectrometer in the pharmaceutical industry in 1995, which indicates that natural stable isotopes were little known or used in this industry. Few pharmaceutical chemists have had any experience with natural or ambient levels of stable isotopes until recently.

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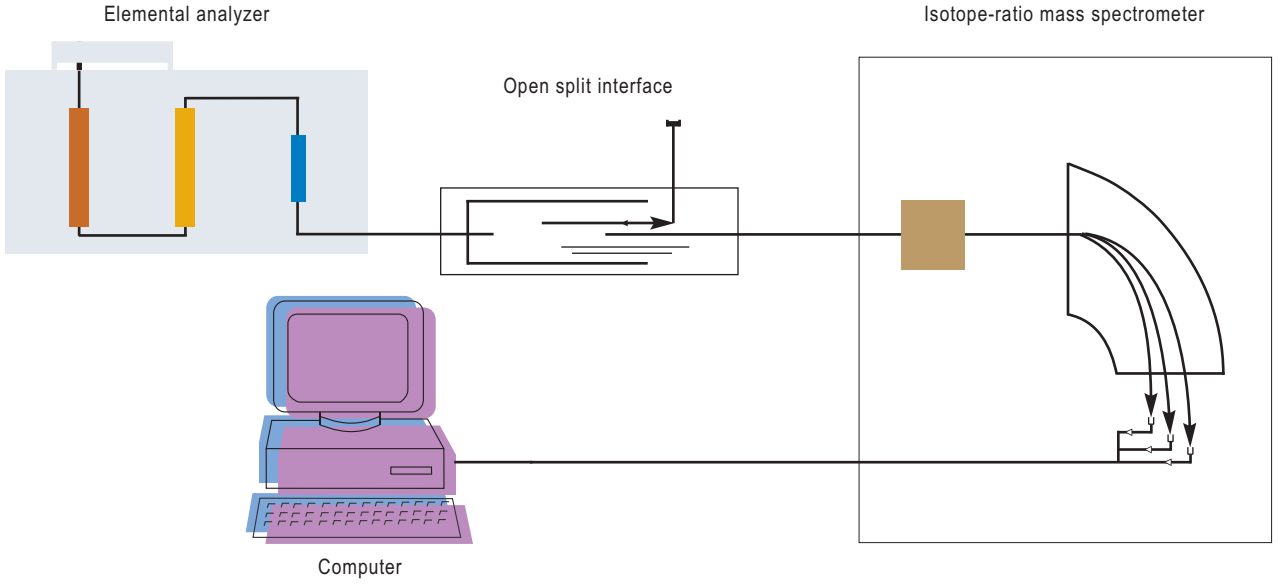
Analyzing stable-isotope ratios

The ratios of stable isotopes in any substance are rarely constant from sample to sample in nature. In fact, the isotopic ratios are typically highly variable. During batch production of pharmaceutical components, however, the raw materials become homogenized. As a result, each batch has a highly specific "isotopic fingerprint." Furthermore, only two factors affect the isotopic ratios in pharmaceutical components: the isotopic composition of the raw materials and the synthetic processes performed upon them. In the language of a chemist, highly specific isotopic ratios are caused by thermodynamic and kinetic processes. There are no other known means for change. To detect these ratios in pharmaceuticals, scientists often use an elemental analyzer/isotope ratio mass spectrometer (EA/IRMS). See Figure 2 [4].

A review of the statistics involved in MSIA shows how the utility of the present technique was developed for tracing pharmaceutical materials. First, let's examine the precision (or uncertainty) of the isotopic measurements. The precision of the isotope ratios (or any other parameter) measured in an analysis in which there were only a

FIGURE 2

Schematic drawing of a Finnigan elemental analyzer/isotope-ratio mass spectrometer (EA/IRMS)[1]



[1] The EA/IRMS analyzes nitrogen andcarbon isotopes. For analysis of hydrogen and oxygen isotopes, a Finnigan thermal conversion elemental analyzer/isotope-ratio mass spectrometer (TCEA/IRMS) is used.

handful (typically between one and five) replicates was recently described [5]. From that data, it's possible to calculate the pooled standard deviations (PSD) and the pooled standard errors (PSE). Next, it is possible to estimate "1σ error bars," and to calculate dynamic ranges (DR). The DR (observed isotopic range / 1σ PSD) is a dimensionless quantitative measure of the number of significantly different measurements that are possible for a given suite of samples with a specified instrument. For example, if a sample suite had a range of 10 per mil (10‰) and the instrument could measure 0.1‰, then the DR would be 100 (10‰ / 0.1‰).

Recent studies

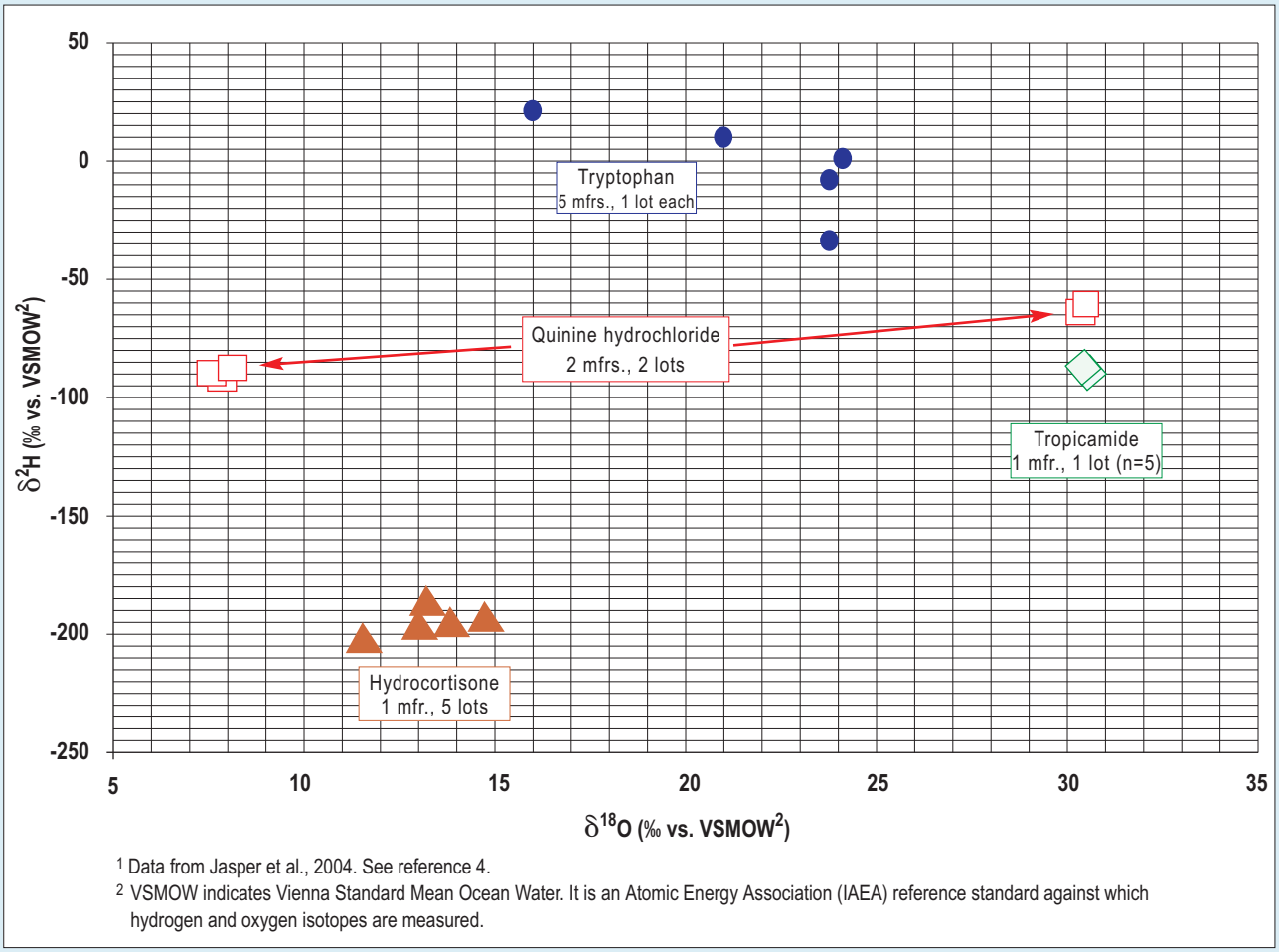
A recent stable-isotopic study performed in collaboration with the FDA's Division of Pharmaceutical Analysis in St. Louis, MO, showed that the isotopic provenance of 20 samples of four APIs could be well differentiated by manufacturer and by individual batches on the basis of their bulk isotopic fingerprints [4]. The ability to trace pharmaceuticals isotopically by the batch is shown in the results for 20 blind samples. See Figure 3. It shows a bivariate plot of oxygen ($\delta^{18}O$) and hydrogen ($\delta^{2}H$) isotopes. This approach is a significant advance in pharmaceutical authentication.

Another study, conducted in cooperation with the FDA, showed that analysis of the stable isotopic composition of a single API (naproxen) enabled the authors to determine differences in 26 blind samples [6]. While the six manufacturers of the API were located in four different countries (Italy, India, Ireland, and USA), it was impossible to differentiate according to the source country. That is to be expected since isotopes are fractionated only by thermodynamic and kinetic processes, not by geography or political processes.

Given the small number of stable isotopes typically analyzed, one may wonder whether the number of combinations is too limited. There is no need for concern. For the sake of analogy, consider a combination lock with four tumblers and ten digits on each tumbler. It has 10^4 (10,000) possible combinations. Similarly, for scale, an isotopic "combination lock" has four isotopes (C, H, N,

FIGURE 3

Bivariate plot of the oxygen-18 (^{18}O) and hydrogen-2 (^{2}H) stable isotopic composition of four APIs (tropicamide, hydrocortisone, quinine hydrochloride, and tryptophan)[1]



[1] Data from Jasper et al., 2004. See reference 4.

[2] VSMOW indicates Vienna Standard Mean Ocean Water. It is an Atomic Energy Association (IAEA) reference standard against which hydrogen and oxygen isotopes are measured.

O), and each of these isotopic "tumblers" has a dynamic range of 100 "digits." That means there is a statistical upper limit of 100^4 (100 million) possible isotopic combinations. Chemists working in the field of pharmaceutical isotopes agree that it would cost more to counterfeit a given isotopic combination accurately than it would cost to purchase the product legally. Thus there is virtually no possible incentive for identity fraud by replicating isotopic combinations.

Conclusion

Overt techniques have a long history in the pharmaceutical industry, and covert techniques have drawn recent attention. Analytical techniques, however, are just now becoming a major area of focus [1]. MSIA offers a method that is digital (numerical), precisely defined, and internationally standardized by the International Atomic Energy Agency (IAEA). Stable isotopic tracers are natural, pre-existing, and ubiquitous in virtually all pharmaceutical materials. Stable isotopic analysis provides highly specific identification of individual batches of pharmaceutical materials. Objective identification of these materials can help mitigate five major problems that are recognized by pharmaceutical manufacturers and regulatory agencies: counterfeiting, diversion, vicarious liability, theft, and patent protection. T&C

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