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Stable Isotopic Characterization of Analgesic Drugs

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Stable isotopic analyses of drug substances are used to identify individual batches of drug substances, thus minimizing the likelihood of counterfeiting, international countertrading, theft, vicarious liability, and patent infringement.

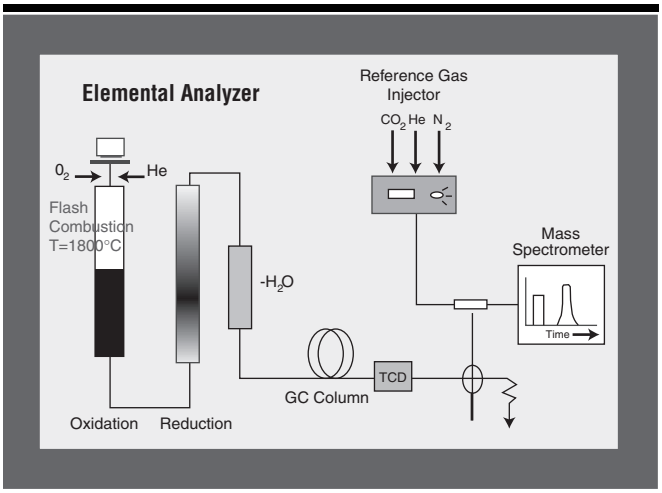

Figure 1: Schematic diagram of elemental analyzer–isotope-ratio mass spectrometer in combustion mode.

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The establishment of chemical homogeneity is a critical goal in manufacturing drug substances and drug products, which are collectively referred to as *pharmaceutical components*. By contrast, manufacturers make no known effort to control these substances' and products' stable isotopic compositions. Because stable isotopes typically are used to characterize the natural materials' origins (1–8), it seems plausible that stable isotopes can be used to quantitatively characterize, or *fingerprint,* individual pharmaceutical batches because they ultimately are derived from natural materials. In fact, this practice and other forensic techniques were topics of particular interest at the recent American Chemical Society's "Pharmaceutical Authentication and Forensic Analysis" meeting in Tampa, Florida (9).

Counterfeiting of pharmaceutical drugs threatens consumer confidence in drug products and pharmaceutical companies' economic well-being. The article explores the potential use of bulk isotopic analyses as a highly specific means to characterize products as they leave the manufacturer and are distributed on the market. The characterization of a product's ambient batch-to-batch stable isotopic variation can be used to differentiate among individual drug batches and subsequently identify counterfeits on the market.

Many fields use isotopic fingerprints to quantitatively characterize various materials (8). Analyzing a wide range of stable isotopes multiplies a given material's specificity of characterization. In cases in which two or more isotopes in a given material are largely independent of each other, specificity (*i.e.,* the ability to differentiate among sources) can be estimated as the product of the analyzed, individual isotopes' dynamic ranges (observed range/1σ analytical variability).

To test for the isotopic variability required to fingerprint ethical pharmaceutical components, the carbon-, oxygen-, and hydrogen-isotopic composition of four over-the-counter analgesic drug products were examined.

Experimental

Samples. The analyzed samples consisted of four commercially available analgesics: two types of acetaminophen and two types of aspirin (see Table Ia–b). The samples were purchased over

the counter from pharmacies in southeastern Connecticut. For simplicity, the samples within any set of drug products are numbered according to their expiration dates. Each isotope's data point corresponds to a different lot number.

Methods. In the preliminary screening, samples of four analgesics were powdered. Their ^{18}O:^{16}O, deutrium:hydrogen (D:H) and ^{13}C:^{12}C ratios were measured by a continuous flow combustion:pyrolysis isotope ratio mass spectrometry using the "IsoPrime" mass spectrometer (Micromass UK, Manchester, UK). A schematic diagram illustrating the principle of the elemental analyzer–isotope ratio mass spectrometer configuration is shown in Figure 1. The typical pooled intrabatch 1σ precisions of these techniques are 0.1‰ for ^{18}O; 0.1‰ for ^{13}C; and 1.5‰ for D:H (7). The isotopic results are given in δ-values (parts per thousand differences from a standard) defined as

$$\delta X\,(‰) = \left(\frac{R_{smpl}}{R_{std}} - 1\right) \times 1000$$

in which R_{smpl} is the ratio (*e.g.,* ^{18}O:^{16}O, ^{13}C:^{12}C, D:H) of the sample material and R_{std} is the ratio of an International Atomic Energy Authority (IAEA) reference standard. For carbon, the standard is known as VPDB, and the ^{13}C:^{12}C ratio is the official zero point of the carbon–isotopic δ-scale. For oxygen and hydrogen, the standard is the Vienna Standard Mean Ocean Water (VSMOW) from the IAEA.

Specificity: the "combination lock" analogy. A combination lock's operational design is similar to using multiple stable isotopes when identifying materials. Such a lock has a set number of dials (d) and the same number of digits (n) on each dial, yielding n^d (*e.g.,* 10^4) combinations. As briefly noted, the dynamic range of a given isotope measurement is the observed range/1σ analytical standard deviation. If various stable isotope ratios occur somewhat randomly in a set of samples (*e.g.,* a set of drug product batches) because (a) either or both the isotopic composition of raw materials or (b) the degrees of fractionation imparted in production synthesis occur somewhat randomly, then the upper limit of specificity of their isotopic characterization can be quantitatively estimated as the product of the dynamic ranges ($D_1 \times D_2 \times \ldots D_x$) of the individual isotopic species. In the present study, the overall specificity would be expressed as ($D_{\delta^{13}C} \times D_{\delta^{18}O} \times D_{\delta D}$). Unlike a combination lock that has a set number of dials and digits on each dial, isotopic specificity is composed of a variable number of isotopes with dynamic ranges that vary in value from ~10 to 1500, with the latter depending on the observed isotopic range and the analytical standard deviations of the measurements (10). Such specificity estimates for pharmaceutical phases are statistical maxima because the isotopic composition of raw materials used in sequential batch production is not wholly random. Despite that caveat, high degrees of differentiation were found in the present suite of analgesic products.

Results and discussion

Stable oxygen, carbon, and hydrogen isotopic values of the four analgesic drugs are shown in Table I. In the whole sample suite, oxygen isotopic values span from 5.1 to 24.3‰ versus VSMOW ($\Delta\delta_{max}$ = 19.2‰). Hydrogen isotopic values span from –73.1 to –48.8‰ versus VSMOW ($\Delta\delta_{max}$ = 24.3 ‰). Carbon isotopic values span from –42.1 to –27.0‰ versus VSMOW ($\Delta\delta_{max}$ = 15.1‰). The isotopic ranges and other statistical parameters for each drug product are presented in Table Ia–b. Batch production-series records of $\delta^{18}O$, $\delta^{13}C$, and δD generally show episodic patterns with varying degrees of temporal continuity (see Figure 2).

Table Ia: Stable isotopic composition of four over-the-counter analgesic drugs.

Sample #	δD (‰ versus VSMOW)	$\delta^{18}O$ (‰ versus VSMOW)	$\delta^{13}C$ (‰ versus VPDB)
Acetaminophen 1			
1	–55.0	7.81	–28.0
2	–73.1	22.6	–30.3
3	–53.9	7.8	–28.8
4	–67.7	23.7	–30.5
5	–62.4	23.7	–30.5
6	–66.2	23.3	–30.4
7	–60.5	22.7	–31.1
8	–64.4	23.7	–30.9
9	–67.9	23.6	–31.2
10	–68.9	23.2	–30.9
11	–65.7	23.8	–31.2
12	–67.7	24.3	–31.1
13	–67.2	23.7	–31.2
14	–64.3	23.4	–31.2
15	–54.4	23.8	–31.0
16	–67.8	23.3	–31.0
17	–59.6	23.0	–31.0
18	–58.1	6.9	–28.8
19	–65.6	6.2	–27.8
20	–63.0	22.7	–30.6
21	–61.4	22.5	–30.8
22	–61.4	22.2	–30.7
23	–59.5	22.5	–31.1
24	–64.3	11.0	–28.7
25	–63.7	22.0	–30.5
26	–61.2	21.2	–30.6
27	–59.0	6.7	–28.2
28	–63.4	21.3	–30.5
29	–64.3	21.6	–30.4
30	–59.7	22.3	–30.5
Acetaminophen 2			
31	–57.1	5.1	–28.3
32	–70.2	14.3	–27.2
33	–56.5	16.1	–27.0

$\delta^{18}O$ records. The bimodal distribution of acetaminophen 1's $\delta^{18}O$ values (6–12‰ versus 20–25‰) demonstrate highly significant differences in either the isotopic composition of the raw materials or fractionation during synthetic processing (see Figure 2a). Because we do not know whether the two groups may have been manufactured at different sites, we cannot assess whether the raw materials have come from markedly different isotopic sources or whether they have been produced by different synthetic pathways. It seems less likely, however, that the isotopic fractionation of synthesis would vary episodically as much as 8–19‰ in what are generally well-constrained chemical processes. The isotopic fractionation imparted by particular synthetic pathways is a topic of interest for active pharmaceutical ingredients manufacturers as a potential means to mitigate patent infringement.

The other three analgesic-drug $\delta^{18}O$ records display a variety of results. The three samples of acetaminophen 2 show a large span in $\delta^{18}O$ ($\Delta\delta_{max}$ = 11‰). The seven samples of aspirin 1 show a rela-

Table Ib: Stable isotopic composition of four over-the-counter analgesic drugs.

Sample #	δD (‰ versus VSMOW)	$\delta^{18}O$ (‰ versus VSMOW)	$\delta^{13}C$ (‰ versus VPDB)
Aspirin 1			
34	–61.4	19.8	–41.9
35	–58.3	18.9	–40.7
36	–51.0	18.9	–39.9
37	–48.8	19.4	–39.8
38	–51.0	18.8	–38.4
39	–61.7	19.5	–42.0
Aspirin 2			
40	–59.6	18.5	–40.0
41	–56.5	22.0	–38.4
42	–55.4	18.6	–42.0
43	—	20.5	–40.0
maxium	–48.8	24.31	–27.0
minimum	–73.1	5.1	–42.1
range	24.3	19.2	15.1
1σ	1.5	0.10	0.10
dynamic range	16.2	192	150
specificity			4.69×10^5

Table II: Perspective on multi-isotope product integrity.

	Isotopic ranges (1σ precision)		
Isotope	**Typical*** **Δδ**	**Maximum**** **Δδ**	**Analgesic**† **Δδ**
$\delta^{13}C$	15(0.1)	140(0.01)	15.1(0.1)
δD	80(1.5)	624(0.2)	24.3(1.5)
$\delta^{18}O$	20(0.1)	160(0.02)	19.2(0.1)
Specificity††	3.6×10^6	349×10^6	469×10^3
log(specificity)	6.6	11.5	5.7

* Typical Δδ is taken to be the commonly occurring natural isotopic ranges (1–2) analyzed by an elemental analyzer/mass spectrometer (EAMS).
** Maximum Δδ is taken to be the maximum observed natural isotopic ranges (1) analyzed by a dual-inlet mass spectrometer.
† Analgesic Δδ represents one hypothetical combined analgesic drug based on the samples reported on in Table I analyzed by an EAMS.
†† Specificities are calculated as the product of the relevant dynamic ranges.

tively limited range of 1.0‰, thereby indicating a well-controlled process in terms of both the raw materials' isotopic composition and differential fractionation resulting from the synthetic processes. The $\delta^{18}O$ record for aspirin 2 is short and discontinuous with a range of 3.5‰.

$\delta^{13}C$ records. Although the $\delta^{13}C$ record of acetaminophen 1 spans only 3.2‰, its isotopic excursions oppose those in the $\delta^{18}O$ record. Acetaminophen 2's $\delta^{13}C$ values of (–28.3 to –27.0‰ versus VPDB) strongly overlap with those of acetaminophen 1. Aspirins 1 and 2 lie within generally overlapping ranges of –42.0 to –38.4‰ versus VPDB. The former generally displays an increasing isotopic value, while the latter record is discontinuous.

δD records. The acetaminophen 1 δD record spans 20‰ and is more episodic than either of its corresponding $\delta^{18}O$ or $\delta^{13}C$ records. This observation is consistent with the fact that different isotopes may have different sources than others and they are plausibly involved in different natural and synthetic reactions. Acetaminophen 1's δD record spans 19‰ and is generally concave up, unlike either its $\delta^{18}O$ or $\delta^{13}C$ records. The aspirin 1 record spans 12‰ and is concave down. Aspirin 2's δD record increases ~5‰ in the middle range of observed isotopic values.

Isotopic bivariate plots. Isotopic bivariate plots of carbon-, oxygen-, and hydrogen-stable isotopes are presented to demonstrate the diversity and clustering of isotopic signals (or specificity) contained within the present data set (see Figure 3). Each box on the graphs' grids represents an area of $2 \times 2\sigma$, yielding a visual illustration of the paired isotopic analyses' specificity.

$\delta^{13}C$ versus $\delta^{18}O$. The graph of the paired $\delta^{13}C$- versus $\delta^{18}O$-values shows a highly resolved data set in terms of measurement precision (see Figure 3a). Because of the large overall ranges of the measurements (~15 and ~20‰, respectively) and their relatively small precisions (~0.1 and ~0.2‰, respectively), their respective overall dynamic ranges are 150 and 192. The product of the individual dynamic ranges is an estimator of the combined analyses' specificity (*e.g.,* $150 \times 192 = 28{,}800$). Thus, if the carbon and oxygen isotopic raw materials of which these analgesic drugs are composed had no isotopic relationship to each other, then the chance that one would randomly reproduce the same set of carbon and oxygen isotopic values in another sample would be ~1 in 28,800. The specificity estimation is based on the assumption that both isotopic variables are not correlated. Although this assumption is not wholly correct in some cases because some paired isotope values cluster together, no apparent functional (*e.g.,* linear or other systematic) correlation exists between the $\delta^{13}C$ and the $\delta^{18}O$ values.

δD versus $\delta^{18}O$. The data on the δD versus $\delta^{18}O$ graph generally are scattered, enabling the differentiation of many individual samples, while a number of samples' isotopic values overlap with only a few neighboring samples. Although the overlap would prevent the differentiation of some small groups of samples, the examination of either isotopic value against a third isotopic value (*e.g.,* $\delta^{13}C$) would very likely permit the differentiation of most or all of the present samples. With a δD-dynamic range of 16.2 and a $\delta^{18}O$-dynamic range of 192, the overall specificity for this combined data set is 3110.

δD versus $\delta^{13}C$. The data on the δD versus $\delta^{13}C$ graph generally are concentrated in small clusters depending on their chemical compositions: aspirins to the left (low $\delta^{13}C$ values) and acetaminophens to the right (high $\delta^{13}C$ values). With a δD-dynamic range of 16.2 and a $\delta^{13}C$-dynamic range of 150, the overall specificity for the combined data set is 2430.

Isotopic trivariate plot: δD versus $\delta^{13}C$ versus $\delta^{18}O$. The data from the analgesic drug products examined in this study are combined in one isotope trivariate plot ($\delta^{13}C$, $\delta^{18}O$, δD, see Figure 4). The acetaminophen values occur in the upper part of the plot. The aspirin values are plotted in the lower left side. The isotopic values of the brand name analgesics (acetaminophen 1, aspirin 1) occurred in relatively concentrated clusters. The relatively few values of the generic analgesics spanned some of the larger clusters of brand-name products.

Condensing three dimensions into such a trivariate or ternary isotope plot yields

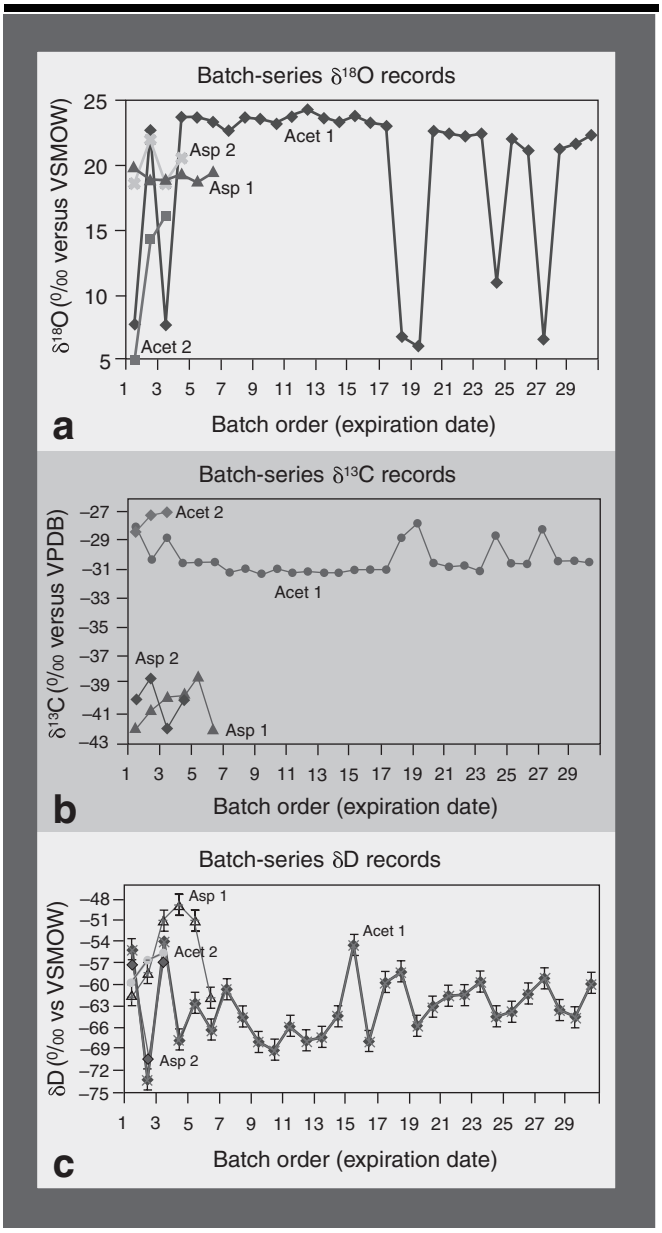

Figure 2: Batch-series records of three stable isotopes of four analgesic drugs (acetaminophens 1 and 2 and aspirins 1 and 2): (a) oxygen isotopic ($\delta^{18}O$) records, (b) carbon isotope ($\delta^{13}C$) records, and (c) hydrogen isotope (δD) records. The isotopic data are ordered according to expiration date, and each represents an individual batch of drug products.

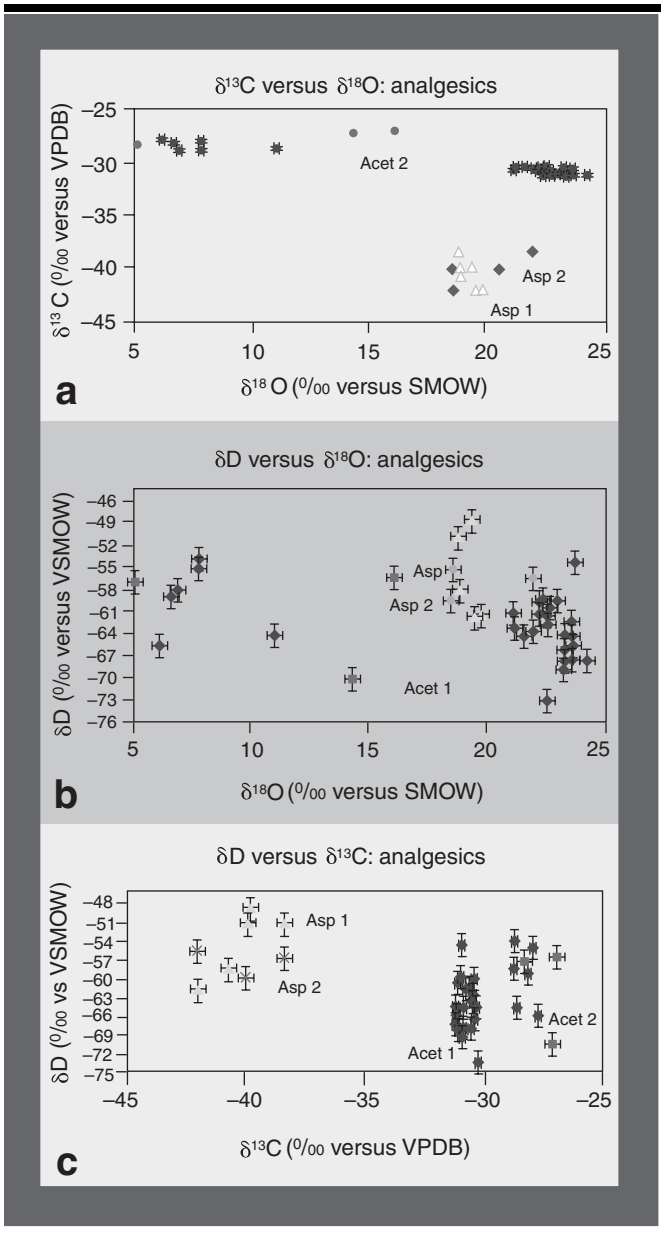

Figure 3: Bivariate isotope plots of the stable-isotopic compositions (*e.g.,* $\delta^{18}O$, $\delta^{13}C$, and δD) of four analgesic drugs (acetaminophens 1 and 2 and aspirins 1 and 2): (a) $\delta^{13}C$ versus $\delta^{18}O$, (b) δD versus $\delta^{18}O$, and (c) δD versus $\delta^{13}C$. The *X*- and *Y*-error bars are typical pooled 1σ estimates of uncertainty. The grid on each graph composed of $2\sigma \times 2\sigma$ boxes represents the error associated with each type of isotopic measurement, thus visually indicating the specificity of each measurement.

a very high content of information about the whole data set and has a very fine 1σ grid spacing. In fact, there would be ~469,000 1σ grid points on Figure 4, if all were shown. In compensation, the sizes of error bars in such a plot are extremely small—on average, only ~1% in each of the three dimensions—and are much smaller than the graph symbols. Similar plotting of other drug components' isotopic compositions in other recent studies has been a useful means to present the clustering of the three most common pharmaceutical stable isotopes ($\delta^{13}C$, $\delta^{18}O$, δD) in one plot, despite the loss of resolution of the uncertainty associated with each measurement.

General discussion. Although the analgesic batch samples are chemically homogeneous because they are manufactured according to precise protocols, the results of this study indicate that they are isotopically heterogeneous. Generally accepted explanations for the observed isotopic heterogeneity include: natural isotopic variation in the raw starting materials, including excipients (*i.e.,* thermodynamic fractionation) and isotopic fractionation during the synthetic processing of the active pharmaceutical ingredients in the drugs (*i.e.,* synthetic variation). Beyond individual isotope plots, the power of this isotopic prod-

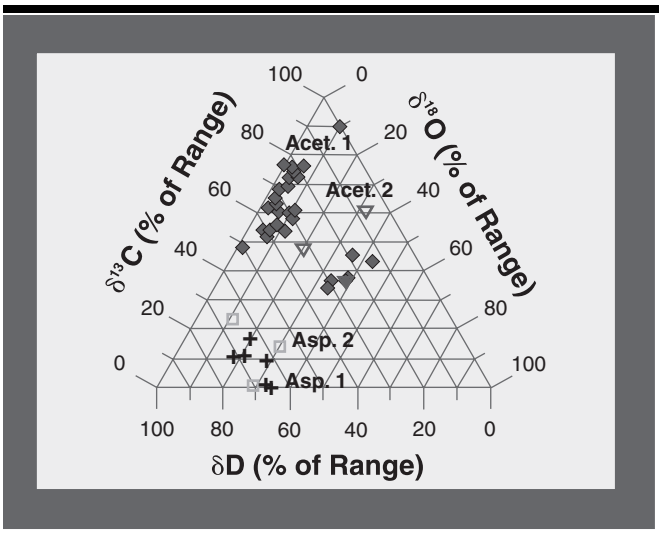

Figure 4: A trivariate or ternary plot of the stable-isotopic composition ($\delta^{13}C$, $\delta^{18}O$, δD) of all the analgesic drug products examined. The observed isotopic δ-values were normalized to 100% of their ranges, thus the units of the axes are all given in "% of range." Because of the fine grid spacing that derives from condensing three dimensions into two, the typical uncertainty on each measurement is ~+1%, markedly smaller than the graph symbols.

uct authenticity technique multiplies when data from the measurement of several different isotopes from the same sample are combined.

Although bivariate- and trivariate-isotope plots are straightforward forms of analysis, more sophisticated approaches such as principle component analyses have already been applied successfully in multi-isotopic approaches to product authentication. Those methods, however, typically require more data and constraints to be significant than in the present cases.

For perspective on the strength of compounding of the dynamic ranges of individual isotopes to produce high combined specificities, consider some plausible ranges of specificity generated by three isotopes (see Table II). The specificities presented are achievable with three isotope values ($\delta^{13}C$, $\delta^{18}O$, and δD) using typical isotopic ranges and analytical standard deviations. Termed *typical, maximum,* and *analgesic* cases, we estimate their respective specificities at 1.2×10^6, 349×10^6, and 469×10^3, depending on the observed isotopic range and the analytical standard deviation generated by two types of isotope–ratio mass spectrometers. Such high specificity is characteristic of a suite of samples that is well identifiable and virtually impossible to counterfeit. Using additional isotopes (*e.g.,* ^{15}N, ^{35}Cl, ^{34}S) would plausibly generate typical specificities of $\sim 10^{12}$ or more.

The relative impenetrability of the combination lock. With the very high specificities ($\sim 10^6$–10^{12} for three isotopes), it is highly unlikely that a given batch's isotopic combination would be randomly reproduced. In fact, it is generally agreed that it would be more costly to reproduce any drug product or drug substance with a prescribed isotopic profile than it would be to purchase the product legally with its manufacturer-imparted profile.

Conclusions

Although specific drug products may be manufactured according to precise protocols for chemical composition, they remain isotopically heterogeneous from batch to batch. Present data support the possibility of using these isotopic differences in a multi-isotopic approach for many kinds of batch-produced product identification, including that of prescription drugs, fine chemicals, munitions, and so forth. Such isotopic product authentication may plausibly become a powerful tool for patent protection and safety controls in drug manufacturing and distribution, thereby discouraging counterfeiting and counter-trading and decreasing manufacturers' exposure to vicarious liability.

Acknowledgement

This article is dedicated to Dr. Bob Williams, chief executive officer of Micromass UK (now, GV Instruments) who died after the substantive drafting of this manuscript. He is acknowledged for his early support of and insight into the utility of stable isotopic tracing of pharmaceutical components. The concepts of quantitative, multistable isotopic characterization of batch-mode produced products (*e.g.,* isotope product authenticity), and natural labeling are subject to pending patents in the G8 countries and in Australia held by Molecular Isotope Technologies, LLC (Niantic, CT).

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