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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 21–30
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Stable isotopic characterization of active pharmaceutical ingredients

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Received 8 July 2003; received in revised form 19 August 2003; accepted 20 August 2003

Abstract

Stable isotopic characterization or "fingerprinting" of active pharmaceutical ingredients (APIs) is a highly-specific means of defining the provenance of these pharmaceutical materials. The isotopic analysts in this study were provided with 20 blind samples of four APIs (tropicamide, hydrocortisone, quinine HCL, and tryptophan) from one-to-five production batch(es) from one-to-five manufacturer(s). Only the chemical identity of the APIs was initially provided to the isotopic analysts. Depending on the API chemical composition, isotopic ratios of either three or four elements ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and/or D/H) were measured by either elemental analyzer/isotope ratio mass spectrometry (EA/IRMS: carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$)) or by thermal conversion-EA/IRMS (TCEA/IRMS; hydrogen (δD) and oxygen ($\delta^{15}N$)); in all cases, the isotopic results are reported in the standard δ-notation which represents part-per-thousand (‰) variations from the isotopic ratios of international standards.

The stable isotopic analyses of the four suites of APIs spanned broad ranges in absolute value ($\Delta\delta$) and in estimated specificity (a product of dynamic ranges (DR, unitless)—note that these are upper limits of specificity because some of these isotope values may be partially interdependent). The five samples of tropicamide from one production batch and one manufacturer demonstrated the narrowest ranges ($\Delta\delta^{13}C = 0.13‰$; $\Delta\delta^{15}N = 0.52‰$; $\Delta\delta^{18}O = 0.24‰$; $\Delta\delta D$=2.8‰) and the smallest specificity of 1:30.9. By contrast, the five samples of tryptophan that came from five separate manufacturers had some of the widest isotopic ranges observed ($\Delta\delta^{13}C = 21.32‰$; $\Delta\delta^{15}N = 5.26‰$; $\Delta\delta^{18}O = 22.07‰$; $\Delta\delta D$=55.3‰) and had the largest specificity of $1{:}19.6 \times 10^6$. The isotopic provenance of the four suites of APIs readily emerged from bivariate plots of selected isotope ratios, particularly δD versus $\delta^{18}O$.

Keywords: Pharmaceutical materials; Active Pharmaceutical Ingredients; Stable isotopes; $\delta^{13}C$; $\delta^{15}N$; $\delta^{18}O$; δD; Isotopic fingerprinting

1. Introduction

Stable isotope ratios have been used as tracers of provenance or, source of natural materials since the 1950s when isotope-ratio mass spectrometers were first developed [1,2]. In fact, 62 of the 112 elements are known to have at least 252 stable isotopes,

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doi:10.1016/S0731-7085(03)00581-8

yielding numerous possible isotopic-ratio tracers. Stable isotopes have been used to characterize different photosynthetic pathways that impart distinctive isotopic compositions to various organic (plant) materials (e.g. [3,4]). Such contemporary organic materials, ancient fossil fuel sources, and inorganic materials are all used as raw materials in the production of active pharmaceutical ingredients (APIs) (drug substances), excipients ("inactive components"), and drug products (final dosage forms).

Purposeful misidentification of drug products or pharmaceutical components (APIs and excipients) threatens the efficacy of and consumer confidence in these commodities, as well as the economic well-being of pharmaceutical companies ([5] and references therein). Characterization of the ambient, or natural batch-to-batch stable isotopic variation of such materials provides a means to isotopically "fingerprint" individual API batches (or, lots). The use of bulk stable isotopic analyses (BSIA) of batch samples of APIs as a highly-specific means of identifying APIs will be explored as they are manufactured and compounded into drug products for distribution in the marketplace.

Recent reports have indicated and shown that the stable isotopic compositions of raw materials impart characteristic and highly-specific isotopic fingerprints in a suite of analgesic drug products [6–8]. In the analgesic-isotope study [8], three isotope ratios (δ^{13}C, δD, and δ^{18}O) were examined in 43 over-the-counter analgesic samples. Dynamic ranges (DR, unitless) of isotope measurements are defined as the observed isotopic range for a given suite of samples divided by the one standard deviation of the measurement (e.g. 10‰/0.1‰ = 100). Multiplication of the dynamic ranges for each of the isotope ratios in that sample suite yielded a specificity of 1:501,000. That is, to a first approximation, there is only ∼1 chance in 501,000 that the observed isotopic fingerprint would be found in a reproduction of the given product from raw materials with randomly-distributed stable-isotopic compositions of the raw materials. There is broad consensus that it would cost more to reproduce, or, counterfeit a specific isotopic fingerprint for a given API or drug product than it would to simply purchase the drug legally. While it is recognized that manufacturing processes do not wholly randomize isotopic compositions, the dynamic-range-based estimates of specificity are a plausible, first-order means by which to quantify the utility of isotopic characterization.

By way of background, there are presently a number of methods used to protect the brand identity of pharmaceutical materials, particularly marketed drug products. They span from extrinsic methods to intrinsic methods. That is, they span from packaging and labeling to the elemental and subatomic (viz. stable-isotopic) composition of the drug products. In the area of package labeling, both the drug-product carton and the immediate container may be marked for product security. Currency-quality printing on either the outer- or inner container acts to impede possible counterfeiting. Holographic images on both containers provide further deterrence. Characteristic stamping of pill shapes, specific colors and sometimes layering, laser-etching of logos are all part of extrinsic identification of drug products. Late-production, added "taggants" have been used in various non-pharmaceutical products, but such added materials are unlikely identifiers of pharmaceutical components whose composition are closely controlled by regulatory authorities. Because pharmaceutical materials are approved on the basis of closely-controlled processes and compositions, it is highly unlikely that an exogenous taggant would be added to a regulatory-authority-approved drug product.

We will show here that the stable-isotopic composition is highly specific to individual batches of pharmaceutical materials. Likely mechanisms for this batch-to-batch variation include equilibrium (thermodynamic) fractionation, kinetic fractionation, or a combination of both mechanisms. Although the present study is one of stable-isotopic products (without examination of the isotopic pathways by which the APIs were determined), it is reasonable to infer that they are substantially the result of (photo-)synthetic processes that produced the raw materials. As is common to isotopic studies, we have begun by surveying the isotopic composition of a suite of bulk-product materials of interest. Further mechanistic studies on the processes that determine the isotopic composition of resultant products (APIs, excipients, and drug products) are planned.

Recent radiocarbon measurements of drug products may plausibly generate a highly-specific criterion (^{14}C/^{12}C) for product identification [9]. When all of the material is derived from a single source (i.e. not

mixed modern and ancient sources), one can determine the natural radiocarbon concentrations of the raw materials and the resultant products. In addition, in combination with the additional specificity of stable-isotopic characterization, these techniques could very specifically determine the identity of materials produced in the batch mode such as pharmaceutical materials discussed here, and biological, batch-produced bacteria such as Anthrax, etc.

In this study, the stable-isotopic composition of a suite of APIs were examined via isotope ratio mass spectrometry (IRMS) to determine whether IRMS can distinguish between (i) APIs produced by different manufacturers and (ii) different batches of the same API produced by the same manufacturer. Depending on their presence, as many as four isotopic ratios (δ^{13}C, δ^{15}N, δD, and δ^{18}O) of APIs were examined and their results presented statistically and graphically. From calculations of the isotopes' respective dynamic ranges, we will estimate the specificity of this suite of API samples.

2. Experimental

2.1. Samples

Twenty samples of four APIs from up to five manufacturers with as many as five unique batches (lots) each was analyzed by Isotech Laboratories Inc. (Champaign, IL, USA) to allow determination of their sources and isotopic provenance. In particular, four sample sets, each representing a particular API material, were created for this study. Each sample subset consisted of five identical vials of ostensibly the same material to be isotopically analyzed. The isotopic analysts were only given the pharmaceutical name of each of the four samples, but no other details regarding the source was given. Known only to the sample providers (viz. the US FDA) before the isotopic analysis, the sample sets were distinguished as follows:

- Set A (tropicamide): Five samples, one manufacturer, one batch.
- Set B (hydrocortisone): Five samples, one manufacturer, five unique batches.
- Set C (quinine HCl): Five samples, two manufacturers, same batch from each manufacturer for a total of two batches.
- Set D (tryptophan): Five samples, five different manufacturers, five unique batches.

2.2. Carbon and nitrogen isotope analyses

2.2.1. Sample preparation for δ^{13}C and δ^{15}N analyses

Individual samples of ~0.4 mg for δ^{13}C analysis and ~1 mg for δ^{15}N analysis were weighed and placed into tin boats that were crimped tightly around the analyte so as to exclude air that might interfere with nitrogen isotopic analysis.

2.2.2. Instrumentation

Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic analysis were performed with a Carlo Erba 1108 Elemental Analyzer interfaced via a Conflo II interface to a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer (EA/IRMS; Fig. 1a; ref. [10]). The EA operated with an oxidation furnace temperature of 1020 °C, reduction furnace temperature of 650 °C, and a packed-column temperature of 60 °C.

2.2.3. Units of stable isotopic measurement

Carbon isotopic results are typically expressed in δ-values (parts per thousand differences from international standards) defined as

$$\delta^{13}\mathrm{C}\ (‰) = \left(\frac{R_{\mathrm{smpl}}}{R_{\mathrm{std}}} - 1\right) 1000$$

where R_{smpl} is the $^{13}C/^{12}C$ ratio of the sample material and R_{std} is the $^{13}C/^{12}C$ ratio of an International Atomic Energy Authority standard (known as "VPDB" whose $^{13}C/^{12}C$ ratio has been defined as the official zero point of the carbon-isotopic scale). Other stable isotope ratios are analogously expressed. The observed isotopic ranges ($\Delta\delta$ in ‰) for all measured isotopes (C, N, O, H), the 1σ pooled standard deviations (±S.D. in ‰) and the resultant dynamic ranges are reported here.

2.2.4. Carbon (δ^{13}C) and nitrogen isotope (δ^{15}N) analyses

Typically, single-to-duplicate measurements of carbon (δ^{13}C) and nitrogen (δ^{15}N) were performed on each API sample, as indicated in Table 1. Thus, averages of two samples, as available, and their pooled

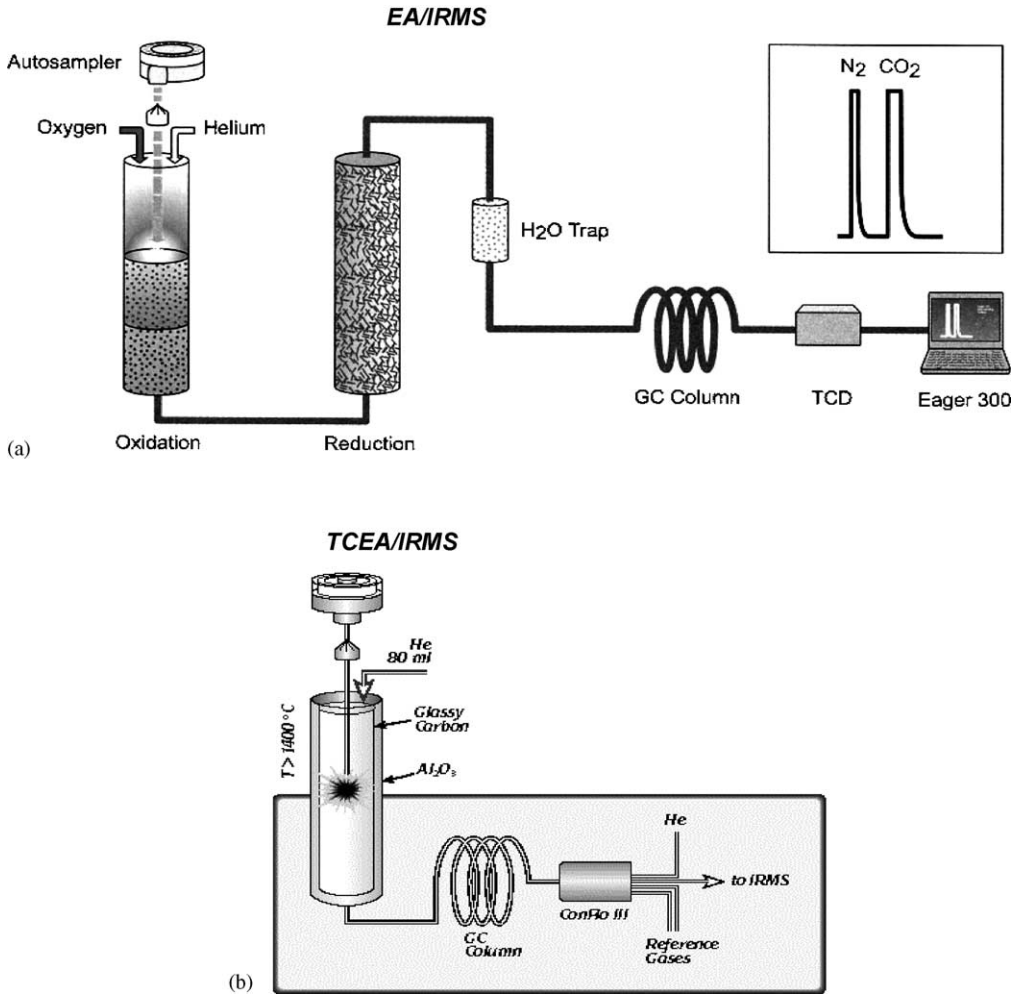

Fig. 1. Schematic drawing of (a) a Finnigan elemental analyzer/mass spectrometer (EAMS) and (b) Finnigan thermal conversion elemental analyzer/mass spectrometer (TCEAMS).

standard deviations [11] are reported here; in the case of one measurement, the individual value is reported with the appropriate pooled standard deviation. $\delta^{13}C$ values are reported relative to the international VPDB standard. $\delta^{15}N$ values are reported relative to atmospheric air, the international standard.

2.3. Hydrogen and oxygen isotope analysis

2.3.1. Sample preparation for hydrogen (δD) and oxygen ($\delta^{18}O$) isotope analysis

Individual samples of ~0.2 mg were weighed and placed into silver boats that had previously been dried in a vacuum oven for 1.5 h to remove surface moisture. The boats were crimped tightly around the analyte.

2.3.2. Instrumentation

Typically, single-to-duplicate hydrogen (δD) and oxygen ($\delta^{18}O$) stable-isotopic analyses of each of the API sample were performed on a Finnigan Thermal Conversion/-Elemental Analyzer (TCEA) interfaced to Finnigan Delta Plus XL isotope-ratio mass spectrometer (IRMS, thus a TCEA/IRMS; Fig. 1b). Analogous to a standard Elemental Analyzer/Isotope Ratio Mass Spectrometer (EAMS; ref. [10]), the TCEA functions with samples sequentially delivered into a

Table 1
Summary of the Isotopic Compositions of API Samples

Sample	All isotopic values given in ‰ vs. Stated standard								Range of Isotope Ratios				Dynamic Ranges				Specificity*
	δ^{13}C (VPDB)	C (S.E.)#	δ^{15}N (air)	N (S.E.)	δ^{18}O (VSMOW)	O (S.E.)	δD (VSMOW)	D (S.E.)	$\Delta\delta^{13}$C	$\Delta\delta^{15}$N	$\Delta\delta^{18}$O	ΔδD	^{13}C	^{15}N	^{18}O	D	(1:*X*)
A-1	−28.93	0.09	−4.51	0.10	30.45	0.12	−77.0	1.0	0.13	0.35	0.24	2.8	1.00	2.39	1.13	1.86	5
A-2	−28.98	0.09	−4.40	0.10	30.53	0.11	−79.8	1.0									
A-3	−29.05	0.13	−4.42	0.10	30.42	0.12	−77.4	1.0									
A-4	−28.93	0.13	−4.16	0.10	30.67	0.12	−79.4	0.7									
A-5	−28.93	0.13	−4.33	0.10	30.50	0.12	−78.6	1.0									
B-1	−28.38	0.03	No Nitrogen		14.05	0.08	−200.8	2.0	1.21		3.40	9.1	26.5		25.0	3.1	2080
B-2	−28.12	0.03	No Nitrogen		14.83	0.08	−199.3	2.0									
B-3	−28.46	0.03	No Nitrogen		13.44	0.07	−193.5	2.0									
B-4	−28.83	0.05	No Nitrogen		13.16	0.08	−201.9	2.0									
B-5	−29.33	0.03	No Nitrogen		11.43	0.07	−202.6	2.0									
C-1	−23.95	0.05	−1.99	0.12	30.45	0.14	−56.4	1.4	0.73	1.02	22.1	39.3	14.7	6.2	88.6	28.2	2.3E+05
C-2	−23.97	0.04	−2.03	0.09	30.53	0.12	−53.0	1.0									
C-3	−24.62	0.05	−1.35	0.12	8.88	0.11	−92.3	1.4									
C-4	−24.64	0.05	−1.31	0.12	8.45	0.11	−91.8	1.4									
C-5	−24.68	0.05	−2.33	0.12	9.11	0.14	−90.4	1.4									
D-1	−31.47	0.12	1.38	0.12	23.88	0.14	−33.8	1.2	21.3	5.26	7.71	55.3	130	32.0	30.7	33.4	4.3E+06
D-2	−17.06	0.12	−1.77	0.12	24.13	0.18	7.3	1.2									
D-3	−10.15	0.12	−2.64	0.12	21.18	0.14	15.4	1.2									
D-4	−19.70	0.16	−3.88	0.12	23.86	0.18	−3.0	1.2									
D-5	−30.20	0.12	0.80	0.12	16.42	0.14	21.5	1.2									
												Max. DR:	130	32.0	88.6	33.4	1.2E+07

Sample Key: A = Tropicamide; B = Hydrocortisone; C = Quinine HCl; D = Tryptophan.

SE: Standard Error (see text); Dynamic Range (DR) = Observed range/Pooled Standard Deviation (Table 2).

* Specificity = Product of all of the dynamic ranges of the observed isotope ratios.

furnace and the effluent gases analyzed by an online IRMS, but with pyrolysis (instead of oxidative combustion as in the EA/IRMS) performed at 1350 °C. The TCEA thermally converts analytes to H_2 and CO rather than combustion into H_2O and CO_2 as in the EAMS. The analyte gases, H_2 and CO, are chromatographically separated on a packed column at 85 °C. The mass spectrometer measures H_2 directly and ^{18}O in the form of CO.

2.3.3. Hydrogen isotope (δD) analyses

Typically, three measurements of hydrogen isotopic composition (δD) were performed on each API sample (Table 1). The first δD value analyzed here was ignored because of the hysteresis (memory) effects from the previous sample. Thus, averages of two measurements of each sample and their pooled standard deviations [11] are reported here. δD values are reported relative to the international VSMOW standard.

2.3.4. Oxygen isotope ($\delta^{18}O$) analyses

Typically, two-to-five measurements of oxygen isotopic composition ($\delta^{18}O$) were performed on each API sample (Table 1). Thus, averages of 3–5 samples and their pooled standard deviations (ref. [11]) are reported here. $\delta^{18}O$ values are reported relative to the VSMOW international standard.

2.4. Estimates of uncertainty

The uncertainty (or precision) of the isotopic measurements in this study is presented in two ways. Pooled standard deviations (S.D.) of raw data were made to derive a representative standard deviation from the whole raw data set in which small numbers of replicates (viz. $n = 1$–5) are pooled to derive an averaged standard deviation that is representative of the whole sample suite ([11]; Table 2). From those pooled standard deviations, standard errors (S.E.) are derived which scale the uncertainty of any given sample measurement to the number of times it was analyzed; more specifically, S.E. = S.D./(square root of n), where n is the number of measurements performed on a given sample ([11]; Table 1). Measurements of the A samples, which were all identical, serve as an index of the intrinsic methodological precision.

Table 2
Pooled S.D. (in ‰) by compound group

Compound*	^{13}C (1σ)	^{15}N (1σ)	^{18}O (1σ)	D (1σ)
A	0.13	0.15	0.21	1.5
B	0.05		0.14	2.9
C	0.05	0.16	0.25	1.4
D	0.16	0.16	0.25	1.7
Average	0.10	0.16	0.21	1.9

* Compounds as footnoted in Table 1.

3. Results and discussion

A summary of the stable isotopic compositional data of the APIs is given in Table 1 and the results are presented graphically in Fig. 2a–d. A statistical summary of the stable isotopic composition, pooled 1σ standard deviation, observed ranges (Δδ), and upper limit of specificity of the suite of four APIs is given in Table 2. Five samples of each of four APIs (tropicamide, hydrocortisone, quinine HCl, and tryptophan) were analyzed in varying degrees of replication ($n = 1$–6; average of ∼3). Upper limits of specificity are given to indicate the relative uniqueness of a given isotopic profile (i.e. the random chance that a given isotopic profile would be expected to recur). Note that the specificities range from 1:30.9 (for five batches of one product lot from one manufacturer) to $1{:}1.96 \times 10^6$ (for five batches of one product lot from five separate manufacturers). As previously suggested, a strict calculation of specificity can only be made on wholly independent variables. However, in considering the natural materials that might plausibly be contributed to pharmaceutical production, there are varying degrees of dependence between natural materials with high degrees of correlation from $\delta^{18}O$ and δD in plants (e.g. ref. [12] and references therein) to lower degrees of correlation of $\delta^{13}C$ to $\delta^{15}N$ in marine organic matter (e.g. ref. [13] and references therein). Unable to give a *unique* degree of specificity because of some potential interdependences, we will use estimated upper limits of specificity to parameterize the observation that exact (+1σ) reproduction of a given multi-isotope profile is highly unlikely. Recognizing the difficulty in synthesizing specific organic compounds with a predetermined and precise value for one isotope, it is widely agreed that it is virtually impossible to precisely and artificially set many

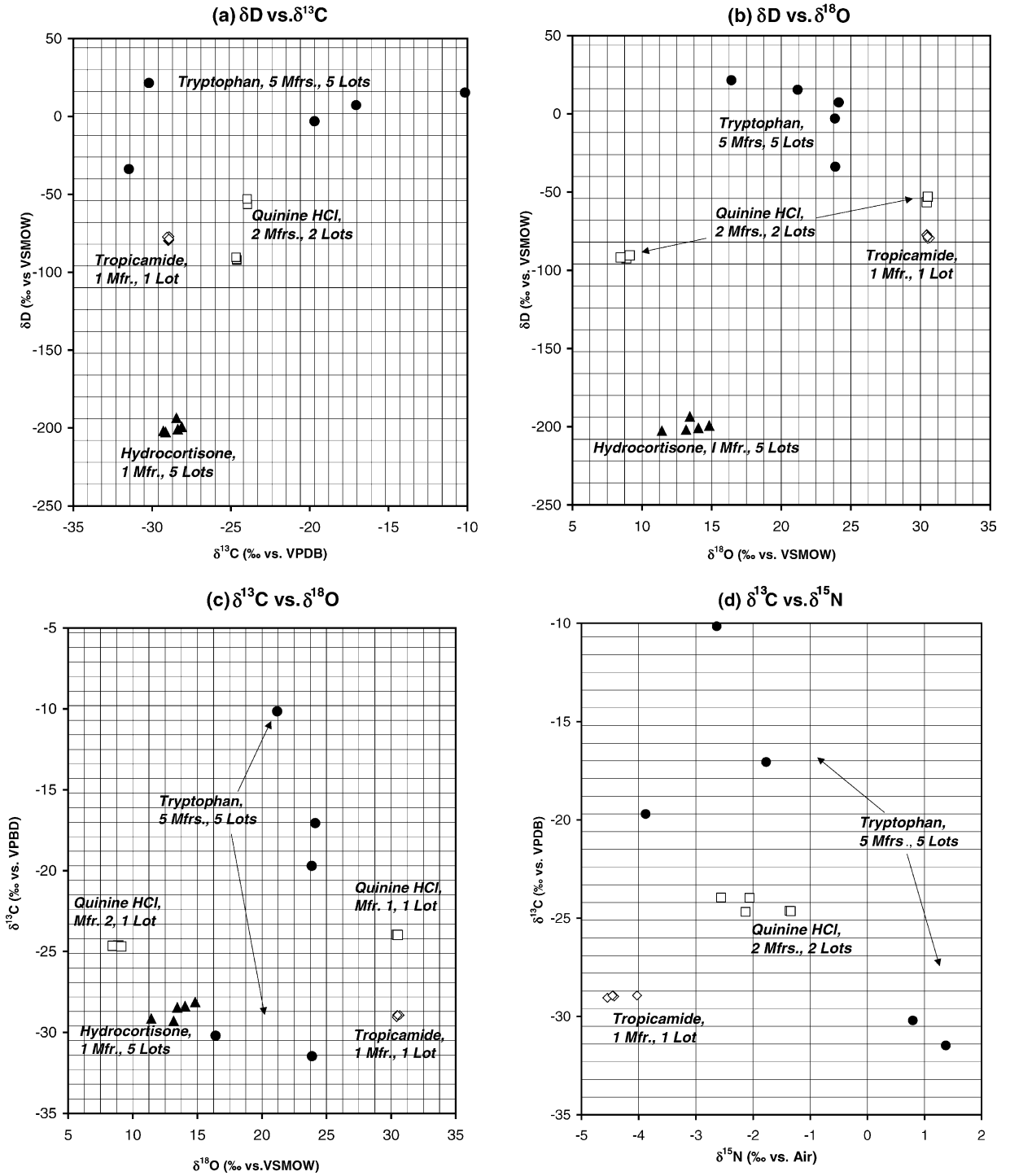

Fig. 2. Bivariate plots of stable isotopic composition of the API sample suite mentioned in Section 2. The plots grids show 10 times the pooled standard deviation for each isotopes.

(e.g. four) isotopes simultaneously in one compound. With that, it is generally agreed that it would be more costly to illicitly produce (counterfeit) a given pharmaceutical material than it would to obtain it legally.

Six bivariate isotope plots encompass all possible combinations of the four isotopes examined in the twenty samples of the four APIs (Fig. 2a–f). Similar relationships of provenance are observed in all six graphs. Typically, all the set A samples (tropicamide: five samples, one manufacturer, one lot) lie very tightly grouped by isotopic values, typically within a few standard deviation units (σ) of other points. The set B samples (hydrocortisone: five samples, one manufacturer, five lots) are relatively closely grouped, typically within 2–10σ. The set C samples (quinine HCl: five samples, two manufacturer, one lot from each) break into two subgroups that are broadly separated. The set D samples (tryptophan: five samples, five manufacturers) are all significantly separated from each other spanning many standard deviation units. In summary, the provenance of the four suites of APIs is apparent in the graphical distributions of their isotopic profiles.

3.1. Isotopic range and specificity

3.1.1. Carbon isotopes

Observed extreme ranges span from 0.13‰ for Tropicamide to 21.32‰ (versus VPDB) for tryptophan (Table 1). Using an averaged 1σ pooled standard deviation from each compound suite in the table, this sample suite exhibits a maximum dynamic range in δ^{13}C of 170. With a natural range of >140‰ [14], the present sample suite records only a small fraction (∼15%) of the naturally-occurring range.

3.1.2. Nitrogen isotopes

Observed extreme ranges span from 0.52‰ for tropicamide to 5.26‰ for tryptophan (Table 1). Using an averaged 1σ pooled standard deviation from each compound suite in the table, this sample suite exhibits a maximum dynamic range in δ^{15}N of 34.0. With a natural range ($\Delta\delta$) of >200‰ [14], the present sample suite records only ∼2.6% of the natural range.

3.1.3. Hydrogen isotopes

Observed extreme ranges span from 2.8‰ for tropicamide to 55.3‰ for tryptophan (Table 1). Using an averaged 1σ pooled standard deviation from each compound suite in the table, this sample suite exhibits a maximum dynamic range in δD of 33.4. Given the observed natural range of 624‰ [14], the present sample suite records only ∼8.9% of the natural range.

3.1.4. Oxygen isotopes

Observed extreme ranges span from 0.24‰ for tropicamide to 22.07‰ for quinine HCl (Table 1). Using an averaged 1σ pooled standard deviation from each compound suite in the table, this sample suite exhibits a maximum dynamic range in δ^{18}O of 88.6. With a natural range of ∼160‰ [14], the present sample suite records only ∼14% of the natural range.

The relatively small range of isotopic values expressed in this sample suite (<2.6–15%) of the natural range shows that only a fraction of the possible total dynamic range for this suite of four isotopes is being expressed here. This observation indicates that there remain broad isotopic ranges that could yet be used for isotopically-identifying this suite of samples, with a concomitant increase in specificity.

Isotopic data for and calculations of specificity shown in Table 3 give a quantitative perspective on the scale of specificity achieved in the present study as compared to other plausible isotopic ranges. Since different types of mass spectrometers have intrinsically-different measurement precisions which

Table 3
Multi-isotope specificity for product integrity

Isotope	Typical[a] $\Delta\delta$	Maximum[b] $\Delta\delta$	Maximum[c] $\Delta\delta$ of APIs[c]
δ^{13}C	15 (0.1)	140 (0.01)	21.3 (0.10)
δD	80 (1.0)	624 (0.2)	55.3 (1.9)
δ^{15}N	10 (0.1)	200 (0.02)	5.3 (0.16)
δ^{18}O	20 (0.1)	160 (0.02)	22.1 (0.22)
Specificity[d]	2.4×10^8	3.5×10^{15}	2.3×10^7
Log (specificity)	8.4	16	7.4

[a] "Typical $\Delta\delta$" are commonly-occurring natural isotopic ranges [1,2] analyzed by an EAMS (with typical S.D.s).

[b] "Maximum $\Delta\delta$" values are maximum observed natural isotopic ranges [14] analyzed by dual-inlet mass spectrometers (with typical S.D.s).

[c] "Max. $\Delta\delta$ of APIs" represents a hypothetical combined API based on the samples reported in this study analysed by an EAMS (with average S.D.s from Table 3).

[d] Specificities are calculated as the product of the relevant dynamic ranges, and dynamic ranges are the ratio of the observed isotopic range/1σ standard deviation.

may differ by an order of magnitude (see table), estimates of specificity may greatly vary: investigators should consider the type of instrument that is most appropriate for their research. Viewed in an aggregate sense for purposes of discussion, these isotopic data for calculations of specificity show the many orders of magnitude (viz. 7.4) are spanned for four sets of isotopic results presented. The specificity of typically occurring organic matter composed of C, N, H, and O is estimated at 2.4×10^8 (based on typical geochemical isotopic data (e.g. ref. [2])). An extreme high specificity of 3.5×10^{15} could be achieved by analysis of isotopically-exotic, naturally-occurring organic matter. The composite suite of four APIs yields a specificity of 2.8×10^7, which while relatively small in comparison to the preceding values, exceeds a typical threshold-for-proof for some physical evidence (1 in 10^5) in criminal matters. While the determination of that threshold is qualitative, it is inarguable that the observed specificity limits the possibility of random—or even intentional attempts at—reproduction of a given pharmaceutical isotopic profile.

3.2. Application and utility of stable isotopic characterization of APIs

Stable isotopic characterization of APIs (and other pharmaceutical materials) provides an innate and highly specific identification for these products. Nothing is added to the pharmaceutical materials, thus avoiding a regulatory challenge regarding natural isotopic composition. Such identification could plausibly mitigate counterfeiting, countertrading (illegal transportation of pharmaceutical materials across national borders to take advantage of price differentials), vicarious liability (misdirected liability suits that seek to take advantage of misidentified pharmaceutical materials), theft, and patent infringement.

In application of such stable-isotopic techniques, we suggest that there are two modes: reactive and proactive. In the reactive mode, a pharmaceutical firm could await until an occurrence of a product of suspect identity occurred, then analyze the suspect material and then the stored reference library materials until they were satisfied whether or not the suspect material was one of their own, thus reacting to the suspected breach of identity. In the proactive mode, samples would be isotopically profiled shortly after their manufacture and the isotopic data would be tabulated and stored in a computerized matrix data format. When a suspect sample was found, it could be isotopically analyzed and its isotopic profile compared to the reference library values. If the isotopic profile of the suspect sample did not match any of the firm's tabulated results, one could plausibly infer that that the sample did not emanate from that firm. Further refined, side-by-side testing of suspect and bona fide materials would produce high-specificity determinations regarding the provenance of suspect materials.

3.3. Natural labeling

Scales of specificity (Table 3), spanning nearly nine orders of magnitude, demonstrate the potential for developing very highly-specific isotopic characterization of pharmaceutical products. In fact, rather than simply accepting the existing isotopic variation in pharmaceutical products that issues from a manufacturing plant (as we have done in this study), a pharmaceutical firm can take the initiative in establishing the isotopic fingerprints of its products by pre-determining the isotopic composition of its raw materials used in product manufacture, then selecting raw materials of known isotopic composition for synthesis of the products. In that way, the firm can substantially influence the isotopic composition of its products. *Natural labeling* of products should presently be unaffected by regulatory authorities because this process simply takes advantage of natural isotopic variations which are implicitly already acceptable. In fact, straightforward calculations on the *absolute* isotope-abundance variations associated with natural isotope variations in typical pharmaceutical materials are so very small (typically, hundredths of one per cent of the absolute abundance ratio), that it is unlikely that biological or chemical assays exist to measure changes in drug efficacy caused by natural isotopic variation in such materials.

In this study, we have simply analyzed the ambient stable-isotopic variations in pharmaceutical materials to quantify their variability and thereby estimate their specificity. We observe that the present suite of samples only represents a tiny fraction ($\sim$0.5 pptr) of the potential isotopic variability observed in nature. With that, we suggest that pharmaceutical and other manufacturers can purposefully take advantage of

pre-measured isotopic composition in raw materials and synthetic intermediates to substantially predetermine the isotopic profile of their pharmaceutical and other products. Such 'natural labeling' of products can, with very little effort and expense, serve to label their products, yet remain within the natural realm of isotopic variation.

4. Conclusions

Stable isotopic analysis of APIs (and drug products) provides a highly-specific means for identifying the provenance or source of these pharmaceutical materials. Graphical and statistical analyses of the present suite of four APIs show that their provenance (number of lots, number of manufacturers) is consistent with their stable isotopic profile. The compounding effect of individual dynamic ranges of a number of stable isotope values generates a very high degree of specificity for the materials. For all practical purposes, the multi-isotopic profile of any given API is so highly specific that it is virtually impossible to precisely (e.g. $\pm 1\sigma$) reproduce it, yielding a high degree of stable-isotopic product authenticity. There is broad agreement in the pharmaceutical community that it would cost more to try to reproduce a specific multi-isotope profile than it would cost to acquire the pharmaceutical product through legal channels.

Acknowledgements

The authors thank D. Coleman, T. Coleman and S. Pelphrey of Isotech Laboratories Inc. (Champaign, IL, USA) for their insights into and the production of the high-quality stable-isotope data used in this study. The authors thank D. Coleman and anonymous reviewers for helpful comments that markedly improved the manuscript. The concepts of quantitative, multi-isotopic characterization of batch-mode produced products ("Isotope Product Authenticity") and of "natural labeling" are subject to pending patents in the G8 Countries and Australia held by Molecular Isotope Technologies, LLC. The views presented in this article do not necessarily reflect those of the Food and Drug Administration.

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