Drug Development and Industrial Pharmacy, 2011, 37(1): 88–92

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI:10.3109/03639045.2010.492397

informa
healthcare

ORIGINAL ARTICLE

Stable isotope-labeled excipients for drug product identification and counterfeit detection

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Abstract

Purpose: Counterfeit drug products have become a major problem worldwide and a number of techniques to detect counterfeit products or reduce the potential for counterfeiting have been investigated. This study examined the use of stable isotope-labeled excipients in solid dosage forms as a method to identify drug products and to detect counterfeits. *Methods*: ^{2}H- and ^{13}C-glucose were used as model excipients and incorporated in wet granulated formulations at a variety of different isotopic ratios. The ratios of $^{2}H/^{1}H$ and $^{13}C/^{12}C$ in each product were then determined by isotope ratio mass spectrometry. *Results*: Results demonstrated the ability to detect the isotope-labeled glucose in both granules and tablets. *Conclusions*: It was possible to use the isotope ratios to differentiate between specific batches of granules, demonstrating the potential of this technique for in-product, batch-specific identification.

Key words: Anticounterfeiting method, in-product identification, labeled excipients, stable isotopes

Introduction

Pharmaceutical counterfeiting is a global concern and the incidence of such counterfeiting continues to increase. Counterfeit drug products include those containing no active pharmaceutical ingredient (API), the wrong API, or an incorrect amount of API[1]. The use of counterfeit drug products containing lower drug content is of particular concern for antibacterial agents because microbial resistance can develop from subtherapeutic dosing[2]. Even if a counterfeit product were to contain the correct medication in the correct strength, good manufacturing practices (GMPs) and other quality standards are likely not followed, further adding to safety concerns.

To detect counterfeit drug products, overt or covert identification features may be used and these include the use of markers or forensic analysis techniques or may involve coding information. Although many techniques employ such identification markers in the packaging, such as radiofrequency identity tags[3], it is desirable to provide identification systems within the dosage forms themselves. Implementing such in-product tags through the addition of new chemical identities would require significant regulatory involvement. The Food and Drug Administration (FDA) has issued a guidance for the incorporation of a substance into solid oral dosage forms for anticounterfeiting purposes[4], demonstrating the interest from the pharmaceutical industry for the implementation of such anticounterfeiting measures.

Elements such as hydrogen, carbon, nitrogen, and oxygen have nuclei that differ in the number of neutrons, and the difference can be readily detected with mass spectrometry. Because of differences in the natural abundance of these isotopes, compounds enriched in the less abundant isotopes have been widely used, for example, as metabolic tracers and even in the pharmaceutical industry to track production methods and sources of active pharmaceutical ingredient (API)[5–9]. The objective of this study was to investigate the use of stable isotope-labeled excipients in the preparation of tablets to allow for the identification and detection of counterfeit drug products based on their ratios of both ^{2}H to ^{1}H and ^{13}C to ^{12}C, using labeled glucose as the model excipient. Because of both the low amounts of labeled excipients required (here, less than 1 part in 2500) and their chemical equivalence to existing

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(Received 22 Feb 2010; accepted 6 May 2010)

excipients, it could be possible to introduce these tags into dosage forms with relatively uncomplicated regulatory involvement and/or limited developmental activities.

Material and methods

Materials

^{2}H-Labeled glucose [Glucose (6,6-$^{2}H_2$), 98% atom] and ^{13}C-labeled glucose [glucose ($^{13}C_6$), 99% atom] were purchased from Isotec (Miamisburg, OH, USA). Caffeine was used as a model drug and was purchased from Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ, USA). Microcrystalline cellulose (Avicel® PH 302) was donated by FMC Corporation (Newark, DE, USA). Polyvinylpyrrolidone (PVP) (Plasdone® K-29/32) was provided by ISP Technologies (Wayne, NJ, USA).

Methods

Preparation of ^{2}H- and ^{13}C-glucose granules

A 4.7% (w/w) solution of PVP was prepared in deionized water. To this solution, various amounts of ^{2}H- and ^{13}C-glucose were added. The PVP solutions containing the labeled isotopes were added dropwise to a blend of caffeine and microcrystalline cellulose to form a wet mass. This wet mass was then manually passed through a 20-mesh sieve. Granules were dried in an oven at 30°C overnight and stored in a plastic bag until analysis or further processing. Several batches of granules with different ratios of the stable isotopes were prepared and the target values of these batches are summarized in Table 1. A batch containing no labeled glucose served as the control.

Tablet preparation

Several batches of ^{2}H- and ^{13}C-glucose-labeled granules were compressed on a single station tablet press (Model 511-7, DT Industries, Bristol, PA, USA) connected to a digital transmitter (DAT 500Series, Precise Instruments Corporation, Woburn, MA, USA). The tablet press was fitted with concaved caplet shaped punches (19 × 8 mm). Tablets weighed approximately 400 mg.

Isotopic ratio measurements of ^{2}H- and ^{13}C-glucose

The extent of ^{2}H- and ^{13}C enrichment in granules and tablets was determined by continuous flow isotope ratio mass spectrometry[10–12]. Samples of 200–400 mg either of granules or from tablets were combusted and either oxidized (carbon) or reduced (hydrogen), and δ^{13}C and δ^{2}H values determined. Hydrogen isotope ratios were measured using the continuous flow high-temperature reduction method using a TC-EA coupled with a Delta Plus XL Thermo-Finnigan mass spectrometer (West Palm Beach, FL, USA)[11]. Carbon isotope ratios were measured using a Costech ECS 4010 Elemental Analyzer coupled with a Thermo-Finnigan Delta Plus mass spectrometer Valencia, CA, USA) through a CONFLO II interface. The graphite laboratory standard was calibrated against NBS 21, NBS 22, and USGS 24. The results are reported using the conventional δ notation; for carbon they are reported relative to Vienna Pee Dee Belemnite and for hydrogen relative to standard mean ocean water, expressed as per mille change:

$$\delta = \left(\frac{R_x - R_{\text{std}}}{R_{\text{std}}} \right) 1000,$$

where R is the ratio of the abundance of the heavy to light isotope, x denotes the sample, and std is an abbreviation for standard. Reproducibility in δ^{13}C was better than 0.1‰ and about 2‰ for hydrogen. As most organic materials have a lower $^{13}C/^{12}C$ ratio than the reference, the δ^{13}C values were negative unless ^{13}C-enriched glucose was added.

Statistical evaluation

Data are presented as the means of three to six replicates, with their SE. A one-way analysis of variance and the Holm–Sidak comparison were employed to determine whether significant differences existed in the data, with a $P < 0.05$ indicating significance. The analyses were performed using SPSS Sigma Stat Version 3.0 (SPSS Inc., Chicago, IL, USA).

Results

Detection of the enrichment in δ^{13}C and δ^{2}H due to isotope-labeled excipients in granules

Figure 1 shows the ratio of enriched to nonenriched hydrogen plotted against the enriched to nonenriched carbon for the granules prepared in this study. The values in each case are given as δ^{13}C or δ^{2}H as described

Table 1. Target values of ^{2}H- and ^{13}C-glucose granules.

	Batch number							
Drug/excipient	1	2	3	4	5	6	7	8
Caffeine (g)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
^{2}H-glucose (mg)	—	2.5	3.5	2.5	3.5	3.0	2.5	3.5
^{13}C-glucose (mg)	—	1.5	1.5	3.0	3.0	2.2	9.0	9.0
PVP (g)	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Deionized water (g)	15	15	15	15	15	15	15	15
Microcrystalline cellulose (g)	21.75	21.75	21.75	21.75	21.75	21.75	21.75	21.75

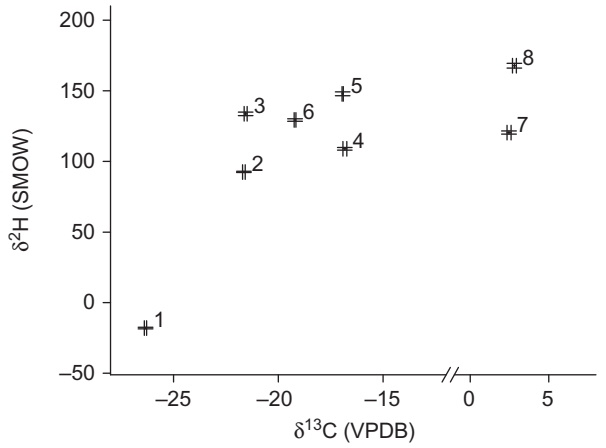

Figure 1. δ^{13}C and δ^{2}H values of batches 1–8 with SEs in both *x* and *y* axes shown.

Table 2. δ^{13}C and δ^{2}H values of 25 g granule batches as a function of added labeled glucose.

Batch ID	^{13}C-Glucose (mg)	^{2}H-Glucose (mg)	δ^{13}C (±SE)	δ^{2}H (±SE)
1	0	0	−26.33 ± 0.06	−18.0 ± 0.55
2	1.58	2.53	−21.64 ± 0.06	92.6 ± 0.34
3	1.58	3.51	−21.56 ± 0.08	133.6 ± 1.33
4	3.07	2.58	−16.83 ± 0.1	108.9 ± 0.88
5	3.01	3.51	−16.93 ± 0.03	147.8 ± 1.35
6	2.27	3.08	−19.21 ± 0.04	129.3 ± 0.94
7	9.04	2.57	2.47 ± 0.13	120.4 ± 1.13
8	9.02	3.54	2.82 ± 0.13	167.7 ± 1.65

in Materials and methods. Data are presented in Table 2. Sample 1 was the control, containing no added isotope material. As is normal for predominantly plant-derived materials, the values for both carbon and hydrogen were slightly negative[13]. Subsequent samples (2–8) contained various amounts of ^{2}H- and ^{13}C-labeled glucose. Adding these excipients shifted the δ to higher values, distinctive from naturally occurring materials.

Batch identification based on enrichment in δ^{13}C and δ^{2}H from isotope-labeled excipients in tablets

To test the ability of this isotope ratio mass spectrometric analysis technique to identify batch-specific products, four granulation formulations were tableted and delivered to the analyst in blinded containers. Table 3 shows the ratio of enriched to nonenriched carbon and hydrogen for these samples. Two tablets from each batch were tested, so variation between samples could be assessed. Importantly, three samples from each tablet were also tested to determine the intra-tablet variance.

The ratio data were evaluated to determine the batch from which the tablet samples were prepared. Using the δ^{13}C and δ^{2}H, samples A, B, and C were correctly identified as being produced from batch 2, batch 7, and batch 1, respectively. When examining the data from sample A, the δ^{2}H ratio demonstrated conclusively that the product came from batch 2 although if only the δ^{13}C ratio was used for the prediction, either batch 2 or batch 3 was the possible source. These data demonstrate the advantages of dual ^{2}H- and ^{13}C-labeling for identification.

Table 3. δ^{13}C and δ^{2}H values of unknown tablets.

'Unknown' sample ID	δ^{13}C	SE	δ^{2}H	SE
A1	−21.12	0.10	88.7	1.1
A2	−21.12	0.05	89.0	1.1
B1	2.01	0.21	117.6	0.8
B2	1.64	0.22	118.6	0.5
C1	−26.13	0.05	−20.1	0.6
C2	−26.20	0.02	−20.3	0.7

Discussion

Stable isotope ratios have been used to verify the legitimacy of a wide range of products and have been used to distinguish the production methods and sources of APIs[5–9]. Obviously, labeling an API could also be used as a technique to detect counterfeit drug products. Labeling an API, however, would be highly problematic from a regulatory perspective. In addition, research has shown that in vivo performance of APIs can be significantly altered by isotope labeling. In fact, such kinetic isotope effects have become a major focus of research activity to improve drug action[14,15]. In this study, isotope-labeled excipients for anticounterfeiting purposes were investigated as a potential, in-product, batch-specific identification technique. According to the FDA guidance, substances added to solid dosage forms as anticounterfeiting measures need to be pharmacologically inactive and toxicologically inert[4]. Excipients are considered to meet these criteria and thus, at least theoretically, the addition of isotope-labeled excipients should not create significant regulatory obstacles. Stable isotopes, because of their nonradioactive nature, have been shown to be safe in humans[16,17]. Moreover, one commercially available product (BreathTek™ from Otsuka, Rockville, MD, USA) has been approved by FDA for the diagnosis of *Helicobacter pylori* infection. More specifically regarding this study, Ruzzin et al. used large amounts of glucose with similar δ^{13}C values to those used in this study safely in a tracing metabolism study[18]. This study showed the practical applications of this technology in that each batch of drug product could be prepared with different amounts of stable isotopes to create a batch-specific identity through unique values of δ^{2}H and δ^{13}C. This approach has clear advantages over the more conventional technologies associated with external packaging. In addition, actual production of stable isotopes requires specialized facilities, which naturally limits access to such labeled excipients, thus making it more difficult for counterfeiters to acquire these chemicals.

As evident in the Results section, there is an inherent wider variance in the δ^2H values which could reduce the discriminating power between different batches using only δ^2H. Combining δ^2H and δ^{13}C, however, could enhance the usefulness of this technique in batch identification. Moreover, one could simply quantize the δ values. In other words, δ^{13}C units between 0 and 0.99 could be coded as A, 1–1.99 would be B; δ^2H would require larger quantization units, for example, 0–24 coded as α, 25–49 coded as β, and so on. An isotopic product code of Aβ would then be matched to a batch number or other identification device and isotope analysis would confirm whether dosage forms bearing that batch number were authentic or counterfeit. Should it be necessary, the exact chemical nature of the isotopic label could also be encoded [i.e., site(s) of deuterium/hydrogen replacement], and determined by LC–MS and this would provide additional layers of security.

In addition to using quantized δ values of ^{2}H- and ^{13}C-glucose, other stable isotope-labeled excipients could be used (e.g., ^{15}N- or ^{18}O-labeled compounds). Stable isotope-labeled compounds are becoming widely used for breath test and metabolic diagnostics, and so many suitable excipients are becoming increasingly available at GMP and European Pharmacopeia metabolic grades. For example, ^{13}C sodium bicarbonate (Cambridge Isotopes Laboratories Inc., Andover, MA, USA), ^{2}H-glucose, glycerol, and leucine, and ^{13}C-labeled leucine (EurisoTop, Saint-Aubin, France) are available to these specifications. In some cases, minor synthetic approaches would be needed to convert available compounds to accepted excipients, such as the conversion of ^{13}C-octanoic acid (Isotec) to tricaprylin by esterification or the conversion of ^{13}C-phenylalanine (Cambridge Isotopes Laboratories Inc.) to neotame. A wealth of simple label introduction chemistry is also possible using $H_2{}^{18}O$ that is widely available at GMP grade for ^{18}F production, and deuteration by exchange with D_2O is also facile. Although not a comprehensive list, it can be seen that stable isotope-labeled excipients are commercially available and many others could readily be made.

There are costs associated with any anticounterfeiting technique and the purchase of isotope-labeled excipients will impact the overall cost per unit dosage form. This study used ^{2}H- and ^{13}C-labeled glucose at a cost of approximately $50/g and $70/g, respectively. For ^{2}H-labeled product, the amount of glucose ranged between 0.01% and 0.014%. Thus, the ^{2}H-glucose would cost approximately $5–7/kg of final granulated material. Assuming a total tablet weight of 300 mg, the addition of the labeled glucose would add approximately 0.2 cents/tablet. The ^{13}C-labeled glucose was slightly more expensive and, using the same calculations and assumptions above, could add up to about 0.8 cents/tablet produced. Purchasing the labeled excipients in bulk could result in significant cost savings. Even so, these costs do not seem excessive for an in-product, batch-specific identification, although this technology may be most beneficial for labeling proprietary products with a high potential for counterfeiting.

An additional cost for implementing stable isotope-labeled excipients in counterfeit detection is the expense associated with product analysis. Currently, the isotope ratio mass spectrometry instrumentation needed to quantify isotopes is relatively large and expensive, although fee-for-testing service providers, already in existence, could be used for initial implementation of such anticounterfeiting measures. Smaller, more compact infrared sensors are being developed for the analysis of both δ^{13}C and δ^2H in CO_2 and H_2O, respectively. These units could be incorporated with a combustion device to convert the solid sample to CO_2 and H_2O vapor for subsequent analysis. Such analytical instrumentation could provide the basis for a portable device for use in the field.

Conclusion

The current study used ^{2}H- and ^{13}C-labeled glucose as stable isotope excipients as a method to identify counterfeit drug products. This study showed the ability of such labeled excipients in different ratios to provide in-product, batch-specific identification using existing technology in the pharmaceutical industry. Although stable isotopes have been administered to humans, regulatory perspectives are needed before this technology could be broadly applied.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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