Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 781–784

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Stable isotopic composition of the active pharmaceutical ingredient (API) naproxen

A.M. Wokovich[a,*], J.A. Spencer[a], B.J. Westenberger[a], L.F. Buhse[a], J.P. Jasper[b]

[a] *Food and Drug Administration, Center for Drug Evaluation and Research, Division of Pharmaceutical Analysis, St. Louis, MO 63101, USA*
[b] *Molecular Isotope Technologies, LLC, 8 Old Oak Lane, Niantic, CT 06357-1815, USA*

Received 12 November 2004; received in revised form 3 February 2005; accepted 11 February 2005
Available online 14 April 2005

Abstract

A survey of the multi-stable isotopic composition of an active pharmaceutical ingredient (API), naproxen, was performed to assess the potential of Isotope Ratio Mass Spectrometry (IRMS) to distinguish the provenance of APIs. Twenty-six lots of naproxen from six manufacturers representing four countries (Italy, India, Ireland, and the USA) were analyzed for three isotope ratios ($^{13}C/^{12}C$, $^{18}O/^{16}O$, and D/H). The samples were analyzed by either Elemental Analyzer/Isotope Ratio Mass Spectrometry (EA/IRMS: carbon ($\delta^{13}C$)) or by Thermal Conversion-EA/IRMS (TCEA/IRMS: hydrogen (δD) and oxygen ($\delta^{18}O$)). Bivariate and trivariate isotope ratio graphs for naproxen show marked clustering of the data for five out of the six naproxen manufacturers, suggesting that IRMS may be a plausible means to screen for manufacturer of given APIs.

Keywords: IRMS; Isotope Ratio Mass Spectrometry; Naproxen; API; Active pharmaceutical ingredient; Isotope ratio; $\delta^{13}C$; $\delta^{18}O$; δD

---

1. Introduction

The geographic or lot-specific identity of botanicals, active pharmaceutical ingredients (APIs), and finished dosages is important for the mitigation of counterfeiting, diversion, reimportation, theft, vicarious liability, and potentially for patent protection [1,2]. Purposeful misidentification of drug products or pharmaceutical components (e.g. APIs, excipients) threatens the efficacy of and consumer confidence in these commodities, as well as the economic well-being of pharmaceutical companies. Characterization of the natural lot-to-lot stable isotopic variation of such materials provides a means to isotopically fingerprint individual lots [1].

Stable isotope ratios have been used as tracers of the source or to provide the “isotopic provenance” of natural materials [1,3–7]. Isotope ratio spectrometry is an accepted technique for the detection of adulteration or establishment of authenticity in food products [7–11]. Isotope Ratio Mass Spectrometry (IRMS) has been used to characterize different photosynthetic pathways that impart distinctive isotopic compositions to various plant organic materials [12,13]. Many excipients used in drug manufacturing are derived from plant sources and so are also expected to show stable isotope ratios influenced by their source and means of production.

The stable isotopic composition of a suite of naproxen API lots was examined via Isotope Ratio Mass Spectrometry to determine whether this method can be used to distinguish the isotopic provenance. Naproxen, an anti-inflammatory drug, was chosen because it is a widely used active pharmaceutical ingredient and manufactured worldwide. In this study, three isotopic ratios ($\delta^{13}C$, δD, and $\delta^{18}O$) of naproxen were examined and their results presented graphically.

2. Experimental

The United States Food and Drug Administration, Center for Drug Evaluation and Research received 26 different lots

---

[*] Corresponding author. Tel.: +1 314 539 3874; fax: +1 314 539 2113.
E-mail address: wokovicha@cder.fda.gov (A.M. Wokovich).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.02.033

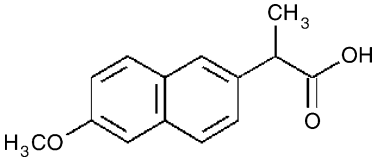

Fig. 1. Structure of naproxen ($C_{14}H_{14}O_3$).

of naproxen API ($C_{14}H_{14}O_3$; Fig. 1) from 6 manufacturers located in 4 countries (India, Italy, Ireland, and the USA) that were distinguished as follows:

- India, Manufacturer A—five different lots.
- India, Manufacturer B—five different lots.
- Italy, Manufacturer C—three different lots.
- Italy, Manufacturer D—three different lots.
- Ireland, Manufacturer E—seven different lots.
- USA, Manufacturer F—three different lots.

These samples were sent to Isotech Laboratories, Inc. (Champaign, IL, USA) as 26 unknown naproxen API samples. Isotech Laboratories, Inc. analyzed the unknown samples to determine their isotopic composition.

2.1. Instrumentation: $\delta^{13}C$

Carbon ($\delta^{13}C$) isotopic analyses were performed with a Carlo Erba 1108 Elemental Analyzer (EA) interfaced via a Conflo II interface to a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer [1]. 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. Instrumentation: δD and $\delta^{18}O$

Hydrogen (δD) and oxygen ($\delta^{18}O$) stable isotopic analyses of each naproxen sample were performed on a Finnigan Thermal Conversion-Elemental Analyzer (TCEA) interfaced to Finnigan Delta Plus XL Isotope Ratio Mass Spectrometer. Analogous to a standard Elemental Analyzer/Isotope Ratio Mass Spectrometer (EA/IRMS) [1], the TCEA functions with samples sequentially delivered into a 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. Sample preparation

Individual samples of ∼0.4 mg for $\delta^{13}C$ analyses were weighed and placed into tin boats that were crimped tightly around the analyte. Single-to-duplicate measurements of carbon ($\delta^{13}C$) were performed on each API sample.

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. The boats were crimped tightly around the analyte. Single-to-quadruplicate hydrogen (δD) and duplicate-to-quadruplicate oxygen ($\delta^{18}O$) measurements of each naproxen sample were performed.

2.4. Units of stable isotopic measurement

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

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

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 reference standard (known as “VPDB”, whose $^{13}C/^{12}C$ ratio has been defined as the official zero point of the carbon isotopic scale). Similarly, δD values and $\delta^{18}O$ values are reported relative to the international Vienna Standard Mean Ocean Water (VSMOW) reference standard.

2.5. Estimations of uncertainty

The uncertainties (or precision) of the isotopic measurements in this study are presented in two ways. Pooled standard deviations (S.D.) of raw data were made to derive a representative standard deviation from the entire raw data set for each isotope ratio. In this calculation, a number of replicates are pooled to give a standard deviation that is representative of the entire sample suite. The standard errors (S.E.) expressed in the table represent the decreased uncertainty in the values due to the multiple determinations. These are calculated as S.E. = S.D./(square root of n), where n is the number of measurements performed on a given sample [14]. The standard errors are shown for the points plotted on the bivariate plots (Fig. 2a–c).

3. Results and discussion

Table 1 summarizes the δD, $\delta^{13}C$, and $\delta^{18}O$ values of the 26 lots of naproxen from 6 manufacturers. Three bivariate isotope plots (Fig. 2a–c) encompass possible combinations of the three isotope ratios examined in this sample suite. Fig. 2a shows a plot of $\delta D/\delta^{13}C$, Fig. 2b shows a plot of $\delta^{13}C/\delta^{18}O$, and Fig. 2c shows a plot of $\delta^{18}O/\delta D$. All three bivariate plots and the trivariate plot (Fig. 3) show general concentration (or “clustering”) of sample points in relatively small spans of the total isotopic ranges ($\Delta\delta$), with one notable exception (one lot from Italian Manufacturer D). The clustering of the data indicates manufacturer-based isotopic provenance.