

Antiretroviral Drug Content in Products from Developing Countries

Scott R. Penzak,¹ Edward P. Acosta,² Michele Turner,² Jorge A. Tavel,³ and Henry Masur⁴

Departments of ¹Pharmacy and ²Critical Care Medicine, Warren G. Magnuson Clinical Center, and ³National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and ⁴Division of Clinical Pharmacology, University of Alabama at Birmingham

Generic and brand name antiretroviral drugs are becoming increasingly available in developing countries. We analyzed 6 antiretroviral medications from 4 international sources for drug content. The active ingredient in tested drug products was within 15% of the labeled amount (range, -12% to +15%) for drugs that were properly stored.

Combination antiretroviral therapy has dramatically reduced morbidity and mortality secondary to HIV infection [1]. In the developing world, at least 6 million of the 42 million HIV-infected patients are in urgent need of antiretroviral medications, yet because of the high costs, <300,000 are receiving treatment. However, generic antiretroviral medications, along with discounted brand name products, are quickly increasing the availability of these drugs [2, 3].

Although generic medications offer affordable treatment for many HIV-infected patients, little information is available regarding the integrity of these medications [4–6]. In a retrospective study, generic nevirapine-containing antiretroviral therapy appeared to be safe and effective in 333 HIV-infected patients in India [5]. We recently reported that several generic nevirapine formulations from 4 developing countries contained the labeled amount of drug ($\pm 3\%$ of 200 mg) [6]. Large-scale studies are necessary to assess all antiretroviral medications from international sources for drug content.

Because of the huge demand for and high cost of antiretroviral medications in developing countries, brand name drugs are a likely target for counterfeiters. In an isolated report, an

HIV-infected man living in Zimbabwe purchased zidovudine tablets that, upon analysis, were found to contain no zidovudine [7]. In addition, 60% of tested antimalarial preparations being sold in Cambodia were found to contain either no active ingredient or inferior substitutes [8]. To this end, we assessed drug content in comparison with label claims for 5 HIV protease inhibitors and the nonnucleoside reverse-transcriptase inhibitor efavirenz from various international sources.

Methods. Drug products were delivered to the National Institutes of Health (NIH; Bethesda, MD), and information on individual drug formulations was recorded (table 1). Drugs were transported to the NIH by physicians participating in an educational program. All products were received at room temperature and had been stored that way for at least several weeks. Upon receipt, saquinavir, ritonavir, and lopinavir-ritonavir were refrigerated, according to manufacturer specifications.

The Uniformity of Dosage Units test was used to assess the dose uniformity of the different antiretroviral formulations [9]. This test specifies that drug content in individual dosage units must be within 85%–115% of the label claim, unless otherwise specified in the drug's US Pharmacopeia/National Formulary (USP/NF) monograph. Saquinavir, the only drug product with a USP/NF monograph in this study, must contain 95%–105% of the label claim, according to its monograph [10]. The Uniformity of Dosage Units test also specifies that the coefficient of variation among dosage units must be $\leq 6.0\%$. Because of the limited number of capsules/tablets available for analysis, 2–4 dosage units (compared with the USP-recommended 10 dosage units) per lot number were assayed; the degree to which this limits the global applicability of our data, if at all, is unclear.

Intact capsules of efavirenz, indinavir, saquinavir, ritonavir, lopinavir-ritonavir, and amprenavir were weighed individually, and the total mass was recorded. After removal of the contents of each capsule, the mass of the empty capsule casing was recorded and subtracted from the total mass to determine the mass of the contents in each capsule and the percentage of active ingredient. Contents from standard capsules (efavirenz and indinavir products) were ground with a mortar and pestle, resulting in homogenous powders. Empty gel caps from saquinavir, ritonavir, lopinavir-ritonavir, and amprenavir formulations were rinsed with methanol and allowed to dry before weighing. Master stock solutions were then prepared by dissolving known weights of capsule powder in precise volumes of aqueous solvent. The exact concentration of active ingredient in each of the stock solutions was calculated using the determined percentage of active ingredient in each capsule. The

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Reprints or correspondence: Dr. Scott R. Penzak, Clinical Center Pharmacy Dept., Bldg. 10, 1N 257, National Institutes of Health, Bethesda, MD 20892 (spenzak@mail.cc.nih.gov).

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Table 1. Antiretroviral preparations analyzed for drug content.

Product ingredient(s) (labeled amount)	Trade name (manufacturer)	Country where product was obtained	Product identification code	Date of manufacture (expiration date)	No. of capsules tested (no. of assays per capsule)	Mean drug content, mg (coefficient of variation, %)	Average accuracy, ^a %
Efavirenz (200 mg)	Sustiva (Bristol-Myers Squibb/DuPont)	Lithuania	NA	NA (NA)	2 (6)	187.5 (4.3)	94
Efavirenz (200 mg)	Stocrin (Merck)	South Africa	HO14400	Apr 2003 (NA)	3 (6)	184.5 (5.9)	92
Indinavir (400 mg)	Crixivan (Merck)	Jamaica	NA	NA (NA)	3 (9)	429.3 (6.0)	107
Indinavir (400 mg)	Crixivan (Merck)	South Africa	HO14910	Mar 2003 (NA)	4 (9)	437.5 (8.8)	109
Indinavir (400 mg)	Crixivan (Merck)	South Africa	NA	NA (NA)	4 (9)	459.8 (8.2)	115
Indinavir (400 mg)	Indivex-400 (Aurobindo Pharma Ltd.)	Zambia	EX 0101	Nov 2001 (Oct 2003)	4 (9)	430.1 (8.1)	108
Lopinavir-ritonavir (133/33 mg) ^b	Kaletra (Abbott)	Lithuania	NA	NA (NA)	2 (6)	117.7 (6)	89
Lopinavir-ritonavir (133/33 mg) ^b	Kaletra (Abbott)	Lithuania	NA	NA (NA)	2 (6)	26.8 (10.1)	81
Amprenavir (150 mg)	Agenerase (GlaxoSmithKline)	South Africa	NA	31 Mar 2001 (NA)	3 (6)	138.5 (4.4)	92
Ritonavir (100 mg)	Norvir (Abbott)	Lithuania	NA	NA (NA)	2 (6)	83.5 (8.1)	84
Ritonavir (100 mg)	Norvir (Abbott)	South Africa	82169ua	Aug 2002	3 (6)	80.8 (6.2)	81
Saquinavir soft-gel capsules (200 mg)	Fortovase (Roche)	Lithuania	NA	NA (NA)	2 (6)	194.2 (4.3)	97
Saquinavir soft-gel capsules (200 mg)	Fortovase (Roche)	South Africa	B1407	May 2003 (NA)	3 (6)	197.4 (7.3)	99

NOTE. NA, not available.

^a Compared with the labeled amount.

^b Same product.

number of capsules sampled and the number of assays per capsule are shown in table 1.

For quantitative analysis, standards of saquinavir, ritonavir, lopinavir, amprenavir, indinavir, and efavirenz were used to prepare calibration and quality-control solutions. The saquinavir standard was provided by Roche Laboratories (Nutley, NJ), ritonavir and lopinavir standards were provided by Abbott Laboratories (North Chicago, IL), the indinavir standard was provided by Merck and Co. (Whitehouse Station, NJ), the efavirenz standard was provided by Bristol-Myers Squibb (Princeton, NJ), and the amprenavir standard was provided by Glaxo-SmithKline (Research Triangle Park, NC). Serial dilutions of each capsule master stock solution provided test solutions within the standard curve range of the assay, which was 25–9000 ng/mL for all drugs except for lopinavir, for which it was 50–9000 ng/mL. High-performance liquid chromatography/ultraviolet analysis of each test solution was performed using a validated assay [11]. The intraday and interday percentage error between nominal and observed concentrations was <10%, and the coefficient of variation was also <10%. Calculation of the concentrations of each test solution, followed by consideration of all dilutions made from capsule to assay, resulted in accurate quantification of active ingredient in the tested products (table 1).

Descriptive statistics were used for data reporting. The mean drug content (table 1) was calculated as the arithmetic mean of the drug amounts from each dosage unit within a given lot.

The drug content for individual dosage units was calculated as the arithmetic mean of multiple (6–9) assays. Average accuracy was determined by dividing the mean drug content by the labeled amount (percent error). The coefficient of variation (relative SD) was used to assess variability among the individual dosage units.

Results. A detailed ingredient analysis, along with specific product information for each of the tested medications, is shown in Table 1. With the exception of ritonavir-containing products, the active ingredient in each of the products was within 15% of the labeled amount (range, –12% to +15%); the absolute value of the mean difference between measured and labeled drug content was 7.7%. The median difference was –2.0%. For efavirenz, indinavir, lopinavir, saquinavir, and amprenavir, expiration dates were available for 5 of the 9 lot numbers sampled (table 1); among these products, only amprenavir (–7.7%, compared with the labeled amount) was analyzed after its expiration date. The coefficient of variation among capsules in individual lots was <9.0% for amprenavir, saquinavir, lopinavir, indinavir, and efavirenz.

Ritonavir content was –19% to –16% of the labeled amount in 1 lopinavir-ritonavir product and 2 ritonavir products (table 1). One of the ritonavir products was analyzed ~1 year after it had expired; expiration dates were unavailable for the other ritonavir product and the lopinavir-ritonavir product. As noted earlier, none of the ritonavir-containing products were stored under continual refrigeration, in accordance with manufacturer

specifications. The coefficient of variation among capsules in individual lots was <10.2% for ritonavir.

Discussion. In this pilot investigation, the active ingredient(s) in several branded and generic antiretroviral medications was quantitated. In accordance with USP standards, none of the products, when stored according to manufacturer specifications, varied by <12% or >15% from the labeled drug amount [9]. Coefficients of variation ranged between 4.3% and 10.1% for all of the tested samples and exceeded the USP-specified limit of 6.0% in 6 of the samples. The relatively few number of dosage units available for analysis likely contributed to this degree of variability, as did the inherent variability in the assay (coefficient of variation, <10% for all drugs). Accordingly, our ability to accurately assess variability with regard to USP specifications is limited.

Saquinavir soft-gel capsules from 2 international sources contained 97%–99% of the labeled drug amount, which satisfied individual USP criteria for saquinavir capsules (95%–105% of the label claim), despite not having been refrigerated in accordance with manufacturer instructions. Similarly, none of the ritonavir-containing products were stored under continual refrigeration; moreover, one of the products had expired. Thus, it is not surprising that ritonavir content did not meet USP content specifications (table 1). These data reinforce the importance of storing ritonavir-containing preparations according to manufacturer specifications. This may be particularly true in warmer climates where the ambient temperature is rarely <25°C (<77°F).

In our analysis of branded and generic antiretroviral medications, the drug content of generic indinavir (Indivex-400; Aurobindo Pharma) was comparable to that of branded products (Crixivan; Merck) and is consistent with USP content specifications (table 1). These data are similar to those we recently reported for generic nevirapine formulations obtained from developing countries [6]. These results are a source of encouragement for HIV-infected patients in developing nations who are relying on generic medications for treatment of their disease. However, these data describing drug content are not meant to imply bioequivalence between generic and branded products. In the future, bioequivalence testing will provide further data on the integrity of specific generic antiretroviral products.

In addition to generic antiretroviral medications, studies to determine drug content among branded products from developing nations are also necessary to prevent the dissemination of counterfeit drug products in the developing world. High cost, coupled with significant demand, suggests that antiretroviral medications may be of particular interest to counterfeiters. Numerous examples exist in which drug products have

been mislabeled, diluted, or substituted with less expensive ingredients [8].

Although the growth in antiretroviral availability is encouraging, it must be accompanied by independent quality-control studies such as this preliminary investigation, not conducted by the sponsoring pharmaceutical company. These investigations, along with bioequivalence testing, are crucial to the optimal care of HIV-infected patients in developing countries.

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References

1. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* **1998**; 338:853–60.
2. UNAIDS Fact Sheet. Access to HIV Treatment and Care. September **2003**. Available at: http://www.unaids.org/html/pub/Publications/Fact-Sheets03/FS_Care_2003_en_doc.htm. Accessed 29 September 2003.
3. World Health Organization. World Health Organization says failure to deliver AIDS medicines is a global health emergency: global AIDS treatment emergency requires urgent response; no more business as usual. 22 September **2003**. <http://who.int/mediacentre/releases/2003/pr67/en/index.html>. Accessed 29 September 2003.
4. Gogtay JA, Manek V, Nayak VG, et al. A pharmacokinetic evaluation of lamivudine, stavudine, and nevirapine given as a fixed dose combination pill versus the same three drugs given separately in healthy human volunteers [abstract PL8.4]. In: Abstracts of the Sixth International Congress on Drug Therapy in HIV (Glasgow). Congress on HIV Infection, the Gardner Coldwell Group, part of the Thomson Corporation. Chesire, UK, **2002**:11.
5. Kumarasamy N, Solomon S, Chaguturu SK, et al. The safety, tolerability and effectiveness of generic antiretroviral drug regimens for HIV-infected patients in south India. *AIDS* **2003**; 17:2267–9.
6. Penzak SR, Acosta EP, Turner M, Tavel JA, Masur H. Analysis of generic nevirapine products in developing countries. *JAMA* **2003**; 289:2648–9.
7. Apoola A, Srisakandabalan PS, Wade AAH. Self medication with zidovudine that was not. *Lancet* **2001**; 357:1370.
8. Newton PN, White NJ, Rozendaal JA, Green MD. Murder by fake drugs. *BMJ* **2002**; 324:800–1.
9. United States Pharmacopeia/National Formulary. Uniformity of dosage units. Rockville, MD: United States Pharmacopeial Convention, **2003**:2227–9.
10. United States Pharmacopeia/National Formulary. Uniformity of dosage units. Rockville, MD: United States Pharmacopeial Convention, **2003**:1665.
11. Tribut O, Arvieux C, Michelet C, Chaplain JM, Allain H, Bentue-Ferrer D. Simultaneous quantitative assay of six HIV protease inhibitors, one metabolite, and two non-nucleoside reverse transcriptase inhibitors in human plasma by isocratic reversed-phase liquid chromatography. *Ther Drug Monit* **2002**; 24:554–62.