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Oral Adherence Monitoring Using a Breath Test to Supplement Highly Active Antiretroviral Therapy

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Abstract

A breath-based adherence system to document ingestion of oral medications (e.g., HAART) was investigated. Specifically, the food additive 2-butanol, which can be easily packaged with a drug, is converted via alcohol dehydrogenase to the volatile metabolite 2-butanone that rapidly appears in breath, indicating adherence. In healthy adults using a portable sensor and GC-MS, the following experiments were performed: yield of 2-butanone in breath following ingestion of 2-butanol, adherence system accuracy, and potential interference of the adherence system by food or misplacement of 2-butanol on the tongue. During feasibility testing, every subject exhaled 2-butanone with 6.6 ± 1.5 min to peak concentrations of 548 ± 235 ppb following ingestion of 2-butanol (40 mg). ROC areas at 5 and 10 min were 0.95 (0.86–1.00) and 3 1.00 (1.00–1.00). Food did not interfere. Tongue application resulted in large concentrations of 2-butanol, but not 2-butanone. A breath test to provide definitive evidence of oral medication adherence appears technically feasible.

Keywords

adherence; antiretroviral therapy; behavioral interventions; prevention of sexual transmission; sexual behavior

INTRODUCTION

Poor adherence to prescribed medications is an important cause of increased HIV transmission and treatment failure with associated morbidity and mortality [1–3]. For example, investigators for the Preexposure Prophylaxis Initiative (iPrEx) trial showed that highly adherent patients (>90%) had a 73% risk reduction for HIV acquisition, whereas

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poorly adherent patients (<90%) had only a 21% benefit from treatment with oral tenofovir disoproxil fumarate and emtricitabine [1, 2]. For HIV-positive patients, intermittent use (<75% adherence) of triple combination therapy was associated with greater mortality when compared to more constant therapy [3]. Beyond reduced HIV transmission, adherence decreases hospitalization costs for HIV/AIDS patients, and reduces the emergence of drug resistance [4, 5]. For these reasons, poor adherence to antiretroviral medication remains a major obstacle to optimal health care delivery. Identification of a successful strategy to monitor medication adherence remains unrealized.

To address the need to find a definitive method of measuring adherence, our goal was to construct a breath-based medication adherence monitoring system for oral HIV/AIDS therapies, such as highly active antiretroviral therapy (HAART). In this system, a chemical taggant (GRAS compound), termed an adherence-enabling marker (AEM), is co-packaged with an active drug into a capsule or tablet in a manner that neither alters its chemical, manufacturing, and controls (CMC) nor bioavailability. Once swallowed, the AEM is absorbed by the stomach and small intestine and is metabolized to a volatile marker that is rapidly exhaled in breath (fig. 1A). The concentration of the metabolite is then measured by a portable miniature gas chromatograph (mGC). By measuring the metabolite in breath, one can be assured that the subject or patient did, indeed, consume the medication, particularly when a biometric such as facial recognition is used to confirm that the breath analyzed by the mGC came from a specific individual. All adherence data, including breath chromatographs, biometric information, and yes/no adherence assessments is stored locally in the mGC device on an internal USB flash drive. Encrypted, HIPAA-compliant data is transmitted real-time via either a standard wireless or cellular router. This adherence measurement system is easily portable and designed to be self-administered by patients or research subjects in their own residences or workplaces which offers significant convenience and economic benefits compared to frequent appointments with healthcare providers for directly observed therapy (DOT). Additionally, since no healthcare staff are required for daily assessments, this adherence system can be used at any time whereas DOT is generally available only during business hours and not during weekends or holidays. Additionally, since gastrointestinal absorption of a taggant is required to exhale a marker, a capsule cannot be “cheeked” and then egested at a later time. Patients’ individual adherence data are automatically uploaded to centralized, computer servers for analysis and possible action by research staff or healthcare providers.

In this study, we hypothesized that a safe food chemical, 2-butanol, would be suitable for use as an AEM because it generates 2-butanone, a ketone with physical characteristics that suggest this metabolite will appear in breath. Next, because the taggant and metabolite can be found in some widely available beverages and foods, we investigated whether the ingestion of foods with known high concentrations of 2-butanol (black tea), 2-butanone (yogurt, beefsteak tomato), or both compounds (cheddar cheese) would cause false adherence signals whereas a high fat meal may reduce uptake of the taggant and generate false non-adherence signals. We then examined the accuracy of the system to discriminate ingestion of encapsulated 2-butanone compared to a sham ethanol capsule. Last, we determined if placement of 2-butanol on the tongue would result in different patterns of breath 2-butanone and 2-butanol concentrations than after swallowing 2-butanol.

METHODS

Five separate studies were performed in five consenting cohorts of healthy adult subjects to examine the: 1) feasibility that the oral administration of 2-butanol would result in measurable concentrations of exhaled 2-butanone; 2) possibility that yogurt or cheddar cheese would alter the exhaled concentrations of 2-butanone and/or 2-butanol in an unblinded,

crossover investigation; 3) prospect that black tea, tomatoes, or a high fat meal would confound the system using a randomized, crossover, un-blinded design; 4) accuracy of the system using a randomized, crossover, double blinded, two limb design to determine if a subject received a tagged capsule compared to sham; and, 5) hypothesis that absorption by tongue would result in 2-butanone and/or 2-butanol concentration similar to those after swallowing. All investigations were approved by the Western Institutional Review Board (Olympia, WA). Subjects were aged >18 years, in general good health, and without known pulmonary disease. Each study is detailed subsequently.

Feasibility of a Breath Test to Measure Adherence

No restriction on oral consumption was observed prior to entry in the study. Once the study commenced, however, all subjects were fasted except for experimental intake. A baseline sample of exhaled breath was collected in a 1.0 L gas impermeable bag (Tedlar™, SKC, Inc., Eighty Four, PA). Thereafter, each subject consumed 2-butanol (40 mg, Penta Manufacturing Company, Fairfield, NJ) in a gelatin capsule (size 0, Capsugel, Inc., Greenwood, SC), followed by tap water (177 mL; 6 ounces) designated as time 0 min. Using frequent sampling intervals to facilitate pharmacokinetic analysis of 2-butanone exhalation, subjects provided additional breath samples at 0.5, 2, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 45, and 60 min following ingestion of the capsule. After 60 min, the experiment was terminated.

Gas Assays—20 mL of gas were aspirated from the bag using a 30 mL glass syringe and transferred for gas chromatography-mass spectroscopy (GC-MS, Trace GC Ultra gas chromatograph interfaced with a DSQ II mass spectrometer, Thermo Fisher Scientific, Waltham, MA). Separation of analytes was achieved using a dimethyl polysiloxane analytical column (60 m length, 0.32 mm internal diameter, 1 µm film thickness, Rxi®-1ms, Restek, Bellefonte, PA) and a gradient program with an initial temperature of 35°C, held for 9 min and then ramped at 3°C/min to 150°C, with a final ramp of 25°C/min to 250°C. The mass spectroscopy transfer line was set to 260°C, and the instrument was configured to scan from 42–180 amu. Samples were introduced into the GC-MS using a thermal desorption system (Markes International, Inc., Cincinnati, OH), which consisted of an auto-sampler (Ultra TD, Markes International Ltd.) and a thermal desorption unit (Unity, Markes International Ltd.). Thermal desorption tubes containing a 2,6-diphenylene oxide polymer resin (Tenax™, SKC, Inc.) were desorbed for 8 min at 280°C. The volatile components were absorbed by a cold trap set to 5°C, and the trap was rapidly heated to 300°C whereby the sample was introduced into the gas chromatography analytical column. All samples were quantitated using extracted ion chromatographs indicative of the more abundant and unique ions in each molecule. Gaseous data are reported at parts-per-billion (ppb) based on molar fractions (i.e., ppb-mole) to account for changes in ambient temperature and pressure as previously detailed [6].

Yogurt and Cheddar Cheese Interference Study

Six subjects (3 men and 3 women) were enrolled to evaluate the effects of yogurt or cheddar cheese on exhaled 2-butanone concentrations. Cheddar cheese typically contains 33.6 parts-per-million (ppm) 2-butanone and 18.1 ppm 2-butanol [7]. Yogurt usually contains 4.0 ppm 2-butanone and minimal-to-no 2-butanol [7]. (Note: unlike ppb units used for gaseous 2-butanone data in this investigation which is based on molar ratios, 1 ppm of solid food equals 1 mg/kg based on mass/mass). All subjects were fasting for at least 6 h prior to testing. In the cheddar cheese studies, subjects consumed 56 g of cheddar cheese (Cracker Barrel Aged Sharp Cheddar Cheese, Kraft Foods Inc., Northfield, IL). In the yogurt studies, subjects consumed 340 g of yogurt (Dannon All Natural Low-Fat Plain yogurt, Dannon, Allentown, PA). Thus, these amounts of cheddar cheese or yogurt contains a mass of 2-

butanone and 2-butanol of 1.9 and 1.0 mg, or 1.4 and 0.0 mg, respectively. The mass of food ingested was equivalent to at least two daily servings, rapidly consumed over a period less than 5 min. Each subject was directed to exhale into a 1 L gas impermeable bag prior to eating the food (time = -5 min). The t = 0 sample was collected immediately after the subjects placed food in their mouth and mixed it around without swallowing. Subsequent breath samples were collected at times 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 15, 17.5, 20, 25, 30, 45, and 60 min. Subjects were directed to exhale the first third of their breath into the room and then fill the sample bags with the remaining breath. Breath samples were transferred to thermal desorption tubes containing Tenax™ using a small vacuum pump (SKC, Inc.) and analyzed using GC-MS as described for the feasibility studies previously.

Black Tea, Tomato, and Feeding Interference Study

Using a randomized, crossover, un-blinded study design, this investigation consisted of two limbs (fasted and fed), with four replicated groups in each limb (2-butanol, black tea, tomato, empty sham capsule). For the fasted limb, each subject was fasted for at least four hours. For the fed limb, each subject consumed a sausage, egg, and cheese biscuit sandwich (Jimmy Dean®, Sara Lee Corporation, Neenah, WI) containing 31 g fat, 27 g carbohydrates, along with fruit juice (177 mL; orange or apple) 15 min prior to ingestion of study test articles. The four replicated groups in each limb were categorized based on ingestion of 1) encapsulated 2-butanol (40 mg) followed by tap water (177 mL); 2) black tea (500 mL; Tetley® Tea, New Providence, NJ); 3) tomato (120 g, *L. esculentum*, beefsteak variety; Publix Super Markets, Lakeland, FL) followed by tap water (177 mL); or 4) an empty sham capsule followed by tap water (177 mL). Each subject participated in each assignment of the study with a minimal 24 h washout between visits. Black tea typically contains no 2-butanone and 4.5 ppm 2-butanol [7], whereas tomato usually contains 4.5 ppm 2-butanone and no 2-butanol [7]. Thus, given the amount of black tea or tomato consumed, a mass of 2-butanone and 2-butanol of 0.0 and 2.3 mg, or 0.5 and 0.0 mg, respectively, were ingested in this study. A 1.0 L sample of exhaled breath was collected in a bag five min before ingestion of any substances to provide baseline concentrations for each subject. Thereafter, each subject began an assigned group for the study trial on that particular day. Following the breakfast sandwich (if applicable) and ingestion of 2-butanol, tea, tomato, or nothing, breath was collected at times: 0, 1, 3, 5, 7, 10, 12.5, 15, 17.5, 20, 30, and 60 min. In this study, time 0 min was designated as the conclusion of consuming the 2-butanol, black tea, or tomato. 2-butanone was measured using the GC-MS method previously described.

Accuracy Investigation

The study employed a prospective, randomized, double-blinded, crossover, controlled design. Subjects fasted 4 h prior for this study and then consumed a meal of their choice 15 min prior to ingestion. At the first visit, subjects were randomized to consume a capsule containing 2-butanol (40 mg) or ethanol (40 mg; sham control) followed by 177 mL water. At times 0, 5, 10, 15, 20, 30, 45 and 60 min after ingestion of the study capsule, the subject exhaled into a mGC (Xhale, Inc., Gainesville, FL) that has been validated to accurately measure 2-butanone [8]. After at least 24 h following this first study, subjects returned to enroll in the opposite limb of the investigation.

Absorption in the Mouth

In separate studies, the concentration-time relationships of 2-butanone and 2-butanol were observed following oral administration on the tongue, but without swallowing. Subjects (n=4) provided a baseline breath at time 0 min. Thereafter, each subject placed 4.0 mg of 2-butanol on the tongue and then exhaled into the mGC at times 5, 10, 15, 20, and 30 min. The concentrations of 2-butanone and 2-butanol were measured in each collected breath.

specimen. These values were compared to subject-matched oral ingestion of 2-butanol (40.0 mg) as measured in the Accuracy Investigation described earlier.

Statistical Analysis

Data are expressed as mean±standard deviation except for sensitivity and specificity data wherein 95% confidence intervals are used. Data were compared using one- or two-way repeated measures ANOVA with Holm-Sidak post-hoc testing, if appropriate (SigmaPlot 11.2, Systat Software, Inc. San Jose, CA). For the feasibility study, a non-compartmental pharmacokinetic analysis was conducted using WinNonlin (Version 5.2; Pharsight Corporation, St. Louis, MO). P values <0.05 were considered significant.

RESULTS

Feasibility of a Breath Test to Measure Oral Adherence

The metabolite, 2-butanone, was observed in the exhaled breath of every subject (n=7) after ingestion of 2-butanol (40 mg) without adverse event. The concentration-time relationship for exhaled 2-butanone is shown in fig. 1B. 2-butanone was always detected within 5–10 min of ingestion. Pharmacokinetic values are presented in Table I.

Potential Interference by Food

Before eating yogurt or cheddar cheese the exhaled concentrations of 2-butanone were low (≈ 3 ppb; fig 2, n=6). Concentrations increased immediately after putting either food in the mouth. In fact, C_{Max} for both foods occurred at the time either food was placed in the mouth (t = 0 min). Whereas the C_{Max} value for yogurt was significantly greater than baseline or other time points, no significant change was observed following cheddar cheese ingestion. In a separate cohort, fasted subjects (n=11) consumed 2-butanol, black tea, tomato, or a sham capsule. In these cohorts, 2-butanone breath levels were less than the limits of detection. Minimal (non-significant) increases in the concentrations of 2-butanone were observed after consuming black tea (fig. 2C) or tomatoes (fig 2D). As with yogurt and cheddar cheese, C_{Max} was observed at time 0 min when food was located in the mouth. The 2-butanone concentration-time relationship observed after swallowing 2-butanol (40 mg) in fed or fasting subjects was much greater than those observed after ingesting black tea ($P<0.001$) or tomato ($P<0.001$). In addition, after consuming 2-butanol following fasting or a high fat meal (fig. 3), no overall difference was observed in the concentration-time relationship for 2-butanone ($P=0.23$).

Accuracy of Breath Test System

Eight subjects enrolled and completed the study without adverse event. 2-butanone was observed every time 2-butanol was ingested, but never after consumption of the sham ethanol capsule. The concentration-time relationship was similar to that observed during the feasibility studies. Representative receiver operative curves (ROC) are shown in fig. 4 for times 0, 5, and 10 min. Additional, tabulated ROC data for all time points are shown in Table II. Sensitivity, specificity, and predictive values for all time points are noted in Table III. Using this measured data, we calculated the positive and negative predictive values for assumed adherence rates of 50%, 70%, and 90% given a cut-off value of approximately 10 ppb as conferring adherent status (Table IV).

Absorption and Metabolism in the Mouth

2-butanol was applied to the tongue directly or swallowed in a capsule. When applied to the tongue directly (n=4), exhaled breath was enriched with 2-butanol, but possessed significantly lower concentrations of 2-butanone (fig. 5A, $P=0.02$). In contrast, when 2-

butanol was swallowed in a capsule, exhaled breath was enriched with 2-butanone while possessing negligible concentrations of 2-butanol (fig. 5B, $P=0.04$). For the purposes of delineating tongue or gastric/duodenal absorption in a particular subject, we calculated the individual subject ratios of 2-butanol to 2-butanone concentrations (fig. 6). The mean ratios of 2-butanol:2-butanone following tongue application were 8,048-fold greater than after swallowing.

DISCUSSION

We describe a feasible method to determine oral medication adherence by using a breath test. In this system, a medication is “tagged” with 2-butanol which is absorbed by the gastrointestinal tract. The 2-butanol is metabolized to 2-butanone which is exhaled and measured by GC-MS or a portable mGC. The appearance of 2-butanone in breath confirms adherence to the medication. The data reported herein demonstrate that this method works for orally administered capsules, and results in easily measured concentrations of 2-butanone following consumption of 2-butanol (40 mg) with available portable technology. The sensitivity, specificity, and predictive values in the time ranges of 10–30 min suggest that this period of time is optimal for breath collection.

We have previously demonstrated the feasibility for a similar system using 2-butyl acetate in tenofovir placebo gel administered by the vaginal route to healthy women to assess for microbicide adherence using breath [8]. In that investigation, the 2-butanone concentrations were approximately 10–100 ppb, values considerably less than reported herein. Several methodological and anatomical differences account for this discrepancy. First, in the former study, the taggants were administered in a gel that likely acted as a reservoir for the parent acetate and reduced the rapidity of vaginal absorption whereas the oral capsules rapidly dissolve in the stomach. Second, acetate was used as a taggant in the vaginal study compared to an alcohol in the present report, which adds an extra metabolic step (i.e., acetate→alcohol→ketone). Third, a smaller taggant dose (15 mg) was used for vaginal investigation. Fourth, vaginal venous drainage occurs by pudendal veins leading directly to the inferior vena cava, which thereby avoids any “first pass” metabolic effects in the liver. In contrast, the major gastric veins lead directly into the portal circulation which favors rapid enzymatic conversion of taggants to metabolites. For these reasons, the concentrations of 2-butanone following oral ingestion exceeded those after vaginal administration.

Potential Food Interference

The concern that food may interfere with exhalation of 2-butanone appears to be unfounded. Because most edible ingested substances are absorbed in the small intestine and fats delay gastric emptying, we hypothesized that a fatty meal may postpone the manifestation of 2-butanone in exhaled breath when compared to a fasting state [9]. The results herein demonstrate, however, that a fatty meal did not slow emanation of 2-butanone in breath. Additionally, the time frame for 2-butanone to be exhaled in maximal concentrations was approximately 5–20 min (in both fed and fasting subjects), a duration far shorter than that necessary for gastric emptying. For example, Hellmig and colleagues’ study in healthy subjects showed that the median time for gastric half-emptying of solids (liquids) measured by ^{13}C breath tests was 127 min (81 min), with a lag time of 82 min (40 min). Taken together, these observations suggest that a significant fraction of 2-butanol absorption, similar to that of ethanol, occurs in the stomach, and not only in the duodenum [10]. For the purpose of adherence monitoring, the tight coupling of medication ingestion to breath testing (10–30 min) is beneficial to minimize delay and provide a convenient time window for subjects.

After consuming double portions of foods enriched with 2-butanol and/or 2-butanone, we observed minimally increased concentrations of 2-butanone at time 0 min or thereafter. These peaks were about 100- to 600-fold less than the concentrations due to 2-butanol consumed in capsules (400–600 ppb). In addition, the time course of 2-butanone exhalation after food consumption (maximal at 0 min) differed from that after capsulized 2-butanol consumption (maximal at 10–20 min). Nevertheless, one must be cognizant of these facts when determining a threshold concentration and time point for 2-butanone exhalation to designate subjects as adherent to consuming oral medication.

Limitations

One physiological limitation of using an alcohol as a taggant may be variation in the rate of ketone exhalation due to genetic polymorphism for enzymes metabolizing alcohols to ketones. For ADH, genetic polymorphisms occur at the ADH2 and ADH3 gene loci, which can significantly alter enzymatic function in ADH isozymes containing the β (β_1 and β_2) and γ (γ_1 and γ_2) subunits, respectively [11–14]. These types of ADH genetic variations should not confound the taggant metabolism described herein because secondary alcohols, such as 2-butanol, are preferentially and efficiently metabolized to ketones by $\alpha\alpha$ -ADH, which is not subject to genetic polymorphisms, unlike the β variation [15–17]. Additionally, in the event of concurrent ingestion of ethanol and a taggant, the system should still remain functional. That is, relative to primary alcohols such as ethanol, secondary alcohols are much more efficiently (V_{Max}/K_M 50–110 fold greater) and preferentially (greater binding affinity: Michaelis constant [K_M] 0.009–0.025 fold lower) metabolized by distinct ADH isozymes (e.g., $\alpha\alpha$ -ADH) [12]. For these reasons, genetic variation and concurrent alcohol ingestion are not anticipated to be problematic, although experimental data to confirm these hypotheses are needed.

The taggant, 2-butanol, appears to be safe as noted by a number of regulatory agencies listings. The agent is included as a direct food additive in the FDA EAFUS (Everything Added to Food in the United States) listing [18], and is noted as the lowest risk Class 3 agent, which implies “...low toxic potential to man; no health-based exposure limit is needed...” in the FDA Q3C Guidance for Industry publication [19]. The permissible daily exposure asserted by the FDA is 300 mg/day [20]. Likewise, 2-butanone (methyl ethyl ketone) is catalogued in the FDA Inactive Ingredients List [21]. Indeed, in 2009 the FDA even suggested adding “... physical-chemical identifiers into solid oral dosage form drug...” for the purposes of augmenting drug anti-counterfeiting efforts, and we suggest that this technique is also useful to measure adherence [22]. Therefore, from a toxicological perspective, 2-butanol is an excellent candidate compound that can be used to document adherence. We hypothesize that 2-butanol and 2-butanone will not interfere with the pharmacokinetics of antiretroviral medications, but additional data is needed to conclusively support this position.

Adherence Monitoring in Clinical Trials and Disease

The current cost of drug development is unsustainable. Recently, Forbes reported that over the last 15 years the top 12 pharmaceutical companies in terms of total research and development expenditures cumulatively spent \$802.5 billion to receive 139 new drug approvals [23]. Although differences were noted among companies, this finding translates to a staggering average \$5.8 billion per new drug approval. Furthermore, Grove cogently observed that clinical trials annually produced 20 new drugs at an expense of \$50 billion [24]. Moreover, Grove wrote that large numbers of clinical trials with many subjects were an important reason for this unsustainable inefficiency that needs better results with fewer subjects. We suggest that real-time adherence measurement in clinical trials may reduce the number of subjects needed and the length of the trial, while providing superior safety and

efficacy data to make key decisions. Furthermore, in disease management, even modest improvements in adherence can markedly impact care. Choudhry and colleagues reported that 4–6% improvement in adherence (baseline rates 36–49%) to cardiovascular medications due to enhanced prescription coverage significantly reduced the frequency of major vascular events after myocardial infarction [25]. The ability to identify subjects/patients who are non-adherent to medication use is a primary step to improve both disease management and clinical trials. Both the tool presented herein or other systems (e.g., capsules tagged with electronics) facilitate this understanding [26].

In summary, we conclude that 2-butanone is generated and exhaled in breath after oral ingestion of 2-butanol. A high fat meal or foods enriched in the taggant, 2-butanol, and/or 2-butanone do not delay or confound the interpretation of 2-butanone concentrations. Placement of 2-butanol on the tongue (vis-à-vis swallowing) can be readily detected. Use of 2-butanol to tag medications to provide definitive evidence of medication adherence appears physiologically and technologically feasible.

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REFERENCES

1. Grant RM, Lama JR, Anderson PL, et al. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med*. 2010; 363:2587–2599. [PubMed: 21091279]
2. Interim Guidance: Preexposure Prophylaxis for the Prevention of HIV Infection in Men Who Have Sex with Men. *MMWR Morb Mortal Wkly Rep*. 2011; 60:65–68. [PubMed: 21270743]
3. Hogg RS, Heath K, Bangsberg D, et al. Intermittent use of triple-combination therapy is predictive of mortality at baseline and after 1 year of follow-up. *AIDS*. 2002; 16:1051–1058. [PubMed: 11953472]
4. Nachega JB, Leisegang R, Bishai D, et al. Association of antiretroviral therapy adherence and health care costs. *Ann Intern Med*. 2010; 152:18–25. [PubMed: 20048268]
5. Gardner EM, Hullsiek KH, Telzak EE, et al. Antiretroviral medication adherence and class-specific resistance in a large prospective clinical trial. *AIDS*. 2010; 24:395–403. [PubMed: 20099399]
6. Morey T, Booth MM, Prather RA, et al. Measurement of Ethanol in Gaseous Breath Using a Miniature Gas Chromatograph. *Journal of Analytical Toxicology*. 2011; 35:134–142. [PubMed: 21439148]
7. Leffingwell JC. GRAS Chemicals in Food. PPM Report: Flavor-Base. 2007
8. Morey TE, Wasdo S, Wishin J, et al. Feasibility of a Breath Test for Monitoring Adherence to Vaginal Administration of Anti-retroviral Microbicide Gels. *J Clin Pharmacol*. 2012 [Epub ahead of print].
9. Little TJ, Horowitz M, Feinle-Bisset C. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. *Am J Clin Nutr*. 2007; 86:531–541. [PubMed: 17823414]
10. Jones AW. Pharmacokinetics of Ethanol - Issues of Forensic Importance. *Forensic Sci Rev*. 2011; 23:91–136.

11. Lieber CS. ALCOHOL: its metabolism and interaction with nutrients. *Annu Rev Nutr.* 2000; 20:395–430. [PubMed: 10940340]
12. Stone CL, Li TK, Bosron WF. Stereospecific oxidation of secondary alcohols by human alcohol dehydrogenases. *J Biol Chem.* 1989; 264:11112–11116. [PubMed: 2738060]
13. Yin SJ, Bosron WF, Magnes LJ, et al. Human liver alcohol dehydrogenase: purification and kinetic characterization of the beta 2 beta 2, beta 2 beta 1, alpha beta 2, and beta 2 gamma 1 "Oriental" isoenzymes. *Biochemistry.* 1984; 23:5847–5853. [PubMed: 6395883]
14. Duester G, Smith M, Bilanchone V, et al. Molecular analysis of the human class I alcohol dehydrogenase gene family and nucleotide sequence of the gene encoding the beta subunit. *J Biol Chem.* 1986; 261:2027–2033. [PubMed: 2935533]
15. Reddy B, Reddy A, Nagaraja T, et al. Single Nucleotide Polymorphisms of the Alcohol Dehydrogenase Genes among the 28 Caste and Tribal Populations of India. *International Journal of Human Genetics.* 2006; 6:309–316.
16. Bosron WF, Magnes LJ, Li TK. Human liver alcohol dehydrogenase: ADH Indianapolis results from genetic polymorphism at the ADH2 gene locus. *Biochem Genet.* 1983; 21:735–744. [PubMed: 6354175]
17. Edenberg, H.; Bosron, WF. Alcohol Dehydrogenases. In: Guengerich, F., editor. *Biotransformation Vol. 3, Comprehensive Toxicology.* New York: Pergamon Press; 1997. p. 119-131.
18. Food and Drug Administration Center for Food Safety and Applied Nutrition. [Accessed: September 28, 2011] Everything Added to Food in the United States (EAFUS). Available at: <http://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?rpt=eafusListing>
19. Food and Drug Administration Center for Drug Evaluation and Research (CDER). [Accessed: December 14, 2011] Guidance for Industry: Q3C - Tables and List. Available at: <http://www.fda.gov/downloads/.../Guidances/ucm128282pdf%20>
20. Food and Drug Administration. [Accessed: September 24, 2011] Q3c Appendix 6 Toxicological Data for Class 3 Solvents. Available at: <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073403.pdf>
21. Food and Drug Administration Center for Drug Evaluation and Research. [Accessed: September 24, 2011] Inactive Ingredient Search for Approved Drug Products. Available at: <http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>
22. Food and Drug Administration Center for Drug Evaluation and Research. [Accessed: December 14, 2011] Guidance for Industry: Incorporation of Physical-Chemical Identifiers into Solid Oral Dosage Form Drug Products for Anticounterfeiting. Available at: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM171575.pdf
23. Herper, M. [Accessed: August 29, 2012] The Truly Staggering Cost Of Inventing New Drugs. Available at: <http://www.forbes.com/sites/matthewherper/2012/02/10/the-truly-staggering-cost-of-inventing-new-drugs/>
24. Grove A. Rethinking clinical trials. *Science.* 2011; 333:1679. [PubMed: 21940863]
25. Choudhry NK, Avorn J, Glynn RJ, et al. Full Coverage for Preventive Medications after Myocardial Infarction. *N Engl J Med.* 2011
26. Au-Yeung KY, Moon GD, Robertson TL, et al. Early clinical experience with networked system for promoting patient self-management. *Am J Manag Care.* 2011; 17:e277–e287. [PubMed: 21819175]

A. **B.** **Figure 1.**

Enzymatic catalysis and resultant exhaled 2-butanone following oral ingestion of 2-butanol (40 mg) in subjects (n=7). Panel A: metabolism of the taggant, 2-butanol, by $\alpha\alpha$ -alcohol dehydrogenase ($\alpha\alpha$ -ADH) to generate the volatile product, 2-butanone. Panel B: breath concentration-time relationship for the exhalation of 2-butanone in breath (measured by gas chromatography/mass spectroscopy) following consumption of 2-butanol at time 0 min. Data shown are mean $\pm$ SD. Note logarithmic scaling of ordinate axis. The absence of error bars indicates that the standard deviation values fell within the width of the symbol. *, $P < 0.05$ for a given time point compared to time point 0 min. The arrow denotes time of capsule ingestion. Concentrations less than the level of 1.0 ppb are noted as "<LOD".

Figure 2.

Concentration-time relationships of exhaled 2-butanone concentrations in humans after eating yogurt (Panel A), cheese (Panel B), black tea (Panel C) or tomato (Panel D). Data shown is mean±standard deviation. *: $P < 0.05$ for 2-butanone concentration at a given time compared to 2-butanone at time -5 min. Note logarithmic scaling of ordinate axis. Concentrations less than the level of 1.0 ppb are noted as “<LOD”.

Figure 3. Concentration-time relationships of exhaled 2-butanone concentrations in humans after fasting or a high fat meal. Data shown is mean±standard deviation. *: $P < 0.05$ for 2-butanone concentration at a given time compared to 2-butanone at time -5 min. Note logarithmic scaling of ordinate axis. Concentrations less than the level of 1.0 ppb are noted as “<LOD”.

Figure 4. Receiver operator curves at times 0, 5, and 10 min for exhalation of 2-butanone to determine if subjects ingested a capsule containing 2-butanol (40 mg) compared to sham ethanol (40 mg) capsules in humans (n=8).

Figure 5.

Concentration-time relationships of 2-butanone and 2-butanol after direct application of 2-butanol to the tongue (Panel A) or swallowing of encapsulated 2-butanol (Panel B) in humans (n=4) at time 0 min. Data shown is mean±standard deviation. Legend applies to both panels. *: P<0.05 for 2-butanone concentration compared to 2-butanol concentration at the same time in a given route of administration. Note logarithmic scaling of ordinate axis. Concentrations less than the level of 1.0 ppb are noted as “<LOD”.

Figure 6.

Ratio of exhaled concentrations of 2-butanol to 2-butanone five min after placement of 2-butanol on the tongue or after swallowing in humans (n=4). Data shown is mean±standard deviation. Note logarithmic scaling of ordinate axis. The horizontal, dashed line notes a ratio of unity.

Table I

Pharmacokinetic parameters for exhaled 2-butanone from human subjects (n=7) after orally consuming 2-butanol (40 mg).

Pharmacokinetic Parameters	Value
LAMBDA_Z (min ⁻¹)	0.21 ± 0.26
Half-life (min)	10.9 ± 9.6
C _{MAX} (ppb)	548 ± 235
T _{MAX} (min)	6.6 ± 1.5
AUC _{0-LAST} (min*ppb)	5227 ± 3858
AUC _{0-∞} (min*ppb)	5585 ± 3976
% AUC Extrapolation	6.69 ± 9.18
MRT _{0-last} (min)	12.9 ± 6.0

Estimates were generated for the following PK parameters (abbreviation, units): first-order elimination rate constant (Lambda_Z), half-life of elimination (Half-life), maximal drug concentration (C_{MAX}), time at maximal drug concentration (T_{MAX}), area under concentration versus time curve from zero to the last time point (AUC_{0-LAST}), area under concentration versus time curve from zero to infinity (AUC_{0-∞}), percentage of area under concentration versus time curve from zero to infinity which is extrapolated from AUC_{0-t} (% AUC Extrapolation) and the mean residence time from zero to the last time point (MRT_{0-last}). The area under the concentration versus time curve (AUC) was calculated using the linear trapezoidal rule. Values are expressed as mean±standard deviation.

Table II

Receiver operator curve (ROC) data for exhaled 2-butanone from human subjects (n=8) after orally consuming a capsule with 2-butanol (40 mg) or a sham capsule with ethanol (40 mg).

Time (min)	ROC Area	95% CI	P _{0.50}	P _{0min}
0	0.42	0.11–0.73	0.60	-
5	0.95	0.86–1.00	<0.001	<0.001
10	1.00	1.00–1.00	<0.001	<0.001
15	1.00	1.00–1.00	<0.001	<0.001
20	1.00	1.00–1.00	<0.001	<0.001
30	1.00	1.00–1.00	<0.001	<0.001
45	0.98	0.94–1.00	<0.001	<0.001
60	1.00	1.00–1.00	<0.001	<0.001

Data shown are ROC areas with 95% confidence intervals (CI). P_{0.50} is the Type I error risk that a ROC area for a given time point is different from a ROC area of 0.50. P_{0min} is the Type I error risk that the ROC area for a given time point is different from the ROC area for time 0 min.

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Table III

Data detailing accuracy over 0–60 min with suggested “cut off” concentrations for exhaled 2-butanone from human subjects (n=8) after orally consuming a capsule with 2-butanol (40 mg) or a sham capsule with ethanol (40 mg).

Time (min)	Cut-off (ppb)	Sensitivity	Sensitivity 95% CI	Specificity	Specificity 95% CI
0	>0	0.88	0.47–0.997	0.00	0.00–0.37
5	1.8	1.00	0.63–1.00	0.75	0.35–0.97
10	9.7	1.00	0.63–1.00	1.00	0.63– 1.00
15	5.4	1.00	0.63–1.00	1.00	0.63–1.00
20	5.2	1.00	0.63–1.00	1.00	0.63–1.00
30	3.7	1.00	0.63–1.00	1.00	0.63–1.00
45	2.9	1.00	0.63–1.00	0.885	0.47–0.99
60	2.4	1.00	0.63–1.00	1.00	0.63–1.00

Cut-off represents the concentration of 2-butanone above which a subject is identified as having orally ingested a capsule containing 2-butanol instead of the sham ethanol capsule.

Abbreviations: CI, confidence interval.

Table IV

Calculated positive and negative predictive values based on data from Table III and assumed adherence rates of 50%, 70%, or 90% with a cut-off value of approximately 10 ppb conferring adherent status.

Time (min)	Assumed Adherence Rate (%) PPV/NPV		
	50	70	90
5	1.00/0.80	1.00/0.63	1.00/0.31
10	1.00/1.00	0.95/1.00	0.99/1.00
15	1.00/1.00	1.00/1.00	1.00/1.00
20	1.00/1.00	1.00/1.00	1.00/1.00
30	1.00/0.89	1.00/0.77	1.00/0.47
45	1.00/0.89	1.00/0.77	1.00/0.47
60	1.00/0.80	1.00/0.63	1.00/0.31

Data shown as positive predictive values (PPV) / negative predictive values (NPV).