

Response of CEDIA[®] Amphetamines Assay After a Single Dose of Bitter Orange

DiemThuy T. Nguyen, PharmD,* Linda T. Bui, PharmD,* and Peter J. Ambrose, PharmD†

Abstract: Bitter orange has recently been substituted as an ingredient in many “ephedra-free” dietary supplements used for weight loss. The primary active ingredient in bitter orange is synephrine. Previous reports have documented false-positive results from ephedrine with urine amphetamine assays. Because of the similarity in chemical structure of ephedrine and synephrine, it is hypothesized that ingestion of a bitter orange supplement may have the potential to cause false-positive results with urine amphetamine assays. The purpose of this study was to determine the response of the CEDIA[®] Amphetamines Assay after ingestion of bitter orange. Six healthy adult male volunteers were administered a single oral dose of Nature’s Way[®] Bitter Orange, a 900-mg dietary supplement extract standardized to 6% synephrine. Urine specimens were collected at baseline and 3 and 6 hours post-administration. Additional urine specimens were collected from 1 subject at 9, 12, and 15 hours after administration. All specimens were analyzed by the CEDIA[®] Amphetamines Assay. Urine specific gravity and pH also were measured. All urine specimens demonstrated a negative response to the CEDIA[®] Amphetamines Assay. Urine specific gravity ranged from 1.007 to 1.028, and pH ranged from 5.0 to 7.0; thus, reducing the possibility that the negative results were caused by diluted specimens or reduced excretion of synephrine into alkaline urine. This information will be of value when health care providers or those who interpret drug screens are asked to provide consultation regarding the interference of bitter orange supplements with the CEDIA[®] Amphetamines Assay. A single-dose of Nature’s Way[®] Bitter Orange was not found to cause a false-positive response to the CEDIA[®] Amphetamines Assay in 6 healthy adult male volunteers.

Key Words: bitter orange, synephrine, *Citrus aurantium*, urine drug testing, amphetamines, CEDIA[®]

(*Ther Drug Monit* 2006;28:252–254)

Received for publication May 16, 2005; accepted October 20, 2005.

From the *Long Beach Memorial Medical Center, Long Beach, California; and †Department of Clinical Pharmacy, School of Pharmacy, University of California, San Francisco, California.

Supported in part by the Vince Isnardi Opportunity Fund as a gift to the School of Pharmacy, University of California, San Francisco, CA.

Poster presentation at the annual meeting of the Drug Information Association, Washington, D.C., June 27, 2005.

Reprints: Peter J. Ambrose, PharmD, Long Beach Memorial Medical Center, Department of Pharmacy Services, 2801 Atlantic Avenue, P.O. Box 1428, Long Beach, CA 90801-1428 (e-mail: pambrose@memorialcare.org).

Copyright © 2006 by Lippincott Williams & Wilkins

Bitter orange extract (*Seville Orange*, *Shangzhou Zhiqiao*, *Sour Orange*, *Zhi qiao*, and *Zhi shi*) is derived from unripe dried fruits and fruit peels of *Citrus aurantium*. Bitter orange recently has been substituted as an ingredient in many “ephedra-free” dietary supplements commonly used for weight loss in the United States. The main active ingredient in bitter orange for this indication is synephrine. Commercially available products often are standardized to 1% to 6% synephrine. Synephrine is a sympathomimetic alkaloid (α_1 -adrenergic agonist) similar in chemical structure to ephedrine and amphetamine (Fig. 1).

Synephrine is found in citrus plants and can be detected in human urine after the ingestion of *Citrus unshiu* fruit.¹ Natural products contain only *l*-synephrine. After ingestion of *Citrus unshiu* fruit, it was found that *l*-synephrine was conjugated, with 10% of conjugated synephrine as *d*-synephrine when excreted in the urine.² Synephrine may undergo oxidation by monoamine oxidase to produce its primary metabolite: *p*-hydroxymandelic acid.³ Other metabolites include hydroxyphenylglycol sulfate, synephrine sulfate, and synephrine glucuronide.⁴

The CEDIA[®] Amphetamines Assay (Microgenics Corporation, Fremont, CA) is a homogenous enzyme immunoassay, which qualitatively and semiquantitatively measures amphetamines in urine, and is commercially available to screen urine samples for the presence of amphetamine and amphetamine derivatives. There is 0.4% cross reactivity of *l*-ephedrine with the CEDIA[®] Amphetamines Assay.⁵

Previous reports have documented that ephedrine produced a false-positive result with other commercially available urine amphetamine assays.^{6,7} Similarities in chemical structure and purported biologic effects of ephedrine and synephrine suggest the possibility that ingestion of bitter orange may cause a false-positive response with amphetamine screening assays. However, there is currently no published information regarding cross-reactivity or compatibility of bitter orange with commercially available urine amphetamine assays. We present the results obtained with the CEDIA[®] Amphetamines Assay after a single dose of bitter orange.

MATERIALS AND METHODS

Six healthy adult male subjects aged 24 to 49 years with a mean BMI of 24.3 (range, 22–28.8) participated in this study. The race and ethnicity of the subjects consisted of

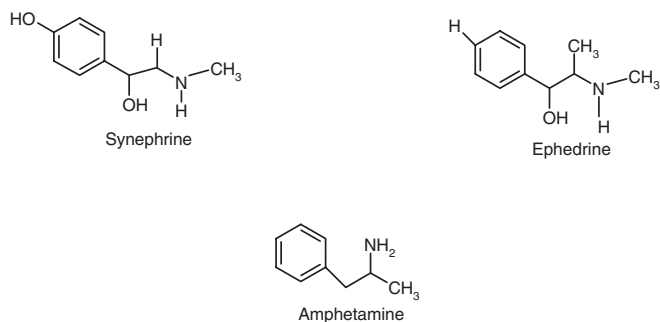


FIGURE 1. Chemical structures of synephrine, ephedrine, and amphetamine.

white (n = 3), Hispanic (n = 2), and Asian (n = 1). Eligible volunteers were men and women at least 18 years of age who demonstrated systolic blood pressure < 140 mm Hg, diastolic blood pressure < 90 mm Hg, and heart rate 60 to 100 beats per minute before enrollment. Exclusion criteria included any known allergy to citrus products; a history of heart, thyroid, liver or kidney disease; diabetes; hypertension; narrow-angle glaucoma; seizures; central nervous system disorders; prostate hypertrophy; or if they were smokers, pregnant, lactating, or had a body mass index ≥ 30 kg/m²; or use of synephrine, ephedrine, or any other supplements or over-the-counter products within 1 month of the study. The study protocol was approved by the Committee on Human Research of the University of California, San Francisco. All volunteers provided written, informed consent before initiation of any study-related procedures.

Study Design

Subjects were required to consume a citrus-free diet for 3 days before and during the study day. Subjects were required to fast for at least 10 hours overnight before the study day. Subjects also were required to refrain from any stimulants, over-the-counter products, or dietary supplements upon enrollment and throughout the study. To prevent urine from being too dilute, subjects were asked to consume no more than 8 ounces of water per hour during the study period.⁸

Six healthy subjects were administered a single, oral dose of Nature’s Way[®] Bitter Orange (Springville, UT), a

900-mg dietary supplement extract standardized to 6% synephrine (2 tablets). The dosing regimen for bitter orange was directly obtained from the manufacturer’s recommended instructions. Urine specimens were collected at baseline (pre-dose) and 3 and 6 hours post-administration. Additional urine specimens were collected from 1 subject at 9, 12, and 15 hours post-administration. The duration and intervals for obtaining urine specimens were based on a previously reported synephrine half-life of 2 hours, a maximum urine excretion of synephrine at 2 to 3 hours after ingestion, and disappearance from the urine at 16 hours.^{2,9} All specimens were analyzed by the CEDIA[®] Amphetamines Assay. Urine specific gravity and pH also were measured.⁸ Urine specimens were refrigerated until analyzed within 1 to 2 days. Kusu et al² reported that *l*-synephrine was stable at temperatures ranging from 30°C to 100°C and at a pH range of 1 to 9.

RESULTS

A total of 21 urine specimens were obtained from 6 healthy adult male volunteers. All urine specimens demonstrated a negative response to the CEDIA[®] Amphetamines Assay. Urine specific gravity and pH ranged from 1.007 to 1.028 and 5 to 7 (Table 1), respectively, thereby reducing the possibility that the negative results were caused by diluted specimens or reduced excretion of synephrine into alkaline urine.

DISCUSSION

As the popularity and use of bitter orange and synephrine for weight loss continues to increase, so will the chances that individuals taking bitter orange will be subject to drug testing for employment, athletics, or other reasons. The information from the present study will be of value when health care providers or those who interpret drug screens are asked to provide consultation regarding the interference of bitter orange with the CEDIA[®] Amphetamines Assay.

The CEDIA[®] Amphetamines Assay uses recombinant DNA technology to produce a homogenous enzyme immunoassay system with β -galactosidase, a bacterial enzyme with 2 inactive fragments that combine to form an active enzyme that cleaves a substrate generating a spectrophotometrically detectable color change. An antibody binds to a drug conjugated on the inactive

TABLE 1. Urine Specific Gravity and pH

Subject	Baseline		Hour 3		Hour 6		Hour 9		Hour 12		Hour 15	
	SG	pH	SG	pH	SG	pH	SG	pH	SG	pH	SG	pH
1	1.019	6.0	1.012	6.5	1.010	6.5	–	–	–	–	–	–
2	1.018	5.5	1.016	5.0	1.007	5.5	–	–	–	–	–	–
3	1.021	5.5	1.025	6.0	1.025	7.0	–	–	–	–	–	–
4	1.018	5.5	1.020	5.5	1.025	5.5	–	–	–	–	–	–
5	1.023	6.5	1.014	7.0	1.018	7.0	–	–	–	–	–	–
6	1.024	5.5	1.028	5.0	1.015	6.5	1.021	6.0	1.023	6.5	1.024	5.0

SG, urine specific gravity.

fragments, inhibiting the reassociation of inactive β -galactosidase fragments. Amphetamines present in the urine will bind to the antibody allowing inactive fragments to join and form active enzyme. The amount of absorbance change and active enzyme formed is directly proportional to the amount of amphetamines present in the urine sample.⁵ All urine specimens collected in our study demonstrated a negative response to the CEDIA[®] Amphetamines Assay.

To prevent urine from being too dilute, subjects were asked to consume no more than 8 ounces of water per hour during the study period.⁸ The specific gravity for all specimens was found to be reasonably concentrated. Alkalinization of the urine reduces the urinary excretion of basic compounds (eg, synephrine and other amphetamine analogues), which can reduce the concentration of the compound in urine.⁸ The pH for all specimens in this study were found to be within the acceptable limits (≤ 7.5).⁸

Because assays require a minimum concentration of a substance to detect the presence of that substance, there may be some limitations to our study. It is possible that a different result may be obtained after the ingestion of higher doses or multiple doses of bitter orange or synephrine.

Ephedrine and synephrine are banned by a number of sports-governing agencies, and several world-class athletes have committed doping violations for consuming ephedrine-related substances commonly present in many dietary supplements. Many dietary supplements contain banned substances that are not adequately disclosed on the product labeling, because they are "hidden" under their botanical names or they are simply not listed. Athletes who take dietary supplements may elect to have their urine screened to make certain that they are complying with regulations. In addition, it also is common practice for employers to require employees to

undergo drug testing before or during employment, which may only include screening assays. Hence, it is important to know whether commonly used substances, such as bitter orange, will yield a false-positive result. Although we did not obtain a positive response to the bitter orange product tested, the response of different bitter orange products with other urine amphetamine assays has not been reported and thus remains to be determined.

CONCLUSION

A single-dose of Nature's Way[®] Bitter Orange was not found to cause a false-positive response to the CEDIA[®] Amphetamines Assay in 6 healthy adult male volunteers.

REFERENCES

1. Stewart L, Newhall WF, Edwards GJ. The isolation and identification of synephrine in the leaves and fruits of citrus. *J Biol Chem*. 1964;239:930-932.
2. Kusu F, Matsumoto K, Arai K, et al. Determination of synephrine enantiomers in food and conjugated synephrine in urine by high-performance liquid chromatography with electrochemical detection. *Anal Biochem*. 1996;235:191-194.
3. Arai K, Jun D, Kusu F, et al. Determination of *p*-hydroxymandelic acid enantiomers in urine by high-performance liquid chromatography with electrochemical detection. *J Pharm Biomed Anal*. 1997;15:1509-1514.
4. Ibrahim KE, Midgley JM, Crowley JR, et al. The mammalian metabolism of R-(-)-m-synephrine. *J Pharm Pharmacol*. 1983;35:144-147.
5. CEDIA[®] Amphetamines Assay [Package Insert]. Freemont, CA: Microgenics Corporation; 2002-2009.
6. Salway JG. *Drug-Test Interactions Handbook*. New York: Raven Press; 1990.
7. Budd R. Amphetamine EMIT-structure versus reactivity. *Clin Toxicol*. 1981;18:91-110.
8. Ambrose PJ. Drug use in sports: a veritable arena for pharmacists. *J Am Pharm Assoc*. 2004;44:501-516.
9. Hengstmann JH, Aulepp H. Pharmacokinetics and metabolism of 3H-synephrine. *Arzneimittelforschung*. 1978;28:2326-2331.