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TECHNICAL REPORT

Exactly which synephrine alkaloids does *Citrus aurantium* (bitter orange) contain?

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Editors' Note: The article below is a technical report of the constituents of bitter orange, a commonly used over-the-counter (OTC) preparation for weight loss. The US Food and Drug Administration has called for the scientific community to assess existing and future OTC weight loss preparations to determine if they contain constituents that might produce adverse events in susceptible individuals. Allison and colleagues have determined that one such preparation of bitter orange contains both *p*-synephrine and *m*-synephrine. Their report confirms that it is not possible to rely on ingredient labels of OTC weight reduction preparations, and additional studies should be performed to determine if ingredients that may cause harm are present.

Following the withdrawal of ephedrine from the dietary supplement marketplace sales of products containing *Citrus aurantium* (*CA*) (bitter orange) for weight loss are believed to have increased dramatically. *CA* contains a number of constituents speculated to lead to weight loss, of which the most frequently cited constituent is synephrine. Concerns have been raised about the safety of products containing synephrine. To develop an adequate basis for clinical and public health recommendations, it is necessary to understand the nature of the synephrine alkaloids in *CA*. There are six possible isomers of synephrine (para, meta, ortho; and for each a *d* or *l* form). Some authors have stated that *CA* contains only *p*-synephrine, whereas other authors have stated that *CA* contains *m*-synephrine. This is an important distinction because the two molecules have different pharmacologic properties, which may differentially affect safety and efficacy. We are unable to identify published data that explicitly show whether *CA* contains *p*-synephrine, *m*-synephrine, or both. In this brief report, we show that at least one product purportedly containing synephrine alkaloids from *CA* contains both *p*-synephrine and *m*-synephrine. We believe this justifies further investigation into which synephrine alkaloids are present in *CA* and products purportedly containing synephrine alkaloids from *CA* and the relative quantities of each of the different isomers.
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Many Americans use over-the-counter dietary supplements for weight loss. However, there are typically few data on the safety and efficacy of products prior to their introduction to the marketplace. Even the composition of products is often open to question.[1]

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With FDA's ban of ephedrine-containing supplements, the sale of dietary supplements containing *Citrus aurantium* (*CA*) is believed to have increased dramatically.[2–4] As noted by Senator Schumer, 'As people switch from ephedra to alternatives that make similar promises and work in similar ways, Bitter Orange use is skyrocketing, and we shouldn't have to wait for years—or for deaths—to act'. Further concern was noted by then FDA Acting Commissioner Dr Crawford, 'As our agency learned from outlawing ephedra, research of this magnitude can place great demands on FDA's resources, and it is in this area where we have a critical need for extramural assistance. …we suggested to ODS four priority topics …One of them is ephedra substitutes,

primarily *Citrus aurantium* or bitter orange…We need your help to identify supplements that are dangerous to human health, and to develop the necessary data to prove their lack of safety' (LM Crawford, Acting Commissioner of FDA, 2004).[5]

The emergence of *CA* on the US market and the public's appetite for its consumption deserves more scrutiny with respect to its chemical composition. *CA* is the Latin name for a plant often called bitter orange, sour orange or Seville orange. The plant is widely used as a medicinal or dietary supplement and contains multiple phytochemicals including octopamine and synephrine alkaloids. These molecules are usually cited as the 'active ingredients' in *CA*.[6] Synephrine alkaloids are α-adrenergic agonists that also have some β-adrenergic properties.[7] Although the effects of *CA*-containing products are not known with certainty, largely because of the synephrine alkaloids contained therein, concerns about their effects have been raised. These primarily relate to cardiovascular-related variables and events. For example, Health Canada reports, from January 1, 1998 to February 28, 2004, that it received '16 reports in which products containing bitter orange or synephrine were suspected of being associated with cardiovascular ARs, including tachycardia, cardiac arrest, ventricular fibrillation, transient collapse and blackout. All cases were considered serious'.[8] Given the postulated ability of the synephrine alkaloids in *CA* to produce such outcomes, careful enumeration of the composition of *CA* seems to be in order.

There is confusion in the literature as to which synephrine alkaloids are present in *CA*. There are several isomers of synephrine. First, there are three positional isomers dependent on the phenolic hydroxyl group: *para*-synephrine (*p-synephrine; p-s*); *meta-synephrine* (*m-synephrine; m-s*);[7] and *ortho-synephrine* (*o-synephrine; o-s*).[9] Each of these synephrine alkaloids have two optical isomers or chiral forms: *d* (dextro) and *l* (levo) forms and it is known that *CA* contains both *d* and *l* forms.[10]

p-s occurs naturally in the human body in small quantities and may act as a neurotransmitter. Since 1927, usually under the name oxedrine, it has been used as a pharmaceutical.[11] *m-s*, often referred to as phenylephrine, also occurs naturally in the human body, is widely used as pharmaceutical, has been studied far more extensively than *p-s*, and is one of the two most widely used over-the-counter decongestants.[12]

Penzak *et al*[13] state that *CA* contains *m-s*, whereas Fugh-Berman and Myers[14] state it contains only *p-s*. However, neither provides data nor a reference to data that explicitly demonstrates whether *CA* contains only *m-s*, only *p-s*, or both. The National Toxicology Program[7] implies (but does not state) that *CA* contains only *p-s* and cites Niemann and Gay[1] as their primary source of information about synephrine content of *CA*-containing products. We contacted Dr Niemann who informed us that he did not test for *m-s vs p-s*. We know of no reports that discuss the presence of *o-s*, or lack thereof, in *CA*.[15] Ibrahim *et al* (p 1699) clearly tested for *m-s vs p-s* in citrus fruits, but the fruits tested did not include *CA*. Thus, we have been unable to find convincing data that *CA* contains only *m-s* or *p-s* despite these statements. This is not a trivial issue as the different synephrine isoforms have fairly different pharmacological properties.[16] Precise knowledge of active constituents is particularly important for substances such as *CA*, which may be taken by millions of individuals who receive only modest (if any) supervision from healthcare professionals.

To address this issue, we recently established a LC-mass spectrometry method to distinguish *m-s* from *p-s*. While determining analysis conditions for *m-s* and *p-s*, we found that both compounds gave the same precursor (parent) ion masses and virtually the same product ions in the positive mode. Although the relative intensities are different, this made any differentiation by mass spectrometry alone impossible due to crosscontaminating product ions (see Figure 1). Monitoring product ions in the negative mode was not feasible due to decreased signal intensity. However, separation of these two compounds with adequate specificity was accomplished by their different retention times during isocratic reverse-phase liquid chromatography combined with mass spectrometry. As shown in the chromatogram, *p-s* elutes slightly ahead of *m-s* and resolution between the two compounds is achievable. This chromatogram was generated with purified standards (Sigma Chemical). Our data indicate that it is possible to determine the presence of either *p-s* or *m-s* or both in an unknown sample.

Having established the technique, we purchased and analyzed an over-the-counter weight loss product containing *CA* for which the advertising explicitly stated that it contained *m-s* from *CA* (*Ultimate Thermogenic Fuel* purchased from Australian Muscle: 3/171 Goodwood Rd., Millswood, South Australia 5034; product produced by Gen-Tec Nutrition (distributed internationally by Optigen: 278 Grange Road, Flinders Park, South Australia 5025 http://www.gen-tec.com.au/)). Our results clearly indicated (as did the product label) that the product contained both *p-s* and *m-s*. Based on these data, we conclude that both *p-s* and *m-s* are available in this product.

This finding raises several interesting questions. First, the marketers of this product may be accurate in stating that *CA* contains *m-s* and is the source of their product's *m-s*. If so, there is a misunderstanding in the literature that needs to be corrected in terms of whether *CA* contains *m-s*. A second possibility is that *CA* contains no *m-s*. Were this true, it needs to be shown unequivocally and the literature clarified. Moreover, if *CA* does not contain *m-s*, then the source of the *m-s* in the product tested must be questioned and the possibility that it has been 'spiked' with synthetic phenylephrine must be considered.

These initial observations speak to the need for a thorough investigation regarding the synephrine alkaloids present in both *CA* itself and products claiming to have synephrine alkaloids from *CA*. Since drafting this report, we have learned that the United States National Institute of Standards and Technology, under contract to the National Institutes of

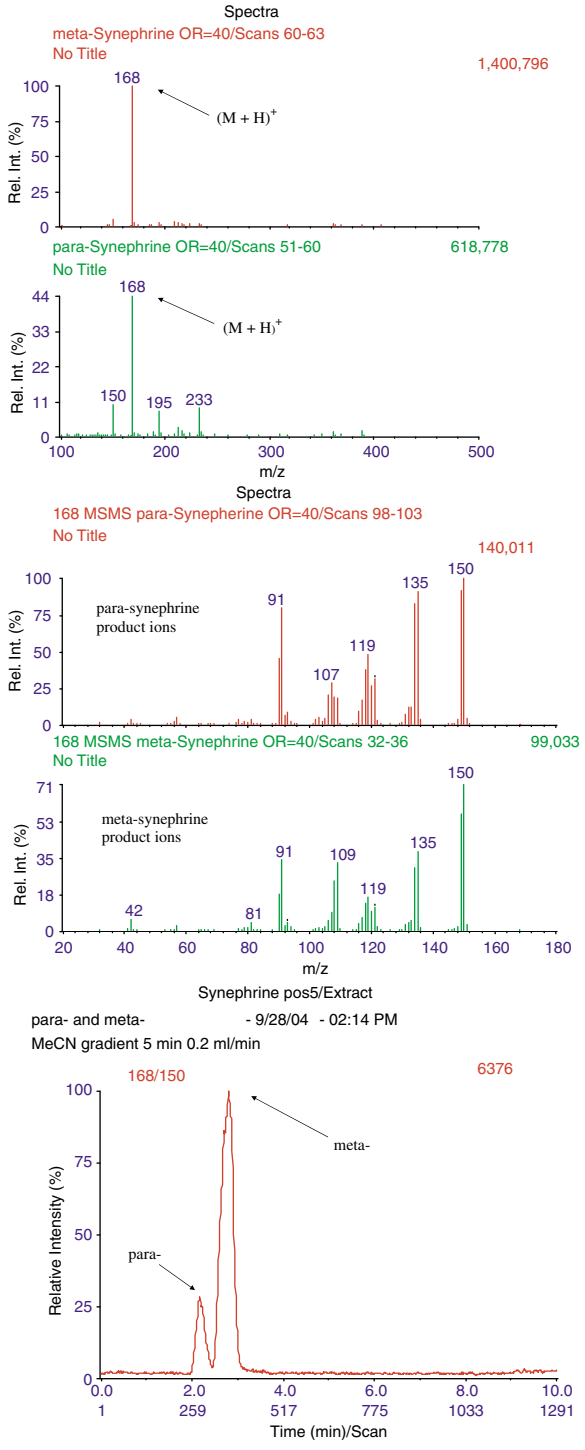

Figure 1 Mass spectrometry analysis of synephrines. In (a), the positive ion mass spectra of authentic *m-s* and *p-s* shows that each produce the same *m/z* 168 $[M+H]^+$ molecular ion. Similarly in (b), the product ion spectra derived from the molecular ions are also essentially the same. However, by carrying out isocratic reverse-phase liquid chromatography, these two synephrine isomers can be resolved and then detected very specifically by examining the eluate by electrospray ionization mass spectrometry by monitoring the combination of the molecular ion/product ion. This shown in (c).

Health's Office of Dietary Supplements, is undertaking just such a rigorous investigation.

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