

Seville (sour) Orange Juice: Synephrine Content and Cardiovascular Effects in Normotensive Adults

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The Seville orange extract *Citrus aurantium* contains *m*-synephrine (phenylephrine) and octopamine; it causes cardiac disturbances in animals and is used by humans for weight loss. Juice from the orange (Seville orange juice [SOJ]) is used to “knock out” intestinal cytochrome P450 (CYP) 3A4 in bioavailability studies. The purpose of this study was to determine synephrine and octopamine concentrations in SOJ and SOJ’s cardiovascular effects in normotensive humans. Subjects consumed 8 ounces of SOJ and water in crossover fashion followed by a repeat ingestion 8 hours later. Hemodynamic (heart rate; systolic, diastolic, and mean arterial pressure) measurements followed. Synephrine and

octopamine were determined by high-performance liquid chromatography. Hemodynamics did not differ significantly between water and SOJ groups. Mean synephrine concentration of SOJ samples was 56.9 ± 0.52 $\mu\text{g/ml}$; octopamine was not detected. SOJ ingestion by normotensive subjects is expected to be safe. Individuals with severe hypertension, tachyarrhythmias, and narrow-angle glaucoma and monoamine oxidase inhibitor recipients should avoid SOJ consumption. Persons taking decongestant-containing cold preparations should also refrain from SOJ intake.

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C*itrus aurantium*, an extract from the fruit/rind of the Seville (sour) orange, is available commercially in herbal dietary supplements that promote weight loss; it is also a natural source of the sympathomimetic amines *m*-synephrine (phenylephrine) and octopamine (Figure 1).¹

Both isolated synephrine and *C. aurantium* extract have been shown to raise blood pressure in animal studies.^{1,2} When administered separately as intravenous doses, unripe fruits of *C. aurantium* (*Fructus aurantii*) and synephrine caused similar dose-dependent elevations in mean arterial pressure in rats.² In another study in rats, repeated oral administration of two *C.*

aurantium extracts resulted in dose-dependent mortality (10%-50%) secondary to cardiovascular toxicity.¹ Collectively, these data suggest that compounds containing *C. aurantium* may be harmful to individuals with cardiovascular conditions such as hypertension or dysrhythmias. Studies in humans, however, are necessary to confirm or refute such speculation.

In contrast to the Seville orange extract *C. aurantium* (obtained from the fruit/rind), freshly squeezed juice from this fruit is frequently used in drug metabolism studies to selectively “knock out” intestinal cytochrome P450 (CYP) 3A4.^{3,4} This is presumably due to 6', 7'-dihydroxybergamottin, a furanocoumarin contained in the juice.⁴ By selectively inhibiting intestinal CYP3A4 without altering the function of the enterocyte efflux protein and *MDR-1* gene product P-glycoprotein, Seville orange juice (SOJ) can assist in determining the relative contributions of intestinal metabolism versus efflux on the systemic availability of coadministered medications.^{3,4} Nonetheless, despite its increasing use in drug metabolism studies, SOJ has not been investigated for its synephrine or octopamine content or for its

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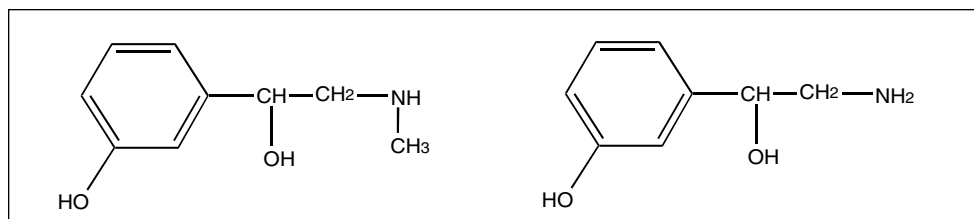


Figure 1. Left: m-syneprine (phenylephrine); right: m-octopamine.

cardiovascular effects in humans. Therefore, the purpose of this study was to quantify the synephrine and octopamine content of SOJ and to characterize its effects on systolic and diastolic blood pressure, mean arterial pressure, and heart rate in healthy volunteers.

SUBJECTS AND METHOD

Subjects

Twelve normotensive subjects (2 females) between 20 and 27 years of age participated in the study. Mean weight for male and female participants was 82.2 ± 19 kg and 61.4 ± 3 kg, respectively; mean height was 72 ± 3 inches and 66 ± 3 inches, respectively. All study volunteers were nonsmokers. Subjects were classified as normotensive based on systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg from two prestudy measurements obtained 1 week prior to study participation. No subject had a medical history positive for hypertension. Participants were not taking any prescription or nonprescription medications, and neither female volunteer was taking contraceptive steroids during the study period. Mercer University's institutional review board approved the study, and all subjects gave written consent. All research was performed at the Mercer University Center for Clinical Research.

Study Design

The study was a two-way, crossover, open-label design with a 7-day washout interval between phases. Each subject participated in both of the following study groups on two different occasions: (1) ingestion of 8 ounces of SOJ prepared from freshly squeezed juice followed by a repeat ingestion 8 hours later (SOJ group) and (2) ingestion of 8 ounces of water followed by a repeat ingestion 8 hours later (control group).

Eight hours prior to their arrival on the study unit (during the previous evening), subjects ingested 8

ounces of SOJ (Phase I) or water (Phase II) prompted by a telephone call from a study investigator. The next morning, subjects arrived at the research unit and presented their empty SOJ/water containers to investigators. Next, duplicate brachial blood pressure (systolic blood pressure [SBP]; diastolic blood pressure [DBP]) measurements were obtained sphygmomanometrically. Heart rate was measured by palpating the radial pulse. Subjects then ingested 8 ounces of SOJ (Phase I) or water (Phase II), and SBP, DBP, and HR were measured at 60-minute intervals over the next 5 hours. In both Phase I and Phase II, subjects refrained from eating and drinking during the 2-hour period following morning SOJ or water ingestion; subjects were then administered a light breakfast consisting of cereal with skim milk and a bagel. Over the remaining 3 hours of the study, subjects ate no additional food but were allowed beverages. Caffeine intake was monitored to ensure that it remained consistent from Phase I to Phase II. Subjects were moderately mobile but remained in the research unit throughout both phases of the study.

Chromatographic Analysis

Instrumentation and chromatographic conditions. A component HPLC system (Shimadzu Scientific Instruments, Columbia, MD) consisted of an LC-600 solvent delivery system, a model SIL-9A autoinjector, and a model SPD-6A UV absorbance detector operating at 208 nm. A prepacked 25 cm \times 4.6 mm (5 μ m particle size) base-deactivated C-18 HPLC column and guard column (Alltima, Alltech Associates, Deerfield, IL) were operated with a mobile phase consisting of acetonitrile, tetrahydrofuran, and water (38:5:57, v/v/v). Sodium lauryl sulfate, an ion-pairing agent, was added to achieve a final concentration of 5 mM. The mobile phase was continuously sparged with helium and delivered at a flow rate of 0.7 ml/min. Column temperature was maintained at 37°C with a model CTO-6A column oven (Shimadzu Scientific Instruments). De-

Table I Hemodynamic Effects of Seville Orange Juice

	Systolic Blood Pressure (mmHg)		Diastolic Blood Pressure (mmHg)		Mean Arterial Pressure (mmHg)		Heart Rate (beats/min)	
	SOJ	Water	SOJ	Water	SOJ	Water	SOJ	Water
Baseline (0 h)	119 ± 8.6	116 ± 9.7	69 ± 6.9	73 ± 9.3	85 ± 7.9	87 ± 8.3	67 ± 13	71 ± 11.2
1 hour	118 ± 14.9	114 ± 10.1	75 ± 11.6	70 ± 9.6	90 ± 11.4	87 ± 7.5	73 ± 10.1	68 ± 7.5
2 hours	119 ± 12	116 ± 7.0	73 ± 7.8	69 ± 7.3	87 ± 6.7	86 ± 6.2	71 ± 7.6	68 ± 10.4
3 hours	120 ± 12.3	118 ± 9.9	72 ± 12.7	69 ± 6.1	88 ± 11.5	86 ± 6.2	71 ± 10.4	73 ± 11.3
4 hours	122 ± 15.1	120 ± 14.9	69 ± 9.9	72 ± 7.9	86 ± 9.8	89 ± 9.7	72 ± 8.6	69 ± 9.2
5 hours	123 ± 16.7	118 ± 12.6	71 ± 8.0	74 ± 11.1	88 ± 9.1	89 ± 10.9	70 ± 7.4	70 ± 9.9

SOJ, Seville orange juice. All measurements are reported as the mean ± standard deviation of determinations in 12 subjects.

tector output was recorded, and chromatograms were analyzed by a CR5-A Chromatopac recorder/integrator (Shimadzu Scientific Instruments).

Standard solutions and sample preparation. Standard curves for synephrine and octopamine were prepared in mobile phase from methanolic stock solutions, covering the range of 6.25 to 400 µg/ml. Eight aliquots of freshly squeezed SOJ, from the same batch administered to study subjects, were analyzed for synephrine and octopamine content by high-performance liquid chromatography (HPLC). SOJ aliquots were directly injected onto the HPLC column using chromatographic conditions previously described by Gurley et al.⁵

Statistical Analysis

Data are presented as the mean ± standard deviation for SBP, DBP, MAP, and HR in 12 subjects. Hemodynamic effects observed during each study phase were analyzed using a two-way repeated-measures analysis of variance (ANOVA) comparing measurements made at each time point with those taken immediately prior to drug administration (time zero). In addition, changes in SBP, DBP, MAP, and HR were compared at each time point between the treatment phases using a repeated-measures ANOVA. Statistical significance was defined a priori as $p < 0.05$.

RESULTS

Subjects

All 12 subjects completed both phases of the study without adverse effects. Changes in SBP, DBP, MAP, and HR (mean ± SD) are summarized in Table I. Baseline hemodynamic measurements observed during

study Phases I and II were similar as analyzed by ANOVA. SOJ had no significant effects on SBP, DBP, MAP, and HR compared with water ($p > 0.05$ for all comparisons).

Chromatographic Analysis

Standard curves for synephrine and octopamine prepared in mobile phase (Figure 2A) were linear over the concentration range of 6.25 µg/ml to 400 µg/ml ($r^2 > 0.999$). Inter- and intraday percent error and relative standard deviation were consistently $< 10\%$ for both compounds. The mean synephrine concentration in eight Seville orange juice samples was 56.9 ± 0.52 µg/ml (Figure 2B); octopamine was not present in any of the tested samples. The synephrine peaks observed in Seville orange juice samples had the same retention times as the synephrine reference standards, confirming that the peaks are likely to be synephrine. Orange juice from frozen concentrate contained neither synephrine nor octopamine (Figure 2C).

DISCUSSION

Cardiovascular indices (SBP, DBP, MAP, and HR) were not significantly altered by SOJ ingestion in 12 healthy subjects, even though the juice contained marked amounts of the sympathomimetic agent, synephrine. Indeed, with each 8-ounce glass of SOJ, subjects ingested approximately 13 to 14 mg of synephrine (57 µg/ml to 240 ml of juice)—an amount comparable to *m*-synephrine (phenylephrine) contained in decongestant-containing cold preparations (as the hydrochloride salt) such as Dimetane™ decongestant caplets and Naldecon™ tablets. Of note, orange juice from frozen concentrate did not contain synephrine (or

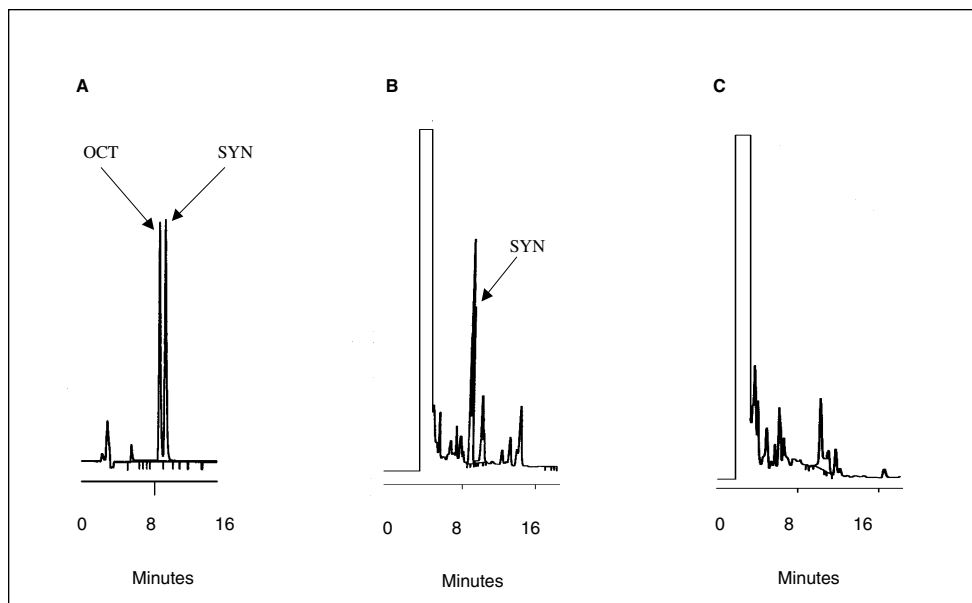


Figure 2. (A) Chromatogram for mobile phase spiked with octopamine (OCT; 50 $\mu\text{g}/\text{ml}$) and synephrine (SYN; 50 $\mu\text{g}/\text{ml}$). (B) Chromatogram for Seville orange juice. (C) Chromatogram for orange juice from frozen concentrate.

octopamine), thereby distinguishing it from Seville orange juice.

Previously, Thomas et al⁶ evaluated the cardiovascular effects of 10 mg oral phenylephrine in healthy volunteers over a 4-hour postdose period by impedance cardiography and forearm plethysmography. Phenylephrine administration resulted in a small but statistically significant elevation in total peripheral resistance in healthy subjects 30 to 60 minutes after dosing; other hemodynamic indices were not appreciably affected. It is possible that hemodynamic alterations such as elevations in total peripheral resistance, which went undetected in this study, may have been observed with the use of more sensitive instrumentation (i.e., impedance cardiography vs. sphygmomanometry).

Poor phenylephrine bioavailability ($\approx 20\%$ - 38%) likely explains the lack of cardiovascular effects observed by Thomas and coworkers and may also explain our observations in this investigation.^{6,7} However, this assumption presupposes that phenylephrine bioavailability is comparable between commercial oral dosage forms and SOJ. After oral administration, phenylephrine undergoes extensive presystemic biotransformation in the intestinal wall and liver by sulfate conjugation and oxidative deamination via monoamine oxidase (MAO).⁷ Although beyond the scope of the current investigation, quantitative analysis of plasma and urine for synephrine and its deaminated and sulfated metabolites will be invaluable in assessing synephrine disposition after SOJ consumption.

The absence of observed changes in hemodynamics with SOJ did not likely arise from the blood pressure

sampling strategy used in this study (every hour for 5 hours after SOJ ingestion). Indeed, peak plasma phenylephrine concentrations and hemodynamic effects are seen within this period following oral dosing.^{6,8} Furthermore, it is unlikely that our method of SOJ administration (8 ounces followed by a repeat ingestion 8 hours later) contributed to our negative hemodynamic findings. Indeed, the influence of SOJ consumed 8 hours previously would not be expected to influence blood pressure the following day. In fact, this method of SOJ administration was chosen simply to mimic that which might be used in a typical pharmacokinetic investigation in which the juice is administered over time. As such, hemodynamic indices were not measured after initial SOJ consumption. Nonetheless, it is unlikely that this initial ingestion of SOJ resulted in hemodynamic changes, given that a subsequent ingestion 8 hours later did not cause hemodynamic changes. Last, the absence of observed changes in hemodynamics with SOJ consumption also suggests that caffeine ingestion, which was monitored in this study—yet allowed—was not of hemodynamic significance in either phase of the investigation. Indeed, a previous study noted that caffeine intake did not contribute to the cardiovascular effects of phenylephrine in healthy volunteers.⁶

An additional factor potentially contributing to our results is that we did not separately quantify the respective stereo (+/-)- and positional (*m* and *p*) isomers of synephrine contained in SOJ. This may be of clinical importance since *m*-synephrine is a much more potent α_1 agonist compared with *p*-synephrine based on ex-

periments conducted with α_1 -adrenoreceptors from rat aorta.⁹ In addition, (–) synephrine is one to two orders of magnitude more potent on α_1 -adrenoreceptors than its (+) stereoisomer. Of note, (–) *m*-octopamine is an equipotent α_1 agonist compared with (–) *m*-synephrine, but we did not detect octopamine in any of the SOJ samples that we tested.

Despite the presence of notable amounts of synephrine in the SOJ samples we analyzed, the lack of an effect on hemodynamic parameters in healthy volunteers strongly suggests that the juice is safe to administer in 8-ounce portions to normotensive, healthy individuals. Nonetheless, the same precautions should be taken when administering SOJ as those taken when administering phenylephrine-containing decongestant products. Specifically, the absorption of phenylephrine from oral dosage forms is markedly increased in the presence of MAO inhibitors; potentiation of synephrine's cardiac and pressor effects results.⁷ Thus, SOJ, with its marked synephrine content, may also cause deleterious cardiac effects when ingested along with MAO inhibitors. Last, similar to phenylephrine-containing oral preparations, SOJ is best avoided in individuals with severe hypertension, tachyarrhythmias, or narrow-angle glaucoma.

In the current study, SOJ from a single batch was analyzed. However, it is possible that Seville oranges obtained from diverse geographical locations and grown and harvested under different conditions may vary with respect to the synephrine and/or octopamine content in their juice (presence vs. absence as well as quantity). This may explain the absence of octopamine in the SOJ we analyzed. Another plausible explanation for octopamine's absence in SOJ—in contrast to its presence in the *Citrus aurantium* extract—may be that it is not quantifiable in the juice. Last, it is unclear whether condiments prepared from Seville oranges (i.e., marmalade) contain appreciable amounts of synephrine and/or octopamine or whether the compound is destroyed during processing.

In conclusion, the SOJ that we tested contained a considerable amount of synephrine but did not alter

cardiovascular indices in normotensive healthy individuals. Caution should be exercised in special patient populations such as those with severe hypertension, tachyarrhythmias, or narrow-angle glaucoma to ensure that they do not participate in drug metabolism studies that require the ingestion of SOJ. In addition, those individuals taking MAO inhibitors and decongestant-containing cold preparations should also refrain from SOJ consumption.

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