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Pharmacokinetic analysis of anti-allergy and anti-inflammation bioactives in a nettle (*Urtica dioica*) extract.

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ABSTRACT

Ayers S, Roschek B Jr, Williams JM, Alberte RS., Pharmacokinetic analysis of anti-allergy and anti-inflammation bioactives in a nettle (Urtica dioica) extract, Online J Pharmacol Pharmacokin, 5: 6-21, 2008. Pharmacokinetic analyses were conducted using DART TOF-MS on the uptake of bioactive components present in a nettle (*Urtica dioica*) extract delivered orally as a lozenge. Previously adenine, synephrine, osthole, and nicotinamide were shown to be key bioactives contributing to the anti-inflammatory and anti-allergy properties of the nettle extract (Roschek et al, 2009). Average serum concentrations of adenine reached 35.2 nmol L^{-1}, nicotinamide reached 8.7 nmol L^{-1}, osthole reached 11.0 nmol L^{-1}, and synephrine reached 107.4 nmol L^{-1}. These serum levels are equivalent to 0.3%, 0.2%, 0.2%, and 1.6% oral bioavailability for adenine, synephrine, osthole and nicotinamide respectively. Urine concentrations for adenine, nicotinamide, synephrine, and osthole reached 4.9 nmol L^{-1}, 2.5 nmol L^{-1}, 6.6 nmol L^{-1}, and 0.2 nmol L^{-1}, respectively. The major pharmacokinetic parameters (C_{max}, T_{max}, $T_{1/2}$) were determined and compared for serum and urine. For all three of the compounds, C_{max} values decreased 2- to 16-fold in urine compared to serum, T_{max} values increased 2- to 12-fold, and $T_{1/2}$ values increased 2- to 10-fold. Molecular modeling revealed that these compounds were not likely to cross the blood-brain barrier, particularly important for the anti-histamine bioactives to ensure a non-drowsy activity. These results show that anti-inflammatory and anti-allergenic compounds in nettle are readily absorbed into the body and excreted in the urine when a nettle extract is delivered orally as a lozenge. In addition, DART

TOF-MS is useful in quantifying multiple components in biological matrices with little or no sample preparation.

Key Words: *Urtica dioica*, nettle, allergies, inflammation, DART TOF-MS

INTRODUCTION

It has been shown recently that there is a strong environmental as well as genetic component to allergy related inflammatory responses (Holgate and Broide 2003; Bousquet *et al.* 2004; Schatz 2007; Schoefer *et al.* 2008). Due primarily to environmental factors there has been a significant increase in seasonal allergies, particularly in children, in the U.S. (Skoner 2001). Many of the current over-the-counter (OTC), prescription, and herbal-based medications on the market for allergies, possess undesirable side-effects like headache, dry mouth and/or drowsiness. However, the treatments for allergies are among the safest drugs in the world, with an extremely low number of adverse effects from use (Scadding 1999; Lipworth 2001; Hore *et al.* 2005; Kawai *et al.* 2007; Bousquet *et al.* 2008).

Nettle (*Urtica dioica* L.) has been used for hundreds of years to treat a variety of disorders ranging from allergic rhinitis to hypertension (Thornhill and Kelly 2000; Legssyer *et al.* 2002). Nettl*e* is a temperate herbaceous species that is a common and aggressive weed found in moist soils throughout the U.S. and Europe and is also cultivated commercially (Whitney and Gibbs 2006). Clinical evidence suggests that freeze-dried extracts of nettle leaves reduce allergy symptoms (Mittman 1990). However, the precise nature of the effectiveness of nettle extracts for allergies is unclear, but clinical data and recent findings suggest the benefits arise from its anti-inflammatory activities (Mittman 1990; Tunon *et al.* 1995; Konrad *et al.* 2005).

Recently Roschek et al. (2009) demonstrated that a proprietary nettle leaf extract possessed anti-inflammatory and anti-allergenic activities including the inhibition of COX-1, COX-2, and Prostaglandin D_2 Synthase enzymes involved in the production of pro-inflammatory prostaglandins as well as the inhibition of tryptase that controls mast cell degranulation leading to the release of cytokines and chemokines that cause allergy symptoms. In addition, the nettle extract possessed antagonist and negative agonist activity against the Histamine 1 Receptor (H1) which controls histamine release from basophils, mast cells and other cells. Recognizing that the efficacy of botanical extracts is based on the presence of bioactives and the bioavailability of these compounds, we examined the pharmacokinetic features of key bioactives in a proprietary nettle extract that possessed activities against key allergy and inflammatory pathways.

Pharmacokinetic analysis of the active components in botanical extracts presents significant analytical challenges because of their chemical complexity, lack of standards for many compounds of interest, and detection limitations of the analytical methods since many bioactives are present in low abundances. Roschek and Alberte (2008) demonstrated the utility of Direct Analysis in Real Time Time-of-Flight Mass Spectrometry (DART TOF-MS) for pharmacokinetic studies that overcomes these limitations. DART TOF-MS has the advantage of

providing instantaneous exact mass data with little or no sample preparation (Cody *et al.* 2005) compared to LC-MS which usually requires lengthy analysis and sample preparation times. In addition, DART TOF-MS offers the ability to accurately calibrate and quantify compounds present in a mixture when no commercial or synthesized standards are available (JEOL 2007; Roschek and Alberte 2008).

We used DART TOF-MS to determine the pharmacokinetic features and bioavailability of four key nettle bioactives, adenine, nicotinamide, synephrine and osthole, recently found to have anti-inflammatory and anti-allergenic activities (Roschek *et al.* 2009). Adenine is a purine with a variety of metabolic roles and is the nucleotide base of adenosine. Adenosine is active in the regulation of cardiovascular function and recent investigations show that adenosine receptors protect against inflammation and vascular adhesion (Linden 2006; Yang *et al.* 2006). Nicotinamide is a water-soluble B-vitamin also known as Vitamin B3 or niacinamide. Nicotinamide has anti-inflammatory activity in skin disorders (Niren 2006), and inhibits the production of inflammation markers including tumor necrosis factor-α (TNF-α) and prostaglandins (Biedron *et al.* 2008). Synephrine is an alkaloid that has been used as a nasal decongestant (Corey *et al.* 1997) and can be found in traditional Chinese medicines where it is used to treat seasonal allergies and other inflammatory disorders (Xue *et al.* 2004). Osthole is an oxycoumarin, and this class of chemicals has been shown to inhibit specific inflammatory mediators in mice macrophages (Nakamura *et al.* 209).

We report here the pharmacokinetics and bioavailability of adenine, nicotinamide, synephrine and osthole, compounds identified in *Urtica dioica* found to have anti-inflammatory and/or anti-allergenic properties (Roschek *et al.* 2009). All four compounds were bioavailable, albeit at low levels, and appeared in serum within 10 minutes of ingestion of the proprietary nettle extract in the form of a slow-dissolve lozenge. The studies indicate significant uptake in the oral cavity which contributes to the rapid appearance of the 4 bioactives in serum.

MATERIALS AND METHODS

Urtica dioica extract: Leaves of stinging nettle (*Utrica dioica* L.) were obtained from Blessed Herbs (Oakham, MA). The leaves were ground to a powder (particle size of 20-40 mesh) and extracted for 2 hours with HPLC grade water (Sigma-Aldrich, St. Louis, MO) at 40^{o}C in several batches. The resultant slurries were filtered through Fisher P4 filter paper (pore size 4-8 μm) and centrifuged at 2000 rpm for 20 minutes. The supernatants were collected and evaporated to dryness at 50°C in a vacuum oven overnight yielding a crystalline powder. One hundred milligrams (100 mg) of this extract was formulated into slow-dissolve lozenges containing magnesium stearate, cyclodextrin, and natural flavorings that do not contain the compounds being monitored.

Experimental design: Five healthy adult females ranging in age from 23 to 57 were subjected to diets free of flavonoids and any NSAIDs for 36 hours prior to the study. All blood and urine samples were collected and handled using approved protocols. Blood samples were collected at defined time intervals between 0 and 480 minutes after ingestion of two 100-mg nettle extract

lozenges. Blood samples were not treated with heparin to avoid any analytical interference and the serum fraction was collected and frozen (-85^{o}C). Urine samples were collected from the same five subjects over the same time course (0 to 480 min) and stored at -85^{o}C prior to analysis.

DART TOF-MS calibration: Separate four-point linear calibration curves were developed using >98% pure adenine, nicotinamide, and synephrine purchased from Sigma-Aldrich (St. Louis, MO) as external standards. Seven replicate analyses were performed for each data point of each curve, giving R^2 values for adenine, nicotinamide, and synephrine of 0.999, 0.988, and 0.995, respectively. For osthole, coumarin was used as an external standard as it has similar chemical and physical properties to osthole yielding an R^2 value of 0.979. The DART TOF-MS intensities of the nettle bioactive compounds, as measured in the serum and urine samples, were converted to molar concentrations using established methods for development of quantitative analyses of drug metabolites in urine using DART TOF-MS (JEOL 2007; Roschek and Alberte 2008).

DART TOF-MS sample preparation: Serum samples were stored frozen until analysis. The serum could be analyzed without sample preparation; however, to remove possible interferences from proteins, peptides, and polysaccharides, an equal volume of 100% ethanol (USP) was added to the serum samples and shaken vigorously. The ethanol/serum solution was centrifuged for 8 min at 13,200 rpm at 20^{o}C to remove insoluble materials. The supernatant was used for DART TOF-MS analyses. Urine was analyzed with no sample preparation.

DART TOF-MS analysis: A JEOL DART^{TM} AccuTOF-mass spectrometer (JMS-T100LC; JEOL USA, Peabody, MA) was used for analysis of the serum and urine samples and was executed in positive ion mode. The needle voltage was set to 3500 V, heating element to 250^{o}C, electrode 1 to 150 V, electrode 2 to 250 V, and helium gas flow to 2.52 L min^{-1}. For the mass spectrometer, the following settings were used: orifice 1 was set to 10 V, the ring lens voltage set to 5 V, and orifice 2 set to 5 V. The peak voltage was set to 1000 V in order to give peak resolution beginning at 100 *m/z*. The microchannel plate detector voltage was set at 2500 V. Mass calibrations were performed internally using PEG 600 (Ultra Chemical, North Kingston, RI), which provided mass markers throughout the required mass range 100-1000 *m/z*. Calibration tolerances were ±10 mmu. Samples (3 □L) were pipetted onto the closed end of a borosilicate glass melting point capillary tube and introduced into the DART by holding the tube in the He plasma stream until the entire sample was consumed.

Absorption, Distribution, Metabolism, and Excretion (ADME) predictions: Accelrys Discovery Studio v2.1 (Accelrys Software Inc, San Diego, CA) was used to predict blood-brain barrier transport and intestinal absorption.

RESULTS

DART TOF-MS analysis of serum and urine: DART TOF-MS was used to identify and quantify 4 known anti-inflammatory and/or anti-allergenic compounds from the nettle extract in serum.

Three additional compounds were identified in urine, but not in serum, indicating that these 3 compounds are bioavailable but rapidly eliminated from the blood (see Table 1; Roschek *et al.* 2009). The four bioactive compounds identified in both serum and urine (nicotinamide, synephrine, adenine, and osthole) were examined in detail.

Table 1. Summary of nettle anti-inflammatory/allergenic compounds detected by DART TOF-MS in serum and in urine

Bioactive Compound	**Present in Serum**	**Present in Urine**	**Earliest Detection in Serum (min)**	**T_{max} serum (min)**	**Target Endpoint**
Nicotinamide	+	+	10	30	COX-1/COX2/H1-receptor
Synephrine	+	+	10	20	H1-receptor
Adenine	+	+	10	120	PGDS*
Vitamin B5	-	+	ND**	ND	PGDS
DL-methyl-m-tyrosine	-	+	ND	ND	H1-receptor
Osthole	+	+	10	20	Tryptase/H1-receptor
4-shogaol	-	+	ND	ND	H1-receptor

*PGDS = Prostaglandin D_2 Synthase; **ND = Not Detected.

Figure 1 shows a representative DART TOF-MS of serum at 40 minutes post-consumption. The presence of adenine (*m/z* = 136.055; red arrow), nicotinamide (*m/z* = 123.048; green arrow), synephrine (*m/z* = 168.095; blue arrow), and osthole (*m/z* = 245.059; purple arrow) as identified by DART TOF-MS indicates that these compounds are bioavailable and readily taken up into the bloodstream. Each of these four compounds was present in serum within 10 minutes of ingestion of the nettle lozenges, indicating rapid absorption into the blood stream via the oral cavity. However, the time at maximum serum concentration (T_{max}) varied for each compound. Synephrine and osthole reached T_{max} concentrations 20 minutes after ingestion, while nicotinamide reached T_{max} at 30 minutes post-ingestion. The T_{max} times for these 3 compounds supports their rapid uptake in the oral cavity. Adenine on the other hand reached T_{max} at 120 minutes post-ingestion. Although the earliest detection of adenine was t at 10 minutes post-ingestion, the delay in T_{max} (compared to the other 3 compounds monitored) suggests that adenine is preferentially absorbed in the stomach and/or lower intestine.

The phenol, 4-shogaol (m/z = 249.115), the amino acid DL-methyl-m-tyrosine (m/z = 196.085), and the Vitamin B5 (m/z = 220.100) were detected in urine, but not in serum. The presence of these compounds in urine indicate that they are taken up into the bloodstream, however, the levels in serum must below detection using DART TOF and/or the serum residence time is extremely short (e.g., <10 minutes).. Sincenicotinamide, adenine, synephrine, and osthole were present in both serum and urine, these compounds were chosen for further examination. These compounds have been identified as nettle bioactives against multiple endpoints associated with inflammation and allergic rhinitis including the inhibition of prostaglandin production via COX-1, COX-2, and PGDS enzymes, and histamine H1 receptor antagonist activity as well as tryptase inhibition (see Table 1; Roschek *et al.* 2009).

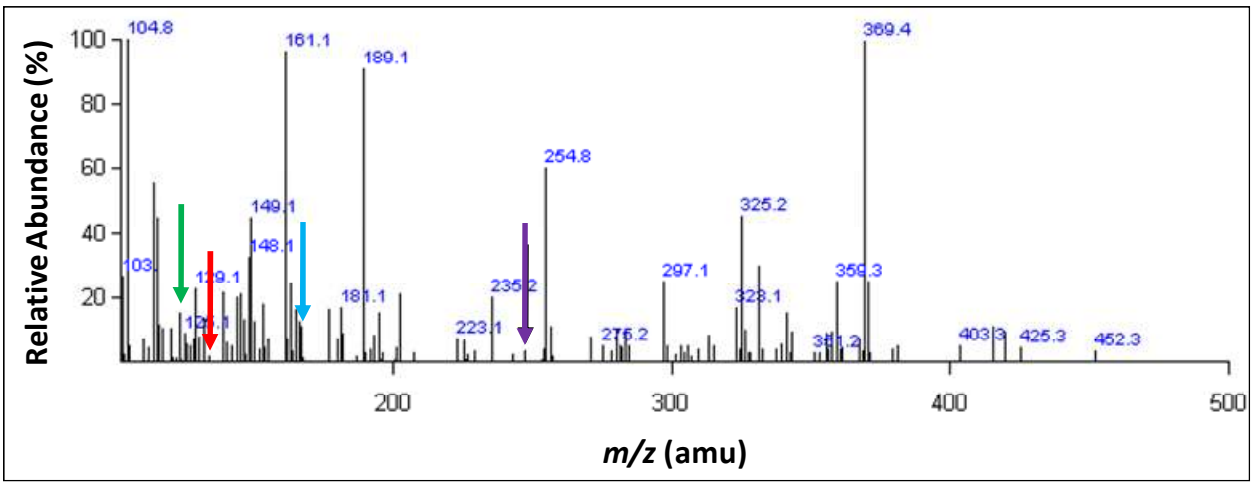

Figure 1. DART TOF-MS of serum sample 40 minutes post-consumption of a nettle extract. Adenine (*m/z* = 136.055; red arrow), nicotinamide (*m/z* = 123.048; green arrow), synephrine (*m/z* = 168.095; blue arrow), and osthole (*m/z* = 245.059; purple arrow) are all detected at this time point.

The bioactive compounds identified in both serum and urine were determined using DART TOF-MS and the elemental composition determination of each of the masses in the DART TOF-MS spectra. The DART TOF mass spectrum of urine at 60 minutes post-consumption is shown in Figure 2 with adenine (red arrow), nicotinamide (green arrow), synephrine (blue arrow), and osthole (purple arrow) identified with arrows. The elemental composition software used here utilizes isotope ratio matching algorithms that allow for the distinction between true compounds and baseline interference so that there is unequivocal identification of mass spectral data and the identification of compounds in low abundance can be separated from the baseline noise related to the mass spectrometer.

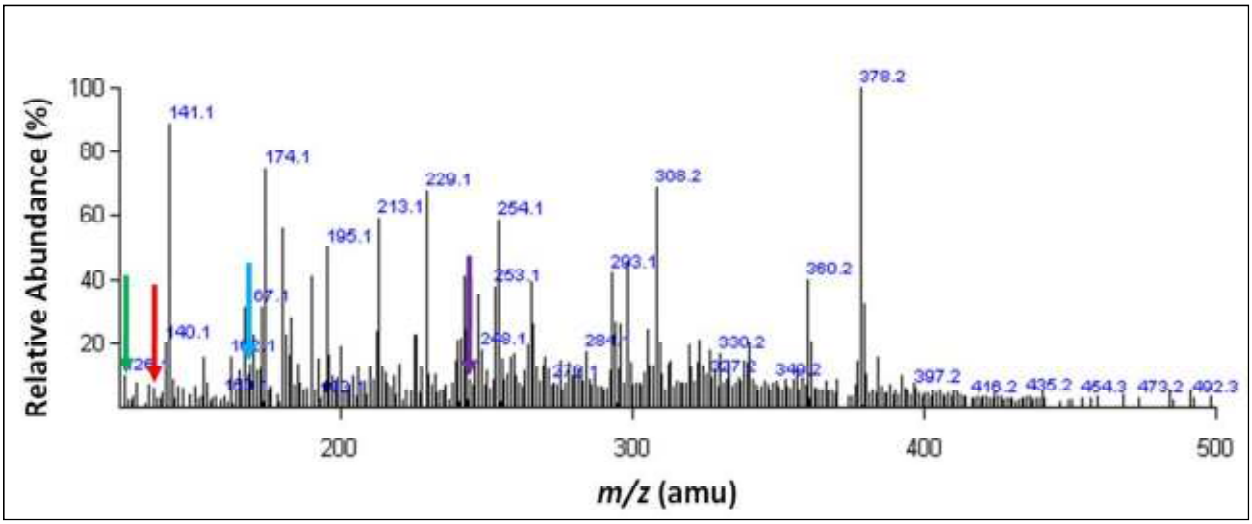

Figure 2. DART TOF-MS of urine sample 60 minutes post-consumption of a nettle extract**.** Adenine (*m/z* = 136.055; red arrow), nicotinamide (*m/z* = 123.048; green arrow), synephrine (*m/z* = 168.095; blue arrow), and osthole (*m/z* = 245.059; purple arrow) are all detected at this time point.

Serum levels of anti-allergenic compounds from a nettle extract: The time course of appearance of adenine, nicotinamide, synephrine and osthole from five subjects that received two 100-mg nettle lozenges (total dose = 200 mg) are shown in Figure 3. For all individuals, the three compounds were first detected 10 minutes after consumption. Variation among individuals for each compound monitored in serum was observed, however the variation was within one standard deviation. Additionally, in all individuals, serum levels of the bioactives were sustained in all of the subjects through 240 minutes and were not detected 8 hours after consumption (Figure 3).

Adenine achieved a maximum serum concentration of 35.2 nmol L^{-1} within two hours of consumption. However, at 40 minutes post-consumption, a spike in serum concentration reaching 30 nmol L^{-1} was observed, indicating that adenine may be taken up in both the oral cavity and the intestine. The maximum serum concentration achieved is equivalent to 0.3% bioavailability of the consumed adenine from the 200-mg dose of the nettle extract. Adenine was detected in serum up to 4 hours post-consumption, albeit at very low levels. Elimination of adenine from serum followed first-order kinetics and was cleared from the serum within 8 hours of ingestion, the last time point collected (Figure 3).

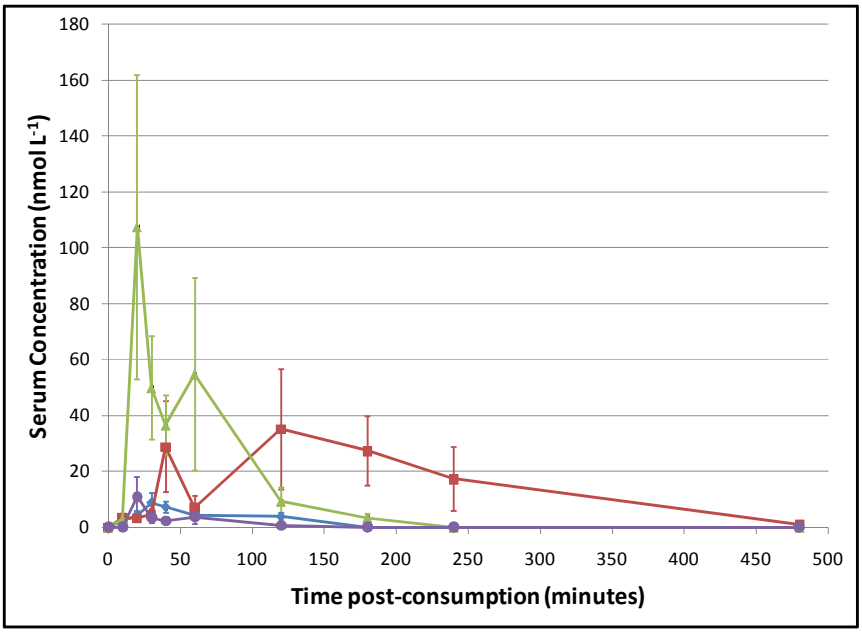

Figure 3. Serum concentrations of nicotinamide (--◆--), adenine (--■--), synephrine (--▲--), and osthole (--●--) as determined by DART TOF-MS analysis of a 200 mg dose of *Urtica dioica extract*. Each data point represents the average serum concentrations from five different subjects with error bars representing ±1 S.D (n=5).

Nicotinamide reached an average maximum serum concentration of 8.7 nmol L^{-1} within 30 minutes of consumption, equivalent to 0.2% bioavailability. Nicotinamide was detected in serum as early as 10 minutes post-ingestion, and elimination from serum followed first-order

kinetics. Detectable levels of nicotinamide in serum were sustained for 3 hours, and nicotinamide was cleared from the serum within 4 hours of consumption (Figure 3). The results obtained here are nearly identical to previous results showing that pure nicotinamide reaches maximum serum concentration 30 minutes post-consumption, is maintained in serum for up to 3 hours, and has a bioavailability of 0.2% (Dragovic *et al.* 1995).

Synephrine, a phenolic amine commonly found in bitter orange, reached a maximum serum concentration of 107.4 nmol L^{-1} within 20 minutes of consumption of the nettle lozenges. Like adenine and nicotinamide, synephrine was first detected in serum within 10 minutes of initial ingestion. Synephrine reached maximum serum concentrations at 20 minutes post-consumption and a secondary spike in serum concentration, lower than the C_{max}, was observed at 60 minutes, however this increase was within one standard deviation of the C_{max} at 20 minutes. Like adenine, this indicates the oral cavity and stomach/intestines are uptake sites for this bioactive. The synephrine maximum serum concentration is equivalent to 1.6% bioavailability, a value greater than previous reports on the pharmacokinetics of this compound provided in a pure form (Hengstmann and Goronzy 1982). Taken together, the findings here and the earlier work, suggests that synephrine is preferentially absorbed in the oral cavity. Synephrine followed zero-order elimination kinetics and was detected 4 hours post-consumption at levels below 1 nmol L^{-1} (Figure 3). Synephrine was not detected at the 8-hour time point.

Osthole, an oxycoumarin commonly extracted from Angelica root, achieved a maximum serum concentration of 11.0 nmol L^{-1} at 20 minutes post-ingestion. This C_{max} is equivalent to 0.23% bioavailability, or 21 µg of the available osthole absorbed in the oral cavity. Osthole followed first-order elimination kinetics throughout the duration of the experiment and was eliminated from both blood and urine within 480 minutes (see Figure 1 and Figure 2). Osthole is also retained in the body for an extended period of time with a determined $T_{1/2}$ of nearly an hour (see Table 2). In fact, all four compounds appear in serum rapidly and elimination is relatively slow, based on half-life for all four compounds.

***Urine levels of anti-allergenic compounds*:** The urine levels of adenine, nicotinamide, osthole, and synephrine are shown in Figure 4. For all subjects, the four monitored compounds were detected in urine at 60 minutes post-consumption, the first time point for urine sample collection. The levels of each of these compounds in urine varied only slightly between the individuals and, except for osthole, were maintained among the subjects through the last sample collection at 8 hours post-consumption (Figure 4). Adenine reached a maximum urine concentration of 14.9 nmol L^{-1} within two hours of consumption. This concentration is equivalent to 0.2% wt/wt (16 □g) of the consumed adenine from the 200 mg dose of the nettle extract. The maximum urine concentration of synephrine was determined to be 6.6 nmol L^{-1} also achieved two hours post-consumption of the nettle extract. This concentration is equivalent to 0.2% wt/wt (8.8 □g), which is 8-times lower than the maximum serum concentration of synephrine indicating that synephrine is very slowly removed from the blood and/or is metabolized/degraded by the liver. The urine concentration of nicotinamide reached 2.5 nmol L^{-1} within two hours of consumption, equivalent to 0.1% wt/wt (2.5 □g) of the

consumed nettle extract. This concentration is nearly 3-times lower than that achieved in serum, again indicating that nicotinamide is likely metabolized by the liver and/or slowly removed from the blood. The concentration of osthole in urine reached 0.22 nmol L^{-1} within 2 hours of ingestion, equating to 0.005% wt/wt (0.43 µg) of the nettle extract. Adenine, nicotinamide, synephrine and osthole all followed first-order elimination kinetics through the final sample time point at 8 hours post-ingestion (Figure 4).

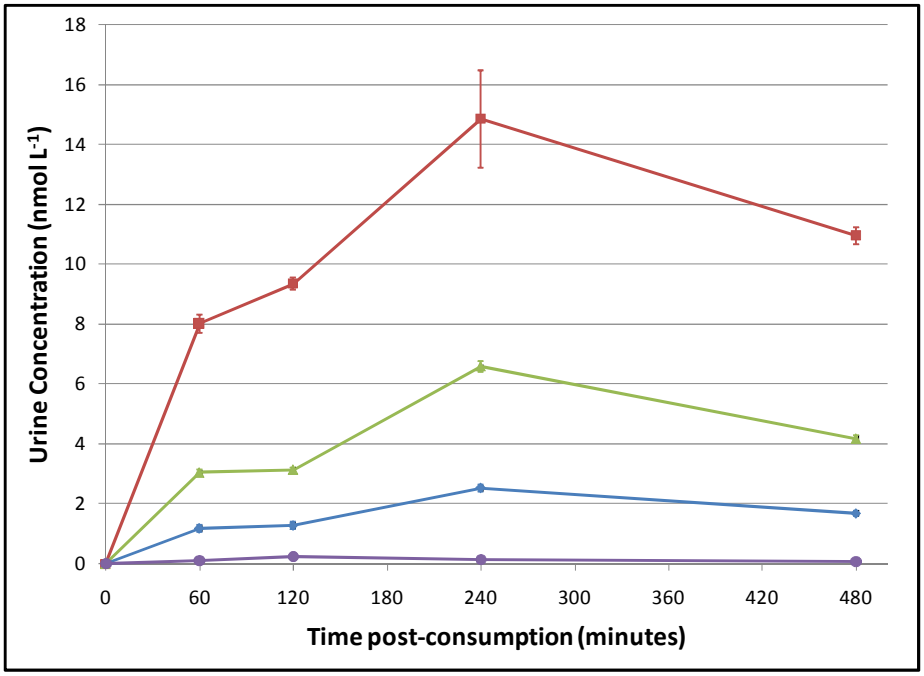

Figure 4. Urine concentrations over time of nicotinamide (--◆--), adenine (--■--), synephrine (--▲--), and osthole (--●--) as determined by DART TOF-MS analysis of a 100 mg dose of *Urtica dioica* extract. Each data point represents the average urine concentrations from five different subjects with error bars representing ±1 S.D. (n =5).

Absorption, Distribution, Metabolism, and Excretion (ADME) Predictions: Accelrys Discovery Studio 2.1 was used to predict ADME properties including intestinal absorption and likelihood that compounds would pass the blood-brain barrier. Adenine, synephrine, osthole, and nicotinamide were predicted to be absorbed in the lower intestine. None of the four bioactive compounds are predicted to cross the blood-brain barrier indicating that they will not directly affect central nervous system receptors such as the histamine H1 Receptor.

Pharmacokinetic analysis of anti-allergenic compounds in the nettle extract: The serum pharmacokinetic parameters for adenine, synephrine, osthole and nicotinamide present in the nettle extract are summarized in Table 2 (serum).

Table 2. The serum pharmacokinetic parameters of nicotinamide, adenine, synephrine and osthole after ingestion of a single 100 mg *Urtica dioica* lozenge. C_{max} values are ±1 S.D., n=5.

Compound Name	C_{max} (nmol L^{-1})	T_{max} (min)	k (nmol L^{-1} min^{-1})	$T_{1/2}$ (min)
Vitamin B3 (niacinamide)	8.7 ± 3.6	30	0.058	75
Adenine	35.2 ± 21.5	120	0.098	180
Synephrine*	107.4 ± 54.4	20	0.021	33
Osthole	11.0 ± 7.0	20	0.105	52

*= zero-order kinetics for elimination

The elimination kinetics for all compounds was first-order in serum, except for synephrine which exhibited zero-order kinetics. The elimination half-life ($T_{1/2}$; the time required for the serum concentration to be reduced by 50%) for the three compounds ranged from 33 to 180 minutes in serum with adenine having the longest residence times (Table 2). In urine, the elimination kinetics for osthole, adenine and nicotinamide were first-order, while synephrine exhibited zero-order kinetics. The elimination half-life ($T_{1/2}$) values for synephrine, osthole, nicotinamide and adenine ranged from 255 to 465 minutes in urine with adenine having the longest residence times for both serum and urine (Table 2 and Table 3), and osthole showing, the most rapid elimination based on k values from Table 2 and $T_{1/2}$ values from Table 3.

Table 3. The urine pharmacokinetic parameters of nicotinamide, adenine, synephrine, and osthole after ingestion of a single 100 mg *Urtica dioica* lozenge. C_{max} values are ±1 S.D., n=5.

Compound Name	C_{max} (nmol L^{-1})	T_{max} (min)	k (nmol L^{-1} min^{-1})	$T_{1/2}$ (min)
Vitamin B3 (niacinamide)	2.5 ± 0.1	240	0.004	313
Adenine	14.9 ± 1.6	240	0.016	465
Synephrine	6.6 ± 0.2	240	0.010	330
Osthole	0.24 ± 0.08	240	4.7×10^{-4}	255

DISCUSSION

Plants and plant products have been used as dietary supplements for thousands of years. Key to the functional role of these dietary supplements is efficacy (Mahady *et al.* 2001) which is strongly dependent on the bioavailability and pharmacokinetic properties of the individual bioactive components, as well as the biochemistry and physiology of the consumer (Holst and Williamson 2008). However, because the gut is a major organ involved in the immune response, unabsorbed phytonutrients may be active locally, rather than systemically, therefore bioavailability can be less critical for certain specific functions (Kanner 2007). For example, phytonutrients like flavonoids are the most abundant polyphenols in the human diet and are readily absorbed in the oral cavity in glycosylated and methylated forms due to the high abundance of sugar transporters in the oral mucosa (Oyama *et al.* 1999), but when introduced into the stomach, bacterial activity can cleave chemical moieties, like sugar residues, that are essential for absorption, thereby rendering the flavonoid unavailable or at best only weakly

absorbed (Schneider *et al.* 1999; Crespy *et al.* 2002; Nemeth *et al.* 2003). Gastrointestinal tract microflora also have a major impact on flavonoid uptake, as they actively transform and metabolize these chemicals (Keppler and Humpf 2005).

Bioaccessibility, the amount of a nutrient potentially absorbed, is a key requirement for bioavailability (Holst and Williamson 2008). Bioaccessibility is limited by the chemical form and properties of the phytonutrients. For example, flavonoid glycosides are present in fruits, but are primarily absorbed as the aglycones after enzymatic hydrolysis of sugar residues (Ross and Kasum 2002).The cleavage of the sugar moieties typically occurs in the stomach via bacterial action. Roschek and Alberte (2008) showed that the aglycones of specific glycosylated flavonoids were readily absorbed in the oral cavity when the extract was supplied in a lozenge form while other flavonoids in the extract were most likely absorbed in the small intestines.

Rochek *et al.* (2009) reported that numerous compounds present in the proprietary nettle extract evaluated here were responsible for the inhibition of key steps in the pro-inflammatory pathways associated with inflammation and allergic rhinitis. Of these compounds, adenine, nicotinamide, osthole, and synephrine were detected in both serum and urine following administration of 200 mg of this nettle extract in the form of a lozenge. The compounds monitored are known to be widespread throughout the plant kingdom and play very unique roles in human physiology, particularly as they relate to inflammation disorders (Corey *et al.* 1997; Linden 2006; Biedron *et al.* 2008). The identification of these compounds in nettle, and their detection in human serum and urine, supports the findings by others that extracts containing these compounds can be useful for relieving symptoms associated with inflammatory conditions (Nakamura *et al.* 209; Corey *et al.* 1997; Xue *et al.* 2004).

Adenine functions in cellular respiration and protein synthesis, and being the purine base of adenosine, it also functions in nucleic acid synthesis and is known to have roles in anti-inflammatory pathways (Linden 2006). Adenine derived from the nettle extract reached maximum serum concentration of 35 nmol L^{-1} two hours post-ingestion and had a half-life of 180 minutes. Bioavailability of adenine was only 0.3%, however adenine may be converted to, and utilized as, adenosine in the body thus lowering the concentration of unchanged adenine that was monitored by DART TOF-MS.

Nicotinamide (Vitamin B3, niacinamide) has been used for insulin-dependent diabetes mellitus and as a chemotherapy agent to improve radiation damage in human tumors (Robinson *et al.* 2000; Gale *et al.* 2004). Nicotinamide has also been reported to have COX-1 and COX-2 inhibitory properties (Jonas *et al.* 1996; Roschek *et al.* 2009). Horsman *et al.* (1993) evaluated the pharmacokinetics properties of pure nicotinamide in humans and mice. When 6 grams of nicotinamide was orally ingested, peak plasma concentrations of 160 µg mL^{-1} were achieved, representing 0.3% bioavailability of the orally administered nicotinamide. In another study using LC-MS (Dragovic *et al.* 1995), it was found that nicotinamide was readily absorbed and well tolerated up to a 6 g oral dose of pure nicotinamide and that the maximum serum concentration increased linearly in a dose-dependent fashion. In addition, maximum serum concentration of nicotinamide was achieved at 30 minutes post-ingestion regardless of dosage,

and nicotinamide was present in serum up to 3 hours post-consumption (Dragovic *et al.* 1995); findings essentially identical to those here when nicotinamide was present as a bioactive in a botanical extract and its kinetic properties monitored by DART TOF-MS. As such, the utility of DART TOF-MS as a useful analytical tool for pharmacokinetics and bioavailability studies of bioactive compounds in biological fluids is further validated.

Synephrine is an ergogenic substance found in bitter orange (*Citrus aurantium*), tangerines, and other citrus fruits (Stewart *et al.* 1964; Hashimoto *et al.* 1992; Haller *et al.* 2008). Many studies have looked at the cardiovascular effects of synephrine when consumed as a pure compound and in dietary supplements (Penzak *et al.* 2001; Haller *et al.* 2008). In addition, synephrine has been studied and used in traditional Chinese medicines for allergy and inflammation relief (Sankawa and Yuito 1985; Miyamoto *et al.* 1990). Pharmacokinetics have been determined for both pure synephrine (Hengstmann and Goronzy 1982) and in a dietary supplement derived from citrus (Haller *et al.* 2005). In both cases, the synephrine was delivered orally as pills where uptake could only be in the stomach or intestines. It was found that the C_{max} of synephrine was 5.4 nmol L^{-1}, while the T_{max} was achieved ca. 90 min post-ingestion, and $T_{½}$ values were between 2 and 3 hours (Hengstmann and Goronzy 1982; Haller *et al.* 2005). These values are dramatically different than those determined here when the synephrine was delivered in an extract in the oral cavity as a lozenge. The C_{max} value obtained here was ca. 20X greater than when the synephrine was delivered as a pill suggesting that that bulk of syneprhine in the nettle extract lozenge is absorbed in the oral cavity, rather than in the stomach and/or intestines. The difference between the experimentally determined $T_{1/2}$ and T_{max} values here (33 and 20 minutes, respectively) and those reported by Haller *et al.* (150 and 90 minutes, respectively), further support the preferential absorption of synephrine in the oral cavity.

Osthole is found in *Angelica pubescens,* and has shown vasorelaxation effects in rats (Fieng-Nien *et al.* 1992). It has also recently been reported that oxycoumarins like osthole, inhibit inflammatory mediators such as NOS and TNF-α (Nakamura *et al.* 2009). Osthole reached a C_{max} of 11.0 nmol L^{-1} within 20 minutes of ingestion of the nettle lozenges. Previous reports show that osthole, present in an extract of *Fructus Cnidii*, achieved a C_{max} in rat plasma of less than 0.01 nmol L^{-1} in 60 minutes of ingestion (Li *et al.* 2005). Collectively, these results indicate that osthole is bioavailable, and is likely more efficiently absorbed in the oral cavity than the stomach and/or small intestines. Physiologically, the improved oral bioavailability of osthole, when delivered as a lozenge, is likely to provide rapid and sustained relief of allergy symptoms associated with tryptase and histamine receptor inhibition.

Nicotinamide and synephrine exert their anti-allergenic effects on the histamine H1 receptor as an antagonist and negative agonist, respectively (Roschek *et al.* 2009). Commercial anti-histamines, such as diphenhydramine, are H1 antagonists (Simmons 2004). However, diphenhydramine readily crosses the blood-brain-barrier and interacts with central nervous system histamine H1 receptors generating the common side-effects of many anti-histamines - drowsiness. Molecular modeling predicts that neither nicotinamide, osthole, nor synephrine will cross the blood-brain barrier, therefore these anti-histamine phytochemicals will likely be active only on peripheral H1 receptors, minimizing or eliminating drowsiness.

As with our previous pharmacokinetic studies of bioactives of elder berry (Roschek Jr and Alberte 2008), DART TOF-MS has again shown to be a reliable and sensitive analytical tool for the detection and quantification of compounds in biological matrices even when they are present at low levels. Further, DART TOF-MS has proven invaluable for pharmacokinetic evaluations of phytonutrients and bioactive compounds in botanical extracts, not only because of the short analysis times and lack of need for sample preparation as compared to traditional analytical techniques (e.g., LC/MS), but because pure chemical standards for the bioactives of interest are not required. This tool is equally valuable for more traditional pharmacokinetic and pharmacodynamic analyses of pharmaceuticals, toxins and other compounds.

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