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Validation of a liquid chromatography–tandem mass spectrometric assay for the quantitative determination of hydrastine and berberine in human serum

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ABSTRACT

A high throughput liquid chromatography/tandem mass spectrometry (LC–MS/MS) method for the simultaneous determination of berberine and hydrastine in human serum, after oral administration of goldenseal (*Hydrastis canadensis* L.), was developed using simple acetonitrile treatment of serum samples. Noscapine served as the internal standard. Lower limit of quantification for both analytes was 0.1 ng mL^{-1} using positive ion electrospray tandem mass spectrometry (MS/MS). The intra-day ($n = 5$) accuracy and precision of the method for hydrastine was 82 ± 8.8%, 97.9 ± 2.4% and 96.2 ± 3.3%, respectively. The inter-day ($n = 4$) accuracy and precision for hydrastine was 90.0 ± 15.17%, 99.9 ± 7.1% and 98 ± 6.54%, respectively. For berberine quantitation intra-day accuracy and precision was 96.0 ± 8.4%, 92.5 ± 4.7% and 94.4 ± 3.7%, respectively. The respective values for inter-day quantitation were 91.0 ± 8.4%, 94.3 ± 4.7% and 94.4 ± 3.7%. The analytical recovery for hydrastine was 82.4–96.2% and for berberine it was 94.4–96.0%. The analytes and noscapine were stable for 24 h at room temperature (CV 5–10%). Matrix ion effects were studied by post-column infusion of hydrastine and berberine, calculation of calibration curve slope precision was obtained using serum from five different subjects, and by comparison of the response of methanol standards and extracted serum samples. The method was further validated by determination of serum pharmacokinetics of hydrastine and berberine after administration of a single oral dose of goldenseal extract containing 77 mg of hydrastine and 132 mg of berberine.

1. Introduction

The increasing use of alternative medicine in western society over the past decade has been well documented. Unpublished sales statistics gathered from various primary and secondary sources by Nutrition Business Journal (NBJ) show that total estimated herb sales in the US market rose 4.1% in 2006 compared to 2005. Preliminary research on 2007 sales indicates a total sale of 4.8 billion dollars, a 4.4% increase over 2006 [1]. Goldenseal (*Hydrastis canadensis* L.) is a popular supplement in U.S. The herb contains a number of alkaloids such as hydrastine, berberine, hydrastinine, berberastine, tetrahydroberberastine, canadine and canadaline [2]. The main alkaloids are the phthalidisoquinoline alkaloid hydrastine and the quaternary protoberberine-type alkaloid berberine. The compound berberine is considered to have a number of potential therapeutic effects in various *in vitro* and *in vivo* models [3–9]. The cholesterol lowering properties of berberine are well documented *in vitro* as well as *in vivo* [10,11]. The mechanism of action of berberine in lowering total cholesterol, LDL cholesterol and total triglyceride levels in the plasma is different from that of statins. The hypolipidemic effects of berberine result from its ability to increase the expression of LDL receptor (LDLR) by stabilizing LDLR mRNA, where as statins achieve the same by increasing the transcription of LDLR mRNA [10]. Many of the studies referenced above were conducted using purified berberine chloride or berberine sulfate. Recently Abidi et al. have shown that the root extract of goldenseal, normalized to berberine dose, was more effective than pure berberine in up-regulation of hepatic LDLR and in reducing plasma cholesterol and low density lipoprotein cholesterol in hyperlipidemic hamsters Goldenseal also contains additional components which are more potent than berberine in increasing LDLR expression [11]. Thus, goldenseal is an attractive herbal supplement that may be a clinically effective hypolipidemic agent.

Many of these diseases, in which berberine is reported to have treatment efficacy, are chronic conditions. As such, it might be expected that patients taking goldenseal would need to take this supplement for an extended period-of-time. A clear understanding

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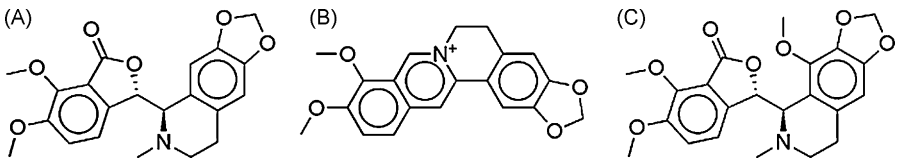

Fig. 1. Chemical structures of (A) hydrastine (B) berberine and (C) noscapine (IS).

of the safety, efficacy, and disposition characteristics of the biologically active isoquinoline alkaloids in goldenseal has not been described in human subjects. Empirical evidence suggests that goldenseal is safe, but there have been no reports of hydrastine and berberine disposition in human subjects following administration of goldenseal.

Since the popularity of herbal supplements is rapidly increasing, there is a need for validated analytical methods for determining tissue concentrations of active components present in these herbal supplements. Many herbal supplements, including goldenseal, contain compounds that can affect the activity of drug metabolizing enzymes or affect the absorption of other drugs from the GI tract and thus pose a risk for herb–drug interactions [12–14]. The study of herb–drug interactions or the use of herbal supplements for treatment of a particular condition requires that the pharmacokinetic parameters for the various compounds present in the herbal supplement be evaluated. A key component and first step in the production of quality clinical pharmacokinetic data is a validated bioanalytical method.

Hua et al. have developed and validated an LC–MS method for the quantitation of berberine in human plasma following an oral dose of berberine hydrochloride [15]. However, the sample clean-up step involved a more cumbersome liquid–liquid extraction (LLE) with ethyl acetate. Zuo et al. also developed a method for the quantitation of berberine in rat plasma following oral administration of berberine chloride, but again a more cumbersome solid phase extraction sample clean-up step was involved [16]. Methods for the quantitation of berberine and other components, following oral administration of herbal supplements containing berberine have also been developed. The method developed by Deng et al. for simultaneous determination of berberine, palmatine, and jatrorrhizine in rat plasma after oral administration of coptis–evodia herb couple involved a simple protein precipitation step using acetonitrile–methanol [1,2]. However, the lower-limit-of-quantitation (LLOQ) was high at 1 ng mL^{-1} [17]. Lu et al. have recently validated an LC–MS based method for the determination of berberine and palmatine in rat plasma following oral administration of a Huang–Lian–Jie–Du decoction [18]. Huang–Lian–Jie–Du decoction is an extract of *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*. Lu and co-workers achieved excellent LLOQ-values for berberine and palmatine (0.3 ng mL^{-1}), but the dynamic range of the method was limited to 0.3–20 ng mL^{-1}. To our knowledge hydrastine and berberine have not been simultaneously determined in human serum after oral administration of goldenseal. Because hydrastine and berberine are distinctive compounds found in goldenseal, several excellent analytical methods have been validated for their determination in various goldenseal extracts [19–21]. But these methods generally lack the analytical sensitivity required for the simultaneous determination of hydrastine and berberine in biological tissues, such as serum. The bioanalytical method described herein uses a simple protein precipitation with acetonitrile for sample clean up and displayed excellent limits of quantitation for both hydrastine and berberine. The method was further validated by determining the serum concentration-time profile for berberine and hydrastine after a single oral dose of 2.7 g of goldenseal supplement in a human subject.

2. Experimental

2.1. Chemicals and reagents

Structures for hydrastine, berberine, and noscapine are shown in Fig. 1. Berberine chloride (purity 99%), β-hydrastine (purity 99%), noscapine (purity 97%) and formic acid (purity 98%) were purchased from Sigma–Aldrich (St. Louis, MO). LC–MS grade methanol and acetonitrile were purchased from Fisher Scientific (Houston, TX). The goldenseal supplement was purchased from Nature's Resource (Lot# 0/10184, Mission Hills, CA). Human serum was purchased from Millipore (Bedford, MA) and Innovative Research (Novi, MI). Water was purified through a Millipore Milli-Q Synthesis A 10 system prior to use.

2.2. Content analysis of herbal supplement

The phytochemical content of the goldenseal (*Hydrastis canadensis* L.) supplement was independently analyzed for specific "marker compounds" by LC-UV methodology by Chromadex Single Laboratory Validation service (Clearwater, FL). The isoquinoline alkaloid content (hydrastine and berberine) of goldenseal was performed using the method of Abourashed and Khan [21] The content of hydrastine and berberine in each supplement capsule was 12.8 ± 0.6 and 22.0 ± 0.7 mg, respectively.

2.3. LC–MS instrument and conditions

LC–ESI–MS analyses were performed using a Shimadzu SIL-HTA liquid chromatographic system (Shimadzu Scientific Instruments, Columbia, MD) and a Waters Quattro Premier triple quadrupole mass spectrometer (Waters, Milford, MA). A Betasil Phenyl-Hexyl column (100 mm × 2.1 mm i.d., 3 μm particle size) (Thermo Scientific, Waltham, MA) was used for analytical separation of the analytes. Instrument control, data acquisition, and data analysis was accomplished using Masslynx v4.1 (Waters). Samples (6 μL) were injected onto the analytical column using a Shimadzu SIL-HTA autosampler equipped with a 100-μL injection loop. Separation was achieved using a binary linear gradient. Mobile phase A was acetonitrile–ammonium formate buffer (pH 2.7; 20 mM) (28:72, v/v) and mobile phase B was acetonitrile–ammonium formate buffer (pH 2.7; 20 mM) (95:5, v/v). The flow rate was 0.4 mL min^{-1}. Solvent B was held at 0% for the first 3.0 min, increased over 2 min to 100%, held at 100% for 3 min, and then decreased to 0% over the next 3 min. The column temperature was maintained at 35 °C. Eluent from the column was directed to waste for the first 0.5 min and again beginning at 5 min until 10.5 min. The total run time was 11 min.

Positive ion electrospray ionization (+ESI) was achieved with the desolvation gas operated at 500 °C and 650 L h^{-1}, and the cone gas at 115 °C and 125 L h^{-1}. Collision-induced fragments were produced in the collision cell with argon at a pressure of 4×10^{-3} Torr. Positive precursor → product ions were monitored in the multiple-reaction-monitoring (MRM) mode with the following *m/z* transitions, 336.5 → 321.5 for berberine, 384.5 → 194.5 for hydrastine and 414.1 → 398.1 for noscapine (IS). Cone voltage and collision energy were optimized for each parent ion by infusing a

500 ng mL^{-1} solution of analyte (10 μL min^{-1}) with mobile phase (0.4 mL min^{-1}), using a tee-connector, into the mass spectrometer. Dwell time and inter-channel delay were 0.15 and 0.1 s, respectively.

2.4. Calibration curves and quality control standards

Analyte standards were prepared in methanol by serial dilution of a 10 μg mL^{-1} of both analytes. Working stock serum standards were obtained by vortex-mixing 100 μL of 100 μg mL^{-1} hydrastine/berberine solution in methanol and 900 μL of serum to obtain a 10 μg mL^{-1} mixture of analytes. The IS stock solution was prepared by weighing the pure standard and dissolving it in methanol to a concentration of 1 mg mL^{-1}. To obtain a working solution of IS, the stock solution was diluted serially in methanol to obtain 250 ng mL^{-1} and stored at 4 °C. Calibration standards of hydrastine and berberine were prepared from serial dilution of the serum working stock. Serum standards containing 1000, 100, 10, 1, 0.5, 0.25, 0.1 ng mL^{-1} of hydrastine and berberine were prepared. Identical standards in methanol were also prepared and used in the assessment of extraction recovery. Quality control standards were prepared by serially diluting the 10,000 ng mL^{-1} spiked serum samples to produce serum samples containing 1, 100, and 500 ng mL^{-1} of berberine and hydrastine. The lower limit of quantitation was determined from the lowest point on the standard curve with a relative standard deviation of less than 15% and signal-to-noise ratio of better than 5 (after smoothing).

2.5. Sample preparation

To 500 μL of human serum samples in a 5-mL borosilicate glass centrifuge tube (Kimble, Vineland, NJ), 40 μL of 250 ng mL^{-1} IS was added. To this 2.16 mL of acetonitrile was added and tubes were vortex-mixed for 1 min. After incubation for 1 h at 4 °C, tubes were centrifuged for 20 min at 3000 × *g* and the supernatants transferred to clean glass tubes. Pellets were resuspended in 2 mL of acetonitrile and after 10 min of incubation at RT, tubes were again centrifuged at 3000 × *g*. Supernatants were combined, and evaporated to dryness under a stream of nitrogen at 40 °C. Residues were reconstituted in 600 μL of methanol by sonication for 2 min followed by vortex mixing for 1 min. Finally, samples were filtered through 0.2-μm centrifugal filters (Millipore) and 6 μL aliquots were injected onto the LC–MS/MS system for analysis.

The efficiency of various organic solvents, pure or in 1:1 combinations, for the simultaneous extraction of hydrastine and berberine from human serum spiked with 100 ng mL^{-1} of hydrastine and berberine was tested. The effects of reconstitution solvent on the extraction recovery were evaluated by comparing mobile phase A to methanol. Chromatographic peak areas of hydrastine and berberine in the samples prepared by organic extractions were compared with those of 100 ng mL^{-1} hydrastine and berberine standard in methanol.

2.6. Application of the method to human serum samples

The method was further validated by determining serum concentrations of hydrastine and berberine in a human subject at various time points after administration of a single oral dose of 2.14 g of goldenseal supplement (total hydrastine and berberine content 77 and 132 mg, respectively). Blood samples (10 mL) were collected prior to goldenseal dose, and then 0.5, 1, 2, 4, 6, 10, and 24 h following goldenseal administration. Blood was allowed to clot for 2 h at 4 °C. Serum was obtained following centrifugation of the clotted sample at 3600 × *g*. Serum samples were stored at −40 °C until analysis by LC–MS/MS. The study protocol was approved by the University of Arkansas for Medical Sciences Institutional Review Board (Little Rock, AR) and participant provided written informed consent before commencing the study.

3. Results and discussion

3.1. Sample preparation, chromatography, and matrix ion effects

The efficiency of various organic solvents, pure or in 1:1 combinations, for the simultaneous extraction of hydrastine and berberine from human serum spiked with 100 ng mL^{-1} of hydrastine and berberine was determined (Table 1). Since both analytes have permanent or pH-dependent positive charge, the relationship between extraction solvent dielectric constant and extraction recovery was investigated. No correlation between hydrastine recovery and extraction solvent dielectric constant was observed. Hydrastine recovery was compromised when chloroform was part of the extraction solvent. Since chloroform cannot interact by hydrogen bonding, this type of solvent:hydrastine interaction may be important for efficient extraction. A second reason for the poor extraction recovery into chloroform may be attributable to matrix ion suppression associated with fatty acids that are also extracted into the chloroform. A correlation between solvent dielectric constant and berberine extraction recovery, when $\sum$ was small (i.e., MTBE and $CHCl_3$) was observed. For solvents with higher dielectric constant values, berberine recovery was effectively the same (>80%). Acetonitrile was chosen as the primary extraction solvent due to its low cost and low vapor pressure, relative to other solvents with equal extraction efficiency. After evaporation of the primary extraction solvent, dissolution of the residue into methanol (MeOH) was

Table 1
Optimization of hydrastine and berberine extraction in various organic solvents and their 1:1 combinations.

Solvent (ε)	Hydrastine		Berberine	
	Recovery (MeOH)	Recovery (MP)	Recovery (MeOH)	Recovery (MP)
MTBE (2.6)	118	85	<1	<1
$CHCl_3$ (4.8)	20	14	20	19
EtOAc (6)	92	84	95	86
MeOH:MTBE (17.8)	122	85	95	44
MeOH:$CHCl_3$ (18.9)	69	24	54	46
MeOH:EtOAc (19.5)	84	85	73	74
Acetone (21)	88	65	99	99
MeOH:Acetone (27.5)	56	88	62	92
MeOH (33)	62	58	53	46
MeOH:CH_3CN (35.5)	76	102	92	75
CH_3CN (38)	98	84	99	87

MeOH = Methanol; MTBE = methyl *tert*-butyl ether; EtOAc = ethyl acetate; MP = mobile phase; ε = dielelctric constant.

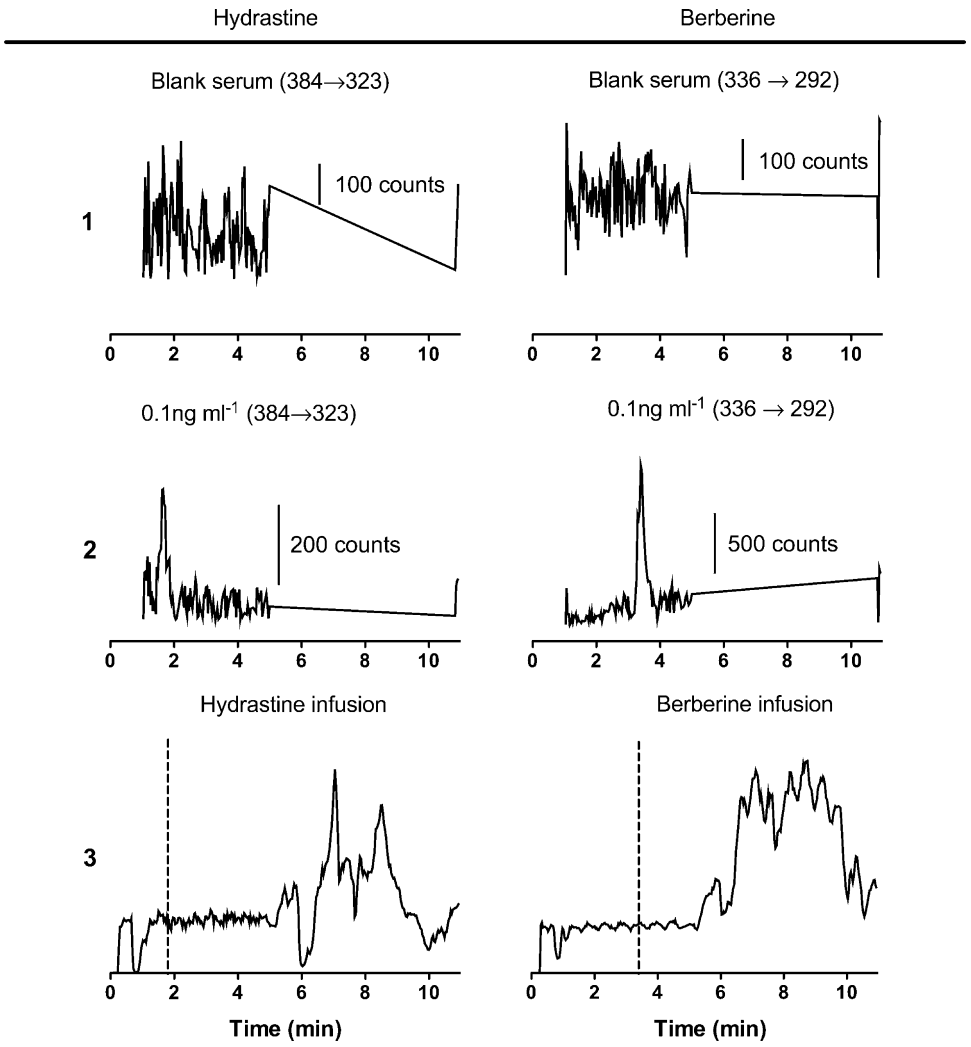

Fig. 2. Representative chromatograms for hydrastine (first column) and berberine. Extracted blank serum, extracted serum standard (0.1 ng mL^{-1}), and infusion of 100 ng mL^{-1} analyte during an injection of extracted blank serum are depicted in rows 1, 2, and 3, respectively. The vertical dashed line shown in the chromatograms on row 3 depict the retention time for hydrastine and berberine.

generally more efficient than dissolution into mobile phase. The mean extraction recoveries of hydrastine and berberine from serum samples were 90.6% (±5) and 95.7% (±5), respectively. Extraction recoveries were calculated by comparing the chromatographic peak areas of berberine and hydrastine in serum samples with that in corresponding methanol standards. Recoveries were determined at 1, 100 and 1000 ng mL^{-1} of hydrastine and berberine.

Elaborate studies on how matrix ion phenomena affect method validation have demonstrated that matrix ion suppression or enhancement frequently leads to deterioration of analytical precision and accuracy [22,23]. The matrix ion phenomena may not be observed for a single serum sample and excellent precision may be obtained for standards prepared in serum from an individual subject. However, precision may be compromised when the same standards are prepared in serum from different individuals. Generally matrix effects have greater impact on the low end of calibration curves than the mid range or high end. In fact, given the high selectivity of mass spectrometers for the compounds of interest, matrix effects are the major bottleneck in the development of validated high-throughput assays. No significant matrix ionization effects where observed for either hydrastine or berberine. This was demonstrated in multiple ways. First, peak areas of hydrastine and berberine in serum samples were not significantly different from the peak areas of these compounds in methanol standards. Second, constant-infusion experiments revealed that the chromatograms are free of matrix ionization effects in the region where hydrastine and berberine elute (Fig. 2). Infusion experiments were performed by infusion of both low (1 ng mL^{-1}) and high (100 ng mL^{-1}) analyte concentrations. Third, the relative matrix ion effect between the different lots of serum was evaluated.

Matuszweski et al. have described a simple experimental approach for evaluating and quantitating the relative matrix ionization interference in LC–MS/MS analyses [22]. These investigators suggested that if the coefficient of variation in the standard curve slope was less than 3–4%, when serum from five different subjects is used as matrix for each curve, then the effects from the matrix would not significantly compromise the analytical data from clinical samples. Thus, this slope precision value can be used as a guide for method applicability to support clinical studies. For the present study, the precision of standard curve slopes for hydrastine and berberine were 6% and 12%, respectively. Although variability in the slope of peak area versus concentration suggested matrix ion effects would compromise the accuracy and precision, the extraction recovery and infusion experiments showed that excellent accuracy and precision were obtained.

When methanol was used for dissolution of the residue obtained after complete drying of supernatant from the protein precipitation step, the recovery of both hydrastine and berberine was better than when mobile phase (20 mM ammonium formate, pH 2.7, 28% acetonitrile) was used. Since matrix ion effects were not observed in the dissolution solvent (no matrix ion effects were seen in the constant infusion experiments, data not shown), the improved recovery observed in methanol was likely due to more favorable analyte solubility in the dissolution solvent.

Chromatographic conditions for the separation of hydrastine, berberine and internal standard (IS) were optimized for rapid elution, good chromatographic selectivity, absence of matrix ion effects in the elution volume of the analytes, and good peak characteristics. Berberine is a quaternary amine and due to its positive charge, some tailing was expected. Several experiments have shown that incorporation of an appropriate concentration of ammonium buffer in the mobile phase improves the chromatographic peak shape of alkaline compounds [24]. Hence, different concentrations of acetonitrile, formic acid, and ammonium formate were evaluated with respect to chromatographic performance. Buffer ionic strength (i.e., 5, 10, and 20 mM) and buffer pH (i.e., 2.7, 3.7, and 4.7) were varied until retention time, peak symmetry, and MRM response were optimized. It was found that 20 mM ammonium formate buffer (pH 2.7) containing 28% acetonitrile (solvent A) enabled separation of hydrastine, berberine and IS within 4 min with good peak characteristics. The retention times for hydrastine, IS, and berberine were 1.69, 2.31, and 3.52, respectively. However, it was observed that some material from the sample injection remained bound to the column or to the LC system components, and eluted when acetonitrile concentration was increased from 28 to 95% using a linear gradient. This material was capable of causing ion suppression of both berberine and hydrastine as shown by the post-column infusion experiments (Fig. 2, row 3). In an isocratic run (28% acetonitrile), these solutes eluted during subsequent runs and caused unpredictable ion-suppression in the analyte elution volume. Hence, a gradient run, as described in Section 2 was performed. No ion-suppression was observed in the subsequent runs in the elution volume of hydrastine, berberine or noscapine. Matrix ion suppression was observed at 1 and 6 min for both hydrastine and berberine (Fig. 2). But since this suppression did not coelute with either compound, the suppression effects did not compromise the method recovery or accuracy. Further evidence for this lack of matrix ion effects can be seen in the high extraction recovery for both hydrastine and berberine.

Deng et al. have recently described validated LC–MS/MS methodology for the determination of berberine, palmatine, and jatrorrhizine following an oral dose of coptis–evodia to rats [17]. Though the analysis was carried out in rat plasma and the herb and the analytes were different than described in the present work, Deng and co-workers employed an approach that was similar to the current methodology. The primary difference in the approach described by Deng was that the isocratic chromatographic run times were only 2.5 min. That was significantly shorter than the 11-min gradient run times reported herein. Gradient elution was necessary with human serum because significant matrix ion suppression was observed under isocratic elution on the second and subsequent injections. Deng et al. reported a lower limit of quantitation of 1.0 ng mL^{-1} from 60 μL of rat plasma, which was an order of magnitude poorer LLOQ when compared to the 0.1 ng mL^{-1} reported herein. The on-column amount was used to compare these LLOQ-values. While others have demonstrated superior lower limits of quantitation, these other methodologies required more extensive sample clean up and were not validated for quantitation in human samples [16,17].

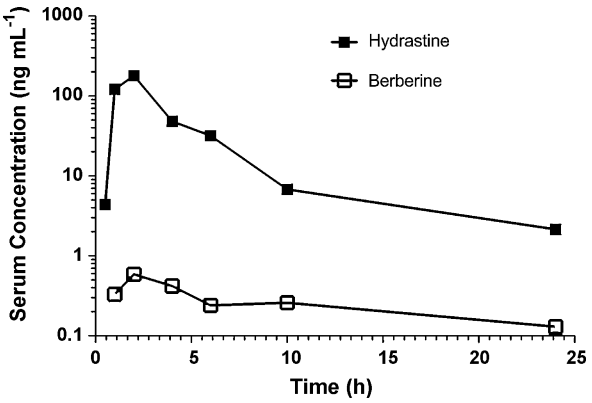

Fig. 3. Serum concentration–time profile of hydrastine and berberine in human serum following administration of a single oral dose of 2.14 g of goldenseal supplement.

3.2. Method validation

3.2.1. Selectivity, calibration, and lower limit of quantitation

Typical chromatograms of blank serum, chromatograms at the lower limit of quantitation (LLOQ) for hydrastine and berberine (0.1 ng mL^{-1}) are shown in Fig. 2. Selectivity of the assay was evaluated by analyzing six different lots of blank human serum. All samples were found to have no interfering peaks at the retention times of hydrastine, berberine or IS (data not shown).

To spiked serum samples (0.1, 0.25, 0.5, 1, 10, 100 and 1000 ng mL^{-1}) of hydrastine and berberine, 40 ng mL^{-1} IS was added. After treatment the samples were analyzed, a standard curve was prepared by plotting the response of hydrastine or berberine versus the nominal concentrations of hydrastine or berberine, respectively. The response was the peak area ratio of analyte to noscapine. A comparison of six such calibration curves showed excellent linearity over the range of 0.1–1000 ng mL^{-1} ($r^2 > 0.999$) and consistent slope values when evaluated by weighted ($1/x$) least squares linear regression for both hydrastine and berberine. Residuals were randomly distributed when plotted against concentration. Calibration curves, generated from regression analysis, for hydrastine and berberine were described by; $y = 2.8 \times 10^{-3}(\pm 7\%)x + 4.4 \times 10^{-4}(\pm 14\%)$, and $y = 8.2 \times 10^{-3}(\pm 9\%)x + 1.6 \times 10^{-3}(\pm 16\%)$, respectively. The LLOQ for both hydrastine and berberine was 0.1 ng mL^{-1}.

3.2.2. Accuracy, precision, and stability

Accuracy and intra- and inter-run precision were determined by analyzing quality control samples at three concentration levels of hydrastine and berberine ($n = 5$ at each concentration level) on three different days. Each concentration was predicted using a calibration curve constructed on the same day. The intra-day accuracy and precision for hydrastine were >82% and <9%, respectively, while the inter-day accuracy and precision were >90% and <15%, respectively. For berberine the intra-day accuracy and precision were >96% and <9%, while the inter-day values were >94% and <9%. Accuracy and precision was evaluated at 1, 100, and 500 ng mL^{-1}.

Storing the QC standards under several different environmental conditions assessed the stability of berberine and hydrastine. Processed samples were stable for 60 h in the auto-sampler tray and when stored at 4 °C. The inter-day variation of the same samples analyzed on three different days with a freeze-thaw step in between each day were not significantly different.

3.2.3. Application of method

The serum concentration-time profiles for hydrastine and berberine are represented in Fig. 3. The serum concentrations of

hydrastine are much higher than those of berberine at various time points and the concentration of berberine at 30 min was below the LLOQ. The peak serum concentrations of hydrastine and berberine were 180 and 0.59 ng mL^{-1} at 2 h. The area under the serum concentration time curve (AUC ($0 \rightarrow t$)) for hydrastine and berberine was 630 and 6 ng h/mL, respectively. A reliable estimation of the elimination half time ($t_{1/2z}$) was not determined for either compound from these data because only two blood samples (e.g., 10 and 24 h) were collected during the apparent elimination phase.

Baoxin et al. have reported significantly different pharmacokinetic parameters for berberine in healthy humans than that reported by Hua and co-workers [25]. Baoxin and co-workers observed a T_{max} of 2.37 h and a C_{max} of 395 ng mL^{-1}. While this C_{max} is 3 orders of magnitude larger than that reported by Hua (i.e., C_{max} = 0.4 ng mL^{-1}) or in the present report (i.e., C_{max} = 0.6 ng mL^{-1}), it would be difficult to compare these values since Baoxin et al. did not report the administered berberine dose.

It appears that the absorption and pharmacokinetics of berberine following an oral dose of goldenseal supplement is different than that following an oral dose of pure berberine. The berberine present in goldenseal supplement appears to be absorbed more rapidly from the gut than the pure compound and thus achieves a higher concentration more rapidly. To test whether berberine or hydrastine were metabolized to glucuronide or sulfate conjugates, serum samples were incubated with ®-glucuronidase/sulfatase. These studies indicated that neither berberine nor hydrastine was conjugated with glucuronide or sulfate (data not shown). The disposition of berberine and hydrastine in humans may be different depending on whether it is administered as a pure compound or as goldenseal supplement. This altered disposition may be due to a number of contributing effects related to the complexity of phytochemicals present in goldenseal extract or to the phenotype of the subjects enrolled in this study. The analytical method described here was validated for the expressed purpose of determining the serum pharmacokinetics of hydrastine and berberine in humans following the oral administration of goldenseal. Interesting differences in the pharmacokinetics of berberine were observed following administration of goldenseal when compared with administration of pure berberine. Future studies should investigate the significance of these differences since human exposure of berberine is primarily from goldenseal supplements.

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