Characterizing "nature's Ozempic™": Non-targeted screening of berberine supplements using ion mobility high-resolution mass spectrometry

Lindsay E. Hatch, Sarah E. Dowd, Gordon T. Fujimoto, Waters Corporation, Milford, MA USA

INTRODUCTION

The natural product, berberine ($C_{20}H_{18}NO_4^+$), is an isoquinoline alkaloid derived from plants, particularly berberis species. Berberine has been increasingly studied in recent years (Figure 1) for its myriad health benefits, including protective effects against several neurological, cardiovascular, and gastrointestinal disorders.[1] It has also been found to help lower blood sugar, effects that can aid in the treatment of diabetes and obesity.[2] Berberine has thus attracted attention as a natural alternative to popular GLP-1 medications, earning the nickname "nature's Ozempic".[3] Many supplement products marketed as berberine or *berberis* extracts are readily available to consumers, with unknown composition. As the bioavailability of berberine is relatively low,[2] the presence of additional components could affect the therapeutic activity of a supplement.

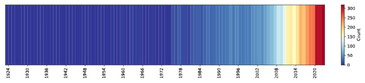

Figure 1. Number of literature publications mentioning berberine over the last century (source: PubChemLite[4])

Objective: Apply non-targeted analysis using high-resolution mass spectrometry coupled to ion mobility spectrometry (IMS) to qualitatively characterize the composition of over-the-counter berberine supplements.

METHODS: SAMPLE PREPARATION

- Seven commercially available supplements in powder-form were purchased from an online retailer. Products included whole root/bark extracts of *berberis vulgaris* or *berberis aristata* and ostensibly more "purified" extracts where berberine was specifically listed as the ingredient (Table 1).
- Authentic standards of berberine and palmatine (Cayman Chemical) were analyzed for reference.
- ~100 g of each powder sample was extracted via sonication in a 50:50 water:methanol solution, followed by centrifugation, filtration, and dilution (100×) prior to analysis. A pooled sample was also analyzed.

Table 1. Summary of products tested

Barberry variety	Sample ID	Ingredient Listing	Extracted Component	Image
Berberis aristata	BA-1	Whole-herb	Root	
	BA-2	Berberine HCl	Bark	
	BA-3	Berberine Phytosome	Root	
	BA-4	Whole-herb	Root	
	BA-5	Berberine HCl	Root	
Berberis vulgaris	BV-1	Whole-herb	Root and bark	
	BV-2	Whole-herb	Root bark	

METHODS: LC-MS ANALYSIS

LC: ACQUITY™ Premier (BSM-FTN)
Column: ACQUITY Premier CSH Phenyl Hexyl (1.7 µm, 2.1 × 100 mm)
MPA: Water + 2mM ammonium acetate & 0.1% acetic acid
MPB: Acetonitrile
Column Temp: 30 °C
Flow rate: 0.3 mL/min
Injection volume: 1 µL

Gradient:

Time (min)	%A	%B
Initial	90	10
10.5	75	25
14.0	5	95
16.0	5	95
16.1	90	10
18.0	90	10

MS: SELECT SERIES™ Cyclic™ IMS
Acquisition Mode: HDMS^E (DIA)
Polarity: Positive
Capillary Voltage: 2 kV
Mass Range: 50-1200 Da
Transfer cell collision energy: 40 – 60 V
MS Analyzer Mode: V Mode (MS Resolution ~60,000 FWHM)

Data were acquired using MassLynx™ and processed using UNIFI™ within the waters_connect™ Software Platform.

Figure 2. The SELECT SERIES Cyclic IMS with an ACQUITY UPLC™ System

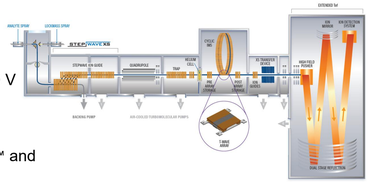

Figure 3. SELECT SERIES Cyclic IMS instrument schematic

RESULTS: SPECTRAL PATTERNS

Berberine and palmatine displayed similar fragmentation patterns, with common constant neutral losses of CH_4, C_2H_4O, and C_3H_8O (Figure 4).[5] Using common neutral loss searching, many other structurally similar compounds were tentatively identified across the tested products. Selected examples are shown in Figure 4.

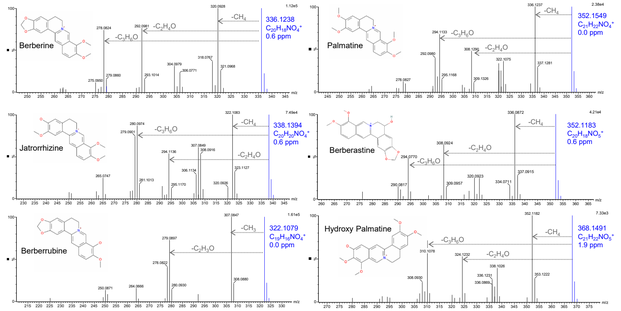

Figure 4. Fragmentation spectra of selected alkaloids detected in the samples. Precursor (product) ions are indicated in blue (black). Berberine and palmatine were positively identified; other assignments are tentative.

RESULTS: ION MOBILITY SEPARATION

The complexity varied significantly among the samples (Figure 5a-g). Berberine was the major component in all samples, with a variable number of additional alkaloids also present. In complex samples, ion mobility provided an additional dimension to better resolve co-eluting components. An example of isobaric separation by IMS is shown in Figure 5h, where the minor component at *m/z* 352.1183 ($C_{20}H_{18}NO_5$+) was separated from the major component at *m/z* 352.1549 ($C_{21}H_{22}NO_4$+, palmatine), demonstrating the benefit of IMS and high-resolution MS for fully characterizing natural products.

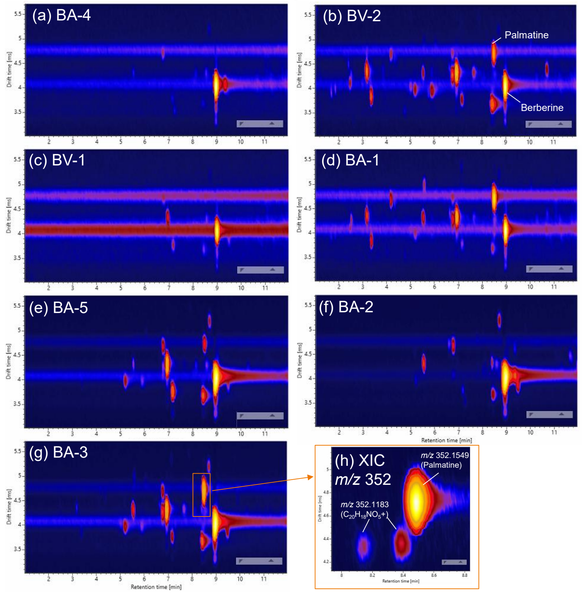

Figure 5. Plots of retention time (RT) vs. drift time (DT) for each supplement sample (IDs list in Table 1). For clarity, the displayed ranges are limited to: RT 1-12 min; DT 3-5.75 ms, 300-400, m/z where most alkaloid species were observed. Panel (h) is a zoomed in XIC of m/z 352.05 – 352.2 in Sample BA-3, demonstrating isobaric resolution by IMS.

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RESULTS: CHEMICAL SPECIATION

Among the tested supplement products, significant variability in the distribution of measured alkaloid compounds was observed (Figures 5 & 6). Berberine contributed between 57 – 98% of the total measured peak area of known and tentatively identified alkaloid compounds. The second most abundant compound was generally either palmatine or jatrorrhizine (Figure 6).

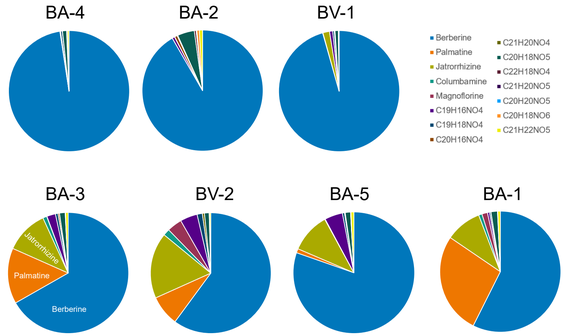

Figure 6. Pie charts displaying the relative amounts of identified and tentatively identified compounds (by peak area) for each sample tested (IDs listed in Table 1). Observed isomers have been grouped by chemical formula. Compounds specified by name were positively identified or previously observed in Berberis *extracts.[6]*

Studies of palmatine and jatrorrhizine pharmacology have reported beneficial effects similar to berberine (e.g., glucose regulation),[7,8] however palmatine may have toxic side effects (e.g., oxidative stress).[7] Synergistic effects have also been observed among combinations of isoquinoline alkaloids.[7] Thus, the particular speciation of isoquinoline alkaloids in a supplement product can affect its efficacy and safety, effects which cannot be readily discerned from product labels.

SUMMARY & CONCLUSIONS

- The isoquinoline alkaloid content was determined for seven supplement products of berberine/*berberis* extracts based on mass spectral fragmentation patterns and online library searching.
- With enhanced separation by ion mobility, the sample complexity was shown to vary significantly with some samples composed of nearly pure berberine and others containing significant fractions of related compounds.
- The measured sample complexity did not appear correlated to the complexity implied by the product label (i.e., those marketed as whole-herb plant extracts vs. berberine specifically), thus making it difficult for consumers to make an informed choice.

Conflict of interest disclosure: the authors are employees of Waters Corporation, presenting on behalf of the company.

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