Natural Food Supplements: Characterization using LC/MS – Ashwagandha, Turmeric and Ginger Examples

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INTRODUCTION

Food supplements are often touted as promoting good health, but these supplements are treated as a food category and do not provide information on the bioactive compounds they contain.

The labels on these food supplements typically list their ingredients based on the amount of raw ingredients in each serving (e.g. ashwagandha (Wintania somifera) root and leaf extract, Organic Turmeric Root (Curcuma longa), Organic Ginger (Zingiber officianle)) and do not list the amounts of compounds purported to have health benefits in their products.

An LC/MS method has been developed for the characterization of food supplements that was tested on turmeric, ginger, boswellic acid and ashwagandha products.

SAMPLE PREPARATION & STANDARDS

Food supplements were purchased from online sources. The majority of the samples came in capsule form, though samples that came in the form of a gummy, a softgel and a tablet were also analyzed.

Capsules & Tablet: 100 mg of the powder contents of the capsules or a 100 mg piece of a tablet was extracted with 20 mL of 50/50 (v/v) water methanol.

Softgel: A softgel capsule was vortexed/sonicated till it broke open in 30 mL water. 10 mL of methanol was then added and the sample vortexed.

Gummy: A gummy was sonicated in 100 mL water till it broke up. Then 100 mL of methanol was then added and the sample vortexed.

Samples were then centrifuged (4000 g for 5 minutes), filtered (through a 0.22 µ PTFE syringe filter) and diluted using 50/50 (v/v) water methanol 10:1, 100:1 or 1000:1 as needed and analyzed.

Standards for 6-Gingerol, 6-Paradol, Curcumin, Boswellic Acid; 3-Acetyl-11Keto-Boswellic Acid (AKBA); 3-Acetyl-Boswellic Acid (ABA), Piperine & Withaferin A were purchased and used to create sets of calibration standards in range from 0.1 to 500 ng/mL.

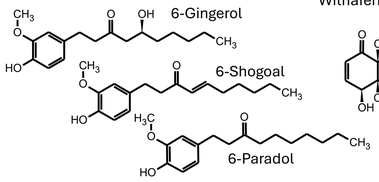
Ashwagandha
Withania somnifera

Turmeric
(Curcuma longa)

Ginger
(Zingiber officinale)

Boswellia serrata

Black Pepper
(Bioperine)



EXPERIMENTAL

MRM Parameters Optimized Using Infusion or Injection Experiments [1]

Compound	RT	Prec	CV	Prod1	CE1	Prod2	CE2
6-Gingerol	6.48	293.18	9	193.09	9	99.08	11
8-Gingerol	7.62	321.21	10	193.09	10	127.11	12
10-Gingerol	8.48	349.24	10	193.09	10	155.14	13
12-Gingerol	9.15	377.27	12	193.09	12	183.18	13
14-Gingerol	9.67	405.30	13	193.09	13	211.21	13
Curcumin	6.90	367.12	30	134.04	34	149.06	18
Demethoxy Cur	6.87	337.11	25	119.05	22	217.05	9
Bisdemeth Cur	6.83	307.10	24	119.05	20	187.04	9
Boswellic Acid	10.38	455.35	48	377.32	34	437.34	35
11-Keto-BA	9.11	469.33	50	391.30	36	407.33	29
3-Acetyl-BA	10.67	497.36	23	59.01	14		
3-Ac-11-K-BA	9.44	511.34	24	59.01	14		
Withafarin A	5.85	469.25	48	122.04	27	451.25	13
Piperine	6.41	286.14	50	115.05	40	135.04	24

'Cur' = Curcumin
Bisdemeth = Bisdemethoxy
BA = Boswellic Acid
RT = Retention Time (min)

Prec = Precursor Ion m/z
Prod = Product Ion m/z
CV = Cone Voltage (V)
CE = Collision Energy (eV)

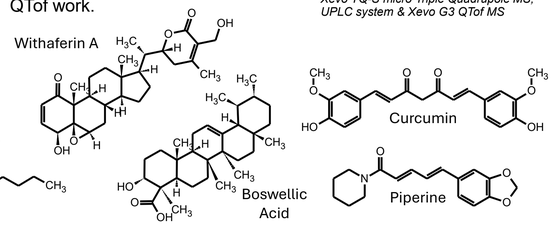
Piperine = Positive Ion ES; Other compounds were Negative Ion ES;

INSTRUMENTATION

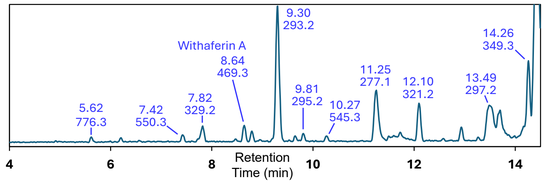
Chromatographic System: ACQUITY™ UPLC™ I-Class Plus
Column: ACQUITY Premier BEH™ C18, 130Å, 1.7 µm, 2.1 x50 mm @35°C
Mobile Phase A: Water w/2.5 mM Ammonium Acetate
Mobile Phase B: Methanol
Gradients: For MRM: 5%B to 95%B in 0 to 10 minutes. Varying gradient times were used for QTof work.

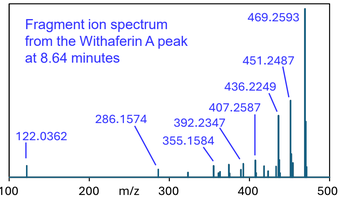
Mass Spectrometer: Xevo™ TQ-S micro & Xevo™ G3 QTof MS
Ionization: ESI+/ESI-
Acquisition: MRM & MSe

Xevo TQ-S micro Triple Quadrupole MS, UPLC system & Xevo G3 QTof MS



RESULTS





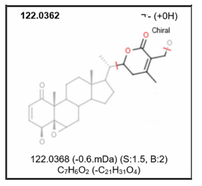
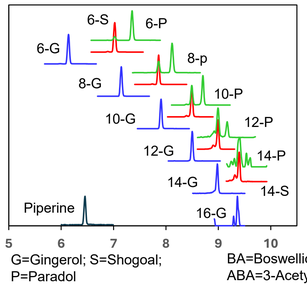
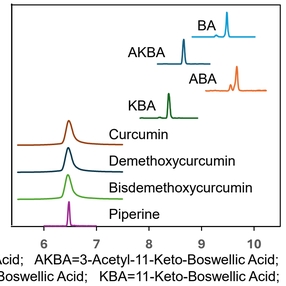


Figure 1. Base peak intensity chromatogram obtained from the injection of an ashwagandha sample analyzed using a Xevo G3 QTof MS operating in the MSe mode. Peaks are annotated with their retention time (minutes) and the m/z of their base peak ion. Fragment ion spectrum and structural analysis of the m/z = 122.0362 fragment ion.

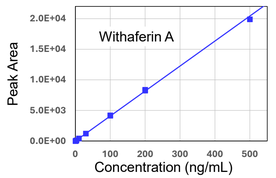


G=Gingerol; S=Shogoal;
P=Paradol



BA=Boswellic Acid; AKBA=3-Acetyl-11-Keto-Boswellic Acid;
ABA=3-Acetyl-Boswellic Acid; KBA=11-Keto-Boswellic Acid;

Figure 2. Example chromatograms (X Axes are Retention Time (minutes))



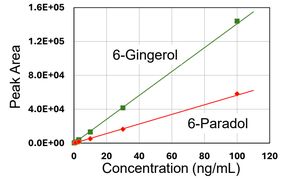


Figure 3. Calibration curves generated for Withaferin A, 6-Gingerol and 6-Paradol

Components measured in Servings of Ginger, Turmeric, Boswellic Acid & Ashwagandha Supplements

	Ging1	Ging2	Ging3	Ging4
mg/Serving	1100	1500	20	1200
6-Gingerol	5.69	2.53	1.51	2.90
8-Gingerol	0.32	0.14	0.10	0.21
10-Gingerol	0.46	0.20	0.15	0.37
12-Gingerol	0.014	0.0047	0.0060	0.0103
14-Gingerol	0.0003	0.0002	0.0003	0.0003
6-Shogaol	5.62	2.36	0.60	0.96
8-Shogaol	0.89	0.36	0.13	0.17
10-Shogaol	0.70	0.35	0.15	0.23
12-Shogaol	0.0125	0.0067	0.0034	0.0034
14-Shogaol	0.0004	0.0003		
6-Paradol	0.200	0.082	0.029	0.074
8-Paradol	0.028	0.0091	0.0050	0.0077
10-Paradol	0.018	0.0080	0.0040	0.0058
12-Paradol	0.0005	0.0003		0.0004

mg/Serving = mg of Ginger Root (Zingiber officinale) extract

Ging = Ginger Root, Turm = Turmeric, BA = Boswellic Acid (Boswellia serrata), Ashw = Ashwagandha
Labels on the packaging that accompanied the supplements provided the serving information.

	Turm-1	Turm-2	Turm-3
mg/Serving	450	750	~500
Curcumin	6	6	8
Demeth	9	14	7
Bisdem	56	153	65
Piperine	8	10.6	11

mg/Serving = mg of Organic Turmeric + Turmeric Extract, supplements also contained Bioperine

	BA-1	BA-2	BA-3	BA-4
mg/Srv	1000	500	1200	600
BA	0.55	0.16	0.062	0.045
KBA	2.78	0.31	0.16	0.152
ABA	0.100	0.016	0.019	
AKBA	0.68	0.05	0.04	0.032

mg/Srv = mg/Serving = mg of Boswellia serrata extract

	Ashw1	Ashw2	Ashw3	Ashw4
mg/Serving	150	1600	125	600
Withaferin A	0.96	0.46	0.022	0.240

mg/Serving =mg of KSM-66 Ashwagandha (Withania sominfera) Root Extract 150 mg (KSM-66 is a registered trademark of Ixoreal Biomed Inc.)

DISCUSSION & CONCLUSION

High resolution mass spectra were acquired using the Xevo G3 QTof MS operating in the MSe mode. The accurate mass data along with analysis of the fragment data provided the means to identify the compounds of interest in the supplement samples. This was especially important in the ashwagandha samples as they contain a large number of compounds that are of 'natural product' interest (withanolides, flavonoids, saponins, etc. [2])

The lack of readily available standards for some of the compounds required the use of 'relative calibration' to determine the concentrations of these compounds. (Gingerol compounds used 6-Gingerol, Shogoal and Paradol compounds used 6-Paradol, 11-Keto-Boswellic Acid used Boswellic Acid.) Piperine is added to some supplements in the form of Bioperine to enhance absorption in the body of nutrients and was also quantified.

LOQ's of down to the 0.1 to 1 ng/mL were achievable in solvent standards for all of the compounds studied. The concentrations of the compounds measured in the extracts of the food supplement samples were corrected for sample size extracted, dilution and the recommended serving size to determine the amount of these compounds one would consume if they followed the recommended serving sizes. Based on the observed LOQ's, the method described here will allow for the quantitation of the selected compounds down to the levels of 0.005 mg/serving.

[1] Full list of MRM parameters available upon request (gordon_fujimoto@waters.com)
[2] P. Mikulska et al., Pharmaceutics. 2023 Mar 24;15(4):1057 and references therein.

CONFLICT OF INTEREST DISCLOSURE: Lindsay Hatch, Sarah Dowd, Nicholas Ellor and Gordon Fujimoto are employees of Waters Corporation and presenting this poster on behalf of the company.

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