APPLICATION

Optimization of the USP HPLC Methodology for *Boswellia serrata* with Advancements in HPLC/UHPLC Column Chemistry

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Scott enjoys surfing and eating. He is crazy about chromatography, because his mom is really into CSI and thinks that is what he does.

Introduction

Indian Frankincense has increased in popularity as a dietary ingredient, and recent investigations suggest that *Boswellia serrata* contains a variety of bioactive compounds contributing to its efficacy. The current USP monograph directs to quantify two keto-derivative β-Boswellic acids on a traditional, fully porous, 5 µm C18 HPLC column within a 45 minute run time.

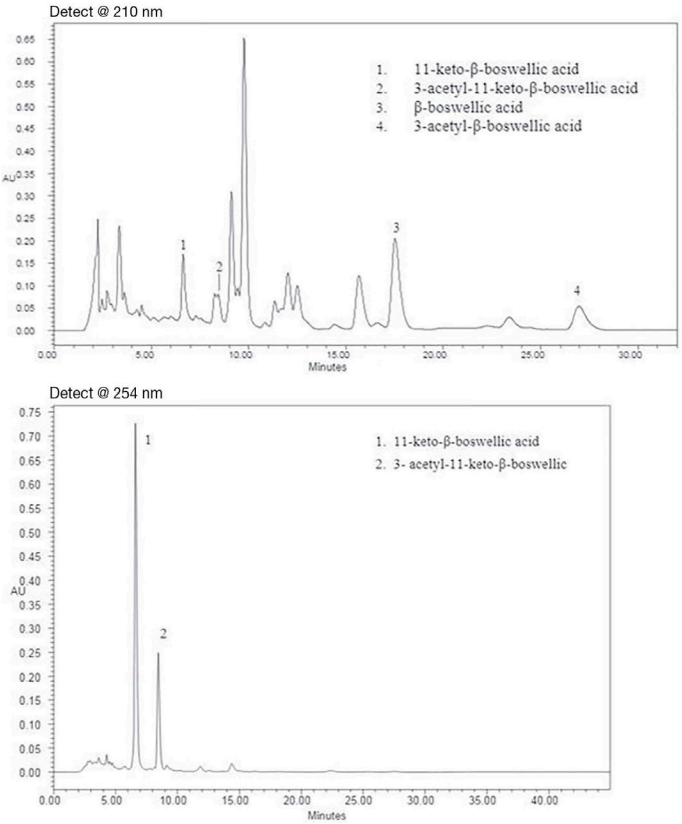
In collaboration with the USP, Alkemist Labs, and Extrasynthese, a core-shell technology and a range of column phase chemistries were screened to establish an optimized separation with run times less than 10 minutes achievable under both conventional and ultra-high performance LC conditions. The method includes four additional analytes to effectively quantify six characteristic boswellic acids. The optimized methodology and expanded method scope provide much-needed enhancements to meet the increasing demand on establishing ingredient authenticity and reliable quantitation of analytical markers.

Materials and Methods

Table 1.
Standards

Name	Supplier	Part Number
11-keto-β-boswellic acid (KBA)	Extrasynthese	2301
3-acetyl-11-keto-β-boswellic acid (AKBA)	USP	1076261
β-boswellic acid (βBA)	Extrasynthese	0036
3-acetyl-β-boswellic acid (AβBA)	Extrasynthese	2311S
α-Boswellic acid (αBA)	Extrasynthese	0049
3-Acetyl-α-boswellic acid (AαBA)	Extrasynthese	0012
Boswellia serrata Extract RS	USP	1076250

Figure 1.
Chromatography in Current USP Monograph Method



Current USP Monograph Chromatographic Conditions

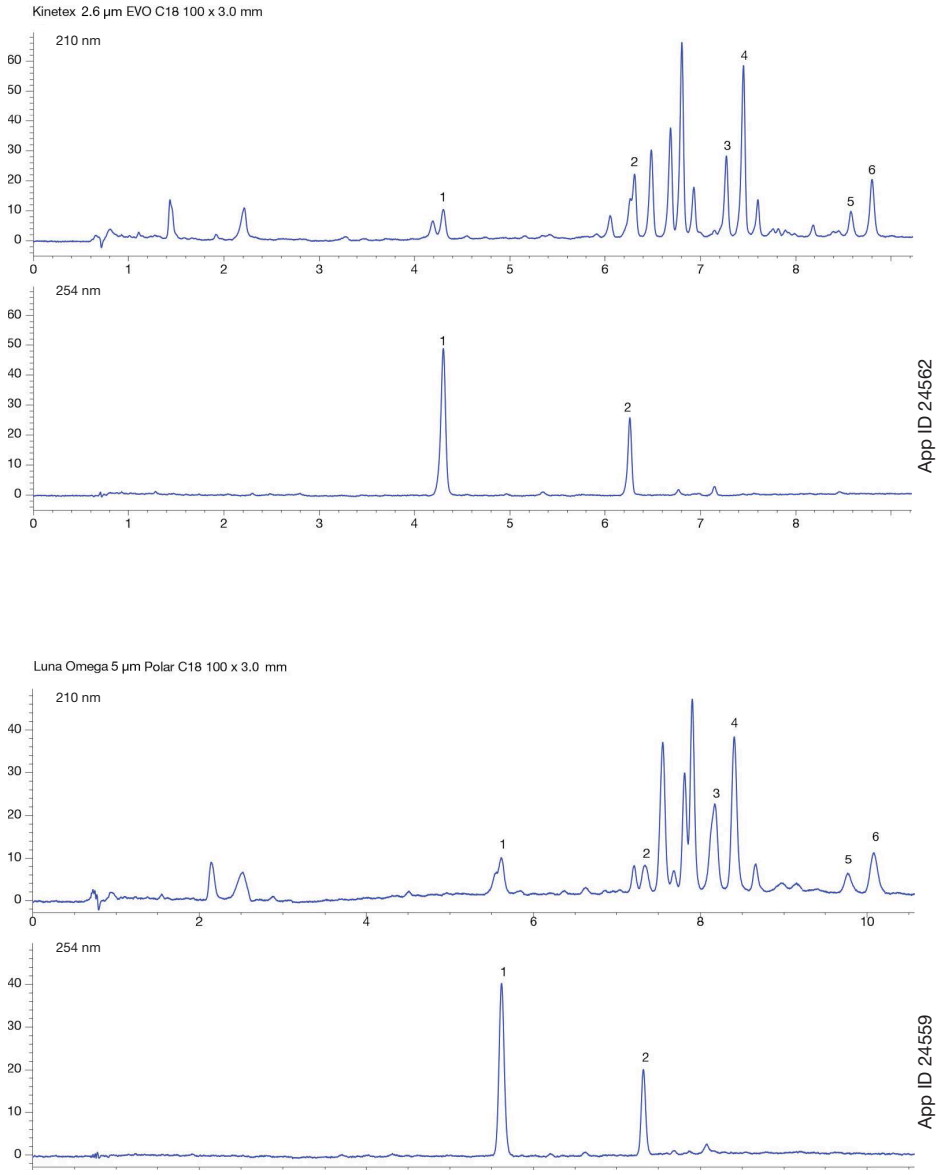
LC-UV Conditions

Column: L1 packing 5 µm
Dimensions: 250 x 4.6 mm
Mobile Phase: Acetonitrile / Water: Glacial Acetic Acid (900:100:0.1)
Flow Rate: 1 mL/min - 2 mL/min
Flow Rate Gradient:

Time (min)	Flow Rate (mL/min)
0	1
5	1.5
10	2
30	2
32	1
45	1

Detection: UV @ 210 nm and 254 nm

Figure 2.
Modern C18 Column Chemistry Screens



Modernized Column Screening Chromatographic Conditions

LC-UV Conditions

Columns: Kinetex® x 2.6 µm EVO C18
Luna® 5 µm Omega Polar C18

Dimensions: 100 x 3.0 mm

Part No.: 00D-4725-Y0
00D-4754-Y0

Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	% B
0	65
0.5	65
5	100
9	100
9.1	65
13	65

Flow Rate: 0.5 mL/min

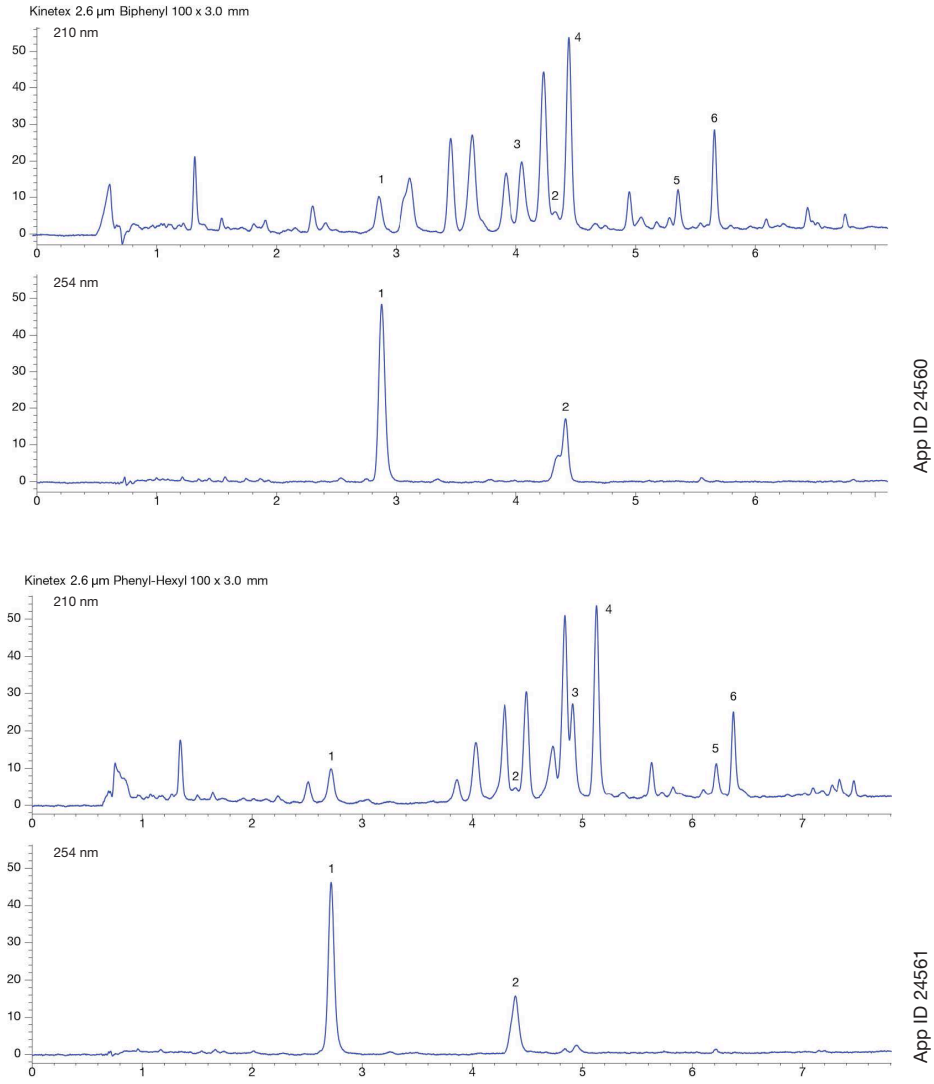
Detection: UV @ 210 nm and 254 nm

Analytes:
1. 11-keto-β-boswellic acid (KBA)
2. 3-acetyl-11-keto -β-boswellic acid (AKBA)
3. α-boswellic acid (αBA)
4. β-boswellic acid (βBA)
5. 3-acetyl-α-boswellic acid (AαBA)
6. 3-acetyl-β-boswellic acid (AβBA)

Acknowledgement

We would like to provide special thanks to Alkemist Labs, the United States Pharmacopeial Convention, and Extrasynthese for their contributions to the application.

Figure 3.
Modern Phenyl Column Chemistry Screens



Modernized Column Screening Chromatographic Conditions

LC-UV Conditions

Columns: Kinetex® 2.6 µm Biphenyl
Kinetex® 2.6 µm Phenyl-Hexyl
Dimensions: 100 x 3.0 mm
Part No.: 00D-4622-Y0
00D-4495-Y0
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Gradient:

Time (min)	% B
0	65
0.5	65
5	100
9	100
9.1	65
13	65

Flow Rate: 0.5 mL/min
Detection: UV @ 210 nm and 254 nm
Analytes: 1. 11-keto-β-boswellic acid (KBA)
2. 3-acetyl-11-keto -β-boswellic acid (AKBA)
3. α-boswellic acid (αBA)
4. β-boswellic acid (βBA)
5. 3-acetyl-α-boswellic acid (AαBA)
6. 3-acetyl-β-boswellic acid (AβBA)

Discussion

The primary goals of the modernized UHPLC method are to reduce the chromatographic run time and to include 2 additional boswellic acid analytes: α-Boswellic acid (αBA) and 3-Acetyl-α-boswellic acid (AαBA).

Several core-shell and fully porous column chemistries were screened in a 100 x 3.0 mm column dimension with an eye towards flexibility with both UV and mass spec detectors, along with a diverse range of front-end HPLC and UHPLC instruments.

The resulting methodology successfully reduced the run time to under 10 minutes with similar selectivity for the original four boswellic acids, while also providing complete separation of the 2 additional boswellic acid standards, α BA and AαBA. KBA and AKBA still coelute with elements of the matrix and will benefit from quantitation at the 254 nm wavelength.

Phenyl column chemistries were also explored in core-shell platforms: Kinetex F5, Kinetex Biphenyl, and Kinetex Phenyl-Hexyl. Kinetex F5 (pentafluorophenyl) did not look promising, but, Kinetex Biphenyl and Kinetex Phenyl-Hexyl showed good resolutions and peak shapes for most components, although with significantly different profiles and elution orders compared to alkyl phase and to each other. This suggests that there is opportunity for improvement with further experimentation and optimization.

Further optimization work is in progress, with the most significant refinements centered on specificity for potential quantification of non-acetyl/keto boswellic acids at 210 nm, where matrix components tend to interfere. If quantification or more firm identification are deemed necessary, having these complementary selectivity options will be a very helpful tool.

Joint efforts of the USP, Alkemist, Extrasynthese, and Phenomenex provide an excellent example of collaboration to yield improved analytical methodology compatible with a variety of modern analytical instrumentation.

APPLICATION

Kinetex® Ordering Information

2.6 µm Minibore Columns (mm)						SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	3/pk
EVO C18	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
Biphenyl	00A-4622-AN	00B-4622-AN	—	00D-4622-AN	00F-4622-AN	AJ0-9209
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
						for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
EVO C18	00B-4725-Y0	00D-4725-Y0	00F-4725-Y0	AJ0-9297
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208
Phenyl-Hexyl	00B-4495-Y0	00D-4495-Y0	00F-4495-Y0	AJ0-8781
				for 3.0 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
EVO C18	00B-4725-E0	—	00D-4725-E0	00F-4725-E0	AJ0-9296
Biphenyl	00B-4622-E0	—	00D-4622-E0	00F-4622-E0	AJ0-9207
Phenyl-Hexyl	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774
					for 4.6 mm ID

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Luna® Omega Ordering Information

5 µm Minibore Columns (mm)					SecurityGuard Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0* (10/pk)
Polar C18	00A-4754-AN	00B-4754-AN	00D-4754-AN	00F-4754-AN	AJ0-7600
				for ID:	2.0 - 3.0 mm

5 µm MidBore™ Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* (10/pk)
Polar C18	00B-4754-Y0	00D-4754-Y0	00F-4754-Y0	AJ0-7600
			for ID:	2.0 - 3.0 mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* (10/pk)
Polar C18	00B-4754-E0	00D-4754-E0	00F-4754-E0	00G-4754-E0	AJ0-7601
				for ID:	3.2-8.0 mm

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000
* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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