Certificate Issued To:

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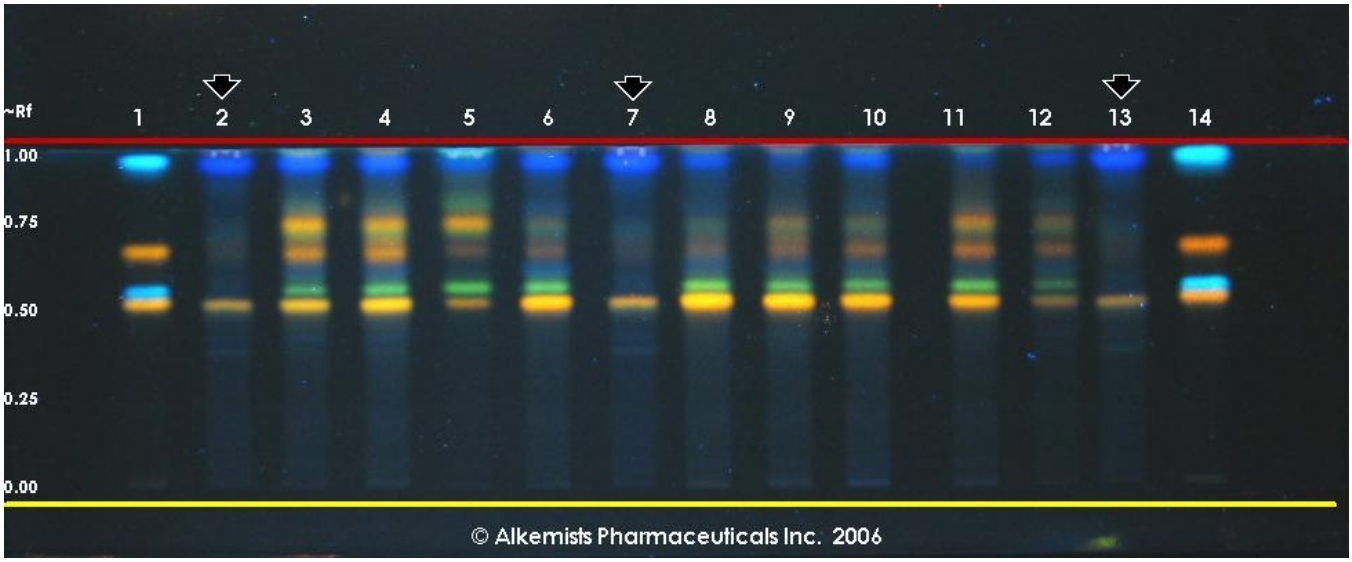
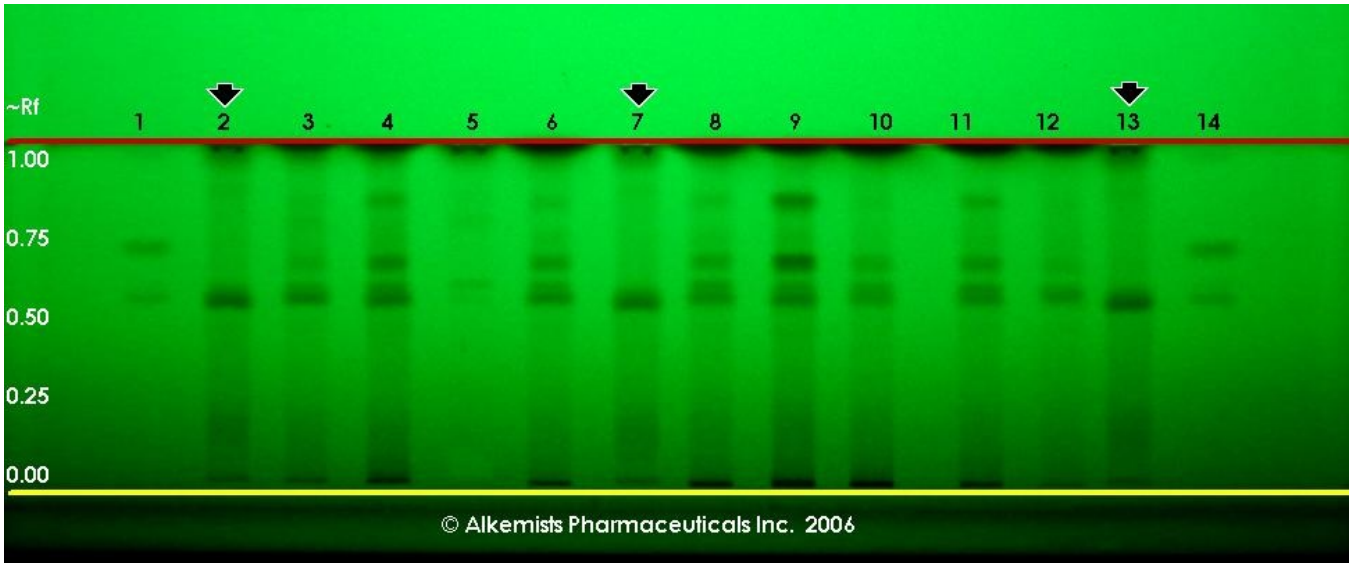
Differentiation of Star Anise and Japanese Star Anise

By High Performance Thin-Layer Chromatography with Photo-Documentation





Client:	American Herbal Products Association
Title:	Differentiation of Star Anise and Japanese Star Anise
Plant Part:	seed/fruit
Latin Binominal:	*Illicium verum* Hook. f. [Illiciaceae]
Examiner:	SS
Sample Prep:	0.5 g raw material with 5 mL CH_3OH added and sonicated for 15 minutes then heated in a dry block incubator at 65° C for about 1 hr. The supernatant is used as the test solution.
Stationary Phase:	Silica gel 60, F_{254}, 20 x 10 cm HPTLC plates, Merck or equivalent
Mobile Phase:	Ethyl acetate: glacial acetic acid: formic acid: water [10/1.1/1.1/2.4]
Detection:	(1) Natural Product Reagent + PEG → UV 365 nm (2) UV light at 254 nm
Reference Standard:	Lanes 1 and 14, a 1µL of a solution of rutin (AE038, Spectrum), chlorogenic acid (03450-001), hyperoside (072605, Chromadex), and caffeic acid (NG0541, Spectrum), ~0.1% in CH_3OH
Reference Source:	Alkemists Pharmaceuticals, Inc.

Samples used for Plates 1 & 2:

Lane 1:	Rutin, chlorogenic acid, hyperoside, and caffeic acid standard
Lane 2:	*I. anisatum* fruit MU16604AHP (3 µL) voucher specimen
Lane 3:	*I. verum* fruit MT16604AHP1 (3 µL)
Lane 4:	*I. verum* fruit MT16604AHP2 (3 µL)
Lane 5:	*I. verum* fruit MT16604AHP3 (3 µL)
Lane 6:	*I. verum* fruit MT13506CS1 (3 µL)
Lane 7:	*I. anisatum* fruit MU16604AHP (3 µL) voucher specimen
Lane 8:	*I. verum* fruit MT13506CS2 (3 µL)
Lane 9:	*I. verum* fruit MT13506CS3 (3 µL)
Lane 10:	*I. verum* fruit MT13506CS4 (3 µL)
Lane 11:	*I. verum* fruit MT13506CS5 (3 µL)
Lane 12:	*I. verum* fruit MT13506CS6 (3 µL)
Lane 13:	*I. anisatum* fruit MU16604AHP (3 µL) voucher specimen
Lane 14:	Rutin, chlorogenic acid, hyperoside, caffeic acid standard

Comments & Conclusions:

The solid yellow lines 10mm from the bottom of the place mark the sample origin. The red line marks the solvent front at 70mm. Lanes 2, 7, and 13 in the above chromatograms are from *Illicium anisatum* voucher specimens while lanes 3, 4, 5, 6, 8, 9, 10, 11 & 12 were made with *Illicium verum*. Lanes 1 and 14 are the above described reference materials.

These chromatograms demonstrate a clear difference between authentic star anise (*I. verum*) fruit and a known adulterant, *I. anisatum* by the following features. The samples in lanes 2, 7, and 13 created from *I. anisatum*, reveal a distinctly different 'fingerprint' from that of *I. verum* in lanes 3 – 5, and 8 – 12. There are no bands or only very light bands between the yellow band at R_f ~ 0.50 corresponding to rutin and the bright blue fluorescent band at R_f ~ 0.95 corresponding to caffeic acid in the *I. anisatum* in image 2 above. There also appears to be no green band in any of the *I. anisatum* as is in the *I. verum*.

These chromatograms clearly reveal the chromatographic differences between (Chinese) star anise (*I. verum*) and its adulterant, Japanese star anise (*I. anisatum*), and the ease with which they may be distinguished by High Performance Thin-Layer Chromatography (HPTLC).

Samples MT16604AHPA, MT16604AHPA1, MT16604AHPA2, and MT16604AHPA3 were obtained from the American Herbal Pharmacopoeia®, Scotts Valley, CA. Alkemist Pharmaceuticals retains samples of each of these in their herbarium.

Authorized by: Sidney Sudberg, Director, Alkemists Pharmaceuticals Report date: 2/23/2007