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$_3$OH 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 Æ 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$_3$OH  
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 MT13506C S1 (3 µL)  
Lane 7: I. anisatum fruit MU16604AHP (3 µL) voucher specimen  
Lane 8: I. verum fruit MT13506C S2 (3 µL)  
Lane 9: I. verum fruit MT13506C S3 (3 µL)  
Lane 10: I. verum fruit MT13506C S4 (3 µL)  
Lane 11: I. verum fruit MT13506C S5 (3 µL)  
Lane 12: I. verum fruit MT13506C S6 (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 plate 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 MT16604AHP A, MT16604AHP A1, MT16604AHP A2, and MT16604AHP A3 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  