

Study On The HPTLC Fingerprints of Bupleurum spp.

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Introduction

The assessment of the chromatographic fingerprints plays much more important role in the standardization, authentication and quality control of Traditional Herbal Medicine than the conventional practice of selecting single marker as quality control target. In this paper, the HPTLC fingerprint study on a commonly used Traditional Chinese Herbal Medicine (TCHM), the root of umbelliferous Bupleurum spp. (Chai Hu) exemplified the powerful chromatographic technique applying to comprehensively identify the herbal drugs.









B. chinense **Habitat: China** Habitat: Japan

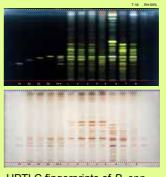
B.marginatum var. stenophyllum Habitat:Tibet,China

Toxic Species

B. longiradiatum

Experimental

Samples: 41 batches of cultivated and wild Bupleurum species viz. B. chinense DC. and B. falcatum L. etc. HPTLC fingerprinting condition: Merck SG60 precoated plate, mobile phase: dichloromethane-ethyl acetate-methanol-water (20:40:20:10), detection: spray with 2% Ehrlich's Reagent (p-DMAB) solution of 20% sulfuric acid in ethanol, heat at 105 until the zones are clear. Examine under daylight and 365nm.



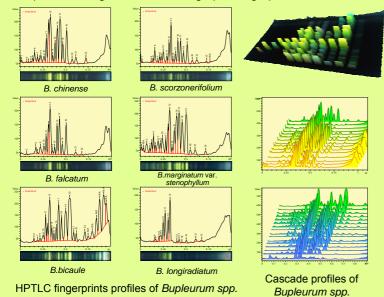


HPTLC fingerprints of B. spp. (top:366nm; bottom: white light)

Saikosaponins - Major Active Constitute in B.spp.

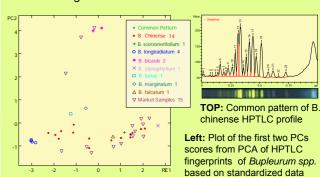
Fingerprint Profile Comparison

Documentation: CAMAG Reprostar3 with winCATs 1.4 **Profile Scan**: Chromap Chromafinger v0.9 chromatographic fingerprint solution.



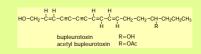
Pattern Recognition

Common Pattern Generation: The common pattern of B. chinense profile was generated through 14 batches of authenticated samples. Multivariate data analysis: similarity, principal component analysis (PCA), clustering etc. pattern recognition methods were used in data analysis. The result strongly supported the chemical differences between the HPTLC fingerprint of various species of Bupleurum spp. by intuitive observation of the HPTLC images.



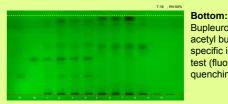
Discussion

- Rich-information obtained from the chromatographic fingerprint generated by colorful HPTLC image can serve comprehensive identification and assessment of multi-components entities of herbal medication.
- In this case study, a poisonous species of Bupleurum longiradiatum which contains toxic constituents like bupleurotoxin and acetyl bupleurotoxin can be easily differentiated from the commonly-used Radix Bupleuri Chinense and other medicinal species by both HPTLC fingerprint identification test (most saikosaponins) and specific identification test (bupleurotoxin and acetyl bupleurotoxin).



Toxic alkines in B. longiradiatum

(63.44% variation accounted)



Bupleurotoxin and acetyl bupleurotoxin specific identification test (fluorescence quenching, UV254nm)