HPTLC illustration of the USP Dietary Supplements Compendium

Eike Reich*[1], Anita Ankli[1], Eliezer Ceniviva[1], Judy Nichols[2], Valeria Widmer[1]

[1]CAMAG Laboratory, Sonnenmattstrasse 11, 4132 Muttenz, Switzerland; *Corresponding author: eike.reich@camag.com

[2]CAMAG Scientific Inc., 515 Cornelius Harnett Drive, Wilmington N.C. 28401, USA;.

Summary

Using Ashwagandha as example we illustrate the process of evaluation and optimization of existing official methods and their conversion into standardized HPTLC methods. This process has been developed in cooperation with the United States Pharmacopoeia as contribution to the Dietary Supplements Compendium, the first official compendium to feature full-color illustrations of chromatograms.

In addition to the illustration of the "old" official TLC methods we have proposed new, equally or better suited HPTLC methods for inclusion in the DSC. This permits the user to choose the new, optimized and less time-consuming HPTLC method while still complying with the cGMP rules for dietary supplements.

Based on submitted data these alternative methods can be rated as "scientifically valid" as required by the cGMP rules.

Introduction

The United States Pharmacopoeia (USP) Dietary Supplements Compendium (DSC) (Fig. 8) is the new comprehensive reference for dietary supplements. The compendium features full-color illustrations of typical TLC and HPTLC chromatograms – for the first time ever in the history of official compendia monographs.

CAMAG Laboratory has been cooperating with the USP DSC since 2007. In the beginning a standardized HPTLC methodology and a suitable standard format for the DSC illustrations have been developed. While continuing with the illustration of existing methods on TLC plates we also contributed HPTLC methods as supplementary information.

For the 2011 revision of the DSC the monographs for Horse Chestnut, Maritime Pine, Stinging Nettle, Valerian, Red Clover, Pygeum, Centella, Bacopa, Bilberry, Saw Palmetto, Senna, Myrrh, Boswellia, Guggul, Garcinia, Malabar Nut Tree, Forskohlii, Andrographis and Ashwagandha (Fig. 1) have been evaluated, the latter is here presented as example.

Fig. 1 Ashwagandha root (Withania somnifera (L.) Dunal)

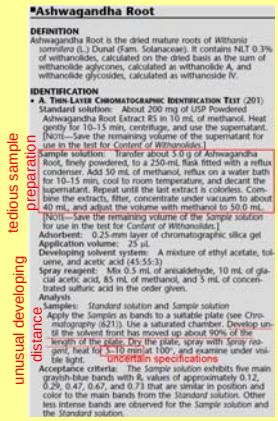


Fig. 2 USP34 monograph

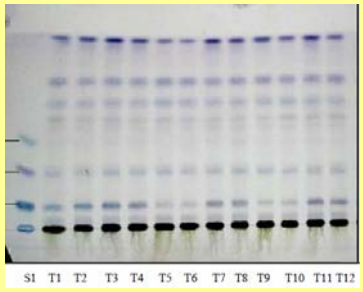


Fig. 3 Representative TLC chromatogram of USP proposed Ashwagandha monographs (source: USP)

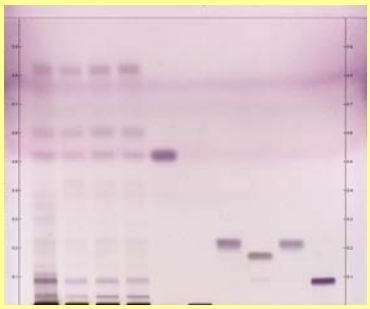
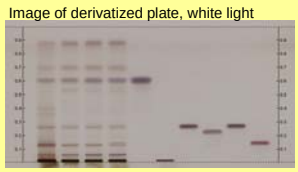


Fig. 4 Standardized TLC method



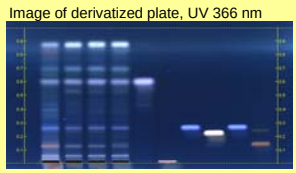
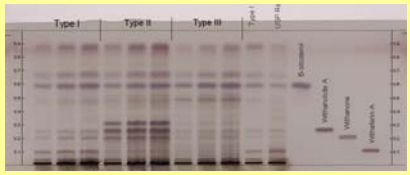


Fig. 5 Optimized method under standard HPTLC conditions [2]



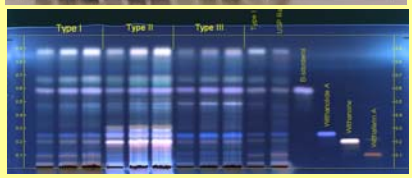


Fig. 7 Discrimination of 3 chemotypes.

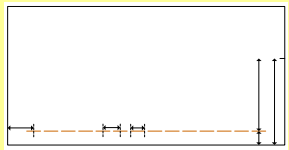


Fig. 6 Suitable HPTLC plate setup:
a=20mm b=10mm c=8mm
d=8mm e=62mm f=70mm

Fig. 8 The United States Pharmacopeia Dietary Supplements Compendium USP-DSC 2009-2010

Material

ADC2 with humidity control, immersion device, plate heater, ATS4, Visualizer (all CAMAG), HPTLC plates Si60 F254 (Merck); p.a. solvents (Acros); standards and plant samples from USP.

Method

1g of powdered plant material is mixed with 10mL of methanol and sonicated for 15 minutes, then centrifuged or filtered. 1mg each of ß-sitosterol, withanolide A, withanone and withaferin A is individually dissolved in 5mL of methanol.

Spray-on application of 10µL as 8mm bands. Development in ADC2, 20min chamber saturation, developing distance 70mm, mobile phase toluene, ethyl acetate, glacial acetic acid 55:45:3 (v/v/v), plates conditioned to 33% RH. Derivatization by immersion into 10% sulfuric acid in methanol, followed by heating at 100°C for 5 minutes.

Acknowledgments

We thank Maged Sharaf, USP, for providing samples and reference substances, and ANCHROM (Mumbai) for help with obtaining samples.

Literature

[1] USP34–NF29 p. 1077 & PF 35(4) p. 885: Monograph Ashwagandha Root, Pwd Ashwagandha Root, Pwd Ashwagandha Root Extract

[2] Reich, E., Schibli, A. (2004) *J. Planar Chromatogr*. 17, 438-443

Results and discussion

Ashwagandha (the dried mature roots of Withania somnifera (L.) Dunal) is commonly used in Ayurvedic medicine and has become popular as a dietary supplement said to relieve stress and enhance body vitality in general.

The USP features monographs on Ashwagandha root, powdered root and powdered root extract [1]. The reference chromatogram (Fig. 3) proposed by the USP as basis for the current method description of the monographs (Fig. 2) is not satisfactory. Sample preparation is tedious and many important method parameters (band length, developing distance) are not sufficiently specified. It is difficult to match results with the given description.

When standardized TLC conditions (Fig. 4) were applied it became clear, that the application volume was not sufficient, sample preparation could be simplified, and detection should be improved. We therefore changed the sample preparation procedure to simple sonication, and adjusted the sample application parameters for use on HPTLC layers (Fig. 5 and 6) following the standard operation procedure for HPTLC [2]. Optimum chromatogram development was achieved in the automatic development chamber ADC2 under controlled relative humidity. The detection step was optimized by changing the derivatization reagent to sulfuric acid reagent and by immersing the plate instead of spraying. Suitable reference standards were withanolide A and ß-sitosterol.

This optimized method allows to discriminate at least 3 chemotypes (Fig. 7). It has been proposed to the USP for inclusion in the DSC 2011 edition.