Analysis of Components in Cold Medicine Using New TLC-FTIR Method

Thin layer chromatography (TLC) is an inexpensive, easy, and highly sensitive separation method used in various fields. Qualitative analysis of separated components in TLC is based on a comparison of rates of migration (Rf). There are great variations depending on the conditions so this method isn't decisive. Infrared analysis is a more decisive qualitative analysis method. In infrared analysis, the “scratch removal” and direct infrared spectral measurement methods have been used until now. Drawbacks of these methods include extensive preparation, concern over sample loss or contamination, and the problem of sensitivity, which make it difficult to obtain accurate spectra.

The following procedure was established to circumvent these problems. A TLC sheet was used which allows transfer of the TLC spots from the sheet by performing redevelopment (transfer) towards a KBr layer formed on the TLC sheet surface. Then IR chromatograms and infrared spectra were measured. The measurement procedure is introduced here.

Principle of New TLC-FTIR Method

The developed TLC plates were set in a stainless steel frame, and KBr powder was loaded onto the sample frame defined by the slit in the stainless steel frame (Figure 1). Next, this sample frame was placed in a porous glass transfer stand, and as the transfer solvent was drawn up through the glass filter pores, the spot components migrated to the KBr powder layer (Figure 2). If the transfer solvent is highly volatile and possesses sufficiently high solubility characteristics, the spot components migrate to the KBr powder layer simultaneously, with each component being transferred in its original separation state.

After transfer, the sample frame was set in a diffuse reflectance accessory, equipped with a motor driven stage for scanning. While this stage was moved at a constant speed, an IR chromatogram was measured using the Gram-Schmidt method. Then, infrared spectra of the separated components were obtained by conducting measurement after the peak positions were verified via the IR chromatogram.

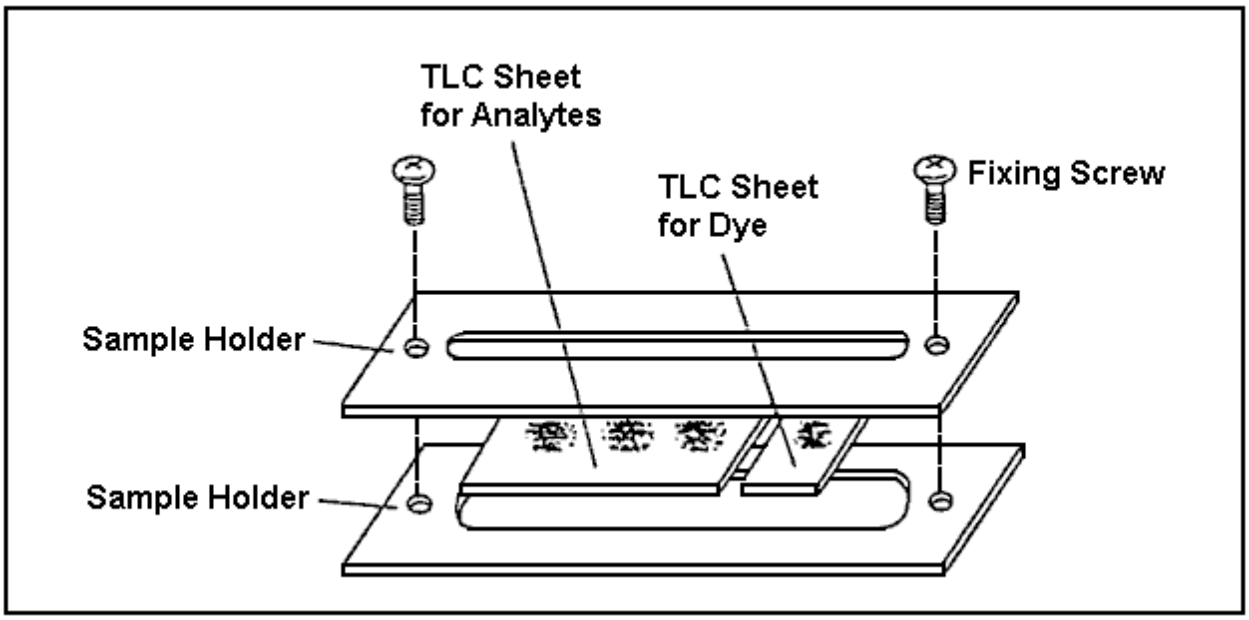

Figure 1 – Sample Holders for Holding the TLC Sheets

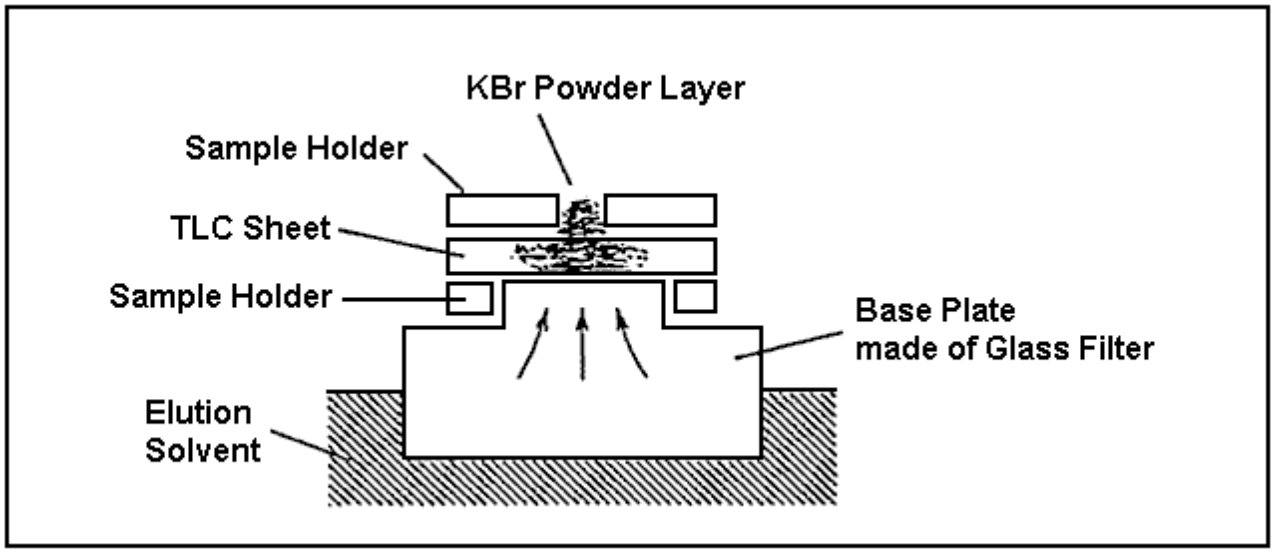

Figure 2 – Cross Section to Show the Transfer Process

Analysis of Cold Remedy Ingredients

15µg each of the cold remedy ingredients A) anhydrous caffeine, B) phenacetin and C) noscapin were developed on an Empore TLC sheet using benzene/acetone (3:2 by volume) as the developing solvent, and then transferred to the KBr powder layer using methanol as the solvent. The pre-transfer TLC plate was measured using the Shimadzu Flying Spot Scanning Densitometer at 220nm to obtain a UV chromatogram ((a) in Figure 3). The post-transfer IR chromatogram is shown in Figure3 (b). The peaks are seen to correspond well, indicating almost no change in the pre- and post-transfer separation states. Figures 4, 5 and 6 show the infrared spectra for each peak (caffeine, phenacetin and noscapin), respectively.

The TLC-FTIR method introduced here is a very simple procedure, providing accurate infrared spectra as well as IR chromatograms - demonstrating its effectiveness in conducting qualitative analysis of TLC-separated components.

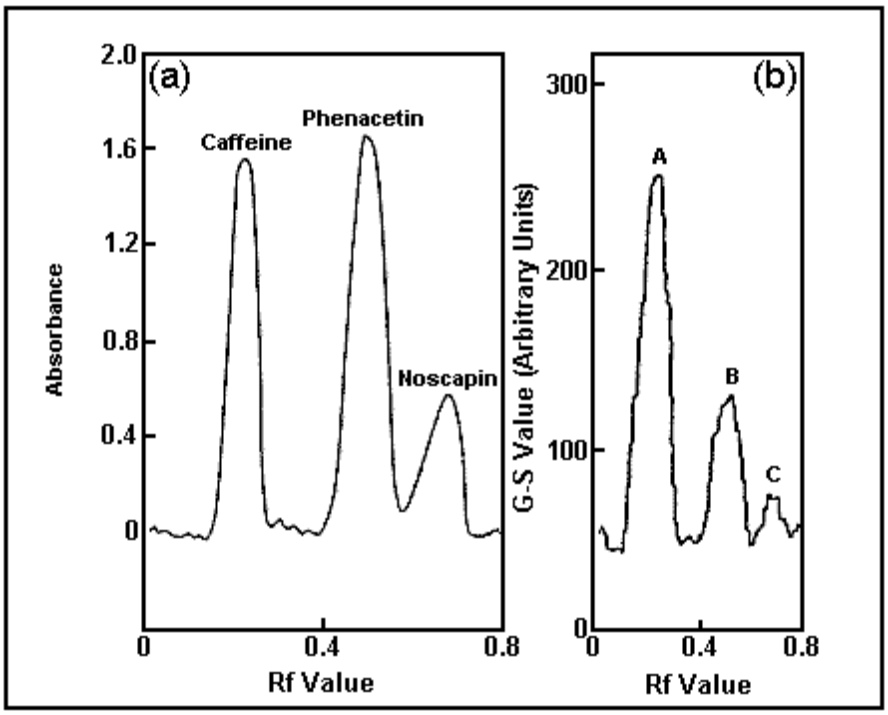

Figure 3 – UV Chromatogram Obtained Before Transfer to KBr Powder Layer (a) and Reconstructed IR Chromatogram after Transfer into the KBr Powder Layer (b)

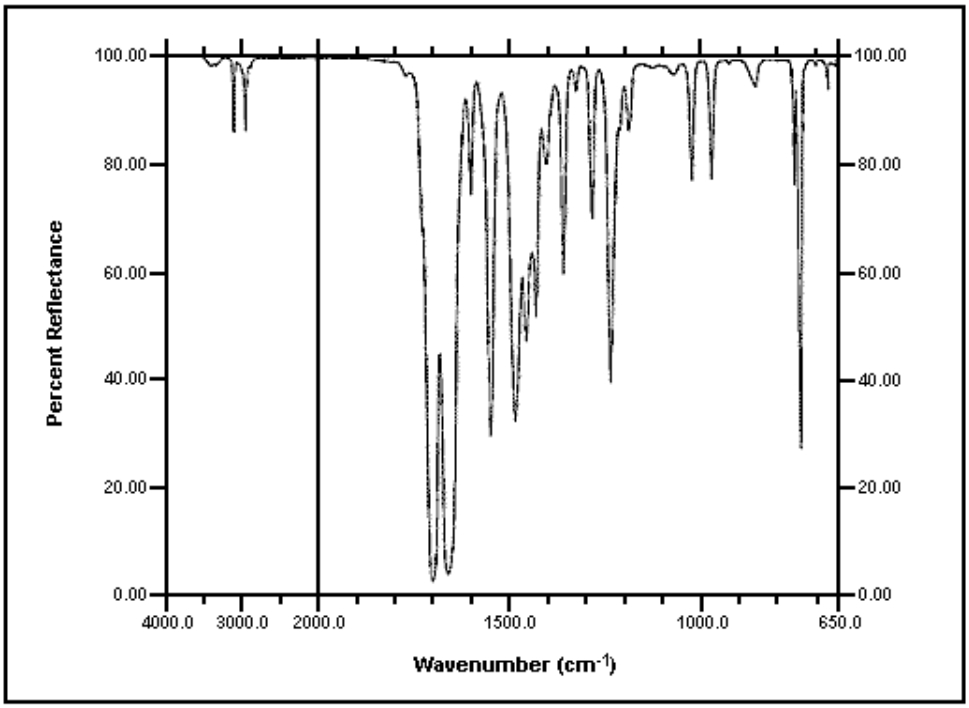

Figure 4 – Infrared Spectrum of Peak A

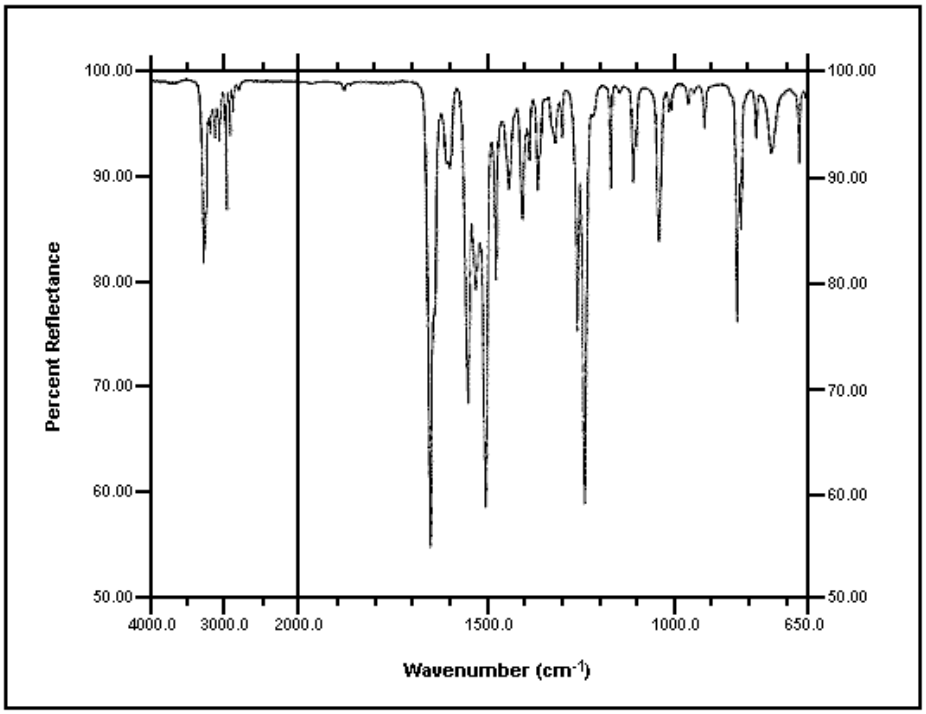

Figure 5 – Infrared Spectrum of Peak B

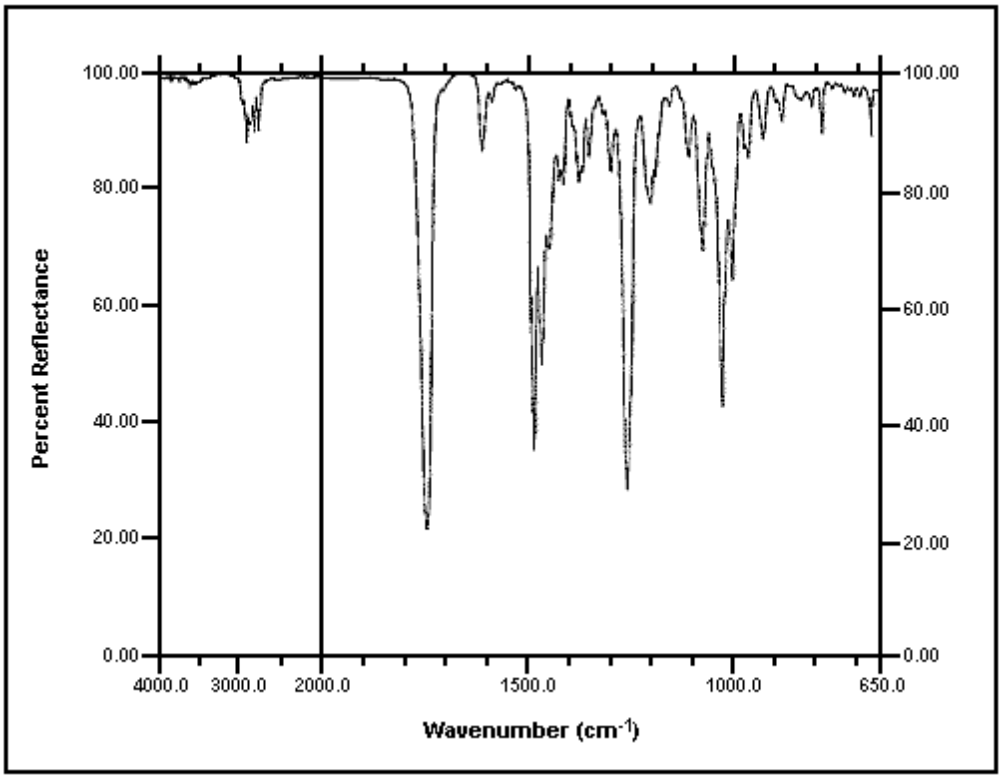

Figure 6 – Infrared Spectrum of Peak C

Resolution	: 4 cm –1
Apodization	: Happ-Genzel
Accumulation	: 100
Detector	: MCT

Table 1 – Analytical Conditions