Possibilities and limitations of the employment of Ψ-spectroscopy in planar chromatography

Valentina Blagodarov, Gerda Morlock, Wolfgang Schwack

Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, 70599 Stuttgart, Germany, wschwack@uni-hohenheim.de

Introduction

Substance identification in HPTLC is usually performed by UV/Vis spectroscopy, besides mass spectroscopy actually an up-coming technique. The influence of substance amount, mobile phase (pH) and layer material on the in situ UV/Vis spectra is well-known [1]. To what extent Ψ-spectroscopy can cope with that drawback was investigated in this study. Ψ-spectra calculated by dividing the derivative spectrum by the original absorption spectrum are principally independent upon concentration [2]. This was demonstrated to be a powerful tool for monitoring of spectrum purity/identity in photometry and HPLC/DAD.

Results and discussion

In situ UV/Vis spectra were ascertained to be not dependent on the instrumental device and the property of the substance, like crystalline or liquid. Further on the mode of substance application (dipping or spraying) and location (low and high R_F value), meaning the distribution of the substance in/on the layer, was shown to have no impact on the concentration dependence of spectra.

The concentration dependence of in situ UV/Vis spectra generated not identical maximal wavelengths as well as broadened (flattened) spectra by increasing concentrations and hence influenced the fine structure of the derivative spectra.
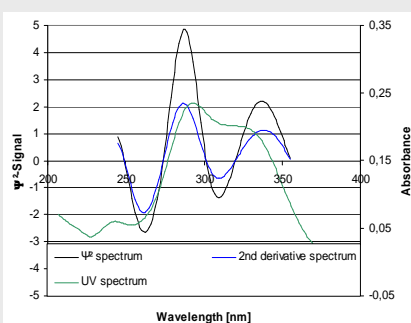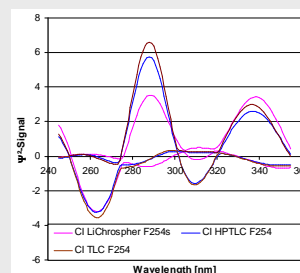

Overlay of UV-spectrum, 2nd derivative and Ψ²-spectra of 1 µg oxybenzone
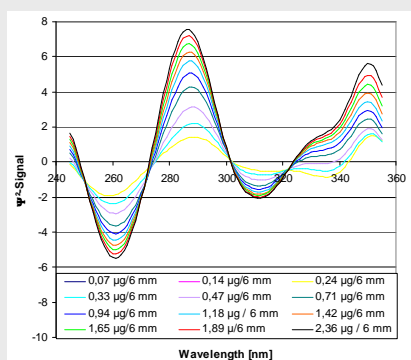
Concentration dependence of the Ψ²-spectra of oxybenzone

Ψ-spectra of HPTLC zones were shown to be concentration dependent (although they are principally concentration independent). The impact of this influence was increasing in the following order: LiChrospher, HPTLC and TLC plates.


Confidence intervall (CI) of the Ψ²-spectra of 1 µg oxybenzone on different layers

However, a change of the background structure by high substance amounts on the plate was not observed and does not explain this effect.
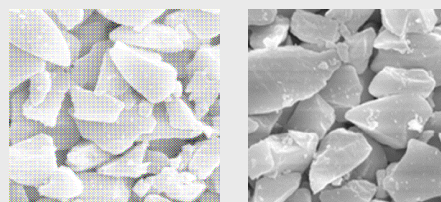
REM pictures (3000 x) of a HPTLC blank position (left)
and an oxybenzone zone (800 ng, right)

Despite this difficulties the employment of Ψ-spectroscopy is possible and illustrated by 4 zones of oxybenzone assigned as samples.
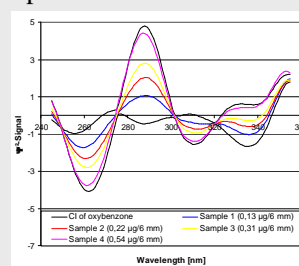
Identity check via Ψ-spectroscopy: the 4 samples are laying within the confidence interval of the external calibration of oxybenzone (0,09 – 0,45 µg/6 mm)

Conclusion

Identical maxima and spectrum slopes are prerequisites for the successful employment of Ψ-spectroscopy. Hence due to the concentration dependence of the spectra, the efficacy of Ψ-spectroscopy is more difficult in planar chromatography than in photometry and HPLC. However, the background influence is minor than for absorbance spectra.

References:
[1] S. Ebel (1990) J. Planar Chromatogr 3, 42-46
[2] W. Schwack, H. van Lishaut, plenary lecture, The Jubilee XXXth Symposium, Katowice (2006)