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A new approach for a multidimensional TLC evaluation

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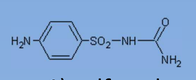
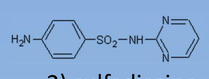
Introduction

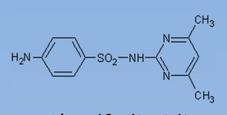
The concept of multidimensional chromatography is a powerful tool for multi component separations. Such separations are difficult to perform in column chromatography. In contrast, it is more suitable to carry out two dimensional separation by high performance thin layer chromatography (HPTLC), using aminopropyl coated silicagel plate with two different solvent mixtures. The HPTLC-plate is simply developed in the first direction, dried, turned upright and then developed in the second direction.

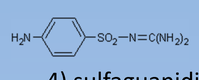
Here we present a data set of four tuples (quaternions) showing light intensities from a sulfonamide 2D-HPTLC separation of 12 sulfonamides, measured at different wavelengths.

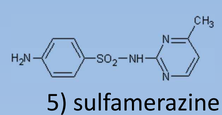
Tab. 1: list of the separated sulfonamide structures and their chemical names

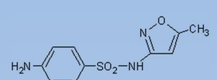

1) sulfacarbamide

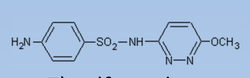

2) sulfadiazine

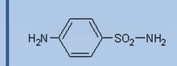

3) sulfadimidine

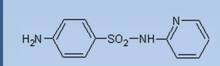

4) sulfaguanidine

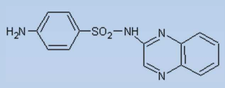

5) sulfamerazine

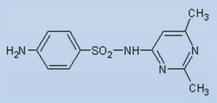

6) sulfamethoxazole

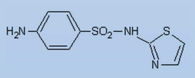

7) sulfamethoxypyridazine


8) sulfanilamide


9) sulfapyridine


10) sulfaquinoxaline


11) sulfisomidine


12) sulfathiazole

Evaluation

A mixture of 12 sulfonamides (2 µg each, tab. 1) is separated by using a cyanopropyl-coated silicagel plate (Merck, 1.16464) with the solvent mix of methyl tert-butyl ether, methanol, dichloromethane, cyclohexane, NH_3 (25%) (48+2+2+1+1, v/v) for the first and a solvent mix of water, acetonitrile, dioxane, ethanol (8+2+1+1, v/v) for the second direction. Both developments are carried out over a distance of 70 mm.

To measure a HPTLC-plate we use a special light fibre interface shown in figur 1. This interface is fixed at a distance of 450 µm above the plate surface. During measurements, the plate is moving underneath this interface with constant velocity. The measurement time for a single spectrum (measured by a Tidas TLC 2010 device) in the wavelengths range from 190 to 1000 nm is 100 msec. A picture of the DAD-scanner is shown in figure 2.

A plate surface with 68.4 by 68.4 mm is measured in 342 rows consisting of 342 different data points. This results in a spatial resolution of 200 by 200 µm. The measurement time is 342*342*0.1 sec = 3 h 25 min.

A scanned TLC-plate needs 400 MB storage space. These data are copied into the computer memory to have rapid access to the data. The evaluation program is written in PureBasic (Ver. 4.50) and needs a 64 bit computer to carry out the program.

Fig. 1: Light fibre interface consisting of a 300 µm inner core, surrounded by nine light fibres with a diameter of 100 µm each.

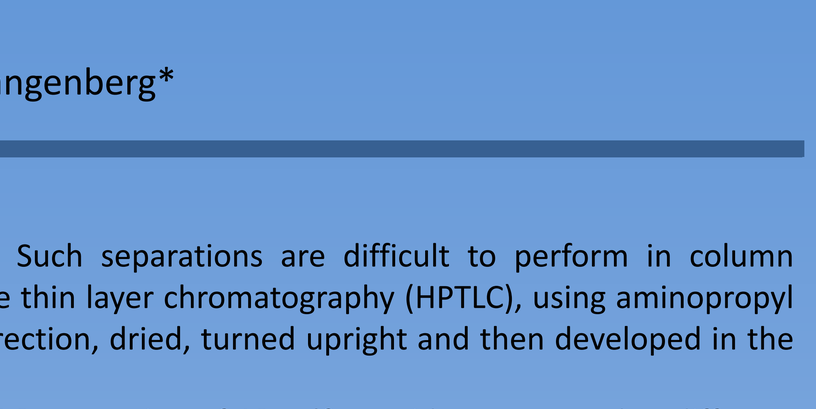


Fig. 2: Assembly of the DAD-scanner. On the left are the tungsten lamp and the DAD-detector and on the right the deuterium lamp and the LED for fluorescence evaluations. In the middle is the X/Y-table with HPTLC-plate and light fibre interface (blue).

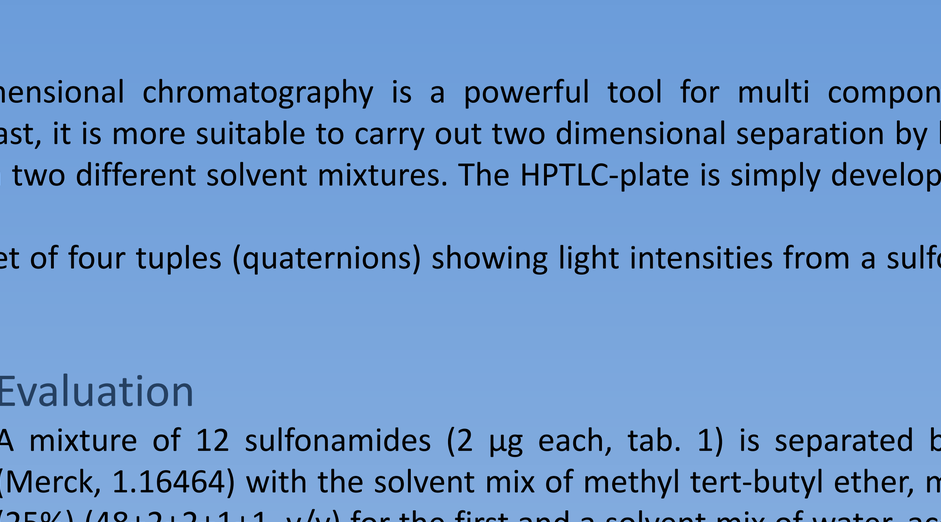


Fig. 3: 3D plot measured at 479 nm. Separation distances are plotted in both directions. Different light intensities are plotted in different colours. The spots of the different sulfonamides are correlated with table 1.

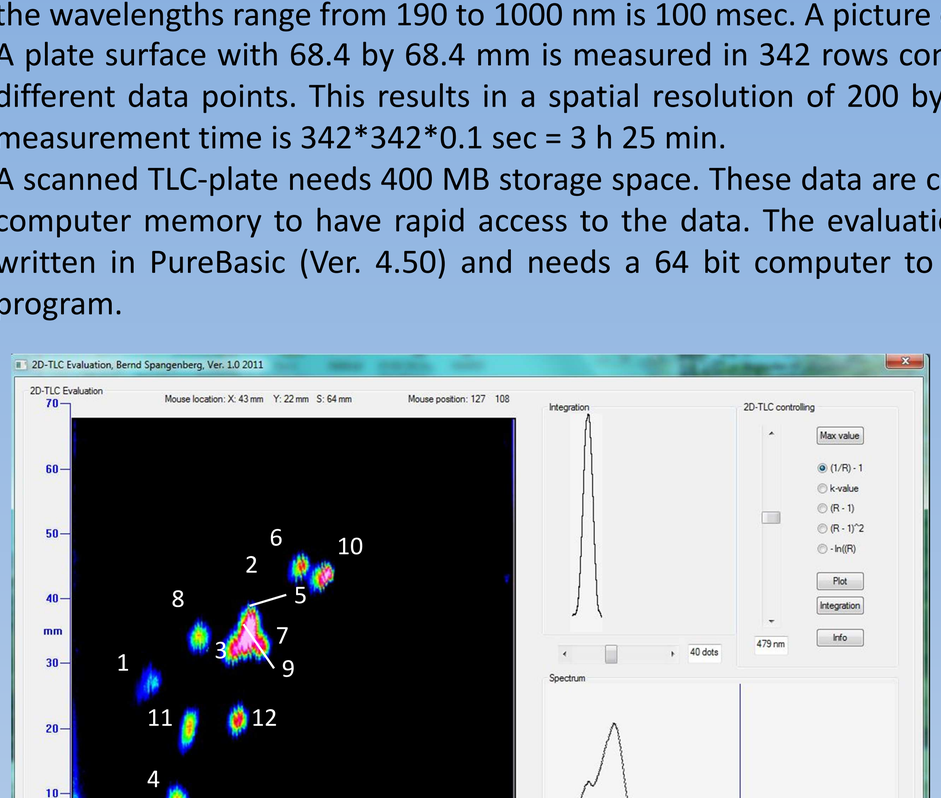


Fig. 4: 3D-plot of a sulfonamide fluorescence measurement. The sulfonamide sulfapyridine, located at 25mm*45mm shows a bright fluorescence.

The characterisation of a 2D-TLC-plate is also possible as quinternions when spectral resolved light is measured in absorption and in fluorescence. The plate is scanned while illuminated by an LED which emits very dense light at 366 nm. The resulting scan is plotted as shown in figur 4 and reveals the fluorescence spectra at a given position instantly. The system is suitable for all kinds of TLC-plates that can be measured in absorption and fluorescence.

Results

Figur 3 shows a 3D plot (plate area and absorption intensities) at a given wavelength. The cursor can be moved over the screen to show the spectrum measured at a particular area on-line.

The plot can be instantly changed by choosing another wavelength. In this way you can resolve neighbouring spots which are not spatially separated. In figure 5 the same area of interest is measured at different wavelengths showing that this method can improve the spot resolution. A spot at a given wavelength can be virtually encircled and the resulting peak is integrated without any problems. This makes the method a quantitative one, unique in 2D-TLC.

The spot diameter (4σ separation) of all peaks is in the range of 3 to 6 mm. Using a new definition for the separation number (SN) in 2D-separation as expression:

$$\mathrm{SN} = \frac{\mathrm{plate-area}}{(\mathrm{peak-area})_{2\sigma}} - 1 = \frac{70\mathrm{mm} * 70\mathrm{mm}}{\pi * 2\mathrm{mm} * 3\mathrm{mm}} - 1 = 259$$

a separation number of 259 was achieved.

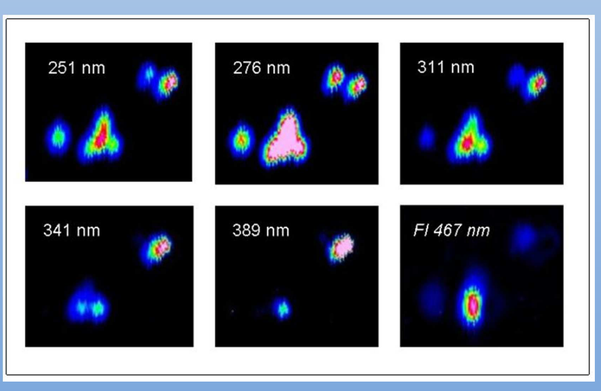


Fig. 5: The same area of interest measured at different wavelengths in absorption (1-5) and fluorescence (6), showing that this method can improve the spot resolution.

Conclusion

We present the first example of a two-dimensional HPTLC-plate, measured by use of a diode-array scanner. A spatial resolution of 200 µm was achieved on plate. The system provides real 2D fluorescence and absorption spectra in the wavelength-range from 190 to 1000 nm with a spectral resolution of better than 1 nm. A separation number of 259 is calculated, which makes this system superior to HPLC.