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A new TLC technique and chamber

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- Aflatoxins are toxic fungal metabolites, they occur in most of the food products.
- Although they are related to a group of chemicals, the most common aflatoxins in foods are Aflatoxin B1, B2, G1 and G2 forms.
- Since the discovery of aflatoxins in 1960, until the mid 1980s, the only analytic technique was TLC for aflatoxin determination, quantification and confirmation.
- Then HPLC have been the most popular technique after 1980s.

Appearance of the four aflatoxins on TLC plate (20X20 cm) under 395 nm UV light is seen below.

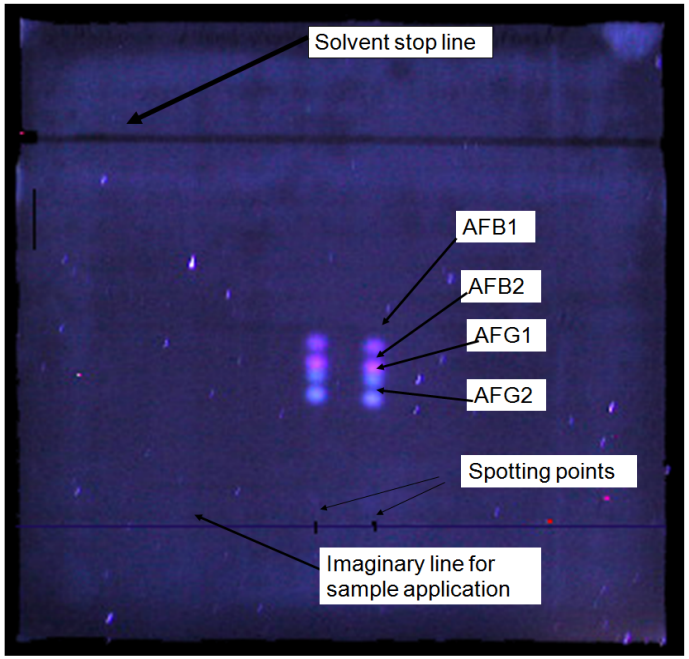


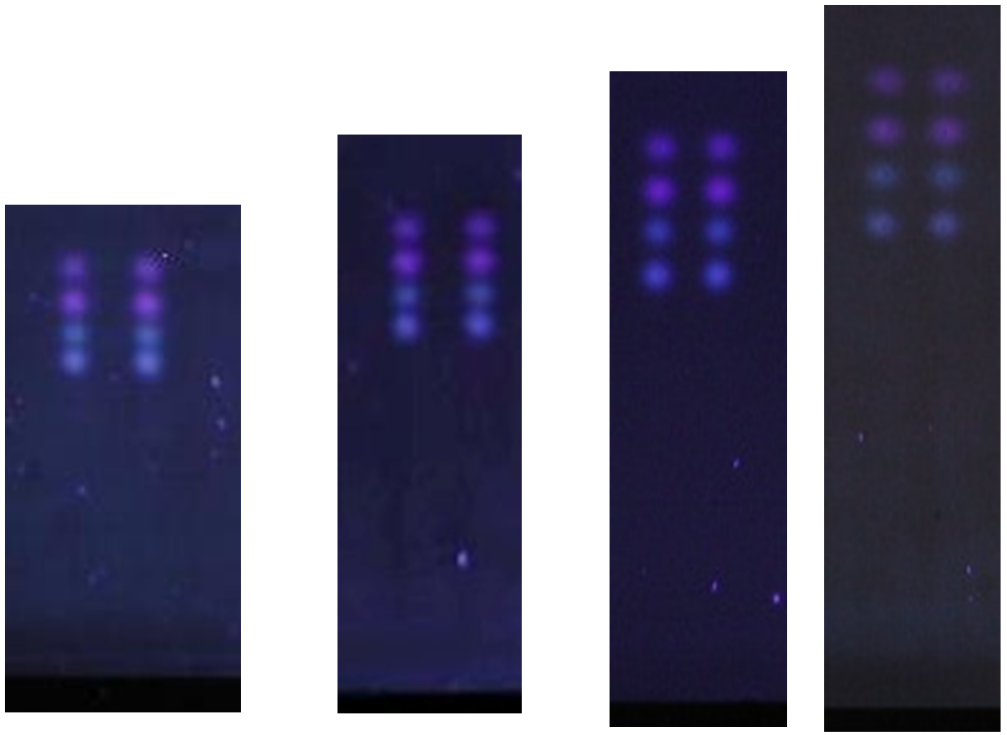
Figure 1: Spots of aflatoxins on TLC plate after development

- Separation of aflatoxin spots from each other is better around 0.5 Rf value, but still, better separation is needed.
- The increasing of the spots migration distance increases the separation.
- But increasing of migration distance of spots, can only be possible by increasing of solvent travelling distance or increasing of plate height.
- Practically, over 20 cm of plate height, chromatographic separation takes a very long time, during this period spots get bigger and separation is still poor.

- In the case of OPTLC, driving force is external solvent pressure (not capillarity) so spots can travel across the plate, then separation is better.
- In Turkey I developed a new, TLC chamber or system (Turkish Patent No. 2007/08721).
- In this system, although driving force is still capilarity, there is not any solvent stop line and chromatographic separation can continue as long as you decide.
- So, you can continue separation until the leading spot (in the case aflatoxins leading spot is AFB1) reach the upper edge of the plate.

A sample about separation durations and spots traveling distance in classic TLC and in new system

By classical TLC, tetr.-methyl butyl ether: methanol: water (480:15:5) mobile phase reached solvent stop line within 83-89 min (distance traveled by solvent was 12 cm; between sample application line to solvent stop line) and AFB1, AFB2, AFG1 and AFG2 spots traveled 4.2, 3.4, 2.8 and 2.3 cm (Rf values were 0.35, 0.28, 0.23 and 0.19), respectively.



Classic one-dimensional TLC; separation time is 89 minutes.

2 h. 3,5 h. 5 h.

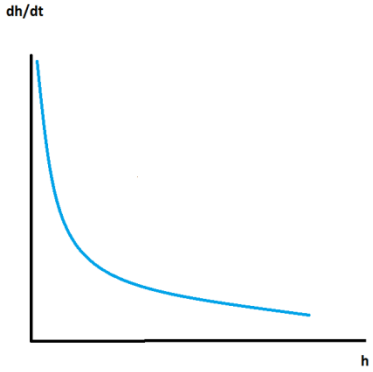
Continuous TLC; plate height : 20 cm

- With the new continuous TLC technique, the spots reached 9.3, 8.3, 7.5 and 6.3 cm in 5 hours, respectively.

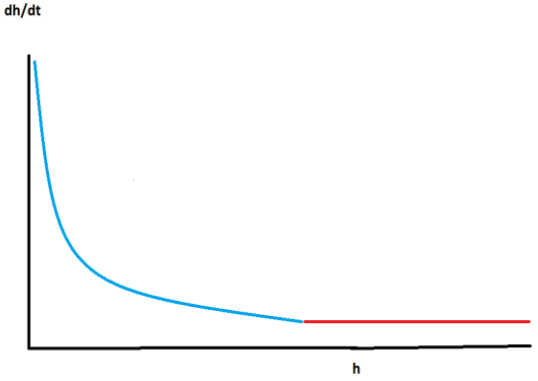
Solvent velocity on the plate versus solvent height on the plate

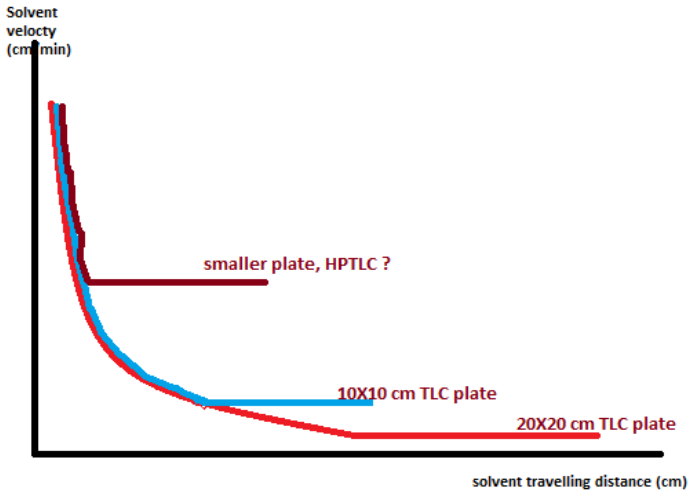
As known, solvent velocity decreases dramatically when solvent height increases. This situation has been explained by diffusion rules. My opinion is that the same situation can be explained by capillarity forces also.

In classic TLC solvent velocity versus solvent height can be displayed as;



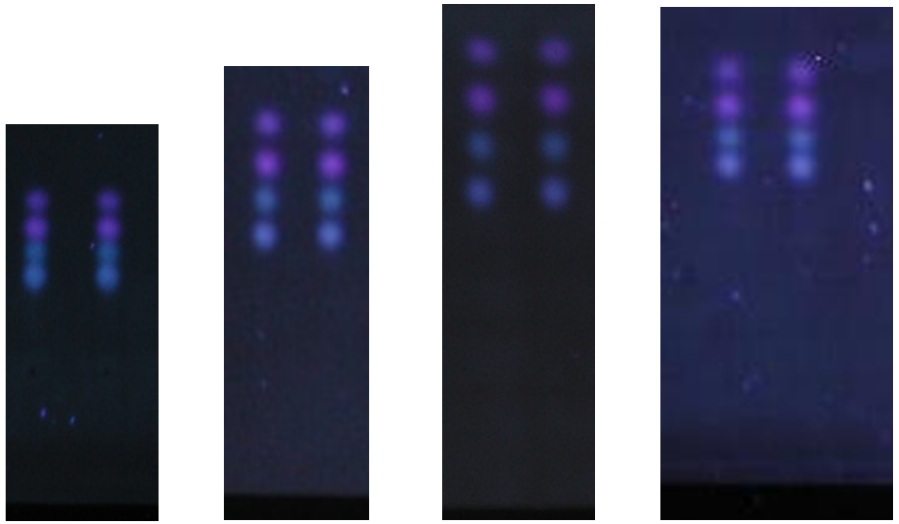
In this new system, when solvent reaches to the upper edge of the plate, h does not increase anymore and velocity becomes constant.





If we decrease the plate height, solvent velocity will not decrease drastically and more effective separation will be obtained in a short duration.

When using 10 cm TLC plate the spots traveled 5.9, 5.0, 4.2 and 3.4 cm, respectively, and the duration of chromatography decreased to 60 min.



30 min. 50 min. 1 h.

Continuous TLC; plate height : 10 cm

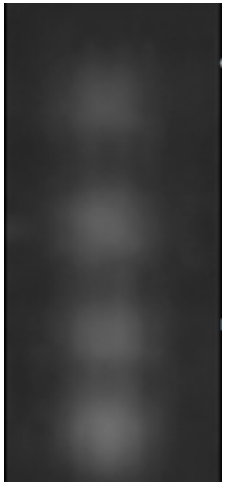
Classic one-dimensional TLC; separation time is 89 minutes.

The resolutions between spots at classical and new developed method were approximately 1.0 and 1.5-2.0, respectively.

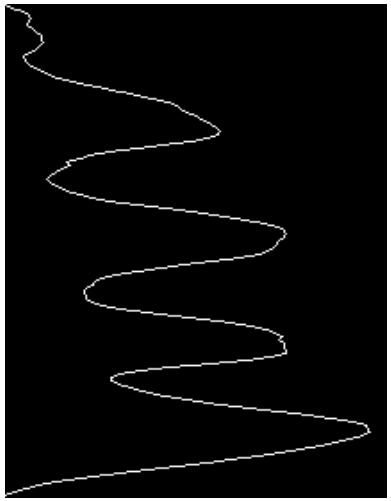
Visualization of chromatograms and calculation of resolutions

Visualization of chromatograms is performed by;

1. Taking the photo of plate under 365 nm UV light by a digital camera,
2. Selecting the spot patterns and inverting this area gray scale by photograph processing program
3. Processing the black and white picture by a Gel electrophorese evaluating program

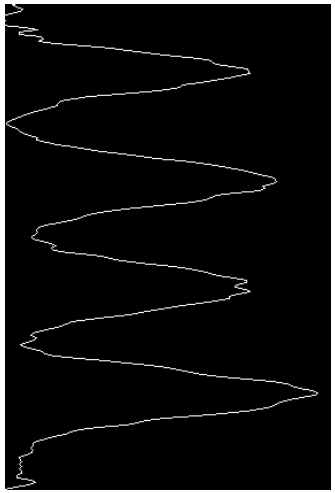


Grey scale picture of aflatoxin spots pattern on the classic TLC plate



Chromatogram of the spots

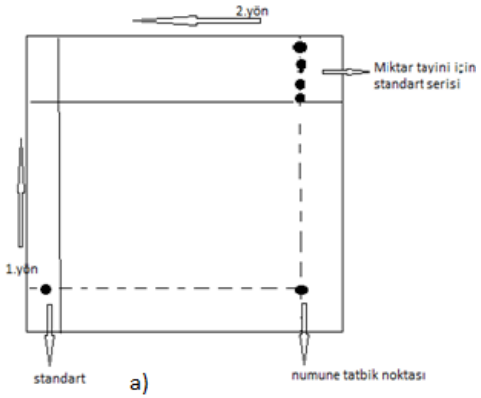
Similar photograph for new continuous technique



Its chromatogram

Quantification and method precision

The new technique is used for aflatoxin analysis in red pepper which is the one of the most difficult food matrix for chromatographic analysis of aflatoxins.
In this study, immune affinity column wasn't used for clean up.
For better separation, two dimensional TLC was performed. A series of calibration standards and a sample spot are applied on each of 12X12 cm silica gel 60 plates.
Two dimensional TLC principal:



Prepared plate

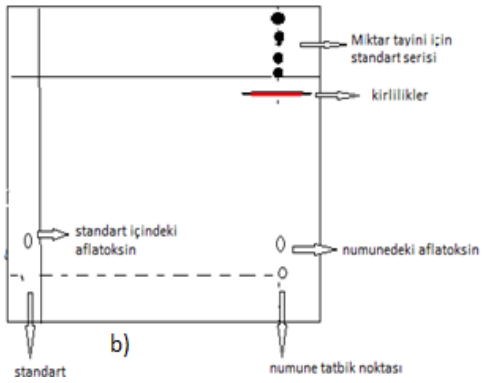


Plate view after development towards direction 1.

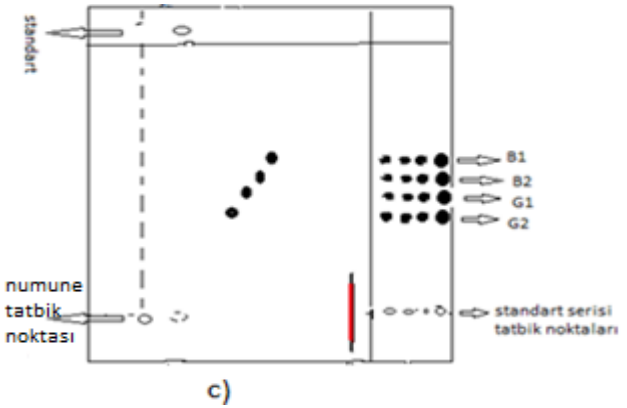
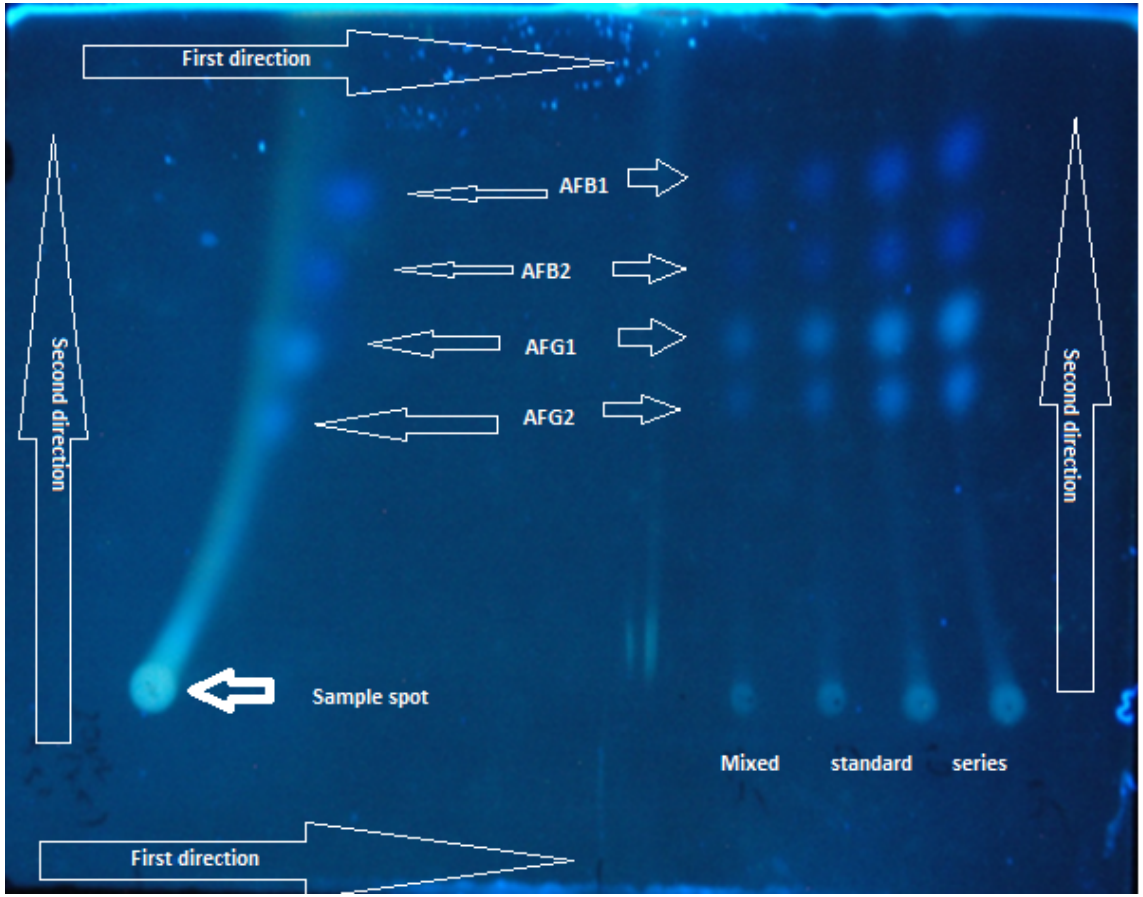


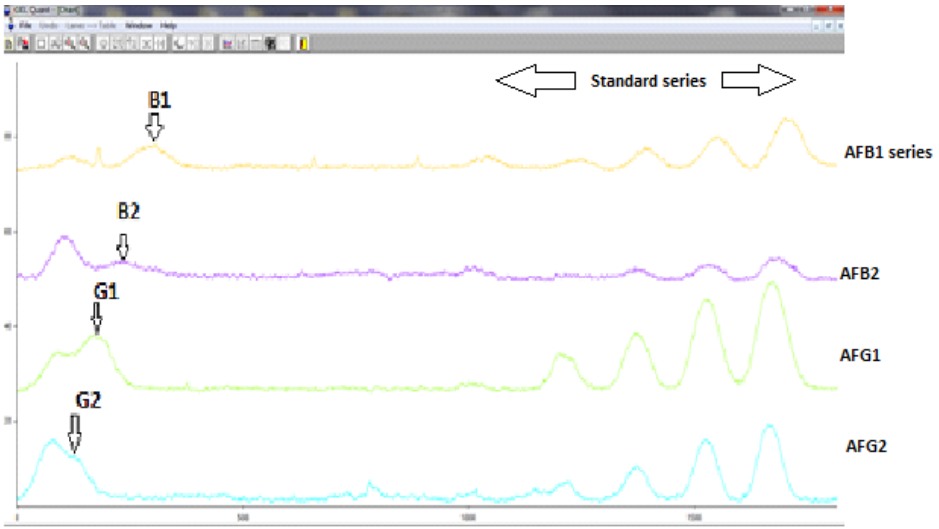
Plate view after development towards direction 2.

(Karakaş, 2011; MsC Thesis)

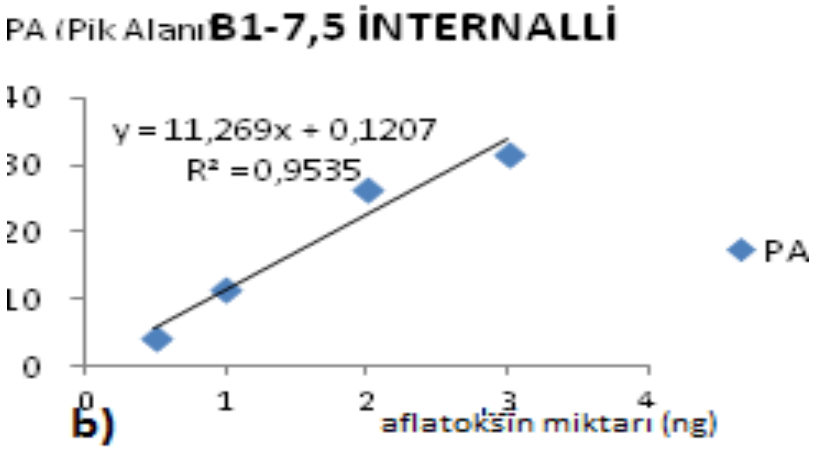
One of the plates;



Sample; red pepper, skipped with 7.5 ppb AfB_1, AfB_2, AfG_1 and $AfG2_1$
Plate: Silica gel G60 coated alliminuum 11X10 cm plate
Solvent 1. drection di ethil ether 165 minutes; 2. drection TBME:meOH:water(480:15:5)



Şekil 4.24. 11.5x10 cm'lik plakada 7.5 ppb AfB_1 içeren örneğin 1. yönde 165 dakika eter ve 2. Yönde 55dakika TBME:met:su(480:15:5) ile developesi ile çift boyutlu ayrımına ait Gelquant programından elde edilen kromotogram.



Note: R2 value of calibration curves are higher then 0.99

- This is a new simple TLC separation technique with some advantages, such as limited time and solvent consumption, lowered cost and higher separation efficiency.
- As a conclusion; the new technique can be easily used for determination of aflatoxins in any food matrix after immunoaffinity column clean-up of the extract.

Thank you for listening...

- If we accept that driving capillarity force is constant and resistance to solvent progress is increases by the solvent height “h” on the plate;
- Solvent progress velocity (dh/dt) is inversely proportional with the solvent height on the plate.

$$\frac{dh}{dt} = k\frac{1}{h}$$

Re arrangement of the equation, integral of both sides

$$\int h\,dh = \int k\,dt$$

gives us;

$$\frac{h^2}{2} + C = kt \qquad \text{or} \qquad t = \frac{h^2}{2k} + \frac{C}{k}$$

Re-parameterization of equation yields;

$$t = ah^2 + h_0$$

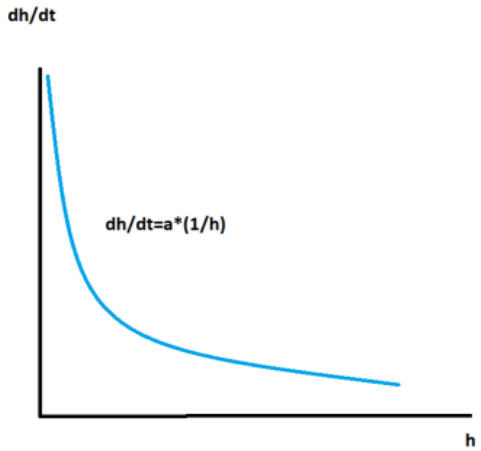
If we accept that at the start of solvent development t=0 and h=0; h0 becomes “0”

Then equation becomes $t = ah^2$ or $h = a't^{0.5}$

These are the known equations. So that, our assumption for solvent velocity is acceptable.

$$\frac{dh}{dt} = k\frac{1}{h}$$

Solvent velocity vs solvent height at classic TLC



At the our system, when solvent reaches to the upper edge of the plate, h does not increase anymore and velocity becomes constant.