Optimization of the immunodetection in planar chromatography

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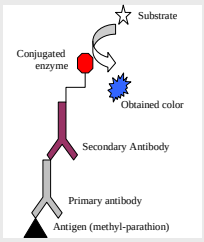
Introduction

Immunodetection (immunostaining) is employed for planar chromatographic analysis of glycolipids, sphingolipids, glycosphingolipids, sulfated lipids and gangliosides [1, 2]. However, it is considered as a time-consuming way of detection in planar chromatography. Hence it has been optimized to use the benefits of both techniques, the immunoassay and the chromatography. The combination has more favourable advantages than the single methods itself and combines substance identification with high selectivity or even specificity.

Steps of detection

The single steps of TLC immunodetection are as follows:

Step	Time
1. Fixation with 0.1% polyisobutyl methacrylate in n-hexane	60 sec
2. Primary incubation in 1:2000 (Parathion Pab Rbx in 0.1% BSA in PBS) at 37 °C	15 min
3. Washing 3 x 15 sec	45 sec
4. Secondary incubation in 1:2000 (alkaline phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) in 0.1% BSA in PBS) at 37°C	15 min
5. Washing 3 x 15 sec	45 sec
6. BCIP/NBT as substrate to visualize the zones	15 min
7. Total time	48 min

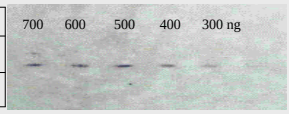


Scheme of immunodetection on a HPTLC plate silica gel 60 F_{254}

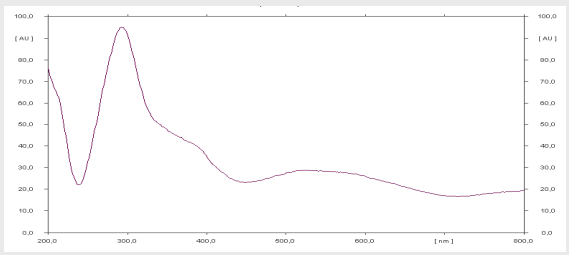
Detection of methyl-parathion

The immunodetection was optimized for the example of the pesticide methyl-parathion regarding detection time, dilution of the antibody, and capability of detection. The incubation time of both antibodies, primary and secondary, was shortened to 15 min in sequence and the overall detection time was reduced to 1 hour inclusively chromatographic development.

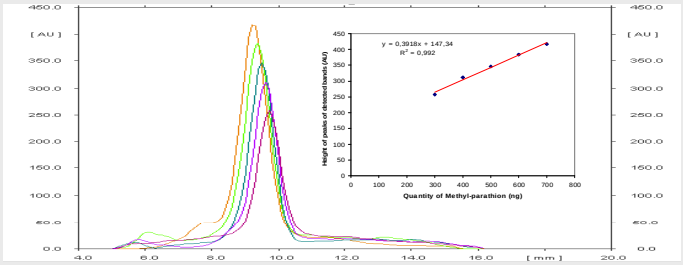
Band No.	1	2	3	4	5
Methyl-parathion (ng)	300	400	500	600	700
Peak height (AU)	258	311	347	383	418



After immunostaining the bands were scanned by absorbance measurement at 265 nm. The limit of detection (S/N of 3) of methyl-parathion was found to be 180 ng.



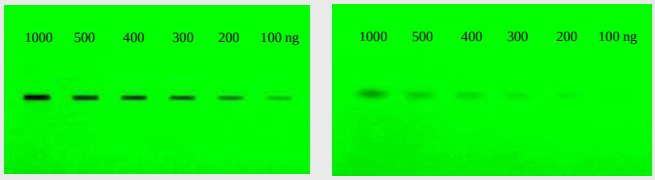
Absorption spectrum (200 - 800 nm) of methyl-parathion on a HPTLC plate silica gel 60 F_{254} after immunodetection with alkaline phosphatase / BCIP/NBT



Absorption scan at 300 nm of 5 concentration levels of methyl-parathion (300 – 700 ng/zone) on a HPTLC plate silica gel 60 F_{254} (left) and respective calibration curve (right)

Remarks

Due to the solubility of methyl-parathion in n-hexane and water, the actually immunodetected amount of methyl-parathion was less than the original one. Therefore, spraying of all incubation solutions was necessary to decrease the loss of methyl-parathion (except for the the fixation step, there dipping without shaking for 60 sec was best). It was observed that chromatography had a strong impact on the capability of detection. Due to diffusion effects during chromatography the intensity of methyl-parathion was highly reduced.



Comparison of the methyl-parathion zones before and after chromatography on a HPTLC plate silica gel 60 F_{254}; visualization by UV 254 nm.

Conclusion

A major advantage is the combination of chromatography with the very selective immunodetection. Compared to other chromatographic methods, planar chromatography can easily be coupled to immunoassay methods due to the local storage of separated zones.

On the example of methyl-parathion the immunodetection time was reduced from generally 5 hours to 1 hour. The limit of detection (LOD) of methyl-parathion was established to be 180 ng/zone.

References:

[1] Irie, T., Watarai, S., Iwasaki, T., Kodama, H., Journal of Veterinary Medical Science 66 (2004) 205-208

[2] Saito, Y., Kimura, A., Sagawa, K., Inoue, H., Yasuda, S., Nosaka, M., Tsuji, T., Japan. Wakayama Igaku 54 (2003) 21-29

Thank is due to CAMAG and Merck for support regarding equipment.