

B. Physical Methods of Detection

1. Visualization by Luminescence

As mentioned in the introduction, current luminescence-based methods for determining pesticides in various sample matrices have been reviewed. The use of fluorescence detection in TLC, HPLC, and FIA and its application to environmental samples has been described (33).

Among the various existing stationary phases, fluorometric detection is used mostly on silica gel plates (174). Theoretical examination has shown that in fluorogenic labeling a fluorophore molecule is fixed to the nonfluorescent analyte, which can be determined fluorometrically (175). Abscisic acid produced by cyanobacteria was determined by fluorescence labeling (176). A luminol-based chemiluminescence flow-injection method for the determination of dichlorvos pesticide has been developed (177).

Detection of fluorescent spots under UV light at 366 and 254 nm is widely applicable. Spraying with $\text{AsCl}_3/\text{HIO}_4$ reagent and UV detection were used in TLC determination of plant growth regulator (178). Rhodamine 6G is commonly used as a reagent before UV detection (179). A biologically active congener of koningin A from *Trichoderma koningii* was visualized under UV light at 254 and 366 nm or by spraying with anisaldehyde and heating (180). HPTLC of about 150 insecticides and fungicides has been carried out. Chromatographic spots were detected by AgNO_3 and UV irradiation or by cholinesterase inhibition (181). TLC of biphenyloxide and metabolites on silica gel using various solvent systems and UV detection at 254 nm has been reported (182). Chlorpyrifos and its by-products were detected with various reagents, and the technique was compared to fluorescence quenching detection (183). In qualitative TLC screening of nitrofurans residues in food, detection was performed by spraying with pyridine and exposing to UV light of 366 nm. Visual comparison was made against a standard equivalent to the maximum residue level (MRL) set by European countries (184). The microbial transformation of prosulfuron was investigated on silica plates with UV (254 nm) detection followed by consecutive spraying and heating with ceric ammonium sulfate and Dragendorff's reagent (185). Numerous hydrolysis products of pyrethroids were detected under UV light and by exposure to iodine vapors (186). In chemical reduction of zoalene to ANOT (3-amino-5-nitro-*o*-toluamide), detection was done under UV light at 366 nm and by exposure to nitrous acid vapors. The plate was then sprayed with a Bratton-Marshall solution (0.4% naphthylethylenediamine dihydrochloride solution in methanol) (187). Detection with 254 nm UV light and spraying with 2% 4-(4-nitrobenzyl)pyridine in acetone, with heating at 110°C, of the OP insecticides in human serum after acute poisoning gave satisfactory results (188).

2. Autoradiography and Related Techniques

Over the last decade, detection by autoradiography was used often, in parallel with UV irradiation and chemical color reactions in agricultural and environmental pesticide analysis. 3-Phenoxybenzaldehyde and its 11 metabolites were detected (189); the metabolism of the insecticidally active GABAA receptor antagonist was investigated (190); radioactive cinmethylin and its degradation products were detected (191); ^{14}C -8-hexachlorocyclohexane was identified after TLC separation (192); and the chromatographic behavior of chlorotoluron and its metabolites was investigated (193). After scraping and elution, quantification was carried out using liquid scintillation counting (LSC) (194–196). The aerobic and anaerobic soil metabolism of dicamba and 2,6-dichlorosalicylic acid were also investigated in this way (197). Structural characterization of [^{14}C]propargite metabolites in goat urine was detected by autoradiography and LSC, followed by FT-NMR and MS (198). In toxicological analysis of food, radioactive zones of moxidectin and metabolites were located by autoradiography (199). In metabolism investigation of pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate in rats, visualization was done under UV or by spraying with bromocresol purple or 2,6-dichlorophenol sodium salt. Radioactive compounds were detected by autoradiography (200). Degradation of commonly used pesticides in Malaysian soils was controlled in the same way (201). Qualitative identification by autoradiography served in the determination of radiochemical purity by elution in preparative TLC of S 53482 and its metabolites (202).