LITERATURE SURVEY FOR THE REAGENTS AND METHODS USED FOR THE DETECTION AND DETERMINATION OF INSECTICIDES AND DRUGS OF FORENSIC INTEREST

LITERATURE SURVEY:

Various reagents are reported in the literature for the detection and determination of organophosphorus insecticides by thin-layer chromatography. Baümler and Rippstein have reported the use of palladium(II) chloride for detection of these insecticides. Whereas the bromine-fluorescein, silver nitrate\(^2,^3\) reagent is used for TLC detection of organophosphorus insecticides. Irudayasmamy and Natarajan\(^4\) have used the congo red hydrogen peroxide reagent for detection of parathion and paroxon insecticides. A sensitive spray reagent 4-(p-nitrobenzyl) pyridine tetra-ethylene-pentamine\(^5\) has been reported for the detection of organophosphorus insecticides. Mercury(I) nitrate\(^6\) and mercury(II) nitrate-diphenyl carbazone reagents\(^7\) which were reported for the detection of derivatives of barbituric acid were further utilized for detection of organothiophosphate insecticides.

Silica gel G (particle size 15 \(\mu\)m) layer impregnated with 0.5 % cresol and developed with hexane-xylene-ethyl acetate-water (50:15:5:18) has been used in the determination of organophosphorus insecticides in vegetables and human blood\(^8\).
Sulphur containing organophosphorus insecticides from biological material has been detected at the 1 μg level by reaction with potassium periodate (KIO₃), the free iodine liberated reacts with starch solution to give blue colour, organochlorine and carbamate insecticides do not interfere. Eight organophosphorus insecticides have been separated and identified in water by silica gel HPTLC and detection by 2-methyl thioacridone spray reagent. Organophosphorus insecticides dichrotophos, ethion or phorate, fensulfothion, oxydemeton-methyl, phosmet, phospholan and trichlorfon have been separated from each other on silica gel foils and detected with silver nitrate-UV reagent.

Abate, from environmental water samples have been quantified on organophosphorus insecticides by densitometry on C-18 layer using N,2,6-trichlorobenzoquinoneimine spray reagent for detection. Parathion and paraoxon have been determined, utilizing cholinesterase inhibition for visualization and a combined TLC-colorimetric method, using 4-amino-N,N'-dimethylaniline-2N-HCl as the colour forming reagent has been described for detection and quantification of parathion in crops. Terbufos and its four oxidative metabolites have been separated on silica gel-60 plates developed with toluene-acetone (85:15) and detected at 50-500 mg levels with palladium(II) chloride-iodine reagent. A new spray reagent, cupric acetate in dilute hydrochloric acid followed by potassium iodide has
been used to detect 10 μg amount of quinalphos and disulfoton\textsuperscript{17} in biological material.

Shivhare, et al. has reported a spectrophotometric method for the determination of methyl parathion residues in plant material and soil. The reaction is based on reduction of nitro-group present in parathion methyl with Zn-HCl to form an amino group, which is subsequently diazotized and coupled with guaiacol in alkaline medium to form an yellow coloured azo-dye, which shows $\lambda_{\text{max}}$ at 470 nm. Other commonly found pesticides do not interfere\textsuperscript{18}. Similarly, Dayananda and Nandakumar have reported a colorimetric enzymic method for determination of methyl parathion and paraoxon. They used egg albumin, 20\% solution, as source of cholinesterase which was incubated with 2 to 28 μg of parathion methyl or 0.2 to 2 μg of paraoxon-methyl in 0.1 ml of acetone for 10 min at 20°C. A solution of 1 μmol of 1-naphthyl acetone in 0.1 ml of acetone and 0.2 ml of aqueous 0.4\% fast blue were added. The mixture was diluted with water and incubated for 25 min at 32°C. The reaction was stopped with acetic acid, and measure the absorption at 545 nm\textsuperscript{19}.

A simple spectrophotometric method has been described for determination of organophosphorus pesticide in plant material. The method is used on the reaction of organophosphorus insecticide with ammonium molybdate in 1:1 $\text{H}_2\text{SO}_4$.
and succinic hydrazide solution to form molybdenum blue complex which was extracted with butanol. The absorbance of the organic phase was measured at 780 nm Vs butanol$^{20}$.

Various workers have reported the gas chromatographic methods for the detection and determination of the residues of organophosphorus insecticides in vegetables plants, fruits, water samples, and soils and also in the formulations$^{21-30}$. Betowski and Jones have described a high performance liquid chromatography-Mass spectrometry method (HPLC-MS) for analysis of organophosphorus insecticides in soil$^{31}$, whereas a thermospray liquid chromatography-mass spectrometry method has been reported for the determination of organophosphorus pesticides and trialkyl and triaryl phosphates. Like the organophosphorus insecticides a number of chromogenic spray reagents has been reported for the detection of carbamate and organochloro insecticides by TLC. Over 100 cholinesterase inhibiting pesticides have been separated by HPTLC and detected by spraying with bovine liver suspension followed by an appropriate ester and chromogenic agent. The method was used to detect pesticides in biological and environmental samples and foods$^{32}$. A novel method for producing coloured zones on a white background, instead of the usual reverse situation, for cholinesterase-inhibiting organophosphorus and carbamate pesticides has been demonstrated using acetylthiocholine as the
substrate for horse serum cholinesterase, and 2,6-dichloro-
indophenol as the redox dye. Low nanogram amount of
pesticides were determined by a combination of this
detection technique and reflectance scanning\textsuperscript{34}.

Kawale et al. have reported the use of Tollens’s
reagent\textsuperscript{35} for TLC detection of carbamate and some organo-
phosphate insecticides in biological materials. The
sensitivity of detection with Tollens’s reagent is about 2\mu g
for these insecticides. It is also reported that carbamate
insecticides, carbaryl, carbofuran and propoxur on alkaline
hydrolysis yields respective phenolic compounds which
further coupled with diazotized aryl amines\textsuperscript{36}. Tiwari and
Singh\textsuperscript{37} have used alkaline fast blue B salt for detection of
carbamate insecticides carbaryl, carbofuran and propoxur.
Rathore, et al. have used p-nitrobenzenediazonium tetra-
fluoroborate for TLC separation, detection and quantitation
of carbaryl in water\textsuperscript{38}.

Cao\textsuperscript{39} reported the rapid detection of carbofuran
pesticide in poisoning cases by TLC by extracting it with
\(\text{CH}_2\text{Cl}_2\) from vomit and feed material and detection by use of
2 % 3,5-dichloro-p-benzoquinonchloramine after alkaline
hydrolysis.

Two specific spray reagents have been described for
detection of carbaryl and its breakdown product, 1-naphthol : 1 % cupric chloride followed by ammonium metavanadate, and
potassium hexacyanoferrate(III) in 0.5 % sodium hydroxide. The selectivity and sensitivity of three detection reagents, silver nitrate-2-phenoxyethanol, 4-(4-nitrobenzyl)-pyridine and 2,6-dibromobenzoquinone-N-chloroimine and its dichloro analog have been examined for visualization of organochloro, organophosphorus insecticides and thiocarbamate herbicides, and residues in vegetables determined by densitometry. Two chromogenic spray reagents viz., diazotized 4-nitroaniline and diazotized 4-aminoacetophenone have been reported for TLC detection of carbaryl in cereal extracts. The sensitivity of the reagents are 5 and 1 μg for carbaryl respectively.

Appaiah et al. have described the spectrophotometric determination of carbaryl in grains, based on hydrolysis of carbaryl with methanolic potassium hydroxide to 1-naphthol, reaction with 4-aminophenazone in the presence of alkaline oxidizing agent, and spectrophotometric measurement at the absorption maximum at 475 nm.

Three spectrophotometric methods have been described for the determination of carbaryl and propoxur in insecticidal formulations, water and grains, based on the formation of coloured species with p-aminophenol, p-N,N-dimethylphenylene diamine dihydrochloride and 1-amino-2-naphthol-4-sulphonic acid respectively under specified experimental conditions.
De Kok and coworkers reported an improved cleanup method for the multiresidue analysis of \(N\)-methylcarbamates in grains, fruits and vegetables by means of HPLC with post-column reaction and fluorescence detection. The grain samples (25 g) were extracted overnight with 100 ml of \(\text{CH}_2\text{-Cl}_2\)-acetone (1:1). The \(\text{CH}_2\text{Cl}_2\) extracts were cleaned up on an aminopropyl-bonded silica column, with \(\text{CH}_2\text{Cl}_2\)-methanol (99: 1) as eluent. The residues were dissolved in 1 ml HPLC mobile phase (aq. 28 % acetonitrile). The carbamates were determined by HPLC with post column reaction.

A fluorometric method for determination of carbaryl and 1-naphthol in hexadecyltrimethyl ammonium bromide in micellar media are described by Ayala et al.\(^{46}\).

For the detection of organochlorine insecticides, by thin-layer chromatography, Kawashira and Hosagai have reported the use of alcoholic o-tolidine or o-dianisidine and irradiation with UV light, giving bluish spots on TLC plates\(^{47}\). An ammonical silver nitrate\(^{48}\) and silver nitrate\(^{49}\) reagents were also reported for the detection of these insecticides by TLC.

Bullachminter and Tolg have used potassium bromide and alkaline rhodamin B reagent for identification of chlorinated pesticides\(^{50}\). Silica gel G impregnated with copper sulphate and ammonia is used for the preparation of TLC plate for
detection of organochlorine, insecticides\textsuperscript{51}. Coutselinis et al. have reported an ethanolic diphenylamine reagent for the detection of organochlorine insecticides by TLC\textsuperscript{52}. Sodium hydroxide followed by methanolic thymol has also been reported for the detection of chlorinated pesticides\textsuperscript{52}. The above described reagents, though sensitive (detection limit 0.2 to 20 \mu g) are susceptible to impurities and found interference with fat co-extracted from biological materials.

A zinc chloride diphenylamine reagent\textsuperscript{54–58} has commonly been used for the identification of organochlorine insecticides, which gives a blue-green spot with this reagent, on heating the TLC plate at 110° C for about 15 min. The formation of blue-green colouration is based on the reaction that when organic compounds containing halogens on heating with zinc chloride, oxidative decomposition occurs and liberates free halogen. The liberated free halogen like other strong oxidants, converts diphenylamine into the blue quinoidal compound\textsuperscript{59}.

A sensitive chromogenic reagent has been described for the detection of endosulfan and phosphamidon in biological material based on the reaction of cobalt(II) acetate and potassium iodide with endosulfan and phosphamidon, followed by starch solution yielding iodine-starch violet coloured complex. Recovery from toxicological material is also carried out and the formation of endosulfan diol is also confirmed\textsuperscript{60}. 
A specific spray reagent for the detection of endosulfan by thin-layer chromatography has been reported by Patil et al. The reagent is based on reaction that endosulfan, containing cyclic sulphite in its structure, is readily hydrolysed by alkali. The sulphite in turn reacts with nickel(II) ammine to give greyish black nickel(IV) oxyhydrate, \( \text{NiO(OH)}_2 \).\(^{61}\)

Patil et al. have also reported a thin-layer chromatographic (TLC) detection of endosulfan and phosphamidon by use of cobalt(II) acetate and o-tolidine reagent. The reaction is based analogous to the formation of black \( \text{Ni(IV)} \), the formation of brown \( \text{Co(III)} \) via \( (\text{COOH})_2\text{SO}_3 \).\(^ {62}\)

Organochlorine pesticides have been extracted from water by solid phase extraction on a C-18 cartridge with ethyl acetate, hexane-benzene (1+1), and hexane-diethyl ether (1+1) as eluents. Pesticides were determined by silica gel TLC with silver nitrate-UV detection and densitometric scanning.\(^ {63}\) Organochlorine insecticides have also been detected with 3,3',5,5'-tetramethylbenzidine, the detection limit was 0.2\( \mu \)g.\(^ {64}\)

A six component organochloro insecticide mixture containing BHC, methoxychlor, heptachlor epoxide, dieldrin, and aldrin has been successfully resolved on a C-18 chemically bonded RP layer by development with acetonitrile-water (75+25). Minimum sensitivity for visual detection and densitometric scanning ranged from 300-900 ng upon detection with o-tolidine reagent.\(^ {65}\)
The chlorinated insecticides chlorpyrifos and its metabolite TPC have been quantified in tap water (5 ppb) and bananas (50 ppb) by pre-adsorbent silica gel TLC, detection with silver nitrate-UV reagent, and scanning of zones.

Deshpande and Bhende described an analytical method for chlorinated and phosphate ester pesticides, I, titrimetric and colorimetric methods. In order to determine aldrin, endrin, DDT and BHC, they used to convert the chloro groups into NaCl by treatment with metallic sodium in propan-2-01, and the NaCl was determined by titrating with 0.01 N-AgNO₃, with as indicator \((\text{NH}_4)\text{}_2\text{SO}_4, \text{Fe}_2(\text{SO}_4)_3, 24\text{H}_2\text{O}\).

Phorate and malathion were hydrolysed with ethanolic NaOH and the resulting diphosphates were determined colorimetrically by measuring the absorbance of their Cu(II) complexes at 420 nm. Carbaryl was hydrolysed to give 1-naphthol which was determined with 4-aminoantipyrine-\(K_3(\text{Fe(CN})_6\) at pH 10. Phosphamidon on oxidation with iodine, \(\text{HNO}_3\) or \(\text{H}_2\text{O}_2\) produced phosphates and could be determined by titrating unreacted iodine with \(\text{Na}_2\text{S}_2\text{O}_3\). Parathion has been determined by reducing it to a primary amine and determining the amine colorimetrically after diazotisation and coupling with \(N-1\)-naphthylethylene diamine.
A gas chromatographic methods for detection and determination of organochlorin pesticides in meat and meat products\textsuperscript{68} in bovine faces\textsuperscript{69}, in mussels\textsuperscript{70}, in milks\textsuperscript{71}, in urine\textsuperscript{72}, in eggs\textsuperscript{73} and in formulations\textsuperscript{74-75} were reported in the literature with their elute and cleanup procedures.

It is well known that organophosphorus, organochlorine and carbamate insecticides are widely being used effectively in agriculture to control the pest, from a long back. Recently a new group of insecticides i.e. Pyrethroid insecticides is introduced in the market. These are effective pest control chemicals and have low mammalian toxicity\textsuperscript{76,77}.

A large number of gas-liquid chromatographic methods\textsuperscript{78-81} for residue analysis of synthetic pyrethroids have been reported, as has autoradiographic thin-layer chromatography (TLC) using \textsuperscript{14-C}-labelled compounds, particularly in metabolic studies, where the unlabelled compounds were detected by visualization on silica gel 60 \textsubscript{F254} chromatographic plates under ultraviolet (UV) light\textsuperscript{82-84}.

Shono et al. have reported the use of phosphomolybdic acid (20 % m/v in ethanol) as a chromogenic reagent for the detection of permethrin, cypermethrin and deltamethrin\textsuperscript{85}. Whereas palladium chloride (0.5 % m/v in 12 mol dm\textsuperscript{-3} HCl) has been described for the detection of deltamethrin\textsuperscript{86}, and silver nitrate impregnated alumina G and irradiation with UV light\textsuperscript{87} for detection of pyrethroid insecticides in general.
Patil et al. have reported a spray reagent for the detection of pyrethroid insecticides containing a nitrile group by thin-layer chromatography. The reaction is based on the alkaline hydrolysis of pyrethroid insecticides containing a nitrile group, yields cyanide ion, which in turn reacts with copper(II) acetate and o-tolidine in an acetic acid medium to give blue colour.\(^88\)

Similarly, Akmal Pasha and Yadathora have described a thin-layer chromatographic method for detection of pyrethroid insecticides. These insecticides, on bromination and treatment with o-tolidine, yield an intensely blue product.\(^89\) 2,4-Dinitrophenylhydrazine and phosphomolybdate has also been used as a chromogenic reagent for thin-layer chromatographic detection of synthetic pyrethroid insecticides in biological materials.\(^90\)

**Literature survey for Benzodiazepines**

Benzodiazepines which are mostly employed as psychotherapeutic, tranquillising, sedatives and hypnotic drugs are widely used in the treatment of different nervous diseases, such as epileptic convulsions, insomnia and anxiety. Owing to their easy availability they are frequently encountered in forensic case work samples involving drug overdoses.
Various methods have been reported for the detection and determination of 1,4-benzodiazepine derivatives. These include gas chromatography$^{91-93}$, polarography$^{94,95}$, high performance liquid chromatography (HPLC)$^{96-98}$ and radio-immunoassay$^{99}$. A fluorescent silica gel G F$_{254}$ has been used for TLC identification of benzodiazepines$^{100}$.

However, a few reagents are available to detect the benzodiazepines by thin-layer chromatography. These are Dragen-Dorff’s reagent$^{101}$, hydrolysis by HCl heating for 30 min and then diazotization and coupling with 1-naphthol$^{102}$, use of 2,5-dimethoxy-tetrahydrofuran heating followed by p-dimethylaminobenzaldehyde has been reported for the detection of nitrazepam, clonazepam and diazepam$^{103}$.

Tiwari et al.$^{104}$ and Schuetz$^{105}$ have described a thin-layer chromatographic method for the detection of some, 1,4-benzodiazepine drugs by exposing the developed plate to NO$_2$ fumes, activating it and then spraying with Bratton-Marshall reagent (0.1 % N (1-naphthyl) ethylene diamine in ethanol).

From the above literature survey it is observed that the various analytical methods i.e. gas chromatography, high performance liquid chromatography, spectrophotometry, polarography, fluorimetry, mass spectrometry etc. were reported for the detection and determination of residues of different pesticides and drugs involved in forensic case
work. But most of these methods were applied for the residue analysis of pesticides in grains, water samples, fruits, vegetables, soil and in insecticidal formulations, where interfering substances are very less. Thus the application of above methods for the detection and determination of residues of these drugs and insecticides in biological materials such as viscera, blood, urine, stomach wash etc. are not very suitable because of the presence of interfering materials like fats, proteins, peptides, amino acids, etc. in extracted material.

However, some workers have reported the use of some of the above methods for the detection and determination of insecticides and drugs in biological materials (blood, urine, stomach-wash etc.) with tedious procedure of extraction, concentration, elution and clean-up procedure where the loss of compound is not to be ruled out. However, these methods are very time consuming, and hence not useful for routine analysis in forensic case work. To overcome these difficulties thin-layer chromatography is the method of choice. The major use of TLC in pesticide and drug analysis is in qualitative screening of residues and as an aid in the identification of gas-chromatographic and high-performance liquid chromatographic peaks. The proper application of TLC methods gives excellent quantitative results at ppm and ppb concentration levels. Numerous quantitative analysis have already been published and many more are expected as
the excellent precision and accuracy of high performance TLC (HPTLC) with densitometric scanning, become more widely recognized by pesticide and drug analysts.

It is also observed from the above literature survey that a very few chromogenic reagents are reported for specific and selective detection of pesticides and drugs of forensic interest by TLC. Among the reported reagents, most of these are described for the detection and determination of these compounds in non-biological materials like grains, vegetables, fruits, water samples, soils etc. shows the interference, with co-extracted biological materials like fats, proteins, peptides and amino acids etc.

Thus, there is a need to develop different chromogenic reagents for specific and selective detection of drugs, insecticides and other organic compounds of forensic interest by thin-layer chromatography, having less/no interference with co-extractives.

Hence, an attempts were made to develop a new chromogenic reagents for selective detection of some of the insecticides and drugs of forensic interest, by use of thin-layer chromatography. The attempts were also made to detect the metabolite products of these compounds found in biological material wherever possible.
This work is distributed in four parts.

Part first is further divided in three sections.

Section A describes the thin-layer chromatographic detection of organophosphorus insecticides containing a nitrophenyl group and their metabolite products in biological materials.

Section B includes the development of a new spray reagent for selective detection of dichlorvos and its metabolite product in biological and non-biological materials by thin-layer chromatography.

Section C describes detection and determination of monocrotofos in biological material by thin-layer chromatography.

Part second is also divided into three sections:

Section A describes a new thin-layer chromatographic spray reagent for the screening of biological materials for the presence of carbaryl.

Section B includes the use of phenylhydrazine hydrochloride for thin-layer chromatographic detection of carbaryl.

Section C describes the spectrophotometric determination of carbaryl in grains and water samples by use of phenylhydrazine hydrochloride reagent.
Part third describes the use of cobalt(II) acetate and o-tolidine reagent for thin-layer chromatographic detection of some pyrethroid insecticides in biological and non-biological materials.

Part fourth deals with detection of certain benzodiazepines in biological samples by thin-layer chromatography with two chromogenic reagents.