

REVIEW ARTICLE

# Forensic Application of Non-destructive ATR-FTIR Spectroscopic Technique for Organophosphorus Pesticides Analysis

Shivpoojan Kori

## ABSTRACT

Over long period organophosphorus pesticides (OPPs) such as Tusk, Dimex, Dysac, Sacban and Monovip contain Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos as active chemicals, respectively are used in agriculture to enhance the crops yield. However, these chemicals are extensively used in criminal cases such as suicidal, accidental, and homicidal throughout the world. Such cases can conveniently be resolved with the help of spectral library as reference/standard to compare with unknown/suspected samples recovered from scene of crime. The spectral library was generated using ATR-FTIR technique which reliable and non destructive in nature. This spectral library has been applied to identify the OPPs in different biological samples and investigate, characterize the OPPs. ATR-FTIR spectroscopic data are, therefore, a prerequisite for their identification. Finally, we believe that the use of ATR-FTIR should make it possible to identify all tested OPPs in one single analysis, even in the low ppm concentration range.

**KEYWORDS** | ATR-FTIR, organophosphorus, biological samples  
non-destructive, spectral library

## INTRODUCTION

PESTICIDES PROTECT THE CROPS from pests but their usage is harmful to humans and other animals due to potential toxicity. Only 1% of the total amount of pesticides (approx. 4 millions tones) annually applied reaches to target pests.<sup>1</sup> On the basis of targets, pesticides are divided into several types such as herbicides, insecticides, fungicides, rodenticides and others. Over long period, organophosphorus pesticides (OPPs) or organophosphates are being used for protecting the crops and also as warfare agents. Organophosphorus pesticides have a pentavalent phosphorous atoms attached to a sulfur or oxygen atoms by double covalent bond predicted by a number of chemicals e.g. Malathion, Dimethoate, Dichlorovos, Chlorpyrifos, Monochrotophos etc.

(Fig. 1). OPPs are named on the basis of type of group attached to phosphorous such as phosphate, thiono, thiol and thithiol type. R1 and R2 predict different substituent groups (alkoxy, alkyl, amino, thioalkyl etc.) present at the skeletal base of OPPs.<sup>2,3</sup> R1 and R2 are replaced by propyl and ethyl groups to manage resistant pests respectively. X represents the alkyl, alkoxy, aryl, heterocyclic, aryl oxy, arylthio and alkylthio groups. OPPs are structurally similar within a class and have a phosphorous atoms with characteristics phosphoryl bond (P=O) OR thiophosphoryl bond (P=S).<sup>1,4</sup> OPPs and their metabolites are used as a pesticide across the world population. The WHO (World Health Organization) was reported that every year 3 million peoples were affected

### Author's Affiliation:

Assistant professor, Department of Chemistry, Biochemistry & Forensic Science, Amity School of Applied Sciences, Amity University Haryana, Gurugram 122413, India.

### Corresponding Author:

Shivpoojan Kori,  
Assistant Professor, Department of Chemistry, Biochemistry & Forensic Science, Amity School of Applied Sciences, Amity University Haryana, Gurugram 122413, India.

Email: [shivpoojan kori@gmail.com](mailto:shivpoojan kori@gmail.com)



### How to cite this article

Shivpoojan Kori. Forensic Application of Non-destructive ATR-FTIR Spectroscopic Technique for Organophosphorus Pesticides Analysis. Indian J Forensic Med and Pathol. 2021;14(2):395-402

by the acute poisoning of OPPs. OPPs are also caused neurotoxicity due to inhibition of acetyl cholinesterase (AChE) enzymes and butyryl cholinesterase (BuChE) enzymes, present in the synaptic membrane of CNS (central nervous system) and PNS (peripheral nervous system) of vertebrates. AChE and BuChE enzymes hydrolyze the neurotransmitter acetylcholine into acetyl and choline which regulates the signal transmission by sodium channel in the CNS and PNS system. OPPs block the active sites of AChE and BuChE and finally deactivate the acetylcholine (ACh), leads to inhibition cholinergic neurotransmission due to accumulation of the acetylcholine. These neurotoxic effects results in neuromuscular paralysis.<sup>5,6</sup> OPPs are also potent to causes immunotoxicity, genotoxicity and carcinogenicity, finally leading to death. Conclusively, OPPs are the most dangerous and determined organic pollutants chemicals due to their potential toxicity. Recently, most of the criminal cases are taking place due to pesticide toxicity. In forensic science set-ups, these types of cases are analyzed from blood, foodstuff, spits and the chemical bottles

pesticides poisoning have been reported in rural area of Asia.<sup>10</sup> WHO (1990) had published the data and found 3 millions/year detectable cases related to pesticides poisoning resulting in 220,000 deaths<sup>11,12</sup> OPPs categories of pesticides had been screened mostly in forensic science laboratory using thin layer chromatography (TLC). Criminal cases related to pesticides are occurring daily and are being forwarded to forensic science laboratory for pesticides analysis using thin layer chromatography and are further confirmed by using FT-IR and GC-MS techniques. GC-MS is destructive, costly and time consuming technique so cannot be used routinely in laboratory. However, FT-IR is non-destructive and reliable technique. Therefore, it has been planned to analyze the ATR-FTIR spectrum of commonly available OPPs, so as to obtain the standard or reference data and this spectral library has been applied in real spiked biological samples. The studies have been carried out using ATR-FTIR spectroscopic technique. It is a powerful technique for analysis of trace and bulk constituents of matrices sample. Infrared absorption technique has efficiently been

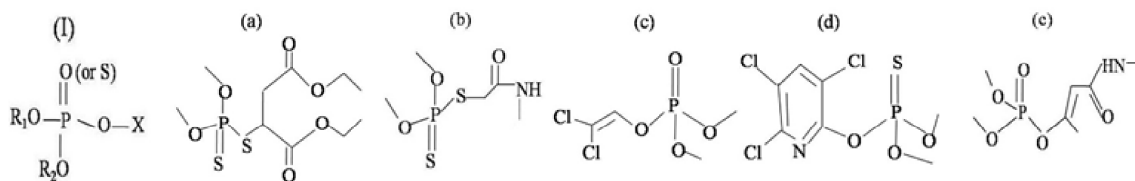


Figure 1: (i) General chemical structure of OPPs and (a) Malathion (b) Dimethoate (c) Dichlorovos (d) Chlorpyrifos (e) Monochrotophos.

Source: Author self.

seized from crime scene to know the cause. Few OPPs such as Malathion, Dimethoate, dichlorovos, Chlorpyrifos and Monochrotophos are highly toxic in nature and have been used in criminal cases.<sup>7</sup> In Brazilian institute, research groups have been analyzed the acetyl cholinesterase enzymes inhibitors such as aldicarb, OPPs chemicals and confirming using FT-IR technique for forensic purpose.<sup>8</sup> In pesticide poisoning cases, OPPs are the most detectable in forensic intoxication viz. 63% and out of it, Quinalphos was the most common OPPs responsible for 28.8% of the total positive cases.<sup>9</sup> Suicidal cases are most common and approximate 60% cases of death due to

used to detect the microscopic pesticide residue.<sup>13</sup> The technique is non-destructive which makes it the best suitable for forensic utility because, the re-analysis of sample can be carried for future purpose.

#### METHOD

Five OPPs—Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos—were analyzed and characterized using ATR-FTIR spectroscopic technique.

#### Sample Preparation

Human biological control samples (free from milk, saliva and vomit materials) were obtained

from healthy volunteer and kept frozen at -20°C. 500µL volume of each biological samples were spiked three times (n=3) with each OPPs (Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos) up to 0.5ppm concentration in a scaled centrifugal vial. Samples were vortexed for FTIR analysis. Each sample was divided into four groups. First three groups were spiked with pesticides at the same concentration (0.5 ppm). However, pesticides were not spiked in the last group and served as a control.

Standard stock solution (2000ppm) was prepared for each OPPs separately. Tusk (50% EC), Dimex (30% EC), Dysac (76% EC), Sacban (20% EC) and Monovip (36% EC) contain Malathion, Dimethoate, Dichlorovos, Chlorpyrifos and Monochrotophos as active chemicals, respectively. OPPs pesticides were purchased from Shivalik agrochemicals at Chandigarh (India).

#### ATR-FTIR Spectroscopy

For ATR-FTIR spectra of each sample, four scans were recorded with a resolution of 4 cm<sup>-1</sup>, in the range from 4,000 to 700cm<sup>-1</sup> using ATR-FTIR, (the Agilent Cary 630 FTIR Spectrometer). The spectral data was analyzed using Agilent Microlab FTIR software. 2.0µL samples were placed directly on the internal reflecting diamond crystal of FTIR with the help of micropipette.

### RESULTS AND DISCUSSIONS

A number of methods had been used to analyse the organophosphorous pesticides (OPPs) residue in suspected samples of forensic interest. Use of infrared spectroscopy in forensic science is best technique because of sample is analysed without destruction & treatment. In spite of these efforts, an attempt in the present study has been equipped to obtain chemical fingerprinting of some commonly encountered OPPs using a non-destructive ATR-FTIR method. The main motive is to generate ATR-FTIR spectra database of these OPPs due to makes it possible to positively identify the major OPPs found at crime scene. Finally, we believe that the use of ATR-FTIR should make it possible to identify all tested OPPs in one single analysis, even in the low ppm concentration range.

This study was to propose the spectral library of OPPs as a reference for any forensic chemist and toxicologist in field of forensic science.

The generated ATR-FTIR spectral library at wavenumber (cm<sup>-1</sup>) was depicted in Table 1 and ATR-FTIR spectrum of spiked OPPs in biological samples was depicted in Table 3. The vibrational assignment of the signature peaks of each OPPs has been discussed in table 2. IUPAC name, Chemical structure and molecular formula of These reference OPPs compounds have been described which are used in this study such as (i) Tusk: The active component of Tusk is Malathion (C<sub>10</sub>H<sub>19</sub>O<sub>6</sub>PS<sub>2</sub>) with IUPAC name Diethyl 2-[(dimethoxyphosphorothioyl) sulfanyl] butanedioate. Chemical structure was depicted in Fig. 1(a). ATR-FTIR spectrum was shown in Fig. 2(a). The vibrational assignment of the principle peaks of ATR-FTIR spectrum has been depicted in Table 2.<sup>14</sup> (ii) Dimex: It contains Dimethoate (C<sub>2</sub>H<sub>12</sub>NO<sub>3</sub>PS<sub>2</sub>) which chemically is designated as O, O-dimethyl S-[2-(methyl amino)-2-oxoethyl] dithiophosphate. Structurally, Dimethoate was depicted in Fig. 1(b) and ATR-FTIR spectrum was shown in Fig. 2 (b). (iii) Dysac: Active component in Dysac is Dichlorovos (C<sub>4</sub>H<sub>7</sub>Cl<sub>2</sub>O<sub>4</sub>P) with specific chemical name as 2, 2-dichlorovinyl dimethyl phosphate. The chemical structure of Dysac was shown in Fig. 1(c). Fig. 2 (c) depicts the FT-IR spectrum.<sup>14-16</sup> (iv) Sacban: The commercial product contains Chlorpyrifos (C<sub>9</sub>H<sub>11</sub>Cl<sub>3</sub>NO<sub>3</sub>PS). It is chemically labeled as O,O-Dethyl O-3,5,6-trichloropyridin-2-ylphosphorothioate [Fig. 1(d)]. The FT-IR spectrum was shown in Fig. 2 (d). Table 2 shows the vibrational assignment of chlorpyrifos.<sup>13,15,17,18</sup> (v) Monovip: It contains Monochrotophos (C<sub>7</sub>H<sub>14</sub>NO<sub>5</sub>P) which chemically is represented as Dimethyl (E)-1-methyl-2-(methylcarbonyl) vinyl phosphate (Fig. 1 (e)). FT-IR spectrum of product was shown in Fig. 2 (e). Table 2 shows the vibrational assignment of monochrotophos.<sup>12,14,15,19,20</sup> This OPPs spectral library has been employed to match the peaks of OPPs residue in spiked biological samples.

In this work, ATR-FTIR spectroscopy has been used to generate a spectral library of some organophosphorous pesticides of forensic interest. These types of pesticides can be seized as bulk market pesticides as well as traces found at the crime scene. There can be legal queries to identify OPPs to forensic expert has to answer. In these

cases, the standard reference database is required for comparison. The modern FTIR systems with diamond ATR proves to be a rapid, sensitive and non-destructive analysis of samples with very little effort. This spectral library can be used as a reference library when an unknown sample is suspected of being organophosphorous. Some of the closely related structures may have spectra very similar to that of particular pesticides, especially for organophosphorous. Thus, it is imperative that appropriate techniques should be used by the forensic chemist and toxicologist to ensure the optimal spectral data are obtained to get a feel for the discriminating features of a spectrum. Screening of pesticides using ATR-FTIR technique is particularly convenient because of both its speed and ability to use without sample preparation.

**CONCLUSIONS**

FT-IR is a very sensitive, rapid, easy, reliable, and non-destructive technique. Application of non-destructive analytical technique to analyze the forensic evidence as samples of forensic interest without destroying the sample. ATR-FTIR is a beneficial to forensic scientist/expert in field of forensic science. This generated spectral library of OPPs by ATR-FTIR can be used as reference/standard for comparison of pesticides products, seized from crime scene.

In this work, ATR-FTIR spectral library was generated for some OPPs of forensic interests which had been frequently used in criminal cases and mostly recovered from crime scene. This

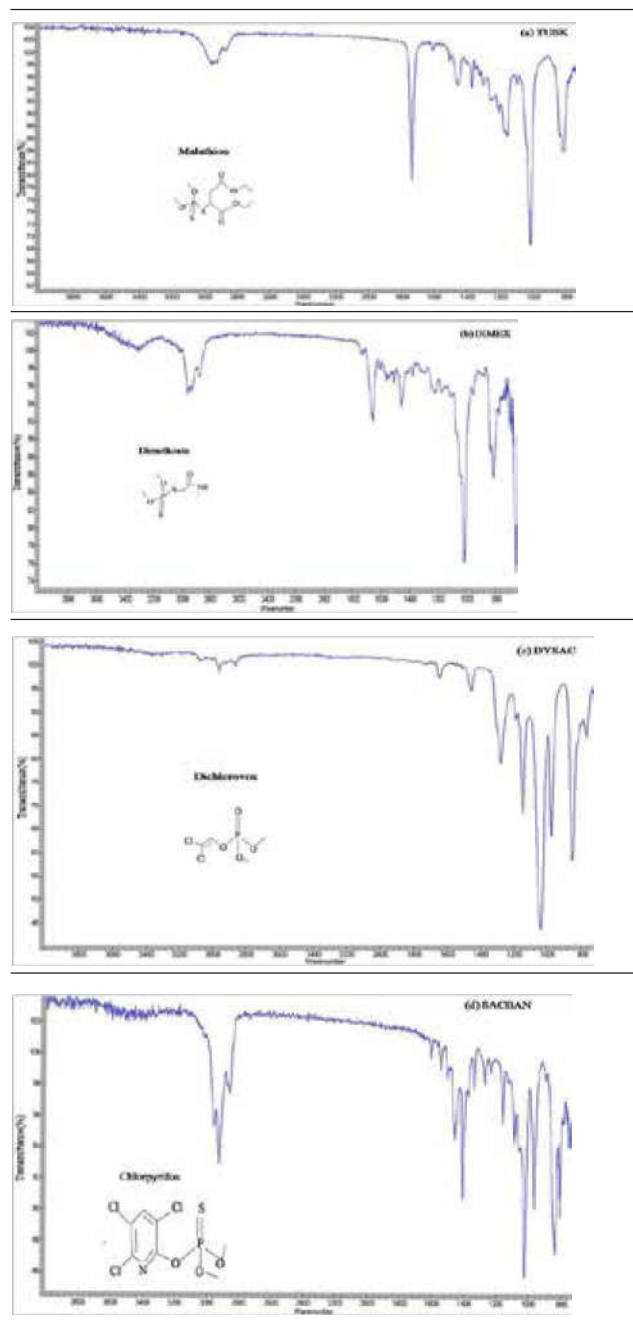
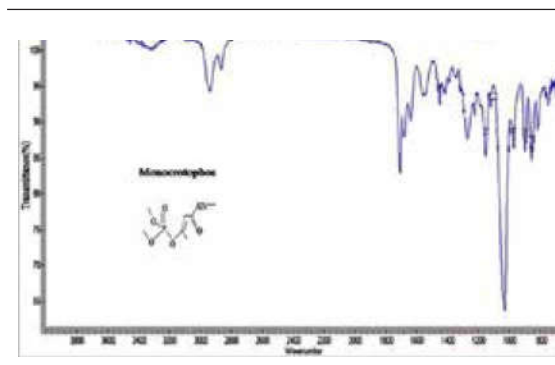


Fig. 2(a). FTIR spectrum of Tusk OPPs product.  
 Fig. 2(b). FTIR spectrum of Dimex OPPs product.  
 Fig. 2(c). FTIR spectrum of Dysac OPPs product.  
 Fig. 2(d). FTIR spectrum of Sacban OPPs product.  
 Fig. 2(e). FTIR spectrum of Monovip OPPs product.  
 Fig. 2(f). FTIR spectrum of Monovip OPPs product.

Source: Author Self

PURE OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1372(w), 1255(w), 1157(m), 1014(s), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1661(s), 1550(w), 1457(m), 1296(w), 1221(w), 1176(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (w), 1456(w), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1607(w), 1544(w), 1506(w), 1457(w), 1410(s), 1338(w), 1214(w), 1162(w), 1024(s), 984(m), 835(s), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1683(w), 1637(w), 1550(w), 1449(w), 1270(m), 1154(m), 1033(s), 970(w), 898(w), 855(w), 811(w)

Table 1: ATR-FTIR spectral peaks of OPPs of standard at transmittance (%) vs. wavenumber (cm-1) in OPPs products.

PURE OPPS	WAVENUMBERS (CM-1)	ASSIGNMENT
Tusk 20% EC (Malathion)	2869cm-1	aliphatic C-H stretching vibration
	1735cm-1	(C=O) carbonyl stretching vibration of ester group
	1456cm-1	C-H stretching vibrations of CH2
	1370cm-1	C-H Vibration of CH3 group
	1255cm-1	C-H rocking vibration of CH2
	1157cm-1	C-O vibration
	1014cm-1, 817cm-1	P-O stretching of P-O-CH3 group P-O vibration of additional phosphates (P043-) group
Dimex 30% EC (Dimethoate)	2948cm-1	aliphatic C-H asymmetric stretching vibration of CH3
	2874cm-1	aliphatic C-H symmetric stretching vibration of CH3
	1661cm-1	(C=O) carbonyl stretching vibration
	1550cm-1	P=S bending vibration
	1457cm-1	C-Hstretching vibrations of CH2
	1310cm-1	C-H Vibration of CH3 group
	810cm-1	P-O stretching of P-O-CH3 group, P-O vibration of additional phosphates (P043-) group bands 1296cm-1, 1221cm-1, 1176cm-1 C-N stretching vibration
Dysac 76% EC (Dichlorovos)	1644cm-1	C=C stretching in alkenes
	1456cm-1	C=H stretching vibrations of CH2
	1039cm-1	P-O stretching of P-O-CH3 group
	977cm-1	C-H bending vibration of CH2
	857cm-1	P-O vibration of additional phosphates (P043-) group
	765 cm-1	C-Cl scissoring vibration
	1279cm-1, 1147cm-1	C-O bending vibration
Sacban 20% EC (Chlorpyrifos)	1607 to 984cm-1	C=N stretching, pyridine stretching, ring vibration, ring breathing, Cl-C stretching, trigonal ring breathing and P=S stretching
	2924cm-1	C-H asymmetric stretching vibration of CH3
	2857cm-1	C-H symmetric stretching vibration of CH3
	1607cm-1	C=C Vibration
	1544cm-1	P=S stretching
	1410cm-1	CH2 bending
	1338cm-1	C-O stretching
	1024cm-1	CH2 in plane vibration
	984cm-1	CH3 wagging vibration, P-O stretching of P-O-CH3 and P-O vibration of additional phosphates (P043-) group bands
	1506cm-1, 1457cm-1	vibration mode of CH2and CH3 group
	1214cm-1, 1162cm-1	C-N stretching vibration
835cm-1, 805cm-1	C-Cl rocking vibration	
Monovip 36% EC (Monochrotophos)	2937cm-1	C-H asymmetric stretching vibration of CH2
	2862cm-1	C-H symmetric stretching vibration of CH2
	1706cm-1	(C=O) carbonyl stretching vibration
	1683cm-1	peaks of additional (C=O) carbonyl stretching vibration
	1637cm-1	C=C stretching in alkenes
	1550cm-1	N-H bending vibration
	1449cm-1	bending vibration of CH2
	1033cm-1	P-O stretching of P-O-CH3
	970cm-1	N-CO bending at out of plane
	898cm-1	out of plane bending CH in C-CN
	1270cm-1, 1154cm-1, 855cm-1, 811cm-1	C-N bending mode of C-CH bending

Table 2: Vibrational assignment of ATR-FTIR spectral peaks corresponding wavenumber (cm-1) of OPPs.

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1372(w), 1157(m), 1014(s),
Dimex 30% EC (Dimethoate)	2948(m), 1661(s), 1550(w), 1457(m), 1176(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (w), 1279(m), 1039, 977(m), 857(m),
Sacban 20% EC (Chlorpyrifos)	2924(s), 1607(w), 1506(w), 1410(s), 1338(w), 1214(w), 984(m), 835(s),
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1883(w), 1637(w), 1449(w), 1154(m), 1033(s), 970(w), 855(w),

(A) ATR-FTIR spectral peaks of OPPs of spiked blood sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1372(w), 1255(w), 1014(s), 817(m)
Dimex 30% EC (Dimethoate)	2874(m), 1661(s), 1550(w), 1457(m), 1221(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (w), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 1544(w), 1506(w), 1457(w), 1338(w), 1214(w), 1162(w), 1024(s), 835(s), 805(s)
Monovip 36% EC (Monochrotophos)	2937(s), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 811(w)

(B) ATR-FTIR spectral peaks of OPPs of spiked urine sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(s), 1735(s), 1372(w), 1157(m), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1550(w), 1457(m), 1296(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (s), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1457(w), 1410(s), 1214(w), 1162(w), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 855(w), 811(w)

(C) ATR-FTIR spectral peaks of OPPs of spiked milk sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(s), 1735(s), 1372(w), 1157(m), 817(m)
Dimex 30% EC (Dimethoate)	2948(m), 2874(m), 1550(w), 1457(m), 1296(w), 1176(w), 1016(s),
Dysac 76% EC (Dichlorovos)	1644 (s), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1457(w), 1410(s), 1214(w), 1162(w), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 2862(w), 1706(s), 1637(w), 1550(w), 1270(m), 1033(s), 970(w), 855(w), 811(w)

(D) ATR-FTIR spectral peaks of OPPs of spiked saliva sample

SPIKED OPPS	WAVENUMBERS (CM-1)
Tusk 20% EC (Malathion)	2869(w), 1735(s), 1456(m), 1255(w), 1157(m), 1014(s),
Dimex 30% EC (Dimethoate)	2948(m), 1550(w), 1457(m), 1296(w), 1221(w), 1016(s), 810(m)
Dysac 76% EC (Dichlorovos)	1644 (m), 1279(m), 1147(m), 1039, 977(m), 857(m), 765(w)
Sacban 20% EC (Chlorpyrifos)	2924(s), 2857(w), 1544(w), 1506(w), 1457(w), 1410(s), 1338(w), 1162(w), 1024(s), 984(m), 805(s)
Monovip 36% EC (Monochrotophos)	2937(w), 1706(s), 1883(s), 1637(w), 1270(m), 1154(m), 1033(s), 855(w), 811(w)

(E) ATR-FTIR spectral peaks of OPPs of spiked vomit sample at transmittance (%) vs. wavenumber (cm-1) in OPs products.

(s): strong peak; (w): weak peak; (m): medium peak

generated ATR-FTIR spectral library has been employed in OPPs spiked biological samples. This ATR-FTIR spectral library could be of great help to forensic scientist to characterize and screen the suspect OPPs for data assessment. It is a practically potential to screen out residual OPPs using ATR-FTIR directly. The present work can be scaled up to grant potential positive contribution to discriminate mysterious OPP. **IJFMP**

## Recommendation

Present work leaves an off-shoot where, we recommend further, to generate ATR-FTIR spectral library of others standard pesticides and drugs as well as OPPs residue in real samples of forensic interest.

## Abbreviations

OPPs: Organophosphorous pesticides, ATR-FTIR: Attenuated total reflection-fourier transforms infra red spectroscopy.

## REFERENCES

- Eleršek T, & Filipi M** (2006) Organophosphorus Pesticides - Mechanisms Of Their Toxicity. National Institute of Biology - Slovenia, 12, 243–260.
- Jose SP, & Mohan S** (2006) FT-IR and FT-RAMAN investigations of nicotinaldehyde. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, 64(1), 205–209.
- Hasegawa T** (2017) Fundamentals of FT-IR. In *Quantitative Infrared Spectroscopy for Understanding of a Condensed Matter* (pp. 37–46).
- Ray AK, & Ghosh MC** (2006) Toxicology of Organophosphate & Carbamate Compounds. *Toxicology of Organophosphate & Carbamate Compounds*.
- Mc Clelland JF, Jones RW, Bajic SJ, et al.** (2002) FT-IR Photoacoustic Spectroscopy. *Handbook of Vibrational Spectroscopy*, 1–45.
- Parolo ME, Savini MC, & Loewy RM** (2017) Characterization of soil organic matter by FT-IR spectroscopy and its relationship with chlorpyrifos sorption. *Journal of Environmental Management*, 196, 316–322.
- Bonilla E, Hernández F, Cortés L, et al.** (2008) Effects of the insecticides malathion and diazinon on the early oogenesis in mice in vitro. *Environmental Toxicology*, 23, 240–245.
- Bruno Sabino HR and AO** (2011) A Forensic View of Pesticide Poisonings in Brazil, Pesticides in the Modern World - Effects of Pesticides Exposure., Dr. Margarita Stoytcheva (Ed.), 251–278.
- Teixeira H, Proença P, Alvarenga M, et al.** (2004) Pesticide intoxications in the Centre of Portugal: Three years analysis. *In Forensic Science International* (Vol. 143, pp. 199–204).
- Ait El Cadi M, Mezzane A, et al.** (2008) Intoxications mortelles aux pesticides au Maroc (2000–2005). *Ann Toxicol Anal*, 20(2), 73–77.
- Konradsen F, Van Der Hoek W, et al.** (2003). Reducing acute poisoning in developing countries - Options for restricting the availability of pesticides. *Toxicology*.
- Ishii K, Suzuki-Yoshihashi S, Chihara K, & Awazu K** (2004) FT-IR analysis of phosphorylated protein. *Biophotonics New Frontier: From Genome to Proteome*, 5461, 17–21/90.
- Neti N, & Zakkula V** (2013) Analysis of chlorpyrifos degradation by *Kocuria sp.* using GC and FTIR, 6(4), 466–472.
- Franckowski RE, & Thompson RA** (2006) Eszopiclone (Lunesta™): An Analytical Profile. *Microgram Journal*, 4, 29–36.
- Armenta S, Quintás G, Garrigues S, & De La Guardia M** (2005) A validated and fast procedure for FTIR determination of Cypermethrin and Chlorpyrifos. *Talanta*, 67, 634–639.
- Bavcon Kralj M, Černigoj U, Franko M, & Trebše P** (2007) Comparison of photocatalysis and photolysis of malathion, isomalathion, malaaxon, and commercial malathion-Products and toxicity studies. *Water Research*, 41, 4504–4514.
- Sylaja B, & Srinivasan S** (2012) Experimental and theoretical investigation of spectroscopic properties of diazepam. *International Journal of ChemTech Research*, 4(1), 361–376.
- Kizil R, Irudayaraj J, & Seetharaman K** (2002) Characterization of irradiated starches by using FT-Raman and FTIR spectroscopy. *Journal of Agricultural and Food Chemistry*, 50, 3912–3918.
- Valsamaki VI, Sakkas VA, & Albanis TA** (2002) Simple determination of 22 organophosphorous pesticides in human blood using headspace solid-phase microextraction and gas chromatography with mass spectrometric detection. *Journal of Chromatographic Science*, 30, 1936–1946
- Singhal RK** (2012) Remediation of Malathion Contaminated Soil Using Zero Valent Iron Nano-Particles. *American Journal of Analytical Chemistry*, 3(January), 76–82.

## REFERENCES

1. **Ab Manan, N. A., Zamari, Z. M., Pillay, I. A., Adnan, A. H. M., Yusof, J., & Raslee, N. N.** (2017). Mother tongue interference in the writing of English as a Second Language (ESL) Malay learners. *International Journal of Academic Research in Business and Social Sciences*, 7(11), 1294-1301.
2. **Ball, G.R., Srihari, S.N.**: Comparison of statistical models for writer verification. In: *Proceedings Document Retrieval and Recognition XVI, San Jose, CA, SPIE* (2009) 7247OE 1{8}
3. **Cheng N, Lee G K, Yap B S, Lee L T, Tan S K and Tan K P.** (2005). Investigation of Class Characteristics in English Handwriting of the Three Main Racial Groups: Chinese, Malay and Indian in Singapore. *J Forensic Sci*, Vol. 50, No. 1, Paper ID JFS2004005, Available online at: [www.astm.org](http://www.astm.org)
4. **Deepani V & Kapoor A K.** (2018). Variability In Human Handwritings: An Indian Understanding. *International Journal of Humanities and Social Sciences (IJHSS)* ISSN(P): 2319-393X; ISSN(E): 2319-3948 Vol. 7, Issue 4, Jun - Jul 2018; 27-32 © IASET
5. **Denizer, E. N.** (2017). Does Mother Tongue Interfere in Second Language Learning? *Journal of Foreign Language Education and Technology*, 39-54
6. **Dweik, B. and Abu Al Hommos, M.** (2007). The effect of Arabic proficiency on the English writing of bilingual Jordanian students, Online Submission, ERIC Number: ED497505.
7. **Dweik, B.** (1986) *Research Papers in Applied Linguistics*. Hebron. Hebron University Press.
8. **Ellis, R.** (1986) *Understanding Second Language Acquisition*. New York. Oxford University Press.
9. **Farooq F, Lorigo L, Govindaraju V.** (2006) On the Accent in Handwriting of Individuals. Guy Lorette. Tenth International Workshop on Frontiers in Handwriting Recognition, Oct 2006, La Baule (France), Suvisoft.
10. **Feder, K.P.**, handwriting, development, -competency, intervention, vol;49, 1991, page(312-317)
11. **Gass, S. M.** (2000). Fundamentals of second language acquisition. In J.W. Rosenthal (Ed.), *Handbook of undergraduate second language education* (pp. 29-46). Mahwah, New Jersey: Lawrence Erlbaum Associate, Inc., Publishers.
12. **Hadhrami AAA** (2017) Regional Variation in Letter Joining Habits in Arabic Writers. *Forensic Res Criminol Int J* 4(2): 00103. DOI: 10.15406/frcij.2017.04.00103
13. **Hilton O.** (1963). Some basic rules for the identification of hand-writing. *Med Sci Law*. 1963 Apr;3:107-17. PMID:13954607
14. **Kapoor, A.K., Saini, M.** (2017). Handwriting as a means of Cultural Identity. *J Forensic Sci & Criminal Inves*. 2017; 3(1): 555605. DOI:10.19080/JFSCI.2017.03.555605.
15. **Karim, K., & Nassaji, H.** (2013). First language transfer in second language writing: An examination of current research. *Iranian Journals of Language Teaching Research*, 1(1), 117-134.
16. **L.E. Baum**, "Inequality and Associated Maximization Technique in Statistical Estimation of Probabilistic Functions of Markov processes", *Inequalities*, vol.3, 1972, pp.1-8.
17. **Mathur S & Choudhary S K.** (2014). Examination Of Handwritten Documents Of Unfamiliar Scripts: A Forensic Viewpoint On Various Indian Languages. *International Journal Of Research In Applied, Natural And Social Sciences*. ISSN(E): 2321-8851; ISSN(P): 2347-4580 Vol. 2, Issue 4, Apr 2014, 137-144
18. **Mehrabi, N.** (2014). The Effect Of Second Language Writing Ability On First Language Writing Ability. *Theory And Practice In Language Studies*, 4(8), 1686-1691.
19. **Osborn A.S.** *Questioned Document 1929* Albany, N.Y., Boyd printing company; London, Sweet & Maxwell, Limited 1929.
20. **Pal, U., Jayadevan, R., and Sharma, N.** (2012). Handwriting recognition in Indian regional scripts: A survey of offline techniques. *ACM Trans. Asian Lang. Inform. Process*. 11, 1, Article 1 (March 2012), 35 pages. DOI = 10.1145/2090176.2090177 <http://doi.acm.org/10.1145/2090176.2090177>
21. **Ramaiah C, Porwal U and Govindaraju V.** (2012). Accent Detection in Handwriting based on Writing Styles. 2012 10th IAPR International Workshop on Document Analysis Systems © 2012 IEEE DOI 10.1109/DAS.2012.13
22. **Saini M, & Kapoor A K.** (2014). Variability In Handwriting Patterns Among Ethnic Groups Of India *International Journal of Humanities and Social Sciences (IJHSS)* ISSN(P): 2319-393X; ISSN(E): 2319-3948 Vol. 3, Issue 1, Jan 2014, 49-60 © IASET
23. **Saini M, et al.** (2015). Impact of Heredity and Environment in Familial Similarity of Handwriting. *International Journal of Computer and Electronics Research* [Volume 4, Issue 1, February 2015]
24. **Saini M, Srinivasan A and Kapoor A K.** (2015). Inheritance of Handwriting Features. *International Refereed Journal of Engineering and Science (IRJES)*. Volume 4, Issue 4 (March 2015), PP.01-05 ISSN (Online) 2319-183X, (Print) 2319-1821
25. **Saini M, Srinivasan A and Kapoor A K.** (2015). Inheritance of Handwriting Features. *International Refereed Journal of Engineering and Science (IRJES)* ISSN (Online) 2319-183X, (Print) 2319-1821 Volume 4, Issue 4 (March 2015), PP.01-05 [www.irjes.com](http://www.irjes.com)
26. **Shabani M, Tahriri A, Ardebili S F.** (2016). The Impact of First and Second Languages on Azerbaijani EFL Learners' Writing Ability. *International Journal of Education & Literacy Studies* ISSN 2202-9478 Vol. 4 No. 1; doi:10.7575/aiac.ijels.v.4n.1p.24 URL: <http://dx.doi.org/10.7575/aiac.ijels.v.4n.1p.24>
27. **Sharma B. R.** (2017). *Handwriting Forensics*. Universal Publication House, Edition 2017
28. **Sharma, Vinny and Jain, A.K. and Johri, Prashant and Jindal, Gunjan,** Offline Handwriting Recognition to Determine the Handwriting Accents – A Pilot Study (February 21, 2020). Available at SSRN: <https://ssrn.com/abstract=3551011> or <http://dx.doi.org/10.2139/ssrn.3551011>
29. **Srihari SN, Cha S-H, Arora H, Lee S.** Handwriting identification: research to study validity of individuality of handwriting & develop computer-assisted procedures for comparing handwriting. Buffalo (NY): University at Buffalo, State University of New York; 2001 TR No.: CEDAR-TR-01-1
30. **Yigzaw, A.** (2013). Students' first language writing skills and their English language proficiency as predictors of their English language writing performance. *Journal of languages & culture* 4 (6), 109-114.

## WEB SOURCE:

31. <http://www.lprinfor.com/history-handwriting-analysis.htm>
32. <https://www.fbi.gov/history/famous-cases/lindbergh-kidnapping>
33. <https://www.translatemedia.com/translation-blog/handwriting-accent-penmanship-can-betray-language-identity/>