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THE SCREENING OF PYMETROZINE INSECTICIDE USINGANOVELTLCMETHODANDITSCONFIRMATION BY GC-MS-MS ANALYSIS

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ABSTRACT

In order to eradicate undesired living forms for profitable agricultural outputs, pesticides are used. Many agrochemicals have been created, and some are currently being tested. One of these agrochemicals is pymetrozine, a substance that belongs to the chemical class of pyridine azomethines. The thin layer chromatography method is not recommended in any literature sources for pymetrozine screening. The first TLC method, developed for the provisional identification of the pymetrozine insecticide, used ethyl acetate, acetonitrile, and methanol (30:15:5) as the mobile phase. The UV visualization in TLC for standard samples revealed two purple spots with *Rf* values of 0.20 and 0.37. Rf values were 0.19 and 0.36 for the visceral spiked samples, nevertheless. Pymetrozine was retained in the column at 16.903 minutes, and GC-MS-MS analysis was used to confirm the findings.

Keywords: Pesticides, Pymetrozine, Thin Layer Chromatography, *Rf*, GC-MS-MS etc.

INTRODUCTION

Pymterozine belongs to the chemical family of pyridine azomethines, and initially registered as insecticide in the year of 1999 [1]. The systemic chemical name of pymetrozine is 4,5-dihydro-6-methyl-4-(3-pyridinylmethylene amino)-1,2,4-traizin-3(2H)-one(CA) with active ingredient code as CGA-215944 (Figure 1. structural formula). The empirical formula is $C_{10}H_{11}N_{50}$ and molecular weight is 217.23g/mol [2,3]. Pymetrozine has a little soil mobility and has a specific mode of action *i.e.,* blockage of stylet penetration of insects [3]. Pymetrozine causes starvation and death preceded by irreversible cessation of feeding by affecting the nerves controlling the salivary suction pump of insects [4,5]. This insecticide targets transient receptor potential ion channel complex of stretch receptor cells (chordotonal stretch receptor neurons) of plant sucking insects and has not direct knockdown effect and is not directly toxic to insects [3,4,6,7]. This attractive toxicity is less toxic (low acute toxicity) for mammals therefore considered as relatively safe for non-target arthropods and other species [3].

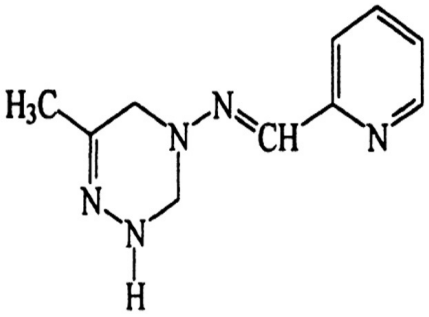


Figure 1. Structural formula of pymetrozine13

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Pesticides are extensively used in India. The consumption of pesticides for the year of 2021-2022, has been reported to be 58720 metric ton as per Directorate of Plant Protection Quarantine & Storage, Ministry of Agriculture and Farmers Welfare, India [8]. Self-ingestion of pesticides is a common mode of suicide in India and pesticide suicide is a constant serious concern for public health problem [9]. Therefore, TLC analysis of unreported insecticides is forensically significant because it is still a method of choice for screening of pesticides in visceral samples. In present study, one of such unreported insecticide *i.e,* pymetrozine, has been selected for TLC analysis. The obtained results were subsequently confirmed by GC-MS-MS.

The study was further expanded to explore the effect of matrix *i.e.,* visceral contents to the TLC analysis. An extraction from visceral sample was spiked with pymetrozine insecticide followed by extraction, and TLC and GC-MS-MS analysis to nearly mimic real life forensic analysis of visceral samples. The proposed method has successfully screened the target analyte and can be used for future Forensic Science laboratory case work.

MATERIALS AND METHODS

TLC plates: pre-coated aluminum backed silica gel G and silica gel 60 F_{254} TLC plates were procured from Merck India and were used in the present study. The plates were cut with a pre-cleaned scissors into dimensions of 5 cm × 12 cm. The plates were activated at 120°C for 30 minutes in a forced-air-circulation oven before use.

TLC Developing tank:A new pre-cleaned TLC chamber (dimensions: L 10 cm × W 10 cm × H 20 cm) with glass lid was used in the present study.

Chemicals and others: All the chemicals used in the current study were of HPLC grade and are as follows: ethyl acetate, acetonitrile, methanol. No separate standard of pymetrozine was purchased for the present study. The locally available pymetrozine insecticide company sealed bottle was purchased with active ingredient of pymetrozine 50% WG.

Microfilter

4.5µ PTFE microfilter was used to filter the samples. The sterile medical plastic 5 mL syringe was used in filter assembly.

Development of Solvent System

The relative solubility and Log P value of pymetrozine were studied [1,2] and three solvents were selected as ethyl acetate, acetonitrile and methanol in the ratio of 30:15:5.

Spot Visualization Reagents: UV (254 nm)

Standard Sample Preparation

As in the current study, commercially available pymetrozine insecticide was considered, therefore active ingredient of the insecticide required reasonable extraction from other ingredients. For standard sample preparation, one gram of pymetrozine 50% WG was mixed with 05 mL of methanol and gently vortexed and kept for five minutes. The eluted supernatant was transferred to a beaker and heated on water bath at 100°C for 2 min and kept aside. Later, beige color crystals (Figure 2) were formed which were re-dissolved in 05 mL methanol and preserved for further study.

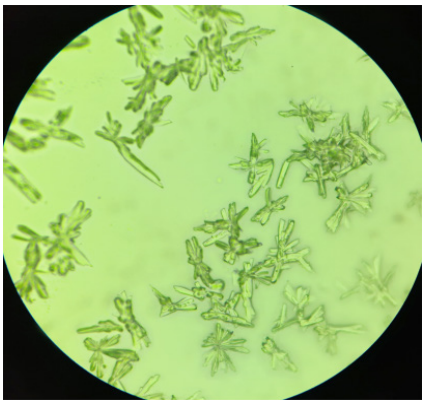


Figure 2. Crystals of pymetrozine

Pymetrozine spiked viscera sample preparation: Direct human viscera sample was not used in the current study. The hexane-acetone (1:1) fraction from the laboratory viscera sample was taken into consideration to estimate the matrix effect on the TLC analysis. 100 µL of the prepared standard sample was mixed with the 25 mL of hexane-acetone fraction (which is a routine protocol for pesticide extraction from viscera samples) to study the matrix effect on TLC run. The mixture was concentrated by heating at 60°C on water bath. The concentrated sample was kept for further study.

GC-MS-MS Parameters

GC-MS-MS: SHIMADZU NEXIS GC-2030, GCMS-TQ8040NX, with column SH-Rxi-5Sil MS (30m, 0.25mm I.D.), autosampler (AOC-20d(S)),

Injection volume – 1 µL, injector temperature 250°C, splitless mode, Carrier gas – Helium, CID gas- Argon, Column flow – 1 mL/min, Purge flow – 3.0 mL/min, column , Initial temperature– 60 ºC, Rate – 15 ºC, final temperature – 300 ºC, Ion source temp – 250 ºC, Interface temp – 300 ºC, Solvent cut time – 2.0 min, Total run time – 24 min, range of molecular weight (m/z) 35-600, Acquisition mode – Q3 scan.

TLC Analysis

50 mL of solvent system was prepared using a graduated measuring cylinder in the following ratio:- Ethyl acetate: Acetonitrile: Methanol : 30:15:5. The solvent system was transferred to the TLC tank and kept undisturbed for 30 minutes to achieve the saturation of the chamber.

TLC Analysis of Standard Sample

An aliquot of 5 µL from the prepared standard sample was spotted on activated TLC plate using 10 µL (micro-volume) micro-syringe. Total 3 spots were spotted on a single TLC plate (5 cm × 12 cm) with inter-spot distance of 1 cm. The spots were placed 1 cm above the bottom side and a complete 10 cm run was performed in the previously saturated TLC tank followed by air drying and visualization under UV. The Rf value of developed spots were measured using standard 10 cm ruler. Total 10 plates were developed similarly.

TLC Analysis of Spiked Sample

1.0 mL Acetonitrile was added to concentrated spiked sample and gently shaken for 10 minutes. The upper Acetonitirle layer was then carefully transferred to a pre-cleaned 10 mL beaker using 1 mL micropipette and further kept for air-drying. The obtained residue was re-dissolved in 20 µL methanol and was slowly (spot by spot) spotted on the TLC plate alongside with the standard 5 µL spot and visualized after complete run similarly as mentioned before. Total 10 plates were developed similarly.

GCMSMS Analysis of Standard Sample

5 µL of the prepared standard solution was diluted with 1 mL of methanol and micro-filtered in 1.5 mL GC vial and subjected to analysis with previously mentioned parameters.

GCMSMS Analysis of Isolated Spots of Spiked Samples From TLC Plate

In order to confirm the migrated spot is the pymetrozine spot on the TLC plate, the area of the developed spots on the TLC plate was scrapped off with pre-cleaned surgical blade and added to 1 mL methanol in a test tube followed by 5 minutes vortexing. The vortexed content was micro-filtered and subjected to GCMSMS analysis as mentioned before.

RESULTS AND DISCUSSION

TLC: The UV visualization revealed purple colored two spots with Rf values of 0.20 and 0.37 for standard samples (Figure 3). However, the Rf value for visceral spiked samples were 0.19 and 0.36 (Figure 4).

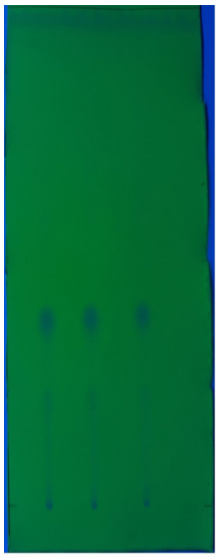


Figure 3

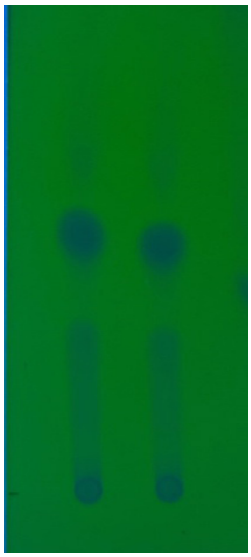


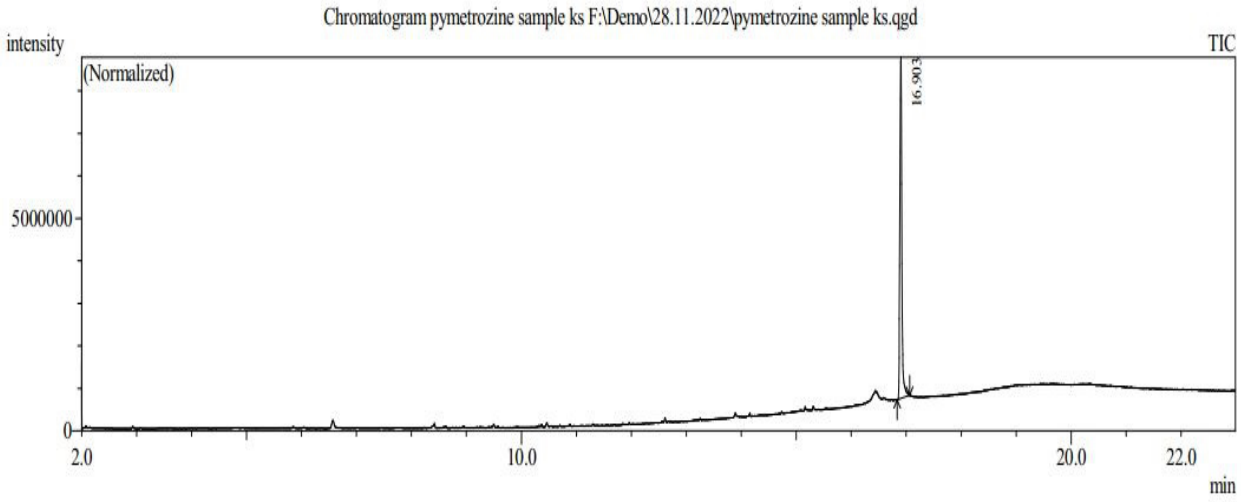
Figure 4

Figure 3. TLC of standard samples

Figure 4. TLC of spiked samples

GCMSMS

The analyzed prepared standard sample revealed the presence of pymetrozine as shown by the inbuilt NSTD library provided by the Shimadzu. The retention time was 16.903 , base peak at 98.05 m/z and similarity 94 % as per the library software (Figure 5, Figure 6). Likewise isolated spots of spiked samples from TLC plate were analyzed using GCMSMS (Figure 7).



Peak Report TIC

Peak#	R.Time	Area	Area%	Base m/z	Name
1	16.903	19314109	100.00	98.05	Pymetrozin
		19314109	100.00		

Figure 5. Chromatogram of Preparaed standard of pymetrozine

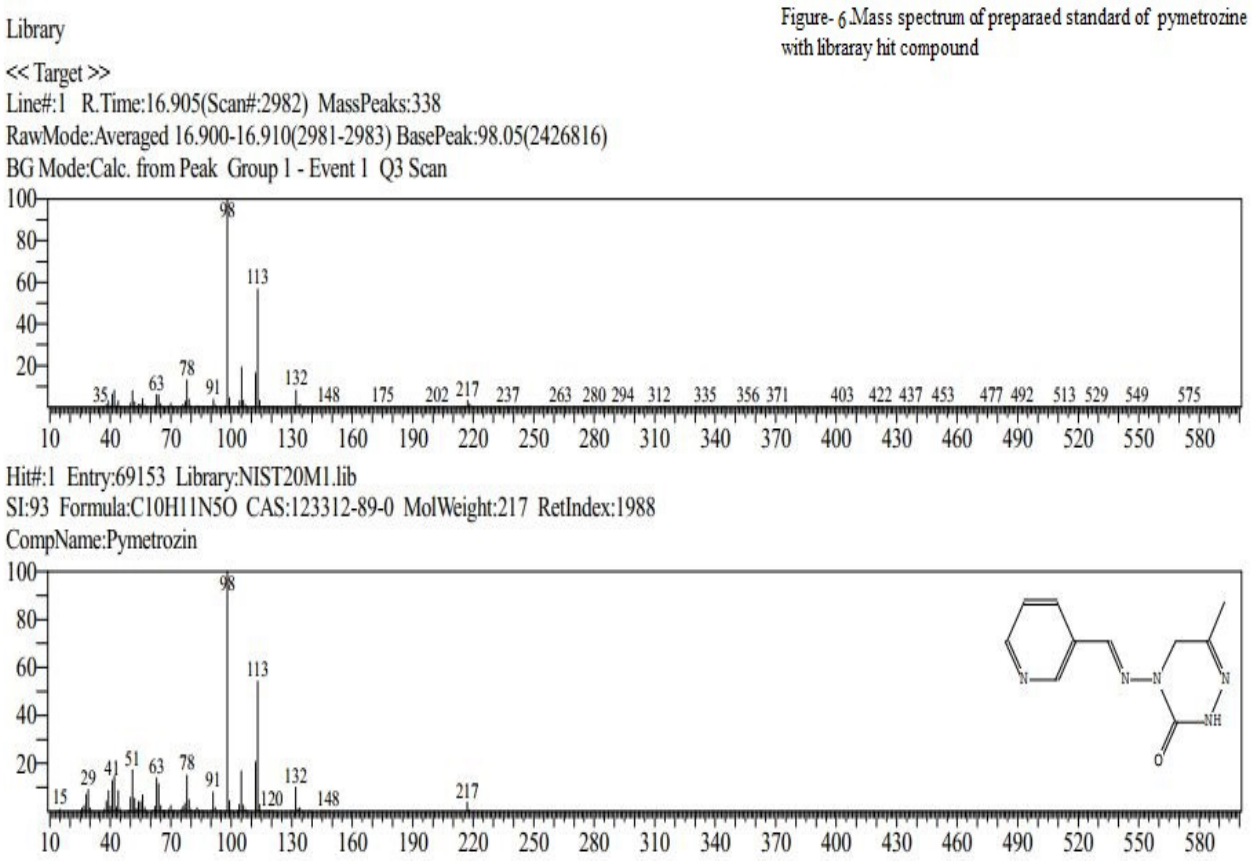
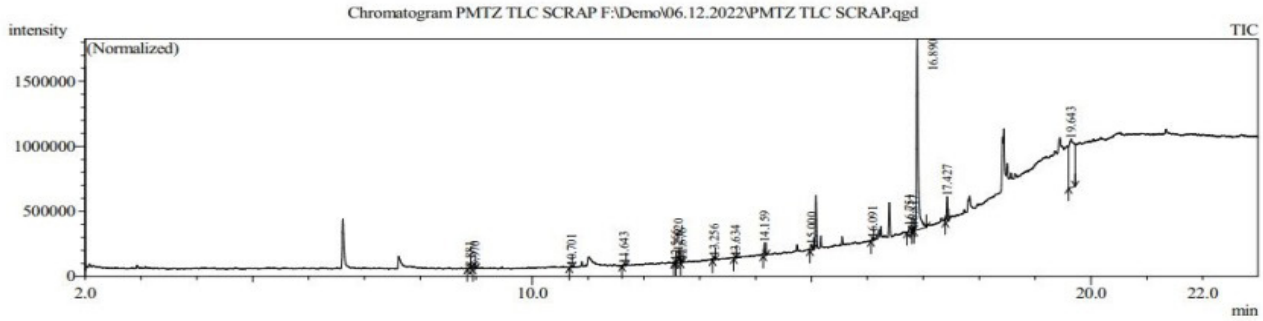


Figure 6. Mass spectrum of preparaed standard of pymetrozine with library hit compound



Peak Report TIC

Peak#	R.Time	Area	Area%	Base m/z	Name
1	8.881	20626	0.30	41.05	1-Undecene
2	8.970	32407	0.48	57.10	Decane, 3,4-dimethyl-
3	10.701	24258	0.36	83.10	Octacosanol
4	11.643	29699	0.44	43.05	1-Dodecanol
5	12.566	18729	0.28	70.05	2-Undecene, 3-methyl-, (Z)-
6	12.620	139730	2.06	83.10	1-Hexadecanol
7	12.676	23174	0.34	57.05	Heptadecane, 2,6,10,15-tetramethyl-
8	13.256	46051	0.68	57.10	8-Pentadecanone
9	13.634	19842	0.29	74.05	Tridecanoic acid, 12-methyl-, methyl ester
10	14.159	132543	1.95	83.10	1-Nonadecene
11	15.000	92391	1.36	214.10	1,1'-Biphenyl, 3,3'-dimethoxy-
12	16.091	43814	0.64	71.10	10-Nonadecanone
13	16.751	95065	1.40	59.05	Hexadecanamide
14	16.817	46719	0.69	97.10	1-Nonadecene
15	16.890	3260700	47.99	98.00	Pymetrozin
16	17.427	301744	4.44	57.05	Myristic acid glycidyl ester
17	19.643	2467570	36.31	81.10	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
		6795062	100.00		

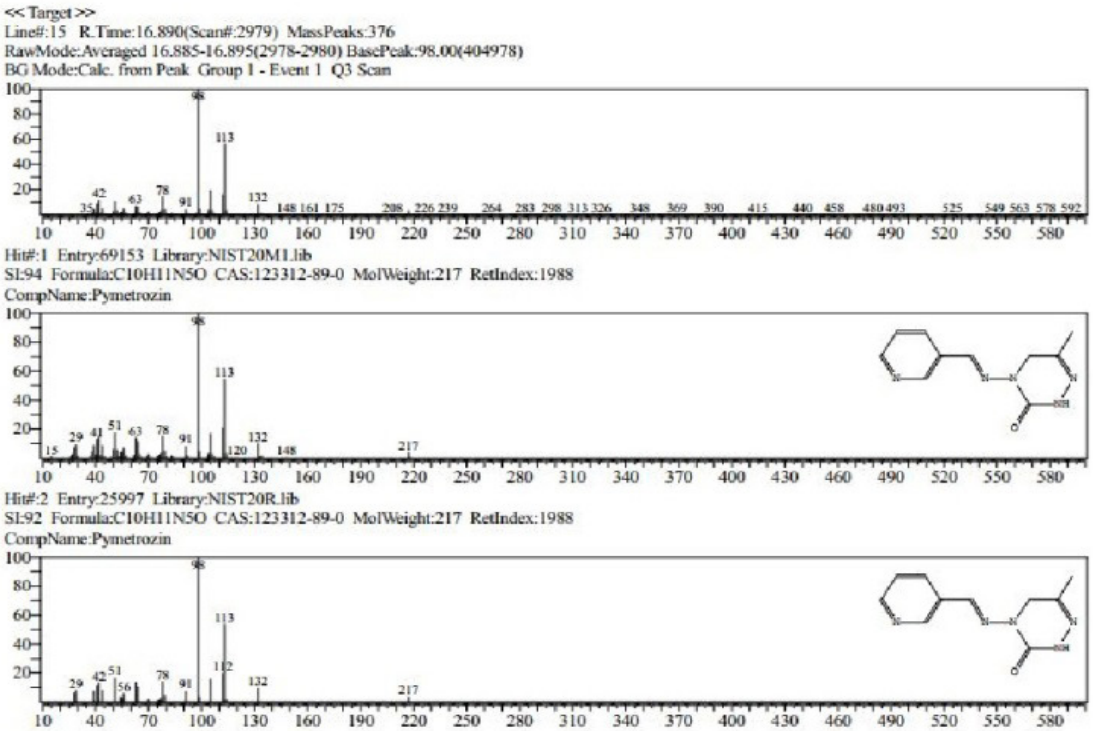


Figure 7 Chromatogram and mass spectram of spkied sample scrapped from the TLC plate

DISCUSSION

Pyridine azomethines based pymetrozine insecticide used against plant-sap sucking insects [7]. This first compound from the chemical class has not been studied using TLC. Therefore, an attempt has been made for development of first TLC method, which can be used for the screening of pymetrozine insecticide. Pymetrozine is more soluble in ethanol and ethyl acetate [2]. The negative Log P (log Kow = -0.18) of pymetrozine indicates its solubility in polar solvents such as water, ethanol, etc. Based on such parameters three solvents were selected as mentioned before. The ratio of the solvents was selected by trial and error approach.

Since the commercial pymetrozine preparation was used as standard in the present study, the commercial preparation needed confirmation by GCMSMS analysis for the purity of pymetrozine. The obtained chromatogram and MS data revealed the only presence of pymetrozine, which ensures the absence of other substances in the commercial preparation. The prepared standard sample was further subjected to TLC analysis. The TLC plates were visualized under short wave UV in a cabinet. UV visualization is rapid, economic and safer as compared to the chemical visualization methods. The quenching fluorescence observed under short wave UV revealing the two spots as evident from the results. Similarly, the spiked visceral samples have shown two spots with slightly lower Rf values possibly due to the matrix effect. However, two spots likewise the standard samples were observed indicating the good separation of the selected mobile phase.

CONCLUSION

First TLC method has been developed for pymetrozine insecticide guided by literature research. The proposed method can effectively be used for real case analysis of viscera samples for the presence of pymetrozine insecticides. This research will be an aid for the forensic toxicologists whenever they encountered a case with pymetrozine poisoning.

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