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DEVELOPMENT AND VALIDATION OF A MODIFIED QUECHERS WITH DERIVATIVE UV-VISIBLE SPECTROSCOPY FOR SIMULTANEOUS DETERMINATION OF CHLORPYRIPHOS AND CYPERMETHRIN

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Abstract

Introduction: Pesticides are often associated with various toxic effects in humans, being additionally accountable for a large number of rural suicides or accidental fatalities. In recent times, for better pest management strategies multiclass pesticides with combination of two or more compounds are extensively used. Simultaneous determination of these pesticides in toxicological analyses is challenging and existing approaches rely on chromatographic techniques. Although derivative UV-visible spectroscopy has been used extensively for multicomponent analysis due to its simplicity and cost-effectiveness, however, simultaneous determination of pesticides using the technique remains less explored. Therefore, the present study aims to develop a method for the simultaneous determination of chlorpyriphos and cypermethrin by first order derivative UV-visible spectroscopy.

Methods: Human blood samples were taken and spiked with standards of chlorpyriphos and cypermethrin in the ratio 10:1. Then the pesticides were extracted using modified citrate buffer QuEChERS-based method. Microvolume of extracts were analyzed using first order derivative UV-Visible spectroscopy and then processed by the zero-crossing point method.

Results: The developed method showed good linearity for both pesticides with correlation coefficients ≥0.99. The method was validated according to ICH Q2(R2) guidelines and the precision study indicated that % RSD was within acceptable limit (<1). LOD for CHL and CYP were 61.905 mg/L and 3.799 mg/L respectively. Whereas, LOQ were 187.593 mg/L and 11.512 mg/L respectively.

Conclusion: The present study showed that 1st order derivative UV-visible spectroscopy is a useful analytical technique for routine toxicological analysis of multiclass pesticides. The developed method of analysis offers significant advantages in terms of simplicity, cost-effectiveness, and in agreement of green chemistry principles.

KEYWORDS: Chlorpyrifos, cypermethrin, QuEChERS, green chemistry, forensic toxicology.

1. INTRODUCTION

The green revolution in the 1960s has resulted in a bloom of the agriculture sector with adequate production of food and fodder. However, this advancement was accompanied by increased use of chemical pesticides for better pest management and higher yield [1]. Although there is no denying of the advantages of greater agricultural productivity, the extensive and frequently uncontrolled use of pesticides in agriculture and home environments has raised concerns related to the detrimental consequences of these agrochemicals on humans, animals, and environment [2]. Their widespread use has led to increased toxicological risks to people, especially in rural communities with little public awareness and regulatory enforcement. Although, the dangers of pesticides to human health have been thoroughly examined; even so, their threats to human well-being remain a significant issue that has received little attention [3].

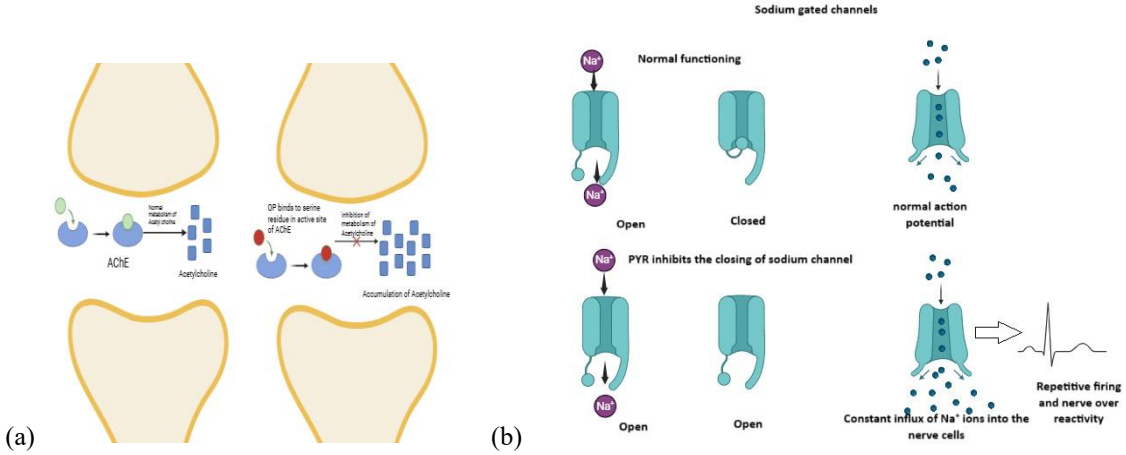
Despite of regulatory measures for the restriction on their application, pesticides are often associated with many poisoning cases being additionally accountable for a large number of both suicidal and accidental poisoning in India [4]. According to the data of NCRB (National Crime Records Bureau) in 2022, poisoning due to insecticides or pesticides was the cause of 25.4% (43,387) suicidal deaths and 5.1% (21,606) accidental deaths [5]. There are numerous factors that influence morbidity and mortality due to pesticide poisoning such as availability, socio-economic development, route and duration of exposure, and the chemical composition of the pesticide formulation [6]. Pesticides are widely available in the rural areas, where agriculture is the primary source of income and, therefore are commonly linked to accidental consumption, occupational exposure, and suicide. [7-10]. Mainly, the low-income households, rural children, and farmers are the most vulnerable groups due to lack of proper medical access, poor storage practices, and unprotective practice of application of the chemicals [11-13].

The chemistry of pesticides has significant impact on their toxicological effects. Pesticides can be classified into two major categories: inorganic and organic. Distinct health effects are associated with different chemical classes of pesticides [17]. Moreover, pesticides also include the substances which are used to control nematodes, fungi, mites, and

rodents. These pesticides can be applied in different physical forms, such as powder, fumes, smoke, and spray. Inorganic compounds with insecticidal properties are sodium fluoride, sodium silicofluoride, boric acid and metallic compounds or metal phosphides such as aluminium phosphide, zinc phosphide etc. Metal phosphides are solid in nature and are generally used as fumigants to protect stored grain from insects and rodents. The phosphide compounds react rapidly with water in the air or stored grain and produce phosphine gas. This phosphine gas is responsible for acute toxicity, and its rate of production is dependent on ambient temperature and moisture [18]. Organic pesticides are further classified into organophosphates, organochlorines, carbamates and pyrethroids. Organochlorines were the first class of synthetically produced organic pesticides used on a large scale, followed by organophosphate and carbamate insecticides [2]. The death rate due compounds like organophosphates and aluminum phosphide are very high. These chemicals are particularly hazardous in situations when there is no quick medical intervention because they are extremely poisonous, fast-acting, and frequently found in farmer-used formulas [6].

Acute exposure to pesticides may cause symptoms of respiratory distress, headache, nausea, vomiting, disorientation, and abdominal discomfort. Additionally, convulsions, twitching of the muscles, excessive salivation, and in cases of severe exposure, central nervous system depression and coma can also be observed [19]. Chronic or repeated pesticide exposure results in long-lasting toxic effects, including persistent neurological, gastrointestinal, and skin problems. Major physiological systems of body including endocrine, immune system, genito-urinary track, respiratory system, cardiac system and muscle-skeletal system are also affected by pesticides. After continuous exposure carcinogenic and mutagenic effects were also observed [3]. These indicate that the toxicological effects of pesticide exposure extend beyond acute clinical effects and may evolve into complex molecular and cellular alterations.

In modern days, multiclass pesticides are used for better pest management and higher effectiveness [20]. As a result, people could be exposed to multiple pesticides at the same time instead of just one active component. As a result, different chemical compouunds acting through many molecular targets may result in additive, interacting, or possibly synergistic biological effects, such combination exposure is of special toxicological concern. Therefore, evaluating multiclass pesticide exposure necessitates taking into account not only each compound's individual toxicity but also its simultaneous presence and possible combination consequences. The combination of organophosphorus (OP) and pyrethroids (PYR) are most commonly used as an insecticide because of their complementary modes of action. OPs inhibit the metabolism of neurotransmitter acetylcholine by binding to the to the serine residue in the active site of acetylcholinesterase enzyme (figure 1a). This results in the accumulation of acetylcholine in the synapse which leads to muscle paralysis, respiratory failure and death [21]. The toxicity of PYRs is also neurological in nature. It acts by binding to the voltage gated sodium channels which facilitates the nerve action potential (figure 1b). It results in a constant influx of sodium ions into nerve cells and nerve overactivity [22, 23]. As both the compounds are neurotoxic, when combined it may lead to a more severe and prolonged toxic effect compared to exposure to either pesticide alone.



(a) (b)

Figure 1 (a) working mechanism of OPs, and (b) working mechanism of pyrethroids.

A popular combination formulation combines chlorpyrifos, an organophosphorus insecticide, and cypermethrin, a synthetic pyrethroid. Commercial formulations of chlorpyrifos 50% and cypermethrin 5% are used for broad-spectrum pest control. Therefore, the inclusion of both substances in biological specimens may have significant effects on toxicological interpretation, especially when exposure is intention ingestion, accidental, or occupational. Determining the type and degree of exposure thus requires accurate identification and simultaneous measurement of these substances in biological matrices.

The intricacy of biological matrices and the variety of physicochemical characteristics of pesticide components make the extraction and analysis of pesticides in biological specimens difficult. The traditional methods of extraction used in toxicological analysis of poisons include liquid-liquid extraction (LLE), solid-liquid extraction (SLE), solid-phase extraction (SPE), accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE). The complexity of the sample matrix and the physicochemical properties of the target analytes play a major role in choosing an appropriate extraction approach. In 2002, the QuEChERS approach, which stands for Quick, Easy, Cheap, Effective, Rugged, and Safe, was introduced and published by Anastassiades et al. in 2003, and then verified by Lehotay et al. in 2005 [25, 26]. The initial technique was designed for the extraction of pesticide residues from fruit and vegetable matrices. Gradually it has demonstrated its importance in analytical chemistry by prevailing in

contemporary fields such as agri-food [27], environmental [28], clinical [29], and forensic toxicological analysis [24, 30]. This method became popular because of its simplicity, speed, and cost-effectiveness relative to older extraction procedures. It employs cost-effective ingredients and necessitates a relatively small quantity of solvents and equipment, in accordance with the principles of green chemistry.

Following the analyte extraction, the selection of instrumental analysis has differed in the literature, employing a variety of complex methodologies and instruments, mostly concentrating on diverse chromatographic techniques (GC, UPLC, UHPLC, LC) in conjunction with mass spectroscopy [31]. Nonetheless, these techniques are destructive, necessitate substantial quantities of expensive and high-quality solvents for comprehensive specimen preparation, and require operation inside an advanced laboratory setting. Several research have employed derivative UV-visible spectroscopy for multicomponent analysis [32, 33]. For simultaneous quantitative analysis of multicomponent samples, 1st order derivative spectra are analysed by "Zero crossing technique" [34]. UV-visible spectroscopy is distinguished as the most economical, easily accessible, and non-destructive method. Although QuEChERS technique is widely used to extract pesticides from biological matrices, its use in conjunction with first-order derivative UV–visible spectroscopy for the simultaneous determination of multiclass pesticides in blood is yet relatively less explored. Therefore, the present study aims to develop and validate a method for the rapid and simultaneous determination of a multiclass pesticide mix of chlorpyriphos and cypermethrin by 1st order derivative UV-visible spectroscopy.

2. MATERIALS AND METHOD

2.1 Chemicals and reagents

Reference standards (purity >99%) of Chlorpyrifos and cypermethrin were purchased from AccuStandard (New Haven, CT, USA). Analytical grade magnesium sulfate, sodium acetate, sodium citrate dibasic sesquihydrate, sodium citrate tribasic dihydrate acetonitrile, toluene and acetone used in the study was procured from Central Drug House (P) Ltd. (India). SPE sorbent ($MgSO_4$, PSA, C18) were purchase from Agilent Technologies (Santa Clara, CA, USA).

2.2 Standard preparation

Stock solutions of standard chlorpyrifos and cypermethrin were prepared of concentration 5000 mg/L in acetonitrile. Five different dilutions of concentrations 100mg/L, 200mg/L, 300mg/L, 400mg/L and 500mg/L for chlorpyriphos and 10mg/L, 20mg/L, 30mg/L, 40mg/L and 50mg/L for cypermethrin were prepared.

2.3 Sample preparation

Two different brands of pesticide mix (50% chlorpyriphos+ 5% cypermethrin) were purchased from local market of Mohali, Punjab belonging to different manufacturers. Five working solutions (WS) of the pesticides in the concentration range of 100 mg/L, 200 mg/L, 300 mg/L, 400 mg/L and 500 mg/L were prepared in acetonitrile. Blank whole blood samples were donated by healthy individuals and stored at –20 °C until analysis. For simulated real case analysis, with 0.9 ml of blank blood 100µl of WS was spiked.

2.4 Sample extraction

Aliquots of 1 ml simulated blood were transferred to 15ml polypropylene centrifuge tubes. 2ml of ice-cold acetonitrile was added followed by vortexing to facilitate protein precipitation. The extraction was done by modified QuEChERS method with salt combination of 1.0 g $MgSO_4$, 0.25 g NaCl, 0.25 g sodium citrate dibasic sesquihydrate, and 0.5 g sodium citrate tribasic dihydrate and then vortexed for 1 min followed by shaking for 5 mins. The solution was then centrifuged at 3000rpm for 10 mins. The upper aqueous layer was separated for dispersive SPE cleanup. To 1 ml of the supernatant 150mg of $MgSO_4$, 50 mg of PSA and 50 mg of C18 was added. The tubes were then again vortexed for 1 min, centrifuged at 10,000 rpm for 5 mins and left to stand for 5 mins. The cleaned extract was then evaporated to dryness under N2 stream at ≤40 °C and then reconstituted to 1ml with toluene: acetone (9:1, v/v).

2.5 Sample analysis

The samples were analyzed using a Hitachi UH3500 double beam UV-visible spectrophotometer connected to a PC with the software. Quartz micro-cuvettes with path length of 1cm and 1ml volume were used for sample analysis. Samples were manually loaded and scanned in the range 200 nm to 600 nm with the scanning speed of 100 nm/minute. The spectra were saved in .csv and .pdf formats. Absorbances were recorded in triplicates. 1st order derivatives of the spectra were obtained with built-in function of the software using Savitzky-Golay method (smoothness=4 & data points=7).

2.6 Method validation

The method adopted for the determination of the selected pesticides were validated according to ICH Q2 R1 guidelines for validation of analytical methods [35] for the determination of linearity, precision, Limit of detection (LOD) and limit of quantitation (LOQ), accuracy and analysis of real samples.

3. RESULTS

3.1 Selection of analytical wavelength

The 1st order derivative of the spectra was processed for the selection of analytical wavelengths such that at zero crossing point (ZCP) of one pesticide measurable absorbance of the other pesticide is observed.

It was observed that CHL shows ZCP at 212nm and CYP showed zero peak at 224nm. At 212nm (ZCP of CHL) CYP showed measurable absorbance, whereas at 224nm (ZCP of CYP) CHL showed measurable absorbance. Thus, 212nm and 224nm was chosen as the analytical wavelengths for the determination of 1st order CYP and CHL respectively by 1st order derivative method.

3.2 Method validation

The method adopted for the determination of the selected pesticides were validated according to ICH Q2 R1 guidelines for validation of analytical methods for the determination of linearity, precision, Limit of detection (LOD) and limit of quantitation (LOQ), accuracy and analysis of real samples.

3.2.1 Linearity

For the preparation of calibration curve of 1st order derivative of absorbance (CHL at 224nm and CYP at 212nm) vs concentration 5 dilutions were analysed (table 1). The calibration curves were prepared in MS Excel. The correlation coefficient of the calibration curves were 0.99 and 0.994 respectively for CHL and CYP (Figure 2).

Table 1 Calibration data of CHL and CYP

CHL (224 nm) (n=3)			CYP (212 nm) (n=3)		
Concentration (mg/L)	**Mean abs ± SD**	**%RSD**	**Concentration (mg/L)**	**Mean abs ± SD**	**%RSD**
100	-0.776 ± 0.005	0.661	10	-0.129 ± 0.001	0.775
200	-0.872 ± 0.003	0.289	20	-0.174 ± 0.002	0.876
300	-0.989 ± 0.007	0.710	30	-0.250 ± 0.002	0.834
400	-1.121 ± 0.003	0.272	40	-0.298 ± 0.003	0.888
500	-1.281 ± 0.004	0.325	50	-0.366 ± 0.003	0.835

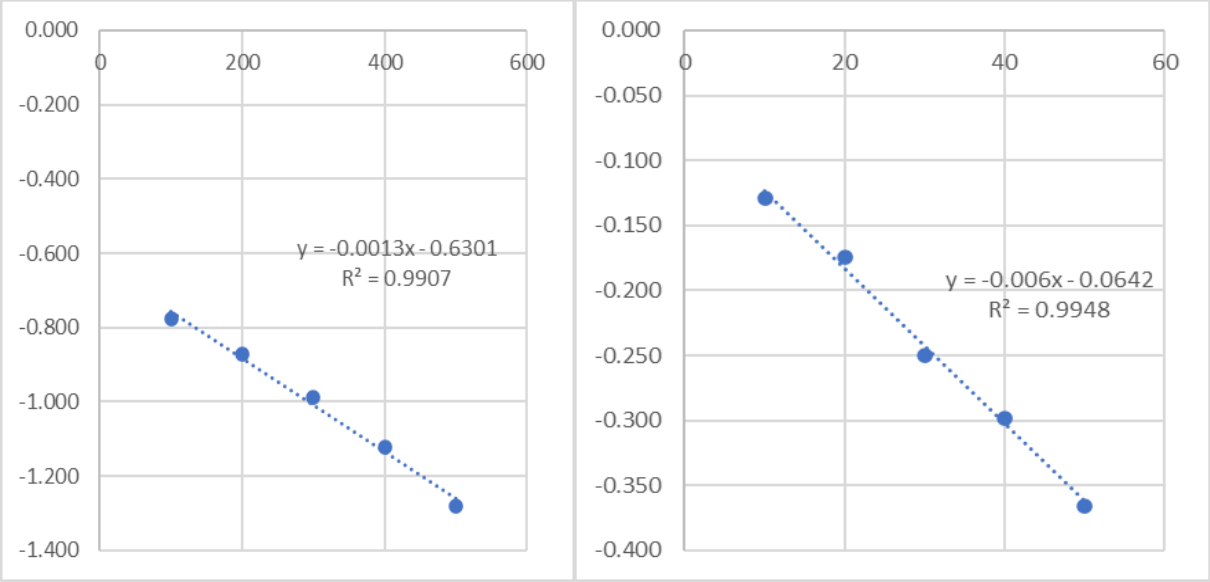


Figure 2 (a) Calibration curve of CHL, (b) Calibration curve of CY

3.2.2 Precision

For the study of precision of the adopted methodology, repeatability was checked with one dilution for each of the pesticide (n=6). Intra-day and inter-day precision of the methodology was also studied with three dilutions for each pesticide (n=3) as given in table 2.

Table 2 Repeatability, Inter-day and Intra-day precision of CHL and CYP

Pesticide	Concentration (mg/L)	Avg Abs ± SD	%RSD
Repeatability (n=6)			
CHL	300	-0.986 ± 0.002	0.197
CYP	30	-0.253 ± 0.002	0.821
Intra-day precision (n=3)			
CHL	200	-0.873 ± 0.001	0.066
	300	-0.989 ± 0.007	0.710
	400	-1.123 ± 0.001	0.103
CYP	20	-0.176 ± 0.002	0.870
	30	-0.248 ± 0.002	0.615
	40	-0.299 ± 0.002	0.697
Inter-day precision (n=3)			
CHL	200	-0.868 ± 0.002	0.176
	300	-0.983 ± 0.003	0.311

	400	-1.121 ± 0.003	0.272
CYP	20	-0.171 ± 0.001	0.585
	30	-0.248 ± 0.001	0.403
	40	-0.294 ± 0.004	1.286

3.2.3 LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were measured using the following formula.

$$\text{LOD} = 3.3 \times \left(\frac{\text{SD}}{\text{Slope}}\right)$$

$$\text{LOQ} = 10 \times \left(\frac{\text{SD}}{\text{Slope}}\right)$$

Where, SD=Standard deviation of Y intercepts of the calibration curves
Slope= mean slope of the calibration curves
The values of LOD and LOQ for CHL were 61.905 mg/L and 187.593 mg/L respectively. Whereas for CYP, the LOD and LOQ were 3.799 mg/L and 11.512 mg/L respectively.

3.2.4 Accuracy

For the study of accuracy of the adopted method, recovery from two different brands of marketed formulations were observed at three different concentration levels (table 3).

Table 3 Analysis of marketed pesticide mix for accuracy

Pesticide	Conc. of dilution (mg/L)	1st order Derivative abs	Conc. detected (mg/L)	% Pesticide detected ± SD (n=3)
CHL (50%)	100	-0.772	109.1538	109.1538 ± 0.001
	200	-0.869	186.0769	93.03845 ± 0.003
	300	-0.982	281.4615	93.8205 ± 0.002
	400	-1.118	379.9231	94.98078 ± 0.001
	500	-1.28	499.9231	99.98462 ± 0.001
CYP (5%)	10	-0.129	11.142	111.42 ± 0.005
	20	-0.173	19.285	96.425 ± 0.003
	30	-0.249	28.44	94.8 ± 0.001
	40	-0.296	39.993	99.982 ± 0.002
	50	-0.365	50.294	100.588 ± 0.001

Percentage of pesticide detected were observed in the range of 93-112%.

3.2.5 Analysis of real samples

For the analysis with real samples, three concentrations of the marketed formulations were spiked with the blood. The extraction of the pesticides was done using QuEChERS method. After extraction from the blood matrix, the recovery % were noted for each concentration given in the table 4.

Table 4 Analysis of real samples

Pesticide	Conc. (mg/L)	Amount recovered (mg/L)	% Recovery ± SD (n=3)
CHL (50%)	200	196.478	93.03845 ± 0.003
	300	299.334	93.8205 ± 0.002
	400	394.67	94.98078 ± 0.001
CYP (5%)	20	19.5	96.425 ± 0.003
	30	28.574	94.8 ± 0.001
	40	39.345	99.982 0.002

4. DISCUSSION

The present study aimed to develop a modified QuEChERS-based analytical method for the simultaneous determination of a multiclass pesticide, containing the combination of chlorpyriphos and cypermethrin using 1st order derivative UV-visible spectroscopy. The selection of wavelength for the analysis of the pesticides was done by zero crossing point technique. This method have been used widely for the simultaneous analysis of combination of drugs. A study by Thula et. al., analysed the combination of two drugs Nebivolol and Clinidine using zero crossing point [34]. In another study by Koba et. al., bisphosphonate drugs were analysed in pharmaceutical formulations using 1st and second order derivative UV-visible spectroscopy. They also applied the technique of peak-zero method for the selection of analytical wavelength for the determination of the drugs [36]. For the analysis of pesticides mainly chromatographic techniques are applied. However, several studies have also demonstrated successful application of UV-visible spectroscopy for pesticide analysis [36-41]. A method for the detection of some common pesticides in simulated beverage samples using derivative UV spectrophotometer was developed by Kaur et. al., [33]. Similarly, Zalat et.al., validated a method for the quantitative determination of chlorpyrifos using UV spectrophotometer and HPLC. Chlorpyriphos showed absorbance at

290nm and linearity was developed in the range of 2.229ppm to 200pppm [41]. In the present study, the linearity was established in the range 100ppm to 500pm for chlorpyriphos and 10ppm to 50ppm for cypermethrin, which is higher than the previous study. The pesticides selected for the present study comes in marketed formulations which have chlorpyriphos and cypermethrin in the ratio 10:1. Based on the concentration requirements of the established extraction process as well as the makeup of the commercial formulations examined, a somewhat greater concentration range was chosen for this study. The successful achievement of linearity for both compounds demonstrates that the established derivative spectrophotometric technique can offer an acceptable quantitative response within the defined ranges. Both intra-day and inter-day precision were used to assess the developed method's precision. The RSD values for intra-day and inter-day precision varied from 0.066 to 0.870 and 0.176 to 1.286 respectively. Intra-day precision was similar to previous studies, however intra-day precision was less due to various factors such as environmental conditions, degradation of the pesticides, or less efficient extraction method. However, the observed RSD values continued to be low and within permitted levels, indicating that the method's precision was adequate.

For the simultaneous measurement of cypermethrin and chlorpyrifos, the developed modified QuEChERS-based extraction in conjunction with first-order derivative UV–visible spectroscopy had satisfactory results. The technique is easy to use, affordable, and appropriate for regular screening and quantification of these pesticides, particularly in labs with restricted access to sophisticated chromatographic equipment.

5. CONCLUSION

In India, pesticide poisoning is still a significant toxicological and public health issue, especially in rural areas where intentional consumption, accidental ingestion, and occupational exposure are frequent. The extensive usage of multiclass pesticides such as the combination of pyrethroid and organophosphorus insecticides highlights the necessity of accurate and reliable analytical techniques for the simultaneous detection of multiple compounds for toxicological analysis. Therefore, in this study a simple, cost-effective modified QuEChERS-based extraction method coupled with first-order derivative UV-visible spectroscopy for the simultaneous determination of Chlorpyrifos (CHL) and Cypermethrin (CYP) was developed. The developed method was validated according to ICH Q2 (R1) guidelines, demonstrating linearity ($R^2 > 0.99$), good precision (RSD < 1.3%), acceptable limits of detection (LOD) and quantitation (LOQ), and satisfactory accuracy with recovery rates ranging from 93% to 112%. The approach was successfully applied to blood samples spiked with commercial pesticide formulations, confirming its practical applicability. The developed method combines the advantages of simplified sample preparation, low solvent and sample requirements and relatively inexpensive instrumentation, thus providing an accessible alternative to more sophisticated chromatographic techniques for the analysis of multiclass pesticide exposure. The ability to accurately determine CHL and CYP simultaneously might be especially useful in toxicology studies involving combined pesticide exposure. The developed method offers a rapid, inexpensive and analytically reliable approach for the simultaneous determination of toxicologically relevant compounds and can be used as a practical complementary technique for the pesticide analysis in laboratories with limited access to sophisticated chromatographic instrumentation.

Conflict of interest

None declared

REFERENCES

1. Unsworth J, Nakagawa Y, Harris C, Kleter G. The battle for a sustainable food supply. In: Springer eBooks. 2019. p. 13–84. Available from: https://doi.org/10.1007/978-3-030-17891-8_2
2. Gupta PK. Pesticide exposure—Indian scene. Toxicology. 2004 Mar 6;198(1–3):83–90. Available from: https://doi.org/10.1016/j.tox.2004.01.021
3. Vera-Herrera L, Sadutto D, Picó Y. Non-Occupational Exposure to Pesticides: Experimental Approaches and Analytical Techniques (from 2019). Molecules/Molecules Online/Molecules Annual. 2021 Jun 16;26(12):3688. Available from: https://doi.org/10.3390/molecules26123688
4. Gupta S, Basera D. Youth Suicide in India: A critical review and implication for the National Suicide Prevention Policy. OMEGA - Journal of Death and Dying. 2021 Sep 10;88(1):245–73. Available from: https://doi.org/10.1177/00302228211045169
5. Home | National Crime Records Bureau. Available from: https://ncrb.gov.in/
6. Peshin SS, Srivastava A, Halder N, Gupta YK. Pesticide poisoning trend analysis of 13 years: A retrospective study based on telephone calls at the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. Journal of Forensic and Legal Medicine. 2013 Dec 18;22:57–61. Available from: https://pubmed.ncbi.nlm.nih.gov/24485423/
7. Srivastava A, Peshin SS, Kaleekal T, Gupta SK. An epidemiological study of poisoning cases reported to the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. Human & Experimental Toxicology. 2005 Jun 1;24(6):279–85. Available from: https://pubmed.ncbi.nlm.nih.gov/16004194/
8. Peshin SS, Gupta YK. Poisoning due to household products: A ten years retrospective analysis of telephone calls to the National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi, India. Journal of Forensic and Legal Medicine. 2018 Jul 10;58:205–11. Available from: https://pubmed.ncbi.nlm.nih.gov/30015223/
9. Vijayakumar L, Jeyaseelan L, Kumar S, Mohanraj R, Devika S, Manikandan S. A central storage facility to reduce pesticide suicides - a feasibility study from India. BMC Public Health. 2013 Sep 16;13(1). Available from: https://pubmed.ncbi.nlm.nih.gov/24041373/

10. Gupta SK, Peshin SS, Srivastava A, Kaleekal T. A study of childhood poisoning at National Poisons Information Centre, All India Institute of Medical Sciences, New Delhi. Journal of Occupational Health. 2003 May 1;45(3):191–6. Available from: https://doi.org/10.1539/joh.45.191
11. Varghese P, Erickson TB. Pesticide poisoning among children in India: the need for an urgent solution. Global Pediatric Health. 2022 Jan 1;9. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8990700/#:~:text=Reducing%20access%20to%20pesticides%20and,life%2Dthreatening%20poisonings%20in%20children.
12. Barbosa N Junior, Huarachi NR, De Francisco N. The link between pesticide exposure and suicide in agricultural workers: a systematic review. Rural and Remote Health. 2024 Jun 26; Available from: https://doi.org/10.22605/rrh8190
13. Dikshit R, Gupta PC, Ramasundarahettige C, Gajalakshmi V, Aleksandrowicz L, Badwe R, et al. Suicide mortality in India: a nationally representative survey. The Lancet. 2012 Jun 1;379(9834):2343–51. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4247159/
14. Mohanraj R, Kumar S, Manikandan S, Kannaiyan V, Vijayakumar L. A public health initiative for reducing access to pesticides as a means to committing suicide: Findings from a qualitative study. International Review of Psychiatry. 2014 Aug 1;26(4):445–52. Available from: https://doi.org/10.3109/09540261.2014.924094
15. Yadav S, K AK, Cunningham SA, Bhandari P, Mishra US, Aditi A, et al. Changing pattern of suicide deaths in India. The Lancet Regional Health - Southeast Asia. 2023 Aug 17;16:100265. Available from: https://doi.org/10.1016/j.lansea.2023.100265
16. Abhijita B, Gnanadhas J, Kar SK, Cherian AV, Menon V. The NCRB Suicide in India 2022 Report: Key time trends and implications. Indian Journal of Psychological Medicine. 2024 Apr 14;46(6):606–7. Available from: https://doi.org/10.1177/02537176241240699
17. Kamath SD, Gautam VK. Study of organophosphorus compound poisoning in a tertiary care hospital and the role of Peradeniya Organophosphorus Poisoning scale as a prognostic marker of the outcome. Journal of Family Medicine and Primary Care. 2021 Nov 1;10(11):4160–7. Available from: https://doi.org/10.4103/jfmpc.jfmpc_518_21
18. Abdelghafar S, Farrag TA, Zanaty A, Alshater H, Darwish A, Hassanien AE. Pattern and predictors of death from aluminum and zinc phosphide poisoning using multi-kernel optimized relevance vector machine. Scientific Reports. 2023 May 22;13(1). Available from: https://doi.org/10.1038/s41598-023-34489-x
19. Thundiyil JG, Stober J, Besbelli N, Pronczuk J. Acute pesticide poisoning: a proposed classification tool. Bulletin of the World Health Organization. 2008 Mar 1;86(3):205–9. Available from: https://doi.org/10.2471/blt.08.041814
20. Srivastava A, Rai S, Sonker AK, Karsauliya K, Pandey CP, Singh SP. Simultaneous determination of multiclass pesticide residues in human plasma using a mini QuEChERS method. Analytical and Bioanalytical Chemistry. 2017 Apr 17;409(15):3757–65. Available from: https://doi.org/10.1007/s00216-017-0317-7
21. Sudan S, Kaur N, Taneja R, Kaur N, Mehmi P. Management of Lambda-Cyhalothrin poisoning in a North Indian healthcare setup: a rare case. Cureus. 2022 Dec 20; Available from: https://doi.org/10.7759/cureus.32746
22. Tsai YH, Lein PJ. Mechanisms of organophosphate neurotoxicity. Current Opinion in Toxicology. 2021 Apr 30;26:49–60. Available from: https://doi.org/10.1016/j.cotox.2021.04.002
23. Hołyńska-Iwan I, Szewczyk-Golec K. Pyrethroids: How they affect human and animal health? Medicina. 2020 Oct 30;56(11):582. Available from: https://doi.org/10.3390/medicina56110582
24. Luzardo OP, Almeida-González M, Ruiz-Suárez N, Zumbado M, Henríquez-Hernández LA, Meilán MJ, et al. Validated analytical methodology for the simultaneous determination of a wide range of pesticides in human blood using GC–MS/MS and LC–ESI/MS/MS and its application in two poisoning cases. Science & Justice. 2015 May 9;55(5):307–15. Available from: https://doi.org/10.1016/j.scijus.2015.04.007
25. Anastassiades M, Lehotay SJ, Štajnbaher D, Schenck FJ. Fast and easy multiresidue method employing acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase extraction" for the determination of pesticide residues in produce. Journal of AOAC International. 2003 Mar 1;86(2):412–31. Available from: https://doi.org/10.1093/jaoac/86.2.412
26. Lehotay SJ, O'Neil M, Tully J, García AV, Contreras M, Mol H, et al. Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study. Journal of AOAC International. 2007 Mar 1;90(2):485–520. Available from: https://doi.org/10.1093/jaoac/90.2.485
27. Wilkowska A, Biziuk M. Determination of pesticide residues in food matrices using the QuEChERS methodology. Food Chemistry. 2010 Oct 8;125(3):803–12. Available from: https://doi.org/10.1016/j.foodchem.2010.09.094
28. Acosta-Dacal, A., Rial-Berriel, C., Díaz-Díaz, R., Del Mar Bernal-Suárez, M., & Luzardo, O. P. (2021). Optimization and validation of a QuEChERS-based method for the simultaneous environmental monitoring of 218 pesticide residues in clay loam soil. The Science of the Total Environment, 753, 142015. https://doi.org/10.1016/j.scitotenv.2020.142015
29. Da Silva MC, Lanaro R, Cabrices OG, Costa JL. A modified Micro-QUECHERS approach for the determination of pesticides found in forensic and clinical blood samples using LC-MS/MS. In: Methods in pharmacology and toxicology. 2021. p. 157–65. Available from: https://doi.org/10.1007/978-1-0716-1928-5_9
30. Dulaurent S, Lachâtre G, Gaulier J m. O28: Use of QuEChERS salts: Biological and forensic toxicological applications. Annales De Toxicologie Analytique. 2014 Jun 1;26(2):S17. Available from: https://doi.org/10.1016/s2352-0078(14)70036-8
31. Santana-Mayor, A., Rodríguez-Ramos, R., Herrera-Herrera, A., Socas-Rodríguez, B., & Rodríguez-Delgado, M. (2023). Updated overview of QuEChERS applications in food, environmental and biological analysis (2020–2023). TrAC Trends in Analytical Chemistry, 169, 117375. https://doi.org/10.1016/j.trac.2023.117375

32. Kus S, Marczenko Z, Obarski N. Derivative UV-Vis spectrophotometry in analytical chemistry. Chemia Analityczna. 1996 Jan 1;41(6):899–929. Available from: http://www.chem.uw.edu.pl/chemanal/PDFs/1996/CHAN1996V0041P00899.pdf
33. Kaur G, Yadav PK, Bumbrah GS, Sharma RM. Derivative UV spectrophotometric detection of some common pesticides in simulated samples of beverages. Journal of Forensic Chemistry and Toxicology. 2019 Dec 15;5(2):93–106. Available from: https://doi.org/10.21088/jfct.2454.9363.5219.1
34. Thula KC, Patel DM, Maheshwari DG. Development and validation of first order derivative UV spectrophotometric method for simultaneous estimation of nebivolol and cilnidipine in pharmaceutical formulation. International Journal of Pharmaceutical Sciences Review and Research. 2015;31(1):243–7. Available from: https://www.globalresearchonline.net
35. European Medicines Agency (EMA). ICH Q2(R2) Guideline on validation of analytical procedures – Step 5 (Revision 1). Amsterdam: EMA; 2023. [cited 2025 Oct 28]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q2r2-guideline-validation-analytical-procedures-step-5-revision-1_en.pdf
36. Koba M, Koba K, Pryborowski L, Polish Pharmaceutical Society. APPLICATION OF UV-DERIVATIVE SPECTROPHOTOMETRY FOR DETERMINATION OF SOME BISPHOSPHONATES DRUGS IN PHARMACEUTICAL FORMULATIONS. Acta Poloniae Pharmaceutica Ñ Drug Research. Polish Pharmaceutical Society; 2008.
37. Venugopal NVS, Sumalatha B. UV-Spectrophotometric Determination of Acephate and Profenofos (organophosphate) Pesticides in Buffer Medium. Asian Journal of Research in Chemistry. 2012 Apr 28;5(4):526–8. Available from: https://ajrconline.org/HTMLPaper.aspx?Journal=Asian%20Journal%20of%20Research%20in%20Chemistry;PID=2012-5-4-16
38. Sahu DK, Rai J, Bhatt C, Rai MK, Goswami J, Sahu AK, et al. UV-Visible spectrophotometric determination of Lambda-Cyhalothrin insecticide in vegetables, soil and water samples. Journal of Ravishankar University (Part-B). 2019 Sep 21;31(1):1–9. Available from: https://doi.org/10.52228/jrub.2018-31-1-1
39. Verma K, Bhattu M, Kathuria D, Verma M. U.V. Spectrophotometric Method towards the Selective Detection of Imidacloprid Using Organic Nanoparticles of (E)-(4-nitrophenyl)-1,1-diamino-2,3-diazabuta-1,3-diene. E3S Web of Conferences. 2024 Jan 1;509:01008. Available from: https://doi.org/10.1051/e3sconf/202450901008
40. Sahu DK, Rai J, Rai MK, Banjare MK, Nirmal M, Wani K, et al. Detection of flonicamid insecticide in vegetable samples by UV–Visible spectrophotometer and FTIR. Results in Chemistry. 2020 Jan 1;2:100059. Available from: https://doi.org/10.1016/j.rechem.2020.100059
41. Zalat OA, Elsayed MA, Fayed MS, Abd El Megid MK, Department of Chemical Engineering, Military Technical College, Kobry Elkobbah, Cairo, Egypt. Validation of UV spectrophotometric and HPLC methods for quantitative determination of chlorpyrifos. International Letters of Chemistry Physics and Astronomy. 2013 Nov.