ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 15, Issue, 12, pp.26573-26575, December, 2023
DOI: https://doi.org/10.24941/ijcr.46315.12.2023

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

REVIEW ARTICLE

LATEST STRATEGIES FOR THE ANALYSIS OF HAZARDOUS CHEMICAL RESIDUES IN MILK

Prabrisha Chatterjee[1] and Sanat Chatterjee[2]

[1]M. Tech in Biotechnology, Kalinga Institute of Industrial Technology (KIIT), Bhubaneswar, Odisha, India;
[2]Associate Professor, The Neotia University, West Bengal, India

ARTICLE INFO

Article History:
Received 20th September, 2023
Received in revised form
27th October, 2023
Accepted 15th November, 2023
Published online 20th December, 2023

Key words:

Multi-residue detection, mycotoxins, QuEChERS, UPLC-QTOF-MS, SELEX, LC-QTOF-MS, pesticides, antibiotics.

***Corresponding author:**
Prabrisha Chatterjee

ABSTRACT

The excessive use and abuse of pesticides and veterinary drugs contaminate the food articles, which can threaten human health and environment. Therefore, there is an extremely urgent need for multi analysis techniques for the detection of pesticide and veterinary drug residues, which can be applied as screening techniques for food safety monitoring and detection. Milk is contaminated with hexachlorocyclohexane (HCH), dichloro-diphenyltrichloro ethane (DDT), endosulfan, cypermethrin, cyhalothrin, permethrin, chloropyrifos, ethion, and profenophos pesticides. Milk is also contaminated by chlorinated pesticides, organophosphates, herbicides, fungicides, antihelminthetic drugs, antiobiotics, hormones, detergents, disinfectants, nitrites, polychlorinated biphenyls, dioxins. Problems associated with antibiotic residues in milk include the risk of allergic reactions and occurrence of antibiotic-resistant bacteria, teratogenicity risk to the foetus, hypoplasia in developing teeth, aplasia of bone marrow, chronic insidious intake lead to elevated cancer risk and disruption of body's reproductive, immune, endocrine and nervous system.

Citation: ***Prabrisha Chatterjee and Sanat Chatterjee.*** **2023.** "Latest strategies for the analysis of hazardous chemical residues in milk*".* *International Journal of Current Research*, 15, (12), 26573-26575.

INTRODUCTION

Pesticides are indispensable for increasing food production as well as improving animal health and aquaculture. Pesticides are widely applied in modern agriculture for controlling weeds, pests, and plant diseases. Veterinary drugs are commonly administered in farming practices for the prevention of diseases. Excessive use and abuse of pesticides and veterinary drugs can lead to health hazards and contaminate the environment. The presence of pesticide residues remain in food articles are considered a worldwide public health hazards and one of the foremost causes of issues related international trade Therefore, latest and effective detection methods for residues in milk have been well established. Being a good source of proteins, fat and minerals, bovine milk is an essential component in the diet of humans of all ages. India's Diary production is about 164 million tonnes in 2016-17 and milk is India's largest crop worth around USD 90 billion which is much more than rice and wheat put together, with higher export potential. Long term exposure to pesticides may cause kidney and liver problems, disruption of endocrine system, neurological and immune system disorders and higher risk of lungs, breast, cervix, and prostate cancer. According to Richhariya*et al.* (2017), Indian milk is contaminated with Organochlorine Pesticides (Lindane, Dieldrin, DDT, Heptachlor, and Endosulfan), Organophosphorus Pesticides (Fenitrothion, Fenthion, 2,4-D, and Atrazine), Carbamate Pesticides (Aldicarb, Carbaryl, and Propoxur), and Synthetic Pyrethroids (Cypermethrin, Detlamethrin, and Permethrin). Indira Debi *et al*. (2022) corroborated that The Food and Agriculture Organization of the United Nations (FAO) data showed that out of the 4.2 million tonnes of pesticides used annually in 2019, herbicides topped the list with 53%, followed by fungicides and bactericides (23%), and insecticides (17%). The top five pesticide consuming countries in the world in 2019 were China, the USA, Brazil, Argentina, and USSR. Organochlorine Pesticides are considered as possible carcinogens. It is also associated with Parkinson Disease, Type-II Diabetes. Fat soluble pesticides are accumulated in animal tissues and are excreted into milk but not into urine. Following pesticides have been detected by Akhtar and Ahad (2017) are DDT, Larvin, Anifose, and Methomyl. Currently there are more than 1100 pesticides registered in the European Union (EU) Market. Bedi *et al.* (2015) estimated that bovine milk from Punjab, India is highly contaminated with chlorpyrifos, DDT, γ-HCH, endosulfan, cypermethrin, cyhalothrin, fenvalerate, deltamethrin, malathion, profenofos, and ethion. Aytenfsu *et al.* (2016) found following dreaded chemicals in milk, viz., chlorinated pesticides, organophosphates, herbicides, fungicides, antihelminthetic drugs, antibiotic, detergents, nitrites, polychlorinated biphenyls, dioxins, mycotoxins, heavy metals, and somatotropin hormone. The use of antibiotics therapy to treat and prevent udder infections in cows is a key component of mastitis control in many countries. Unauthorized antibiotic use may result in residues of these substances in milk. During 1962, a marine biologist Rachel Carson wrote an extraordinary beautiful book, "*Silent Spring*"which is the first authenticated book in the world against pesticide residues in the environment and food. After that, the USA Government banned twelve pesticides including DDT in 1972.Régo *et al.* (2019) reported from Brazil that commercial milk are contaminated with persistent organic pollutants(POP), organochlorine pesticides (OCP), polychlorinated biphenyls (PCB), and poly-aromatic hydrocarbons

(PAH). They also found OCPs like DDT, Hexachlorobenzene (BHC), Aldrin, Dieldrin, Heptachlor and Toxaphene. Most of the Veterinary treatment of dairy cattle involves intra-mammary infusion of antibiotics for the management of mastitis. Some drugs apply to control endoparasites, ectoparasites, and several illnesses and to increase milk production. Antihelminthic drug, albendazole is readily absorbed from the gut and rapidly transformed to various metabolites, the major metabolites being albendazolesulfoxide, albendazolesulfone, and albendazole 2-amino sulfone. These metabolites have been found in milk and dairy products.

Bovine Growth Hormone (BST): Bovine Growth Hormone or Bovine Somatotropin (BST), which is administered to animals, is a genetically engineered protein hormone either identical or similar to the natural Bovine Growth Hormone produced by pituitary gland. Its primary function is to increase milk production of dairy lactating cattle which can increase milk production in cows between 10 to 15%. BST increases activity of mammary secretory cells, probably via Insulin-like Growth Factor (IGF)-I produced by the liver or the mammary gland. IGF-I is not destroyed by the pasteurization but the heating of milk for the preparation of infant formula denatures IGF-I significantly.

Mycotoxins: Some of the fungus produces various toxic metabolites under appropriate temperature and moisture conditions. These metabolites, which may be hazardous for human health, called mycotoxins. Catheyet *al.* (1994) detected that Aflatoxin M-1 (AFM-1) found in the milk of animals that fed with Aflatoxin B-1 (AFB-1) containing feed. AFM-1, metabolite of AFB-1 occurs in liver and it secretes into milk in the mammary gland of dairy cows. International Agency for Research on Cancer (IARC) of WHO included AFB-1 as primary and AFM-1 as secondary groups of carcinogenic compounds. Heat treatments like pasteurization were not effective in the reduction of formation of AFM-1. It is mainly found in cheese samples because of its affinity to casein fraction in milk and due to the water solubility of this toxin lower level of AFM-1 found in cream and butter.

Nitrates and Nitrites: Nitrates and Nitrites are chemicals used in fertilizers, in rodenticides, and as food preservatives. WHO during 2006 reported that nitrates and nitrites come in various forms, but when dried are typically a white or crystalline powder. Nitrates (NO_3-) and Nitrite (NO_2-) are also naturally occurring compounds that are a metabolic product of microbial digestion of wastes containing nitrogen, for example, animal faeces or nitrogen based fertilizers. The use of nitrate fertilizer on crops that result in higher concentration in some crop residue of animal feed.

Heavy Metals: Heavy metals enter the human and animal body mainly by the routes of inhalation and ingestion. With increase in environmental pollution, a heavy metal exposure assessment study is necessary. Licata *et al.* (2004) reported that heavy metals produce toxic effects by replacing essential metal ions existing in the chelates present in our body. Lead (Pb), and Cadmium (Cd) are toxic for human and children are more sensitive to these heavy metals than adults. Copper (Cu) and Zinc (Zn) are essential micronutrients and have a variety of biochemical functions in all living organisms. While Cu and Zn are essential micronutrients, they can be toxic if taken in excess; both toxicity and necessity vary from element to element. Milk is the fundamental food for infants and the daily intake of heavy metals lead, cadmium, copper, zinc can be determined by different age groups of infants through different milk products and baby foods.

Analytical Methodologies For Chemical Residues: Analytical data quality is a key factor for the success of the program dealing with residues in food stuffs. There are varieties of methods for identifying, confirming, and quantifying analytes, which can be used individually or coupled to each other in a suitable way. These methods grouped into Bioassay, Microbial Assay, Immunochemical Assay, and Physicochemical

Bioassay: Biological methods for determining toxic residues in food stuffs can be used both *in-vivo* and *in-vitro*.

The mouse bioassay is the most used one and accepted by the regulating body. FAO during 2004 published a paper regarding bioassay that a toxin extractsintraperitoneally injected into mice having around 20 gram body weights in the mouse bioassay and their survival has been monitored from 24-48 hours. One mouse unit (MU) defined as being the minimum quantity of toxin needed to kill a mouse within 24 hours. Sample toxicity (MU/g whole tissue) is determined from the smallest dose at which two mice or more in a group of three die within 24 hours. The regulatory level is set at 0.05 MU/g whole tissues in many countries.

Microbial Assay: The microbial methods used for detecting antimicrobial residues in food stuffs based on inhibiting microbial growth, microbial receptor activity and enzymatic reactions and applied to all types of matrix usually milk, meat, eggs, and honey. Maria and Mary (2012) described microbial inhibition methods involving culture of micro-organisms from a standard strain, usually *Bacillus stearothermophilus, Bacillus subtilis, Bacillus sereus, Micrococcus luteus, Escherichia coli, Bacillus megatherium, Sarcinalutea, Streptococcus thermophiles.* The analysed milk sample applied on the agar surface either directly or with a paper disk called Disk assay Plate Methods. In the course of incubation, the diffusion of the sample into the medium takes place (the Agar Diffusion Principle), and if the sample contains inhibitor agents, reduction or total inhibition occurs of the tasted micro-organism growth. Depending on the method used the presence of inhibitor agents in the tested sample indicated by the formation of a clear zone of inhibition around the disk.

Immunochemical Assay: Accurate determination of trace target analytes in complex food material is a major challenge for the development of rapid detection methods. Enzyme Linked Immunosorbent Assay (ELISA) is a routine technique for the detection of pesticides and veterinary drug residues in food and environmental samples. Generic antibodies are generated by immunization with general-structure haptens, which exhibit the common features of an entire class of related analytes. Zhao *et al.* (2015) synthesized eight kinds of O,O-diethyl O-(3-carboxyphenyl) phosphorothioate with different carboxy groups in the meta position of the benzene ring, in order to prepare immunogens or coating antigens. Due to low molecular weight of the target analytes, Competitive ELISA is commonly used for pesticide and veterinary drug residue determination. Li *et al.* (2018) have identified a monoclonal antibody for five antibacterial synergist, 5C4, with uniform broad specificity and high affinity was obtained and used to develop an Indirect Competitive ELISA (icELISA) method. Zhao, G.M. *et al.* (2018) has identified a lateral flow immunoassay (LFIA) is another conventional immunochemical method, which has been widely used for the on-site detection of residues. Shi *et al.* (2018) applied AuNPs conjugated to 4-aminothiophenol as a surface-enhanced Raman scattering (SERS) label to develop a SERS based multiple LFIA for the simultaneous detection of neomycin and quinolones antibiotics. Song *et al.* (2015) has quantisized quantum dots (QDs) and fluorophores with different emission wavelengths can also be used in multi-label strategies. A multi-coloured QD-based microtiter plate array analysis has been used for the sensitive and visual detection of streptomycin, tetracycline and penicillin G in milk. Aptamers serve as an ideal alternative to an antibody for the analysis of pesticide and veterinary drug residues. Alkhamis*et al.* (2019) has developed aptamers which are single-stranded oligonucleotides (RNA or DNA) selected by the systemic evolution of ligands by the exponential enrichment (SELEX) method. Yoo *et al.* (2020) has quantified 18 anthelmintics (Closantel, Nitroxynil, Niclosamide, Rafoxanide, Eprinomectin, Emamectin, Levamisole, Cymiazole, Praziquantel, Tetramisole, Thiophanate, Morantel, Pyrantel, Fluazuron, Guaifenesin, Carbendazim, Cambendazole, and Tricholfon.) with the help of liquid chromatography-tandem mass spectrometry (LCMS). Zhang *et al.* (2015) have developed a simple and rapid multiclass multi residue analytical method for the screening and quantification of veterinary drugs in milk by ultra-performance liquid chromatography coupled to quadrupole time- of- flight mass spectrometry (UPLC-QTOF-MS). A total of 90 veterinary drugs have

been investigated belong to almost 20 classes including lincomycins, macrolides, sulphonamides, quinolones, tetracyclines, β-agonist, β-lactams, sedatives, β-receptor antagonists, sex hormones, glucocorticoids, nitroimidazoles, benzimidazoles, and nitrofurans. The linearity, sensitivity, accuracy, repeatability, and reproducibility of the method were fully validated. To ensure human food safety, the European Union has set a maximum residue limit (MRL) of 4 to 30 microgram/kg of penicillins, 20 to 100 microgram/kg of cephalosphorins, 30 to 100 microgram/kg of quinolones, and 100 microgram/kg for sulphonamides (SA) in milk. Liquid chromatography-quadrupole-time-of-flight-mass spectrometry (LC-QTOF-MS) was used for the analysis of sulfamethazine, flunixin, cephapirin, and enrofloxacin. However, the sensitivity of these methods needs to be improved further for trace residue analysis. Despite the advances in separation and detection techniques, sample extraction is still cornerstone of the analytical process, and effective sample preparation is essential to achieve reliable results and maintain instrument performance. Because milk is a complex matrix due to its high protein and fat content, which often interfere in analytical procedures, special attention is to be focused on sample preparation and extraction. To simplify the sample pre-treatment procedure, the quick, easy, cheap, effective, rugged and safe (QuEChERS) method is a streamlined approach that makes it easier and less expensive for analytical chemist to examine pesticide residues in milk. It should be pointed out that QuEChERS method needs to be modified further, including the type and amount of extraction solvent, the appropriate acid, salting out procedure, and the type and amount of sorbent, for the analysis of multi-residue, multiclass veterinary drugs with physical and chemical performances in complex matrices.

CONCLUSION

The residues of pesticides and veterinary drugs threaten human health and environment. Therefore, several screening assays have been developed for the rapid multi-residue detection of pesticides and veterinary drugs. With the development of genetic engineering and computer modelling technology, recognition elements with the desired broad specificity and high affinity to multiplex target can be designed and obtained. The modified QueChERS procedure was quick, easy, cheap, effective, rugged, and safe and no further clean up steps were necessary. Furthermore, utilizing the UPLC technology shortened analysis times for all analytes. The UPLC-QTOF-MS offers the best performance for screening purposes and can also effectively provide concentration values. It is accurate enough to differentiate between positive and negative samples or drug concentration s below or above the MRL (Maximum Residue Level)

Ethics Approval And Consent To Participate: We have maintained the human and animal ethics in our manuscript as per as we concern. We are hereby giving our full consent for participating in any kind of controversy.

Consent For Publication: We are giving our full consent for publication of our manuscript in this reputed journal.

Availability of data and materials: We have collected all the data and materials from all the renowned review papers.

Competing Interest: We hereby declare that there is no competing interest

Funding: We have not received any funding from any institution.

Author Contributions

Conceptualization, Investigation, Resources, Original Draft Preparation: P. Chatterjee. Review and Editing, Supervision: S. Chatterjee.

ACKNOWLEDGEMENTS

We are deeply indebted to Shri Achyuta Samanta, founder of Kalinga Institute of Industrial Technology, Bhubaneswar, Odisha, India.

REFERENCES

Akhtar, S and Ahad, K. 2017. Pesticides Residue in Milk and Milk Products: Mini Review. *Pak. J.Anal.Environ.Chem*.18(1):37-45.

Alkhamis. O. *et al.* ., 2019. Innovative engineering and sensing strategies for aptamer-based small-molecule detection. *Trse.Trends Anal.Chem*.121: 115699.

Aytenfsu, S. *et al.* ., 2016. Review on chemical Residues in Milk and their Public Health Concern in Ethiopia. *J.Nutr.Food Sci*. 6(4).

Bedi, J.S. *et al.* Pesticide Residues in Bovine Milk in Punjab, India: Spatial Variation and Risk Assessment to Human Health (2015). *Arch Environ ContamToxicol.*

Cathey, C.G. *et al.* 1994. Development and Evaluation of a Minicolumn Assay for the detection of Aflatoxin M-1 in Milk. *JDairy Sci*. 77:1223-1231.

Fao. Marine Biotoxins. 2004. Fao Food and Nutrition Paper 80, Rome, Italy.

Gill, J.P.S *et al.* ., 2020. Pesticide Residues in Peri-Urban Bovine Milk from India and Risk Assessment: A Multicenter Study. *Scientific Reports*.10: 8054.

Indira Devi, P. *et al*. 2022. Agrochemicals, Environment, and Human Health. Annu. Rev. Environ. Resour. 47 : 399- 421.

Jeong, I. *et al.* 2012. Determination of pesticide residues in milk using a QuEChERS-based method developed by response surface methodology. *Food Chemistry*. 133:473-481.

Jia, M. *et al.* ., 2020. Rapid Multi-Residue Detection Methods for Pesticides and Veterinary Drugs. *Molecules*.25:3590.

Li, H. et.al. 2018. Generic Hapten Synthesis, Broad-Specificity Monoclonal Antibodies Preparation, and Ultrasensitive ELISA for Five Antibacterial Synergists in Chicken and Milk. *J.Agric.Food Chem*. 66: 11170-11179.

Licata, P. *et al.* ., 2004. Labels of "Toxic" and " Essential" Metals in Samples of Bovine Milk from Various Dairy Farms in Calabria, Italy. *Environ Int*30: 1-6.

Maria, CL and Mary, T. 2012. Chemical Residues in Animal Food Products: An Issue of Public Health, Public Health Methodology, Environmental and System Issues. National University of Columbia, Columbia 51: 641-648.

Régo, I.C.V. *et al.* ., 2019. Organochlorine Pesticides Residues in Commercial Milk: A Systematic Review. *ActaAgronómica*. 68(2).

Richhariya, N. *et al.* ., 2017. Pesticide Residues Contamination of Liquid milk in India- a Review. *World Journal of Pharmacy and Pharmaceutical Sciences*.6(4):549-562.

Shi, Q. eta al. 2018. A SERS-based multiple immune-nanoprobe for ultrasensitive detection of neomycin and quinolone antibiotics via a lateral flow assay. *Microchim.Acta*.185:84.

Song, E *et al.* 2015. Multi-colour quantum dot-based fluorescence immunoassay array for simultaneous visual detection of multiple antibiotic residues in milk. *Biosens.Biolectron*.72:320-325.

Stachniuk, A. and Fornal, E. 2016. Liquid Chromatography-Mass Spectrometry in the Analysis of Pesticide Residues in Food. *Food Analytical Methods*.9:1654-1665.

WHO International Program on Chemical Safety, Environmental Health Criteria 5: Nitrates, Nitrites and N-Nitroso Compounds (2006). Geneva, Switzerland.

Yoo, K. *et al.* 2020. Development of an analytical method for multi-residue quantification of 18 anthelmintics in various animal-based food products using liquid chromatography-tandem mass spectrometry (2020). *Journal of Pharmaceutical Analysis*.

Zhang, Y. *et al.* 2015. Multi-class, multi-residue analysis of trace veterinary drugs in milk by rapid screening and quantification using ultra-performance liquid chromatography-quadrupole time-of- flight mass spectrometry.

Zhao, F. *et al.* 2015. Development of Mab-Based Immunoassay for the Simultaneous determination of O, O-diethyl and O, O-dimethyl Organophosphorus Pesticides in Vegetable and Fruit samples Pretreated with Qu EChERS. *Anal. Bioanal.Chem*. 407: 8959-8970.