ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(3):
Pg. 979-983

Brief communication

Advancements in Sample Preparation Techniques for Quantitative Pharmaceutical Detection in Biological Samples

MAHAVIR M. SHARMA[1]*, HARSHKUMAR BRAHMBHATT[1] and ASHIM KUMAR SEN[2]

[1*,2]Department of Pharmacy, SumandeepVidhyapeeth Deemed to be University, Vadodara, Gujrat, India.
*Corresponding author E-mail: mahavir.svp@gmail.com

http://dx.doi.org/10.13005/ojc/410331

(Received: January 11, 2025; Accepted: May 23, 2025)

ABSTRACT

Researchers have recently shifted their attention to the bio-analysis of pharmaceuticals as a result of the growing demand for medication development and the growing worry about the presence of pharmaceuticals in the environment. The very low quantities of medicines in biological samples and the complicated structure of sample matrices provide substantial obstacles, nevertheless. As a result, a lot of work has gone into finding solutions to these problems, mostly by improving methods of sample preparation. High throughput, automated, on-site, non-invasive, and in vivo analysis focusing sample preparation techniques is a major growing trend. This trend has substantial implications for both biomedical and environmental analyses. This review explores recent progress in sample preparation techniques over the past decade for the quantitative bio-analysis of pharmaceuticals. The discussion includes an overview and summary of these advances, along with a comparative analysis of the merits associated with each sample preparation technique. Additionally, the review addresses innovative approaches such as electro membrane extraction and micro fluidic devices. Furthermore, the focus extends to future prospects, emphasizing sample preparation techniques with the potential for in vivo pharmaceutical analysis. The discussion of future trends highlights aspects such as miniaturization, high specificity, and portability, underscoring the ongoing evolution of sample preparation methodologies in the field of pharmaceutical analysis.

Keyword: Bio-analysis, Biological samples, Principle of solubility, Immiscible phases.

INTRODUCTION

Bio-analysis of pharmaceuticals, which involves pharmaceutical analysis in biological samples, includes studying in vivo concentration, metabolic pathways, pharmacodynamics, pharmacokinetics, point-of-care testing, and pharmaceutical sites of action. It also includes studying single-cell pharmacology. This analytical method is essential for drug discovery, therapeutic drug monitoring, and pharmaceutical efficacy assessment since it increases our knowledge of how drugs are taken in, distributed, metabolised, and excreted by living organisms.[1-2]

Although there is no denying the usefulness

of pharmacological analysis in biomedicine, the growing demand for medicines is causing serious problems for the environment. Soil, groundwater, and drinking water are contaminated with pharmaceuticals when they reach the environment by many means, including effluent from the pharmaceutical sector, home sewage, and breeding water.[3] Toxic effects on ecosystems caused by these pharmaceutical pollutants may interfere with how creatures normally go about their lives, which in turn can affect human health as they make their way up the food chain.[4] Disposal of medical waste, which includes disinfection chemicals and medications, also poses additional environmental dangers and ecological damage within the context of the COVID-19 pandemic. To assess the safety of medicines, it is essential to study their behaviour in the environment and their biological consequences. In addition to improving our knowledge of pharmacodynamics and pharmacokinetics, bio-analysis of medicines helps us investigate the hazards, environmental fates, and toxicological impacts of pharmaceuticals.[5-8]

Pharmaceutical analysis relies on the accurate measurement of target pharmaceuticals from complex biological matrices, which is a tough but vital task. Contaminated equipment, impaired sensitivity, and impacted sampling and extraction devices are all possible outcomes of sample matrix interference.[9] Efforts have so far concentrated on refining methods of sample preparation, such as isolation and preconcentration. Because it takes up a considerable chunk of the overall time for quantitative analysis, selecting suitable sample preparation procedures is crucial.

Despite the long-standing prominence of liquid-liquid extraction (LLE) and solid-phase extraction (SPE) in pharmaceutical bioanalysis, new methods have evolved to improve sensitivity, streamline procedures, and tackle new challenges. These methods include liquid-phase microextraction (LPME), micro-dialysis (MD), solid-phase micro extraction (SPME), and microfluidic devices. More practical and eco-friendly LLE and SPE techniques are also appearing in the modern day.[10-13] Methods for pharmaceutical bioanalysis that are non-invasive, on-site, automated, and have a high throughput are currently trending. Discussing their evolution, relative strengths in pharmaceutical quantitative analysis, and showcasing innovative methods for in vivo analysis, this paper offers a thorough outline of sample preparation procedures for pharmaceutical bioanalysis. Miniaturisation, high specificity, and mobility are some of the future possibilities that are discussed.

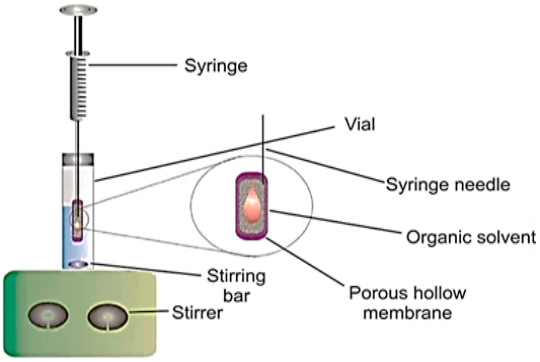

Fig. 1. Liquid-phase microextraction technique

Section snippets[14-16]

Introduction to Pharmaceutical Analysis in Biological Samples: Research into the concentration of medicines in living organisms, metabolic pathways, pharmacodynamics, pharmacokinetics, single-cell pharmacology, point-of-care diagnostics, and sites of action is all part of bioanalysis, the study of pharmaceuticals in biological samples. Medication development, therapeutic drug monitoring, and the assessment of pharmacological efficacy rely heavily on this analytical method.

Environmental Implications of Pharmaceutical Consumption: Domestic sewage, wastewater from the pharmaceutical sector, and breeding water are the main entry points for these compounds into the environment, which poses environmental concerns because to the increased usage of medications. Polluting soil, groundwater, and drinking water sources has a domino effect on ecological toxicity, which in turn may harm creatures and humans via the food chain. The disposal of medical waste during the COVID-19 pandemic further exacerbates environmental risks.

Significance of Bioanalysis in Both Biomedical and Environmental Fields: Toxicological effects, environmental fates, and dangers associated with medications may be better understood using bioanalysis, which also improves our knowledge of pharmacodynamics and pharmacokinetics. It serves as a bridge between biomedical and environmental

fields, addressing both human health concerns and environmental sustainability.

Challenges in Quantification and Sample Preparation: Accurate quantification of target pharmaceuticals from complex biological matrices presents challenges, including interference from sample matrix constituents that affect sampling and extraction devices. Sample preparation techniques, such as preconcentration and isolation, become essential in overcoming these challenges, considering their significant contribution to the overall quantitative analysis time.

Evolution of Sample Preparation Techniques: Although solid-phase extraction (SPE) and liquid-liquid extraction (LLE) have been widely used, they have limitations including slow processing times and poor sensitivity. To overcome these disadvantages, new methods such as microfluidic devices, solid-phase microextraction (SPME), micro dialysis (MD), and liquid-phase microextraction (LPME) been evolved. Modern LLE and SPE methods are also adapting to be more convenient and environmentally friendly.

Trends in Pharmaceutical Bioanalysis: Methods for preparing samples that are non-invasive, on-site, automated, and have a high throughput are currently trending. With a focus on enhancing sensitivity, streamlining methods, and solving growing obstacles, these improvements are essential for the development of pharmaceutical bioanalysis.

Future Directions and Prospects: The review anticipates future trends, highlighting the development of sample preparation techniques for in vivo pharmaceutical analysis. Prospects include miniaturization, high specificity, and portability, reflecting the ongoing evolution of methodologies in pharmaceutical analysis.

Liquid-liquid extraction (LLE)[17-19]

Analytical chemists and bioanalyses often use the sample preparation method of liquid-liquid extraction (LLE). It is employed to separate a target compound or compounds from a liquid mixture by exploiting differences in their solubilities in two immiscible liquid phases. Typically, the immiscible liquids involved are an organic solvent and an aqueous phase.

Here's an overview of the key features and steps involved in liquid-liquid extraction:

Key Features

Principle of Solubility

LLE is based on the principle that different compounds have varying solubilities in different solvents.[20]

The choice of solvents is crucial to ensure that the target compound preferentially partitions into one of the liquid phases.

Immiscible Phases

Two immiscible liquid phases are utilized, commonly an organic solvent and an aqueous phase.

The organic solvent is chosen based on its ability to dissolve the target compound, while the aqueous phase may contain impurities or interfering substances.[21]

Partitioning Process

The sample is mixed with the two immiscible phases.

The target compound partitions between the organic and aqueous phases based on its solubility in each.

Separation

After sufficient mixing, the two liquid phases are allowed to separate into distinct layers due to their immiscibility.

The layer containing the target compound is then isolated for further analysis.

Steps Involved

Extraction

The sample is mixed with the organic and aqueous phases.

The target compound selectively moves into one of the phases based on its solubility.

Separation

The mixture is allowed to settle, and the organic and aqueous phases separate into distinct layers.

Collection

The layer containing the target compound (organic phase or aqueous phase, depending on solubility) is carefully collected.

Additional Wash or Back Extraction (Optional)

Further purification steps may be employed, such as washing the collected phase or performing a back extraction to transfer the target compound into a different solvent.

Concentration and Analysis

Before analysing the collected phase using analytical methods like chromatography or spectrometry, it is common practise to evaporate it in order to concentrate the desired chemical.[22]

Advantages

Simple and widely applicable

Effective for the extraction of a wide range of compounds.

Versatile in adapting to different sample types.

Drawbacks

Requires careful optimization of solvent choice and extraction conditions.

Time-consuming compared to some modern techniques.

May involve the use of large volumes of organic solvents, rising environmental and safety concerns.

Liquid-liquid extraction remains a valuable tool in analytical chemistry, especially when dealing with complex sample matrices or when specific selectivity is required for target compound isolation.[23]

The principle behind liquid-liquid extraction (LLE), a popular sample preparation procedure in pharmaceutical bioanalysis, is that analytes have distinct solubilities in two solvents that are incompatible with one another. Consequently, the most important part of LLE is choosing the extractants. Common extractants used in LLE of biological samples include ethyl acetate, methanol, and chlorinated alkanes. One example is the anthelmintic medication that He et al. extracted using ethyl acetate.[24]

CONCLUSION

In conclusion, the versatility of classic sample preparation methods like linear gradient electrophoresis (LLE) and solid phase extraction (SPE) makes them well-suited for a wide range of pharmaceutical analytical applications. Additionally, the device's enrichment and purification capacities may be significantly enhanced by the development of custom-made SPE materials. Conventional LLE, on the other hand, is laborious, takes a long time to prepare samples, and uses a lot of organic solvents.

ACKNOWLEDGMENT

We extend our heartfelt gratitude to all authors, whose contributions made this review article possible. We are particularly thankful to Mr. Mahavir M. Sharma for their valuable insights, feedback, and encouragement throughout the preparation of this manuscript.

We also wish to acknowledge Mr. Mahavir M. Sharma for their financial support, which facilitated this research and writing process. Additionally, we express our appreciation to Sumandeep Vidyapeeth deemed to be university that provided access to resources critical for the comprehensive exploration of the subject.

Finally, we thank the anonymous reviewers for their constructive comments and suggestions, which have greatly enhanced the quality of this work.

Conflict of interest

No any Conflict of interest

REFERENCES

1. Bylda C.; Thiele R.; Kobold U.; Volmer DA.; Recent advances in sample preparation techniques to overcome difficulties encountered during quantitative analysis of small molecules from biofluids using LC-MS/MS., *Analyst.*, **2014**, *139*, 2265–2276.
2. Medvedovici A.; Bacalum E.; David V. Sample preparation for large-scale bioanalytical studies based on liquid chromatographic techniques., *Biomed Chromatogram.*, **2018**, *32*.

3. Vaghela A.; Patel A.; Patel A.; Vyas A.; Patel N. Sample preparation in bioanalysis: A review. *Int J Sci Tech Res.*, **2016**, *5*, 6–10.
4. Kohler I.; Guillarme D. Multi-target screening of biological samples using LC-MS/MS: Focus on chromatographic innovations., *Bioanalysis.*, **2014**, *6*, 1255–1273.
5. Lopes BR.; Barreiro JC.; Cass QB. Bioanalytical challenge: A review of environmental and pharmaceuticals contaminants in human milk., *J Pharm Biomed Anal.*, **2016**, *130*, 318–325.
6. Deda O.; Gika HG.; Wilson ID.; Theodoridis GA. An overview of fecal sample preparation for global metabolic profiling., *J Pharm Biomed Anal.*, **2015**, *113*, 137–150.
7. Niu Z.; Zhang W.; Yu C.; Zhang J.; Wen Y. Recent advances in biological sample preparation methods coupled with chromatography, spectrometry and electrochemistry analysis techniques., *TrAC Trends Anal Chem.*, **2018**, *102*, 123–146.
8. Nunes de Paiva MJ.; Menezes HC.; de Lourdes Cardeal Z. Sampling and analysis of metabolomes in biological fluids., *Analyst.*, **2014**, *139*, m3683–3694.
9. Rosenthal TB. The effect of temperature on the pH of blood and plasma *in vitro.*, *J Biol Chem.*, **1948**, *173*, 25–30.
10. Fernández-Peralbo MA.; Luque de Castro MD. Preparation of urine samples prior to targeted or untargeted metabolomics mass-spectrometry analysis., *TrAC Trends Anal Chem.*, **2012**, *41*, 75–85.
11. Inoue K.; Harada K.; Takenaka K.; Uehara S.; Kono M.; Shimizu T.; Takasuga T.; Senthilkumar K.; Yamashita F.; Koizumi A. Levels and concentration ratios of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers., *Environ Health Perspect.*, **2006**, *114*, 1179–1185.
12. Nunes LAS.; de Macedo DV. Saliva as a diagnostic fluid in sports medicine: Potential and limitations., *J Bras Patol Med Lab.*, **2013**, *49*, 247–255.
13. de Giovanni N, Fucci N. The current status of sweat testing for drugs of abuse: A review. *Curr Med Chem.*, **2013**, *20*, 545–561.
14. Wan JY.; Liu P.; Wang HY.; Qi LW.; Wang CZ.; Li P.; Yuan CS. Biotransformation and metabolic profile of American ginseng saponins with human intestinal microflora by liquid chromatography quadrupole time-of-flight mass spectrometry., *J Chromatogram* A., **2013**, *1286*, 83–92.
15. Roseboom IC.; Rosing H.; Beijnen JH.; Dorlo TP. Skin tissue sample collection, sample homogenization, and analyte extraction strategies for liquid chromatographic mass spectrometry quantification of pharmaceutical compounds., *J Pharm Biomed Anal.*, **2020**, 191.
16. Smith KM.; Xu Y. Tissue sample preparation in bioanalytical assays., *Bioanalysis.*, **2012**, *4*, 741–749.
17. Buszewski B.; Szultka M. Past, present, and future of solid phase extraction: A Review. *Crit Rev Anal Chem.*, **2012**, *42*, 198–213.
18. Alexovi M.; Dotsikas Y.; Bober P.; Sabo J. Achievements in robotic automation of solvent extraction and related approaches for bioanalysis of pharmaceuticals., *J Chromatogr B Analyt Technol Biomed Life Sci.*, **2018**, *1092*, 402–421.
19. Arthur CL.; Pawliszyn J. Solid-phase microextraction with thermal desorption using fused silica optical fibers., *Anal Chem.*, **1990**, *62*, 2145–2148.
20. Boyacı E.; Rodríguez-Lafuente Á.; Gorynski K.; Mirnaghi F.; Souza-Silva ÉA.; Hein D.; Pawliszyn J. Sample preparation with solid phase microextraction and exhaustive extraction approaches: Comparison for challenging cases., *Anal Chim Acta.*, **2015**, *873*, 14–30.
21. Vas G.; Vékey K. Solid-phase microextraction: a powerful sample preparation tool prior to mass spectrometric analysis., *J Mass Spectrom.*, **2004**, *39*, 233–254.
22. de Oliveira FM.; Scheel GL.; Augusti R.; Tarley CR.; Nascentes CC. Supramolecular microextraction combined with paper spray ionization mass spectrometry for sensitive determination of tricyclic antidepressants in urine., *Anal Chim Acta.*, **2020**, *1106*, 52–60.
23. Jalili V.; Barkhordari A.; Ghiasvand A. A comprehensive look at solid-phase microextraction technique: A Review of reviews. *Microchem J.*, **2020**, 152.
24. Matys J.; Gieroba B.; Jóźwiak K. Recent developments of bioanalytical methods in determination of neurotransmitters in vivo., *J Pharm Biomed Anal.*, **2020**, 180.