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Editor-in-Chief

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From Editor-in-Chief's Desk

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It is my pleasure to present Volume 1, Issue 3 of Advances in Multidisciplinary Health Research (AMHR). As our journal continues to grow, we are encouraged by the increasing number of high-quality submissions from researchers, clinicians, academicians, and healthcare professionals across diverse disciplines. This issue reflects our commitment to publishing scientifically rigorous, clinically relevant, and impactful research that contributes to advancing healthcare knowledge.

The current issue features a strong collection of original research articles, showcasing innovative methodologies, meaningful clinical findings, and evidence-based approaches that address important challenges in medicine, pharmacy, public health, nursing, dentistry, biotechnology, and allied health sciences. Each manuscript has undergone a thorough peer-review process to ensure scientific quality, integrity, and relevance.

We are particularly proud to highlight several outstanding contributions that exemplify excellence in research design, analytical rigor, and practical significance. These articles not only advance scientific understanding but also provide valuable insights for clinical practice, healthcare policy, education, and future research.

I extend my sincere appreciation to our authors, reviewers, editorial board members, and publishing team for their dedication and unwavering support in maintaining the highest standards of scholarly publishing. Their collective efforts continue to strengthen the credibility and global reach of AMHR.

As we move forward, we remain committed to fostering innovation, encouraging multidisciplinary collaboration, and providing an accessible platform for high-impact research that benefits the scientific community and society at large.

Dr. Anupama Koneru
Editor-in-Chief
Advances in Multidisciplinary Health Research (AMHR)

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EDITORIAL ARTICLE

Visionary leadership for 2030: Preparing health research for the next decade

Authors: Prof. Dr. Pramod Kumar Rajput
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Abstract:

Health research is entering a transformative era driven by artificial intelligence, real-world evidence, digital technologies, and interdisciplinary collaboration. However, technological advancement alone is insufficient without visionary leadership that ensures ethical governance, systems thinking, agility, and global equity. This editorial examines the evolving leadership competencies required to prepare health research for 2030. It highlights five foundational pillars: ethical stewardship of emerging technologies, systems-based leadership, strategic mentorship of future researchers, crisis preparedness through adaptive research ecosystems, and inclusive, equity-focused scientific practice. The article further advocates redefining research success beyond traditional bibliometric indicators to encompass meaningful societal and health system impact, while emphasising the critical role of academic journals in fostering responsible, transparent, and implementation-oriented scholarship. Cultivating visionary leadership today is essential for advancing trustworthy, ethical, and impactful health research in the coming decade.

Keywords:

Visionary leadership, Health research; Artificial intelligence; Research ethics; Systems thinking.

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Opening Perspective

Over the past decade, health research has undergone a profound transformation. Previously confined to disciplinary silos, it has now evolved into interconnected, data-driven ecosystems facilitated by advancements in artificial intelligence, genomic analytics, wearable technologies and real-world evidence platforms.

The use of digital twins allows for the simulation of patient responses prior to clinical exposure, while machine learning algorithms discern patterns within vast datasets. Furthermore, open science initiatives have expedited the dissemination of knowledge across international borders.

In the midst of this rapid technological advancement, a significant gap has become apparent. Human readiness has not advanced in tandem with the progression of digital technology. While we have developed machines capable of swift learning, we have not similarly cultivated leaders equipped to govern their ethical application with equivalent speed and wisdom.

The leadership challenge we encounter extends beyond mere managerial competence. It encompasses issues of foresight, moral courage, and systemic intelligence.[1]

The coming decade will not require more research. This will require better leadership in research. As we approach 2030, leadership will be defined not by authority or hierarchy but by foresight, ethics and convergence.

The Changing Landscape of Health Research

Health research now operates within three powerful, interwoven transformations.

Digital acceleration is fundamentally transforming each phase of discovery and implementation. Artificial intelligence facilitates drug development, predictive modelling and diagnostic algorithms. The use of digital twins and simulation platforms expedites development timelines.

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Wearable and remote monitoring technologies produce continuous streams of real-world data. Nevertheless, this abundance presents risks, including algorithmic bias, data privacy vulnerabilities, opaque decision-making systems and the inclination to prioritize speed over scientific rigor.

The convergence of multiple disciplines has effectively dissolved the traditional boundaries separating medicine, pharmacy, data science, engineering, and public health. Translational research now necessitates proficiency across these fields, while implementation science requires a focus on context, culture and system dynamics.

Complex health issues, such as chronic diseases, antimicrobial resistance, and climate-related illnesses, cannot be addressed within the confines of individual departments.[2]

The phenomenon of global interconnectedness has rendered research activities increasingly borderless. Cross-continental collaborations are thriving, and open science initiatives are facilitating democratized access to data and publications.

Nevertheless, this interconnectedness also heightens complexity. Challenges such as regulatory heterogeneity, debates over data sovereignty, misinformation and inequitable research participation pose significant threats to the integrity and inclusiveness of global health sciences.

Traditional hierarchical leadership models that are departmental, authority-driven, and risk-averse are insufficient for this dynamic ecosystem. The complexity of 2030 demands leaders who can orchestrate systems rather than managing silos.

Core Pillars of Visionary Leadership for 2030

Pillar 1: Ethical Stewardship in the Age of Artificial Intelligence

Artificial intelligence presents unparalleled opportunities alongside significant ethical responsibilities. Algorithmic bias has the potential to perpetuate existing disparities, while data breaches may undermine public trust. Furthermore, predatory publishing and research misconduct threaten the credibility of scientific endeavours. It is imperative for visionary leaders to ensure that innovation does not surpass integrity.[3]

Ethical governance must be integrated at every stage, from algorithm design to data curation, interpretation and dissemination. Transparent methodologies, explainable artificial intelligence systems, reproducibility frameworks and rigorous peer-review mechanisms constitute the foundational pillars of trust.

By 2030, leadership will be evaluated not by the speed of technological adoption but by the responsibility with which these technologies are governed.

Pillar 2: Systems Thinking Over Departmental Thinking

The health challenges of the next decade, including pandemics, aging populations, and climate-related disease burdens, are system problems. Addressing these issues requires a systems thinking approach.

Visionary leaders design ecosystems rather than managing isolated teams. They integrate academia, industry, policymakers and the community into coherent research pathways. They ensure that laboratory discoveries are translated into clinical practice and inform policies. They break institutional silos through collaborative platforms and shared accountability.

Systems thinkers recognize feedback loops, unintended consequences, and the interconnectedness of health determinants. Sustainable solutions emerge not from fragmented excellence but from coordinated intelligence.

Pillar 3: Mentorship as Strategic Investment

The sustainability of health research depends on the next generation. However, early-career researchers face mounting pressures such as funding insecurity, publication competition, and burnout.

Visionary leadership reconceptualizes mentorship as a strategic investment. Institutions should develop structured mentorship programs, digital literacy training, ethical scholarship education and interdisciplinary exposure to realize this vision.

Reverse mentorship, wherein junior researchers introduce emerging tools and digital innovations, can enhance institutional adaptability.[4]

Leadership in 2030 is about succession, not self-promotion. The true measure of a leader is not their personal publication count, but the strength and integrity of the scholars they nurture.

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Pillar 4: Agility and Crisis Preparedness

The COVID-19 pandemic has exposed both the resilience and vulnerability of global research systems. Effective preparedness necessitates more than mere infrastructure; it demands capability. The capacity to conduct rapid evidence synthesis, translate findings into policy and adapt protocols amidst uncertainty differentiates truly capable systems from those that are merely well-equipped.

Visionary leaders build agile research frameworks, including rapid review teams, flexible funding mechanisms, adaptive trial platforms, and policy-engaged networks, established before crises occur.

Speed must be balanced with rigour to ensure accuracy. Preparedness must be proactive, rather than reactive.

In a world where emerging threats are inevitable, leadership must institutionalize agility to respond to them.

Pillar 5: Inclusivity and Global Equity

Health disparities persist not only in outcomes but also in research participation. Low- and middle-income countries remain underrepresented in clinical trials and genomic databases. Marginalized populations are frequently studied without meaningful engagement.

Visionary leadership necessitates the democratization of research participation. Equity must be integrated into study protocols, recruitment strategies, authorship policies and funding allocations. Community engagement must transition from token consultation to co-creation. Health equity does not occur by chance; it is achieved by design. Leadership in 2030 must ensure that scientific progress benefits all populations, not solely the privileged few.[5]

Redefining Metrics of Success

For decades, academic leadership has been measured using impact factors, h-indices, and grant acquisition. These proxies reward volume over value and visibility over a verifiable societal impact.

The next decade will demand a broader evaluation framework. Real-world outcomes, policy influence, implementation uptake, workforce development, and societal benefits must complement traditional bibliometrics.

Research impact evolves incrementally and often manifests through translation, rather than citation.

Journals and institutions must resist metric-driven distortions that incentivize superficial productivity. Instead, they should cultivate a culture that rewards rigour, relevance and ethical scholarship.

Excellence in 2030 will not be defined by citation density but by meaningful changes in health systems and communities.

The Role of Academic Journals in Shaping 2030 Leadership

Academic journals are not passive archives. These are leadership institutions that shape the scientific culture.

As we approach the year 2030, academic journals must function as platforms for multidisciplinary dialogue, uphold ethical accountability and ensure implementation-oriented scholarship.

They are required to enforce transparent reporting standards, promote responsible disclosures regarding artificial intelligence, and reject predatory practices. Furthermore, they should amplify the voices of early-career researchers and incorporate diverse global perspectives.

An advance in Multidisciplinary Health Research is particularly well-positioned to exemplify this model of leadership. By prioritising interdisciplinary scholarship, promoting equity-focused research and valuing societal impact alongside methodological rigour, AMHR can contribute to redefining research excellence.

Journals do not merely chronicle scientific advancements. They catalyze it.

A Call to Action: Leadership as Responsibility

The next decade will test the moral courage of health research leaders. Artificial intelligence will become more powerful. Climate instability reshapes disease patterns. Global inequity demands deliberate redress.

Leadership in this era requires balance, including innovation with wisdom, speed with rigour, collaboration with conviction, and growth with governance. Authority alone is insufficient. Influence arises from integrity, inclusiveness, and the capacity to inspire a collective purpose.

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Every leader guiding a laboratory, institution, or journal must ask: Are we designing systems that future generations can trust? Are we preparing scholars to think ethically and systemically? Are we aligning our progress with our purpose?

The future of health research will not be shaped by the fastest technologies but by the wisest leaders who guide them. The time to cultivate leadership is now.

Declaration:

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Conflict of Interest (COI)	The author declare that there are no conflicts of interest regarding the publication of this paper.
Artificial Intelligence (AI) Use Declaration	The author declare that artificial intelligence tools were used only for language editing, grammar refinement, and formatting assistance during manuscript preparation. All conceptualization, data collection, analysis, interpretation, and final manuscript approval were performed solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

References:

1. Joshi S. Leadership in the Age of AI: Review of Quantitative Models and Visualization for Managerial Decision-Making. SSRN Electronic Journal [Internet]. 2025 Jan 1 [cited 2026 Feb]; Available from: https://doi.org/10.2139/ssrn.5223882
2. Huang TT -K., Callahan EA, Haines ER, Hooley C, Sorensen D, Lounsbury DW, et al. Leveraging systems science and design thinking to advance implementation science: moving toward a solution-oriented paradigm. Frontiers in Public Health [Internet]. 2024 May 15 [cited 2025 Sep];12. Available from: https://doi.org/10.3389/fpubh.2024.1368050
3. Pellegrina D, Helmy M. AI for scientific integrity: detecting ethical breaches, errors, and misconduct in manuscripts. Frontiers in Artificial Intelligence [Internet]. 2025 Sep 2 [cited 2026 Jan];8:1644098. Available from: https://doi.org/10.3389/frai.2025.1644098
4. Mamun MA, Huraira MdA, Begum M, Jitu MdHI, Muntashir N, Khan MM, et al. Research literacy and its predictors among university students and graduates identified by machine learning and spatial analysis. Scientific Reports [Internet]. 2025 Oct 13 [cited 2025 Oct];15(1). Available from: https://doi.org/10.1038/s41598-025-19488-4
5. Heath V, Price CC. Addressing Health Disparities: How Having a More Diverse Biomedical Workforce Can Contribute to Addressing Health Disparities in Communities that Are Often Underrepresented in the Healthcare System. British Journal of Biomedical Science [Internet]. Taylor & Francis; 2025 Sep 22 [cited 2026 Feb];82:14973. Available from: https://doi.org/10.3389/bjbs.2025.14973

REVIEW ARTICLE

Vaccination and its impact on public health

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Abstract:

Vaccination is one of the most effective and cost-efficient public health interventions, significantly reducing the burden of infectious diseases and improving population health worldwide. This review highlights the broad impact of immunization in preventing vaccine-preventable diseases, lowering morbidity and mortality, and strengthening healthcare systems. It discusses the historical achievements of vaccination programs, including the control and elimination of diseases such as smallpox, polio, measles, and Haemophilus influenzae type b infections, while emphasizing recent advances in vaccine development for emerging infectious diseases. The review also explores current global immunization coverage, persistent challenges such as vaccine hesitancy, inequitable access, and under-vaccination, and the need for sustained public health efforts to improve vaccine uptake. Furthermore, it underscores the importance of life-course immunization, including vaccination during pregnancy, adulthood, older age, and travel, as well as the role of herd immunity in protecting vulnerable populations. The economic value of vaccination, including reductions in healthcare costs, productivity losses, and antimicrobial resistance, is also discussed. Strengthening immunization programs through improved accessibility, awareness, and policy support remains essential to achieving global health goals. Continued investment in innovative vaccine technologies and equitable vaccination strategies will play a crucial role in preventing infectious diseases and ensuring healthier communities worldwide.

Keywords: Vaccination, Immunization, Public Health, Vaccine-Preventable Diseases, Herd Immunity, Global Immunization Programs

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Introduction

Vaccination is among the most successful public health interventions and has played a central role in reducing the burden of infectious diseases worldwide. Over the past several decades, immunization programs have significantly lowered illness, disabilities and death associated with many preventable diseases. Vaccines continue to be a cornerstone of modern medicine by limiting disease transmission and preventing millions of deaths annually. Childhood immunization schedules, seasonal vaccines, and vaccinations administered across different stages of life have become essential components of healthcare systems globally.

Currently, vaccines provide protection against more than 30 potentially life-threatening diseases.1 Nearly all 194-member states of the World Health Organization (WHO) have incorporated immunization programs targeting major diseases such as pneumococcal infections, rotavirus-associated diarrhea, cervical cancer, typhoid, cholera, and meningitis.2 Continuous advances in vaccine science have also expanded preventive options for diseases that previously relied primarily on supportive management. Vaccines for malaria, dengue, and Ebola have emerged as major developments in recent years. Ongoing research involving broadly neutralizing antibodies, therapeutic vaccines, immunization beyond childhood, maternal vaccination, vaccination for older adults, needle-free delivery methods, and improved vaccine storage and supply systems is expected to further strengthen immunization strategies in the coming years.3

Vaccines function by stimulating the body's immune system to recognize and respond to harmful microorganisms. They typically contain weakened,

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inactivated, or selected components of pathogens that trigger protective immune responses without causing disease. Following vaccination, the immune system develops antibodies and immunological memory capable of recognizing future exposure to the same pathogen. This memory enables a rapid and effective response that can either prevent infection or reduce disease severity. Some vaccines provide long-lasting protection after a single series, while others require repeated doses or booster vaccinations because immunity may decline over time. This article highlights the role and importance of vaccines in promoting public health.

Pre- and Post-Introduction of Vaccines:

Prior to the widespread use of vaccines, infectious diseases caused substantial illness and mortality among both children and adults. Diseases such as smallpox, measles, mumps, rubella, pertussis, and rotavirus infection were responsible for considerable health burdens worldwide. Smallpox alone caused an estimated 400 million deaths during the twentieth century before global vaccination efforts led to its complete eradication since 1978.4 Likewise, before the introduction of measles-containing vaccines, outbreaks occurred frequently with approximately 30 million cases and 2.6 million annual deaths worldwide.5

Vaccination initiatives have prevented approximately 57 million deaths globally between 2000 and 2022.6 In addition to common childhood illnesses, severe conditions such as paralytic poliomyelitis, diphtheria, and Haemophilus influenzae type b (Hib) infections caused significant morbidity and mortality. During major polio outbreaks between 1948 and 1955, fear of infection substantially affected daily life, limiting social interactions and public activities. However, global immunization efforts have brought the world close to complete eradication of polio, with only two countries (Pakistan and Afghanistan) continuing to report endemic transmission.7

Similarly, before Hib vaccination became widely available, the incidence of meningitis in patients younger than 5 years was 57/100,000, and for all Hib diseases except nonbacteremic pneumonia, it was 71/100,000, indicating 357,000 and 445,000 cases per year, respectively and at least 108,500 of these children died.8 The introduction of Hib vaccines in the 1970s dramatically reduced these conditions. Another important example is the COVID-19 pandemic, during which vaccination programs contributed substantially by averting 2.5 million deaths during 2020-2024, saving 15 million life-years9 and mitigating broader health and economic consequences.

Overall, immunisation against various infectious diseases saves an estimated 4 to 5 million lives globally every year.10 An additional 1.5 million deaths could be avoided by improving global vaccination coverage.11 Between 2001 and 2020, vaccines averted an estimated 20 million deaths, 500 million cases of illness, and 9 million cases of long-term disability globally.12 The list of important childhood vaccines and their recommended doses is shown in Table 1.

Table 1: Important childhood vaccines and recommended doses

Vaccine	Protect against disease/infection	Recommended doses
BCG	Protects against tuberculosis	At birth
Hepatitis B	Protects against liver cancer	4 doses (at birth, at 6-10-14 weeks)
DTP	Protects against diphtheria, tetanus, and pertussis (whooping cough)	5 doses (at 6-10-14 weeks, 15-18 months, & 4-6 years)
Polio	Protects against disabling poliovirus paralysis.	4 doses (at 6-10-14 weeks, 15-18 months)
Hib	Protects against severe pneumonia and meningitis.	4 doses (at 6-10-14 weeks, 15-18 months)
Pneumococcal	Protects against pneumonia and ear infections	3 doses (at 6 &14 weeks, 9-12 months)
Rotavirus	Protects infants from severe, dehydrating diarrhoea	3 doses (at 6-10-14 weeks)
MMR	Protects against measles, mumps, and rubella	2 doses (first at 12-15 months, & second at 4-6 years)
Varicella	Protects against chickenpox	2 doses (first at 12-15 months, & second at 4-6 years).

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Vaccine	Protect against disease/infection	Recommended doses
Hepatitis A	Protects against serious liver disease	2 doses (typically given between 12 & 23 months).
HPV	Protects against human papillomavirus-related cancers	1 or 2 doses for most children starting at age 9 years.
Influenza (Flu)	Protect against sessional flu complications	1 dose annually (starting at 6 months)

Note: The recommended dose/s & type of vaccine may differ as per individual county's National Immunization Schedule

Immunization Coverage:

Although vaccines have demonstrated remarkable effectiveness in preventing infectious diseases, ensuring equitable access and improving vaccination coverage remain major public health priorities. Global childhood immunization coverage has generally remained high for several key vaccines. Recent estimates indicate that coverage for diphtheria-tetanus-pertussis (DTP) remains high, wherein an estimated 89% of the world's population had received the first dose and 85% had received the third dose of DTP vaccine13. Similarly, measles vaccination rates have shown gradual improvement in many regions, with increasing numbers of children receiving both the first dose (84%) and second dose (76%) of the vaccine. In the same period, 31% of eligible adolescent girls globally received at least 1 dose of the HPV vaccine wherein most doses were administered in countries using a single-dose schedule. While far from the 90% coverage target by 2030, it represents a substantial increase from the 17% coverage in 201914. Global immunization coverage among 1-year olds during year 2024 is displayed in Figure 1.

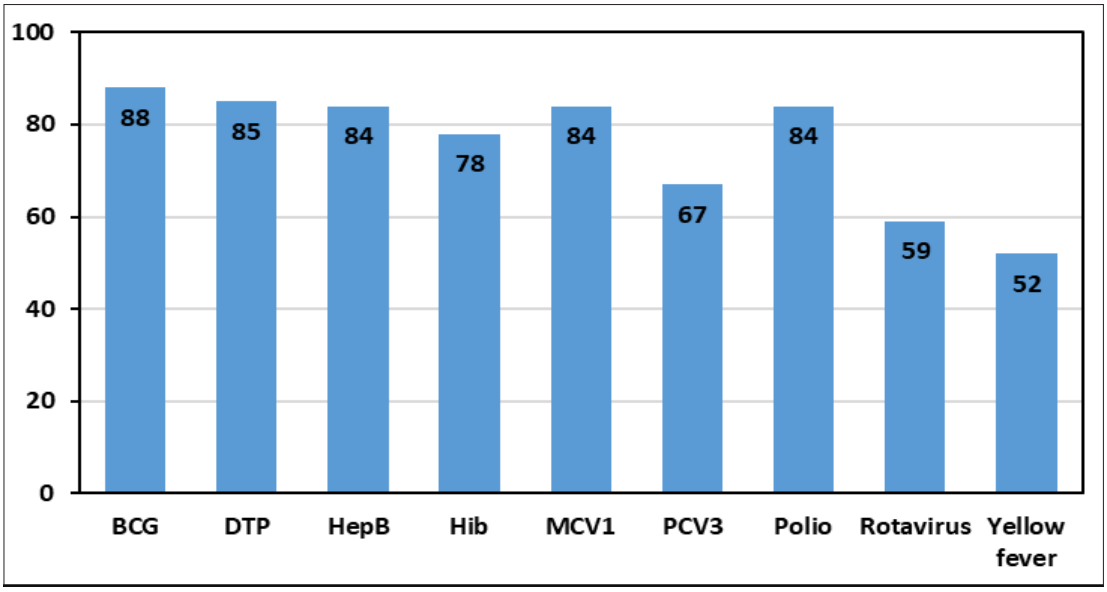

Figure 1: Immunization coverage among 1-year olds (%)

Source: https://www.who.int/data/gho/data/themes/topics/immunization-coverage

Vaccination coverage indicators such as DTP3 administration by 12 months of age are frequently used to assess the performance of immunization programs. Coverage rates for measles-containing vaccines and polio vaccines also provide important measures of the effectiveness of national vaccination systems.15

Despite overall progress, outbreaks of vaccine-preventable diseases continue to occur in areas with inadequate immunization coverage. Though global coverage for DTP is improving, but outbreaks of these diseases were continuously reported during intervals in regions with low vaccine coverage, such as Yemen, Bangladesh and Venezuela.16 In Germany, 25,271 pertussis cases were officially recorded in 2024, indicating a more than sevenfold increase compared to the preceding year; whereas, France also showed an upward trend in pertussis cases with cyclical waves every 3–5 years, with a peak in the summer months.17 Also, in case of measles, there were an estimated 95,000 deaths reported globally in 2024, mostly among

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unvaccinated or under vaccinated children under the age of 5 years.18 These findings emphasize that vaccine-preventable diseases continue to represent significant public health concerns despite the availability of safe and effective preventive measures. Therefore, strengthening immunization policies and implementing targeted strategies to address gaps in coverage are essential to ensure that all populations benefit from life-saving vaccines.

Vaccination Throughout Life

Vaccination should not be considered exclusively a childhood intervention. Adolescents, adults, and older individuals also require immunization to maintain protection against various infectious diseases throughout life. The risk of infection may be particularly elevated among older adults and immunocompromised individuals because of age-related decline in immunity, chronic health conditions, nutritional factors, environmental exposure, and limited healthcare access.19 Adult vaccine uptake remains suboptimal in many regions because of multiple barriers, including inadequate awareness, sociocultural influences, resource limitations, vaccine hesitancy, insufficient policy frameworks, and healthcare accessibility issues. In addition, inadequate disease surveillance often results in underestimation of disease burden among adults.

Recognising such challenges, the CDC has recommended that adults should receive the seasonal flu vaccine annually, as well as vaccines for diseases like shingles, pneumonia, and tetanus along with booster shots of certain vaccines to help protect against new and emerging viruses.20 High-risk adult groups like health workers and members of displaced communities also benefit from immunisations. For outbreak-prone diseases like typhoid, cholera, Ebola, and yellow fever, vaccines protect community members of all ages. Vaccination during adolescence is also important for preventing diseases such as human papillomavirus infection and meningococcal disease. Appropriate vaccination during these years can reduce future disease risk and contribute to long-term population health outcomes. List of recommended vaccines for adults is given in Table 2.

Table 2: Recommended adult vaccines for different age groups

Vaccine	18-26 years	27-49 years	50-64 years	≥ 65 years
Influenzae	Every year			
Covid-19	During the pandemic			
RSV	If pregnant during RSV session		If age 50-74 years	If age 75 years/ above
Hep A	If additional risk factors			
Hep B	Through 59 years			
HPV				
MMR				
Tdap/Td	Tdap every pregnancy. Td/Tdap every 10 years for all adults.			
Chickenpox				
Shingles				
Pneumococcal				
MenACWY				
Chikungunya				
Cholera				
Meningococcal				
Hib				

At-risk | If additional risk factors | Based on benefit-risk ratio

Note: The requirement of vaccines among adults may vary depending upon age group, country and associated risk factors.

Source: https://www.cdc.gov/vaccines/imz-schedules/adult-easyread.html; https://japi.org/article/japi-73-5-s1-21

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Maternal immunization has become an important strategy for protecting both pregnant women and newborn infants from infectious diseases. Maternal and neonatal tetanus historically represented a major cause of mortality, particularly in resource-limited settings with inadequate maternal healthcare and unhygienic delivery conditions. In 1988, the WHO estimated that 787,000 newborns died of neonatal tetanus.21 Maternal immunization has the potential to reduce the burden of infectious diseases in the pregnant woman and her infant. Expanded immunization initiatives during 2000-2022 have substantially reduced disease incidence and mortality associated with maternal (89%) and neonatal tetanus (84%).22 Additionally, vaccination during pregnancy against influenza and pertussis has shown important benefits in reducing disease risk among both mothers and infants. Thus, since 2012, the WHO has recommended influenza immunization during pregnancy in any trimester in many high resource countries23 along with the recently approved tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine, adsorbed (Tdap) vaccine for immunization during the third trimester of pregnancy to prevent pertussis in infants younger than 2 months of age. 24

Traveling for any reason to certain areas may raise the risk of infection because of differences in hygienic settings, food and water sources, disease patterns, environment, and immunization exposure.25 Thus, use of travel vaccines is important to minimize travel-related illnesses and concerns while maximizing a safe and successful journey. For example, the yellow fever immunization is requisite for travel to parts in sub- Saharan Africa and tropical South America, where it is endemic and intermittently epidemic.26 Similarly, Meningococcal vaccination is required by the Saudi Arabian government but only for travel during the hajj, or annual pilgrimage to Mecca.27

Beyond individual protection, vaccination contributes significantly to community-level disease prevention through the development of herd immunity, also known as population immunity. Herd immunity occurs when a sufficiently large proportion of individuals within a population become immune to a disease, reducing opportunities for transmission and limiting the spread of infection within the community. This indirect protection is particularly important for individuals who cannot receive vaccines because of medical contraindications, including certain allergic conditions, severe immune deficiencies, or specific health-related limitations. By reducing the circulation of infectious agents, vaccinated individuals help protect vulnerable groups who remain at increased risk of serious complications.

The effects of herd immunity have been demonstrated in several vaccination programs worldwide. Following the introduction of oral vaccines against diseases such as cholera and rotavirus, in United States (2006) and Austria (2007), substantial reductions in disease incidence have been observed not only among vaccinated children but also among older age groups and unvaccinated individuals.28 These reductions suggest that vaccination can decrease pathogen transmission within households and communities by lowering infection rates and reducing pathogen shedding. The broader public health impact of herd immunity extends beyond disease control by contributing to reduced healthcare burdens and supporting disease elimination efforts. Achieving and maintaining high vaccination coverage, therefore, remains essential for sustaining community protection and minimizing the risk of future outbreaks.

Economic impact of vaccines:

Infectious diseases can impose substantial economic burdens on individuals, families, healthcare systems, and national economies. Costs associated with disease outbreaks extend beyond direct medical expenses and may include productivity losses, absenteeism from work or school, long-term disability, and broader social and economic disruptions. Preventive healthcare strategies such as vaccination can significantly reduce these financial consequences. Each dollar spent on vaccination saves $52.2.29 It was reported that, for each US birth cohort that received a set of vaccines against 10 different diseases in 2010, an estimated $43 billion was saved, including $9.9 billion in direct savings from medical costs and $33 billion saved indirectly through reduction in social costs like missed work.30 Similarly, a 2016 study conducted by Johns Hopkins University and published in Health Affairs found that for every dollar invested in vaccination in the world's 94 lowest-income countries, US$16 is

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expected to be saved in healthcare costs, lost wages and lost productivity due to illness and death.31 The major impact of vaccination was reported by reducing direct and indirect costs associated with cancers caused by HPV and HepB. In 2019, an estimated economic impact of $106.3 billion was reported due to vaccine-preventable cancer-associated deaths globally. Improved coverage and adaptation of gender-neutral vaccination against HPV is important for countries with limited resources.32 By reducing disease-related costs and supporting healthier populations, vaccination programs strengthen both healthcare systems and national economies, reinforcing their value as one of the most cost-effective preventive health measures available.

Management of the healthcare system:

With a growing proportion of older adults in many countries and with age being one of the risk factors for many severe illnesses and chronic diseases, pressure on healthcare systems is rapidly increasing. Vaccines can help to ease this pressure. For example, vaccines can reduce hospitalizations, benefiting patients and allowing hospitals to free up vital space to treat others. In Spain, hospitalisations due to chickenpox decreased by 78% after routine vaccination of 15-18-month-old infants between 2006 and 2010.33 In the case of influenza, vaccination can reduce hospitalizations and deaths by 45% and 38%, respectively, in older people with diabetes, and is also associated with reduced risk of cardiovascular death.34,35

Antimicrobial Resistance (AMR) may already cause 700,000 deaths per year globally, and in a worst-case scenario, this number could reach 10 million by 2050.36 This is equivalent to one death every three seconds. Evidence shows that vaccines are critical in the fight against AMR. They prevent disease, both bacterial and viral, and reduce demand and misuse of antibiotics, safeguarding their effectiveness.37 For example, vaccines can reduce the use of antimicrobials by an estimated 47% with universal pneumococcal vaccine coverage in children.38

Conclusion:

Immunization is an important pillar of the primary healthcare system, and the most cost-effective way to support a healthier and safer world. Globally, millions of children—often referred to as “zero-dose” or under-vaccinated—miss routine immunizations primarily due to systemic supply constraints, conflict, poverty, and misinformation. Addressing these complex challenges is critical to achieving health equity and ensuring that everyone has access to life-saving vaccines. To improve access, public health organizations have implemented strategies such as community vaccination clinics, mobile vaccination units, and targeted outreach programs in minority and historically underserved communities. Three pillars—affordable pricing, sustainable breakthrough supply, and modernized distribution and administration technologies—form the core of global public health equity strategies spearheaded by organizations like the World Health Organization (WHO), GAVI, and CEPI. Moving forward, the development of newer therapeutic vaccines is also important for combating deadly diseases and cancers. This will help in achieving WHO’s Immunization Agenda (IA2030), where everyone, everywhere, at every age, fully benefits from vaccines to improve health and well-being.

Declaration:

Author Contribution	**Conceptualization: Hitt Sharma, Sameer Parekh, Pramod Pujari, Sunil Shewale**
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References:

1. World Health Organization. Vaccines and immunization. https://www.who.int/health-topics/vaccines-and-immunization#tab=tab_1 (Accessed May 2026).
2. World Health Organization. Immunization dashboard. https://immunizationdata.who.int/ (Accessed May 2026).
3. Immunization Agenda 2030 Partners. Immunization agenda 2030: A global strategy to leave no one behind. Vaccine. 2024 Apr 8;42 Suppl 1:S5-S14. doi: 10.1016/j.vaccine.2022.11.042.
4. Meyer H, Ehmann R, Smith GL. Smallpox in the Post-Eradication Era. Viruses. 2020 Jan 24;12(2):138. doi: 10.3390/v12020138.
5. Altindag O, Greve J, Tekin E. Impact of Measles, Mumps, and Rubella Vaccination on Hospitalizations and Human Capital: Evidence from Copenhagen School Health Records. Vaccines (Basel). 2025 Mar 11;13(3):302. doi: 10.3390/vaccines13030302.
6. Minta A.A. Progress toward measles elimination—Worldwide, 2000–2022. MMWR Morb. Mortal. Wkly. Rep. 2023;72:1262–1268. doi: 10.15585/mmwr.mm7246a3.
7. Cochi SL, Freeman A, Guirguis S, et.al. Global polio eradication initiative: lessons learned and legacy. J Infect Dis. 2014 Nov 1;210 Suppl 1(Suppl 1):S540-6. doi: 10.1093/infdis/jiu345.
8. Peltola H. Worldwide Haemophilus influenzae type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. Clin Microbiol Rev. 2000 Apr;13(2):302-17. doi: 10.1128/CMR.13.2.302.
9. Ioannidis JPA, Pezzullo AM, Cristiano A, et.al. Global Estimates of Lives and Life-Years Saved by COVID-19 Vaccination During 2020-2024. JAMA Health Forum. 2025;6(7):e252223. doi:10.1001/jamahealthforum.2025.2223
10. Carter A, Msemburi W, Sim SY, et.al. Modeling the impact of vaccination for the immunization Agenda 2030: Deaths averted due to vaccination against 14 pathogens in 194 countries from 2021 to 2030. Vaccine. 2024 Apr 8;42 Suppl 1:S28-S37. doi: 10.1016/j.vaccine.2023.07.033.
11. World Health Organization. Immunization. https://www.who.int/news-room/facts-in-pictures/detail/immunization (Accessed May 2026).
12. Ozawa S, Clark S, Portnoy A, et.al. Estimated economic impact of vaccinations in 73 low- and middle-income countries, 2001-2020. Bull World Health Organ. 2017 Sep 1;95(9):629-638. doi: 10.2471/BLT.16.178475.

13. Stala O, Patel S, Donlon C, et.al. Tetanus Control in the United States and Global Disaster Settings: Public Health Disparities and Prevention Strategies. Medicina (Kaunas). 2026 Feb 7;62(2):338. doi: 10.3390/medicina62020338.
14. WHO. Global childhood vaccination coverage holds steady, yet over 14 million infants remain unvaccinated – WHO, UNICEF. 15th Jul 2025. https://www.who.int/news/item/15-07-2025-global-childhood-vaccination-coverage-holds-steady-yet-over-14-million-infants-remain-unvaccinated-who-unicef (Accessed May 2026).
15. Report MW. Global routine vaccination coverage, 2013. Wkly Epidemiol Rec. 2014;89:517–22.
16. Lim DW, Au AJH, Chavatte JM, et.al. Seroprevalence of diphtheria, tetanus, pertussis among healthcare workers (HCWs) - A cross-sectional study at the National Centre for Infectious Diseases and Tan Tock Seng Hospital, Singapore. Hum Vaccin Immunother. 2026 Dec;22(1):2641318. doi: 10.1080/21645515.2026.2641318.
17. Duda-Madej A, Łabaz J, Topola E, et.al. Pertussis-A Re-Emerging Threat Despite Immunization: An Analysis of Vaccine Effectiveness and Antibiotic Resistance. Int J Mol Sci. 2025 Oct 1;26(19):9607. doi: 10.3390/ijms26199607.
18. World Health Organization. Fact Sheet of Measles. https://www.who.int/news-room/fact-sheets/detail/measles (Accessed May 2026).
19. Vaid A, Rastogi N, Doherty TM, et.al. Review of the unmet medical need for vaccination in adults with immunocompromising conditions: An Indian perspective. Hum Vaccin Immunother. 2023 Aug 1;19(2):2224186. doi: 10.1080/21645515.2023.2224186.
20. Centers for Disease Control and Prevention. https://www.cdc.gov/vaccines/imz-schedules/adult-easyread.html (Accessed May 2026).
21. Marshall H, McMillan M, Andrews RM, et.al. Vaccines in pregnancy: The dual benefit for pregnant women and infants. Hum Vaccin Immunother. 2016 Apr 2;12(4):848-56. doi: 10.1080/21645515.2015.1127485.
22. Jones CE, Yusuf N, Ahmed B, et.al. Progress Toward Achieving and Sustaining Maternal and Neonatal Tetanus Elimination — Worldwide, 2000–2022. MMWR Morb Mortal Wkly Rep 2024;73:614–621. DOI: http://dx.doi.org/10.15585/mmwr.mm7328a1.
23. Loubet P, Tsatsaris V, Launay O. Influenza immunisation in pregnancy is efficacious and safe, but questions remain The Lancet Respiratory Medicine. 2020;8(6):533-534
24. Rubin R. Vaccine Approved in Pregnancy to Protect Young Infants. JAMA. 2022;328(19):1898. doi:10.1001/jama.2022.18478.
25. Sharma H, Parekh S, Shewale S. Travel Vaccination. Journal of Vaccines and Immunology. 2015;1(1): 103.
26. Gianchecchi E, Cianchi V, Torelli A, et.al. Yellow Fever: Origin, Epidemiology, Preventive Strategies and Future Prospects. Vaccines (Basel). 2022 Feb 27;10(3):372. doi: 10.3390/vaccines10030372.
27. Alasmari A, Houghton J, Greenwood B, et.al. Meningococcal carriage among Hajj pilgrims, risk factors for carriage and records of vaccination: a study of pilgrims to Mecca. Trop Med Int Health. 2021 Apr;26(4):453-461. doi: 10.1111/tmi.13546.
28. CMC, Plotkin SA. Impact of Vaccines; Health, Economic and Social Perspectives. Front Microbiol. 2020 Jul 14;11:1526. doi: 10.3389/fmicb.2020.01526.
29. Sim SY, Watts E, Constenla D, et.al. Return on Investment From Immunization Against 10 Pathogens In 94 Low- And Middle-Income Countries, 2011-30. Health Aff (Millwood). 2020 Aug;39(8):1343-1353. doi: 10.1377/hlthaff.2020.00103.
30. Schuchat A. Human vaccines and their importance to public health. Procedia in Vaccinology. 2011;5:120-126. doi: https://doi.org/10.1016/j.provac.2011.10.008
31. Ozawa S, Clark S, Portnoy A, et.al. Return On Investment From Childhood Immunization In Low- And

Middle-Income Countries, 2011-20. Health Aff (Millwood). 2016 Feb;35(2):199-207. doi: 10.1377/hlthaff.2015.1086.

32. Bencina G, Oliver E, Meiwald A, et al. Global burden and economic impact of vaccine-preventable cancer mortality. J Med Econ. 2024;27(sup2):9-1. doi: 10.1080/13696998.2024.2350877.
33. Lo Presti C, Curti C, Montana M, et.al. Chickenpox: An update. Med Mal Infect. 2019 Feb;49(1):1-8. doi: 10.1016/j.medmal.2018.04.395.
34. Chevalier-Cottin EP, Ashbaugh H, Brooke N, et.al. Communicating Benefits from Vaccines Beyond Preventing Infectious Diseases. Infect Dis Ther. 2020 Sep;9(3):467-480. doi: 10.1007/s40121-020-00312-7.
35. Modin D, Claggett B, Køber L, et.al. Influenza Vaccination Is Associated With Reduced Cardiovascular Mortality in Adults With Diabetes: A Nationwide Cohort Study. Diabetes Care. 2020 Sep;43(9):2226-2233. doi: 10.2337/dc20-0229.
36. Mantel C, Cherian T. New immunization strategies: adapting to global challenges. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2020 Jan;63(1):25-31. doi: 10.1007/s00103-019-03066-x.
37. F, Bagnoli F, Rappuoli R, Serruto D. The role of vaccines in combatting antimicrobial resistance. Nat Rev Microbiol. 2021 May;19(5):287-302. doi: 10.1038/s41579-020-00506-3. European Commission (2017).
38. A European One Health Action Plan against Antimicrobial Resistance (AMR). https://health.ec.europa.eu/document/download/353f40d1-f114-4c41-9755-c7e3f1da5378_en (Accessed May 2026).

CASE REPORT

Under-recognized comorbid features in polycystic ovary syndrome: A case report

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ABSTRACT

Polycystic ovary syndrome (PCOS), also known as polycystic ovarian disease, is a common endocrine disorder. It arises in women of reproductive age. The prevalence of PCOS in the mentioned age group is estimated to range from 5% to 18% and it affects the quality of life of the patients due to the complications associated with it. Various treatment plans are targeting it, but certain symptoms such as sleep apnoea, vitamin D deficiency, hyperhidrosis, and altered mood are usually ignored. The case study on PCOS presented here shows that these symptoms were not considered in the treatment plan. This study confirms that the most common symptoms are treated while these other important symptoms are ignored, which may worsen the case. Hence, in this report, we have discussed a treatment plan with better symptomatic relief and provided new approaches towards the disease.

Keywords, Ignored symptoms; Sleep apnoea; Vitamin D deficiency; Depression; Hyperhidrosis.

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Introduction:

Polycystic ovary syndrome (PCOS), a common endocrine disorder, arises in women of reproductive age. Its prevalence in the mentioned age group is estimated from 5% to 18%.[1] PCOS affects the quality of life of the patients as it shows various symptoms such as oligomenorrhea (irregular periods), amenorrhea (absence of periods), infertility, obesity, hyperandrogenism, hirsutism (unwanted growth of hair on face and body), acanthosis nigricans. It can be diagnosed by hyperandrogenism, ovarian disorder, and polycystic ovaries, though with significant distinctions between the individuals.[2]

Various treatment plans are targeting it but certain symptoms such as sleep apnoea, vitamin D deficiency, hyperhidrosis, and altered mood are usually ignored. So, here we are writing a case report to discuss a treatment plan along with better symptomatic relief and provide new approaches towards drug delivery targeting all the associated complications of PCOS. This study mainly focuses on a case wherein other symptom like sleep apnoea, Vit. D deficiency, hyperhidrosis (excessive sweating), and altered mood was observed.

Case History

2.1 Medical history

A 29-year-old woman weighing 83 kgs with 161 cm height (BMI = 31.5 kg/m sq.) was suffering from symptoms of obesity, oligomenorrhea (irregular periods), acne, difficulties conceiving a baby and was given medical treatment accordingly. She was diagnosed with PCOS in 2012, which was confirmed with an ultrasound. On presentation, she also suffered from other symptoms like vitamin D deficiency, hyperhidrosis (excessive sweating), and

altered mood; also, her husband reported that she had obstructive sleep apnoea (as the symptoms were suggestive of OSA, however, formal evaluation was not done). These symptoms were not considered in her treatment plan. Initially, before having her first baby, she faced a miscarriage. Later, she conceived a baby who is now 5 years old and again, recently, one year ago she had another miscarriage.

2.2 Family history

Her mother is suffering from type 2 diabetes mellitus.

2.3 Exercise and diet

Being a lawyer by profession and practicing a sitting job of 9 am to 5 pm, she does not practice any kind of exercise. She follows a normal diet plan consisting of morning juice (cucumber, carrot, mint); fruits, omelette, dosa, idli, etc. for breakfast; sabji (veg or non-veg) and roti for lunch and dinner.

2.4 Diagnosis and treatment prescribed

Diagnosis method: Primary diagnosis was done by ultrasound, and the patient was complaining about irregular menstruation, unable to conceive, and obesity.

As per recent pelvic sonography, her uterus was retroverted and measured 5.1 X 3.8 X 3.2, the myometrial echoes were homogenous and normal. There was no evidence of fibroids. The endometrial echo thickness measured 7 mm. Multiple small cystic follicles were seen at the periphery of both ovaries. (right ovaries measured 2.8 X 2.4 X 2.3 cm and their volume was 8.8 cm^3; left ovaries measured 2.6 X 2.2 X 1.6 cm, and their volume was 5.5 cm^3). A cystic area was seen in the right adnexa, measuring 25 X 22 X 17 mm. This could be a parapelvic cyst. Minimal free fluid was seen in the pouch of Douglas. The sonography picture is shown in fig 1.

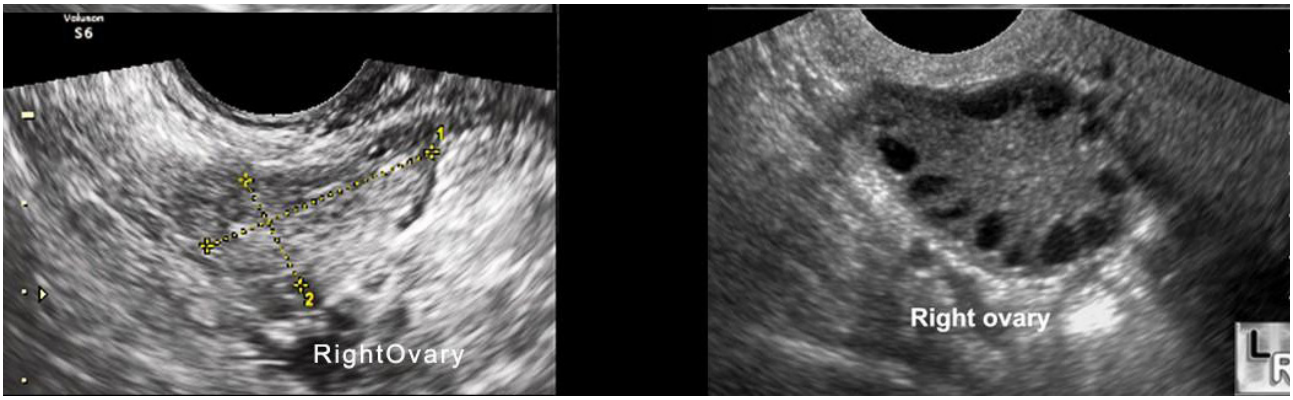

Figure 1. Sonography of the patient

Several blood and hormonal tests were conducted showing the following results:

- FSH: 6.80 mIU/mL
- LH: 14.10 mIU/mL
- CA-125 (Glycoprotein): 21 U/mL
- Blood glucose (fasting): 81 mg/dL
- Blood glucose (post prandial): 115.7 mg/dL
- Insulin (fasting): 8.70 µLU/mL
- Creatinine: 0.5 mg/dL
- Prolactin: 4.80 ng/mL
- 25(OH)D: 48.2 nmol/L

The patient was prescribed Metformin (250 mg TID).

DISCUSSION

In the given case, there are some important tests, such as, anti-mullerian hormone (AMH) test and testosterone hormone test, which were not conducted. In addition to the lack of diagnostic tests, some symptoms, such as obstructive sleep apnoea and psychological factors, were ignored during the initial treatment.

It was observed that the patient's vitamin levels were low; however, no treatment was prescribed for this condition. Low vitamin levels led to excessive

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tiredness and sweating in the patient, which the patient did not mention directly to the doctor (possibly due to hesitation). Thus, doctors need to initiate this conversation and motivate the patient to share the symptom. Sweating is also caused by elevated cortisol levels. High cortisol levels lead to anxiety and, hence, nervous sweating.[3] Depression is another complication caused by vitamin D deficiency. Therefore, it is very important to manage vitamin D deficiency in women with PCOS.

Obstructive sleep apnoea is another symptom that needs special attention, especially in women with PCOS. It leads to reduced oxygen supply to the organs, including the brain, which is a cause of depression.

After consulting other gynaecologists and a respiratory consultant, the following treatment is suggested:

- Adjuvant to medication, lifestyle modification refers to a combination of diet and exercise
- Execution of a new lifestyle could be enhanced by counselling sessions, psychological support and meditation
- Vitamin D rich diet/supplements
- Weight loss up to 70 Kgs in 12 months
- Cyproterone acetate

Treatment for sleep apnoea [4]

- Continuous positive airway pressure (CPAP)
- Mandibular advanced splints (MAS)
- In some cases, Uvulo-palatopharyngoplasty (UPPP) may be an option
- Weight control and bariatric surgery
- Avoid smoking, drinking alcohol, and using sedatives and hypnotics

Vitamin D [5]

The normal range for Vitamin D is 75-250 nmol/L). Vitamin D deficiency may be linked to depression and ovulatory dysfunction. The ovulatory dysfunction in PCOS as shown in figure 2.

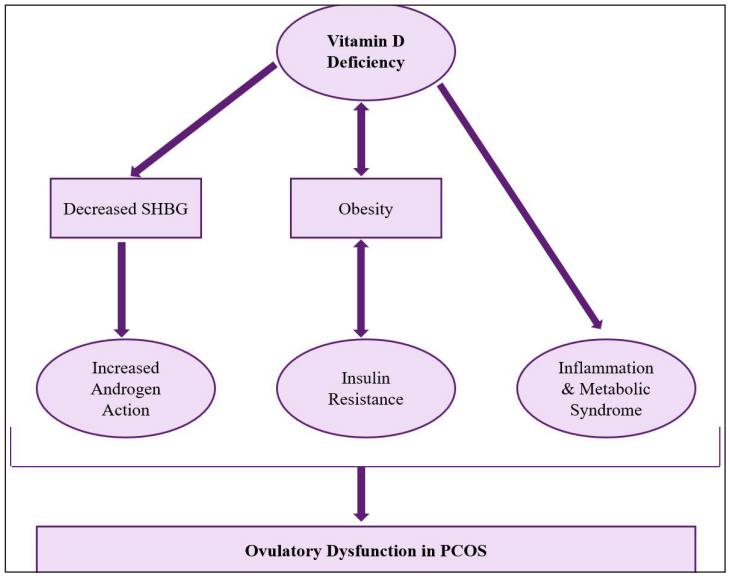

Figure 2. Relationship between vitamin D deficiency and PCOS

To compensate vitamin D deficiency, foods rich in it can be consumed, which include fatty fish, like tuna, mackerel, and salmon; foods fortified with vitamin D, like some dairy products, orange juice, soy milk, and cereals; beef liver; cheese; and egg yolks.

Exercise

Studies have shown statistical benefits of exercise for various metabolic, anthropometric, and cardiorespiratory fitness-related outcomes.[6] Hence, exercise such as brisk walking, cycling, moderate dancing, race walking, jogging, and mountain climbing should be practised to decrease the abdominal and total body fat, improve menstrual frequency, and decrease serum testosterone concentration.

It has been observed that the treatment of PCOS includes only the primary symptoms and not the

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other symptoms and complications associated with it. The secondary symptoms and complications worsen the condition of the patient and intensify PCOS. Hence, it is important to highlight commonly untreated symptoms and to include a treatment plan that considers all criteria. This will provide better symptomatic relief, thereby improving patients' quality of life.

Conclusion:

This case highlights that the clinical management of polycystic ovary syndrome should extend beyond the treatment of menstrual irregularities and metabolic disturbances. Under-recognized comorbid conditions, including vitamin D deficiency, suspected obstructive sleep apnoea, excessive sweating, and psychological symptoms, may substantially influence disease burden and overall quality of life if left unaddressed. A comprehensive patient assessment, open communication about overlooked symptoms, and a multidisciplinary treatment strategy that incorporates lifestyle modification, nutritional support, mental health evaluation, and appropriate specialist referrals may improve long-term outcomes. Although this report represents a single patient, it underscores the need for clinicians to adopt a more holistic and individualized approach to PCOS management and encourages further research to evaluate the impact of addressing these often-neglected clinical features.

Declaration:

Author Contributio	Conceptualization: Chetashri N. Patil & Ohj Dawood Noori Writing original draft: Chetashri N. Patil & Ohj Dawood Noori Review and editing: Shivani Desai, Hemant G. Deshpande, Chandrakant S. Madkar, Mansi L. Patil Paper finalization: All authors
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 | doi: 10.XXXX/amhr.[2026].XXXXX

References:

1. Ding T, Hardiman PJ, Petersen I, Wang FF, Qu F, Baio G. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: A systematic review and meta-analysis. Oncotarget. 2017; 8: 96351-96358
2. Bazarganipour F, Taghavi SA, Montazeri A, Ahmadi F, Chaman R, Khosravi A. The impact of polycystic ovary syndrome on the health-related quality of life: A systematic review and meta-analysis. Iran J Reprod Med. 2015; 13: 61-70
3. Hoehn-Saric R, McLeod DR. The Peripheral Sympathetic Nervous System: Its Role in Normal and Pathologic Anxiety. Psychiatric Clinics of North America. 1988;11(2): 375-386
4. Spicuzza L, Caruso D, Di Maria G. Obstructive sleep apnoea syndrome and its management. Ther Adv Chronic Dis. 2015; 6(5): 273-285
5. Lin MW, Wu MH. The role of vitamin D in polycystic ovary syndrome. Indian J Med Res. 2015; 142(3): 238-240
6. Kite C, Lahart IM, Afzal I. Exercise, or exercise and diet for the management of polycystic ovary syndrome: A systematic review and meta-analysis. Syst Rev. 2019; 8(1): 51

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RESEARCH ARTICLE

Evaluating the risk of falls in the elderly population at a public teaching tertiary care hospital

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Abstract:

The published literature on falls and their risk among the elderly in India is limited. The need to investigate fall risk in the elderly has increased with the rise in unexplained deaths.

This cross-sectional, prospective study, conducted at the outpatient department (OPD) of a public teaching tertiary care hospital, evaluated real-time risk factors among elderly outpatients. The Johns Hopkins Fall Risk Assessment Tool (JHFRAT) was used. The results are based on data obtained from 105 eligible patients.

The average age of the patients was 69.3±0.6 years (range 60-85 years; median 68 years, mode 62 years). The average number of diagnoses was 2.2±0.8, and the average number of prescribed medications was 6.7±0.2.

The fall history analysis showed that 17 of 105 patients had experienced a fall before their OPD visit. The average age for this set was 71.5±1.6 years. Using the JHFRAT, patients were categorized into low-, moderate-, and high-risk groups (6%, 67%, and 27%, respectively). Increasing age, female gender, and various comorbidities were identified as risk factors for fall-related hospital admissions.

These results indicate that high fall-risk drugs are one of the major contributors to fall risk among the elderly population.

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Introduction

Falls and imbalance are common in the elderly and falls or instability are considered among the giants in geriatric medicine.[1] After the age of 60 years, the rate of falling and prevalence of risk factors for falling increase gradually.[2]

One of the most pressing public health challenges in today's ageing society is preventing falls among the elderly.[3] Multiple reports from within India show that the prevalence of falls among the elderly population is in the range of 26-37%.[4]

According to the World Population Ageing Highlights 2017, the global population aged 60 or over numbered 962 million in 2017 and is expected to double again by 2050, reaching nearly 2.1 billion. In 2030, the elderly are expected to outnumber children under 10, and by 2050, they are expected to outnumber adolescents and youth ages 10-24.[5]

The published literature confirms that patients who report falls are more likely to report poor self-rated health and more chronic conditions. Florence and co-workers[6] showed in 2018 that the estimated medical costs attributable to fatal and non-fatal falls were approximately US$50bn. Moreover, the life expectancy of the people is increasing, and thus the population living with chronic disorders such as cardiovascular diseases, arthritis and diabetes are on the rise. These, along with the medications used to treat them, are associated with increased fall risk among older individuals.[7] Some drugs might increase a person's risk of falling because they create dizziness or disorientation as side effects.

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The published literature on falls and their risk among the elderly in India is scattered and limited. The rapid increase in the number of unknown deaths in the elderly has foregrounded the need to investigate the risk of falls. This study has evaluated real-time risk factors among elderly outpatients at a public tertiary care teaching hospital.

Methodology:

Study design, sampling, and setting – This cross-sectional prospective study was conducted at the outpatient department (OPD) of a public teaching tertiary care hospital.

Data collection procedure – The study was initiated after approval from the "Institutional Ethics Committee (Human Studies)" of the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, and the "Ethics Committee" of the public teaching hospital. The purpose of the study and the data collection method were explained verbally to the patients, and written informed consent was obtained from the patients or their legal caretakers prior to enrolling them in the study.

The definition of fall in this study was "an event which results in a person coming to rest inadvertently on the ground or floor or other lower level".[8]

Patients aged 60 years or more, either sex, with one or more concurrent diseases, on one or more drugs, and willing to participate were included. Patients with incomplete documentation, those completely immobilised, or those unable to answer the questions were excluded from the study.

All the relevant information was collected from the patient record file, and a patient interview was conducted for each patient in the study. Patient-related information included demographic characteristics (age, gender, body weight, and height), co-morbidities, diagnoses, and prescribed drugs. All this information was collected in a standard case record form.

Patient's rights were respected, and confidentiality was maintained.

The Johns Hopkins Fall Risk Assessment Tool (JHFRAT; 14) was used; it consists of seven variables including age (1-3 points), fall history (0 or 5 points), elimination of bowel and urine (2 or 4 points), medications and sedative procedure (3, 5 or 7 points), patient care equipment (1-3points), mobility (2, 4 or 6points) and cognition (1-7points). Based upon these inputs, the total score was obtained for all patients. These scores formed the basis of classifying the patients into 'low risk' (<6 points), 'moderate risk' (6-13 points) and 'high risk' groups (>13 points).

The fall risk score calculation was not processed further when any of the following conditions were met:

- history of more than one fall within 6 months before admission;
- The patient has experienced a fall during this hospitalization.
- patient is deemed high fall-risk per protocol (e.g., seizure precautions);
- complete paralysis or being completely immobilized.

The patients who met the "not processed further" criteria were excluded from the study. Permission to use the Johns Hopkins Fall Risk Assessment tool, a pre-validated instrument for evaluating fall risk among elderly patients, was obtained from the developer. The frequencies of fall risk factors were calculated from individual scores for each parameter, as measured by the Johns Hopkins Fall Risk Assessment Tool (JHFRAT). The individual items/parameters, i.e., elimination of bowel and urine, mobility, and cognition, were evaluated using predetermined scores for their sub-parameters.

Elimination, bowel, and urine parameters were assessed using three sub-parameters: incontinence, urgency/frequency, and urgency/frequency and incontinence.

Likewise, the mobility was assessed by using three sub-parameters, i.e.

1. requires assistance or supervision for mobility, transfer, or ambulation;
2. unsteady gait;
3. visual or auditory impairment affecting mobility.

The cognition parameter was assessed using three sub-parameters: altered awareness of the immediate physical environment, impulsivity, and lack of understanding of one's physical and cognitive limitations.[9]

All the data obtained during the study were organized into a spreadsheet. The descriptors of

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central tendency and variations were computed using Microsoft excel® and the free version of SPSS. The results were presented as percentages, and the average was supported with the standard error of the mean (SEM). The likelihood ratio test was used to evaluate the overall relationship between an independent variable and a dependent variable. $p<0.05$ was considered statistically significant.

Results

In this cross-sectional study, 117 elderly patients were evaluated using a personal interview and validated scales/questionnaire to predict fall risk in the outpatient (OPD) setting at a public teaching hospital. 12 patients were excluded from the final analysis of results (7 with incomplete information and 5 unwilling to participate). Therefore, the results of this study are based on data obtained from 105 patients.

Of the 105 patients, there were 52 males and 53 females. The average age of the patients was 69.3±0.6 years (range 60-85 years). The median age was 68 years, and the mode was 62 years. The average age of male patients was 71.1±1.0 years, and that of female patients was 67.7±0.7 years ($p<0.0001$).

For better understanding, the patients were categorized into three age groups: "young-old" (60- 69 years), "middle-old" (70- 79 years), and "very-old" (≥80 years), according to internationally accepted criteria.[15]

The average number of diagnoses was 2.2±0.8. Approximately 85% patients had two or more diagnoses. The most common diagnoses of the patients were hypertension, diabetes mellitus, cardiovascular disorders, chronic kidney disease, chronic obstructive pulmonary disease, chronic liver disease, Parkinson's disease, rheumatoid arthritis, and Benign Prostate hyperplasia was common in males. Osteoporosis was also seen to be more common in the patients, and this very likely contributes to the risk of falls.

The JHFRAT was used to assess the fall risk, and the patients were categorized into 'low', 'moderate' and 'high' fall risk categories (6%, 67% & 27%, respectively).

1. 'Low' Fall Risk Category: (n=5; 1 female and 4 males). The average age of these patients was 73.2±3.9 years. 2 belonged to the 60–69-year age group, 1 to the 70–79-year age group, and 2 were 80 years or more.
2. 'Moderate' Fall Risk category: (n=71; 38 females and 33 males) The average age of these patients was found to be 68.0±0.6 years. Of these 71, 44 were in the 60-69 year age group, 23 in the 70-79 year age group, and 4 were over 80 years old.
3. 'High' Fall Risk Category: (n=29; 14 females and 15 males). Of these, 13 were in the 60-69 year age group, 7 were in the 70-79 year age group, and 9 were over 80 years of age. The average age of these patients was 71.7±1.4 years.

Analysis of the fall history confirmed that 17 patients had experienced a fall prior to their OPD visit. The average age of these patients was 71.5±1.6 years. Eight belonged to the 60-69-year age group, 5 to the 70-79-year age group, and 4 were over 80 years of age (Table 1).

Table 1: Fall risk assessment based on Socio-demographic details

Socio-demographic Details		N	"Low risk" (0-5 points)	'Moderate risk' (6-13 points)	"High risk" (>13 points)
No. of Patients- 52 males; 53 females		105	5	71	29
Smokers (52 Males only)	Yes	7	0	5	2
	No	45	4	28	13
Alcoholic (52 Males only)	Yes	47	3	31	13
	No	5	1	2	2
Treatment funding	RI	15	0	10	5
	None	90	5	6	24

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Medication history –

The average number of medications prescribed was 6.7±0.2. The drugs that were prescribed most to the patients included antihypertensives, supplements, antidiabetics, diuretics, antimicrobials, antipsychotics, antidepressants, cardiovascular drugs, and analgesics. 50.4% of patients were prescribed 6-10 medications, and only 11.4% were prescribed more than 10. Table 2 shows the frequency of high fall risk (HFR) drugs.

Table 2: Frequency of HFR-drugs prescribed

No. of drugs	No. of Patients	Average JHFRAT score
2	2 (1.9%)	4.5
3	11 (10.4%)	8.6
4	15 (14.2%)	9.8
5	12 (11.4%)	12.1
6	12 (11.4%)	11.3
7	14 (13.3%)	14
8	12 (11.4%)	11.3
9	10 (9.5%)	10.7
10	5 (4.7%)	14
11	6 (5.7%)	11.2
12	3 (2.8%)	13.7
13	1 (0.9%)	8
14	1 (0.9%)	15
15	1 (0.9%)	12

Polypharmacy is also a major cause of falls in the elderly. In this study, 77 (73.3%) patients were prescribed 5 or more drugs. This could be a potential contributing factor to the increased fall risk score in the entire population.

Discussion

In this study, data from 105 patients show that 16% have experienced a fall event and 84% have not. These results align well with those of Puvvada et al.[10]

Risk factors for fall-related hospital admissions included increasing age, female gender, and various comorbidities. According to the Pearson Correlation analysis, there was no statistically significant difference in gender between the fall risk categories (p=0.31).

Consumption of certain high fall risk drugs is a major factor. This study shows that with an increase in the number of HFR drugs, there is an increment in the average JHFRAT score, confirming that HFR drugs are one of the major contributors to fall risk among the elderly population.

It was seen that antihypertensive agents were the most prescribed drugs under the HFR-drugs category, with amlodipine being the most common. The results of Juraschek and team in 2019 cautioned against the use of amlodipine in the elderly.[11] Further, Insulin was seen to be associated with an increased risk of falls, possibly due to the severity of disease and hypoglycemic episodes, further leading to reduced balance, strength, and gait.[12] two antidiabetics, metformin and glimepiride, also showed a moderate risk of falls. The risk of falls is thus associated with glycemic control and the strength of the drugs being used.[13]

Mamoto et. al. [14] did not find any higher incidence of falls in people with rheumatoid arthritis.[15] Individuals suffering from osteoporosis have skeletal fragility, which leads to an exponential

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increase in risk of fractures with age. This is related to both decreased bone density and other factors, such as decreased muscle strength and increased risk of falls in the elderly.[16]

Rashid et al, using Multinomial logistic regression from a dataset of 235 patients, demonstrated two salient facts: one the mobility functions, cognitive functions, high fall risk drugs and advanced age are major contributing factors to falls in the elderly and second, A positive correlation between high fall risk drugs and average fall risk score existed.[17]

This study, like any other scientific investigation, is not free from limitations. In the first place, the sample size is small and, the learned reader is expected to exercise caution before extrapolating upon the findings. Secondly, the study was performed at a single site. The heterogeneity of prescriptions of the elderly is another limitation.

Conclusions

The results of this cross-sectional study, conducted at the outpatient department of a public teaching hospital, are based on the data collected from 105 elderly patients. The assessment of risk of falls, using the Johns Hopkins fall risk assessment tool, showed that a little over a quarter of the patients were at "high fall risk" (27.6%); 67.7% patients were at "moderate fall risk", and another 4.7% patients were at "low fall risk".

The risk factors observed were age, followed by mobility functions, high-fall-risk medications, cognitive functions, patient care equipment, elimination (bowel and urine), and fall history. High fall risk drugs -like antihypertensives, followed by anticonvulsants, followed by opioid analgesics, antidepressants, diuretics, and antianginals- were most frequently used. A significant direct association was observed between HFRDs and average JHFRAT scores.

The results of this study also point to an association between falls and medications and diagnoses. It also proves that both these factors are directly proportional to the increased risk of falls.

Declaration:

Author Contribution	Conceptualization: Trinamjot Kaur
	Writing original draft: Trinamjot Kaur
	Review and editing: Pramil Tiwari
	Paper finalization: Pramil Tiwari & Sarabmeet Singh Lehl
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Copyright Declaration	Author declare that the manuscript titled "Evaluating the risk of falls in the elderly population at a public teaching tertiary care hospital" is an original work and has not been published previously nor is it under consideration for publication elsewhere. Upon acceptance, the copyright of this article will be transferred to the journal. The author grant the journal the right to publish, reproduce, distribute, and archive the article in all forms and media.
Conflict of Interest (COI)	The author declare that there are no conflicts of interest regarding the publication of this paper.
Artificial Intelligence (AI) Use Declaration	The author declare that artificial intelligence tools were used only for language editing, grammar refinement, and formatting assistance during manuscript preparation. All conceptualization, data collection, analysis, interpretation, and final manuscript approval were performed solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

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References:

1. Willeboorsde F, Hugtenburg HG, van Dijk, Bosmans JE, de Vries OJ, Schellevis FG, Elders PJM. Opti-Med: the effectiveness of optimised clinical medication reviews in older people with 'geriatric giants' in general practice; study protocol of a cluster randomised controlled trial. BMC Geriatrics. 2014 Dec (1):1-9.
2. Cuevas-Trisan R. Balance problems and fall risks in the elderly. Clinics in geriatric medicine. 2019 May ;35(2):173-83.
3. Park SH. Tools for assessing fall risk in the elderly: a systematic review and meta-analysis. Ageing Clin Exp Res. 2018 Jan;30(1):1-6.
4. Joseph A, Kumar D, Bagavandas M. A Review of Epidemiology of Fall among Elderly in India. Indian J Community Med. 2019 Apr-Jun;44(2):166–168
5. Garbaccio JL, Tonaco LA, Estevao WG et al. Ageing and quality of life of elderly people in rural areas. Revista Brasileira de Enfermagem. 2018;71:724-32.
6. Florence CS, Bergen G, Adam Atherly A, Burns E, Stevens J, Drake C: Medical Costs of Fatal and Nonfatal Falls in Older Adults, J Am Geriatr Soc, 2018 Apr;66(4):693-698.
7. Krishnaiah S, Ramanathan RV. Impact of blindness due to cataract in elderly fallers: findings from a cross-sectional study in Andhra Pradesh, South India. BMC research notes. 2018 Dec;11(1):1-6.
8. Falls. World Health Organization. 2021. Apr 26, [Last accessed on 2026 Jun 22]. pp. 1–2. Available from: http://www.who.int/news-room/fact-sheets/detail/falls.
9. Öztürk ZA, Özdemir İ, Türkbeyler H, Zeynep D. Quality of life and fall risk in frail hospitalised elderly patients. Turk. J. Med. Sci. 2017;47(5):1377-83.
10. Puvvada RK, Undela K, Parthasarathi G. Prevalence, risk factors, and cost burden of fall-related hospital admissions in India. Sr. care pharm. 2021 Jul ;36(7):343-9.
11. Juraschek SP, Simpson LM, Davis BR, Beach JL, Ishak A, Mukamal KJ. Effects of antihypertensive class on falls, syncope, and orthostatic hypotension in older adults: the ALLHAT trial. Hypertension. 2019 Oct;74(4):1033-40.
12. van Poelgeest EP, Handoko ML, Muller M, van der Velde N, EUGMS Task & Finish group on Fall-risk-increasing drugs. Diuretics, SGLT2 inhibitors and falls in older heart failure patients: to prescribe or to deprescribe? A clinical review. European geriatric medicine. 2023 Feb 3:1-6.
13. Fick, D.M., Semla, T.P., Steinman, M., Beizer, J., Brandt, N., Dombrowski, R., DuBeau, C.E., Pezzullo, L., Epplin, J.J. and Flanagan, N., 2019. The American Geriatrics Society 2019 updated AGS Beers Criteria® for potentially inappropriate medication use in older adults. Journal of the American Geriatrics Society,67(4), pp.674-694.
14. Cheng LY, Leung SY, Leung MK. The association of glycemic control and fall risk in diabetic elderly: a cross-sectional study in Hong Kong. BMC Primary Care. 2022 Aug 1;23(1):192.
15. Mamoto K, Inui K, Okano T, Sugioka Y, Tada M, Koike T, Nakamura H.Incidence rate of falls and its risk factors in patients with rheumatoid arthritis compared to controls: four years of the TOMORROW study. Mod. Rheumatol. 2017 Jan ;27(1):8-14.
16. Poe S.S., Dawson P.B., Cvach M., Burnett M., Kumble S., Lewis M., Thompson C.B., Hill E.E. The johns Hopkins fall risk assessment tool: A Study of Reliability and Validity. J. Nurs. Care Qual. 2018;33:10–19. doi: 10.1097/NCQ.0000000000000301
17. Rashid I, Tiwari P, Lehl SS. Prevalence, and determinants of fall risk among elderly inpatients at a tertiary care public teaching hospital. J. Gerontol. Geriatr. 2019;67:96-102.

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RESEARCH ARTICLE

Curcumin attenuates oxidative stress and nitric oxide-mediated alterations in experimental models of schizophrenia

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Abstract:

Background:
Schizophrenia is a chronic neurodevelopmental disorder characterized by positive, negative, and cognitive symptoms. Despite advances in antipsychotic therapy, suboptimal outcomes and adverse effects remain significant challenges. Oxidative stress and nitric oxide (NO) dysregulation are implicated in schizophrenia pathophysiology. Curcumin, a polyphenolic compound derived from Curcuma longa, exhibits antioxidant, anti-inflammatory, and inducible nitric oxide synthase (iNOS) inhibitory properties. This study evaluated the potential therapeutic role of curcumin in experimental animal models of schizophrenia.

Methods:
Male Swiss albino mice and Wistar rats were allocated into five groups (n=6 per group). Curcumin (100 mg/kg) was administered intraperitoneally for 28 days. On day 28, haloperidol (2 mg/kg, p.o.) or apomorphine (1 mg/kg, s.c.) was administered to induce catalepsy and stereotypic climbing behavior, respectively. Behavioral assessments were followed by biochemical estimations, including dopamine (LC–MS/MS), nitrite levels (Griess assay), thio barbituric acid reactive substances (TBARS), reduced glutathione (GSH), and catalase activity. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test.

Results:
Curcumin significantly reduced haloperidol-induced catalepsy duration ($P < 0.001$). However, it did not significantly inhibit apomorphine-induced climbing behavior ($P>0.05$). Biochemically, curcumin significantly decreased thioarbituric acid reactive substances (TBARS) and nitrite levels while increasing reduced glutathione (GSH) and catalase activity ($P < 0.05$). Dopamine levels were not significantly altered.

Conclusion:
Curcumin attenuated oxidative stress and nitrosative alterations in experimental schizophrenia models and improved haloperidol-induced motor impairment. These findings suggest its potential as an adjunctive therapeutic agent targeting oxidative and inflammatory pathways in schizophrenia. Further long-term and dose-response studies are warranted.

Keywords, Curcumin; Schizophrenia; Oxidative Stress; Nitric Oxide Synthase; Catalepsy; Antioxidants

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Introduction:

Schizophrenia is a well-known neurodevelopmental disorder. Schizophrenia usually develops in late adolescence or early adulthood. More than 21 million people worldwide are affected by schizophrenia.1 Schizophrenia mostly starts in adolescence; however, around 30% of the first onsets are past the age of 40 years. Though the incidence is low, the lifetime prevalence of schizophrenia is 1%.2

Many patients reported having suboptimal outcomes with recently developed antipsychotics. Incidents of refractory and treatment-resistant schizophrenia are reported despite well-intentioned and thoughtful therapies.3–5 Poor adherence to antipsychotic drugs is another major setback in patients with

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psychotic disorders, which is linked to increased rates of readmissions in hospitals; higher treatment costs can triple the costs of external services. 25% to 80% of patients fail to take their drugs correctly at some point during their treatment.6

Haloperidol-induced catalepsy is a widely used method to screen neuroleptic drugs in rodents. It is a robust behavioural rodent model to assess the nigrostriatal function and their modulation by cholinergic, nitrergic, serotonergic and other neurotransmitter pathways.7 The positive symptoms of schizophrenia were evaluated using apomorphine induced climbing behaviour which is due to the stimulation of dopamine receptors. It has been utilised as an appropriate method for the assessment of antipsychotic drugs.8,9

Schizophrenia is assumed to have immune and inflammatory implications. Nitric oxide (NO) plays a major role in the pathophysiological mechanism of schizophrenia and is closely associated with dopamine and glutamate, two neurotransmitters that are dysfunctional in schizophrenia. It has been observed that the NO metabolism is altered in the postmortem brain of schizophrenic patients. Preliminary studies suggest that antipsychotics may decrease the synthesis of nitric oxide synthase (NOS)10. Also, it was shown that the NO levels of erythrocytes from schizophrenic patients are significantly increased.11,12 Inducible nitric oxide synthase (iNOS) is the enzyme responsible for the generation of NO and has a possible potential role in schizophrenia.13

Curcumin (diferuloylmethane) [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is extracted from turmeric, the powdered rhizome of the plant Curcuma longa is a naturally occurring polyphenolic compound and comprises of 3% to 5% of turmeric, which is its pharmacologically active constituent.14 Curcumin is well known for its anti-inflammatory properties. Curcumin, even at smaller doses, targets multiple sources of oxidative stress and has potent antioxidant activities.15 Curcumin has neurogenerative and neuroprotective properties and can influence memory processes.16,17 A variety of studies have reported that curcumin inhibits inducible nitric oxide synthase (iNOS) in animal models.18–21 Also, curcumin has been demonstrated as a promising agent for the prevention as well as for the treatment of both NO and microglial cell-mediated neurodegenerative disorders.18

Considering the above evidence into account, our hypothesis is inhibition of inducible nitric oxide synthase (iNOS) by curcumin which would be an effective treatment for schizophrenia due to its neurogenerative, and neuroprotective properties. The present study will test the effect of curcumin on the animal models of schizophrenia.

Methods:

Study Design

Experimental preclinical study using established rodent models of schizophrenia.

Animals

Male Swiss albino mice (25–35 g) and Wistar rats (250–300 g) were procured from the Central Animal House, Monad University. Animals were acclimatized for one week prior to experimentation under controlled environmental conditions (temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12-hour light/dark cycle). Standard pellet diet and water were provided ad libitum throughout the study duration. Animals were housed in polypropylene cages with sterile bedding, which was changed regularly to maintain hygienic conditions.

All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Ethical Approval:

Approved by Institutional Animal Ethics Committee, Monad University (Registration No. 1933/PO/Re/S/17/CPCSEA; Project Code: ARFC/SOP/IAEC/15/18-19). CPCSEA guidelines were followed.

Drugs and chemicals:

Curcumin, haloperidol, and apomorphine were procured from Sigma Aldrich (USA) in pure form. Curcumin was dissolved in peanut oil and diluted to the desired concentration (100 mg/kg; p.o.) with peanut oil on the day of drug administration, whereas other drugs were dissolved in normal saline. All the chemicals and reagents used were of analytical grade.

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Methodology

All the animals were divided into five groups of six animals each. Group I animals were peanut oil treated; group II, normal control saline-treated; group III, toxic control (haloperidol or apomorphine); group IV, curcumin per se; group V, co-administration of curcumin with haloperidol or apomorphine. The dose of curcumin was selected following preliminary experiments.[22] Curcumin (100 mg/kg) was administered daily intraperitoneally (i.p.) for 28 days, while haloperidol was administered per orally (2 mg/kg, p.o) and apomorphine subcutaneously (1 mg/kg, s.c.) as a single dose on the 28th day. Behavioral tests were performed on the 28th day, 1hour after the last dosing regimen. Animals were sacrificed after behavioural tests, and the brains were removed for biochemical estimations.

Experimental Groups

Five groups (n=6 each):

1. Peanut oil control
2. Normal saline control
3. Toxic control (haloperidol or apomorphine)
4. Curcumin per se
5. Curcumin + toxic control

Behavioral Assessments

Haloperidol-Induced Catalepsy[7,23]

Haloperidol (2 mg/kg p.o) was used to induce catalepsy, and it was determined every hour up to 4 hours by using a standard bar test[7,23]. The phenomenon was calculated as the time when the mouse continued in an imposed position with both front limbs extended and resting on a 4 cm high bar (0.4 cm diameter). The total time throughout which the animal stayed on the bar (even if it climbed back up) was recorded for a maximum period of 300 seconds.

Apomorphine-Induced Climbing Behaviour[8,9]

Apomorphine-induced climbing behaviour was followed as previously explained with few modifications[8,9]. Vehicle and curcumin were administered 1 hour and 2 hours before the administration of apomorphine (1 mg/kg s.c). Immediately after injection, the rats were individually placed in cylindrical wire mesh cages (height 13 cm, diameter 14 cm, mesh size 3 mm). During the trial, the time when each rat climbed on the inside of the wire cage was recorded for 30 minutes. Maximum time (maximum time spent in a single climb all through the duration of the apomorphine effect) and percent climbing index (the percent of time spent climbing during a 30 minutes period following the first climb) were recorded.

Biochemical Estimations

Dopamine (LC–MS/MS)[24]

Animals were decapitated, and their brains were removed and kept on ice without delay. Within 4 minutes, striata were removed and wrapped in aluminium foil and kept in a deep freezer at -80°C until dopamine analysis via liquid chromatography-mass spectrometry (LC-MS-MS) was conducted. 100 mg striatum was homogenised in 500 µl ice-cold methanol by vortex mixing for 10 minutes. One millilitre of acetonitrile was mixed with homogenate into a 2.5 ml conical plastic centrifuge tube and centrifuged at 14,000 rpm for 20 minutes. After this, the supernatant was evaporated to dryness by nitrogen evaporator at 14°C temperature. Then the dry residue was reconstituted with 400 µl mobile phase 0.05% formic acid acetonitrile (92: 8, v/v) and vortex-mixed for 10 seconds; the mixture was then centrifuged at 14,000 rpm for another 20 minutes. The upper aqueous layer was injected into the LC-MS-MS. The sample volume injected was 10 µl for dopamine.[24]

Nitric Oxide Synthase (NOS) activity

The preserved whole brains were incubated in cell-culture media and using the principles of the Griess reagent reaction, the nitrate levels were assessed. After the end of the incubation period, the Grace's medium (at least 45 µl) was transferred from the microtiter wells into the first set of centrifuge tubes corresponding to each group. Nitrate reductase and NADPH were added at this step to convert nitrates to nitrites. A gentle centrifuge (around 8,175 x g) was done for 2 minutes to pellet any brain or tissue particulates that may have been aspirated from the wells.

30 µl of the supernatant was pipetted from the first set of centrifuge tubes into the second set of corresponding tubes, making sure not to disrupt the pellet after spinning down the samples. Griess reagent was added in a 1:1 ratio and incubated for 5-10 minutes, and the assay was performed using a microplate reader at an absorbance wavelength of 540 nm with 620 nm reference wavelength.[25,26]

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Thio Barbituric Acid Reactive Substances (TBARS)[27]

One millilitre of suspension medium was taken from the supernatants of the 10% tissue homogenate and centrifuged at 10,000 rpm with 0.5 ml of 30% TCA, followed by adding 0.5 ml of 0.8% TBA. The tubes were covered with aluminium foil and kept in a shaking water bath for 30 minutes at 80°C. After 30 minutes, tubes were taken out and kept in ice-cold water for 10 minutes. They were then centrifuged at 3000 rpm for 15 minutes. The absorbance of supernatants was read against an appropriate blank at 540 nm at room temperature. The blank consists of 1.0 ml distilled water, 0.5 ml TBA, and 0.5 ml TCA.

Reduced Glutathione (GSH)[28]

Brain tissue was homogenized in 0.02 M of EDTA (6%) and 5 ml of homogenate was mixed with 4 ml of distilled water and 1 ml of 50% TCA. The tubes were shaken for 10 to 15 min and then centrifuged at 300 rpm for 1 minute. Following this, 2 ml of supernatant was mixed with 4 ml of 0.4 M tris buffer (pH 8.9) and 0.1 ml of DTNB. The absorbance was read within 5 minutes of the addition of DTNB at 410 nm against a reagent blank with no homogenate.

Catalase Activity[29]

In 2ml of potassium phosphate buffer (pH 7.4), a 10% tissue homogenate was prepared. This homogenate was centrifuged at 3000 rpm for 15 minutes. Catalase activity was measured in the supernatant obtained after centrifugation. 2.95 ml of 19 mM H_2O_2 was put in the cuvette. 0.05 ml of cytosolic supernatant was added to it, and a change in absorbance at 240 nm was recorded at the 1-minute interval for 3 minutes. The presence of catalase decomposes H_2O_2, leading to a decrease in absorbance.

Statistical Analysis

The sample size (n = 6 per group) was determined based on commonly accepted practices in preclinical pharmacological studies and prior published literature using similar experimental models.[7],[23]. This sample size has been demonstrated to provide adequate power to detect statistically significant differences in behavioral and biochemical parameters while adhering to the principles of reduction in animal use as per CPCSEA guidelines.

Although the sample size per group was relatively small, data were expressed as mean ± SEM and assumed to follow a normal distribution based on consistency with previously validated experimental models and literature. One-way analysis of variance (ANOVA) was therefore considered appropriate for comparing group means. Additionally, ANOVA is widely used in similar pharmacological studies involving small sample sizes when data variability is controlled and experimental conditions are standardised.

Dunnett's multiple comparison test was employed as the post hoc analysis because the study design primarily involved comparisons of multiple treatment groups against a single control group. Dunnett's test is specifically designed for such comparisons and provides greater statistical power while controlling Type I error. In contrast, the Games–Howell test is typically used when comparing all possible pairwise differences under conditions of unequal variances and sample sizes, which was not the objective of the present study. A P-value < 0.05 was considered significant.

Results:

Effect of Curcumin on Haloperidol-Induced Catalepsy

Treatment of haloperidol (2 mg/kg, p.o) in mice caused a highly significant cataleptic state within 1 hour of administration, reaching a maximal plateau after 2 hours and lasting for 4 hours ($P < 0.001$). Vehicle-treated animals did not display catalepsy as they remained for about less than 10 seconds on the bar at each time point.

Pretreatment with curcumin (100 mg/kg p.o.) reduced the catalepsy score in haloperidol-treated mice significantly as compared to toxic control ($P < 0.001$) (Refer to Supplementary Appendix 1, Table 1).

Effect of Curcumin on Apomorphine-Induced Climbing Behaviour in Rats

Administration of apomorphine (1 mg/kg, s.c) caused a significant increase in the climbing index and climbing time ($p < 0.001$) in rats. Pretreatment with curcumin (100 mg/kg p.o.) 2 h before the administration of apomorphine showed an inhibitory effect on climbing behaviour but failed to achieve a significant effect on the maximum time of single climb and climbing index in rats ($p > 0.05$) (Figures to Supplementary appendix 1, Table 2).

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Effect of Curcumin on TBARS, GSH and Catalase Levels

Administration of curcumin (100 mg/kg i.p.)) alone and in combination with different treated groups such as haloperidol and apomorphine showed a significant reduction in thio barbituric acid reactive substances (TBARS) levels, an increase in reduced glutathione (GSH) and catalase levels in both models used in the present study ($P < 0.001$) (Refer to Supplementary appendix 2, Figure 1 and 2).

Effect of Curcumin on Dopamine Levels

Administration of curcumin (100 mg/kg, i.p) alone displayed a non-significant slight increase in dopamine level as compared to vehicle control in both models. The effect of curcumin in combination with toxic control (haloperidol or apomorphine) failed to produce any significant effect ($P < 0.05$) (Refer to Supplementary appendix 2, Figure 3 and 4).

Effect of Curcumin on Nitrite Levels

A significant decrease in nitrite levels was reported in the curcumin group in all two models as compared to the vehicle control group ($p<0.05$). Pre-treatment with Curcumin (100 mg/kg) with toxic control significantly attenuated the plasma nitrite levels as compared to toxic control in haloperidol-induced catalepsy ($p<0.05$) and apomorphine-induced climbing model ($p<0.001$) (Refer to Supplementary appendix 2, Figure 5 and 6).

Discussion:

The current study is the first preclinical investigation evaluating the potential role of curcumin in both cataleptic and stereotypic models of schizophrenia. In this study, curcumin demonstrated anti-schizophrenic effects in the haloperidol-induced catalepsy model; however, no remarkable beneficial effects were observed in the apomorphine-induced climbing model. Our findings are consistent with the published clinical trial results, which reported promising benefits of curcumin in the management of negative symptoms of schizophrenia.[30]

Curcumin may have potential as an alternative or adjunctive treatment for patients with schizophrenia. Previous preclinical and clinical studies have demonstrated that curcumin possesses antioxidant, anti-inflammatory, neuroprotective, and pro-cognitive properties.[30]

In the present study, haloperidol was used as a neuroleptic agent known to exert multiple effects on dopaminergic signalling and to induce catalepsy. Blockade of D_1 and D_2 dopamine receptors in the nigrostriatal pathway, along with the involvement of other neurotransmitter systems, is considered responsible for haloperidol-induced catalepsy. The current findings further demonstrate that haloperidol increases oxidative stress in the brain, contributing to the development of catalepsy. Increased oxidative stress has also been reported in chronic schizophrenic patients and is primarily attributed to altered antioxidant enzyme levels.[31,32]

Oxidative stress in this study was evaluated by measuring thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), and nitrite levels in brain tissue. Curcumin, known for its potent antioxidant properties, targets multiple sources of oxidative stress even at lower doses and acts as an effective free radical scavenger of reactive oxygen species, including superoxide, peroxyl, and hydroxyl radicals.[15,33] These antioxidant effects likely contributed to the attenuation of cataleptic behavior observed in our study. Several previous experimental investigations have similarly confirmed the antioxidant and free radical scavenging properties of curcumin, supporting our findings.[34,35]

Apomorphine, a dopamine receptor agonist, induces climbing behavior in rats through stimulation of dopamine receptors in the striatum. Previous studies have demonstrated that nitric oxide synthase (NOS) inhibitors, such as 7-nitroindazole, can prevent apomorphine-induced stereotypy and climbing behavior. Additionally, myricitrin and thymoquinone, both inducible nitric oxide synthase (iNOS) inhibitors, have shown antipsychotic-like effects in this model.[9,36] Another study reported that nitric oxide donors potentiate apomorphine-induced climbing behavior, which can be suppressed by NMDA receptor antagonists. Furthermore, inducible nitric oxide synthase (iNOS) inhibitors were found to prevent apomorphine-induced climbing behavior, and this effect was enhanced by NMDA receptor agonists.[37]

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In the present study, pre-treatment with curcumin (100 mg/kg, i.p.) reduced the maximum time of a single climb and climbing index compared with the control group, possibly due to its inducible nitric oxide synthase (iNOS) inhibitory activity. However, this inhibition did not reach statistical significance ($P < 0.05$).

The negative symptoms of schizophrenia share similarities with depressive symptomatology. In several previous studies, curcumin has demonstrated antidepressant effects, which may logically explain the improvement observed in schizophrenia models. A recent randomised clinical trial reported a significant improvement in the negative symptom subscale of schizophrenia with curcumin adjunct therapy, although no significant differences were observed in positive symptoms.30 These clinical findings align closely with the results of the present study and further support the potential role of curcumin as an adjunctive therapy in schizophrenia.

However, no remarkable improvements were observed in the positive symptom model of schizophrenia. Therefore, further investigations using varying doses of curcumin are necessary to comprehensively evaluate its effects in experimental models of schizophrenia.

Conclusion:

Curcumin demonstrated significant antioxidant and nitrosative modulation in experimental schizophrenia models and improved haloperidol-induced motor impairment. These findings support further investigation of curcumin as an adjunctive therapeutic agent in schizophrenia.

Table 1: Effect of curcumin on haloperidol induced catalepsy in mice

Groups (N=6)	Drug treatment	Dosage	Catalepsy Duration (Sec)			
			After 1 hr	After 2 hr	After 3 hr	After 4 hr
I	Peanut oil	1 ml	10.0 ± 0.18	11.43 ± 5.8	10.32 ± 1.2	9.29 ± 5.2
II	Normal Saline	1 ml	9.45± 2.6	10.78±5.7	10.54±3.6	7.19±6.5
III	HAL	2 mg/kg	159.17±18.42$^{***1, ***2}$	248.33±17.77$^{***1, ***2}$	274±22.13$^{***1, ***2}$	279±27.68$^{***1, ***2}$
IV	CUR	100 mg/kg	8.92±4.5	10.56±3.7	13.67±9.7	11.45±5.4
V	HAL + CUR	2 mg/kg + 100 mg/kg	113±25.68 $^{***1, ***2, ***3}$	182±21.57$^{***1, ***2, ***3}$	173±33.62$^{***1, ***2, ***3}$	167±21.94$^{***1, ***2, ***3}$

CUR: curcumin, HAL: haloperidol, hr: hour, sec: second. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

Table 2: Effect of curcumin on apomorphine induced climbing behaviour in rats

Groups (N=6)	Drug treatment	Dosage	Maximum time (sec)	% Climbing index
I	Peanut oil	1 ml	12.43±0.57	7.6±1.52
II	Normal Saline	1 ml	13.86±1.24	8.25±0.97
III	APO	1 mg/kg	309.37±20.52^{***1,***2}	61.06±4.57^{***1,***2}
IV	CUR	100 mg/kg	16.81±2.15	10.53±1.11
V	APO + CUR	1 mg/kg + 100 mg/kg	292.57±10.34	53.28±8.45

APO: apomorphine, CUR: curcumin, hr: hour, sec: second. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

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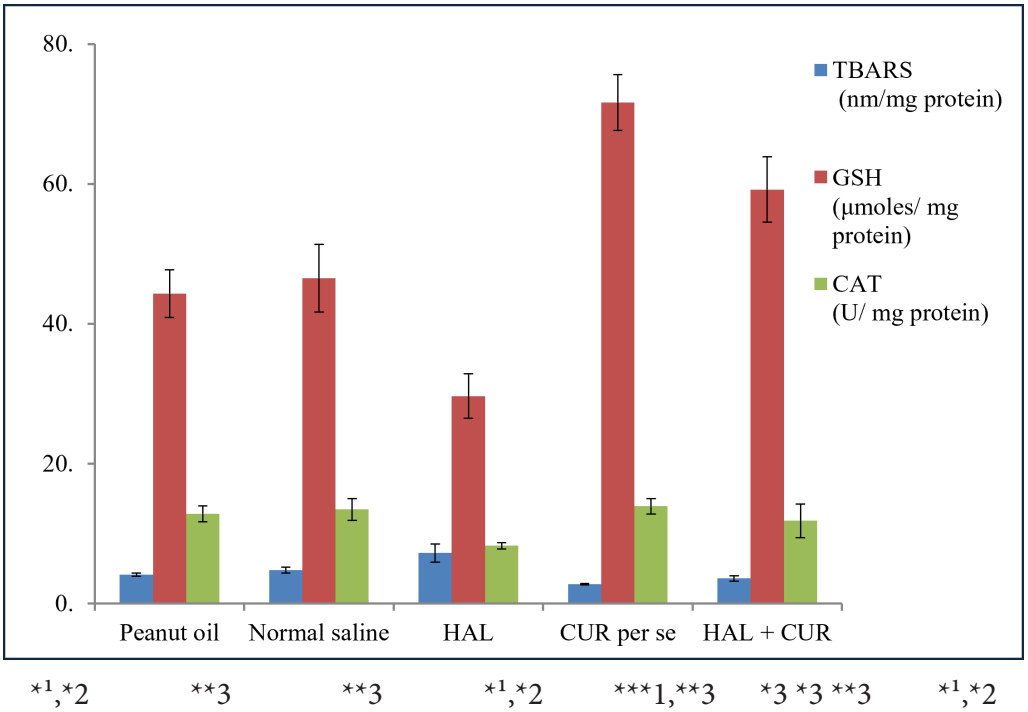

Figure 1: Effect of curcumin on TBARS, GSH and CAT levels in haloperidol-induced catalepsy in mice

X-axis: Animal groups; **Y-axis:** Catalepsy Duration (seconds)

HAL: Haloperidol, CUR: Curcumin. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

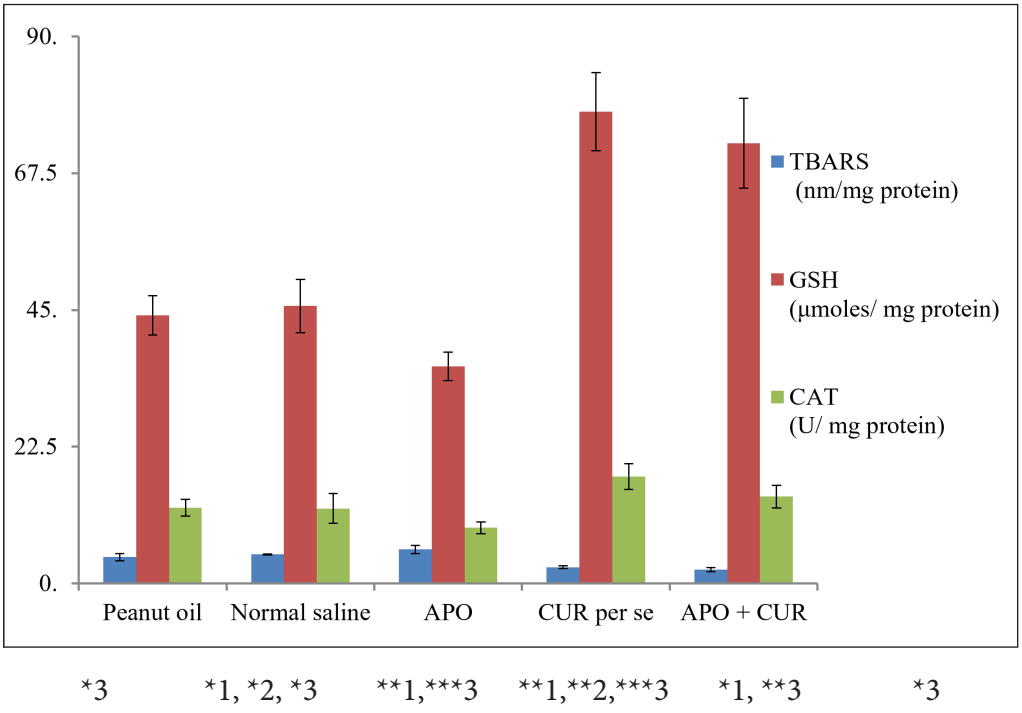

Figure 2: Effect of curcumin on TBARS, GSH and CAT levels in apomorphine-induced climbing behaviour in rats

X-axis: Animal groups; **Y-axis:** Climbing time (seconds)

APO: Apomorphine, CUR: Curcumin. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

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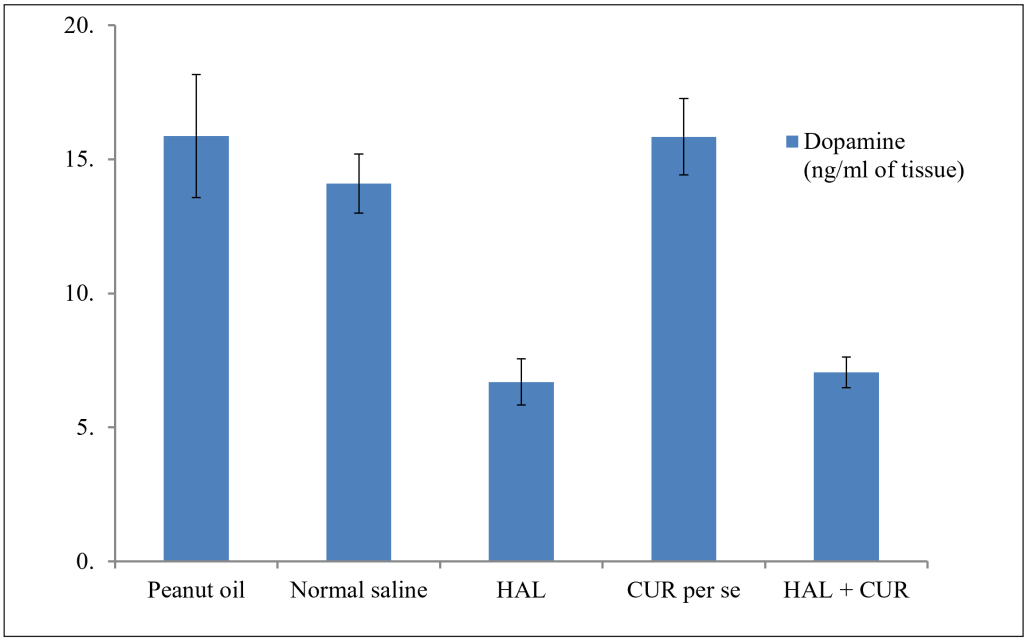
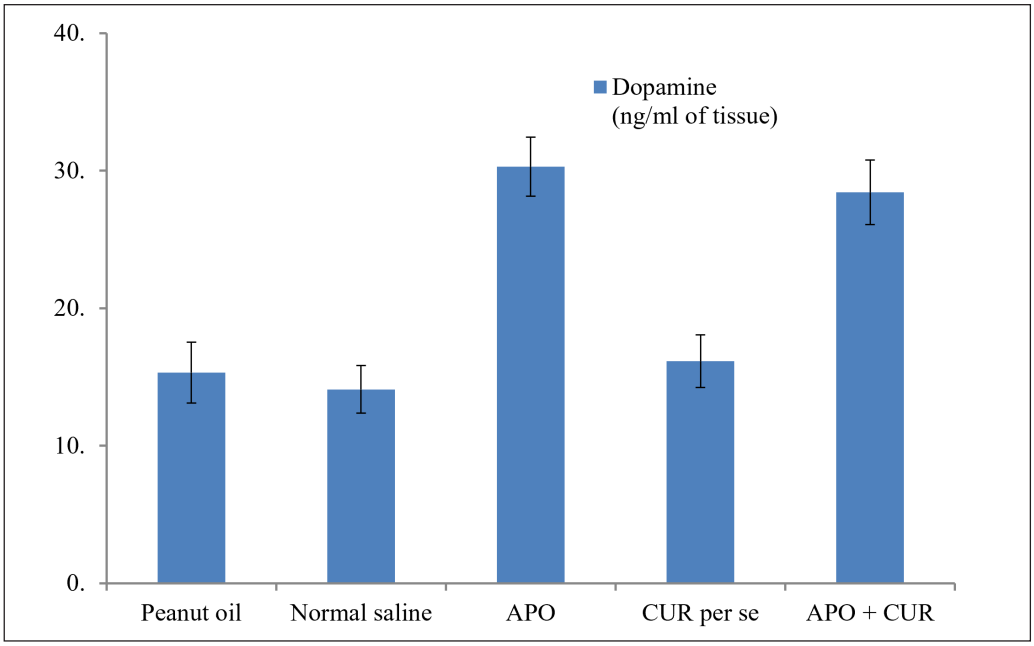


1,2 ***1,***2

Figure 3: Effect of curcumin on dopamine levels in haloperidol-induced catalepsy in mice

X-axis: Animal groups; **Y-axis:** Catalepsy Duration (seconds)

HAL: Haloperidol, Cur: Curcumin All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).



1,2 ***1,***2

Figure 4: Effect of curcumin on dopamine levels in apomorphine-induced climbing behaviour in rats

X-axis: Animal groups; **Y-axis:** Climbing time (seconds)

APO: Apomorphine, CUR: Curcumin. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

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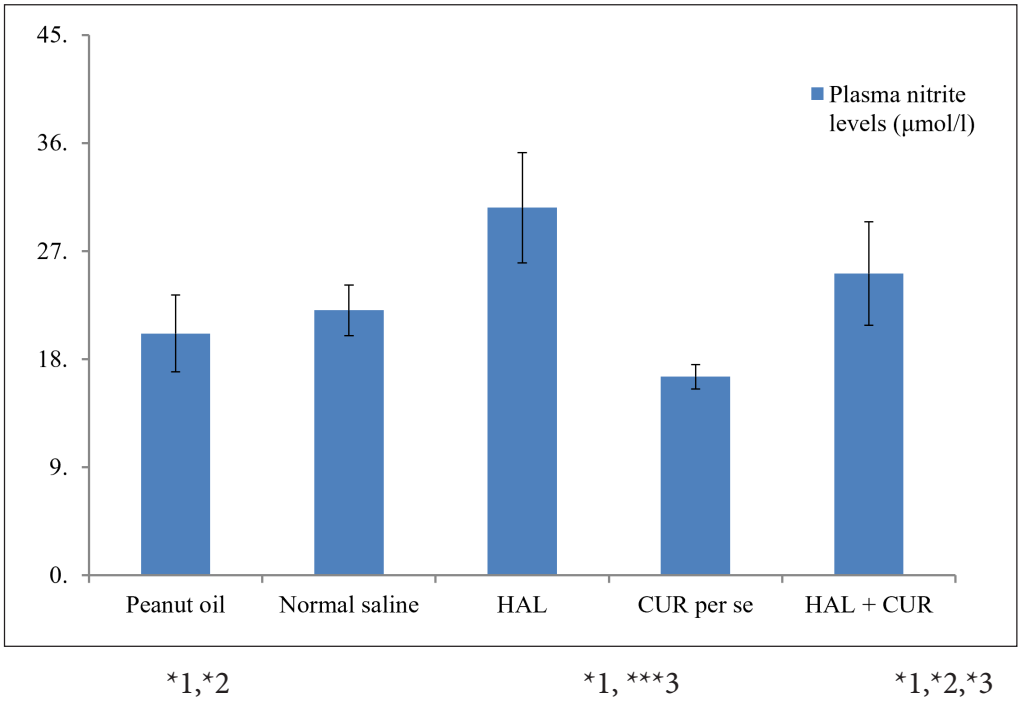

Figure 5: Effect of curcumin on nitrite levels in haloperidol-induced catalepsy in mice

X-axis: Animal groups; **Y-axis:** Catalepsy Duration (seconds)

HAL: Haloperidol, CUR: Curcumin All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control)

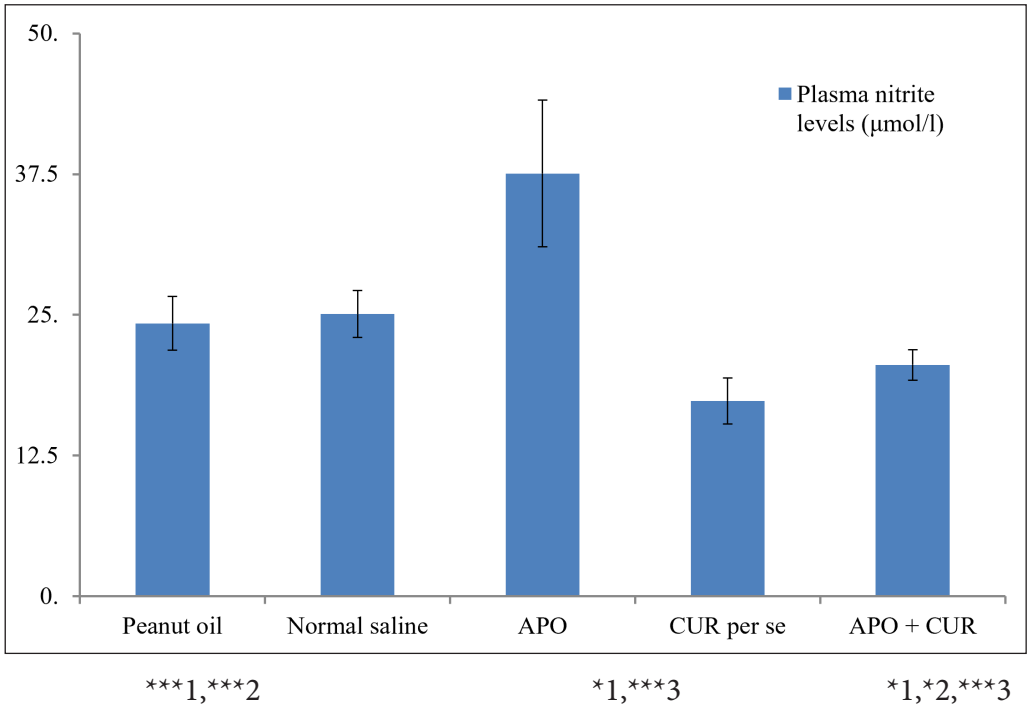

Figure 6: Effect of curcumin on nitrite levels in apomorphine induced climbing behaviour in rats

X-axis: Animal groups; **Y-axis:** Climbing time (seconds)

APO: Apomorphine, CUR: Curcumin. All values were expressed as mean ± standard error of mean (SEM), analysed by ANOVA followed by Dunnett's multiple comparison test. n = 6, number of animals in each group. * P<0.05, **P<0.01, *** P<0.001 (¹ Compared with peanut oil control, ² Compared with saline control, ³ Compared with toxic control).

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Declaration:

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Copyright Declaration	Author declare that the manuscript titled "Curcumin attenuates oxidative stress and nitric oxide-mediated alterations in experimental models of schizophrenia" is an original work and has not been published previously nor is it under consideration for publication elsewhere. Upon acceptance, the copyright of this article will be transferred to the journal. The author grant the journal the right to publish, reproduce, distribute, and archive the article in all forms and media.
Conflict of Interest (COI)	The author declare that there are no conflicts of interest regarding the publication of this paper.
Artificial Intelligence (AI) Use Declaration	The author declare that artificial intelligence tools were used only for language editing, grammar refinement, and formatting assistance during manuscript preparation. All conceptualization, data collection, analysis, interpretation, and final manuscript approval were performed solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

References

1. Schizophrenia https://www.who.int/news-room/fact-sheets/detail/schizophrenia.
2. Löhrs, L., and Hasan, A. (2019). [Risk factors for the development of schizophrenia]. Fortschr. Neurol. Psychiatr. *87*, 133–143. https://doi.org/10.1055/A-0836-7839.
3. Huhn, M., Nikolakopoulou, A., Schneider-Thoma, J., Krause, M., Samara, M., Peter, N., Arndt, T., Bäckers, L., Rothe, P., Cipriani, A., et al. (2019). Comparative efficacy and tolerability of 32 oral antipsychotics for the acute treatment of adults with multi-episode schizophrenia: a systematic review and network meta-analysis. The Lancet *394*, 939–951. https://doi.org/10.1016/S0140-6736(19)31135-3.
4. Kennedy, J.L., Altar, C.A., Taylor, D.L., Degtiar, I., and Hornberger, J.C. (2014). The social and economic burden of treatment-resistant schizophrenia: A systematic literature review. Int. Clin. Psychopharmacol. *29*, 63–76. https://doi.org/10.1097/YIC.0B013E32836508E6.
5. Hert, M. De, van Winkel, R., Wampers, M., Kane, J., van Os, J., and Peuskens, J. (2007). Remission criteria for schizophrenia: Evaluation in a large naturalistic cohort. Schizophr. Res. *92*, 68–73. https://doi.org/10.1016/J.SCHRES.2007.01.010.
6. Priebe, S., Yeeles, K., Bremner, S., Lauber, C., Eldridge, S., Ashby, D., David, A.S., O'Connell, N., Forrest, A., and Burns, T. (2013). Effectiveness of financial incentives to improve adherence to maintenance treatment with antipsychotics: cluster randomised controlled trial. BMJ *347*. https://doi.org/10.1136/BMJ.F5847.

7. Khan, R.A., Kalam Najmi, A., Hussain Khuroo, A., Goswami, D., and Akhtar, M. (2014). African Journal of Pharmacy and Pharmacology Ameliorating effects of thymoquinone in rodent models of schizophrenia. *8*, 413–421. https://doi.org/10.5897/AJPP2013.3968.
8. Costall, B., Naylor, R.J., and Nohria, V. (1978). Climbing behaviour induced by apomorphine in mice: A potential model for the detection of neuroleptic activity. Eur. J. Pharmacol. *50*, 39–50. https://doi.org/10.1016/0014-2999(78)90251-0.
9. Moore, N.A., Tye, N.C., Axton, M.S., and Risius, F.C. (1992). The behavioral pharmacology of olanzapine, a novel "atypical" antipsychotic agent. Journal of Pharmacology and Experimental Therapeutics *262*, 545–551. https://doi.org/10.1016/s0022-3565(25)10792-1.
10. Wiley, J.L. (1998). Nitric Oxide Synthase Inhibitors Attenuate Phencyclidine-Induced Disruption of Prepulse Inhibition. Neuropsychopharmacology *19*, 86–94. https://doi.org/10.1016/S0893-133X(98)00008-6.
11. Deutsch, S.I., Rosse, R.B., Paul, S.M., Tomasino, V., Koetzner, L., Morn, C.B., and Mastropaolo, J. (1996). 7-Nitroindazole and methylene blue, inhibitors of neuronal nitric oxide synthase and NO-stimulated guanylate cyclase, block MK-801-elicited behaviors in mice. Neuropsychopharmacology *15*, 37–43. https://doi.org/10.1016/0893-133X(95)00153-5;KWRD.
12. Herken, H., Uz, E., Özyurt, H., Söğüt, S., Virit, O., and Akyol, Ö. (2000). Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Molecular Psychiatry 2001 6:1 *6*, 66–73. https://doi.org/10.1038/sj.mp.4000789.
13. Förstermann, U., and Sessa, W.C. (2012). Nitric oxide synthases: regulation and function. Eur. Heart J. *33*, 829–837. https://doi.org/10.1093/EURHEARTJ/EHR304.
14. Salvioli, S., Sikora, E., Cooper, E.L., and Franceschi, C. (2007). Curcumin in Cell Death Processes: A Challenge for CAM of Age-Related Pathologies. Evidence-Based Complementary and Alternative Medicine *4*, 181–190. https://doi.org/10.1093/ECAM/NEM043.
15. Fujisawa, S., Atsumi, T., Ishihara, M., and Kadoma, Y. (2004). Cytotoxicity, ROS-generation Activity and Radical-scavenging Activity of Curcumin and Related Compounds. Anticancer Res. *24*, 563–570.
16. Kang, S.K., Cha, S.H., and Jeon, H.G. (2006). Curcumin-induced histone hypoacetylation enhances caspase-3-dependent glioma cell death and neurogenesis of neural progenitor cells. Stem Cells Dev. *15*, 165–174. https://doi.org/10.1089/ SCD .2006.15.165; WEBSITE:WEBSITE:SAGE; WGROUP:STRING:PUBLICATION.
17. Kim SoJung Kim SoJung, T.G.S.P.H.P.H.P.M.P.M.K.M.K.M.K.H.K.H.C.H.C.H.M.P.M.L.J.L.J. (2008). Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. Journal of Biological Chemistry *283*, 14497–14505.
18. Jung, K.K., Lee, H.S., Cho, J.Y., Shin, W.C., Rhee, M.H., Kim, T.G., Kang, J.H., Kim, S.H., Hong, S., and Kang, S.Y. (2006). Inhibitory effect of curcumin on nitric oxide production from lipopolysaccharide-activated primary microglia. Life Sci. *79*, 2022–2031. https://doi.org/10.1016/J.LFS.2006.06.048.
19. Wei, S., Xu, H., Xia, D., and Zhao, R. (2010). Curcumin attenuates the effects of transport stress on serum cortisol concentration, hippocampal NO production, and BDNF expression in the pig. Domest. Anim. Endocrinol. *39*, 231–239. https://doi.org/10.1016/J.DOMANIEND.2010.06.004.
20. Gilhotra, N., and Dhingra, D. (2010). GABAergic and nitriergic modulation by curcumin for its antianxiety-like activity in mice. Brain Res. *1352*, 167–175. https://doi.org/10.1016/J.BRAINRES.2010.07.007.
21. Song, M.Y., Yim, J.Y., Yim, J.M., Kang, I.J., Rho, H.W., Kim, H.S., Yhim, H.Y., Lee, N.R., Song, E.K., Kwak, J.Y., et al. (2011). Use of curcumin to decrease nitric oxide production during the induction of antitumor responses by IL-2. Journal of Immunotherapy *34*, 149–164. https://doi.org/10.1097/CJI.0B013E3182056EC4.

22. Dhouib, I.B., Annabi, A., Doghri, R., Rejeb, I., Dallagi, Y., Bdiri, Y., Lasram, M.M., Elgaaied, A., Marrakchi, R., Fazaa, S., et al. (2017). Neuroprotective effects of curcumin against acetamiprid-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: biochemical, histological, and behavioral changes. Environmental Science and Pollution Research 2017 24:35 *24*, 27515–27524. https://doi.org/10.1007/S11356-017-0331-5.

23. Akhtar, M., Devi, P.U., Ali, A., Pillai, K.K., and Vohora, D. (2006). Antipsychotic-like profile of thioperamide, a selective H 3-receptor antagonist in mice. Fundam. Clin. Pharmacol. *20*, 373–378. https://doi.org/10.1111/J.1472-8206.2006.00411.X;CTYPE:STRING:JOURNAL.

24. Su, F., Wang, F., Zhu, R., and Li, H. (2008). Determination of 5-Hydroxytryptamine, Norepinephrine, Dopamine and Their Metabolites in Rat Brain Tissue by LC–ESI–MS–MS. Chromatographia 2008 69:3 *69*, 207–213. https://doi.org/10.1365/S10337-008-0879-9.

25. Tsikas, D. (2007). Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the l-arginine/nitric oxide area of research. Journal of Chromatography B *851*, 51–70. https://doi.org/10.1016/J.JCHROMB.2006.07.054.

26. Hall, C.N., and Attwell, D. (2008). Assessing the physiological concentration and targets of nitric oxide in brain tissue. Journal of Physiology 586, 3597–3615. https://doi.org/10.1113/ JPHYSIOL.2008.154724; WGROUP:STRING:PUBLICATION.

27. Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. *95*, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

28. Ellman, G.L. (1959). Tissue sulfhydryl groups. Arch. Biochem. Biophys. *82*, 70–77.

29. Claiborne, A. (1985). Catalase Activity. CCRC Handbook of Methods for Oxygen Radical Research, 283–284. https://doi.org/10.1201/9781351072922-57.

30. Miodownik, C., Lerner, V., Kudkaeva, N., Lerner, P.P., Pashinian, A., Bersudsky, Y., Eliyahu, R., Kreinin, A., and Bergman, J. (2019). Curcumin as add-on to antipsychotic treatment in patients with chronic schizophrenia: A randomized, double-blind, placebo-controlled study. Clin. Neuropharmacol. *42*, 117–122. https://doi.org/10.1097/WNF.0000000000000344.

31. Grabnar, I., Vovk, T., Kores Plesnicar, B., and Boskovic, M. (2011). Oxidative Stress in Schizophrenia. Curr. Neuropharmacol. *9*, 301–312. https://doi.org/10.2174/157015911795596595/CITE/REFWORKS.

32. Flatow, J., Buckley, P., and Miller, B.J. (2013). Meta-Analysis of Oxidative Stress in Schizophrenia. Biol. Psychiatry *74*, 400–409. https://doi.org/10.1016/J.BIOPSYCH.2013.03.018.

33. Menon, V.P., and Sudheer, A.R. (2007). Antioxidant and anti-inflammatory properties of curcumin. Adv. Exp. Med. Biol. *595*, 105–125. https://doi.org/10.1007/978-0-387-46401-5_3.

34. Ak, T., and Gülçin, I. (2008). Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. *174*, 27–37. https://doi.org/10.1016/J.CBI.2008.05.003.

35. Kulkarni, S.K., Bhutani, M.K., and Bishnoi, M. (2008). Antidepressant activity of curcumin: involvement of serotonin and dopamine system. Psychopharmacology 2008 201:3 *201*, 435–442. https://doi.org/10.1007/S00213-008-1300-Y.

36. Pereira, M., Siba, I.P., Chioca, L.R., Correia, D., Vital, M.A.B.F., Pizzolatti, M.G., Santos, A.R.S., and Andreatini, R. (2011). Myricitrin, a nitric oxide and protein kinase C inhibitor, exerts antipsychotic-like effects in animal models. Prog. Neuropsychopharmacol. Biol. Psychiatry *35*, 1636–1644. https://doi.org/10.1016/J.PNPBP.2011.06.002.

37. Hong, J.T., Kim, H.C., Kim, H.S., Lee, Y.M., and Oh, K.W. (2005). The role of nitric oxide on glutaminergic modulation of dopaminergic activation. Pharmacol. Res. *52*, 298–301. https://doi.org/10.1016/J.PHRS.2005.05.002.

RESEARCH ARTICLE

Development and validation of stability-indicating reverse-phase high-performance liquid chromatographic method for the estimation of Sevabertinib in bulk drug

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Abstract:

A simple method was created and evaluated to accurately measure the amount of Sevabertinib in its raw drug using a type of liquid chromatography called reverse-phase high-performance liquid chromatography (RP-HPLC). Chromatographic separation was performed using an Inertsil ODS C18 column (4.6 × 150 mm, 5 µm) with a mobile phase consisting of 0.1% ortho phosphoric acid buffer, acetonitrile, and methanol in a 30:60:10 v/v/v ratio, at a flow rate of 1.5 ml/min and detected at 290 nm. The method was evaluated using ICH Q2(R1) guidelines, showing satisfactory linearity with an $R^2 % value of 0.9979 and an average recovery of 100.50%. The precision was evaluated and found to be very good, with a %RSD of 0.2%, the limit of detection was 2.97, and the limit of quantification was 9.97. Robustness studies showed that small changes in flow rate and mobile phase composition did not significantly impact system suitability. This tested method is very precise, reliable and validated for regular quality checks and testing how stable Sevabertinib is over time.

Keywords, RP-HPLC, Sevabertinib, Method development, non-small cell lung cancer (NSCLC)

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Introduction:

Sevabertinib is a novel, reversible tyrosine kinase inhibitor that selectively targets HER2(ERBB2) mutations and demonstrated promising therapeutic potential in HER2-mutant cancers, particularly non-small cell lung cancer (NSCLC), selectively inhibits HER2 tyrosine kinase activity, thereby blocking aberrant signaling pathways responsible for tumor cell proliferation and survival[1-2], that received accelerated approval from the U.S Food and Drug Administration (FDA) in November 2025 for the treatment of adult patients with locally advanced or metastatic NSCLC characterized by HER2 tyrosine kinase domain mutations, this approval underscores its clinical importance as a targeted therapeutic option in precision oncology[3]. RP-HPLC is commonly used for analyzing non-volatile, Lipophilic drugs like tyrosine kinase inhibitors, and has shown reliable performance with drugs such as afatinib and erlotinib in terms of sensitivity and reproducibility[4-5]. However, for Sevabertinib, the available studies are still limited to basic method development and validation[6], with no detailed or stability-indicating analytical methods reported so far. Hence, the development of a stability-indicating method is essential to ensure accurate quantification of the drug under stress conditions. This study aims to develop and validate

a simple, rapid, and stability-indicating RP-HPLC method for the estimation of Sevabertinib in bulk drug. The method offers high sensitivity, selectivity and routine quality control and stability studies. Validation performed in accordance with ICH Q2 (R1) guidelines to ensure accuracy, precision, and robustness[7]. Emphasis is placed on effective separation of the drug from its degradation products formed under various stress conditions, particularly alkaline and oxidative environments.

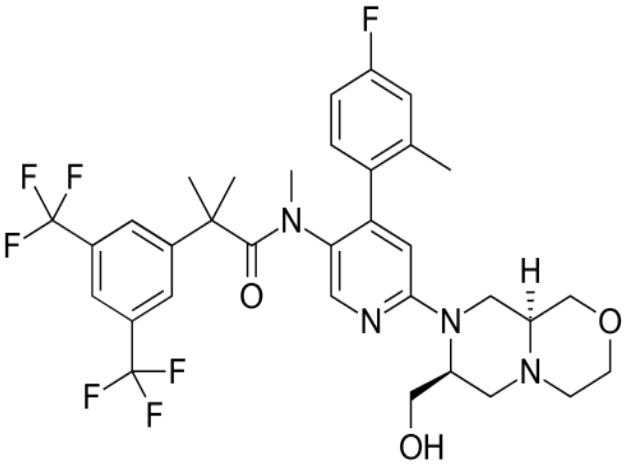

Figure 1: chemical structure of Sevabertinib

Experimental

Materials

Sevabertinib from Pharmatrain, orthophosphoric acid from FINAR Chemical Ltd., HPLC-grade water, methanol, and acetonitrile from Standard Solutions Ltd., and hydrochloric acid (HCl), hydrogen peroxide ($H2O_2$), and sodium hydroxide (NaOH) from MERCK are among the chemicals and reagents provided and used in this investigation.

Instruments

An Empower software-equipped WATERS High-Performance Liquid Chromatography (HPLC) system, a 2695 separation module, and a UV detector were among the tools and software utilized in this study. A LABINDIA UV3000+ UV/Vis spectrophotometer was employed for spectroscopic analysis. pH measurements were conducted using an Adwa–AD1020 pH meter. For precise weighing, an Afcoset ER-200A weighing machine was utilized. Additionally, standard laboratory glassware such as pipettes, burettes, and beakers from Borosil were used throughout the experimental procedures.

Preparation of Solutions

0.1% Ortho Phosphoric acid buffer

Take 1000 ml flask, add orthophosphoric acid (1ml), make up with HPLC water, sonicated for 10 min, followed by filtered through a 0.45µm membrane filter under vacuum. The pH of the buffer was measured using a calibrated pH meter and found to be 2.8 ± 0.05. pH was adjusted using dilute NaOH.

Preparation of mobile phase

Make a buffer, ortho phosphoric acid, acetonitrile, and methanol mixture that has been filtered and degassed in the following ratio: 300:600:100 v/v. (used as diluents).

Stock solution of Sevabertinib

After precisely weighing and transferring 10 mg of the working standard of Sevabertinib into a 10 ml dry flask, add the diluent, sonicate it until it dissolves completely then top with the same solvent (stock solution). In addition, transfer 1 ml and 3 ml of the stock solution into separate 10 ml flasks, then dilute to the required volume with diluent.

Preparation of working standard solution

Transfer precisely 10 mg of Sevabertinib into a dry, clean 10 ml flask. After adding around 7 ml of the diluent and shaking it until the drug is completely dissolved, add additional of the same liquid until the flask is filled. This creates the stock solution. Furthermore, pipette 1 mL of the stock combination into a 10 mL flask and dilute it to the proper concentration using diluent. Similarly, pipette 3 mL of the stock mixture into a 10 mL flask and dilute it to the proper amount using diluent.

Methodology

Method Development

A tested method using RP-HPLC was created to measure Sevabertinib. A Waters HPLC with an auto sampler and DAD/UV detector was used to perform the analysis. The inertsil ODS C18 column (4.6 × 150 mm, 5 µm) was chosen due to its good peak shape and resolution. 290 nm was selected as the detection wavelength due to its good absorbance after scanning the UV spectra of 10 µg/mL Sevabertinib in diluent (mobile phase composition) between 200 and 400 nm. 30% orthophosphoric acid buffer, 60% acetonitrile and 10% methanol made up the mobile phase, which

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had a flow rate of 1.5 ml/min. With a run time of 10 min and an injection volume of 20 µl was selected to achieve peak response and system suitability parameters. The analysis was conducted with the column and the temperature was maintained at 30^0 C throughout the analysis. The acidic pH of the buffer was selected to improve peak symmetry, enhance retention behaviour, and ensure reproducible chromatographic performance of Sevabertinib. During method optimization, chromatographic conditions were adjusted to obtain acceptable peak shape and reproducibility within a short run time, rather than maximizing theoretical plate count.

Method Validation

The ICH Q2 (R1) guidelines were followed in the validation of the analytical method,

focusing on things (parameters) such as how well the system works (system suitability), how accurate the results are(accuracy), how precise the measurements are (precision), how the results line up with expected values (linearity), how reliable the method is under different conditions (robustness), and the lowest and highest levels of a substance that can be reliably measured [limit of quantification (LOQ) and limit of detection (LOD)].

System suitability

The prepared standard solution was injected six times to test the system's suitable attributes, including theoretical plates, retention time, tailing factor, and %RSD.

Accuracy

Accuracy is the degree to which a measured value matches the actual value. Three analyte concentrations (50%, 100%, 150) were created, and their chromatograms were recorded in order to assess accuracy. Weigh 5mg, 10mg, and 15mg of the working standard for Sevabertinib into different 10 ml flasks. Add about 7 ml of diluent, sonicate to dissolve, and make up to the mark with diluent. Take 1.0 ml and 3.0 ml of the stock preparation and put each into a separate 10 ml container. Then add diluent to each container until it reaches the 10 ml mark.

Specificity

An analytical method's ability to clearly find and measure the desired substance without being affected by other substances like impurities, degradation products, or other components present in the sample.

Precision

The degree of agreement between different outputs of the same quantity is measured as precision. To prepare the stock solution, precisely weigh 10 mg of Sevabertinib working standard into 10 ml flask, add diluent, sonicate to dissolve, and make up to the mark. Next, aliquot 1 ml and 3 ml of this stock into different 10 ml flasks, then use the diluent to dilute to the appropriate level.

Linearity and range

The method is used to produce a linear response when the analyte concentration is within the predetermined range. Take exactly 10 mg of Sevabertinib working standard and carefully put it into a 10 ml clean and dry flask. Add the diluent to the flask and use sonication to fully dissolve the sample. Then add more of the same diluent until the solution reaches the marked line on the flask. This creates a stock solution.

LOD:

Calculate the LOD and LOQ levels by accurately weighing 10 mg of Sevabertinib in a 10 ml flask, adding diluent and sonicating until completely dissolved, and then making up to the mark to prepare the stock solution. Introduce 1ml, 3ml, 0.5, and 0.4 ml of this stock into different 10 ml flasks, then dilute with diluent to the required volume.

LOQ:

Precisely weigh 10 mg of Sevabertinib working standard into 10 ml flask, add diluent, sonicate to dissolve, and make up to the mark. Next, aliquot 1 ml and 3 ml of stock into different 10 ml flasks, then use the diluent to dilute them to the required level.

Robustness

Robustness was assessed by slightly modifying the parameters of the approach and tracking the impact on the method through the results of system compatibility tests. By altering the chromatographic conditions, the standard and sample of Sevabertinib were injected. Resolution, tailing factor, asymmetry factor, and plate count did not significantly alter.

Degradation Studies:

Stress testing is recommended by ICH guidelines for the stability of new medicines and ingredients

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to check how stable the main active substance is under different conditions. The goal of this study was to apply the proposed method to determine how stress affects the breakdown of Sevabertinib.

Results and Discussion:

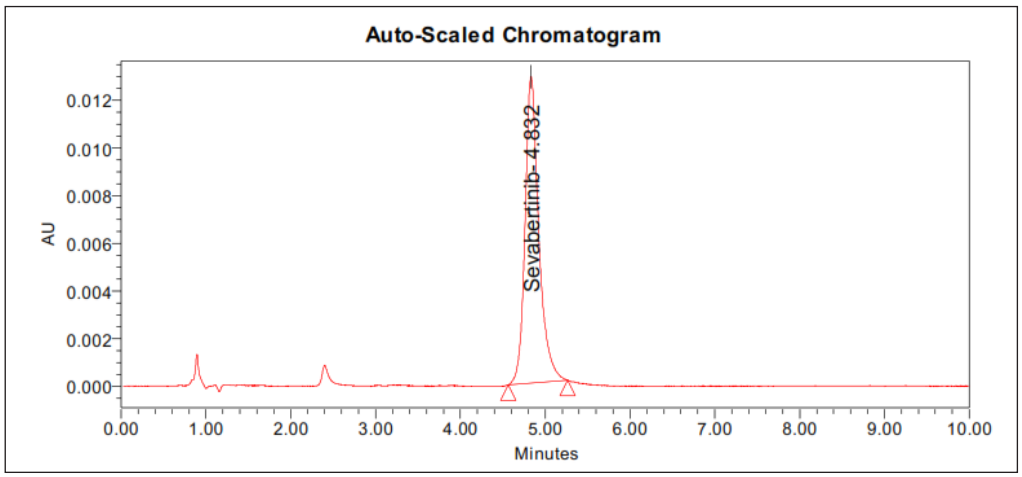

Figure 2: Optimized chromatogram of Sevabertinib

System Suitability

System suitability tests are conducted by injecting the prepared standard solution five times and measuring parameters such as theoretical plates, retention time, tailing factor, and %RSD. Although the theoretical plate count value is relatively lower than that typically observed for 150 mm C18 column, it was considered acceptable for the intended analytical purpose.

Table 1: Results of system suitability

S. No	Injection	RT (min)	Area (µV sec)	Height (µV)	USP tailing	USP plate count
1	1	5.00	1336390	100298	1.75	2502
2	2	5.01	1335205	100120	1.74	2498
3	3	4.99	1337102	100410	1.76	2508
4	4	5.00	1336020	100250	1.75	2501
5	5	5.01	1336488	100330	1.74	2499

Linearity:

Linearity of the method was evaluated over the selected concentration range. The calibration curve was constructed by plotting peak area versus concentration. The regression equation was found to be: y=mx+c, where m represents the slope and c as intercept. The correlation coefficient (R^2) was found to be 0.9979, indicating a good linear relationship between concentration and peak area.

Table 2: Results of Linearity by RP-HPLC method

S. No	Sevabertinib	
	Concentration (µg/ml)	Area
1	15	978094
2	30	1775358
3	45	2820363
4	60	3834947
5	75	4565230

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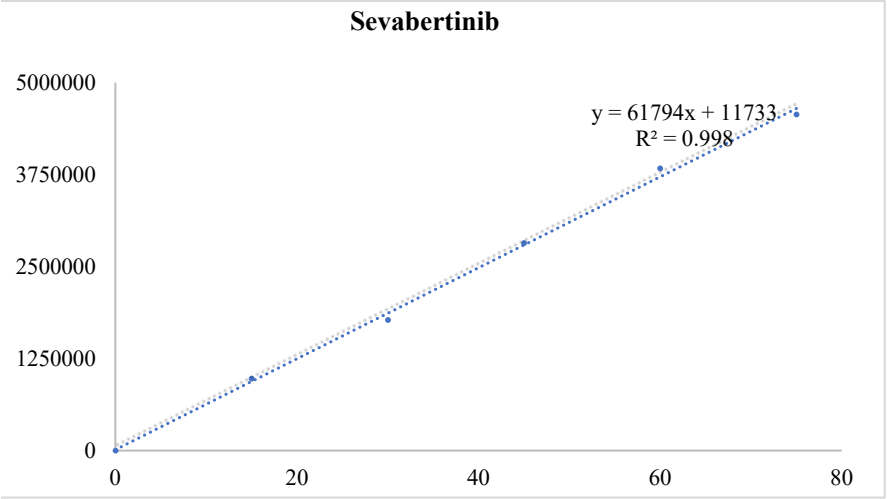

Figure 3: Calibration curve of Sevabertinib

Precision:

The system precision results show consistent peak areas for Sevabertinib with a %RSD of 0.2%, indicating good repeatability and precision of the analytical method.

Table 3(a): Results of Precision for Sevabertinib

Injection	Area
Injection-1	2820068
Injection-2	2806192
Injection-3	2803181
Injection-4	2805321
Injection-5	2810736
Injection-6	2806028
Average	2808587.8
Standard Deviation	6141.9
%RSD	0.2

Table 3(b): Results of Intermediate precision for Sevabertinib

Injection	Area
Injection-1	2812450
Injection-2	2809185
Injection-3	2811022
Injection-4	2807568
Injection-5	2813340
Injection-6	2808895
Average	2810410
Standard Deviation	2165
%RSD	0.08

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Sensitivity:

Limit of Detection (LOD)

The signal-to-noise (S/N) ratio for Sevabertinib was found to be **2.97**, indicating the detectable signal obtained in comparison with the baseline noise.

Table 4(a): Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Concentration
Sevabertinib	61	181	2.97	0.072 µg/mL

Limit of Quantification (LOQ)

The signal-to-noise (S/N) ratio for Sevabertinib was found to be **9.97.**

Table 4(b): Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio	Concentration
Sevabertinib	61	608	9.97	0.219 µg/mL

Accuracy:

The accuracy study for Sevabertinib shows recoveries ranging from 99.21% to 101.43% across 50%, 100%, and 150% concentrations, with a mean recovery of 100.50%, demonstrating the method's reliability.

*Table 5: Accuracy (recovery) data for Sevabertinib *Average of three determinations*

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1387761.8	5	5.07	101.43	
100%	2842114.8	10	9.92	99.21	100.50
150%	4251422.6	15	15.13	100.88	

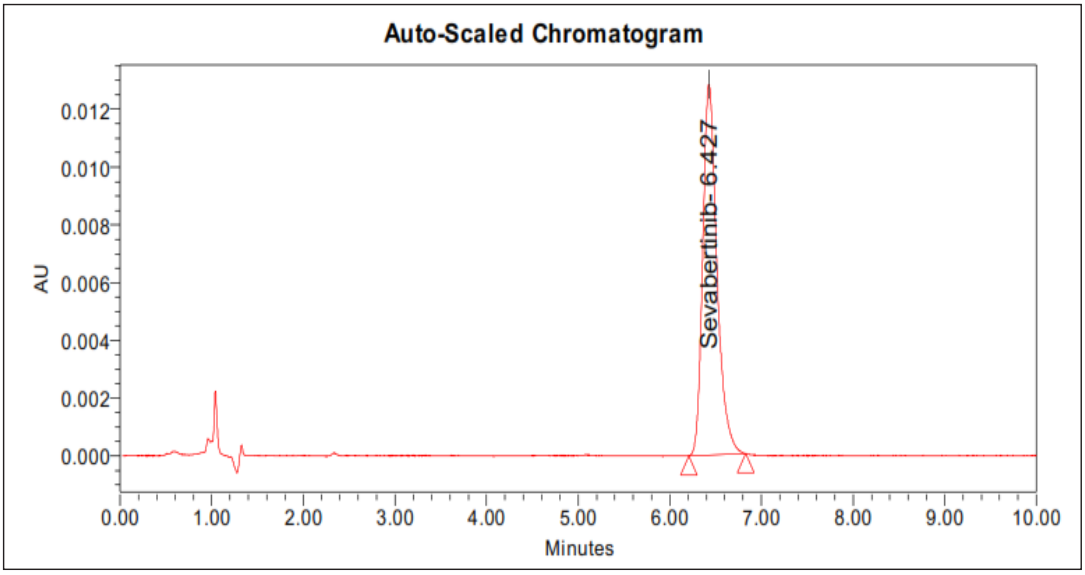

Figure 4.1: Chromatogram for Accuracy 50%

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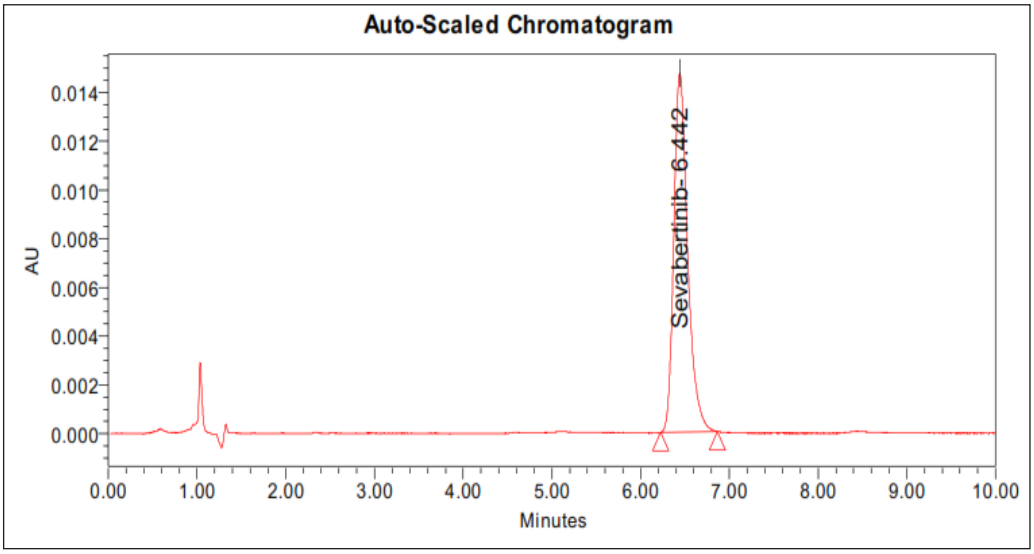

Figure 4.2: Chromatogram for Accuracy 100%

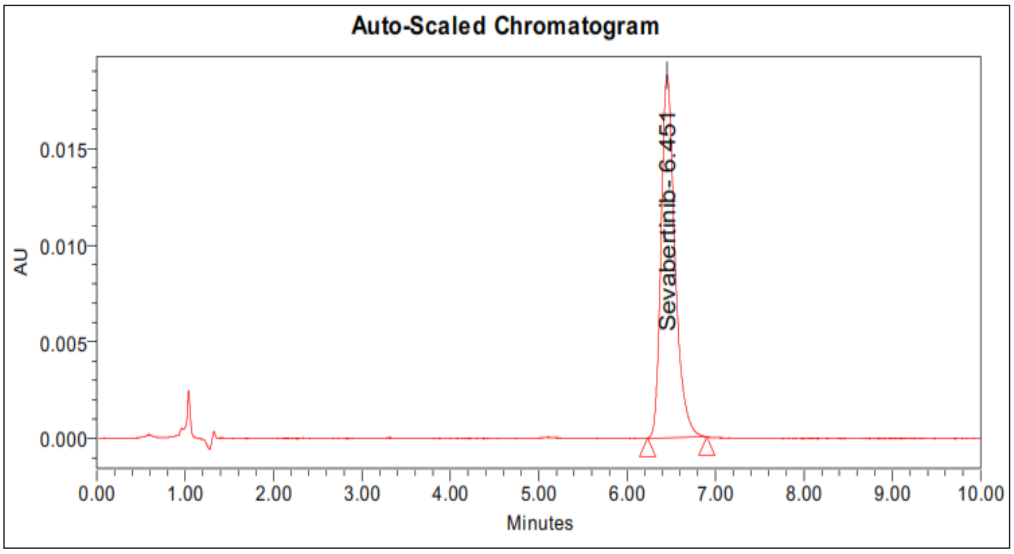

Figure 4.3: Chromatogram for Accuracy 150%

Robustness

The robustness study for Sevabertinib shows that varying the flow rate from 1.4 to 1.6 mL/min resulted in USP plate counts of 2374–2503 and USP tailing factors of 1.32–1.75, demonstrating that the method remains reliable under small deliberate changes.

Table 6(a): Results for variation in flow for Sevabertinib

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	1.4	2491.77	1.55
2	1.5	2502.76	1.75
3	1.6	2374.78	1.32

Table 6(b): Results for variation in mobile phase composition for Sevabertinib

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S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	1456.04	1.23
2	*Actual	2502.76	1.75
3	10% more	1395.08	1.18

Specificity:

Specificity evaluated by injecting blank and standard solutions. The blank show no interfering peaks at the retention time of Sevabertinib. The standard chromatogram exhibits a well-defined peak at retention time of 5.0 min.

Table 7: Results of specificity showing no interference at retention time

Injection	Retention time	Interference at RT
Blank	-	No interference
Standard	5.0	No interference

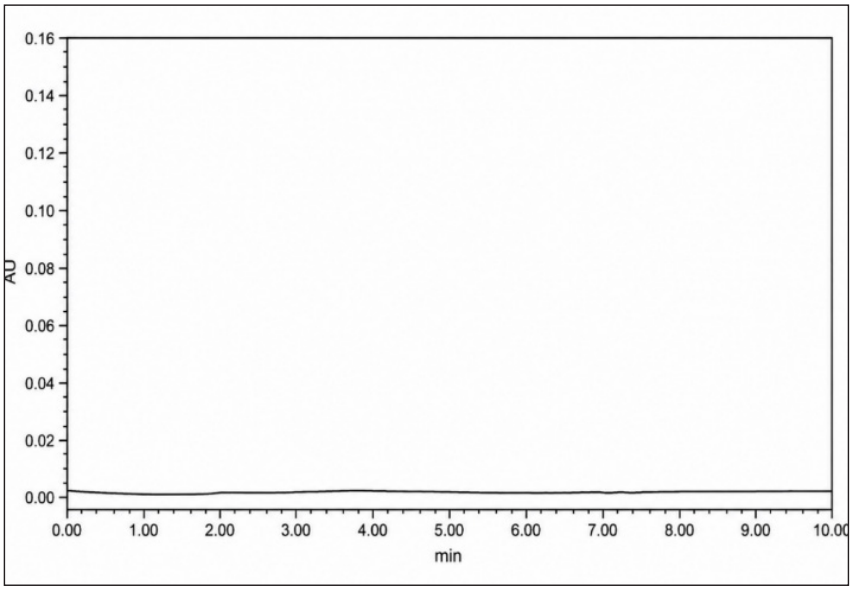

Figure 5(a): blank chromatogram

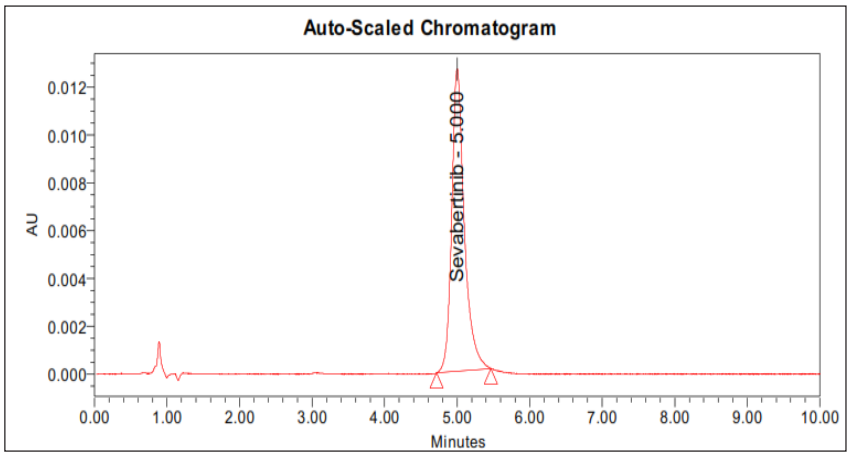

Figure 5(b): standard chromatogram

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Degradation Studies:

Forced degradation studies were performed to evaluate the stability-indicating capability of the developed RP-HPLC method Sevabertinib. The drug was subjected to various stress conditions, such as acidic, alkaline, oxidative, thermal, and photolytic degradation.

Acid degradation:

For acid degradation, Sevabertinib was treated with 0.1 HCL and refluxed for 2 hours, followed by cooling, neutralization (if required), then diluted with the mobile phase, filtered through a 0.45 µm membrane filter and subjected to RP-HPLC analysis.

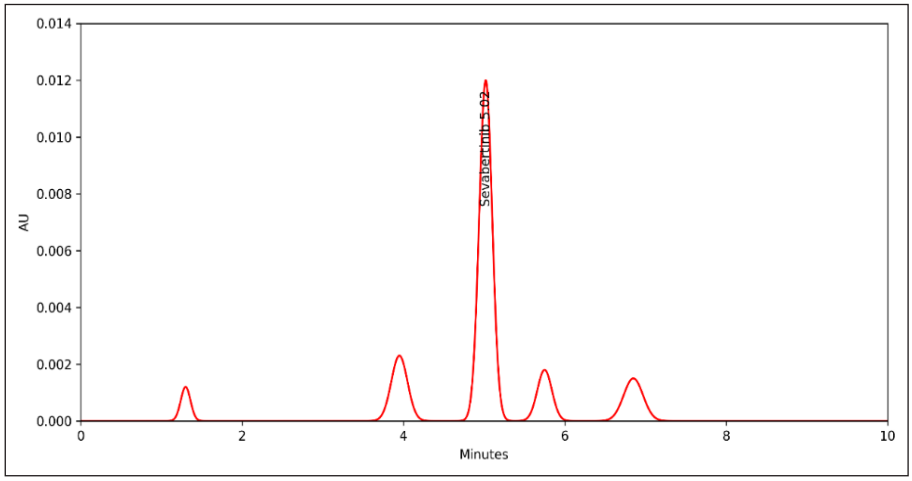

Figure 6(a): Chromatogram showing Acidic degradation

Table 7(a): Results of acid degradation

Parameter	observation
Retention time(min)	5.02
%Assay remaining	92.4
%Degradation	7.6
Degradation peaks	Minor peaks observed

Alkaline degradation:

Alkaline degradation was performed under similar conditions using 0.1N sodium hydroxide (NaOH) in place of hydrochloric acid.

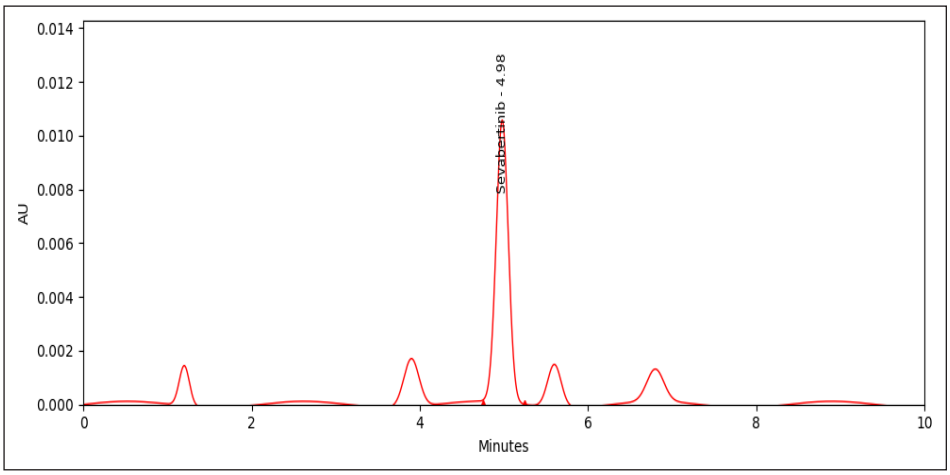

Figure 6(b): Chromatogram showing Alkaline degradation

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Table no 8(b): Results of alkaline degradation

Parameter	observation
Retention time(min)	4.98
%Assay remaining	88.6
%Degradation	11.4
Degradation peaks	Additional peaks observed

Oxidative degradation:

Oxidative degradation was carried out by treating the drug with 3% hydrogen peroxide and allowing it to react for 2 hours under controlled conditions, followed by dilution with the mobile phase, filtration, and RP-HPLC analysis.

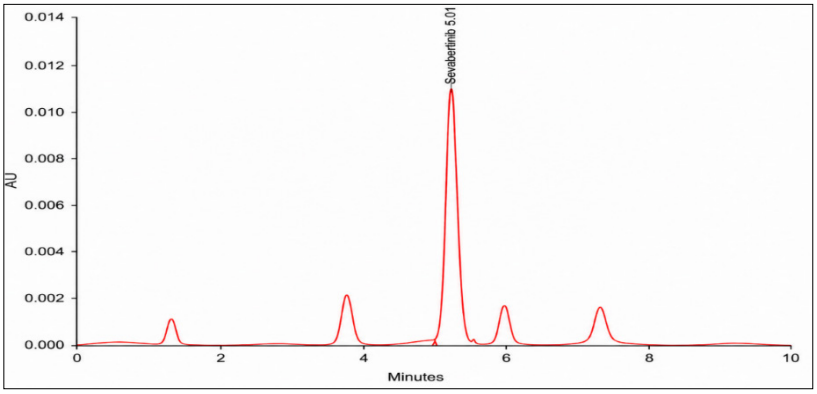

Figure 6(c): Chromatogram showing Oxidative degradation

Table no 8(c): Results of oxidative degradation

Parameter	observation
Retention time(min)	5.01
%Assay remaining	90.2
%Degradation	9.8
Degradation peaks	Noticeable oxidative peaks

Thermal degradation:

For thermal degradation studies, the sample was exposed to a temperature of 60^0 C in a hot air oven for 24 hours. After the exposure period, the sample was allowed to cool to column temperature, suitably diluted and analyzed.

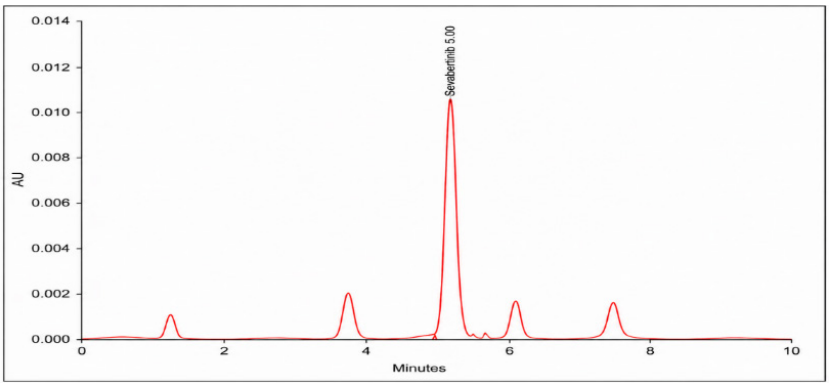

Figure 6(d): Chromatogram showing Thermal degradation

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Table no 8(d): Results of thermal degradation

Parameter	Observation
Retention time(min)	5.00
% Assay remaining	95.6
% Degradation	4.4
Degradation peaks	Negligible

Photolytic degradation:

In photolytic degradation studies, the sample was exposed to UV light for 24 hours, followed by appropriate dilution and analysis.

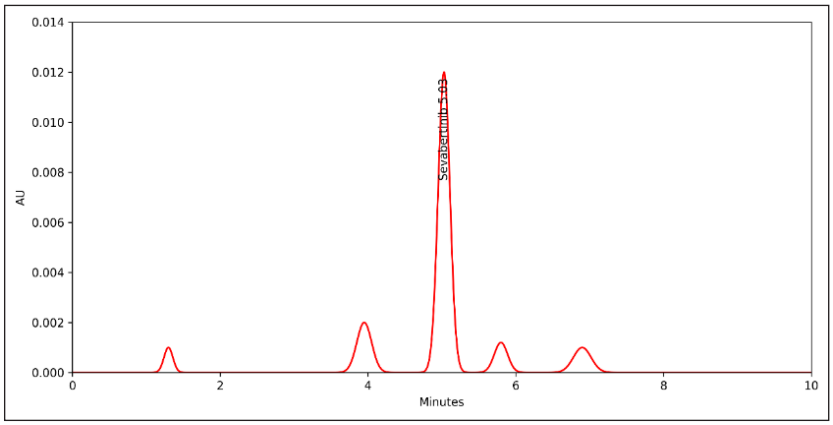

Figure 6(e): Chromatogram showing Photolytic degradation

Table no 8(e): Results of photolytic degradation

Parameter	Observation
Retention time(min)	5.03
%Assay remaining	96.2
%Degradation	3.8
Degradation peaks	Very minor peaks

Conclusion:

The RP-HPLC method for Sevabertinib was tested and confirmed to work well for measuring the drug in medicine forms. The method was appropriate for routine quality control, stability investigations. Linearity was excellent (R^2 = 0.9979), precision and intermediate precision were within limits (RSD 0.6% and 0.4%), and accuracy was within 97–103% (mean recovery 100.50%). Limit of detection and Limit of quantification were 2.97 and 9.97 µg/mL, respectively. Tests on how well the method works when there are changes in mobile phase and flow rate showed that the method is reliable (Robustness) and the system is suitable. Forced degradation studies indicates that it is more susceptible to alkaline and oxidative degradation, while it is relatively stable under thermal and photolytic conditions.

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Declaration:

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Patient and Public Involvement	Not applicable
Copyright Declaration	Author declare that the manuscript titled "Development and validation of stability-indicating reverse-phase high-performance liquid chromatographic method for the estimation of Sevabertinib in bulk drug" is an original work and has not been published previously nor is it under consideration for publication elsewhere. Upon acceptance, the copyright of this article will be transferred to the journal. The author grant the journal the right to publish, reproduce, distribute, and archive the article in all forms and media.
Conflict of Interest (COI)	The author declare that there are no conflicts of interest regarding the publication of this paper.
Artificial Intelligence (AI) Use Declaration	The author declare that artificial intelligence tools were used only for language editing, grammar refinement, and formatting assistance during manuscript preparation. All conceptualization, data collection, analysis, interpretation, and final manuscript approval were performed solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

References:

1. Le X, et al. Sevabertinib in advanced HER2-mutant non-small cell lung cancer.N engl j Med. 2025.
2. Siegel F, et al. Sevabertinib, a reversible HER2 inhibitor with activity in lung cancer.2025
3. U.S. Food and Drug Administration. FDA grants accelerated approval to Sevabertinib for non-squamous non-small cell lung cancer; 2025.
4. Ankita Dhiware Development and Validation of a Rapid RP-HPLC Method for the Analysis of Afatinib in Tablet Dosage Form Using AQBD Approach INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES [ISSN: 0975-4725; CODEN(USA): IJPS00]
5. v. kalyana Chakravarthy Development and validation of RP-HPLC method for estimation of erlotinib in bulk formulation Vol.4, No.2 (2011), 393-399 ISSN: 0974-1496
6. Prasada Rao M, Narasimha Rao BV, Udaya Krishna Veni A, Lavanya D, Ambika G, Manasa J, et al. RP-HPLC method development and validation for the estimation of Sevabertinib in bulk and pharmaceutical dosage form. *Int J Med Pharm Sci.* 2026;2(5):641–647.
7. International Conference on Harmonisation (ICH). ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology; ICH: Geneva, 2005.

RESEARCH ARTICLE

Method development and validation for simultaneous estimation of Tipiracil and Trifluridine in pharmaceutical dosage form by using reverse phase -ultra performance liquid chromatography

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Abstract:

A rapid, accurate, and stability-indicating RP-UPLC method was developed for the simultaneous quantitative estimation of Tipiracil and Trifluridine in pharmaceutical dosage forms. They were chromatographed on a BEH C18 (4.6 × 100 mm, 3.0 µm) column, quantified in 6 min with a simple mobile phase of phosphate buffer (pH 3.5) and methanol (30:70 v/v) and a flow rate of 0.3 mL/min, and detected at 251 nm. The novelty of this method is that it can measure both drugs in a single analysis, making it more efficient, less expensive, and more robust compared to existing methods. Excellent calibration (r^2 = 0.999), excellent reproducibility (%RSD < 2.0%), and excellent accuracy (98%-102%) have been observed across a range of concentrations. The results indicate the Limit of Detection (LOD) at Signal-to-Noise ratios of 2.97 and 2.96 for Tipiracil and Trifluridine, respectively, yielding Limit of Quantification (LOQ) values of 9.97 and 9.91, respectively. It is fit for purpose for validations as per ICH Q2(R1) validation specifications and is a single methodology that combines speed, sensitivity, and reproducibility. This new approach could be adopted as a robust routine quality-control and regulatory tool, as well as for assessing the stability of Tipiracil/Trifluridine dual-drug oncology drugs.

Keywords, Tipiracil, Trifluridine, Stability-Indicating Method, Pharmaceutical Dosage Form

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Introduction:

Fluoropyrimidines, which have been used in the treatment of several carcinomas for decades, especially mCRC. Of these, the one that stands out is Ultra Performance Liquid Chromatography (UPLC), which came of age in 2004.[1] It is a technique that is more precise, faster, and more sensitive, with the capacity to use very small column-filled particle sizes.[2] Tipiracil is an oral chemotherapeutic drug designed to treat metastatic colorectal cancer (mCRC) and gastric cancer in patients who have previously been treated. The adverse effects of TAS 102 (Trifluridine/Tipiracil) are generally acceptable and consist mainly of haematological toxicity (anemia, neutropenia, and thrombocytopenia).[3] Trifluridine is a nucleoside analog of thymidine that is antineoplastic by virtue of its incorporation into DNA, thereby interfering with normal DNA replication and synthesis. By interfering with DNA integrity and function, trifluridine effectively inhibits cellular proliferation, thereby exerting its cytotoxic effects against malignant cells.[4] The FTD/TPI combination,

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marketed as Lonsurf, was first approved in Japan in 2014, followed by the United States in 2015 and the European Union in 2016, for patients with metastatic colorectal cancer (mCRC) who had previously received standard chemotherapy regimens including fluoropyrimidines, oxaliplatin, and irinotecan; the recommended oral dosing schedule of 15 mg/6.14 mg or 20 mg/8.19 mg twice daily for five consecutive days, followed by two days of rest and a two- week respite repeated every four weeks, balances efficacy with tolerability in heavily pretreated patient populations.[5] Given the clinical importance of FTD/TPI, robust analytical methodologies are essential to ensure accurate quantification, stability assessment, and quality control in pharmaceutical dosage forms. The FTD has a fast metabolism and an absolute requirement for therapeutic activity of the parent compound; stability-indicating and sensitive HPLC-MS methods are required to differentiate the parent compound from inactive metabolites. These techniques play a key role in the validation of a formulation's integrity, in regulatory compliance, and in clinical pharmacokinetic studies.[6] Therefore, the present study aimed to develop and validate a rapid, simple, accurate, precise, and stability-indicating RP-UPLC method for the simultaneous estimation of Tipiracil and Trifluridine in pharmaceutical dosage forms in accordance with ICH Q2(R1) guidelines. The chemical structures of Tipiracil and Trifluridine are shown in Figure 1.

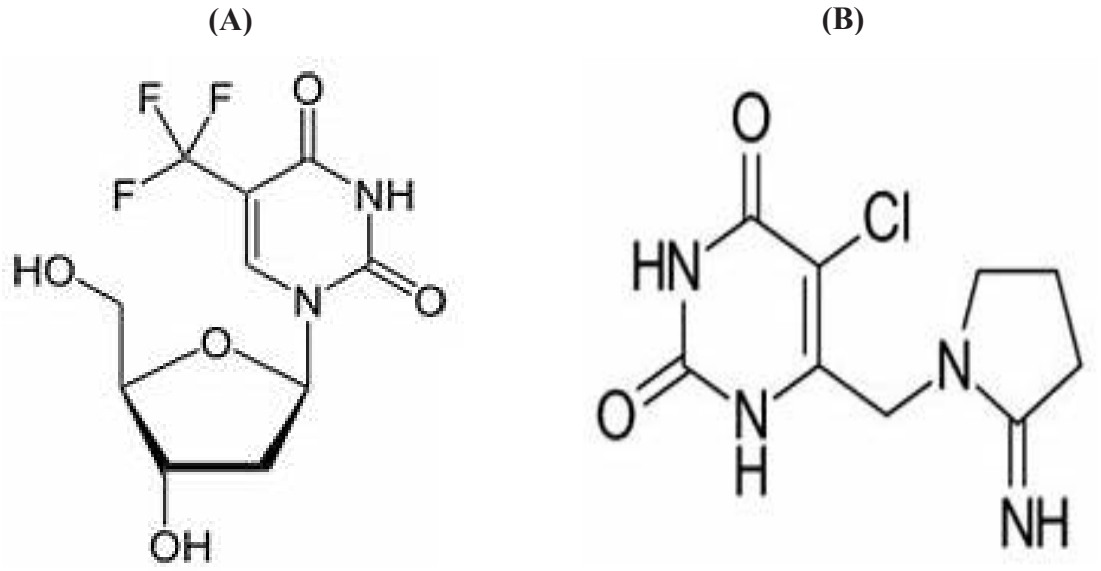

Figure1: Chemical Structure: A) Tipiracil and B) Trifluridine

Experimental

Materials

Pharmaceutical-grade Tipiracil and Trifluridine were procured from MSN Laboratories and used throughout the study. Analytical-grade triethylamine was obtained from FINAR Chemicals Ltd. Hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), and sodium hydroxide (NaOH) were procured from Merck. HPLC-grade water, methanol, and acetonitrile were purchased from Standard Solutions Ltd. All chemicals and solvents used were of analytical or HPLC grade and were used without further purification.

Instruments

Chromatographic analysis was performed using a Waters UPLC system equipped with Empower software, a Waters 2695 separation module, and a Waters 2487 UV detector. UV spectrophotometric measurements were carried out using a LAB INDIA UV-3000+ UV/Visible spectrophotometer. The pH of the solutions was measured with an ADWA AD1030 pH meter (Adwa Instruments), and sample weights were measured with an AFCOSAT ER-200A

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analytical balance.[7] Borosil volumetric glassware, including pipettes, burettes, volumetric flasks, and beakers, was used for solution preparation and other laboratory procedures.

Preparation of Solutions

Phosphate Buffer

A phosphate buffer solution was prepared by dissolving 6.4 g of phosphate salt in HPLC-grade water and diluting to a final volume of 1000 mL in a volumetric flask.

Mobile Phase

The mobile phase consisted of phosphate buffer and methanol in a 30:70 (v/v) ratio. It was prepared by mixing 300 mL of phosphate buffer with 700 mL of methanol. The prepared mobile phase was sonicated for 5 minutes to remove dissolved gases and filtered through a 0.45 µm membrane filter using vacuum filtration before use.

Standard and Stock Solutions

Tipiracil

An accurately weighed quantity of 6 mg of Tipiracil was transferred into a 10 mL volumetric flask. The drug was dissolved in the diluent using sonication, and the volume was made up to the mark to obtain a stock solution of 600 µg/mL. Subsequently, 0.3 mL of this stock solution was diluted to 10 mL with the diluent to obtain a working standard solution containing 18 µg/mL.

Trifluridine

An accurately weighed quantity of 15 mg of Trifluridine was transferred into a 10 mL volumetric flask. The drug was dissolved in the diluent using sonication, and the volume was adjusted to the mark to obtain a stock solution of 1500 µg/mL. Thereafter, 0.3 mL of the stock solution was diluted to 10 mL with the diluent to obtain a working standard solution containing 45 µg/mL.

Methodology

Method Development

A simple, rapid, and stability-indicating RP-UPLC method was developed for the simultaneous estimation of Tipiracil and Trifluridine. Chromatographic separation was achieved using a Waters UPLC system equipped with Empower software and a BEH C18 column (4.6 × 100 mm, 3.0 µm).

The mobile phase consisted of phosphate buffer and methanol (30:70, v/v) and was delivered at a flow rate of 0.3 mL/min. Detection was performed at 251 nm using a UV detector.

Standard and sample solutions were prepared using the mobile phase as the diluent, then sonicated to ensure complete dissolution. The solutions were filtered through a membrane filter before injection into the UPLC system.

The developed method was validated in accordance with ICH Q2(R1) guidelines by evaluating system suitability, specificity, accuracy, precision, linearity, intermediate precision, robustness, limit of detection (LOD), and limit of quantification (LOQ).

The optimized chromatographic conditions produced well-resolved peaks with excellent linearity, reproducibility, and precision, demonstrating that the developed method is suitable for routine pharmaceutical analysis.

Method Validation

The developed analytical method was validated in accordance with the International Council for Harmonisation (ICH) Q2(R1) guidelines. Validation parameters included system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System Suitability

System suitability was evaluated by injecting the standard solution six consecutive times before analysis. Parameters, including retention time, theoretical plate count, tailing factor, and percentage relative standard deviation (%RSD) of the peak area, were assessed to confirm the suitability and performance of the chromatographic system.

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Accuracy

Accuracy was evaluated by recovery studies at three concentration levels: 50%, 100%, and 150% of the target concentration.

Working standards of Tipiracil (6 mg) and Trifluridine (15 mg) were accurately weighed and transferred into a clean, dry 10 mL volumetric flask. The drugs were dissolved in the diluent using sonication, and the volume was adjusted to the mark to prepare the stock solution. Subsequently, 0.3 mL of the stock solution was diluted to 10 mL with the diluent to obtain the working standard solution. Recovery studies were performed at the three concentration levels, and the percentage recovery was calculated.

Specificity

Specificity was evaluated to demonstrate that the method could accurately quantify Tipiracil and Trifluridine in the presence of excipients, degradation products, impurities, and other potential interfering substances without any significant interference.

Precision

Method precision (repeatability) was evaluated by preparing a standard solution containing accurately weighed amounts of 6 mg Tipiracil and 15 mg Trifluridine in a 10 mL volumetric flask. After dissolution by sonication, the volume was adjusted to the mark with the diluent. Subsequently, 0.3 mL of the stock solution was diluted to 10 mL with the diluent to obtain the working standard solution.

Multiple replicate injections were performed, and the relative standard deviation (%RSD) was calculated to assess the method's repeatability.

Linearity and Range

Linearity was evaluated by preparing a series of standard solutions over the required concentration range from the stock solution prepared using 6 mg of Tipiracil and 15 mg of Trifluridine. Calibration curves were constructed by plotting peak area against concentration, and linear regression analysis was performed to determine the correlation coefficient.

Limit of Detection (LOD)

The limit of detection was determined to establish the lowest concentration of analyte that could be reliably detected by the developed method.

Stock solutions of Tipiracil (600 µg/mL) and Trifluridine (1500 µg/mL) were prepared. Aliquots of 0.1, 0.3, 0.4, 0.5, and 1.0 mL were transferred separately into 10 mL volumetric flasks and diluted to volume with the diluent. The resulting solutions were analyzed, and the LOD was determined based on the signal-to-noise ratio.

Limit of Quantification (LOQ)

The limit of quantification was determined as the lowest concentration of the analyte that could be quantified with acceptable accuracy and precision.

Stock solutions of Tipiracil and Trifluridine were prepared as described previously. Aliquots of 0.1, 0.3, 0.5, and 1.2 mL were transferred separately into 10 mL volumetric flasks and diluted to volume with the diluent. These solutions were analyzed to determine the LOQ of the developed method.

Robustness

Robustness was evaluated by introducing small, deliberate variations in chromatographic conditions, including flow rate, mobile phase composition, column temperature, and detection wavelength. The effects of these variations on chromatographic performance were examined.[8]

The developed RP-UPLC method demonstrated consistent performance across all modified conditions, indicating robustness and suitability for routine quality control analysis.

Results and Discussions

Optimised Chromatogram:

The optimized chromatographic conditions produced well-resolved peaks for Tipiracil and Trifluridine with satisfactory peak symmetry and retention times. The representative optimized chromatogram is shown in Figure 2.

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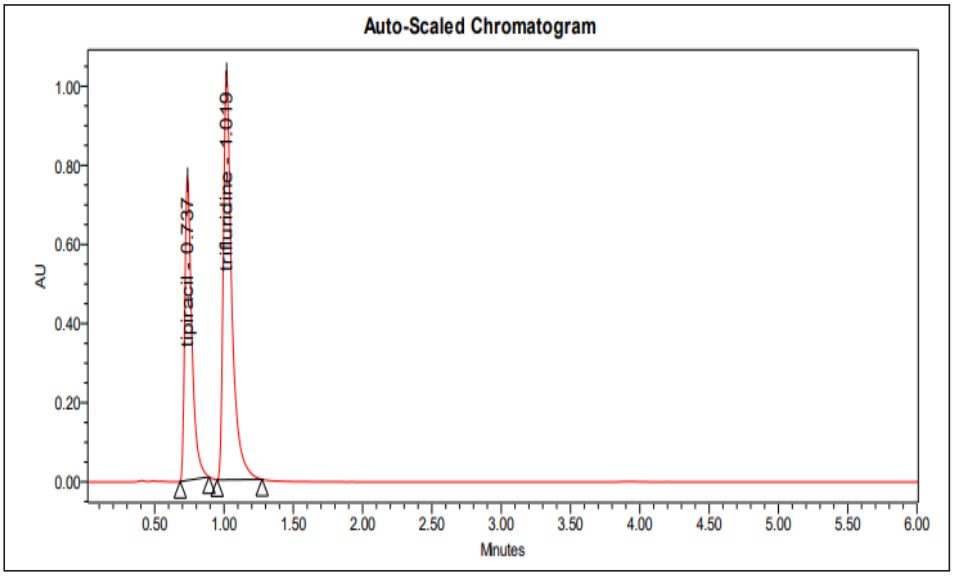

Fig 2: Optimized chromatogram of Tipiracil and Trifluridine

The optimized RP-UPLC chromatographic conditions resulted in well-resolved and symmetrical peaks for Tipiracil and Trifluridine with retention times of 1.913 min and 2.838 min, respectively (Figure 2). The short analysis time (<3 min) demonstrates the rapid nature of the developed method while maintaining adequate chromatographic resolution between the analytes.

Specificity:

The specificity was evaluated by injecting blank (diluent), placebo, standard, and sample solution into the chromatographic system. The blank and placebo chromatograms showed no peaks at the tirapicillin (≈1.9 min) and trifluoridine (≈2.8 min) retention times, indicating no interference from other formulation components at these times. The separation between the two analytes was > 2.0, indicating good resolution and method specificity.

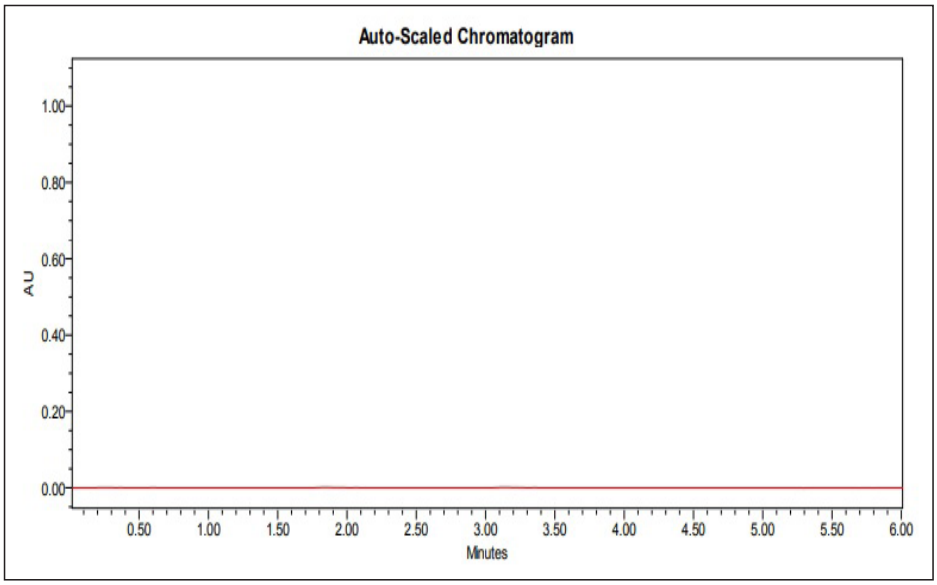

Fig 3: Chromatogram of Blank

No interfering peaks were observed in the blank and placebo chromatograms at the retention times of either analyte (Figures 3–5), confirming that the excipients present in the pharmaceutical formulation did not interfere with the analysis. The resolution between the two analytes was greater than 2.0, indicating complete chromatographic separation and excellent specificity of the developed RP-UPLC method.

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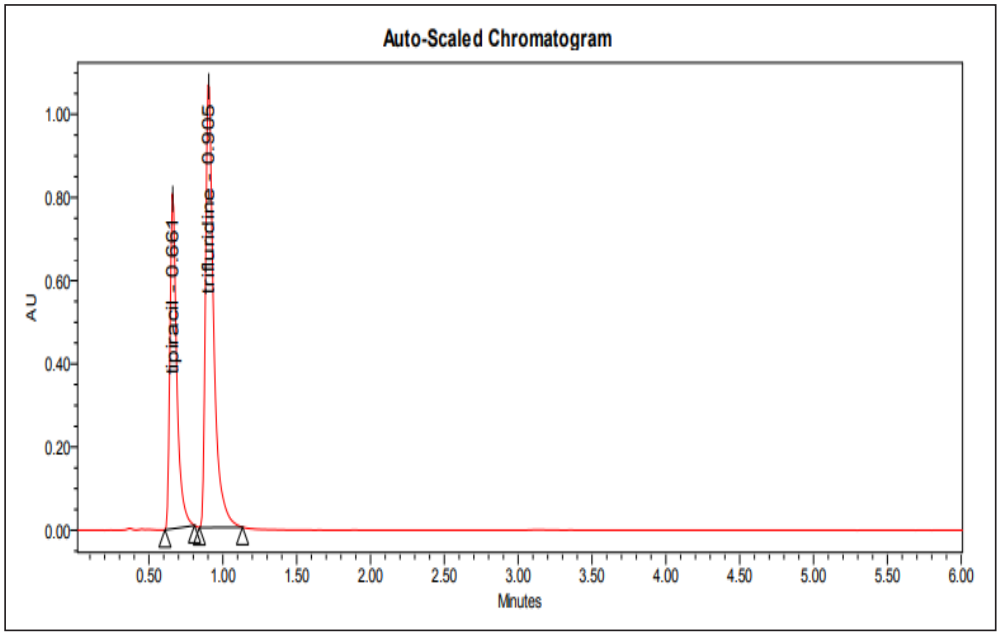

Fig 4: Chromatogram of Standard

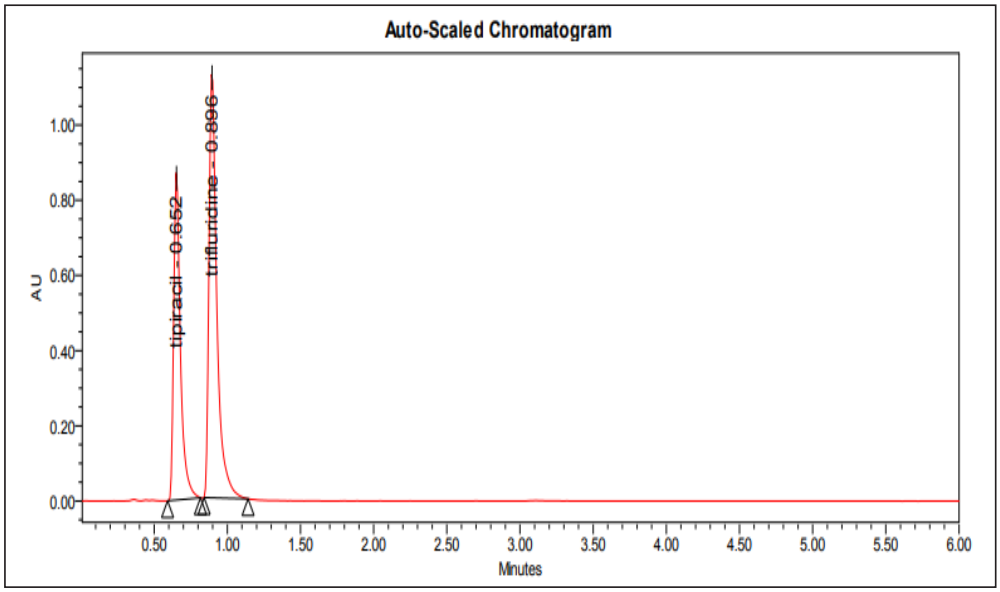

Fig 5: Chromatogram of Sample

SYSTEM SUITABILITY:

Under the optimized RP-UPLC conditions, Tipiracil was eluted at a retention time of 1.913 minutes. The peak showed an area of 1,446,550 µv·sec and a height of 138,723 µv. The system suitability parameters showed a resolution of 2.81, a tailing factor of 1.34, and a plate count of 8801.5, indicating good peak resolution and high system efficiency.

The peak area of trifluridine was 7,674,456 µV.sec, the peak height was 516,995 µV, and the elution time was 2.838 min. The compound had a tailing of 1.18 and a plate count of 7,859.78, which had good peak shape and good run-to-run column performance. The representative chromatogram obtained during system suitability testing is presented in Figure 6.

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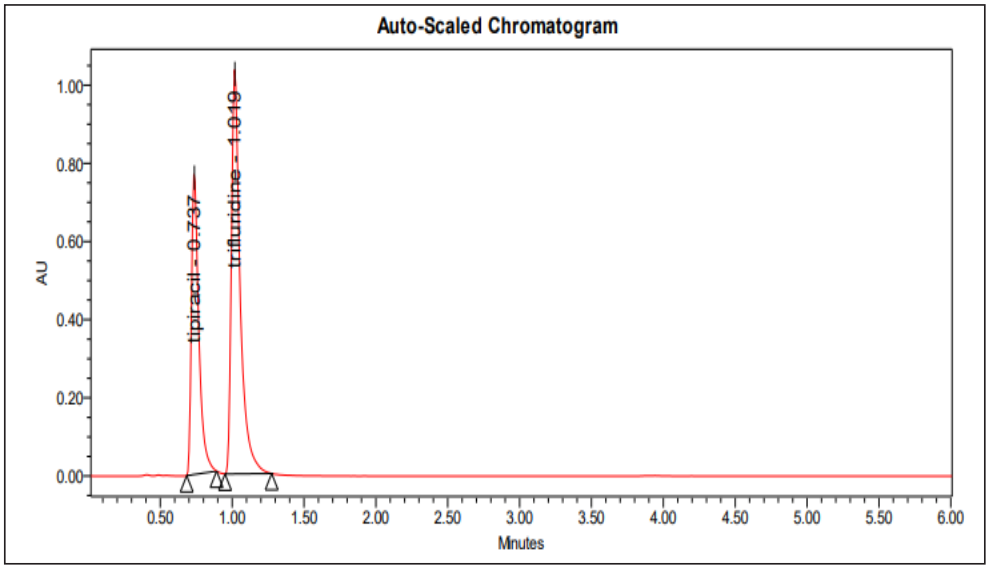

Fig 6: Chromatogram for system suitability

The system suitability results are summarized in Table 1.

Table 1: Results of system suitability parameters

S. No	Name	RT(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Tipiracil	1.913	1446550	138723	2.81	1.34	8801.50
2	Trifluridine	2.838	7674456	516995		1.18	7859.78

The system suitability parameters complied with the acceptance criteria recommended by ICH guidelines. Theoretical plate counts greater than 7000 indicated excellent column efficiency, while tailing factors below 2.0 confirmed satisfactory peak symmetry. The obtained resolution demonstrated complete separation of Tipiracil and Trifluridine, indicating that the chromatographic system was suitable for routine analysis.

S.No : Serial Number

RT [min] : Retention Time [Minutes]

Area [µV sec] : Peak Area [microvolt-seconds]

Height [µV**] :** Peak Height

USP Resolution : United States Pharmacopeia Resolution

USP Tailing : United States Pharmacopeia Tailing

USP Plate Count : United States Pharmacopeia Theoretical Plate Count

µV **:** Microvolt

Sec : Second

Min : Minute

USP : United States Pharmacopoeia

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Linearity:

The calibration data show that the peak area of Tipiracil and Trifluridine increases proportionally with concentration in the range of 6-30 µg/mL of Tipiracil and 15–75 µg/mL of Trifluridine, indicating good linearity of the method. The calibration plots for Tipiracil and Trifluridine are shown in Figures 7 and 8, respectively.

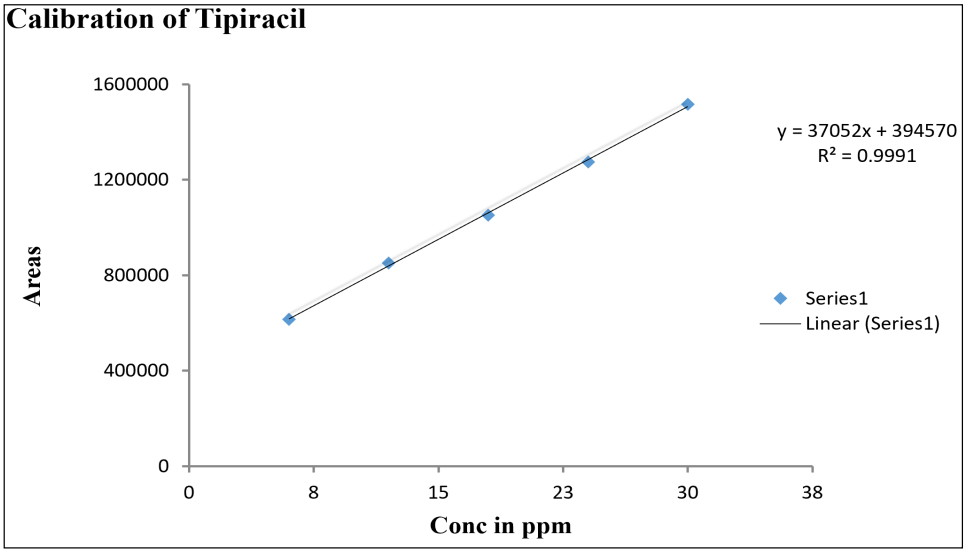

Fig 7: Calibration graph for Tipiracil

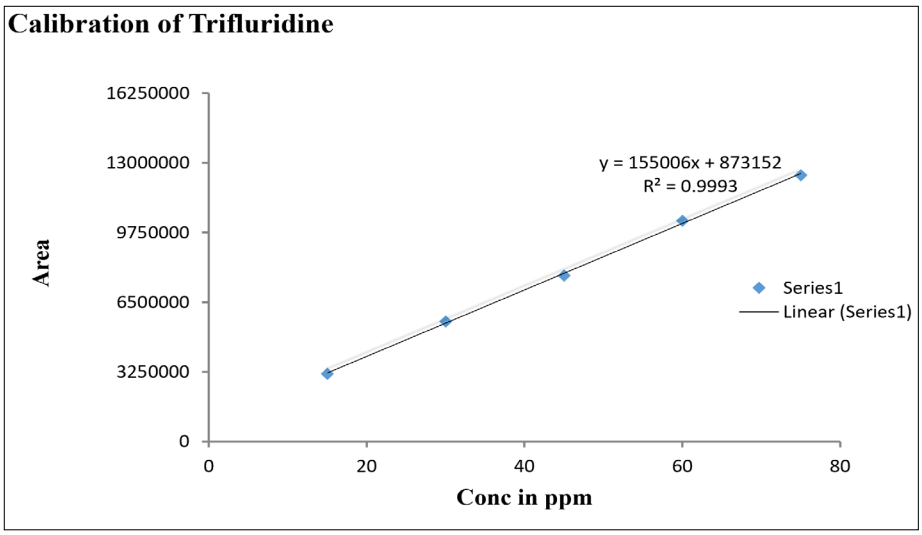

Fig 8: Calibration graph for Trifluridine

The corresponding linearity data are presented in Table 2.

Table 2: Results of Linearity by RP-UPLC method

S. No	Tipiracil		Trifluridine	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	6	615618	15	3160168
2	12	851274	30	5596971
3	18	1050645	45	7747596
4	24	1274352	60	10306499
5	30	1515639	75	12430844

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Excellent linearity was observed over the concentration ranges of 6–30 µg/mL for Tipiracil and 15–75 µg/mL for Trifluridine. Correlation coefficients greater than 0.999 demonstrated a strong linear relationship between concentration and peak area, confirming that the developed method is suitable for accurate quantitative estimation.

Precision:

All six injections were performed on the UPLC using the standard solution, and the areas were measured for all injections. The %RSD for the area of six replicate injections was within the specified limits. The results of intraday and interday precision are summarized in Table 3.

Table 3: Results for Comparison of method precision and intermediate precision

S.No.	Tipiracil		Trifluridine	
	Intraday precision Area	**Interday precision Area**	**Intraday precision Area**	**Interday precision Area**
1	1453371	1468131	7839785	7918737
2	1455487	1472532	7852682	7941013
3	1456408	1481186	7857421	7987808
4	1459838	1477046	7873389	7969309
5	1462389	1478247	7888982	7973993
6	1473262	1416996	7899723	7971501
Mean	1460126	1465690	7862452	7960394
Std Dev	7193.504	24292.6	19092	25496
%RSD	0.25	1.7	0.24	0.32

The intraday and interday precision studies produced %RSD values below 2.0% for both analytes, demonstrating excellent repeatability and intermediate precision. These findings indicate that the developed RP-UPLC method provides consistent and reproducible analytical performance.

Accuracy:

The Accuracy study for Tipiracil yielded an average recovery of 99.63% at concentrations of 50%, 100%, and 150%, with values ranging from 99.5% to 99.6%, indicating the method's reliability. The recovery results for Tipiracil and Trifluridine are presented in Tables 4.1 and 4.2, respectively.

Table 4.1: Accuracy (recovery) data for Tipiracil

%Concentration (at specification Level)	Area*	Amount Added(mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	720720	5	4.98	99.6	99.63
100%	1448126	10	9.95	99.5	
150%	2185492	15	14.97	99.8	

This method of Trifluridine is reliable because the percent recovery during the accuracy study ranged from 99.4 % to 99.6 % from 50 – 150 % concentration with a mean of 99.53 %.

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Table 4.2: Accuracy (recovery) data for Trifluridine

%Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	3907546	5	4.97	99.4	99.53
100%	7805590	10	9.96	99.6	
150%	11717302	15	14.95	99.6	

Recovery values ranged from 99.4% to 99.8% for both analytes, which fall within the acceptable recovery range of 98–102% recommended by ICH guidelines. These results confirm the excellent accuracy of the developed method and indicate that formulation excipients did not interfere with analyte quantification. Representative chromatograms obtained during the recovery studies at concentrations of 50%, 100%, and 150% are shown in Figures 9–11.

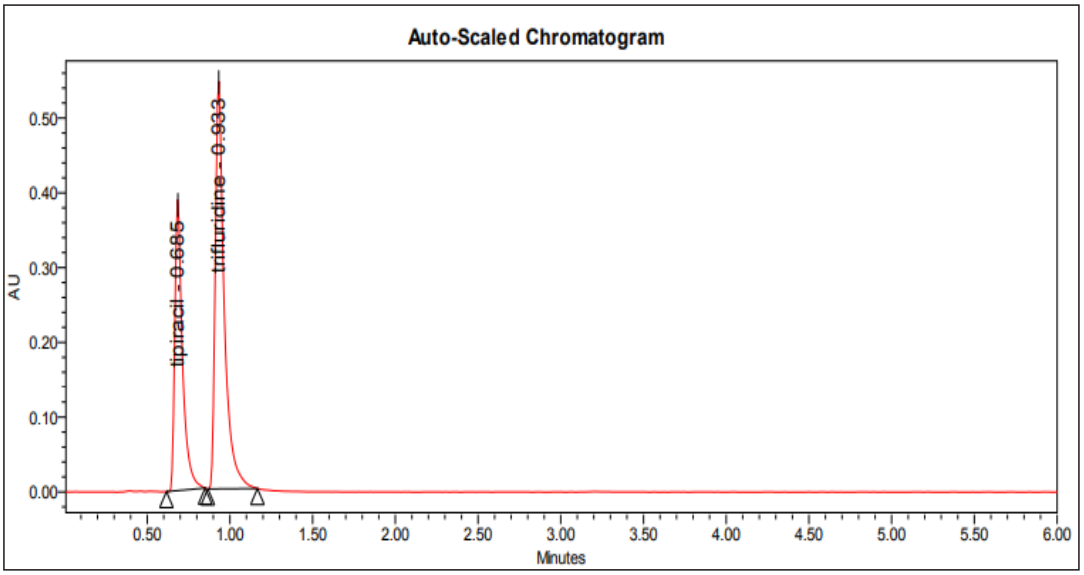

Fig 9: Chromatogram for Accuracy 50%

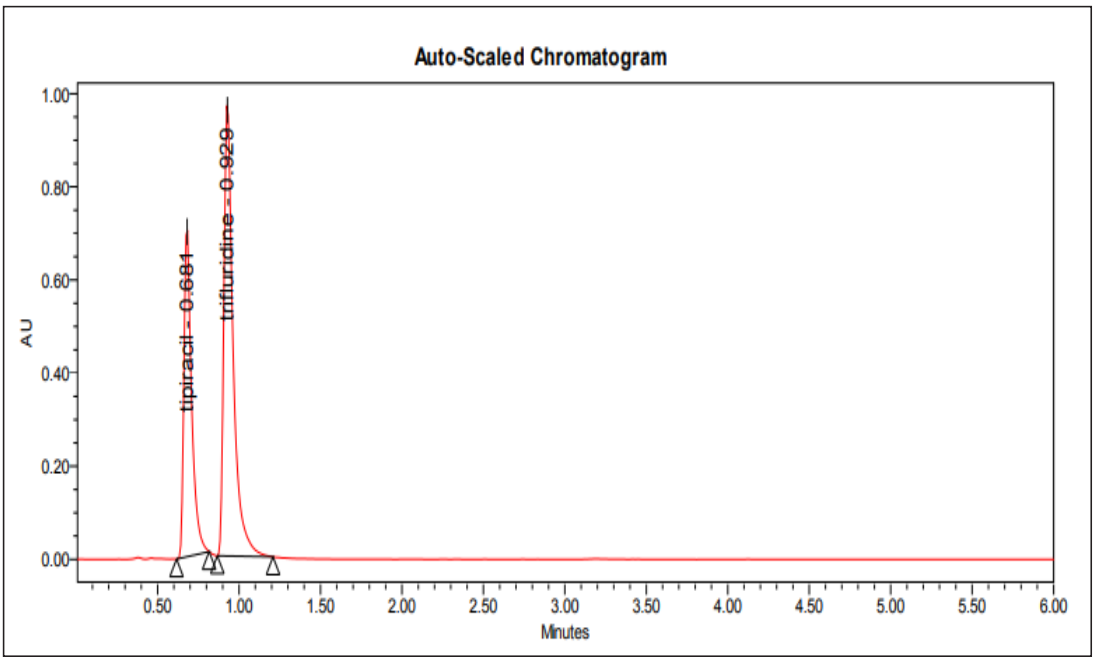

Fig 10: Chromatogram for Accuracy 100%

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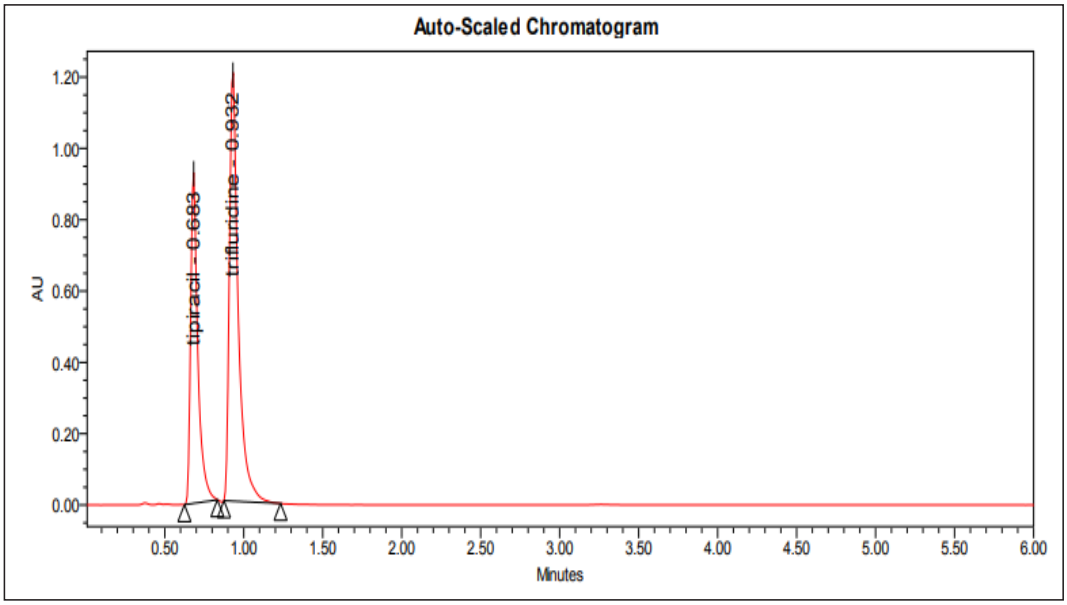

Fig 11: Chromatogram for accuracy 150%

Sensitivity:

Limit of Detection (LOD):

The sensitivity of the developed RP-UPLC method was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ), and the corresponding results are presented in Tables 5.1 and 5.2, respectively.

The signal-.to-noise (S/N) ratio for Tipiracil & Trifluridine was found to be 2.97&2.96.

Table 5.1: Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tipiracil	61	181	2.97
Trifluridine	59	175	2.96

The low LOD values demonstrate the high sensitivity of the developed RP-UPLC method, enabling reliable detection of very small quantities of both analytes.

Limit of quantification:

The signal-to-noise ratio (S/N) for Tipiracil & Trifluridine was determined to be 9.97 and 9.91, respectively.

Table 5.2: Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Tipiracil	61	608	9.97
Trifluridine	59	585	9.91

The obtained LOQ values indicate that the developed method can accurately quantify low concentrations of Tipiracil and Trifluridine with acceptable precision and accuracy, making it suitable for routine quality-control applications.

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Robustness:

The robustness study was performed by introducing deliberate variations in flow rate and mobile phase composition. The corresponding system suitability results are summarized in Tables 6.1–6.4. However, the method showed good robustness, as the system suitability was acceptable across the set variations in flow rate.

Table 6.1: Results for variation in flow for Tipiracil

S. No	Flow Rate (ml/min)	System Suitability Results	
	d	USP Plate Count	USP Tailing
1	0.8	8930.69	1.42
2	1.0	8801.50	1.34
3	1.2	8881.64	1.38

Table 6.2: Results for variation in flow for Trifluridine

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	8992.19	1.29
2	1.0	9859.78	1.18
3	1.2	9985.19	1.14

Overall, acceptable system suitability values were obtained under deliberate changes in the mobile phase composition, confirming the robustness of the method.

Table 6.3: Results for variation in mobile phase composition for Tipiracil

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	8972.15	1.41
2	*Actual	8201.50	1.34
3	10% more	9202.62	1.24

Table 6.4: Results for variation in mobile phase composition for Trifluridine

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	8142.67	1.20
2	*Actual	8592.78	1.18
3	10% more	9912.09	1.15

Deliberate variations in chromatographic conditions, including flow rate and mobile phase composition, did not produce significant changes in system suitability parameters. Theoretical plate count and tailing factor remained within acceptable limits, confirming that the developed method is robust and reliable under normal laboratory operating conditions.

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Conclusion

A rapid, simple, precise, accurate, robust, and stability-indicating RP-UPLC method was successfully developed and validated for the simultaneous estimation of Tipiracil and Trifluridine in pharmaceutical dosage forms. The developed method demonstrated excellent chromatographic performance with well-resolved peaks, short retention times, and satisfactory system suitability parameters, including theoretical plate count, tailing factor, and resolution. Validation studies performed in accordance with ICH Q2(R1) guidelines confirmed excellent linearity, specificity, precision, accuracy, sensitivity, and robustness. The recovery values were within the acceptable range of 98–102%, while the low LOD and LOQ values demonstrated the method's high sensitivity. Furthermore, deliberate variations in chromatographic conditions did not significantly affect analytical performance, confirming the method's robustness and reliability. Owing to its rapid analysis time, simplicity, reproducibility, and cost-effectiveness, the validated RP-UPLC method is suitable for routine quality control, stability studies, and the simultaneous quantitative estimation of Tipiracil and Trifluridine in pharmaceutical formulations. It may also serve as a reliable analytical tool for regulatory and industrial quality assurance applications.

Declaration:

Author Contributio	Conceptualization: Akkili Lekha Sree Reddy Writing original draft: Akkili Lekha Sree Reddy Review and editing: M. Mahesh Paper finalization: C. Gopinath
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Data Availability	None
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Patient and Public Involvement	Not applicable
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Conflict of Interest (COI)	The author declare that there are no conflicts of interest regarding the publication of this paper.
Artificial Intelligence (AI) Use Declaration	The author declare that artificial intelligence tools were used only for language editing, grammar refinement, and formatting assistance during manuscript preparation. All conceptualization, data collection, analysis, interpretation, and final manuscript approval were performed solely by the authors. The authors take full responsibility for the integrity and accuracy of the work.

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5. References:

1. Victorino AP, Meton F, Mardegan L, Festa J, Piranda DN, Araujo KB. Trifluridine/Tipiracil (FTD/TPI) and regorafenib in older patients with metastatic colorectal cancer. *Journal of Geriatric Oncology.* 2023 May 1;14(4):101477.
2. Ahuja S, Scypinski S, editors. Handbook of modern pharmaceutical analysis. Elsevier; 2001 Aug 2.
3. Huang F, Yang H, Bao W, Bin Y, Zhou S, Wang M, Lv X. Efficacy and safety of trifluridine/tipiracil (TAS-102) in patients with metastatic colorectal cancer: a systematic review and meta-analysis. *Clinical and Translational Oncology.* 2024 Feb;26(2):468–76.
4. Pinto C, Lonardi S, Maiello E, Martinelli E, Prisciandaro M, Salvatore L, Sartore-Bianchi A, Scartozzi M, Aprile G, Cremolini C, Sobrero A. Trifluridine/tipiracil regimen in combination with bevacizumab for metastatic colorectal cancer in the third line: an expert opinion. *Frontiers in Oncology.* 2025 Jan 22;14:1502185.
5. Talaat W, Kaddah MM, Keshk RM. Selective sensing of the nucleoside analogue, trifluridine and tipiracil in dosage form and biological matrices. *Talanta.* 2022 Feb 1;238:123009.
6. Zaniboni A, Bertocchi P, Barni S, Petrelli F. TAS-102 (Lonsurf) for the treatment of metastatic colorectal cancer. A concise review. *Clinical Colorectal Cancer.* 2016 Dec 1;15(4):292–7.
7. Enevold Thaulov Andersen J, Menong M. Uncertainty of Routine pH Measurements Evaluated by the Principle of Pooled Calibrations. IJCA. 2025 Mar 30;8(1):18–30.
8. Khan PDA. A Comprehensive Review on Advantages of Ultra Performance Liquid Chromatography (UPLC) for Analytical Method Development, Validation, and Stability Studies. IJSREM. 2024 May 16;08(05):1–5.

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