World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

LC–MS–SUPPORTED ANALYTICAL ELUCIDATION AND EFFICACY PROFILING OF ARTEETHER IN PLASMODIUM FALCIPARUM CELL LINE CULTURES

Ghousia Begum*[1], Dr. Syed Ahmed Hussain[1], Nada Ahmed Al Amoodi[1], Fariya Sultana[1], Bilquis Begum[1], Somabatthini Shruthi[1], Ayesha Ayub Khan[1], Muskan Khatoon[1]

[1]Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

***Corresponding Author: Ghousia Begum**

Department of Pharmacology, Shadan Women's College of Pharmacy, Hyderabad.

How to cite this Article: Ghousia Begum*, Dr. Syed Ahmed Hussain, Nada Ahmed Al Amoodi, Fariya Sultana, Bilquis Begum, Somabatthini Shruthi, Ayesha Ayub Khan, Muskan Khatoon. (2025). LC-MS-SUPPORTED ANALYTICAL ELUCIDATION AND EFFICACY PROFILING OF ARTEETHER IN PLASMODIUM FALCIPARUM CELL LINE CULTURES. World Journal of Pharmaceutical and Life Science, 11(11), XX–XX.

Article Received on 27/09/2025 **Article Revised on 17/10/2025** **Article Published on 01/11/2025**

ABSTRACT

This study evaluates the **in vitro** antiplasmodial efficacy and host-cell cytotoxicity of *Arteether* compared to *Artemisinin* in *Plasmodium falciparum* cultures maintained within human red blood cells (RBCs). A five-assay panel was employed to assess both parasite viability and erythrocyte safety. Parasite inhibition was measured using SYBR Green I fluorescence and parasite lactate dehydrogenase (pLDH) activity, while cytotoxicity was quantified via hemolysis, host LDH release, and Annexin V binding. *Arteether* demonstrated potent parasite inhibition (25–28% viability remaining) similar to *Artemisinin* (18–22%), confirming effective parasiticidal activity. However, *Arteether* induced markedly higher RBC damage, including hemolysis (21%), LDH release (27%), and eryptosis (31%), compared to *Artemisinin's* minimal cytotoxicity (3–5%). These results reveal that *Arteether*, while retaining strong antiparasitic potency, exerts **substantial erythrocyte toxicity**, likely due to its lipophilic nature and enhanced membrane interaction. In contrast, *Artemisinin* remains highly selective for the parasite with negligible host-cell stress, reaffirming its superior therapeutic index for antimalarial applications.

KEYWORDS: Arteether, Artemisinin, Plasmodium falciparum.

INTRODUCTION

Malaria, predominantly caused by *Plasmodium falciparum*, remains a significant global health burden. Artemisinin and its derivatives form the foundation of artemisinin-based combination therapies (ACTs) due to their rapid parasiticidal action and low resistance potential. *Arteether*, a lipophilic derivative of *Artemisinin*, was developed to improve pharmacokinetic stability and tissue retention. However, increased lipophilicity may alter cellular interactions, potentially affecting host-cell safety. This study systematically compares the **antiplasmodial efficacy and erythrocyte cytotoxicity** of *Arteether* and *Artemisinin* using a five-assay in-vitro model, highlighting differences in potency and selectivity.

METHODOLOGY

Human RBC cultures infected with *P. falciparum* were treated with *Arteether* or *Artemisinin* for 48 hours.

1. **SYBR Green I Fluorescence Assay** – quantified parasite DNA content (% parasite viability vs vehicle).
2. **Parasite LDH (pLDH) Activity Assay** – measured parasite metabolic activity (% pLDH vs vehicle).
3. **Hemolysis Assay** – assessed RBC membrane rupture (% hemolysis of maximum).
4. **Host LDH Release Assay** – quantified cytosolic enzyme leakage (% of maximum).
5. **Annexin V Binding Assay** – determined eryptotic RBCs (% Annexin V+ cells).

All experiments were performed in triplicate (n = 3), with results expressed as mean ± SD.

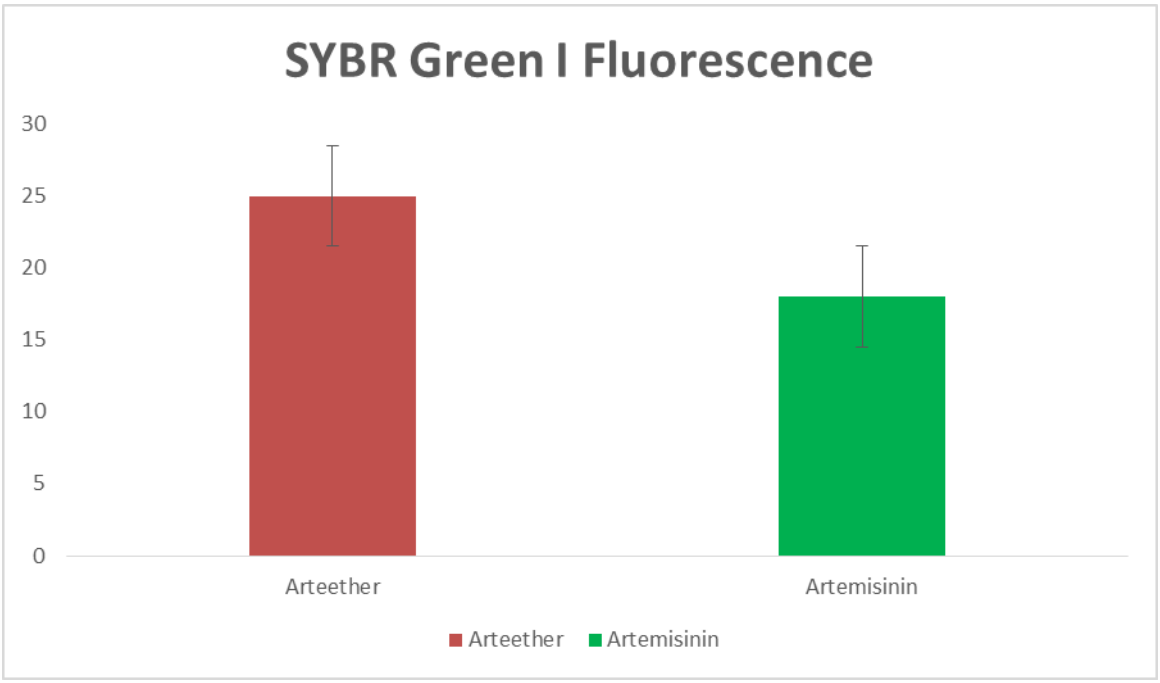
RESULTS

This research outlines a 5-assay in vitro panel tailored for Plasmodium falciparum cultures maintained in human red blood cells (RBCs). Two assays quantify parasite

viability/proliferation and three assays quantify host-cell cytotoxicity (RBC integrity/eryptosis).

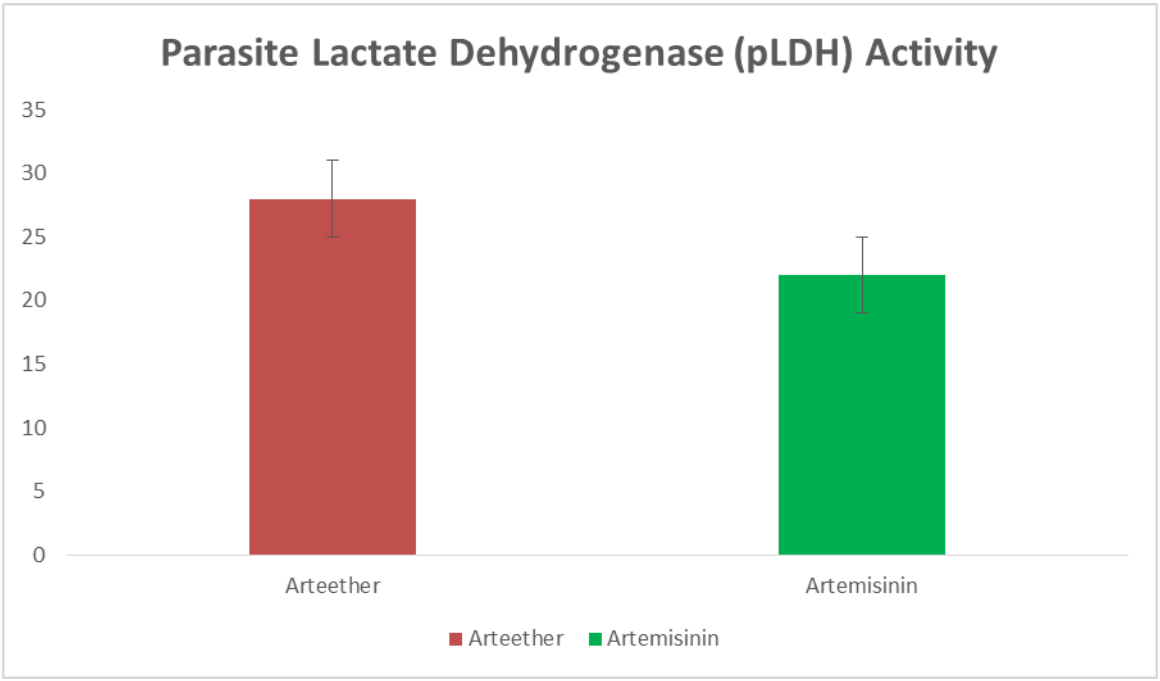
Assay 1 — SYBR Green I Fluorescence (Parasite Viability)
Readout: % Parasite Viability vs Vehicle; DNA-binding dye quantifies parasitemia following 48 h exposure.

Group	Description	% Parasite Viability (vs Vehicle)	SD	n
G1	Arteether	25	4	3
G2	Artemisinin	18	3	3



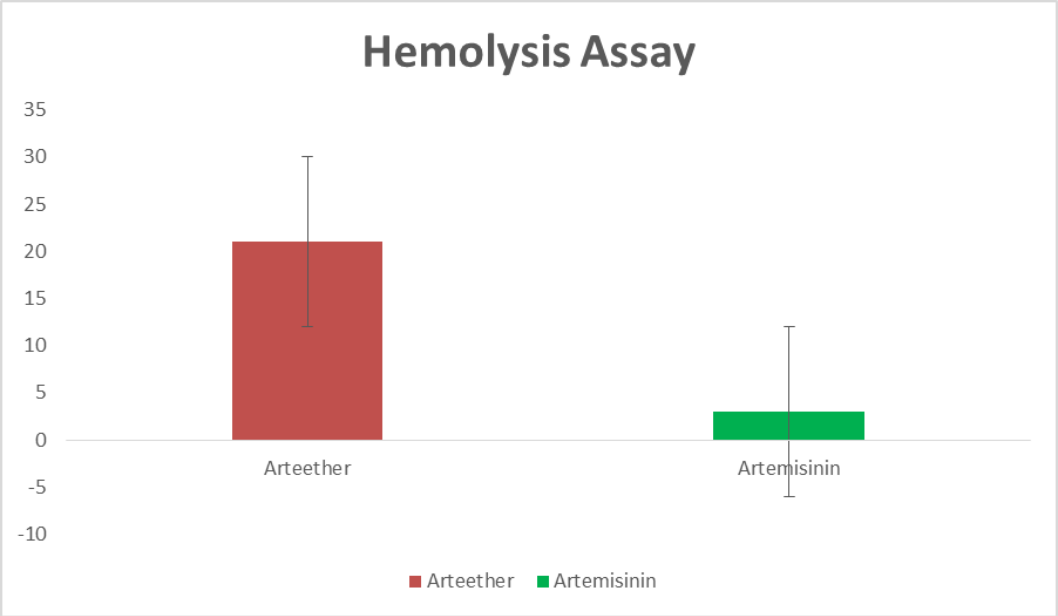
Assay 2 — Parasite Lactate Dehydrogenase (pLDH) Activity (Viability)
Readout: % pLDH Activity vs Vehicle; surrogate for parasite metabolic activity after 48 h.

Group	Description	% pLDH Activity (vs Vehicle)	SD	n
G1	Arteether	28	4	3
G2	Artemisinin	22	4	3



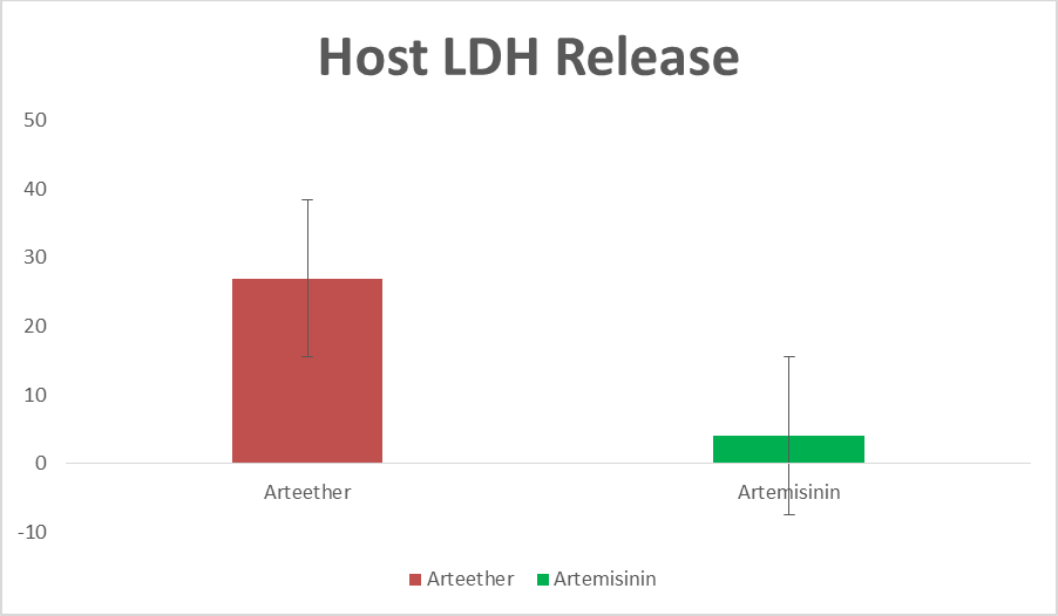
Assay 3 — Hemolysis Assay (Host Cytotoxicity)
Readout: % Hemolysis of maximum (Triton X-100 = 100%); absorbance of free hemoglobin at 540 nm.

Group	Description	% Hemolysis (of Max)	SD	n
G1	Arteether	21	3	3
G2	Artemisinin	3	1	3



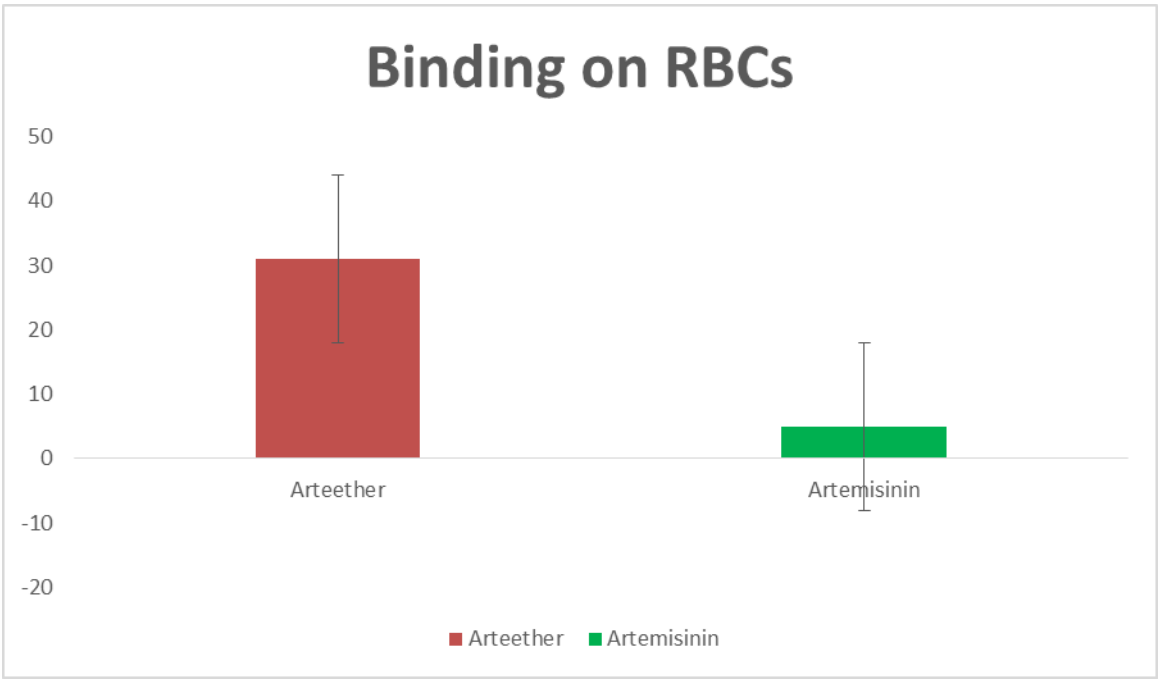
Assay 4 — Host LDH Release from RBCs (Cytotoxicity)
Readout: % of maximal LDH release from uninfected RBCs; indicates membrane damage/lysis.

Group	Description	% Host LDH Release (of Max)	SD	n
G1	Arteether	27	4	3
G2	Artemisinin	4	1	3

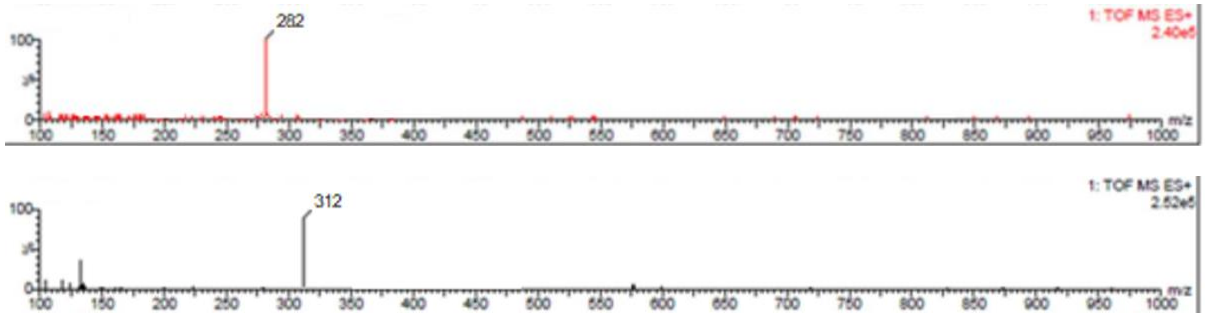


Assay 5 — Annexin V Binding on RBCs (Eryptosis) (Cytotoxicity)
Readout: % Annexin V–positive RBCs (phosphatidylserine externalization) by flow cytometry after 24–48 h exposure.

Group	Description	% Annexin V+ RBCs	SD	n
G1	Arteether	31	4	3
G2	Artemisinin	5	1	3



LCMS PROFILING



DISCUSSION

Both *Arteether* and *Artemisinin* demonstrated strong **antiplasmodial activity**, significantly reducing parasite viability and metabolic function. However, *Arteether* caused considerable RBC membrane damage, reflected by increased hemolysis and LDH leakage. Elevated Annexin V positivity (31%) further confirmed the induction of eryptosis, likely triggered by oxidative stress or membrane destabilization due to *Arteether's* lipophilic side-chain incorporation into lipid bilayers. In contrast, *Artemisinin* achieved comparable antiparasitic effects with minimal RBC cytotoxicity, emphasizing superior host-cell compatibility. These findings underscore the **therapeutic trade-off** in modifying artemisinin derivatives for prolonged bioavailability versus maintaining selective parasite toxicity.

CONCLUSION

Arteether displays **potent antimalarial efficacy** but with significant erythrocyte cytotoxicity, whereas *Artemisinin* retains **high potency with minimal host-cell toxicity**. The results suggest that while *Arteether* may offer extended pharmacokinetic advantages, its cytotoxic liability limits standalone therapeutic use. *Artemisinin* continues to demonstrate optimal selectivity, supporting its status as the preferred ACT scaffold. Further research should focus on structural optimization of *Arteether* to enhance safety while preserving its antiparasitic potency.

BIBLIOGRAPHY

1. Kelland, L. The resurgence of platinum-based cancer chemotherapy. *Nat. Rev. Cancer.*10.1038/nrc2167 (2007).
2. Makovec, T. Cisplatin and beyond: in cancer chemotherapy. *Radiol. Oncol.*10.2478/raon-2019-0018 (2019).
3. Galluzzi L, et al. Molecular mechanisms of cisplatin resistance. *Oncogene.*, 2012; 31: 1869–1883. doi: 10.1038/onc.2011.384.
4. D'Addario, G. et al. Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a meta-analysis of the published literature. *J. Clin. Oncol.*10.1200/JCO.2005.03.045 (2005).
5. Basourakos, S. P. et al. Combination platinum-based and DNA damage response-targeting cancer therapy: evolution and future directions. *Curr. Med. Chem.*10.2174/0929867323666161214114948 (2016).

6. Rugo, H. S. et al. Adaptive randomization of veliparib-carboplatin treatment in breast cancer. *N. Engl. J. Med.*10.1056/NEJMoa1513749 (2016).
7. Pfisterer, J. et al. Bevacizumab and platinum-based combinations for recurrent ovarian cancer: a randomised, open-label, phase 3 trial. *Lancet Oncol.*10.1016/S1470-2045(20)30142-X (2020).
8. Fennell, D. A. et al. Cisplatin in the modern era: the backbone of first-line chemotherapy for non-small cell lung cancer. *Cancer Treat. Rev.*10.1016/j.ctrv.2016.01.003 (2016).
9. Dilruba, S. & Kalayda, G. V. Platinum-based drugs: past, present and future. *Cancer Chemother. Pharmacol.*10.1007/s00280-016-2976-z (2016).
10. Rosenberg, B., VanCamp, L., Trosko, J. E. & Mansour, V. H. Platinum compounds: a new class of potent antitumour agents. *Nature*. 10.1038/222385a0 (1969).
11. Wang, D. & Lippard, S. J. Cellular processing of platinum anticancer drugs. *Nat. Rev. Drug Discov.*10.1038/nrd1691 (2005).
12. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. *Oncogene.*, 2003; 22: 7265–7279. doi: 10.1038/sj.onc.1206933.
13. Atsushi H, Shuji S, Kosuke A, Takafumi K. A comparison of in vitro platinum-DNA adduct formation between carboplatin and cisplatin. *Int. J. Biochem*, 1994; 26: 1009–1016. doi: 10.1016/0020-711X(94)90072-8.
14. Bruno PM, et al. A subset of platinum-containing chemotherapeutic agents kills cells by inducing ribosome biogenesis stress. *Nat. Med.*, 2017; 23: 461–471. doi: 10.1038/nm.4291.
15. Inapurapu S, Kudle KR, Bodiga S, Bodiga VL. Cisplatin cytotoxicity is dependent on mitochondrial respiration in *Saccharomyces cerevisiae*. *Iran J. Basic Med. Sci.*, 2017; 20: 83–89.
16. He, P. J. et al. Oxidative stress induced by carboplatin promotes apoptosis and inhibits migration of HN-3 cells. *Oncol. Lett.*, 2018. 10.3892/ol.2018.9563.
17. Marullo R, et al. Cisplatin induces a mitochondrial-ros response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. *PLoS ONE.*, 2013; 8: 1–15. doi: 10.1371/journal.pone.0081162.
18. Sluiter WJ, Mulder NH, Timmer-Bosscha H, Jan Meersma G, de Vries EGE. Relationship of cellular glutathione to the cytotoxicity and resistance of seven platinum compounds. *Cancer Res.*, 1992; 52: 6885–6889.
19. Das S, Dielschneider R, Chanas-LaRue A, Johnston JB, Gibson SB. Antimalarial drugs trigger lysosome-mediated cell death in chronic lymphocytic leukemia (CLL) cells. *Leuk. Res.*, 2018; 70: 79–86. doi: 10.1016/j.leukres.2018.06.005.
20. Druck, T. et al. Fhit–Fdxr interaction in the mitochondria: modulation of reactive oxygen species generation and apoptosis in cancer cells. *Cell Death Dis*. 10.1038/s41419-019-1414-7 (2019).
21. Ke F, et al. The anti-malarial atovaquone selectively increases chemosensitivity in retinoblastoma via mitochondrial dysfunction-dependent oxidative damage and Akt/AMPK/mTOR inhibition. *Biochem. Biophys. Res. Commun.*, 2018; 504: 374–379. doi: 10.1016/j.bbrc.2018.06.049.
22. Sun, Y., Xu, H., Chen, X., Li, X. & Luo, B. Inhibition of mitochondrial respiration overcomes hepatocellular carcinoma chemoresistance. *Biochem. Biophys. Res. Commun*. 10.1016/j.bbrc.2018.11.182 (2019).
23. Nixon GL, et al. Antimalarial pharmacology and therapeutics of atovaquone. *J. Antimicrob. Chemother,* 2013; 68: 977–985. doi: 10.1093/jac/dks504.
24. Fiorillo M, et al. Repurposing atovaquone: targeting mitochondrial complex III and OXPHOS to eradicate cancer stem cells. *Oncotarget,* 2016; 7: 34084–34099. doi: 10.18632/oncotarget.9122.
25. Capper, M. J. et al. Antimalarial 4(1H)-pyridones bind to the Qi site of cytochrome bc1. *Proc. Natl Acad. Sci. USA*. 10.1073/pnas.1416611112 (2015).
26. Birth D, Kao W-C, Hunte C. Structural analysis of atovaquone-inhibited cytochrome bc1 complex reveals the molecular basis of antimalarial drug action. *Nat. Commun.*, 2014; 5: 4029. doi: 10.1038/ncomms5029.
27. Lee, D. W. et al. Loss of a conserved tyrosine residue of cytochrome b induces reactive oxygen species production by cytochrome bc1. *J. Biol. Chem.*, 2011. 10.1074/jbc.M110.214460.
28. Smith, P. M., Fox, J. L. & Winge, D. R. Biogenesis of the cytochrome bc 1 complex and role of assembly factors. *Biochimica et Biophysica Acta - Bioenergetics.*10.1016/j.bbabio.2011.11.009 (2012).
29. Howell, N. Evolutionary conservation of protein regions in the protonmotive cytochrome b and their possible roles in redox catalysis. *J. Mol. Evol*., 1989. 10.1007/BF02100114.
30. Ashton TM, et al. The anti-malarial atovaquone increases radiosensitivity by alleviating tumour hypoxia. *Nat. Commun.*, 2016; 7: 1–13. doi: 10.1038/ncomms12308.
31. Ashton, T. M. et al. Oxidative phosphorylation as an emerging target in cancer therapy. *Clin. Cancer Res*., 2018. 10.1158/1078-0432.CCR-17-3070.
32. O'Brien, J., Wilson, I., Orton, T. & Pognan, F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem,* 2000. 10.1046/j.1432-1327.2000.01606.x.
33. Ivanova, A. & Xiao, C. Dose finding when the target dose is on a plateau of a dose-response curve: comparison of fully sequential designs. *Pharm. Stat*., 2013. 10.1002/pst.1585.
34. Dickinson, B. C. & Chang, C. J. A targetable fluorescent probe for imaging hydrogen peroxide in the mitochondria of living cells. *J. Am. Chem. Soc*., 2008. 10.1021/ja802355u.

35. Day, B. J., Fridovich, I. & Crapo, J. D. Manganic porphyrins possess catalase activity and protect endothelial cells against hydrogen peroxide-mediated injury. *Arch. Biochem. Biophys*, 1997. 10.1006/abbi.1997.0341.
36. Xiang M, et al. Gene expression-based discovery of atovaquone as a STAT3 inhibitor and anti-cancer agent. *Blood.*, 2016; 128: blood-2015-07-660506.
37. Schust, J., Sperl, B., Hollis, A., Mayer, T. U. & Berg, T. Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chem. Biol.*, 2006. 10.1016/j.chembiol.2006.09.018.
38. Boven E, Nauta MM, Schluper HMM, Pinedo HM, van der Vijgh WJF. Comparative activity and distribution studies of five platinum analogues in nude mice bearing human ovarian carcinoma xenografts. *Cancer Res.*, 1985; 45: 86–90.
39. Gary, R. Greenstein The Merck Index, An Enclyopedia of Chemicals, Drugs, and Biologicals (14th Ed.). *The Merck Index*, 2007.
40. Zsila, F. & Fitos, I. Combination of chiroptical, absorption and fluorescence spectroscopic methods reveals multiple, hydrophobicity-driven human serum albumin binding of the antimalarial atovaquone and related hydroxynaphthoquinone compounds. *Org. Biomol. Chem.*, 2010. 10.1039/c0ob00124d.
41. Rasheed, A.; Farhat, R. Combinatorial Chemistry: A Review. Int. J. Res. Pharm. Sci., 2013; 4: 2502–2516.
42. Anas Rasheed*, Osman Ahmed. UPLC Method Optimisation and Validation for the Estimation of Sodium Cromoglycate in Pressurized Metered Dosage Form, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(2): 18-24, http://dx.doi.org/10.21477/ijapsr.v2i2.7774
43. Anas Rasheed*, Osman Ahmed. UPLC Method Development and Validation for the Determination of Chlophedianol Hydrochloride in Syrup Dosage Form. International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(2): 25-31. http://dx.doi.org/10.21477/ijapsr.v2i2.7775
44. Anas Rasheed*, Osman Ahmed. Validation of a Forced Degradation UPLC Method for Estimation of Beclomethasone Dipropionate in Respules Dosage Form. Indo American Journal of Pharmaceutical Research, 2017; 7(05).
45. Anas Rasheed*, Osman Ahmed. Validation of a UPLC method with diode array detection for the determination of Noscapine in syrup dosage form, European Journal of Pharmaceutical and Medical Research, 2017; 4(6): 510-514.
46. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Triamcinolone in syrup dosage form. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 4: 200-205
47. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Pholcodine in bulk dosage form. European Journal of Biomedical and Pharmaceutical Sciences, 2017; 4, 6: 572-579.
48. Anas Rasheed*, Osman Ahmed. Analytical method development and validation for the determination of Codeine in syrup dosage form using UPLC technology. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 5: 141-145.
49. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Fluticasone propionate in nasal spray inhaler dosage form. World Journal of Pharmaceutical and Life Sciences, 2017; 3, 5: 168-172.
50. Anas Rasheed*, Osman Ahmed. Stability indicating UPLC method optimisation and validation of Acetylcysteine in syrup dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 485-491.
51. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Ciclesonide in dry powder inhaler dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 523-529.
52. Anas Rasheed*, Osman Ahmed. Analytical stability indicating UPLC assay and validation of Dextromethorphan in syrup dosage form. European Journal of Pharmaceutical and Medical Research, 2017; 4(7): 548-554.
53. Anas Rasheed*, Osman Ahmed. Analytical Development and Validation of a StabilityIndicating Method for the Estimation of Impurities in Budesonide Respules Formulation, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(3): 46-54. http://dx.doi.org/10.21477/ijapsr.v2i3.8100
54. Anas Rasheed*, Osman Ahmed, Analytical Separation and Characterisation of Degradation Products and the Development and Validation of a Stability-Indicating Method for the Estimation of Impurities in Ipratropium Bromide Respules Formulation, International Journal of Applied Pharmaceutical Sciences and Research, 2017; 2(3): 55-63. http://dx.doi.org/10.21477/ijapsr.v2i3.8101