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Review Article

METHOD DEVELOPMENT AND VALIDATION OF ERYTHROMYCIN AND OLAPARIB IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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ABSTRACT

The liquid chromatography–tandem mass spectrometry (LC-MS)/MS methodology was used to develop and validate a method for detecting erythromycin and olaparib in human plasma. Antibiotics such as erythromycin and olaparib fall into this category. Liquid chromatography is used to separate stationary and mobile phases based on differences in their affinities as well as to remove unwanted contaminants. It improves repeatability, sensitivity, resilience, and low-level protein detection. A C18 (C18, 5 m, 100×4.6 mm) column is utilized for high resolution and peak area. The calibration curve is created using linear regression. Internally, telmisartan is utilized as a benchmark. The flow rate of the mobile phase is 0.5 mL/min. Erythromycin and olaparib have mass-to-charge ratios of 735.43–115.97 and 435.08–102.04, respectively. Erythromycin in combination with olaparib resulted in a 98% recovery rate. The precision and accuracy of the results determined by interday QC samples are within acceptable limits. There was no evidence of instability.

Keywords: Erythromycin, Olaparib, Human plasma, Liquid chromatography–tandem mass spectrometry, Electrospray Ionization.

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INTRODUCTION

Macrolide antibiotics [1] include erythromycin. Macrolide antibiotics decrease the development of bacteria, which is beneficial for the production of proteins necessary for the survival of bacteria and are used to treat bacterial infections. This includes skin diseases and infections of the respiratory tract. Protein synthesis, which is mediated by ribosomal proteins, is necessary for bacterial reproduction. In vulnerable bacterial organisms, it works as a protein synthesis inhibitor in the 50S ribosome component. It prevents bacterial protein synthesis by preventing the translocation phase in protein synthesis and the gathering of the 50S ribosomal subunit, which leads to the control of various illnesses.

Olaparib is an antineoplastic medicine that inhibits the nuclear enzyme Poly ADP Ribose Polymerase (PARP). It is used to treat refractory and advanced ovarian cancer [2]. PARP inhibition may increase the cytotoxicity of DNA-damaging drugs and reverse chemoresistance and radio resistance in tumor cells. The FDA authorized olaparib as the first PARP inhibitor for gBRCAm metastatic breast cancer. The commercial chemotherapeutic medication Lynparza has a generic name of Olaparib. Structure of Erythromycin and Olaparib is shown in (Fig. 1a and 1b).

According to the literature survey, several techniques were used for the analysis of erythromycin and olaparib. Erythromycin is analyzed by ultraviolet-spectrophotometry [3], high-performance thin-layer chromatography [4], high-performance layer chromatography (HPLC) [5,6], liquid chromatography–mass spectrometry (LC-MS) [7-10] and olaparib is analyzed by HPLC [11], ultraperformance LC-MS [12], LC-MS [13-15].

EXPERIMENT

Chemicals and reagents

Working standards for erythromycin and olaparib were obtained from Sigma-Aldrich in Bengaluru, India. For analysis, acetonitrile HPLC grade from Merck, ammonium acetate AR grade from Qualigens fine chemicals, and water HPLC grade from the Milli-Q RO system were utilized for analysis.

Chromatography

An Acquity SM sample manager, an Acquity BSM binary solvent manager, and a thermostated column compartment were included in the system (Waters, Milford, USA). The chromatography was carried out at 40°C on a Phenomenax Luna column (C18, 5 m, 100×4.6 mm). It is set up as 75% A and 25% B for the first 0.5 min, then 10% A and 90% B for the next 3.5 min. Then, it steadily increased to 75% A and 255% B by 3.75 min and stayed there at 5.0 min at a flow rate of 0.5 mL/min.

LC-MS/MS conditions and parameters

Multiple reaction monitoring was used to perform mass spectrometric detection on a Quattro Micromass quadrapole instrument (Waters, Milford, USA) (MRM). In the turbo electrospray interface, a positive ionization mode was adopted. Table 1 summarizes the functioning parameters of a mass spectrometer.

Standard sample preparation

To make a 1.0 mg/mL erythromycin and olaparib solution, pour 100 mg of the working standard solution into a 100 ml volumetric flask and dissolve it with acetonitrile; the final solution is prepared with a 1:1 ratio of water and acetonitrile. The erythromycin and olaparib solutions were obtained and kept in the refrigerator at temperatures below 8°C.

RESULTS AND DISCUSSION

Validation

In technique validation, the second stage of the analysis, precise, and accurate data was achieved. Otherwise, it is unfit and inappropriate, according to FDA rules.

For concentration determination, calibration curves in the range of 1–1000 ng/mL should be used. Erythromycin and olaparib standards were extracted in the presence of an internal standard and then evaluated using MRM transitions. The analyte to internal standard peak area ratio was computed from the chromatograms. Human plasma was examined without the use of analytes to determine the stability of the sample at room temperature for 24 h.

Construction of calibration curve

Five sets of quantitation standards were prepared using the same plasma on alternate days to determine linearity, and a dilute stock solution with six different concentration levels was prepared using the same plasma. Intra- and inter-day samples were prepared on alternate days to determine the lowest level. Fig. 2a and 2b shows the calibration curve of erythromycin and Olaparib.

Table 1: Working parameters

Capillary (v)	1.0
Cone (v)	30.0
Extractor	4
RF lens (V)	0
Source temperature (°C)	150
Desolvation temperature (°C)	500
Dwell time per transition	200
Cone gas flow (L/hr)	30
LM1 resolution	3.6
HM1 resolution	14.6
Ion energy 1	0.8
Entrance	1.0
Collision	23
Exit	0
LM2 resolution	10.5
HM2 resolution	14.6
Ion energy 2	0.5

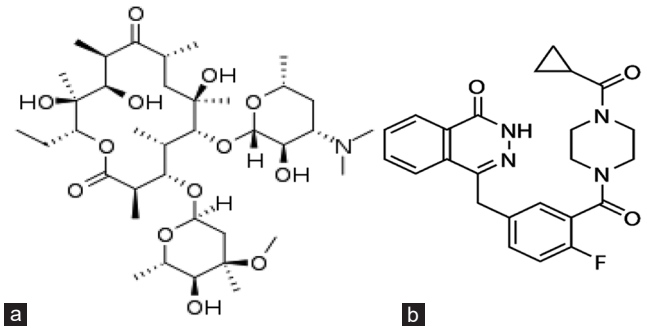

Fig. 1: (a) Erythromycin structure, (b) olaparib structure

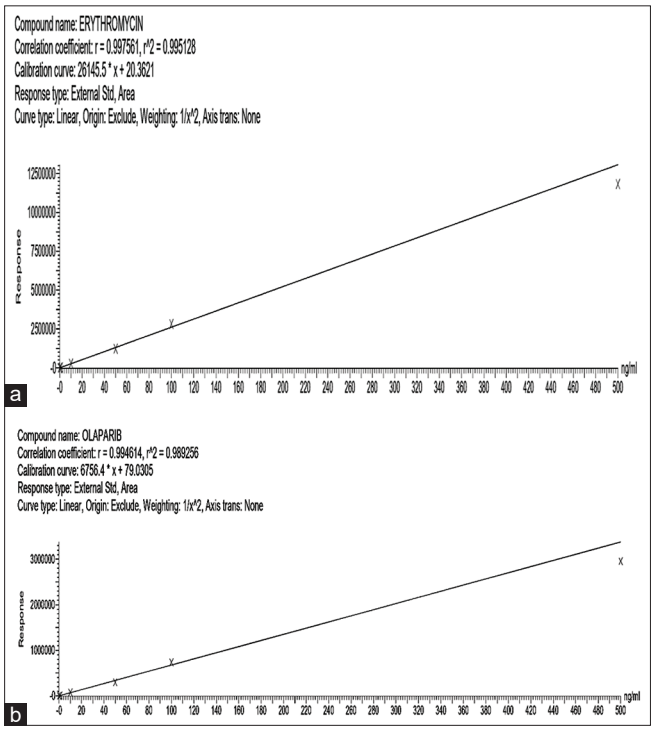

Fig. 2: (a) Calibration curve of erythromycin, (b) Calibration curve of olaparib

MRM transitions

When switching from a precursor to a product ion in positive ion mode, multi reaction monitoring is performed. Erythromycin has a mass-to-charge ratio of 435:811, while olaparib has a mass-to-charge ratio of 734:158. Fig. 3a and 3b depict product ion spectra. Table 2 shows the MRM transition conditions summary.

Linearity

A 1.5 software calibration curve was created by the analyst. The back computed values for erythromycin and olaparib are shown in Tables 3 and 4. The accuracy should be between 85% and 115%. The correlation coefficient for the calibration curve was 0.99.

Accuracy and precision

Tables 5 and 6 show the intra- and inter-batch precision and accuracy of erythromycin, whereas Tables 7 and 8 show the precision and accuracy of olaparib. The results of the multi-step preparation technique are acceptable, with nominal concentrations ranging from 90% to 110%. The coefficient of variation is <15%, which meets the nominal criterion. Figs. 4 and 5 shows the representative examples of Erythromycin and Olaparib.

Recovery

For erythromycin recovery of LQC area 97.3%, MQC area 99.1% and HQC area is 99.7% and for olaparib LQC area is 96.5%, MQC area 102.7% and HQC area 95.2%. The average recovery of erythromycin is 98.7% and that of olaparib is 98.2%, respectively shown in Tables 9 and 10.

Stability

To acquire LQC, MQC, and HQC values, three cycles of freezing and thawing were performed. For 24 h, the samples are maintained in

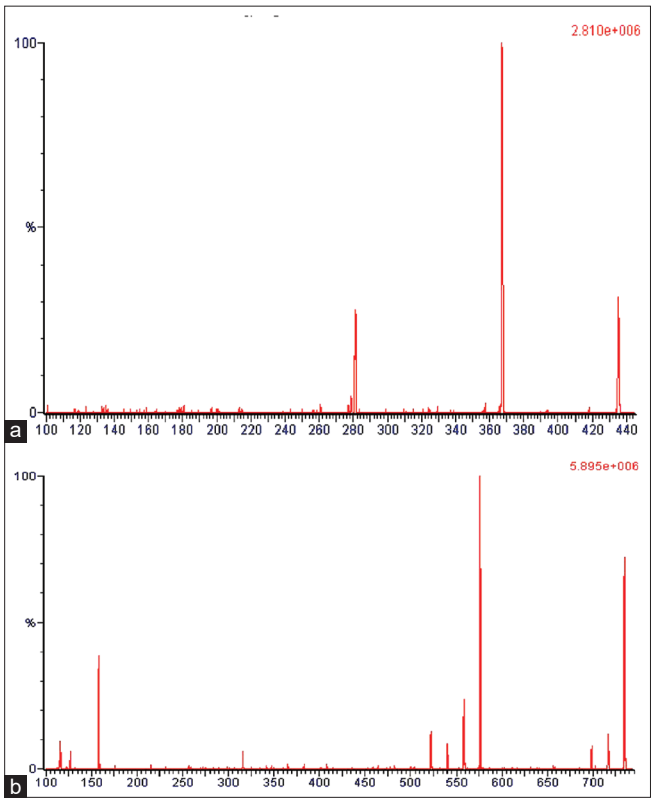

Fig. 3: (a) Product ion spectra of erythromycin, (b) Product ion mass spectra of olaparib in positive ionization mode

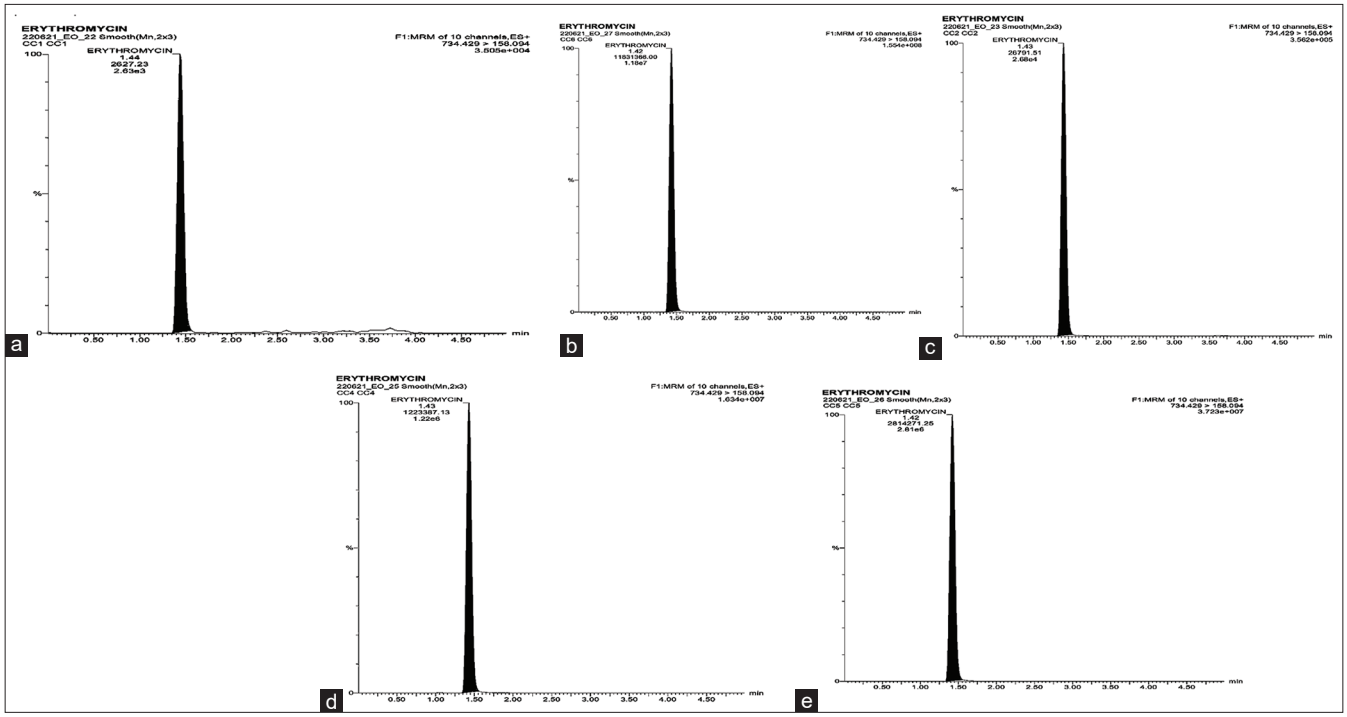

Fig. 4: (a) Representative example of 0.1 ng/mL chromatogram of erythromycin, (b) representative example of 500 ng/mL chromatogram of erythromycin, (c) representative example of 1 ng/mL chromatogram of Erythromycin, (d) representative example of 50 ng/mL chromatogram for erythromycin, (e) chromatogram 100 ng/mL for erythromycin

Table 2 : Summary of MRM transition conditions

Compound	Mode of ionization	Q1 mass (m/z)	Q3 mass (m/z)	Cone voltage	CE	Dwell (SEC)
Erythromycin	Positive	435	281	46	28	0.038
Olaparib	Positive	734	158	44	44	0.038

Table 3: Erythromycin back-calculated standard curve data

Concentration (ng/mL)							
Std conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
0.1	0.08	0.09	0.1	0.09	0.01	11.1	90
1	0.9	0.82	0.12	0.61	0.43	69.9	61.3
10	9.6	8.9	10.2	9.57	0.65	6.8	95.6
50	49.6	48.9	50.1	49.5	0.6	1.2	99.0
100	99.7	98.6	99	99.1	0.56	0.5	99.1
200	196	189	188	191	4.36	2.2	95.5
400	386	392	398	392	6	1.5	98
500	489	491	497	492	4.16	0.8	98.4
1000	990	982	989	987	4.36	0.4	98.7

Table 4: Olaparib back-calculated standard curve data

Concentration (ng/mL)							
Std conc.	**Batch-1**	**Batch-2**	**Batch-3**	**Mean**	**SD**	**% CV**	**% Accuracy**
0.1	0.09	0.1	0.08	0.09	0.01	11.11	90
1	0.9	0.8	0.9	0.87	0.06	6.662	86.67
10	9.1	8.9	9.2	9.07	0.15	1.685	90.6
50	49	47	46	47.3	1.53	3.227	94.6
100	96	97	92	95	2.65	2.785	95
200	187	191	188	189	2.08	1.103	94.3
400	391	389	396	392	3.61	0.92	98
500	491	489	497	492	4.16	0.846	98.4
1000	960	985	989	978	15.7	1.607	97.8

Table 5: Erythromycin calculated concentrations obtained for precision and accuracy batches

S. No.	Batch-1			Batch-2			Batch-3		
	LQC	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
1	3.6	520	813	4.1	489	810	4.1	496	820
2	3.4	517	780	3.8	511	796	3.9	515	797
3	3.2	518	794	3.6	521	800	3.8	516	812
4	3.1	515	802	4.2	516	803	4.2	513	810
5	3	521	791	3.7	521	789	3.6	520	807
6	3.9	519	805	3.5	520	812	4.3	518	815
MEAN	3.3	518.3	797.5	3.8	513.1	801.6	3.9	513.9	810.1
SD	0.3	2.1	11.6	0.2	12.3	8.6	0.2	8.6	7.8
%CV	10.0	0.4	1.4	7.3	2.4	1.0	6.6	1.6	0.9

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

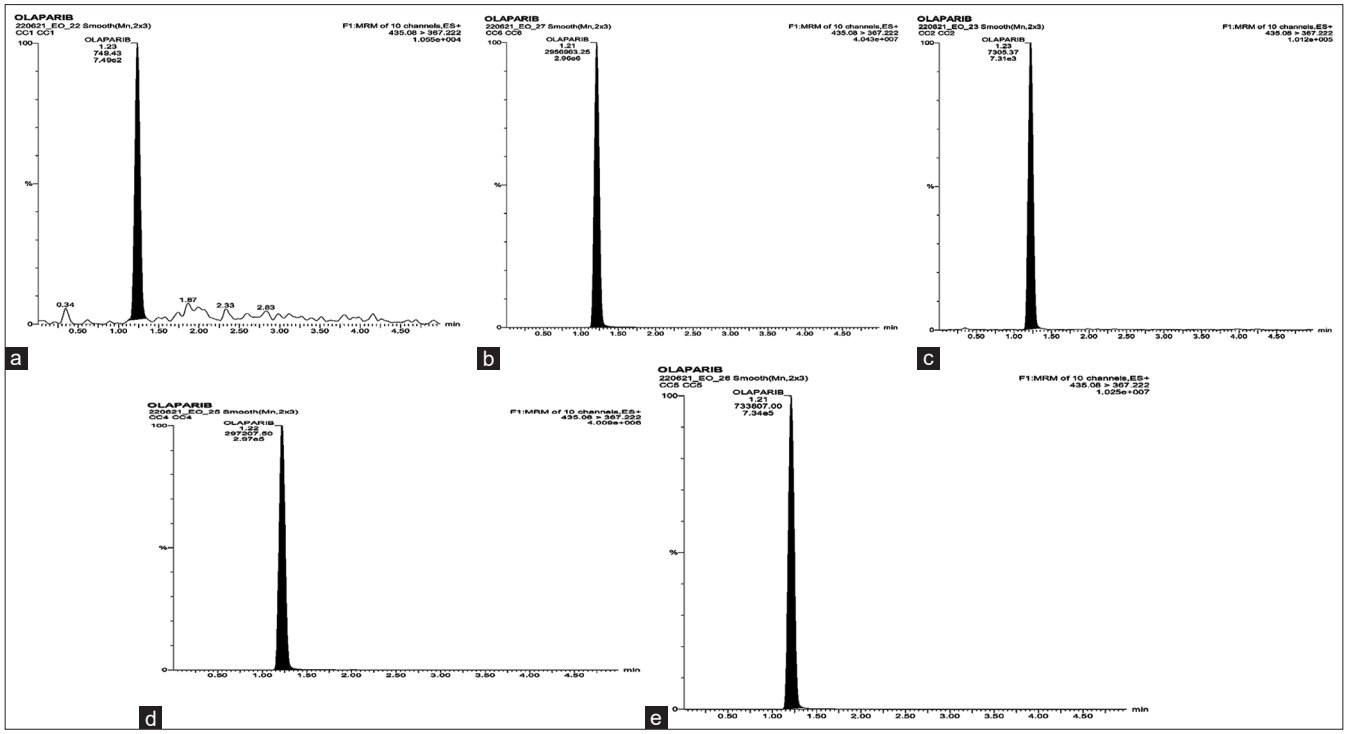

Fig. 5: (a) 0.1 ng/mL chromatogram for olaparib, (b) 500 ng/mL chromatogram for olaparib, (c) 1 ng/mL chromatogram for olaparib, (d) 50 ng/mL chromatogram for olaparib, (e) 100 ng/mL chromatogram for olaparib

Table 6: Intra- and inter-run precision and accuracy for erythromycin in human plasma

	Concentration (ng/mL)		
	LQC	**MQC**	**HQC**
BATCH-1 (n=6)			
Intra-run mean	3.37	518.3	797.5
Intra-run SD	0.34	2.1	11.6
Intra-run % CV	10.1	0.4	1.4
Intra-run % Accuracy	84.2	103.6	99.6
BATCH-2 (n=6)			
Intra-run mean	3.82	513.1	801.6
Intra-run SD	0.28	12.3	8.6
Intra-run % CV	7.3	2.4	1.0
Intra-run % Accuracy	95.4	102.6	100.2
BATCH-3 (n=6)			
Intra-run mean	3.98	513	810.1
Intra-run SD	0.26	8.6	7.8
Intra-run % CV	6.63	1.6	0.9
Intra-run % Accuracy	99.6	102.6	101.2
INTER-BATCH (n=18)			
Inter-run mean	3.72	514.7	803.1
Inter-run SD	0.39	8.6	10.4
Inter-run % CV	10.4	1.6	1.3
Inter-run % Accuracy	93.1	102.9	100.3

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

Table 7: Olaparib calculated concentrations obtained for precision and accuracy batches

S. No.	**Batch-1**			**Batch-2**			**Batch-3**		
	LQC	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
1	4.1	516	791	3.6	505	823	4.1	510	797
2	3.9	518	807	3.8	510	808	3.9	511	801
3	3.6	519	789	3.7	509	817	3.6	509	810
4	3.7	521	800	3.5	511	799	3.5	505	816
5	3.4	517	793	4.1	513	806	3.5	503	814
6	3.3	512	796	3.9	515	810	4	512	799
Mean	3.6	517	796	3.7	510.5	810.5	3.7	508.3	806.1
SD	0.3	3.06	6.6	0.2	3.45	8.4	0.2	3.5	8.1
% CV	8.21	0.59	0.8	5.7	0.676	1.0	7.0	0.7	1.0

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

Table 8: Intra-and inter-run precision and accuracy for olaparib in human plasma

	Concentration (ng/ml)		
	LQC	**MQC**	**HQC**
BATCH-1 (n=6)			
Intra-run mean	3.67	517.1	796
Intra-run SD	0.3	3.0	6.6
Intra-run % CV	8.21	0.5	0.8
Intra-run % Accuracy	91.7	103.4	99.5
BATCH-2 (n=6)			
Intra-run mean	3.77	510.5	810.5
Intra-run SD	0.22	3.4	8.4
Intra-run % CV	5.74	0.6	1.0
Intra-run % Accuracy	94.2	102.1	101.31
BATCH-3 (n=6)			
Intra-run mean	3.77	508.3	806.1
Intra-run SD	0.27	3.5	8.1
Intra-run % CV	7.06	0.7	1.0
Intra-run % Accuracy	94.2	101.6	100.7
INTER-BATCH (n=18)			
Inter-run mean	3.7	512	804.2
Inter-run SD	0.2	4.9	9.6
Inter-run % CV	6.7	0.9	1.1
Inter-run % Accuracy	93.3	102.4	100.5

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

Table 9: Erythromycin recovery data

S. No.	LQC Area (Counts)		MQC Area (Counts)		HQC Area (Counts)	
	Aqueous	**Extracted**	**Aqueous**	**Extracted**	**Aqueous**	**Extracted**
1	7901	7282	896121	874161	1703411	1712120
2	8201	7986	877126	862121	1821504	1801891
3	7634	7531	789143	791722	2123083	1928602
4	7501	7632	802162	799023	1911202	2023124
5	7821	7621	823168	807860	2023161	2018926
6	7932	7704	791260	801251	1900121	1967961
MEAN AREA	7831.66	7626	829830	822689	1913747	1908770
% RECOVERY	97.3		99.1		99.7	

AVG. %RECOVERY=98.75, SD=1.229, %CV=1.245, LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

Table 10: Olaparib recovery data

S. No.	LQC Area (Counts)		MQC Area (Counts)		HQC Area (Counts)	
	Aqueous	**Extracted**	**Aqueous**	**Extracted**	**Aqueous**	**Extracted**
1	12168	11962	993612	976017	1876321	1924561
2	10796	9893	891230	890214	1902318	1971206
3	11384	10321	913216	973205	2134781	1999012
4	10916	10124	981024	970134	2345671	2109307
5	9962	10016	892106	901289	2294301	2102175
6	10614	11284	791260	900631	1967071	1824179
Mean Area	10973.333	10600	910408	935248	2086743	1988406
% RECOVERY	96.5		102.7		95.2	

AVG. %RECOVERY=98.2, SD=3.972, %CV=4.044, LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

Table 11: Stability data showing the % CV of analytes

Analyte name	Fresh samples	Freeze-thaw stability samples	Auto-sampler stability samples	Bench-top stability samples
Erythromycin				
LQC	7.8	3.3	5.6	3.6
MQC	5.4	4.6	5.4	7.4
HQC	6.5	8.1	6.0	5.3
Olaparib				
LQC	4.8	8.8	6.4	8.3
MQC	6.2	5.1	3.2	2.2
HQC	7.5	3.9	2.5	4.1

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control

the auto sampler. To achieve bench-top stability, the samples are maintained at room temperature for 4 h. During the entire procedure, no instabilities were discovered. Table 11 shows the stability of analytes.

CONCLUSIONS

The current study uses liquid chromatography and mass spectrometry to create and validate erythromycin and olaparib in human plasma. Telmisartan is utilized as an internal standard, and it should be operated in a positive ion mode. Erythromycin has a 98.7% recovery rate, while olaparib has a 98.2% recovery rate. Linearity is measured in ng/mL increments ranging from 0.1 to 1000. The process was found to be stable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Amin MM, Zilles JL, Greiner J, Charbonneau S, Raskin L, Morgenroth E. Influence of the antibiotic erythromycin on anaerobic treatment of a pharmaceutical wastewater. Environ Sci Technol 2006;40:3971-7. doi: 10.1021/es060428j, PMID 16830570
2. Washington CR, Richardson DL, Moore KN. Olaparib in the treatment of ovarian cancer. Future Oncol 2019;15:3435-49. doi: 10.2217/fon-2019-0271, PMID 31478762
3. Anindya B. Development and validation of UV spectrophotometric method for estimation of erythromycin in bulk drug and pharmaceutical formulation. Int Adv Pharm 2015;5:71-6.
4. Rathore A, Lohidasan S, Mahadik K. Validated HPTLC method for simultaneous estimation of isotretinoin and erythromycin in bulk drug and topical gel form. Am J Anal Chem 2010;1:144-9. doi: 10.4236/ajac.2010;13018
5. Taninaka C, Ohtani H, Hanada E, Kotaki H, Sato H, Iga T. Determination of erythromycin, clarithromycin, roxithromycin, and azithromycin in plasma by high-performance liquid chromatography with amperometric detection. J Chromatogr B Biomed Sci Appl 2000;738:405-11. doi: 10.1016/S0378-4347(99)00512-5
6. Stubbs C, Haigh JM, Kanfer I. Determination of erythromycin in serum and urine by high-performance liquid chromatography with ultraviolet detection. J Pharm Sci 1985;74:1126-8. doi: 10.1002/jps.2600741027, PMID 4078714
7. Mahmoudi A, Boukhechem MS. Novel liquid chromatographic method for the simultaneous evaluation of erythromycin and azithromycin in human urine. J Mater Environ Sci 2017;8:1953-9.
8. Li YX, Neufeld K, Chastain J, Curtis A, Velagaleti P. Sensitive determination of erythromycin in human plasma by LC-MS/MS. J Pharm Biomed Anal 1998;16:961-70. doi: 10.1016/s0731-7085(97)00095-2, PMID 9547699
9. Xiao W, Chen B, Yao S, Cheng Z. Simultaneous determination of erythromycin propionate and base in human plasma by high-performance liquid chromatography-electrospray mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2005;817:153-8. doi: 10.1016/j.jchromb.2004.11.055, PMID 15686979
10. Gu Y, Wang G, Sun J. Simultaneous determination of erythromycin ethylsuccinate and its metabolite erythromycin in human plasma using liquid chromatography-electrospray ionization mass spectrometry for clinical study. J Pharm Biomed Anal 2006;40:737-43. doi: 10.1016/j.jpba.2005.11.005, PMID 16376051
11. Daumar P, Dufour R, Dubois C, Penault-Llorca F, Bamdad M, Mounetou E. Development and validation of a high-performance liquid chromatography method for the quantitation of intracellular PARP inhibitor Olaparib in cancer cells. J Pharm Biomed Anal 2018;152:74-80. doi: 10.1016/j.jpba.2018.01.036. PMID 29414021
12. Krens SD, van der Meulen E, Jansman FG, Burger DM, van Erp NP. Quantification of cobimetinib, cabozantinib, dabrafenib, niraparib, olaparib, vemurafenib, regorafenib and its metabolite regorafenib M2 in human plasma by UPLC–MS/MS. Biomed Chromatogr 2019;34:1-12. doi: 10.1002/bmc.4758
13. Nijenhuis CM, Lucas L, Rosing H, Schellens JH, Beijnen JH. Development and validation of a high-performance liquid chromatography-tandem mass spectrometry assay quantifying Olaparib in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2013;940:121-5. doi: 10.1016/j.jchromb.2013.09.020, PMID 24145016
14. Jolibois J, Schmitt A, Royer B. A simple and fast LC-MS/MS method for the routine measurement of cabozantinib, olaparib, palbociclib, pazopanib, sorafenib, sunitinib and its main active metabolite in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2019;1132:121844. doi: 10.1016/j.jchromb.2019.121844, PMID 31678788
15. Roth J, Peer CJ, Mannargudi B, Swaisland H, Lee JM. A sensitive and robust ultra HPLC assay with tandem mass spectrometric detection for the quantitation of the PARP inhibitor olaparib in human plasma for pharmacokinetic application. Chromatography 2014;1:82-95. doi: 10.3390/chromatography1020082