RJC

RASĀYAN *J. Chem.*
Vol. 16 | No. 1 |494-501| January - March | 2023
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATION OF RELUGOLIX IN PLASMA SAMPLES BY LC-MS/MS: APPLICATION TO BIOAVAILABILITY STUDY IN RABBITS

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ABSTRACT

A novel, sensitive, and specific LC-MS/MS technique was developed for the quantification of Relugolix in plasma and validated as per the regulatory guidelines. Elution of Relugolix and Canagliflozin was achieved on Inertsil C18 (150mm × 2.10mm i.d, 5.0 µm particles size) column with acetonitrile and 0.1% HCOOH as a mobile phasic system in the fraction of 90:10 with a flowrate of 0.8 mL/min. The sciex API 6000 triple quadrupole mass equipment was executed with the mode of multiple reactions and m/z 624.18/127.03 for Relugolix and m/z 445.14/267.12 for the Canagliflozin, were the optimized transitions. Relugolix exhibited a rectilinear plot in the range of 3.9 – 1500.0 ng/mL concentrations. The LC–MS/MS validated methodology was efficiently utilized for the assessment of Relugolix in the rabbit plasma. From the pharmacokinetics data, the mean of C_{max} and T_{max} were 42.01 ± 0.99 ng/mL and 5.922 ± 0.157, individually. Plasma conc. reduced with $t_{1/2}$ of 8.28 ± 0.174. $AUC_{0\rightarrow Last}$ value obtained was 303.437 ± 8.01 ng. h/ml, respectively. In short, the method that was produced has been successfully tested, and pharmacokinetic parameters were shown after healthy rabbits were given Relugolix orally.

Keywords: Relugolix, Prostate Cancer, LC-MS/MS, Method Development, Rabbits, Kinetics.
RASĀYAN *J. Chem.*, Vol. 16, No.1, 2023

INTRODUCTION

A GnRH (gonadotropin-releasing hormone) receptor antagonist called relugolix is utilized to treat a number of hormonally sensitive disorders. It was originally authorized in Japan in 2019 for the symptomatic management of uterine fibroids beneath the name brand Relumina6, and more recently by the FDA in the United States (US) in 2020 for the prevention of advanced prostate cancers under the brand name Orgovyx. Additionally, relugolix has been investigated for the treatment of endometriosis symptoms.[1,2] Since degarelix and other comparable therapies require subcutaneous administration, the drug is the 1st orally directed antagonist for GnRH receptors agreed for the management of prostate cancer. This makes relugolix a low burdensome therapeutics choice for the patient who may otherwise need to visit a clinic for administration by medical staff.[3-5]

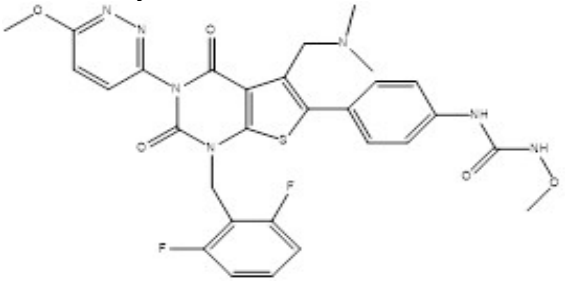

Fig.-1: Relugolix Chemical Structure

Relugolix was shown to be better than leuprolide, another androgen deprivation drug used to treat prostate cancer, in the depression of testosterone levels in addition to being very simple to apply. The actions of testosterone seem to be at least partially responsible for the etiology and development of prostate cancer. 5 Androgen deprivation therapy(ADT) has become the standard in the management of prostate cancer,

especially in advanced illnesses, since it has been shown to cause cell death and tumor shrinkage in several well-differentiated prostate cancer cell lines. It is designated chemically as 1-[4-[1-[(2,6-difluorophenyl)methyl]-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxothieno[2,3-d]pyrimidin-6-yl]phenyl]-3-methoxyurea with molecular formula and molecular weight of $C_{29}H_{27}F_2N_7O_5S$ and 623.64 g/mol correspondingly(Fig.-1).[6-8]

Literature on Relugolix shows only a single technique was reported for the quantitation of Relugolix by LC–MS/MS.[9] The reported method has low recovery rates. So, LC-MS/MS skills are needed to analyze biological samples, which can help with forensic, pharmacodynamics, and pharmacokinetic studies. In this work, we made a method that used plasma and then used the same methodology on the healthy rabbit.

EXPERIMENTAL

Reagents

Relugolix (99.81% pure), and Canagliflozin (99.92% purity) were procured from Metrochem API Private Limited, India. Analyte-free plasma having K_2EDTA was acquired from RedCross blood bank, Rangareddy, Telangana, India. Purified water from Milli-Q equipment (U.S.A) was employed for the water of LC-grade in the present study. LC-grade acetonitrile (ACN) and HCOOH were gained from local markets. The pharmacokinetics on healthier rabbit animals was permitted by the Institutional Ethical Committee with Ethical no: 1447/PO/Re/S/11/24/A.

Liquid chromatography and Mass Systems

Shimadzu LC20 ADvp, Japan consisting of a SILHTC autosampler, LC20 AD mobile phase delivery system combined with sciex API6000 tandem mass system, Canada were utilized for the current study. All the data obtained from the system was processed with applied biosystems version 1.4.2 software.

Standards for Calibration Curve

Stock solutions for Relugolix and Canagliflozin were processed individually in diluent (mobile phase). This stock was subjected to dilutions by taking Relugolix diluted solutions (20µL) along with K_2EDTA pooled plasma (960 µl) samples. Finally, 20µL of Canagliflozin was mixed with all solutions to get concentration levels of 3.9-1500.0 ng/ml, which was monitored at-20 °C.

Quality Controls

Three levels of low-quality controls (L.Q.C), median quality controls (M.Q.C), and highest-quality controls (H.Q.C) were processed. Blank plasma was spiked with Relugolix to acquire conc. (concentration) of 11.0 ng/mL, 750.0ng/mL and 1100.0ng/mL for L.Q.C, M.Q.C, and H.Q.C separately and warehoused at-20^0C.

Liquid Chromatography

Chromatographic isolation was achieved on C18-Inertsil (150mm × 2.10mm i.d, 5.0µm) column with HCOOH(0.1%) and acetonitrile in a quotient of 100: 900, as a movable system having a speed of flow at 0.8mL/min.

Mass Optimization

ESI source with a +ve ionization approach was utilized in the mass instrument. MRM (multiple reaction monitoring) modes were executed with the parameters of (Table-1): voltage of capillary at-2000(V); nebulizer gas flow 20 psi; voltage of tube lens-100(V); the flow of auxiliary gas at 25 psi. The source temperature was set at 500°C. The parent/product ion transitions in MRM mode were 624.18/127.03 m/z for Relugolix and m/z 445.14/267.12 for Canagliflozin.[10-13]

Preparation of Sample

Processing of the sample was executed by utilizing blank plasma, analyte, and IS stock solutions, which were subjected to vortexing upto 5Min. 4 mL of acetonitrile was added to the resulting solutions and centrifuged at 4500 rpm for 10 minutes at 04.0 °C. The organic segment from that was dried using an evaporator, and 500 µL of mobile phase was added to a dried remainder. Additionally, that solution was put into auto-sampler vials before being infused into the chromatographic apparatus.

Table-1: Parameters of Mass System

Parameter	Relugolix	Canagliflozin
DP (Declustering potential) (V)	60	50
Dwell time (MS)	100	100
EP (Entrance potentials) (V)	10	10
CUR (Curtain gas flows) (psi)	35	35
CE (Collision energies) (V)	25	32
Ion source voltages (V)	5500	3500

Method Validation

Optimized and developed technique was executed for the validation with respect to the FDA and EMA guidelines.[14-17]

Sensitivity and Linearity

The rectilinear graph was created between peak response quotients of Relugolix and canagliflozin in contrast to the actual concentration of Relugolix standard concentrations with drug concentration oscillating from 3.9-1500.0 ng/ml.[13]

Specificity

8 variables of human K_2EDTA blank plasma sample solutions were analyzed to establish the nonexistence of interfering constituents at retaining timings (RT) of Relugolix and canagliflozin.

Accuracy and Precision (A and P)

A and P parameters were analyzed from 6 replicate infusions of control samples (3 batches). These parameters were evaluated within 3 successive days.

Recovery Studies

Recoveries were quantified by contrasting of findings of sample extracts with that of plasma-spiked sample solutions afterward the extraction process. Recovery processing was executed at 3 QC concentrations and dilute standard controls were evaluated against the 6 mean sample extracts.

Matrix and Carryover Effect

The matrix effect was evaluated at low and high QC levels with 6 infusions into LC-MS/MS system. The effect of carryover was analyzed by the estimation of response peak by the infusion of matrix blank after the infusion of the higher calibration standards.[15-18]

Stability

LQC and HQC standards were subjected to a stock solution, freeze-thaw stability, short-term temperature, bench-top, autosampler, and wet extraction stabilities. Respective solutions were evaluated against the fresh sample solutions.

Pharmacokinetics in Rabbits

Six healthy white rabbits of both sexes were used in the study (2–3 kg). Animals were kept in a room with a temperature of 25.0°C, a relative humidity of 45.0%, a light/dark cycle of 12.0 hours, and a 100% fresh air exchange. The rabbits were given water and their normal food. After taking 15 mg of Relugolix by mouth, blood samples were taken at set times and put into a polypropylene tube with EDTA solution until 24 h after the last dose. The sample was spun at 6000 rpm for ten minutes to separate the plasma, which was then kept at –20.0°C. Almost 10µL of the sample was put into the LC-MS/MS, and the non-compartmental method analysis with WinNonlin 03.30® software (Pharsights Mountain Views, CA, USA)[14,17-22] was used to figure out the pharmacokinetic constraints.

RESULT AND DISCUSSIONS

Specificity

The specificity of the study showed that there are no interferences at the elution time of Relugolix and canagliflozin when the response of peak in sample blanks was made compared with to response of the LLOQ sample that contains canagliflozin mixture (Fig.-2 and 3).

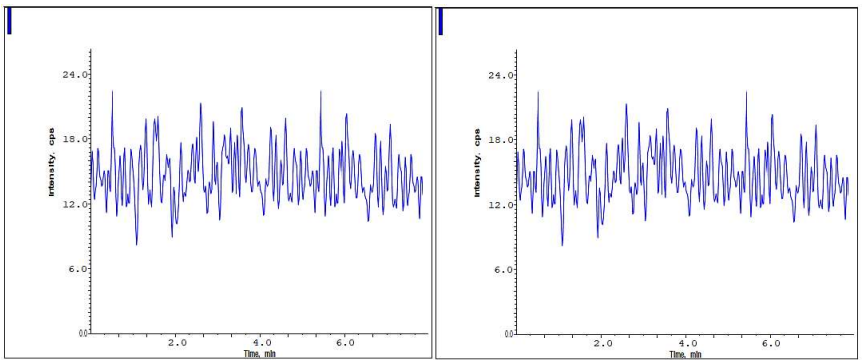

Fig.-2: Relugolix Blank Chromatogram

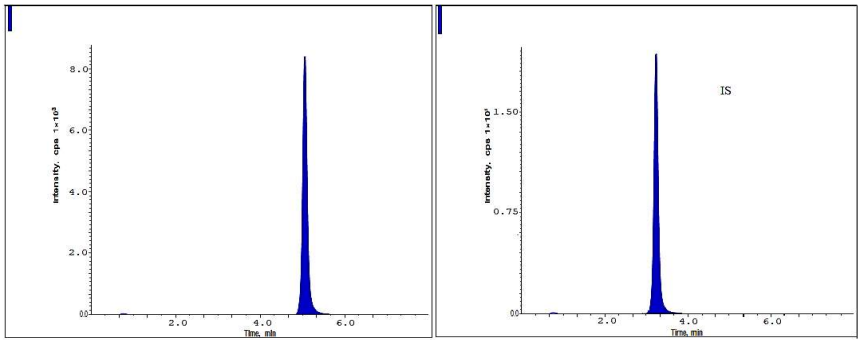

Fig.-3: Relugolix LLOQ Chromatogram

Sensitivity and linearity

The linearity plot was straight, with the equation y = 0.001610x + 0.0052, and the r^2 value was greater than 0.999(0.9997). For Relugolix, the LLOQ level concentration for the methodology was 3.9 ng/ml. The outcomes showed how important the process was. At these concentrations, the better signal-to-noise ratio showed that LLOQ can be lowered even more.

Recovery Study

The evaluated mean recovery values for the 3 control standards for Relugolix were 97.92%–98.88% (Table- 2). Canagliflozin recovery was estimated by comparing it with the reference solutions. The average recovery of canagliflozin was 97.69% (Fig.-4 to 6 and Table-2).

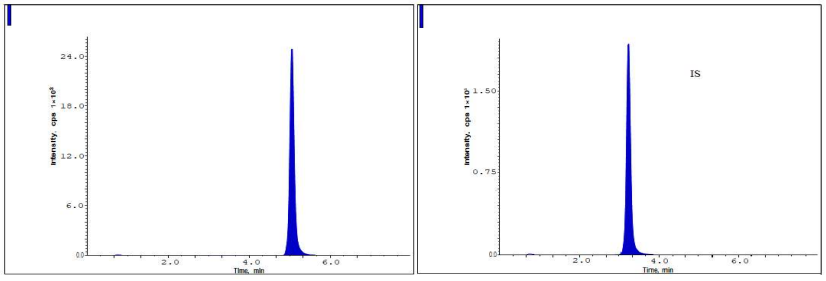

Fig.-4: Relugolix LQC Chromatogram

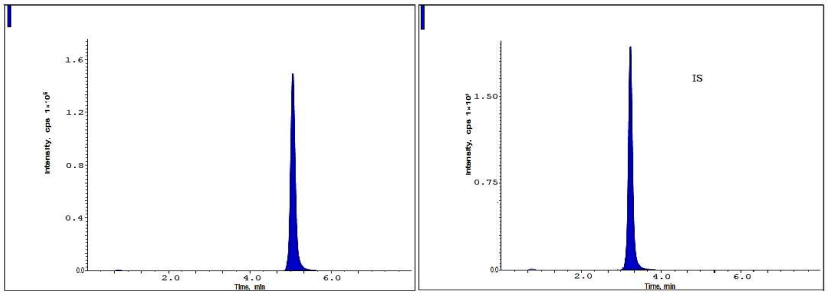

Fig.-5: Relugolix MQC Chromatogram

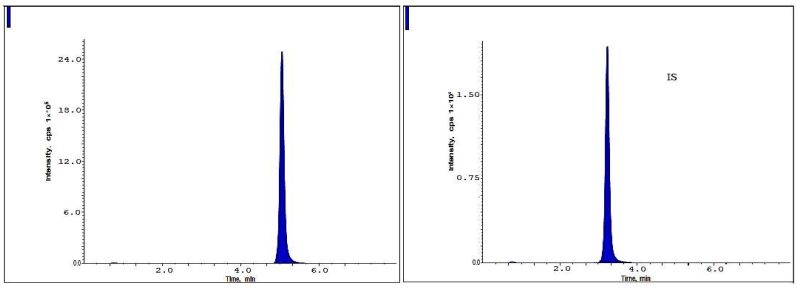

Fig.-6: Relugolix HQC Chromatogram

Table-2: Relugolix and Canagliflozin Recovery Study

S. No.	L.Q.C	M.Q.C	H.Q.C	Canagliflozin
1.	93.65	103.25	97.28	98.24
2.	94.87	95.36	98.15	99.18
3.	102.72	96.22	103.26	101.03
4.	104.18	101.69	102.84	98.64
5.	96.77	100.86	95.19	97.35
6.	95.34	94.11	96.54	100.37
Mean % Recovery	97.92	98.58	98.88	99.135
Standard Deviation	4.42	3.81	3.378	1.37
%CV	4.52	3.86	3.43	1.38

Carryover and Matrix Effects

No peak response was obtained in the blank after the higher calibration standard. The % C.V for the matrix effect of L.QC and H.QC was 2.39 % and 2.23 % for Relugolix (Table-3).

Table-3: Relugolix Matrix Effect

S. No	LQC			HQC		
	Relugolix	Canagliflozin	IS normalized	Relugolix	Canagliflozin	IS normalized
1.	1.07	1.01	1.06	1.09	1.02	1.07
2.	1.06	1.03	1.03	1.06	1.03	1.03
3.	1.04	1.02	1.02	1.05	1.02	1.03
4.	1.02	1.01	1.01	1.04	1.02	1.02
5.	1.09	1.01	1.08	1.03	1.01	1.02
6.	1.05	1.03	1.02	1.02	1.03	0.99
Avg			1.04			1.03
S.D			0.03			0.02
% C.V			2.39			2.23

The A and P

Intra-batch accuracy findings in between 96.11–103.09% and precision outcomes were between 2.22–4.95%. Inter-batch accuracy findings in between 94.62–102.54% and the precision outcome were between 0.92–4.57% (C.V) (Table-4).

Table-4: Relugolix Intrabatch and Interbatch P and A

	QC	Conc. (ng/ml)	Conc. found[a] (ng/ml)±S.D	% Accuracy	% C.V
Intra-batch	LLOQ	3.9	3.781±0.084	96.95	2.22
	LQC	11	11.34±0.38	103.09	3.35
	MQC	750	731.29±21.84	97.50	2.99
	HQC	1100	1057.21±52.37	96.11	4.95
Inter-batch	LLOQ	3.9	3.69±0.034	94.62	0.92
	LQC	11	11.28±0.42	102.54	3.72
	MQC	750	762.82±32.74	101.71	4.29
	HQC	1100	1083.54±49.57	98.50	4.57

a: Average of 6 replicates

Stability

From the stability tests, it was found that Relugolix was stable under the conditions tested. The outcomes of the test stabilities were shown in Table-5. The P value was less than 15%, and the analyte was stable between 85% and 115% of the time.

Table-5: Relugolix Stability Findings.

Parameters	Q C	Concen.(ng/mL)	Mean Concen(ng/mL) of Relugolix	%C.V	%Recovery
Freeze–thaw stability	LQC	11	11.22	4.37	101.96
	HQC	1100	1125.96	3.84	102.36
Benchtop stability	LQC	11	10.59	2.64	96.25
	HQC	1100	1088.80	3.74	98.98
Wet extract stability	LQC	11	11.53	1.99	104.83
	HQC	1100	1050.35	3.85	95.49
Autosampler stability	LQC	11	10.53	4.18	95.77
	HQC	1100	1150.73	3.75	104.61

Concen.- concentration

Pharmacokinetics in Rabbits

The plasma concentrations–time profile graph of Relugolix, after the administration of the drug through the mouth was represented in Fig.-7 and Table-6. The mean of C_{max} and T_{max} were 42.01 ± 0.99 ng/mL and 5.922 ± 0.157, respectively. Plasma conc. reduced with $t_{1/2}$ of 8.28 ± 0.174. $AUC_{0\rightarrow Last}$ value obtained was 303.437 ± 8.01 ng. h/ml, respectively (Table-7).

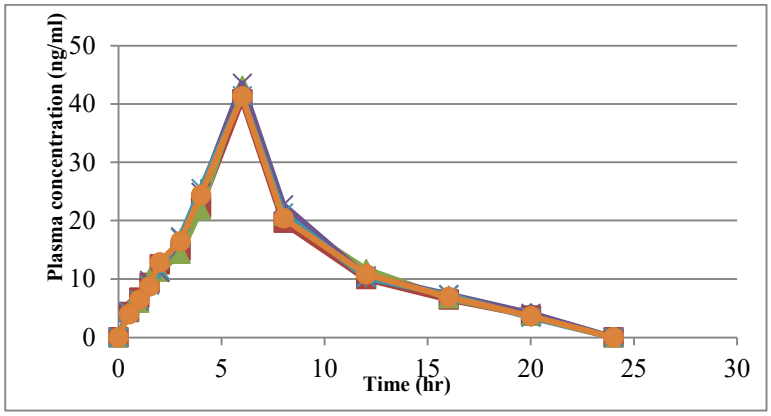

Fig.-7: Relugolix Plasma-Time Profile in Rabbits

Table-6: Concentration of Plasma at Several Time Intervals of 6 Rabbits

	Concentrations (ng/mL)							
Time in hrs	AL -1	AL -2	AL -3	AL -4	AL -5	AL -6	Avg	S.D
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	4.78	4.35	4.57	4.46	4.35	3.93	0.282989	6.420681
1	6.69	6.79	5.95	6.27	6.37	6.41	0.305355	4.765494
1.5	9.66	9.34	10.09	9.77	8.92	8.71	0.527467	5.601378
2	11.89	12.53	11.26	11.15	11.58	12.85	0.689644	5.806486
3	15.93	14.87	14.34	17.21	16.99	16.46	1.156923	7.246179
4	24.21	22.3	21.88	24.85	25.49	24.43	1.444789	6.055158
6	41.84	40.78	43.12	43.54	41.42	41.31	1.090854	2.597047
8	21.35	19.65	20.82	22.73	21.35	20.39	1.043648	4.958873
12	10.83	9.98	11.68	10.51	10.19	10.83	0.59984	5.619909
16	6.9	6.48	6.69	7.33	7.22	6.9	0.318021	4.595048
20	4.14	3.72	3.72	4.14	3.39	3.61	0.297876	7.863807
24	0	0	0	0	0	0	0	0

AL - animal; Avg: average; S.D- Standard deviations.

Table-7: Rabbit Pharmacokinetics

Parameter	AL -1	AL -2	AL -3	AL -4	AL -5	AL -6	Mean	STD
C_{max}	41.84	40.78	43.12	43.54	41.42	41.31	42.01	0.99
log C_{max}	1.62	1.61	1.63	1.64	1.62	1.62	1.62	0.01
T_{max}	5.78	5.88	6.21	6.05	5.79	5.82	5.92	0.16
logT_{max}	00.76	00.77	00.79	00.78	00.76	00.76	00.77	0.01
Half time($t_{1/2}$)	8.12	8.26	8.02	8.55	8.41	8.28	8.27	0.17
log $t_{1/2}$	00.91	00.92	00.90	00.93	00.92	00.92	00.92	00.01
Ke	00.051	00.054	00.058	00.06	00.056	00.054	00.06	00.01
log^{Ke}	-01.29	-01.27	-01.24	-01.23	-01.25	-01.27	-01.26	00.02
$AUC_{0\rightarrow last}$	306.94	289.19	302.97	316.29	303.82	301.42	303.44	8.01
log AUC $_{0\rightarrow last}$	2.49	2.46	2.48	2.50	2.48	2.48	2.48	0.01

STD-Standard deviation; AL - animal.

CONCLUSION

A unique, easy, and specific LC-MS/MS methodology was made for the Relugolix quantitation in biological plasma and validated as per the regulatory guidelines. Elution of Relugolix and Canagliflozin was achieved on Inertsil C18 (150mm × 2.10mm i.d, 5.0 µm particles size) column with acetonitrile and 0.1% HCOOH as mobile phase system in the fraction of 90:10 with a flow rate of 0.8 mL/min. The sciex API 6000 triple quadrupole mass equipment was executed with the mode of multiple reactions and m/z 624.18/127.03 for Relugolix and m/z 445.14/267.12 for the Canagliflozin, were the optimized transitions. Relugolix exhibited a rectilinear plot in the range of 3.9 – 1500.0 ng/mL concentrations. The LC–MS/MS validated methodology was efficiently utilized for the assessment of Relugolix in the rabbit plasma. From the pharmacokinetics data, the mean of C_{max} and T_{max} were 42.01 ± 0.99 ng/mL and 5.922 ± 0.157, individually. Plasma conc. reduced with $t_{1/2}$ of 8.28 ± 0.174. $AUC_{0\rightarrow Last}$ value obtained was 303.437 ± 8.01 ng. h/ml, respectively.

ACKNOWLEDGMENTS

The authors are grateful to the GITAM Deemed to be University, Medak for the support, and successful completion of the research work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. J. E.Michaud, K. L. Billups, A. W. Partin, *Therapeutic Advances in Urology*, **7(6)**, 378(2015), https://doi.org/10.1177/1756287215597633
2. A. Markham, *Drugs*, **79(6)**, 675(2019), https://doi.org/10.1007/s40265-019-01105-0
3. K. Miwa, T. Hitaka, T. Imada, S. Sasaki, M. Yoshimatsu, M. Kusaka, A. Tanaka, D. Nakata, S. Furuya, S. Endo, K. Hamamura, T. Kitazaki, *Journal of Medicinal Chemistry*, **54(14)**, 4998(2011), https://doi.org/10.1021/jm200216q

4. F. Barra, M. Seca, L. Della Corte, P. Giampaolino, S. Ferrero, *Drugs Today*, **55(8)**, 503(2019), https://doi.org/10.1358/dot.2019.55.8.3020179
5. D. B. MacLean, H. Shi, H. M. Faessel, F. Saad, *Journal of Clinical Endocrinology and Metabolism*, **100(12)**, 4579(2015), https://doi.org/10.1210/jc.2015-2770
6. N. D. Shore, F. Saad, M.S. Cookson, D. J. George, D. R. Saltzstein, R. Tutrone, H. Akaza, A. Bossi, D. F. Veenhuyzen, B. Selby, X. Fan, V. Kang, J. Walling, B. Tombal, *New England Journal of Medicine*, **382(23)**, 2187(2020), https://doi.org/10.1056/NEJMoa2004325
7. L. Goenka, M. George, M. Sen, *Biomedicine and Pharmacotherapy*, **90**, 575(2017), https://doi.org/10.1016/j.biopha.2017.03.092
8. R. Elancheran, V. L. Maruthanila, M. Ramanathan, S. Kabilan, R. Devi, A. Kunnumakara, Kotoky Jibon, *Medicinal Chemistry Communications*, **6(5)**, 746(2017), https://doi.org/10.52711/2231-5691.2021.00043
9. Liying Xing, Ya-nan Liu, Hongye Yao, Tingting Wang, Fuchen Xie, Shunbin Luo, Pingping Luo, Shengling Tang, *Frontiers in Pharmacology*, **13**, 874973(2022), https://doi.org/10.3389/fphar.2022.874973
10. X. Fan, G. Yang, W. Cui, Q. Liu, Z. Zhang, Z. Zhang, *Biomedical Chromatography*, **34**, e4768(2020), https://doi.org/10.1002/bmc.4920
11. N. Saraner, A. Karagoz, B. Guney, O. Saglam, *International Journal of Analytical and Bioanalytical Methods*, **1**, 2(2019), https://doi.org/10.35840/2633-8912/2402
12. Guodong He, Liping Mai and Xipei Wang, *Hindawi International Journal of Analytical Chemistry*, **6959761**, 1(2018), https://doi.org/10.1155/2018/6959761
13. Darshan Bhatt, B. Rajkamal, *International Journal of Applied Pharmaceutics*, **9(1)**, 30-36(2017), http://dx.doi.org/10.22159/ijap.2017v9i1.15652
14. Chunling Zhou, Jinmiao Tian, Peng Lin, Tianzhu Liu, Aiping He, Lina Fang, Lingling Sun, *Bioanalysis*, **12(5)**, 285(2020), https://doi.org/10.4155/bio-2020-0011
15. ICH Guidelines for Validation of Analytical Procedures: Text and Methodology, Q2(R1) ICH, Geneva, 2005, p.1-14.
16. US FDA, Guidance for Industry Bioanalytical Method Validation, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Rockville, Maryland, USA, 2001.
17. E. D. L. Putra, N. Nazliniwaty, F. R. Harun and N. Nerdy, *Rasayan Journal of Chemistry*, **13(2)**, 968(2020), http://dx.doi.org/10.31788/RJC.2020.1325645
18. C.H. Shankar, D.V.R.N. Bhikshapathi, *International Journal of Pharmaceutical Research*, **13(1)**, 6513(2021), https://doi.org/10.31838/ijpr/2021.13.01.830
19. I. Sopyan, D. Dwiputri and M. Muchtarid, *Rasayan Journal of Chemistry*, **13(4)**, 2207(2020), http://dx.doi.org/10.31788/RJC.2020.1346045
20. Ulrike Glaenzel, Yi Jin,Regine Hansen, Kirsten Schroer, Gholamreza Rahmanzadeh, Ulrike Pfaar, Jan Jaap van Lier, Hubert Borell, Axel Meissner, Gian Camenischand Sylvia Zhao, *Drug Metabolism and Disposition*, **48(10)**, 873(2020), https://doi.org/10.1124/dmd.119.090324
21. V. Jaivik, A. Shaha Priyanka, V. Shaha Priya, Shahb Mallika, S. Sanyalc Pranav, Shrivastav, *Journal of Pharmaceutical Analysis*, **7**, 163(2017), https://doi.org/10.1016/j.jpha.2016.11.004
22. K. Chandrasekhar and A. Manikandan, *Rasayan Journal of Chemistry*, **14(2)**, 665(2021), http://dx.doi.org/10.31788/RJC.2021.1425740

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