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Utility of green chemistry for native spectrofluorimetric quantification of darolutamide as a modern anti-neoplastic drug in its market form and biological fluids

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A simple, new, green, and sensitive approach was established and validated for assay of the recently approved antineoplastic medication; darolutamide (DAR) in its authentic form, pharmaceutical formulation, and biological fluids fluorimetrically. This experiment relied on the native fluorescence of the cited drug and detects the ideal solvent utilized throughout the approach. The proposed approach was validated regarding linearity, accuracy, and precision. The calibration graph showed linearity over the range of 0.1–2.0 $\mu\text{g mL}^{-1}$. The limit of detection and quantitation (LOD and LOQ) were 0.032 $\mu\text{g mL}^{-1}$ and 0.09 $\mu\text{g mL}^{-1}$, respectively. Because of the approach's high sensitivity, it was decided to spike the mentioned drug in plasma and urine samples. At last, checking for content uniformity was performed regarding the United States Pharmacopoeia (USP) by adjusting the proposed approach.

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1. Introduction

Prostatic cancer (PC) is the second most frequent solid cancer in males and the fifth-biggest cause of cancer-related mortality in men. PC tumor advancement and progression are helped by androgen receptor (AR) signaling, with androgens promoting PC multiplication and survival.^{1,2} It has been detected that androgen stimulation is a cause for up to 80% of prostate carcinomas. Androgen deprivation treatment or surgical castration is the most common therapy for metastatic prostate cancer. DAR is a recently developed drug that is recommended for that type of tumor. Darolutamide (DAR) is chemically *N*-[(2*S*)-1-[3-(3-chloro-4-cyanophenyl)pyrazol-1-yl]propan-2-yl]-5-(1-hydroxyethyl)-1*H*-pyrazole-3-carboxamide (Fig. 1), its brand name is Nubeqa, and is a novel second-generation orally active nonsteroidal antiandrogen for the treatment of castrate-resistant, non-metastatic prostate cancer.

In the literature review, DAR has only a few reported approaches with certain techniques, such as chromatographic techniques^{3–12} and recently only one spectrofluorimetric technique,¹³ in which depends on quantitative fluorescence quenching of mercurochrome reagent with the studied drug. Chromatographic techniques such as LC-MS and HPLC have many requirements as, sample pretreatments, including protein precipitation and solid-phase extraction, which drain

huge volumes of solvents, which raise costs and have a bad effect on the environment and are hard to get, so they may not be useable in numerous laboratories. On the other hand, spectrofluorimetric approaches are generally utilized in quality control and other research because of their high sensitivity, selectivity, simplicity, and wide application. As mentioned previously, only one spectrofluorimetric approach for the estimation of DAR is present. As a result, this work aims to establish a new, non-extractive, sensitive, and cost-effective fluorimetric approach for quantification of the cited drug in its authentic, pharmaceutical form, and biological fluids to meet the green chemistry requirements. Using ethanol as a diluent without further extraction and disuse of hazard solvents makes this approach environmentally friendly without any harmful impact. As a consequence, this approach can be considered for that moment the method of choice for quantification of DAR in pure form, dosage form, and biological fluids

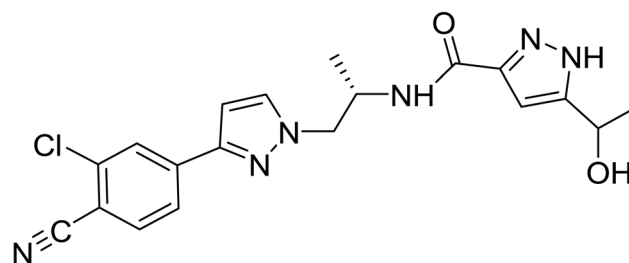


Fig. 1 Chemical structure of darolutamide.

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due to its simplicity, environmentally friendly, and sensitivity than the reported one.

2. Experimental

2.1 Apparatus

A 150 W xenon streak lamp and a 1 cm quartz cell were used in Agilent Cary Overshadow Fluorescence Spectrofluorimeter (USA). The excitation and emissions opening slits were 10 nm in diameter, and the Cary overshadow search program software version 1.2 was used. To assess pH, a HANNA pH 211 chip pH meter with double intersection glass anode was employed, A 4000 rpm (Bramsen ECCO, Germany) centrifuge was also utilized.

2.2 Materials and dosage forms

DAR was kindly supplied by Bayer HealthCare; Dana-Farber Cancer Institute; Latin American Cooperative Oncology Group; Orion. Its purity was found to be 99.75% according to its comparison spectrofluorimetric approach.¹³ Nubeqa® oral tablets, as a dosage form is claimed to contain 300 mg of DAR (Bayer Co., USA).

Phosphoric acid, citric monohydrate acid, sodium hydroxide, disodium hydrogen phosphate, hydrochloric acid, sodium dodecyl sulfate (SDS), Tween 80, ethanol, methanol, acetonitrile, and dimethylformamide (DMF) were among the other chemicals used, all of which were purchased from the ELNasr Chemical Co. (Cairo, Egypt). McIlvaine buffer was produced by combining various amounts of 0.2 M disodium dihydrogen phosphate and 0.1 M citric acid to yield a set of buffers with a pH range of (3.0–7.0). To produce solutions with a pH ranging from 7 to 12, Teorell & Steinhagen buffer was employed. Hence, 1 M sodium hydroxide, 1 M phosphoric acid, and 1 M citric acid were mixed, then adjusted with 0.1 M HCl. Plasma and urine samples were obtained from Minia University Hospital, blood bank, Minia, Egypt, and were kept in solid form until use after careful defrosting.

2.3 Standard solution

In a volumetric flask, 10.0 mg of DAR was dissolved in 100 mL ethanol to provide a stock solution of 100 $\mu\text{g mL}^{-1}$. Further dilutions of the used stock were made using the same diluent to give concentrations within the range of 1–20.0 $\mu\text{g mL}^{-1}$ to output the working solutions. When kept at 4 °C, the solutions stayed stable for about a couple of weeks.

2.4 Procedure

Using a set of 10.0 mL volumetric flasks, further dilutions of 1.0 mL of DAR's working solutions were performed using ethanol as the diluent. Each flask's content showed native fluorescence intensity, which was observed at λ_{em} 355 nm following excitation at 310 nm for the cited drug. In addition, ethanol was tested individually to illustrate the blank experiment.

2.5 Quantification of the medication in the market formula

A certain weight of the mixed fine authentic powder from 20 tablets of Nubeqa® tablets was dissolved in ethanol that was equivalent to 10.0 mg of DAR. The solution was then filtered into a volumetric flask with a capacity of 100.0 mL, and ethanol was added up to the mark. This filtrate was diluted *via* the same diluent to finally make working solutions with a concentration range of 1–20 $\mu\text{g mL}^{-1}$, then followed by the previously mentioned procedure.

2.6 Quantification of the investigated medication in biological fluids

2.6.1 For spiked human plasma. A volume of 5 mL drug-free blood sample was taken from the forearm of a healthy volunteer into a previously heparinized tube. The separation of needed plasma is done through centrifugation of the obtained blood sample for 20 min at 4000 rpm in centrifuge tubes. 1 mL of human plasma was spiked with 1 mL of DAR containing 1–20 $\mu\text{g mL}^{-1}$, along with 2 mL acetonitrile that was utilized for deproteinization purposes and stayed for 10 minutes. At the same speed and time, centrifugation was performed once again. Next, the supernatant was diluted with ethanol to obtain 0.1–2 $\mu\text{g mL}^{-1}$, the determined concentrations were (1.0, 1.5, and 2.0 $\mu\text{g mL}^{-1}$) then the general procedures were performed in this mixture. A blank experiment was determined utilizing plasma free from the cited drug.

2.6.2 For urine samples. The sample was made up of 1.0 mL urine spiked with 1 mL standard DAR solution 10 $\mu\text{g mL}^{-1}$ and 2 mL of acetonitrile (protein precipitating agent) was added and completed to 10 with distilled water centrifuge equipment was used to spin the mixture for 10 minutes at 3000 rpm and then, after diluting the supernatant with ethanol to get 0.1–2 $\mu\text{g mL}^{-1}$, the tested concentrations were (1, 1.5, and 2 $\mu\text{g mL}^{-1}$). Finally, the general procedures were carried out in this mixture. A blank experiment was evaluated using plasma free from the cited drug.

2.7 Test of tablet content uniformity

To get a workable concentration of 10 $\mu\text{g mL}^{-1}$, ten individually crushed (Nabeqa® 300) mg tablets were dissolved and filtered using the suggested solvent. Within the prescribed technique, 1.0 mL of this solution was used to allow the final working concentration of 1 $\mu\text{g mL}^{-1}$, which was within the linear range of the suggested experiment. The content uniformity testing was connected to understanding the United States Pharmacopoeia (USP) rules.¹⁴ After individually assessing ten pills, the acceptability value (AV) was evaluated.

3. Discussion and approach's results

DAR displayed native fluorescence at (355 nm) after excitation at 310 nm (Fig (2)). Our technique presented a simple, green as well as fast spectrofluorimetric measurement method for the recently approved studied drug, DAR, in its market formulation and biological fluids.



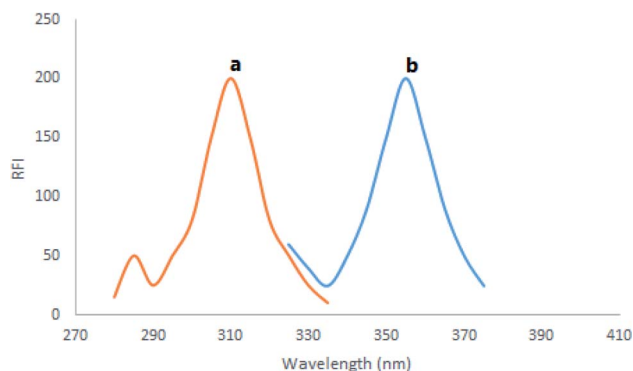


Fig. 2 Excitation (a) and emission (b) spectra of darolutamide ($0.5 \mu\text{g mL}^{-1}$).

3.1 Optimization

In order to find the optimum conditions, the experimental factors that may influence the native fluorescence or its stability were studied. The values of each of these parameters were altered independently, while the values of the other parameters remained constant.

3.1.1 pH optimization. To see how pH affects the native fluorescence of the investigated drug, an acidic medium utilizing 2 M HCl (0.5 mL) and buffer of McIlvaine with a pH ranging from 3.0 to 7.0 was created. On the other side, (0.5 mL) of 1 N NaOH and buffer of Teorell & Steinhagen with a pH ranging from 7.0 to 12.0 (ref. 15) were used to create basic media. The relative fluorescence intensity (RFI) values of DAR were not altered by pH changes, as they appeared to be roughly the same at pH 3.0–12.0. In any event, the strong acidic media (2 M HCl) appeared to affect the investigated drug, as a modest decrease in DAR's fluorescence was observed. Consequently, the use of no buffer systems was the most ideal condition in this case. Fig. 3, shows the usage buffer systems and their effect on fluorescence intensity of DAR.

3.1.2 Effect of surfactants. Utilizing numerous quantities of two various surfactants as Tween 80 & SDS in concentration 2% at various values of pH, the influence of additional chemicals to the quantification medium on the fluorescence's native of the stated drug was studied. The utilizing of SDS did not observe any enhancement on the RFI levels, while the utilizing

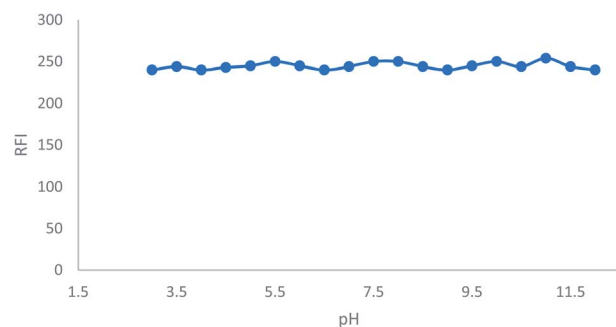


Fig. 3 Effect of pH on the native fluorescence of darolutamide ($0.7 \mu\text{g mL}^{-1}$).

of Tween 80, RFI was significantly reduced as a result of this. Subsequently, no surfactants were utilized in this assay (Fig. 4).

3.1.3 Solvents optimization. Finally, numerous solvents were employed to dilute the stated drug, including distilled water, ethanol, methanol, DMF, and acetonitrile. While keeping the other variables constant. The best solvents were revealed to be both ethanol and methanol, which created the most RFI. Because ethanol is the least hazardous of all the alcohols, it was

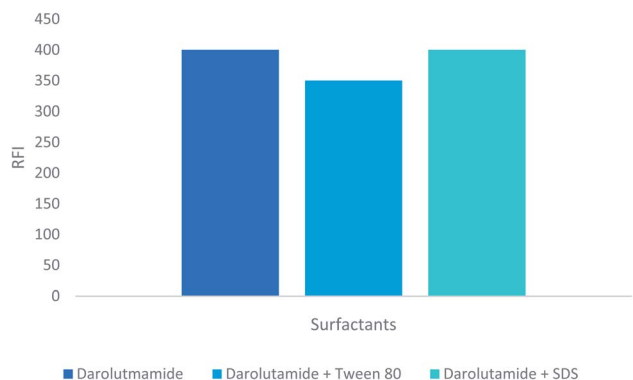


Fig. 4 Effect of the presence of surfactants on the native fluorescence of darolutamide ($1.0 \mu\text{g mL}^{-1}$).

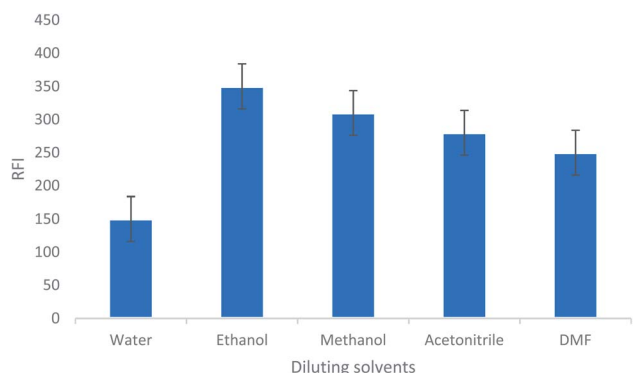


Fig. 5 Effect of diluting agent on native fluorescence of darolutamide ($0.7 \mu\text{g mL}^{-1}$).

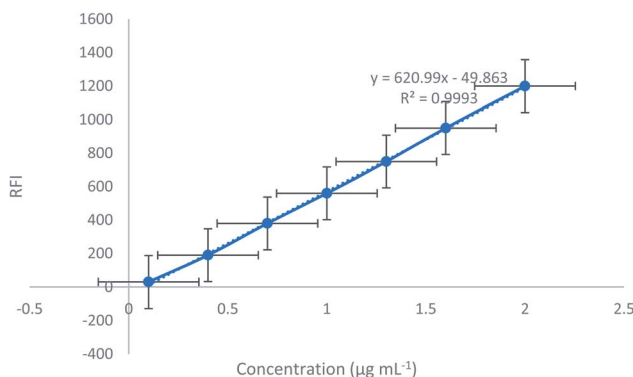


Fig. 6 Calibration curve construction of darolutamide ($90.1\text{--}2.0 \mu\text{g mL}^{-1}$).



Table 1 Analytical parameters obtained from the fluorimetric method for analysis of DAR

Parameters	Proposed method
λ_{ex} (nm)	310
λ_{em} (nm)	355
Linear range ($\mu\text{g mL}^{-1}$)	0.1–2.0
Correlation coefficient (r)	0.9996
Determination coefficient (r^2)	0.9993
Intercept \pm SD ^a	-49.863 ± 2.091
Slope \pm SD	620.99 ± 2.149
LOD ($\mu\text{g mL}^{-1}$)	0.032
LOQ ($\mu\text{g mL}^{-1}$)	0.09

^a SD: standard deviation.

Table 2 Evaluation of the accuracy of the proposed method at five concentration levels within the linear range of DAR

Sample no.	Taken conc. ($\mu\text{g mL}^{-1}$)	% ^a mean recovery \pm SD
1	0.3	99.30 ± 1.13
2	0.5	98.63 ± 0.99
3	0.7	100.75 ± 1.35
4	0.9	99.16 ± 0.95
5	1.2	101.43 ± 1.01

^a Mean of five replicate determinations.

perfect for use in our developed approach. As it appears in Fig. 5, the bar errors were very small, that's accept the usage of the suggested solvent.

3.2 Assay validation

The suggested technique was validated using the International Council for Harmonization (ICH) criteria,¹⁶ and the results were as follows.

3.2.1 Calibration linearity and range. More than five different concentrations of the studied drug were attempted under optimum conditions to construct a calibration curve. When measured at 355 nm (taking after excitation at 310 nm), these concentrations were matched to RFI measurements on a parallel plot ranging from 0.1 to 2 $\mu\text{g mL}^{-1}$ with a correlation value (r) of 0.9996 and determination value (r^2) of 0.9993

Table 3 Intraday and interday precisions for the proposed method for fluorimetric analysis of DAR

Concentration level ($\mu\text{g mL}^{-1}$)	% ^a mean recovery \pm ^b RSD	
	Intra-day	Inter-day
0.6	99.75 ± 0.17	99.22 ± 1.88
1	100.34 ± 0.98	100.36 ± 0.46
1.4	99.76 ± 1.85	100.93 ± 0.55
1.8	100.51 ± 1.11	100.01 ± 1.73

^a Mean of four determination. ^b RSD, relative standard deviations.

illustrated in (Fig. 6). The additional calculated parameters were shown in (Table 1).

3.2.2 Approach's precision & accuracy. Inside the direct range, five concentrations of DAR (0.3, 0.5, 0.7, 0.9, and 1.2 $\mu\text{g mL}^{-1}$) were tested, yielding five results to check the reliability of the suggested technique. The mean recoveries (% R) computed were excellent, indicating that the measured values were near to the true value, and implying that the suggested approach was extremely accurate (Table 2). On the other hand, four replicates at four different concentrations (0.6, 1, 1.4, and 1.8 $\mu\text{g mL}^{-1}$) were tested on the same day and on three consecutive days to determine its precision on both interday and intraday basis. The calculated percent relative standard deviation (% RSD) was less than 2%, showing the high precision of the developed approach (Table 3).

3.2.3 Sensitivity. The LOD was determined to be 0.032 g mL^{-1} , while the LOQ was 0.09 g mL^{-1} . These data demonstrated that the recommended approach was more sensitive than the only fluorometric reported paper¹³ (Table 4).

3.3 Application of the prescribed approach

3.3.1 Quantification of dosage form. The described method was successfully developed and validated for rapid quantification of the investigated drug DAR contained in Nubeqa® tablets 300 mg and yielded a remarkable % R (99.65 ± 0.88). When the obtained results were compared to those of the previously published technique,¹³ the calculated t - and F -value values were both smaller than their hypothetical values at a 95% confidence probability interval showing the precision and accuracy of the demonstrated approach (Table 5).

Table 4 A comparison between the reported method¹³ and the proposed method

Item	Proposed method	Reported method ¹³
Reagent	Absent	Present (mercurochrome)
Linear range	0.1–2.0 $\mu\text{g mL}^{-1}$	0.4–10.0 $\mu\text{g mL}^{-1}$
Correlation coefficient	0.9992	0.9990
Wavelength	λ_{ex} 310 nm, λ_{em} 355 nm	λ_{ex} 350 nm, λ_{em} 583 nm
Heating	Absent	Absent
Extraction step	Absent	Present
Buffer	Absent	Present
Surfactant	Absent	Absent
Application	Dosage form, content uniformity testing, spiked human plasma and urine sample	Dosage form, spiked human plasma and urine sample



Table 5 Proposed and reported methods used to determine DAR spectrofluorimetrically

Dosage form	Labeled content	Mean of % recovery ^a ± SD		<i>t</i> -value ^b	<i>F</i> -value ^b
		Proposed method	Reported method		
Nubeqa® tablet	300 mg	99.65 ± 0.88	100.21 ± 1.44	0.609	2.677

^a Mean of five replicate determinations. ^b The tabulated *t*-value and *F*-value at the 95% confidence limit are 2.78 and 6.39, respectively.

Table 6 Application of the proposed spectrofluorimetric method for analysis of DAR in spiked human plasma and urine samples

Concentration added (µg mL ⁻¹)	Plasma samples	Urine samples
	% recovery ^a ± SD	% recovery ^a ± SD
0.5	99.06	97.59
1	98.37	98.69
1.5	99.44	99.19
Mean	99.08	98.86
RSD	1.04	2.04
% error	0.97	1.75

^a Mean of five replicate determinations.

Table 7 Application of the proposed method to determine content uniformity of DAR in tablet form

Tablet number	% recovery ^a of the claimed content Nubeqa® 300 mg tablet
1	99.75
2	99.05
3	98.54
4	100.95
5	96.48
6	99.75
7	99.12
8	100.26
9	98.68
10	97.68
Mean	99.03
±SD	1.11
% RSD	1.12
Acceptance value (AV)	4.01
Maximum allowed AV (L1)	15

^a Mean of five replicate determinations.

3.3.2 Investigation of biological fluids. The proposed method may be used to quantify DAR in spiked human plasma and urine samples since it was so sensitive. Human plasma and urine samples were spiked with three different concentration levels of the investigated drug (1, 1.5, and 2 µg mL⁻¹) found in the experiment's linear range. For each concentration, the % *R* was calculated, and the mean % *R* was 99.08% (RSD = 1.04) & 98.86% (RSD = 2.04) for DAR assay in plasma and urine samples respectively. These findings showed that the proposed approach might be used to assay drugs in human plasma &

urine without causing considerable matrix-related interference (Table 6).

3.3.3 Content uniformity testing. The individual content of Nubeqa® in each tablet was determined for application of content uniformity testing to confirm that each tablet contained the claimed drug content based on USP methods.¹⁷ The estimated AV was less than 15 (the highest severe accepted esteem), demonstrating the consistency of DAR testing (Table 7).

4. Conclusion

The new approach was shown to be more sensitive, simple, and fast than the only published fluorimetric paper for the investigated drug without usage of either reagents, buffers or surfactants. Moreover, utilizing a single excitation step with green solvent may be conveniently employed in quality control laboratories because it takes less time and effort. Other methodologies with comparable sensitivity, such as LC and HPLC, used more complicated steps, very costly equipment, complex instrumentation, expensive organic solvents, and time-consuming procedures.

Ethical statement

All experiments were performed in accordance with the Guidelines "Ethics Requirements", and approved by the ethics committee at Deraya university. Informed consents were obtained from human participants of this study.

Author contributions

All authors contributed to the study's conception and design. Data collection and analysis were performed by all authors. The first draft of the manuscript was written by Hesham Salem and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing financial interests.

References

- 1 R. L. Siegel, S. A. Fedewa, K. D. Miller, A. Goding-Sauer, P. S. Pinheiro, D. Martinez-Tyson and A. Jemal, *Ca-Cancer J. Clin.*, 2015, **65**, 457–480.



- 2 J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J. W. W. Coebergh, H. Comber, D. Forman and F. Bray, *Eur. J. Cancer*, 2013, **49**, 1374–1403.
- 3 V. Kiran, A. Dixit, B. B. Gabani, N. R. Srinivas and R. Mullangi, *Biomed. Chromatogr.*, 2020, **34**, DOI: 10.1002/BMC.4938.
- 4 N. Balaji, S. P. Sulochana, N. K. Saini, A. Siva Kumar and R. Mullangi, *Biomed. Chromatogr.*, 2018, **32**, e4173, DOI: 10.1002/BMC.4173.
- 5 S. P. Sulochana, N. K. Saini, P. Daram, S. B. Polina and R. Mullangi, *J. Pharm. Biomed. Anal.*, 2018, **156**, 170–180.
- 6 A. Zakkula, V. Kiran, U. Todmal, S. P. Sulochana and R. Mullangi, *Drug Res.*, 2019, **69**, 537–544.
- 7 N. K. Saini, S. P. Sulochana, M. Zainuddin and R. Mullangi, *ADMET DMPK*, 2018, **6**, 242–257.
- 8 K. Fizazi, M. R. Smith and B. Tombal, *Clin. Genitourin. Cancer*, 2018, **16**, 332–340.
- 9 S. Dittakavi, P. K. V. S. P. Nagasuri, S. P. Sulochana, S. M. Saim, S. R. Mallurwar, M. Zainuddin, P. Dewang, S. Rajagopal and R. Mullangi, *J. Pharm. Biomed. Anal.*, 2017, **145**, 454–461.
- 10 N. Matsubara, H. Mukai, A. Hosono, M. Onomura, M. Sasaki, Y. Yajima, K. Hashizume, M. Yasuda, M. Uemura and C. Zurth, *Cancer Chemother. Pharmacol.*, 2017, **80**, 1063–1072.
- 11 K. Fizazi, L. Albiges, Y. Loriot and C. Massard, ODM-201, *Expert Rev. Anticancer Ther.*, 2015, **15**, 1007–1017.
- 12 K. Mori, H. Mostafaei, B. Pradere, R. S. Motlagh, F. Quhal, E. Laukhtina, V. M. Schuettfort, M. Abufaraj, P. I. Karakiewicz, T. Kimura, S. Egawa and S. F. Shariat, *Int. J. Clin. Oncol.*, 2020, **25**, 1892–1900.
- 13 H. Salem, N. Abdal-Karim, B. Omran, A. R. Abdel-Gayed, M. Atef and M. Abdelgaleel, *Luminescence*, 2022, 1–9, DOI: 10.1002/bio.4206.
- 14 *The National Formulary 25 US Pharmacopeial convention*, Electronic version, 2007.
- 15 M. Pesez and J. Bartos, *Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs*, Marcel Dekker, Madison Avenue, New York, 1974.
- 16 ICH Harmonized Tripartite Guideline, *Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Current Step 4 Version, Parent Guidelines on Methodology*, Dated 6 November 1996, Incorporated, 2005.
- 17 M. Rockville, *The United States Pharmacopoeia 30, The National Formulary 25 US Pharmacopeial Convention*, Electronic version, 2007.

