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Research Article

Spectrofluorometric Determination of Baricitinib in Pure Form and Application on Pharmaceutical Dosage Form; Green Profile Evaluation via Eco-scale and GAPI Tools

Ahmed M. Saleh[a*], Amira M. El-Kosasy[b], Nermine V. Fares[b]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Horus University, 34518, Egypt*

[b]*Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo 11566, Egypt*

Abstract

FDA-approved baricitinib for the treatment of COVID-19 for hospitalized patients. So far, there is no previously published spectrofluorometric method for the analysis of baricitinib. To this purpose, a first spectrofluorometric method with an environmentally friendly approach for the determination of baricitinib in its pure and tablet dosage form has been developed and validated. The spectrum of baricitinib in methanol shows λex at 224 nm and λem at 414 nm. The calibration graph was found to be linear (r= 0.9998) over the concentration range of (0.500-1.000 µg/mL). The proposed method was validated with a percentage recovery of 100.32 % ± 1.284 and a relative standard deviation of less than 2.00 demonstrating the accuracy and precision of the method. The method was found to have a LOD of 0.102 µg/mL and a LOQ of 0.309 µg/mL respectively. The study established showed that the proposed method can be adopted in the routine analysis since there is no specific monograph available in official pharmacopeia up to this date for the determination of baricitinib.

Keywords: *Baricitinib, COVID-19, Spectrofluorometry, Eco-scale, GAPI.*

***Correspondence** | Ahmed M. Saleh; Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Horus University, 34518, Egypt.
Email: asaleh@horus.edu.eg

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1. Introduction

The world has faced a global crisis after the discovery of the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2/coronavirus disease-2019 [COVID-19]) pandemic **[1]**. Epidemiological data from the World Health Organization (WHO) shows that since 2019, the number of deaths attributable to COVID-19 has risen to over 6.3 million, with over 500 million cases having been documented around the world **[2]**.

The primary cause of mortality with COVID-19 is the cytokine storm, which causes lung failure, resulting in acute respiratory distress syndrome which is connected to Janus kinases with signal transducer and activator of transcription (JAK-STAT) signaling system **[3]**. The JAK-STAT signaling system is a prominent cellular mechanism involved in the inflammatory response linked with the release of cytokines such as interleukin (IL)-6 and tumor necrosis

factor-α with a considerable increase in COVID-19 patients **[4]**. To date, blocking this immunological pathway has been the most effective method of reducing COVID-19-related mortality **[5]**. On November 19, 2020, using baricitinib (BCT) in conjunction with remdesivir has been given emergency approval by the US Food and Drug Administration (FDA) for the treatment of hospitalized patients with COVID-19 **[6]**. On May 10, 2022, Baricitinib is approved alone by FDA for treating COVID-19 in people who are hospitalised and need oxygen therapy or extracorporeal membrane oxygenation (ECMO), as per FDA approval it is a selective Janus kinase inhibitor for (JAK1) and (JAK2), leading to blocking of the JAK-STAT signaling pathway to stop the cytokine storm making it the frontline treatment for patients suffering from COVID-19 **[7, 8]**. Baricitinib; **(Fig. 1)** is 2-[1-(ethanesulfonyl)-3-(4-{7H-pyrrolo[2,3-d]-pyrimidin-4-yl}-1H-pyrazol-1-yl)-azetidin-3-yl]-acetonitrile **[9]**.

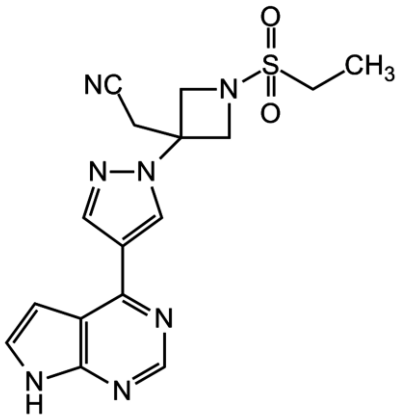


Fig.1. Chemical structure of Baricitinib.

A review of the literature revealed a limited number of techniques to determine baricitinib, including LC-MS/MS **[10, 11]**, HPLC **[12]**, and UV spectrophotometric **[13]** approaches. As far as we can tell, there isn't yet a spectrofluorometric technique for determining BCT alone or in its formulation has been published. The current research article aims at developing the first spectrofluorimetric method that is simple, selective, and sensitive for the determination of BCT in its pure powdered raw material form and its formulated dosage form.

2. Experimental

2.1. Instrumentation

A Cary Eclipse® fluorescence spectrophotometer equipped with a Xenon lamp from Agilent Technologies (5301 Stevens Creek Blvd. Santa Clara, CA 95051, United States). Quartz sample cell (1 cm). The slit width of excitation and emission monochromators = 5 nm.

A Jenway® 3510 pH meter (Jenway, Staffordshire, United Kingdom).

2.2. Materials

Baricitinib (99.90% certified purity) was kindly supplied by Hikma Pharmaceuticals (Sixth of October City, Giza, Egypt).

2.3. Pharmaceutical Dosage form

Baritava®, batch no: 00113, containing 4 mg baricitinib per tablet was provided by Hikma Pharmaceuticals (Sixth of October City, Giza, Egypt).

2.4. Chemicals

Organic solvents; Acetonitrile, methanol, acetone, 1-propanol, and DMSO were HPLC grade and purchased from Fisher Chemical (Loughborough, UK).

Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, phosphoric acid, trisodium citrate dehydrate, citric acid monohydrate, boric acid, hydrochloric acid, and tris-[hydroxymethyl] aminomethane-an (Tris) were all analytical grades and purchased from El-Nasr Pharmaceutical Chemicals, Cairo, Egypt.

Buffers with varying pH values were freshly

prepared according to USP [14]:

1. Acetate buffer pH range from 2.0 to 6.0
2. Phosphate buffer pH range from 6.0 to 8.0
3. Tris buffer pH range from 6 to 8
4. Borate buffer pH range from 8.0 to 10.0

De-ionized water (Millipore, Milford, MA, USA).

2.5. Standard solutions

2.5.1. BCT stock standard solution (100.00 µg⁄mL)

Dissolving quantitively 0.01 g of BCT in 100 mL methanol yielded a stock solution (100 µg/mL).

2.5.2. BCT working standard solution (10.00 µg⁄mL)

One mL of the stock standard solution of BCT 100 µg/mL was transferred into a 10 mL volumetric flask and completed with methanol to the mark.

2.6. Procedures

2.6.1. Spectral characteristics of BCT

An accurately measured volume of 0.5 mL of BCT working standard was added into a 10 mL volumetric flask. Then, 2 ml of phosphate buffer (10 mM, pH 7.5) was added and mixed well. Methanol was added to get the volume to the mark. After excitation at 224 nm, the fluorescence intensity was measured at 414 nm and the blank samples underwent the same procedure.

2.6.2 Optimization and development of the spectrofluorometric method

2.6.2.1. Choice of excitation and emission wavelengths

The proposed procedure described in section (2.6.1) was performed using an aliquot of 0.5 mL working BCT solution and a fluorescence scan was applied to identify the wavelength with the maximum emission intensity and its matching excitation wavelength.

2.6.2.2. Effect of Diluting Solvents

Using a fixed volume of working BCT (0.5 mL) and various diluents, such as 0.1 HCl, 0.1 NaOH, 1-propanol, acetonitrile, DMSO, water, and methanol, the overall process described in section (2.6.1) was repeated.

2.6.2.3. Effect of buffer's pH

The general procedure described in section (2.6.1) was repeated using a constant volume of working BCT (0.5 mL) and several buffers with pH values ranging from 2 to 10.

2.6.2.4. Effect of buffer's Volume

Using a fixed volume of working BCT (0.5 mL) and varying quantities of phosphate buffer ranging from 1 to 7 mL at pH= 7.5, the general procedure under (2.6.1.) was repeated.

2.7. Method validation

The proposed methodology was validated as per International Conference on Harmonization (ICH) guidelines **[15]**.

2.7.1. Linearity

Appropriate drug working standard solutions were delivered into a series of 10 mL volumetric flasks, each of which held 2 mL of phosphate buffer (10 mM, pH 7.5) to prepare different solutions with concentrations in the range of (0.5–1.0 µg/mL) then the volume was completed with methanol. The regression equation was calculated by plotting the concentrations against the fluorescence intensity at 414 nm following excitation at 224 nm.

2.7.2. Accuracy

The previously mentioned procedure under (2.6.1) was repeated for the determination of different concentrations of pure samples of BCT in triplicates. The concentrations were calculated from the corresponding regression equation and the mean recovery percentages and standard deviations were then computed.

2.7.3. Precision

2.7.3.1. Repeatability (Intraday precision)

The intraday variation was examined using the previously mentioned procedure under (2.6.1.) for the analysis of (0.65, 0.75, and 0.85 µg/mL) BCT, all on the same day, three times (n= 9). The concentrations were determined by applying the related regression equation and the relative standard deviations were calculated.

2.7.3.2. Intermediate precision (Interday precision)

Using the procedure mentioned above (2.6.1.), the above-mentioned concentrations of BCT were measured on three days in a row (n= 9)

2.7.4. Limit of detection and limit of quantification

The corresponding calibration curve was used to figure out the LOD and LOQ of BCT for the proposed method. The estimate was based on the standard deviation of response, which is part of the ICH guideline for figuring out LOD and LOQ, thus $LOD = 3.3 \times \sigma/S$.

$$LOQ = 10 \times \sigma/S.$$

Where σ is the response standard deviation and S is the calibration curve's slope. In this case, the standard deviation of the y-intercept of the regression line can be utilized as the response standard deviation.

2.7.2.5. Selectivity

It was studied by checking the incidence of any interference coming from the excipients. It was clear that these compounds did not interfere with the results of the proposed method which ensure the high selectivity of the proposed method for BCT.

2.7.2.6. Robustness

The robustness of the proposed method was validated by evaluating the ability to remain unaffected by small, but deliberate variations. The excitation and emission wavelengths were altered by ± 2 nm for investigation.

2.7.2.7. Application of the proposed method for the determination of BCT in pharmaceutical formulation (Baritava®)

Ten tablets were weighed and pulverized then a weighed amount of the powder equivalent to 10.0 mg of BCT was placed in a 100 mL volumetric flask, followed by 40 mL of methanol. Sonication was conducted for 15 min, then the flasks were filled to mark with methanol, followed by filtering. The first quantity of filtrate was discarded and appropriate dilution was performed before repeating the processes in (2.6.1). By testing the pharmaceutical formulation spiked with varied quantities of pure reference medication, the standard addition procedure was applied. These BCT concentrations were estimated using the corresponding regression equation, followed by the mean recovery percentages and standard deviations.

3. Results and Discussion

From the literature in hand, the proposed method is the first spectrofluorometric method for the determination of baricitinib. Different solvents were investigated including 0.1 HCl, 0.1 NaOH, 1-propanol, acetonitrile, DMSO, water, and methanol.

Native fluorescence of baricitinib can be observed in methanol and its emission can be measured at 414 nm (λ emission) after excitation at 224 nm (λexcitation). The emission and excitation spectra of BCT in methanol were shown in **(Fig.2)**.

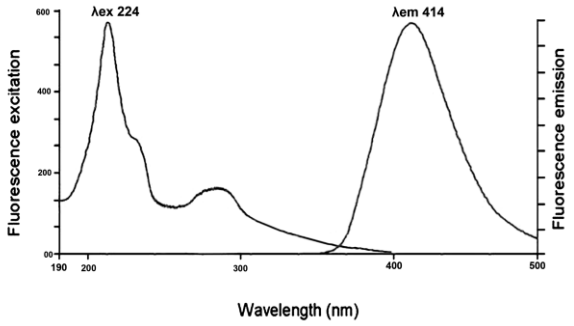


Fig.2. Excitation and Emission spectra of baricitinib in methanol at (λex 224) and (λem 414), respectively using the proposed spectrofluorometric method.

Methanol was the best-diluting solvent **(Fig.3)** which is suggested to be due to the intermolecular hydrogen bonds (HB) between methanol and the high electronegative atoms (N, S, O) in the structure of Baricitinib as the internal conversion (IC) can be facilitated by the hydrogen bond strengthening as the energy gap between ππ* and nπ* states of nitrogen-containing heterocycles can be significantly widened in methanol by hydrogen bonding and this sort of hydrogen bond is enhanced in the excited ππ* state resulting in increased fluorescence while the drop in fluorescence intensity of baricitinib in DMSO on the other hand, is most likely owing to the quenching impact of DMSO as a solvent and its usage as a quencher for [pyrrolopyrimidine-] based compounds such as baricitinib, due to the low energy gap between the ππ* and nπ* states **[16-20]**. After testing with acetate, phosphate, borate, and tris buffers, we found that pH has a crucial effect on BCT fluorescence switch-on results, with the lowest fluorescence intensity being found in acidic media. This is thought to be because the high electronegative atoms of Baricitinib are easy to protonate and ionize which makes the fluorescence intensity go down **[21]**.

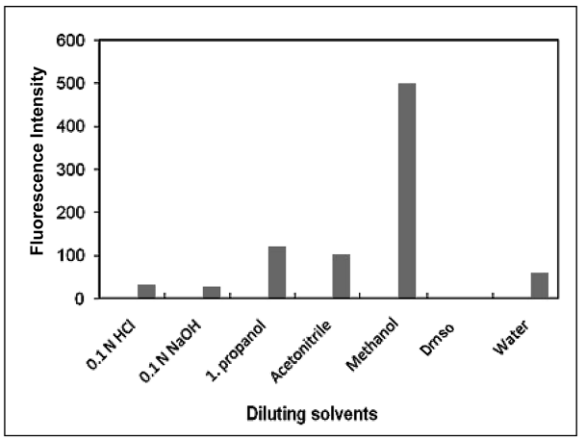


Fig.3. Effect of different diluting solvents on fluorescence intensity of Baricitinib.

The fluorescence intensity steadily rose with increasing pH value up to 7.5 and stayed steady up to 8, above this value the fluorescence intensity marginally decreased with increasing medium alkalinity as shown in **(Fig. 4)**.

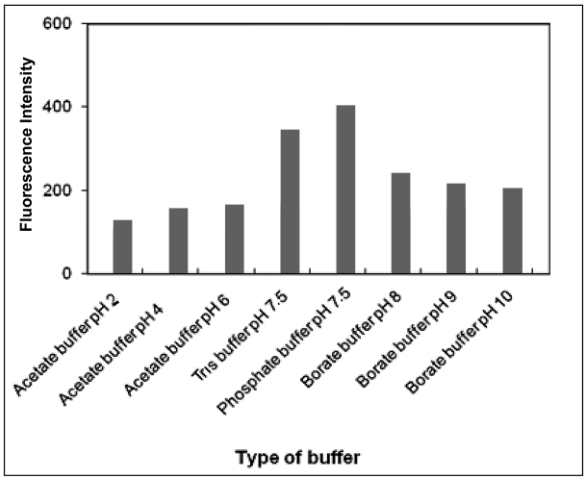


Fig.4. Effect of different buffer types on fluorescence intensity of baricitinib.

As a result, a pH of 7.5 was employed for the developed method, and the highest fluorescence intensity was attained by using 2

mL of the buffer solution, as shown in **(Fig.5)**. The calibration plot was found to be linear in the (0.5-1.0 µg/mL) range with a narrow range which supposed to be due to the inner filter effect of the terminal acetonitrile and its drawbacks on the quantum yield **[22]**. The correlation coefficient (r) for the BCT calibration curve was determined to be 0.9998. The graph's linear regression equation was:

$$F = 583.72 X - 21.627 \quad (r = 0.9998)$$

Where (F) is the fluorescence intensity, (X) is the drug concentration in µg⁄mL, and (r) is the correlation coefficient.

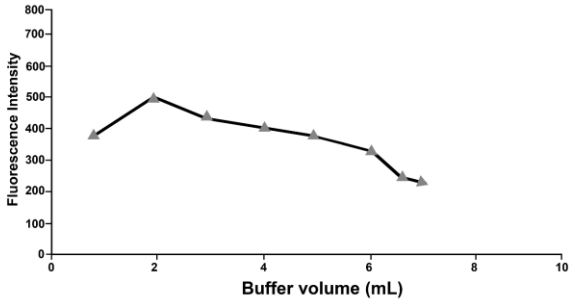


Fig.5. Effect of volume of phosphate buffer (10 mM, pH 6.7) on fluorescence intensity of baricitinib.

A high percentage of recovery (100.32± 1.284) was obtained indicating that the developed work was accurate. LOD was determined to be 0.102 µg/mL, whereas LOQ was determined to be 0.309 µg/mL. The low LOD and LOQ values imply good sensitivity. The proposed procedure's assay parameters for determining pure BCT samples were given in **(Table 1)**. The low % RSD results (less than 2%) suggested satisfactory precision of the proposed method as demonstrated in **(Table 2)**. A high percentage of recovery (100.32±1.284) was obtained which indicated good accuracy of the developed method **(Table 2)**. Baritava® tablets' BCT concentrations were accurately determined using the suggested approach, which was found to be unaffected by excipients as shown in **(Table 3)**. For robustness testing the slight variations did not have a significant effect on the measured fluorescence as the recoveries obtained were acceptable and RSD% with low values **(Table 4)**. When comparing the proposed method with the reported method statistically, there was no discernible difference as shown in **(Table 5)**.

Table 1. Assay parameters and method validation for the determination of pure sample of BCT by the proposed spectrofluorometric method

Parameters	
λ *excitation (nm)*	224.0
λ *emission (nm)*	414.0
Concentration µg⁄mL	0.50 – 1.00
Slope	583.72
Intercept	-21.627
Significance F	1.218×10^{-7}
S_{a*}	*7.218*
S_{b**}	*9.227*
RSD% of the slope (S_b%)	*1.14*
$S_{y/x***}$	*3.827*
Correlation coefficient(r)	0.9998
Accuracy (mean ± S.D.)	100.32 ± 1.284
LOD [a] µg⁄mL	0.102
LOQ [a] µg⁄mL	0.309

[a]; Limit of detection and limit of quantification.

*; Standard deviation of the intercept.

**: Standard deviation of the slope.

***: Standard deviation of residuals.

Table 2. Accuracy and precision results for the determination of BCT using the proposed method

Accuracy [a] conc. (µg/mL)	**% recovery**	**Found conc.** (µg/mL)
0.65	99.69	0.648
0.75	100.80	0.756
0.80	101.75	0.814
0.85	100.94	0.858
0.90	98.44	0.886
n=6	Mean ± S.D 100.32 ± 1.284	

[a] Average of triplicate injections per quality control concentration (n = 3)

Precision conc. (µg/mL **)**	**Intra-day** [b]		**Inter-day** [c]	
	mean ± S.D	RSD	mean ± S.D	RSD
0.65	100.66 ± 0.976		100.61 ± 1.381	
0.75	100.75 ± 0.951	1.144	100.80 ± 1.415	1.703
0.85	100.05 ± 0.997		100.27 ± 1.442	

[b]; The intraday (n= 3), an average of three different concentrations three times within the day.

Table 3. Determination of BCT in its formulation by the proposed spectrofluorometric method and application of standard addition technique

Dosage form	**Drug**					
Baritava®		**Taken** (µg/mL)	**recovery (%) ±S.D.**	**Added** (µg/mL)	**Found** (µg/mL)	**% Recovery***
labeled to contain 4 mg Baricitinib per tablet	BCT	0.5	100.33 ± 0.112	0.25	0.249	99.60
				0.35	0.351	100.29
				0.45	0.449	99.78
				Mean ± S.D.		99.89±0.356

* Average of three determinations.

Table 4: Robustness of the proposed spectrofluorimetric method

Parameter	Recovery(%) ± SD [a]	RSD(%) [b]
λex 224 ± 2 nm	99.73 ± 1.18	0.73
λem 414 ± 2 nm	100.12 ± 0.97	0.81

[a]: Average of three determinations.

[b]: % Relative standard deviation.

Table 5. Statistical comparison of the results obtained by applying the proposed method and the reported method for the analysis of pure BCT

Value	Proposed method	Reported method[13]
Mean	100.32	99.23
SD	1.284	0.992
RSD%	1.280	0.991
n	5	5
Variance	1.648	0.984
Student's t-test [a]	1.508 (2.306)	
F value [b]	1.674 (9.60)	

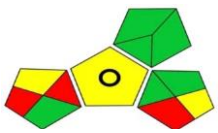
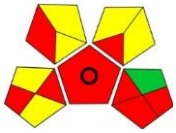
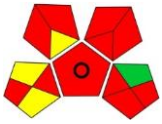
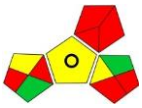
[a,b]; The values in parenthesis are the corresponding theoretical values of t and F

4. Assessment of the analytical method of greenness using analytical eco-scale and green analytical procedure index (GAPI)

Recently, there has been a rise in both the acceptance and interest in the "Green Analytical Chemistry" (GAC) approach from researchers in the scientific fields **[23-25]**. The proposed method's greenness profile was eva luated with two different guidelines namely; Analytical Eco-scale (AES) and Green Analytical Procedure Index (GAPI) **[26-30]**. Analytical Eco-Scale is an approach where the ideal green method is scored with a value of 100, then, penalty points are subtracted based on the number of reagents, hazards, energy, and waste and the results are ranked on a scale, where a score higher than 75 ranks the method as excellent green, a score higher than 50 represents an acceptable green method while a score less than 50 signifies an inadequate green method **[31]**. With only 12 points deducted, the produced work's analytical technique is excellent and eco-friendly, producing less waste and using fewer potentially dangerous reagents **(Table 6)**. (GAPI) is another method for gauging the eco-friendliness of an analysis. The environmental impact of each stage of the analysis process is measured in terms of five pentagrams of fifteen

parameters, all of which are graphically represented using a color coding, with green, yellow, and red indicating low, medium, and high environmental impact, respectively **[32]**. The proposed method as illustrated in **(Table 6)**, is a direct method that did not require any extraction steps. Due to the offline sampling, obligatory transfer between pharmaceutical manufacturing sites and QC labs, and no waste treatment has been undertaken, the analytical technique only reveals three red zones and their associated sections (1 & 3 & 15). The green pictograms represent the environmentally friendly nature of the developed work.

Table 6. Greenness assessment of the proposed and reported methods using Analytical Eco-Scale and GAPI guidelines

Technique	*Proposed Method* Spectrofluorimetry	*Reported Method* LC-Ms/Ms	*Reported Method* LC-Ms/Ms	*Reported Method* RP-HPLC	*Reported Method* HPLC-UV
Analytical Eco-Scale	**Penalty Points**				
• Reagents					
1-Methanol	12	12	12	-	12
2- Acetonitrile	-	8	8	8	-
3-Ammonium acetate buffer	-	2	-	-	-
4- DMSO	-	-	-	-	5
5-Formic acid	-	-	6	-	-
6-dichloromethane	-	-	2	-	-
7- phosphoric acid	-	-	-	2	-
8-n-hexane	-	-	2	-	-
9- Phosphate buffer	0	-	-	-	-
• Energy consumption	0	1	1	1	0
• Occupational Hazard	0	0	0	0	0
• Waste	6	6	6	8	6
• Total penalty points	18	29	37	19	23
• Analytical Eco-Scale score *	82	71	63	81	77
GAPI					

*Analytical Eco-Scale score

Conclusion

A spectrofluorometric method with an excellent green profile was developed for the determination of baricitinib in bulk and its tablet dosage form with nano-level sensitivity. The greenness of the proposed method has been successfully assessed using analytical Eco-Scale and GAPI guidelines. The present work was fully validated following ICH guidelines.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

No competing interests were declared by the authors

Funding statement

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