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A SIMPLE METHODS TO DEVELOPMENT AND VALIDATION OF ATORVASTATIN AND BEZAFIBRATE IN THE TABLETS DOSAGE FORMS BY SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC.

Nabil A Alhemiary* [1, 3], Ibrahim A. Alhagri [2, 3]

[1]Department of Chemistry, College of Science and Arts – Sharurah, Najran University, Sharurah, Saudi Arabia.,
[2]Department of Chemistry, College of Science, Qassim University, Buraidh, Saudi Arabia.,
[3]Department of Chemistry, College of Science, Ibb University, Ibb, Yemen.
*E-mail: dralhemiary@gmail.com

Abstract:
Developed and validated for the determination of Atorvastatin (ATV) and Bezafibrate (BZF) in the tablets dosage forms using spectrophotometric and Spectrofluorimetric methods. The spectrophotometric methods were based on derivatization of the investigated drugs with two reagents NQS and NBD-Cl. Beer's law was obeyed over the ranges of 0.3 - 6 and 0.5- 9 µg/mL for NQS reaction, and the ranges of 0.5 - 5 and 1-10 µg/mL for NBD-Cl reaction, the absorbance readings of ATV and BZF, respectively. Spectrofluorimetric methods were developed based on the fact, that the derivative explored drugs with NBD-Cl reagent is very fluorescent items. The fluorescence concentration plots have been linear above the ranges of 0.05–0.5 and 0.5–4 µg /mL for ATV and BZF, respectively. The fluorescence measurement enabled the detection of ATV and BZF at concentrations of about 0.011 and 0.079 µg/mL, respectively. The proposed methods have been validated and efficaciously applied in accordance with the dedication of the investigated drugs of the tablets. The results obtained had been statistically compared to those obtained from reference methods.

Keywords: *Spectrophotometric, Spectrofluorimetric, Atorvastatin, Bezafibrate, Pharmaceutical analysis.*

Corresponding author:
Nabil A Alhemiary,
Department of Chemistry, College of Science and Arts – Sharurah, Najran University, Sharurah, Saudi Arabia.

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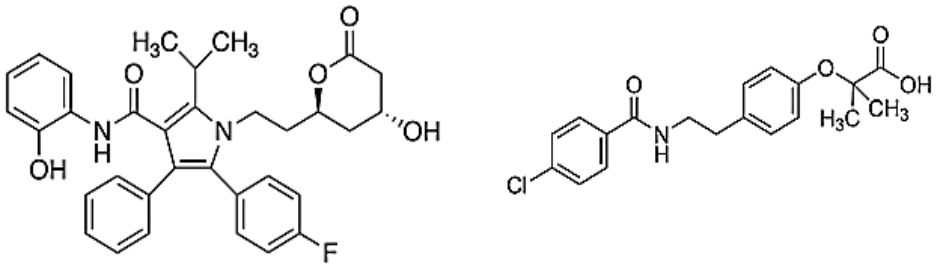
INTRODUCTION:

Atorvastatin (ATV), (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-yl-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid; (Fig 1.a) is administered as the calcium salt of its active acid form, and is a member of statin group medicine that reduces lowering blood cholesterol; thus it is used to treat hypercholesterolemia. Independent of the cholesterol-lowering property of statins they also have anti-inflammatory and immunomodulation effects[1]. Atorvastatin is metabolized by cytochrome P4503A4 to form active ortho- and para-hydroxylated metabolites, and also works by inhibiting of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reeducate), an enzyme found in liver tissue that plays a key role in production of cholesterol in the body[2]. The mean plasma elimination half-life of inhibitory activity for HMG-CoA reeducates is approximately 20 to 30 hours due to the contribution of the active metabolites [3]. Several methods have been described for the quantitative determination of ATV by HPLC in different pharmaceutical preparations, either alone [4-8] or with other active ingredients [9-14]. HPTLC[15], LC/MS-MS[16], UPLC-MS/MS[17] electrochemical[18-21], capillary electrophoresis[22] spectrofluorimetric[23, 24] and spectrophotometric[5, 25-31].

Bezafibrate (BZF), 2-[2-(p-[2-chlorobenzamido] ethyl] phenoxy] 2- methylpropionic acid (Fig1.b), It represents along with fenofibrate, ciprofibrate, gemfibrozil, and etofibrate, is a fibric acid derivate [32]. Bezafibrate is an agonist of the peroxisome proliferator receptor selective for α receptors (PPAR-α) that potently decreases plasma low density lipoprotein LDL and triglycerides and improving HDL levels in the blood [33]. Thus, several analytical methods have been described for the quantitative determination BZF by capillary electrophoresis [34], chromatography [32, 35, 36], HPLC-MS[37] spectrophotometric[38, 39] and voltammetry[40]. Among the various methods available for determination of drugs, spectrophotometric and Spectrofluorimetric, continue to be the most convenient analytical techniques, because of their inherent simplicity, low cost and wide availability in most quality control laboratories [41]. Chromatographic strategies require very sophisticated and costly instruments and solvents that may not be available in some quality control research facilities in the creating nations. 1, 2-Naphthoquinone-4-sulphonate (NQS) [42-45] and 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) [46-49] have been utilized as derivatizing reagents in development of both spectrophotometric and spectrofluorimetric methods for determination of some drugs amines. ATV and BZF are amines; there is a possibility of their interaction both NQS and NBD-Cl. The present study was devoted to investigating the reaction of ATV and BZF with both reagents, and development of new simple spectrophotometric and Spectrofluorimetric methods for determination of both drugs in its tablets dosage forms.



(a) (b)

Fig 1: Chemical structures for (a) Atorvastatin (ATZ) and (b) Bezafibrate (BZF).

MATERIAL AND METHODS:

Instruments and chemicals:

Double beam 6850 UV-Visible Spectrophotometer, Jenway Ltd, UK, with 1cm quartz cell was used for all the spectrophotometric measurements. Fluorescence spectrometer Lumina, Thermo Scientific, USA. With 1cm quartz cells and a 150 W xenon lamp, was used for the fluorimetric measurements. Also, pH meter 3030, Jenway Ltd., UK. All reagents and solvents were of analytical reagent grade. Atorvastatin (ATV) Pure sample was kindly provided by Jamjoom pharma, Raiyah, Saudi Arabia, Purity, 99%. ATV tablets: Ator tablets 10 mg, Batch No. 2G325, the product of EIPICO Pharmaceutical, 10th of Ramadan City, Egypt; Lipigard tablets 20 mg, Batch No.203904, the product of Julphar Pharm. Ind., Ras al-Khaimah, UAE. Obtained from commercial sources in the local market. Bezafibrate (BZF) pure sample was kindly provided by Jamjoom pharma, Raiyah, Saudi Arabia, Purity, 99.5%. BZF tablets: Bezalip@ Retard tablets 400 mg,

Batch No. HE45325, product of Roche Pharma, Basle, Switzerland; Bezafibrate tablets 200 mg, Batch No.L19754 product of ASIA Pharm. Ind., Damascus, Syria. Obtained from commercial sources in the local market. 1,2-Naphthoquinone-4-sulfonic acid sodium salt (NQS) solution of 0.5% (w/v) was prepared by dissolving 500 mg in 100 mL distilled water. The solution was freshly prepared and protected from light during use. 4-Chloro-7-nitrobenzofurazan (NBD-Cl) solution of 0.2% was freshly prepared by dissolving 200 mg in 100 mL methanol[41]. The borate buffer solution 0.2M alkaline of pH values were prepared by mixing 100 mL of 0.2M aqueous solution of boric acid and potassium chloride (500 mL contains 6.184 g of boric acid and 7.45g of potassium chloride) with 42.6mL of 0.2M sodium hydroxide in 400 mL standard flask [41, 50].

ATV and BZF stock solutions (1mg/mL) arranged independently by dissolving 100 mg of each medication in 100 mL methanol. The standard solutions have been stable for 7 days then kept into the refrigerator. The stock solutions had been similarly dilute together with distilled methanol (for reactions with NBD-Cl) or water (for reactions with NQS) to obtain working solutions in the concentration ranges detailed in table 1.

Spectrophotometric method with NQS or NBD-Cl reagents:

1mL of standard solutions of ATV and BZF (over the concentration ranges shown in Table 1) wear transferred into separate 10 mL calibrated flasks. 1 mL of borate buffer solution of pH 10.0 for ATV and 1.5 mL of pH 9.5 for BZF was added followed by 1mL of NQS solution, 1mL of borate buffer solution of pH 9.0 for ATV and 1.5 mL of pH 10.0 for BZF was added followed by 1mL of NBD-Cl solution. The solution (for reactions with NQS) was allowed to proceed for 15 min at room temperature 25 ºC ATV and was heated in a thermostatically controlled water bath at 50°C for 20 min for BZF; the mixture was diluted quantitatively with distilled water. The solution (for reactions with NBD-Cl) was heated in a thermostatically controlled water bath at 70ºC for a settled time of 20 min for ATV and at a similar temperature for a settled time of 15min for BZF; the mixture was quenched by cooling under tap water. Then, it was acidified by adding 0.1 mL of 4 M hydrochloric acid solution and completed to volume with methanol. The absorbance of the solutions were measured at 520 and 493 nm for ATV and BZF (for reactions with NQS), 480 and 485 nm for ATV and BZF (for reactions with NBD-Cl), respectively, against a reagent blank prepared in the same manner but no drugs.

Spectrofluorimetric method:

1 mL of standard solutions of ATV and BZF (over the concentration ranges shown in Table 1) was transferred into separate 10 mL calibrated flasks. 1mL of borate buffer solution of pH 8 and 9.5, respectively, was added followed by 1mL of 0.2% (w/v) NBD-Cl solution. The mixture was allowed to proceed in a thermostatically controlled water bath at 70 ºC for 20 min, and then cooled to room temperature 25ºC. After cooling, mixture was acidified by adding 1mL of 4M hydrochloric acid, and completed to volume with acetonitrile[51]. The relative fluorescence intensity (RFI) of the resulting solution was measured at λ_{ex} = 480 nm, λ_{em} = 520 nm for ATV and at λ_{ex} = 480 nm, λ_{em} = 510 nm for BZF against a reagent blank prepared in the same manner with 1mL water instead of 1mL sample solution [52].

Procedure for the tablets:

The contents of 10 tablets were emptied and well mixed. An accurately weighed amount equivalent to 50 mg was transferred into 50 mL calibrated flask and dissolved in about 20 mL in methanol. The contents of flask was swirled, sonicated for 5 minutes, and then completed to volume with methanol [53]. It were mixed good and filtered; the first portion of the filtrate was rejected, and solution was diluted quantitatively with distilled methanol (for reactions with NBD-Cl) or water (for reactions with NQS) to obtain suitable concentration for the analysis by the spectrophotometric and spectrofluorimetric methods.

Stoichiometric ratio:

The stoichiometric ratio of the formed products was scrupulous by Job's method[54]. Equimolar stock solutions of each drug, NQS, and NBD-Cl were prepared in previously specified solvents. The drug and both reagents were prepared as 3.47×10^{-3} and 6.9×10^{-3} M for ATV and BZF, respectively. Series of 5 mL portions of the stock solutions of the drug and the reagent were made up comprising different complementary proportions in 10 mL standardize flasks containing 1mL of convenient buffer[41]. The solutions were moreover manipulated as described under generally recommended procedures described above.

RESULTS AND DISCUSSION:

Absorption and fluorescence spectra:

The absorption concerning ATV or BZF was separately recorded in opposition to water (Fig 2 and 3). It was found that the maximum absorption peaks (λ_{max}) of both drugs were 270 and 235 nm and their molar absorptivity ε were 1.61×10^3 and 0.96×10^3 L/mol. cm for ATV 1.11×10^3 and 0.353×10^3 L/ mol.

cm for BZF, respectively. . The reaction between the two medications and NQS or NBD-Cl was performed, and absorption spectra of products were record against reagents blank. For the reaction with NQS, the products were red-colored exhibiting λ_{max} at 520 and 493 nm for ATV and BZF, respectively (Fig. 2 and 3), with regard to reaction with NBD-Cl, the products were orange-colored exhibiting λ_{max} at 480 and 485 nm for ATV and BZF, respectively (Fig 4 and 5). As well, the sensitivity (ε) was greatly enhanced to be $2.17x10^3$ and $1.14x\ 10^3$ L/ mol. cm for ATV; and $1.78x\ 10^3$ and $1.34x\ 10^3$ L/ mol. cm for BZF using NQS and NBD-Cl reagents, respectively (Table 1). Therefore, the spectrophotometric measurements were carried out at 520 and 493 nm for ATV; and 480 and 485 nm for BZF utilizing NQS and NBD-Cl, respectively.

ATV and BZF don't have a native fluorescence; accordingly, their derivatization with NBD-Cl reagent was vital for their Spectrofluorimetric determination, because NBD-Cl frames highly fluorescent subordinate with secondary amines utilizing moderately mellow reaction status[46]. Owing to the presence of labile chloride in the chemical structure of NBD-Cl, a daily fresh solution was prepared and tested in the present study, It was found that ATV and BZF reacts with NBD-Cl and forms yellow-colored fluorescent derivative. The derivatives exhibited maximum fluorescence intensity at λ_{em} of470 nm and 480 nm after their excitation at λ_{ex} of 520 and 510 nm for ATV and BZF, respectively, show in figures. 6 and 7.

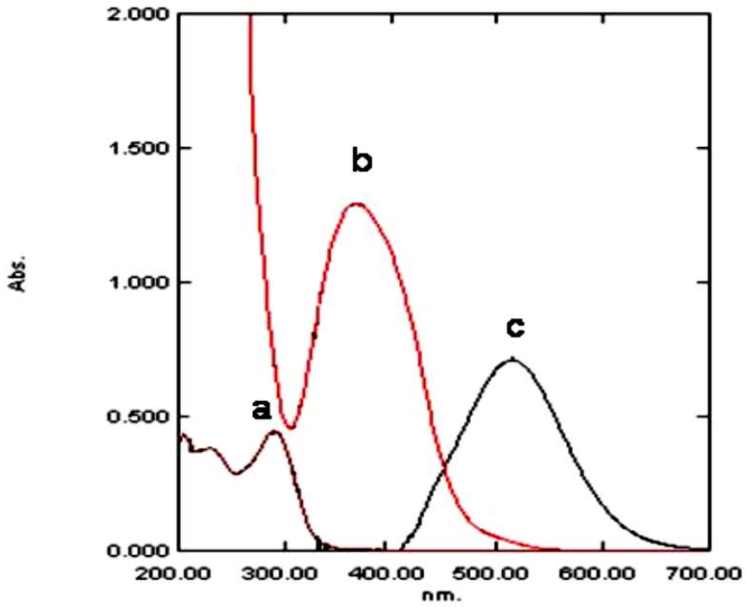


Fig 2: Absorption spectrum of 2µg/mL ATV against water, b- absorption spectrum of NQS against water, c- absorption spectrum of reaction of ATV with NQS.

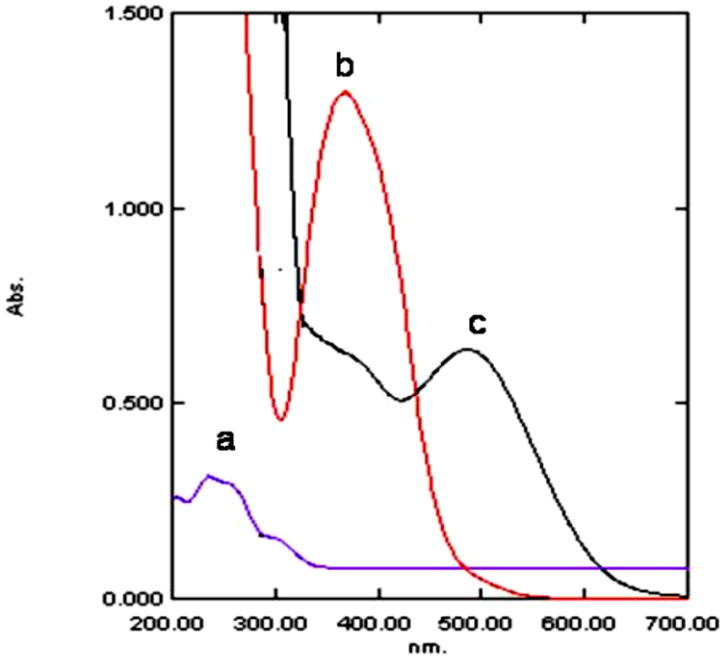


Fig 3: Absorption spectrum of 3µg/mL BZF against water, b-absorption spectrum of NQS against water, c-absorption spectrum of reaction of BZF with NQS.

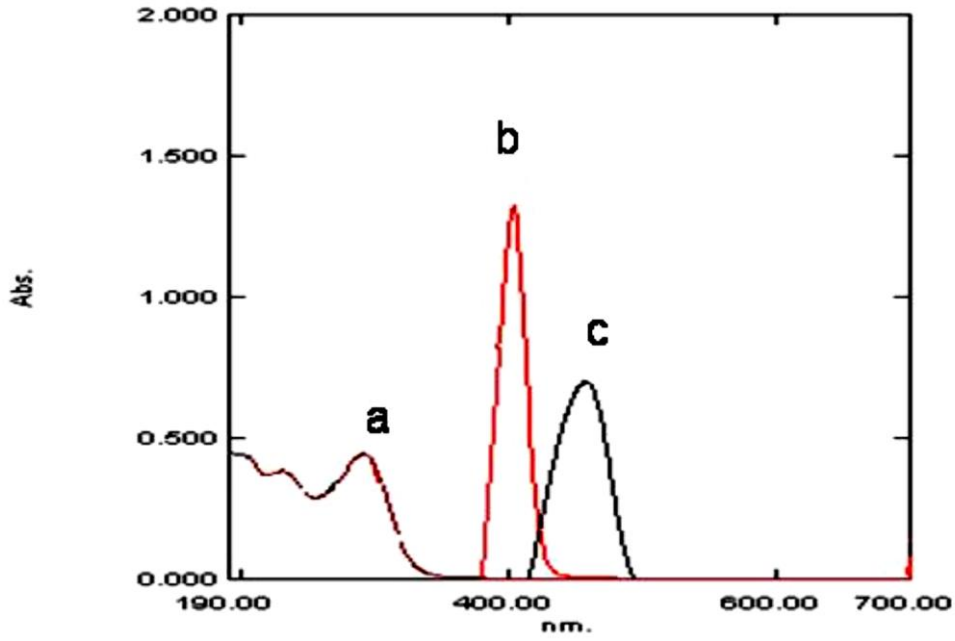


Fig 4: Absorption spectrum of 2µg/mL ATV against water, b- absorption spectrum of NBC-Cl against water, c-absorption spectrum of reaction of ATV with NBC-Cl.

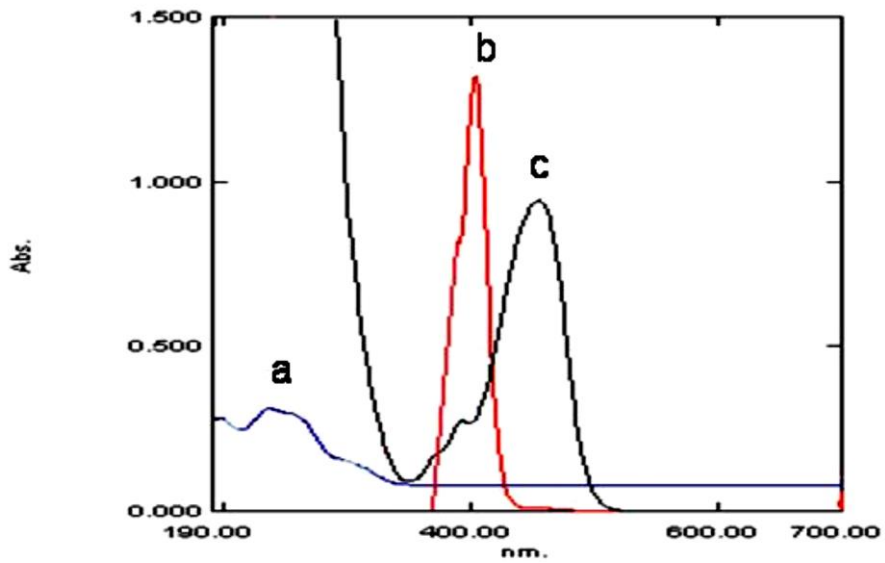


Fig 5: Absorption spectrum of 3 µg/mL BZF against water, b- absorption spectrum of NBC-Cl against water, c- absorption spectrum of reaction of BZF with NBC-Cl.

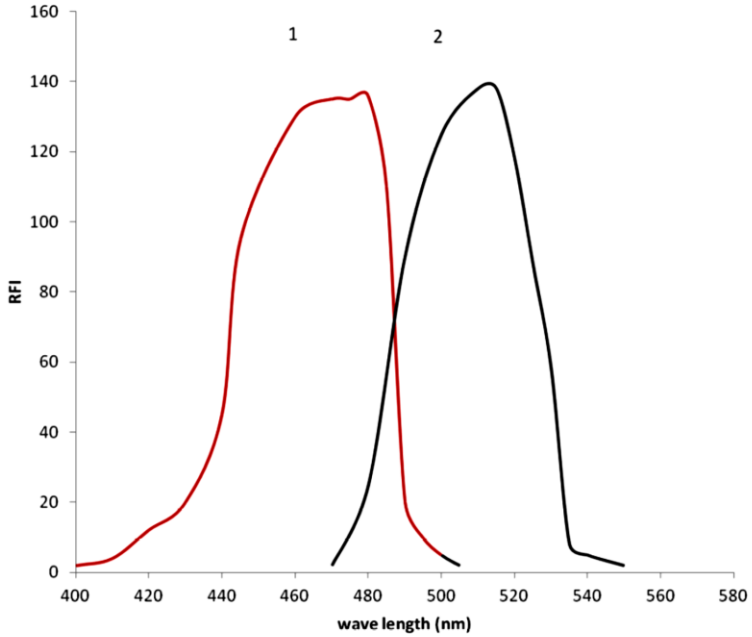


Fig 6: 1-Excitation and 2- Emission spectra of 0.1 µg/mL ATV and it is reaction product with NBC-Cl against reagent blank

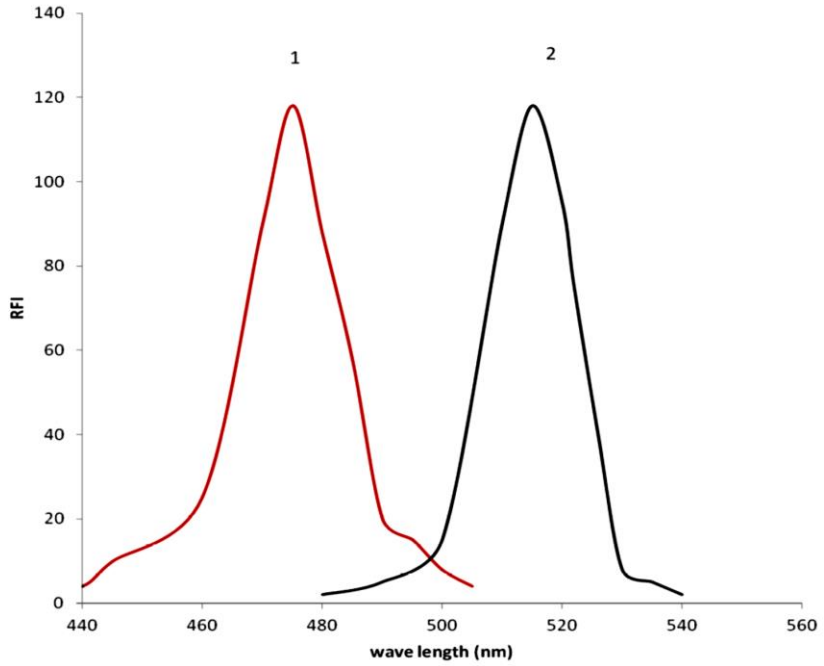


Fig 7: 1-Excitation and 2- Emission spectra of 1µg/mL BZF and it is reaction product with NBC-Cl against reagent blank

Reaction Variables:

Diversified exploratory parameters influencing the shading improvement and its dependability were cautiously examined and upgraded. These factors were changed separately while keeping others consistent. These variables incorporate pH, reagent fixation, time, temperature and weakening dissolvable.

Effect of Reagent.

The observed of the effect of NQS and NBD-Cl concentrations on their reactions with ATV or BZF, that the reactions were depending on the reagent concentration because the readings accelerated with the increase within the reagent concentration (Fig 8). The highest readings had been attained at ranges of 0.1- 1% (w/v) for NQS and NBD-Cl, respectively. For high values in addition experiments were carried out using 0.5% and 0.2% for NQS and NBD-Cl, respectively, show in table 1.

Table 1: Summary of optimization variables affecting the reaction of ATV and BZF with NQS and NBD-Cl reagents employed in the methods proposed.

Parameter	Used range	Spectrophotometric method				Spectrofluorimetric method	
		NQS		NBC-Cl		NBC-Cl	
		ATV	BZF	ATV	BZF	ATV	BZF
pH of borate buffer	7 – 12	10	9.5	9	10	8	9.5
Volume of borate buffer mL	0.5-3	1	1.5	1	1.5	1	1.5
NQS/NBD-Cl (%w/v)	0.2-1.5	1mL 0.5%	1mL 0.5%	1mL 0.2 %	1mL 0.2 %	1mL 0.2 %	1mL 0.2 %
Temperature (°C)	20 - 90	25	50	70	70	70	70
Reaction time (min)	2 - 40	15	20	20	15	20	20
Diluting solvent	Different *	Water	Water	Water	Water	Methanol	Methanol
Measuring wavelength λmax (nm)	200 -700	520	493	480	485	470 (λex), 520 (λem)	480 (λex), 510(λem)

*Solvents tested: water, methanol, 1,4-dioxane, acetonitrile, isopropanol, ethanol, acetone ,and dimethylsulphoxid.

Effect of pH:

The effect of pH at reaction of ATV or BZF with both NQS and NBD-Cl investigated by using out the reaction in a buffer solution of varying pH values. The outcomes found out that ATV or BZF has difficulty to react with both NQS and NBD-Cl in acidic media (Fig 9). This was potentially because of presence of the amino group of ATV or BZF as hydrochloride salt, for this reaction, it loses its nucleophilic substitution functionality. As the pH increased the readings increased swiftly, as the amino group of ATV or BZF (in the hydrochloride salt) transforms into the free amino group, therefore facilitating the nucleophilic substitution[44]. The pH was changed over the pH range of 7 to12 using 0.2M borate buffer. At increasing the pH, higher absorbance were acquired with maximum absorbance at pH values 10, 9 and 8 for ATV; and 9.5,10 and 9.5 for BZF using NQS and NBD-Cl, spectrophotometric and spectrofluorimetric, respectively, show in table1. At higher pH values, a sharp decrease in the readings befell. This into attributed possibly to the increase in concentration of hydroxide ion that holds back reaction of the investigated drugs with both reagents[52]. At pH 2 to 6 was found to less absorption different supports having a similar pH values, for example, phosphate and citric acid-phosphate (Mcllvaine's buffer) were tried. Borate support was observed to be unrivaled because it brought about increasingly stable exceedingly shaded arrangements.

Effect of temperature and reaction time:

The effect of temperature on reaction was thought about through finishing the reaction at different temperatures at rang 20 to 90 ^{o}C with steady warming time. It was found that reaction of ATV or BZF with NQS wear superfluously affected by raising the temperature. The greatest absorbance perusing wear accomplished in 15 min at 25 ^{o}C for ATV but longer reaction time up to 30 min didn't affect the reaction, and the reaction of BZF with NQS at room temperature was observed to be slow. Increasing temperature of the water bath produced an increase in the product absorbance up to 40 ^{o}C, therefore maximum absorbance reading was attained in 10 min at 40 ^{o}C. It was found that heating the reaction mixture of TAV with NQS in the range of 40 to 50 ^{o}C had a slight positive effect on the reaction. Reaction of ATV and BZF with NBD-Cl has not been finished at 25 ^{o}C for no under than 1h, increasing the temperature of the water bath up to 70 ^{o}C produced an increase in the absorbance of the reaction product. However, heating for longer periods up to 40 min had a negligible effect on the obtained readings. Therefore, further experiments of ATV and BZF with NBD-Cl reagent had been accomplished at 70 ^{o}C for 20 and 15 min, respectively, show in figures 10 and 11. The outcomes showed that the reaction was reliant on temperature, and the ideal conditions were accomplished by warming the response at 70 ^{o}C for 20 min.

Effect of solvent:

Diverse solvents, such as water, isopropanol, 1,4-dioxane, methanol, acetone, ethanol, and acetonitrile had been tested as capacity diluting media. Water was found to be the most efficient solvent for each tablet, as the best absorbance values have been obtained, for NQS reactions. For NBD-Cl reactions, the highest readings have been obtained while methanol was used for dilution. Significantly high fluorescence background was observed with NBD-Cl, this was attributed to the hydrolysis of NBD-Cl to the corresponding hydroxyl derivative, namely, 4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH)[52]. The fluorescence of NBD-OH changed into to be quenched via reducing the pH of the response medium to less than two the reaction product was not affected hence the sensitivity more was enhanced[55]. This step was accomplished with of the addition of one mL of 4M hydrochloric acid solution to the reaction mixtures of both drugs [41].

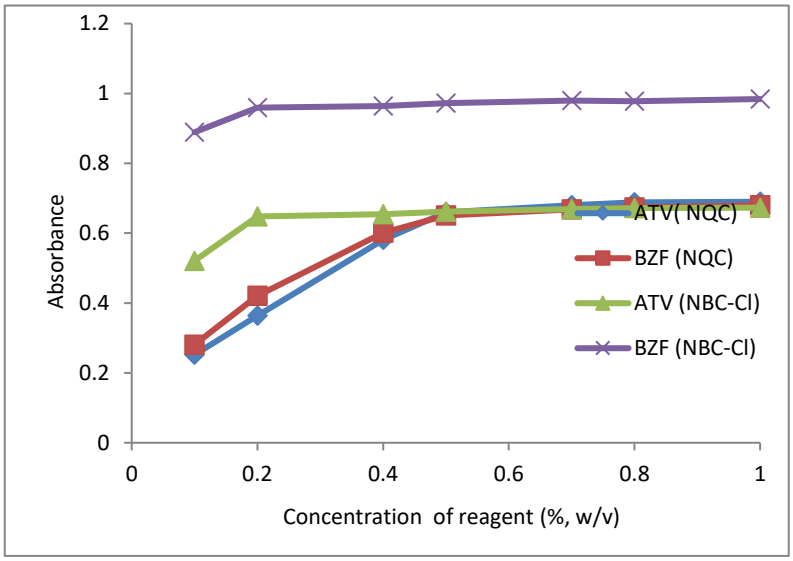


Fig 8: Effect of reagent concentrations NQS and NBD-Cl on their reactions with ATV and BZF.

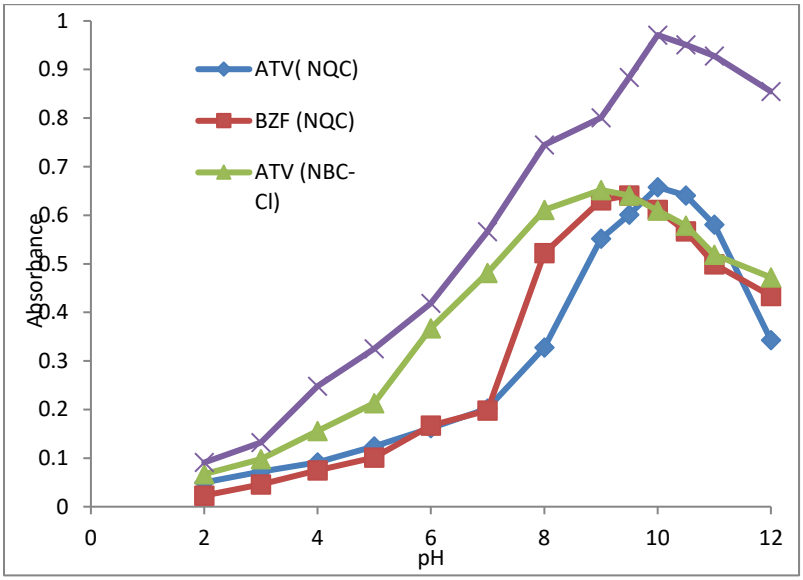


Fig 9: Effect of pH on the reaction of ATV and BZF with NQS and NBD-Cl, respectively.

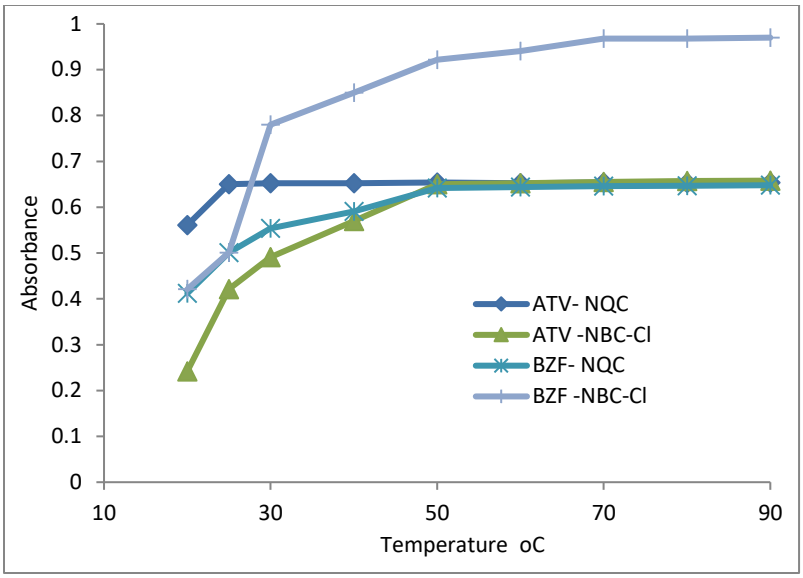


Fig 10: Effect of temperature on the reaction of ATV and BZF with NQS and NBD-Cl.

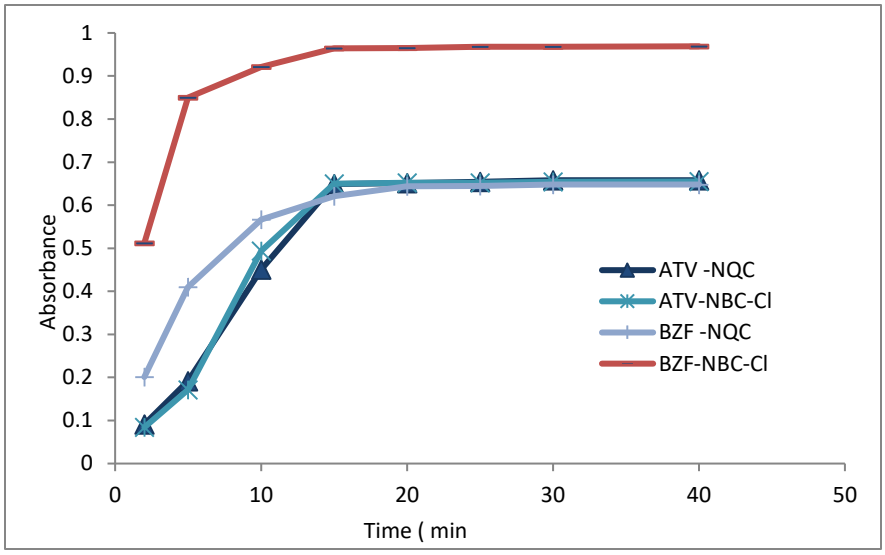


Fig 11: Effect of Time on the reaction of ATV and BZF with NQS and NBD-Cl.

Stability of the reaction:

After dilution the reaction solutions, it was observed that absorbance of the chromophores for ATV-NQS, BZF-NQS, ATV-NBD-Cl and BZF-NBD-Cl and the fluorophores for ATV-NBD-Cl and BZF-NBD-Cl. These complexes are stable for at the least 4h at room temperature and at least for 48h while stored at 4°C within the darkish, this allowed the processing of massive batches of samples, as well as making it applicable to a large number of samples.

The stoichiometry of the reactions:

Under the optimum conditions, the stoichiometry of the reactions among ATV and BZF was investigated by Job's method[47]. The symmetrical state shape of Job's plot (not appeared) indicated that the medication: reagent proportion was 1:1 for the two medications utilizing the two reagents show in figure 12. This ratio was normal as the examined medications contain just only one center (a secondary amino group in ATV and BZF) accessible for the reactions. In view of this ratio, the reactions pathways were proposed to continue as appeared in scheme 1 and 2.

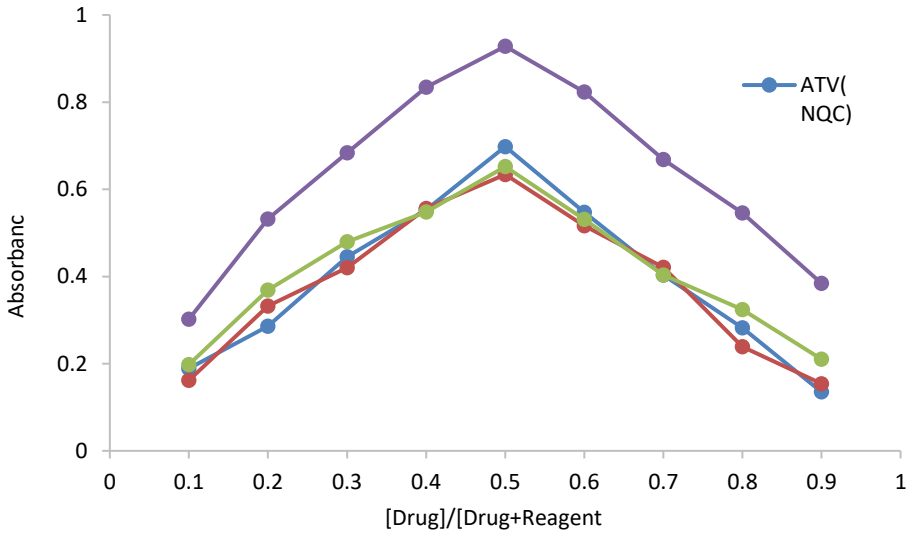
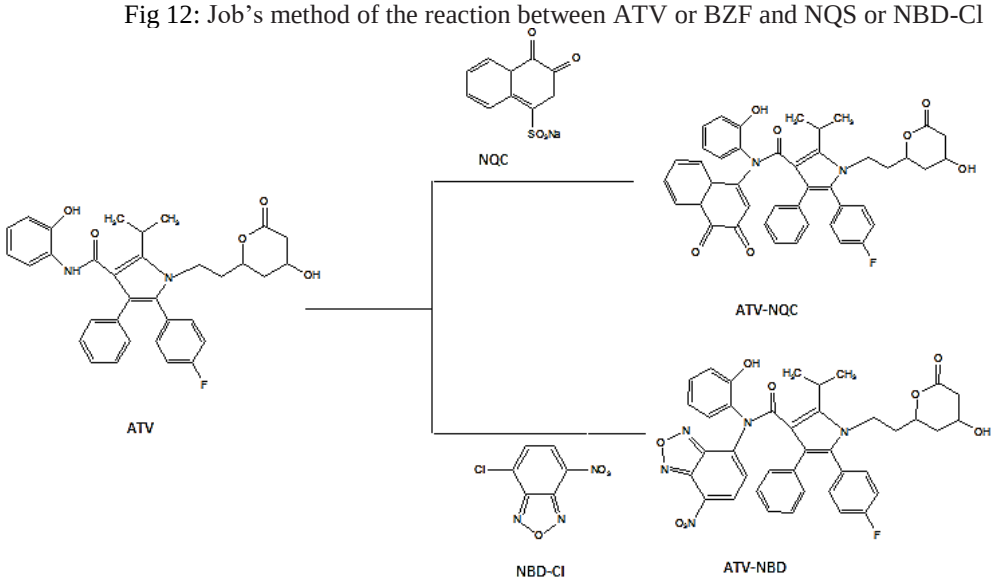
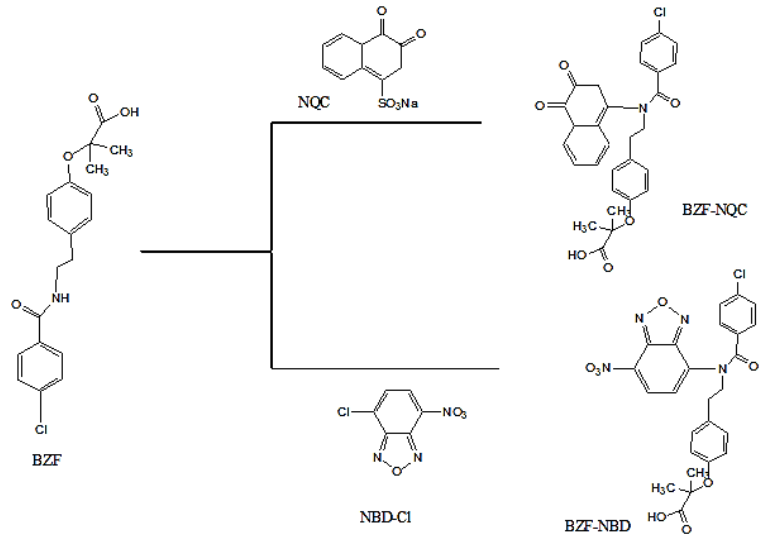


Fig 12: Job's method of the reaction between ATV or BZF and NQS or NBD-Cl



Scheme 1: Stoichiometric ratio of ATV with NQS and NBD-Cl.



Scheme 2: Stoichiometric ratio of BZF with NQS and NBD-Cl.

Validation of methods:

The suggested analytical technique used to was validated in by ICH guidelines[56], regarding certain parameters for example linearity, limit of identification (LOD), limit of quantitation (LOQ), precision, accuracy, specificity, and solidness.

Linearity:

A linear relationship between the responses of ATV and BZF and their concentrations was obtained. The regression equation y = bC ± a for each drug was also computed[51]. In this study, was used at least five concentrations for ATV or BZF. Linearity of the calibration curves used to be validated by the values correlation coefficients (r) on the regression equation, tiny values of the honor deviation regarding residuals (Sy/x), and a small value of the percentage relative error in table 2. The good linearity the calibration graphs the negligible scatter of the empirical points are clear by means of the high values on the correlation coefficients and standard deviations.

Table 2 The parameters for the proposed methods for the determination of ATV and BZF.

Parameter	Spectrophotometric method				Spectrofluorimetric method	
	NQS		NBC-Cl		NBC-Cl	
	ATV	BZF	ATV	BZF	ATV	BZF
Linear range (µg/mL)	0.3 – 6	0.5 - 9	0.5 – 5	1 - 10	0.05 – 0.5	0.5 – 4
Intercept	0.0006	0.0386	0.0153	0.0011	2.0176	-0.5750
Sa[a]	0.0038	0.0022	0.0023	0.0046	1.9249	0.3970
Slope	0.1356	0.0940	0.0623	0.0633	0.5793	0.8180
Sb[b]	0.0014	0.0004	0.0010	0.0005	0.0084	0.0505
Sy/x[c]	0.0043	0.0023	0.0104	0.0006	0.0018	0.3970
Correlation coefficient	0.9997	0.9999	0.9992	0.9999	0.9992	0.9999
LOD (µg/mL)[d]	0.11	0.07	0.12	0.24	0.011	0.0794
LOQ (µg/mL)[e]	0.34	0.23	0.353	0.73	0.033	0.2410

[a] Sa is the standard deviation of the intercept. [b]Sb is the standard deviation of the slop. [c]Sy/x is the standard deviation of the residual. [d]LOD is the limit of detection. [e]LOQ is the limit of quantification.

Limit of detection (LOD) and limit of quantification (LOQ):
In accordance with the guidelines of ICH[56], the limit of detection LOD =3.3 σ/s and limit of quantification LOQ =10 σ/s, where σ is the standard deviation of replicate determinations of the blank and s is the sensitivity [57]. The LOD and LOQ of the proposed techniques were exhibited in table.2. Clearly, the LOD and LOQ values are lower for the spectrofluorimetric and spectrophotometric methods because of the sensitivity of these methods.

Accuracy and precision:
The recovery percentage results for the assay of ATV and BZF in their laboratory prepared mixtures to indicate and accuracy of the technique, repeatability (intra-day) and inter-mediate precision (inter-day) were assessed using 3 concentrations and 5 replicates of each drug. The recovery %, RSD % and RF% as shown in table 3, and the values indicate the good accuracy and precision of methods.

Specificity:
Specificity of the methods was evaluated by assay the interference liabilities from the common excipients that may be including amidst during drugs. Samples were prepared by through blending known amount (20 mg ATV/50 mg BZF) with numerous quantities of the common excipients: glucose, starch, acacia, lactose, magnesium stearate and talc. These laboratory-prepared samples had been analyzed by the proposed methods applying the normally endorsed manner. The recoveries was good of both drugs ranging from 98.82±0.62 to 101.97± 1.24 and 98.07± 1.42 to 101.11±1.07 for the spectrophotometric and Spectrofluorimetric techniques, respectively, show in table 4. These data certain the absence of interference from any of common excipients with determination of ATV or BZF by both methods.

Table 3: Accuracy and precision of the proposed methods for the determination of ATV and BZF.

Method	Drug	Amount taken (µg/mL)	Intra-days (n=5)			Inter-days (n=5)		
			Recovery (mean% ± SD)*	RSD%	RF%	Recovery (mean% ± SD)*	RSD%	RF%
NQC Spectrophotometric	ATV	0.3	100.46 ± 0.03	0.29	0.47	98.93 ± 0.18	1.20	-1.05
		2	101.00 ± 0.01	0.01	0.98	100.13 ± 0.33	1.12	0.14
		5	100.94 ± 0.31	0.31	0.94	99.12 ± 0.53	0.97	-0.87
	BZF	0.5	99.00 ± 0.32	0.32	-1.01	100.10 ± 0.30	0.30	0.46
		5	99.30 ± 0.60	0.60	-0.70	100.10± 0.2	0.19	0.10
		9	100.50 ± 50	0.49	0.50	101.40 ± 0.31	0.31	1.05
NBC-Cl Spectrophotometric	ATV	0.5	101.01 ± 0.11	0.11	1.00	99.60 ± 0.61	0.61	-0.40
		2	99.10 ± 0.32	0.32	-.090	101.05 ± 0.35	0.34	1.01
		5	99.59 ± 0.22	0.22	-0.51	100.50 ± 0.32	0.32	0.50
	BZF	1	100.50 ± 0.49	0.49	0.50	99.01 ± 0.50	0.51	-1.00
		5	98.33 ± 0.81	0.82	-1.70	99.67 ± 0.12	0.12	-0.20
		10	98.00 ± 0.72	0.73	-2.00	99.10 ± 0.39	0.39	-1.00
NBC-Cl Spectrofluorimetric	ATV	0.05	100.01 ± 0.24	0.75	0.94	100.10 ± 0.34	1.13	0.57
		0.1	99.90 ± 0.61	0.61	-0.44	100.08 ± 0.70	0.76	1.12
		0.5	100.24 ± 0.43	0.24	0.67	100.20 ± 0.43	0.24	0.14
	BZF	0.5	100.33 ± 0.17	0.17	0.33	98.67± 0.12	0.12	-1.30
		2	102.10 ± 0.15	0.15	-0.02	99.97 ± 0.35	0.35	-0.03
		4	100.87 ± 0.22	0.20	-0.86	99.96 ± 0.43	0.43	-0.04

*Each result is the mean of 5 experiments.

Ruggedness:
Ruggedness was also experienced by applying methods to investigate of ATV and BZF using the same operational conditions yet utilizing two various instruments a two different laboratories and different elapsed time. Outcomes obtained from lab-to-lab and day-to-day differences have been reproducible, as % RSD didn't exceed 3%, this provided an clarification of reliability of the proposed methods throughout they routine application for analysis of ATV and BZF.

Table 4: Analysis of ATV and BZF in presence of common excipients by the proposed methods.

Excipient	Recovery %± SD * Spectrophotometric method				Recovery %±SD * Spectrofluorimetric method	
	NQS		NBC-Cl		NBC-Cl	
	ATV	BZF	ATV	BZF	ATV	BZF
Starch (50)**	100.21 ± 1.02	99.61 ± 0.80	101.61 ± 0.92	99.58± 0.83	101.07 ±1.24	100.42 ± 1.31
Glucose (10)	99.84± 0.52	100.10 ± 1.00	100.09 ± 1.07	99.04 ± 0.19	98.74±1.16	99.25 ±0.95
Lactose (10)	98.91 ± 1.18	99.96± 0.57	99.96 ± 0.57	100.78 ± 0.95	99.62± 1.92	98.73 ±0.62
Acacia (10)	101.47 ± 0.43	101.28 ± 0.62	101.28 ± 0.62	101.97 ± 1.24	98.70 ± 1.46	98.07 ±1.42
Talc (5)	100.37 ± 0.67	98.99 ± 1.29	98.99 ± 1.29	98.82± 0.62	99.48 ± 1.58	101.11 ±1.07
Magnesium stearate (10)	99.07 ± 0.71	99.39 ± 0.79	99.39 ± 0.76	99.45± 0.73	97.09 ± 1.34	99.58 ±0.79

*Values are mean of 3 determinations. **Figures in parenthesis are the amounts in mg added to 20 mg and 50 mg of ATV or BZF, respectively.

Robustness:

Robustness was examined by evaluating influence of small variation in the methods variables on its analytical performance of the proposed methods. In these analyses one parameter was changed through the others were kept remaining the same, and % recovery was determined each time[53]. The studied parameters were NQS or NBD-Cl concentration, heating temperature, buffer pH, time on the method sensitivity and suitability. It turned into discovered that small variant inside the techniques variables did no longer considerably affect the tactics; recovery values were 97.67–101.13 ± 0.95–1.81 %. Variation of heating temperature by ±7 ºC, buffer pH by ±0.3 pH units, the reagent volume by ±0.1 mL, reaction time by ±8 min, and measurement wavelength by ±5 nm did not significantly affect the developed methods. This provided an evidence for the reliability of the proposed methods in the course of their habitual application for evaluation of ATV and BZF.

Pharmaceutical applications:

The proposed methods were successfully applied to the determination of ATV and BZF in two brands of tablets and the results were gathered in Table 5. The results were statistically compared to those obtained by the reference methods[23, 27, 38, 39] with respect to accuracy t-test and precision F-test. The values of calculated t and F at 95% confidence level were less than the tabulated ones. This an evidences that there are no significant differences between the proposed methods and the reference method with respect to accuracy and precision[58].

Table. 5: Statistical evaluation of results by the proposed methods for the determination of ATV and BZF in tablets

Drug	Statistical parameter	Spectrophotometric method		Spectrofluorimetric method	Reference method [23, 27, 38, 39]
		NQC	NBC-Cl	NBC-Cl	
ATV	Ator tablets 10 mg % Recovery ± SD[a]	99.90 ± 0.54	99.85 ± 1.24	99.42 ± 1.50	100.36 ± 0.67
	T-test[b]	0.76	2.13	2.47	
	F-test[c]	1.73	2.35	1.49	
	Lipigard tablets 20 mg % Recovery ± SD[a]	100.01 ± 0.49	100.04 ± 0.45	99.29 ± 0.56	99.06 ± 0.51
	T-test[b]	0.42	1.87	1.10	
	F-test[c]	1.43	2.20	3.47	
BZF	Bezalip@ Retard tablets 400 mg	99.91 ± 1.40	100.22 ± 0.41	100.10 ± 1.31	100.13 ± 0.41
	% Recovery ± SD[a]				
	T-test[b]	1.17	0.73	1.12	
	F-test[c]	1.68	1.53	0.19	
	Bezafibrate tablets 200 mg	100.17 ± 1.31	99.80 ± 1.30	99.80 ± 0.69	98.77 ± 0.68
	% Recovery ± SD[a]				
	T-test[b]	1.27	1.10	1.92	
	F-test[c]	2.62	1.01	0.35	

[a] Mean value of 5 determinations. [b] Tabulated at t-value at the 95% confidence level is 2.78.
[c] Tabulated at F-value at the 95% confidence level is 6.39.

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CONCLUSIONS:

Present spectrophotometric and spectrofluorimetric methods describe evaluation of NQS and NBD-Cl as analytical reagents in the development of simple, sensitive, and accurate methods for determination of ATV and BZF in the tablets dosage form. The reagents used within the proposed methods are cheap and without difficulty to be had and the procedures do not involve any critical reaction conditions or tedious sample preparation. Proposed methods have comparable analytical performances and without any capability interference from the regularly encountered excipients and additives. The good analytical performance regarding validation parameters was achieved and all the validated data attained are in agreement with the ICH guidelines. Statistical analysis proves that the proposed methods could be applied for the analysis of ATV and BZF in their pure forms and in the tablets dosage forms. Therefore, these methods can be recommended for the routine analysis of ATV and BZF in quality control laboratories.

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