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Novel analytical method development for some amide group containing drugs using *Bougainvillea spectabilis* bract extracts

Suresh Killedar[1*], Anuja Pawar[2], Sameer Nadaf[3], Ashwini Nale[2], Umarfarukh Tamboli[3], Sachin Pishawikar[4]

[1]*Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur–416013, India [M.S.]*

[2]*Department of Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Kolhapur–416013, India [M.S.]*

[3]*Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur–416013, India [M.S.]*

[4]*Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur–416013, India [M.S.]*

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ABSTRACT

Objective: To develop and validate a simple, accurate and precise colorimetric method using *Bougainvillea spectabilis* (*B. spectabilis*) bract color previously not exploited for estimation of amide group containing drugs *i.e.* lidocaine and ranolazine in pharmaceutical formulations.
Methods: Methanolic extract of *B. spectabilis* was prepared and evaluated for stability of its color at different pH and temperature for a period of 3 weeks. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision, limit of detection, limit of quantitation and specificity according to International Conference on Harmonization guidelines. About 0.5% of *B. spectabilis* bract color was added to the working standard solutions of the drugs separately and after formation of color complex, and absorbances were noted at 418 nm.
Results: For color complexes of lidocaine and ranolazine, linearity was found to be in the range of 4 to 24 and 5 to 25 μg/mL respectively. The % relative standard deviation was found to be within specification limits. Presence of lone pair of electron on nitrogen of amide group of both drugs shows basic nature, contributed in formation of color complex between amide and the color pigment obtained from *B. spectabilis* bracts.
Conclusions: It can be concluded that the method is simple, accurate, economic, and rapid hence can be employed for routine analysis.

1. Introduction

Colorimetry is concerned about the measurement of the intensity of electromagnetic radiation in the visible spectrum transmitted through a solution or transparent solid. Passing of electromagnetic radiation through object or solution leads to absorption of certain wavelength, leaving unabsorbed wavelength to be transmitted or reflected. This observed as color which is complementary to absorbed color[1].

Bougainvillea spectabilis (*B. spectabilis*) belonging to Nyctaginaceae family is a popular ornamental plant, commonly known as "paper flower" due to the bracts are thin and papery. *B. spectabilis* is used in several countries to prepare extracts with antibacterial activity[2–4]. Its aqueous and methanolic extracts show good oral glucose tolerance and significantly reduce the intestinal glucosidase activity. Study on leaves revealed its potential as anti-inflammatory, anti-diabetic, anti-fertility, antiviral, antibacterial and so on[5–11].

Lidocaine [2-(diethylamino)-N-(2,6-dimethylphenyl) acetamide] is a first amino amide-type local anaesthetic, white or slightly yellowish, crystalline powder, having molecular formula $C_{14}H_{22}N_2O$ and molecular weight 234.34 g/mol (Figure 1). Lidocaine is used topically to relieve itching, burning and pain from skin inflammations, injected as a dental anesthetic or as a local anesthetic for minor surgery. It acts by blocking the fast voltage gated sodium (Na^+) channels

*Corresponding author: Dr. Suresh Killedar, Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur-416013, Maharashtra State, India.
Tel: 91-231-2637286
Fax: 91-231-2638833
E-mail: sureshgk64_rediffmail.com

in the neuronal cell membranes that are responsible for signal propagation and stabilizes the membrane[12,13].

Ranolazine [(RS)-N-(2,6-dimethylphenyl)-2-[4-[2-hydroxy-3-(2-methoxyphenoxy)-propyl] piperazin-1-yl] acetamide] is an antianginal medication, white or slightly yellow, crystalline powder having molecular formula $C_{24}H_{33}N_3O_4$ and molecular weight 427.537 g/mol (Figure 1). Ranolazine affects the sodium-dependent calcium channels during myocardial ischemia in rabbits and alters the transcellular late sodium current. It is useful in the treatment of neuropathic pain[14,15].

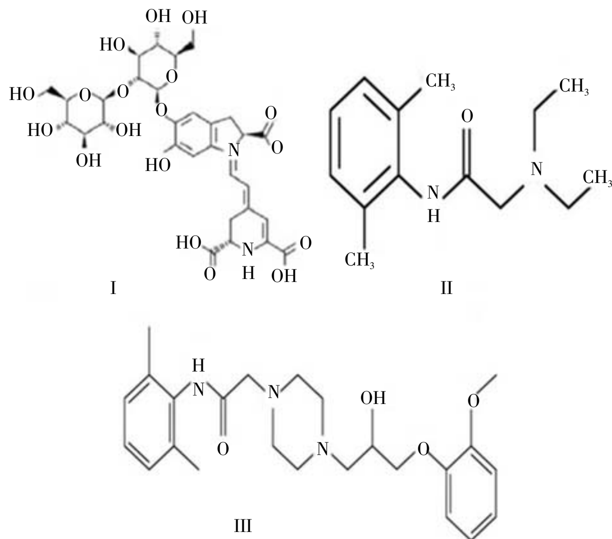

Figure 1. Structure of bougainvillein-V (I), lidocaine (II) and ranolazine (III).

Literature survey reveals that previously work has been carried out on estimation of lidocaine either single or in combination using UV spectrophotometer[16-18]. Similar type of work has also been reported for estimation of ranolazine in bulk and tablet dosage form[19,20]. Colorimetric method for estimation of both lidocaine and ranolazine using synthetic chemicals has been already reported[21,22]. However, no attempt has been previously made for colorimetric estimation of aforesaid drugs using a natural color pigment. So in present work, colorimetric method using extract of *B. spectabilis* bracts was developed for estimation of lidocaine and ranolazine based on principle that the amide group due to presence of lone pair of electron on nitrogen is going to show basic nature which contributes in formation of color complex between amide and the color pigment obtained from *B. spectabilis* bracts.

2. Materials and methods

2.1. Plant material

B. spectabilis bracts were collected from local area (NH4 highway between Kolhapur and Peth Vadgaon) during the flowering season in the month of April to July. The collected material was air dried and packed in airtight bags. Identification and authentication of plant was done with the help of herbarium sheet by Dr. M. Y. Cholekar-Bachulkar, Principal, Shri Vijaysinha Yadav Arts and Science College, Peth Vadgaon. Further, the herbarium sheet was submitted to the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur.

2.2. Chemical

Lidocaine and ranolazine were procured from pharmaceutical companies. Sodium hydroxide, hydrochloric acid, ascorbic acid, methanol and other chemical used were of analytical grade. Further dilutions were made as per the requirement.

2.3. Extraction

The extracts were obtained by immersing fresh bracts, dried bracts and dried bracts powder of *B. spectabilis* in 1% methanolic HCl for 24 h at room temperature. The extract was further concentrated under reduced pressure. To the concentrated solution, a mixture of diethyl ether and light petrol in 2:1 ratio was added when the colouring matter separates. The supernatant liquid was decanted after a couple of hours and lastly the deep blue-red coloured viscous residue was dried in a vacuum dessicator over anhydrous calcium chloride.

2.4. Spectrophotometric analysis of extract solution

A Jasco spectrophotometer (model: UV-630) was used to measure the λmax and absorbance of extracts of *B. spectabilis* bracts solutions at pH values of 3, 5 and 8. For this sample, 0.06 g each of various extracts of *B. spectabilis* bracts were dissolved in 10 mL of methanol and pH were adjusted using 0.1 mol/L HCl and NaOH. The λmax and absorbances of the solutions were measured using the spectrophotometer in the visible light spectra (400-800 nm) (Figure 2). Values of λmax and absorbance at different pH are reported in Table 1.

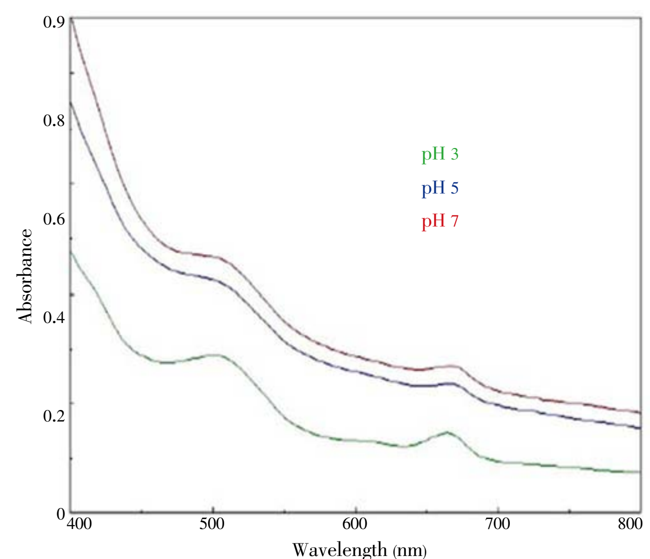

Figure 2. UV overlain spectra of *B. spectabilis* colour at different pH solution.

Table 1
Absorbance and λmax of 0.12% (w/v) solutions of *B. spectabilis* bracts colour at different pH values.

pH	λ (nm)	Absorbance
3	516.0±0.2	0.290±0.032
5	516.2±0.2	0.420±0.050
8	515.5±0.3	0.440±0.100

Values are expressed as mean±SD, n=3.

2.5. Stability study of *B. spectabilis* bracts colour

Reversibility of the colour change was also tested for this natural colour. The extract was subjected to various treatments including thermal treatment, pH, antioxidant additives and light exposure. Colour changes of the treated samples were monitored from Week 0 to 3 with a UV-visible spectrophotometer. The stability study of *B. spectabilis* bracts colour was carried out by considering the following factors.

2.5.1. Additional of antioxidant

Ascorbic acid of various concentrations, 0.1% (w/v), 0.5% (w/v) and 1.0% (w/v) was added into the extracts separately. Extracts along with ascorbic acid were then subjected to pH adjustment. Control was prepared without addition of ascorbic acid.

2.5.2. pH treatment

Samples added with different percentages of ascorbic acid were subjected to pH adjustment using 1 mol/L HCl or 1 mol/L NaOH. Each set of samples in section was adjusted to pH 3.0, pH 5.0 (original pH of *B. spectabilis* bracts) and pH 7.0 respectively. These samples were further treated at different temperature.

2.5.3. Heat treatment

Each set of the sample was finally kept at 4 °C, 25 °C in dark and 25 °C with light exposure.

2.5.4. Spectrophotometric analysis

Colour changes of the treated samples were monitored weekly at 516 nm with a UV-visible spectrophotometer from Week 0 to 3.

2.6. Colorimetric estimation of lidocaine and ranolazine formulation

2.6.1. Selection of drug

Phytochemical investigation of extract of *B. spectabilis* bract shows presence of betalain group of compounds which have polyphenolic nature similar to synthetic dyes like methyl orange, bromocresol green, *etc*. The synthetic dyes generally show reactivity with amide linkage whenever colorimetric method has to be developed. As lidocaine and ranolazine contain amide linkage, the selection of drug was done for colorimetric method development.

2.6.2. Preparation of standard stock solutions

2.6.2.1. Lidocaine

Standard stock solution of lidocaine was prepared by dissolving 50 mg of lidocaine in few mL of methanol by sonication and the final volume was made up to 100 mL using methanol to get a stock solution having concentration 500 μg/mL.

2.6.2.2. Ranolazine

Standard stock solution of ranolazine was prepared by dissolving 20 mg of ranolazine in few mL of methanol by sonication and the final volume was made up to 100 mL with methanol to get a stock solution having concentration 200 μg/mL.

2.6.3. Preparation of standard working solutions

2.6.3.1. Lidocaine

A volume of 0.4 mL of stock solution was pipetted out in 10 mL graduated volumetric flask. Volume was made up to 10 mL with methanol to give standard working solution of 20 μg/mL of lidocaine.

2.6.3.2. Ranolazine

A volume of 1 mL of stock solution was pipetted out in 10 mL graduated volumetric flask. Volume was made up to 10 mL with methanol to give standard working solution of 20 μg/mL of ranolazine.

2.6.4. Optimization of reagents and reaction condition

2.6.4.1. Lidocaine

Temperature of reaction, quantity, concentration and sequence of addition of reagents were optimized after several experimental trails. Using extract, solutions of different concentration like 0.5%, 1%, 1.5% and 2% were prepared in methanol. About 1 mL of standard working solution of lidocaine having concentration 20 μg/mL was pipetted out in different 10 mL graduated volumetric flasks. To this 1 mL of extract, solution having different concentrations was added. All the solutions were screened at identified λmax (418 nm). It was found that 0.5% solution of extract gives excellent color complex.

The optimization of volume of 0.5% solution was the next step. About 0.48 mL volume of 0.5% extract of *B. spectabilis* bract solution was found to be optimum for completion of reaction. The intensity of the colour formed, reached maximum in 10 min after mixing the reagent at room temperature and was stable up to 2 h.

2.6.4.2. Ranolazine

Using extract, solutions of different concentration like 0.5%, 1%, 1.5% and 2% were prepared in methanol. Stock solution of 1 000 μg/mL of ranolazine was prepared. From this, 0.1 mL was pipetted out in 10 mL volumetric flask. To this 1 mL of above extract, solution with different concentration was added.

Identification of λmax for developed complex was carried out, which was found to be 418 nm.

2.6.5. Procedure for plotting calibration curve

2.6.5.1. Lidocaine

Serial volumes of standard working solution were pipetted out into a series of 10 mL volumetric flasks. To each volumetric flask, 1 mL of 0.5% extract solution of *B. spectabilis* bract was added and the volume was then made up to 10 mL with methanol to get final concentrations of 4 to 24 μg/mL. Flasks were kept aside for 5 to 10 min for completion of formation of reaction complex in dark. The absorbance of the red colored complex was measured at 418 nm against reagent blank.

2.6.5.2. Ranolazine

Appropriate aliquots of standard drug solution were taken into a series of 10 mL volumetric flasks. To each volumetric flask, freshly prepared 0.5% solution of extract of *B. spectabilis* bracts was added and the volume was then made up to 10 mL with methanol to get final concentrations of 5 to 25 μg/mL and kept aside for 10 min for completion of formation of color complex. The absorbance of the pale yellow colored complex was measured at 418 nm against reagent blank.

2.6.6. Spectrophotometric analysis of drug-extract complexes

A Jasco spectrophotometer (model: UV-630) was used to measure the λmax and absorbance of lidocaine-extract color complex and ranolazine-extract color complex.

2.6.7. Fourier transform infrared spectroscopy (FTIR) study

Study of possible interaction between *B. spectabilis* extract and drug *i.e.* lidocaine and ranolazine has also been carried out using an infrared spectrophotometer (Jasco-V-730 model).

2.7. Method validation

2.7.1. Analysis of formulation

An accurately measured volume equivalent to 10 mg of lidocaine was dissolved in 10 mL of methanol by sonication. The solution was then filtered through Whatman filter paper No. 41. Appropriate aliquots within the Beer's law limit were analyzed by the proposed method using the procedure described earlier. The concentration of lidocaine present in the sample solution was calculated by using the formula:

Absorbances=A+B×C

Where, A=-0.00532, B=0.0003278 and C=concentration of lidocaine.

2.7.2. Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were conducted by adding different amounts (80%, 100% and 120%) of the pure drug to the pre-analysed formulation. The solutions were prepared in triplicates and the % recovery was calculated.

2.7.3. Precision

Precision studies were carried out to determine the reproducibility of the proposed method. Repeatability was determined by preparing nine replicates of three different concentrations of the sample and the absorbance was measured. For lidocaine sample, concentration was 4, 12 and 20 μg/mL whilst for ranolazine it was 5, 10 and 25 μg/mL. Intraday precision study was conducted by preparing drug solution of aforesaid concentration and analyzing it at three different times in a day. To determine interday precision, the same procedure was followed for three different days. Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample, it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

2.7.4. Specificity

Analytical techniques that can measure the analyte response in the presence of all potential sample components should be used for specificity validation.

2.7.5. Limit of detection (LOD)

LOD is the lowest amount of analyte in the sample that can be detected. LOD may also be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (S) at levels approximating the LOD according to the formula:

LOD=3.3(SD/S)

2.7.6. Limit of quantitation (LOQ)

LOQ is the least possible quantity of analyte in the sample that can be quantitatively determined by suitable precision and accuracy. The calculation method is again based on the standard deviation of the response (SD) and the slope of the calibration curve (S) according to the formula:

LOQ=10(SD/S)

2.7.7. Linearity

The linearity of the analytical method was its capability to bring forth test results which are directly proportional to analyte concentration in samples within a given range. It may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components. The response for the complex was strictly linear in the investigated concentration range.

3. Results

Extract was successfully isolated from *B. spectabilis*

Table 2

Effect of pH, temperature and antioxidant on the degradation of extract of *B. spectabilis* colour at 25 °C.

pH	Concentration of ascorbic acid (%)	25 °C (light)				25 °C (dark)			
		Weak 0	Weak 1	Weak 2	Weak 3	Weak 0	Weak 1	Weak 2	Weak 3
3	0.1	0.7±0.3	7.0±0.1	45.9±0.4	60.8±0.6	0.3±0.5	6.8±0.5	43.6±1.4	60.5±0.6
	0.5	0.7±0.5	8.5±1.2	40.4±0.2	68.2±0.4	0.2±0.4	6.6±1.4	38.7±0.7	56.7±1.3
	1.0	0.5±0.3	8.7±0.3	32.5±1.2	66.9±0.5	0.3±0.2	6.2±0.3	27.5±1.0	50.3±1.1
5	0.1	0.6±0.4	10.0±1.6	48.1±0.3	72.7±0.5	0.2±0.8	9.7±0.3	36.4±0.5	64.3±0.5
	0.5	0.4±0.5	10.5±0.5	39.3±0.5	70.3±1.5	0.4±0.6	10.0±0.4	30.5±0.8	61.4±0.8
	1.0	0.4±0.9	9.3±1.0	30.0±1.0	68.4±0.3	0.3±0.1	9.1±1.1	22.7±1.0	59.1±0.2
7	0.1	0.7±0.4	25.0±0.4	54.6±0.9	80.1±0.6	0.6±0.3	19.4±1.2	48.0±0.4	48.0±0.4
	0.5	0.6±0.3	22.4±2.5	43.3±0.6	74.6±0.8	0.3±0.4	19.0±0.9	36.2±0.7	65.8±0.9
	1.0	0.5±0.2	21.6±0.3	35.5±2.5	71.1±1.4	0.1±0.8	17.3±0.6	30.6±0.3	66.0±1.3

Data are expressed as mean±SD, n=3. Degradation (%)=(original concentration−determined concentration)×100.

bracts and percentage yield was found to be in the range of 18.3%–22.5% in methanol.

3.1. Stability study of B. spectabilis bracts colour

3.1.1. Additional antioxidant

Addition of 0.1% ascorbic acid to *B. spectabilis* bracts colour managed to preserve the colour in all dark storage conditions. Unfortunately, 0.1% ascorbic acid was not enough to stop colour degradation in sample exposed to light. Contrast to this, addition of 1.0% ascorbic acid managed to preserve the colour of the pigment, though the extract was exposed to light. Results are shown in Tables 2 and 3.

3.1.2. pH treatment

Sample at pH 7, stored in the dark at 4 °C showed approximately 25% degradation from the initial reading after 3 weeks. In contrast, sample at pH 3 managed to maintain 80% of the colour at the end of Week 3. The results indicated that betacyanin seems to be in favor of acidic pH region (Tables 2 and 3).

Table 3

Effect of pH, temperature and antioxidant on the degradation of extract of *B. spectabilis* colour at 4 °C.

pH	Concentration of ascorbic acid (%)	4 °C (dark)			
		Week 0	Week 1	Week 2	Week 3
3	0.1	0.3±0.1	11.0±1.2	16.3±0.6	20.2±0.8
	0.5	0.2±0.3	10.9±1.5	14.7±0.3	19.6±1.1
	1.0	0.3±0.2	9.5±1.4	15.8±0.2	19.2±2.3
5	0.1	0.4±0.9	12.0±0.5	17.1±0.7	22.1±1.4
	0.5	0.5±0.4	11.8±0.6	16.1±1.0	21.6±0.6
	1.0	0.4±0.2	10.3±0.3	16.5±1.0	21.1±0.4
7	0.1	0.6±0.4	13.5±0.5	19.8±0.4	25.0±0.2
	0.5	0.5±0.6	13.2±0.4	19.3±0.6	24.7±0.6
	1.0	0.4±0.5	12.6±1.0	18.9±0.3	21.7±2.1

Data are expressed as mean±SD, n=3. Degradation (%)=(original concentration−determined concentration)×100.

3.1.3. Heat treatment

Results revealed light as the major factor of betalain pigment degradation. Refrigeration storage (4 °C) condition without light exposure managed to preserve the colour up to 3 weeks than storage at 25 °C with exposure to light (Tables 2 and 3).

3.2. Optimization of reagents and reaction condition

For lidocaine, the method employs 0.5% solution of *B. spectabilis* bracts prepared by making use of freshly prepared extract. The absorption peak of complex was located at 418 nm. Temperature of reaction, quantity, concentration and sequence of addition of reagents were optimized after several experimental trials. For lidocaine, the optimum quantity and concentration of *B. spectabilis* bracts dried extract solution was found to be 0.48 mL of 0.5% solution. The concentration of lidocaine over a range of 4 to 24 μg/mL was found to obey Beer's law (Table 4).

Table 4

Absorbance values for calibration curve of lidocaine colour complex and ranolazine color complex.

Complex	Concentration (μg/mL)	Absorbance
Lidocaine	4	0.017
	8	0.042
	12	0.065
	16	0.090
	20	0.114
	24	0.142
Ranolazine	5	0.053
	10	0.069
	15	0.082
	20	0.095
	25	0.111
	–	–

For ranolazine, the optimum quantity and concentration of *B. spectabilis* extract solution was found to be 0.48 mL of 0.5% solution. The concentration of ranolazine over a range of 1–25 μg/mL was found to obey Beer's law in the

stated range employed for analysis (Table 4). The correlation coefficient value for this curve was 0.998. The intensity of the colour formed, reached maximum in 1 min after mixing the reagent at room temperature and was stable up to 1 h. The results showed that optimal condition for the reaction of *B. spectabilis* bracts extract solution with ranolazine was in an alkaline medium. General mechanism of complex formation is reported in Figure 3.

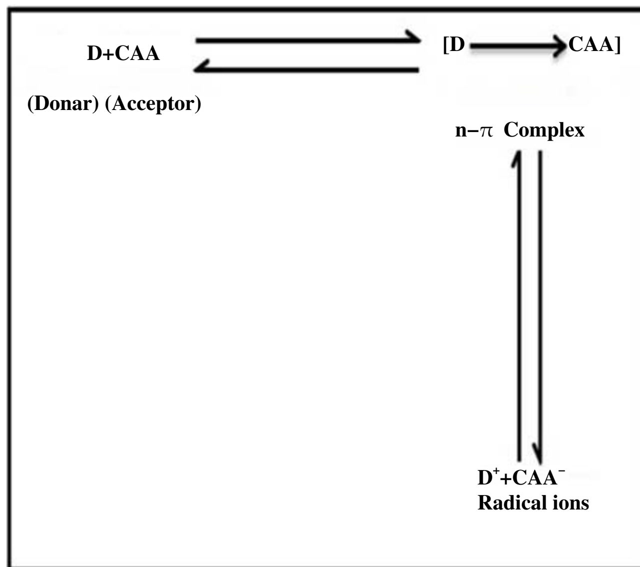

Figure 3. Possible mechanism action of colorimetric reaction.

3.3. Spectrophotometric analysis of drug-extract complexes

UV spectra of *B. spectabilis* color, lidocaine and lidocaine-color complex has been reported in Figure 4. While UV spectra of *B. spectabilis* color, ranolazine and ranolazine-color complex has been reported in Figure 5. UV overlain spectra of lidocaine color complex and ranolazine-color complex has shown in Figure 6 A and B respectively.

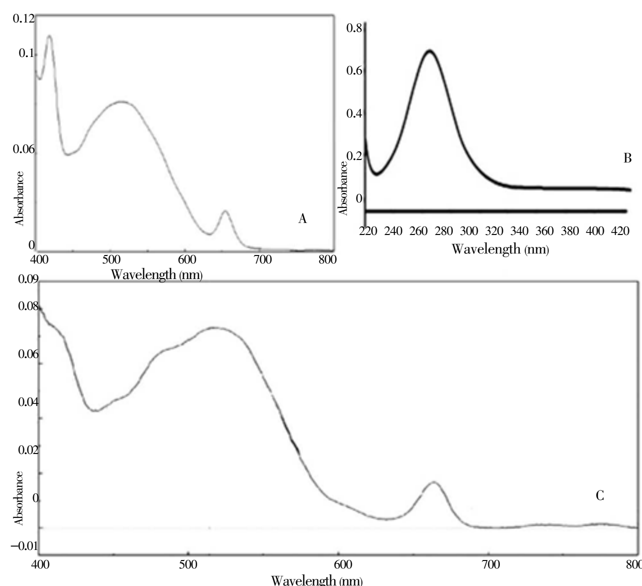

Figure 4. UV spectra of *B. spectabilis* extract (A), lidocaine (B) and lidocaine-color complex (C).

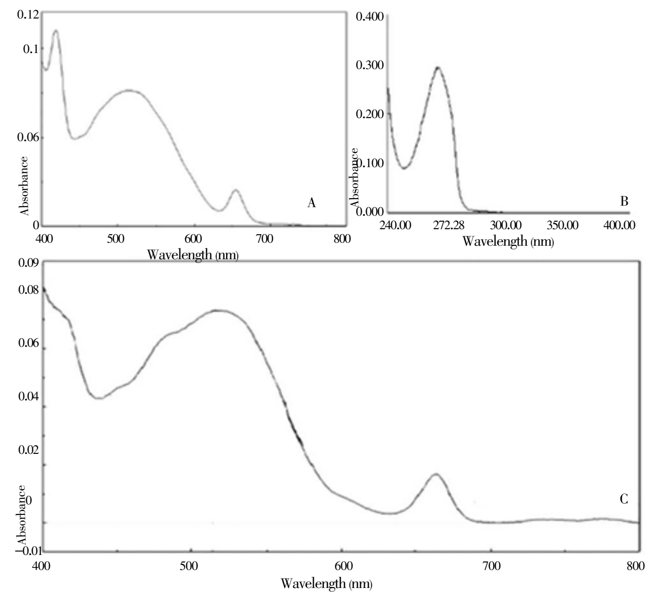

Figure 5. UV spectra of *B. spectabilis* extract (A), ranolazine (B) and ranolazine-color complex (C) .

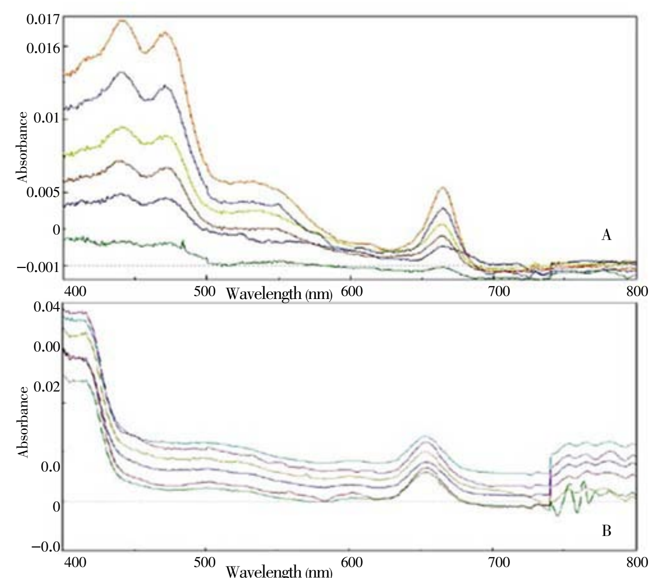

Figure 6. UV overlain spectra of lidocaine-colour complex (A) and ranolazine-colour complex (B) .

3.4. FTIR study

FTIR study of pure drugs (lidocaine and ranolazine), extract of *B. spectabilis* and complex of extract and drug indicated there were no incompatibilities between extract and drugs. *B. spectabilis* color showed major peak was at 3 375.89 wavenumber cm^{-1} which corresponds to phenols, -OH stretching. Even as peak was found to be retained in *B. spectabilis* color-lidocaine complex was at 3 322.17 wavenumber cm^{-1} which also corresponds to phenols, -OH stretching. While in case of *B. spectabilis* color-ranolazine complex peak was at 3 222.15 wavenumber cm^{-1} and

corresponded the same. So the major peak has found be retained both in color and complexes (Figures 7 and 8).

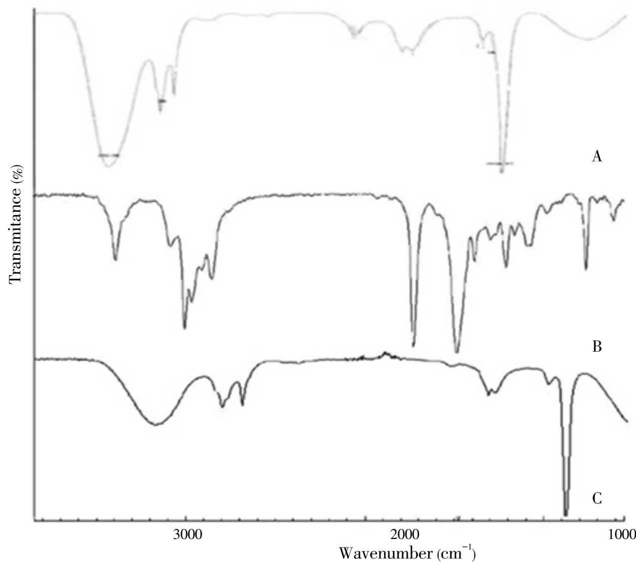

Figure 7. FTIR spectra of *B. spectabilis* extract (A), lidocaine (B) and lidocaine-colour complex (C).

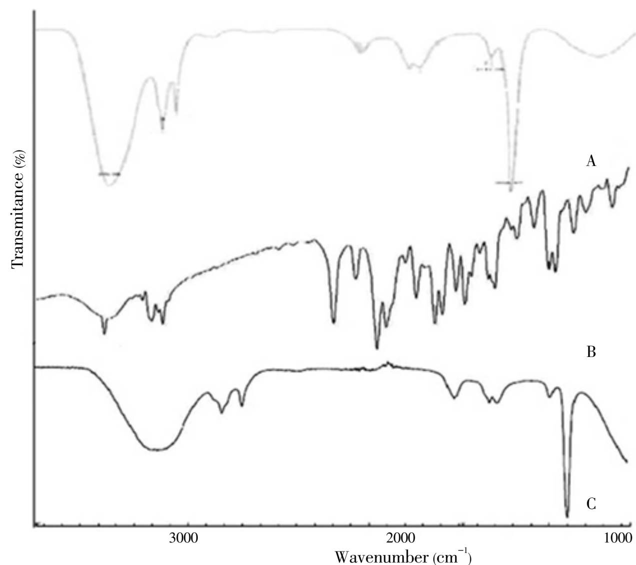

Figure 8. FTIR spectra of *B. spectabilis* extract (A), ranolazine (B) and ranolazine-colour complex (C) .

3.5. Method validation

3.5.1. Accuracy

The results indicated excellent recoveries ranging from 98.33% to 99.60% and 98.33% to 99.50% for lidocaine and ranolazine respectively and reliability of the method (Table 5). Recoveries obtained for the drug did not differ significantly from 100% showing that there was no interference from common excipients used in the formulation and thus indicating accuracy and reliability of the method.

Table 5

Results of accuracy.

Level		Concentration added (mg)	Concentration found (mg)	% Recovery	% RSD
Lidocaine	80%	34.16	34.02	99.60	0.253
	100%	42.70	41.83	97.95	0.266
	120%	51.24	50.98	98.33	0.592
Ranolazine	80%	8.00	7.87	98.33	0.898
	100%	10.00	9.82	98.73	1.386
	120%	12.00	11.94	99.50	0.971

RSD: relative standard deviation.

3.5.2. Precision

Intraday and interday precision was found to be approximately 99% for both lidocaine and ranolazine. Results of repeatability have reported as % relative standard deviation in Table 6.

Table 6

Results of repeatability (intraday and interday).

Analyte		Concentration (μg/mL)	Concentration estimated (%)
Lidocaine	Intraday	4	99.410±0.102
		12	99.720±0.160
		20	99.010±0.225
	Interday	4	96.750±0.212
		12	99.080±0.110
		20	99.410±0.189
Ranolazine	Intraday	5	98.730±0.060
		10	99.590±0.062
		25	99.620±0.086
	Interday	5	98.600±0.014
		10	99.480±0.066
		25	99.440±0.040

Data are expressed as mean±SD, n=9.

3.5.3. LOD

LOD was found to be 0.088 and 0.26 μg/mL for lidocaine and ranolazine respectively.

3.5.4. LOQ

LOQ was found to be 0.266 and 0.80 μg/mL lidocaine and ranolazine respectively.

4. Discussion

Formation of color complex between drugs and extract is contributed to the keto-enol tautomerism. Electrons on the phenolics oxygen get simply delocalized in aromatic ring, making it acidic due to easy removal of proton (H^+). While amide due to presence of lone pair of electron on nitrogen is going to show basic nature which contributes in formation of color complex between amide and the color pigment obtained from *B. spectabilis*. Study revealed that increased concentration of ascorbic acid directly contributed in

stability of color. Extract mainly contains betalains, stable at acidic pH so it is essential to store up this extract at acidic pH to maintain its activity.

Betalains are the compound mainly responsible for color of *B. spectabilis* bract which mainly contains bougainvillein-V. *B. spectabilis* bracts color was found to be less stable at 25 °C exposed to light and at pH 7. While, refrigeration storage (4 °C) condition without light exposure managed to preserve the color up to 3 weeks than storage at 25 °C with exposure to light. The proposed method for colorimetric estimation of lidocaine and ranolazine is accurate, precise, economical and convenient, yielding reproducible results. Moreover, the method is economic, simple and rapid, hence can be employed for routine analysis in quality control laboratories as it is specific without interference of excipients. If the *B. spectabilis* bract extact is stored properly, this could be useful in colorimetric estimation of aforesaid amide containing drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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