DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF IRBESARTAN BY THE HYDROTROPHY TECHNIQUE**

R. Biyani, K. S. Yadav*

Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, V. L. Mehta Road, Vile Parle-West, Mumbai-400 056, Maharashtra, India;
e-mail: khush.yadav@gmail.com; khushwant.yadav@nmims.edu

Irbesartan is a class II crystalline drug according to the Biopharmaceutical Classification System, the solubility of which in water is less than 0.1 mg/mL, and thus it possess a challenge for developing methods of its analysis. Hydrotrophy is one such phenomenon in which water-soluble agents enhance the solubility of hydrophobic compounds, precluding the use of toxic organic solvents. Among various hydrotropes screened, 1 M sodium acetate showed solubility enhancement of irbesartan by 33 times. After checking the effect of molarity from 0.5 to 4 M, 1 M sodium acetate showed characteristic absorption peaks and obeyed the Beer-Lambert law for 10–20 μg/mL solution of irbesartan hydrotrope mixture. Based on the plot of the concentration v/s absorbance, the calibration curve equation was found to be y = 0.040x – 0.013 with a correlation coefficient of 0.998. The LOD and LOQ values were found to be 0.3 and 1. The proposed method was validated for accuracy and precision at three suitable levels of concentration, i.e., 10, 14, and 18 μg/mL, for which the RSD was found to be 0.09, 0.07, and 0.1%, respectively. It was found that the method is robust and specific in the presence of other excipients and shows RSD values of 0.4, 0.5, and 0.6%, which are within acceptable limits.

Keywords: *irbesartan, hydrotrophy, solubility enhancement.*

РАЗРАБОТКА И ВАЛИДАЦИЯ УФ-СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА ДЛЯ ОЦЕНКИ СОДЕРЖАНИЯ ИРБЕСАРТАНА С ИСПОЛЬЗОВАНИЕМ ГИДРОТРОФИИ

R. Biyani, K. S. Yadav*

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Школа фармацевтического и технологического менеджмента Shobhaben Pratapbhai Patel, Мумбаи-400 056, Махарашtра, Индия;
e-mail: khush.yadav@gmail.com; khushwant.yadav@nmims.edu

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Исследовано влияние гидротропов на растворимость ирбесартана. Из гидротропов выбран ацетат натрия 1 М, позволяющий увеличить растворимость ирбесартана в 33 раза. В результате исследования влияния молярности в пределах 0.5–4.0 М установлено, что 1-М ацетат натрия обладает характерными максимумами поглощения и подчиняется закону Ламберта-Бера при концентрации раствора гидротропной смеси ирбесартана 10–20 мкг/мл. На основании зависимости концентрации от оптической плотности получено уравнение калибровочной кривой y = 0.040x – 0.013 с коэффициентом корреляции 0.998. Пределы обнаружения (LOD) и количественного определения (LOQ) 0.3 и 1. Предложенный метод проверен на точность и достоверность при трех концентрациях 10, 14, 18 мкг/мл, для которых установлено относительное стандартное отклонение RSD = = 0.09, 0.07 и 0.1% соответственно. Результаты показывают, что метод является надежным и чувствительным к присутствию других наполнителей и обеспечивает относительные стандартные отклонения 0.4, 0.5, 0.6 %, которые находятся в допустимых пределах.

Ключевые слова: *ирбесартан, гидротрофия, увеличение растворимости.*

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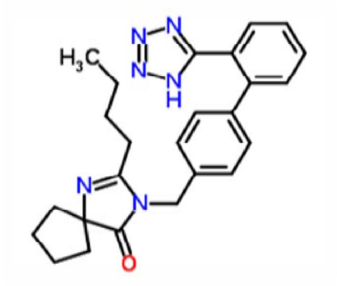
Introduction. According to IUPAC, irbesartan is 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl] phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one, $C_{25}H_{28}N_6O$, molecular weight: 428.54 g/mol:



Irbesartan is said to exert its antihypertensive effect by selectively inhibiting the conversion of angiotensin I to angiotensin II, which influences the aldosterone secretion responsible for the retention of sodium salts and water, thus decreases arterial wall pressure. Irbesartan is recommended as mono-therapy or with other synergistic drugs of the same class used in conditions such as high blood pressure responsible for arterial disease, diabetic renal failure, etc., with initial and maintenance dose of 150 and 300 mg, respectively [1]. Irbesartan is classified as a category-II crystalline drug with a lower aqueous solubility, which in water is less than 0.1 mg /ml. One of the important prerequisites for the analysis of a drug is its solubility, and a poorly water soluble drug poses a wide challenge for qualitative and quantitative analysis by spectroscopic methods such as UV-visible spectrophotometry [2].

The development of an analytical method is one of the significant quality control functions, in which a method for the quantification of analyte in its pure form and dosage form is developed. Thus, the instrumental method of analysis involves the use of spectrophotometric techniques such as UV-visible spectrophotometry, mass spectrophotometry, etc. The development of a UV-visible spectrophotometric method to evaluate a drug for routine quality functions is preferable if the developed method is validated and found to be precise and accurate.

Most analytical methods involve the use of organic solvents and reagents that are synthesized from complex chemical processes; the use of sophisticated instruments involves the investment of skill, time, money, and other resources. The determination of an active analyte is necessary to know its authenticity, strength, and quality, which are vital for compliance assessment.

Hydrotropy is a phenomenon first coined by Neuberg in the year 1916, which describes the addition of alkali salts or acids as solubilizing agent that increases the solubility of water-insoluble compounds [3]. It is simply an addition of hydrotropes that enhances the solubility of water-insoluble compounds. Hydrotropes usually consist of a hydrophobic part and an ionic moiety responsible to form a solution. Intermolecular hydrogen bonding orients a structure making or breaking phenomenon and influences solution properties such as solubility and surface tension. The mechanism of action of hydrotropes is generally based on the minimum concentration of the hydrotrope required to bring the aqueous solubility of the solute, which is based on the volume fraction solubility, the structure of the hydrotrope, and the drug together, which are formed either due to structure breaking and making, hence a polar group of the hydrotrope, which affects the water depression and the solute interactions [4, 5].

Hydrotropic agents are stated as ionic organic salts that help to increase or decrease the solubility of the solute in a given solvent via the ‘salt in’ or ‘salt out’ effects, respectively. Salts that show ‘salt in’ of nonelectrolytes are called “hydrotropic salts”, and the phenomenon is known as “hydrotropism”. They do not exhibit any colloidal properties, but they improve solubility via a weak interaction with solute molecules. A hydrotropic molecule interacts with a less water-soluble molecule via weak van der Waals interactions such as π–π or attractive dipole-dipole interaction. They show weak Van der waals interaction and intermolecular hydrogen binding, which helps to bring remarkable solubility and efficiency of the hydrotrope based on its solubility enhancement ratio and its influence on solvent properties [6]. When two or more hydrotropes are used, it is a ‘mixed hydrotropy’ phenomenon, by which the total concentration of individual agents is reduced with a substantial increase in the solubility of the poorly soluble drug [7].

Hydrotrophy involves the use of easily water-soluble compounds that helps to preclude the use of organic solvents. Hence, the objective of the present study was to develop and validate a UV-visible spectrophotometric method to evaluate irbesartan by using the hydrotrophy technique. Such developed analytical method would be useful in the quantitative estimation of irbesartan.

Experimental. Irbesartan was received from Yarrow Pharma, Mumbai, Maharashtra, India. All reagents and chemicals used were of analytical grade. Purified water was sourced from a Merck Millipore assembly system. Ammonium acetate and sodium acetate were sourced from Research Lab Fine Chem. Ltd., Mumbai.

The hydrotropes such as sodium acetate and ammonium acetate in varying molarities and the hydrotropic mixtures of 1 M sodium acetate and 1 M ammonium acetate were screened based on the solubility of irbesartan and scanned in the UV-visible spectrophotometer between 200 to 400 nm for the selection of appropriate wavelengths for the detection.

Solubility is a physicochemical process where a solute is dissolved into the solvent phase and forms a solution; thus hydrophilic compounds show enhanced solubility compared to poorly soluble drugs in water.

Solubility enhancement ratio is the ratio of the solubility of the drug in the hydrotrope and the solubility of the drug in purified water, based on which an increase in solubility is observed.

Shake flask method. In this solubility determination method, an excess amount of the drug is added to a suitable volume of medium in which the solubility has to be determined by keeping this solution at room temperature for a period of 24 h, after which the solution is filtered and analyzed spectrophotometrically at the appropriate wavelength [8]. For determining the solubility of irbesartan in a hydrotropic mixture of the screened hydrotropes, the same method was employed, and the solubility in mg/mL was found and its enhancement ratio was calculated.

Calibration curve for irbesartan in hydrotropes. Based on screening of the hydrotropes, the solubility enhancement of the drug, and the UV-spectrum of irbesartan, a standard calibration curve was developed for estimating irbesartan from the derived equation.

Sodium acetate as hydrotropic agent. Aliquots of a standard drug solution of 1 to 5 mL were withdrawn and transferred into a series of 10-mL volumetric flasks, and the volume was made up to the mark with 1 M sodium acetate to get an equivalent sample solution of 10–50 μg/mL, which was analyzed at λ_{max} = 232 nm.

Ammonium acetate as hydrotropic agent. Aliquots of a standard drug solution of 1 to 5 mL were accurately transferred into a series of 10 mL volumetric flasks, and the volume was adjusted to the mark with 1 M ammonium acetate to get an equivalent sample solution of 8–50 μg/ml, which was analyzed at λ_{max} = 234 nm. Based on the screening of the individual hydrotropes, a hydrotropic mixture of 1 M sodium acetate and 1 M ammonium acetate was also studied.

Hydrotropic mixture of 1 M sodium acetate and 1 M ammonium acetate. Aliquots of a standard drug solution of 0.6 to 1.4 mL were accurately transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the 1 M hydrotropic mixture to get an equivalent 6–14 μg/mL sample solution, which was analyzed at λ_{max} = 234 nm.

Validation of the analytical method. Based on the screening by the UV-visible spectrophotometer and the solubility values, 1 M sodium acetate was selected as the hydrotrope and λ_{max} was determined. Further, the concentration of the sample solution, which obeyed the Beer-Lambert law, was found, and the standard calibration curve was plotted to develop a method of analysis. As per the ICH guidelines, an analytical method developed has to be validated for use in routine analysis; thus, the method needs to be precise, accurate, specific, and robust for quantitative estimation.

Linearity. It was performed to check whether the sample solutions, which are analyzed spectrophotometrically by the standard calibration curve method, are appropriate for an assay at the selected wavelength.

Precision. This was studied to check the sample solutions, which are analyzed spectrophotometrically, for closeness of agreement between the exact and known values, when the measurements are performed on the same day and subsequent days.

Specificity. The developed method should be able to detect the analyte in the presence of inactive ingredients, which makes it suitable for quantitative analysis in bulk or final form. Inactive ingredients such as PEG-4000 and maize starch were thoroughly mixed in sample solutions and analyzed spectrophotometrically at the selected wavelength. Thus, the developed method is said to be specific when it expresses the known and exact value of the analyte present.

Robustness. The developed analytical method must be able to detect the analyte even when changing experimenting conditions, such as changing the analytical instrument and changing the detection wavelength, and must show closeness of agreement between the known and expected values to ensure that the developed method is suitable for the intended use.

For examining changes in wavelength, the sample solutions were analyzed at three different wavelengths in the range of ±2 of λ_{max} of the detection of the UV-visible spectrophotometer.

For examining changes in the instrument, the sample solutions were analyzed by using different UV-visible spectrophotometers.

Accuracy. It was determined to check whether the sample solutions that are analyzed spectrophotometrically are the same for the expected and known values in quantification of analyte.

Limits of detection (LOD) and quantification (LOQ). These are the minimum amount of analyte that can be detected and quantified by the developed analytical method designed to analyze the sample. LOD and LOQ allow assessing the sensitivity of the method. They were calculated based on the standard deviation of the response and the slope from the calibration curve using the formulas for the detection limit and quantification limit:

$$DL = 3.3s/S, \quad QL = 10s/S,$$

where s is the standard deviation of the response and S is the slope of the calibration curve.

Results and discussion. Irbesartan's official monograph appears in both the United States Pharmacopoeia (USP) and the Indian Pharmacopoeia (IP) [9, 10]. However, there are no UV-spectrophotometric methods reported for its quantitative determination in its pure form and pharmaceutical formulations.

Screening of hydrotropes by UV-visible spectrophotometry. *1 M sodium acetate as a hydrotropic agent.* Scanning of irbesartan in 1 M sodium acetate using the UV-spectrophotometer showed λ_{max} at 232 nm in different concentrations used, as shown in Fig. 1. The absorbance of irbesartan at 232 nm obeyed the Beer-Lambert law and was found to be linear in the concentration range of 10–20 µg/mL. Sodium acetate as hydrotrope did not show intrinsic absorbance at this λ_{max}, and hence any interference in the desired spectrum of the drug was not expected due to the presence of hydrotrope.

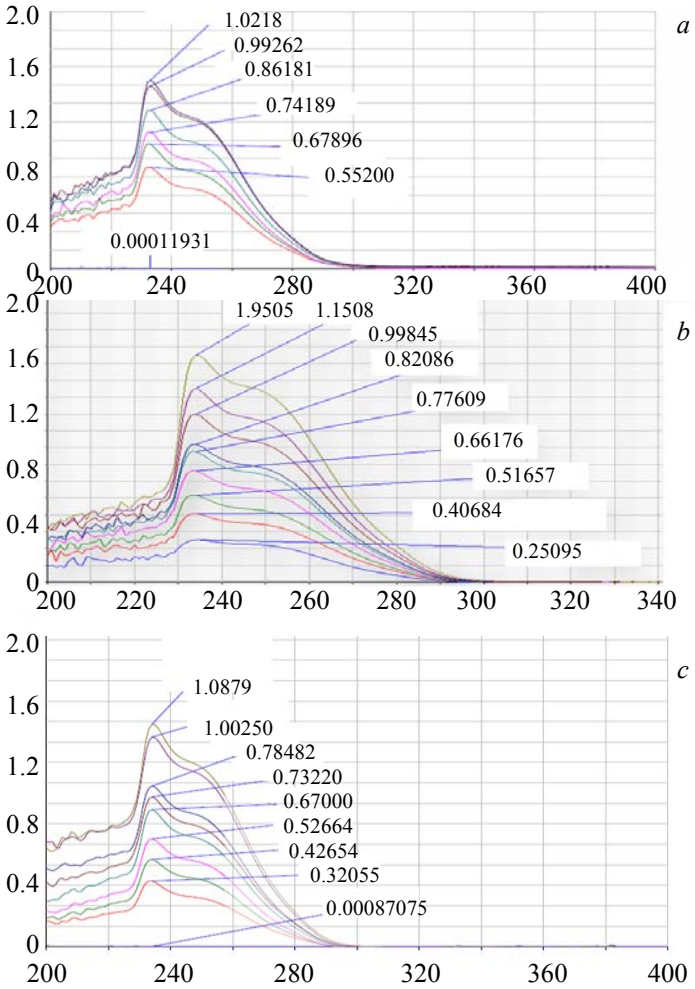


Fig. 1. Scan of 1 M sodium acetate irbesartan in the UV-spectrophotometer at 232 nm (a), 234 nm (b), and (c) of 1 M ammonium acetate and sodium acetate irbesartan at 234 nm in different concentrations.

1 M ammonium acetate as a hydrotropic agent. With 1 M ammonium acetate, irbesartan showed the absorbance peak at 234 nm, and the Beer Lambert concentration range was found to be linear from 8–18 μg/mL. Ammonium acetate as hydrotrope did not show any intrinsic absorbance and any interference in the absorbance spectrum of the drug.

Hydrotropic mixture of 1 M sodium acetate and ammonium acetate. In a 1:1 mixture of 1 M sodium acetate and ammonium acetate, irbesartan showed the absorbance peak at 234 nm and obeyed the Beer-Lambert law at the concentration range from 6 to 18 μg/ml. In addition, there was no interference in the absorbance spectrum of the drug.

Table 1 lists the various hydrotropes studied with the solubility enhancement ratio. It is seen that sodium acetate gives the highest solubility enhancement ratio of 33.37 times. This was also supported by the higher solubility of irbesartan in sodium acetate.

TABLE 1. Solubility Enhancement Ratio of Selected Hydrotropes and their Mixture

Hydrotrope	Solubility of Irbesartan, mg/mL	Solubility Enhancement Ratio
Sodium Acetate	1.3351	33.37
Ammonium acetate	1.1158	27.89
Sodium acetate + ammonium acetate	0.41519	10

N o t e. Molarity 1 M.

Solubility enhancement ratio of selected hydrotropes and its mixture. The solubility of irbesartan in purified water was found to be 0.04 mg/mL. Hence, 1 M sodium acetate was screened as the better hydrotrope based on its higher solubility.

Standard calibration curve. *Sodium acetate as a hydrotropic agent.* The method was developed by taking a series of solutions in the Beer-Lambert concentration range. They were used in triplicate and analyzed at 232 nm. Table 2 gives the data of the different absorbance values obtained for sodium acetate as hydrotrope. The standard calibration curve of irbesartan in 1 M sodium acetate can be used to estimate the amount of the drug by calculating it from the standard curve equation $y = 0.0401x - 0.0137$ showing the regression value r as 0.9987. The limit of detection and the limit of quantification were calculated by the standard deviation of the response and slope from the calibration curve. Table 3 summarizes the proposed method for evaluating irbesartan in 1 M sodium acetate using the UV-spectrophotometer.

TABLE 2. Standard Curve of Irbesartan in 1 M Sodium Acetate

Concentration, μg/mL	Absorbance ± S.D
10	0.3835 ± 0.01
12	0.4689 ± 0.006
14	0.5536 ± 0.001
16	0.6218 ± 0.007
18	0.7076 ± 0.004

TABLE 3. Proposed Method Development for Estimating Irbesartan in 1 M Sodium Acetate

Parameter	Proposed method
Maximum wavelength λ_{max}, nm	232
Linear range, μg/mL	10–18
Intercept	0.013
Slope	0.040
Regression coefficient R^2	0.998
LOD, μg/mL	0.3
LOQ, μg/mL	1

*LOD AND LOQ calculated by the standard deviation of the response and a slope of the calibration curve.

1 M ammonium acetate as a hydrotropic agent. The method was developed by taking a series of solutions in the Beer-Lambert concentration range used in triplicate and analyzed at 234 nm. Table 4 provides information on the absorbance obtained to develop the standard calibration curve for irbesartan in 1 M ammonium acetate as hydrotrope. The standard calibration curve of irbesartan in 1 M ammonium acetate can be used to estimate the amount of the drug by calculating it from the standard curve equation $y = 0.0492x - 0.0728$ showing the regression value r as 0.9992. The limit of detection and limit of quantification were calculated by the standard deviation of the response and the slope of the calibration curve. Table 5 gives a summary of the proposed method for estimating irbesartan in 1 M ammonium acetate by the UV-spectrophotometer.

TABLE 4. Standard Calibration Curve for Irbesartan in 1 M Ammonium Acetate.

Concentration, μg/mL	Absorbance ± S.D
8	0.3229 ± 0.003
10	0.4169 ± 0.014
12	0.5184 ± 0.007
14	0.609 ± 0.007
16	0.7185 ± 0.009

TABLE 5. Method Development for Estimating of Irbesartan in 1 M Ammonium Acetate

Parameter	Proposed method
Maximum wavelength λ_{max}, nm	234
Linear range, μg/mL	8–16
Intercept	0.072
Slope	0.049
Regression coefficient R^2	0.999
LOD, μg/mL	0.4
LOQ, μg/mL	1.4

The analytical method for estimating irbesartan in the 1 M SA and 1 M AA hydrotropic mixture was developed, and the Beer-Lambert law was obeyed in the concentration of 6–16 μg/mL; also, the calibration curve equation was found to be $y = 0.053x + 0.001$ with a regression coefficient value of 0.990.

Based on the solubility enhancement ratio of irbesartan in various hydrotropes, 1 M sodium acetate showed an increase of 33.3 times; thus, it was found to be ideal, and hence the developed method was further validated.

The literature reveals that not many methods based on UV-spectrophotometry are available for estimating irbesartan [11]. Hydrotropic agents have been used to develop spectrophotometric methods for evaluating drugs [5]. The increase in solubility by using hydrotrope molecules is due to the specific aggregation that these agents produce by stacking in a manner that solubilizes the solute in an aqueous solution [12]. It has been reported that hydrotropes are very useful because they are solubilized in high concentration [13]. However, in our case, a lower molar concentration (1 M) of sodium acetate has shown a better solubility of irbesartan as compared to higher ones (4 M).

The proposed method was validated for linearity, precision, accuracy, robustness, specificity, and sensitivity parameters such as detection and quantitation limits [14, 15]. The method developed was validated for linearity, and % RSD values were obtained under the acceptable limits. Table 6 gives the values obtained for the linearity study of the developed method for irbesartan in 1 M sodium acetate. The precision of the method was calculated in terms of intraday and interday (repeatibility) % RSD values. The intraday and interday study showed satisfactory precision. Table 7 gives information on the interday and intraday values of the concentration of the drug analyzed, illustrating precision. Values of % RSD below 2 are said to be precise, and all the values obtained were well below 1.0.

TABLE 6. Linearity Study of the Developed Method for Irbesartan in 1M Sodium Acetate

Concentration taken, μg/mL	Concentration found, μg/mL	% RSD
10	9.9	0.01
14	14.1	0.1
18	18.01	0.04

TABLE 7. Interday and Intraday Precision

Concentration, μg/mL	Interday precision		Intraday precision	
	Concentration found ± S.D, μg/mL	% RSD	Concentration found ± S.D, μg/mL	% RSD
10	9.9 ± 0.001	0.01	9.3 ± 0.009	0.09
14	14.1 ± 0.01	0.1	13.43 ± 0.01	0.07
18	18.01 ± 0.032	0.04	18.1 ± 0.032	0.17

TABLE 8. Specificity of the Developed Analytical Method

Concentration, μg/mL	Concentration, μg/mL ± SD	% RSD, *n*=3
10	9.79 ± 0.079	0.4 %
14	14.10 ± 0.09	0.6 %
18	18.73 ± 0.568	0.5%

TABLE 9. Robustness (Change in Wavelength)

Concentration μg/mL	Concentration, found ±SD, μg/mL	% RSD *n* = 3 at 230 nm	Concentration found ±SD, μg/mL	% RSD *n* = 3 at 232 nm	Concentration found ± SD, μg/mL	% RSD *n* = 3 at 234 nm
10	8.42 ± 0.046	0.54	9.3 ± 0.009	0.09	9.08 ± 0.02	0.22
14	11.90 ± 0.035	0.29	13.43 ± 0.01	0.074	13.08 ± 0.017	0.1
18	15.59 ± 0.0321	0.2	17.63 ± 0.02	0.11	17.6 ± 0.02	0.1

The proposed method was found to be specific for the estimation of irbesartan by the hydrotrophy technique in the presence of the excipients PEG 4000 and maize starch. Table 8 depicts the values obtained for the specificity of the developed analytical method. Herein the RSD values were much less than 2%, indicating that the method is specific. The robustness of the developed method was tested by changing the wavelengths of 232 ± 2 (Table 9) and by changing the instrument (Table 10).

The proposed method was found to be robust at selected appropriate wavelength with RSD ≤ 1%. Also, it was shown to be accurate with RSD ≤ 1 %, thus making it suitable for the quantitative analysis of irbesartan in bulk form (Table 11).

TABLE 10. Robustness (Change in Instrument)

Concentration, μg/mL	Concentration found ± SD *n* = 3, μg/mL	% RSD *n* = 3 at 232 nm
10	9.3 ± 0.009	0.09
14	13.43 ± 0.01	0.07
18	17.63 ± 0.02	0.1

TABLE 11. Accuracy of the Developed Method

Concentration, μg/mL	Concentration found ± SD, *n* = 3, μg/mL	% RSD *n* = 3 at 232 nm
10	9.5 ± 0.01	0.10
15	14.8 ± 0.02	0.13
20	20.7 ± 0.03	0.14

The linearity of the method was established in the concentration range of 10–18 μg/mL. The precision of the method was determined by the repeatability (intraday) and (interday), with RSD ≤ 1%. The specificity of the method was achieved by adding excipients to the sample solutions and it was assayed at RSD ≤ 1%. The accuracy of the method was also found to be satisfactory with the assay determined. The wavelength and instrument were varied to check the robustness of the method, and the % RSD obtained at different levels was ≤1. Hence, the developed analytical method for estimating irbesartan in 1 M sodium acetate was validated. Thus, the sensitive, specific, accurate, and robust method can be used for the spectrophotometric estimation of a drug by enhancing the drug solubility in water without the use of organic solvents, thus making it a cost-effective eco-friendly method.

Conclusion. Various hydrotropes and their mixtures that are easily accessible and economical were evaluated, and it was found that 1 M sodium acetate showed an increase in solubility of irbesartan, and hence it was utilized to develop the analytical method. A UV-spectrophotometric method for estimation of irbesartan in bulk form by the hydrotropic solubilization technique was developed that precludes the use of organic solvents, thus lowering the risk of residual toxicities and hazards. The developed hydrotrophy method is a selective, specific, sensitive, robust, and cost-effective analytical method for evaluating irbesartan in its pure form by using 1 M sodium acetate as hydrotrope.

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