BOOK CHAPTER

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Chapter: 13

DETERMINATION OF RIOCIGUAT USING NINHYDRIN AS A CHROMOGEN

Giri Prasad Gorumutchu, Venkata Nadh Ratnakaram, Balamurali K

Introduction: Riociguat is useful to treat both forms of pulmonary hypertension (PH) viz., CTEPH (chronic thromboembolic pulmonary hypertension) and PAH (pulmonary arterial hypertension) [1] Bayer markets it with a brand name of Adempas. Methyl *N*-[4,6-Diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-b]pyridin-3-yl]-5-pyrimidinyl]-*N*-methyl-carbaminate (Fig. 1) is the chemical name of it with molecular formula $C_{20}H_{19}FN_8O_2$ and molar mass of 422.415 g/mol. It is white to yellowish non-hygroscopic agent. It is a potent, oral stimulator of sGC (soluble guanylate cyclase), an enzyme in the cardiopulmonary system and the receptor for nitric oxide (NO) [2] Bayer Healthcare Pharmaceuticals got approval for it in 2013. Its function includes dual mode of action on sGC i.e., stimulates it directly and also its sensitivity is enhanced towards NO. It has very rapid absorption and high bioavailability (94.3%). At its therapeutic levels, it has negligible effect on transporter proteins as well as major CYP isoforms. Moreover, multiple cytochrome P450 (CYP) enzymes clear it quickly. Hence, clinical risk pertaining to drug interactions is low [3].

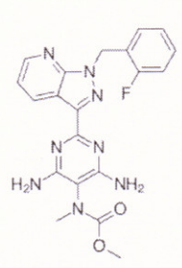

Fig. 1: Riociguat Chemical Structure

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A thorough literature collection shows that LCMS/MS [4], HPLC–MS [5], visible [6], HPLC [7, 8] and UV [9] methods were proposed to estimate Riociguat. However, visible spectrophotometric methods are scanty. Due to presence of amine groups in Riociguat, it is capable to form Ruhemann purpole with ninhydrin. Therefore, the present method describes visible spectrophotometric determination of Riociguat using ninhydrin.

Materials and Methods:

Ninhydrin solution: Prepared by dissolving 0.2 g of Ninhydrin in 100 mL of water. On exposure to sunlight, it becomes red. Hence, this solution was refrigerated at 4 °C and used for a period of 3 days.

Citrate buffer: 4.2 grams of citric acid was dissolved in 40 mL of 0.1 N NaOH and then diluted to 500 mL.

Preparation of standard drug solution: The standard drug of Riociguat (50 mg) was weighed accurately and transferred to 50 mL volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg mL^{-1} (stock solution). This solution was diluted for further suitably.

Results and Discussions: In the present study, ninhydrin is selected as chromogenic reagent because it reacts with aromatic amines to produce a chromophore with λ_{max} 597 nm (Fig. 2).

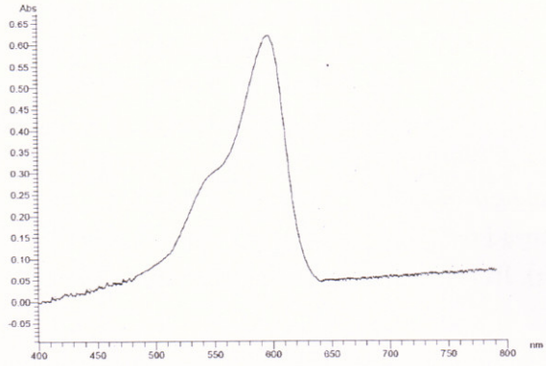

Fig. 2: Visible spectrum of chromophore formed due to Riociguat

Recommended Analytical Procedure and Stoichiometry: Various aliquots of standard Riociguat solution were transferred into a series of 10 mL boiling test tubes. Citrate buffer (0.5 mL) and ninhydrin (2 mL) were added to each test tube. Then the solution mixture was heated at 100 ± 2 °C for 15 min. The contents were cooled and the volume was made up to the mark with water in 10 mL volumetric flasks. The absorbance was measured at 597 nm against a reagent blank. Stoichiometric mole ratio of the drug - Riociguat and ninhydrin was determined by Job's method [10]. Developed maximum intensity of colour had deviated from 1:2 and skewed towards higher values of ninhydrin. This observation might be likely due to reasons like photolytic instability, side reactions, slower rate of reaction, and interferences [11, 12].

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Chromophore Formation and Chemistry: Ninhydrin is one of the well-known chromogenic agent which is used to estimate the amount of certain amines, amino acids and thiophenes [13]. Literature shows the successful usage of it for assay of different molecules of pharmaceutical importance and containing nitrogeneous functional groups like amino acids, amines, amides, hydrazine's, piperazines and cyanides. Chemically it is triketohydrindene hydrate. *In most of the cases,* a typical purple colour formation is the basis for its use in spectrophotometric measurements [14] . Its popular name is Ruhemann's reagent due to formation of Ruhemann's purple [15]. Colour, intensity, reaction and the mechanism involved in colour formation is based on the functional group in the substrate (amino acid, pyrrole, imino acid, primary amine, secondary amine and ammonium salt) [16]. Consecutive oxidative deamination and condensation of the reduced ninhydrin are involved in the formation of Ruhemann's purple colour due to reaction between ninhydrin and pharmaceutical drugs bearing primary amines like gabapentin [17], lisinopril [18], pregabalin [15], Amlodipine [19] and, famotidine [20, 21].

In all the above cases, Ruhemann's purple formation is possible as it happens only whenever there exists at least one hydrogen on the adjacent carbon to amine group. Whereas in the present case, riociguat is an aromatic amine and hence, the involved reaction is entirely different. To substantiate it a thorough literature collection was carried out and listed down.

According to Moubasher [22] and Ruhemann [23], most of the aromatic amines give compound 3a by reacting one aromatic amine molecules with ninhydrin. Substituted anilines form different products compared to aniline. Compound 3b forms due to reaction between two molecules of aniline and one molecule of aniline. These reactions were taken as basis by Robert Suffis [24]for the spectrophotometric estimation of p-phenylenediamines and p-aminophenols with ninhydrin because these compounds form blue or purple colour with high molar absorptivities compared to other anilines. Their high reactivity might be due to existence of a stable quinonoid resonant form 3d with Schiff base (III) 3c which in turn formed by dehydration of 3a (Fig.3). In addition to stability, high intensity and high λmax are also due to the quinoid form.

Midori Yano et al [25] carried out reaction between different substituted aromatic amines with ninhydrin. The nature of the condensation reaction involved depends on the substituents. A carbinolamine 3e was isolated with aniline with removal of one water molecule. But concerned Schiff bases 3f are formed with p-aminophenol and xylidines (2,4- and 2,3-xylidine) with removal of two water molecules.

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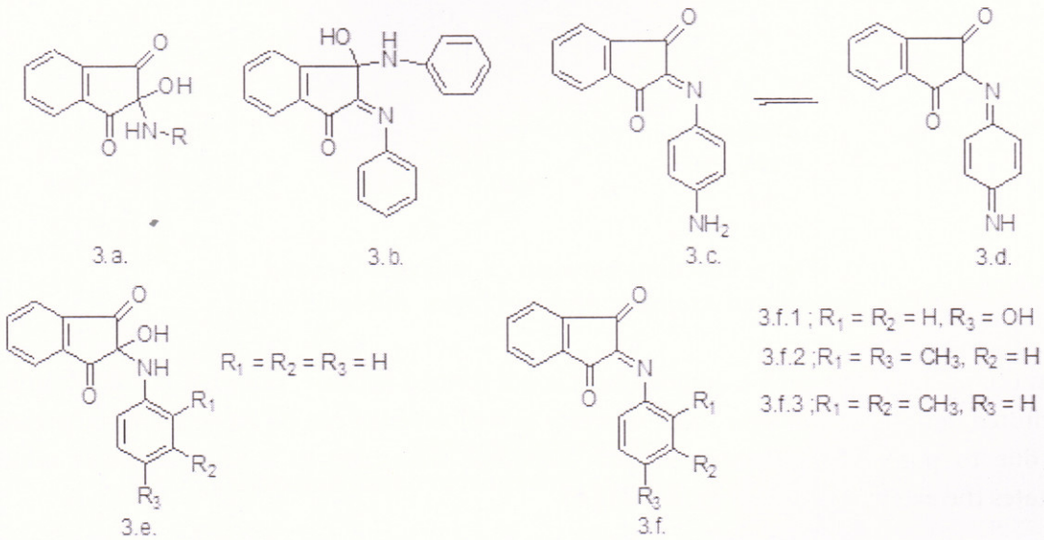

Fig. 3: Products in literature for reaction between ninhydrin and aromatic amines [22-25]

A range of condensation products were reported due to reaction between ninhydrin hydrate and nucleophilic reagents inclusive of aromatic amines [26]. A nucleophilc displacement with a removal of one water molecule occurs due to reaction between ninhydrin hydrate and amino group present in aniline / p-chloroaniline / 2-aminopyridine / m-aminophenol to yield 4a (Fig.4). Whereas, a spontaneous dehydration takes place in the case of o- & p-aminophenol and p-phenylenediamine. Products (4b) stabilization through resonance interactions was considered to be the driving forces for this dehydration [26].

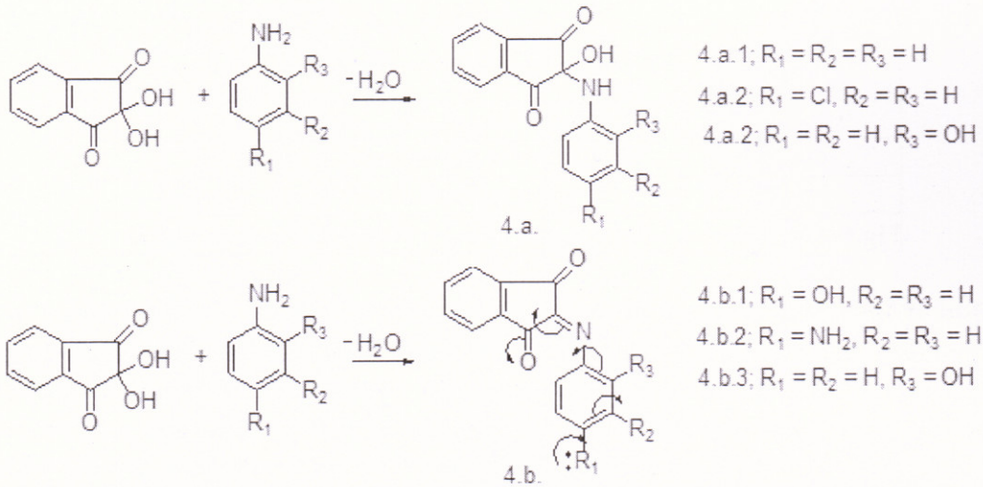

Fig. 4: Reactions between ninhydrin and substituted aromatic amines (Friedman et al) [26]

In similar, formation of a tautomeric mixture (imine 5c and enamine 5d, Fig.5) was reported in the reaction between 1,2-indanediones 5a and 3,5-dimethoxyaniline 5b [27].

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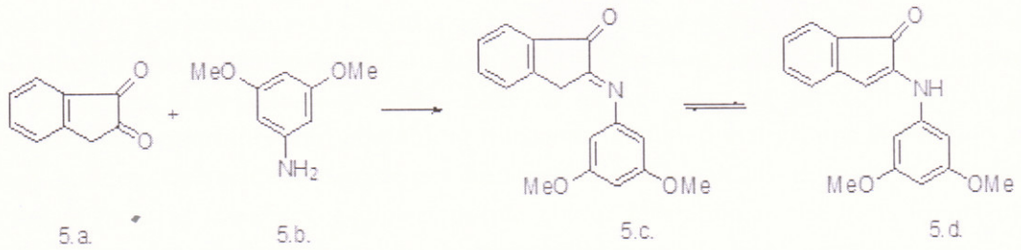

Fig. 5: Reactions between 1,2-indanediones and Substituted Aromatic Amines (Taylor and Joullié) [27]

But in the present case, such a quinoid form is not formed because, riociguat is meta diamine substituted derivative. However, high intensity as well as λmax can be expected in the present case due to possible attachment of two ninhydrin molecules to a drug molecule which facilitates the extension of conjugation (Fig.6).

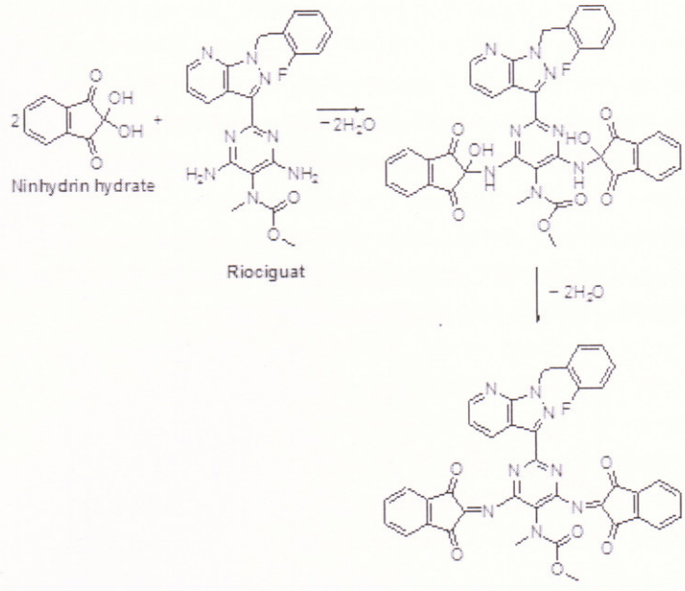

Fig.6: Chromophore Formation between Ninhydrin and Riociguat

Validation of Method:

Linearity and range: Linearity of the method was tested in the concentration in the range of 5.0 – 30.0 μg mL^{-1} (Fig. 7) and confirmed from high correlation coefficient (> 0.999). y = 0.0316x+0.001 is the linear regression equation. Optical as well as regression parameters are shown in Table 1.

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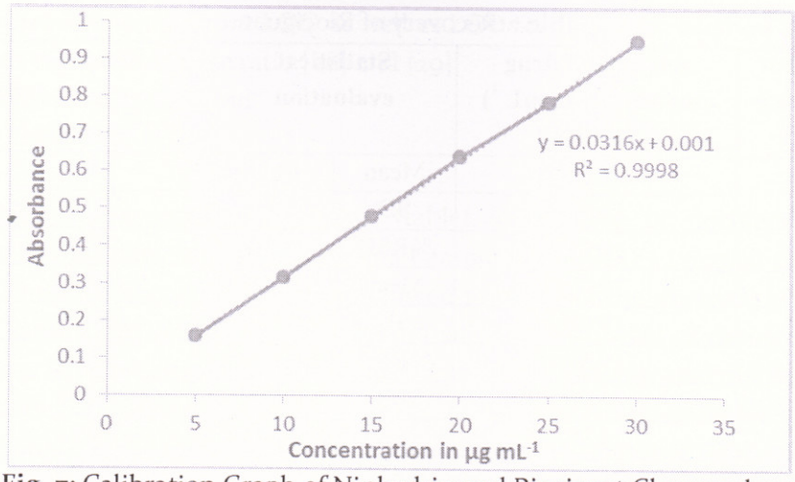

Fig. 7: Calibration Graph of Ninhydrin and Riociguat Chromophore

Table 1: Optical, Regression and Validation Parameter Values

S. No.	Parameter	Observation
Optical characteristics		
1.	Apparent molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	1.3×10^4
2.	Sandell's sensitivity ($\mu g\ cm^{-2} A^{-1}$)	0.0316
Regression analysis		
1.	Slope	0.0316
2.	Intercept	0.001
3.	Regression coefficient (r)	0.9998
Validation parameters		
1.	λ_{max}	597 nm
2.	Beer's Law Limit (Linearity, $\mu g\ mL^{-1}$)	5.0–30.0
3.	Limit of detection ($\mu g\ mL^{-1}$)	0.15
4.	Limit of quantitation ($\mu g\ mL^{-1}$)	0.50
5	Stability period	24 hours

Accuracy: Low values of SD and %RSD are ascertained the accuracy of the method from the studied recovery levels of 50%, 100% and 150%. Table 2 shows the % recoveryvalues range as 99.87 – 100.06.

Precision: Three dissimilar concentrations of Riociguat were chosen to endorse the both precision studies and confirmed the precision from low % RSD values which falls under acceptable limit (below 1%). Table 3 is a compilation of estimated values of six each on the same day in addition to sequential days.

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Table 2:Recovery of Riociguat

Level of recovery (%)	Amount of drug recovered (µg mL^{-1}) (Practical)	Statistical evaluation		% Recovery = Practical x 100/ Theoretical
50	14.99	Mean	14.99	99.93
	15.01	SD	0.012	100.06
	14.98	%RSD	0.083	99.87
100	20.01	Mean	19.99	100.05
	19.99	SD	0.012	99.95
	19.98	%RSD	0.062	99.90
150	24.99	Mean	24.99	99.96
	24.98	SD	0.005	99.92
	24.99	%RSD	0.019	99.96

Selected nominal concentration (a): 10.0 µg mL^{-1}
Added amount of drug (b): 5.0, 10.0 and 15.0 µg mL^{-1} respectively for 50%, 100% and 150% recovery levels
Theoretical amount:Total amount of drug (a + b) = 15.0, 20.0, 25.0 µg mL^{-1} respectively for 50%, 100% and 150% recovery levels

Table 3: Precision Data

Concentration of Drug (µg mL^{-1})	Concentration*			
	Intraday (Mean ± SD) (µg mL^{-1})	% RSD	Inter-day (Mean ± SD) (µg mL^{-1})	% RSD
5.0	5.001±0.0014	0.028	5.025±0.0011	0.022
15.0	15.008±0.002	0.013	15.025±0.016	0.106
30.0	30.121±0.007	0.023	30.124±0.013	0.043

* Average of six determinations

Ruggedness: Low %RSD values show the reproducibility and hence confirm the ruggedness of the above method (Table 4).

Table 4: Ruggedness Data

Test Concentration of Drug (µg mL^{-1})	Concentration*	
	Analyst change	
	Mean ± SD (µg mL^{-1})	% RSD
5.0	5.121±0.008	0.156
15.0	15.024±0.026	0.173
30.0	30.151±0.011	0.036

* Average of six determinations

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Detection limits determination: LOQ as well as LOD for the above method are given below from the values of signal to noise ratio [28] and [29].

$LOD = 3.3 \times \sigma / S = 0.15\ \mu g\ mL^{-1}$ and

$LOQ = 10 \times \sigma / S = 0.50 \mu g\ mL^{-1}$

Analysis Tablet Formulations: Adempas® (tablet formulation of riociguat) powder was sonnicated for ten minutes in presence of small amount of DMSO to extract the API content present in it. Then the above method was followed to estimate the riociguat present in tablet formulation (Table 5). In general, in industrial quality control units of developing countries, the best option is visible spectrophotometric method for routine analysis by using simple chromogenic reactions like Diazo coupling / oxidative coupling reactions [30-34], red-ox reactions [35], ion pair / ion association / charge transfer complex formation [36-44], in addition to simple UV-Visible spectroscopic methods [45-46]. Hence, ninhydrin can be used as a chromogenic agent to determine the amount of Riociguat in pure and tablet formulations by the above established visible spectrophotometric method.

Table 5: Assay of Pharmaceutical Formulation

Formulation	Labeled amount (mg)	Amount found* (mg)	% Drug Recovered	%RSD
Adempas®	1	1.0102±0.0002	101.02	0.020
* Average of three determinations				

Conclusions: The observed high intensity as well as λmax of the generated chromophore might be due to attachment of two ninhydrin molecules to drug molecule which facilitates the extension of conjugation. According to the present guidelines of ICH, the proposed method was validated. In quality control laboratories this method can be applied for routine analysis of Riociguat (bulk drug and tablet formulation) as a substitute to the expensive and sophisticated instrumental methods.

References:

1. Mittendorf, J., Weigand, S., Alonso-Alija, C., Bischoff, E., Feurer, A., Gerisch, M., Kern, A., Knorr, A., Lang, D., Muenter, K., Radtke, M. Discovery of riociguat (BAY 63-2521): a potent, oral stimulator of soluble guanylate cyclase for the treatment of pulmonary hypertension. Chem. Med. Chem. 4(5) (2009): 853-865.
2. Ghofrani, H.A., D'armini, A.M., Grimminger, F., Hoeper, M.M., Jansa, P., Kim, N.H., Mayer, E., Simonneau, G., Wilkins, M.R., Fritsch, A., Neuser, D. Riociguat for the treatment of chronic thromboembolic pulmonary hypertension. N. Engl. J. Med. 369(4) (2013): 319-329.
3. Frey, R., Becker, C., Saleh, S., Unger, S., van der Mey, D., Mück, W. Clinical pharmacokinetic and pharmacodynamic profile of Riociguat. Clin. pharmacokinete. 2017: 1-15.

ISBN 978-93-86435-86-6

4. Gnoth, M.J., Hopfe, P.M., Czembor, W. Determination of riociguat and its major human metabolite M-1 in human plasma by stable-isotope dilution LCMS/MS. Bioanalysis. 7(2) (2015): 193-205.
5. Grimminger, F., Weimann, G., Frey, R., Voswinckel, R., Thamm, M., Bölkow, D., Weissmann, N., Mück, W., Unger, S., Wensing, G., Schermuly, R.T. First acute haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension. Eur. Respir. J. 33(4) (2009): 785-792.
6. **Prasad, G.G.**, Nadh, R.V., Sireesha, M. Determination of Riociguat by Oxidative Coupling using Visible Spectrophotometry. Oriental J. Chem. 35(spl 1) (2019): 48-53, DOI: org/10.13005/ojc/35Specialissue107.
7. Sirisha, P., Sharma, J.V., Nikhitha, S., Likitha, R., Uday, K.B., Durga, P.S. Method development and validation of Riociguat by RP-HPLC. *Int. J. Pharm. Pharm. Scie.*7(4) (2016): 3060-3062.
8. Temgire, P.R., Sobia, G., Kumar, M.V., Sayali, W., Mohan, S.R., Smita, N., Vaidhun, B. Development and validation of reverse-phase high-performance liquid chromatography method for quantitative estimation of riociguat in tablet dosage form. J. Pharm. Res. 12(4) (2018): 461.
9. Ashok, C.V., Sailaja, B.B., Praveen, K.A. Method development and validation of UV-visible spectroscopic method for the estimation of assay of sugammadex sodium, apremilast, riociguat and vorapaxarsulfate drugs in API form. Asian J. Pharma. Clin. Res. 10 (2017): 241-250.
10. Job, P. Formation and stability of inorganic complexes in solution. Ann. Chim. 2 (1928): 113.
11. Pundarikakshudu, K., Shah, P.B. Spectrophotometric, difference spectroscopic and RP-HPLC methods for the determination of cefixime in pharmaceutical formulations. J. Pharm. Pharm. *Sci.* 89 (2006): 987-994.
12. Wani., Y.B., Patil., D.D. An experimental design approach for optimization of spectrophotometric method for estimation of cefixime trihydrate using ninhydrin as derivatizing reagent in bulk and pharmaceutical formulation. J. *Saudi.* Chem. Soc. 21(2013): S101-S111, http://dx.doi.org/10.1016/j.jscs.2013.11.001.
13. Fiegel., F. Spot Tests in Organic Analysis, Elsevier Publishing Co, London, 1960.
14. Papanna., R.K., Krishnegowda, J.B., Nagaraja, P. Spectrophotometric method for the determination of cefepime, cefazolin sodium and cefalothin sodium in pure and pharmaceutical dosage forms by using ninhydrin. J. Pharm. Pharm. *Sci.* 7(5) (2015): 194-199.
15. Gupta, C., Puri, R., Hussain, A., Jain, S.K. Development and validation of ninhydrin based colorimetric spectrophotometric assay for determination of pregabalin in different dissolution mediums. J. Anal. Chem. 8(2) (2012): 90-98.
16. Mccaldin, D.J. The chemistry of ninhydrin. Chemical Review. 60(1) (1959): 39-52.
17. Abdellatef, H.E., Khalil, H.M. Colorimetric determination of gabapentin in pharmaceutical formulation. J. Pharm. Biomed. Anal. 31(1) (2003): 209-214.
18. Raza, A., Ansari, T.M. Spectrophotometric determination of lisinopril in pure and pharmaceutical formulations. J. Chin. Chem. Soc. 52(5) (2005): 1055-1059.

ISBN 978-93-86435-86-6

19. Rahman, N., Azmi, S.N. Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. Il Farmaco. 56(10) (2001): 731-735.
20. Rahman, N., Kashif M. Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations. Il Farmaco. 58(10) (2003): 1045-1050.
21. Hosseinimehr, S. J., Pourmorad, F., Moshtaghi, E., Amini, M. Colorimetric determination of Baclofen with ninhydrin reagent and compare with HPLC method in tablet. *Asian.* J. Chem. 22(1) (2010): 522-526.
22. Moubasher, R. J . Chem. SOC. 1949, 1238-1239.
23. Ruhemann, S. CXXXII.—Cyclic di-and tri-ketones. J. Chem. SOC.Transactions. 97 (1910): 1438-1449.
24. Suffis, R., Levy, A., Dean, D.E. Spectrophotometric Determination of p-Phenylenediamines and p-Aminophenols with Ninhydrin. Anal. Chem. 36(3) (1964): 636-637.
25. Yano, M., Hayashi, T., Namiki, M. Formation of Free Radical Products by the Reaction of Dehydro-ascorbic Acid or Ninhydrin with Aromatic Amines. Agric. Biol. Chern. 42(4) (1978): 809-817.
26. Friedman., M. Mechanism of the ninhydrin reaction. II. Preparation and spectral properties of reaction products from primary aromatic amines and ninhydrin hydrate. Can. J. Chem. 45(19) (1967): 2271-2275.
27. Taylor, B.M., Joullié, M.M. Reaction of 1, 2-indanedione with 3, 5-dimethoxyaniline. Tetrahedron. 54(50) (1998): 15121-15126.
28. Sethi, P.D. HPLC quantitative analysis of pharmaceutical formulations. CBS publications. India. 2001.
29. ICH guidelines.,Validation of Analytical Procedures: Text and Methodology.Q2(R1) (2015): 8-13.
30. **Prasad, G.G.,** Nadh, R.V. Oxidative Coupling: A tranquil approach for determination of selexipag by visible spectrophotometry. Oriental J. Chem. 34(6) (2018) 3112- 3117. DOI:10.13005/ojc/340656.
31. **Prasad, G.G.,** Nadh, R.V. Diazocoupling for Determination of Selexipag by Visible Spectrophotometry. Int. J. Green Pharm. 12(4) Suppl (2018):S822-S828, DOI: org/10.22377/ijgp.v12i04.2261.
32. **Prasad, G.G.,** Nadh, R.V., Sireesha, M. Ninhydrin based Visible Spectrophotometric Determination of Gemigliptin. Oriental J. Chem. 35(1) (2019): 363-369, DOI:org/10.13005/ojc/350145.
33. Sudhir, M.S., Nadh, R.V. Diazo-Coupling: A facile mean for the spectrophotometric determination of rasagiline hemitartrate. Oriental J. Chem. 29(4) (2013): 1507-1514, DOI:10.13005/ojc/290429.
34. Sudhir, M.S., Venkata Nadh, R., Manjunatha, H., Oxidative coupling reaction for the determination of lurasidone. J. Anal. Chem.74(6) (2019): 528–533, DOI: 10.1134/S1061934819060121;
35. **Prasad, G.G.,** Nadh, R.V. Res. Determination of mianserine using Fe^{3+}-phenanthroline by visible spectrophotometry. Research J. Pharm and Tech 2019; 12(1) (2019) : 209-212. DOI: 10.5958/0974-360X.2019.00038.6

ISBN 978-93-86435-86-6

36. **Prasad, G.G.,** Nadh, R.V. Determination of Mianserine using Tropaeoline-OOO by Ion Pair Formation.Int. J. Appl. Pharmaceutics. 11(1) (2019): 168-173, DOI:10.22159/ijap.2019v11i1.30125.
37. **Prasad, G.G.,** Nadh, R.V., Kiran, K.K. *Piperacillin* estimation by ion-associative complex formation.*Asian J. Pharm. Clinical Res.*12(3) (2019): 1-5,DOI: 10.22159/ajpcr.2019.v12i3.29289.
38. Kiran, K.K., Nadh, R.V., Nagoji, K.E.V. Extractive spectrophotometric determination of nicergoline through ion-pair complexation reaction. Oriental J. Chem. 29(1) (2013): 263-269, DOI:10.13005/ojc/290142.
39. Kiran, K.K., Nadh, R.V., Nagoji, K.E.V. Determination of bendamustine hydrochloride in pure and dosage forms by ion-associative complex formation reaction.Oriental J. Chem. 30(2) (2014): 905-910, DOI:10.13005/ojc/300272.
40. **Prasad, G.G.,** Nadh, R.V., Sireesha, M. IonPairFormationforDeterminationofMianserineusingFastSulphonBlack F. Asian J. Pharm. 12 (4) (Suppl) (2018): S1390- S1396, DOI:10.22377/ajp.v12i04.2940.
41. **Prasad, G.G.,** Nadh, R.V., Kiran, K.K. Ion-associative complex formation for estimation of piperacillin. Int. Res. Pharm. Sci. 10(1) (2019): 117-124, DOI:10.26452/ijrps.v10i1.1787.
42. **Prasad, G.G.,** Nadh, R.V. Extractive spectrophotometric determination of ulipristal acetate using naphthol blue black. Research J. Pharm and Tech. 12(3)(2019): 1347-1352, DOI: 10.5958/0974-360X.2019.00226.9.
43. **Prasad, G.G.,** Nadh, R.V., Kishore V.N.V. Visible Spectrophotometric Determination of Gemigliptin using Charge Transfer Complex. Oriental J. Chem. 35(Special Issue) (2019): 694-699, DOI: 10.13005/ojc/350226.
44. Kiran Kumar, K., Venkata Nadh, R., Siva Kishore, M., Giri Prasad, G. Determination of Satranidazol through Ion-Associative Complex Reaction, Asian J. Org. Med. Chem. 4(1) (2019): 23-27, DOI: 10.14233/ajomc.2019.AJOMC-P166
45. Sudhir, M.S., Ratnakaram, V.N. Simple and validated ultraviolet spectrophotometric method for the estimation of lurasidone in bulk form. Res. J. Pharm. Bio.Chem. Sc. 4(1) (2013): 609-617.
46. Sudhir, M.S., Mohan, P.M., Venkata Nadh, R. Simple and validated ultraviolet spectrophotometric method for the estimation of febuxostat in bulk and pharmaceutical dosage forms. Oriental J. Chem. 29(1) (2013): 235-240, DOI:10.13005/ojc/290137

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DETERMINATION OF RIOCIGUAT USING NINHYDRIN AS A CHROMOGEN

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Abstract: A simple method is described to determine the amount of riociguat in bulk and tablet formulation by visible spectrophotometry. Formation of a chromophore with λ_{max} of 597 nm, due to the reaction between the aromatic amine groups present on riociguat and ninhydrin in citric acid medium forms the basis for the current method. Extension of conjugation due to attachment of two ninhydrin molecules to a riociguat molecule explains the noticed high intensity as well as λmax of the generated chromophore. Current ICH guidelines were followed to validate the method. The obtained regression equation (y = 0.0316x+0.001) has a good correlation coefficient (> 0.999) in the studied range of 5.0-30.0 µg mL^{-1}. Due to lack of separation steps in the method, it is found to be rapid as well as simple. The recovery levels of riociguat were in the range of 99.87 – 100.06.

Key words: Determination, ninhydrin, riociguat, validation, visible spectrophotometry

Introduction: Riociguat is useful to treat both forms of pulmonary hypertension (PH) viz., CTEPH (chronic thromboembolic pulmonary hypertension) and PAH (pulmonary arterial hypertension) [1] Bayer markets it with a brand name of Adempas. Methyl *N*-[4,6-Diamino-2-[1-[(2-fluorophenyl)methyl]-1*H*-pyrazolo[3,4-b]pyridin-3-yl]-5-pyrimidinyl]-*N*-methyl-carbaminate (Fig. 1) is the chemical name of it with molecular formula $C_{20}H_{19}FN_8O_2$ and molar mass of 422.415 g/mol. It is white to yellowish non-hygroscopic agent. It is a potent, oral stimulator of sGC (soluble guanylate cyclase), an enzyme in the cardiopulmonary system and the receptor for nitric oxide (NO) [2] Bayer Healthcare Pharmaceuticals got approval for it in 2013. Its function includes dual mode of action on sGC i.e., stimulates it directly and also its sensitivity is enhanced towards NO. It has very rapid absorption and high bioavailability (94.3%). At its therapeutic levels, it has negligible effect on transporter proteins as well as major CYP isoforms. Moreover, multiple cytochrome P450 (CYP) enzymes clear it quickly. Hence, clinical risk pertaining to drug interactions is low [3].

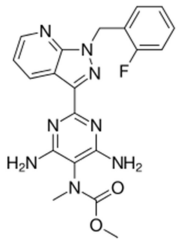


Fig. 1: Riociguat chemical structure

A thorough literature collection shows that LCMS/MS [4], HPLC–MS [5], visible [6], HPLC [7, 8] and UV [9] methods were proposed to estimate Riociguat. However, visible spectrophotometric methods are scanty. Due to presence of amine groups in Riociguat, it is capable to form Ruhemann purpole with ninhydrin. Therefore, the present method describes visible spectrophotometric determination of Riociguat using ninhydrin.

Materials and Methods:

Ninhydrin solution: Prepared by dissolving 0.2 g of Ninhydrin in 100 mL of water. On exposure to sunlight, it becomes red. Hence, this solution was refrigerated at 4 °C and used for a period of 3 days.

Citrate buffer: 4.2 grams of citric acid was dissolved in 40 mL of 0.1 N NaOH and then diluted to 500 mL.

Preparation of standard drug solution: The standard drug of Riociguat (50 mg) was weighed accurately and transferred to 50 mL volumetric flask. It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg mL^{-1} (stock solution). This solution was diluted for further suitably.

Results and Discussions:

In the present study, ninhydrin is selected as chromogenic reagent because it reacts with aromatic amines to produce a chromophore with λ_{max} 597 nm (Fig. 2).

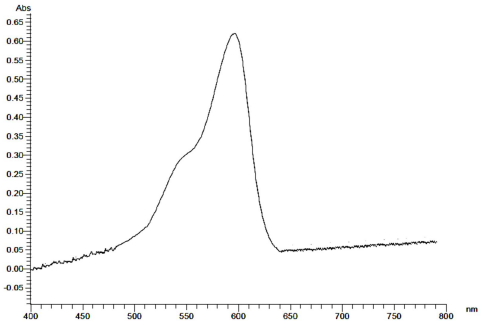


Fig. 2: Visible spectrum of chromophore formed due to Riociguat

Recommended analytical procedure and stoichiometry:

Various aliquots of standard Riociguat solution were transferred into a series of 10 mL boiling test tubes. Citrate buffer (0.5 mL) and ninhydrin (2 mL) were added to each test tube. Then the solution mixture was heated at 100 ± 2 °C for 15 min. The contents were cooled and the volume was made up to the mark with water in 10 mL volumetric flasks. The absorbance was

measured at 597 nm against a reagent blank. Stoichiometric mole ratio of the drug - Riociguat and ninhydrin was determined by Job's method [10]. Developed maximum intensity of colour had deviated from 1:2 and skewed towards higher values of ninhydrin. This observation might be likely due to reasons like photolytic instability, side reactions, slower rate of reaction, and interferences [11, 12].

Chromophore Formation and Chemistry:

Ninhydrin is one of the well-known chromogenic agent which is used to estimate the amount of certain amines, amino acids and thiophenes [13]. Literature shows the successful usage of it for assay of different molecules of pharmaceutical importance and containing nitrogeneous functional groups like amino acids, amines, amides, hydrazine's, piperazines and cyanides. Chemically it is triketohydrindene hydrate. In most of the cases, a typical purple colour formation is the basis for its use in spectrophotometric measurements [14] . Its popular name is Ruhemann's reagent due to formation of Ruhemann's purple [15]. Colour, intensity, reaction and the mechanism involved in colour formation is based on the functional group in the substrate (amino acid, pyrrole, imino acid, primary amine, secondary amine and ammonium salt) [16]. Consecutive oxidative deamination and condensation of the reduced ninhydrin are involved in the formation of Ruhemann's purple colour due to reaction between ninhydrin and pharmaceutical drugs bearing primary amines like gabapentin [17], lisinopril [18], pregabalin [15], Amlodipine [19] and, famotidine [20, 21].

In all the above cases, Ruhemann's purple formation is possible as it happens only whenever there exists at least one hydrogen on the adjacent carbon to amine group. Whereas in the present case, riociguat is an aromatic amine and hence, the involved reaction is entirely different. To substantiate it a thorough literature collection was carried out and listed down.

According to Moubasher [22] and Ruhemann [23], most of the aromatic amines give compound 3a by reacting one aromatic amine molecules with ninhydrin. Substituted anilines form different products compared to aniline. Compound 3b forms due to reaction between two molecules of aniline and one molecule of aniline. These reactions were taken as basis by Robert Suffis [24] for the spectrophotometric estimation of p-phenylenediamines and p-aminophenols with ninhydrin because these compounds form blue or purple colour with high molar absorptivities compared to other anilines. Their high reactivity might be due to existence of a stable quinonoid resonant form 3d with Schiff base (III) 3c which in turn formed by dehydration of 3a (Fig.3). In addition to stability, high intensity and high λmax are also due to the quinoid form.

Midori Yano et al [25] carried out reaction between different substituted aromatic amines with ninhydrin. The nature of the condensation reaction involved depends on the substituents. A carbinolamine 3e was isolated with aniline with removal of one water molecule. But concerned Schiff bases 3f are formed with p-aminophenol and xylidines (2,4- and 2,3-xylidine) with removal of two water molecules.

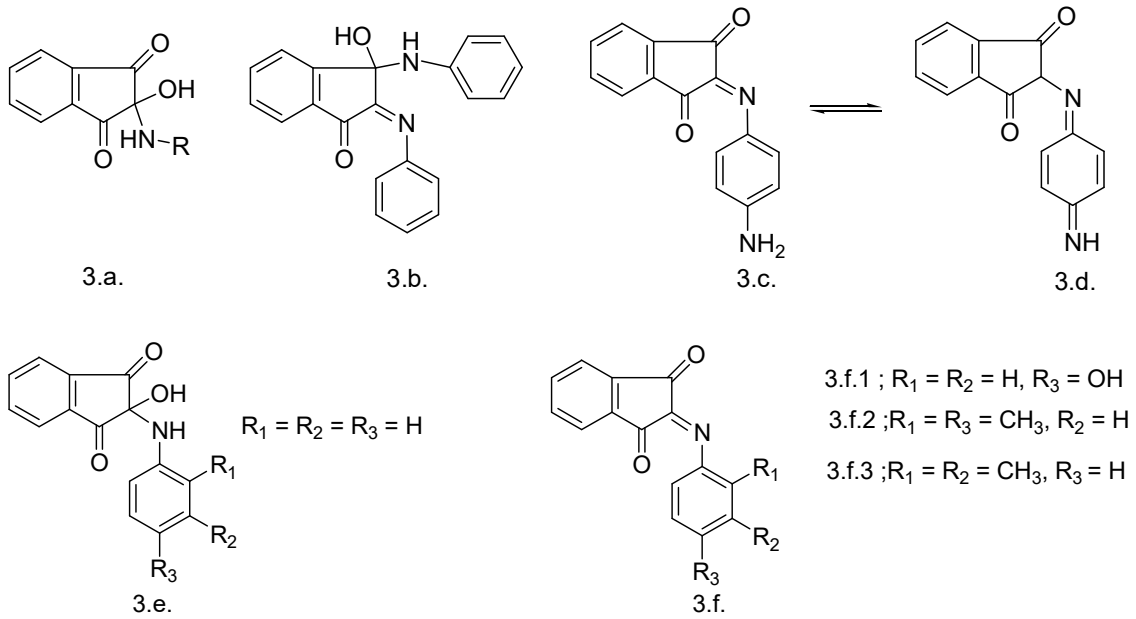


Fig. 3: Products in literature for reaction between ninhydrin and aromatic amines [22-25]

A range of condensation products were reported due to reaction between ninhydrin hydrate and nucleophilic reagents inclusive of aromatic amines [26]. A nucleophilc displacement with a removal of one water molecule occurs due to reaction between ninhydrin hydrate and amino group present in aniline / p-chloroaniline / 2-aminopyridine / m-aminophenol to yield 4a (Fig.4). Whereas, a spontaneous dehydration takes place in the case of o- & p-aminophenol and p-phenylenediamine. Products (4b) stabilization through resonance interactions was considered to be the driving forces for this dehydration [26].

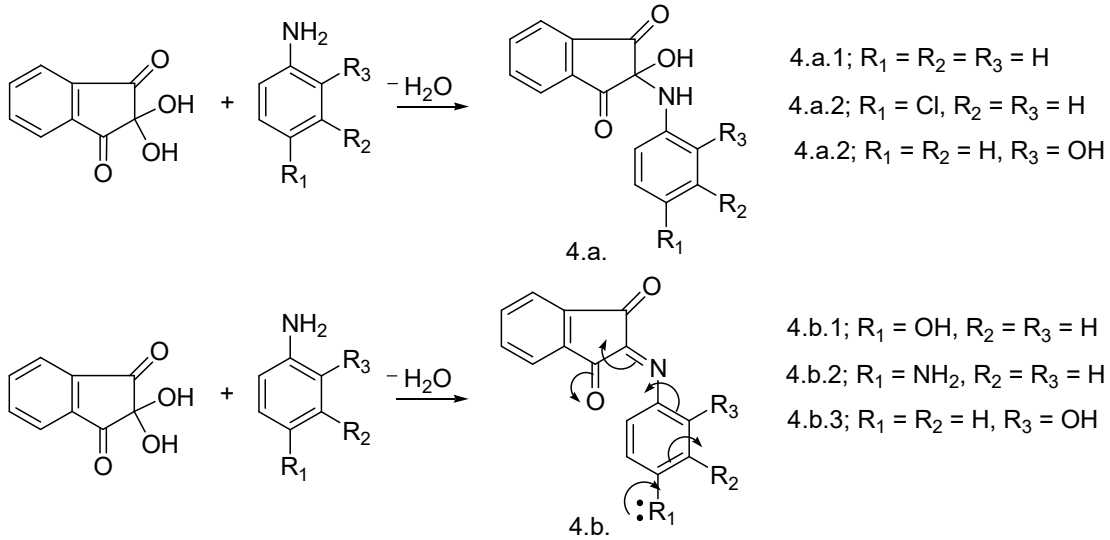
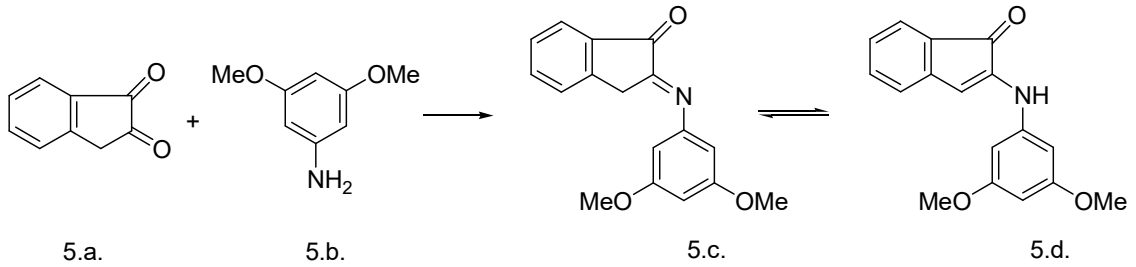


Fig. 4: Reactions between ninhydrin and substituted aromatic amines (Friedman et al) [26]

In similar, formation of a tautomeric mixture (imine 5c and enamine 5d, Fig.5) was reported in the reaction between 1,2-indanediones 5a and 3,5-dimethoxyaniline 5b [27].



5.a. 5.b. 5.c. 5.d.

Fig. 5: Reactions between 1,2-indanediones and substituted aromatic amines (Taylor and Joullié) [27]

But in the present case, such a quinoid form is not formed because, riociguat is meta diamine substituted derivative. However, high intensity as well as λmax can be expected in the present case due to possible attachment of two ninhydrin molecules to a drug molecule which facilitates the extension of conjugation (Fig.6).

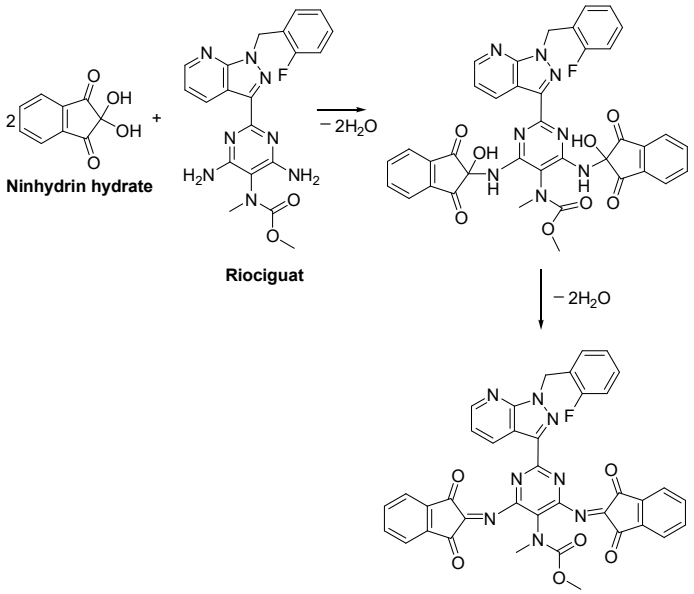


Fig.6: Chromophore formation between ninhydrin and riociguat

Validation of Method

Linearity and range: Linearity of the method was tested in the concentration in the range of 5.0 – 30.0 µg mL^{-1} (Fig. 7) and confirmed from high correlation coefficient (> 0.999). y = 0.0316x+0.001 is the linear regression equation. Optical as well as regression parameters are shown in Table 1.

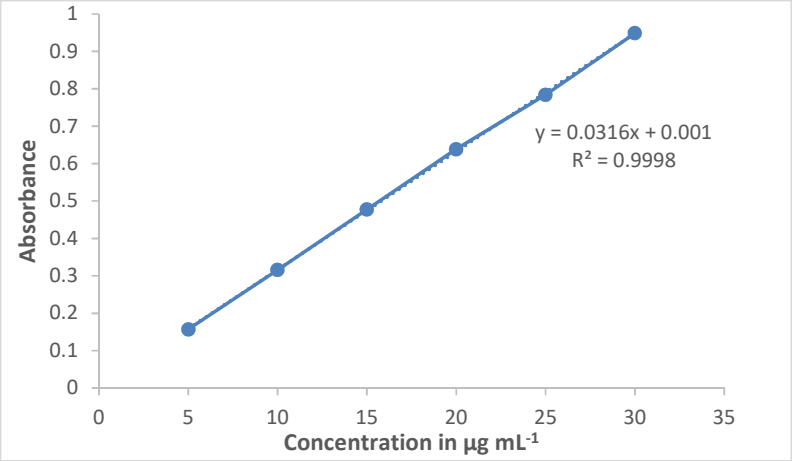


Fig. 7: Calibration graph of ninhydrin and riociguat chromophore

Table 1: Optical, regression and validation parameter values

S. No.	Parameter	Observation
	Optical characteristics	
1.	Apparent molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	1.3×10^4
2.	Sandell's sensitivity ($\mu g\ cm^{-2} A^{-1}$)	0.0316
	Regression analysis	
1.	Slope	0.0316
2.	Intercept	0.001
3.	Regression coefficient (*r*)	0.9998
	Validation parameters	
1.	λ_{max}	597 nm
2.	Beer's Law Limit (Linearity, $\mu g\ mL^{-1}$)	5.0–30.0
3.	Limit of detection ($\mu g\ mL^{-1}$)	0.15
4.	Limit of quantitation ($\mu g\ mL^{-1}$)	0.50
5	Stability period	24 hours

Accuracy: Low values of SD and %RSD are ascertained the accuracy of the method from the studied recovery levels of 50%, 100% and 150%. Table 2 shows the % recovery values range as 99.87 – 100.06.

Precision: Three dissimilar concentrations of Riociguat were chosen to endorse the both precision studies and confirmed the precision from low % RSD values which falls under acceptable limit (below 1%). Table 3 is a compilation of estimated values of six each on the same day in addition to sequential days.

Table 2. Recovery of Riociguat

Level of recovery (%)	Amount of drug recovered (µg mL^{-1}) (Practical)	Statistical evaluation		% Recovery = Practical x 100/ Theoretical
50	14.99	Mean	14.99	99.93
	15.01	SD	0.012	100.06
	14.98	%RSD	0.083	99.87
100	20.01	Mean	19.99	100.05
	19.99	SD	0.012	99.95
	19.98	%RSD	0.062	99.90
150	24.99	Mean	24.99	99.96
	24.98	SD	0.005	99.92
	24.99	%RSD	0.019	99.96

- Selected nominal concentration (a): 10.0 µg mL^{-1}
- Added amount of drug (b): 5.0, 10.0 and 15.0 µg mL^{-1} respectively for 50%, 100% and 150% recovery levels
- Theoretical amount:Total amount of drug (a + b) = 15.0, 20.0, 25.0 µg mL^{-1} respectively for 50%, 100% and 150% recovery levels

Table 3: Precision data

Concentration of Drug (µg mL^{-1})	Concentration*			
	Intraday (Mean ± SD) (µg mL^{-1})	% RSD	Inter-day (Mean ± SD) (µg mL^{-1})	% RSD
5.0	5.001±0.0014	0.028	5.025±0.0011	0.022
15.0	15.008±0.002	0.013	15.025±0.016	0.106
30.0	30.121±0.007	0.023	30.124±0.013	0.043

* Average of six determinations

Ruggedness: Low %RSD values show the reproducibility and hence confirm the ruggedness of the above method (Table 4).

Table 4: Ruggedness data

Test Concentration of Drug (µg mL^{-1})	Concentration* Analyst change	
	Mean ± SD (µg mL^{-1})	% RSD
5.0	5.121±0.008	0.156
15.0	15.024±0.026	0.173
30.0	30.151±0.011	0.036

* Average of six determinations

Detection limits determination: LOQ as well as LOD for the above method are given below from the values of signal to noise ratio [28] and [29].

LOD = $3.3 \times \sigma / S$ = 0.15 µg mL^{-1} and

LOQ = $10 \times \sigma / S$ = 0.50 µg mL^{-1}

Analysis Tablet Formulations: Adempas® (tablet formulation of riociguat) powder was sonnicated for ten minutes in presence of small amount of DMSO to extract the API content present in it. Then the above method was followed to estimate the riociguat present in tablet formulation (Table 5). In general, in industrial quality control units of developing countries, the best option is visible spectrophotometric method for routine analysis by using simple chromogenic reactions like Diazo coupling / oxidative coupling reactions [30-34], red-ox reactions [35], ion pair / ion association / charge transfer complex formation [36-44], in addition to simple UV-Visible spectroscopic methods [45-46]. Hence, ninhydrin can be used as a chromogenic agent to determine the amount of Riociguat in pure and tablet formulations by the above established visible spectrophotometric method.

Table 5. Assay of Pharmaceutical Formulation

Formulation	Labeled amount (mg)	Amount found* (mg)	% Drug Recovered	%RSD
Adempas®	1	1.0102±0.0002	101.02	0.020

* Average of three determinations

Conclusions: The observed high intensity as well as λmax of the generated chromophore might be due to attachment of two ninhydrin molecules to drug molecule which facilitates the extension of conjugation. According to the present guidelines of ICH, the proposed method was validated. In quality control laboratories this method can be applied for routine analysis of Riociguat (bulk drug and tablet formulation) as a substitute to the expensive and sophisticated instrumental methods.

References

1. Mittendorf, J., Weigand, S., Alonso-Alija, C., Bischoff, E., Feurer, A., Gerisch, M., Kern, A., Knorr, A., Lang, D., Muenter, K., Radtke, M. Discovery of riociguat (BAY 63-2521): a potent, oral stimulator of soluble guanylate cyclase for the treatment of pulmonary hypertension. Chem. Med. Chem. 4(5) (2009): 853-865.
2. Ghofrani, H.A., D'armini, A.M., Grimminger, F., Hoeper, M.M., Jansa, P., Kim, N.H., Mayer, E., Simonneau, G., Wilkins, M.R., Fritsch, A., Neuser, D. Riociguat for the treatment of chronic thromboembolic pulmonary hypertension. N. Engl. J. Med. 369(4) (2013): 319-329.
3. Frey, R., Becker, C., Saleh, S., Unger, S., van der Mey, D., Mück, W. Clinical pharmacokinetic and pharmacodynamic profile of Riociguat. Clin. pharmacokinete. 2017: 1-15.
4. Gnoth, M.J., Hopfe, P.M., Czembor, W. Determination of riociguat and its major human metabolite M-1 in human plasma by stable-isotope dilution LCMS/MS. Bioanalysis. 7(2) (2015): 193-205.
5. Grimminger, F., Weimann, G., Frey, R., Voswinckel, R., Thamm, M., Bölkow, D., Weissmann, N., Mück, W., Unger, S., Wensing, G., Schermuly, R.T. First acute

haemodynamic study of soluble guanylate cyclase stimulator riociguat in pulmonary hypertension. Eur. Respir. J. 33(4) (2009): 785-792.
6. Prasad, G.G., Nadh, R.V., Sireesha, M. Determination of Riociguat by Oxidative Coupling using Visible Spectrophotometry. Oriental J. Chem. 35(spl 1) (2019): 48-53, DOI: org/10.13005/ojc/35Specialissue107.
7. Sirisha, P., Sharma, J.V., Nikhitha, S., Likitha, R., Uday, K.B., Durga, P.S. Method development and validation of Riociguat by RP-HPLC. *Int. J. Pharm. Pharm. Scie.* 7(4) (2016): 3060-3062.
8. Temgire, P.R., Sobia, G., Kumar, M.V., Sayali, W., Mohan, S.R., Smita, N., Vaidhun, B. Development and validation of reverse-phase high-performance liquid chromatography method for quantitative estimation of riociguat in tablet dosage form. J. Pharm. Res. 12(4) (2018): 461.
9. Ashok, C.V., Sailaja, B.B., Praveen, K.A. Method development and validation of UV-visible spectroscopic method for the estimation of assay of sugammadex sodium, apremilast, riociguat and vorapaxarsulfate drugs in API form. Asian J. Pharma. Clin. Res. 10 (2017): 241-250.
10. Job, P. Formation and stability of inorganic complexes in solution. Ann. Chim. 2 (1928): 113.
11. Pundarikakshudu, K., Shah, P.B. Spectrophotometric, difference spectroscopic and RP-HPLC methods for the determination of cefixime in pharmaceutical formulations. J. Pharm. Pharm. *Sci.* 89 (2006): 987-994.
12. Wani., Y.B., Patil., D.D. An experimental design approach for optimization of spectrophotometric method for estimation of cefixime trihydrate using ninhydrin as derivatizing reagent in bulk and pharmaceutical formulation. J. *Saudi.* Chem. Soc. 21(2013): S101-S111, http://dx.doi.org/10.1016/j.jscs.2013.11.001.
13. Fiegel., F. Spot Tests in Organic Analysis, Elsevier Publishing Co, London, 1960.
14. Papanna., R.K., Krishnegowda, J.B., Nagaraja, P. Spectrophotometric method for the determination of cefepime, cefazolin sodium and cefalothin sodium in pure and pharmaceutical dosage forms by using ninhydrin. J. Pharm. Pharm. *Sci.* 7(5) (2015): 194-199.
15. Gupta, C., Puri, R., Hussain, A., Jain, S.K. Development and validation of ninhydrin based colorimetric spectrophotometric assay for determination of pregabalin in different dissolution mediums. J. Anal. Chem. 8(2) (2012): 90-98.
16. Mccaldin, D.J. The chemistry of ninhydrin. Chemical Review. 60(1) (1959): 39-52.
17. Abdellatef, H.E., Khalil, H.M. Colorimetric determination of gabapentin in pharmaceutical formulation. J. Pharm. Biomed. Anal. 31(1) (2003): 209-214.
18. Raza, A., Ansari, T.M. Spectrophotometric determination of lisinopril in pure and pharmaceutical formulations. J. Chin. Chem. Soc. 52(5) (2005): 1055-1059.
19. Rahman, N., Azmi, S.N. Spectrophotometric method for the determination of amlodipine besylate with ninhydrin in drug formulations. Il Farmaco. 56(10) (2001): 731-735.
20. Rahman, N., Kashif M. Application of ninhydrin to spectrophotometric determination of famotidine in drug formulations. Il Farmaco. 58(10) (2003): 1045-1050.

21. Hosseinimehr, S. J., Pourmorad, F., Moshtaghi, E., Amini, M. Colorimetric determination of Baclofen with ninhydrin reagent and compare with HPLC method in tablet. *Asian*. J. Chem. 22(1) (2010): 522-526.
22. Moubasher, R. J . Chem. SOC. 1949, 1238-1239.
23. Ruhemann, S. CXXXII.—Cyclic di-and tri-ketones. J. Chem. SOC. Transactions. *97* (1910): 1438-1449.
24. Suffis, R., Levy, A., Dean, D.E. Spectrophotometric Determination of p-Phenylenediamines and p-Aminophenols with Ninhydrin. Anal. Chem. 36(3) (1964): 636-637.
25. Yano, M., Hayashi, T., Namiki, M. Formation of Free Radical Products by the Reaction of Dehydro-ascorbic Acid or Ninhydrin with Aromatic Amines. Agric. Biol. Chern. 42(4) (1978): 809-817.
26. Friedman., M. Mechanism of the ninhydrin reaction. II. Preparation and spectral properties of reaction products from primary aromatic amines and ninhydrin hydrate. Can. J. Chem. 45(19) (1967): 2271-2275.
27. Taylor, B.M., Joullié, M.M. Reaction of 1, 2-indanedione with 3, 5-dimethoxyaniline. Tetrahedron. 54(50) (1998): 15121-15126.
28. Sethi, P.D. HPLC quantitative analysis of pharmaceutical formulations. CBS publications. India. 2001.
29. ICH guidelines., Validation of Analytical Procedures: Text and Methodology. Q2 (R1) (2015): 8-13.
30. Prasad, G.G., Nadh, R.V. Oxidative Coupling: A tranquil approach for determination of selexipag by visible spectrophotometry. Oriental J. Chem. 34(6) (2018) 3112- 3117. DOI: 10.13005/ojc/340656.
31. Prasad, G.G., Nadh, R.V. Diazocoupling for Determination of Selexipag by Visible Spectrophotometry. Int. J. Green Pharm. 12(4) Suppl (2018): S822-S828, DOI: org/10.22377/ijgp.v12i04.2261.
32. Prasad, G.G., Nadh, R.V., Sireesha, M. Ninhydrin based Visible Spectrophotometric Determination of Gemigliptin. Oriental J. Chem. 35(1) (2019): 363-369, DOI: org/10.13005/ojc/350145.
33. Sudhir, M.S., Nadh, R.V. Diazo-Coupling: A facile mean for the spectrophotometric determination of rasagiline hemitartrate. Oriental J. Chem. 29(4) (2013): 1507-1514, DOI:10.13005/ojc/290429.
34. Sudhir, M.S., Venkata Nadh, R., Manjunatha, H., Oxidative coupling reaction for the determination of lurasidone. J. Anal. Chem. 74(6) (2019): 528–533, DOI: 10.1134/S1061934819060121;
35. Prasad, G.G., Nadh, R.V. Res. Determination of mianserine using Fe^{3+}-phenanthroline by visible spectrophotometry. Research J. Pharm and Tech 2019; 12(1) (2019) : 209-212. DOI: 10.5958/0974-360X.2019.00038.6
36. Prasad, G.G., Nadh, R.V. Determination of Mianserine using Tropaeoline-OOO by Ion Pair Formation. Int. J. Appl. Pharmaceutics. 11(1) (2019): 168-173, DOI: 10.22159/ijap.2019v11i1.30125.
37. Prasad, G.G., Nadh, R.V., Kiran, K.K. *Piperacillin* estimation by ion-associative complex formation. *Asian J. Pharm. Clinical Res.* 12(3) (2019): 1-5,

DOI: 10.22159/ajpcr.2019.v12i3.29289.

38. Kiran, K.K., Nadh, R.V., Nagoji, K.E.V. Extractive spectrophotometric determination of nicergoline through ion-pair complexation reaction. Oriental J. Chem. 29(1) (2013): 263-269, DOI:10.13005/ojc/290142.
39. Kiran, K.K., Nadh, R.V., Nagoji, K.E.V. Determination of bendamustine hydrochloride in pure and dosage forms by ion-associative complex formation reaction. Oriental J. Chem. 30(2) (2014): 905-910, DOI:10.13005/ojc/300272.
40. Prasad, G.G., Nadh, R.V., Sireesha, M. Ion Pair Formation for Determination of Mianserine using Fast Sulphon Black F. Asian J. Pharm. 12 (4) (Suppl) (2018): S1390-S1396, DOI: 10.22377/ajp.v12i04.2940.
41. Prasad, G.G., Nadh, R.V., Kiran, K.K. Ion-associative complex formation for estimation of piperacillin. Int. Res. Pharm. Sci. 10(1) (2019): 117-124, DOI: 10.26452/ijrps.v10i1.1787.
42. Prasad, G.G., Nadh, R.V. Extractive spectrophotometric determination of ulipristal acetate using naphthol blue black. Research J. Pharm and Tech. 12(3) (2019): 1347-1352, DOI: 10.5958/0974-360X.2019.00226.9.
43. Prasad, G.G., Nadh, R.V., Kishore V.N.V. Visible Spectrophotometric Determination of Gemigliptin using Charge Transfer Complex. Oriental J. Chem. 35(Special Issue) (2019): 694-699, DOI: 10.13005/ojc/350226.
44. Kiran Kumar, K., Venkata Nadh, R., Siva Kishore, M., Giri Prasad, G. Determination of Satranidazol through Ion-Associative Complex Reaction, Asian J. Org. Med. Chem. 4(1) (2019): 23-27, DOI: 10.14233/ajomc.2019.AJOMC-P166
45. Sudhir, M.S., Ratnakaram, V.N. Simple and validated ultraviolet spectrophotometric method for the estimation of lurasidone in bulk form. Res. J. Pharm. Bio.Chem. Sc. 4(1) (2013): 609-617.
46. Sudhir, M.S., Mohan, P.M., Venkata Nadh, R. Simple and validated ultraviolet spectrophotometric method for the estimation of febuxostat in bulk and pharmaceutical dosage forms. Oriental J. Chem. 29(1) (2013): 235-240, DOI:10.13005/ojc/290137

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