RASĀYAN *J. Chem.*
Vol. 15 | No. 2 |1403-1410| April - June | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

NEW METHOD DEVELOPMENT AND VALIDATION FOR HYDRAZINE IN PANTOPRAZOLE SODIUM SESQUIHYDRATE USING RP-HPLC

SudharshanaCharyulu S.[1,2], T. Krishnamohan[1], N. Sundara Rao[3], V.V.K.P.L.N.Murty[3], Y.L.N. Murthy[3,✉] and J. V. Shanmukha Kumar[2]

[1] Dr. Reddy's Laboratories Ltd. Active Pharmaceutical Ingredients, Chemical and Technical operations-2, Bollaram, Hyderabad-502325, Telangana, India.

[2] Department of Chemistry,KoneruLakshmaiah Education Foundation (KLEF), Green Fields, Vaddeswaram, Guntur, Andhra Pradesh, India-522502.

[3]Department of Chemistry, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India

✉Corresponding Author: murthyyln@gmail.com

ABSTRACT

Pantoprazole sodium sesquihydrate is a white crystalline powder. Quantification of hydrazine through derivatization with salicylaldehyde was investigated by RP-HPLC analysis and found to be accurate and robust in achieving detection limits as low as 3.1 ppm. HPLC analysis of hydrazine was done using Inertsil ODS-3V (250 mm ×4.6 mm) 5µm column, and ammonium dihydrogen phosphate (10 gm) in water (1000 mL) was used as a buffer. The mobile phase used was in the ratio of buffer and methanol (25:75 v/v). With a constant flow rate (1.0 mL/min), elution at 360 nm was monitored. The developed method was validated as per International Conference on Harmonization (ICH) guidelines with reference to system suitability, linearity, precision, accuracy, and robustness.

Keywords: Low-Level Determination, Hplc, Hydrazine, Pantoprazole Sodium Sesquihydrate, Hydrazine.

RASĀYAN*J. Chem.*, Vol. 15, No.2, 2022

INTRODUCTION

Hydrazine is widely used in the pharmaceutical industry as a hydrazine hydrate. Based on International Conference on Harmonization (ICH) guideline for impurities, other than the active moiety remaining impurities must be regulated with appropriate limits in the drug material, regardless of its hazardous character. Hydrazine (N_2H_4) is an inorganic compound that is used to make indazoles[1,2] (a common moiety found in a wide range of small molecule drugs) used in Knorr Synthesis,[3,4] Gabriel Synthesis,[5] and Wolff-Kishner reaction[6] in pharmaceutical drug API processes.[7,8] As a result, the threshold for analytical methods[9-15] to measure hydrazine in low ppm levels is raised. The industry is looking for sensitive ways to properly measure hydrazine during the synthesis process and in the finished drug component. Several studies utilizing HPLC techniques for drug detection and method validation have been published.[16-22] The present research study was focused on the development of a sensitive and rapid HPLC method for the quantification of hydrazine due to its higher selectivity and sensitivity.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade methanol, ammonium dihydrogen phosphate and salicylaldehyde [Merck, Mumbai, India], Hydrazine hydrate analytical grade [Qualigens Chemicals Ltd., Mumbai, India]. Ultra-Pure water [Millipore, Milfordford, MA, USA].

Standard Preparation

Hydrazine hydrate standard 156 mg (equivalent to about 100 mg of hydrazine) is taken in a volumetric flask (10 ml) containing water (5 ml) and diluted to 10ml. 6.25 µL of this solution was transferred into a volumetric flask (100ml), dissolved, and diluted with diluent (methanol) upto 100ml. Again this solution (10 ml) was transferred into a volumetric flask (100ml) containing diluent (50 ml) and added

http://doi.org/10.31788/RJC.2022.1526856
This work is licensed under a CC BY 4.0 license.

Salicylaldehyde (0.5 ml) and cyclomix for 20 minutes and make up with water. This solution contains 6.25 ppm with respect to test concentration.

Sample Preparation

Accurately weighed 100 mg of the test sample is taken into a volumetric flask (10 ml) containing diluent (5 ml), added to 50 µL of Salicylaldehyde and cyclomix for 20 minutes, and makeup to the mark with diluent.

Instrumentation and Method Conditions

Waters HPLC (Model Alliance 2695 separation module) (Waters Corporation, Milford, MA, USA) equipped 2998 Photo Diode Array (PDA) detector was employed. Waters empower software was used to process the data. An isocratic analytical method with Inertsil ODS-3V column (250 x 4.6 mm, particle size 5 mm, GL Sciences Inc., Japan). A mobile phase of 25:75 v/v ratios of buffer and methanol is employed. Buffer preparation consists of dissolving 10 grams of ammonium dihydrogen phosphate into 1000 ml milli-Q water. The temperature at 30°C in the column was maintained. For 40-minute duration, the flow rate (1.0 mL/min) was maintained constantly and at 360 nm column eluent was examined.

Method Validation

The following validation factors have to be examined according to validation recommendations for limit test methods: system suitability, Limit of quantitation (LOQ) and Limit of detection (LOD) precision at LOQ, accuracy at LOQ, linearity, method precision, accuracy at TAL, test and mobile phase stability, range, ruggedness, and robustness.

RESULTS AND DISCUSSION

Method Validation

The developed analytical method was validated for hydrazine impurity as ICH guidelines and parameters such as linearity, LOQ, LOD, accuracy, precision, selectivity, recovery, and ruggedness/robustness, details were illustrated in Tables-1 to16.

Linearity

Linearity of the method was established at a low level as performed by preparing six different solutions (3.1 ppm to 9.4 ppm) from the LOQ to 150% (LOQ, 60, 75, 100, 125, and 150%) of hydrazine impurity in relation to the concentrations of the target analyte. For linear regression analysis, the graph was plotted between peak area and concentration. For hydrazine impurity, the calibration plot was obtained across the calibration ranges tested from LOQ to 150 percent. For hydrazine impurity, the correlation value was 0.998 and is depicted in Fig.-1. The details of the results are presented in Table -1.

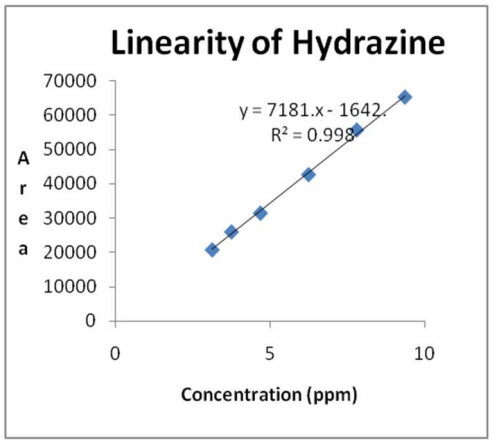

Fig.-1: Linearity of Hydrazine

Table-1: HydrazineLinearity and Graph

Level	Concentration (ppm)	Area
LOQ	3.125	20705
60%	3.7500	25935
75%	4.6875	31389
100%	6.2500	42636
125%	7.8125	55634
150%	9.3750	65207
Correlation Coefficient		0.9991
Slope		7181.78
Intercept		-1642.72
% Y-Intercept		-3.85

System Suitability

Before beginning each validation parameter, prepared standard solution injected to HPLC System in 6 replicates. In a diluted standard solution, the percent RSD of the peak owing to hydrazine impurity was recorded. The results are shown in Table -2.

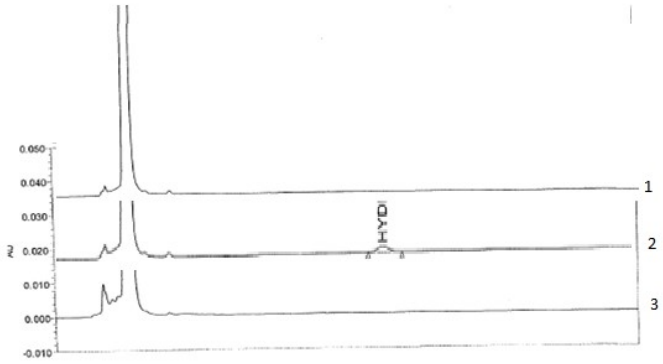

Fig.-2: Chromatogram Overlays Showing derivatized Pharmaceutical Compound
1. Analysis of Blank, 2. Analysis of Target Analytical Level-6.25 ppm Hydrazine Solution
3. Analysis of the Test Sample

Table-2: System Suitability

Reference solution	Area of Hydrazine
Injection-1	41226
Injection-2	40928
Injection-3	42082
Injection-4	41062
Injection-5	41234
Injection-6	41770
Mean	41383.7
STDEV	446.16
% RSD	1.08
Acceptance criteria: The % RSD for the Hydrazine area shall not be more than 5.0	

Limits of Detection and Quantitation

The lowest quantity of the analyte in the sample can be quantitatively measured with precision and accuracy is the limit of quantitation. Formula for calculating LOQ is LOQ = 10σ/S. The values of Theoretical plates. The least concentration that can be detected, but is not always defined as an exact amount, is referred to as the LOD.

The formula LOD = 3.3 σ/S is used to compute LOD

Where, σ= Standard deviation of the response,

S = Slope of the calibration curve.
LOQ gives a signal-to-noise ratio of 10. LOD of hydrazine impurity is 0.0000103 mg/ml (1.03 ppm). Under the same conditions, the LOQ was 0.000031 mg/ml (3.1 ppm). The details of LOQ and LOD are presented in Table-3 and 4.

Table-3: Limit of Detection Results

Impurity	Concentration in mg/ml	Concentration w.r.to test conc. in ppm	S/N Ratio	Acceptance criteria
Hydrazine	0.0000103	1.03	3.0	The signal to noise ratio should be

Table-4: Limit of Quantification Results

Impurity	Concentration. in mg/ml	Concentration w.r.to test conc. in ppm	S/N Ratio	Acceptance criteria
Hydrazine	0.000031	3.1	9.67	The signal to noise ratio should be between 9.5 and 10.4

Precision
Six Individual measures of hydrazine impurity in pantoprazole were performed. For each of the preparations, the impurity content of hydrazine was obtained, and the technique accuracy was assessed by calculating the percentage RSD of the impurity content in six preparations. In order to determine the intermediate precision of the devised technique, experiments were conducted in the same laboratory using another analyst, column, and equipment. %RSD for the content of hydrazine impurity was less than 5.30%, indicating the high precision of the method. The analytical data are presented in Table -5.

Table-5: Precision at Limit of Quantification Level Results

Preparation	Area of Hydrazine at LOQ
1	19629
2	18386
3	18352
4	18883
5	18658
6	18713
Mean	18770
STDEV	466.61
% RSD	2.49
Acceptance criteria: % RSD for the Hydrazine peak area shall not be >10.0	

Accuracy
The impurity was evaluated at three concentrations LOQ, 100%, and 150%.The results are shown in Table -6.

Table-6: Accuracy at Limit of Quantification Level Results

Preparation	% Recovery of Hydrazine
1	98.21
2	100.18
3	97.80
Average	98.73
Acceptance criteria: % recovery should range between 80.0 - 120.0	

Ruggedness and Robustness Test
A robustness evaluation was done throughout the development of the analytical method, as suggested by ICH Guidelines and Dutch Pharmacists Guidelines. The method's robustness is determined by a

comparison of intra and inter-day tests of hydrazine impurity assay findings done by two analysts. These tests of hydrazine impurity in drug compounds done in the same laboratory by two analysts had RSD values of less than 3.8 percent, confirming the method's robustness. The method's resilience was further tested under a range of circumstances, including variations in the eluent's pH, buffer composition, and flow rate. Findings degree of repeatability is found to be the consequence of modest purposeful alterations in the method parameters and analytical operator change has indicated the robustness of the technique. The data is presented in Tables-7, 8, 9, 10, 11, 12, 13, and 14.

Table-7: Ruggedness: System Suitability (1st System, 1st Column, and 1stAnalyst)*

Reference solution	Area of Hydrazine (1st System, 1st Column, and 1st Analyst)*	Area of Hydrazine (2nd System, 2nd Column, and 2nd Analyst)
Injection-1	40353	40185
Injection-2	40521	41090
Injection-3	40995	41079
Injection-4	40834	41095
Injection-5	40694	41647
Injection-6	41089	41502
Mean	40747.7	41099.7
STDEV	281.12	509.63
% RSD	0.69	1.24
Acceptance criteria: The % RSD for the Hydrazine area shall not be more than 5.0		

Table-8: Ruggedness: Hydrazine Precision for Area and the Hydrazine Content (ppm)

Preparation	Hydrazine			
	1st System*,1st Column*, and 1st Analyst*		2nd System, 2nd Column, and 2nd Analyst	
	Area	ppm	Area	ppm
1	41747	6.403	40267	6.123
2	40871	6.269	40277	6.125
3	40526	6.216	40179	6.110
4	40758	6.252	41889	6.370
5	40800	6.272	41831	6.361
6	40892	6.272	41804	6.357
Mean	40932.33	6.278	41041.17	6.241
STDEV	420.019	0.064	877.632	0.33
% RSD	1.03	1.02	2.14	2.14
Acceptance criteria: The % RSD for the area and Hydrazine content shall not be more than 10.0				

Table-9: Robustness: Variation in Mobile Phase Composition

Mobile phase composition	Buffer(mL)	Methanol(mL)
Ideal composition	250	750
90%	325	675
110%	175	825

Table-10: Robustness: System Suitability

Reference solution	Area of Hydrazine (Ideal)	Area of Hydrazine (90% Organic)	Area of Hydrazine (110% Organic)
Injection-1	41226	42580	43457
Injection-2	40928	43495	43698
Injection-3	42082	42456	43739
Injection-4	41062	40838	43788
Injection-5	41234	42834	43187
Injection-6	41770	41410	42255
Mean	41383.7	42268.8	43354.0
STDEV	446.16	937.62	583.30
% RSD	1.08	2.30	1.35
Acceptance criteria: The % RSD for the Hydrazine area shall not be more than 5.0			

Table-11: Robustness: Different Flow Rate

Parameter	Ideal condition	Condition-1	Condition-2
Flow rate	1.0 ml/min	0.8 ml/min	1.2 ml/min

Table-12: Robustness: System Suitability

Reference solution	Area of Hydrazine (Ideal)	Area of Hydrazine (0.8 ml/min)	Area of Hydrazine (1.2 ml/min)
Injection-1	41226	55309	41416
Injection-2	40928	56647	41046
Injection-3	42082	57121	41803
Injection-4	41062	56275	41158
Injection-5	41234	57580	40701
Injection-6	41770	56891	40610
Mean	41383.7	56637.2	41122.3
STDEV	446.16	785.21	446.46
% RSD	1.08	1.39	1.09
Acceptance criteria: The % RSD for the Hydrazine area shall not be more than 5.0			

Table-13: Robustness: Different Temperatures

Parameter	Ideal condition	Condition-1	Condition-2
Temperature	30°C	25°C	35°C

Table-14: Robustness: System Suitability

Reference solution	Area of Hydrazine (Ideal)	Area of Hydrazine (25° C)	Area of Hydrazine (35° C)
Injection-1	41226	44286	45297
Injection-2	40928	44377	44122
Injection-3	42082	44964	44062
Injection-4	41062	44427	44822
Injection-5	41234	43310	44218
Injection-6	41770	44199	44890
Mean	41383.7	44260.5	44568.5
STDEV	446.16	537.42	505.39
% RSD	1.08	1.21	1.13
Acceptance criteria: The % RSD for the Hydrazine area shall not be more than 5.0			

Stability

Test samples and impurity spiked samples are shown to be stable under conditions of room temperature for 48 hours from the time of preparation. The results are shown in Tables -15 and16.

Table-15: Solution Stability Results (Spiked Test Sample)

Impurity Name	Solution Stability		
	Initial	1st day	2nd day
Hydrazine	6.20	6.21	6.21
±Variation(ppm)	-	0.01	0.01

Table-16: Mobile Phase Stability Results

Impurity Name	Mobile Phase Stability		
	Initial	1st day	2nd day
Hydrazine	6.20	6.23	6.21
±Variation(ppm)	-	0.03	0.01

CONCLUSION

Employing a facile derivatization process and RP HPLC method for precise and sensitive quantification of hydrazine in pharmaceutical materials has been developed. The choice of salicylaldehyde as the derivatization agent was an important step in developing this analytical method, which results in a derivatized product that fulfills the particular needs of analytical procedures. The derivatization caused the effective shift to higher UV wavelengths for the resulting hydrazone product, in which API matrix components have not interfered in the analysis. Hence, the developed methodology indicates its high adaptability for routine application.

REFERENCES

1. N. Venugopal, A. Vijaya Bhaskar Reddy, K. Gangadhar Reddy, V. Madhavi, G. Madhavi, *Journal of Pharmaceutical and Biomedical Analysis,* **70**, 592(2012), https://doi.org/10.1016/j.jpba.2012.05.031
2. Safwan Ashour, Soulafa Omar, *Arabian Journal of Chemistry,* **9**, S114(2016), https://doi.org/10.1016/j.arabjc.2011.02.010
3. Deepak Bageshwar, Vineeta Khanvilkarn, Vilasrao Kadam, *Journal of Pharmaceutical Analysis*, **1(4)**, 275(2011), https://doi.org/10.1016/j.jpha.2011.09.012
4. Balasekhara R. Challa, Sai H.S. Boddu, Bahlul Z. Awen, Babu R. Chandu, Chandrasekhar K. Bannoth, Mukkanti Khagga, Kanchanamala Kanala, Rihana P. Shaik, *Journal of Chromatography B,* **878**, 1499(2010), https://doi.org/10.1016/j.jchromb.2010.03.049
5. B.L. Bhaskara, U.R. Anil Kumar, K. Basavaiah, *Arabian Journal of Chemistry*, **4**, 163(2011), https://doi.org/10.1016/j.jchromb.2010.03.049
6. Senthamil Selvan Perumal, Sanmuga Priya Ekambaram, Samundeswari Raja, *Journal of Food and Drug Analysis,* **22**, 520(2014), https://doi.org/10.1016/j.jfda.2014.05.003
7. Meixia Chen, Yu Xia, Zhiyu Ma, Liang Li, Dafang Zhong, Xiaoyan Chen, *Journal of Chromatography B,* **906**, 85(2012), https://doi.org/10.1016/j.jchromb.2012.08.019
8. Cherukuru Nagaraju, Uttam Kumar Ray, Sharma Hemant Kumar and Siddaiah Vidavalur, *Der Pharmacia Letters,* **7(11)**, 286(2015).
9. Jin-Aa Oh, Ho-Sang Shin, *Journal of Chromatography A,* **1395**, 73(2015), https://doi.org/10.1016/j.chroma.2015.03.051
10. Gracy Elias, William F. Bauer, *Journal of Separation of Science,* **29(3)**, 460(2006). https://doi.org/10.1002/jssc.200500380
11. Jenny Wang, Samuel Yang, Kelly Zhang, *Journal of Pharmaceutical and Biomedical Analysis,* **126**, 141(2016), https://doi.org/10.1016/j.jpba.2016.04.038
12. Lan Yu, Xinshen Zhang, Linyun Yu, C*ommun. Information Science and Management Engineering*, **4(1),** 13(2014).
13. Mary George, K. S. Nagaraja, N. Balasubramanian, *Indian Journal of Chemistry,* **46A**, 1621(2007).

14. K. Venkata Rao, Raja Gorla, B. Sreenivasulu, N. Sreenivas, Sharma Hemant Kumar, Raghubabu Korupolu, *International Journal of Pharmacy and Pharmaceutical Sciences,* **6(9)**, 119(2014).
15. David T. Fortin and Raymond Chen, *Journal of Chromatographic Science*, **48(4)**, 299(2010), https://doi.org/10.1093/chromsci/48.4.299
16. Boyka G. Tsvetkova, P. Lily, *Journal of Chemical and Pharmaceutical Research*, **5(1)**, 168(2013).
17. Olabemiwo, Ojeyemi Mathew, Mahesh, Pottabattula Rao, A.B.N. Nageshwarrao, V. Jayathirtha, *Asian Journal of Chemistry*, **24(6)**, 2762(2012).
18. B. Brahmaiah, K. Sujana , A. Prameela Rani, Asian *Journal of Pharmaceutical Clinical Research*, **6(1)**, 23(2013).
19. Shradhanjali Singh, Kirti S. Topagi, Mrinalini C. Damle, *Research Journal of Pharmacy and Technology*, **2(4)**, 749(2009).
20. Bilal Yilmaz, *International Journal of Pharmaceutical Sciences Review and Research*, **1(1)**, 39(2010).
21. Lakka Narasimha Swamy, Nishant Goswami, Penugonda Balakrishna, Vaddi Sailaja, International Journal of Research in Pharmaceutical Sciences, **2(4)**, 608(2011).
22. A. Patel, C Macwana, V. Parmar, S. Patel, *Journal of AOAC International*, **95(2)**, 419(2012).

[RJC-6856/2020]