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Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form

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Abstract

A highly sensitive, selective, reproducible, rapid and stability indicating RP-HPLC and spectrophotometric method has been developed and validated successfully for analysis of a new anti angina agent Ivabradine HCL in solid dosage form. Separation and detection of Ivabradine HCL by HPLC was achieved by Inertsil ODS-3V [250 mm x 4.6mm] 5µ column and U.V. detector at λ 286 nm respectively. In HPLC method the retention time was about 7 minutes. Complete validation study for both the methods was carried out according to ICH guideline. Linearity of both the methods was achieved in the range 4.2 to 31.6 µg mL^{-1} with a correlation coefficient (r^2) ≥ 0.999. The limit of detection and the limit of quantification were 0.06 µg mL^{-1} and 0.2 µg mL^{-1} respectively. The intra-day and inter-day precision and accuracy values for both methods were within the assay variables as per the ICH guideline. The HPLC method can be used for the routine quantitative determination and stability study of Ivabradine HCL in Pharmaceutical dosage forms, since it proved to be stability indicating also.

Keywords:

Ivabradine HCL, Pharmaceutical Dosage Form, RP-HPLC. Spectrophotometric, UV detector

1. Introduction

Ivabradine HCL is a novel medication used for symptomatic management of stable angina pectoris. Ivabradine acts by reducing the heart rate in a mechanism different from beta-blockers. It acts on the I_f ion current, which is highly expressed in sinoatrial node. I_f is a mixed Na^+ - K^+ inward current activated by hyperpolarisation and modulated by autonomic nervous System. Preliminary animal study indicate that ivabradine unlike beta- blocking agents, does not have any vasodilatory effect on inotropic properties[3, 4, 5, 6]. Originally a liquid chromatography (LC) method using fluorimetric detection and a LC method using mass spectrometric detection was validated to quantify compounds in urine and plasma respectively.[1, 2] No method for determination and quantification of Ivabradine in bulk drug and pharmaceutical dosage form has been reported. There fore it is very important to have a specific, selective, reliable and cheap method for determination of Ivabradine in bulk drug and pharmaceutical dosage form. In this paper we describe a very simple yet rapid, selective, and highly sensitive HPLC and spectrophotometric method, not requiring sample treatment, for

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determination of Ivabradine. We have also laid emphasis on the stability indicating assay method of the same by HPLC.

2. Experimental

2.1. Materials

Ivabradine HCL, Active Pharmaceutical Ingredient (API) and working standard was supplied by Cadila Health Care Limited (Ankleshwar, India). Tablets of Ivabradine as a marketed formulation Ivangin (5 mg) was provided by Zydus Cadila Health Care Limited (Ahmdabad, India)

2.2. Chemicals and Reagents

GR Grade Formic Acid of, MERCK Ltd. India, HPLC Grade Ammonia Solution of, Spectrochem Pvt. Ltd. India, HPLC Grade Methanol of, Spectrochem PVT. Ltd. India

HPLC Grade Acetonitrile of, Spectrochem PVT. Ltd. India

2.3. Apparatus and Equipment

Photo stability chamber, Hot air oven: Proto-Tech oven, Analytical balance: AX205, METTLER TOLEDO., pH Meter: Thermo Orion, model 420, Sonicator: Oscar Ultra Sonics, OU-72(SPL), UV 1700, Shimadzu

2.4. Chromatographic Conditions

The HPLC system (Shimadzu Corporation, Japan), model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010, Shimadzu), UV-VIS detector (LC 2010C UV PHARMASPEC 1700), Shimadzu and CLAS-VP software version==SPI, binary pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and Chem station (software). The chromatographic parameters are shown in Table 1.

Table 1. The chromatographic Parameters

Column	Inertsil ODS-3V [250 mm * 4.6mm] 5µ
Detector	286 nm
Injection Volume	10 µL
Flow Rate	0.7 mL min^{-1}
Temperature	30º C
Run Time	12 minutes
Mobile Phase	0.5% Formic Acid (pH=7.0): Acetonitrile (65: 35 v/v)
Diluent	Methanol

2.5. Preparation of Mobile Phase

5 mL formic acid in 1 L Milli Q water (0.5%) with pH 7.0 done by concentrated Ammonia solution was the buffer. The composition of mobile phase was adjusted to maintain the separation conditions using stressed samples.

Forced degradation study was performed using the buffer pH 7.0 of mobile phase. Acetonitrile was used to reduce the run time. The ratio of buffer and acetonitrile was finalized

as 65:35 v/v after analyzing all the degraded samples and identifying the peak purity, specificity, and a stability indicating nature of the method

2.6. Preparation of Stock and Standard solution

The standard stock solution of Ivabradine HCL (500 μg mL^{-1}) was made in 200 ml volumetric flask. 10 ml of 0.1 N HCL was added and made volume up to the mark with Water: Acetonitrile (50: 50 v/v). Further dilution was made in Methanol to achieve 20 μg mL^{-1}. This was used as standard for both the methods HPLC and Spectrophotometric.

2.7. Preparation of test solution for Assay

Accurately 20 intact tablets were weighed to determine average weight of tablets. Tablets powder eq. to 40 mg of Ivabradine was weighed and transferd in to 200 mL volumetric flask. 10 ml 0.1 N HCL was added and sonicate for 10 minutes. 100 mL of Water: Acetonitrle (50: 50 v/v) was added and sonicate for another 20 minutes. The solution was cooled and volume made up to the mark with Water: Acetonitrle (50: 50 v/v). This was filtered through 0.45μ (Pressure and Vacuum Driven Fiteration - PVDF Millipore Fiter). A further dilution was made in Methanol to achieve 20 μg mL^{-1}. This was used as sample solution for both the methods.

2.8. Stress Studies

In order to prove the selectivity of analytical method, the Ivabradine HCL API, Placebo and its formulation were studied under various stressed conditions to perform forced degradation studies. Stress studies were carried out under the condition of acid/base hydrolysis, oxidation, thermal, humidity and photolytic, as mentioned in ICH guidelines[7, 8]. The stress conditions are mentioned in Table 2.

Table 2. Degradation Conditions

Condition	Detail
Thermal	105º C for 24 Hrs
U.V.	254 nm for 48 Hrs
Acid	2 mL 5 N HCL and heated for 30 min at 60º C
Alkali	2 mL 5 N NaOH and heated for 30 min at 60º C
Oxidation	(i) 2 mL 0.3% H_2O_2.Heated for 30 min at 60º C (ii) 2 mL 3% H_2O_2.Heated for 30 min at 60º C
Humidity	40 ºC / 75% RH for 24 Hrs

3. Result and Discussion

Literature review revealed that since the drug is very new so there is no method developed for analysis of Ivabradine HCL in solid dosage form. The methods given here were found to be highly specific and linear. The peaks of the active ingredients and other additives as well as the degradation products were separated out by the HPLC method. Therefore, HPLC method is highly specific and stability indicating for degradation products and formulation excipients. Both methods can apply for routine analysis of Ivabradine HCL in solid dosage form. HPLC method was also found to be stability indicating and very specific for estimation of Ivabradine HCL in presence of other degradation products and various excipients used in solid dosage form.

Complete validation studies for both the methods proved them to be specific, linear, robust and reproducible.

3.1 Method Validation

Validation was done with respect to various parameters, as required under ICH guideline [7, 8, 9]. The method was validated with respect to parameters such as linearity, precision, accuracy, specificity, solution stability, ruggedness and robustness.

3.2. System Suitability

System Suitability was daily performed during the entire validation by both the methods. The system suitability and system precision results of HPLC method, are given in Table 3.

Table 3. System suitability and System Precision

Retention time (Mean ± SEM)	Theoretical Plates (n)	Capacity factor (k')	Asymmetry/Tailing (T)
8.67 ± 0.0376	10990.42	85.12	1.29

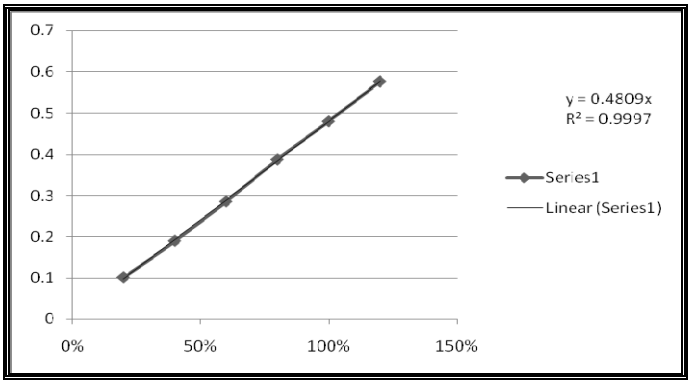
SEM = Standard error mean (Standard deviation / Under rout N (comment: SEM is a step ahead of standard Deviation)
RSD in Spectrophotometric Method (n=5) = 0.4%
RSD in HPLC Method (n=5) = 0.1%

3.3. Linearity

To achieve linearity and range for both the methods, stock solution of 500 μg mL^{-1} was diluted to yield solutions in the range 4.2 to 31.6 μg mL^{-1}. The solutions were prepared in triplicate and analyzed by using 10 μL into HPLC. The linearity and range results of both the methods are given in Table 4. The calibration curve of U.V. method and HPLC method are shown in Fig.1 and Fig.2, respectively.

Table 4. Characteristics of the method from Standard calibration curve

	U.V	HPLC
Correlation (r^2)	0.99974	0.9989
Residual std.	0.00460	1990.9926
Slope of Range	0.0161362	12313.05138



X Axis = concentration in % Y Axis = UV Absorbance (QAs)

Fig 1. Calibration Curve of Ivabradine HCL in U.V. Spectrophotometric method

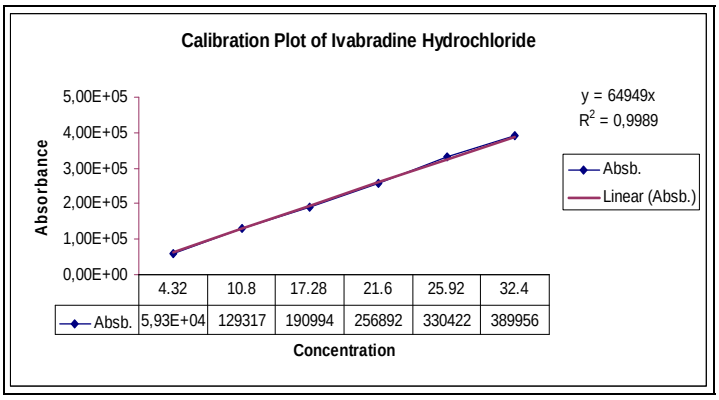


Fig 2. Calibration Curve of Ivabradine HCL in RP-HPLC Method (QAs)

3.4. Specificity

Specificity of developed method was established by determining peak purity of active component in standard preparation, test preparation and spiked sample preparation using PDA detector. Interference of placebo was showed within 2% by the U.V. spectrophotometric method, there by proving it to be specific and suitable for analysis of Ivabradine HCL in solid dosage form.

In HPLC method there was no interference from placebo and peak purity of Ivabradine HCL was found to be 0.998. Results of specificity of HPLC method are shown in Table 5. Overlay chromatograms, indicating the specificity are shown in Fig. 3.

Table 5. Peak Purity in HPLC method

Sets	3 point peak purity
Standard Solution	0.99785
Placebo spiked with API	0.99888
Test Solution	0.99849

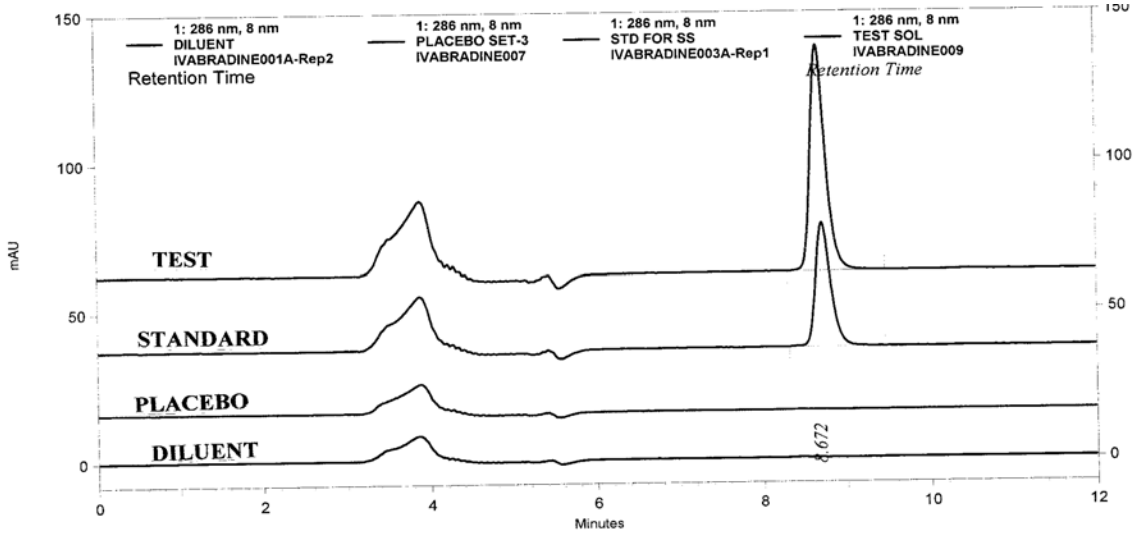


Fig.3. HPLC – Overlay chromatograms of Diluent, Placebo, Standard and Test

3.5. Stability indicating nature of the Developed Method

Placebo preparation, Ivabradine HCL API, and tablets exposed to various stress conditions showed the peak purity as that of the normal condition. The degradation study clearly indicated that Ivabradine HCL degrades in acid and oxidation condition and it also degrade in photolytic condition. The overlay chromatograms of Ivabradine API degradation and Tablets degradations were shown in Figure IV and Figure V respectively. The degradation results were shown in Table V. The results clearly indicate that there is no merging of the impurity peak with the main peak of drug. The % assay of the degraded samples sum up with % of observed impurities peaks (by area normalization) bring close to 100%, suggests the HPLC method to be stability indicating.

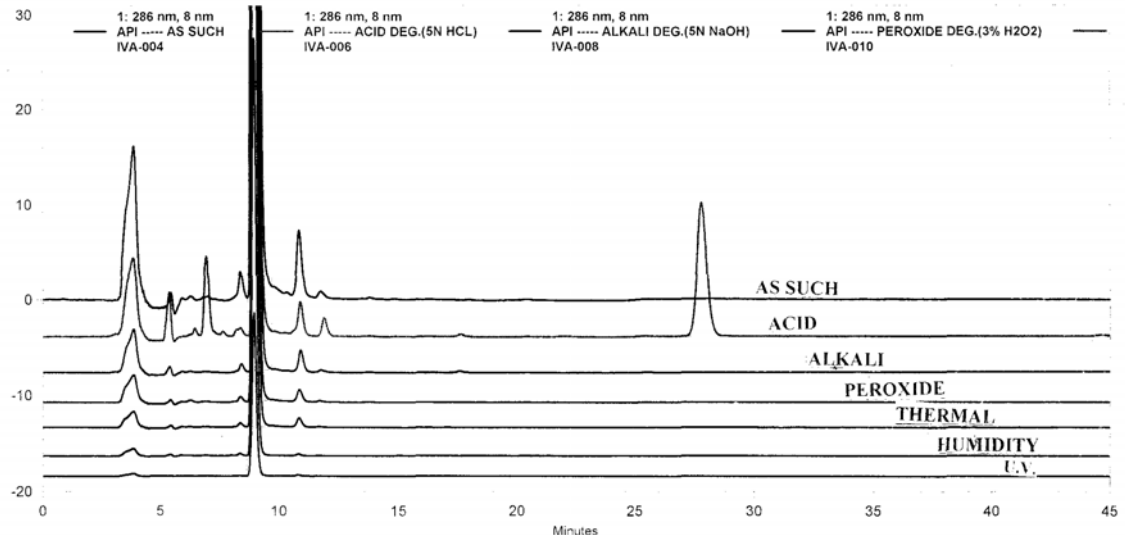


Fig 4. Overlay Chromatograms of API degradation

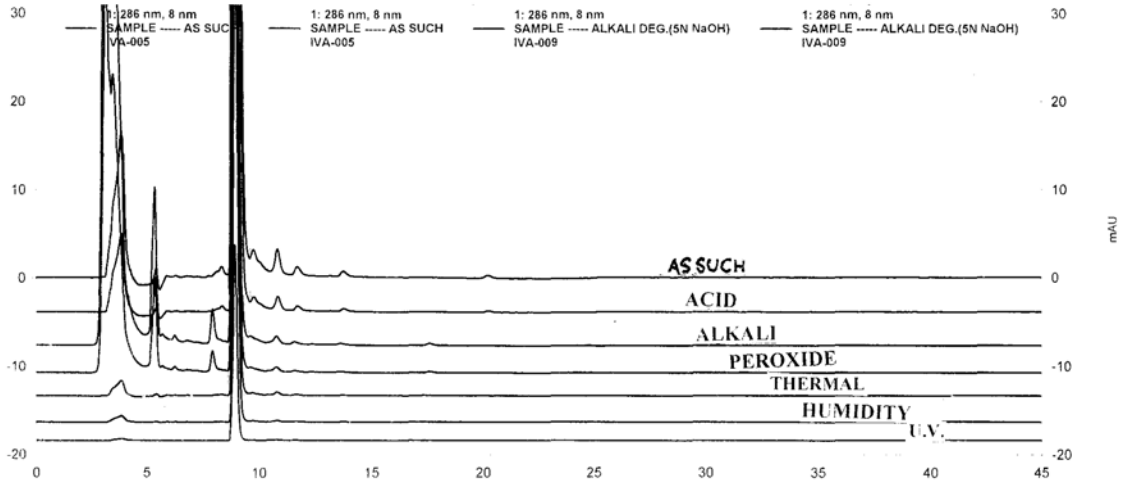


Fig 5. Overlay Chromatograms of Sample degradation

3.7. Method Precision

The method precision (repeatability) was obtained by determining the assay by preparing six-sample preparation. The low value of standard deviation proved the method to be very precise. The method precision both Interday and Intraday results in Table 7.

Table 6. Forced Degradation

S. No	Treated Parameters	Assay%	Total Impurity%	3 Point Peak purity
1.	As Such	100.0	0.76	0.9989
2.	Thermal 105°C 24 hrs	100.0	0.79	0.9989
3.	Acid-2mL 5N HCL(Heat)	88.0	10.96	0.9959
4.	Alkali-2 mL 5 N NaOH (Heat)	99.7	1.00	0.9965
5.	Oxidation-2 mL 3% H_2O_2 (Heat)	90.0	3.71	0.9981
6.	U.V. light 48 Hrs. 254 nm	99.8	0.72	0.9981

Table 7. Method Precision

	U.V	HPLC
Intraday Precision		
Assay (Mean ± SEM)	101.5 ± 0.4224	0.2097
% RSD of Assay	1.0%	1.1%
Interday Precision		
Assay (Mean ± SEM)	105.0 ± 0.2651	101.8 ± 0.4490
% RSD of Assay	1.3%	0.5%

3.8. Method Ruggedness

Ruggedness test was determined by two different analysts and instruments in U.V. method while two different analysts, instruments and columns in HPLC method. The results of both the methods are shown in Table 8.

Table 8. Method Ruggedness

	U.V.		HPLC	
	Assay (Mean ± SEM)	RSD	Assay (Mean ± SEM)	RSD
Day I: Analyst I, Instrument I & Column I	101.5 ± 0.4242	0.6%	101 ± 0.2189	0.3%
Day II: Analyst II, Instrument II & Column II	102.3 ± 0.2556	0.56%	102.0 ± 0.4540	1.1%

3.10. Method Recovery

To ensure the reliability and accuracy of the method recovery studies were carried out in triplicate at 50%, 100% and 150% of target concentration. Results of accuracy study are within the range of 98% to 102% and RSD < 1% in both the methods. Recovery results of UV method and HPLC method are shown in Table 9 and Table 10 respectively.

Table 9. U.V. Method Recovery (QAs)

Level	Amount of Drug Added (mg mL^{-1})	Recovery (%)	Mean ± SEM(%), n=3	%RSD
50%	23.0	99.0	99.0±0.3551	0.8%
	23.6			
	23.9			
100%	43.8	98.9	98.9±0.2160	0.5%
	43.5			
	43.8			
150%	65.0	99.9	99.9±0.0849	0.2%
	65.1			
	64.9			

Table 10. HPLC Method Recovery (QAs)

Level	Amount of Drug Added (mg mL^{-1})	Recovery (%)	Mean ± SEM(%), n=3	%RSD
50%	23..2	100.7%	100.7±0.3958	0.9%
	23.5			
	23.2			
100%	40.8	100.9%	100.9±0.0987	0.3%
	40.7			
	40.5			
150%	61.8	99.2%	99.2±0.0214	0.2%
	61.7			
	61.9			

3.11. Method Robustness

Robustness of the method was determined by small deliberate changes in flow rate, organic phase ratio, buffer pH and column oven temperature. Even after these changes content of the drug did not affect adversely. The low values of relative standard deviation indicating that the HPLC method is robust In the U.V. method the wavelength and instrument was changed in order to study robustness. The results of U.V. Method and HPLC Method are shown in Table 11 and Table 12, respectively.

Table 11. Method Robustness (U.V.)

	Condition	RSD,%
Wavelength	Normal	0.62
	-2 λ	0.21
	+2 λ	0.56

3.12. Solution Stability

Standard and sample solutions both in U.V. and HPLC methods were evaluated at room temperature for 24 Hours. The solutions were analyzed after 2, 4, 6, 12, 18 and 24 Hours the relative standard deviation was found to be below 2.0% in both the methods. It proves the solution stability of standard and sample solution at room temperature. The results of solution stability U.V. method and HPLC method are shown in Table 13 and Table 14, respectively.

Table 12. Method Robustness (HPLC)

	Condition	RSD,%
Mobile Phase ratio	Normal	0.33
	-2%	0.30
	+2%	0.33
Flow Rate	Normal	0.33
	-10%	0.26
	+10%	0.23
pH	Normal	0.33
	-0.2 units	0.12
	+0.2 units	0.20
Temperature	Normal	0.33
	+5º C	0.31
	-5 º C	0.28

Table 13. Solution stability of U.V. Method

Time	Std Absorb	% Difference	Sample Absorb	% Difference
Initial	0.335	---	0.345	---
6 Hrs	0.335	0.0	0.344	-0.3
10 Hrs	0.336	0.3	0.347	0.6
18 Hrs	0.338	0.9	0.349	1.2
24 Hrs	0.340	1.5	0.350	1.4

Table 14. Solution Stability of HPLC Method

Time	Std Absorb	% Difference	Sample Absorb	% Difference
Initial	286943	----	318766	----
6 Hrs	286943	0.0	318847	0.0
10 Hrs	286568	-0.1	319359	0.2
18 Hrs	280743	-1.9	329156	0.4
24 Hrs	285359	-0.6	320037	0.4

3.13. Assay

Both the developed and validated methods were successfully applied for the estimation of Ivabradine in tablet dosage form. The assay results was100%. The mean retention time was about 8.6. The results of assay indicate that the method is specific for the analysis of Ivabradine without interference from the excipients used to prepare and formulate these tablets

3.14. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection (LOD) determines the lowest limit that could be detected by the detector immaterial of its quantification. Limit of Quantification determines the lowest concentration with acceptable precision and accuracy. The LOD came to be 0.06 µg mL^{-1} and LOQ came to be 0.2 µg mL^{-1}.

4. Conclusion

The above-developed methods are suitable for determination of Ivabradine HCL in solid dosage form. Both methods were found to be highly sensitive, precise, accurate for determination of Ivabradine HCL in pharmaceutical dosage form. These methods are quite useful and reasonably satisfy all the standards. Therefore these methods can be used for routine analysis of Ivabradine HCL in pharmaceutical dosage form. More over the HPLC method has also proved to be stability indicating because it can separate degradation peaks from the man peaks and accurately quantifies it in the stability samples.

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