

Research Article

A Validated Bioanalytical Method for Quantification of Ziprasidone in Human Plasma by RP-HPLC: Application to a Pharmacokinetic Study.

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ABSTRACT

A simple and robust analytical reversed-phase high-performance liquid chromatography method was developed and validated for Ziprasidone (ZPS) with Albendazole as internal standard drug. The method was developed in human plasma and dosage formulation with high-quality chromatographic separation between the drug peaks by using a stainless steel Reverse Phased analytical column C18 (25 cm, 4.6 mm id, 5 mm particle size). The system was operated at 25°C using a mobile phase consisting of Phosphate buffer: acetonitrile [60:40, v/v] with pH adjusted 2.5 with ortho phosphoric acid in isocratic flow rate of 1 mL min⁻¹ with ultraviolet detection, UV 730 D monitored at 230 nm. The parametric statistics, i.e., correlation coefficient of 0.999, was assessed for all the drugs having linearity over the tested concentration range (15-500 ng mL⁻¹) in plasma using calibration curve. The accuracy of samples for six replicate measurements at lower limit of quantitation level was within limit. The method was applicable for the quality control of the mentioned drugs in raw material, bulk drug and pharmaceutical formulations as well as in pharmacokinetic studies.

KEYWORDS

Ziprasidone, Bioanalytical Method Validation, RP-HPLC

1. INTRODUCTION

Ziprasidone (Figure-I) is the fifth atypical antipsychotic drug¹ approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia, and acute mania and mixed states associated with bipolar disorder. Its intramuscular injection form is approved for acute agitation in schizophrenic patients. Ziprasidone is also used off label for depression, bipolar maintenance, and post-traumatic stress disorder². The oral capsule form of ziprasidone is the hydrochloride salt as ziprasidone hydrochloride. The intramuscular form, on the other hand, is the mesylate salt, ziprasidone mesylate trihydrate, and is also provided as a lyophilized powder. Ziprasidone is a full antagonist of D2 receptors and of 5-HT_{2A} receptors and is a partial agonist of 5-HT_{1A} receptors and a partial antagonist of 5-HT_{2C} receptors and 5-HT_{1D} receptors³. Chemically it is belonging to benzisoxazole derivatives have chemical name is 5-[2-[4-(1, 2-benzisothiazol-3-yl)-1-piperazinyl] ethyl]-6 chloro-1,3-dihydro-2H-indol-2-one. Its molecular formula is C₂₁H₂₁ClN₄OS and its molecular weight is 412.94^{4,5}. Literature survey reveals that, there are various methods reported for quantification of Ziprasidone by dielectric spectroscopy⁶, estimation in pharmaceutical formulation¹⁴, HPLC in pharmaceutical dosage form⁷⁻¹⁰, Liquid- liquid extraction by internal standard¹¹, LC-MS method¹², Method Validation Manager (MVM)¹³, RP-HPLC with densitometric HPLC with spectrophotometric detection¹⁵, chemometric study and quantitative structure retention relationship modelling of liquid chromatography¹⁶, Solid Phase Extraction with HPLC¹⁷, Simultaneous determination of five antipsychotic drugs in rat plasma¹⁸, HPLC with pharmacokinetic study in rat plasma¹⁹, UPLC²⁰, LC-MS-MS²¹.

1.1. Need of Method Validation

Although sensitive, the previously mentioned methods lack simplicity in the sense that they use sophisticated instruments such as HPLC–MS-MS and UPLC–MS-MS, and require a high volume of plasma and lengthy extraction procedure that leads to high cost. Moreover reported methods in the literatures have issue of sample recovery and longer run time. Thus there was a need to develop a new, simple, rapid, economical, precise and accurate analytical method with special emphasis on sample recovery for those components which are generally have lower pKa Value by protein precipitation or liquid-liquid extraction in biological matrix as well as pharmaceutical formulation along with its validation study. This work describes the validation parameters stated by the International Conference on Harmonization (ICH) guidelines which include specificity, precision, linearity, accuracy, range, stability of analytical solution, robustness and system suitability suitably extracted from human plasma.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

A reference Internal standard Albendazole was received as gift sample from Blue Cross and Ziprasidone bulk was gifted from Zydus Cadila which were characterized by physicochemical characteristics like solubility, M.P., λ^{\max} and these were considered as pure drug. The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. Potassium dihydrogen orthophosphate, Trichloroacetic Acid (TCA) triethylamine, hydrochloric acid, ortho phosphoric

acid, methanol, acetonitrile, (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

2.2. Instrument and Chromatographic conditions

Younglin (S.K) gradient system UV Detector with Autochro-3000 database software, RP C₁₈ column (250×4.6 mm), particle size 5μ) was used. The separation was performed with freshly prepared mobile phase consist of Phosphate buffer: acetonitrile [60:40, v/v] with pH adjusted 2.5 with ortho phosphoric acid at a working temperature of 25°C.

The phosphate buffer was 0.05 M KH₂PO₄ solution with an addition of 10 mL triethylamine/L solution, adjusted to pH 2.5 with ortho phosphoric acid. Both acetonitrile and phosphate buffer were filtered through 0.45 μ membrane filter and sonicated before use. The flow rate was 1.0 mL/min, and elute was monitored at 230 nm.

2.3. Biological matrix employed in bioanalysis

Various methods have been employed for extraction of drug from plasma which include A. Liquid-liquid extraction (LLE), B. Solid phase extraction (SPE) and C. Protein Precipitation method²². Protein precipitation is the simple method of extraction as compared to the LLE and SPE. This can be carried out by using the suitable organic solvents which has good solubility of the analyte and protein precipitating properties. Acetonitrile is the first choice of solvent for protein precipitation due to its complete flocculent precipitation of proteins which settles down after centrifugation at high speed and gives clear supernatant. The aim of Protein precipitation is to minimize the protein interferences form plasma and serum and subsequently reduces the deterioration of HPLC columns.

2.4. Preparation of standard solution

The protein precipitation was carried out by 1 part of plasma (300 μl drug free human Plasma), 0.1 part of drug (30 μl having concentration 10 ppm) and 0.05 parts of I.S. (15 μl having concentration 10 ppm). Plasma previously spiked with I.S.in a 1.5 mL capacity micro centrifuge tube. The blend was subjected to vortex for ~3.0 minute. The mixture was allowed to stabilize for 2 minutes, then 100 μl cold ACN was added and mixture was subjected to vortex for 10 minutes followed by centrifugation for 10 min at 15,000 rpm at 4°C. About 200 mL of supernatant was collected and mixed with equal volume of 0.1% phosphoric acid (pH 2.5) and subjected to vortex for 3 min. About 20 mL of the buffered supernatant was injected into the HPLC system. At the end of precipitation, supernatant was mixed with buffer of mobile phase to make the sample more compatible with the developed method. The % mean recoveries for all the analyte were ranging from 90 to 95% in the developed method.

2.5. Bioanalytical method validation

The developed HPLC conditions were validated as per the ICH guideline for bioanalytical method validation. The developed method was validated for its specificity, sensitivity, calibration range, recovery, precision and accuracy and stability as per the bioanalytical method validation guideline suggested by ICH.

2.5.1. Specificity

Specificity of the method is the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample. For this method blank plasma from five different lots and spiked plasma samples were analyzed. Selectivity was established by injecting six samples at the lower limit of quantitation (LLOQ) level and each of the six blank plasma samples were tested for interference by comparing the mean peak response obtained by injecting blank plasma samples with that of mean peak response of LLOQ (15 ng/mL for ZPS and 25 ng/mL for ABZ). Representative chromatograms were generated to show that other components that could be present in the sample matrix are resolved from the selected analyte.

2.5.2. Calibration curve

A calibration curve was prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. The standard calibration curve was determined on each day of the 6-day validation period; the slope, intercept and the correlation coefficient were determined. The calibration curve had to have a correlation coefficient (r^2) of 0.999 or better.

2.5.3. Sensitivity

The sensitivity (LLOQ) was determined by signal-to-noise ratio. The resolution solution were serially diluted and spiked to the plasma, and injections were made to obtain chromatogram. Similarly, blank plasma samples were also processed and injected into chromatograph. The LLOQ was expressed for the analyte concentration having a response at least five times more compared with a blank response.

2.5.4. Precision and accuracy

The precision of an analytical method describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple aliquots of a single homogeneous volume of biological matrix. Precision should be measured using a minimum of five determinations per concentration. A minimum of three concentrations in the range of expected concentrations is recommended. These concentrations were chosen from the low, medium and high range of the standard curve as quality control (QC) samples. Plasma samples spiked at three concentrations, that is, low QC (LQC), medium QC and high QC (HQC) (25, 50 and 100 ng/mL Ziprasidone while 50, 100 and 200 ng/mL for Albendazole), were analyzed at each day of the 6-day validation. Intraday precision, interday precision and the repeatability were calculated from data obtained during a 6-day validation. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV).

2.5.5. Recovery

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the pure authentic standard. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery of the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible. Recovery experiments should be performed by comparing the analytical results for extracted samples at three concentrations (low, medium, and high) with unextracted standards that represent 100% recovery.

3. RESULTS AND DISCUSSION

The objective of this work was to develop a reversed-phase (RP)-HPLC simultaneous estimation method for separation of ZPS and ABZ drugs. The optimized mobile phase consisted of Phosphate buffer: acetonitrile [60:40, v/v] with pH adjusted 2.5 with ortho phosphoric acid at a working temperature of 25°C. The results of system suitability studies in the final and optimized HPLC method are given in Table-I.

3.1. Development of protein precipitation method

The protein precipitation was carried out by 1 part of plasma, 0.1 part of drug and 0.5 parts of I.S. Plasma previously spiked with ISs was used in precipitation. At the end of precipitation, supernatant was mixed with buffer of mobile phase to make the sample more compatible with the developed method. The % mean recoveries for all the analyte were ranging from 90 to 95% in the developed method.

3.2. Bioanalytical method validation

The developed method was validated for its specificity, sensitivity, calibration range, recovery, precision and accuracy and stability as per the bioanalytical method validation guideline suggested by ICH Guidelines.

3.2.1. Specificity

The specificity of optimized bioanalytical RP-HPLC method was evaluated by observing interference due to matrix.

3.2.1.1. Standard curve and linearity

A calibration curve was prepared in the same biological matrix as the samples in the intended study by spiking the matrix with known concentrations of the analyte. Based upon the results of lower limit of quantification of Ziprasidone Hydrochloride and Albendazole, it was noted that percent C.V. for Ziprasidone Hydrochloride and Albendazole at concentrations below 15 and 25 ng/ml respectively, was found to be more than 20%. Thus 15 and 25 ng/ml were selected as a LLOQ of Ziprasidone Hydrochloride and Albendazole respectively. Calibration curve consisted of calibration standards from 15-500 ng mL⁻¹ ZPS, 25-800 ng mL⁻¹ ABZ (internal standard). Calibration curves were calculated by linear regression analysis. The correlation coefficient (r²) was determined to assess linearity of calibration curves. Observations of ZPS and ABZ linearity curves are shown in Table-II and Table-III respectively. Standard curve for ZPS and ABZ are shown in Figure-II and Figure-III and chromatograms for ZPS and ABZ linearity are shown in Figure-IV.

The standard curve was determined on each day of the 6-day validation, and the slope, the intercept and the correlation coefficient were determined. Percentage relative standard deviation (%RSD) was found to be less than 2% that proves method is precise

3.2.2. Accuracy and precision

The values obtained, during the 6-day validation period, for plasma intraday and interday precision and accuracy are summarized in Table IV. All values of accuracy and precision were within the recommended limits. Intraday precision ranged from 1.04 to 7.75%, whereas the interday precision was from 1.38 to 14.09%. The intraday mean error was from 21.98 to 9.5%, whereas the interday mean % relative error was from 22.88 to 210.82%.

3.2.3. Recovery

The extraction recovery was calculated at LLOQ, QC samples and ULOQ level. The highest recovery at 500 ng mL⁻¹ concentration was obtained ZPS using the protein precipitation method ACN alone was used which is taken as final precipitating media and data for mean % recovery in the finally optimized protein precipitation method are given in Table-V.

3.3. Stability

Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix, and the container system. The stability of an analyte in a particular matrix and container system is relevant only to that matrix and container system and should not be extrapolated to other matrices and container systems. For this purpose stock solution of ABZ and ZPS were stable at room temperature for 24 h and at 2–8°C for 48 h. ABZ and ZPS analytes in control human plasma at room temperature were stable at least for 24 h and for minimum of three freeze and thaw cycles. Spiked plasma samples, stored at -20°C for long-term stability experiment, were stable for minimum of 90 days. Different stability experiments in plasma with values for precision and percent change are summarized in Table-VI.

3.4. Pharmacokinetic application

The proposed HPLC method enables a rapid assay of the ABZ and ZPS human plasma in a single run. The method consists of a protein precipitation procedure and simple isocratic chromatography conditions using a reversed-phase LC column. Furthermore, the method was shown to be highly reproducible and can be considered valuable for rapid and reliable preclinical and clinical and pharmacokinetic studies of the ABZ and ZPS in biological fluid. To get optimum conditions, various method development strategies were systematically optimized. The method was aimed with effective separation of ABZ and ZPS from each other and peaks of plasma. Various mobile phase combinations of ACN/ MeOH with phosphate buffer and without buffer (i.e., with 0.1% phosphoric acid) were investigated to optimize sensitivity, speed and peak shape. At relatively basic pH 7 of mobile phase, ZPS was eluted with peak asymmetry factor more than 2.2 owing to full protonation. The ionization constant (pKa in conjugated acid form) values for ABZ and ZPS were 9.51 and 12.05 while (pKa in conjugated base form) 4.27 and 7.09; therefore, the pH of mobile phase kept 2.5–3.5 to get unionized form of all the drugs. Further when the ratio of mobile phase was set to 50 parts for ACN and 50 parts for buffer, the peak of ZPS eluted before 5 min (i.e., the peak of rat plasma may interfere before 5.0 min). On setting higher part of buffer, retention time of ZPS increased and the peak symmetry was also 1.5. However, when ABZ and ZPS were chromatographed under the same HPLC conditions as that for XYZ, the retention time was increased more than 30 min for both the drugs and quality of peak symmetry for ABZ and ZPS decreased. A mobile phase consisting of ACN and phosphate buffer with pH, 2.5, in isocratic elution pattern, was found most suitable for eluting selected combination drugs in a single chromatographic run. The selected stationary phase (RP-C18 Octadecyle column) has shown better system suitability parameter. ACN gave a better response than MeOH in isocratic HPLC method development. The method has been successful in determining the P ZPS drug in concentration as low as 25 ng mL⁻¹, whereas 10 ng mL⁻¹ for ABZ.

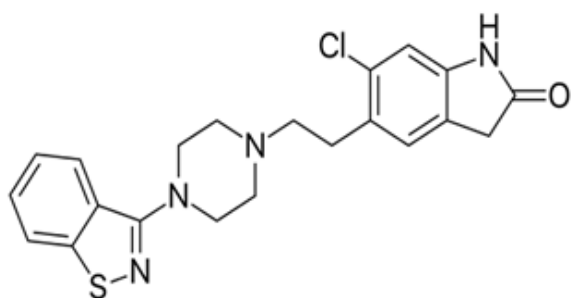
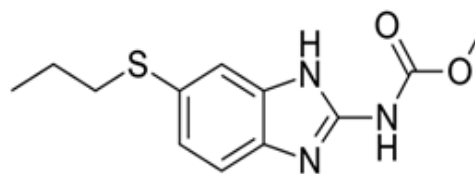


Fig. 1a- Structure of Ziprasidone.



1b- Structure of Albendazole.

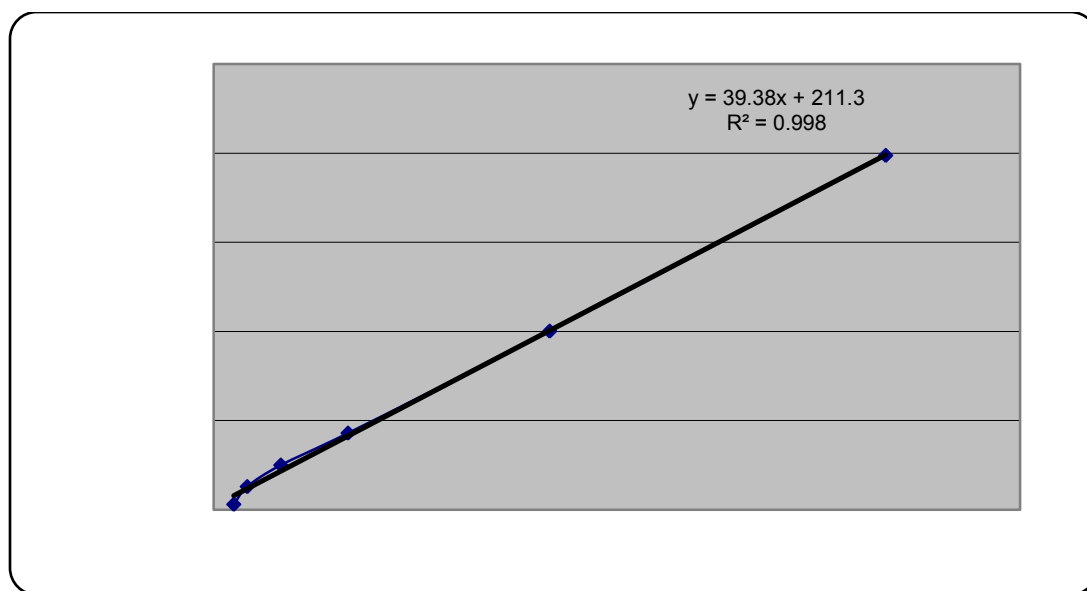


Fig.2-Calibration curve for Ziprasidone Hydrochloride.

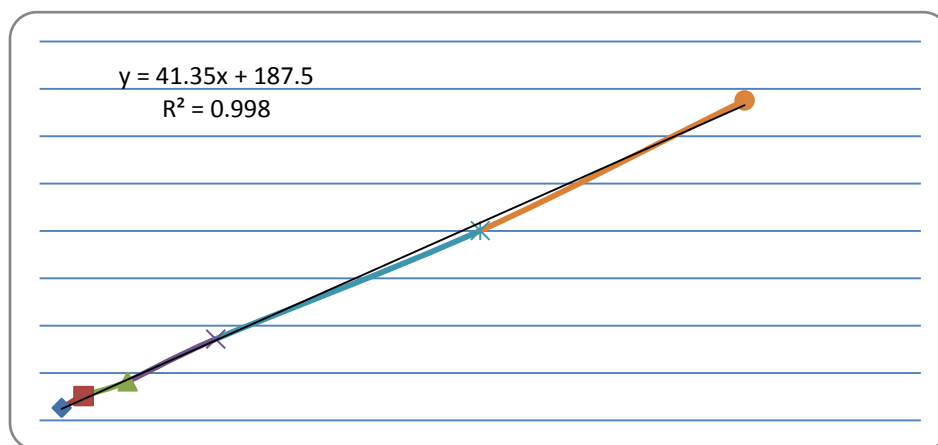


Fig. 3-Calibration curve for Albendazole.

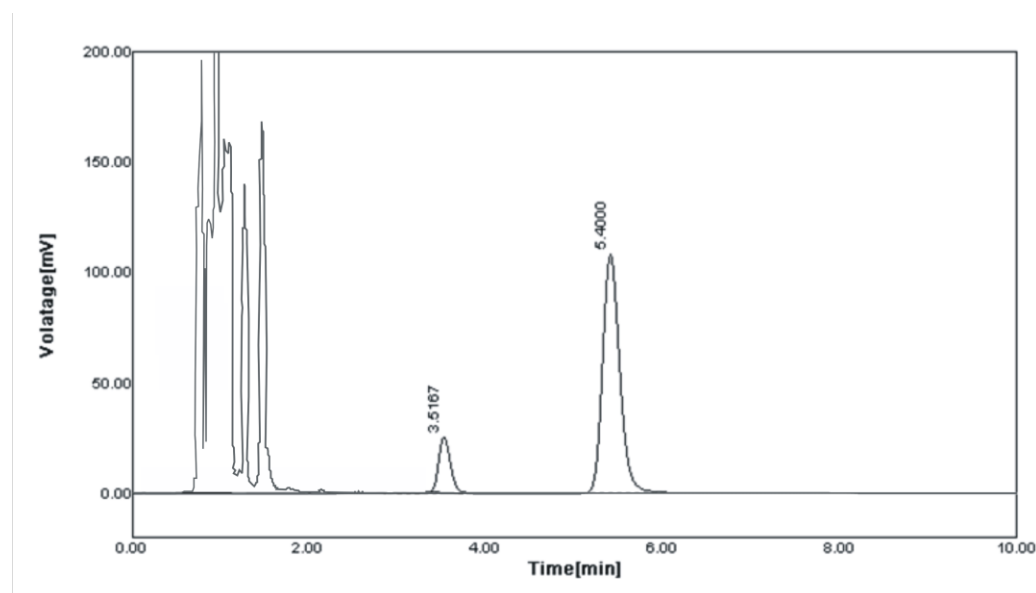


Fig. 4- Typical chromatogram of Ziprasidone and Albendazole with retention time 3.5167 and 5.400 minutes respectively.

Table-1: System suitability studies for resolution of ZPS and ABZ (n=6) in optimized chromatographic condition.

System suitability Parameters	USP Limit	ZPS	ABZ
Retention time	---	3.5167	5.400
% RSD of Peak Area	≤ 2.0	1.02	0.90
Capacity Factor (K')	0.5 to 2.0	0.99	1.04
Mean Number of theoretical plate	≥ 2000	3164.5	3487.1

Table-2: Calibration Curve for ZPS and ABZ.

Particulars	ZPS		ABZ	
	Concentration (ng/mL)	Peak Area (± SD)	Concentration (ng/mL)	Peak Area (± SD)
	15	301.8±5.81	25	1323.4±24.74
	25	1295.8±21.29	50	2566.6±22.21
	50	2504.2±25.73	100	4129.4±25.92
	100	4299.0±25.29	200	8584.0±59.25

	250	10017.2±59.78	500	20025.4±161.12
	500	19871.6±73.60	800	33767.8±188.14
Equation	Y = 39.38X+211.3		Y= 41.35X+187.5	
Regression coefficient (R²)	0.999		0.998	

Table-3: Precision parameters for ZPS and ABZ.

Drug	Precision (Intraday)				Precision (Interday)			
	Concentration (ng/mL)	Mean Peak Area	SD	% RSD	Concentration (ng/mL)	Mean Peak Area	SD	% RSD
ZPS	25	1685.33	43.32	0.86	25	1588.66	72.84	1.53
	50	4074.66	12.42	0.10	50	4093.00	28.21	0.23
	100	8997.66	30.09	0.11	100	9031.00	75.97	0.28
ABZ	50	2850.33	69.17	0.81	50	2882.33	15.50	0.18
	100	5985.66	62.50	0.35	100	6150.33	82.14	0.45
	200	12897.67	26.18	0.24	200	12878.66	35.08	0.09

Table-4: % mean Recoveries for ZPS and ABZ

Concentration (ng/mL)	% Mean Recovery for ZPS	% Mean Recovery for ABZ
LLOQ	96.83 ± 1.35	90.92 ± 2.39
LQC	95.52 ± 2.48	91.92 ± 2.25
MQC	96.75 ± 1.25	92.99 ± 1.89
HQC	94.03 ± 2.09	94.41 ± 1.89
ULOQ	90.83 ± 2.15	93.59 ± 2.36
	94.792 ± 1.864	92.766 ± 2.156

Table-5: Stability of ZPS and ABZ at the LQC and HQC level.

Stability	Condition	Level	Mean % Change	
Bench top	Room temperature (24 h)	LQC	1.41	1.61
		HQC	2.61	2.19

Freeze and thaw	After third cycle at -20°C	LQC	2.34	3.94
		HQC	4.24	-1.12
Long-term stability	90 days at -20°C	LQC	1.07	-2.42
		HQC	0.47	-2.1

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