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Research Article

Stability Indicating Method Development and Validation for Simultaneous Estimation of the Netupitant and Palonosetron in Bulk and Pharmaceutical Dosage Form by RP-HPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in Pharmaceutical dosage form. Chromatogram was run through Phenomenex C18 150 mm (4.6 x 150mm, 5µm) Mobile phase containing Buffer 60% 0.01N KH2PO4: 40% Acetonitrile was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected was 230.0 nm. Retention time of Palonosetron and Netupitant were found to be 2.266 min and 2.945 min. %RSD of the Netupitant and Palonosetron were and found to be 0.8 and 0.8 respectively. %Recovery was obtained as 101.08% and 100.35%for Netupitant and Palonosetron respectively. LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.27, 3.86 and 0.002, 0.006. respectively. Regression equation of Netupitant is y =18431x + 50471.and y = 13091x + 11.98. of Palonosetron. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Netupitant, Palonosetron, RP-HPLC

INTRODUCTION

Netupitant is an antiemitic drug approved by the FDA in october 2014 for use in combination with palonosetron for the prevention of acute and delayed vomiting and nausea associated with cancer chemotherapy including highly emetogenic chemotherapy. Netupitant is a neurokinin 1 receptor antagonist. The combination drug is marketed by eisai inc. And helsinn therapeutics (u.s.) Inc. Under the brand akynzeo.

Palonosetron (inn, trade name aloxi) is an antagonist of 5-ht3 receptors that is indicated for the prevention and treatment of chemotherapy-induced nausea and vomiting (cinv). it is the most effective of the 5-ht3 antagonists in controlling

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delayed cinv nausea and vomiting that appear more than 24 hours after the first dose of a course of chemotherapy and is the only drug of its class approved for this use by the u.s. food and drug administration. as of 2008, it is the most recent 5-ht3 antagonist to enter clinical use.

MATERIALS AND METHODS

Chemicals and Reagents: Netupitant and Palonosetron pure drugs (API), Combination Netupitant and Palonosetron tablets **(Akynzeo)**, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments and Chromatographic Conditions: Electronics Balance-Denver, P^H meter-BVK enterprises, India, Ultrasonicator-BVK enterprises, WATERS HPLC WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software, UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Netupitant and Palonosetron solutions.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately Weighed and transferred 150mg of Netupitant and 0.25mg of Palonosteron working Standards into a 50ml clean dry volumetric flask, add 3/4th volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (3000ppm of Netupitant and 5ppm of Palonosteron)

Preparation of Standard working solutions (100% solution): 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml. (300ppm of Netupitant and 0.5ppm of Palonosteron)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (3000µg/ml of Netupitant and 5µg/ml of Palonosetron)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (300µg/ml of Netupitant and 0.5µg/ml of Palonosetron)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100ml volumetric flask, 5 ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (3000µg/ml of Netupitant and 5µg/ml of Palonosetron).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (300µg/ml of Netupitant and 0.5µg/ml of Palonosetron)

Preparation of buffer:

Buffer:

0.01N Potassium dihyrogen Ortho phosphate

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.3 with dil. Orthophosphoric acid solution

Method Validation

As per ICH guidelines the method was validated and the parameters like Linearity, Specificity, Accuracy, Precision, Limit of Detection (LOD) and Limit of Quantitation (LOQ) were assessed.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: Stock solutions of Netupitant and Palonosetron is taken in to 6 different volumetric flasks and diluted to 10ml with diluents. Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml.

Accuracy: Accurately weighed 150mg of Netupitant, 0.25mg of Palonosetron and transferred to 150ml flasks and 3/4th of diluents was added to this flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (3000µg/ml of Netupitant and 5µg/ml Palonosetron)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution*:* 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Netupitant, Palonosetron, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Netupitant, Palonosetron, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Netupitant (50ppm) and Palonosetron (25ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should be not more than 2%.

Assay of Abiraterone: Assay of the marketed formulation was carried out by injecting sample corresponding to equivalent weight into HPLC system.

RESULTS & DISCUSSIONS

Optimization of Chromatographic Conditions: To develop and establish a suitable RP-HPLC method for estimation of Netupitant and Palonosetron in bulk and tablet dosage forms, different preliminary tests were performed and different chromatographic conditions were tested and optimized chromatographic conditions were developed which were given in Table-1.The final analysis was performed by using 65% 0.01N Kh2po4:35% Acetonitrile at a flow rate of 1ml/min, samples were analyzed at 230nm detector wave length and at an injection volume of 10µL using Phenomenex C18 (4.6 x 150mm, 5µm) with run time of 10min. The proposed method was optimized to give sharp peak with good resolution and the optimized chromatogram was obtained as shown in (Figure- 3).

Validation: Linearity was established at Six linear concentrations of Netupitant (75-450µg/ml) and Palonosetron (0.125-0.75µg/ml) were injected in a duplicate manner. Average areas were mentioned and linearity equations obtained for Netupitant was y = 18431x + 50471. and of Palonosetron was y = 13091x + 11.98. Correlation coefficient obtained was 0.999 for the both drugs. The linearity calibration curves were plotted as shown in (figure-4,5) Retention time of Netupitant 2.266min and Palonosteron was 2.945min. No interfering peaks in blank and placebo were found in this method. So, this method holds its specificity. Three levels of Accuracy samples 50%, 100%, 150% were prepared by standard addition method. Triplicates injections were given % Recovery was obtained as 101.08% and 100.35% for Netupitant and Palonosetron respectively. % RSD for system precision for Netupitant was 0.8% and for Palonosetron was 0.2%. %RSD for repeatability for Netupitant was 0.8% and for Palonosetron was 0.5%. %RSD for intermediate precision for Netupitant was 0.8% and for Palonosetron was 0.4%. Since %RSD is was less than "2" the system precision was passed in this method shown in (Table-3). The LOD and LOQ values were evaluated based on Relative standard deviation of response and slope of the calibration curve Abiraterone. The detection limit value for Netupitant was 1.27 and for Palonosetron was 0.002. The Quantification limit value for Netupitant was 3.86 and for Palonosetron was 0.006 as given in (Table-4). Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus (70B:30A), mobile phase plus (60B:40A), temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner (Table-5). System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit (Table-6). Netupitant and Palonosetron pure drug (API) was obtained from Spectrum Pharma research solutions Rhodes pharmaceuticals, bearing the label claim Netupitant

300mg, Palonosetron 0.5mg. Assay was performed with the above formulation. Average % Assay for Netupitant and Palonosetron obtained was 100.62% and 100.47%respectively the result was shown in (Table-7) and the pharmaceutical dosage form were shown in (4, 5 Figure) respectively.

Degradation studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Netupitant and Palonosetron in Tablet dosage form. Retention time of Palonosetron and Netupitant were found to be 2.266 min and 2.945 min. %RSD of the Netupitant and Palonosetron were and found to be 0.8 and 0.8 respectively. %Recovery was obtained as 101.08% and 100.35%for Netupitant and Palonosetron respectively.

LOD, LOQ values obtained from regression equations of Netupitant and Palonosetron were 1.27, 3.86 and 0.002, 0.006. respectively. Regression equation of Netupitant is y =18431x + 50471.and y = 13091x + 11.98. of Palonosetron. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

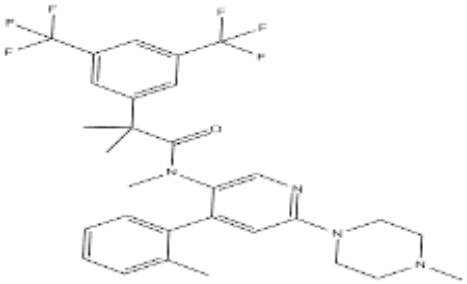

Figure-1: Chemical Structure of Netupitant

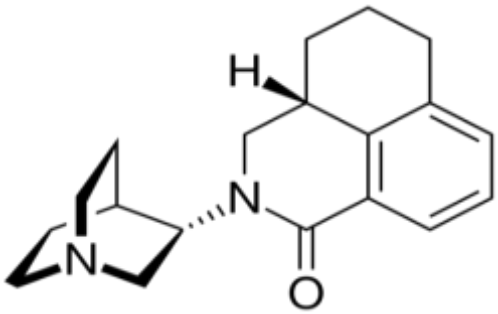

Figure-2: Chemical Structure of Palonosetron

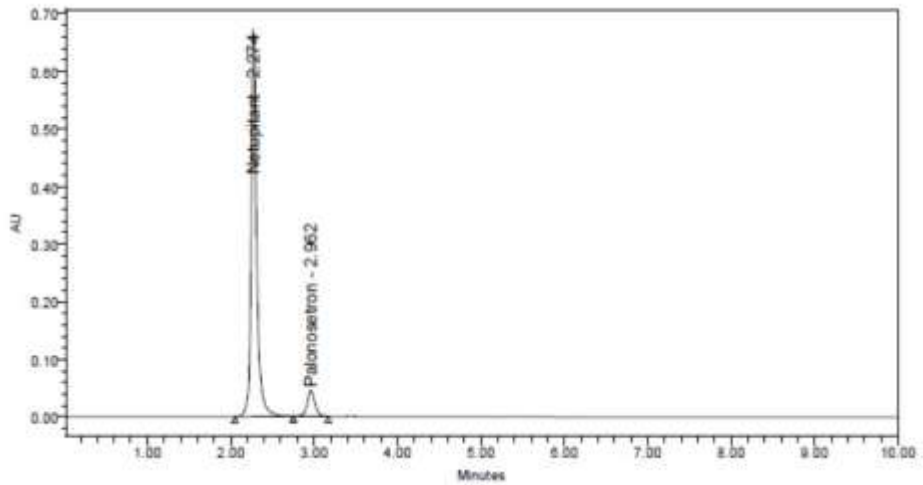

Figure-3: Optimized chromatogram

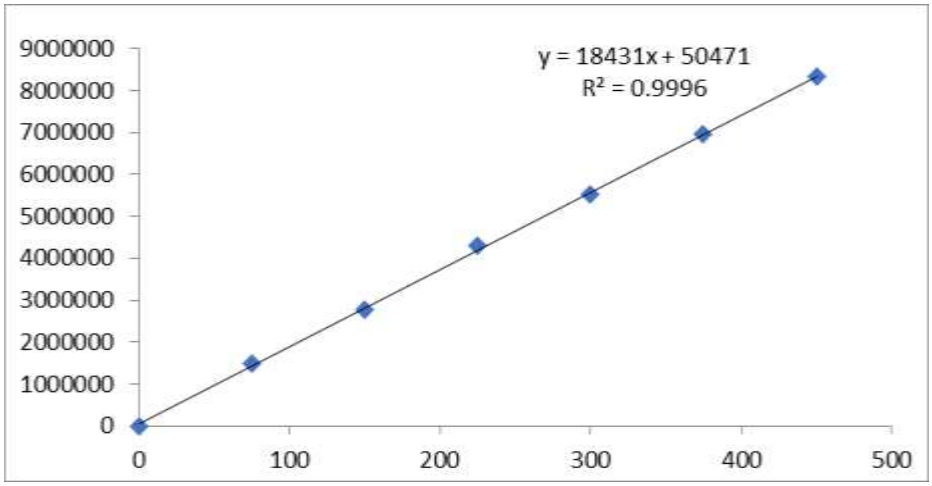

Figure-4: Linearity curve of Netupitant

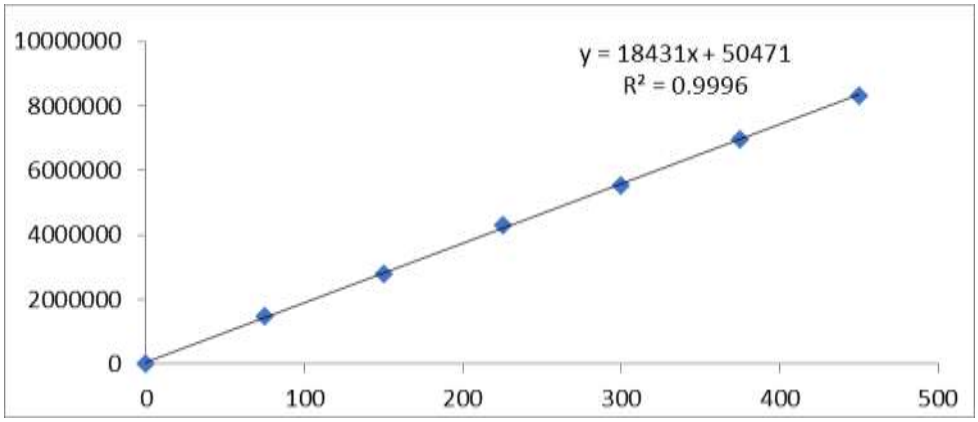

Figure-5: Linearity curve of Palonosetron

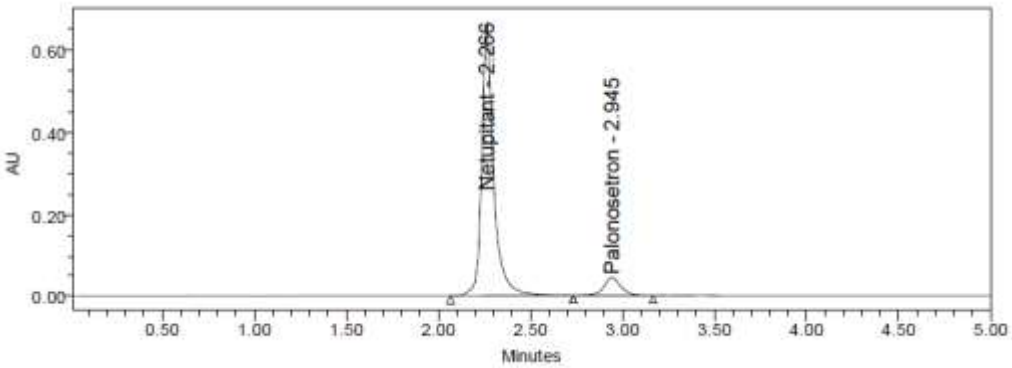

Figure-6 Standard Chromatogram of working standard solution

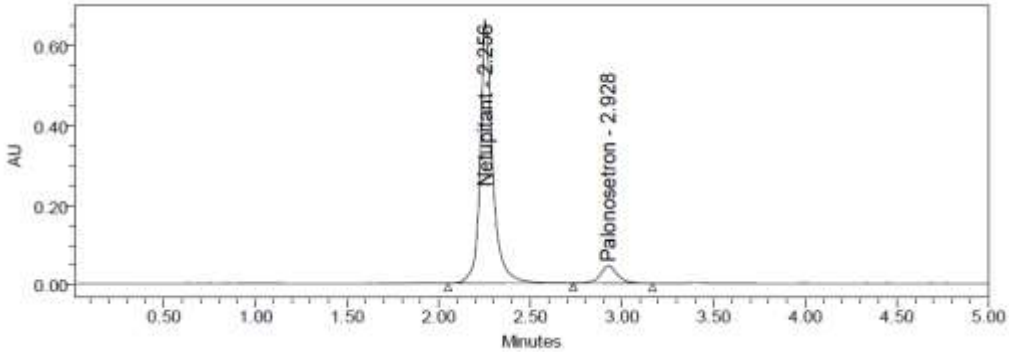

Figure-7 Sample Chromatogram of working sample solution

Table-1: Optimized method Chromatographic conditions:

Parameter	Condition
RP-HPLC	WATERSHPLC2695 SYSTEM equipped with quarternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 software
Mobile phase	65% 0.01N Kh2po4: 35% Acetonitrile
Flow rate	1ml/min
Column	Phenomenex C18 (4.6 x 150mm, 5µm)
Detector wavelength	230nm
Column temperature	30°C
Injection volume	10µL
Run time	10min
Diluents	Water and Acetonitrile in the ratio 50:50
Results	Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

Table-2: Accuracy results of Netupitant (Drug1) and Palonosetron (Drug2)

%Level	Amount Spiked(µg/ml)		Amount Recovered(µg/ml)		% Recovery		Mean % Recovery	
	Drug 1	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**	**Drug 1**	**Drug 2**
50%	150	0.25	152.0	0.249	101.4	99.50	101.08%	100.35%
	150	0.25	151.7	0.249	101.1	99.72		
	150	0.25	150.9	0.250	100.6	100.08		
100%	300	0.5	303.4	0.503	101.1	100.54		
	300	0.5	300.5	0.503	100.2	100.64		
	300	0.5	301.1	0.500	100.4	100.09		
150%	450	0.75	456.9	0.759	101.5	101.24		
	450	0.75	457.9	0.755	101.8	100.70		
	450	0.75	457.6	0.755	101.7	100.64		

Table-3: Precision Results of Netupitant and Palonosetron

s.no	System precision		Repeatability		Intermediate precision	
	Netupitant	**Palonosetron**	**Netupitant**	**Palonosetron**	**Netupitant**	**Palonosetron**
1	5503029	6692	5527946	6690	5604238	6675
2	5507986	6663	5632235	6728	5503029	6663
3	5563117	6659	5552218	6690	5514537	6610
4	5514537	6681	5647050	6700	5597050	6668
5	5527836	6661	5579846	6778	5566059	6681
6	5617176	6690	5566059	6728	5538287	6629
mean	5538947	6674	5584226	6719	5553867	6654
S.D	43981.7	15.1	46460.5	33.7	42248.6	28.3
%RSD	0.8	0.2	0.8	0.5	0.8	0.4

Table4: LOD and LOQ Values of Netupitant and Palonosetron

Molecule	LOD	LOQ
Netupitant	1.27	3.86
Palonosetron	0.002	0.006

Table-5: Robustness Data of Netupitant and Palonosetron

S.no	Condition	%RSD of Netupitant	%RSD of Palonosetron
1	Flow rate (-) 0.9ml/min	0.7	1
2	Flow rate (+) 1.1ml/min	0.2	0.6
3	Mobile phase (-) 70B:30A	0.9	0.8
4	Mobile phase (+) 60B:40A	1.1	0.9
5	Temperature (-) 25°C	0.1	0.8
6	Temperature (+) 35°C	0.4	0.2

Table-6: System Suitability Parameters for Netupitant and Palonosetron

S.no	Netupitant			Palonosetron			
Inj	**RT(min)**	**USP Plate Count**	**Tailing**	**RT(min)**	**USP Plate Count**	**Tailing**	**Resolution**
1	2.248	6905	1.39	2.922	5357	1.07	4.8
2	2.248	7009	1.38	2.923	5221	1.08	4.8
3	2.253	7390	1.39	2.934	5170	1.08	4.9
4	2.254	7250	1.40	2.935	5149	1.08	4.8
5	2.254	7441	1.39	2.936	5263	1.08	4.9
6	2.266	7198	1.36	2.945	5253	1.05	4.9

Table-7: Assay Results of Netupitant and Palonosetron

S.no	% Assay Netupitant	%Assay Palonosetron
1	99.60	100.03
2	101.48	100.60
3	100.04	100.03
4	101.75	100.18
5	100.54	101.35
6	100.29	100.60
Avg	100.62	100.47
Stdev	0.84	0.5
%RSD	0.8	0.5

Table-8: Degradation data for Netupitant and Palonosetron

Type of degradation	Netupitant		Palonosetron	
	%RECOVERED	% DEGRADED	%RECOVERED	% DEGRADED
Acid	94.06	5.94	94.11	5.89
Base	95.96	4.04	95.34	4.66
Peroxide	95.72	4.28	95.34	4.66
Thermal	97.53	2.47	97.13	2.87
Uv	99.01	0.99	99.32	0.68
Water	99.45	0.55	99.80	0.20

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