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Oxidative Coupling Method for the Estimation of Darunavir and Capecitabine by Visible Spectrophotometry

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Abstract : Objective This proposed work describes two simple and fast colorimetric methods for the estimation of oxidation complexes of Darunavir and Capecitabine with 2, 2'-bipyridyl and ferric chloride.

Methods Both the methods were developed on Perkin Elmer LAMBDA 25 UV –VIS spectrophotometer interfaced with UV Win lab software and 1cm quartz cells. The functional groups susceptible to oxidation gets oxidized with ferric chloride and couples with 2, 2'-bipyridyl. The method was optimized as per standard optimization parameters.

Results By the optimized method the λ max of the reddish pink colored chromogen of DNV was found to be at 522 nm and orange colored complex of CAP at 382 nm. The linearity range of CAP is 40-160 µg/ml and DNV is 10-60 µg/ml; LOD and LOQ was found to be 6.51069 and 21.7023 µg/ml for CAP; 0.6901and 2.3004 µg/ml for DNV. The colorimetric methods were extensively validated as per ICH guidelines and all the parameters were within the acceptance criteria with the correlation of 0.999 and % RSD less than 2 for both the methods.

Conclusion The methods were proved to be more accurate, simple, precise and rapid by statistical validation as well as recovery studies and could be used for routine laboratory analysis.

Key words : Darunavir (DNV), Capecitabine (CAP), 2, 2'-bipyridyl, ferric chloride, Oxidation complex.

Introduction for Dnv and Cap

DNV[1], chemically is [1S,2R]-3-[[(4aminophenyl)sulfonyl] (2-methylpropyl)amino]-2-hydroxy-1-(phenyl methyl)propyl]-carbamic acid(3R,3aS,6aR)hexahydrofuro[2,3-b]furan-3-yl ester mono ethanolate (fig. 1), is a protease inhibitor which prevents HIV replication by binding to the enzyme's active site, thereby preventing the dimerization and the catalytic activity of the HIV-1 protease. Combination therapy is the current standard of care for anti retroviral therapy. Since combination of antiretroviral agents may be synergistic, additive/antagonistic.

Capecitabine[2], (5-deoxy-5-fluoro-N-[(pentyloxy) carbonyl]-cytidine, Xeloda) (fig. 2), is a fluoropyrimidine carbamate, which is converted in liver and tumour to the active agent 5-fluorouracil (5-FU). It is used in the chemotherapeutic treatment of patients with breast and colon cancer. Carboxylesterases located in the liver and plasma of humans and liver of rodents convert capecitabine to 5'-deoxy-5-fluorocytidine (DFCR). DFCR is then converted by cytidine deaminase both in liver and tumour to 5'-deoxy-5- fluorouridine (DFUR).

Literature survey of this drug revealed that there are few methods available for the determination of DNV/CAP by RP-HPLC[3,4], HPTLC[15], spectrophotometric methods[16-18,20-23]. Single UV spectrophotmetric method and few HPLC methods were available for CAP. For DNV estimation HPLC, HPTLC and only two visible spectrophotometric methods were available. MBTH with ferric chloride and 1,2 naphthaquinone 4-sulphonate has been used for visible spectrophotmetric estimation of DNV. The official pharmacopoeia did not recommend any methods for CAP and DNV estimation. The available HPLC and HPTLC methods suffer from the need of most expensive instruments, tedious experimental procedures and expensive solvents. In evidence with all the drawbacks, there is a need for sensitive, reliable and cost effective analytical methods for the estimation of selected drugs. For its simplicity, sensitivity and low cost, spectrophotometry is the instrumental technique of choice for under developed and developing nations for quantitative estimation. Hence in view with the need of more accurate and sensitive time effective methods, this work was aimed for the validated colorimetric methods by exploring the specificity of oxidation complexes with ferric chloride and 2, 2' bipyridyl for the quantification of Method A and Method B.

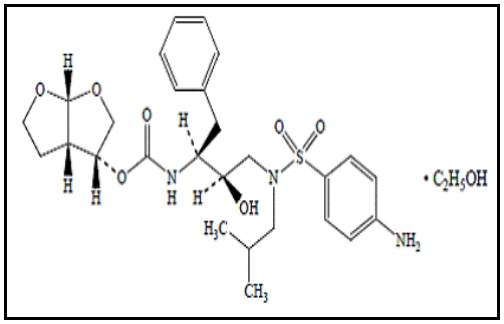

Fig. 1: Structure of DNV

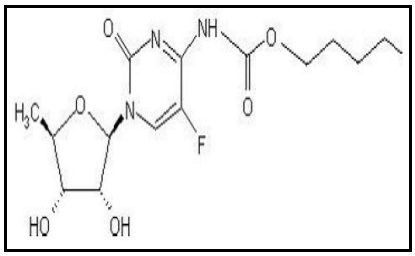

Fig. 2: Structure of Capecitabine

Materials and Methods

Equipment

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer interfaced with UV WIN lab software and 1 cm quartz cuvettes was used for spectral measurements. Sartorius balance was used for weighing the samples.

Chemicals

Both DNV and CAP were obtained as a gift sample from Aurobindo pharma Ltd, Hyderabad. Ferric chloride and 2,2'-bipyridyl of AR grade were used for the experimental work.

Preparation of stock solution for estimation of DNV

Accurately weighed 25 mg of DNV was transferred to a 25 ml volumetric flask, dissolved and diluted to volume with ethanol to reach the concentration of 1 mg/ml. Calibration standard were further diluted using stock solution.

Preparation of stock solution for estimation of CAP

Accurately weighed 10 mg of CAP was transferred to a 10 ml volumetric flask, dissolved and diluted to final volume with ethanol. The resulting solution has a concentration of 1 mg/ml. Calibration standards were further diluted using stock solution.

Preparation of reagents: Ferric chloride (1 % w/v) for DNV and (0.5 % w/v) for CAP was prepared by using water. 2, 2'-bipyridyl (0.5 % w/v) was prepared by dissolving 500 mg of 2,2'bipyridyl in 5 ml 0.1M hydrochloric acid and the final volume to 100 ml was made with distilled water. Ortho phosphoric acid (5 %) was prepared using water as diluent.

Procedure for calibration standards for DNV

In a series of 10 ml volumetric flasks, aliquots of 0.1-0.6 ml of working standard solution of DNV was pipetted out followed by 1.4 ml of 2, 2'bipyridyl solution and 1.2 ml ferric chloride solution, mixed well and

warmed at 70 ºC for 5 min, then kept aside for 30 min and final volume was made to 10 ml with water. The absorbance of the reddish pink colored chromogen was measured at 522 nm against the reagent blank. The amount of DNV present in the sample solution was computed from its calibration curve.

Preparation of calibration standards for CAP

In a series of 10 ml volumetric flasks, 1 mg/ml of working standard solution of CAP was pipetted out followed 0.4 ml of 2,2'bipyridyl solution and 1.4 ml ferric chloride solution and Ortho phosphoric acid 0.4 ml were added, kept at room temperature, then kept aside for 30 min and final volume was made to 10 ml with water. The absorbance of the reddish pink colored chromogen was measured at 382 nm against the reagent blank. The amount of CAP present in the sample solution was computed from its calibration curve.

Assay procedure for DNV/CAP

Twenty tablets of commercial samples of DNV/CAP were accurately weighed and powdered. Tablet powder equivalent to 25 mg of DNV and 100 mg of CAP were accurately weighed, dissolved and diluted to 25 ml with ethanol and filtered separately. Then the solution was subjected to above respective procedure and measured the absorbance at corresponding λmax of the drug.

Results and Discussion

Method optimisation

The selection of the suitable reagent was based on the functional groups present in the drug entity by trial and error method as per the standard optimization protocol. Both methods were studied for method optimization parameters like order of addition and concentration of reagent, effect of time, temperature and reaction time, stability of the color product etc. It was observed from the results given in Table 1 established for method A, third order addition of reagent has shown enhanced intensity of absorbance and it is similar in the case of method B.

Table 1: Order of addition

S. No	Order of Addition	Absorbance
1.	DNV+$FeCl_3$+2,2'bipyridyl	0.26
2.	DNV+2,2'bipyridyl+ $FeCl_3$	0.35
3.	$FeCl_3$+2,2bipyridyl DNV	0.29
Optimised order	DNV+2,2' bipyridyl + $FeCl_3$	
1.	CAP + $FeCl_3$ + OPA+2,2'bipyridyl	1.18
2.	$FeCl_3$+CAP+2,2'bipyridyl+OPA	0.42
3.	OPA+2,2'bipyridyl+ $FeCl_3$+CAP	0.79
4.	2,2'bipyridyl+ $FeCl_3$+CAP+ OPA	0.86
Optimised order	CAP+ $FeCl_3$+OPA+2,2'bipyridyl	

Effect of concentration of reagents were studied and shown in Table2. It was evidenced that 1.4 ml of 2, 2'bipyridyl and 1.2 ml of $FeCl_3$ for DNV; 0.4 ml of 2, 2'bipyridyl and 1.4 ml of $FeCl_3$ has shown better absorptivity in case of CAP, when compared to the lower and higher volumes of reagent. The effect of time on absorptivity was studied and found that between 20 to 30 min all the samples showed greater absorptivity compared to less time and longer time intervals. Effect of temperature on absorptivity was studied at various temperatures from 20 ºC to 80 ºC. DNV has shown good absorptivity at 70 ºC and CAP at room temperature. For stability of colored product, the products were found to be stable for up to 2 h. The scheme of reaction for selected drugs was given in (fig. 3 and 4)

Table 2: Fixing the reagent concentration for Method A and B

Reagent	Optimization of reagent volume		Volume optimised for procedure	
	Method A	Method B	Method A	Method B
2,2'bipyridyl	0.2-2.4 ml	0.2-1.6 ml	1.4 ml	0.4 ml
$FeCl_3$	0.2-1.4 ml	0.2-1.8 ml	1.2 ml	1.4 ml

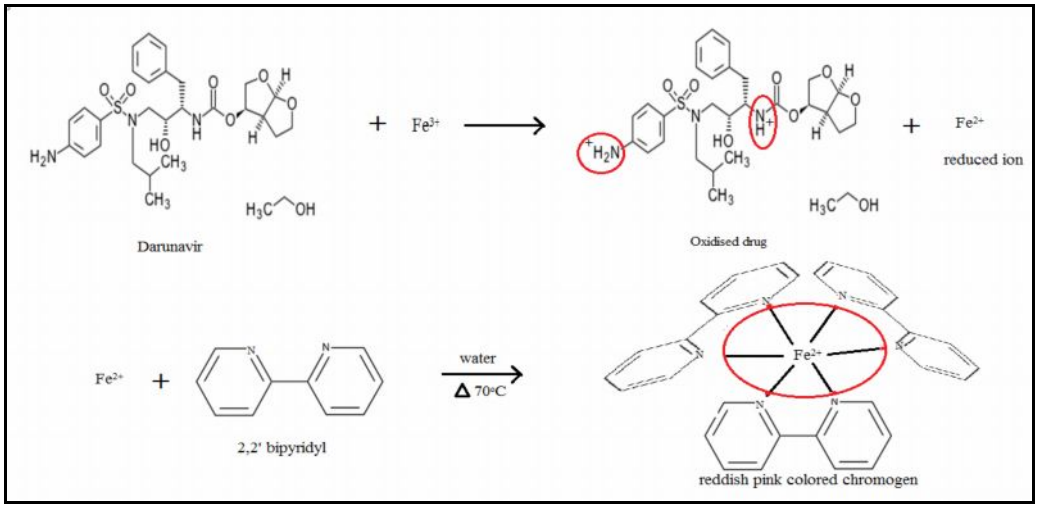

Fig. 3: Schematic reaction of DNV

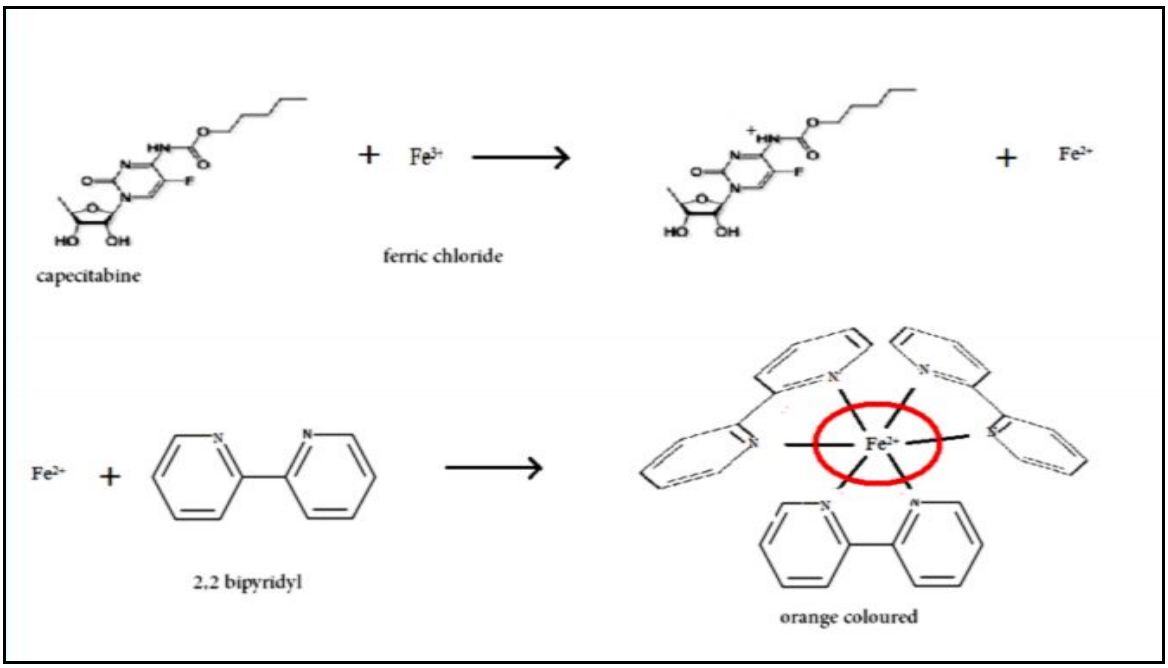

Fig. 4: Schematic reaction of CAP

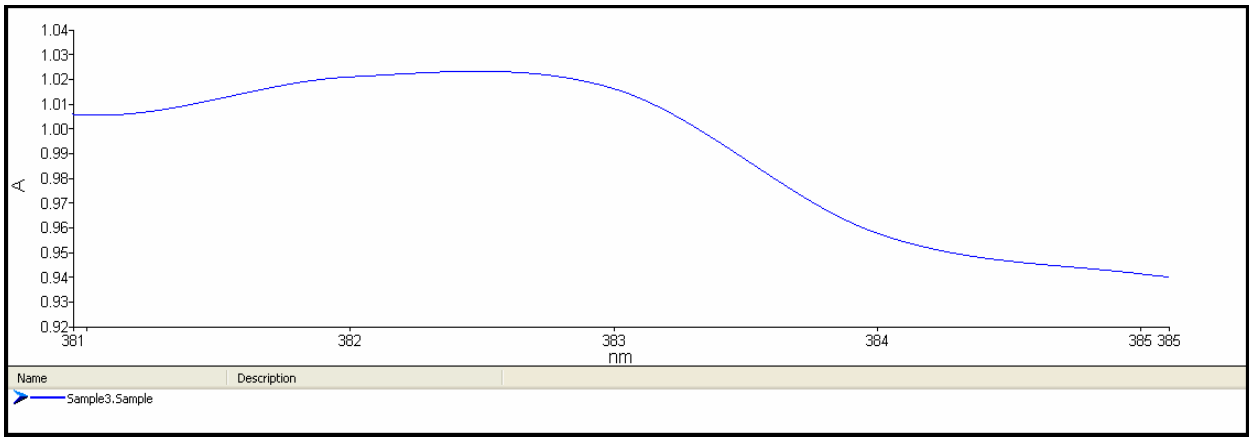

Fig. 5: Absorption spectrum of CAP with 2,2-bipyridyl

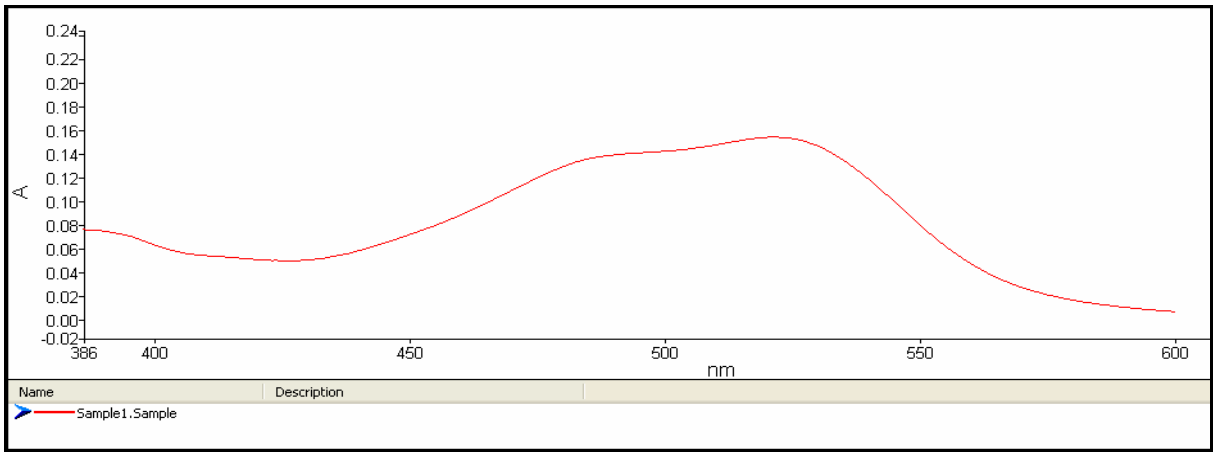

Fig. 6: Absorption spectrum of DNV with 2,2-bipyridyl

Method validation

All the methods were validated for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory as per ICH guidelines.

Regression parameters were presented in Table 3.

Table 3: Optical and regression parameters for Method A and B

Parameters	**Method A**	**Method B**
λmax, nm	522	474
Beer's law range (µg/ml)	10-60	2-10
Molar extinction coefficient ($L.mole^{-1}.cm^{-1}$)	1.5×10^{4}	3.7×10^{-4}
Sand ell's sensitivity ($\mu g/cm^{2}$)/0.001 absorbance unit	3.0×10^{-4}	4.2×10^{-4}
LOD, µg/ml	0.6901	0.8046
LOQ, µg/ml	2.3004	2.682
Slope (m)	0.002483	0.04979
Intercept (b)	0.0005857	0.01619
Correlation coefficient (r)	0.9999	0.9992

Linearity and range

At the described experimental conditions for DNV/CAP the standard calibration curves were constructed by plotting the increase in absorbance with concentration presented in (fig. 6 and 7).

A linear correlation was found between absorbance and concentration of DNV/CAP and all the optical and regression parameters were presented in Table 4. The statistical parameters given in the regression equation were calculated from the calibration graphs. The high values of the regression coefficients and low values of y-intercepts of the regression equations, proved the linearity of the calibration curve.

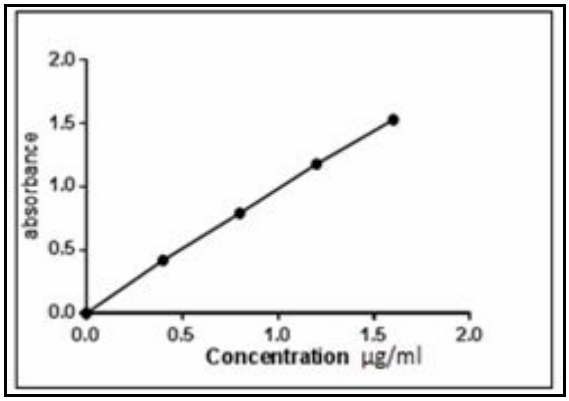

Fig. 7: Linearity plot of CAP with 2,2'bipyridyl

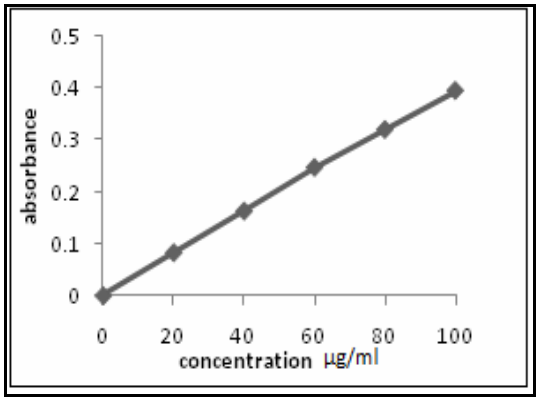

Fig. 8: Linearity plot of DNV with 2, 2'bipyridyl

Table 4: Table showing linearity plot

Method A		**Method B**	
Conc. (µg/ml)	**Absorbance**	**Conc. (µg/ml)**	**Absorbance**
10	0.0258	2	0.23
20	0.0500	4	0.42
30	0.0759	6	0.62
40	0.0998	8	0.80
50	0.125	10	1.01
60	0.149	-	-

Precision

Precision of the proposed methods was assessed by determining the relative standard deviation (RSD) of six replicate analysis on the same solution containing a fixed concentration of DNV (within Beer's Law limit). The low % RSD of the intraday and interday repeatability studies corroborates precision of the method. Table 5 represents the results of precision studies.

Table 5: Results showing Precision for Method A and B

Parameter	**Method A**		**Method B**	
	Inter day*	**Intra day***	**Inter day***	**Intra day***
Conc (µg/ml)	50	50	8	8
Mean abs	0.1275	0.1253	0.692	0.696
SD	0.000837	0.000516	0.0052	0.0068
% RSD	0.6564	0.4118	0.692	0.986

**N= Six determinations*

Robustness

Robustness was checked by narrow alteration of the optimized parameters and the % RSD was satisfactory for both methods and reported in Table 6.

Table 6: **Results showing Robustness for Method A and B**

Drug	**λmax nm**	**% RSD N=3**	**2,2bipyridyl ml**	**% RSD N=3**	**$FeCl_3$ ml**	**% RSD**
Method A	522±2	0.5701	1.4±0.1	0.806	1.2±0.1	0.8
Method B	474±2	0.093	1.8±0.1	0.457	1.4±0.1	0.187

Ruggedness

System to system/ analyst to analyst/ variability study was conducted on different colorimeters and the results for DNV/CAP were reported in Table 7.

Table 7: **Results showing Ruggedness for Method A and B**

Parameters	Method A	Method B
Analyst-1	0.125	650
Analyst-2	0.126	655
Mean absorbance	0.1255	0.652
SD	0.000707	0.0035
% RSD	0.5633	0.541

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ, presented in Table 3 was determined by analyzing progressively lower concentrations of standard solution using optimized conditions and the results showed lower LOD and LOQ values proving the sensitivity of the method.

Accuracy

The validity and accuracy of the proposed methods were further assessed by recovery studies using the standard addition technique. For this purpose, a known amount of pure drug at three different levels was spiked to the fixed and known quantities of pre analyzed formulation samples and the nominal values of drug was estimated by the proposed methods. The results for DNV/CAP given in Table 8 establish that the methods were reproducible by low SD and % RSD. No interference was evidenced from the commonly encountered formulations excipients.

Table 8: Accuracy table for Method A and B

Drug	Std added	Sample added	% Recovery	% RSD N=3
	50	40	99.97	0.010
Method A	50	50	99.12	0.0058
	50	60	99.59	0.0100
Method B	2	4	98.91	0.01
	4	4	99.82	0.01
	6	4	99.23	0.01

Application of the proposed method to formulation

To evaluate the proposed methods, they were applied to the determination of DNV/CAP in commercial formulations. The recoveries are close to 100%, indicating that there is no serious interference of excipients present in the samples. The good agreement between these results and known values indicate the successful applicability of the proposed methods for the determination of DNV/CAP in formulations. The results are given in Table 9.

Table 9: Assay results for Method A and B

S. No	Formulation	Label claim (mg)	Amount found (mg)	% Recovery
1.	Daruvir	300	299.4	99.76
2.	Capecitabine	500	499.6	99.8

Conclusion

The new, cost–effective, simple and sensitive visible spectrophotometric methods, using 2, 2'bipyridyl reagent, was developed for the determination of DNV/CAP in bulk and pharmaceutical formulations. The developed methods were also validated. From the statistical data , it was found that the proposed methods was accurate, precise and reproducible and can be successfully applied to the analysis of the same and could make a better alternative to the existing methods.

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