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QUANTITATIVE MEASUREMENT OF TRACE LEVELS OF RESIDUAL HYDROXYLAMINE HYDROCHLORIDE BY A SIMPLE GAS CHROMATOGRAPHIC METHOD AND ITS APPLICATION IN DRUG SUBSTANCE

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ABSTRACT

A simple and rapid gas chromatographic method has been developed for the determination of residual Hydroxylamine hydrochloride in drug substance as Paliperdione. Hydroxylamine hydrochloride is treated as Potentential Genotoxic impurity (GTI) and its control is shown less than 1.8 ppm level. The proposed method is based on a simple gas chromatographgic technique employing flame ionization detector and 100% Dimethylpolysiloxane as a stationary phase. The proposed method is simple, cost effective, sensitive, and specific, and showed reproducible results. Linearity of detector response was established up to 2.7µg/ml and the detection limit was 0.45 µg/ml for Hydroxylamine hydrochloride. The presented method proposes simple derivatization of Hydroxylamine hydrochloride to Acetone oxime using diluent as composition of water and acetone. Specificity of Method was checked and no interference of organic solvents (used in the paliperidone process) was observed. The proposed method has a potential to check and control the traces of hydroxylamine hydrochloride in drug substance. The results from all parameters proved that the method was suitable for determining the residual Hydroxylamine hydrochloride in drug substance. The novelty of the proposed method lies in attributes such that it uses a simple flame ionization detector unlike use of high end Mass spectrometer detectors described in most of the methods reported in literature[7]. The method is simple, yet accurate and efficient, and can be used in any Research or Industrial laboratory where Gas Chromatograph with Flame ionization detector is available.

KEY WORDS: Gas chromatography, Genotoxic, Hydroxylamine hydrochloride, FID.

INTRODUCTION

Hydroxylamine is a key raw material used in Intermediates and Active pharmaceutical ingredients (APIs). Hydroxylamine is used as a reducing agent in synthesis of intermediates and drug substances. Hydroxylamine chloride is soluble in water and its molecular formula is $NH_2OH.HCl$ and molecular weight is 69.49[1,2]. Hydroxylamine hydrochloride is reported as carcinogen[3]. Therefore, these residual impurities in a drug substance should be controlled to limits permitted by threshold of toxicological concern (TTC). This value was estimated to be 1.5 microgram/person/day intake of a genotoxic impurity and considered as an acceptable risk for drug substances as per EMEA guidelines and as well as risk assessment literature[4,5]. Trevicta (Active substance is Paliperdione) is available in suspension form in the market and in dose size from 175 mg, 263 mg, 350 mg and 525 mg by Jansen[6]. The maximum daily dose for the drug is 525 mg and the permitted value of hydroxylamine, based on the TTC calculation, is 2.85 µg/ml. However, in this case, stringent and lowest limit of 1.8 µg/ml for hydroxylamine hydrochloride was considered with respect to test concentration. The molecular structures of Hydroxylamine hydrochloride and Paliperidone are shown in Figure 1.

Many analytical methods have been reported in literature for determination of Hydroxylamine hydrochloride by derivatization methods[8,9,10], Ion chromatography[11], Indirect determination by Headspace GC[12], LC with ECD detector[13], LC with UV and Fluorescence detection[14]. The novelty of the presented method is that it uses commonly and cheaply available simple flame ionization detector unlike high end Mass spectrometer detectors used by most of the researchers for such measurements. Further, the method is economical because it does not involve use of expensive chemical derivatization reagents and thus saves enormous time engaged in cumbersome derivatization process. The proposed method is very sensitive and can achieve detection level of 0.45 µg/ml. Till date, no method has been reported to achieve the detection level as low as reported in present work. The prime objective of present work was develop a simple, efficient and cost effective method which would enable quantification of trace levels of hydroxylamine hydrochloride in a drug substance so that it can be easily applied in any research or industrial labs.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, Isopropyl alcohol, Acetonitrile, Toluene, Acetone and dichloromethane were procured from Merck Ltd. (All are GC grade with 99.0% purity) Hydroxylamine hydrochloride was purchased from Sigma Aldrich Inc. (99%purity) All reagents used were of analytical GC reagent grade. Paliperdone was prepared in our R&D analytical lab.

Instrumental

Gas chromatographic system of Perkin Elmer with FID and combipal auto sampler with chromeleon software was used. The separation was performed on DB-1 (100% dimethylpolysiloxane) 60 meter long, 0.32 mm I.D. and 5µm film thickness capillary column. The carrier gas (Nitrogen) flow was maintained at 2.5 ml/min in a flow mode and the sample was introduced with the split ratio of 2:1. The capillary injector temperature was set at 180°C and that of FID at 230°C. The column oven temperature was kept at 60°C with the initial hold time of 8 minutes, further increasing with the ramp rate of 10°C/min to attain 100°C with the hold time of 8 minutes and then increasing with the ramp rate of 45°C/min to attain 220°C with the final hold time of 5 minutes. With this program, the total runtime emerges out to be 28 minutes. It is shown in Table-1.

Standard stock solution

The standard stock solution was prepared by weighing about 1000 mg Hydroxyl amine hydrochloride standard taken into a 100 ml volumetric flask and dissolving it in 1 ml of water and further diluting it with acetone to the total volume of 100 ml. Further, 1 ml of this diluted solution was diluted to 100 ml with acetone.

Standard solution

The standard solution was prepared by transferring 0.36 mL of above Standard stock solution into 100 mL volumetric flask and diluting it by acetone to the total volume.

Sample solution

500 mg of drug substance sample was taken into 20 ml headspace vial and added 5 ml of diluent, shaked it for one minute and filtered through 0.45µ filter and injected.

LOQ solution

0.18 mL of Standard stock solution was diluted to 100.0 mL with acetone.

OD solution (0.45 ppm with respect to sample concentration)

5.0 mL of LOQ solution (prepared above) was taken in to 10.0 mL volumetric flask and diluted to volume with acetone.

RESULTS

Method development and optimization summary

Preliminary experiments were carried out based on the retention of Acetone oxime. Acetone oxime formed during reaction was measured in terms of hydroxylamine hydrochloride .Reaction scheme is shown in figure No.2. The elution pattern of acetone oxime (derivative of Hydroxylamine) including other solvents, used in the synthesis of paliperdione, was investigated by using non polar to mid polar stationary phases like DB-1(100% dimethylpolysiloxane), DB-5 (5% Phenyl,95%Dimethylpolysiloxane and DB-624 (6% cyanopropyl,94% dimethylpolysiloxane) capillary columns The separation was achieved on DB-1 column (100 % Dimethylpolysiloxane) of 60 meter length, 0.53 mm internal diameter and film thickness of 5µm with Nitrogen as a carrier gas. The diluent was used as a composition of water and acetone. (1:9). As Hydroxylamine is not completely soluble in organic solvents, certain amount of water was added for its dissolution during sample preparation. Both the peak shape and peak response were satisfactory and found well within symmetry. For recovery purpose, hydroxylamine solution was spiked in paliperidone drug substance with varying amounts, shaked for one minute and filtered through 0.45µ, Filtered solutions were run on chromatographic system and recovery was investigated. The determined % recovery values ranged from 80 to 120.

Validation of the method

In order to establish the suitability of the presented method for determining traces of hydroxylamine hydrochloride in a drug substance, the validation parameters like specificity, limit of detection, limit of quantification, linearity, accuracy and precision were investigated as per the ICH guidelines[15].

Specificity

To assess the ability of the method, individual solutions were prepared with known amount of Hydroxylamine hydrochloride with respect to drug substance concentration and injected into the gas chromatograph and the chromatograms were recorded. The sample solution was prepared as per given sample preparation method and injected into the gas chromatograph. The drug substance shows no peaks either due to Hydroxylamine

hydrochloride in the sample solution, So it reveals that the drug substance is highly pure and free from hydroxylamine hydrochloride.

Therefore, the sample solution was spiked with known amount of Hydroxylamine hydrochloride reference standard at target level, and injected into the chromatograph (Spiked sample). No interference of blank was observed corresponding to Hydroxylamine hydrochloride peaks and the analyte peaks were well resolved.

To widen the scope of the method, commercially employed solvents namely Methanol, Isopropyl alcohol, Acetonitrile, Toluene, Acetone, dichloromethane and trimethylamine, which were used during the synthesis of drug substance, were injected into gas chromatograph at known concentration level and their retention times were confirmed by respective individual chromatograms.

The resolution between Acetone oxime and triethylamine peak was found to be 2.5. There are no co-eluting peaks or interference of process solvents peaks with Acetone oxime peak. Thus, a system suitability criterion was established from the specificity experiment. Figure 3 show the chromatograms obtained from blank, Standard, Spiked sample with standard at LOQ and Limit level, Figure 4 show Blank, LOD and LOQ level and Figure 5 show Spiked sample with solvents used in drug substance process.

Limit of detection and Limit of quantification

For determining the limit of detection (LOD) and limit of quantification (LOQ) values, The solutions of Hydroxylamine hydrochloride used for LOD and LOQ evaluation were prepared at predicted concentration levels and precision was checked by analyzing it six times. The data is presented in Table 2.

Linearity

The linearity of the method was determined by preparing a series of solutions by using Hydroxylamine hydrochloride (Acetone oxime) at concentration levels from LOQ level to 150% of the target level (2.7µg/ml). The concentration studied ranges between 0.9 to 2.7µg/ml. The statistical parameters like slope, intercept and correlation co-efficient values were calculated and reported in Table 2.

Precision

The method precision was studied using repeatability and reproducibility (ruggedness). The method performance was evaluated with replicate injections of the standard and sample solutions. Standard solution was analyzed six times for checking the performance of the gas chromatographic system under the chromatographic conditions on the day tested (System precision). The values of % RSD for LOQ and Limit level were found to be 8.5 and 2.1, respectively. The data is reported in Table 2.

Accuracy

The accuracy of the method was evaluated by recovery experiments using standard addition technique. The recoveries were determined by spiking the respective hydroxylamine hydrochloride at three different levels ranging from 50 to 150% into drug substance with respect to sample concentration. The samples were prepared as per the methodology, analyzed in triplicate and percentage recoveries were calculated. It was found to be in the range of 100 to 104%. .The data is reported in Table 2.

Robustness

To assess the robustness of the method, experimental conditions were deliberately altered. The study was carried out with respect to flow rate of carrier gas ± 5% and initial temperature of the column oven 60°C± 2°C and ramp temperature 10°C/min ± 2°C. There is not much variation observed in the retention time of hydroxylamine hydrochloride obtained at different deliberately varied conditions from the developed methodology. Hence, it concludes that the test method is robust for all varied conditions.

Application of Analytical Method in drug substance (Paliperidone)

Thee batches of drug substance were tested for hydroxylamine hydrochloride content and it was detected in none of the batches. The data is given in Table 3.

Table I: Chromatographic conditions

Column	60 meters 0.32 mm ID column that contains 6% cyanopropyl phenyl and 94% dimethyl polysiloxane of 5 mm film thickness. (DB-1 of J & W Scientific

	column)
Injector temperature (°C)	180
Detector temperature(°C)	230
Carrier gas	Nitrogen
Column flow (ml/min)	2.5
Oven ramp (°C)	60°C for 8 minutes then increased to 100°C at the rate of 10°C/min and hold for 8 minutes then increased to 220°C at the rate of 45°C/min for 5 minutes.
Split ratio	2:1
Detector	Flame Ionisation
FID Attenuation	1
FID Range	1
Injection Volume	3 µl

Table II: Statistical parameters of Hydroxylamine (Acetone oxime

Parameter	Hydroxylamine hydrochloride (Acetone oxime)
Retention time	14.42
Linearity	
Correlation coefficient(R)	0.9922
Slope	8374
Detection limit(in ppm with respect to test concentration)	0.45
Quantification limit(in ppm with respect to test concentration)	0.90
Precision at LOQ level (%RSD)	8.5
Precision at Working level (%RSD)	2.1
Accuracy	
Accuracy at LOQ level (n=3)	102
Accuracy at 100% level (n=3)	100
Accuracy at 150% level (n=3)	104

Table III: The Hydroxyl amine hydrochloride content in Paliperdione API samples are given below

Sr.No	Batch Number	Hydroxylamine hydrochloride content Result in (ppm)
1	FDX160001	Not detected
2	FDX160015	Not detected
3	FDX160018	Not detected

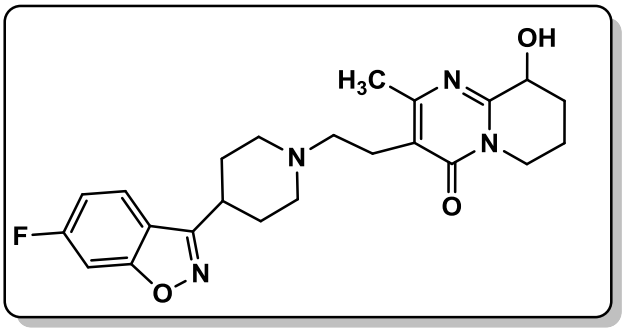
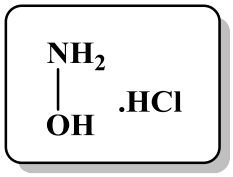


Figure 1: The Chemical structure of Paliperdione.

The Chemical structure of Hydroxylamine hydrochloride



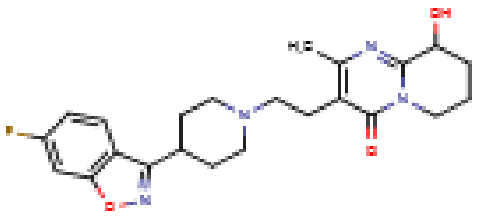
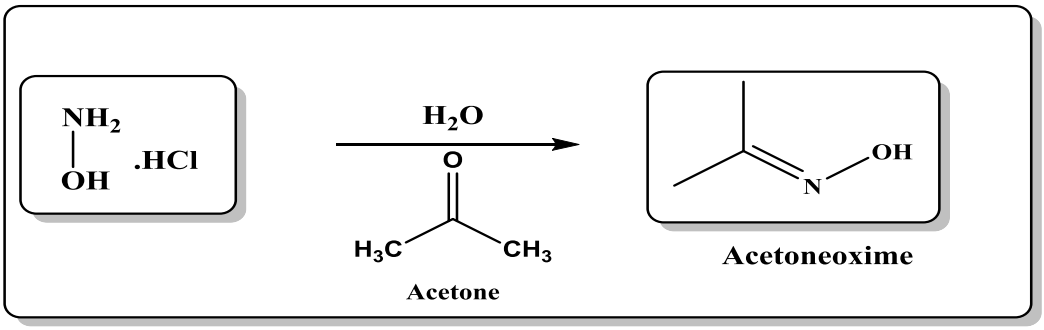


Figure 2:



Reaction scheme of formation of Acetone oxime by the condensation of acetone and hydroxylamine in the presence of Hydrochloride.

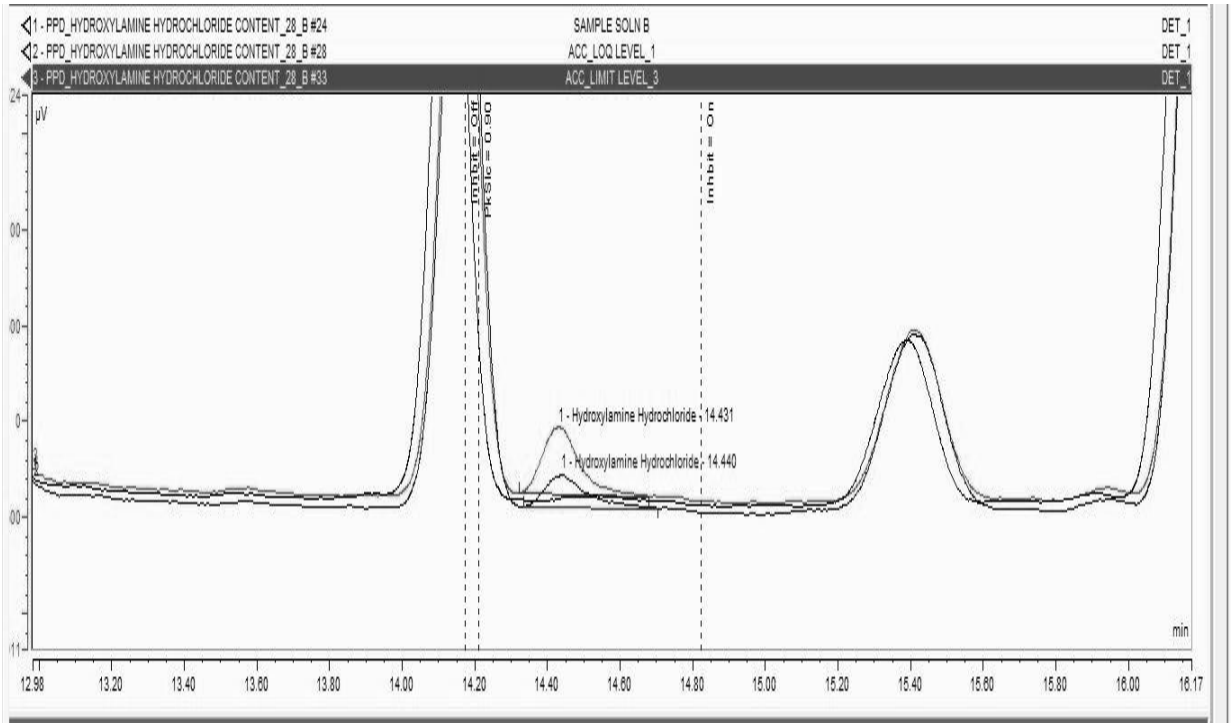


Figure 3. Representative overlay chromatogram of Sample, spiked sample with LOQ and spiked sample with limit level

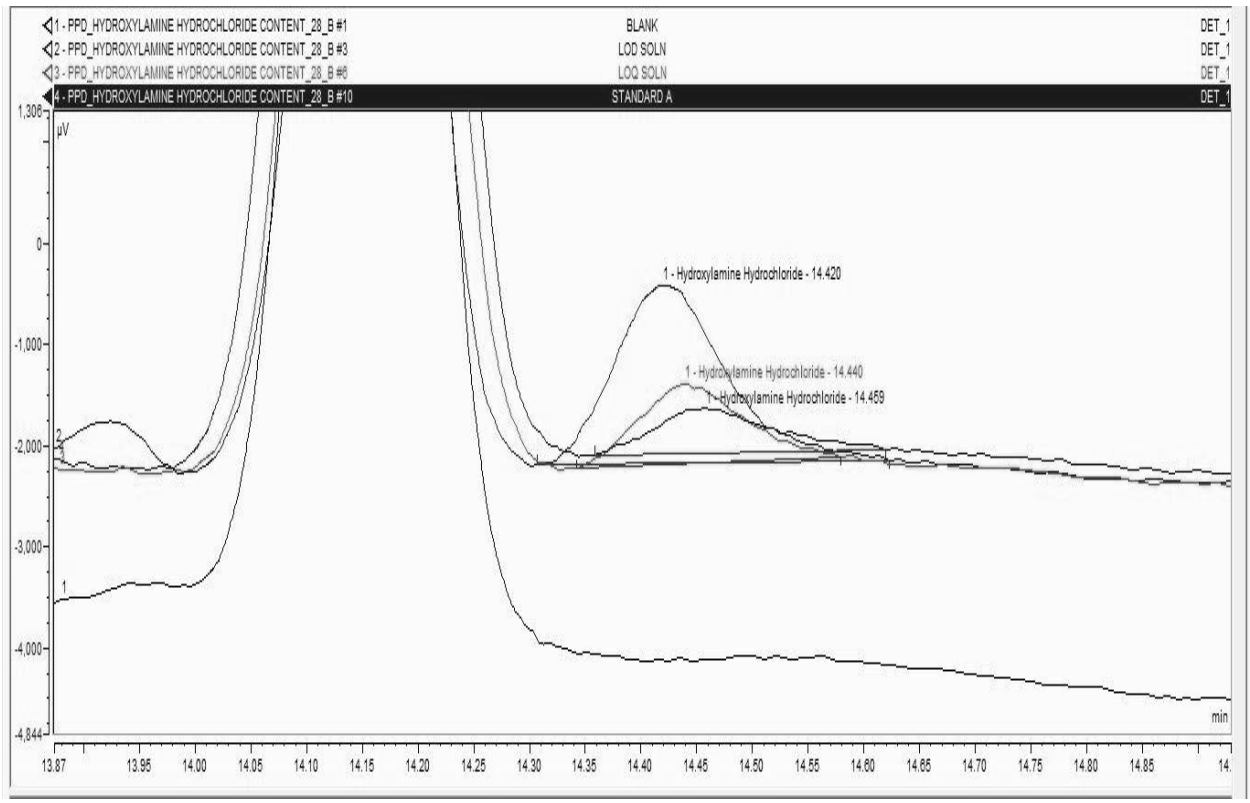


Figure 4. Representative overlay chromatogram of Blank, LOD and LOQ level.

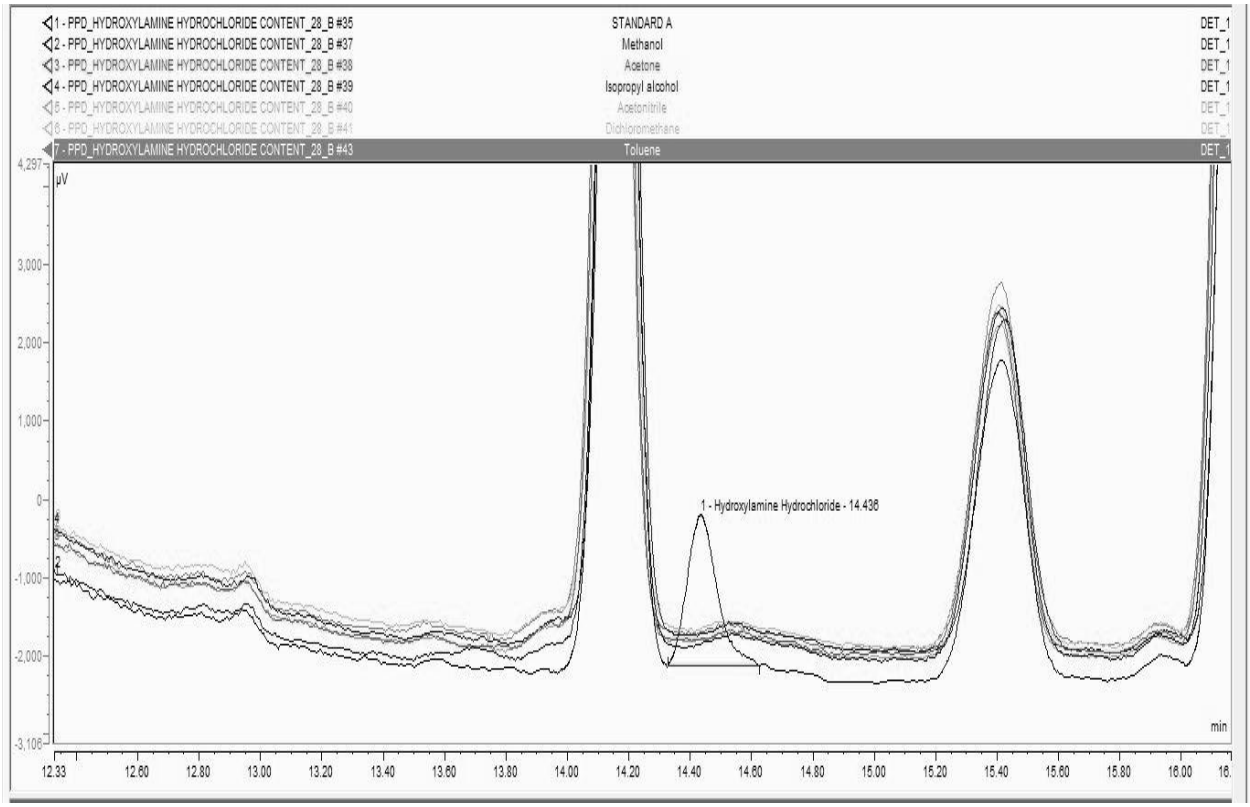


Figure 5. Representative overlay chromatogram of process solvents and hydroxylamine (Specificity)

CONCLUSION

The presented gas chromatographic method is useful for determination of low level traces of Hydroxylamine hydrochloride present in the drug substance. The results of various validation parameters confirmed that the method is specific, robust, linear, precise and accurate. The method is very simple to adapt in research as well in any industrial analytical lab as it does not demand use of derivatising reagents and high end mass detectors. The experimental data show that the method offers potential application for the quantitative determination of hydroxylamine hydrochloride present in the drug substances at very low level.

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