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## MICELLAR LIQUID CHROMATOGRAPHIC METHOD DEVELOPMENT FOR DETERMINATION OF 2,4,5,6-TETRAAMINO PYRIMIDINE SULPHATE SALT

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### **Abstract:**

*A rapid, simple and sensitive liquid chromatographic procedure that use micellar mobile phase containing only Tween-20 and n-butanol, is reported for the determination of 2,4,5,6-Tetraaminopyrimidine sulfate salt. The determination of 2,4,5,6-Tetraamino pyrimidine sulfate salt could be achieved with a micellar mobile phase of 5% n-butanol in 0.05 mol L<sup>-1</sup> Tween-20, with retention time below 3 minute. The proposed method involved separation of 2,4,5,6-Tetraamino pyrimidine sulfate salt on a reversed phase Nucleodur, C-18 Pyramid column and determination with UV detection at 215 nm. The working standard curve was linear (R=0.9990) over the concentration range of 25 ppm to 125 ppm with detection limit of 0.69 µg/ml and quantification limit 2.10 µg/ml. The method was environment friendly and economical in term of time taken and amount of solvent used.*

### **Keywords:**

*Micellar liquid chromatography, Surfactant, 2,4,5,6-Tetraaminopyrimidine sulfate salt, Tween-20*

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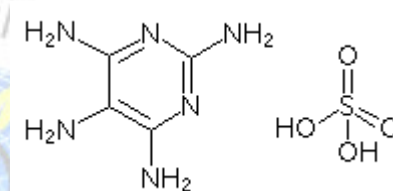
## Introduction:

Micellar liquid chromatography has been reported as a suitable technique for pharmaceuticals and intermediate for drug and cosmetics interest [1]. Micellar solution can replace conventional aqueous organic mobile phase with good results. Micellar liquid chromatography (MLC) is a reversed phase liquid chromatographic (RPLC) mode with mobile phases containing a surfactant (Ionic or Non ionic) above its critical concentration (CMC) [2]. In these conditions the stationary phase is modified with an approximately constant amount of surfactants monomers, and solubilizing capability of mobile phase is altered by the presence of micelles, giving rise to diverse interactions (Hydrophobic, ionic and satiric) with major implications and selectivity. This technique has evolved up to becoming a real alternative in some internsace to classical RPLC with hydro-organic mixtures, owing to its peculiar features and unique advantages. The idea of using pure micellar solution as mobile phase is very attractive owing to the lower cost and toxicity, and the reduced environmental impact. In practice, however, the addition of small amount of organic to the micellar solution is needed to achieve retention in particular time window.

Micellar mobile phases have been used with different bonded stationary phases (mostly C8, C18 and cyanopropyle). The most common surfactant are the anionic Sodium dodecyl sulphate (SDS) cationic Cetytrimethylammonium bromide (CTAB), and non-ionic Tween-20, several organic solvents have been used as modifiers, short/medium chain alcohols and Acetonitrile being the most suitable. The presence of micellar contributes well above their solubility

in water. Also, the risk of evaporation is diminished.

2,4,5,6-Tetraaminopyrimidine sulfate salt CAS No. 5392-28-9 is a intermediate of many pharmaceuticals and cosmetic like Fludarabine phosphate and Methotrexate which is Antineoplastic and antirheumatic. The 2,4,5,6-Tetraaminopyrimidine sulfate salt is ingredient of oxidative and non oxidative hair dye formulations. This molecule has very low solubility character in water and other organic solvent which are used in reversed phase chromatography.



**Fig.1 Chemical structure of 2,4,5,6-Tetraamino pyrimidine sulfate salt**

The development of meaningful dissolution procedure for compounds with limited water solubility has been a great challenge. It has been seen that surfactant play very important role in solubilizing organic and in-organic salt by reducing interfacial tension and contract angle between solid particles and aqueous media. Thus improving compounds adaptability and increasing surface availability for compounds dissolutions [3-6].

Aqueous micellar solutions are surfactant based self organized system that can be used as less hazards substituted for organic solvent in HPLC separation [7]. The amphiphillic structure of surfactant and their assembly in aqueous solution proved a multifunctional environment for the solubilization and partitioning of aqueous soluble and insoluble compounds. The literature survey revile that no Micellar liquid chromatographic

method have been published concerning for 2,4,5,6-Tetraaminopyrimidine sulfate salt. There are certain advantages associated with this method such as dissolution, high selectivity, sensitivity, low cost, less time consuming, less hazardous and low limit of detection.

## Material and Methods:

### Reagents & standards

Tween-20, n-butanol and water were obtained from Merck. All reagents were of HPLC grade unless otherwise specified. Working standard 2,4,5,6-Tetraaminopyrimidine sulfate salt were obtained from commercial source from Sigma Aldrich. The following standard pharmaceutical grade like 2,4,5,6-Tetraaminopyrimidine sulfate salt, CAS No: [5392-28-9], Certified 97.0 % pure is obtained from Sigma Aldrich.

### Apparatus

Sample analysis were performed on an chromatographic system of Waters alliance series chromatograph equipped with an in-built solvent degasser, quaternary pump and Waters-2996 Photo diode array detector with variable injector and auto sampler. The chromatographic column utilized in these studies was a Nucleodur, C-18 Pyramid (150 X 4.6 X 5.0 $\mu$ m). The UV detection wavelength was 215 nm. Mobile phase flow rate was 1.0 ml/min. twenty micro liters of sample were injected into the HPLC for each analysis. A Waters column heater module was used to maintain a constant column temperature of 25<sup>0</sup>C. Photodiode array spectra were obtained from Waters separation module equipped with a model 2696 photo diode array detector. Peak purity analysis was carried out over a wavelength range 208-350 nm through the use of the Empower<sup>TM</sup>-2 Build-2154 software. The stability chamber utilized during forced degradation studies was a

controlled by temperature controller. All measurements were carried out at room temperature (25 $\pm$ 0.1<sup>0</sup>C). The pH metric studies were carried out on Decibel, Db-1011 digital pH meter fitted with a glass electrode as an indicator and saturated calomel electrode as reference electrode.

## Result and Discussion

### Method Development

Optimal separation of related substances from each other and from 2,4,5,6-Tetraamino pyrimidine sulfate salt was achieved with an Isocratic mobile phase. A mobile phase temperature of 25 <sup>0</sup>C was employed for the separation. No significant degradation of 2,4,5,6-Tetraaminopyrimidine sulfate salt was observed at 25 <sup>0</sup>C temperature during its elution time. Typical chromatogram with retention time and elution order observed for 2,4,5,6-Tetraamino pyrimidine sulfate salt is presented in Fig. -2.

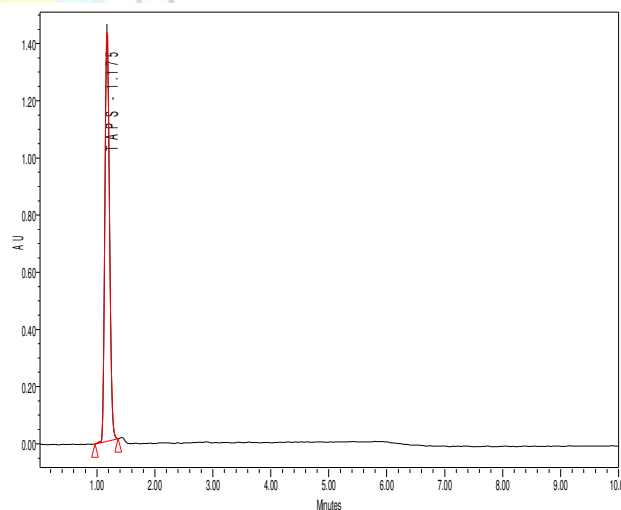
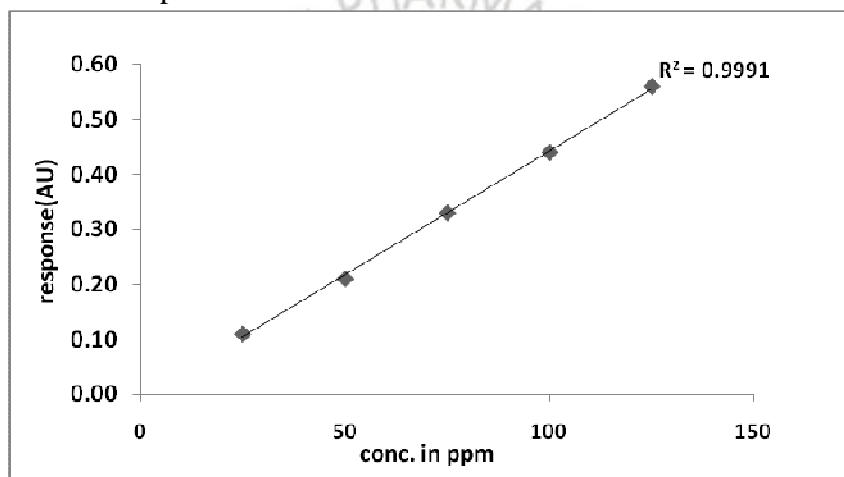


Fig. 2 Chromatogram of 2,4,5,6-Tetraamino pyrimidine sulfate salt obtained using micellar mobile phase 5% n-butanol in 0.05 mol L<sup>-1</sup> Tween-20, wavelength- 215 nm, flow rate 1.0 ml/min.

### Solution and sample preparation

The required amount of the sample and working standard should weigh accurately about 25 mg of test sample in a dry 50 ml volumetric flask and add 10-20 ml of mobile phase then sonicated it to dissolved then make up the volume 50 ml with mobile phase. Working standard and sample solution should be filtered; however, the filtration should always be performed directly into the auto sampler vials through 0.45  $\mu\text{m}$  nylon membranes of 13 mm diameter. In this study after many experiments a new mobile phase with a higher eluting strength 5% n-butanol in 0.05 mol  $\text{L}^{-1}$ , Tween-20 was found satisfactory. In this work, it is demonstrated that mobile phase based on

Tween-20 with n-Butanol are suitable for the analysis of 2,4,5,6-Tetraaminopyrimidine sulfate salt. The two main advantages of micellar procedure are the elimination of organic solvents and simplification of sample preparation step. The seven point's calibration graphs were constructed covering a concentration range. 0.5 to 5 mg/ml. Linear relationship was obtained between the peak area ratio of 2,4,5,6-Tetraaminopyrimidine sulfate salt in the concentration range 25 ppm to 125 ppm. The correlation coefficient was found 0.9990. According to International Conference on Harmonization (ICH) guidelines [8] the following expression is used to evaluate LOD and LOQ.



**Fig. 3 Plot of different 2,4,5,6-Tetraaminopyrimidine sulfate salt conc. v/s response in micellar mobile phase**

#### 1. Sensitivity/detection limit:

The detection limit was calculated by the equation  $\text{LOD} = 3.3\text{S.D.}/b$ , [9] where S.D. is the standard deviation of the intercept and  $b$  is the slope of the regression line. The calculated detection limit for the standard solution was  $0.69 \mu\text{g mL}^{-1}$ .

#### 2. Quantification limit:

The quantification limit was examined by the equation  $\text{LOQ} = 10 \text{ S.D.}/b$ . The lower limit of

quantification for the standard solution was found to be  $2.1 \mu\text{g mL}^{-1}$ .

#### 3. Specificity:

Specificity [10] is the ability of the method to measure the analytical response in the presence of all potential impurities. For the specificity test, Chromatogram of the standard solution of 2,4,5,6-Tetraaminopyrimidine sulfate salt were recorded under selected conditions. The response of the analyte in this mixture was compared with the response of pure 2,4,5,6-Tetraaminopyrimidine

sulfate salt. It was found that assay results were not changed.

#### 4. Stability:

In this study, 2,4,5,6-Tetraamino pyrimidine sulfate salt stock solution were kept in the -dark at +4°C for 15 days and were analyzed at different times (every day). It has been seen that repeatable

peak currents [11] of 2,4,5,6-Tetraamino pyrimidine sulfate salt stock solution occurred up to 15 days and after that the peak current decreased significantly. So the solutions were found to be stable for 15 days.

**Table 1. Showing different parameters and results**

Parameters	Results
Concentration range in ppm	25-125
LOD (µg/ml)	0.69
LOQ(µg/ml)	2.10
Correlation coefficient	0.9990
Recovery	99%
Intercept	0.009
% RSD	0.29

#### Conclusion

The proposed micellar chromatographic method has been evaluated over the linearity, precision, accuracy, specificity and proved to be convenient and effective for the quality control [12] of 2,4,5,6-Tetraamino pyrimidine sulfate salt. There are certain advantages associated with this method such as dissolution, high selectivity, sensitivity, low cost, less time consuming, less hazardous and low limit of detection. Moreover, the lower solvent consumption along with the short analytical run time of 1.17 minutes leads to a cost effective and environment friendly chromatographic procedure. Consequently the proposed method has a high potential of good analytical alternative for determining quality of 2,4,5,6-Tetraamino pyrimidine sulfate salt.

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