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Development And Validation Of HPLC Method For Estimation Of Ceftriaxone Sodium

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Abstract-

The Validation are growing at a tremendous rate due to greater patient acceptability, multiple actions, increased potency, quicker relief and fewer side effects .The arena of pharmaceutical analysis calls for simple, rapid, accurate and specific methods of analysis which ensure the quality of the products. Form the analytical point of view, the drug present in a multi- component formulation suffer from severe spectral overlapping .The currently available methods for the analysis of multi-component formulations are different chromatographic techniques like HPLC. Although, these methods provide accurate and precise results, these suffer due to some drawbacks like time consuming nature, need expensive and solvents and the procedures are generally complex in nature. The aim of the present work is therefore to develop a simple, fast, convenient, economical, accurate and reliable method for the simultaneous determination of the formulations. Experimental designs, a fascinating area of chemo metrics are also applied in the chromatographic technique HPLC, proving the method is highly robust.

Keywords: Ceftriaxone, HPLC, ICH guidelines, Mobile phase.

1.0 INTRODUCATION

Chromatography is a separation technique that uses the size, shape, chemical properties or charge of molecules in a sample to separate the sample into its constituent components. It is often used to detect one, or a number of, components in a complex mixture. High Performance Liquid Chromatography principal instruments applications will be described in this article. The components of an HPLC device are a mobile phase, pump/compressor, injector, column, and detector. Due to the pressure needed to push the mobile phase and sample through the tightly packed columns, HPLC was initially known as high-pressure chromatography. Unlike traditional liquid chromatography, which depends on gravity, HPLC uses a pump to transport the mobile phase and sample through the column. Concentrations below the ppt threshold are simple to find. HPLC usually uses a variety of stationary phases, a pump to transport the mobile phase(s) and analyte through the column, and a detector to provide an analyte‟s distinctive retention time.

Principle of High-Performance Liquid Chromatography (HPLC)

- The fundamental principle behind HPLC, a sample is broken down into its individual components based on the respective affinities of various molecules for the stationary phase and mobile phase that are being used to perform the separation.

- It uses two pumps to pass a solvent referred to as a "mobile phase (eluent)" and a sample mixture known as "stationary phase (packing material).

Applications of High-Performance Liquid Chromatography :

- Analysis of drugs.
- Analysis of synthetic polymers .
- Analysis of pollutants in environmental analytics.
- Determination of drugs in biological matrices .
- Isolation of valuable products .
- Product purity and quality control of industrial products and fine chemicals.
- Separation and purification of biopolymers such as enzymes or nucleic acids .
- Water purification.
- Pre-concentration of trace component.

2.0 Materials and Methods

2.1 Materials: Chemicals and reagents

S.No	Drugs/Reagents	Supplier Name
1	Ceftriaxone Sodium	Alkem Laboratories, Sikkim, India.
2	Menthol	S.D Fine Chem Ltd., Mumbai, India
3	Potassium phosphate	S.D Fine Chem Ltd., Mumbai, India
4	Triethylamine	S.D Fine Chem Ltd., Mumbai, India
5	Acetonitrile HPLC Grade	Rankem, India.

2.2 Methods:

2.2.1 Chromatographic conditions

- **Chromatographic separation**: Xterra C18 (4.6 x 150mm),
- **Software:** Empower
- **Detection:** UV at 242 nm at ambient temperature.
- **Injection volume:**20µl
- **Flow rate :**1.0 ml/mil.
- **Run time**: 5 minutes.
- **Mobile phase :** methanol, potassium phosphate buffer (pH 7.0) and triethylamine
- **Ratio:**23:77:0.2,
- **Flow rate**: 1.15mL/min

2.2.2 Mobile phase and solutions

The mobile phase consisting of a binary mixture of acetonitrile and buffer adjusted to pH 3.5 with orthophosphoric acid in a ratio of 65:35. Degassed by ultrasonic water bath and filtered through 0.45µ membrane file.

2.2.3 Standard and Sample Solution Preparation

Preparation of standard solution: -

- Weighed accurately 24.8 mg and transferred 100mg of Ceftriaxone in a 100 ml volumetric flask.
- Dissolved with 70 ml of mobile phase
- Sonicated for 10 minutes and made up the volume with the mobile phase.
- Pipetted out 5 ml from this solution and diluted to 50ml with the mobile phase.

Preparation of sample solution:-

- An appropriate weight of the sample containing 100mg of ceftriaxone was transferred in a 100ml volumetric flask.
- Dissolved with 70 ml of mobile phase
- Sonicated for 10 minutes and made up the volume with the mobile phase.
- The solution was filtered through 0.22µ filter
- filtrate was diluted with the mobile phase to give a final concentration of 100µg/mL of Ceftriaxone.

2.2.4 Validation Parameters

As per ICH guidelines Q2 (R1), the method validation parameters were studied: Accuracy, Linearity and Precision, Limit of detection and Limit of quantization.

2.2.4.1Linearity: - Several aliquots of standard stock solutions of Ceftriaxone were transferred into 10ml volumetric flasks and diluted up to the mark by diluents to achieve the concentrations of 10 to 30µg/ml for Ceftriaxone. Each sample solution was injected into HPLC system and the peak areas were measured. A graph of peak areas vs. concentrations was plotted and the correlation coefficient was calculated.

2.2.4.2 Precision :-

Precision Preparation of stock solution: Accurately weighed and transferred 26 mg of Ceftriaxone working standard into a 100 mL volumetric flask, added about 70 mL of mobile phase and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock solution).

Preparation of 50 µg/ml solution:

Further pipetted 2 ml of the above stock solution out into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

2.2.4.3Accurary :

Preparation of Standard stock solution

Accurately weighed and transferred 26 g of Ceftriaxone Working standard into a 100 mL volumetric flask, added about 70 mL of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent (Stock solution).

Preparation of 50% solution

- Accurately weighed and transferred 15mg of Ceftriaxone API sample into a 100 mL volumetric flask, added about 70 mL of diluent and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution).
- Further pipetted 2 ml of the above stock solution out into a 10ml volumetric flask and diluted up to the mark with diluent. Mixed well and filtered through 0.45µm filter.

Preparation of 100% solution

- Accurately weigh and transfer 26.1 mg of Ceftriaxone into a 100 mL volumetric flask add about 70 mL of Distilled water and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
- Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

For preparation of 150% solution (With respect to target Assay concentration)

- Accurately weigh and transfer 44.5 mg of Ceftriaxone API sample into a 100 mL volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).
- Further pipette 2 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.
- Calculate the Amount found and Amount added for Ceftriaxone and calculate the individual recovery and mean recovery values.

2.2.4.4 Specificity:-

It is the ability of the method to measure the analyte of interest specifically in presence of matrix and other components. Samples of blank and placebo were injected as per the test procedure.

2.2.4.5 Limit of detection:-

Preparation of 10µg/ml solution: Accurately weigh and transfer 10 mg of Ceftriaxone API sample into a 100 mL volumetric flask add about 70 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Pipette 1 ml of stock solution into a 10 ml of volumetric flask dilute up to the mark with diluents.

2.2.4.6 Limit of quantification:-

Preparation of solution was same as in case of Limit of detection As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method.

a) The flow rate was varied at 0.7 ml/min to 0.9 ml/min.

b)The Organic composition in the Mobile phase was varied from 30% to 40%

Forced Degradation study

Forced degradation studies include the degradation of new drug substance and drug product at conditions more severe than accelerated conditions. These studies illustrate the chemical stability of the molecule which further facilitates the development of stable formulation with suitable storage conditions. ICH guidelines demonstrate certain degradation conditions like light, oxidation, dry heat, acidic, basic, hydrolysis etc. ICH Q1A, QIB and Q2B exemplify the forced degradation studies.

3.0 Results and Discussion

3.1 Standard and Sample Solution Preparation

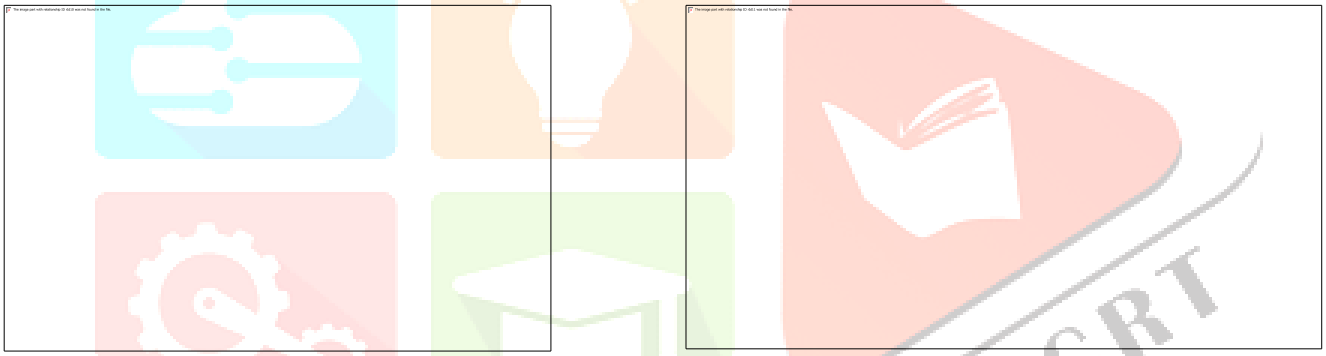


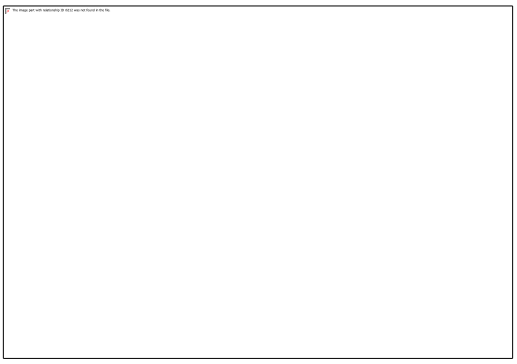
Figure 01: Standard and sample

3.2 Validation Parameters

3.2.1. Linearity

Concentration(ug/ml)	Mean Peak Area (n=3)
5	0.0543
10	0.1230
15	0.0134
20	0.0154
25	0.2341
30	0.2543
Correlation coefficient	0.99945

Table 1: Result of Linearity



n=Number of replicate injections

Figure 02. Linearity spectras of ceftriaxone sodium

3.2.2 Precision

S.no	Peak Area	Retention time (min)	Tailing factor
1	4476.4	3.876	1.312
2	4572.65	3.880	1.334
3	4534.203	3.934	1.345
4	4502.65	3.962	1.356
5	4516.52	3.973	1.358
6	4502.61	3.983	1.367
Mean	45.05.14	3.941	1.324
SD	24.122	Limit: %RSD for area NMT 2.0%	
%RSD	0.5641		

Table no 2: Results of Repeatability

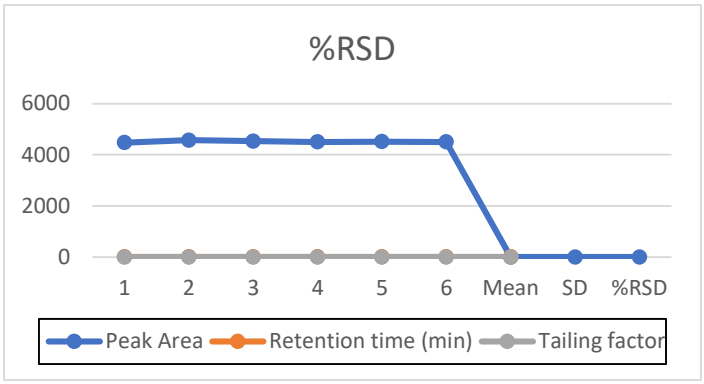


Figure 03. Repeatability spectra of ceftriaxone sodium

Drug	Concentration (ug/ml)	Inter-day area mean(n=3) ±SD	%RSD
Cetroxime	5	2221.34 ±12.34	0.92
	10	4460.43±50.21	1.12
	15	5645.32±32.12	1.24
	20	6432.41±12.23	1.32
	25	6821.23±41.34	1.46
	30	7234.45±76.34	1.52

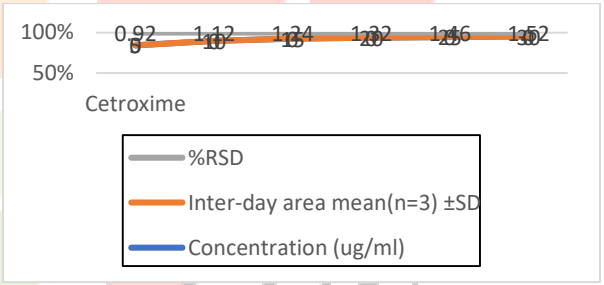


Figure 04. Intermediate Precision of ceftriaxone sodium

Table 3: Result of Intermediate Precision

3.3. Accuracy

Table 04: Accuracy for 50%, 100 % and 150 % solution-percentage RSD value

S.No	Peak Name	RT	Area	Height	RT	Area	Height	RT	Area	Height
1	Ceftriaxone	2.865	536612	71984	2.868	912212	121923	2.864	1561026	208192
2		2.865	536682	71804	2.868	913332	122194	2.862	1559472	208264
3		2.865	537015	70912	2.868	914672	122066	2.862	1561156	209915
Mean			536764			913442			1560552	
SD			215.0			1234.5			936.2	
%RSD			0.04			0.12			0.04	

Acceptance Criteria for accuracy

The % Recovery for each level should be between 98.0 to 102.0%. From the experimental value the recovery was found to be within the limit.

Table 05. Percentage Recovery

% Conc	Mean Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50	536765	15.3	15.36	101.03	
100	913405	26.2	26.13	100.3	100.5
150	1560553	44.6	44.63	100.5	

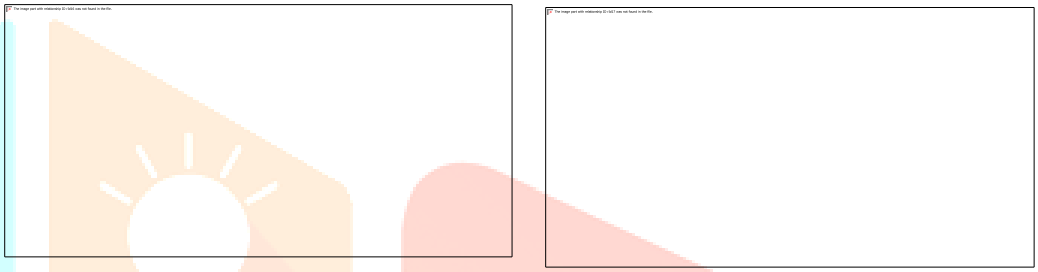


Figure 05. Accuracy spectras for 50% solution

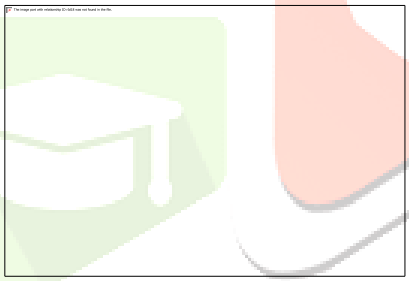


Figure 06. Accuracy spectras for 100% solution

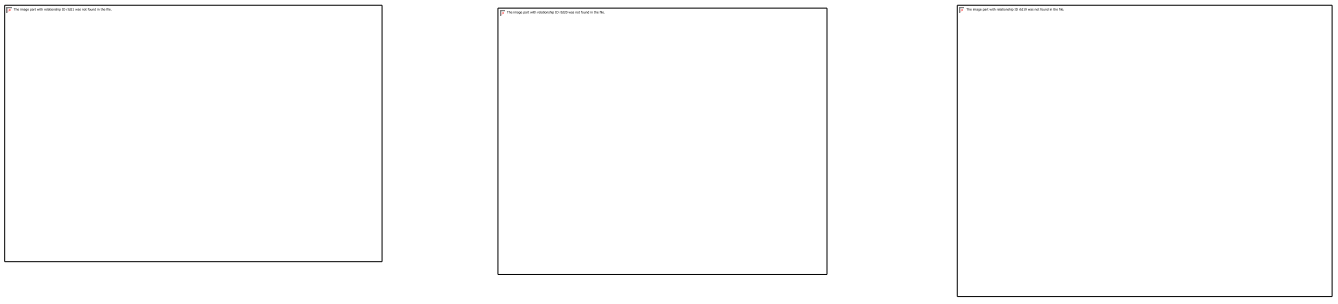


Figure 07. Accuracy spectras for 150% solution

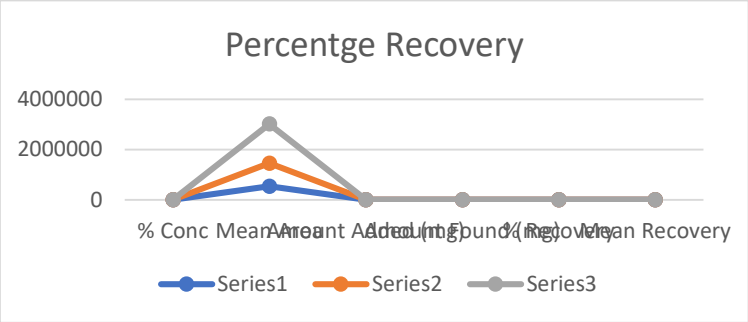


Figure 08 : Percentage Recovery

3.4. Specificity

It is the ability of the method to measure the analyte of interest specifically in presence of matrix and other components. Samples of blank and placebo were injected as per the test procedure. The chromatograms of blank and placebo are represented as (Fig. 08).

Figure 09: Chromatogram of Blank and Placebo

3.5.Limit of detection

S/N Ratio value should be 3 for LOD. Calculation of S/N Ratio: Average Baseline Noise obtained from Blank: 24 µV Signal Obtained from LOD solution (0.2% of target assay concentration): 69 µV S/N = 69/24 = 2.87. From the experimental value the S/N ratio was found to be within limit i.e. 2.87 (Figure 09

Figure:10. Spectra for limit of detection

3.6. Limit of quantification S/N Ratio value should be 10 for LOQ solution Calculation of S/N Ratio: Average Baseline Noise obtained from Blank: 24 µV Signal Obtained from LOD solution (0.8% of target assay concentration): 253µV S/N = 253/24 = 10.54 From the experimental value the S/N ratio was found to be within limit i.e. 10.54 (Figure 10).

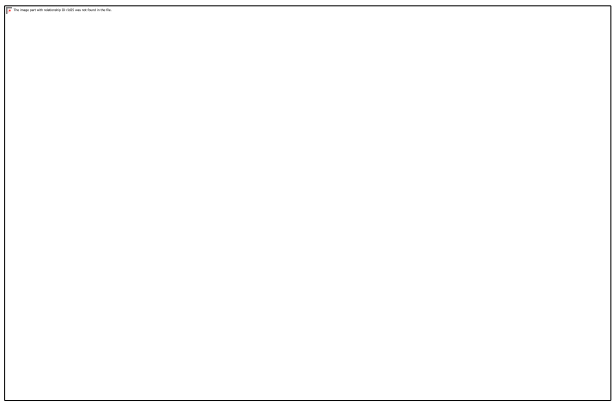


Figure 11. Spectra for limit of quantification

3.7. Forced Degradation study

Stress Parameters	**Sample treatment**	**Assay (%)**	**Degradation(%)**
Reference	Fresh solution	98.03	0
Acid hydrolysis	0.1 M HCl for 30 mins	91.52	6.63
Base hydrolysis	0.1 M NaOH for 10 mins	83.12	15.03
Oxidation	5.0% H2O2 for 30 mins	60.72	38.21
Light degradation	UV-Light for 24 h	74.82	22.61

Table no : 06 :Results from the Forced Degradation Study of the Method

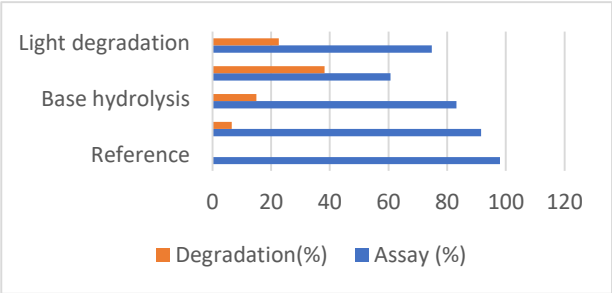


Figure11: Results from the Forced Degradation Study of the Method

3.8. Recovery Method

Table:07 Recovery Result

S.No	Standard drug conc.(ug/ml) (a)	Sample drug conc.(ug/ml) (b)	Total drug conc.(ug/ml) (c)	Total amount found*(ug/ml) (d)	% Recovery of standard (d-b)/a*100
1	2.5	2.5	5	5.02	102.32
2	7.5	2.5	10	9.82	98.43
3	12.5	2.5	15	15.14	100.93

***Mean of three replicate studies**

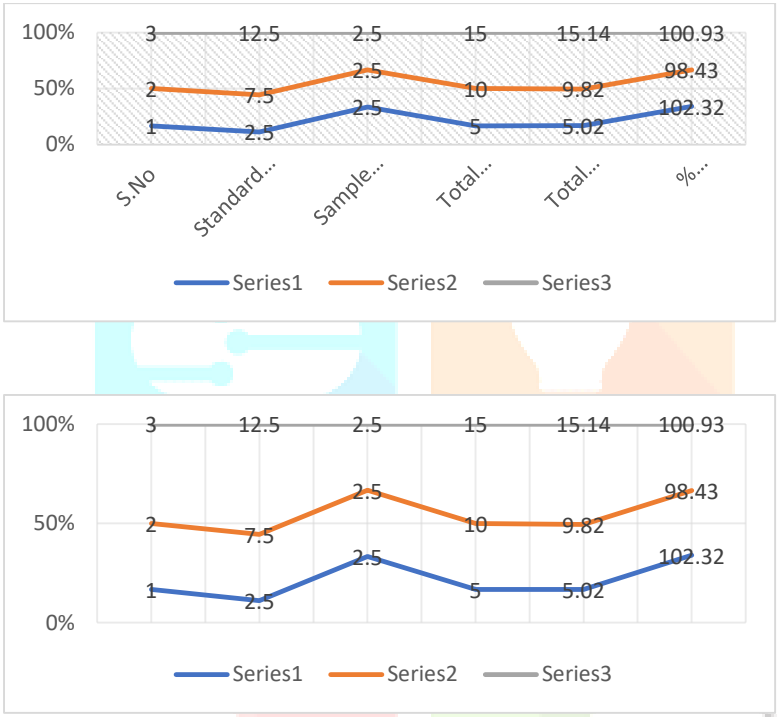


Figure12: Recovery Result

4.0 Conclusion: -The use of isocratic HPLC methods for simultaneous quantification is most popularity because they not only reduce costs but also boost analysis speed. For the quantitative analysis of Ceftriaxone sodium, a more quick, precise, specific, sensitive, economical, reproducible, isocratic reverse phase HPLC technique was designed and validated. Some Validation parameters are:

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S.No	Parameters	Values
1	Linearity	0.9945
2	Precision	0.564
3	Accuracy	0.04,0.12 and 0.04
4	% Recovery	100.5

5	Specificity	3.23
6	LOD	2.87
7	LOQ	10.54
8	Forced degradation	98.03
9	Recovery method	102.32,98.43 and 100.93

Thus, the proposed analytical method was simple and represents specific procedure for assay of Ceftriaxone Sodium and this HPLC method successfully applicable for regular analysis of Ceftriaxone sodium in quality control laboratories.

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Conflict of Interest

Author declairs that there is no conflict of interest.

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