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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

SIMULTANEOUS DETERMINATION AND VALIDATION OF PARACETAMOL, TRAMADOL AND SERRATIOPEPTIDASE BY RP-HPLC IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Paracetamol, Tramadol, Serratiopeptidase and RP-HPLC.

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ABSTRACT

A new simple, accurate, precise and Rapid RP -HPLC method for the determination of Paracetamol, Tramadol and Serratiopeptidase was developed and validated as per ICH guidelines. Paracetamol(PAR), Tramadol(TMD) and Serratiopeptidase(SRT) were separated by RP - HPLC using a Shimadzu C_{18} column (5µm, 250mm x 4.6mm i.d.) and isocratic elution with a flow rate of 1 mL/min. Mixture of Phosphate buffer and Acetonitrile in the ratio (55:45) with pH=4.4 was used as mobile phase and UV detection at 278 nm. The retention time of Paracetamol, Tramadol and Serratiopeptidase was found to be 2.872 min, 4.110 and 1.782min respectively. The linear range of determination for Paracetamol, Tramadol and Serratiopeptidase were 20-100µg/mL, 4-20 µg/mL and 2-10 µg/mL respectively. LOD of above drugs was found to be 0.561057 µg/mL, 0.134477 µg/mL and 0.185634 µg/mL respectively and LOQ of 1.700172 µg/mL, 0.40706 µg/mL and 0.562528 µg/mL for Paracetamol, Tramadol and Serratiopeptidase respectively. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision and Robustness.

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INTRODUCTION:

Paracetamol exerts analgesic and antipyretic effect like salicylates. It has weak activity on COX in the inflamed peripheral tissues, it does not produce GI irritation, acid-base imbalance, electrolyte disturbances, nor does it affect platelet activity. Chemically Paracetamol is N-(4-hydroxyphenyl) acetamide[5]

The analgesic properties of Tramadol can be attributed to norepinephrine and serotonin reuptake blockade in the CNS, which inhibits pain transmission in the spinal cord. Chemically Tramadol is (1R,2R)-2-[(dimethyl amino) methyl]-1-(3-methoxyphenyl)

Cyclohexan-ol[4]

Serratiopeptidas is an enzyme derived from the bacteria belonging to genus Serratia. Serratiopeptidase binds to α2-macroglobulin in the blood in a1:1 ratio. The mechanism of action of Serratiopeptidase appears to be hydrolysis of histamine, bradykinin and serotonin. Serratiopeptidase also has a proteolytic and fibrinolytic effect. Chemically Serratiopeptidase is Ca_7Zn^{+16} [6]

The combination of Paracetamol, Tramadol and Serratiopeptidase is prescribed for treating to relieve severe pain.

MATERIALS AND METHODS:

Instrument used: A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software.

Chemicals and reagents: Acetonitrile (HPLC grade) was procured from Merck Ltd, double distilled water (HPLC grade), Potassium di-hydrogen o-phosphate. All other chemical reagents were of analytical grade.

Drug sample: Standard Paracetamol, Tramadol and Serratiopeptidase were obtained as gift sample from micro labs, Bangalore.

Preparation of mobile phase: The mobile phase (1000 ml) was prepared by mixing of Buffer solution and Acetonitrile in the ratio of 55:45v/v and adjust the pH 4.4 with Triethyleneamine. The mobile phase was filtered through a 0.45 µm filter paper then it was sonicated for 10 min.

Preparation of buffer solution: 2.72 g of Potassium di-hydrogen o-phosphate was dissolved into 1000 ml of HPLC water, and adjusted pH to 4.4 with Triethylenamine.

Preparation of standard stock solution: 50 mg Paracetamol, Tramadol and Serratiopeptidase were weighed separately and transferred into three different 50ml volumetric flasks. All the above drugs were

dissolved in 35ml of mobile phase by sonication and then volume was made up to the mark with mobile phase to obtain final concentration of 1000µg/ml of each component (stock A A_1 and A_2 solution). From the above stock A solution 10ml of aliquot was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with mobile phase to obtain the final concentration of 100µg/ml of each component (stock B B_1 and B_2 solution).

Preparation of sample solution

20 tablets of Paracetamol, Tramadol and Serratiopeptidase in combination were weighed and powdered. Tablet powder equivalent to 100 mg of Paracetamol was weighed accurately and dissolved in 70 mL of mobile phase and sonicated for 15 min then filtered and the filtrate was diluted upto 100 mL with mobile phase (stock A).

From the above stock A solution, 10 ml of aliquot was pipetted out in a 100 mL volumetric flask and the volume was made up to the mark with mobile phase (stock B).

From the stock B further dilutions were made to get the concentration of the drugs within the range.

RESULTS AND DISCUSSION

The selected drugs Paracetamol, Tramadol and Serratiopeptidase were estimated in Bulk and Formulation by using RP-HPLC methods. The methods were validated for all validation parameters as per ICH guidelines. The linearity range for PARA, TMD and SRT was 20-100µg/ml, 4-20µg/ml and 2-4µg/ml respectively. The % RSD for intraday and inter-day precision was found to be less than 2%. The methods have been validated in assay of active pharmaceutical ingredients. The accuracy of the methods were validated by recovery studies and was found to be significant and under specification limits, with % recovery 99-102%. The assay results were found to be within the acceptable limits.

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. The solution containing 40 µg/ml of PAR, 8 µg/ml of TMD and 4 µg/ml of SRT was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate and wavelength.

Ruggedness

The evaluation of ruggedness has been considered during the development phase and depends upon the type of procedure under study. It shows the reliability of analysis with respect to deliberate variations in analyst. The solution containing 40 µg/ml of PAR, 8 µg/ml of TMD and 4 µg/ml of SRT was injected into sample injector of HPLC three times by different analysts.

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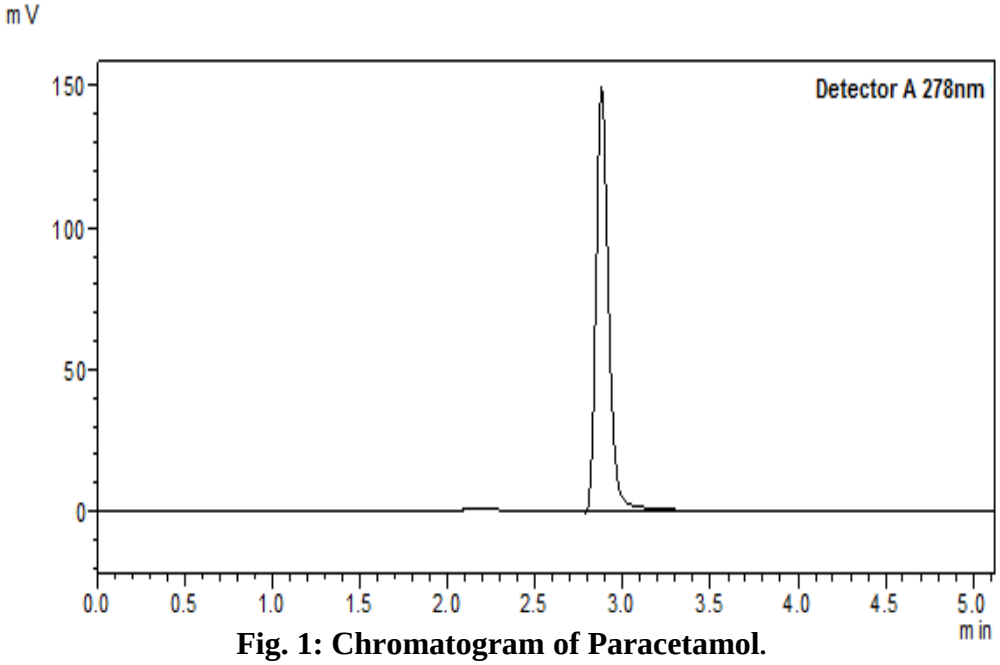

Fig. 1: Chromatogram of Paracetamol.

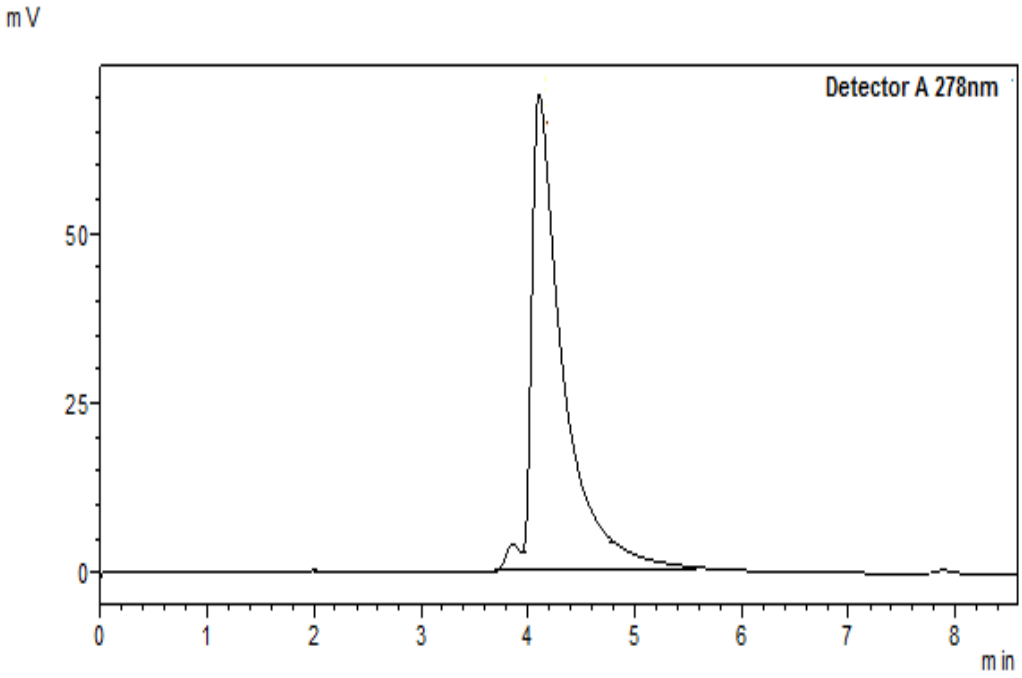

Fig. 2: Chromatogram of Tramadol.

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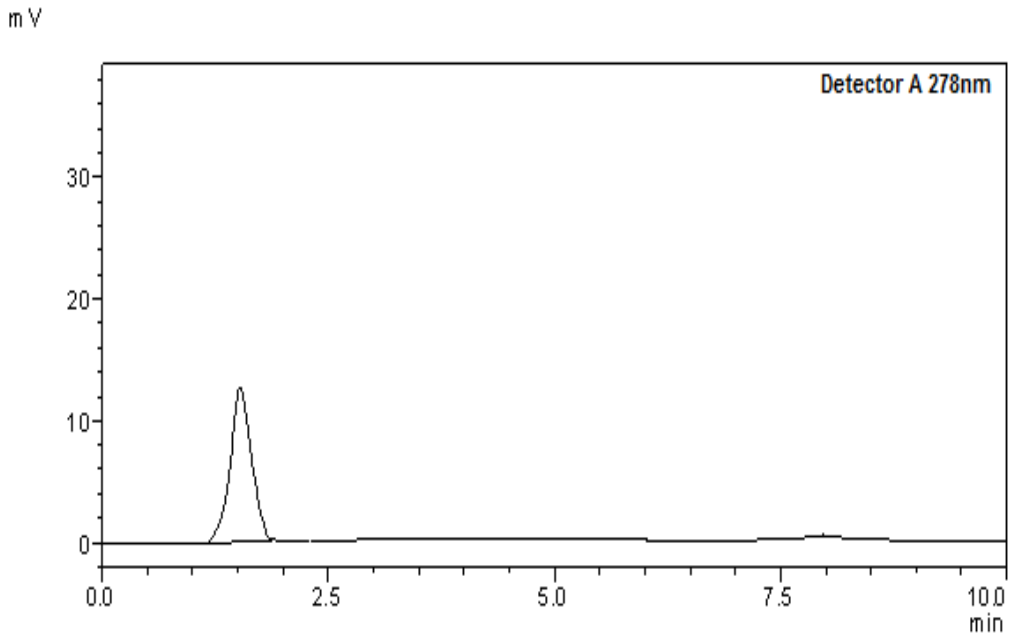

Fig. 3: Chromatogram of Serratiopeptidase.

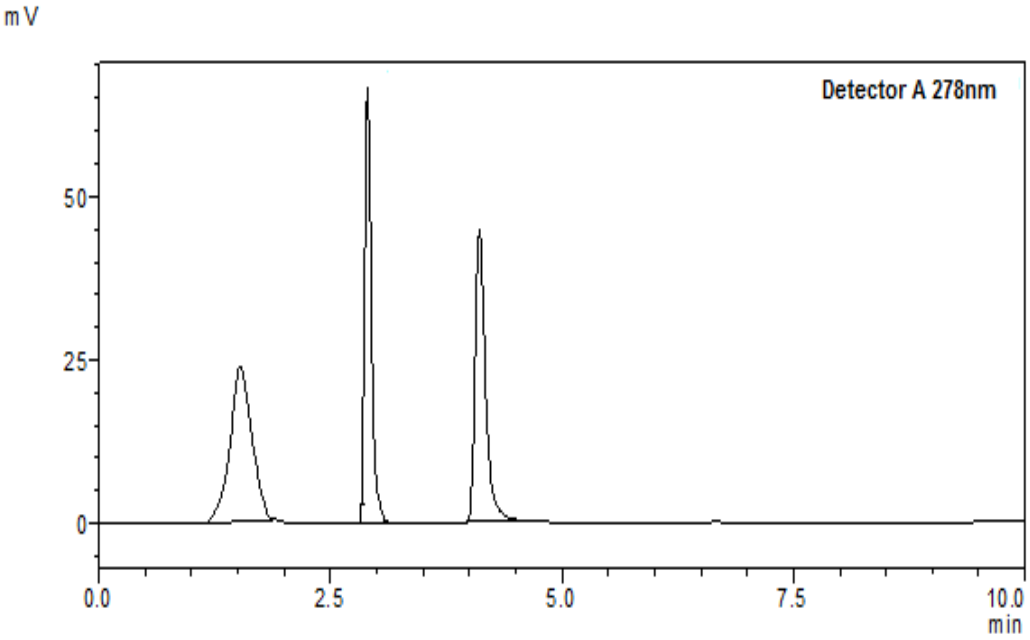

Fig. 4: Chromatogram of Paracetamol, Tramadol and Serratiopeptidase.

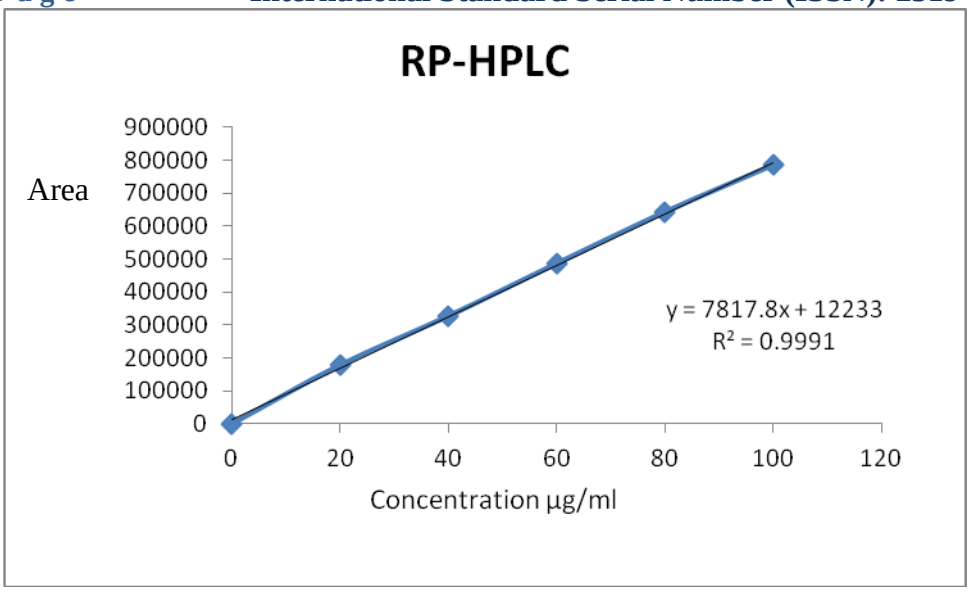

Fig. 5: Calibration Curve of PAR at 278 nm by RP – HPLC Method.

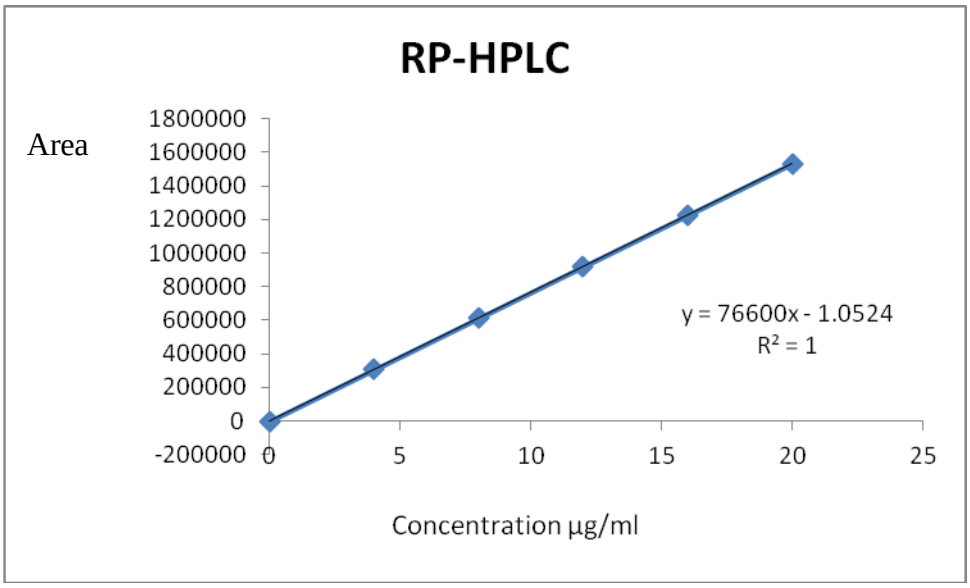

Fig. 6: Calibration Curve of TMD at 278 nm by RP – HPLC Method.

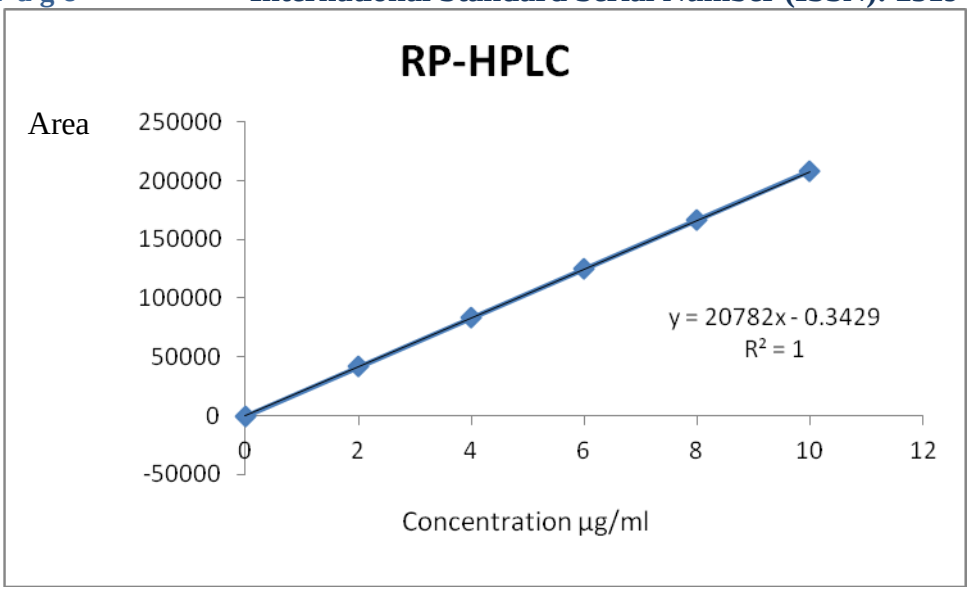

Fig. 7: Calibration Curve of SRT at 278 nm by RP – HPLC Method.

Table no. 1: Summary of Validation and System Suitability Parameters of Paracetamol, Tramadol and Serratiopeptidase.

Parameters	PAR	TMD	SRT
Linear Range (µg/ml)	20-100	4-12	2-10
Slope	7817.8x	76600 x	20782x
Intercept	12233	1.0524	0.3429
Regression coefficient (r^2)	0.9991	1	1
Limit of Detection (µg/ml)	0.561057	0.134477	0.185634
Limit of Quantification (µg/ml)	1.700172	0.407506	00.562528
Retention time (min)	2.878	4.110	1.782
Tailing Factor	1.259	1.483	1.063
Resolution Factor	21.142		
Theoretical Plate	6516	6384	2750

Table 2: Statistical Validation Data for Accuracy Determination

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Total amount recovered (µg)	% Recovery	RSD
80%	PAR	40	32	72.3	100.416	0.138313
	TMD	8	6.4	14.5	100.694	1.055893
	SRT	4	3.2	7.3	101.38	1.38889
100%	PAR	40	40	80.2	100.25	0.260317
	TMD	8	8	16.1	100.625	0.950742
	SRT	4	4	8.1	101.25	1.25
120%	PAR	40	48	88.2	100	0.236195
	TMD	8	9.6	17.7	100.65	0.564972
	SRT	4	4.8	8.7	98.863	1.136364

Table no. 3: Statistical Validation Data for Intra-day Precision.

Component	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
PAR	99.958	0.020412	0.020421	0.008334
TMD	100.06	0.377921	0.377658	0.154291
SRT	99.194	1.765146	1.779481	0.720644

Table no. 3: Statistical Validation Data for Inter-day Precision.

Component	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
PAR	100.01	0.07722	0.077212	0.031526
TMD	99.944	0.08611	0.086114	0.035138
SRT	99.888	0.17213	0.172324	0.070275

Table no. 5: Robustness Results for Variation in Flow Rate (ml/min).

Method Parameter Flow Rate (mL/min)	Level	Retention Time			Tailing Factor		
		PAR	**TMD**	**SRT**	**PAR**	**TMD**	**SRT**
0.9	-1	2.889	4.124	1.794	1.269	1.494	1.075
1.0	0	2.878	4.110	1.782	1.259	1.483	1.063
1.1	+1	2.866	4.123	1.793	1.268	1.494	1.074

Table no. 6: Robustness result for variations in Wavelength (nm).

Method Parameter Wavelength (nm)	Level	Retention Time			Tailing Factor		
		PAR	TMD	SRT	PAR	TMD	SRT
276	-2	2.766	4.012	1.671	1.168	1.372	1.052
278	0	2.878	4.110	1.782	1.259	1.483	1.063
280	+2	2.987	4.231	1.893	1.378	1.594	1.174

Table no. 7: Ruggedness result for variations in Analyst.

Method Parameter	Retention Time			Tailing factor		
Analysts	PAR	TMD	SRT	PAR	TMD	SRT
Analysts 01	2.878	4.110	1.782	1.259	1.483	1.063
Analysts 02	2.876	4.109	1.783	1.258	1.482	1.064

CONCULSION:

An accurate and precise HPLC method with UV detection for the simultaneous estimation of Paracetamol, Tramadol and Serratiopeptidase was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for above drugs is less than 10 min and shows high degree of accuracy and precision. It is convenient for simultaneous estimation of PARA, TMD and SRT in tablet formulation.

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