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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DUTASTERIDE AND TAMSULOSIN HYDROCHLORIDE BY RP-HPLC METHOD IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The present work was undertaken with the aim to develop and validate a rapid and consistent RP-HPLC method in which the peaks will be appear with short period of time as per ICH Guidelines. The Purpose of this work to develop an accurate, simple, sensitive and precise RP-HPLC method was developed for the determination of dutasteride and tamsulosin hydrochloride in tablet dosage form. The RP-HPLC separation was achieved on BDS Hypersil C18 column (250 mm, id 4.6 mm, 5 µm) using mobile phase CH3COONH4: Methanol (55:45)v/v) at a flow rate of 0.8 ml/min at an 30^{0}C temperature. Quantification was achieved with photodiode array detection at 254 nm over the concentration range 80µg/ml. The proposed method was validated for its linearity. Statistically, accuracy ,precision and robustness. This method can be employed for routine quality control analysis of Dutasteride and Tamsulosin Hydrochloride in tablet dosage form, and also applied successfully for the determination of dutasteride and tamsulosin Hydrochloride in combination of pharmaceutical dosage form. The method was simple, precise, accurate and

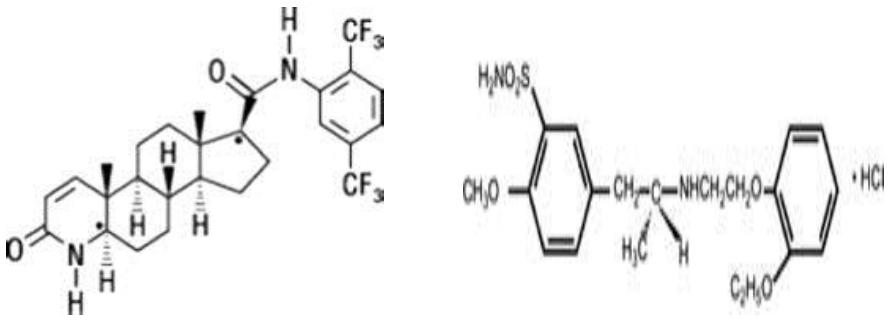
sensitive and applicable for the simultaneous determination of Dutasteride and Tamsulosin hydrochloride in bulk drug and in combined dosage forms.

KEYWORDS: Tamsulosin Hcl, dutasteride, RP-HPLC.

INTRODUCTION

Dutasteride inhibits the conversion of testosterone to 5 alpha-dihydrotestosterone (DHT), which is the androgen primarily responsible for the initial development and subsequent enlargement of the prostate gland. Testosterone is converted to DHT by the enzyme 5 alpha-reductase, which exists as 2 isoforms, type 1 and type 2. Dutasteride is a competitive and specific inhibitor of both type 1 and type 2 5 alpha-reductase isoenzymes, with which it forms a stable enzyme complex. Dissociation from this complex has been evaluated under *in vitro* and *in vivo* conditions and is extremely slow. Dutasteride does not bind to the human androgen receptor. For the treatment of symptomatic benign prostatic hyperplasia (BPH) in men with an enlarged prostate gland to improve symptoms, and reduce the risk of acute urinary retention and the need for surgery. Highly bound to albumin (99%) and α-1 acid glycoprotein (96.6%). Dutasteride is extensively metabolized in humans. Dutasteride and its metabolites were excreted mainly in feces.

Dutasteride is Freely soluble in water, slightly soluble in methanol and alcohol, practically insoluble in acetone and in methylene chloride. The IUPAC name is (1S,2R,7R,10S,11S,14S,15S)-N-[2,5-bis(trifluoromethyl)phenyl]-2,15-dimethyl-5-oxo-6-azatetracyclo[8.7.0.0^{2},^{7}.0^{11},^{15}]heptadec-3-ene-14-carboxamide (Fig.1.0).



A **B**

Fig.1: Chemical Structures of A) Dutasteride B) Tamsulosin Hcl.

Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Approximately 70% of the alpha1-

receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate, and thus decreases urinary outflow resistance in men. It was Used in the treatment of signs and symptoms of benign prostatic hyperplasia (reduction in urinary obstruction and relief of associated manifestations such as hesitancy, terminal dribbling of urine, interrupted or weak stream...etc.)**.** Absorption of tamsulosin HCI from capsules 0.4 mg is essentially complete (>90%) following oral administration under fasting conditions.

Absorption of tamsulosin HCI from capsules 0.4 mg is essentially complete (>90%) following oral administration under fasting conditions. Tamsulosin, a sulfamoylphenethylamine-derivative alpha-adrenoceptor blocker with enhanced specificity for the alpha-adrenoceptors of the prostate, is commonly used to treat benign prostatic hyperplasia (BPH). The drug is commercially available in a racemic mixture of 2 isomers, and is pharmacologically related to doxazocin, prazosin, and terazosin. However, unlike these drugs, tamsulosin has a higher affinity for the alpha-1A- adrenergic receptors, which are located in vascular smooth muscle. Studies show that tamsulosin has about 12 times greater affinity for alpha-1 adrenergic receptors in the prostate than those in the aorta, which may result in a reduced incidence of adverse cardiovascular effects. Tamsulosin hydrochloride is extensively metabolized by cytochrome P450 enzymes in the liver and less than 10% of the dose is excreted in urine unchanged. The metabolites of tamsulosin hydrochloride undergo extensive conjugation to glucuronide or sulfate prior to renal excretion. On administration of the radiolabeled dose of tamsulosin hydrochloride to four healthy volunteers, 97% of the administered radioactivity was recovered, with urine (76%) representing the primary route of excretion compared to feces (21%) over 168 hours.

5-[(2R)-2-{[2-(2-ethoxyphenoxy)ethyl]amino}propyl]-2-methoxybenzene-1-sulfonamide. It is Very soluble in water, soluble in methanol, practically insoluble in methylene chloride. The molecular formula and molecular weight $C_{20}H_{28}N_2O_5S$ and 408.512.

This drug is not official in any pharmacopoeia; hence no official method was available for the estimation of this drug in the pharmaceutical formulations. Literature survey revealed several Bioanalytical methods for its estimation which include Reversed Phase-High Performance Liquid Chromatography [RPHPLC] with fluorescence detection, HPLC - electrospray tandem mass spectrometry, LC-MS, liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry and RPHPLC method[19-20]. The developed method

was unique advantage over the above mentioned methods, as it is simple, economical, faster, precise, accurate and specific for quantitative determination of Tamsulosin hydrochloride in pharmaceutical dosage form.

Till date, there was no single RP-HPLC method was developed for the simultaneous estimation of Tamsulosin and Dutasteride in bulk drug as well as in pharmaceutical dosage forms. The present work deals with RP-HPLC method development and validation for the quantitative analysis of Tamsulosin and Dutasteride and its stress degradation products. The aim of the present work was to develop an economic, accurate, specific, reproducible RP-HPLC method using PDA detection for the determination of Tamsulosin and Dutasteride, either in bulk form or in Pharmaceutical dosage form. The chemical structures of the drugs were represented in fig. no. 1 & 2.

MATERIALS AND METHODS

Materials and Chemicals

The following chemicals were procured for the process: Water [HPLC Grade], Methanol [HPLC Grade], Acetonitrile [HPLC Grade], Tamsulosin and Dutasteride [Working standards] & KH2PO4 all the chemicals were procured from STANDARD SOLUTIONS and the tablets were collected from the Local market.

Instrument Configuration

WATERS HPLC, Model: Aglient 2695, Photodiode array detector (PDA), with an automated sample injector. The output signal was monitored and integrated using Empower 2 software. ELIPSE C8 (150mm x 4.6, 5 µm, Make: Waters) column was used for separations.

Methods

Preparation of mobile phase

Transfer 1000ml of HPLC water into 1000ml of beaker and add Ammonium Acetate.

Transfer the above solution 700ml of CH3COONH4, 300ml of Methanol is used as mobile phase. They are mixed and sonicated for 20min.

Preparation of the dutasteride and tamsulosin standard and sample solution:

Accurately weigh and transfer 10 mg of Dutasteride and 8.13mg TamsulosinHclinto50ml of volumetric flask and add 10ml of Methanol and sonicate 10min (or) shake 5min and make with water.

Transfer the above solution into 5ml into 25ml volumetric flask and dilute to volume with water.

Preparation of sample stock solution

Commercially available 20 tablets were weighed and powdered the powdered equivalent to the 2650.5mg of Dutasteride and Tamsulosin Hcl of active ingredients were transfer into a 25ml of volumetric flask and add 10ml of Methanol and sonicate 20min (or) shake 10min and makeup with water.

Transfers above solution 5ml into 25ml of the volumetric flask dilute the volume with Water. And the solution was filtered through 0.45µm filter before injecting into HPLC system.
All additional reagents were prepared to their closest concentrations utilizing HPLC-grade water.

Optimized Method Chromatographic parameters

Mobile Phase: CH3COONH4: Methanol (550:450)
Column: BDS Hypersil 250X4.6mm, C18, 5um
Flow Rate: 0.8ml/min
Temperature: 30^0c
Volume: 10ul
Detector: PDA

METHOD VALIDATION

System suitability

Tailing factor for the peaks due to Dutasteride and Tamsulosin Hcl in standard solution should not be more than 2.0. Theoretical plates for the peaks Dutasteride and Tamsulosin Hcl in standard solution should not be less than 2000.

Specificity

Solution of standard, sample, blank, and placebo were prepared as per test procedure and injected into the HPLC system.

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Linearity

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Statistical Evaluation

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

Precision

Preparation of sample

Transfer the 2650.5mg of sample into a 50ml of volume at flask and add 10ml of water and 10ml of Methanol and sonicate 20min and makeup with water. Transfer the above solution into 5ml into 25ml volume metric flask dilute to the volume with water. The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections.

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention time is are not more than 2.0.

Recovery/Accuracy

Recovery study can be performed in the concentration range of 80% to 120% of the target concentration of the test. Minimum 3 concentrations are recommended.

Limit Of Detection

The sensitivity of measurement of Dutasteride and Tamsulosin Hcl by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noice level. The LOD was calculated as three times the noise level.

$$LOD = 3.3\ \sigma / S$$

Limit Of Quantitation

The sensitivity of measurement of Dutasteride and Tamsulosin Hcl by the use of proposed method was estimated in terms of limit of quantitation (LOQ). The LOQ was calculated by

the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

$$LOQ = 10\ \sigma / S$$

Robustness

Effect of variation in flow rate

Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

Effect of variation in wavelength

Prepare the system suitability solution as per the test method and injected into the HPLC with ±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.

RESULTS AND DISCUSSION

System suitability study are summarized in the above table. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis. Chromatograms explain that retention time for standard, sample and commercial product of Dutasteride and Tamsulosin Hcl are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 100.00% for Dutasteride and 100.00% for Tamsulosin Hcl All the results indicate that the method is highly accurate**.** For percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.** A linear relationship between peak areas versus concentrations was observed for Dutasteride and Tamsulosin Hcl in the range of 50% to 150% of nominal concentration. Correlation coefficient was 0.999 for both Dutasteride and Tamsulosin Hcl which prove that the method is linear in the range of 50% to 150%. The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce

significant changes in analytical results which were presented in the above table. As the changes are not significant we can say that the method is Robust.

Table 1: System suitability data of Dutasteride and Tamsulosin Hcl.

parameter	Dutasteride	Tamsulosin Hcl	Acceptance criteria
Retention time	3.153	7489323	±10
Theoretical plates	39801	3808	>2500
Tailing factor	1.36	1.22	<2.00
% RSD	0.3	0.5	<2.00

Table 2: Accuracy (%recovery) results of Dutasteride.

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	1325.25	19.800	19.83	100	100
		2	1325.25	19.800	19.89	100	
		3	1325.25	19.800	19.86	100	
2	100%	1	2650.50	39.600	39.72	100	100
		2	2650.50	39.600	39.75	100	
		3	2650.50	39.600	39.71	100	
3	150%	1	3975.75	59.400	59.53	100	100
		2	3975.75	59.400	59.55	100	
		3	3975.75	59.400	59.55	100	

Table 3: Accuracy (%recovery) results of Tamsulosin HCL.

S.NO	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	1325.25	16.000	15.98	100	100
		2	1325.25	16.000	15.99	99	
		3	1325.25	16.000	15.99	100	
2	100%	1	2650.50	32.000	31.94	100	100
		2	2650.50	32.000	31.95	100	
		3	2650.50	32.000	31.95	100	
3	150%	1	3975.75	48.000	47.87	100	100
		2	3975.75	48.000	47.82	100	
		3	3975.75	48.000	47.90	100	

Table 4: Precision data for Dutasteride.

S. no.	RT	Area	%Assay
injection1	2.043	3733623	99
injection2	2.047	3730257	99
injection3	2.050	3738735	99
injection4	2.049	3732083	99
injection5	2.024	3739693	99
injection6	2.033	3737411	99
Mean			99

Std. Dev.			0.10
% RSD			0.10

Table 5: Precision data for Tamsulosin HCL.

S. no.	RT	Area	%Assay
injection1	3.756	7466690	100
injection2	3.761	7466163	100
injection3	3.751	7463225	100
injection4	3.743	7463689	100
injection5	3.707	7469873	100
injection6	3.718	7468240	100
Mean			100
Std. Dev.			0.03
% RSD			0.03

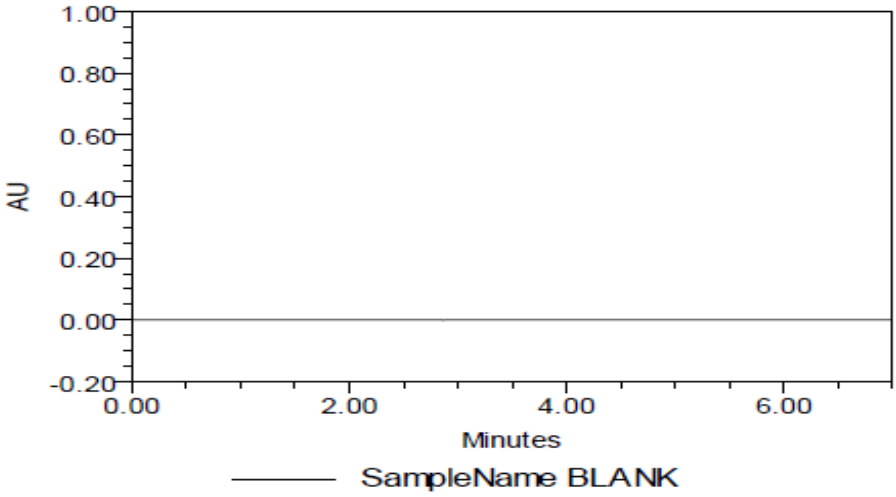


Fig 2:Typical chromatogram of the blank.

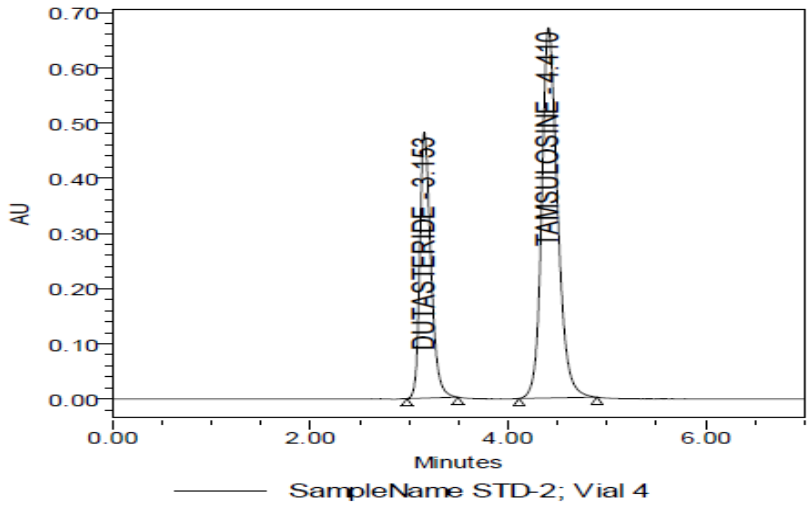


Fig 3. Chromatogram of Standard.

Table 6:Linearity data for Dutasteride.

s.no	Conc(µg/ml)	RT	Area
1.	50	3.132	1866130
2.	75	3.126	2795946
3.	100	3.128	3735679
4.	125	3.149	4661797
5.	150	3.152	5596661
Correlation coefficient (r^2)			0.999

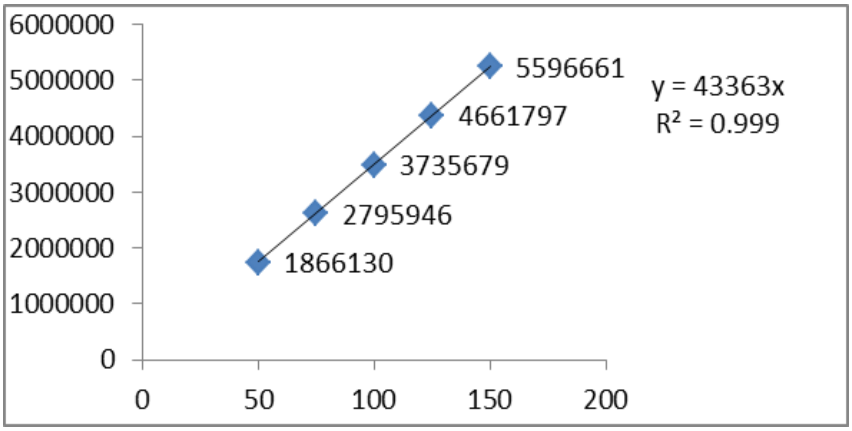


Fig 4: Linearity plot of Dutasteride.

Table 7: Linearity data for Tamsulosin Hcl.

s. no.	Conc(µg/ml)	RT	Area
1.	50	4.297	3731645
2.	75	4.287	5590588
3.	100	4.282	7465490
4.	125	4.317	9322618
5.	150	4.330	11143282
Correlation coefficient (r^2)			0.999

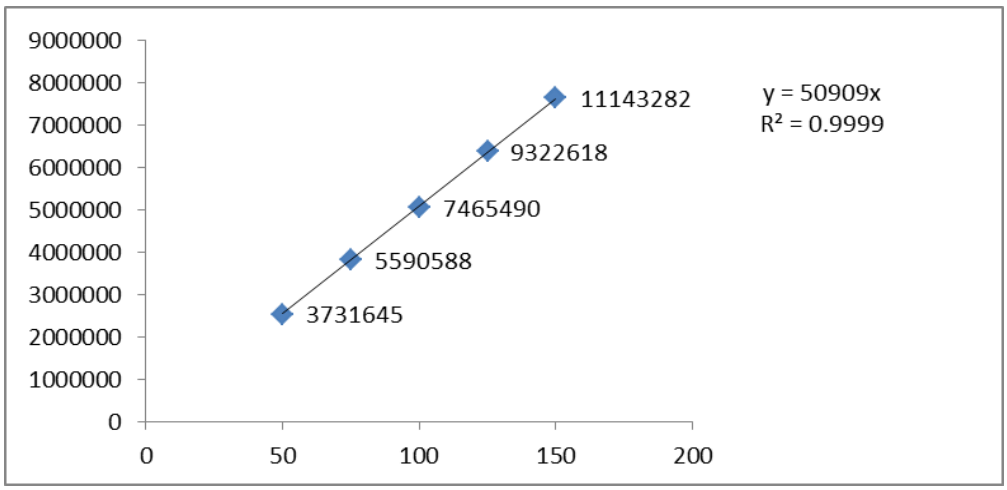


Fig 5: Linearity plot of Tamsulosin HCL.

CONCLUSION

From the reported literature, there were few methods established for the determination of Dutasteride and Tamsulosin Hcl individual and in combination with other drug. It was concluded that there was reported for the simultaneous estimation of the above selected multi component dosage form, which promote to pursue the present work. The scope and objective of the present work is to develop and validate a new simple RP-HPLC method for simultaneous estimation of Dutasteride and Tamsulosin Hcl combined dosage form. In simultaneous RP-HPLC method development, Waters HPLC with PDA detector and column used is BDS Hypersil 250X 4.6mm, C18with 5-micron particle size. Injection volume of 10 µL is injected and eluted with the mobile phase selected after optimization was 0.1M CH3COONH4 and Methanol in the ratio of 55:45 was found to be ideal. The flow rate was found to be optimized at 0.8 mL/min. Detection was carried out at 254nm. Quantitation was done by external standard method with the above mentioned optimized chromatographic condition. This system produced symmetric peak shape, good resolution and reasonable retention times of Dutasteride and Tamsulosin Hcl were found to be3.118 and4.296 minutes respectively.

The Dutasteride and Tamsulosin Hcl showed linearity in the range of 1000-3000 µg/mL and 50-150 respectively. The slope and correlation coefficient values for Dutasteride were found to be 43363 and 0.999 respectively and 13168 and 0.999 respectively for Tamsulosin Hcl which indicates excellent correlation between response factor Vs concentration of standard solutions.

Precision of the developed method was studied under system precision and method precision. The %RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The %RSD value for percentage recovery Dutasteride and Tamsulosin Hcl were found to be within the acceptance criteria. The results indicate satisfactory accuracy of method for simultaneous estimation of the Dutasteride and Tamsulosin Hcl.The forced degradation study showed the method was highly specific.

Hence, the chromatographic method developed for the Dutasteride and Tamsulosin Hcl said to be rapid, simple, sensitive, precise and accurate that can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing

laboratories, bio-pharmaceutics and bio-equivalence studies and in clinical pharmacokinetic studies.

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CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this research.

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