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Development and Validation of A Stability Indicating Reverse Phase HPLC Method For The Analysis of Montelukast Sodium

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Abstract:

Montelukast sodium**,** a cysteinyl leukotriene type-1 receptor (CysLT1) antagonist, is widely used in the form of oral tablets and granules as a second-line therapy for the management of chronic asthma. Already developed HPLC method are having certain drawbacks like long run time and use of excess amount of solvents as mobile phase. To combat these problems, a new reverse phase HPLC method is developed and validated by us. The method validation performed as per ICH Q2(R1) guidelines by using parameters like Linearity, Precision, Accuracy, LOD, LOQ, Robustness, Ruggedness, Specificity, System suitability etc. In this method, the run time is less and the retention time of montelukast sodium is at 4.56 minutes. The method is showing % RSD for area, theoretical plates and tailing factor 1.076, 0.651 and 1.187 respectively, which is less than 2%. The percent recovery was obtained from the analysis of Montelukast sodium is in the range of 99.14049% - 102.6854%.

Keywords: Montelukast sodium; Reverse phase HPLC; Robustness; Specificity; Theoretical plates, Percent recovery

1. INTRODUCTION

Montelukast Sodium, a cysteinyl leukotriene type-1 receptor antagonist, is generally utilised as tablets and granules as a second-line treatment to cure Asthma. The chemical name is 2-[1-[1(R)-[3-[2(E)-(7-chloroquinolin-2-yl)vinyl]phenyl]-3[2-(1-hydroxy-1methylethyl)phenyl]propyl sulfanyl methyl] cyclo-propyl] acetic acid[1]. It is a CysLT1 antagonist; that is, in the bronchial tubes and lungs, it binds and it obstructs the action of leukotriene D4. This decreases the bronchoconstriction generally

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brought by LT & reduces inflammation. Decreasing inflammation reduces swelling that narrows airways. Bronchial tube walls are relaxed by this drug[2].

It is taken orally, once tablet in a day subsequently expanding the compliance of the asthma patients.it does not have any adverse drug interaction. it shows efficacy against the allergens and it is the only LT- modifier which is approved by the USFDA for children ranging from 2-12 years for the treatment of asthma[3].

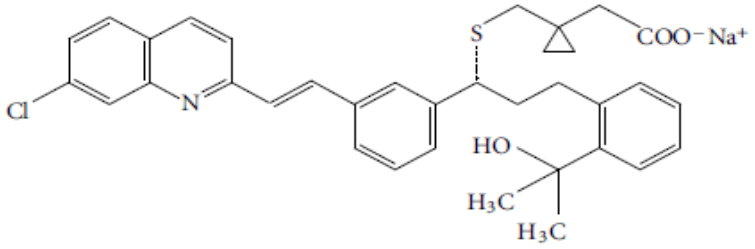

Figure 1: Chemical structure of Montelukast Sodium[4]

Montelukast sodium is an anti- asthmatic drug. The dose is 10 mg & the molecular weight is 608.2 g/mol. Itis soluble in ethanol, water and methanol. It is white in colour. It is quickly absorbed after oral administration, having 64% oral bioavailability. The volume of distribution (V_d) recorded for montelukast sodium is 8-11L and the protein binding is > 99%. It is mainly metabolized by three enzymes: CYP2C8, CYP2C9 and CYP3A4. CYP2C8 plays vital role in metabolism of drug. Half-life of drug varies from 3-6 hours. Faeces and bile are the primary sites for the excretion of montelukast sodium as well as the metabolites[5]. Montelukast sodium is available in various dosage forms. It is mainly present in the form of tablets[6], capsules[7], fast dissolving films[8]. Solid lipid nanoparticles[9], and buccal patches[10]. Other dosage forms like oral granules[11], oral suspensions[12] and implant are also available[13].

Montelukast sodium is analysed by different analytical instruments. It is mainly analysed by HPLC by using both Reversed phase[2] and normal phase[14]. In other methods, the solvents used are costlier like as more organic phase is used[4]. In one more method, the run time is more[15]. Montelukast sodium is also analysed by UV spectrophotometry[16]. Fluorometric spectroscopy is sometimes used to analyse montelukast sodium in dosage forms[17]. But there are drawbacks associated with these analytical instruments. Sophisticated instruments are now available with sensitivity up to micro-gram(µg) and pico-gram. And for fluorometric spectroscopy derivatization agents are required which is also a problem. Here, we have better and optimized High performance liquid chromatography method for analysis of drug.

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3. MATERIALS & METHODS

3.1 Materials:

Polyvinyl alcohol and Span 80 were kindly supplied by Qualikem Laboratory Reagent, Vadodara, India. Poloxamer 188 is obtained from Kolliphor P-188, BASF, Germany. Eudragit RS 100 and Eudragit RL 100 is given as a token of gift from Evonik Rohm GmbH, Germany. Potassium dihydrogen Orthophosphate and Sodium dihydrogen orthophosphate are received from Thomas Baker Pvt.. Ltd., Mumbai, India. The other reagents like methanol & n- octanal is procured from SD Fine-chem Ltd, Mumbai, India.

3.2 Equipment's Used:

HPLC (Shimadzu, Japan) equipped with autosampler, column oven, PDA detector, software LC solutions was used to quantify the samples. A pH meter (Lab India Ltd., Mumbai, India) was used to check the pH of mobile phase. UV spectrophotometer (Shimadzu, Japan) was used to check wavelength of the compound. Other instruments like FTIR (Bruker Alpha, Berlin, Germany), Electronic weighing balance (Shimadzu, Japan) were used.

3.3 Chromatographic Conditions:

For the development of reverse phase HPLC method, we used High performance liquid chromatography (Shimadzu) contains 2 LC-10 ATVP pumps, SPD-10AVP UV-Visible detector and Rheodyne injector. For data acquisition & processing Shimadzu LC-solution version 6.42 software was used. Different combinations of methanol, acetonitrile and buffers were tried as a mobile phase to get less run time and good peak shape. We tried both types of column for better separation and good shape of peak. Column temperature also varied from 25 °C to 40 °C to get fast analysis.

3.4 Sample solutions-Preparation:

- **Standard stock solution preparation**: Weigh accurately 10 mg drug and transferred it to flask having 6 milli litre of diluents and then keep it on sonicator for 15 minutes, followed by making up of volume.
- **Standards sample solution preparation:** Test solution of various concentrations ranging from 1-10 µg/ml was prepared from the stock solution & further dilution was done using diluents.

3.5 Parameters for method validation

There are eight analytical method validation parameters. It includes system suitability, Linearity, precision, accuracy, LOD, LOQ, robustness, ruggedness & specificity.

1. **System suitability-** It was checked by taking 6 inj. of concentration 5µg/ml of Montelukast sodium and parameters like R.T, tailing factor, no. of theoretical plates and repeatability were used and relative standard deviation is determined
2. **Linearity**: It is analysed with the help of standard curves of concentration 1 to 10 µg/ml from stock solution of Montelukast sodium (10 µg/ml) in suitable mobile phase in triplicate.

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To prepare the calibration curve, the following concentrations are needed to be prepared (1, 2, 3, 4 5, 6, 7, 8, 9 and 10 μg/ml). From linear regression analysis, the linearity is calculated.

3. **Precision**: Repeatability, intra-day and inter-day variation is done in the same day, using same conc. and then the % Relative standard deviation was determined.
4. **Accuracy**: It is calculated by % recovery. Recovery study is performed by the addition of drug sample with is standard at higher conc. And level of lower medium of every drug in initially analysed test sample.
5. **Limit of detection and Limit of quantification:** The LOD and LOQ of optimized method of montelukast sodium were considered according to ICH rules. To check the LOD and LOQ various methods are used that may be instrumental or not.
6. **Robustness-** It can be checked by doing some intentional changes in the parameters of method. With the help of relative standard deviation, the response of montelukast sodium was noted. By doing some alterations in the wavelength & flow rate, the robustness was checked. The Percentage RSD should not be more than 2.
7. **Ruggedness**- It is calculated by analysing 6 test conc. Ranging from 1-5 μg/ml in the same laboratory by the 2 different analysts to check whether the results are reproducible or not.
8. **Specificity:** To check the specificity of the HPLC, the partition of the analytes from other potential segments, for example, pollutions, degradants or excipients was done (Eudragit RS100, Poloxomer 188 and ethyl cellulose).20μl solution of every ingredient & excepient was injected and the results were seen through chromatogram.

4. RESULTS AND DISCUSSION

4.1 Optimized HPLC method

- Instrumentation: High performance liquid chromatography (Shimadzu) contains 2 LC-10 ATVP pumps, SPD-10AVP UV-Visible detector and Rheodyne injector. For data acquisition & processing Shimadzu LC-solution version 6.42 software was used.
- Mobile phase and diluent: mixture of ACN, 0.1% v/v trifluoroacetic acid & methanol in 10:10:80 ratio
- Flow rate: 1.3 ml/min.
- Detector: Ultraviolet visible
- Wavelength: 347 nm
- Volume of injection Volume: 20μl
- Chromatographic column: Nucleodur, C18 endcapped (250mm × 4.6 mm I.D., 5 μm)
- Column temperature: 40°C

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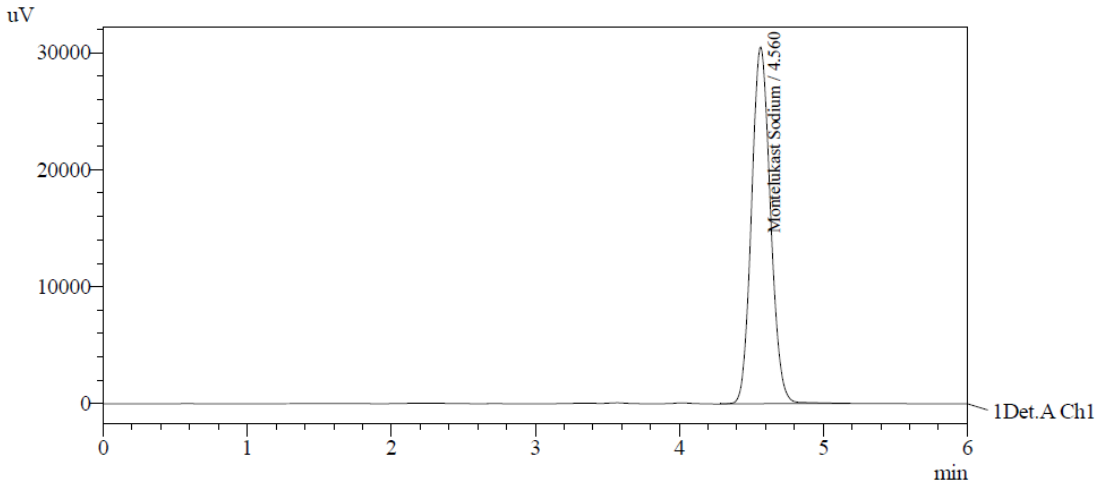

Figure 2: HPLC chromatogram with optimized method

4.2 System suitability

For this optimized method the system suitability parameters were set as number of theoretical plates is greater than 35000 and tailing factor for main peak must be not more than 1.2. The data for the same were shown in Table 4.

4.3 Linearity

By plotting concentration on x- axis & area on y- axis, we obtained linear response with equation, $y = 27411x + 5667$. The correlation coefficient was found to be $R^2 = 0.999$ as shown in Figure 3.

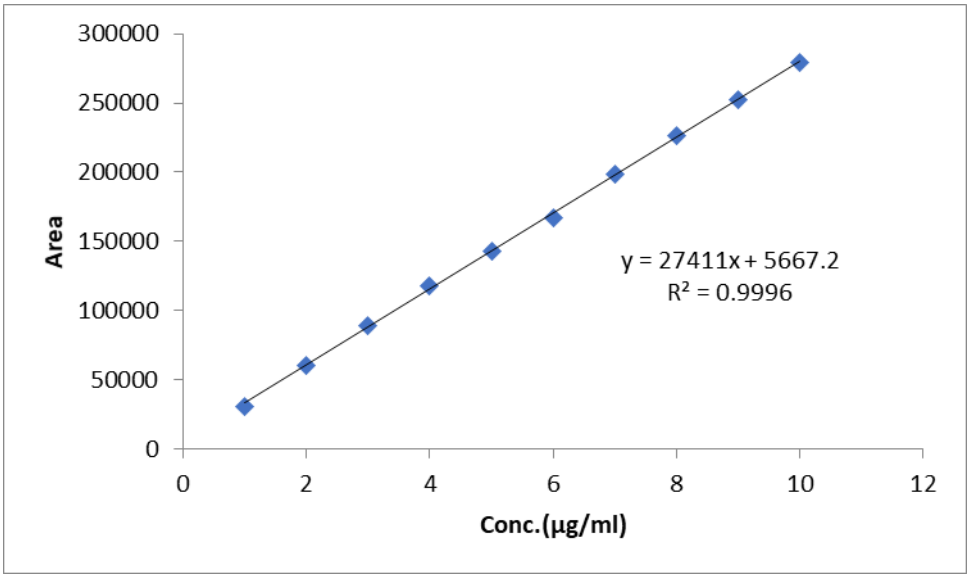

Figure 3: Standard curve of Montelukast Sodium

4.4 Precision

Precision studies were performed for system, method, intraday, interday precision and repeatability. The data for all parameters are given in Table 4.

4.5 Accuracy (Recovery)

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The range of 99.14% - 102.69% for recovery is obtained from the analysis of Montelukast sodium that shows that the method is sensitive enough to analyse the sample. Results for recovery studies are represented in Table 4.

4.6 LOD AND LOQ

Table 1: Limit of detection & Limit of quantification

Drug	**Limit of detection (µg/ml)**	**Limit of quantification(µg/ml)**
Montelukast Sodium	0.065	0.196

Based upon the slope & SD of response, LOQ & LOD results are shown in table 1.

4.7 Robustness

The percentage relative standard deviation (RSD) was found for alterations in column temperature, flow rate & wavelength. All the results are illustrated in table 4 and shows robustness of the method.

4.8 Ruggedness

Table 2: Analysis done by Analyst -1

Concentration (µg/ml)	**Area**	**Recovered quantity (µg/ml)**	**Recovery in %**
5	146053	5.122	102.430
5	144650	5.070	101.407
5	143012	5.011	100.211
5	144926	5.080	101.608
5	145209	5.091	101.815
5	144979	5.082	101.647
Mean	**144804.8**	**5.076**	**101.520**
SD	**1000.451**	**0.036**	**0.730**
%RSD	**0.691**	**0.719**	**0.719**

Table 3: Analysis done by Analyst-2

Concentration (µg/ml)	**Area**	**Recovered quantity (µg/ml)**	**Recovery in %**
5	145798	5.112	102.244
5	143905	5.043	100.863
5	144304	5.058	101.154
5	149023	5.2230	104.597
5	147570	5.177	103.537

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5	147587	5.177	103.550
Mean	**146364.5**	**5.133**	**102.658**
SD	**2031.309**	**0.074**	**1.482**
%RSD	**1.388**	**1.444**	**1.444**

The results shown above, indicating the ruggedness of the current method and it is within the limits as per the ICH guidelines.

4.9 Specificity

The calculated % RSD for specificity was 0.195, which is < 2.0, shows good specificity of the method and the data is given in table 4.

Table 4. Data of all validation parameters (Mean, Standard deviation and Percent relative standard deviation). All the parameters are within limits as per the ICH (International council for Harmonization) guidelines[18] **that shows that this HPLC method is better to analyse Montelukast sodium.**

S. No.	Validation Parameter	Coefficient of regression	Mean	SD	%RSD (<2%)
1	**System suitability**				
	Retention time (min)		4.556	0.003	0.069
	Area		145636	1569.589	1.077
	Theoretical plates		35349.71	230.005	0.65
	Tailing factor (%)		1.139	0.013	1.187
2	**Linearity-1**	0.999			
	Linearity-2	0.999			
	Linearity-3	0.999			
3	**Accuracy (% Recovery)**				
	80%		102.685	0.086	0.084
	100%		99.140	0.034	0.035

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	120%		97.074	0.634	0.653
4	**Precision**				
	Interday		144064	1628.230	1.130
	Intraday		144659	1091.680	0.755
	Repeatability		144405.170	450.993	0.313
5	**Ruggedness (%)**				
	Analyst-1		101.519	0.729	0.719
	Analyst-II		102.657	0.482	1.443
6	**LOD (µg/ml)**		0.064		
7	**LOQ (µg/ml)**		0.195		
8	**Specificity (%)**		10046.100	0.204	0.202
9	**Robustness**				
	Column temperature (±5°C)				
	35°C		145864.700	1591.899	1.091
	40°C		142203.700	1449.006	1.019
	45°C		146710.700	1236.739	0.842
	Wavelength (±5 nm)				
	342 nm		140463.700	2196.369	1.563
	347 nm		142203.700	1449.006	1.019

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	352 nm		153577	2632.495	1.714
	Flow rate (±10%)				
	1.17 ml/min		147504.17	1176.893	0.797
	1.3 ml/min		142203.700	1449.006	1.019
	1.43 ml/min		147197.500	695.912	0.472
	Mobile phase ratio (Methanol: ACN:0.1%TFA v/v)				
	82:10:8 v/v		147596.167	1888.646	1.280
	80:10:10 v/v		142203.700	1449.006	1.019
	78:10:12 v/v		146031.667	2156.882	1.477

4.10 Forced degradation

Acid degradation

In a volumetric flask, add 1 ml of sample containing Montelukast sodium and then 0.1 M Hydrochloric acid is transferred to the above solution. Keep it isolated for 60 minutes at 60°C ± 2°C and then neutralisation is done by the addition of 0.1 M sodium hydroxide, followed by dilution, sonication, and filtration with 0.22µm and then further proceed to inject in High performance liquid chromatography**.**

Base degradation

In a 10ml volumetric flask, 1ml test (5 µg/ml) sample of Montelukast sodium is transferred and then mixed in 0.1 M NaOH (1ml) & keep it for 1 hour at 60°C ± 2°C. After warning, 0.1 M HCl is added to make sample neutral followed by dilution up to 10ml with diluents. Further, sonication and filtration are done and the sample is ready to inject in HPLC.

Photolytic degradation by UV light

Photolytic degradation was studied by placing an API/Product solution (5 µg/ml) in a volumetric flask and then keep it in a UV radiation for not less than 1 hour. Triplicate injections were prepared and then run the chromatogram.

Oxidative degradation

Test solution (5 µg/ml) containing 1 ml of Montelukast sodium was added in a 10 ml flask, after that blended in with 1 ml of 1% H_2O_2 & keep it untouched for 1 hour at 60 °C ± 2°C followed by

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warming on water bath, and then test solutions were diluted by M.P to 10ml. Each of three solutions were infused in triplicate & then follow the chromatographic procedure.

Thermal degradation:

1ml of a prepared sample solution containing Montelukast sodium was moved to a 10 or 20ml light protected flask & afterward warmed for 1 hour, 2 hour & 4 hours at 60 °C ± 2 °C. The resultant solutions were diluted in M.P up to 10 ml and infused in triplicate & then follow the chromatographic procedure as stated above.

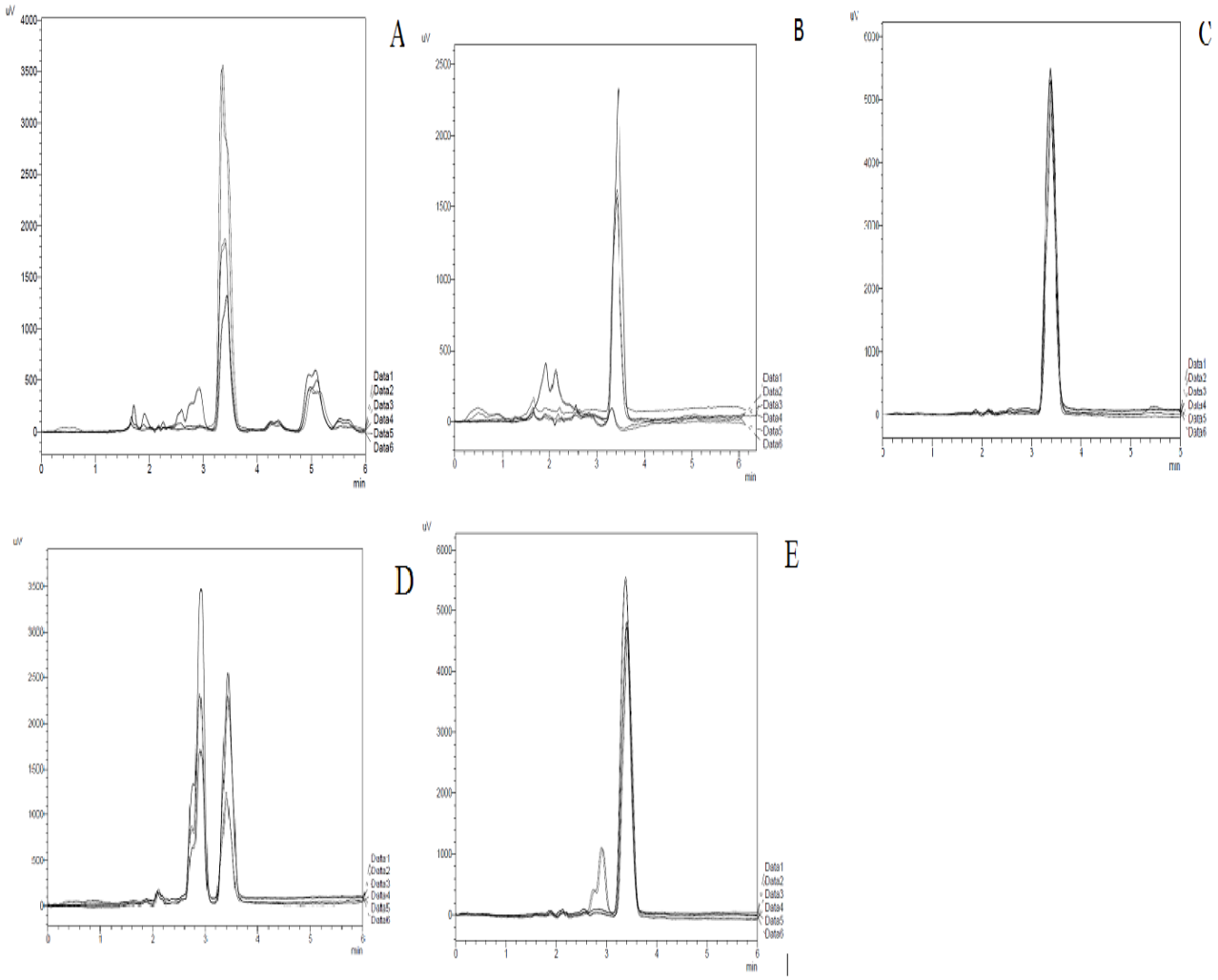

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Figure 3: Shows A, B, C, D, and E Chromatographs. A is chromatogram of Montelukast sodium during acid degradation at 1 hr, B is chromatogram of Montelukast sodium during base degradation at 1 hr, C is **:** chromatogram of Montelukast sodium during Photolytic degradation by UV at 1 hr, D is chromatogram of Montelukast sodium during Oxidative degradation at 1 hr**, E is** chromatogram of Montelukast sodium during Thermal degradation at 1 hr, 2 hr and 4 hr.

Table 5: Percent change in area of Montelukast sodium for forced degradation studies. (n=3)

S. No.	Type of degradation	% Change in area	
		Mean	**SD**
1	Acid degradation (I hr)	71.239	0.102
2	Base degradation (1hr)	80.884	0.021
	UV degradation (1hr)	50.597	0.029
4	Oxidative degradation (1 hr)	78.930	0.088
5	Thermal degradation (1 hr)	50.908	0.326
	Thermal degradation (2 hr)	57.7188	0.014
	Thermal degradation (4 hr)	60.973	0.160

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