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**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Development and Validation of a Stability Indicating RP-HPLC Method for the Quantitative Analysis of Budesonide in Bulk Drug and Capsule Formulations

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**ABSTRACT:**

A robust Precise, stability-indicating RP-HPLC method was successfully optimized & validated to ensure reliable analysis of budesonide quantitative in raw form& capsule dosage forms. The components were effectively resolved using octadecylsilane column using eluent consisting of acetonitrile and H2O (pH 3.0) was altered H3PO4. With a flow rate maintained at 1.0ml/min detection was performed at a wavelength of 244nm. The procedure was thoroughly verified according to validation parameters. (LOD),(LOQ) and robustness. Budesonide was subjected to various forced degradation studies. The method successfully resolved budesonide apart from its breakdown products. Demonstrating it stability to selectively identify the drug and indicate its stability. The results confirm that the method is appropriate for regular quality assessment and stability testing of Budesonide within medical preparations.

Key words: Budesonide, Reverse phase-high pressure liquid chromatography

## INTRODUCTION:

"Pharmaceutical Analysis" involves the process of creating "Quantitative" and "Qualitative" studies. Quantification of analytes in intricate samples typically demands analytical thinking and troubleshooting.[1]

Pharmaceutical analysis facilitates the analytes in minute quantities of substances in specific sample types. Raising awareness about environmental quality & the potential impact of even tiny traces of chemicals on biological process as driven scientists to create practical methods that can detect & analyze substances at very low concentrations such as ppm & ppb. Pharmaceutical analysis is mainly divided into 2 key areas, the choice of method for quantitative analysis of drug substances depends on the quantity of substance present & the level of accuracy needed.[2]

## Materials and Methods:

Equipped with Auto sampler, Photodiode array detector and Quartenery gradient system, and waters HPLC 2998 system with Empower 2 software. The absorbance of budesonide measured was carried out using a Shimadzu UV 1800 double beam UV/Visible spectrophotometer.

***Materials:***

Budesonide active ingredient, purified water, ACN, KH2PO4, K2HPO4, Methanol, Ethanol, GAA , Sodium Hydroxide, Orthophosphoric acid.[3]

**To prepare 0.25M KH2PO4 buffer of Ph 7.0:**

34.02 gm of KH2PO4 were accurately weighed & mixed thoroughly in purifiedH20 in a 1000 ml measuring flask. Final capacity with same solvent the pH was brought to 7.0 using 0.1N hydrochloric acid.[4]

**To prepare the eluent:**

300 mil/lit (30%) of 0.25 M KH2PO4 buffer with a pH of 7.0 was combined with 700 ml. (70%) Methanol was blended together in a 1000Ml measuring flask & then filtered using 0.46p passed through a membrane filter under reduced pressure. To eliminate dissolved gases the solution was sonicated in an ultrasonic bath for 10min. The eluent also served as the diluent.

**To prepare the primary stock solution of budesonide:**

0.1g of BDN were accurately weighed & transferred to a 0.1L measuring flask. Solvent were transferred and diluted to obtain a final volume concentration of1000 µg/ml.

**To prepare Budesonide working standard solution:**

A volume of 1.4ml of primary BDN stock solution was taken &diluted to 25 ml with solvent in 25 ml measuring flask. The solvent were made to the indicated level to get final concentration of 140µg/ml.

**Preparation of sample solution (Assay**):

20 tablets content wars precisely measured, and the mean weight was calculated and powdered in a motor & pestle. Powder corresponding to the amount of 25 mg (653.44 mg) of BDN and accurately weighed portion was transferred to a 25mL volumetric flask. Ten milliliters of mobile phase were added and the solution was sonicated for 10 minutes. The volume was then made up to the mark with the same mobile phase to obtain a sample stock solution of 1000 microgram per milliliter. The resulting solution was filtered using a 0.45µm membrane filter. Subsequently 1.4 ml of this solution was transferred to a 25 mL volumetric flask and diluted to volume with mobile phase. 20 µL aliquot the final solution was injected into the chromatograph. The Peak area and RT were recorded from the chromatogram and the amount of BDN was calculated accordingly.[5]

***Method validation:***

The method, once optimized, was subjected to validation in line with the parameters specified by ICH guidelines.

**Preparing standard solutions for System suitability:**

Six replicates of diluted standard solution of BDN were made and introduced into HPLC system. Various system suitability characteristics like theoretical plates count. Average peak area, peak height, Retention time, tailing factor, resolution, and signal to noise ratio determined.[6]

**Preparation of solutions for Precision:**

Intra and day to day precision were determined using optimized concentration (100%). For intraday precision, 6 replicates of prepared samples of BDN was prepared and introduced into HPLC at three different times of a same day. Similarly, for inter day precision. Six replicates of sample solutions of BDN were prepared and injected into HPLC in three consecutive days. Peak areas of BDN were determined. Average, the mean, standard deviation, %RSD of the peak area values were determined.

**Preparation of solutions for Accuracy:**

Accuracy was evaluated through recovery studies by preparing solutions at 3 concentration levels 50, 100, & 150 percent using the sample solution.[7]

**Preparation of 50% accuracy solution:**

259. 35 milli grams of sample had been accurately weighed out and shifted to 25 milli/lit. measuring flask .10 milliliters of eluent were introduced and subjected to sonication for 5 minutes. The volume was adjusted to the mark using eluent. From this solution1.4 milliliters were made up to 25 milliliters to reach the required concentration of 70 micrograms. Six replicates of 50% solutions was made ready and analyzed using high pressure liquid chromatography, peak areas was determined, and quantity introduced, percentage recovered was calculated**.**

**Preparation of 100% accuracy solution:**

518.71 milligrams ofsample were accurately measured and moved into 25 milli liters of measuring flask. 10 milli liters of eluent introduced and subjected to sonication for 5 minutes. The volume was adjusted to the mark using eluent. Using the same solution1.4 milli liters were withdrawn and made upto 25 milli liters to reach the required concentration of 140 micrograms per milli liters. Three replicates of 100%solutionswas made ready and analyzed using high pressure liquid chromatography. Peak areas was determined, and quantity introduced, percentage recovered was calculated.

**Preparation of 150% accuracy solution:**

778.06 milligrams of sample had been accurately weighed out & shifted to 25 milliliters measuring flask. 10 millilitres of eluent was added and then sonicated for 5 minutes. The volume was adjusted to the mark using eluent.Using the same solution 1. 40 milli liters were withdrawn and made upto 25 milli liters to reach the required concentration of 210 micrograms per milliliter. Six replicates of 150% solutions was ready & analyzed using high pressure liquid chromatography. Peak areas was determined, and quantity introduced, percentage recovered was calculated.

**Preparation of calibration standards at concentrations between 50 –150 for linearity study:**

- Linearity correlation 50%

0.7 milli liters of BDN primary stock solution (1000 microgram per milliliter)were accurately pipetted out and moved to 10 milli liter measuring flask. Final volume was brought up to the graduation mark using diluent (mobile phase) for attaining the final concentration of 70 microgram per milliliter.

- Linearity correlation 75%

1.05 milliliters of BDN primary stock solution (1000 microgram per milliliter) were accurately aspirated with a pipette and moved to 10 milli liters of measuring flask. Volume was brought up to the graduation mark using diluent (mobile phase) for attaining the final concentration of 105 microgram per milliliter.

- Linearity correlation100%

1.40milli liters of BDN primary stock solution (1000 microgram per milliliter) were accurately aspirated with a pipette and moved to10 milli liters of measuring flask. Final volume was brought upto the graduation mark using diluent (mobile phase) for attaining the final concentration of 140 microgram per milliliter.

- Linearity correlation 125%

1.75 milliliters of BDN primary stock solution (1000 microgram per milliliter) were accurately aspirated with a pipette and moved to 10 milliliters of measuring flask. Final volume was brought upto the graduation mark using diluent (mobile phase) for attaining the final volume concentration of 175 microgram per milliliter.

- Linearity correlation 150%

2.10 milli liters of BDN primary stock solution (1000 microgram per milliliter) were accurately aspirated with a pipette and moved to10 milliliters of measuring flask. Final volume was brought up tothe graduation mark using diluent (mobile phase) for attaining the final concentration of 210 microgram per milliliter.

**LOD & LOQ:**

The LOD & LOQ for the developed method were established by repeatedly injecting standard solutions at low concentrations using the developed reverse phase high pressure liquid chromatography method. These values were calculated as per the international conference harmonization guidelines;

$$LOD = 3.3 \times SD / S$$

$$LOQ = 10 \times SD / S$$

The standard deviation (SD) represents the variability in the response data, and the slope (S) is the gradient of the calibration curve obtained from plotting the data.[8]

**Preparation of sample for robustness**:

Robustness was evaluated by introducing variations in the optimised chromatographic parameters via; flow rate varied by the plus or minus0.2 milliliter, temperature fluctuated within plus or minus 5°C & pH changed by plus or minus 0.21. Three replicates of sample solution at 100% concentrative were prepared for the determination of each parameter and injected in HPLC at robustness conditions as specified above. Retention time, peak areas and % assay were determined.[9]

**Stability stress studies:**

The regulatory guidelines by ICH guidelines titled “stability testing of new drug substances and products” the fundamental stability profile of the active ingredient. The project aimed to analyzing the sample under adverse conditions by applying proposed method.

**Acid degradation:**

From primary stock sample solution, 1.4 ml was withdrawn and then moved to 25 milli liter standard flask. 2 ml of 1N HCL included. Subjected to reflux conditions at 75°C for 3h. The solution was neutralized with 2 ml of 1N NaOH made upto volume with diluent to achieve the final concentrations of 140 microgram per milliliter. The obtained solution was cooled down to ambient temperature. Strained through 0.22milli meters syringe and injected 10 micro liter from each solution into HPLC system and measured the area under the peak for BDN % assay and degradation was calculated.[10]

**Alkaline degradation:**

From primary stock sample solution. 1.4 and was withdrawn then moved to 25 milli liter standard flask. 2 milli liter of 01 NaOH was included mixed well &subjected to reflux conditions at 90 °C for 2 hours. The solution neutralized with 2 milliliter of 0.1N Hydrochloric acid, made up to volume with diluent to achieve the final concentrations of 140microgram per milliliter. The obtained solution was cooled down to ambient temperature. Strained through 0.22millimeters using a syringe, 10 microliter of each solution was injected into the HPLC system for analysis the area under the peak for BDN% assay and degradation was calculated.[11]

**Thermally induced degradation:**

From primary stock sample solution. 1. 40 ml was withdrawn and moved into a 25 ml standard flask. Diluent was added dilute to the mark to obtain the terminal concentrations of140 microgram per milliliter. The solution were mixed well and kept in a hot air over at 90°C for 6 h. The prepared solution was allowed to cool at ambient conditions filtered through 0.22mm syringe and injected 10 µl of from each solution injected into the HPLC system and measured the area under the peak BDN, % assay and % degradation was calculated.

**Oxidative degradation:**

1.40 ml of the Primary stock sample solution was pipetted into a 25milliliter standard flask. Added 3 milliliter of 10% hydrogen peroxide and the volume was made up to the mark with diluents to get the final concentrations of 140µg/ml. The standard flask was then refluxed at 75°C for 5 H. The resulting solution was cooled, filtered through 0.22 mm syringe and injected 10 µl of each solution into the HPLC system and measured the peak area for BDN. %assay and % degradation was calculated.

**Photo degradation:**

From primary sample stock solution. Pipette out 1.40 ml to a Petri dish and kept in a photostability chamber and UV exposure was conducted in a chamber at 200 Wh/m and 1.2 million 1xh for 24 h. Finally, standard flask was made up to 25 ml. with diluents to get the final concentrations for140µg/ml. The resulting solution filtered through 0.22mm syringe and in injected 10 µl. of each solution into the HPLC system and measured the peak area for BDN.% assay and % degradation was calculated.[12]

## Conclusion:

A reliable, specific, and precise RP-HPLC method was developed and validated for the quantitative determination of budesonide in bulk and capsule formulations. The method demonstrated excellent validation parameters in accordance with ICH guidelines. Importantly the method proved to be stability-indicating by effectively separating budesonide from its degradation products under various degradation studies including acid, base, oxidative. thermal and photolytic degradation. Therefore, this RP-HPLC method is suitable for routine analysis and quality control of budesonide in pharmaceutical dosage forms. and for use in stability studies.

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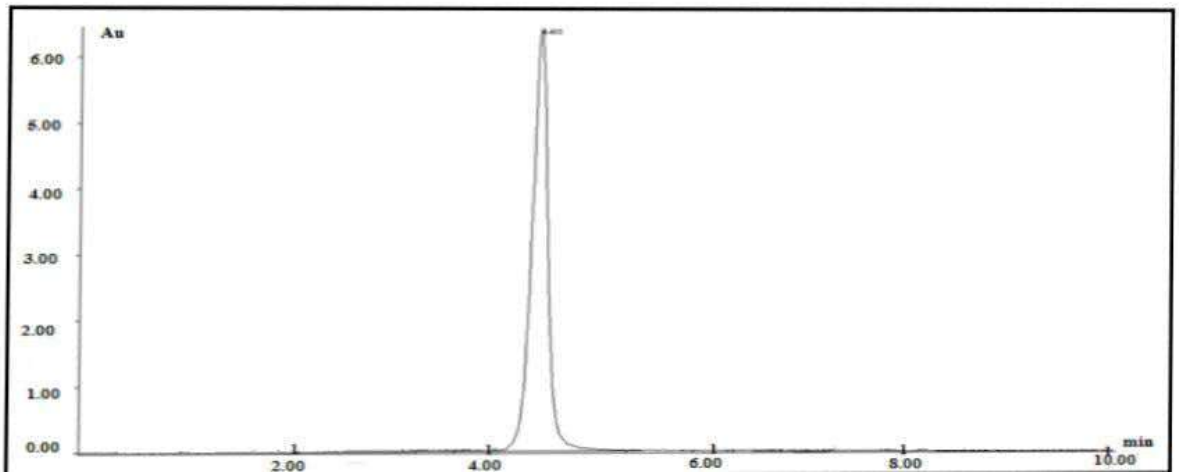
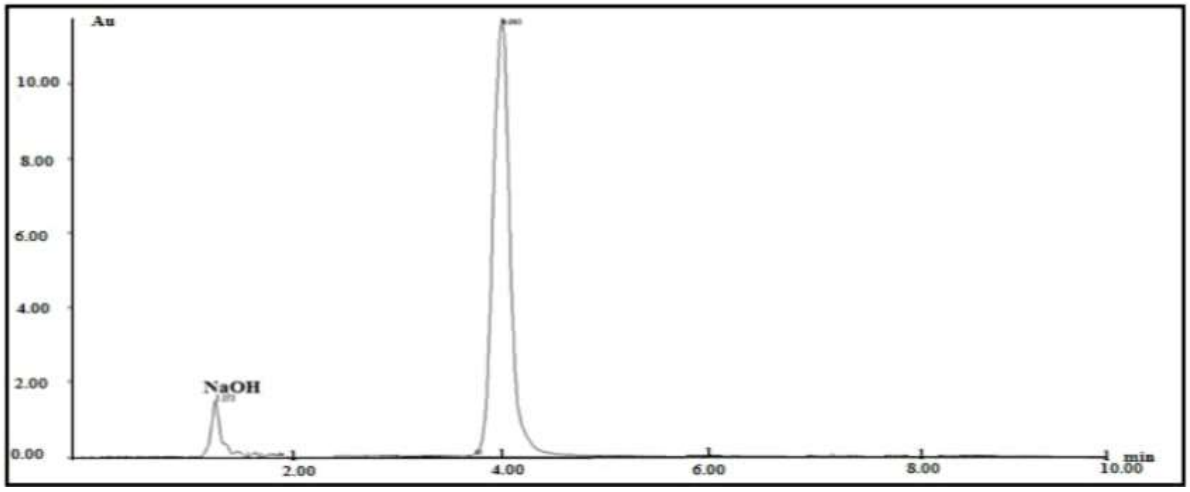
Assay results of Budesonide:

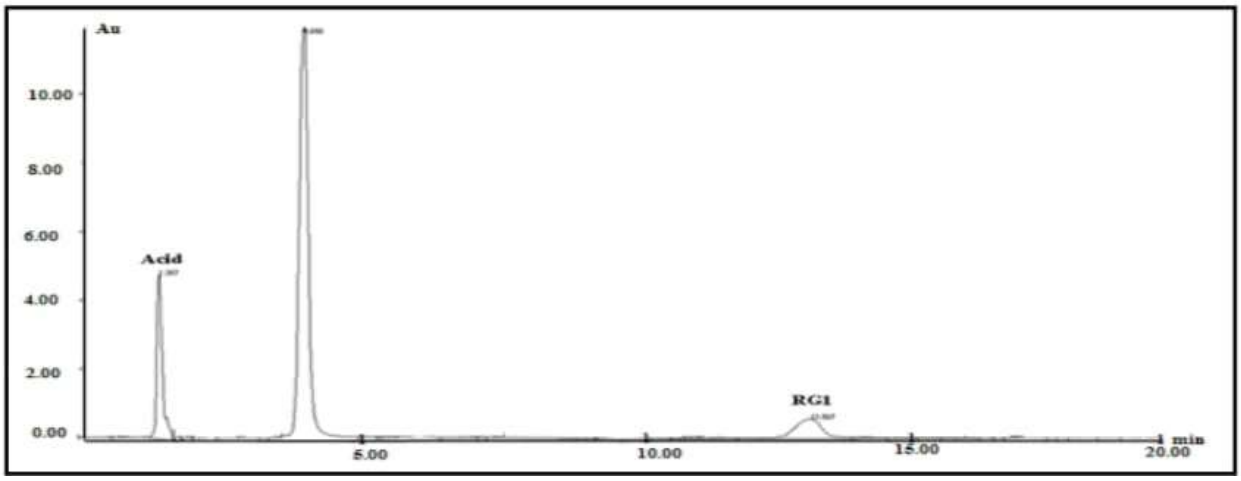
| sample | Label claim | Amount found | % assay | % RSD (n=3) |
|---|---|---|---|---|
| Bulk drug | 100 | 99.1 | 99.1% | 0.78 |
| Bulk drug | 100 | 98.7 | 98.7% | 0.65 |
| Capsule-A | 3 | 2.96 | 98.7% | 0.92 |
| Capsule-B | 3 | 2.94 | 98.0% | 1.04 |
| Capsule-C | 3 | 2.99 | 99.7% | 0.88 |



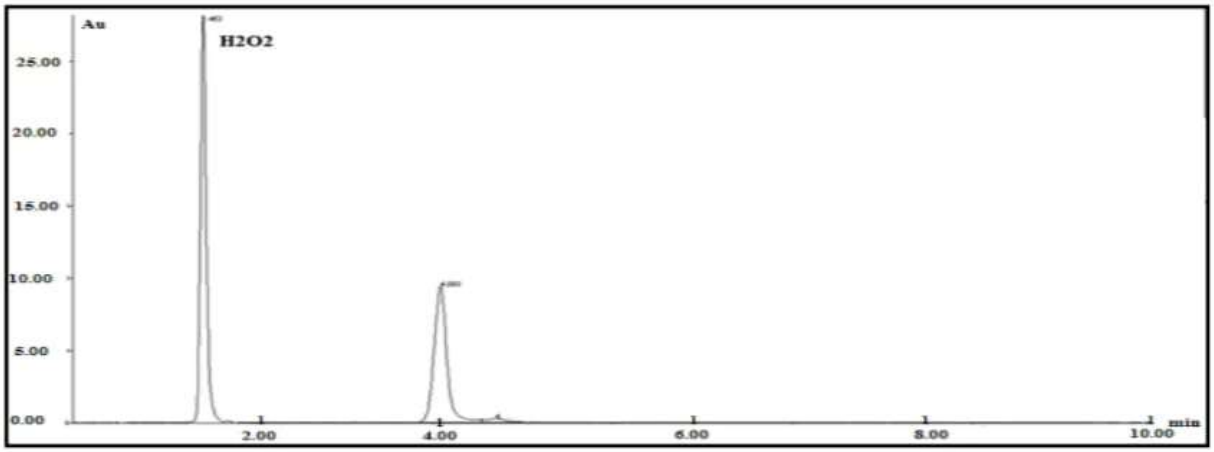

Standard chromatogram of budesonide



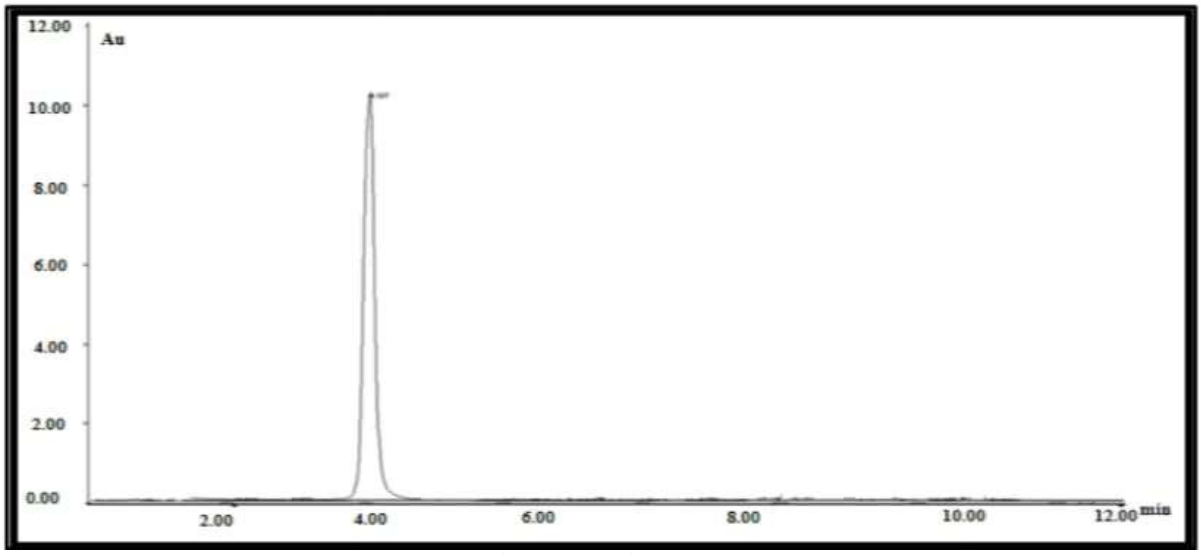

Chromatogram of Budesonide after alkaline degradation



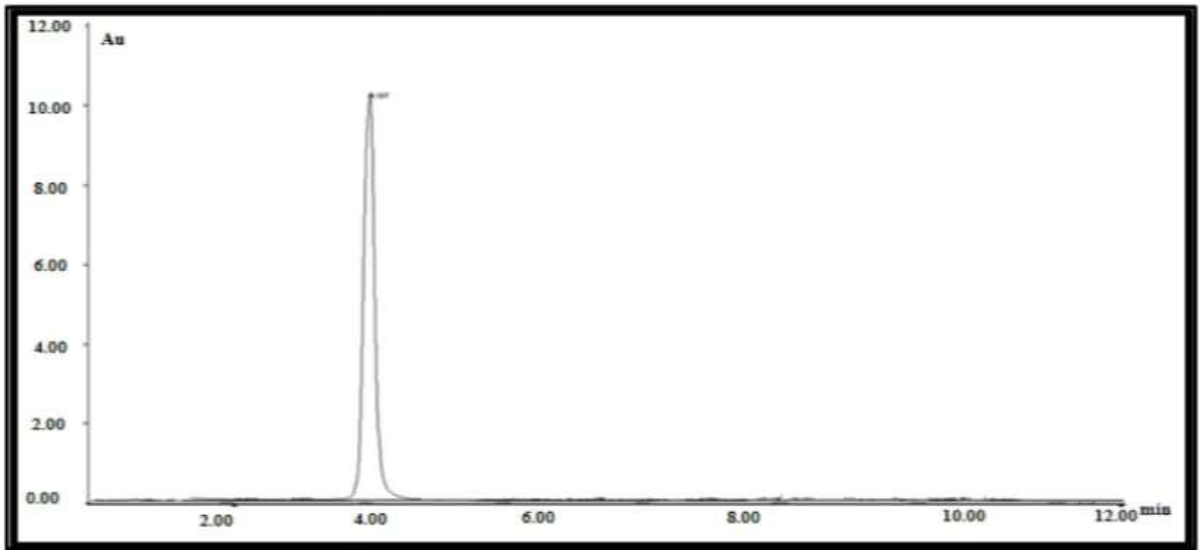

Chromatogram ofBudesonideafter acid degradation




Chromatogram of Budesonide after oxidation with 30% v/v $H_2O$




chromatogram of Budesonide after Thermal degradation




chromatogram of Budesonide after photolytic degradation