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Analytical Development and Validation of a Stability-Indicating Method for the Estimation of Impurities in Budesonide Respules Formulation

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Abstract:

A short selective, precise, accurate and sensitive stability-indicating LC-MS/MS^n method was developed for the quantitative determination of process-related impurities and degradation products of Budesonide in pharmaceutical respules formulations. During the stress study, the degradation products of Budesonide were well-resolved from Budesonide and its impurities and the mass balances were found to be satisfactory in all the stress conditions, thus proving the stability-indicating capability of the method. The developed method was validated as per ICH guidelines with respect to specificity, linearity, limit of detection and quantification, accuracy, precision, ruggedness and robustness. During the stability analysis of the drug product, all known impurities were detected by the above stability-indicating method. The flow rate was 0.8 ml/min and effluent was monitored at 247nm. Retention time was found to be 17.329±0.75 min and18.439±0.65 min of epimers (22R and 22S)respectively. The LOD and LOQ values for were found to be 0.20936 (µg/ml) and 0.6344 (µg/ml) respectively.

1. Introduction

Budesonide is a glucocorticoid used in the management of asthma, the treatment of various skin disorders, and allergic rhinitis. Budesonide is provided as a mixture of two epimers (22R and 22S). Interestingly, the 22R form is two times more active than the 22S epimer. The two forms do not interconvert. Chemically it is, (1S,2S,4R,8S,9S,11S,12S,13R)-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0^{2},^{9}.0^{4},^{8}.0^{13},^{18}]icosa-14,17-dien-16-one.

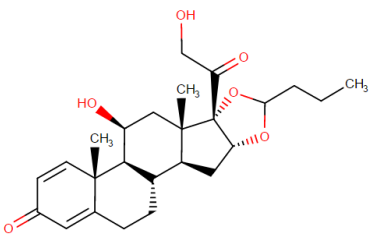

Figure 1: Molecular Structure of Budesonide

Chemical stability of Budesonide is a matter of great concern as it affects the safety and efficacy of the finished drug product. Forced degradation studies provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the Budesonide molecule and validation of stability indicating analytical procedures. (ICH Q2 (R1), 2005)

A detailed literature revealed that several analytical methods have been reported for the determination of Budesonide in pharmaceutical pressurized inhalation dosage forms (KatariSrinivasaro et al., 2012, NandiniPai et al., 2013, Patil A.T et al., 2011). In our present knowledge, there is no method reported for the estimation forced degradation studies of Budesonide in pharmaceutical pressurized inhalation dosage form by UPLC (Michael E Swartz, 2005).

As per the stringent regulatory requirements recommended by the ICH and regulatory agencies, it is mandatory and important to identify and structurally characterize any impurity formed during production and stability testing, exceeding the identification threshold. Various analytical instruments and advanced hyphenated techniques are routinely used to carry out the impurity profile study.

The present work aims with the development of method to separate the degradation product by preparative UPLC and subjected to ESI-MS/MS. The

study describes the separation of different impurities of Budesonide, as well as the development and validation of a stability-indicating RP-UPLC method for the estimation of degradation and process-related impurities of Budesonide. Forced degradation studies were performed on the drug product to show the stability-indicating nature of the method. These studies were performed in accordance with established ICH guidelines.

2. Experimental

2.1 Materials

Budesonide (98.40 % purity) used as analytical standard was procured from Spectrum Labs (Hyderabad).

HPLC grade methanol, acetonitrile (HPLC grade) was purchased from Qualigens fine chemicals, Mumbai, India. Distilled, 0.45 µm filtered water was used for UPLC quantification and preparation of buffer. Buffers and all other chemicals were analytical grade. The aqueous suspension for nebulizer (PulmicortRespules) labeled to contain each 2.5 ml contains 0.5 mg of Budesonide. All chemicals used were of pharmaceutical or special analytical grade.

2.2 Instrumentation

Acquity, Waters UPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Water 2996 wavelength absorbance detector (PDA) was employed throughout the analysis.

The data was collected using Empower 2 software. The column used was Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm). A Band line sonerexsonicator was used for enhancing dissolution of the compounds. A Labindia pH System 362 was used for pH adjustment.

The electrospray ionization and MS-MS studies were performed on the triple quadrupole mass spectrometer PE Sciex Model: API 3000.

2.3 Chromatographic conditions

Table 1: Chromatographic Conditions of the validating method

Parameter	Value
Column	Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm)
Mobile Phase	Ammonium formate and Methanol in the ratio of 35:65%v/v
Flow rate	0.8mL/min
Run time	30 Min.
Column Temperature	Maintained at ambient temperature
Injection volume	5 µL
Detection wavelength	247nm
Diluent	Mobile Phase

2.4 Preparation of Standard Stock Solution

2.4.1 Preparation of Diluent

In order to achieve the separation under the optimized conditions after experimental trials that can be summarized, stationary phase like Hypersil BDS C18 (100 mm x 2.1 mm, 1.7 µm) column was most suitable one, since it produced symmetrical peaks with high resolution and a very good sensitivity and with good resolution. The flow rate was maintained 0.8 mL min-1 which shows good resolution. The PDA detector response of Budesonide was studied and the best wavelength was found to be 247 nm showing highest sensitivity.

The mixture of two solutions Ammonium formate and Methanol in the ratio of 35:65%v/v was used as mobile phase at 0.8mL/min was found to be an appropriate mobile phase for separation of Budesonide. The column was maintained at ambient temperature.

2.4.2 Preparation of internal standard solution

Weighed accurately about 10 mg of Nimusolide working standard and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter.

2.4.3 Preparation of Budesonide standard solution:

Weighed accurately about 10 mg of Budesonide and transfer to 100 ml volumetric flask, add 50 ml of mobile phase and sonicate to dissolve it completely and then volume was made up to the mark with mobile phase to get 100 µg/ml of standard stock solution of working standard. Then it was ultrasonicated for 10 minutes and filtered through 0.20 µ membrane filter. Linearity was determined in the range of 2- 10 µg mL-1.

2.5 Stability indicating studies

Stability indicating studies like acid hydrolysis, basic hydrolysis, wet heat degradation and oxidative degradation were carried out.

2.6 Mass Spectrometry conditions for MS/MS

The samples (5 µL) is injected directly into the source by the flow injection method using Ammonium formate and Methanol in the ratio of 35:65%v/v as mobile phase at a flow rate of 0.8 mL/min. The mass spectra were recorded in ESI negative mode. Ultra-high purity nitrogen and helium were used as curtain and collision gas respectively. The typical ion source conditions were: nebulizer gas, 60 psi; dry temperature, 325˚C; dry gas, 5.0 mL/min; capillary voltage, 5kV; capillary current, 80.243 nA; vapourizer temperature, 400°C; dwell time, 200 ms. For the collision-induced dissociation (CID) experiments, the precursor ion was selected using the quadrapole analyzer and product ions were analyzed by the time-of-flight analyzer. HRMS data acquisition was performed by the following source conditions: capillary voltage, 5 kV; declustering potential (DP) and collision energy (CE) were −60 V and −10 V, respectively; focusing potential, 220 V; resolution 40,000 (FWHM).

3. Results and discussions

3.1 Validation

The analytical method was validated with respect to parameters such as linearity, precision, specificity, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness in compliance with ICH guidelines.

3.2 Linearity and Range:

The linearity of an analytical procedure is the ability to obtain test results that are directly proportional to the concentration of an analyte in the sample.

The calibration curve showed good linearity in the range of 2 – 10 µg/ml, for Budesonide (API) with correlation coefficient (r^2) of 0.9996. A typical calibration curve has the regression equation of y = 1141.25x +40250.1for Budesonide. Results are given in Table 2.

3.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ):

The LOD and LOQ of Budesonide were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. The LOD of Budesonide was found to be 0.20936 (µg/ml) and the LOQ of Budesonide was found to be 0.6344 (µg/ml). Results are given in Table 2.

3.4 Precision:

The Precision of the method was studied in terms of intraday and interday precision of sample injections (4 µg/ml). Intraday precision was investigated by injecting six replicate samples of each of the sample on the same day. The % RSD was found to be 0.46%. Interday precision was assessed by analysis of the 6 solutions on three consecutive days. The % RSD obtained was found to be 0.47%. Low % RSD values indicate that the method is precise. The results are given in Table 3.

3.5 Accuracy:

To study the accuracy of method, recovery studies were carried out by spiking of standard drug solution to pre-analyzed sample at three different levels i.e., at 50, 100, and 150%. The resultant solutions were then reanalyzed by the proposed method. At each level of the amount, six determinations were performed. From the data obtained, the method was found to be accurate. The % recovery and %RSD were calculated and presented in Table 4.

3.6 Robustness:

Small deliberate changes in chromatographic conditions such as change in temperature (± 2°C), flow rate (± 0.1ml/min) and wavelength of detection (±2nm) were studied to determine the robustness of the method. The results were in favor of (% RSD < 2%) the developed UPLC method for the analysis of Budesonide. The results are given in Table 5.

3.7 Results of Stability Indicating Studies

According to Singh and Bakshi, 2000, the stress testing suggests a target degradation of 20-80 % for establishing stability indicating nature of the method. UPLC study of samples obtained on stress testing of Budesonide under different conditions using mixture Ammonium formate and Methanol in the ratio of 35:65%v/v as a mobile solvent system suggested the following degradation behaviour.

3.7.1 Acid hydrolysis

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry round bottom flask (RBF). 30 ml of 0.1 N HCl was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. Allowed to cool at room temperature. The sample was then neutralized using 2N NaOH solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Ammonium formate and Methanol in the ratio of 35:65%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure 4.

3.7.2 Basic hydrolysis:

An accurate 10 ml of pure drug sample solution was transferred to a clean and dry RBF. 30 ml of 0.1N NaOH was added to it. It was refluxed in a water bath at 60°C for 4 hours. Drug became soluble after reflux which was insoluble initially. It was allowed to cool at room temperature. The sample was then neutralized using 2N HCl solution and final volume of the sample was made up to 100ml with water to prepare 100ppm solution. It was injected into the UPLC system against a blank of Ammonium formate and Methanol in the ratio of 35:65%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure 5.

3.7.3 Wet heat degradation:

Accurate 10 ml of pure drug sample was transferred to a clean and dry RBF. 30 ml of HPLC grade water was added to it. Then, it was refluxed in a water bath at 60°C for 6 hours uninterruptedly. After the completion of reflux, the drug became soluble and the mixture of drug and water was allowed to cool at room temperature. Final volume was made up to 100 ml with HPLC grade water to prepare 100 ppm solution. It was injected into the UPLC system against a blank of Ammonium formate and Methanol in the ratio of 35:65%v/v after optimizing the mobile phase composition, chromatogram was recorded and shown in Figure 6.

3.7.4 Oxidation with (3%) H_2O_2:

Approximately 10 ml of pure drug sample was transferred in a clean and dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble and then kept as such in dark for 24 hours. Final volume was made up to 100 ml using water to prepare 100 ppm solution. The above sample was injected into the UPLC system. The chromatogram was recorded and shown in Figure 7.

In all degradation studies, there was a significant formation of degradation products when compared to that of a standard. This indicates that, the drug may be degraded to low molecular weight non-chromophoric compounds.

3.8 Structure and separation of the known/ unknown impurities

No unknown impurities with respect to Budesonide were observed during the stability study of the drug product and we tried to enhance the api by using the forced

degradations to quantify it. But the unknown impurities were not observed in any trial. All the known impurities were separated by preparative UPLC from stability samples with a purity of > 98% and used for its characterisation by LC-MS -MS^n studies.

The positive ESI-MS spectrum of all the known impurities showed their peaks from m/z 372.68 to 429.61 amu [M+H]+ (Fig. 8) which were 86.82-30.44 amu less than that of Budesonide (m/z 460.05). The comparison of MS/MS studies of the unknown impurity and Budesonide showed common fragment ions at m/z 402.12. The common fragment ion peak suggests that 1RS-Butylidenebis(oxy) was intact and changes were at the hydroxypregna atom.

3.9 Impurity profile:

The impurity profile shows us the amount of respective impurities obtained in the studied formulations Table 7.

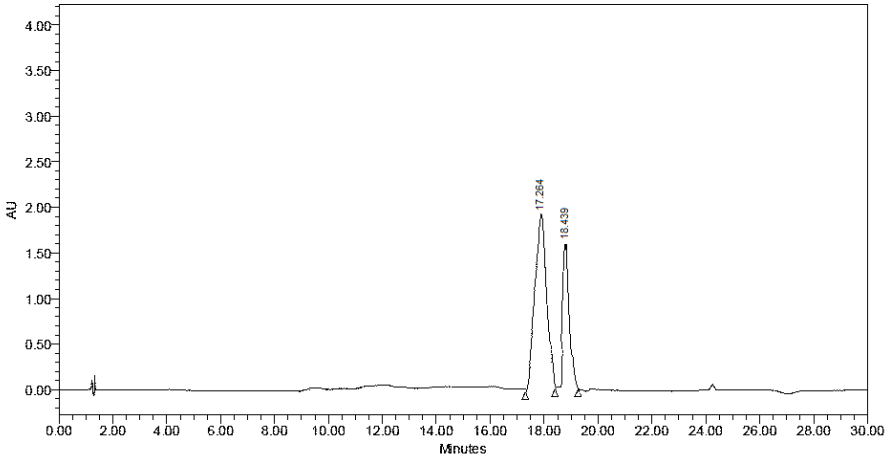

Figure 2: Standard Chromatogram of Budesonide, using mobile phase of Ammonium formate and Methanol in the ratio of35:65%v/v

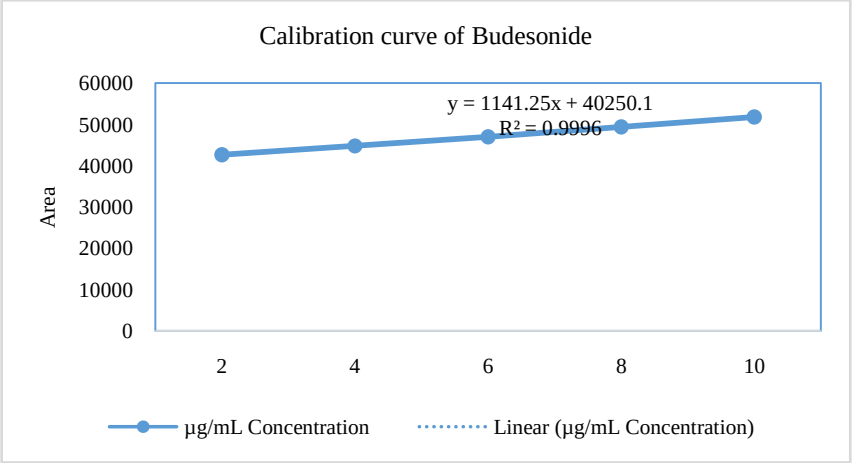

Figure 3: Calibration curve of Budesonide

Table 2: Summary of validation parameters for the proposed method

Parameter	Budesonide
Linearity	2 – 10 µg/ml
Intercept (c)	40250.1
Slope (m)	1141.25
Correlation coefficient	0.9996
LOD	0.20936 (µg/ml)
LOQ	0.6344 (µg/ml)

Table 3: Results of Precision Studies

Replicate		*Budesonide*	
S.No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	04.00	44776	99.99%
2		44789	99.96%
3		44802	99.93%
4		44834	99.86%
5		44856	99.81%
6		44878	99.76%
Average			99.88%
Std.Dev			0.090055
% RSD			0.09%
Standard weight			4mcg
Standard potency			99.60%

Table 4: Results of accuracy study

Budesonide						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	02.05	02.03	99.02	99.34%	0.28005	0.28%
100	04.10	04.08	99.51			
150	06.15	06.12	99.50			

Table 5: Results of Robustness Studies

Robustness Studies			
Parameter	**Value**	**Peak Area**	**% RSD**
Flow Rate	**Low**	45234	0.11%
	Actual	45285	
	Plus	45336	
Temperature	**Low**	44649	0.67%
	Actual	44921	
	Plus	45250	
Wavelength	**Low**	44837	0.07%
	Actual	44858	
	Plus	44902	

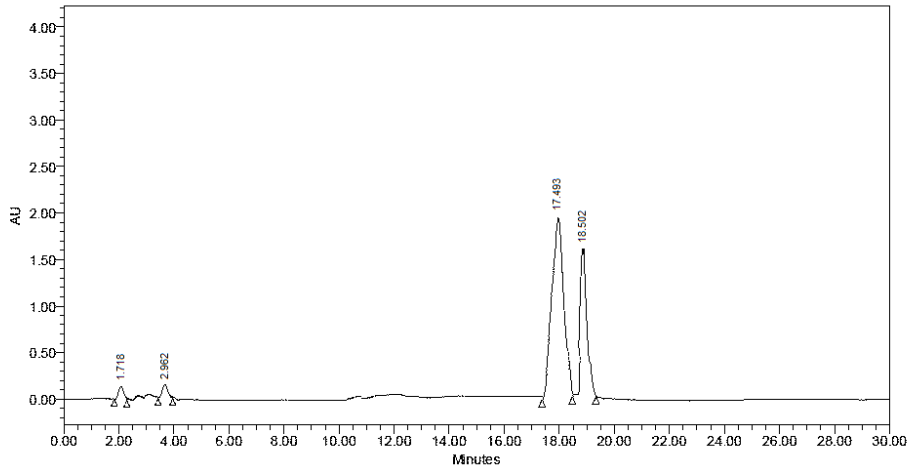

Figure 4: Chromatogram showing the degraded products in Acidic degradation

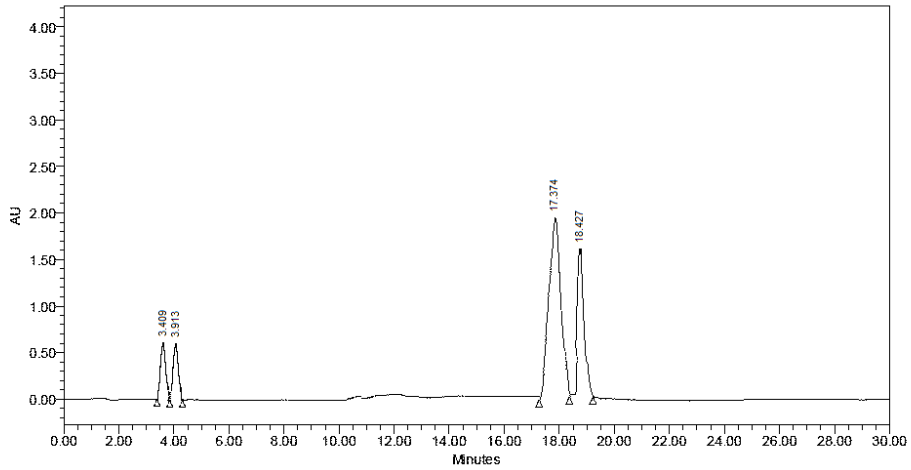

Figure 5: Chromatogram showing the degraded products in Basic degradation

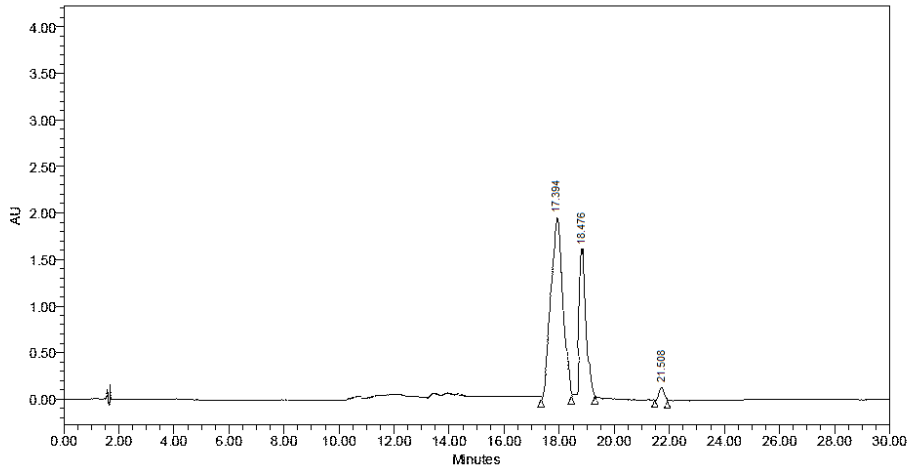

Figure 6: Chromatogram showing the degraded products in Wet heat degradation

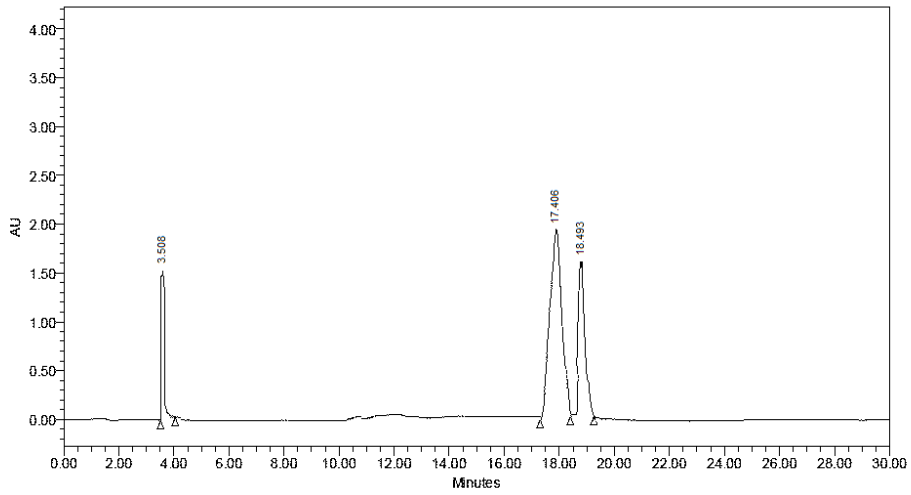

Figure 7: Chromatogram showing the degraded products in H_2O_2

Table 6: Stability Indicating study for the developed method

Nature of Stress	Degradation condition	Time(h)	Number of degradation products (Rt)
Acidic	60°C	3	2 (0.316, 1.819)
Basic	60°C	9	1 (0.269)
Oxidative	RT	48	1 (5.689)
Wet Heat	105°C	24	1 (4.486)

Table7: Impurity Profile of Budesonide

S.No	Impurity Name	Active Pharmaceutical Ingredient	Relative Retention Time
1	16α-Hydroxylprednisolone	Budesonide	0.790
2	D-Homobudesonide		0.725
3	21-Dehydrobudesonide		0.667
4	14,15-Dehydrobudesonide		0.624

Table 8:, Compositions of Budesonidein MS/MS spectra

Analyte	Observed ion mass (Da)	Proposed formula	Calculated mass (Da)	Error (ppm)
Budesonide	460.05	$C_{25}H_{34}O_6$	460.03	-1.87
	372.68	$C_{21}H_{28}O_6$	372.62	-2.65
	402.22	$C_{23}H_{30}O_6$	402.18	-2.84
	428.36	$C_{24}H_{30}O_6$	428.34	1.26
	429.61	$C_{25}H_{32}O_6$	429.57	-2.13

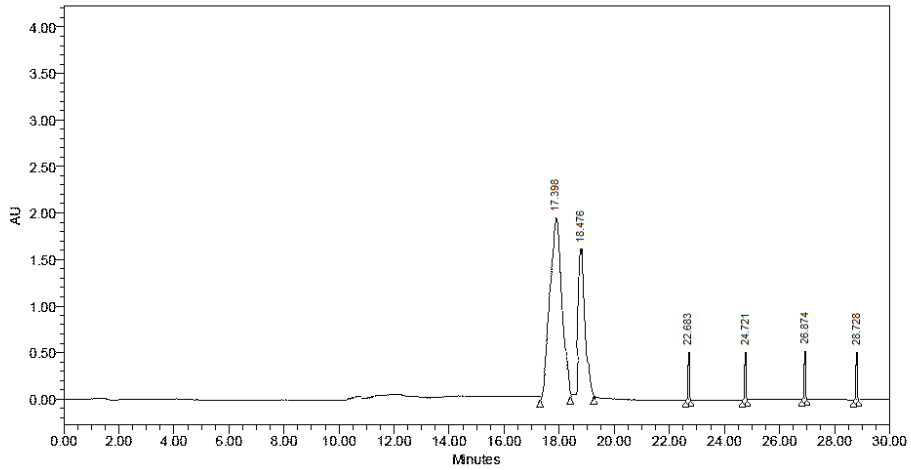

Figure 7: Chromatogram showing the impurities of Budesonide

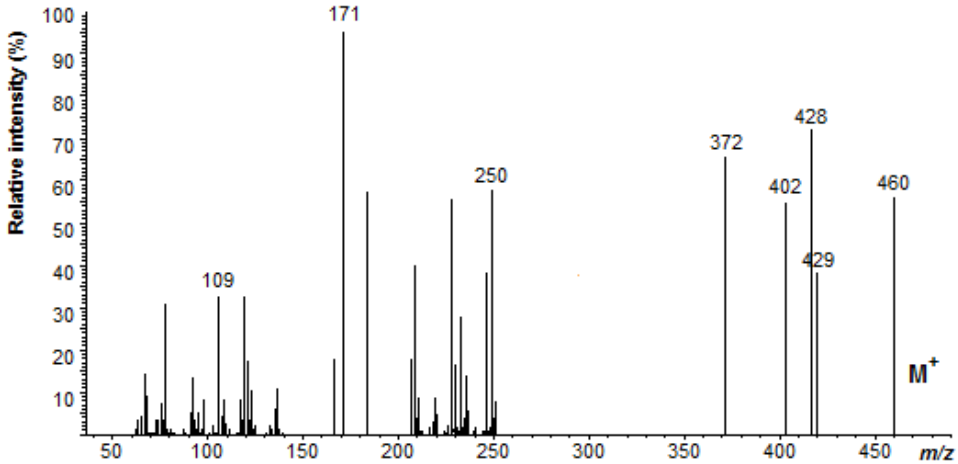

Figure 8: MS/MS^ncharacterisation of impurities in Budesonide

4. Conclusion

This research paper describes the separation and characterization of impurities in Budesonide pharmaceutical respules formulations. The impurities were isolated by preparative liquid chromatography and characterized by using spectroscopic techniques. A simple and efficient RP-UPLC method development and validation was discussed. The degradatedproducts were formed during the study and was well-resolved from Budesonide by the proposed RP-UPLC method. The proposed structure of Budesonide was characterized by

MS/MS^n analysis and was further confirmed to be accurate mass measurements.

Conflict of interest

None declared

5. References

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