Research Article

Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Balsalazide disodium dihydrate in Bulk and Its Pharmaceutical Formulations

Manish Manoharan*, P.D. Hamrapurkar, Askshay S, Sunil Patil, Atul Kadam
Department of Pharmaceutical Analysis, Prin. K.M Kundnani College Of Pharmacy, Colaba, Mumbai.
***Corresponding author's E-mail:** manish_manoharan@hotmail.com

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ABSTRACT

An isocratic reverse phase High Performance liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Balsalazide disodium dihydrate in Bulk and its pharmaceutical formulation. Separation was achieved with CrestPack RP C_{18} (Make: Jasco; 250 mm x 4.6 mm I.D; particle size 5 µm) Column and Ammonium formate buffer (pH adjusted to 3.5 with diluted formic acid) : Acetonitrile : MeOH : (65:25:10) v/v as eluent at a flow rate of 1.0 ml/min. The Photo-Diode Array (PDA) was used for detection purpose and detection was performed at 361 nm. The method is simple, rapid, and selective. The described method of Balsalazide disodium dihydrate is linear over a range of 2.5 µg/ml to 7.5 µg/ml. The correlation coefficient (r^2) was found to be 0.999. The method precision for the determination of assay was below 1.73 % RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 98.95 to 102.13%. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of Balsalazide disodium dihydrate in bulk, and its dosage forms.

Keywords: Balsalazide disodium dihydrate, Dosage form, RP-HPLC, Validation.

INTRODUCTION

Balsalazide disodium dihydrate is an oral Non-Steroidal anti-inflammatory drug and is indicated for the treatment of ulcerative colitis and IBD, 5-aminosalicylic the active metabolite of Balsalazide disodium dihydrate exerts its anti-inflammatory effects locally (in the GI tract) rather than systemically. Mucosal production of arachidonic acid metabolites, both through the cyclooxygenase pathways, and through the lipoxygenase pathways, is increased in patients with chronic inflammatory bowel disease. 5-aminosalicylic acid diminishes inflammation by blocking production of arachidonic acid metabolites in the colon through both the inhibition of cyclooxygenase and lipoxygenase.

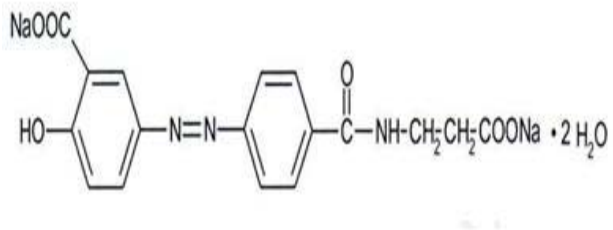

Figure 1: Chemical Structure of Balsalazide disodium dihydrate

For drug such as Balsalazide disodium dihydrate that is not intended to be absorbed into the bloodstream, Bioequivalence needs to be established by conducting a study with Pharmacodynamic endpoints. A comparison of In-Vitro dissolution profiles in different pH medium is an appropriate method for evaluating the bioequivalence of generic oral tablets.

MATERIAL AND METHODS

Instrumentation

The analysis of the drug was carried out on a Jasco LC system equipped with 2089 pump and photo-diode array detector (PDA) was used and a Reverse phase HPLC column CrestPack RP C_{18} (Make: Jasco; 250 mm x 4.6 mm I.D; particle size 5µm) was used. The output of signal was monitored and integrated using Chromnav software.

Chemicals and solvents

Milli-Q Water, Acetonitrile (ACN) (HPLC Grade), Methanol (MeOH) (HPLC Grade), Formic acid (GR Grade), Ammonium Formate (GR Grade) was obtained from Merck, Mumbai.

Buffer preparation

Accurately weigh and transfer about 630 mg of Ammonium Formate in 1000 ml of purified water and mix. Adjust pH to 3.5 (±0.05) with dilute Formic acid solution. Filter the solution through 0.45 µm membrane filter.

Mobile phase preparation

Prepare a filtered and degassed mixture of Buffer Acetonitrile and Methanol in the ratio of 65:25:10 v/v respectively.

Standard preparation

Accurately weigh and transfer about 10 mg of Balsalazide disodium dihydrate into a 10 ml volumetric flask, add 4 ml of Methanol, sonicate to dissolve. Cool the solution to room temperature and dilute to volume with Methanol (1000 µg/ml) (Solution A). Transfer 1.0 ml of solution A

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into a 10 ml volumetric flask and dilute to volume with Methanol to give 100 µg/ml solution (Solution B). Dilute 1.0 ml of Solution B to 10 ml with methanol to give 10 µg/ml solution (Solution C). Further dilute 5 ml of Solution C to 10 ml with methanol to give 5 µg/ml solution which is the standard solution.

Sample preparation: (For Balsalazide disodium dihydrate Capsules 750 mg)

Weigh the contents of 5 Capsules. Accurately weigh and transfer equivalent to 10 mg of Balsalazide disodium dihydrate into a 10 ml volumetric flask add about 4 ml of Methanol, and sonicate for 30 minutes with intermittent shaking at controlled temperature and dilute to volume with MeOH and mix (Solution A). Filter the solution through 0.45 µm membrane Filter. Transfer 1.0 ml of (Solution A) into a 10 ml volumetric flask and dilute to volume with MeOH to give (Solution B). Take 1.0 ml of (Solution B) into a 10 ml volumetric flask and and dilute to volume with MeOH to give (Solution C). Finally dilute 5.0 ml of (Solution C) into a 10 ml volumetric flask and and dilute to volume with MeOH to give Test solution.

Chromatographic conditions

CrestPack RP C_{18} (Make: Jasco; 250 mm x 4.6 mm I.D; particle size 5 µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The sample injection volume was 20 µl. The photodiode array detector was set to a wavelength of 361 nm for the detection and Chromatographic runtime was 10 minutes.

RESULTS AND DISCUSSION

Method development[4-6]

Choice of Column

Balsalazide disodium dihydrate is acidic in nature (ionizes in basic medium), it also has two bulky phenyl groups so reverse phase chromatography is the best choice. The efficiency of two different reverse – phase column C_8 and C_{18} was evaluated. C_{18} column being hydrophobic was preferred for separation of drug because drug retention was a problem on C_8 column. Sufficient HETP was obtained for the drug peak.

Mobile phase

The pKa of Balsalazide disodium dihydrate is 3.06, so the working range for pH of buffer was kept at pH 3.0 ± 1.0. Buffer was used to maintain pH and to overcome pH variations observed while using water. Ammonium formate 10mM was used as the buffer as it has a buffering range of 2.8 to 4.8 which is the desired buffering range. The pH of the buffer was further adjusted to 3.5 with Formic acid and Flow rate was kept at 1 ml/min. Initially Buffer: MeOH: 65: 35 was tried which gave long retention time about 15 minutes with broad peak and tailing. Buffer: ACN: 65: 35 caused peak splitting, Buffer: MeOH: 20: 80 and 30: 70 resulted in overlap of mobile phase peak and the analyte peak, Buffer: ACN 50: 50 and 40: 60 were tried both gave peaks with retention time below 3 minutes and peak splitting. It was thus noted that ACN causes fast elution but with peak splitting and MeOH delays elution but at high ratio causes merging with solvent peak. So Buffer: ACN: MeOH: 65: 25: 10 was tried with pH kept at 3.5 resulting in peak with symmetry and tailing factor (≤ 1.5).

Effect of mobile Phase composition

Various compositions of the mobile phase Ammonium formate (buffer) : ACN : MeOH : (75 : 15 : 10) (75 : 5 : 20) (70 : 10 : 20) were tried which gave retention time above 15 minutes and broad peaks. Also resolution between the main peak and impurities 4-NO_2 Benzoic acid and Phenol-2-Carboxylic acid was less than 1.

Effect of mobile phase pH

The optimized mobile phase composition of buffer: ACN: MeOH: (65: 25: 10) and Flow rate of 1ml/min was kept constant and the pH was varied. Higher pH of 5 caused higher retention time above 20 min and peak broadening and pH of 2.5 gave peak shape problems and poor reproducibility. pH range of 8 gave retention time of 27 minutes with run to run variations as the pH was beyond the buffering range.

Effect of flow rate

It was observed that theoretical plates were highest at flow rate 1 ml/min with asymmetry less than 1.3. Increasing the flow rate to 1.2 ml/min and 1.5 ml/min resulted in poor resolution due to rapid mass transfer and higher back pressure. A low flow rate of 0.8 ml/min resulted in drug retention time beyond 12 min that was more time consuming along with broad peaks. Hence, the flow rate of mobile phase was optimized at 1.0 ml/min with the retention time of drug around 7.5 mins.

Detection system

The presence of chromophore i.e., substituted benzene ring makes UV-visible detection possible for determination. Selection of λmax was done based on Spectrophotometric scan of compound. Balsalazide disodium dihydrate showed λmax at 361 nm.

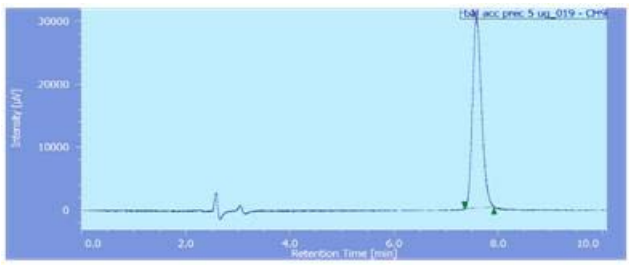

Figure 2: A typical HPLC Chromatogram showing the Peak of Balsalazide disodium dihydrate

Method validation[7-8]

The developed RP-HPLC method extensively validated for the determination Balsalazide disodium dihydrate Content using the following Parameters.

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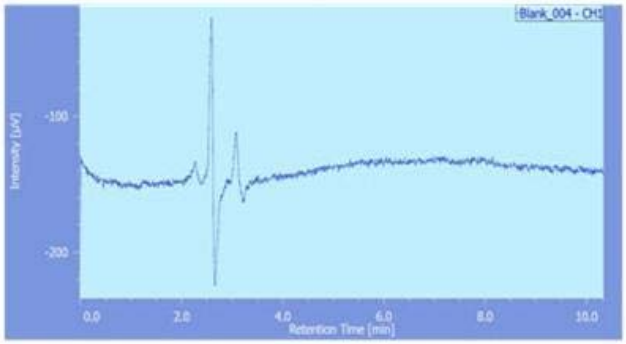
Table 1: System suitability parameters for Balsalazide disodium dihydrate by proposed method

Name Of Compound	Number Of Theoretical Plates	Retention time	Symmetry Factor
Balsalazide disodium dihydrate	9313	7.582	1.213

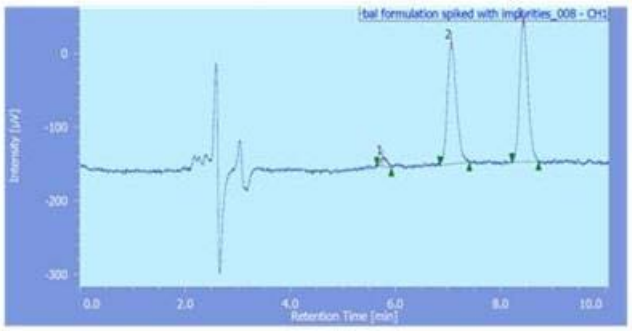
Specificity

Blank interference

A study to establish the interference of blank was conducted. Methanol was injected into the HPLC system as defined in above chromatographic conditions and the blank chromatogram was recorded. Chromatogram of Blank showed no peaks at the retention time of Balsalazide disodium dihydrate peak. This indicates that Methanol used in sample preparation do not interfere in estimation of Balsalazide disodium dihydrate in Balsalazide disodium dihydrate Capsules. The chromatogram of Balsalazide disodium dihydrate Blank using the proposed method is shown in Figure 3 (a).



(A)



(B)

Figure 3: (a) typical HPLC Chromatogram showing the no interference of Solvent for Balsalazide disodium dihydrate (b) Chromatogram showing resolution of Impurities, Peak 1: Phenol-2-carboxylic acid (Rt 5.758), Peak 2: Balsalazide (Rt 7.067), Peak 3: 4-NO_2 Benzoic Acid (Rt 8.433).

Resolution of Impurities

The method developed was able to resolve Process related impurities like 4-NO_2Benzoic Acid and Phenol-2-Carboxylic acid from the peak of Balsalazide disodium dihydrate as shown in Figure 3 (b), which makes the method more specific.

LOD and LOQ

The limit of detection (LOD) was obtained by successively decreasing the concentration of Balsalazide disodium dihydrate as long as a signal to noise ratio of not less than 3:1 is maintained. The LOD of Balsalazide disodium dihydrate was found to be 100 ng/ml.

The LOQ of Balsalazide disodium dihydrate was found to be 300 ng/ml

System and Method Precision

In the study of the instrumental system precision where, a RSD of 1.04% was obtained for the standard area. The method precision study for six sample preparations in marketed samples showed a RSD of 1.73 % and with the recovery range of 98.95 - 102.13 % with an average recovery of 99.97 %. For the intermediate precision, a study carried out by the same analyst working on different day. The results calculated as intra-day RSD corresponded to 1.41 % (For Standard) (Intermediate Precision). The same study was carried out for different analysts (*n* = 6 number of samples per analyst) obtaining a RSD of 1.51 % and with the recovery range of 98.7 - 101.5 with an average recovery of 99.3. The Overall % RSD for n = 12 for the three concentration levels (4.0, 5.0, 6.0 µg/ml) is 1.23 (Intraday). Both results together with the individual results are showing that the proposed analytical technique has good intermediate precision.

Table 2: Precision data table

Sr No.	4.0	5.0	6.0
	(Conc in µg/ml) Day one (Intraday)		
1	290183	359835	451146
2	290688	368140	451329
3	288521	360065	446389
4	295988	366233	458449
5	292192	367385	451942
6	292230	367066	449227
	4.0	5.0	6.0
	(Conc in µg/ml) (Interday)		
1	291089	359921	444127
2	289402	353672	448098
3	287530	357831	430134
4	289833	358703	449732
5	289109	356093	448136
6	288095	355232	450742
Mean	290405	360848	448287.6
RSD	0.79	1.41	1.49
Average RSD (Intraday precision)	1.23		

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Table 3: Accuracy data

	Levels in µg/ml					Levels in µg/ml				
	2.5	4.0	5.0	6.0	7.5	2.5	4.0	5.0	6.0	7.5
Sr No	Area					Recovery				
1	183320	290183	359835	451146	541302	2.49	3.94	4.88	6.12	7.35
2	180670	290688	368140	451329	542717	2.45	3.95	5.00	6.13	7.37
3	184360	288521	360065	446389	540853	2.50	3.92	4.89	6.06	7.34
4	187312	295988	366233	458449	552468	2.54	4.02	4.97	6.22	7.50
5	189250	292192	367385	451942	556951	2.57	3.97	4.99	6.14	7.56
6	187284	292230	367066	449227	552783	2.54	3.97	4.98	6.10	7.50
Mean	185366	275489	364787.3	451413.7	548540.3	2.51	3.96	4.95	6.13	7.44
RSD	1.70	0.87	1.04	0.88	1.28	1.70	0.87	1.04	0.88	1.28
Accuracy %	----	----	----	----	----	100.60	98.95	99.03	102.13	99.16

Accuracy

The accuracy of the method was determined on five concentration levels by recovery experiments. The recovery studies were carried out on six preparations of each concentration level and analyzed as per the proposed method.

Table 4: Linearity Data

Parameter	Slope	Intercept	Correlation coefficient (r^2)
Value	73636	170.3	0.999

Table 5: Forced Degradation Data

	Peak name	Rt	Area	NTP	Resolution	Symmetry
Control	Balsalazide	7.282	48520	10252	N/A	1.180
Acid	Degradant 1	4.350	982	14391	10.135	1.110
	Degradant 2	6.217	1058	12290	6.501	1.265
	Balsalazide	8.058	41750	8789	3.852	1.152
	Degradant 3	11.525	4398	996	N/A	1.291
Base	Degradant 1	6.650	1986	9479	2.727	1.139
	Balsalazide	7.550	40589	6042	14.925	1.152
	Degradant 2	16.433	5892	6667	N/A	0.858
Peroxide	Balsalazide	7.542	43501	10943	11.004	1.174
	Degradant 1	10.725	4919	21623	N/A	1.031
Thermal	Balsalazide	7.450	43693	10349	10.327	1.256
	Degradant 1	10.567	4703	18323	N/A	1.201
Photolytic	Balsalazide	7.808	49724	9963	N/A	1.245

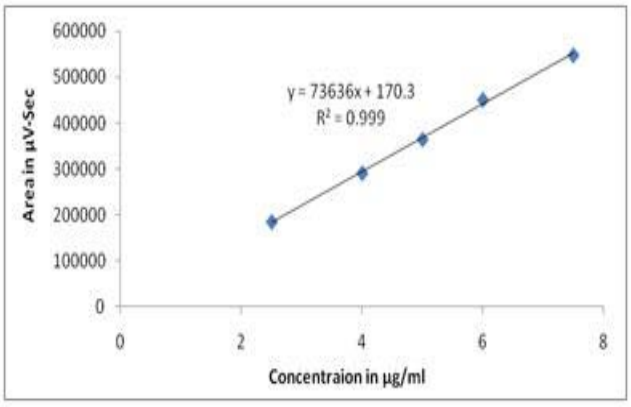

Figure 4: Calibration curve for Balsalazide disodium dihydrate

Linearity of detector response

The standard curve was obtained in the concentration range of 2.5 - 7.5 µg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r^2] of standard curve were calculated and given in Table 4.

Forced Degradation

Stock Solution

10 mg of working standard of Balsalazide disodium dihydrate was accurately weighed and dissolved in 10 ml of Methanol to give a solution of 1000 µg/ml (Solution A). 5ml of Solution A was diluted to 100 ml to give the stock solution of concentration 50 µg/ml.

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Acid Degradation Sample

For Acid Hydrolysis, 10 ml of 1 N HCl was added to 5 ml of stock solution (50 µg/ml) in round bottom flask. The mixture was refluxed on water bath for 4 Hrs at 100°C. Before loading the stress sample on HPLC, it was neutralised with corresponding base.

Base Degradation Sample

10 ml of 1 N NaOH was added to 5 ml of stock solution (50 µg/ml) in round bottom flask. The mixture was refluxed on water bath for 1 Hr at 80°C. Before loading the stress sample on HPLC, it was neutralized with corresponding acid.

Peroxide Degradation Sample

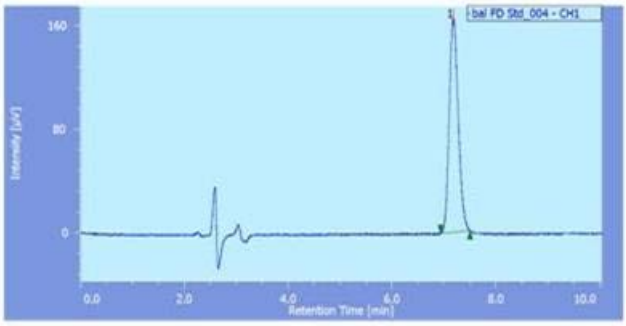
For oxidative degradation, 5 ml of stock solution was treated with 10 ml of 0.5 % (v/v) H_2O_2 solution and this mixture was heated at 80°C for 1 Hr on water bath.

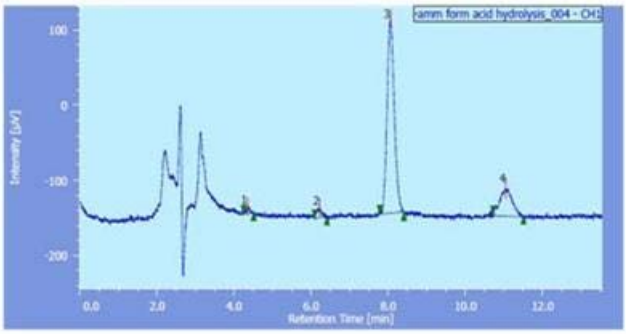
Thermal Degradation Sample

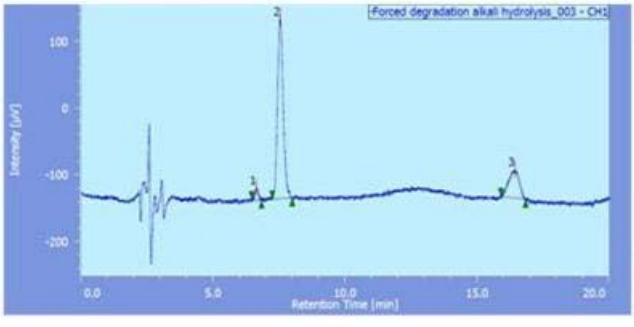
For thermal degradation, 5 ml of stock solution was refluxed for 4 Hrs on water bath at 100°C.

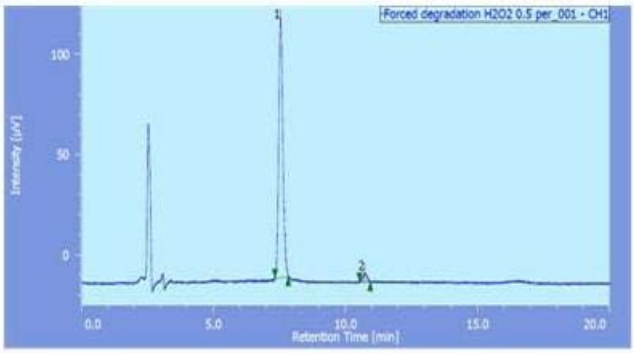
Photolytic Degradation

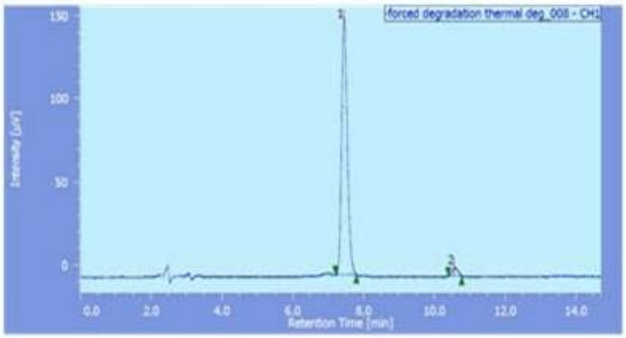
The photolytic degradation was carried out by exposing drug substance under UV light at 290 nm for one week.











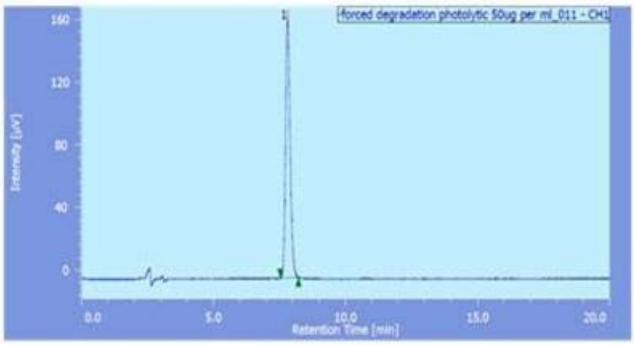


Figure 5: a) Control Sample b) Acid Degradation, c) Base Degradation, d) Peroxide Degradation, e) Thermal Degradation, f) Photolytic Degradation

Application on Marketed Formulation

The Developed Stability Indicating RP-HPLC method was successfully applied for the estimation of Balsalazide disodium dihydrate from the marketed formulation of Balacol capsules of Torrent Pharma which was found to contain 97.44 % of the Label Claim.

CONCLUSION

We have developed a fast, simple and reliable analytical method for determination of Balsalazide disodium dihydrate in pharmaceutical preparation using RP-LC. The method is specific as there is no interference of blank at the retention time of Balsalazide disodium dihydrate and process related Impurities 4-NO_2 Benzoic Acid and Phenol-2-Carboxylic acid could be resolved very well. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliable analysis of Balsalazide disodium dihydrate in bulk and its pharmaceutical dosage forms.

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