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Stress degradation studies on betahistine and development of a validated stability-indicating assay method

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ABSTRACT

The purpose of this work was to study the stability of betahistine (BET) at different stress conditions and to develop a sensitive stability-indicating high-performance liquid chromatographic (HPLC) assay method. The stress conditions applied were including the effect of heat, moisture, acid–base, and ultra-violet (UV) light. Betahistine and its decomposition products were derivatized by reaction with dansyl chloride (Dan-Cl) and analyzed by HPLC equipped with fluorescence detector (FL) set at 336 and 531 nm as excitation and emission wavelengths, respectively. The drug was particularly labile at UV light and oxygen rich media. Two potential degradation products could be separated and identified by spectral methods. The chromatographic method involved Zorbax Eclipse XDB-C_{18} column kept at $30 \pm 2\,^{\circ}C$ and a gradient elution with mobile phase composed of acetonitrile and 0.02 mol L^{-1} sodium acetate. The response factor of dansylated BET monitored by fluorescence detection was 32 times more than its UV response. The calibration curve of BET in bulk form was linear from 0.005 to 4.2 ng μL^{-1}. Intraday and interday precision were less than 0.04% (CV), and accuracy was between 99.2% and 100.9% over 2.0 ng μL^{-1}. The limit of detection was 0.002 ng μL^{-1}. The method was also validated for sample stability during reaction, robustness and selectivity. The method was applied for purity testing of betahistine in tablet form.

1. Introduction

Betahistine hydrochloride [*N*-methyl-2-(pyridin-2-yl)ethanamine dihydrochloride] is vasodilator and most commonly used to treat the symptoms of Ménière's disease and vertigo [1]. It also used to treat the symptoms of tinnitus [1]. A limited number of publications were found in the literature concerning the determination of betahistine either in dosage forms or in biological matrix. Among those, the British Pharmacopoeia 2007 (BP) cited a liquid chromatographic method for the determination of the related substances of BET and potentiometric assay method (in bulk form) [2]. A liquid chromatographic–tandem mass spectrometric assay method has been reported for the analysis of the major metabolite of betahistine, 2-pyridylacetic acid, in human plasma [3]. El-Walily et al. reported a spectrophotometric, atomic absorption spectrometric and HPLC procedures for the determination of betahistine hydrochloride (BET-2HCl) and captopril [4]. Douglas and Hohing described a gas chromatographic method for the determination of BET in serum in the range of 0.6–6.0 $\mu g\,mL^{-1}$ [5]. No reports were found for the analysis of BET in presence of its possible degradation products. However, the potential impurities of BET and their limits are cited in BP 2007 [2] and United States Pharmacopoeia [6] (Fig. 1). Both compendia cited an HPLC separation methods using reversed phase column and monitoring at the UV range. Described here is a fully validated sensitive liquid chromatographic analytical method for the detection and assay of BET and its possible degradation products after pre-column derivatization with Dan-Cl. A gradient HPLC with fluorescence detection was designed to fulfill the requirements of Food and Drug Administration (FDA)/International Conference on Harmonisation (ICH) regarding the selectivity, suitability, linearity, accuracy, robustness, ruggedness and sample solution stability [7–10]. The developed method was applied for determination of BET and its degradation products in marketed pharmaceutical dosage form. The reaction conditions and the chromatographic procedure were optimized to achieve complete derivatization, no side reaction, and very low detection limits of drug and its degradation products.

2. Experimental

2.1. Chemicals and reagents

All solvents were of HPLC grade, Merck, Darmstadt, Germany. All other materials were of analytical grade. 5-Dimethyl-

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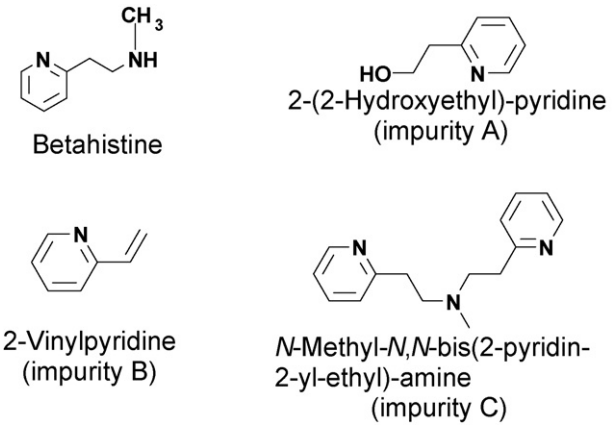

Fig. 1. Chemical structure of betahistine and its impurities cited in BP 2007 and USP-29 NF-24.

aminonaphthalene-1-sulfonyl chloride (Dansyl chloride, yellow form, HPLC grade 99.0%) was purchased from Sigma–Aldrich, and verified for identity and purity as per specifications published on the web page of Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO, USA). Betahistine dihydrochloride was obtained as gift from Solvay Pharmaceuticals (Weesp, Holland) and verified for related substance by applying the BP 2007 analytical method [2]. Betaserc® tablets (16 mg BET-2HCl per tablet) were purchased from the local market in Saudi Arabia (manufactured by Solvay Pharmaceuticals, Weesp, Holland), batch number: 325010, expiration date: June 2008.

2.2. Equipment

The HPLC system consisted of an Alliance Waters separations module 2695, photodiode array detector model 2996, multi λ fluorescence detector model 2475 (Milford, MA, USA). Column heater was set to 30 ± 2 °C. The control of HPLC system and data processing were performed by Empower software (Build 1154, Waters). Screw-capped reaction vials 1.8-mL and V-shaped autosampler vials 300-μL were used (Alltech, GmbH, Unterhaching, Germany). Heating oven adjusted to 55 ± 2 °C (Heraeus, Kendro, Hanau, Germany). Digital micro-transfer pipettes 5–250 μL were used (Brand, Wertheim, Germany). The UV-lamp used for photostress testing was 125 cm long (General Electric, Cincinnati, OH, USA). This lamp is installed in Laminar flow cabinet (SterilGard Hood, Class II), designed as germicidal UV light source (The Baker Co. Inc., Sanford, Maine, USA). GC/MS Clarus 500 Gas Chromatograph, Clarus 500 Mass spectrometer (PerkinElmer, Shelton, CT, USA). Software controller/integrator; TurboMass, version 4.5.0.007, PerkinElmer, Shelton, CT, USA). Elite 5MS GC capillary column, 30 m × 0.25 mm × 0.5 μm (PerkinElmer, Shelton, CT, USA). Carrier gas; Helium (purity 99.9999%), flow; 2 mL min^{-1}, 32 p.s.i., flow initial; 55.8 cm s^{-1}, split; 1:40. Inlet line temperature; 200 °C, source temperature; 150 °C, trap emission; 100 °C, electron energy; 70 eV. Column temperature program; 50 °C for 1 min, to 180 °C (rate; 20 °C min^{-1}), hold for 2 min. Injector; 220 °C. MS scan from 50 to 650 *m/z*. ^{1}H NMR spectra were recorded on a JEOL JNM-AL 300 FT-NMR system (JEOL, Tokyo, Japan). Chemical shifts are expressed as δ units (part per million) downfield from tetramethylsilane (TMS). $CDCl_3$ was used as solvent.

2.3. Chromatographic conditions

Analytes were separated on Agilent Zorbax Eclipse XDB-C_{18} (250 mm × 4.6 mm i.d., 5 μm particle diameter) protected with Agilent Zorbax XDB-C_{18} pre-column (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was programmed to deliver 30% acetonitrile and 70% sodium acetate (0.02 mol L^{-1}, pH 4.5) with a flow rate of 1.0 mL min^{-1}, for 1 min and linear gradient elution from 1 to 21 min to end with 70% acetonitrile and 30% sodium acetate solution. The UV detector was set at 254 nm and the fluorescence detector set at 336 and 531 nm (as excitation and emission wavelengths, respectively).

2.4. Solutions

Dansyl chloride (Dan-Cl) solution was prepared by dissolving 10.0 mg of reagent in 10.0 mL of acetonitrile. This solution was prepared freshly everyday. A volume of 50 μL was used for derivatization. Sodium bicarbonate (0.5 mol L^{-1}) solution was prepared in water and adjusted to pH 9.5 ± 0.1 with 5 mol L^{-1} sodium hydroxide and checked by calibrated pH-meter. Standard stock solution of BET-2HCl was prepared by dissolving 10.0 mg of substance in 10.0 mL of water.

2.5. Derivatization procedure

A volume of 50 μL from BET standard solution and 50 μL Dan-Cl solution were transferred to autosampler glass vial (capacity = 300-μL) using digital micropipette. A volume of 200 μL $NaHCO_3$ solution was added. The vial was capped, swirled, and left to stand at 55 ± 2 °C for 20 min. A volume of 10 μL was injected for HPLC analysis with fluorescence detection.

Two blank experiments were prepared using 50 μL water instead of sample solution, or 50 μL acetonitrile instead of Dan-Cl solution. These blank solutions were prepared to identify any peak(s) due to reagent. The underivatized BET was monitored by HPLC-diode array detector at 254 nm.

2.6. Calibration curves

Six standard solutions of betahistine dihydrochloride were prepared in water, spanning the range 41.5–0.046 μg mL^{-1}. A volume of 50 μL from each solution was derivatized as described under derivatization procedure. A volume of 10 μL was injected for HPLC analysis. The peak area of the dansylated betahistine (Dan-BET) was plotted versus the corresponding concentration in ng μL^{-1} (as base) of the final derivatized sample solution. The final concentrations tested were spanning the range 4.50–0.005 ng μL^{-1} BET base. The calibration curve was constructed using a least square regression equation for the calculation of the slope, intercept and correlation coefficient.

2.7. Forced degradation

A standard solution of BET (40 ng μL^{-1}) was derivatized and injected for HPLC analysis before and after each sample analysis. The derivatized-degraded samples were monitored by HPLC-fluorescence mode. Relatively high concentration solutions of degraded BET were directly injected for monitoring the underivatized and derivatized forms using UV and FL detectors, simultaneously. In all stress experiments, the amounts of BET remained (not degraded) and the generated degradation products were calculated from the regression line of Dan-BET and then expressed as percents of initial BET mass concentration.

2.7.1. Effect of heat and moisture

Two screw-capped reaction vials were used for this experiment. The first vial contained 16 mg of BET; the second vial contained 16 mg BET-2HCl with 10 μL water as a source of moisture. Both vials were kept at 60 ± 2 °C for 24 h in a hot-air oven. The contents of the

vials were dissolved and diluted in water to give a concentration of 20.6 ng μL^{-1} as base. A volume of 50 μL was derivatized with dansyl chloride as described under derivatization procedure. A volume of 10 μL was injected for HPLC analysis. The same procedures were repeated at 70 and 80 ± 2 °C (the claimed concentration of intact BET in the final reaction mixture was 3.43 ng μL^{-1} as base).

2.7.2. Alkali and acid hydrolysis

A weight of 16 mg of BET-2HCl and a volume of 1 mL sodium hydroxide (1 mol L^{-1}) solution were mixed in a screw-capped reaction vial. The vial was capped, heated at 100 ± 5 °C for 5 min, cooled, and neutralized with 1 mol L^{-1} hydrochloric acid. The vial content was then diluted with water to give a claimed concentration of 20.6 ng μL^{-1} of intact BET as base. A volume of 50 μL was derivatized with Dan-Cl as described under derivatization procedure. A volume of 10 μL was injected for HPLC analysis.

The same procedure was repeated but using 1 mL, 1 mol L^{-1} HCl for hydrolysis, and then neutralized with 1 mol L^{-1} NaOH. Both 1 mol L^{-1} HCl and 1 mol L^{-1} NaOH solutions were standardized and tested for neutralization before use. The claimed concentration of intact BET injected in both cases is 3.43 ng μL^{-1} (as base).

2.7.3. Oxidation

In a 2-mL reaction vial, a volume of 1 mL of aqueous standard BET solution (132 ng μL^{-1} as base) was mixed with 0.1 mL 20% hydrogen peroxide. This vial was left to stand at room temperature for 30 min. The reaction mixture was then diluted to 20 mL with water, bubbled with nitrogen gas for 30 min to expel dissolved oxygen, and adjusted again to 20 mL with water. A volume of 50 μL was used for derivatization as described previously. A volume of 10 μL was injected for HPLC analysis. The claimed concentration of intact BET injected is 1.1 ng μL^{-1} (as base).

2.7.4. Effect of UV light on solid form

A sample film of BET-2HCl powder of approximately 0.1-mm thickness in a flat glass dish was subjected to the UV light. The dish including sample spread was kept inside small transparent glass-desiccator to protect sample from absorption of moisture because of its hygroscopic property. The distance between UV-lamp and sample was 5-cm. A suitable concentration of BET (11.0 ng μL^{-1} as base) was prepared in water after exposure time of 6, 12 and 24 h. A volume of 50 μL was used for derivatization as described previously. A volume of 10 μL was injected for HPLC analysis. The claimed concentration of intact BET injected is 1.85 ng μL^{-1} (as base).

2.7.5. Effect of UV light on Betaserc® 16 mg tablets

Five Betaserc® 16 mg tablets were powdered and spread on glass dish (10-cm diameter) and exposed to UV light as described under effect of light on solid form. An equivalent amount of one tablet powder was extracted in water. A suitable claimed concentration (11.0 ng μL^{-1}) was prepared in water. A volume of 50 μL was used for derivatization as described previously. A volume of 10 μL was injected for HPLC analysis. The claimed concentration of intact BET injected is 1.85 ng μL^{-1} (as base).

In both photo-stress experiments, control experiments were carried out. The control samples were wrapped by aluminum foil before exposure to UV light.

2.8. Thin-layer chromatography (TLC) of photodecomposed BET-2HCl

A portion of UV-degraded pure BET-2HCl (50 mg) was reconstituted in a 10 mL acetonitrile and derivatized with Dan-Cl (1 part of BET with 20 part of Dan-Cl, w/w). A volume of 5 mL was spotted onto TLC silica-gel plates, 20 cm × 20 cm, 0.25 mm layer thickness (non-fluorescent background). The TLC plate was developed in a mixture of acetonitrile:dichloromethane (1.0:9.0, v/v). Migration distance of the developing system was not less than 15 cm. The bands were located by viewing under UV light at 254 and 365 nm. The separated bands were scratched, extracted in acetonitrile, analyzed by HPLC using the developed method, and identified by gas chromatography–mass spectrometry (GC–MS), and ^{1}H Nuclear Magnetic Resonance (^{1}H NMR).

2.9. Purity testing of Betaserc® 16 mg tablets

Two procedures were carried including the British Pharmacopeial and the developed methods. The procedure cited in BP 2007 under the related substances of betahistine dihydrochloride was applied to detect any known impurity. Also, the developed method was applied to analyze the degradation products, as follow: ten tablets were weighed, powdered, and an average weight (260 mg) equivalent to one Betaserc® tablet was introduced into a 100-mL stoppard conical flask, and shacked for 5 min with 70 mL water. This solution was sonicated for 2 min, completed to volume with water, mixed, and a portion of about 10 mL was filtered through nylon-syringe filtration disks. A further dilution was prepared from this solution (34.8 μg mL^{-1} as base), and then a volume of 50 μL was derivatized as described above and analyzed by HPLC with fluorescence detection.

3. Results and discussion

3.1. Optimization and confirmation of the derivatization

The compendial analytical methods cited for BET [2,6] are claimed to be stability indicating, however, it was necessary to study the effect of environmental factors so as to know the source of possible degradation products and their detection limits. The official methods were unable to detect the most of the generated degradation products (Fig. 2). However, the developed method was able to detect all the potential degradation products at very low mass concentrations.

Here below, described a sensitive stability-indicating analytical method to analyze the potential degradation products of BET. Detector response for dansylated degradation products was approximately equal as for the dansylated BET. This was provided by the calibration data and standard deviations of the recovered amounts calculated. This is expected since the fluorescence property is attributed to the dansyl moiety. Two blank experiments were carried to confirm that the detected peaks are due to dansylated BET

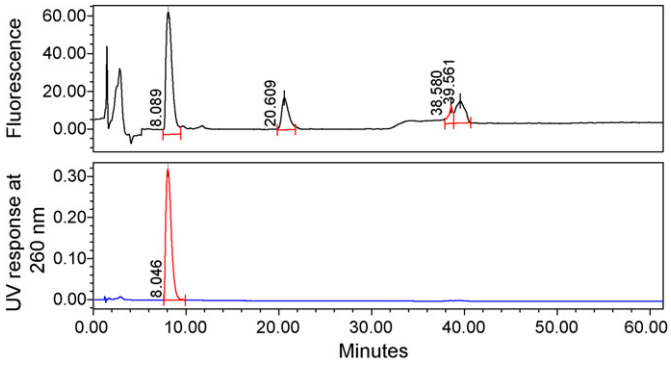

Fig. 2. Chromatograms of photodecomposed BET monitored by; UV detector at 260 nm (applying the BP 2007 analytical method) and fluorescence detector at 265 nm (ex)/311 nm (em).

and dansylated degradation products, but not reaction byproducts. The first blank was prepared to contain photodegraded-BET without adding Dan-Cl, but the second blank experiment was prepared to contain Dan-Cl without adding BET. The first blank experiments have shown no fluorescent peaks at the adjusted wavelengths. Also, the second blank has shown only one fluorescent peak corresponds to Dan-Cl. More confirmation was done by injecting high concentration of derivatized UV-degraded sample and each single peak was UV-scanned by using diode array detector (DAD) matching capability. All peaks were in perpendicular pattern, from 200 to 400 nm. This confirmed that all detected peaks were coupled with the same fluorofor (dansyl derivatives). The reaction completeness and reagent stability was investigated. The amount ratio of Dan-Cl to drug was about 10 to 1. However, a ratio of 24 to 1 mol L^{-1} (Dan-Cl to BET) was used at the highest calibration level. This reagent amount was found enough for complete dansylation. The effect of pH and heating time on derivatization was also studied. The chromatogram of dansylated pure BET-2HCl showed no peaks due to degradation of Dan-Cl or any evidence for BET side reaction.

3.2. Selectivity, precision, and performance parameters

Forced degraded BET samples were injected 6 repetitive times for HPLC analysis. The eluted analytes were first passed to UV detector, and then goes through the fluorescence detector. All peaks corresponding to degradation products were completely separated from each other and from the principle peak (BET) eluted at about 18.4 min (Fig. 3). The relative standard deviation (R.S.D.) of the calculated mass concentrations of BET and its dansylated degradation products were not more than 0.4%. The chromatographic performance parameters are listed in Table 1 using UV-degraded BET sample as a representative case. The chromatographic performance parameters were of acceptable values.

3.3. Linearity and range

A linear UV response of peak areas for BET was observed over the range, 0.005–4.2 ng μL^{-1}. The squared regression coefficient was 0.9999. The response factor (RF) of Dan-BET measured by fluorescence detector was 161120.0, while its UV-RF value was only 5033 (peak area unit per each one nanogram injected). That mean, the fluorescence response of Dan-BET is 32 fold more than its UV response. The lower limit of quantitation (LOQ) was estimated by satisfying two criteria: the S/N ratio is not less than 9 and the relative standard deviation (R.S.D.) of five replicate injections of the LOQ solution is less than 6%. LOQ of BET was 0.005 ng μL^{-1}, and the limit of detection (LOD) was 0.002 ng μL^{-1}. The mass concentration of each degradation product was calculated from the calibration curve of BET-2HCl. The total calculated amount of degradation products was approximately equal to the amount lost by BET-2HCl. This concept of calculation was followed as per ICH guidelines (7–10).

3.4. Accuracy

Analyzing placebo solutions spiked with known amounts of BET showed the accuracy of the method (claimed, 0.005, 0.2, 0.5, 1.0, 2.0, and 4.2 ng μL^{-1}; found, 0.005 ± 0.002, 0.2 ± 0.012, 0.5 ± 0.005, 1.0 ± 0.012, 2.0 ± 0.017, and 4.2 ± 0.01 ng μL^{-1}, respectively). Because the results obtained were within the acceptable range of $100 \pm 5\%$ the method was deemed to be accurate.

3.5. Robustness

For the evaluation of the method robustness, one chromatographic parameter was changed while the other parameters were kept unchanged. A standard solution (claimed, 1.85 ng μL^{-1}) of photodecomposed BET-2HCl powder (exposed to UV light for 12 h) was injected three times after each change. The chromatographic parameters (including retention factor, k, retention time, tR, peak asymmetry, As, resolution, Rs, and USP width, $W_{0.05}$) were calculated and compared with those of the system suitability (Table 1). The method robustness was tested after changing the pH of the acetate solution (4.0–4.8), changing gradient profile, and upon using Agilent Zorbax Extended C_{18} HPLC column. The results revealed that the method was robust for these small changes in pH but not robust for different gradient system. However, at pH > 4.8, the retention times and fluorescence response of the decomposition products were markedly fluctuated. The method was robust upon using end capped C_{18} HPLC column from Agilent (Zorbax-Eclipse); however, the non-end capped column (Zorbax-Extended) has shown an acceptable resolution with longer retention time for all separated peaks. The effect of using 0.1% counter ion in acetate solution was investigated (tetrabutylammonium bromide, and *n*-hexanesulfonic acid sodium salt). Both counter ions have shown dramatic shift of the all peaks to higher tR with tailing and negligible fluorescence response. Besides, the method robustness toward the reaction conditions was tested. No extra peaks or different responses were observed upon using reagent amount in the range of 30–70 μL (1 mg mL^{-1}), or varying heating temperature in the range of 50–60 °C for 30 min.

3.6. Ruggedness

The ruggedness of the method was evaluated by applying the HPLC procedure using two different analysts with two different HPLC systems (Waters Alliance system and 1100 Hewlett Packard HPLC system). Both HPLC systems were equipped with DAD and fluorescence detectors. The R.S.D. of tR, k, As, and peak areas obtained with the two chromatographic systems were not more than 0.2 and 0.4% for UV and fluorescence detection, respectively.

3.7. Sample solution stability

Sample solution stability was tested by a repetitive daily injection of BET calibration solution (1 ng μL^{-1}) and photodecomposed BET (1.85 ng μL^{-1}) for four days. The derivatized samples were stable at room temperature for 24 h. Samples left in the autosampler for more than 2 days have shown different results. Also, the prepared reagent (Dan-Cl) was freshly prepared every day. To apply this method successfully, a freshly prepared solution should be used and the analysis should be done within 12 h.

3.8. Forced degradation of BET

The results of the forced degraded BET are presented in Fig. 3 and Table 2. The powder of BET-2HCl and Betaserc® tablet were turned yellowish to brown color upon exposure to UV light, and showed matched chromatographic profiles. Table 2 illustrated the percentage mass concentrations of BET and its potential degradation products released at different stress conditions. It is clear that BET was very sensitive toward UV light and oxidation. Both, peaks eluted at 11.2 and 15.2 min were the most potential degradation products released due to the effect of light. Also, the maximum amount of the peak eluted at 19.8 min was released due to the effect of oxidation. Both, peak at tR 11.2 and 19.8 min were generally observed in all stress cases including Betaserc® tablets. The

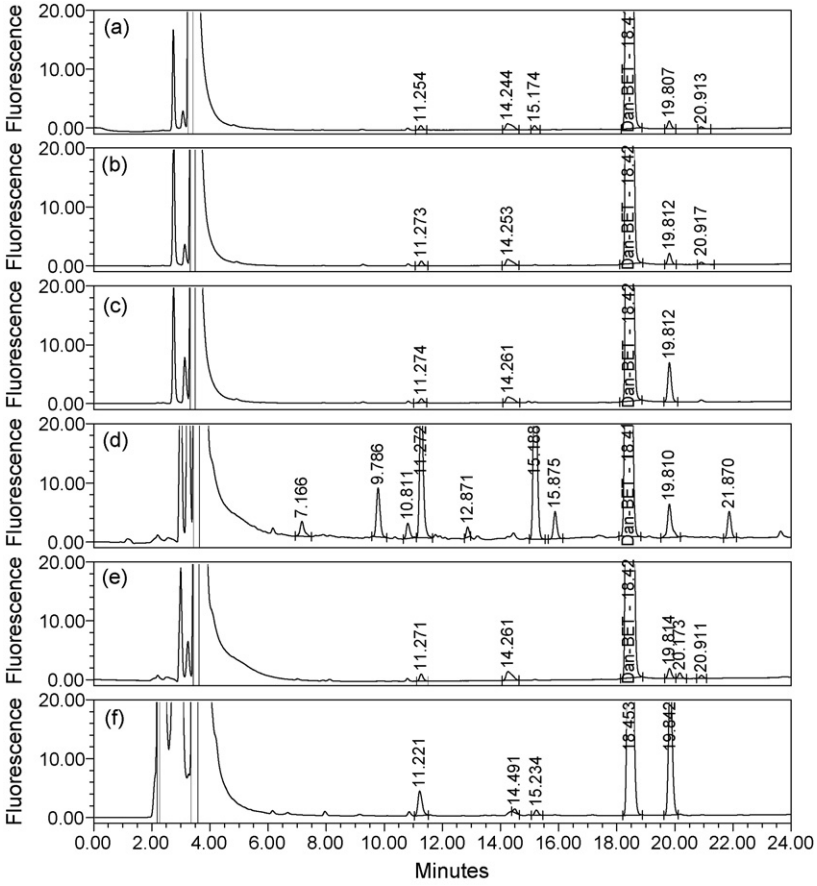

Fig. 3. Representative chromatograms of betahistine hydrochloride; heated in solid state [a], boiled in 1 mol L^{-1} NaOH [b], boiled with 1 mol L^{-1} HCl [c], exposed to UV light [d], extracted from Betaserc® tablets [e], and oxidized with H_2O_2 solution [f].

Table 1
Chromatographic parameters of betahistine and its photodecomposition products analyzed by HPLC (initial betahistine mass concentration 1.85 ng μL^{-1}, $n = 6$)[a]

Retention time (min)	Area	Mass concentration (ng μL^{-1})	%[b]	$W_{0.05}$ (min)	k	α	USP resolution	USP tailing	USP plate count
7.17	25,538	0.016	0.86	0.56	1.87			1.31	15,902
9.79	72,378	0.045	2.43	0.52	2.91	1.56	11.00	1.22	29,491
10.81	22,378	0.014	0.75	0.46	3.32	1.14	4.47	1.17	37,042
11.27	218,772	0.136	7.35	0.55	3.51	1.06	2.04	1.31	41,451
12.87	11,116	0.007	0.37	0.21	4.15	1.18	7.90	0.99	79,065
15.19	327,900	0.204	11.01	0.55	5.08	1.22	11.59	1.18	78,460
15.88	37,859	0.024	1.27	0.50	5.35	1.05	3.11	1.13	86,936
(Dan-BET) 18.41	2,165,868	1.346	72.74	0.76	6.37	1.19	10.36	1.01	80,514
19.81	58,224	0.036	1.96	0.66	6.92	1.09	5.44	1.15	112,723
21.87	37,693	0.023	1.27	0.44	7.75	1.12	8.78	1.09	151,129

[a] $W_{0.05}$ Peak width at 95% height from peak apex; k, retention factor; and α, selectivity coefficient.
[b] Percents of initial betahistine mass concentration.

Table 2
Amounts of BET and its degradation products generated at different stress conditions: percents of initial BET mass concentration

Condition	BET (peak at tR = 18.4 min)	Peak at tR = 11.2 min	Peak at tR = 15.2 min	Peak at tR = 19.8 min
Heat	99.25	0.11	0.09	0.20
NaOH, heat	99.33	0.11	0.00	0.25
HCl, heat	98.54	0.12	0.00	1.02
UV light	72.74	7.35	11.01	1.96
H_2O_2	84.62	2.35	0.00	12.33
Tablets[a]	99.38	0.11	0.00	0.15

[a] Marketed Betaserc® tablets (for purity testing).

Table 3
Chromatographic parameters of Betaserc® tablets analyzed by HPLC (initial betahistine mass concentration 5.8 ng μL^{-1}, $n = 6$)[a]

Retention time (min)	Area	Mass concentration (ng μL^{-1})	%[b]	$W_{0.05}$ (min)	k	α	USP resolution	USP tailing	USP plate count
11.27	9,909	0.006	0.11	0.39	3.51			1.19	42,554
14.26	22,399	0.014	0.24	0.57	4.70	1.34	8.77	1.53	14,793
(Dan-BET) 18.42	9,304,705	5.764	99.38	0.76	6.37	1.35	12.02	1.18	100,440
19.81	14,051	0.009	0.15	0.39	6.93	1.09	5.99	1.18	125,346
20.17	7,308	0.005	0.08	0.36	7.07	1.02	1.65	1.18	139,276
20.91	4,400	0.003	0.05	0.35	7.36	1.04	3.37	1.08	140,602

[a] $W_{0.05}$ Peak width at 95% height from peak apex; k, retention factor; and α, selectivity coefficient.
[b] Percents of initial betahistine mass concentration.

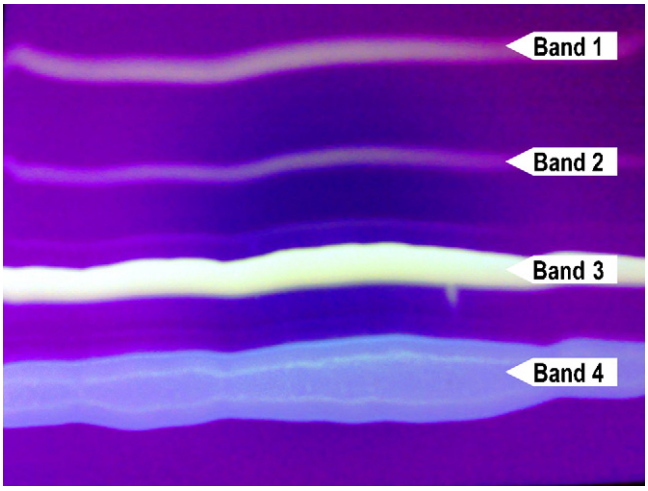

Fig. 4. Preparative-TLC of dansylated UV-degraded BET-2HCl (acetonitrile:CH_2Cl_2; 1:9, v/v), Photographed under UV-254 nm.

developed method was applied for the purity testing of commercial betahistine tablets. The percent of total degradation products was equal to 0.62%. Table 3 proved that Betaserc® tablets have been exposed to light, because of the peaks recorded at tR, 11.2 and 19.8 min.

3.9. Separation and identification of UV-degradation products

The dansylated products of UV-degraded BET were well separated by thin-layer chromatography (Fig. 4). All separated bands were scratched and extracted in acetonitrile for HPLC analysis. Band-1 and Band-2 were assigned to the major photodegradation products as confirmed by HPLC analysis (tR 11.2 and 15.2 min, respectively). However, Band-3 and Band-4 were assigned to Dan-BET and Dan-Cl, respectively. The spectral data and GC–MS analysis confirmed that Band-2 is pre-defined in BP 2005 and USP 29 [2,6] as impurity-A (identified as Dan-impurity-A). However, Band-1 is a new dansylated impurity and assigned as Dan-N-oxide of BET (Fig. 5). The ^{1}H NMR and MS data are presented as below.

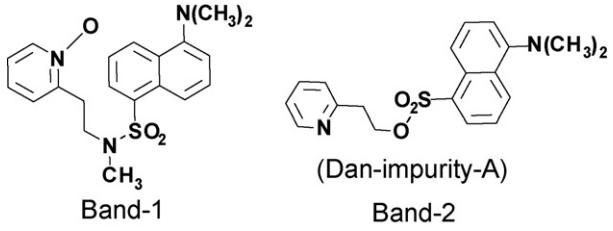

Fig. 5. Chemical structure of the identified two potential dansylated-photodegradation products.

3.9.1. Dansylated betahistine-N-oxide spectral analysis (Band-1)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.48 (s, 3 H, N-C*H*$_3$ betahistine), 2.83 (s, 6 H, N-(C*H*$_3$)$_2$, 2.81 (t, 2 H, C*H*$_2$ ethyl, J = 15.1 Hz), 3.58 (m, 2 H, CH_2-N), 6.76 (d, 1 H, Ar *H*, J = 17.2 Hz), 7.05 (m, 1H, ArH), 7.11–7.25 (m, 4 H, Ar *H*), 7.61–7.72 (m, 2 H, *Ar-H*), 8.01 (d, 1 H, *Ho*—SO_2, J = 15.1 Hz), 8.15 (d, 1 H, Ho-N-O, J = 18.5 Hz). MS; m/z 386.18 (M + H)$^+$.

3.9.2. O-Dansylated β-pyridin-2-yl-ethanol spectral analysis (Band-2, Dan-impurity-A)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.85 (s, 6 H, N-(C*H*$_3$)$_2$, 2.95 (t, 2 H, C*H*$_2$ ethyl, J = 12.7 Hz), 3.88 (m, 2 H, CH_2-O), 6.60 (d, 1 H, Ar *H*, J = 17.5 Hz), 7.17–7.29 (m, 4 H, Ar *H*), 7.66–7.75 (m, 2 H, *Ar-H*), 8.05–8.11 (m, 2 H, *ArH*), 8.65 (d, 1 H, CH═N, J = 17.8 Hz). MS; m/z 357.25 (M + H)$^+$.

3.9.3. Dansylated betahistine spectral analysis (Band-3)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.62 (s, 3 H, N-C*H*$_3$ betahistine), 2.88 (s, 6 H, N-(C*H*$_3$)$_2$, 3.05 (t, 2 H, C*H*$_2$ ethyl), 3.55 (m, 2 H, CH_2-N), 6.82 (m, 1 H, Ar *H*), 7.15–7.30 (m, 4 H, Ar *H*), 7.59–7.72 (m, 3 H, *Ar-H*), 8.17 (d, 1 H, *Ho*—SO_2, J = 12.0 Hz), 8.91 (d, 1 H, CH═N, J = 15.5 Hz). MS; m/z 370.38 (M + H)$^+$.

4. Conclusion

The developed method is enough sensitive and precise to analyze betahistine in bulk and tablet form. Betahistine is very sensitive to light and oxidation. Three potential degradation products were identified (as their dansyl derivative) one of them is defined as N-oxide of betahistine and the other one is officially pre-defined as impurity-A. The method is capable to test the tablet purity for stability study and estimation of expiration date.

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