Preliminary Impurity Profile Study of Desloratadine Used in Toxicological Studies

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Desloratadine is a potent and selective peripheral H_1 receptor antagonist, a major active metabolite of loratadine, used in treatment of allergic symptoms. The aim of this study was to identify and analyse the impurity profile of desloratadine used for quantitative determination in biological fluids by performant chromatographic methods. Materials and methods: Fourier transform infrared (FT-IR) spectrum of desloratadine was recorded using a Tensor 27 Opitcs FT-IR spectrophotometer. The method for related substances was performed using System HPLC Agilent 1100. Chromatographic profile was recorded using a ChemStation software. Validation parameters considered in order to assess preliminary method performance were linearity, precision, specificity. The study was able to verify the purity profile of desloratadine used in toxicological studies.

Keywords: desloratadine, FT-IR spectroscopy, HPLC

Desloratadine, 8 - chloro - 11 - (piperidin - 4 - ylidene) - 6, 11 - dihydro - 5H - benzo [5, 6] cyclohepta [1, 2 - b] pyridine, is a potent and selective peripheral H_1 receptor antagonist. It is a major active metabolite of loratadine used in treatment of allergic symptoms by blocking the histamine activity. Desloratadine is official for the first time in European Pharmacopoeia 8.0 [1]. It is official in British Pharmacopoeia (2) and United States Pharmacopoeia. European Pharmacopeia 8.0 describes infrared absorption spectrophotometry method for identification of desloratadine and liquid chromatography method for related substances test [1].

The aim of this study was to identify and analyse the impurity profile of desloratadine used for quantitative determination in biological fluids of its by performant chromatographic methods.

European Pharmacopoeia 8.0 describes liquid chromatography method. As of now, several analytical methods are available for determination of desloratadine levels in biological fluids. Wen J et al and Ponnuru VS et al reported a sensitive LC-MS/MS method for simultaneous determination of rupatadine and its metabolite desloratadine in human plasma and a LC-ESI-MS/MS method for quantification of desloratadine in human plasma, respectively [3, 4]. Vlase L et al developed a high-performance liquid chromatography with mass spectrometry detection for determination of loratadine and its active metabolite, desloratadine in human plasma [5].

Spectrophotometric [6, 7], HPLC (high performance liquid chromatography) [8, 9], gradient ion pair chromatography [10], stability indicating UPLC (ultra performance liquid chromatography) [11] and densitometric methods [12] were also developed and described for the determination of desloratadine in bulk and solid pharmaceutical formulation.

Experimental part

Instrumentation

Fourier transform infrared spectrum of desloratadine was recorded using a Tensor 27 Opitcs FT-IR (fourier transform infrared) spectrophotometer from Bruker, Germany with Spectral range 7500 – 370 cm^{-1} using KBr beamsplitter; resolution < 1cm^{-1} (apoptised), interferometer - ROCKSOLID™, permanently aligned, scan speed – 3 speeds, 2,2-20 KHz, detector – DigiTech, DATGS. The method for related substances was performed using System HPLC (High Performance Liquid Chromatography) Agilent 1100 equipped with a pump (400 barr), autosampler, diode array detector. Chromatographic profile was recorded using a ChemStation software.

Samples preparation

Samples were prepared according to European Pharmacopoeia: 2 mg of desloratadine were triturated in about 300-400 mg of potassium bromide, previously conditioned by heating at 200 °C for 24h. The mixture was pressed to form a disc at a maximum pressure of 10 MPa.

Degradation studies were performed in different stress media, using a solution of 1M hydrochloric acid, sodium hydroxide 1M, hydrogen peroxide 30% and for photodegradation it was used a UV lamp at 366 nm wavelength.

Prepared tablets were subject to FT-IR measurements. Recorded spectra were further processed to eliminate differences between the amplitudes of the baselines and to achieve normalization with respect to differences between the minimum and maximum of absorption. The tableting press was used for sample preparation and was provided by Specac-UK.

Sample were prepared in mobile phase. A concentration of 0.2 mg/mL was prepared for sample solution and a

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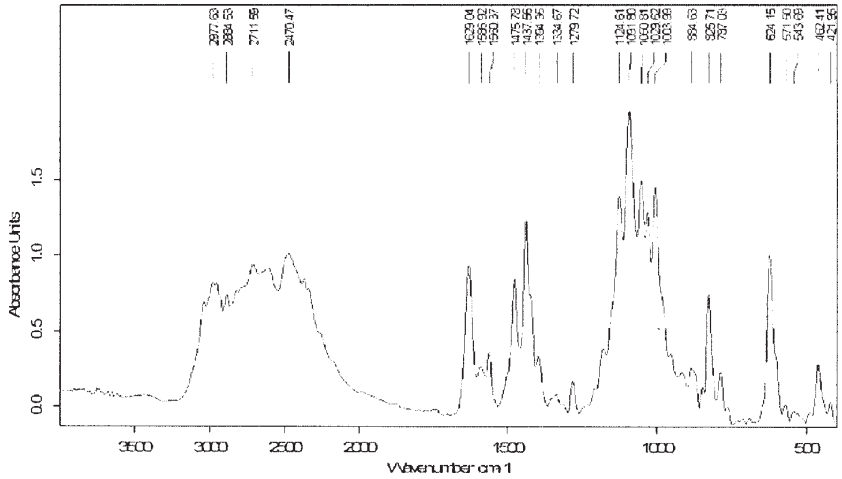

Fig. 1. FT-IR spectrum of desloratadine

reference solution of 0.1% of the sample solution was obtained by dilution of the first solution. Also, a solution of desloratadine for system suitability was injected.

Method validation

Method optimization employed several modifications. In this condition, some validation parameters were considered in order to assess preliminary method performance. Injection repetability was performed on the system suitability solution in order to assess the stability of the signals (area and retention time) for impurity A, impurity B and desloratadine. Linearity was monitored only for desloratadine in a range from the concentration of 0.3 µL towards the limit of quantification. Recovery was monitored using spiked samples of desloratadine in a pharmaceutical liquid solution in order to evaluate the influence of the placebo on the recovery and linearity of signals. Precision was evaluated using the same conditions from the repeatability and the confidence intervals were evaluated for desloratadine and known impurities. Method specificity was evaluated according to the ICH guideline using different degradation media as acidic, basic, UV and oxidation.

Results and discussions

Spectrophotometric analysis

By Fourier transform infrared spectroscopy we can identify the characteristic bands to pyrazine compounds such as the broad bands at 2470-2977 cm^{-1}, positions which depends on the adjacent hydrogen bonds. Also, the spectra shows bands at 1600-1400 cm^{-1}, which are dependent on the nature of the substituents and the vibration mode. A possible result is determined by the deformation vibration outside the plan of the C-H bond what determines the characteristic line at 787 cm^{-1}. Important is also the line at 624 cm^{-1} characteristic to the stretching vibration of the chlorine substituent (fig. 1).

Chromatographic analysis

Method optimization

Method optimization employed some modifications at the level of the mobile phase. Octadedcylsilyl C18 column – Agilent Zorbax Eclipse DB was used with: 250 mm length, 4.6 mm internal diameter, 5µm particle size, 180 mm /g surface area, carbon load 10%, 80Å° pore size. As reference was considered a method from USP for loratadine, the parent compound of desloratadine. A gradient elution was chosen in order to fulfill the performance of the method, relative retention of 0.8 for impurity A and 0.9 for impurity B relative to desloratadine chromatographic peak. Also, it was modified the type of ion pair agent and also the concentration was increased because of the shorter chain (sodium decansulfonate). The detection was maintained at the 280 nm because it is more selective and is corresponding to the absorption maxima of the UV signal. The method translation is presented in the table I.

Optimized method	European Pharmacopeea Method
❑ Mobile phase	❑ Mobile phase
S1: sodium decansulfonate (0,2%), pH=3	S1: sodium dodecylsulphate (0,086% m/V),
S2: acetonitrile	trifluoracetic acid 0.005% V/V
❑ Gradient elution	S2: acetonitrile
S1 S2	❑ Isocratic elution
0 75 25	S1 S2
45 30 70	0 57 43
50 75 25	45 57 43
❑ Temperature 40°C	❑ Temperature 35°C
❑ Flow 1,2 mL/min	❑ Flow 1,0 mL/min
❑ Detection: spectrophotometer at 280 nm	❑ Detection: spectrophotometer at 280 nm
Injection volume – 100 µL	Injection volume – 100 µL

Table 1
COMPARATIVE ANALYTICAL METHODS (OPTIMIZED METHOD / EUROPEAN PHARMACOPOEIA METHOD)

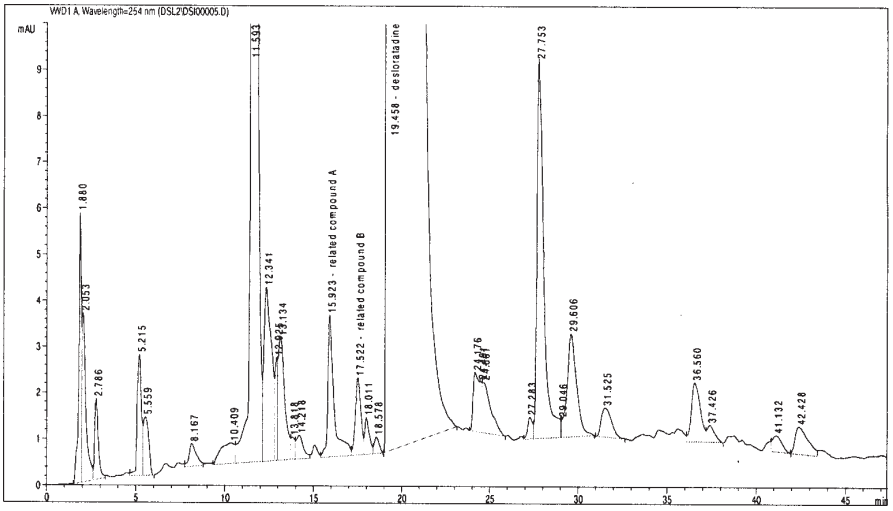

Fig. 2. System suitability chromatogram (contain desloratadine, desloratadine related compound A, desloratadine related compound B)

Method validation

Method validation evaluated the principal parameters. System compatibility and injection repeatability showed a good stability of the signals. For a series of 10 determinations the maximum value of relative standard deviation was of 1.98 for impurity A regarding the area and 0.105 for the peak of desloratadine for the retention time. For system suitability, important were the signal-to-noise and resolution parameters for the specified impurities. The values for signal-to-noise were of 14.2 for impurity A and 19.4 for impurity B and the resolution was 8.57 from impurity A to B and 1.23 from impurity B to desloratadine. The following chromatograms show the chromatographic profile for sample solution, reference solution and placebo (fig. 3-5).

Linearity was monitored from the concentration of 0.3 μg/mL for desloratadine and 0.05 μg/mL for 3 consecutive series. The correlation factor was of minimum 0.9913 and the overall standard error of the calibration curve was of 1.07.

Recovery was evaluated in the conditions of the method for three consecutive determinations from 0.3 to 0.1 μg/mL in triplicate and the maximum recovered concentration using the calibration curve was of 0.104μg/mL, corresponding to 104.14% for the lowest concentration. The highest value for percentual deviation was of 3.76% for the 0.3 μg/mL solution.

The precision of the method calculated the average values for desloratadine at the level of reference solution (0.2 μg/mL) and specified impurities and also the

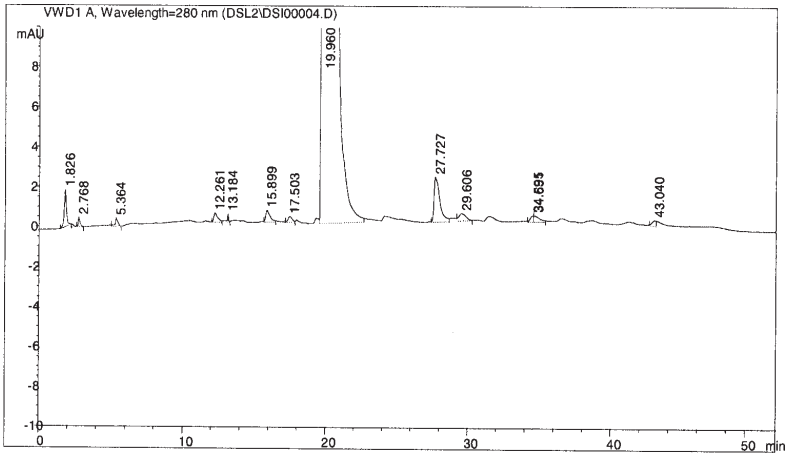

Fig. 3. Sample solution chromatogram

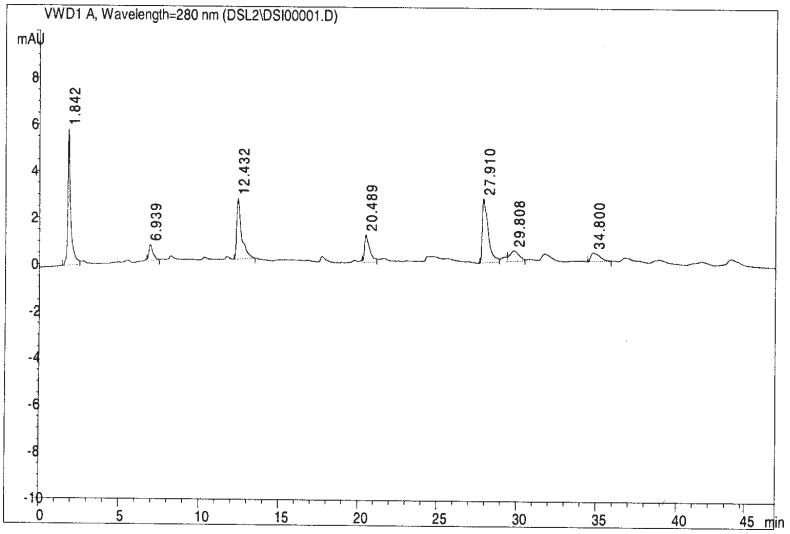

Fig. 4. Reference solution chromatogram (DSL – 20.49 min) and placebo solution

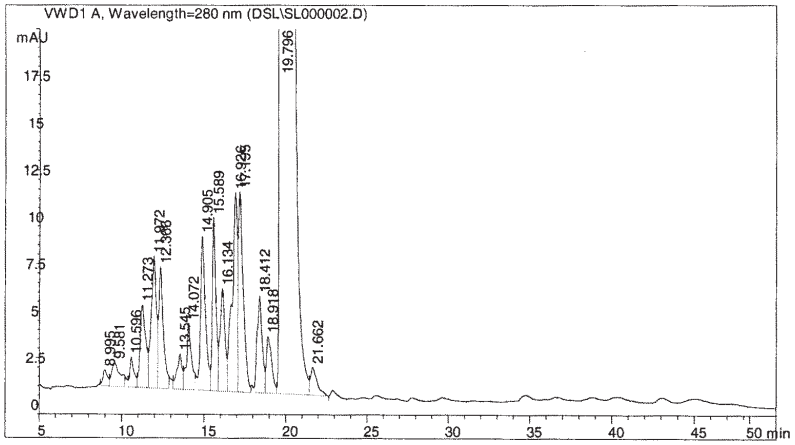

Fig. 5. Sample solution chromatogram (DSL – 19.7 min) in oxidative media

	Imp.1	Imp.2	Imp.3	Imp.4	Imp.5	Imp.6	Imp.A	Imp.7	Imp.8	Imp.B	Imp.9
Rt / *Area*	11.2	11.9	12.26	14.9	15.58	16.13	16.9	17.19	18.4	18.9	21.72
Acidic media										67.3	51.54
Oxidative media	81.2	130.2	93.18	127.8	111.7	86.99	210.8	199	99.63	53.7	
UV media											49.20

Table 2
AREA AND RETENTION TIME OF IMPURITIES

confidence level. In these conditions the confidence level (95%) was 0.37 for desloratadine, 0.05 for impurity B and 0.09 for impurity A.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated using the slope, stadandard error of the slope and the equivalence factors 3.3 for LLOD and 10 for LLOQ. Using the specified values, the LOD for desloratadine was of 0.02μg/mL and theLOQ was 0.067 μg/mL.

Method specificity was evaluated regarding the placebo corresponding to a liquid pharmaceutical formulation and also in relation with the degradation products resulting from the stress media. Up to 9 possible supplementary impurities were identified. Also, the impurities A and B were produced during the degradation studies. The area and retention times (Rt) are presented in the table 2.

Conclusions

The study was able to verify the purity profile of desloratadine used in toxicological studies. The identification using spectrophotometric method showed the characteristic bands of the molecule. Using the module for spectra identification we were able to determine the similarity match of minimum 99.9% against the standard used for the evaluation.

The method for related substances showed the absence of the related compounds A and B from the chromatogram corresponding to the test solution.

The validation of the method showed good linearity, stability and no matrix effect on the recovery and accuracy. The limit of quantification was lower than the disregard limit which in general is established at the half concentration of the reference solution, namely 0.05%.

The method specificity revealed a high instability of desloratadine regarding the oxidation media, but the presence of these compounds did not affect the performance of the method because in all the cases the purity of the desloratadine signal showed no interference. Specified impurities were revealed in the acidic and oxidative media. The chromatographic profile of the unknown impurities showed a level less than 0.1% from the concentration of reference solutions.

The method gave the same results in terms of the relative retention in respect with the requirements of the desloratatine monography from European Pharmacopoeia (impurity A- about 0.8, impurity B- about 0.9).

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