



Identification of photodegradation product of amisulpride by ultra-high-pressure liquid chromatography–DAD/ESI-quadrupole time-of-flight-mass spectrometry

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ARTICLE INFO

Article history:

Received 2 May 2011

Received in revised form 10 July 2011

Accepted 22 July 2011

Available online 30 July 2011

Keywords:

UHPLC

Q-TOF

Photodegradation

Amisulpride

UVA irradiation

ABSTRACT

Photostability of amisulpride under UVA irradiation in methanol solution was investigated and structural elucidation of its photodegradation products was performed. For the purpose of the quantitative and qualitative analysis of amisulpride and the stress degradation products elucidation, the reversed phase UHPLC–DAD system coupled with accurate mass hybrid ESI–Q–TOF mass spectrometer was used. During one run (10 min) with the use of auto MS/MS mode all essential data for the determination of photodegradation kinetics and for the structural formulas elucidation of the products was collected. Four degradation products were found and their masses with high accuracy (0.53–3.05 ppm) and formulas were obtained – 258.0666 ($C_{10}H_{14}N_2O_4S$), 367.1564 ($C_{17}H_{25}N_3O_4S$), 341.1412 ($C_{15}H_{23}N_3O_4S$) and 385.1665 ($C_{17}H_{27}N_3O_5S$). For all the analyzed compounds MS/MS fragmentation spectra were obtained (collision energy 19.8–26.1 V) allowing structural elucidation of unknown degradation products and indicating photodegradation pathways of amisulpride. UHPLC–DAD/ESI–Q–TOF system was found to be a powerful analytical tool for the fast and accurate stability analysis of pharmaceutical substances.

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1. Introduction

Amisulpride (4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxy-benzamide) is an atypical antipsychotic drug whose pharmacological activity is based on the selective binding to D2 and D3 dopaminergic receptors. This drug is characterized by a lower risk of extrapyramidal side-effects (EPS) and relatively better toleration than conventional antipsychotic drugs. Nowadays, amisulpride is first of all used for the treatment of different kinds of schizophrenia [1], as well as in a low dose it can be used for the treatment of depression patients [2,3]. The newest research informs that amisulpride can also be used in the therapy of chronic fatigue syndrome (CFS) [4].

Photostability testing is an integral part of the stability study of the drugs and today it must be considered during the development and registration process of pharmaceutical products [5–7]. Photodegradation of the drugs is a very important subject of investigation because this kind of a process can result in the loss of the activity of the drug and also in adverse effects due to the formation of toxic degradation products. The knowledge what exactly is formed from the drug during this process can be very useful for the manufacturing, storage and administration of phar-

maceuticals and may significantly improve the safety of therapy [8,9].

In literature data there are many papers concerning the analysis of amisulpride in biological materials [10–23] and pharmaceuticals [23,24] with the use of various methods: LC [11–20,24], TLC [24], CE [24], spectrophotometric [24], MS [21,22], RIA [10] and voltametric [23]. Nevertheless, there is no paper describing the stability study including the photostability of amisulpride and also the ultra-high-pressure liquid chromatography method and hybrid Q-TOF mass spectrometer were not used for the analysis of this drug so far.

Taking into account the above fact and our previous work [24] where it was observed during the method validation process that amisulpride is very labile under UV 254 nm radiation (UVC), that is necessary to carry out the photostability study of this substance. Taking into consideration the second fact that most of the published papers concerning the photostability investigation of the drugs required using at least a few analytical methods (LC, GC, MS, spectrophotometry, IR, etc.) [25–27] simultaneously in order to obtain satisfactory results, the aim of this work was to develop a simple, fast and accurate method enabling the whole photostability analysis during one run. For this purpose a new analytical method with the use of new generation hybrid MS/MS spectrometer combined with UHPLC–DAD including powerful auto MS/MS functions and algorithms was developed.

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2. Experimental

2.1. Materials

Amisulpride was kindly supplied by Synthelabo Groupe, Quetigny, France. Methanol hypergrade for LC–MS and 98% formic acid p.a. were purchased from Merck (Darmstadt, Germany). Water for GC and LC was obtained from Honeywell Burdick & Jackson (Muskegon, USA).

Amisulpride solution for photodegradation tests (at concentration 2.4×10^{-5} M) was prepared in hypergrade methanol.

2.2. Photodegradation conditions

Amisulpride methanolic solution was placed in quartz capped cell ($l = 1$ cm) and irradiated with UVA radiation. As a UVA source Haland HA-05 (Warsaw, Poland) ultraviolet laboratory lamp equipped with Philips TL 8W BLB tube emitting radiation at 365 nm (1.3 W of UVA radiation) was used. The distance between the UVA lamp and the sample was 10 cm and the temperature in the chamber was controlled and kept below 26 °C. The UVA intensity was controlled with MKVI Magnaflux radiometer (Athens, Greece) and the average irradiation intensity was 7 W/m². The dark control sample was used by exposing the amisulpride sample in quartz cell wrapped in aluminium foil for the same period of time.

2.3. UHPLC–DAD/ESI–Q–TOF–MS analysis

LC–MS/MS analysis was performed with the use of Agilent Accurate–Mass Q–TOF LC/MS G6520B system with dual electrospray (DESI) source and Infinity 1290 ultra-high-pressure liquid chromatography system consisting of: binary pump G4220A, FC/ALS thermostat G1330B, autosampler G4226A, DAD detector G4212A, TCC G1316C module and Zorbax Eclipse–C18 (2.1 mm \times 50 mm, dp = 1.8 μ m) Rapid Resolution HD column (Agilent Technologies, Santa Clara, USA). A mixture of methanol (A) and water (B) with addition of 0.1% solution of formic acid in both media was used as a mobile phase. The gradient elution was carried out at constant flow 0.4 ml/min from 12%A (88%B) 0–3 min and then 12%A to 20%A 3–10 min. 1 min post time (12%A) was performed to return to initial conditions. The injection volume was 1.5 μ l and the column temperature was maintained at 25 °C. MassHunter workstation software in version B.04.00 was used for the control of the system, data acquisition, qualitative and quantitative analysis.

The optimization of the instrument conditions was started with the proper tuning of Q–TOF detector in a positive mode with the use of Agilent ESI–L tuning mix in high resolution mode (4 GHz). Next, the main parameters were optimized and the following settings were applied: gas temp.: 300 °C, drying gas: 10 l/min, nebulizer pressure: 40 psig, capillary voltage: 3000 V, fragmentor voltage: 200 V, skimmer voltage: 65 V, octopole 1 RF voltage: 250 V.

In order to make the qualitative and quantitative analysis in one run, data acquisition was performed in auto MS/MS mode with spectral parameters: mass range: 50–950 m/z and acquisition rate: 1.40 spectra/s (for MS and MS/MS data). In this mode the maximum data for structure elucidation was collected, and it was not necessary to repeat the analysis in different modes. Collision energy was also calculated with auto algorithm with formula: 5 V (slope) \times (m/z)/100 + 6.8 V (offset) and in this case it ranged: 19.8 V–26.1 V. Maximum 2 precursors per cycle were selected with an active exclusion mode after 1 spectra for 0.1 min.

To ensure accuracy in masses measurements, reference mass correction was used. Mass 121.0508 and 922.0097 were used as lock masses.

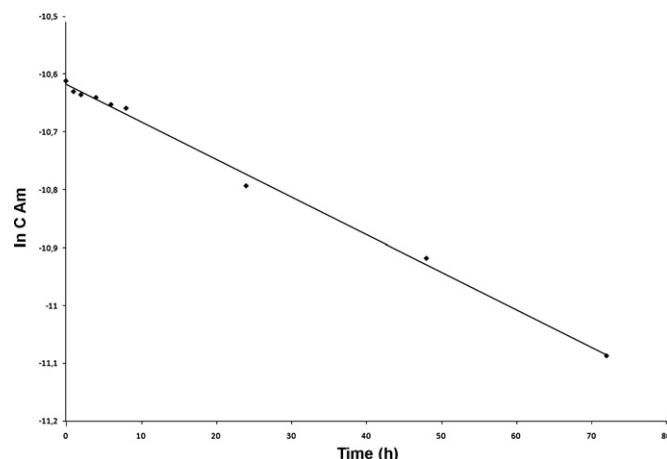


Fig. 1. First-order kinetics of the photodegradation of amisulpride in methanol solution.

Diode array detector collected data in the range 200–400 nm, and 270 nm was selected for the quantitative analysis of amisulpride.

2.4. Quantitative analysis and photodegradation kinetics

The method calibration for the determination of the concentration of amisulpride in tested samples was performed with the use of DAD detection. Calibration curve was obtained by plotting the peak area against the amount of the drug in the range: 5.4×10^{-7} to 2.7×10^{-5} M and studied by fitting the results to linear least-squares regression. All calibration standards were analyzed five times and the average calibration curve with statistic parameters was calculated.

The obtained calibration curve was used for the determination of photodegradation kinetics of amisulpride in methanol solution. During the irradiation procedure 100 μ l of the tested solution was collected and analyzed by UHPLC–DAD/ESI–Q–TOF–MS system in the time: 0, 1, 2, 4, 6, 8, 24, 48 and 72 h of exposing to UVA radiation. The photodegradation kinetics parameters: rate constant (k) and $t_{1/2}$ were calculated with the use of equation:

$$\ln c = \ln c_0 - kt,$$

where c_0 is the concentration in the time 0, c is the remaining concentration, k the rate constant (h^{-1}) and t is the time (h).

The dark control sample concentration was measured in the same time period.

3. Results and discussion

3.1. Quantitative study of the photodegradation process

The calibration of the quantitative analysis method for the determination of amisulpride was performed on DAD detector at 270 nm maximum band in the range: 5.4×10^{-7} to 2.7×10^{-5} M. The obtained calibration curve: $y = 2.92 \times 10^6 (\pm 6185)x - 0.9681 (\pm 0.0616)$ was linear over the concentration range ($r = 0.9999$) and the limits of detection (LOD) and quantification (LOQ) were 1.4×10^{-7} M and 4.1×10^{-7} M respectively. The obtained results were used to calculate the concentration of amisulpride at proper time intervals during UVA irradiation. As shown in Fig. 1 the decomposition of amisulpride followed apparent first-order kinetics reaction according to the equation: $\ln c = \ln c_0 - kt$. The rate constant value, correlation coefficient and the half-life time of photodegradation process were respectively: $k = 6.5 \times 10^{-3} \text{ h}^{-1}$, $r = 0.9983$, $t_{1/2} = 103.1$ h.

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