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Identification of the specified impurities of silver sulfadiazine using a screening of degradation products in different stress physico-chemical media

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ABSTRACT

Determination of silver sulfadiazine degradation products in several stress media was carried out by high pressure liquid chromatography (HPLC) with diode array detector (DAD) and hybrid mass spectrometer triple quadrupole-linear trap. The optimal chromatographic method used a Hypercarb column with a stationary phase 100% carbon, a mobile phase composed by a mixture 45:55 formic acid 1% solution and acetonitrile and detection at 275 nm. Structure elucidation was carried out on the mass spectrometry system using same chromatographic conditions and based on MS/MS techniques. Under these conditions up to 9 possible impurities were demonstrated to be degradation products respecting silver sulfadiazine evolution under different stress conditions: temperature, acid, basic, oxidation, reduction and catalyzed photodegradation. Sulfacetamide, sulfanilic acid (4-aminobenzenesulfonic acid), aniline, pyrimidin-2-amine, 4-aminobenzenesulfonamide, 4-methylidenesulfanilaniline, 4-aminophenol, 4-amino-n-methyl benzenesulfonamide and benzenesulfonic acid were identified by mass spectrometry in order to cover the possible degradation paths of silver sulfadiazine. Kinetics were also evaluated to obtain the prediction of shelf life of the substance. The linearity domain for the method was between 0.0005 mg/ml and 0.25 mg/ml for each compound. Recovery factors in accuracy determination were between 95 and 105% relative to target concentrations of silver sulfadiazine and the quantitation limit was 0.00025 mg/ml.

1. Introduction

Silver sulfadiazine is a sulfonamide compound used for the treatment and prevention of infections caused by severe burns and other skin disorders such as leg ulcers and for prophylaxis of infection in skin tattooing. Silver sulfadiazine, unlike sulfadiazine has a broader antimicrobial activity against gram positive, gram negative bacteria and some yeast, because of the fact that silver salts have a higher influence on the cell membrane and cell wall [1]. Under these conditions, there was reported a quite small systemic absorption, only a concentration of 10–20 micrograms/ml being achieved. The quantity could rise if the treated area is increased and the time of application is prolonged [2].

Noda et al. [3] showed that the type of excipients used in pharmaceutical formulations and the conditions of formulation are essential for the therapeutic efficiency. Also, can predict possible reactions which employs nucleophilic interactions with electrophilic excipients or for interactions between electrophilic substances (carboxylic acid derivates, esters, amides, halogens) and nucleophilic excipients.

According to the ICH Q3A guideline [4], different type of media can be applied in order to synthesize and simulate the rate of the degradation of active pharmaceutical substance such as basic and acidic, oxidation and reduction media, photo-degradation or temperature [5].

In the most topical formulations, silver sulfadiazine is dispersed, but the presence of different substances with specific character produce the redistribution of silver ions, so the problem of the study of the behavior in different stress media is reduced to the monitorization of the sulfadiazine.

Recent scientific studies on sulfadiazine and sulfonamides are concerned on the determination of the active pharmaceutical ingredients (APIs) and degradation products from watersamples [6–11], elimination from soil [12], food [13] and biological samples [14]. For better elimination of sulfonamides derivates from different matrixes it was concluded that there is a special need to involve some catalyzers for speeding up the reactions.

Presence of metal ions could create the conditions of acceleration of the instability of a certain substance based exactly on the

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catalization properties. Metal salts as $FeCl_3$, $FeNO_3$were used for the elimination of some sulfonamides derivates from wastewater; the concentration of the ions was about just 10 mM and the reaction was performed on exposure to ultraviolet irradiation. Also in these cases compounds like titanium dioxide were used for complementary of reaction [15]. Using titaniumas excipient should be with high concern on the photo instable drugs based on its properties to produce hydrolysis reactions, similar with basic or acidic media [16]. Temperature and temperature/humidity are used in stability studies of pharmaceutical sunder accelerated conditions (e.g. 40 °C and 80%) for shelf life prediction of the active pharmaceutical ingredient in formulated products.

In all these previously mentioned studies there were used hyphenated techniques such as high performance liquid chromatography coupled with triple quadrupole or high resolution mass spectrometry in order to determine the concentration of the analytes and identification of the degradation products. Most of the methods were performed on reversed phase chromatographic columns using as mobile phase mixtures of acetonitrile and solutions of formic acid, an organic pH modifier compatible with mass spectrometry technology. Some degradation products such as sulfanilic acid, aniline, sulfacetamide, benzenesulfonamide, benzenesulfonic acid were identified [16].

The scope of the present study was to identify the impurities and degradation products of silver sulfadiazine under advanced stress conditions in order to identify the degradation products and their structure elucidation with an ion trap mass spectrometer, as well as establishing the best selectivity of the method by experimental design methodology using a high efficient chromatographic column suitable for conventional high performance liquid chromatography but also for ultrahigh pressure chromatography.

2. Experimental

2.1. Materials and reagents

All chemicals were of analytical-reagent grade or better. All solutions and dilutions were prepared with ultrapure water from a Milli-Q Plus water purification system (Millipore, Billerica, MA, USA). Acetonitrile-HPLC grade, formic acid, triethylamine, sodium acetate, nitric acid (65% purity), hydrochloride acid (37% purity) and sodium hydroxide, hydrogen peroxide (35%), ammonia (35%), formaldehyde (37%) and iron (III) nitratedecahydratewere supplied by Merck KGaA, (Germany). Silver sulfadiazine (SSD) was obtained from BioMolekula(Germany). Methyl p-hidroxibenzoate 99.3% (NPG) and propyl p-hydroxybenzoate 99.5% (NPS) were supplied by Sigma-Aldich Chimica (Romania).

Standard 5 mg mL^{-1} stock solutions of SSD used for forced degradation studies were prepared by dissolving 50 mg SSD to 10 mL of each degradation media. Solutions of 0.25 mg $mL^{-1}$and 1 μg mL^{-1} prepared by adequate dilution of standard stock solution with mobile phase (formic acid 1%: acetonitrile 45:55 %v/v) were used for HPLC-DAD and HPLC–MS analyses, respectively. All solutions were filtered through nylon 0.45 μm filter previously to HPLC injection.

2.2. Equipment and experimental conditions.

Analytical profiling of the degradation products of SSD was performed on an Agilent 1100 series equipped with a photodiode array UV–vis (DAD) and fluorescence detectors in series mode. Temperature was maintained using column thermostat at 25 °C, isocratic elution was performed on a quaternary pump at 0.2 mL/min and 10 μL were injected using a manual Rheodyne injector. The method was applied on a 3 μm particle size Hypercarb™Thermo Fisher chromatographic column (100 mm length × 2.1 mm i.d.). Mobile phase consisted of a mixture of acetonitrile and 1% formic acid solution (55:45, v/v) that was filtered (0.45 μm) and degassed before use.

Mass spectrometry analyses were carried on a UPLC Agilent 1290 system coupled with a triple quadrupole linear ion trap hybridQtrap3200 MS system from ABSCIEX, USA. Same chromatographic conditions were used. The mass spectrometer operated using electrospray interface system with Turbo V™ source with TurboIonSpray® probe (300 °C) in positive polarity mode with a mass/charge (m/z) ratio in the range of 50–300 m/z. The curtain gas was set at 20 (relative units). Spray voltage (IS) was set at 5.5 kV. Three different types of scanning modes (EMS+, ER+ and EPI+) were considered for the elucidation of the intermediate products chemical structure considering the precursors and the characteristics of the product ions. For EPI+ experiments a declustering potential (DP) of 50 V and a collision energy (CE) of 35 V were applied.

2.3. Stress conditions assays.

Forced degradation studies were performed under stress conditions according to the ICH Topic Q1A (R2) Stability Testing of New Drug Substances and Products [17] and the limit of the degradation products were established according with the daily intake at maximum 1% without any information about toxicological testing [5]. The following assays were performed:

1. Acid–basic hydrolysis degradation study was performed using SSD solutions in nitric acid or sodium hydroxide media at a concentration interval ranged between 1 and 5 M. Obviously, the use of hydrochloric acid was not considered due to the low solubility of silver chloride salts. Solutions were neutralized with nitric acid or sodium hydroxide before chromatographic analysis.
2. Oxidation–reduction media assay was performed exposing SSD solutions to a reduction media obtained with formaldehyde of a concentration of 35% (v/v). Oxidation media was obtained with hydrogen peroxide with a concentration of 20% (v/v).
3. Temperature stability test were performed by exposing a SSD solution to a temperature interval between 40 °C and 100 °C. Additionally, the dry substance was exposed to a temperature variation between 40 °C and 140 °C to check if there are relevant differences on the stability of the molecule.
4. Temperature/humidity test monitorization was performed on dry substance and solution by considering 80% humidity and a temperature variation between 40 °C and 100 °C.
5. Ultraviolet (UV) and visible (vis) light exposure under room conditions (23 °C) test was performed on SSD solution. UV and vis catalyzed test were performed according to the ICH Q1B [18] guideline using a radiant exposure flow with a spectral range of 320 nm and an intensity of 200 W h/m^2.

Aqueous 5 mg mL^{-1} SSD solutions were submitted to the different stress conditions test. A volume of 0.5 mL was considered for each sampling time and diluted to 10 mL with mobile phase. Monitorization was performed using a solution of 50 mg SSD dissolved in 1 mL ammonia (25%) and 9 mL of each degradation media except for acid hydrolysis degradation and catalyzed photodegradation studies where 50 mg SSD were dissolved in 1 mL nitric acid (1 M) and 9 mL of each degradation media. Every degradation study was monitored by a total time interval of 10 h. In every case, blank solutions were prepared according to the sample protocol previously described and injected.