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Short communication

Stress degradation studies on ezetimibe and development of a validated stability-indicating HPLC assay

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Abstract

Ezetimibe was subjected to different ICH prescribed stress conditions. Degradation was found to occur in hydrolytic and to some extent in photolytic conditions, while the drug was stable to oxidative and thermal stress. The drug was particularly labile under neutral and alkaline hydrolytic conditions. A stability-indicating HPLC method was developed for analysis of the drug in the presence of the degradation products. It involved a C-8 column and a mobile phase composed of ammonium acetate buffer (0.02 M, pH adjusted to 7.0 with ammonium hydroxide) and acetonitrile, which was pushed through the column in a gradient mode. The detection was carried out at 250 nm. The method was validated for linearity, range, precision, accuracy, specificity, selectivity and intermediate precision.

Keywords: Ezetimibe; Degradation; Stress testing; Stability-indicating; Assay; Validation

1. Introduction

The parent drug stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) [1] suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and they should be fully validated.

Accordingly, the aims of the present study were to establish inherent stability of ezetimibe through stress studies under a variety of ICH recommended test conditions [1,2], and to develop a stability-indicating assay [3]. The drug is chemically 1-(4-flurophenyl)-(3*R*)-[3-(4-flourophenyl)-(3*S*)-hydroxypropyl]-4*S*-(4-hydroxyphenyl)-2-azetidinone (Fig. 1). It is a white, crystalline powder that is freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water. It melts at about 163 °C and is reported to be stable at ambient temperature [4]. It is one of the first new classes of lipid-lowering compounds that selectively inhibit the intestinal absorption of cholesterol and related phytosterols, and is generally prescribed as an oral tablet containing 10 mg drug.

In literature, analytical methods reported for this drug include quantization of ezetimibe–glucuronide complex by LC-MS [5] and a reversed-phase HPLC method for determination of the drug in pharmaceutical dosage forms [6]. The latter report includes limited stress testing, in which a single product was indicated to be formed under alkali conditions. More intensive stress studies in our laboratory instead showed that the drug was decomposed to almost seven products under different stress conditions. Accordingly, a stability-indicating method was developed, which could separate various degradation products.

2. Experimental

2.1. Materials

Ezetimibe was supplied by Ind-Swift Laboratories Limited, Bhagwanpur, India and used as such. Acetonitrile (HPLC grade) was purchased from J.T Baker (Xalostoc, Mexico). The other

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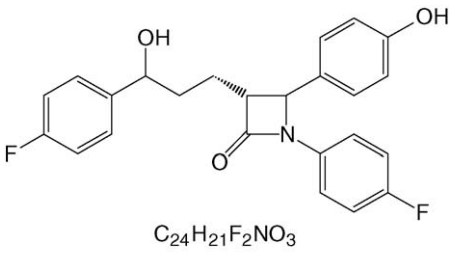

$C_{24}H_{21}F_2NO_3$

Mol. Wt.: 409.43

Fig. 1. Structure of ezetimibe.

chemicals and solvents used in the studies were of analytical grade. Ultra-pure water, obtained from an ELGA (Bucks, UK) water purification unit, was used in making solutions.

2.2. Instrumentation

Precision water baths equipped with MV controller (Julabo, Seelbach, Germany) were used for hydrolytic studies. Stability studies were carried out in humidity chamber (KBF 760, WTB Binder, Tuttlingen, Germany) and photo stability studies were carried out under the sunlight. Sunlight intensity during the studies was tested using a lux meter (ELM 201, Escon, New Delhi, India). Thermal stability studies were performed in a dry air oven (NSW Limited, New Delhi, India). The HPLC system consisted of an on-line degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10AL$_{VP}$), solvent delivery module (LC-10AT$_{VP}$), auto injector (SIL-10AD$_{VP}$), column oven (CTO-10AS$_{VP}$), UV-visible dual-wavelength detector (SPD-10A$_{VP}$), system controller (SCL-10A$_{VP}$) and CLASS-VP software (all from Shimadzu, Kyoto, Japan). The chromatographic separations were carried out on a Merck (Lichrospher, Darmstadt, Germany) C-8 column of 250 mm × 4.0 mm i.d. with particle size of 5 μm. Additionally, intermediate precision studies were also carried out on Inertsil (GL Sciences, Tokyo, Japan) C-8 column (250 mm × 4.6 mm i.d., particle size 5 μm).

2.3. Degradation studies

All stress decomposition studies were performed at an initial drug concentration of 0.5 mg ml^{-1} in water containing 30% acetonitrile. Acid hydrolysis was performed in 0.1 and 1 M HCl at 80 °C for 8 h. The study in alkaline condition was carried out in 0.1 M NaOH at 80 °C for 8 h. These were repeated at lower temperature of 40 °C keeping all the other conditions constant. For study in neutral condition, drug dissolved in water was heated at 80 °C for 8 h. Oxidative studies were carried out at room temperature in 3 and 20% hydrogen peroxide for 24 h. Photo-degradation studies were performed in water and in 1 M HCl. The solutions were exposed to sunlight during the daytime (60,000–70,000 lux) for 2 d. Suitable controls were kept under the dark. Additionally, the drug powder was exposed to dry heat at 50 °C for 45 d and at 60 °C for 7 d. Samples were withdrawn at appropriate time and subjected to HPLC analysis after suitable dilution.

2.4. Separation studies

HPLC studies were carried out first on all reaction solutions individually, and then on a mixture of those solutions in which decomposition was observed.

Separations were achieved by gradient elution using buffer (0.02 M ammonium acetate, pH adjusted to 7.0 with ammonium hydroxide)-acetonitrile as the mobile phase. Organic modifier was increased linearly from 30 to 100% in 80 min and brought back to initial in next 10 min. The mobile phase was filtered through 0.45 μm nylon membrane and degassed before use. The injection volume was 20 μl and the mobile phase flow rate was kept constant at 1 ml min^{-1}. The analyses were carried out at 250 nm.

2.5. Validation of the method

2.5.1. Linearity and range

A stock solution of the drug was prepared at strength of 1 mg ml^{-1}. It was diluted to prepare solutions containing 5–500 μg ml^{-1} of the drug. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20 μl)

2.5.2. Precision

Six injections, of three different concentrations (25, 100 and 500 μg ml^{-1}), were given on the same day and the values of relative standard deviation (R.S.D.) were calculated to determine intra-day precision. These studies were also repeated on different days to determine inter-day precision.

2.5.3. Accuracy

Accuracy was evaluated by fortifying a mixture of degraded solutions with four known concentrations of the drug. The recovery of the added drug was determined.

2.5.4. Specificity and selectivity

The specificity of the method was established through study of resolution factors of the drug peak from the nearest resolving peak, and also among all other peaks.

2.5.5. Intermediate precision

The intermediate precision was established through a study on a different chromatographic system using a different column.

3. Results and discussion

3.1. Degradation behaviour

HPLC studies on ezetimibe under different stress conditions suggested the following degradation behaviour:

3.1.1. Acidic condition

The drug gradually decreased with time on heating at 80 °C in 1 M HCl, forming degradation products at RRT 0.48 and 1.14. The rate of hydrolysis in acid was slower as compared to that of alkali or water.

3.1.2. Neutral (water) condition

Upon heating the drug solution in water at 80 °C for 1 h, steep fall in the drug peak area was observed. At the end of 1 h, almost complete degradation of the drug was observed with the corresponding rise in the major degradation peak at RRT 1.14.

3.1.3. Degradation in alkali

The drug was found to be highly labile to alkaline hydrolysis. The reaction in 0.1 M NaOH at 80 °C was so fast that whole of the drug was degraded in 0 min. Subsequently, studies were performed in 0.01 M NaOH at 40 °C. Drug degradation was associated with rise in a major degradation product at RRT 1.14. Complete degradation of the drug was observed in 4 h. Minor degradation products at RRT 0.48 and 1.88 emerged after 4 h.

3.1.4. Oxidative conditions

The drug was stable to hydrogen peroxide (3 and 20%) at room temperature.

3.1.5. Photolytic conditions

No major degradation product was observed after exposure of drug solution in 1 M HCl to sunlight for 2 d, only minor degradation products at RRT 0.48, 1.09, 1.15 and 1.88 were formed. The nature of degradation in light and dark was found to be similar, indicating that light had no effect on the degradation of the drug in acid. On the other hand, the samples in water degraded under sunlight for 2 d to a major product at RRT 1.14, along with minor degradation products at RRT 0.48, 0.61, 0.87 and 1.60. Corresponding rate of degradation in dark was much slower.

3.1.6. Solid-state study

The solid-state studies showed that ezetimibe was stable to the effect of temperature. When the drug powder was exposed to dry heat at 50 °C for 45 d and at 60 °C for 7 d, no decomposition of the drug was seen.

3.2. Development and optimization of the stability-indicating method

The method was optimized to separate major degradation products formed under various conditions. Resolution was also checked on mixture of the degradation solutions to confirm the separation behavior. The resulting chromatogram is shown in Fig. 2. It indicates that the gradient method was successful in separation of drug and all chromophoric degradation products.

It may be pertinent to add here that the product V at RRT 1.14 (Fig. 2) formed almost as a single compound in water was isolated and characterized as (2*R**,3*R**,6*S**)-*N*,6-bis(4-fluorophenyl)-2-(4-hydroxyphenyl)-3,4,5,6-tetrahydro-2*H*-pyran-3-carboxamid through crystallographic studies [7].

3.3. Validation of developed stability-indicating method

The response for the drug was strictly linear in the concentration range between 5 and 500 μg ml^{-1}. The mean (± %R.S.D.) values of slope, intercept and correlation coefficient were 48467 (±1.123), 123107 (±1.745) and 0.9997 (±0.015), respectively.

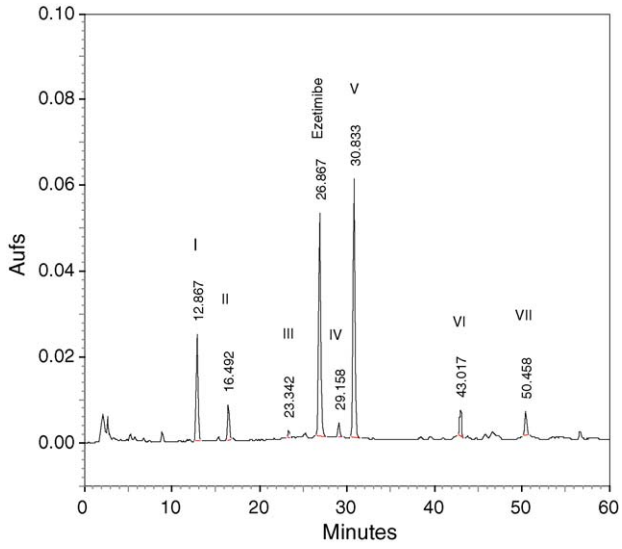

Fig. 2. Chromatograms showing separation of different degradation products of ezetimibe contained in a mixture of reaction solutions. Key: I, formed in all types of hydrolytic and photolytic conditions; II, formed in water under dark and photolytic conditions; III, photolytic product in water; IV, photolytic product in acid; V, formed in all types of hydrolytic and photolytic conditions; VI, photolytic product in water; VII, hydrolytic degradation product in alkali and photolytic product in acid.

The data obtained from precision experiments are given in Table 1 for intra-and inter-day precision studies. The %R.S.D. values for intra-day precision study were <1.0% and for inter-day study were <2.0%, confirming that the method was sufficiently precise.

Percentage recovery was calculated from differences between the peak areas obtained for fortified and unfortified solutions. As shown from the data in Table 2, excellent recoveries were made at each added concentration.

Table 1
Reproducibility and precision data evaluated through intra-day and inter-day studies

Actual concentration (μg ml^{-1})	Intra-day measured concentration (μg ml^{-1}) ± S.D.; R.S.D.% (n = 6)	Inter-day measured concentration (μg ml^{-1}) ± S.D.; R.S.D.% (n = 3)
25	27.132 ± 0.271; 0.997	26.738 ± 0.088; 0.331
100	95.843 ± 0.737; 0.769	95.745 ± 0.813; 0.850
500	492.975 ± 2.353; 0.477	494.326 ± 5.068; 1.025

Table 2
Recovery studies

Actual concentration (μg ml^{-1})	Calculated concentration (μg ml^{-1}) ± S.D.; R.S.D.% (n = 3)	Recovery (%)
50	50.403 ± 0.372; 0.738	100.81
100	103.851 ± 0.477; 0.459	103.85
250	244.444 ± 2.005; 0.820	97.78
500	496.968 ± 7.636; 1.537	99.39

Fig. 2 shows that the method was sufficiently specific to the drug. The resolution factor for the drug peak was >3 from the nearest resolving peak.

Intermediate precision was performed to confirm that separation was satisfactory under conditions external to the method. Good separations were always achieved, indicating that the method remained selective for all components under the tested conditions.

4. Conclusions

The study shows that ezetimibe is a labile molecule in water and alkali, and also shows lability in water under light conditions. It is stable to oxidation and dry heat. A stability-indicating method was developed, which separates all the degradation products formed under variety of conditions. The method proved to be simple, accurate, precise, specific and selective. Hence it is recommended for analysis of the drug and degradation products in stability samples by the industry.

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