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High performance liquid chromatographic method for determination of ezetimibe in pharmaceutical formulation tablets

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Abstract

Ezetimide belongs to a class of lipid lowering compounds that selectively inhibits intestinal absorption of cholesterol and related phytosterols. The purpose of this study is to establish a reliable and quick method for the assignment of ezetimibe in tablets form by high performance liquid chromatography with ultraviolet detection (HPLC-UV). A rapid and sensitive HPLC method has been developed for determination of ezetimibe in tablets formulation. Mobile phase was composed of acetonitrile-ammonium acetate (10 mM, pH 3.0), 75:25 (v/v) with a flow rate of 1 ml/min. The eluted peaks were detected by a UV detector was set at wavelength of 240 nm. The method results in excellent separation with good resolution of analyte. Standard curves were linear (r = 0.996) over the wide ezetimibe concentration range of 10-60.0 µg mL^{-1} with acceptable accuracy and precision. The limits of detection (LOD) and quantitation (LOQ) of the method were 5 and 10 µg/ml, respectively. The average drug recovery was 95.3% throughout the linear concentration range. Statistical assessment of various in vitro dissolution parameters and assay results was also conducted to establish if there were any significant difference among them. The validated HPLC method has been used successfully to study ezetimibe. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

Keywords: Ezetimibe, HPLC, assay, dissolution, tablets

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Introduction

Ezetimibe (Fig. 1) is an anti hyperlipidemic and is usually categorized as HMG CoA reductase inhibitor (1-2). Ezetimibe belongs to a class of lipid lowering compounds that selectively inhibits intestinal absorption of cholesterol and related phytosterols .It potentially inhibits the transport of cholesterol across the intestinal walls there by reducing plasma cholesterol (3). Ezetimibe co-administered with HMG CoA reductase inhibitors is licensed for the treatment of primary hypercholesterolemia in patients, and for homozygous familial hypercholesterolemia (4). Various methods have been reported for estimation of ezetimibe in pharmaceutical formulations which includes the muse of spectrophotometry (5-12), Capillary Zone Electrophoresis (13), HPLC (14-17), and LC -MS (18-19) methods. Eranda was analysed the ezetimibe by HPLC but theirs method have a long time for run time for HPLC (16). Pawer applied the HPLC method but this method was a low recovery of ezetimibe(5). Sistla used the HPLC method for analysis of ezetimibe but this method was not high accuracy (14). Singh used for analysis of ezetimibe from HPLC method but this method was not simple and no precision (15). Although these methods were sufficiently sensitive, they were not suitable for most laboratories to perform studies involving samples in high through-put for therapeutic monitoring. The problems of these methods are, the long analysis time, large volume of sample, or low extraction recovery may not meet the requirement for high throughput, speed and sensitivity in bio sample analysis for quantitative analysis. As a result, a simple method that can determine ezetimibe in tablets formulation was required. Present studies involves

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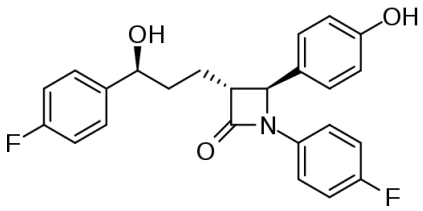

Figure 1 Chemical structure of ezetimibe

development of RP HPLC method using simple mobile phase containing acetonitrile and buffer for quantitative estimation of ezetimibe in tablet dosage forms which is sensitive and requires shorter analysis time. The developed method was validated as guidelines based on our pervious works (18). We study the analysis study of some drugs such as clonidine, amlodipine, atorvastatin, enalapril, cellcept by LC–MS and HPLC methods in human plasma (20-30). In this paper, we describe a simpler, selective and highly sensitive method by using high performance liquid chromatography for the determination of ezetimibe in tablet dosage forms.

Materials and methods

Materials

Ezetimibe 10 mg tablets (batch no. 014) provided by Bakhtar Biochemi (Kermanshah, Iran) and Ezetimibe 10 mg tablets manufactured by (MSD; Merk Sharp and Dohms, haar, Germany) (batch no. 291302) were used as test and reference products, respectively. Ezetimibe reference standard (99.9% purity) was kindly donated by Bakhtar Biochemi (Kermanshah, Iran). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

Instrument and HPLC method

The HPLC system to include of pump (KNAUER, model 1000, Germany), wavelength UV detector (KNAUER, model 2800 (DAD), Germany) used at a wavelength of 240 nm with the outputs to record and analyze using with a software (ChromGate, KNAUER, Germany). The drug analization was performed using a C18 analytical column (250mm × 4.6mm, particle size 5µm; Perfectsill, MZ-Analysentechnik, Germany) equipped by a guard column of the same packing. The mobile phase was composed of ammonium acetate buffer (pH 3)-acetonitrile (75:25 v/v) with a flow rate of 1 ml/min. Sample injection to system (50µl) was made by a loop injector (Rheodyne®7725i, Cotati, CA, USA).

Preparation of stock solutions

Stock solutions of ezetimibe was prepared in HPLC mobile phase at concentrations of 1mg/ml and were stored at 4 °C. Working solutions of ezetimibe were prepared daily in HPLC mobile phase by appropriate dilution at 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 55, and 60 µg/ml.

Estimation of ezetimibe in tablet dosage form

Each tablet contains 10 mg of ezetimibe. Twenty tablets were taken and weighed accurately. The average weight of one tablet was calculated and powdered. Equivalent to 1mg of ezetimibe of powder was taken and transferred to a 100 mL volumetric flask and about 75 ml of water and acetonitrile was added and sonicated to dissolve. The volume was made up to the mark with and acetonitrile. The solution was filtered through a membrane filter (0.22 µm) and sonicated to degas. Then 5 mL of above solution was pipetted out in 50 mL volumetric flask and volume was made up to the mark with and acetonitrile. The prepared solution was injected into the HPLC system and the observation was recorded.

Dissolution test

In vitro dissolution of EZE tablets was studied in USP dissolution apparatus II employing a paddle stirrer at 50 rpm. 500 mL of 4.5 acetate buffer was used as dissolution medium. The temperature of the dissolution medium was previously warmed to 37 ± 0.5 °C and was maintained throughout the experiment. One tablet is placed in each of the baskets, lower down the baskets into each dissolution vessel, start and run the apparatus immediately.5 ml of the sample of dissolution medium was withdrawn by means of a syringe fitted with pre filter, at 0, 10, 20, 30, 40, 50 and 60 min and replaced with equal volume to

maintain sink condition.. The sample was analyzed for drug release by measuring HPLC after suitable dilutions. The volume withdrawn at each interval was replaced with same quantity of fresh dissolution medium. The study was conducted in triplicate and the results of in-vitro release profile obtained for all the formulations were plotted in modes of data treatments as follows. The concentration of each sample was determined from a calibration curve obtained from pure samples of ezetimibe.

Method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, stability, detection limit and quantization limit according to the principles of the FDA industry guidance (31).

Assay specificity

To evaluate the matrix effect on the ionization of analytes, five different concentration levels of ezetimibe (10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 55, and 60 µg/ml) were prepared in the mobile phase as five sample series using five different lots of the mobile phase and the samples were processed, as described, and injected to HPLC. The same concentrations were prepared in mobile phase and analyzed for drug concentration using the same procedure. A comparison of the matrix effects of the two variants was made as an indicator of the method specificity.

Linearity

Standard curves of ten concentrations of ezetimibe ranged 10–60.0 µg/ml were assayed. The limit of detection (LOD) was estimated from the signal-to-noise ratio. This parameter was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The limit of quantification (LOQ) was defined as the lowest concentration level that provided a peak area with a signal-to- noise ratio higher than 5, with precision (%CV) within ± 20% and accuracy (%recovery) between 80−120%.

Within-run variations

In one run, three samples with concentrations of 10, 30, and 60µg/ml (from high, middle, and low regions of the standard curve) were prepared in triplicate and analyzed by developed HPLC method. Then, the coefficient of variations (%CV) of the corresponding determined concentrations were calculated in each case.

Between-run variations

On three different runs, samples from upper, intermediate, and lower concentration regions used for construction of standard curve (the same as within-run variations test) were prepared and analyzed by HPLC method. Then, the corresponding %CV values were calculated.

Extraction recovery

Three samples with concentrations of 10, 30, and 60 µg/ml (from high, middle, and low regions of the standard curve) were prepared in triplicate and analyzed by developed HPLC method. Then, the ratio of the recorded peak heights to the peak heights resulted from the direct injection of the aqueous solutions of ezetimibe with the same concentrations were determined as percentage in each case.

Stability

Freeze and thaw stability

Three concentration levels of QC samples were stored at the storage temperature (−20 °C) for 24 h and thawed unassisted at room temperature. When completely thawed the samples were refrozen for 24 h under the same conditions. The freeze-thaw cycle were repeated 3 time, then the samples were tested after three freeze (−20 °C)-thaw (room temperature).

Short-term temperature stability

Three concentration levels of QC samples were kept at room temperature for a period that exceeded the routine preparation time of samples (around 6 h).

Long-term stability

Three concentration levels of QC samples kept at low temperature (−20 °C) were studied for a period of 4 weeks.

Post-preparative stability

The auto sampler stability was conducted reanalyzing extracted QC samples kept under the auto sampler conditions (4 °C) for 12 h.

Results

Method development

In response to lack of an accessible, consistent, and simple to use analysis method for ezetimibe assay as an vital part of pharmacokinetic and bioequivalence estimate projects on the drug we urbanized a simple and offered HPLC method with UV detection based on the available equipment's found in most pharmaceutical laboratories. To this end, initially a series of isocratic as well as gradient conditions using different usual mobile phase compositions, polarities, ionic strengths, and pH values were tested in order to determine the best condition for the analyte separation.

System suitability tests

The number of theoretical plates (N), peak symmetry, and retain ability (K') of the method for ezetimibe were 1296, 1.143, and 2.75, respectively. These data show that the developed method is of appropriate separation efficiency and peak shape, both of which are important factors in estimate of the chromatographic method outputs. Typical chromatograms produced from the developed method are shown in figure 2. The HPLC chromatogram for a blank plasma sample indicating no endogenous peaks at the retention positions of ezetimibe was shown in figure 2. A.

Linearity

The method produced linear responses throughout the ezetimibe concentration range of 10-100µg/ml, which is suitable for intended purposes.

A typical linear regression equation of the method was: y = 7.345x + 0.0234, with x and y representing ezetimibe concentration (in mcg/ml) and peak height (in arbitrary units), respectively, and the regression coefficient (r) of 0.9942. The lower limit of quantification for ezetimibe was proved to be 10 µg/ml and the lower limit of detection (LOD) was 5 µg/ml. Figure 2.B shows the chromatogram of an extracted sample that contained 5 µg/ml (LOD) of ezetimibe. Figure 2.C shows the chromatogram of an extracted sample that contained 30 µg/ml of ezetimibe.

Within-run variations, between-run variations and extraction recovery

The within-run variations, between-run variations of the developed HPLC method and extraction recovery for ezetimibe are shown in table 1.

Stability

Table 2 summarizes the freeze and thaw stability, short term stability, long-term stability and post-preparative stability data of ezetimibe. All the results showed the stability behavior during these tests and there were no stability related problems during the samples routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies. The stability of working solutions was tested at room temperature for 6 h. based on the results obtained; these working solutions were stable within 6 h.

Estimation of ezetimibe in tablet dosage form

The percent content of ezetimibe in tablet dosage form was found to be 107.01 ± 2.06 with RSD 1.93. The USP specifications for assay are that the ezetimibe content should be less than 90 % and not more than 110%.

In vitro drug release study

The release profiles of different brands of ezetimibe tablets are shown in figure 3. All dissolution data are based on the actual drug content of the test tablets as calculated from the

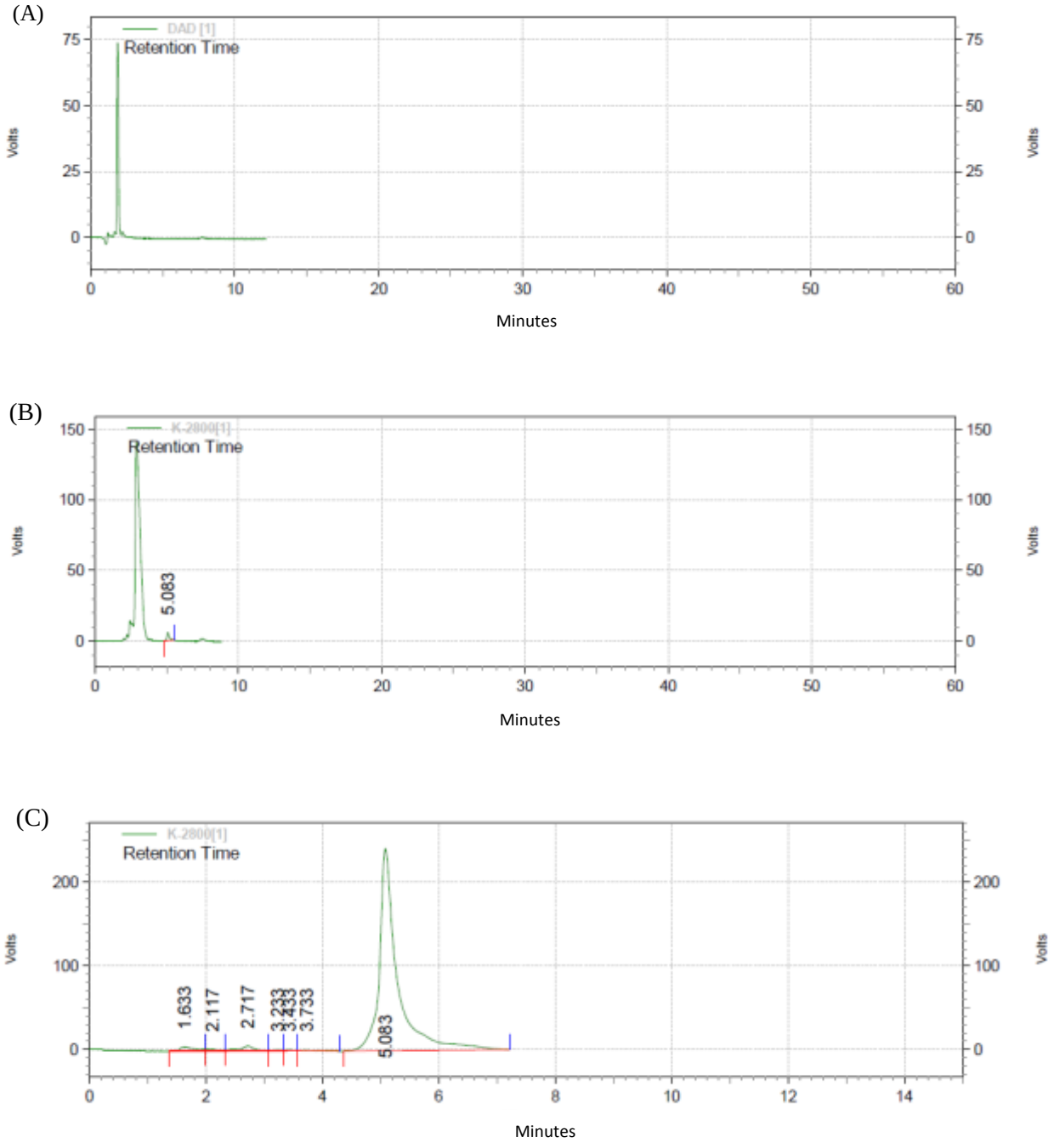

Figure 2 chromatogarm of samples: A: Blank B: chromatogram of an extracted sample that contained 5 µg/ml (LOD) of ezetimibe. C: chromatogram of an extracted sample that contained 30 µg/ml of ezetimibe.

Table1 Within–run variations, variations and relative recovery of the HPLC method for quantitation of ezetimibe (n = 3).

Nominal added concentration (µg/ml)	Sample number	Mean ± SD between–run	RSD	Mean ± SD within–run	RSD	Mean ± SD recovery	RSD
10	1 2 3	10.12 ± 0.35	3.45	9.97 ± 0.031	0.38	98.32 ± 0.84	0.85
30	1 2 3	29.76 ± 0.13	0.44	30.21 ± 0.21	0.61	95.43 ± 1.08	1.13
60	1 2 3	60.31 ± 0.27	0.44	59.98 ± 0.14	0.23	97.67 ± 0.15	0.15

Table 2 Data showing stability of ezetimibe at different QC levels (n = 5)

	10(µg/ml) Mean ± SD	**30(µg/ml)** Mean ± SD	**60(µg/ml)** Mean ± SD
Short-term stability	96.21 ± 1.12	94.65 ± 0.98	95.11 ± 1.02
Freeze and thaw stability	97.46 ± 2.23	94.78 ± 0.58	94.60 ± 2.31
Long-term stability	96.18 ± 0.45	98.45 ± 1.52	95.31 ± 1.09
Post-preparative stability	94.82 ± 0.63	91.65 ± 1.36	96.56 ± 0.65

assay results. Around 80% drug was released within 30 min and almost 100% drug was released within 60 min from all the brands in phosphate buffer.

Discussion

Ezetimibe is an Antihyperlipidemic and is usually categorized as HMG-CoA reductase inhibitor (1). Ezetimide belongs to a class of lipid lowering compounds that selectively inhibits intestinal absorption of cholesterol and related phytosterols. It potentially inhibits the transport of cholesterol across the intestinal walls there by reducing plasma cholesterol (2-3). Ezetimibe is rapidly absorbed and primarily metabolized in the small intestine and liver to its glucuronide, both of which undergo enterohepatic recycling in humans (4, 5). Since ezetimibe does not influence the activities of

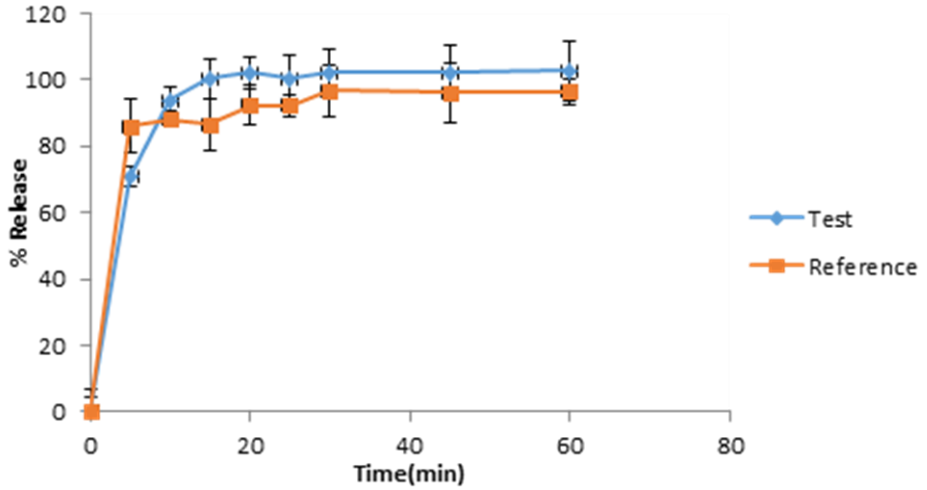

Figure 3 Diagram dissolution of ezetimibe for test and reference sample

CYP 450 enzymes, significant pharmacokinetic interactions with other medications including statins, fibrates, digoxin and warfarin have not been found (5). Ezetimibe complements the lipid lowering effects of other therapies, such as statins. Several bioanalytical methods are reported to determine ezetimibe in tablet dosage form (6-10). Eranda was analysed the ezetimibe by HPLC but theirs method have a long time for run time for HPLC (16). Pawer applied the HPLC method but this method was a low recovery of ezetimibe (5). Sistla used the HPLC method for analysis of ezetimibe but this method was not high accuracy (14). Singh used for analysis of ezetimibe from HPLC method but this method was not simple and no precision (15). Although these methods were sufficiently sensitive, they were not suitable for most laboratories to perform studies involving samples in high through-put for therapeutic monitoring. A rapid, specific isocratic HPLC method has been developed for the determination of ezetimibe using a UV detector. The method was validated for accuracy, precision, linearity and stability. The method uses a simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 5 min and the relatively low flow rate (1 ml/min) allows the analysis of large number of samples with less mobile phase that proves to be cost-effective.

Hence, this HPLC-UV method can be used for the routine drug analysis. The HPLC method developed is sensitive and specific for the quantitative determination of ezetimibe. Also, the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms.

Conclusion

A sensitive, selective, accurate and precise HPLC method was developed and validated for determination of ezetimibe in tablets. The reported method offers several advantages such as a rapid and simple extraction scheme, and a short chromatographic run time, which makes the method suitable for the analysis of large sample batches resulting from the pharmacokinetic, bioavailability or bioequivalent study of ezetimibe.

Conflict of interest

The authors report no conflicts of interest.

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