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Determination of enantiomeric purity of esomeprazole pharmaceutical products using validated HPLC method

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Abstract:

This method was developed to perform a comparative study of enantiomeric purity of some products of esomeprazole (capsules, tablets) manufactured locally and in some neighboring countries for the determination the percentage of R enantiomer. A product of under license company was used for comparison of the results. The study was performed by using a validated HPLC method on chiral column Nucleocel Alpha S and a mixture ethanol: hexane (70:30, v/v) as a mobile phase, the flow rate was 0.65 ml/min, and the detection was carried out using UV detector at 302nm. The temperature of column was set at 25°C.The study showed that some samples were not polluted with R enantiomer, while the others contained this enantiomer in the range of 0.1-2.24%.

Key Words: Enantiomeric Purity, Omeprazole, Esomeprazole, HPLC.

1. Introduction:

Esomeprazole magnesium trihydrate (ES), bis (5-methoxy-2- [(S) - [(4-me thoxy-3,5-dime thyl -2-pyridinyl)methyl]sulfinyl-1H-benzimidazole-1-yl) magnesium trihydrate is the S isomer of racemic omeprazole approved in February 2001 for use as a new pharmacological entity designed to improve the clinical outcome of available proton pump inhibitors in the management of acid-related disorders[1,2].

Proton Pump Inhibitors are synthetic compounds that inhibit the enzyme H^+/K^+ ATPase that is responsible for pumping protons in the stomach and they used to treat gastric acid disorders such as ulcers that exist in the esophagus, stomach, and duodenum. PPIs include Omeprazole, Lansoprazole, Dexlansoprazole, Esomeprazole, Pantoprazole, Rabeprazole[1-3].

The chemical structure of these compounds is derived from pyridyl methyl benzimidazole-sulfoxide, and their structures are contain an asymmetric sulfur atom, which allows them to present in the form of Enantiomers.

These drugs are used in the treatment in the form of racemic mixtures except of omeprazole and Lansoprazole (a racemic mixture of R and S Enantiomers), which are also given in the form of a single enantiomer called Esomeprazole and Dexlansoprazole, respectively[1-3].

Since 1992 the FDA and the European Committee for Proprietary Medicinal Products have required that the properties of each enantiomer be studied separately before decisions are taken to market the drug as one of the enantiomers or as a racemate.

Several methods have been developed to allow the determination of enantiomers and enantiomeric purity of the chiral drugs, the most important of which are the chromatographic methods (eg, HPLC, GC) and electrical methods (eg. CE)[4,5].

The methods of determination of R and S omeprazole in pharmaceutical forms and human

plasma focused on the use of HPLC, chiral column, and UV detector. Most of these methods were conform on plasma samples after oral administration of omeprazole, while a few of them applied on samples of pharmaceutical preparations[6-13].

The United States Pharmacopoeia (USP38) issued in 2015 contains a monograph for the determination of enantiomeric purity of esomeprazole as a raw material using Chiral HPLC and L41 (á1-Acidglycoprotein) as a column, and a mobile phase of phosphate buffer pH = 6.0 and acetonitrile 15 : 85, v/v. In this method, a chiral column Nucleocel Alpha S was used which contains amylose triphosphate (5,3 diethyl phenylcarbamate).

The aim of this study was to development a validated HPLC analytical method that allows the separation and determination of S and R enantiomers of omeprazole. So that it can be used in a comparative study of the enantiomeric purity of pharmaceutical preparations for esomeprazole (tablets, capsules) that obtained from national industries and the industries of neighboring companies by determining the percentage of enantiomer R compared to a reference product obtained from an under- license company.

2. Materials & Methods:

2.1. Equipment and instruments: Use HPLC system with model of Agilent 1100, with G1311A Quaternary Pump, G1313A Autosampler, UV / Vis Detector, and G1379A Vacuum Degasser. Ultrasonic Device for Elma.Filters 0.45 μm of Millipore Millex-LCR.Chiral column: Nucleocel Alpha S, (250mmx4.6mm, 5 μm). This column contains amylose tri (5,3 Dimethyl phenylcarbamate) from Macherey-Nagel company, CN guard column (4mmx4mm, 5 μm) fromMacherey-Nagel company, analytical Balance model Shimadzu AUW220D, with accuracy 0.1 mg.

2.2. Reagents and solvents: HPLC-Grade solvents were used:ethanol from Panreac, methanol from Panreac, Hexane from Sigma-Prich, isopropanol from Riedel de Haën AG, Heptane from Scharlau, and diethylamine Extra Pure.

2.3. Samples:

2.3.1. Standard Materials: Omeprazole and esomeprazole Standards: Purchased from the European Council of the European Pharmacopoeia (EPH) Strasbourg, France.

2.3.2. Pharmaceutical Products: Esomeprazole capsules and tablets were bought from pharmacies in Damascus and belong to several national pharmaceutical companies and companies from neighboring countries, including the Nexium product produced by under-license company.

2.4. Preparation of solutions:

2.4.1. The mobile phase was amixture of ethanol and normal hexane 70:30, v/v.

2.4.2. The stock solution of omeprazole (1 mg / ml): Weigh 100.0 mg of omeprazole standard and take it into a 100 mL volumetric flask, dissolve it by adding 10 mL methanol and about 70 ml of mobile phase, add 0.1 ml diethyl amine, sonicate for 15 minutes, add mobile phase to volume. Stored this solution at -20 ° C away from direct light[7].

2.4.3. The standard solution of omeprazole (100 mcg / ml):

Diluent 5.0 ml of stock solution to 50 ml Using mobile phase as a diluent.

2.4.4. The stock solution of esomeprazole (0.5 mg / ml): Weigh 55.5 mg of esomeprazole magnesium trihydrate standard and take it into a 100 ml volumetric flask, dissolve it by adding 10 mL methanol and about 70 ml of mobile phase, add 0.1 ml diethyl amine, sonicate for 15 minutes, add mobile phase to volume. Store this solution at -20 ° C away from direct light[7].

2.4.5. The standard solution of esomeprazole (50 mcg / ml): Diluent 5.0 ml of stock solution to 50 ml using mobile phase as a diluent.

2.4.6. The sample solutions of esomeprazole: These solutions were prepared to contain a

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concentration similar to the concentration of the standard solution of esomeprazole.

2.4.7. The chromatographic conditions to determination the percentage of R and S enantiomers of omeprazole:

Mobile phase: A mixture of Ethanol – normal Hexane 70:30, v/v.

Flow rate: 0.65 ml / min.

Injection volume: 20 µl.

Column temperature was set at 25 °C.

Detector: UV at a wavelength of 302 nm.

Figure 2: shows the Chromatogram of omeprazole standard solution.

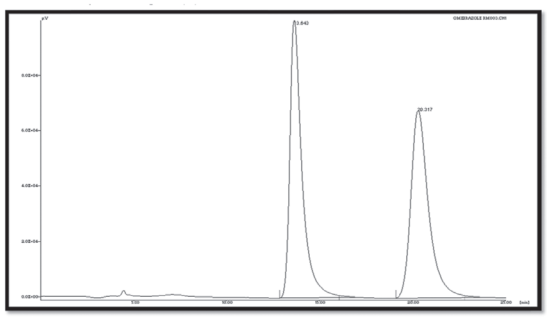


Figure 1: show the Chromatogram of omeprazole, standard solutions.

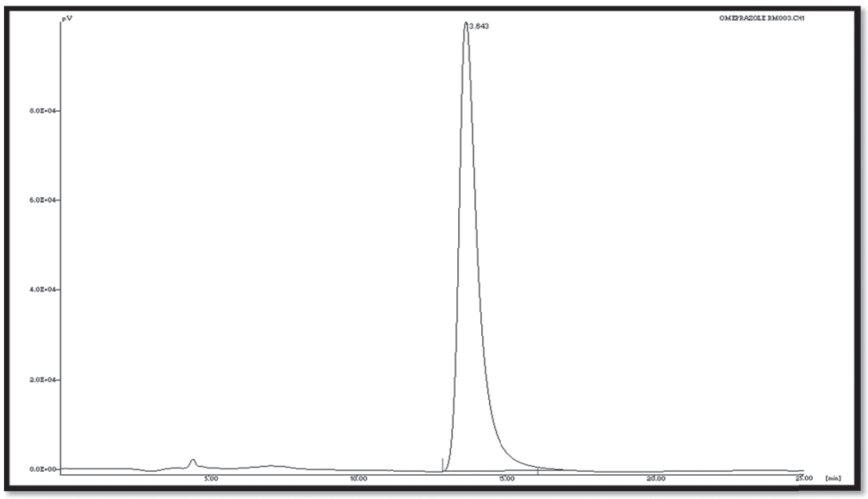


Figure 2: show the Chromatograms of esomeprazole standard solutions.

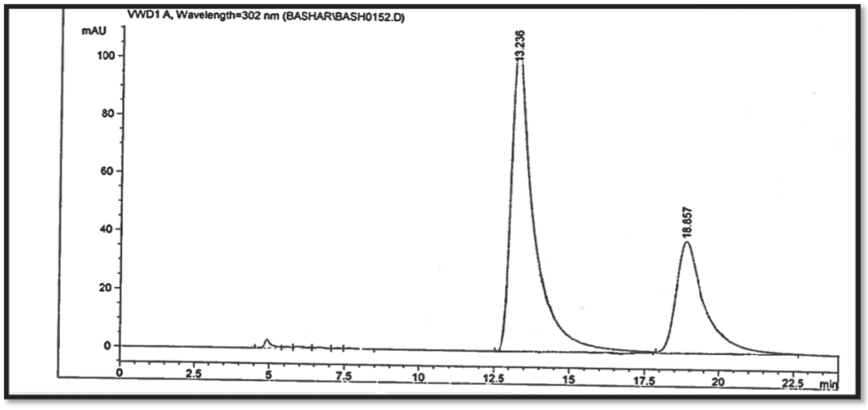


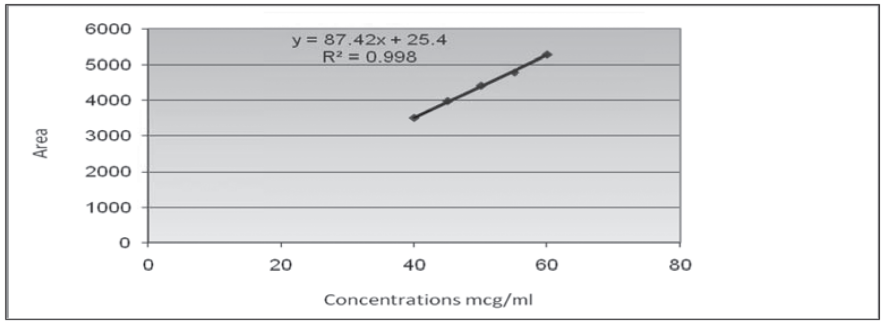
Figure (3): the Chromatogram of the mixture of esomeprazole standard solution and omeprazole standard solution.

Determination of enantiomeric purity of esomeprazole

2.5. Determination of the peaks of omeprazole enantiomers in the chromatogram:

The S and R peaks were confirmed by injecting a standard esomeprazole solution and observing the resulting chromatogram (Fig. 2), and by injecting a mixture of omeprazole and esomeprazole (the chromatogram in Fig. 3).

From the above, it was noted that the first peak with a retention time of 13.51 minutes was to the enantiomer S and the second peak with a retention time was 19.17 minutes was to the enantiomer R, table (1) shows the parameters of omeprazole enantiomers peak in figure (1).



The figure (4): the calibration curve of S-enantiomer.

Table (1): the parameters of peaks in Chromatogram of Figure (1).

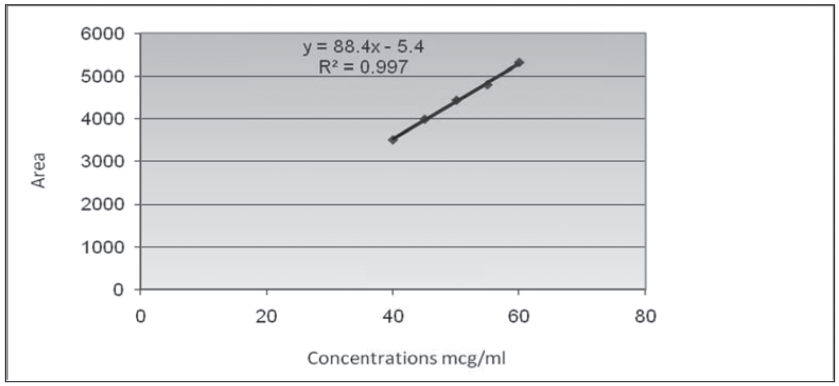
Compound	Retention time	Asymmetry	Theoretical plates	Capacity factor	resolution	Area %	Enantiomer
Enantiomer S	13.51	1.77	2551	2.52	-	5088	50.20
Enantiomer R	19.17	1.68	2390	3.40	5.55	5032	49.80

3. Results:

3.1. Validation of method:

The table (2) discusses the parameters of method validation.

Parameter	**Enantiomer S**	**Enantiomer R**
Retention Time (min)	13.64	20.32
Linearity (r^2), (figure .4 and 5).	0.9966	0.9958
Accuracy (Recovery %)	99.55±1.63	99.37±1.81
Repeatability (RSD, %)	1.52	1.80
Intermediate Precision (RSD, %)	1.16	0.99
Selectivity (Recovery %)	101.69±0.26	100.02±0.2
Robustness 0.55 ml/min	0.998	0.991
(Relative Retention 0.65 ml/min	0.999	0.998
Time) 0.75 ml/min	1.001	0.999
Detection Limit (ng/ml)	69.99	48.27
Quantitation Limit (ng/ml)	233.3	160.74



The figure (5): the calibration curve of R-enantiomer.

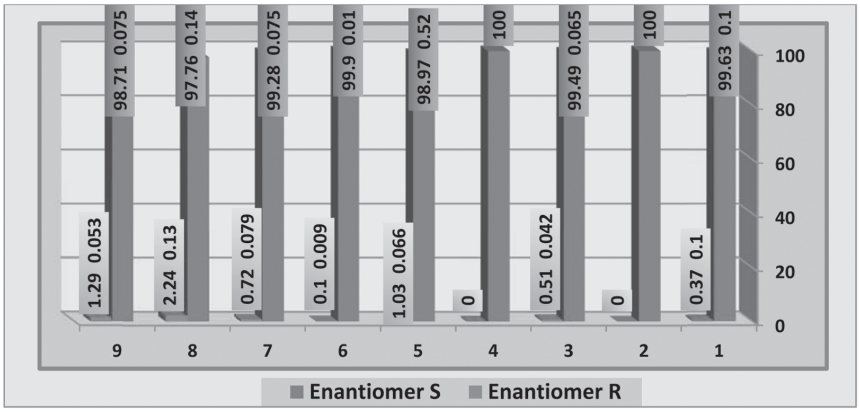


Figure (6): The percentages of S-enantiomer and R-enantiomer in pharmaceutical products of esomeprazole.

3.2. Study of enantiomeric purity of esomeprazole preparations:

The study was conformed on 9 pharmaceutical products of esomeprazole from 1 to 9, including the reference product (No 2). Figure 6 shows the percentages of both S and R enantiomers in the products.

The percentage of enantiomer-R was calculated in pharmaceutical products of esomeprazole by the following formula: **100 * (r_u / r_s)**

r_u : the peak response for the R-enantiomer

r_s: the sum of the responses of the both the esomeprazole and R-enantiomer peaks.

The monograph of esomeprazole in USP 38, 2015 determined the enantiomeric purity of the material as a limit (NMT 0.2% of the R-enantiomer)[14].

3. Results and discussion

A validated analytical method was developed using Chiral HPLC to determine the enantiomeric purity of esomeprazole products and the percentage of the R-enantiomer. The results of enantiomeric purity showed that two pharmaceutical product were free from enantiomer-R, while enantiomeric purity of other products was between 0.1-2.24 %.

The table 2 showed the parameters of validation of the analytical method, and the figures. 4,5 showed the calibration curves of S and R-enantiomers.

3.3. Discussion

1 - A validated analytical method was developed using Chiral HPLC to determine the enantiomeric purity of esomeprazole products and the percentage of the R-enantiomer.

2- The results of enantiomeric purity showed that two pharmaceutical product were free from enantiomer-R, while enantiomeric purity of other products was between 0.1-2.24 %.

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