Research Article

Analytical assay of indomethacin in presence of quercetin for indomethacin induced peptic ulcer prevention

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Abstract

Indomethacin becomes the first-choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other NSAIDs. Quercetin, a flavonoid, can prevent from gastric damage and protects against NSAIDS-induced ulcers using several mechanisms. This study aimed to analyze indomethacin in presence of Quercetin for Indomethacin induced peptic ulcer prevention.

Indomethacin alone and quercetin alone showed a strong absorption in the ultraviolet region. It was not possible to use UV analysis to quantify indomethacin in presence of quercetin. HPLC technique was used to quantify indomethacin in presence of quercetin.

Introduction

Non-steroidal anti-inflammatory drugs such as indomethacin are widely prescribed in clinical practice because they exert excellent efficacy in the management of pain, fever and inflammation (Rainsford, 2007). However, the use of NSAIDs is associated with severe gastrointestinal events such as gastric mucosal erosion, ulceration, bleeding and perforation (Wolfe et al., 1999). Indomethacin becomes the first-choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other NSAIDs (Sigthorsson et al., 2000).

Flavonoids are a group of naturally occurring compounds that are widely distributed as secondary metabolites in the plant kingdom. Quercetin, a flavonoid, prevents gastric damage and protects against NSAIDS-induced ulcers using several mechanisms, such as scavenging oxygen radicals (Abrahamse et al., 2005).

The aim of the present study is to analyze indomethacin quantitatively in presence of Quercetin.

Experimental

1. Materials

- Indomethacin was provided by Kahira Pharmaceuticals & Chemical Industries Company, Egypt.
- Quercetin and ethano werel purchased from Sigma Aldrich, (Germany).

2. Equipment

- UV/VIS double beam spectrophotometer Spctronic® GeneSys. (Milton Roy Co., USA). The equipment operates with Winspec® software.

3. Methodology

A.1. UV assay of indomethacin

Stock solution of indomethacin (1mg/ml) was prepared dissolving 25mg of the drug in minimum amount of absolute ethanol and the mixture was sonicated for few minutes then diluted with absolute ethanol in 25 ml volumetric flask. Standard solution (1) of indomethacin (20µg/ml) was prepared by dilution of 2ml of stock solution with 5ml absolute ethanol, and then the volume was completed using 50% ethanol in 100 ml volumetric flask. Spectra were recorded over the wavelength range of 200-400 nm, using 1 cm quartz cells at a slow speed.

From standard solution (1) (20µg/ ml) several concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18 µg/ml) were prepared by serial dilution. The absorbance of the drug was determined at λmax of 265 nm and 319 nm. Standard calibration curves were constructed.

A.2. UV assay of quercetin

Stock solution of quercetin (1mg/ml) was prepared by dissolving 25mg of the drug in minimum amount of absolute ethanol and

the mixture was sonicated for few minutes then diluted with absolute ethanol in 25 ml volumetric flask. Standard solution (2) of quercetin (20µg/ml) was prepared by dilution of 2ml of stock solution with 5ml absolute ethanol, then the volume was completed using 50% ethanol in 100 ml volumetric flask. Appropriate dilutions of standard drug solution were prepared with 50% alcohol. Spectra were recorded over the wavelength range of 200-400 nm, using 1 cm quartz cells at a slow speed.

From standard solution (2) (20µg/ ml) several concentrations (2, 4, 6, 8, 10, 12, 14, 16, 18 µg/ml) were prepared by serial dilution. The absorbance of the drug was determined at λmax of 256 nm and 373 nm. Standard calibration curves were constructed.

A.3. UV assay of indomethacin and quercetin mixture

Mixtures of indomethacin and quercetin were prepared in different ratios from their standard solutions (1) and (2) by appropriate dilutions. Spectra were recorded over the wavelength range of 200-400 nm, using 1 cm quartz cells at a slow speed using zero, first, second and forth derivative UV analysis.

The absorbances of the drug mixtures were determined at λmax of 256, 265, 319 and 373 nm and compared with that of each drug in the same λ.

B- High-Performance Liquid Chromatographic Simultaneous Determination of indomethacin in presence of quercetin:

Materials and Reagents

All solvents were of HPLC grade, Merck (Darmstadt, Germany). All other materials were of analytical grade.

- Phosphate buffer (30 mmol L-1, pH 3), was prepared according to BP.
- Double distilled water was obtained through SAWS-1004D automatic double distillatory (Shin Saeng Scientific Co. Ltd., Korea) and used throughout the work.

Instruments

Chromatographic system

- The chromatographic system consisted of a Knauer HPLC system (Knauer, Berlin, Germany), which consisted of K-500 solvent delivery pump, injector valve with a 20 µl loop and K-2500 UV variable wave length detector. The HPLC system control and data processing were performed by computer integration software (EuroChrom 2000® Knauer).
- Analytes were separated using a Gemini RP-C18 column (250 x 4.6 mm, 5 µm), (phenomenex, USA) protected with a precolumn (guard column with Gemini C18 precolumn inserts) (Phenomenex, USA) fitted just before the inlet junction of the analytical column.
- The eluent was filtered through a 0.45 µm membrane filter (Gelman instrument Co.) using vacuum filtration unit (phenomenex, USA).
- Ultrasonic cleaner (Cole-Parmer, Chicago, USA).
- Auto vortex (Stuart Scientific, London, UK).
- Sartorius handy balance – H51 (Hanover, Germany).
- pH meter, model 3305 (Jenway, London, UK).
- Digital micro-transfer pipettes 5-200 µL (Acura, Socorex, Switzerland).

Standard solutions

Standard stock solutions of 100 µg mL-1 quercetin and endomethcin were prepared by dissolving 10 mg of quercetin and endomethcin separately in 100 mL methanol and protected from light using aluminum foil. Working standard solutions containing both drugs were prepared from the stock solutions by appropriate mixing and dilution with water.

HPLC procedure

The mobile phase consisted of a mixture of acetonitrile: 30 mM phosphate buffer in a ratio of 65:35, v/v.

The mobile phase was filtered through a 0.45 mm membrane filter (Phenomenex, USA) using vacuum filtration unit (Phenomenex, USA) and was degassed in an ultrasonic cleaner (Cole-Parmer, Chicago, IL, USA) and delivered at flow rate 1 mL/min. The injection sample volume was 20 µL. The detector wave length was set at 320 nm. The chromatography was performed at room temperature.

Results

A.1. UV assay of indomethacin

The proposed method is simple, safe, economic, accurate and cost-effective and can be successfully employed in routine analysis of the drug.

The UV spectrum of indomethacin was shown in (figure 1-1). Indomethacin shows a strong absorption in the ultraviolet region, with a maximum absorption at wavelength of 265 and 319 nm (λmax).

A.2. UV assay of quercetin

Quercetin shows a strong absorption in the ultraviolet region, with the maximum absorption peaks at the wavelength of 256 nm and 375 nm (figure 1-1), and showed a straight line within the studied concentration range (2–18µg/ml) with regression coefficient (r)=0.9995 and a slope of 0.0578.

A.3. UV assay of indomethacin and quercetin mixture

The results of the UV spectra of indomethacin/quercetin combination were illustrated in (figure 1-1). From the figure, it could be noticed that the λmax of indomethacin (at 265 and 319 nm) and that of quercetin (at 256 and 375 nm) were affected. The absorbance at each λmax (256, 265, 319 and 365 nm) was found to be greater than it should be expected from each drug alone.

It was not possible to estimate indomethacin in presence of quercetin by UV assay even by first, second and forth derivative UV analysis (figures 1-4, 1-5 and 1-6).

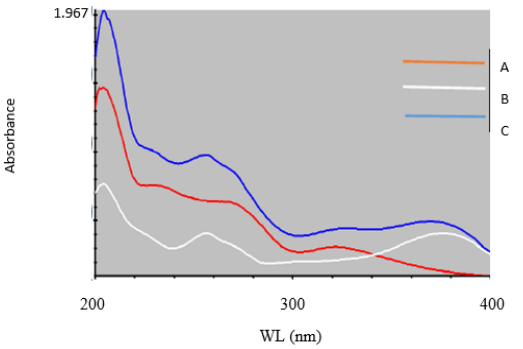

Figure 1-1: Zero order Ultraviolet spectrophotometric scan for A: Indomethacin (12µg/ml), B: Quercetin (6µg/ ml) and C: Mixture of Indomethacin and Quercetin (12µg/ml IM: 6µg/ ml Q)

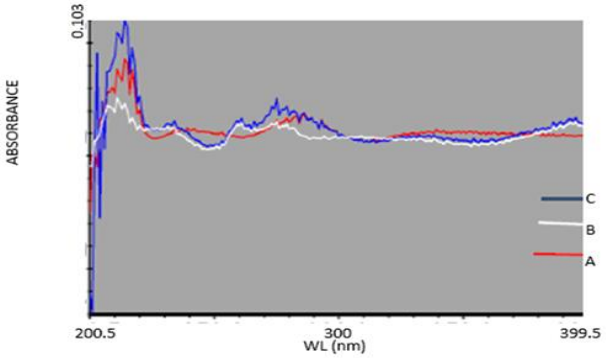

Figure 1-4: First order Ultraviolet spectrophotometric scan for A: Indomethacin (12µg/ml), B: Quercetin (6µg/ ml) and C: Mixture of Indomethacin and Quercetin (12µg/ml IM: 6µg/ ml Q).

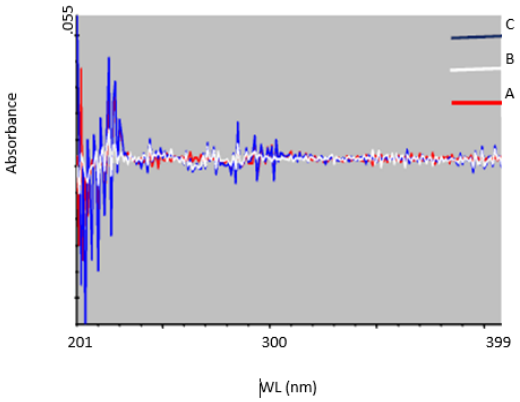

Figure 1-5: Second order Ultraviolet spectrophotometric scan for A: Indomethacin (12µg/ml), B: Quercetin (6µg/ ml) and C: Mixture of Indomethacin and Quercetin (12µg/ml IM: 6µg/ ml Q).

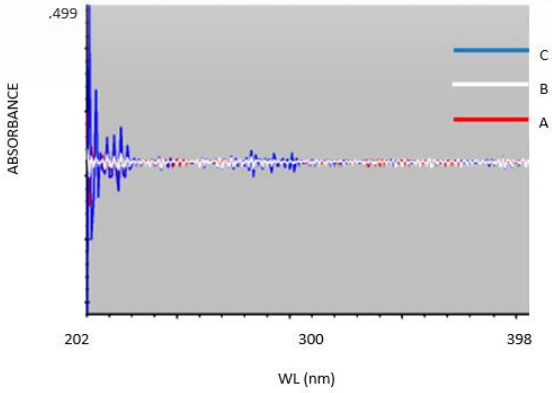

Figure 1-6: Forth order Ultraviolet spectrophotometric scan for A: Indomethacin (12µg/ml), B: Quercetin (6µg/ ml) and C: Mixture of Indomethacin and Quercetin (12µg/ml IM: 6µg/ ml Q)

B- High-Performance Liquid Chromatographic Simultaneous Determination of indomethacin in presence of quercetin:

Indomethacin could be detected in presence of quercetin by the used HPLC method figure 1-7

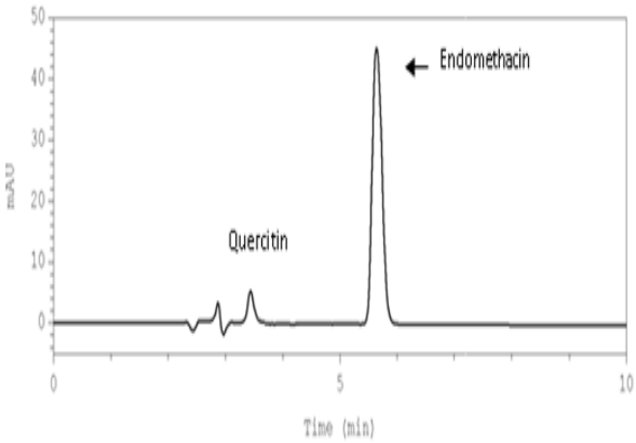

Figure 1-7: HPLC estimation of indomethacin release in presence of quercetin in phosphate buffer pH 7.2 at 37 ºC.

Discussion and conclusion

Indomethacin alone and quercetin alone showed a strong absorption in the ultraviolet region. It was not possible to use UV analysis to quantify indomethacin in presence of quercetin. HPLC technique was used to quantify indomethacin in presence of quercetin.

References

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