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ORIGINAL ARTICLE

A validated stability-indicating HPLC method for simultaneous determination of Silymarin and Curcumin in various dosage forms

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Abstract A new, reliable, sensitive and stability-indicating gradient HPLC method was introduced for the simultaneous determination of two anti-hepatotoxic polyphenolic drugs (Silymarin and Curcumin). The method was adapted to analyze both drugs in their dosage forms (tablets and capsules) with no interference from common excipients. The photo diode array detector was used as a tool for peak identification and purity confirmation especially that both drugs have several reported peaks. In order to assess the stability-indicating power of the assay procedure, SIL and CUR were subjected to different forced degradation studies: acidic, alkaline and neutral hydrolysis, photo-degradation, oxidative degradation and dry heat. The developed method could efficiently separate the parent drug peak from the degradation products peaks. The method was validated according to the ICH guidelines with respect to linearity, detection and quantitation limits, accuracy, precision, specificity, and robustness. Finally, the results of the proposed method for determination of SIL were statistically compared to the official BP method and no significant difference was found between them.

1. Introduction

Silymarin (SIL) is an anti-hepatotoxic polyphenolic substance isolated from the milk thistle plant, *Silybum marianum*, family (Asteraceae) (United States Pharmacopeia, 2011). SIL (Sweetman, 2009), which is a mixture of flavonolignans including the isomers silibinin, silicristin, and silidianin -of which silibinin is the major component- is claimed to be a free radical scavenger and to have hepatoprotectant properties. It has been used in

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various liver disorders, as well as to prevent hepatotoxicity associated with poisoning (Sweetman, 2009).

Curcumin (CUR) which is a polyphenolic compound present in the rhizomes of the turmeric (*Curcuma longa* Linn.) family (Zingiberaceae) (Sweetman, 2009), has a wide biological and pharmacological profile. It is reported to have antioxidant and hepatoprotective activity (Park et al., 2000). This compound exhibits numerous biological activities including anti-inflammatory, antiprotozoal, antibacterial, anti-HIV and anti-cancer activities against several malignancies. Also, hepato, neuroprotective, hypoglycemic, and antirheumatic effects of curcumin were reported (Anand et al., 2007). The safety of CUR at very high doses has been proved in various animal and human studies (Anand et al., 2007). These studies led to the approval of CUR as a 'Generally Regarded as Safe' ingredient by the Food and Drug Administration (FDA) of the United States of America, by the Natural Health Products Directorate of Canada and the Expert Joint Committee of the Food and Agriculture Organization/World Health Organization (FAO/WHO) on food additives (JECFA) (Basnet and Skalko-Basnet, 2011).

The USP 34 (United states Pharmacopeia, 2011) monograph of SIL (Powdered Milk Thistle Extract) describes a gradient HPLC-UV method for its assay in pure form, in capsules and in tablets, with a limit for resolution between Silybin A and B not less than 1. However, the BP 2012 (British Pharmacopoeia, 2012) monograph of SIL (Milk-thistle Fruit) describes also a gradient HPLC method for its analysis in pure form, with a limit for resolution between Silibinin A and B not less than 1.8.

Various methods are available for the determination of SIL, including stability-indicating HPTLC method for quantitative estimation of Silybin in bulk drug and pharmaceutical dosage form (Parveen et al., 2010). HPLC and capillary electrophoresis have been used for the determination of SIL from dried fruits (Quaglia et al., 1999).

UPLC-UV method was reported for the simultaneous determination of active compounds in SIL (Liu et al., 2009). Several reversed phase HPLC-UV methods have been published for the analysis of Silibinin in rat plasma and bile (Wu et al., 2007), in the seed extract of some Milk Thistle (Radjabian et al., 2008), in pharmaceutical preparations (Hadad et al., 2009), and in human plasma (Kosina and Bartek 2000). UPLC-MS methods were also used to analyze and separate the active constituents of the silymarin extract (Wang et al., 2010).

A differential pulse voltammetric method has been used for the determination of SIL and vitamin E acetate mixture in pharmaceuticals (Hassan et al., 2008).

Determination of SIL using spectrophotometry in bulk drug and pharmaceutical formulations (Moin et al., 2010) has also been found.

A survey of the literature showed that different analytical techniques have been used for the analysis of CUR. CE methods have been used for the detection and determination of CUR (Lechtenberg et al., 2004), also different TLC and HPTLC methods have been reported for the simultaneous quantification and determination of Curcuminoids in Curcuma longa (Phattanawasin et al., 2009). In addition, a stability-indicating HPTLC method for the determination of CUR in bulk drug and pharmaceutical formulations (Ansari et al., 2005) was published.

Several fluorimetric methods have been used for the determination of CUR (Wang et al., 2008). NMR methods have also been reported for the rapid quantitation of CUR (Gören et al., 2009).

Different HPLC methods have been described for the analysis of CUR, such as HPLC methods with fluorescence detector which have been used for its quantification in biological samples (Schiborr et al., 2010). HPLC-MS methods have been used for the assay of CUR in plasma (Yang et al., 2011).

HPLC-UV methods were also used, such as: HPLC methods for the quantitative determination of CUR in biological fluids (Han et al., 2011). Simultaneous determination of Curcuminoids in different types of extracts (Wichitnithad et al., 2009) and in food products was done.(Nagappan et al., 2009).

A stability-indicating LC method dealing with the quantitative determination of CUR in laboratory samples has been found (Dandekar and Patravale, 2009).

A literature survey revealed that no analytical method has been reported for the simultaneous analysis of SIL and CUR and for their simultaneous analysis in the presence of their forced degradation products.

In the present work, a new validated stability-indicating HPLC method has been developed for the simultaneous analysis of SIL and CUR. Furthermore, the different conditions for optimization of the HPLC parameters for the simultaneous identification and determination of both drugs in their dosage forms (tablets and capsules) were studied using HPLC- DAD. The diode array detector which is an elegant part of the HPLC instrument enhances the performance of the instrument and is very efficient in confirming the peak purity of the several reported peaks of SIL and CUR.

To validate the stability-indicating power of the developed analytical method, SIL and CUR were subjected to forced degradation studies including the effect of hydrolysis (acidic, alkaline and neutral), oxidation, photolysis and dry heat. The proposed method could effectively separate the drug peaks from those of their degradation products.

2. Experimental

2.1. Materials and reagents

Silymarin and Curcumin were kindly supplied by EMITCO Pharmaceuticals (Alexandria, Egypt). Methanol and acetonitrile used were of HPLC grade and were obtained from LAB-SCAN (UK). Other reagents were of analytical grade including: potassium dihydrogen phosphate and orthophosphoric acid obtained from LOBA-Chemie, NaOH, HCl and H_2O_2 obtained from EL-NASR company-ADWIC for chemical industries (Egypt).

2.2. Pharmaceutical formulations

Legalex® Tablets labeled to contain 70 mg Silymarin per tablet were manufactured by the Alexandria Company for Pharmaceutical and Chemical Industries (Alexandria, Egypt). Hepapro® Capsules labeled to contain 100 mg Silymarin and 50 mg Curcumin per capsule were manufactured by EMITCO Pharmaceuticals (Alexandria, Egypt).

2.3. Apparatus

A Waters HPLC system consisting of: Alliance e HPLC with a 2695 separation module consisting of a solvent management module, a sample management module and a column oven.

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