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Separation and characterization of silybin, isosilybin, silydianin and silychristin in milk thistle extract by liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A selective and sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS) method has been developed for the characterization of silymarin in commercially available milk thistle extract. In this study, six main active constituents, including silydianin, silychristin, diastereomers of silybin (silybin A and B) and diastereomers of isosilybin (isosilybin A and B) in silymarin, were completely separated on a YMC ODS-AQ HPLC column using a gradient mobile phase system comprised of ammonium acetate and methanol/water/formic acid. Identification and characterization of the major constituents were based not only on the product ion scan, which provided unique fragmentation information of a selected molecular ion, but also on the specific fragmentation of multiple reaction monitoring (MRM) data, which confirmed the retention times of LC chromatographic peaks. The method was applied in the analysis of human plasma samples in the presence of silymarin and appeared to be suitable for the pharmacokinetic studies in which the discrimination of silymarin constituents is essential.

Keywords: Silymarin; Milk thistle; Silybin; Isosilybin; Diastereomers; LC/MS/MS

1. Introduction

Silymarin, derived from the milk thistle plant, *Silybum marianum*, has been used for centuries as a natural remedy in the treatment of hepatitis and cirrhosis, as well as in the protection of the liver from toxic substances [1–5]. Recent reports have demonstrated that silymarin also has exceptionally high anti-tumor promoting activity [5–8]. The main bioactive constituents in silymarin are composed of a mixture of flavonolignans, silychristin, silydianin, silybin and isosilybin [9–12]. In nature, silybin and isosilybin have been reported to contain two groups of diastereoisomeric flavonolignans, silybin A and silybin B, and isosilybin A and isosilybin B, respectively [13–15]. The chemical structures of the six main active constituents in *Silybum marianum* are shown in Fig. 1. Among commercial products currently available, silymarin, the standardized extract obtained from the dried fruits of *Silybum marianum* contains approximately 70% to 80% of the silymarin complex and approximately 20% to 30% of a chemically undefined fraction comprised mostly of polymeric and oxidized polyphenolic compounds [12].

The different biological activities have been reported for these six main components in silymarin [16–22]. Moreover, controversy also surrounds the activity of these compounds in animal studies. Many pharmacological studies on silymarin conducted using standardized plant extract fail to identify the manufacturing source of silymarin and quantitate silymarin contents and its individual active components in the extract [12,22–24], making the evaluation of dose-exposure relationships impossible and likewise ensure that dose relationships continue to be poorly-defined. Therefore, there is a pressing need for the development of a sensitive and selective analytical method to characterize and quantify each component of silymarin in commercial products, and furthermore, to quantify these active components in biologic matrices. This is especially true in light of recent requests to define exposure-response relationships for silymarin. Such relationships could be used

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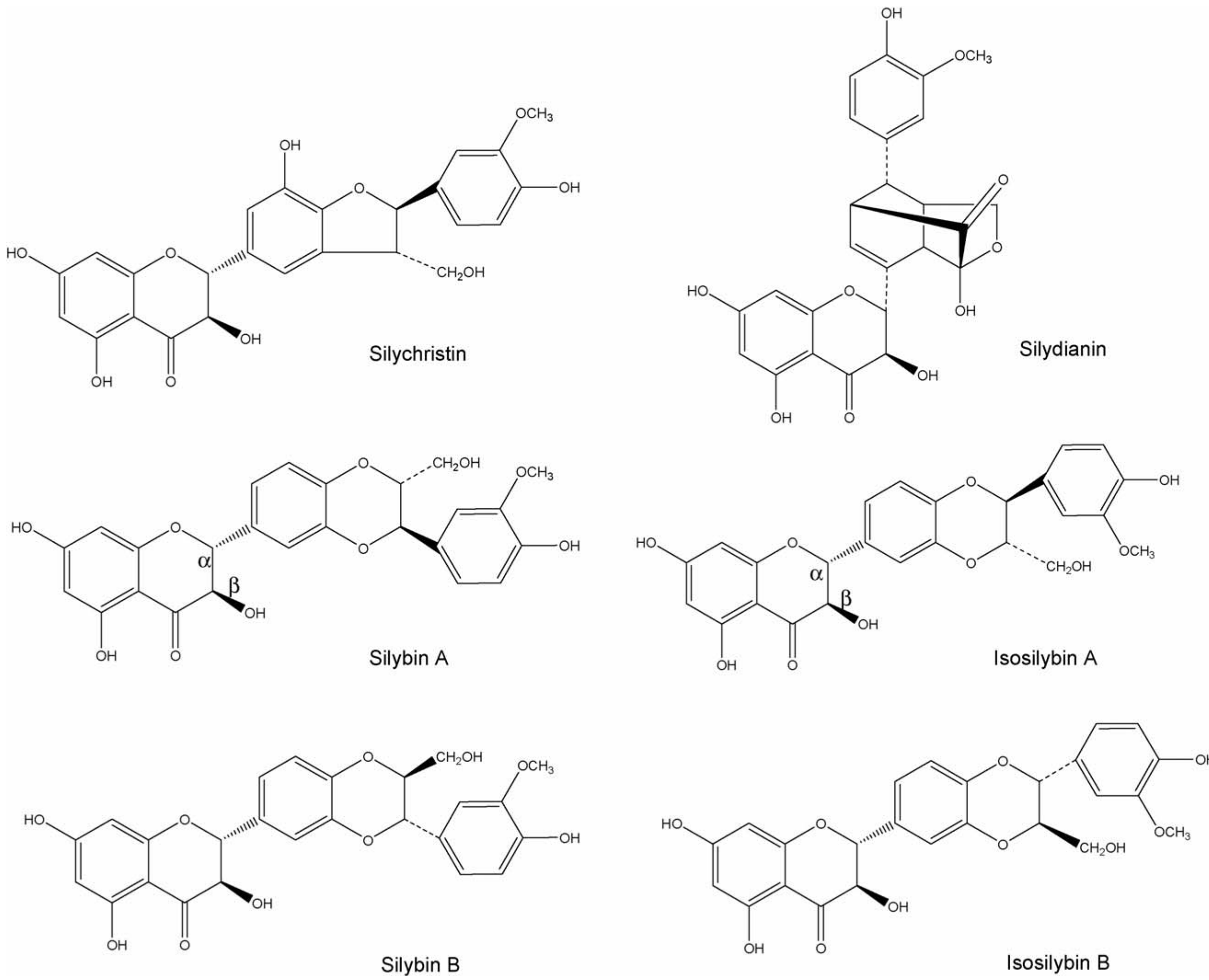

Fig. 1. Chemical structures of the main active constituents in *Silybum marianum*: silychristin, silydianin, diastereomers of silybin (silybin A and B), and diastereomers of isosilybin (isosilybin A and B).

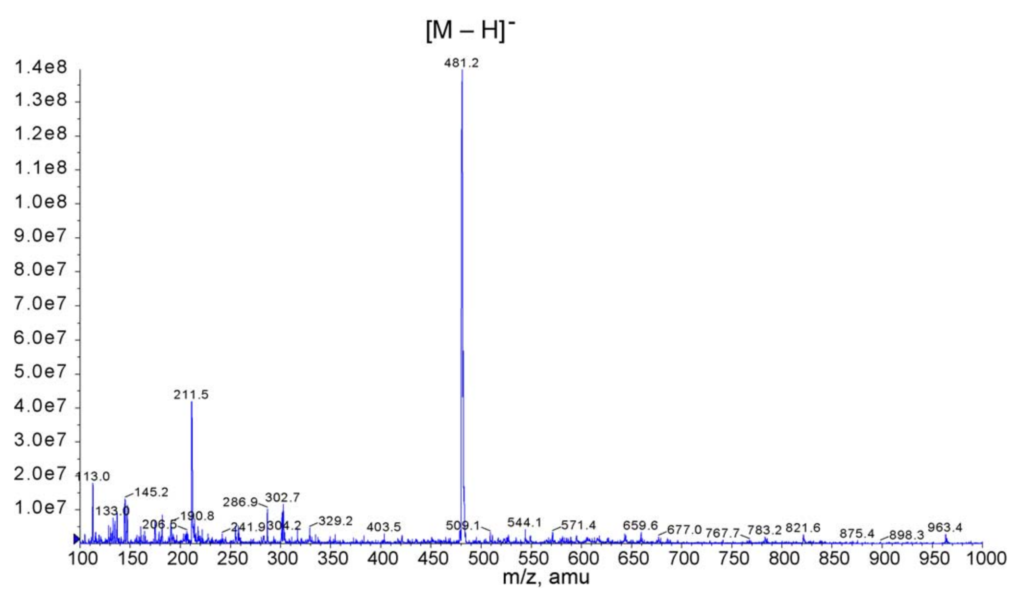

Fig. 2. Q1 full scan mass spectrum of silymarin in a commercial milk thistle seed extract by negative TurboIonSpray ionization.