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Quantification of lipoic acid in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry

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Abstract

A sensitive and specific liquid chromatography electrospray ionization mass spectrometry (LC–ESI-MS) method has been developed and validated for the identification and quantification of lipoic acid (LA) in human plasma. LA and the internal standard, naproxen, were extracted from a 500 μl plasma sample by one-step deproteination using acetonitrile. Chromatographic separation was performed on a Zorbax SB-C_{18} Column (100 mm × 3.0 mm i.d. with 3.5 μm particle size) with the mobile phase consisting of acetonitrile and 0.1% acetic acid (pH 4, adjusted with ammonia solution) (65:35, v/v), and the flow rate was set at 0.3 ml/min. Detection was performed on a single quadrupole mass spectrometer by selected ion monitoring (SIM) mode via electrospray ionization (ESI) source. The method was linear over the concentration range of 5–10,000 ng/ml for LA. The intra- and inter-day precisions were less than 7% and accuracy ranged from −7.87 to 9.74% at the LA concentrations tested. The present method provides a relatively simple and sensitive assay with short turn-around time. The method has been successfully applied to a clinical pharmacokinetic study of LA in 10 healthy subjects.

Keywords: Lipoic acid; HPLC–ESI-MS

1. Introduction

α-Lipoic acid (LA), which plays an essential role in mitochondrial dehydrogenase reactions, has recently gained considerable attention as an antioxidant. LA, or its reduced form, dihydrolipoate, reacts with reactive oxygen species such as superoxide radicals, hydroxyl radicals, hypocholorous acid, peroxyl radicals, and singlet oxygen. It also protects membranes by interacting with vitamin C and glutathione, which may in turn recycle vitamin E. LA administration has been shown to be beneficial in a number of oxidative stress models such as ischemia-reperfusion injury, diabetes, cataract formation, HIV activation, neurodegeneration, and radiation injury. Furthermore, LA can function as a redox regulator of proteins such as myoglobin, prolactin, thioredoxin and NF-B transcription factor [1]. The drug is rapidly absorbed and extensively metabolized in the liver and great individual variability in LA concentration occurs among patients. A sensitive and simple LA determination method for pharmacokinetics studies and for therapeutic drug monitoring is then desired. Various methods have been developed for the determination of LA. GC methods based on prior derivatization were the most useful techniques for the determination of LA in biological samples [2]. However, all these methods needed a derivatization procedure and the sample preparation was very laborious. Determination of LA by HPLC with electrochemical detection [3,4] or, more selectively with a dual gold–mercury electrode [5] was highly sensitive and the sample preparation was relative simple, but the concentration-response curve was only linear in a narrow concentration range, and the reconstitution of the Hg-electrode was necessary. Although the sensitivity of the HPLC-ultraviolet detection method for LA was low, due to

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the lack of a strong chromophore, LA can be sensitively detected by HPLC-fluorescence detection based on fluorogenic labeling. Witt et al. [6] and Haj-Yehia et al. [7] reported HPLC methods with fluorescence detection after pre-column derivatization with monobromobimane or strong fluorophore 2-(4-aminophenyl)-6-methylbenzothiazole to analysis LA in human plasma, respectively. However, all these methods included a very tedious sample preparation procedure. In recent years, Teichert et al. developed an isocratic reversed-phase HPLC method for the quantitation of LA in human plasma employing simple solid-phase extraction and pulsed amperometric detection (PAD) and the limit of quantification was 10 ng/ml [8]. In this paper, no internal standard was used and in order to separate LA from endogenous components and its metabolites, the total run time was 22 min per sample. More important, a disadvantage of PAD was the large anodic background current and the fact that the kinetics of surface oxide formation could be influenced by the adsorbed analyte. The calibration curve was strongly influenced by the absorption isotherm of the analyte and therefore, it deviated from linearity at high concentrations. Due to the high concentrations appearing after administration of therapeutic doses and short elimination half-lives of LA, the method must cover a broad concentration range. So in order to minimize the influence of the adsorbed analyte, the author performed all measurements in the same detector in range of 0.1 μA and most of the samples had to be diluted differentially and analyzed repeatedly. In this paper, we describe a more simple, selective and highly sensitive method by using high performance liquid chromatography coupled with electrospray ionization (ESI) single quadrupole mass spectrometry (MS) in the negative selected ion monitoring (SIM) mode for the determination of LA in human plasma and this method has been successfully used for clinical LA pharmacokinetic studies.

2. Experimental

2.1. Reagents and chemicals

LA was obtained from Shanghai Institute of Pharmaceutical Industry (Shanghai, China). Naproxen, used as internal standard (I.S.), was obtained from Shanghai Institute for Drug Control (Shanghai, China). Chemical structures were presented in Fig. 1. The purity of LA and Naproxen were all >99.5%. Acetonitrile was chromatographic pure grade and purchased from Merck (Merck Company, Germany), acetic acid and ammonia solution (analytical reagent grade) were purchased from Shanghai Chemical Reagent Company (Shanghai, PR China). Double distilled water was purified by Millipore Simplicity™ (Millipore, Bedford, MA, USA). The drug-free human heparinized plasma was obtained from Shanghai Blood Center (Shanghai, PR China).

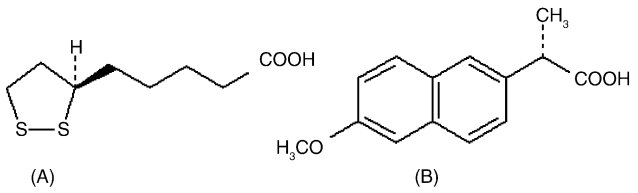

Fig. 1. Chemical structures of LA and naproxen.

2.2. Preparation of standard solution

The primary stock solutions of LA were prepared by dissolving 50.0 mg of LA in 50 ml methanol, producing a concentration of 1.0 mg/ml and was stored at 4 °C. Working solutions of LA were prepared by appropriately diluting the stock solution with water at the concentrations of 0.1, 0.5, 1, 5, 10, 50 and 200 μg/ml. The internal standard stock solution was prepared by dissolving 10.0 mg of naproxen in 10 ml methanol, producing a concentration of 1.0 mg/ml and was stored at 4 °C. This solution was further diluted with the same solvent to prepare the internal standard working solution containing 20 μg/ml of naproxen. All these solutions were stored at 4 °C and no change in stability over a period of 1 month was observed.

2.3. Equipment

The experiments were carried out with a HP1100 system (Agilent Technology, Palo Alto, CA, USA). The system consisted of a G1312A binary pump, a mobile phase vacuum degassing unit, a G1329A autosampler, a temperature-controlled column compartment, and a HP1100 single-quadrupole mass spectrometric (MS) detector equipped with an electrospray source. Data were acquired and integrated by the ChemStation software runn on a HP Vectra 150/PC with a Windows NT operating system. The stationary phase was composed of Zorbax SB-C_{18} material (Agilent Technology) packed in a stainless steel column (100 mm × 3.0 mm i.d. with 3.5 μm particle size).

2.4. Chromatographic and MS conditions

Chromatographic separations were achieved using a mobile phase consisting of acetonitrile and 0.1% acetic acid (pH 4, adjusted with ammonia solution) (65:35, v/v), with a flow rate set at 0.3 ml/min. The analytical column was kept at 40 °C. The column effluent was connected to an electrospray ionization MS interface without splitting. Electrospray ionization was performed using nitrogen as nebulizing gas at 10 l/min flow rate, 40 psi nebulizing pressure, and 350 °C drying gas temperature. Capillary voltage was set at 3000 V. Fragment voltage applied between capillary outlet and the first skimmer produced fragment ions by in-source collision-induced dissociation by nitrogen. Optimum fragment voltage of 70 V was selected after varying between 50 and 150 V. Negative-ion selected ion monitoring (SIM) mode was used to detect *m/z* 205.0 ($[LA-H]^-$), and *m/z* 229.0 ($[Naproxen-H]^-$).