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A novel micellar *per* aqueous liquid chromatographic method for simultaneous determination of diltiazem hydrochloride, metoprolol tartrate and isosorbide mononitrate in human serum

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ABSTRACT

A novel micellar *per* aqueous liquid chromatographic method was investigated to simultaneously determine diltiazem hydrochloride, metoprolol tartrate and isosorbide mononitrate in human serum. Separation and determination of the analytes were performed on a Pinnacle II Cyano column as the stationary phase using the mobile phase consisted of aqueous solution (4.15×10^{-2} mol/L sodium dodecyl sulfate and 0.02 mol/L sodium dihydrogen phosphate) with 10% (*v/v*) of 1-propanol at pH 7.0. This method was validated by linearity, lower limit of quantification, extraction recovery, stability, precision, and accuracy. The main analytical parameters were linearity ($r > 0.9950$), intra- and inter-day precisions (intra-day RSD 2.2–3.5%, and inter-day RSD 3.7–9.5%), lower limit of quantification (20 ng mL^{-1} for isosorbide mononitrate, metoprolol tartrate and diltiazem hydrochloride). The extraction recovery was 63.3% (0.1 μg/mL), 65.6% (1.0 μg/mL), and 69.5% (25 μg/mL) for isosorbide mononitrate; 65.1% (0.1 μg/mL), 69.5% (1.0 μgmL) and 73.5% (2.5 μg/mL) for metoprolol tartrate; 67.1% (0.1 μg/mL), 68.8% (1.0 μg/mL) and 73.8 % (2.5 μg/mL) for diltiazem hydrochloride. The relative error of stability was <6.4% at the room temperature for 24 h, <3.8% at 4 °C for 1 week, <4.6% at −20 °C for 1 month, and <6.7% for freeze/thaw cycles ($n = 3$). The results indicated that the proposed method was rapid, sensitive, and accurate for determination of the three antianginal drugs in human serum. The possible separation mechanism of the method was also discussed, and a model of separation mechanism for the analytes was established.

1. Introduction

Cardiovascular diseases are common in clinic and cause the wide public concerns because of the major reason of global morbidity and mortality [1]. Currently, calcium channel blockers, β-blockers and nitrates are common drugs in the angina therapy and are frequently administered in combination [2]. Some of these drugs can relieve and prevent angina, and some can reduce the risk of heart attack and sudden death. In fact, because of the difference of individual resistant ability and the great variation of individual conditions, the dosage of these drugs should be adjusted appropriately by the routine observation of clinical symptoms.

For diltiazem hydrochloride (DI), the dosage requirements may vary considerably from patient to patient, if a patient fails to respond sufficiently, the plasma drug concentration should be determined to see if the adequate concentration has been attained [3]. For metoprolol tartrate (ME), the individual variation of the serum concentration is great. Thus, the individualized administration of ME through clinical monitoring is important [4]. Some drugs [5] (nitrates, β-blockers, calcium channel blockers, potassium channel openers, antiplatelet agents, and cholesterol lowering agents) are used in the management of angina and are frequently taken in combination. The main reason why these drugs are frequently taken in combination is that it is assumed that a small dose of a second drug is preferable than increasing the dose of the first drug. This allows all drugs to be used in the low dose range that is more likely to be free of side effects. However, the improper combination of isosorbide mononitrate (ISMN), DI, and ME might cause bradycardia and atrioventricular block. So the clinical motoring of these drugs in human serum concentration is important.

Determination of drugs in biological fluids is essential in toxicological and clinical chemistry research because the therapeutic efficacy is related to the concentration of drugs in biological fluids and tissues. The simple and rapid methods for simultaneous analysis of the drugs, which have severe contraindications or possible

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toxicity used concurrently [6,7], are interesting for therapeutic drug monitoring purposes.

A number of liquid chromatography (LC) methods have been developed for analysis of antianginal drugs successfully [8]. The researchers adopted the different methods to quantify the ISMN [9,10]. Siddiqui et al. [11] developed a new assay method for simultaneous analysis of DI, metformin, pioglitazone and rosiglitazone in pharmaceuticals and human serum. García Alvarez-Coque et al. [12–14] built a micellar liquid chromatography (MLC) for determination of β-antagonists and other antihypertensive drugs in pharmaceuticals and urine samples. Soltani and Jouyban [15] developed an MLC method to simultaneously determine furosemide, ME, and verapamil in human plasma. Esteve-Romero et al. [16] determined five antianginal drugs in pharmaceuticals by MLC. Ruiz-Ángela et al. [17] developed submicellar and micellar reversed-phase liquid chromatographic methods to separate β-blockers. In 2010, our group [18] developed a gradient reversed-phase LC (RPLC) method to separate and determine DI, ME and ISMN in human serum simultaneously. However, the gradient elution was too complex and the analysis time was too long (20 min) and consumed more acetonitrile (ACN) in this method. In order to overcome these disadvantages, we established a new method, micellar *per* aqueous liquid chromatography (MPALC), to separate and determine DI, ME and ISMN in human serum simultaneously. The proposed method was also applied to monitor the antianginal drugs in clinical practice successfully.

2. Experimental

2.1. Instruments and reagents

A Dionex UltiMate 3000 HPLC (Ultimate 3000 model system, Dionex, Sunnyvale, USA) consisted of a photodiode array detector (DAD), column compartment, and a 10 μL loop manual injector. System control and data analysis were carried out by the chromatographic workstation Chromeleon Client (Version Chromeleon 680, Dionex Corporation, Sunnyvale, USA). FE20 pH meter (Mettler Toledo Instrument Co., Ltd, Shanghai, China) was used to measure the pH of the mobile phase. A KH-300DB ultrasonic cleaner (Kun Shan He Chuang Ultrasonic Instruments Co., Ltd, Kunshan, China) was employed for controlling ultrasonic cleaning device. AP-01P vacuum pump (Autosci Ence Instruments Co., Ltd, Tianjin, China) was used to filter solution by 0.22 μm nylon membrane. Pinnacle II Cyano column (150 × 4.6 mm, 5 μm, ResTEK, Belleforte, Sunnyvale, USA) was used as the stationary phase.

Chromatographic-grade ACN and methanol (MeOH) were purchased from Yuwang Reagent Factory (Yucheng, China). Sodium dodecyl sulfate (SDS), sodium dihydrogen phosphate (NaH_2PO_4), and 1-propanol were analytical grade and bought from Tianjin Chemical Industrial Company (Tianjin, China). 1-propanol was distilled before use. Distilled water was from the GLP lab of Lanzhou University (Lanzhou, China) and was used to prepare kinds of solution.

Reference standards of ISMN (Batch No. 100694-200401), DI (Batch No. 10161-0102), ME (Batch No. 100084-200101) and pseudoephedrine hydrochloride (PE) (Batch No. 171237-200304)were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPP, Beijing, China). The human serum samples were from The Third People's Hospital of Gansu Province (Lanzhou, China). All human serum samples were stored under freezer at −20 °C until analysis.

2.2. Preparation of mobile phase and standard solutions

The mobile phase consisted of a buffer solution and 1-propanol according to the proportion of the buffer solution/1-propanol (90/10, *v/v*). The buffer solution was prepared by dissolving 3.632 g SDS and 0.9234 g NaH_2PO_4 in 270 mL distilled water, and the pH was adjusted to 7.0 with 0.1 M NaOH solutions.

Individual stock solutions of ISMN, DI, ME and internal standard (IS) were prepared by dissolving each one in distilled water at the concentration of 1.0 mg/mL. All standard stock solutions were stored until use under refrigeration at 4 °C.

2.3. Preparation of standard samples and quality control samples (QC samples)

All standard samples were prepared by dissolving the moderate mixture standard solutions in 500 μL human serum into a standard polypropylene microtube. Final concentrations of the standard samples were 0.02, 0.1, 0.5, 1.0, 2.5 and 5 μg/mL for DI, ME, and ISMN in human serum, respectively.

All QC samples were prepared by dissolving the concentrated mixture standard solutions in about 2500 μL human serum into a standard polypropylene microtube. The concentrations of QC samples in serum were 0.1, 1.0, and 2.5 μg/mL for DI, ME, and ISMN, respectively. Then equally split them to five multiple vials (each vial volume is 500 μL) and then stored them under −20 °C as the clinical samples stored conditions.

2.4. Preparation of the serum samples

Before analysis, the serum sample was thawed to room temperature. In centrifuge tube, an aliquot of 10 μL of the internal standard working solution (1.0 mg/mL) was added to 0.5 mL of collected serum sample followed by the addition of 2 mL of ACN. The tubes were vortex mixed for 10 min. After centrifugation at 10,800 rpm for 10 min, the supernatant was transferred to another standard polypropylene microtube and evaporated to dryness in a water bath at 35 °C under a stream of nitrogen gas. Then the dried extract was redissolved in 100 μL of water.

All preparation solutions were stored at 4 °C and filtered by 0.22 μm nylon membrane filtration prior to injecting to chromatographic system.

2.5. Optimized chromatographic conditions

Separation and determination of ME, DI and ISMN were performed on a Pinnacle II Cyano column as the stationary phase, and a mobile phase consisted of an aqueous solution (4.15×10^{-2} mol/L SDS and 0.02 mol/L NaH_2PO_4) with 10% (*v/v*) of 1-propanol at pH 7.0, running at 0.8 mL/min at 40 °C. The detection wavelength was set at 225 nm. The injection volume was 10 μL.

2.6. Method validation

To evaluate the linearity, the standard samples of DI (0.02–5 μg/mL), ME (0.02–5 μg/mL), and ISMN (0.02–5 μg/mL) were extracted and assayed, and the linearity regression curve for DI, ME and ISMN were calculated by internal standard method. The lower limit of quantification (LLOQ) was defined as the lowest concentration on the calibration curves.

The precision of the method were assessed by determination of the QC samples at different concentrations (0.1, 1.0, and 2.5 μg/mL for DI, ME and ISMN in human serum, respectively), in a single day for the intra-day precision and across three different days for the inter-day precision. The accuracy of the method was determined by injecting the QC samples including concentrations of 0.1, 1.0, and 2.5 μg/mL, respectively.

The extraction recoveries, which are used to evaluate the ability that analytes are extracted from biological samples by pretreatment method, were evaluated by comparing the peak area ratios of