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Determination of five antiarrhythmic drugs in human plasma by dispersive liquid–liquid microextraction and high-performance liquid chromatography

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ABSTRACT

A fast and sensitive high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection was developed and validated for the simultaneous quantitation of five antiarrhythmic drugs (metoprolol, propranolol, carvedilol, diltiazem, and verapamil) in human plasma samples. It involves dispersive liquid–liquid microextraction (DLLME) of the desired drugs from 660 μL plasma and separation using isocratic elution with UV detection at 200 nm. The complete separation of all analytes was achieved within 7 min. Acetonitrile (as disperser solvent) resulting from the protein precipitation procedure was mixed with 100 μL dichloromethane (as an extraction solvent) and rapidly injected into 5 mL aqueous solution (pH 11.5) containing 1% (w/v), NaCl. After centrifugation, the sedimented phase containing enriched analytes was collected and evaporated to dryness. The residue was re-dissolved in 50 μL de-ionized water (acidified to pH 3) and injected into the HPLC system for analysis. Under the optimal conditions, the enrichment factors and extraction recoveries ranged between 4.4–10.8 and 33–82%, respectively. The suggested method was linear ($r^2 \geq 0.997$) over a dynamic range of 0.02–0.80 μg mL^{-1} in plasma. The intra- and inter-days relative standard deviation (RSD%) and relative error (RE%) values of the method were below 20%, which shows good precision and accuracy. Finally, this method was applied to the analysis of real plasma samples obtained from the patients treated with these drugs.

1. Introduction

Cardiovascular diseases (CVDs) are common causes of morbidity and mortality in all countries of the world and are caused by risk factors such as high blood pressure, coronary thrombosis, strokes, and renal failure [1]. Pharmacological treatment can reduce the risk of CVDs. Cardiovascular medications comprise the largest number of drugs that are used in controlling heart diseases [2]. Beta blockers (BBs) and calcium channel blockers (CCBs) are clinically important drugs and are used to treat a multitude of CVDs such as hypertension, angina pectoris, and arrhythmia [3–5]. According to the literature, the combination of a beta-blocker and non-dihydropyridine CCBs such as verapamil or diltiazem is not recommended, because their additive negative effects on heart rate and atrioventricular (AV) conduction may result in severe bradycardia or heart block [6]. Hence, these antihypertensive agents should be used alone. Furthermore, a common situation in optimal therapy is the lack of universal methods for trace level and quantitative drug analysis in a wide variety of sample matrices. Simultaneous determination of several cardiovascular drug groups is, therefore, highly desirable in the cases of intoxication, controlling the therapy compliance of patients, and pharmacokinetic interactions with other drugs (lipid-soluble beta-blockers with calcium antagonists). For these studies, efficient, selective, and reproducible bioassay methods are, therefore, essential in order to effectively monitor levels and to make proper dose adjustments. Different bioanalytical methods have been reported for antiarrhythmic drug determination in biofluids (including human plasma, whole blood, and urine). These methods include conventional high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) [7,8], tandem

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MS/MS [9–11], fluorimetry (FL) [12,13], and ultraviolet (UV), particularly those in a diode-array configuration detection system [14–17]. Moreover, gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and capillary electrophoretic (CE) methods [18–20] have also been used. GC–MS analysis for the less or semi-volatile analytes (such as BBs and CCBs) is often accomplished after derivatization to improve their gas chromatographic behavior [21,22]. This step not only complicates the analysis but also increases the time required to analyze these drugs. CE methods suffer from a few problems when compared with HPLC for therapeutic drug monitoring (TDM). The major problem in drug analysis by CE is the sub-optimal detection limits, especially for analysis of drugs that tend to be present in a low concentration [23]. Unlike HPLC, CE is greatly affected by the sample matrix that include salts and proteins which can affect the enhancement of analyte detection in stacking methods [24]. In addition, injection repeatability in CE is generally not as good as that of HPLC. Therefore, improving precision in order to get reproducible data is necessary [25]. However, many of these problems can be solved by using HPLC methods, but most of the published HPLC assays are associated with tedious and time-consuming extraction steps. Hence, for TDM studies, a simple and fast procedure is preferred. Liquid–liquid extraction (LLE) [26], solid-phase extraction (SPE) [27], and protein precipitation (PPT) [28] are the main extraction techniques used to monitor levels of antiarrhythmic drugs in plasma, serum, and urine samples. In addition, salt-assisted liquid–liquid extraction (SALLE) [29], microwave-assisted liquid–liquid extraction (MALLE) [30], solid-phase microextraction (SPME) [31], hollow fiber-liquid phase microextraction (HF-LPME) [32], carrier-mediated liquid phase microextraction (CM-LPME) [33], and exhaustive electromembrane extraction (EME) [34] have also been reported. Recently, some DLLME-based methods combined with spectrofluorimetry [35], HPLC–UV [36,37], CE–MS [38], and FASS–CE–DAD [39] have been reported for the determination of these drugs in human plasma and urine. It should be noted that these methods except the last method were developed for determination of individual drug concentration. In our previous work, low sensitivity limitation associated with CE was improved through the use of hybrid preconcentration methods. LLE is time consuming, labor intensive and requires relatively large quantities of toxic solvents [40]. In most cases, the resulting extract is evaporated to dryness and the residue is reconstituted with a suitable solvent before analysis. SPE is a very popular technique for rapid and selective sample preparation that involves multiple steps such as sorbent conditioning, sample application, washing, and elution. In addition, an extra step, solvent exchange, may be required to preconcentrate the analytes further into smaller volumes [41]. SPE has several advantages when compared with the other sample preparation techniques, such as PPT and LLE. It can be easily automated, gives more efficient separation of interferences from analytes, reduces organic solvent consumption, and is more efficient in analyte recovery. Nevertheless, potential variability of SPE packing, irreversible adsorption of some analytes on SPE cartridges, and more-complex method development are some of the drawbacks of this technique [42]. In order to overcome these problems, microextraction-based techniques can be considered an alternative to the conventional extraction method but all of them have some limitations. In generally, solvent microextraction is defined as an equilibrium-based non-exhaustive sample preparation technique compared with LLE and SPE [43]. In this technique, absolute recoveries are low due to only a portion of the analytes present in the samples that are extracted. In addition, some of these methods require a longer equilibrium time in extraction as compared with DLLME. Thus, DLLME not only reduces equilibration time but also increases the sample throughput within a working day. In this study, the applicability of the DLLME method as an efficient microextraction technique for bioanalysis was assessed. This method was used for analysis of three BBs (metoprolol, propranolol, and carvedilol) and two CCB drugs (diltiazem and verapamil) in human plasma samples. Different factors affecting the DLLME procedure such as type and volume of extraction and dispersive solvents, ionic strength, and sample pH were investigated and optimized. Finally, the developed method was validated according to the Food and Drug Administration (FDA) bioanalytical method validation and then used for the determination of the analytes in real plasma samples. To date, to the best our knowledge, there are no reported studies on the use of DLLME method for simultaneous determination of five antihypertensive agents in plasma samples.

2. Experimental

2.1. Reagents

The reference substances of the studied drugs were kindly supplied by the following Iranian Pharmaceutical Companies: metoprolol by Alborz Darou, propranolol hydrochloride by Rouzdarou, carvedilol by Salehan Chemi, and diltiazem and verapamil by Darou Pakhsh. Molecular structures, log P, p*K*a values, and therapeutic levels of these drugs are reported in Table 1. Acetonitrile (HPLC grade) and methanol, ethanol, acetone, chloroform, dichloromethane, and carbon tetrachloride (analytical grade) were obtained from Scharlau (Barcelona, Spain). Analytical-grade sodium dihydrogen phosphate, sodium chloride, hydrochloric acid (37%, $d=1.18$ g mL^{-1}), and sodium hydroxide were obtained from Merck Company (Darmstadt, Germany). Deionized water was purchased from Shahid Ghazi Pharmaceutical Company (Tabriz, Iran) and was used in this study.

2.2. Preparation of solutions

Individual stock standard solutions (1000 mg L^{-1}) and mixed standard solution (10 mg L^{-1}) of the studied drugs were prepared in methanol. These solutions were stored in a refrigerator at 4 °C for two weeks and used to prepare working solutions in acidified water (pH 3.0). Drug-free (blank) human plasma samples were used for DLLME optimization and method validation of the proposed method, and they were obtained from healthy donors (Iranian Blood Transfusion Research Center, Tabriz, Iran). Human plasma matrix lots were aliquoted into polypropylene microtubes and stored in a freezer at −20 °C. Plasma sample standards that included the therapeutic plasma levels of the studied drugs were prepared daily by dilution of appropriate amounts of the mixed standard solution with the blank plasma. The exact concentrations are found in the text or figures. In addition, real blood samples were collected from cardiac patients who had signed consent forms approved by the ethics committee, Tabriz University of Medical Sciences. These were collected in heparinized tubes and centrifugated (3000 rpm, for 10 min) immediately after collection, and the plasma was separated and stored at −20 °C until assay.

2.3. Chromatographic conditions

HPLC–UV analyses were performed using an Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an isocratic pump, a manual sample injector with a 20.0 μL loop (Rheodyne, USA), and a UV variable wavelength detector (VWD). Separations were performed at room temperature on an Agilent ZORBAX Eclipse column (4.6 mm × 100 mm, 3.5 μm particle size) preceded by a guard column (Waters, Milford, PA, USA). ChemStation version B.04.02 (Waldbronn, Germany) was used to