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Comparison of counter-current chromatography and preparative high performance liquid chromatography applied to separating minor impurities in drug preparations

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ABSTRACT

Drug impurity profiling and identification are carried out along with the drug discovery process. Due to its inherent low concentration in drug products, the isolation and purification of impurities present a challenge to drug development processes. In our development of honokiol and quercetin as anticancer drug candidates, counter-current chromatography (CCC) and preparative HPLC were used for the impurity profiling and identification of honokiol and quercetin. Several performance parameters such as separation column volume, maximum sample loading, separation time, solvent consumption and sample throughput were investigated in order to compare the separation efficiency. We found that the sample loading capacity and therefore the throughput of preparative HPLC were not satisfactory, while CCC provided larger sample loading (especially for a sample with poor solubility), consumed less solvent and produced higher throughput than preparative HPLC. Six impurities of honokiol including one new compound were isolated in the present work.

1. Introduction

Impurity means any component of the drug substance that is not the chemical entity defined as the drug substance [1]. The identification and quantification of impurities in active pharmaceutical ingredients (APIs) and pharmaceutical products are very important in the drug discovery process [2]. Impurities in APIs are of significant concern. Their activity might be responsible for eventual undesirable side effects or toxicity and even interfere with the drug's activity. Thus, monitoring impurities in APIs and drug products is a prerequisite to ensure drug safety and quality. In order to understand the complete impurity profile and to check the characterization of analytical performance, reference compounds are in great need. However, isolation of impurities presents a challenge to drug development processes due to their inherent low concentrations. Chromatographic methods with high sample capacity, good sample recovery, minor irreversible adsorption and excellent resolution are strongly suggested in the application of impurity separation.

Preparative HPLC developed in the 1970s is a powerful tool to isolate and purify compounds with excellent efficiency and high recovery [3]. Preparative HPLC has become a standard tool in the pharmaceutical industry and been widely used in impurity separation [4–6]. CCC is a support-free liquid–liquid partition chromatography technique, with a long history of effective application in the area of natural products isolation [7]. Due to the absence of solid stationary phase, adsorption losses are minimized and hence an apparently 100% sample recovery is guaranteed [8]. In particular, CCC has been increasingly used to isolate and purify the impurities of APIs and drug products [6]. Recently, Weisz et al. successfully separated impurities from the color additive D&C Green No. 8 by pH-zone-refining method of CCC [9]. Chen et al. separated and purified three impurities of sodium tanshinone IIA sulfonate in a one-step isolation with CCC [10].

However, very few comparative studies for impurity separation by CCC and preparative HPLC have been reported. In our present studies of developing honokiol and quercetin into anticancer drug candidates, the impurity separations of these two drug candidates were performed by two methods independently. The performance

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parameters such as separation column volume, flow rate, mobile phase, separation mode, maximum sample loading, injection volume, separation time, solvent consuming and sample throughput were all investigated and discussed. The separation efficiency of CCC and preparative HPLC were further compared.

2. Experimental

2.1. Apparatus

The Midi-DE HPCCC (high performance counter-current chromatography, Dynamic Extractions Ltd., Slough, UK) employed in this paper had two coils on two bobbins all integrated in one machine. The preparative columns used a 4.0 mm ID polyfluoro alkoxy (PFA) tubing with volumes for coils 1 and 2 being 500.5 and 499.5 ml, respectively. The β value ranges were 0.52–0.86. A SH150-2100 constant temperature regulator (LabTech Ltd., Beijing, China) was used to control the separation temperature. The Midi-DE HPCCC system was equipped with HPLC Pump 1800 (Knauer, Berlin, Germany) and was monitored by a Smartline UV 2500 detector (Knauer, Berlin, Germany). EuroChrom®2000 basic version 3.05 (Knauer, Berlin, Germany) was used as the workstation.

Preparative HPLC separations were performed on a PACK-N-SEP™ dynamic axial chromatographic column: LC50.340.VE100 PS TH (I.D. 50 mm, length 340 mm, NovaSep, Pompey, France). Packing material was ODS (*S*—10 μm, YMC co., Ltd., Japan). The column yielded a bed volume of 625 ml and void volume of 212 ml. The preparative HPLC system was equipped with HPG500 Pump (Sunyear Scientific Inc., Shanghai, China) and was monitored by a Smartline UV 2500 detector (Knauer, Berlin, Germany). Calesep workstation version 2.22 was used as the workstation (Sunyear Scientific Inc., Shanghai, China).

Analytical HPLC was performed on a Waters 2695 Separations Module equipped with a Waters 2996 phodediode array detector and Empower workstation software (Waters, Milford, MA, USA). The chromatographic column was a SunFire C_{18} (150 mm × 4.6 mm I.D., 5 μm) (Waters, Milford, MA, USA).

2.2. Reagents and materials

All the organic solvents used for CCC were of analytical grade and purchased from Chengdu Changzheng Chemical Factory, Sichuan, China. Reverse osmosis Milli-Q water (18 MX) (Millipore, Bedford, MA, USA) was used for all the solutions and dilutions. Methanol and acetonitrile used for analytic and preparative HPLC were chromatographic grade and purchased from Fisher Chemical (Loughborough, UK)

Two drug candidates of honokiol and quercetin were used for the impurities investigation. Honokiol was initially isolated from the *Magnolia officinalis* Rehd. Et Wils by silica gel column chromatography and crystallized with heptane. The original purity of honokiol was 99.13% measured by analytical HPLC. Forty grams of honokiol was stored at room temperature in open environment for two years for the stability testing. The honokiol crystal changed from white to red due to the impurities. The purity was reduced to 86.77% (see Figs. 1 and 2). To investigate the impurities of honokiol, we used preparative HPLC and CCC for the enrichment and purification of impurities derived from honokiol.

Quercetin was obtained by hydrolysis with hydrochloric acid of rutin at 90 °C according to reference [11]. Its purity was 97.12%. Two impurities were indentified, by comparing with the reference substances, as kaemferol and isorhamnetin (see Fig. 3).

2.3. CCC procedure

2.3.1. Preparation of the two-phase solvent system and sample solution

The *n*-hexane–ethyl acetate–methanol–water (HEMWat) phase systems at various volume ratios were thoroughly equilibrated in a separatory funnel at room temperature. The two phases were separated into organic and aqueous phases just before the experiments.

The sample solutions for Midi-DE HPCCC were prepared by dissolving solute in the lower phase of the solvent system.

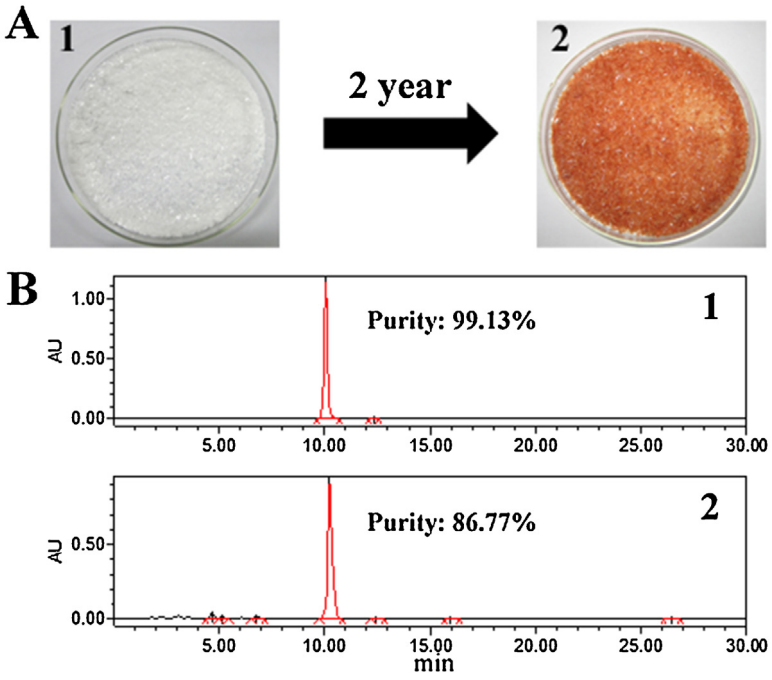

Fig. 1. The detection of honokiol sample. (A) Appearance change of honokiol. (B) Purity descend of honokiol sample. Column: SunFire C18 column (150 × 4.6 mm I.D., 5 μm, Waters, USA); mobile phase: acetonitrile–0.1% formic acid (acetonitrile: 0–25 min, 45–95%); flow rate: 1 ml/min; wavelength: 254 nm.