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Two-dimensional preparative liquid chromatography system for preparative separation of minor amount components from complicated natural products

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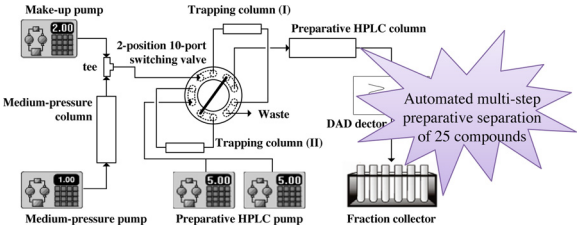
HIGHLIGHTS

- Preparative MDLC system was developed for separation of complicated natural products.
- Medium-pressure LC and preparative HPLC were connected by interface of SPE.
- Automated multi-step preparative separation of 25 compounds was achieved by using this system.

GRAPHICAL ABSTRACT



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ABSTRACT

An on-line comprehensive two-dimensional preparative liquid chromatography system was developed for preparative separation of minor amount components from complicated natural products. Medium-pressure liquid chromatograph (MPLC) was applied as the first dimension and preparative HPLC as the second one, in conjunction with trapping column and makeup pump. The performance of the trapping column was evaluated, in terms of column size, dilution ratio and diameter-height ratio, as well as system pressure from the view of medium pressure liquid chromatograph. Satisfactory trapping efficiency can be achieved using a commercially available 15 mm × 30 mm i.d. ODS pre-column. The instrument operation and the performance of this MPLC × preparative HPLC system were illustrated by gram-scale isolation of crude macro-porous resin enriched water extract of *Rheum hotaoense*. Automated multi-step preparative separation of 25 compounds, whose structures were identified by MS, ^{1}H NMR and even by less-sensitive ^{13}C NMR, could be achieved in a short period of time using this system, exhibiting great advantages in analytical efficiency and sample treatment capacity compared with conventional methods.

1. Introduction

Natural products have been a major resource for the investigation of naturally-occurring biologically active substances. The traditional way of studying natural products, in most cases, is pretreatment and fractionation of a complex matrix, separation and isolation of the individual components using repeat liquid chromatography, in an off-line mode. The off-line approach is very easy but presents several disadvantages: it is time-consuming, operationally intensive, and difficult to automate and to reproduce. Moreover, sample contamination or formation of artifacts can occur [1]. Techniques developed in recent years for the purpose of

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analysis and structure elucidation, such as HPLC-SPE-NMR [2] and on-line multidimensional liquid chromatography (MDLC) [3], have shown a significant acceleration in the isolation and identification of natural products.

MDLC, usually refers to two-dimensional liquid chromatography (2D-LC), has played an important role in complex sample analysis. 2D-LC combines two independent columns with different separation mechanisms in tandem mode. Depending on whether components can be eluted from the first dimension into the second dimension directly or not, the methods can be classified as on-line [4–6] or off-line [7–9], respectively. Furthermore, depending on whether first dimensional components are eluted into the second dimension completely or not, the methods can be classified as comprehensive [10] or heart cutting [11], respectively. On-line comprehensive 2D-LC is an ideal mode for the analysis of complex samples. Automatic performance in the on-line mode avoids sample contamination, deterioration and personal error, and comprehensive separation enables maximum information to be obtained. Such separation systems have been extensively used to increase the number of compounds separated in complex samples encountered in proteomics, analysis of biopolymers and synthetic polymers, pharmaceutical and environmental analysis and in the analysis of naturally-occurring compounds [12–14].

Nevertheless, several problems occurred when MDLC is used. First, the term "analysis" is added as constraint to the MDLC definition. Preparative separations, with a goal of isolating material, were suggested to be excluded from the definition, because it was considered not providing analytical information at each step [15]. Secondly, substantial amounts of analytes are required in many cases while MDLC system used presently can not meet the demands. For example, ^{13}C NMR and hyphenated two dimensional (2D) experiments (like heteronuclear multiple bond correlation – HMBC), which are highly important for elucidation of structures of novel compounds, still require at least 100 μg of analytes, even if using HPLC-SPE-NMR system or CapNMR probe [16]. The total injected amount of sample on the HPLC column should be in the range of a few decade-milligrams (10–100 mg). Normally an analytical MDLC system cannot handle such amounts of sample. Moreover evaluation of the efficacy and safety of nature products, which are a central issue for the lead compound investigators and pharmaprojects researchers, always demands one to provide adequate amount of individual pure compound in gram-grade.

Interface techniques, including trapping columns [17], sample loops [18], parallel columns [4] and vacuum solvent evaporation [19], were developed as the application of on-line comprehensive MDLC. Trapping columns are used in liquid chromatography to trap samples eluted from the first dimension under the first dimensional mobile phase. The samples trapped by trapping columns are eventually eluted by the mobile phases of the second dimension. Trapping columns can delay the elution of samples from the first dimension to the second dimension. Switching the mobile phase on the trapping column can avoid incompatibility between two-dimensional mobile phases. Thus, trapping column interface could be valuable to overcome the above-mentioned problems [20].

We report here an on-line comprehensive medium-pressure liquid chromatography (MPLC) × preparative HPLC system for preparative separation of natural products, where a medium-pressure column was applied as the first dimension and preparative HPLC column as the second one, interfaced by a makeup pump and a 10-port switching valve in conjunction with two reversed-phase trapping columns (Fig. 1). After evaluation in terms of column size, dilution ratio and trapping volume, as well as flow rate and system pressure from the view of medium pressure liquid chromatograph, 15 mm × 30 mm i.d. column packed with ODS was selected as trapping column. In this case, analytes present in the MPLC elution bands are captured online onto the trapping column. The analytes retained on the trapping column are then eluted into the preparative HPLC column process with the corresponding mobile phase. Higher sample processing capability was provided by the 1st dimension MPLC; meanwhile, separation ability was preserved by using a 250 mm × 20 mm i.d. preparative HPLC column as the 2nd dimension. Macro-porous resin enriched water extract of *Rheum hotaoense* L. was applied as model complex mixture to evaluate the operation and performance of this MPLC × preparative HPLC system. A practical procedure for selection of isolating condition was established, regarding the factors of sample weight, 1st dimensional medium-pressure column size, trapped volume of elution and preparative HPLC column volume. This MPLC × preparative HPLC integrated system was successfully applied in automated multi-step preparative separation of 26 compounds, 25 of which were identified by MS, ^{1}H NMR and even by less sensitive ^{13}C NMR, from the macro-porous resin enriched water extract of *R. hotaoense*, exhibiting great advantages in analytical efficiency

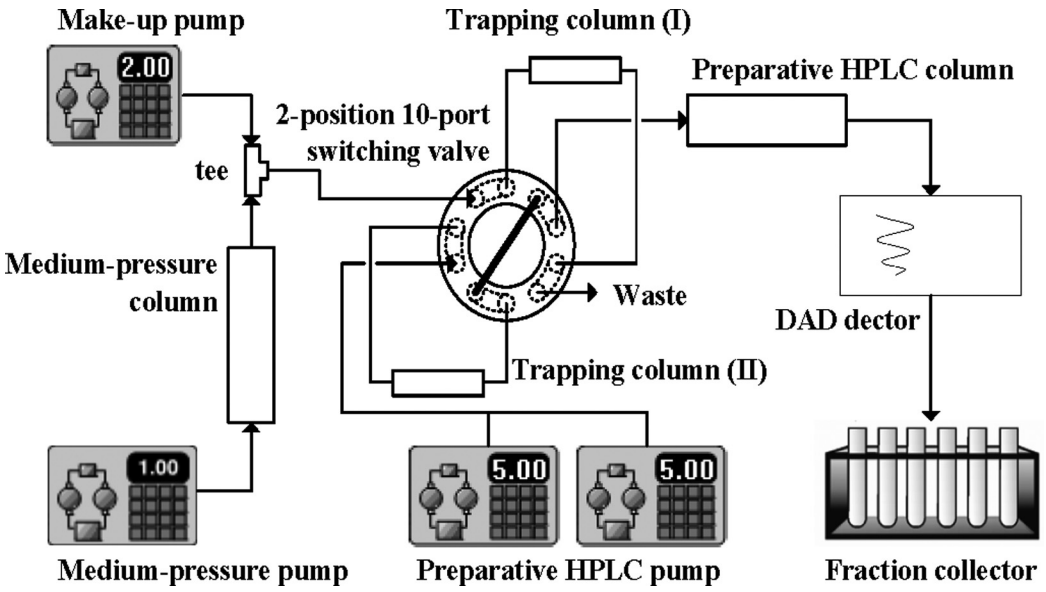

Fig. 1. Scheme for MPLC × preparative HPLC system.