On-Line Sample Treatment LC System for MS Compatibility

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Abstract

In the present work, a new liquid chromatography–mass spectrometry (LC–MS) system with on-line pretreatment using column switching and a dilution function was developed. This system can be used under conventional high-performance liquid chromatography (LC) separation conditions, including mobile phases containing phosphate buffer. The built-in autodilution function greatly improves the trapping efficiency for target compounds, followed by desalination that optimizes the ionization conditions for MS analysis. This fully automated two-dimensional LC system interfaced with mass spectrometry provided a powerful tool for the determination of impurity profiles in pharmaceutical research and the identification of traditional Chinese medicine in natural products.

Keywords

Column liquid chromatography–mass spectrometry
On-line sample pretreatment
Natural products
Coptidis Rhizoma

Introduction

Liquid chromatography–mass spectrometry (LC–MS) is now a widely used analytical technique, specially as a powerful tool for analyzing small analytes such as drugs in biological samples and for studying impurity profiles in phar-

maceutical research and identifying compounds in natural products research [1, 2]. Phosphate buffer was used widely to improve chromatographic performance in liquid chromatographic applications because of its wide buffering range and lack of UV absorbance at short wavelengths. However, many

problems arise when phosphate solutions as conventional buffer solutions are used in the LC–MS mobile phase because phosphate buffer results in ion suppression and salt precipitation in the ion source [3]. Generally, volatile buffers were used and replaced phosphate buffers for LC–MS, which lead analytical conditions to be changed and optimized once again when MS is used for detection instead of UV. When phosphate buffer was necessary for separation, it is invalid. Thus, the LC–MS system must be improved to satisfy various requirements.

During the last decade, many high-performance liquid chromatography (LC) systems with automated sample pretreatment have been reported to enhance analytical productivity [4–8]. Most of these techniques mainly concerned a sample concentration for enhancement of detection, such as on-line solid phase extraction (SPE) coupled to LC–MS technologies [9], which is advantageous in terms of accuracy, reproducibility, reliability, and capacity for multi-components determination. The automated sample pretreatment system reported here was composed of column switching and a dilution function, which not only on-line preconcentrated the analytes to improve the limit of detection of

Fig. 1. Schematic diagram of the LC-MS system

analytical methods but also allowed the use of conventional LC analytical conditions such as phosphate buffers for LC-MS analysis because of the function of salts removal. In a word, the new valve-switching LC-MS system improved MS compatibility and was convenient for identifying compounds utilizing virtues of MS, since conventional LC conditions based on phosphate buffer can be utilized. In addition, a dilution function that enhances trap efficiency was also discussed and applied to analyze traditional Chinese medicine (TCM) extracted from a Chinese herb.

Experimental

Solvents and Reagents

LC-grade water was obtained by purification of de-ionized water through a Milli-Q system (Waters, Bedford, MA, USA). Methanol and acetonitrile (LC grade) were purchased from Fisher (Fair Lawn, NJ, USA). Diethylether and methanol for sample preparation were analytical grade from Beijing Chemical Reagents (Beijing, China). All reagents (analytical grade) and caffeine standard were purchased from Wako (Osaka, Japan). Coptidis Rhizoma samples were purchased from a local pharmacy in Beijing.

Sample Preparation

A sample of 1.0 g of Coptidis Rhizoma was weighed into a 50-mL conical flask and 10 mL diethyl ether was added. The

sample was vigorously mixed on a Vortex mixer for 10 min and then allowed to stand for 10 min before filtering through filter paper (Advantec, Tokyo, Japan). The residue was collected and transferred to a 50-mL conical flask and extracted with 10 mL ethanol. The solution was again vigorously mixed by vortexing for 10 min and left to stand for 10 min. The supernatant was then filtered through a 0.45- μ m membrane filter.

Instrumentation and Conditions

The LC-MS system consisted of three LC-20AD pumps, an LC-20AB gradient pump, two DGU-20A3 degassers, an SIL-20AC auto-sampler, a CTO-20A column oven, an SPD-20A UV detector and an LCMS-2010EV single quadrupole MS analyzer equipped with an ESI ionization interface (Shimadzu, Kyoto, Japan). The ionization interface can be operated in positive ion mode. Data acquisition and processing were performed on an LCMS-Solution Version 3.0 workstation. Chromatographic separation was performed on a Shim-pack VP-ODS column (5 μ m, 4.6 mm i.d. $\times$ 250 mm) from Shimadzu. A linear gradient of A (20 mmol L⁻¹ phosphate buffer, pH 2.6, 100 mmol L⁻¹ sodium per chlorite) and B (acetonitrile) was used for elution as follows: first, 80% (v/v) A hold for 15 min, then, the content of A was decreased to 10% and held for 5 min, finally, the content of A was increased to 80% again and held for 5 min. The flow rate was 1.0 mL min⁻¹ and the column temperature was set at 40 °C. The sample

injection volume was 10 μ L and the UV detection wavelength was set at 260 nm. The ESI conditions were as follows: positive ion mode, drying with N₂ at 4 L min⁻¹, heater block temperature 200 °C, CDL temperature 250 °C, detector voltage 1.5 kV, scan range 50–750 amu.

A flow diagram of the LC-MS system is shown in Fig. 1. A 250- μ L sample loop installed in switching valve 1 collects the target compound separated under phosphate buffer conditions. The analyte together with phosphate buffer in the sample loop was transferred to the sample trap column (Shim-pack GVP-ODS, 5 μ m, 4.6 mm i.d. $\times$ 10 mm, from Shimadzu), attached to switching valve 2 using 0.1% aqueous formic acid. To enhance the trap efficiency of the sample trap column, an additional pump was added for dilution of the sample loop solvent. In most cases, the flow rate of the sample introduction and dilution pumps was set at 0.4 and 3.6 mL min⁻¹, respectively. As a result, the target compound in the phosphate buffer-containing organic solvent was diluted ten-fold by aqueous solution prior to delivery to the trap column. Therefore, the efficiency of sample trapping or focusing on the column was significantly increased [10–12]. Since aqueous solution is used to trap the target compound, phosphate buffer can easily be removed and shunted to waste. A desalting time of 10 min was employed. Finally, the target compound was eluted from the sample trap column using methanol containing 0.1% formic acid delivered by the sample elution pump (0.3 mL min⁻¹) to the MS detector for identification. This system has the advantage of selecting the most suitable solvent composition for optimal ionization of analytes for MS detection.

Results and Discussion

Trap Efficiency

The effect of the injection sample volume on column performance is shown in Fig. 2. Several different standard solutions were prepared in a combination of water and methanol using 100 μ g of

caffeine as the standard. When using solvents with strong elution capability, such as 100% methanol, the performance of the column significantly decreased with increasing injection volume. On the other hand, column performance was maintained using solvents with weak elution capability such as pure water, regardless of the injection volume. However, the increase in volume led to a deterioration of the peak shape. Therefore, a sample solvent with weak elution capability (less organic) such as nearly 100% water had to be used to increase the trap efficiency. The dilution pump delivers 0.1% aqueous formic acid to dilute the trapped sample inside the sample loop. The dilution factor is normally 10, so although the trapped sample is in 100% methanol, during sample transfer, this is diluted to 10% methanol, resulting in much better trap efficiency which is critical for this application.

Desalination Efficiency

Desalination is often used for bioanalysis of proteins [13]. The solution eluted from the LC-MS inlet pipe was fractionated to analyze the residual phosphate ion. Ion chromatography results showed that the concentration of PO_4^{3-} is 2.064 mg L^{-1} . In this case, 50 mmol L^{-1} phosphate buffer, pH 2.5 (equivalent to $5,000 \text{ mg L}^{-1}$ phosphate ion) was used as the separation mobile phase. The 2.064 mg L^{-1} residual phosphate ion concentration indicates 99.95% removal efficiency.

Application

The method was used for analysis of a real sample, *Coptidis Rhizoma*, which contains several active ingredients. A chromatogram of *Coptidis Rhizoma* is shown in Fig. 3. Since berberine is the main ingredient, the main peak is supposed to be berberine [14]. Four small peaks in front of main peak are supposed to be jateorrhizine, epiberberine, coptisine and palmatine. To confirm the identification of these compounds, each peak was fractionated. Fractions eluting at 9.56–9.81, 9.72–9.97, 9.94–10.19,

Fig. 2. Effect of sample volume on column performance (Mobile phase: solid line, 100% water; dashed line, 30% water/70% methanol (v/v); dotted line, 100% methanol)

Fig. 3. Chromatogram of a *Coptidis Rhizoma* sample. Peaks: 1 = jateorrhizine; 2 = epiberberine; 3 = coptisine; 4 = palmatine; 5 = berberine

10.69–10.94 and 10.90–11.15 min were collected.

Based on molecular weight information (see Fig. 4) in MS spectra, peaks 1–5 might be considered to be jateorrhizine, epiberberine, coptisine, palmatine and berberine, respectively.

A carry-over effect was evaluated by injecting $10 \mu\text{L}$ ethanol after every sample injection, since ethanol has relatively strong elution capability. Therefore, if the contaminant remains in the system, the contaminant peak can be easily observed as a carry-over evaluation. In addition, methanol (containing 0.1% formic acid) was used for washing the pre-column. No carry-over peaks were observed. Since this system allows the

injection of $10 \mu\text{L}$ of sample compared to the usual $2 \mu\text{L}$ for LC-MS injection, a five times better sensitivity was easily achieved. However, peak fractionation directly affects quantitative results and therefore, this system may not be suitable for a quantitative study.

Conclusions

A new LC-MS system with automated on-line sample pretreatment using column switching for multi-dimensional LC separation and desalination as well as dilution to optimize conditions for MS analysis were evaluated. This system allows automated analyte/impurity

	MW	R ₁	R ₂	R ₃	R ₄
Berberine	335	-O-CH ₂ -O-		OCH ₃	OCH ₃
Epiberberine	335		OCH ₃	-O-CH ₂ -O-	
Coptisine	319	-O-CH ₂ -O-		-O-CH ₂ -O-	
Jateorrhizine	337	OH	OCH ₃	OCH ₃	OCH ₃
Palmitine	351	OCH ₃	OCH ₃	OCH ₃	OCH ₃

Fig. 4. Molecular structure of the main ingredient in *Coptidis Rhizoma*

separation, enhanced trapping and optimization of ionization conditions for target components. This is a useful instrument configuration for studying impurity profiles which is critical in pharmaceutical research, and for the identification of compounds in natural products research which is important as a tool in drug discovery.

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