Optimising speed and selectivity for the fast separation of sulphonamides and trimethoprim by applying a simultaneous solvent and temperature gradient

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1 Introduction

In conventional liquid chromatography temperatures between 30 °C and 60 °C are usually applied for the separation of analytes. In contrast to this high temperature liquid chromatography is a relatively new variant of liquid chromatography, where both the mobile and stationary phase is heated up to 200 °C. Due to a significant decrease of the viscosity of the mobile phase at high eluent temperatures, the system back-pressure decreases. Furthermore, at higher temperatures the mass transfer of the analytes between the mobile and stationary phase is enhanced, so that higher flow rates can be applied without a loss in efficiency. Due to the decreased back-pressure it is now possible to use columns with smaller particles (sub-2 µm) at higher flow rates without the need of a special HPLC system. In this study the separation of a mixture of eight sulphonamides and trimethoprim was performed by applying a simultaneous solvent and temperature gradient. A conventional liquid chromatography system could be used with a column containing 1.8 µm particles.

2 Experimental

2.1 System set up

The HPLC separations were carried out on an Agilent 1100/1200 HPLC system consisting of a G1312A binary pump, a G1315A degasser, a G1313A autosampler and a G1315C diode array detector (Agilent, Waldbronn, Germany). For data acquisition and analysis, the Agilent ChemStation for 3D LC software (version B.01.03) was used. A Zorbax StableBond C8 column (50 x 3.0 mm, 1.8 µm) was used. HPLC solvents (Promochem, Wesel, Germany) were of HPLC grade. For MS measurements a Qtrap 3200 mass spectrometer (Applied Biosystems, Darmstadt, Germany) and a Unique MS (LECO, Münchenbergbach, Germany) were used. For column heating a prototype column oven (SIM GmbH, Oberhausen, Germany) was employed. The heating system was developed for high temperature liquid chromatography and consists of three modules, which can be independently controlled. The heating range of this system extends from 15 °C to 225 °C with maximum heating rates of 40 °C/min. This system can be used for isothermal and temperature programmed operations and is described in detail elsewhere [1]. To keep the mobile phase in the liquid state, even at high temperatures, a 500 psi back pressure regulator (GermananalyseTechnik, Bremerhaven, Germany) was connected behind the UV.D

Figure 1 shows the coupling of the high-temperature liquid chromatographic system with the mass spectrometers.

3 Results and Discussion

3.1 Separation of sulphonamides and trimethoprim with UV DAD

Eight sulphonamides and trimethoprim were separated in less than 2 minutes. Due to the low viscosity and thus lowered system pressure at the starting temperature of 70 °C, the flow rate could be adjusted to 1.4 mL/min resulting in a very fast separation. The overall system pressure was below 400 bar, therefore, the separation can be easily transferred to any commercially available HPLC system.

3.2 Separation of sulphonamides and trimethoprim with MS/MS

Figure 2 shows the TOF-MS chromatogram of the separation of eight sulphonamides and trimethoprim. As was anticipated, the scan rate of the mass spectrometer was high enough to perform an accurate data analysis and quantification. This is underlined by the insertion of the selected peak in figure 4.

4 Conclusions

It could be shown that very fast separations can be achieved on a standard HPLC system using elevated temperature liquid chromatography. This has been demonstrated for the separation of eight sulphonamides and trimethoprim using a silica-based reversed-phase stationary phase. No loss in efficiency was observed for this column during the completion of this study. The fast chromatographic resolution was obtained when a simultaneous solvent and temperature gradient was applied. In this study, the hyphenation of the high-temperature liquid chromatography system with a UV diode array detector, a Q Trap mass spectrometer and a time-of-flight mass spectrometer was realised. The problem we encountered was that an accurate quantitation of the narrow peaks could not be made using the Qtrap 3200 mass spectrometer in MRM mode due to the limited mass scan rate. In contrast to this, when using a Time-of-Flight mass spectrometer approximately 200 scans per second were obtained. Therefore, when developing fast LC methods the suitability of the detection system is of utmost importance.

5 Acknowledgements

LECO Instrumente GmbH, Münchenbergbach, Germany, for the measurements on the time of flight mass spectrometer is gratefully acknowledged. The financial support by the German Federation of Industrial Cooperative Associations Otto von Guericke e. V. (Pro Inn o II), project number KP008704FPRO and the Working Committee Separation Science in the German Chemical Society (DDCh) is gratefully acknowledged. The Agilent 1100 HPLC system was provided by Scientific Instruments Manufacturer GmbH (SIM) for the duration of this study and is also gratefully acknowledged.

6 Literature


Figure 1: Coupling of an Agilent 1100/1200 HPLC system with a Qtrap 3200 mass spectrometer (left) and a time of flight MS (right).

Figure 2: Chromatogram of the separation of eight sulphonamides and trimethoprim with (a) simultaneous solvent and temperature gradient, (b) isothermal at 70 °C and solvent gradient, (c) isothermal at 90 °C and solvent gradient. Chromatographic conditions: Column: Zorbax StableBond (50 mm x 3 mm, 1.8 µm), flow rate: 1.4 mL/min, eluent A: acetonitrile + 0.1 % formic acid, eluent B: deionised water + 0.1 % formic acid, eluent gradient: 7 % A to 15 % A in 2.5 minutes, temperature gradient: 70 °C to 90 °C in 2 minutes, detector: UV DAD at 270 nm.

Figure 3: Chromatogram of the separation of eight sulphonamides and trimethoprim with simultaneous solvent and temperature gradient. Chromatographic conditions: Column: Zorbax StableBond (50 mm x 3 mm, 1.8 µm), flow rate: 1.4 mL/min, eluent A: acetonitrile + 0.1 % formic acid, eluent B: deionised water + 0.1 % formic acid, eluent gradient: 7 % A to 15 % A in 2.5 minutes, temperature gradient: 70 °C to 90 °C in 2 minutes, detector: MS/MS in MRM mode, source: ESI in positive mode, dwell time: 5 msec, Q1 cut-off; Q1: 100, Q2: 760, TOF: 790 C.

Figure 4: Chromatogram of the separation of eight sulphonamides and trimethoprim using a silica-based reversed-phase stationary phase. Chromatographic conditions: Column: Zorbax StableBond (50 mm x 3 mm, 1.8 µm), flow rate: 1.4 mL/min, eluent A: acetonitrile + 0.1 % formic acid, eluent B: deionised water + 0.1 % formic acid, eluent gradient: 7 % A to 15 % A in 2.5 minutes, temperature gradient: 70 °C to 90 °C in 2 minutes, detector: MS/MS in MRM mode, source: ESI in positive mode, dwell time: 5 msec, Q1 cut-off; Q1: 100, Q2: 760, TOF: 790 C.