Optimization of Jet Stream ESI Parameters When Coupling Agilent 1260 Infinity Analytical SFC System with Agilent 6230 TOF LC/MS

Technical Overview

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Abstract

Supercritical Fluid Chromatography (SFC) with Mass Spectrometric detection (SFC/MS) is suitable for a broad range of applications. In this Technical Overview, the Agilent 1260 Infinity Analytical SFC system was coupled to an Agilent 6230 Time-of-Flight LC/MS (6230 TOF). The Agilent Jet Stream ESI was used as an ion source. It is compatible with the SFC system, and, using a super-heated nitrogen sheath gas that collimates the nebulizer spray, results in higher MS sensitivity. The main ion source parameters, such as temperature and flow rate of the drying gas and sheath gas, as well as nebulizer pressure and capillary voltage, were optimized, showing the relationships among the parameters. Of all the parameters analyzed, drying gas temperature and drying gas flow rate had the largest impact on MS sensitivity, leading to a significant (3x) improvement.

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Introduction

In recent years, SFC with packed columns has received renewed interest for the analysis of both polar and nonpolar compounds, primarily, but not exclusively, for pharmaceutical applications.[1] SFC selectivity is complementary to reversed phase liquid chromatography (RPLC), and similar to normal phase liquid chromatography (NPLC) or hydrophilic interaction liquid chromatography (HILIC). While HILIC may require the use of large amounts of acetonitrile, SFC is considered a green chromatographic technique, because the mobile phase is mainly comprised of CO_2. For this reason, SFC has also been described as green HILIC. In comparison to NPLC, SFC enables high analysis speed and fast equilibration times. Based on these characteristics, SFC is ideal for the analysis of a large amount of samples.

The hyphenation of SFC with atmospheric pressure ionization MS detection can widen the application range of SFC. In particular, coupling with Agilent 6230 TOF can lead to more accurate mass data, important for compound screening and identification. During the optimization of an SFC/MS method, particular attention should be paid to the selection of the ion source parameters, in particular, the expansion of CO_2 entering the source at high flow rates may prevent the ionization process due to temperature reduction[2].

The Agilent Jet Stream source design is based on the use of a super-heated nitrogen sheath gas that collimates the nebulizer spray. This source provides higher sensitivity and desolvation in comparison to the conventional ESI sources[3]. The Jet Stream source can be effectively used for SFC/MS coupling, controlling the cooling process caused by CO_2 expansion.

This technical overview describes the optimization of the Jet Stream source parameters to increase SFC/MS sensitivity. Diclofenac, a common anti-inflammatory drug, was selected as a test molecule.

Experimental

Chemicals

Carbon dioxide (CO_2) and nitrogen (N_2) were purchased from Linde AG. Methanol was sourced from VWR. Diclofenac sodium salt and ammonium acetate were purchased from Sigma-Aldrich.

Solutions

A stock solution of diclofenac was prepared at a concentration of 1 mg/mL in methanol and diluted 1:10 in methanol prior to the analysis.

Instrumentation

Analyses were performed on an Agilent 1260 Infinity Analytical SFC System coupled to an Agilent 6230 TOF mass spectrometer equipped with the Agilent Jet Stream source. A make-up flow delivered by an Agilent 1260 Infinity Isocratic Pump was added between the SFC and the MS ion source, as shown in Figure 1.

Software

- Agilent OpenLAB CDS (ChemStation Edition); version A.01.05
- Agilent MassHunter Workstation; Version B 05.00

At the time this work was performed, MassHunter Software could not control the SFC system. Instead, OpenLAB CDS ChemStation was used. Since then, software that allows control of both the SFC and TOF under MassHunter is available.

Description	Model number
Agilent 1260 Infinity Analytical SFC System consisting of:	G4309A
Agilent 1260 Infinity HiP Degasser	
Agilent 1260 Infinity SFC Binary Pump	
Agilent 1260 Infinity SFC Autosampler	
Agilent 1260 Infinity TCC (Oven)	
Agilent 1260 Infinity DAD VL+	
Agilent 1260 Infinity SFC Control Module	
Agilent 1260 Infinity Isocratic Pump	G1310B
Agilent 6230 TOF-MS with Jet Stream Technology	G6230A

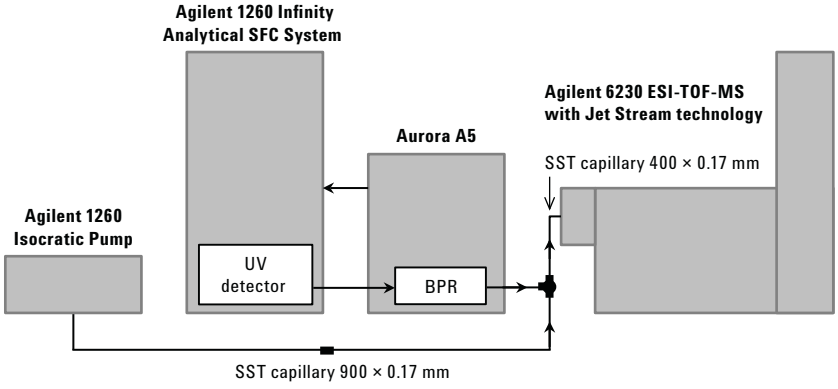

Figure 1. Configuration of the Agilent 1260 Infinity Analytical SFC System with an Agilent 6230 TOF and an Agilent 1260 Infinity Isocratic pump.

Results and Discussion

In LC/MS method development, ion source conditions should be carefully optimized to obtain the best sensitivity. Typical parameters adjusted in ESI techniques are drying gas temperature, flow rate, nebulizer pressure, and capillary voltage.

When using the Jet Stream source, the sheath gas temperature and flow rate also require optimization.

Method development can be performed by applying a one-variable-at-a-time approach, where a single parameter is varied, while all the others are kept constant. However, this approach is often insufficient for comprehensive studies since it does not take into account the interactions among the individual parameters. A more careful approach is represented by an experimental design that emphasizes the relationships among the various parameters and requires a lower number of analyses.

In the first set of experiments, drying gas temperature and flow rate (L/min) were optimized at two different nebulizer pressures.

Figure 2 shows that the drying gas temperature had the greatest influence on peak intensity. In the studied gas temperature range of 275–350 °C, the variation of peak area was above 50 %. Drying gas flow rate and nebulizer pressure had a significant influence on MS sensitivity. In particular, lower drying gas temperatures and flow rates, as well as lower nebulizer pressure, allowed increased sensitivity. These effects were not independent, for example, the influence of the gas temperature on decreasing MS signal intensity was stronger at higher nebulizer pressure.

Chromatographic conditions

Parameter	Setting
Column	Agilent ZORBAX RX-Sil, 4.6 × 150 mm, 5 µm (p/n 883975-901)
Mobile phase	A) CO_2 B) 10 mM ammonium acetate in methanol (LC/MS-grade)
Elution	20 %B (isocratic condition)
Make-up flow	Methanol 0.2 mL/min
Flow rate	1 mL/min
BPR pressure	150 bar
BPR temperature	60 °C
Column temperature	40 °C
Injection volume	5 µL
Detection	DAD, 250 nm; MS scan 80–1,000, Agilent Jet Stream ESI (–)

TOF conditions

Parameter	Setting
Fragmentor	175 V
Skimmer	65 V
OCT1RF Vpp	750 V
Gas temperature	275/300/325/350 °C
Drying gas	5–7 L/min
Nebulizer	45/60 V
Sheath gas temperature	250/275/300 °C
Sheath gas flow	6/8/10 L/min
Capillary voltage	500/1,000/2,000/3,000/4,000 V
Nozzle voltage	2,000 V

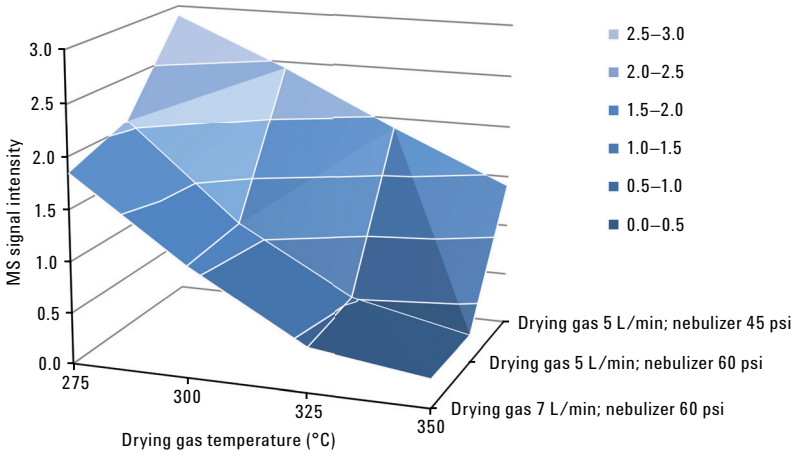

Figure 2. Response surface of diclofenac MS signal intensity (peak area) as a function of drying gas temperature (°C) and flow rate (L/min) at two different nebulizer pressures (psi). Constant settings: sheath gas temperature 275 °C, sheath gas flow rate 6 L/min; capillary voltage 3 kV. Peak areas were the results of three measurements with RSD < 15 %.

Figure 3 shows the extracted ion chromatograms of diclofenac acquired at different drying gas temperatures (°C).

Optimized drying gas settings of 275 °C, flow rate of 5 L/min, and nebulizer at 45 psi were selected as conditions for further experiments. Sheath gas temperature and flow rate were the next parameters optimized. Sheath gas temperature was varied between 250 and 300 °C, and flow rates between 6 and 10 L/min. Figure 4 shows that sheath gas influences MS sensitivity to a lesser extent than the drying gas settings. Lower sheath gas flow rates and lower temperatures led to higher signal intensities.

The influence of capillary voltages was also tested and optimal values were found to be approximately 1–2 kV (Figure 5). At 3–4 kV, a decrease of peak area of up to 35 % was observed, whereas at 0.5 kV the decrease was most significant, above 99 %.

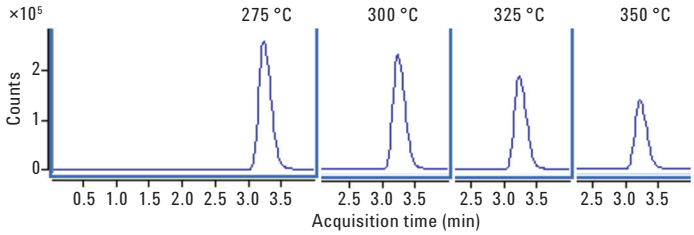

Figure 3. Extracted ion chromatograms of diclofenac acquired at different drying gas temperatures (°C). Drying gas flow rate 5 L/min; nebulizer pressure 45 psi; other conditions are the same as those described in Figure 1.

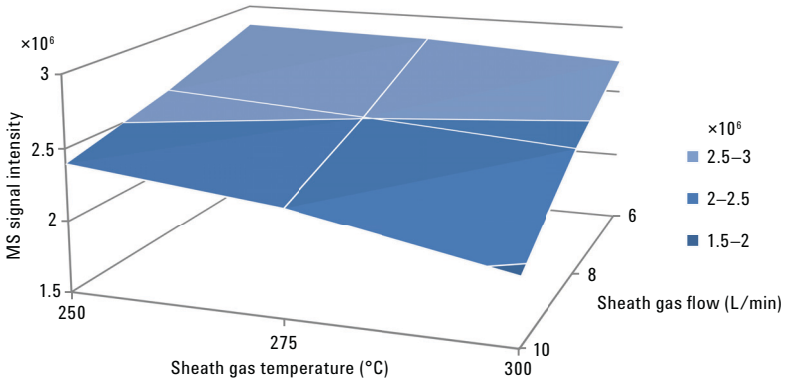

Figure 4. Response surface of diclofenac MS signal intensity (peak area) in function of sheath gas temperature (°C) and flow rate (L/min). Constant settings: nebulizer pressure 45 psi; drying gas temperature 275 °C, drying gas flow rate 5 L/min; capillary voltage 3 kV. Peak areas were the results of three measurements with RSD < 10 %.

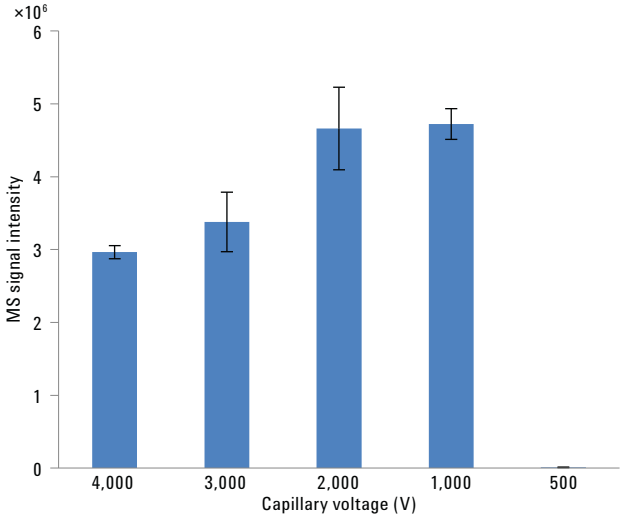

Figure 5. Diclofenac MS signal intensity (peak area) in function of capillary voltage (V). Constant settings: drying gas temperature 275 °C, drying gas flow rate 5 L/min; nebulizer pressure 45 psi; sheath gas temperature 275 °C; sheath gas flow rate 6 L/min. Peak areas were the results of three measurements with RSD < 10 %.

Conclusion

The Agilent 1260 Infinity Analytical SFC system coupled to an Agilent 6230 TOF offers a unique platform for fast and accurate analyses of polar and nonpolar molecules. The Agilent Jet Stream source is compatible with the SFC system, and may assist in focusing the CO_2 entering the source, and prevent cooling. Optimization of the ion source parameters, such as temperature and flow rate of the drying gas and sheath gas, is an important step that should always be performed during method development. This Technical Overview has demonstrated that they can greatly increase MS sensitivity.

References

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