INTERFACING UPLC TO MASS SPECTROMETRY VIA A LIQUID-JUNCTION, SECONDARY ULTRASONIC API SOURCE

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INTRODUCTION

In primary ultrasonic API sources, the liquid flow from a chromatographic column passes through a capillary that is directly agitated by an ultrasonic transducer. Historically, these nebulisers have included the addition of a high voltage and nitrogen gas flows at the capillary tip while other examples have utilised arrays of multi emitters. In secondary ultrasonic API sources, the liquid capillary is typically remote from an ultrasonically agitated surface. A stable, uninterrupted jet must be formed between the two components for chromatographic integrity to be preserved. However, the jet conditions are critically dependent on liquid flow velocity and hence capillary diameter. In this study, we have made use of a liquid-junction between the liquid capillary and the ultrasonic surface to enable stable UPLC/MS operation with high chromatographic fidelity for a wide range of flow rates. The data have been directly compared to data obtained from a conventional, nebuliser-assisted ESI source.

METHODS

Liquid Junction, Secondary Ultrasonic Source

A schematic of the ultrasonic source is shown in Figure 1. The source was constructed by positioning a Ø80µm i.d. stainless steel capillary at a distance of <0.5mm from the chamfered end (active surface) of a Ø3mm ultrasonic horn that was vibrated at a frequency of 40kHz with a maximum p-to-p axial displacement of approximately 30µm. The transducer/horn assembly was designed and supplied by Sonic Systems UK Ltd (Ilminster, UK) and is shown in operation in Figure 2. The active surface was positioned approximately X=6mm from the ion inlet orifice of the MS. An annular heater (350°C) was used to deliver nitrogen at a flow rate of 150L/hr. This gentle heating was not critical from a sensitivity viewpoint but was required to prevent excessive condensation on the source surfaces. In contrast, the ESI source required a heater temperature of 550°C and a heater gas flow rate of 1200L/hr in order to optimise signal response. It should also be noted that although the ultrasonic horn could be biased to 1kV, this was found to have no significant effect on source performance and was thus grounded as standard.

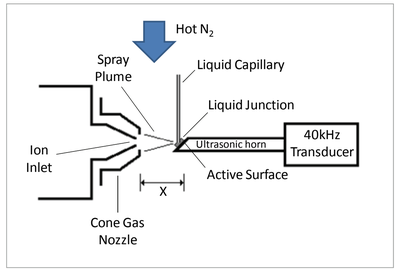

Figure 1. A schematic of the liquid junction, secondary ultrasonic API source.

UPLC/MS/MS

A test mixture was prepared and consisted of 7 analytes at the following concentrations: acetaminophen 20pg/µL, caffeine 20pg/µL, sulphadimethoxine 10pg/µL, verapamil 5pg/µL, 17-α hydroxyprogesterone 500pg/µL, β-estradiol 1ng/µL and δ-tocopherol 1ng/µL. 10µL samples of the mixture were separated on a Waters UPLC column (Acquity BEH, C18, 1.7µm, 2.1 x 50mm) at a flow rate of 0.6mL/min. Mobile phase A was 100% water + 0.01% formic acid and B was 100% acetonitrile + 0.01% formic acid. MS/MS data were obtained on a Waters Xevo TQ-S system. All analyte responses were monitored in MRM mode (one transition per analyte, dwell=30ms, inter channel delay = Auto).

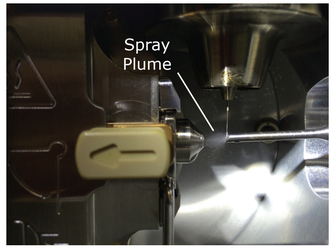

Figure 2. A photograph of the ultrasonic source where the jet plume is clearly visible (0.6mL/min liquid flow).

RESULTS AND DISCUSSION

For the geometry used in this study, it was observed that the angle of the active surface was important in terms of beam stability and ion signal intensity. In the case of a near vertical surface, it is found that the majority of the liquid flow drips off the end of the surface before significant nebulisation can occur. It was determined that an angle of between typically 22° and 45° to the vertical gave rise to the most efficient spray plume.

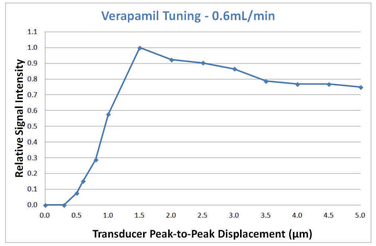

Figure 3. Ultrasonic transducer displacement versus relative ion signal for verapamil.

Figure 3 shows a typical tuning characteristic for verapamil as the p-p displacement of the ultrasonic transducer is increased from zero to its maximum value. It should be noted that the horn amplifies the p-p displacement at the active surface by a factor of approximately x6 such that the apex of the curve in Figure 3 corresponds to a displacement of 9µm at the liquid junction. The tuning characteristic was obtained for a 50/50 acetonitrile/water mobile phase where a similar response curve was obtained for all the test analytes used in this study. Figure 4 shows reconstructed ion chromatograms obtained with the ultrasonic source for the seven analytes in the test mixture. With the exception of sulphadimethoxine, which exhibits some peak tailing, the ultrasonic source gives rise to stable chromatographic peaks with peak widths that are comparable to ESI (FWHM of between 1.0 and 2.2 seconds). The ultrasonic source was compared directly with a conventional ESI source where the ratios of electrospray ion signal to ultrasonic ion signal and the corresponding signal-to-noise (S:N) ratios are tabulated in Table 1. Thus, it is observed that, with the exception of δ-tocopherol, ESI gives rise to typically 10-400 times more ion signal when compared with the ultrasonic source under these conditions. However, with the exception of sulphadimethoxine and verapamil which are known to be ESI sensitive, it is observed that the S:N is more comparable between the two sources, particularly in the case of caffeine, β-estradiol and δ-tocopherol. In stark contrast to the general relative behaviour between the sources, δ-tocopherol is shown to produce the same ion signal as ESI and may indicate that a different ionisation mechanism is involved for the non-polar, late eluting compounds which are known to be problematic for ESI-based analyses. Since δ-tocopherol elutes at the highest organic percentage of the chromatographic gradient (99% acetonitrile), the experiment was repeated by adding a post-column flow of 0.2mL/min of water to the existing UPLC flow to determine whether this would influence the source sensitivity.

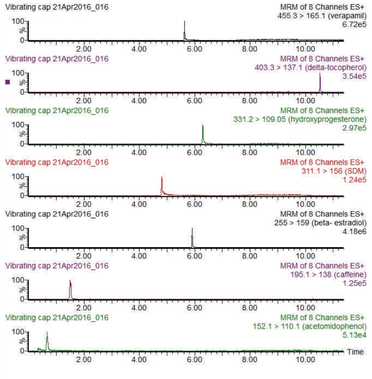

Figure 4. Typical UPLC/MS/MS chromatograms obtained with the ultrasonic API source.

0.6mL/min — No Post-Column Addition

	ESI Response / Ultrasonic Response		
Analyte	Area	Height	S:N
Acetaminophen	356	432	19
Caffeine	169	174	4
Sulphadimethoxine	132	286	160
Verapamil	89	142	133
β-estradiol	8	9	4
Hydroxyprogesterone	216	334	19
δ-tocopherol	0.9	1.0	1.4

Table 1. Comparison of ESI versus Ultrasonic MRM response for chromatographic peak area, height and S:N.

Table 2 summarises the relative response ratios for the ESI and ultrasonic sources with the post column addition of a 0.2mL/min flow of water. The most significant observation is that the water gives rise to a large increase (x20) in the δ-tocopherol signal with the ultrasonic source whilst the effect on the other analytes is only marginal. Inversely, it has been shown that the post column addition of 0.2mL/min of acetonitrile with the ultrasonic source will result in increased sensitivity for the two earliest eluting analytes (data not shown).

With 0.2mL/min Post-Column Addition of Water

	ESI Response / Ultrasonic Response		
Analyte	Area	Height	S:N
Acetaminophen	497	578	24
Caffeine	214	212	5
Sulphadimethoxine	126	292	136
Verapamil	116	160	124
β-estradiol	12	15	6
Hydroxyprogesterone	255	380	28
δ-tocopherol	0.04	0.05	0.63

Table 2. Comparison of ESI versus Ultrasonic MRM response with post-column addition of 0.2mL/min of water.

As previously mentioned, the use of a hot-nitrogen gas flow for intersecting the spray plume is critically important for the ESI source sensitivity at these chromatographic flow rates. On the other hand, the ultrasonic source signal intensity is found to be weakly dependent on the temperature of the gas and can operate at room temperature. However, the ultrasonic source is found to be strongly dependent on the temperature of the MS inlet system, i.e. the internal and external surfaces that constitute the main source components such as the cone gas nozzle, the ion inlet cone and the ion block that supports these components. This temperature dependence is different for each analyte and can result in two orders of magnitude signal loss for acetaminophen as the inlet temperature is decreased from 150°C to 80°C. This would suggest that gas phase ions can be formed downstream of the point of nebulisation. It also follows that we could expect to see sensitivity enhancements by combining the ultrasonic source arrangement of Figures 1 with a REIMS-type MS inlet system, where a hot bead or other such obstruction with a temperature in the range 100-1000°C is placed in the first vacuum region such that droplets from the spray plume impact onto the bead downstream of the ion inlet orifice.

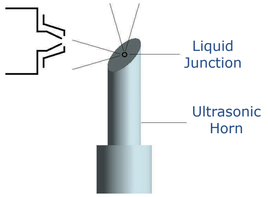

Figure 5. A schematic of an alternative ultrasonic horn arrangement that allows access from the front of an API source

Results from this preliminary investigation into the use of a liquid-junction, secondary ultrasonic nebuliser as an ion source for UPLC/MS analysis suggest that a simple, compact and reliable device that delivers high chromatographic fidelity is entirely feasible. From a pragmatic viewpoint, this type of source is highly adaptable to suit a number of source geometries. Figure 5 shows an alternative geometrical arrangement that has been successfully demonstrated on a Waters Xevo TQ-S MS system where the transducer/horn assembly enters from the front as opposed to the side of the API source. The transducers used in this study generate an axial displacement along the horn. Since the initial trajectories of droplets will be normal to the active surface, we can use a 45° chamfered surface that is axially rotated by 45° to direct the spray plume towards the ion inlet of the MS system for front-mounted transducer/horn assemblies.

CONCLUSION

- **A liquid junction, secondary ultrasonic API source has been constructed that allows UPLC/MS analyses to be conducted with high chromatographic fidelity at flow rates of 0.1-1.0mL/min.**
- **The source can produce S:N data for some analytes that approaches that obtained by conventional ESI.**
- **The ultrasonic source sensitivity may exceed that of ESI for some non-polar analytes.**
- **Alternative heating and gas flow methods will be investigated with a view to further improving the source performance.**

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