

A COMPARISON OF MATRIX EFFECTS WITH STANDARD AND MODIFIED ESI PROBES

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OVERVIEW

- To compare the effects of coeluting matrix on the signal intensity observed with a standard ESI probe and a modified probe containing a central wire (CWESI)
- Matrix effects characterised by LC-MRM analysis of sirolimus-spiked whole blood extracts or post-column infusion chromatograms
- Modified CWESI probe is found to significantly reduce the effects of both ion enhancement and ion suppression in the presence of coeluting matrix

INTRODUCTION

ESI is the most favoured ionisation technique for clinical MS assays but is known to suffer from deleterious matrix effects where coeluting endogenous material can enhance or suppress the analyte signal. In practice, this can severely limit the sensitivity and accuracy of analysis. The degree of matrix interference is known to be influenced by chemical factors such as chromatographic buffer or surfactant concentration and experimental parameters such as flow rate and ESI capillary voltage. Recent models of matrix suppression suggest that it is beneficial to produce small, highly charged ESI droplets. Here, we report on a comparison of matrix effects with a standard open tubular ESI capillary and a novel central wire electrospray (CWESI) design.

Sirolimus is a relatively new addition to a group of immunosuppressive drugs that are difficult to chromatographically elute with satisfactory peak shapes. The additional requirement of fast analysis times has led to the adoption of a standard clinical assay where the analyte is eluted via a simple step gradient from 50% to 100% organic mobile phase. High organic conditions have been known to exacerbate matrix effects in such analyses. In this study, matrix effects were characterised by either spiking the matrix with a sirolimus standard prior to analysis or by the post column infusion of sirolimus during elution of the matrix components. All data were obtained using LC or UPLC and triple quadrupole mass spectrometry.

METHODS

Sample Preparation

Matrix samples were produced by diluting 50 μ L of whole blood with 200 μ L of 0.1M aqueous ZnSO₄. The sample was protein precipitated by the addition of 500 μ L of acetonitrile prior to centrifuging at 13000 rpm for a period of 5 mins. The resulting supernatant was drawn for analysis.

Chromatography

All data were obtained using a Waters Acuity UPLC system with a mobile phase flow rate of 0.6mL/min. Solvent A = H₂O, solvent B = methanol, 1:1 for 0 to 0.4 mins, gradient to 100% B from 0.4 to 1.0 mins. A and B contained 2mM ammonium acetate and 0.1% formic acid. A 2.1 x 10mm, 6 μ m, C18 column was used for all matrix experiments.

Mass Spectrometry

Unless otherwise stated, all data were obtained on a Waters Quattro Micro triple quadrupole mass spectrometer. Data were acquired in MRM mode for the sirolimus transition m/z 931 to m/z 864 (source temp=140°C, desolvation gas flow=900L/hr, dwell time=200ms, inter scan delay=50ms, cone voltage=22V, collision energy=18eV and gas cell pressure=3.3x10⁻³ mbar).

ESI Probes

The standard ESI and CWESI probe geometries are shown schematically in Figure 1. The standard ESI probe basically consists of a concentric open tubular design with an inner liquid capillary (127 μ m i.d., 230 μ m o.d.) and an outer nitrogen nebulization gas capillary (330 μ m i.d., 640 μ m o.d.). In these experiments, the same probe was converted into a CWESI probe by inserting a 60mm long, 90 μ m o.d. sharp-tipped wire into the central bore of the liquid capillary. The central wire was held in position by introducing a "kink" at a distance of 15mm from the blunt end of the wire prior to insertion.

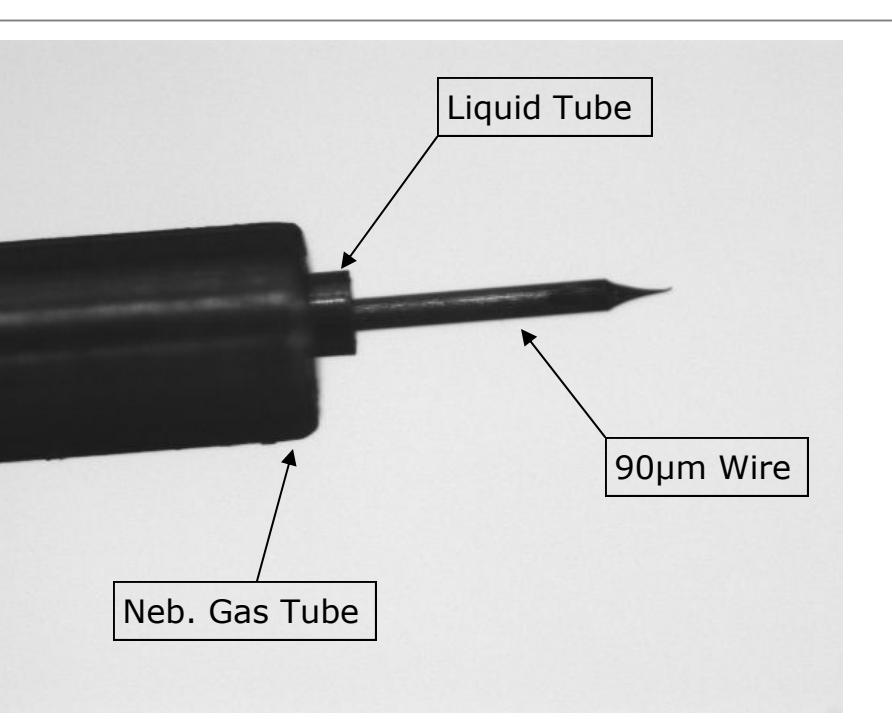


Figure 1. Schematic of the standard ESI and CWESI electrospray probe tips.

RESULTS

A number of studies in this laboratory have shown that the use of a central wire can lead to sensitivity gains over a standard open tubular ESI probe design. Figure 3 shows a comparison of ESI and CWESI linearity plots obtained from a UPLC-MRM analysis of sulphadimethoxine standards. These data were obtained using a Waters Quattro Premier XE MS system. A standard fast gradient (0 - 0.6mins, 0-100% acetonitrile, 0.1% total formic acid) was employed.

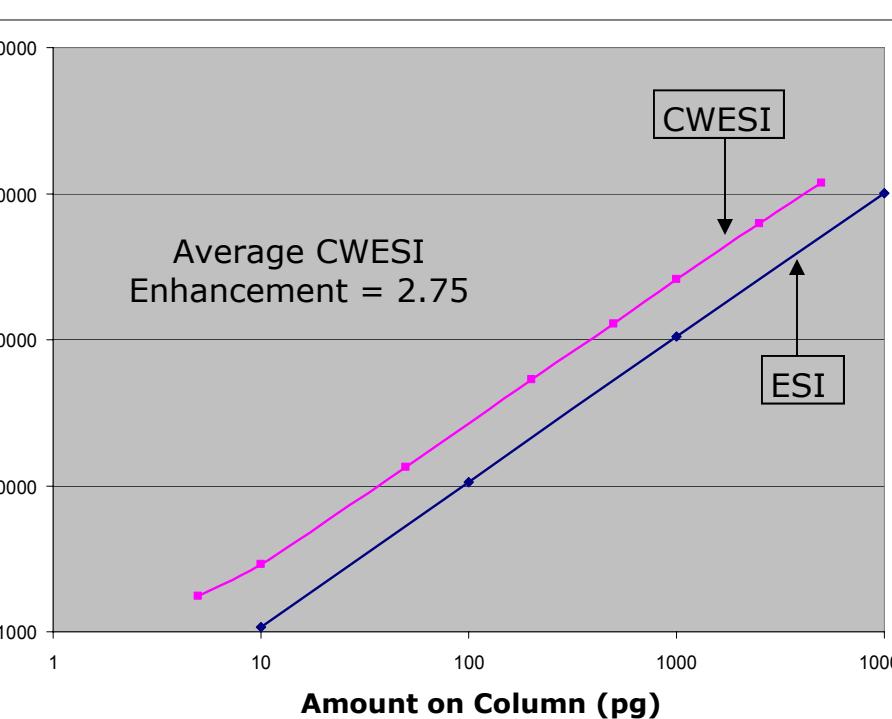


Figure 3. UPLC-MRM linearity plots for sulphadimethoxine

The UPLC column was a Waters Acuity C18, 2.1 x 50mm, 1.7 μ m. Figure 3 compares the average MRM chromatographic peak areas obtained for the same analysis conducted with a standard ESI probe (each point, n=5) and a CWESI probe (each point, n=3). Thus, it is shown that the CWESI probe leads to an average signal enhancement of 2.75 over the three orders of sample concentration used in this study.

To evaluate the performance of the two probe types under conditions of matrix interference, a series of experiments were conducted using the infusion chromatogram technique [1]. Figure 4 shows the sirolimus infusion chromatograms obtained with both probe types in the absence of matrix, i.e. for a 10 μ L injection of clean acetonitrile on the column (refer to chromatography conditions described in Methods section).

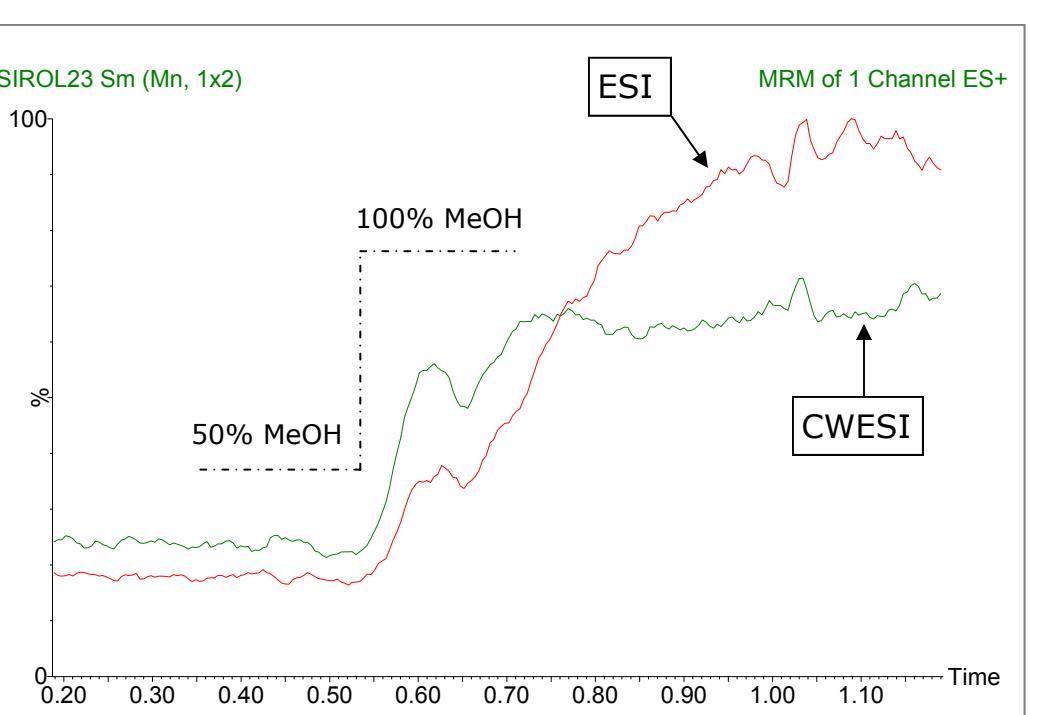


Figure 4. Sirolimus infusion chromatograms in the absence of matrix (blank injection) for the standard ESI and CWESI probes

In this experiment, a 100 μ g/ μ L solution of sirolimus was infused at a flow rate of 10 μ L/min into the 0.6mL/min column eluent. The profiles represent the signal response of each probe type to the changing solvent gradient. The position of the step gradient is shown in Figure 4 (corrected for the system dead volume). Thus, it is shown that the CWESI probe exhibits a significantly faster response to the solvent gradient, such that a signal equilibrium is attained in approximately 40% of the time taken with the standard ESI probe. Although response differences are observed, it should be noted that analytes will elute on the gradient front where the CWESI response is higher (see Figure 7).

This type of infusion experiment was used to quantify the susceptibility of the two probe types to matrix interference. Thus, Figure 5 shows representative response data obtained from the standard ESI probe during elution of a matrix and a blank methanol standard (both 10 μ L injections). All data were obtained in triplicate (only one trace shown). Here, it is shown that the presence of eluting matrix components leads to an initial period of signal enhancement (~100% max. at t=0.68mins) followed by a region of signal

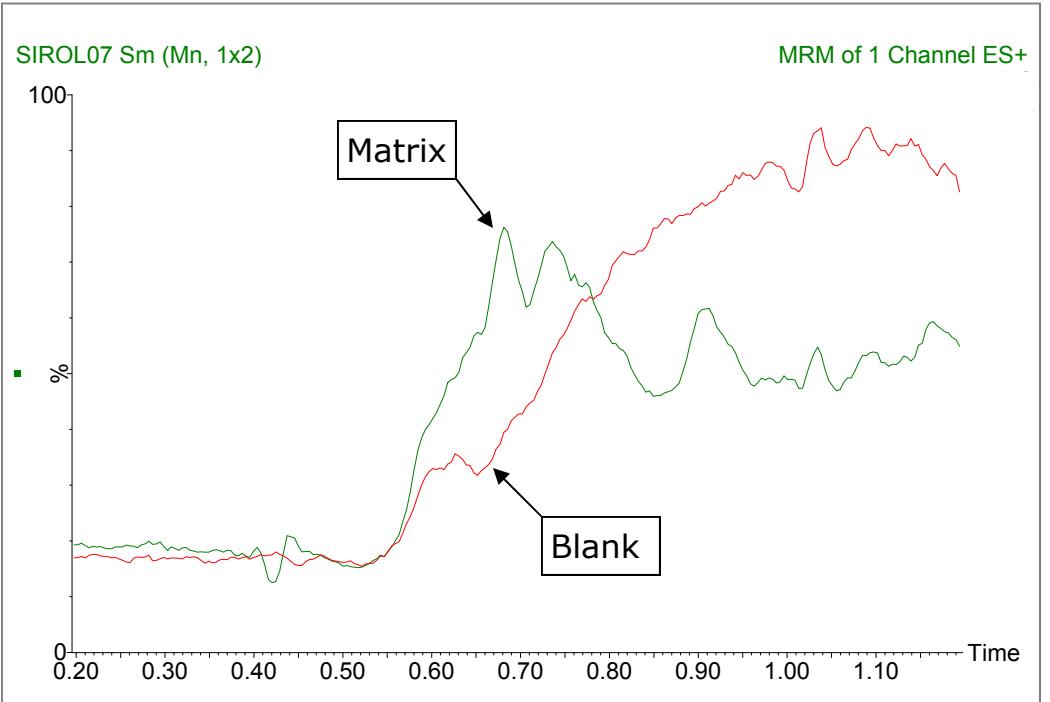


Figure 5. Susceptibility to matrix effects with the standard ESI probe

suppression (~45% max. at t=1.05mins). In contrast, Figure 6 shows the signal response to matrix obtained using the CWESI probe. Here, we observe no matrix enhancing effect and a region of only minor signal suppression (~15% max. at t=0.98mins).

To evaluate the effects of matrix interference during chromatographic analysis, sirolimus standards and spiked matrix samples were prepared at a concentration of 10 μ g/ μ L. The upper trace of Figure 7 shows the MRM response obtained for the duplicate analysis of 10 μ L injections of the standard and the spiked matrix sample with the ESI probe. The retention time of 0.63mins correlates to the enhancing region for the ESI probe (see Figure 5) and results in a clear difference in response between the standard and spiked matrix samples (RSD=16.5% for the 4 injections). In contrast, no matrix interference was observed with the CWESI probe (RSD=1.9%).

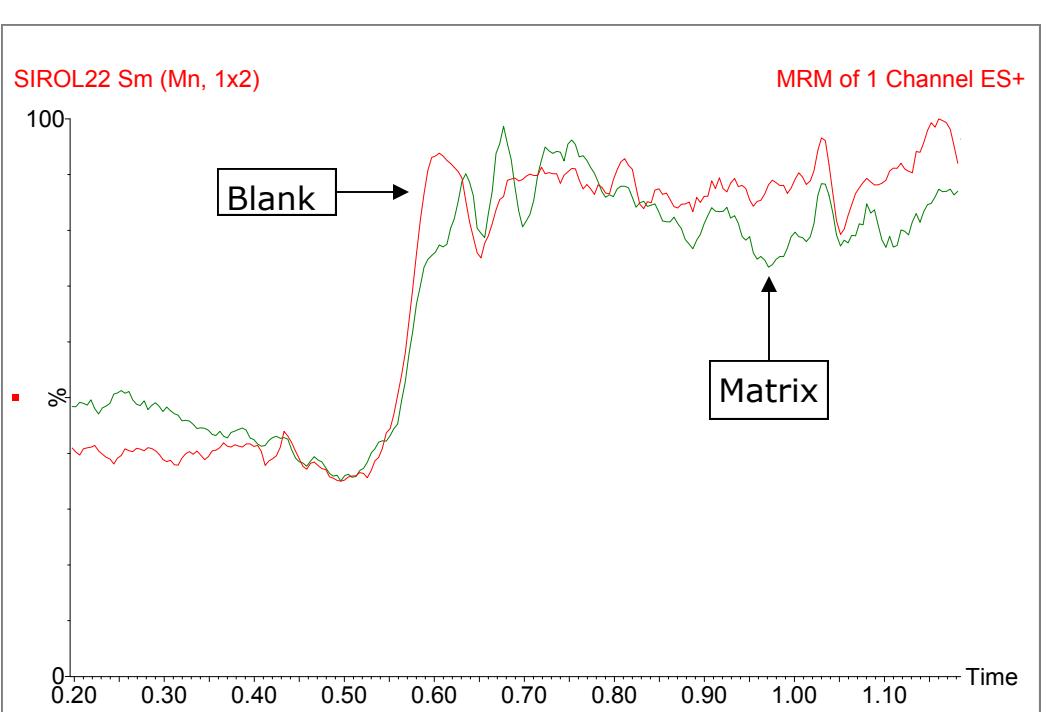


Figure 6. Susceptibility to matrix effects with the CWESI probe

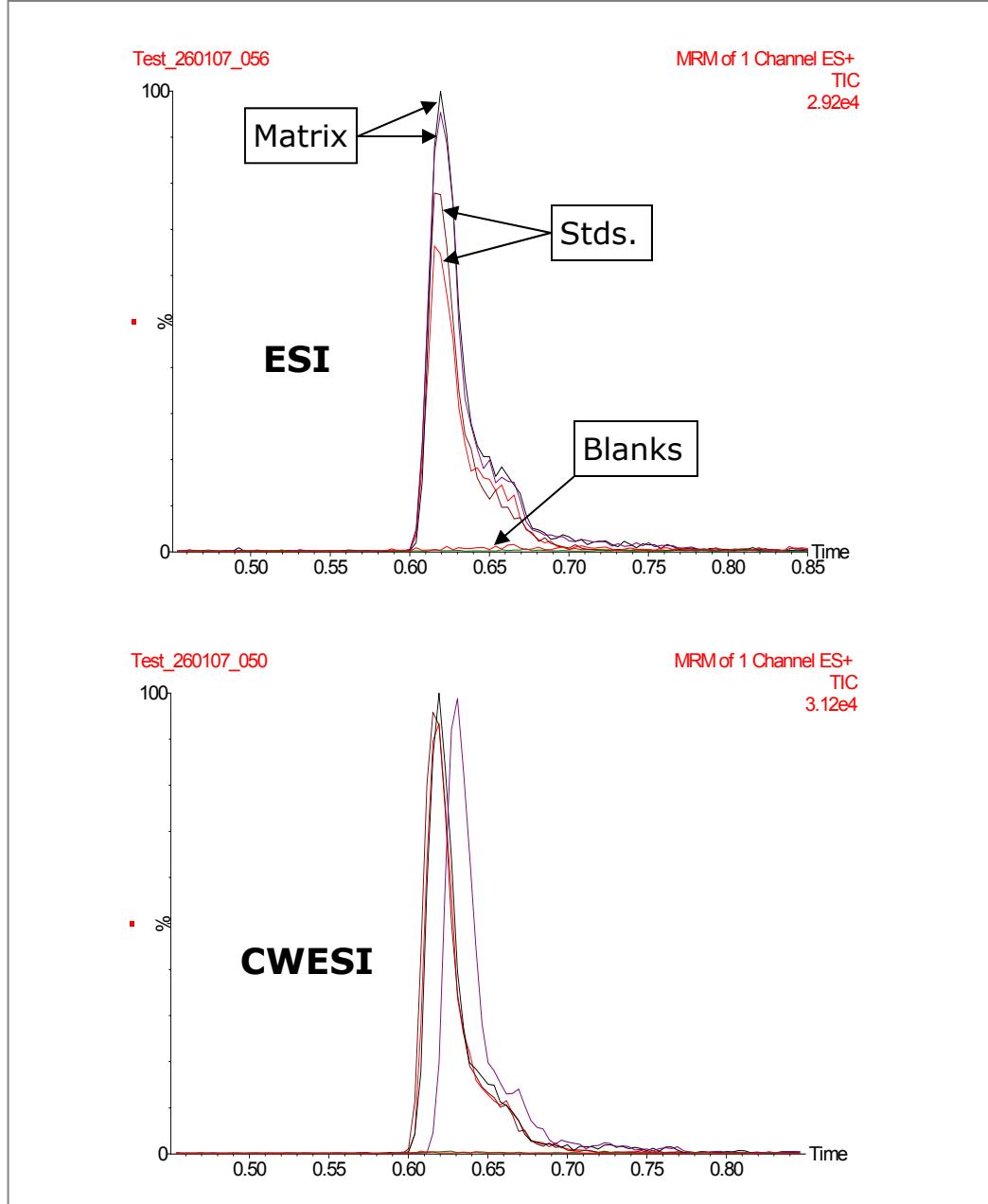


Figure 7. On-column MRM chromatograms for the analysis of sirolimus standards and spiked matrix samples

CONCLUSIONS

Comparisons between a standard open tubular ESI probe and a modified central wire electrospray (CWESI) probe have shown that CWESI can

- increase the sensitivity of UPLC-MRM analyses
- exhibit a faster signal response to a rapidly changing mobile phase gradient
- significantly reduce the effects of matrix suppression or enhancement

Further studies are required to evaluate the susceptibility to matrix effects over a wider range of chromatographic conditions and analyte classes

Reference [1] Bonfiglio et al, Rap. Comm. Mass Spec. 13 1175-1185 (1999)