

to the solution sprayed from ES probe tip **6** while a sample solution is sprayed from ES probe tip **7**. If the calibration and sample solutions are sprayed simultaneously from ES probe tips **6** and **7** respectively, the mass spectrum acquired from the resulting ion mixture contains a set of internal calibration peaks corresponding to the known molecular weight compounds included in the calibration solution. Using this embodiment of the invention a mass spectrum can be acquired containing an internal standard set of peaks without having mixed the calibration and sample compounds in solution. Known component and sample component ion mixing occurs in the gas phase prior to mass analysis. Alternatively, the solution flow through ES probe tips **6** and **7** can be turned on sequentially. If one ES probe contains a calibration solution, sequential spraying of ES probes **6** and **7** allows acquisition of a mass spectrum which can be used as an external standard close in time to the acquisition of the subsequent sample mass spectrum. The probe positions remain fixed during Electrospraying with MS acquisition while spraying simultaneously or separately in time. Including internal standards in an acquired mass spectrum allows increased accuracy in assignment of the molecular weights of sample related peaks contained in the spectrum. Internal standards in a mass spectrum can also serve to improve quantitative accuracy.

[0040] Conventionally, to acquire a mass spectrum which includes an internal standard, calibration compounds are mixed with sample bearing solution prior to Electrospraying. Typically when acquiring an external calibration mass spectrum, the calibration solution is delivered through the same ES probe that the following sample solutions will flow through. Calibration compounds contaminate the transfer lines and ES probe tip internal bore and can result in unwanted peaks in a mass spectrum acquired from a sample solution. Mixing calibration compounds in solution, directly or through a layered flow Electrospray probe configuration, to create an internal standard in the resulting acquired mass spectrum, can also cause suppression of sample ion signal during the Electrospray ionization process. Mass calibration compounds contaminate sample delivery lines and are often difficult to eliminate when switching between applications that require internal standards, external standards or no calibration peaks in the acquired mass spectrum. Long flushing time may be required to remove calibration compounds from transfer lines and ES probe assemblies, adding to analysis time. Due to this contamination problem, mixing calibration solutions with sample solutions in the liquid phase does not allow rapid application and removal of calibration compounds during API source operation. The invention overcomes the analytical disadvantages of mixing calibration and sample solutions to acquire mass spectra containing internal standards. Simultaneous operation of multiple ES probes produces ions from independently spraying solutions that mix in the gas phase prior to mass analysis. Each independent ES probe spray can be rapidly turned on and off with no residual unwanted compound contamination appearing in subsequently acquired mass spectrum. The Electrospray generated ions are produced from charged droplets produced from separate sprayers. Any sample or calibration ion interaction is limited to those processes occurring in the gas phase. As the ions produced are of the same polarity, chemical interference through interaction in the gas phase is minimal. By varying relative solution component concentrations and compositions, the invention

allows independent control of the intensities and m/z locations between the calibration and sample component peaks in an acquired mass spectrum.

[0041] Adjusting the location of the ion mixing region **43** relative to nose piece opening **28** and capillary entrance orifice **28**, varies the ratio of ions from each spray which enter capillary bore **23**. For a given calibration solution concentration, the calibration peak intensities relative to the sample peak intensities can be changed by moving probe assembly **5** in the x direction and locking with locking knob **19**. Depending on the relative liquid flow rates and nebulization gas flow rates through probe ES tips **6** and **7** rotational adjustment of ES probe assembly **5** can also be used to change the placement of ion mixing region **43** relative to capillary entrance orifice **48** to optimize performance. For many analytical applications, it is desirable to maximize sample ion signal even while adding calibration component related peaks to the acquired mass spectrum. Adjustment of the position of ES probe assembly **5** with fixed relative ES probe tip positions allows introduction of calibration peaks in an acquired spectrum with minimum sample signal loss. The parallel ES tip configuration allows a wide range of liquid flow rates to be sprayed independently from each tip with efficient mixing of ions produced. Consequently, optimal performance over a wide range of analytical applications can be achieved using a parallel sprayer configuration without the need to re-adjust the position probe assembly **5**. An example of a mass spectrum acquired while simultaneously Electrospraying solutions delivered at two different liquid flow rates through two ES tips is shown in **FIG. 4**.

[0042] An Electrospray probe assembly, similar to ES probe assembly **2**, configured with two ES tips oriented to spray approximately in a parallel direction as diagrammed **FIGS. 1 and 3**, was used during acquisition of the mass spectra shown in **FIGS. 4a through 4c**. Electrospray ion source **1** was interfaced to a quadrupole mass spectrometer for the data acquired in **FIGS. 4a through 4c**. **FIG. 4a** shows mass spectrum **60** acquired from a 10 ng/ul gramicidin S, in a 1:1 methanol: water sample solution, continuously infused through delivery line **9**. The solution containing the gramicidin S sample was Electrosprayed with pneumatic nebulization assist from ES tip **3** at a liquid flow rate of 50  $\mu$ l/min. The doubly charge peak **61** of Gramicidin S is the dominant peak in the spectrum with a relative abundance of 3,100 as shown by ordinate **62**. The orientation of the axis of ES probe tips **3** and **4** was approximately 60 degrees angled up from the horizontal (z-x) plane which intersects ES source centerline **24**. For the data acquired in **FIG. 4**  $\theta_2=60$  degrees where  $\theta_2$  is the angle formed by the ES probe tip axis relative to the z axis and is axially symmetric around the z axis. The axis of ES tips **3** and **4** were positioned approximately parallel and each tip was positioned an equal distance from the z-x plane during spraying. ES tips **3** and **4** were separated by fixed distance of approximately 8 mm during acquisition of mass spectra **60**, **64** and **68**. ES tips **3** and **4** were positioned approximately 1.5 cm along the z axis and up approximately 1.0 cm along the y axis as shown by dimensions **Z** and **r** respectively in **FIG. 3**. The position of ES tips **3** and **4** along the x axis was adjusted to optimize performance after which the dual ES tip positions were locked in position during acquisition of the mass spectra series shown in **FIGS. 4a through 4c**. A mixture of calibration compounds valine (50 ng/ul), tri-tyrosine (25 ng/ul) and hexa-tyrosine (50 ng/ul) in a 79% water, 19% iso-propanol and 2%