Capillary Electrospray Ionization (CESI) is the integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device (Figure 1). CESI-MS analysis of nontoxic ions from normal cell cultures having several advantages including increased ionization efficiency and a reduction in ion suppression. In this study we describe the use of CESI-MS in negative ion mode for the analysis of plant metabolites including phosphorylated sugars and low molecular weight metabolites. These data were generated using Capillary Electrospray Ionization with negative ion electrospray ionization for the analysis of plant metabolites. Until recently, only mass spectrometry techniques typically relied on traditional liquid chromatography systems enabling CESI-MS and ESI-MS experiments. PIL-3, 5600, and 5800/6500, which are available in the laboratory. In Figure 1, the scan rate was increased for the analysis of the 3 phosphorylated sugars. At these times, the data and the scan rate (10-15 ppm) corresponded to concentrations in the pMolar range.

RESULTS

Initially both normal and reverse polarity were investigated. However, preliminary trials showed that reverse polarity provided the best separation on the CEG-MS system in negative ion mode. Under these conditions a lower background was obtained than using normal polarity and the sensitivity for detecting transition increases. Using a bare fused silica capillary, Carb mobil, and 1mM acid as a background electrolyte, low milliliters to microliters of plant metabolites were detected. These metabolites included low molar mass compounds, including sugars, nucleic acids, and peptides. These sugars include monosaccharides, disaccharides, and oligosaccharides, sugars and their glycosides and carbohydrates. In the preliminary phase, the reverse polarity of the liquid chromatography systems enabled CESI-MS experiments. This study investigated both separation reproducibility and sensitivity for the novel pilosus metabolites difficult to analyze by other means.

MATERIALS AND METHODS

CE Conditions: A method and set of procedures to obtain spectra of unknown metabolites using negative ion CESI-MS. This work used a Source on a SCIEX AX500 triple quadrupole mass spectrometer interfaced with a Sciex Model 1000 electrospray interface. Electrophoretic separation was performed using a 50 cm column inside a 1.0 mm OD fused silica capillary at a separation voltage of 30 kV. The temperature of the oven was held at 30°C (B). A pressure of 100 psi was applied to the reverse electrophoretic solvent from the bare fused silica capillary used for these separations.

Between each injection the capillary was conditioned by rinsing with 1.0 ml methanol/water, 1:1 mixture, and air-drying at 37°C and finally the background electrolyte.

PIL-3 Conditions: Bennet Courter (50:50 60s flow through SILC Separations) was coupled to a ThermoQ-500 60s multi-spectrum SCIEX operating in negative ion electrospray mode using 13.6,20K dalton background electrolyte. This was used for the plant metabolites (20 m. mass accumulation. The peak width with a mass range from 60-66 was 10% at half height. The flow rate was 90 KHz and the temperature was 30°C. The detection voltage was set at 1000 mV and the scan was held at a pressure of 40 psi. Due to low flow rate used by the CESI, this certain gas was turned around 15 ps and the temperature of the nozzle spray interface was set at 60°C.

To check on the linearity of response for this method standards were prepared from Sigma-Aldrich. These standards were prepared for mass spectrometric evaluation and the calibration line was evaluated for the linearity of response. No internal standards were used in this test. Figures 6 and 7 show the calibration line for 2 of the 3 phosphorylated sugars. At these times were done and the range tested (10-15 ppm) corresponded to a concentration in the pMolar range.

Accurate, precise, mobile, tuneable and complete detection was in addition to elution using this approach. This technique is characterized by a high degree of sensitivity, allowing the detection of as low as 0.1 ng of a compound. In the separation of all three of these shown in Figure 8 and an example of a calibration line for ascorbic acid at 15.5 minutes is shown (Figure 11). In order to test the versatility of this system the analysis of underivitised amino acids as well as amino acids with polar and charged side chains was performed. This was done by switching the polarity of the CE and MS systems (B). CESI-MS could be used to detect underivitised amino acids and partially charge plant metabolites in the same samples. This approach is therefore an ideal way to assessing the biological and negatively charged plant metabolites in a combination approach and it is very important to do normal phase chromatography.

CONCLUSIONS

A novel, fast, and reliable method, for the detection over 26 negatively charged polar plant metabolites has been developed. This method uses a simple pressure injection and has been shown to produce reproducible results for the analysis of polar and organic molecules, amino acids, and other polarized sugars which is not possible by reverse phase HPLC separation. In addition to simply switching the polarity of the CE and MS systems, CESI-MS can be used to detect underivitised amino acids and partially charge plant metabolites in the same samples. This approach is therefore an ideal way to assessing both positively and negatively charged plant metabolites in a combination approach and it is very important to do normal phase chromatography.

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