The application of Capillary Electrospray Ionization with negative ion electrospray ionization for the analysis of plant metabolites

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INTRODUCTION

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 operates at low nL/min flow rates offering 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 organic acids. These are generally small polar compounds typically not retained on traditional liquid chromatography columns making LC-separations difficult.

This study investigated both separation reproducibility and sensitivity for numerous plant metabolites difficult to analyze by other means.

MATERIALS AND METHODS

CE Conditions:
A method was set up for detection of over 40 plant metabolites using negative ion CESI-MS. This work used reverse polarity with a pressure injection (5 sec. at 5 psi) and a background electrolyte of 10% acetic acid. Electrophoretic separation was performed using a 90 cm bare fused silica capillary and a separation voltage of 30 kV creating a field strength of 333 V/cm. A pressure of 2 psi was applied to counter the reverse electro osmotic flow from the bare fused silica capillary used for these separations.

Between each injection the capillary was conditioned by rinsing with 0.1 molar sodium hydroxide, water, 0.1 molar hydrochloric acid and finally the background electrolyte.

MS/MS Conditions:
Beckman Coulter CESI 8000 (sold through SCIEX Separations) was coupled to a TripleTOF® 5600+ mass spectrometer (SCIEX) operating in negative ion electrospray mode using looped dedicated product ion scan for each of the plant metabolites (20 msec. accumulation time for each product ion with a mass range from 40 – 50 amu above the parent ion mass) together with a general TOF scan (100 -1000 amu for 100m sec) to screen for unknown targets. The total scan time for each cycle was approximately 1 second. The ionspray voltage used was set to -1200V and the spray position and MS source were tuned using direct pressure injection at 10 psi.

Due to low flow rate used by the CESI, the curtain gas was lowered to around 4-5 psi and the temperature of the nano-spray source interface was set at 50 ºC.

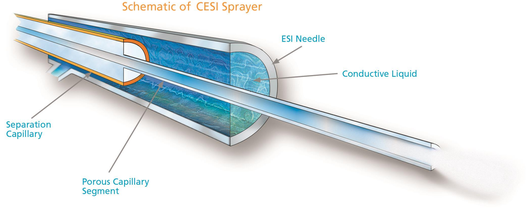

Figure 1. Schematic for CESI Sprayer which connects to MS systems via an adapter fitted to a nano spray source

RESULTS

Initially both normal and reverse polarities were investigated. However, preliminary trials showed that reverse polarity provided the best separation on the CESI-MS system in negative ion mode. Under these conditions a lower ion spray voltage could be used which reduced the potential for discharge formation.

Using a bare fused silica OptiMS Cartridge and 10% acetic acid as a background electrolyte, low nMoles/10 µL concentration of plant metabolites were detected. These metabolites included low molecular weight organic acids including succinate and malate. In addition, separation of the isobaric phosphorylated disaccharides of Sucrose-6-Phosphate and Trehalose-6-Phosphate was possible. Figure 2 shows the separation of these two plant metabolites. The increase of pressure during the separation speeded up the analysis and increased the peak height but did not have a negative effect on the overall resolution. Sucrose-6-Phosphate migrated first and both sucrose and trehalose have nearly identical MS fragmentation patterns so separation of these metabolites is needed to clearly distinguish these compounds.

Migration times are shorter for phosphorylated monosaccharides as these compounds are smaller. Baseline separation of all three mono-phosphorylated sugars was not totally achieved (Figure 3) but Gluctose-1-phosphate (G-1-P), glucose -6-phosphate (G-1-P), fructose-1-phoshate (F-1-P) were distinguished by CESI. Baseline resolution of G-1-P (migrating at 9.57 minutes) and G-6-P (migrating at 9.75 minutes) was possible but complicated by F-1-P which eluted between these isomers at 9.66 minutes. Again fragmentation patterns were very similar but some diagnostic ions were possible as soon in the bottom panes of Figure 3. Again the separation of these mono-phosphorylated sugars is not possible by standard reverse phase LC. When this method was then applied to a plant extract two phosphorylated monosaccharides were identified (G-1-P and F-1-P) at low µMolar concentration in the extract (Figure 4).

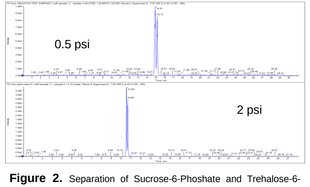

Figure 2. Separation of Sucrose-6-Phoshate and Trehalose-6-Phosphate by CESI and the effect of pressure on the separation

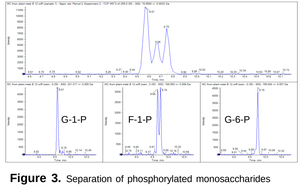

Figure 3. Separation of phosphorylated monosaccharides by CESI at a pressure of 2 psi.

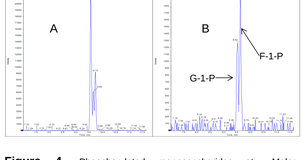

Figure 4. Phosphorylated monosaccharides at uMolar concentration (A) and adetected by CESI in a plant extract containing 6% methanol (B).

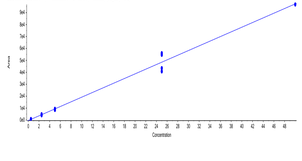

Figure 5. Calibration line for Sucrose-6-Phosphate.

To check on the linearity of response for this method standards several compounds from Sigma Aldrich were purchased and calibration standards were prepared in water. These standards were then injected in triplicate to test linearity of response (no internal standards were used in this test). Figures 5, 6 and 7 show the calibration lines for 3 out of the 5 phosphorylated sugars. All lines were linear over the range tested (0.5- 50 µg/ml) corresponds to concentrations in the µMolar range.

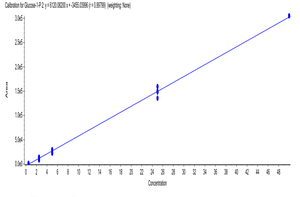

Figure 6. Calibration line for Glucose-1-Phosphate.

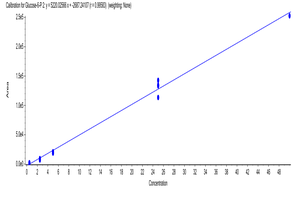

Figure 7. Calibration line for Glucose-6-Phosphate.

An important feature of this method is it's ability to separate the isobaric species of citrate and isocitrate within the same analysis. These compounds only differ by the position of the hydroxy-position and are highly polar and again very difficult to separate by reverse phase LC analysis. CESI-MS produced a baseline separation of these two species (Figure 8).

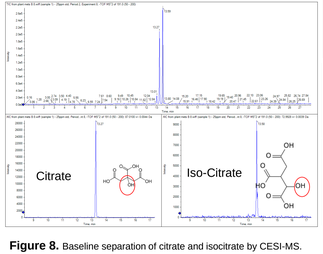

Figure 8. Baseline separation of citrate and isocitrate by CESI-MS.

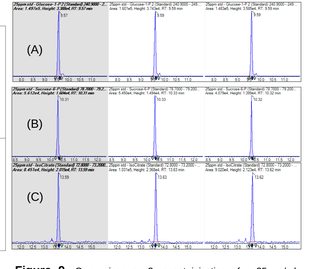

Figure 9. Comparison on 3 repeat injection of a 25 µg/ml standard (A=Glucose-1-Phosphate, B= Sucrose-6-Phosphate and C=Isocitrate)

To show reproducibility each standard was injected in triplicate. Figure 9 shows the electropherograms for 3 compounds and show the high reproducibility of the migration times. Key to this was the conditioning of the capillary between injections.

Ascorbate, pyruvate, malate, fumarate and succinate were detected in addition to citrate using this approach. This preliminary method has been set to detect over 30 negatively charged polar metabolites. An example of the separation of just three of these is shown in Figure 10 and an example of a calibration line for ascorbate whose migration time 13.38 minutes is shown in Figure 11.

Figure 10. Electropherograms for Fumarate (migration time of 13.4 minutes), malate (migration time of 14 minutes) and succinate (migration time of 14.5 minutes).

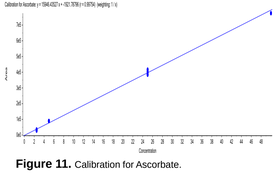

Figure 11. Calibration for Ascorbate.

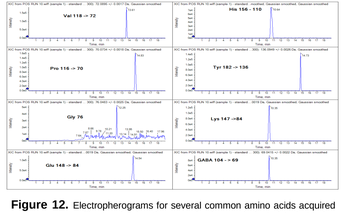

Figure 12. Electropherograms for several common amino acids acquired on the same capillary and using the same background electrolyte but using normal polarity and a different CE method.

In order to test the versatility of this system the analysis of underivitised amino acids as well as other positively charged plant metabolites was attempted using the same capillary and background electrolyte system. In this test the MS was switched to positive mode and the polarity for CE separation changed to normal mode. An example of the results is shown in Figure 12. Using this approach, all underivitised amino acids tested were detected in just a 20 minute window.

CONCLUSIONS

A fast, robust, and reliable method, for the detection over 30 negatively charged polar plant metabolites has been developed. The method uses a simple pressure injection and has been shown to produce reproducible migration times and separation of isobaric species and geometric isomers of phosphorylated sugars which is not possible by reverse phase HPLC separation

In addition by simply switching the polarity of the CE and MS systems, CESI-MS can be used to detect underivitised amino acids and positively charged plant metabolites in the same samples. This approach is therefore an ideal way for screening both positively and negatively charged polar metabolites in an automated approach which is very difficult to do using normal phase chromatography.

TRADEMARKS/LICENSING