Analysis of Chlorpheniramine, Codeine, and Methadone by CE/MS

Summary

The drugs chlorpheniramine, codeine, and methadone were separated using capillary electrophoresis/mass spectrometry (CE/MS). The use of linear polyacrylamide coated capillaries for the CE/MS separation of these drugs as cations is discussed. Finally, high-speed scanning UV absorbance detection in conjunction with mass spectrometric detection is demonstrated.

Conditions

Sample and Buffers

Separations were performed using 10 mM or 20 mM acetic acid, pH 3.4 and 3.2 respectively. Sheath liquids were 60/39/1 methanol/water /acetic acid or 70/29.5/0.5 isopropanol/water/formic acid. The sample solution of chlorpheniramine maleate (36 µM), codeine (39 µM), and methadone hydrochloride (32 µM) was prepared in 1 mM acetic acid.

Capillary Electrophoresis

Capillary electrophoresis was performed using the BioFocus® 3000 CE system. The capillary used for the separation was a 50 µm ID x 365 µm OD BioCAP™ linear polyacrylamide (LPA) coated capillary of 75 cm total length. The BioFocus system was connected to the Finnigan Atmospheric Pressure Ionization (API) electrospray source using the BioFocus CE/MS Interface.

Mass Spectrometry

A Finnigan MAT TSQ 700 fitted with a Finnigan API electrospray source was used to perform mass spectrometry. Auxiliary and sheath gasses were not used in this experiment. Mass data were collected from 50 to 650 mass-to-charge (m/z) units at a rate of 1 scan/sec.

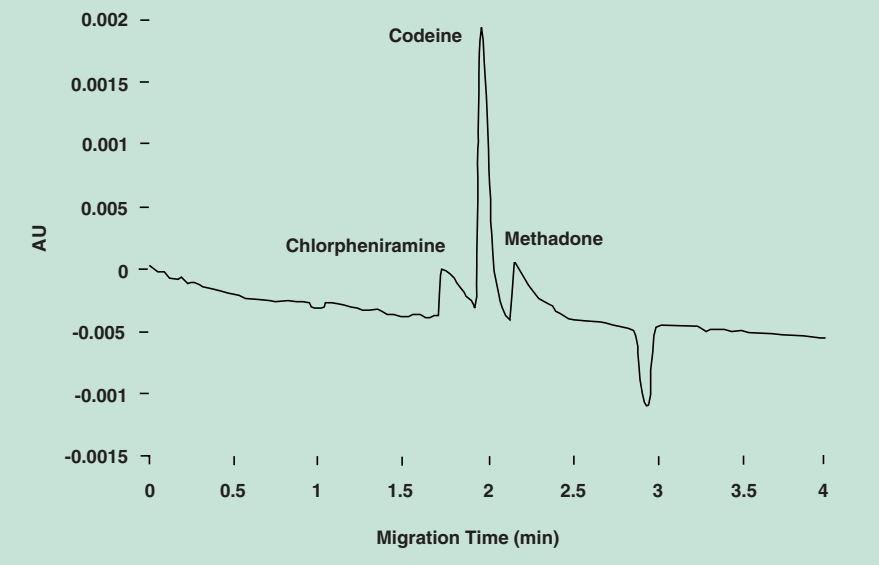

Fig. 1. Separation of chlorpheniramine, codeine, and methadone using an uncoated capillary and UV absorbance detection. Conditions: capillary, 50 µm ID x 365 µm OD with a total length of 50 cm; inlet buffer, 20 mM acetic acid, pH 3.2; outlet buffer, 70/29.5/0.5 isopropanol/water/formic acid; injection, 5 kV for 5 sec; separation voltage, 30 kV (+to-); detection, UV absorbance at 215 nm.

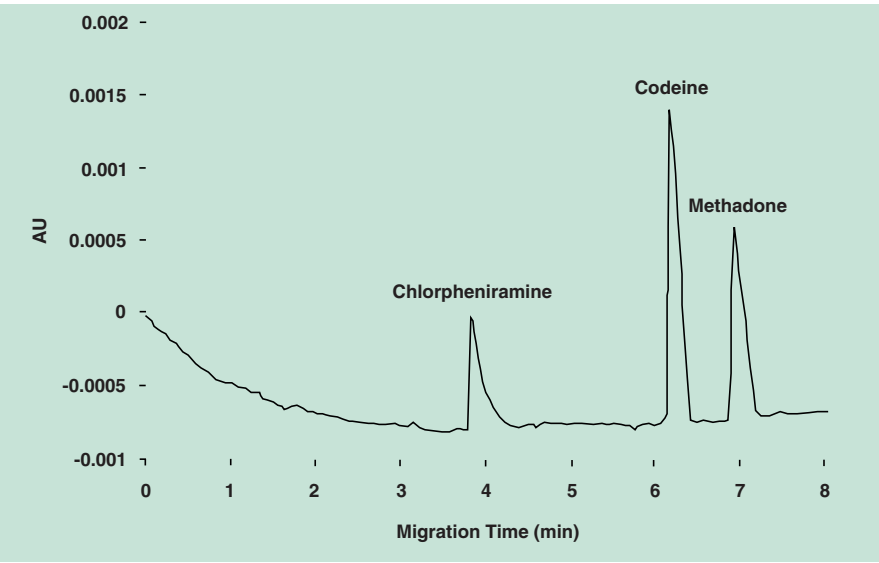

Fig. 2 . Separation of chlorpheniramine, codeine, and methadone using a coated capillary and UV absorbance detection. Conditions: capillary, 50 µm ID x 365 µm OD with a total length of 50 cm; inlet buffer, 20 mM acetic acid, pH 3.2; outlet buffer, 70/29.5/0.5 isopropanol/water/formic acid; injection, 5 kV for 10 sec; separation voltage, 30 kV (+to-); detection, UV absorbance at 215 nm.

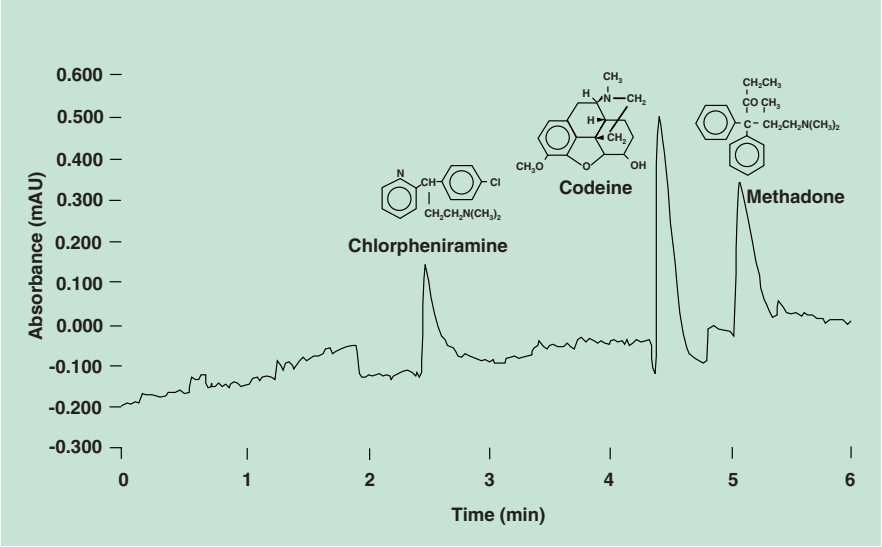

Fig. 3. UV absorbance electropherogram of chlorpheniramine, codeine, and methadone separated on a coated capillary in CE/MS mode. Conditions: capillary, 50 µm ID x 365 µm OD with a total length of 75 cm; inlet buffer, 10 mM acetic acid, pH 3.4; sheath liquid, 60/39/1 methanol/water/acetic acid delivered at a flow rate of 6 µl/min; injection, 12 kV, 20 sec with electrospray voltage (+4 kV) applied; separation electric field, 213 V/cm; electrospray voltage, +4 kV.

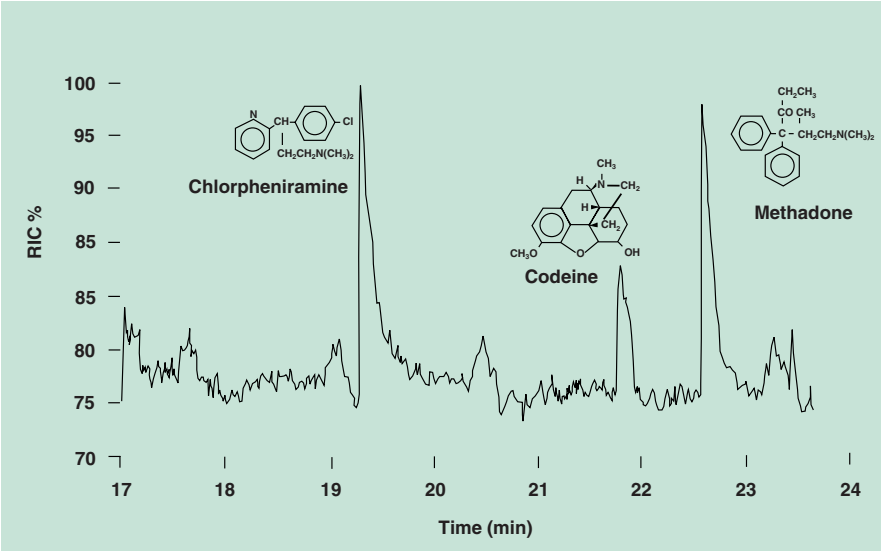

Fig. 4. Relative ion current electropherogram of chlorpheniramine, codeine, and methadone separated on a coated capillary. Conditions are the same as in Figure 3.

References

1. Foret, F., Thompson, P. V., Karger, B. L., Gebauer, P., Bocek, P., "Liquid Sheath Effects on the Separation of Proteins in Capillary Electrophoresis/Electrospray Mass Spectrometry", *Anal. Chem.*, **66**, 4450-4458 (1994).

Results and Discussion

Analysis of organic cations by CE is a straight forward technique. Therefore, it was initially predicted that the separation of a mixture of chlorpheniramine, codeine, and methadone would be routine. The only special condition was the exclusive use of volatile buffers. Our first attempts at this separation using an uncoated capillary revealed that chlorpheniramine and methadone interact with the capillary wall, resulting in severe peak distortion (Figure 1). Rather than use buffer additives to reduce or eliminate this interaction and possibly decrease the mass spectrometer sensitivity, this problem was nearly eliminated by the use of a BioCAP coated capillary (Figure 2).

After workup of the method using CE with UV absorbance detection, the method was transferred to CE/MS. In this mode, the drugs are adequately separated by the time they reach the UV absorbance detector (Figure 3), which was 17.5 cm from the inlet end of the capillary. The slight baseline instability is attributed to anions from the sheath liquid migrating into the capillary.[1] The relative ion current (RIC) electropherogram, based on those ions with an m/z ratio of 174 to 313, (Figure 4) reveals a peak pattern similar to that in Figure 3. Because the drugs have an additional 57.5 cm of capillary to migrate through between UV detector and the mass spectrometer, the analytes are better resolved in Figure 4 than in Figure 3.

To reduce the effects of ions entering the capillary from the sheath liquid, the conductivity of the sheath liquid was increased by using 0.5% formic acid rather than 1% acetic acid. In this configuration, the conductivity of the sheath liquid was greater than that of the buffer in the capillary. This resulted in increased baseline stability and allowed the full detection capabilities of a BioFocus 3000 system connected to a Finnigan mass spectrometer to be demonstrated.

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