Optimized parameters for the analysis of tropane alkaloids by CE-ESI-MS

Application Note

Pharmaceutical

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Abstract

The study of chiral molecules is of critical importance during drug characterization. Stereoisomers usually behave differently in biological systems, for example, they may have different pharmacokinetic properties. Regulatory agencies have recently changed their policies and only approve chiral compounds when both enantiomers and the racemate are characterized. Capillary electrophoresis (CE) is very suitable for quantitative evaluation of the isomeric composition of pharmacokinetic samples. CE with electrospray-ionization mass spectrometry (ESI-MS) for additional confirmation may then become the method of choice because of the greater specificity and sensitivity in selected ion mode (SIM).

Experimental

CE-ESI-MS analysis was performed using the Agilent Capillary Electrophoresis system with CE-MS capillary cassette coupled to the Agilent MSD equipped with electrospray source and orthogonal sprayer for CE-MS. Sheath liquid was delivered by an Agilent binary pump equipped with a 1:100 flow splitter. The Agilent ChemStation software was used for instrument control.

Sample preparation: five Datura seeds were crushed and taken into 1 mL ammonia in methanol (1 % v/v). After ultrasonication for 3 minutes the mixture was filtrated through a 0.22 µm filter. 200 µL of the filtrate was vacuumdried and dissolved in 100 µL water.

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Results

Atropine and scopolamine belong to the major active tropane alkaloids found in *Solanaceae* plants. Atropine consists of a racemic mixture of D- and L-hyoscyamine. Scopolamine is also known as l-hyoscine. All three tertiary amines show different antimuscarinic potencies. The separation of racemic mixtures by CE is achieved by adding chiral selectors, for example cyclodextrins (CD), to the buffer. However, during electrospray ionization these non-volatiles decrease the ionization efficiency. This effect can be minimized by using an orthogonal spraying device and by detecting ions in the more sensitive selected ion mode. When a method will be transferred from CE with UV-detection to CE with mass spectrometric detection, other electrophoretic and electrospray parameters must also be optimized.

Figure 1A shows the analysis of an alkaloid standard by CE-MS. Using optimized method parameters as given in the figure caption, detection limits in the mid-ppb range could be achieved. Reproducibility was < 3 % RSD for area and < 1 % RSD for migration time. The assay was linear ($r^2 > 0.999$) in a range of 1–100 mg/L. The method was then applied to the analysis of tropane alkaloids in Datura seeds. Figure 1B shows the analysis of an extract from *Datura tatula L.* seeds. Quantitative results are given in table 1.

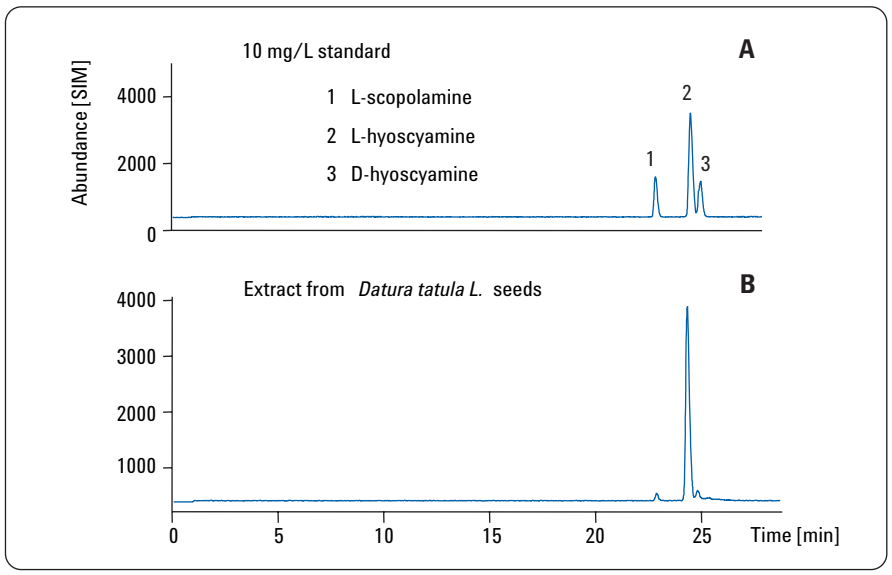

Figure 1
A: Analysis of tropane alkaloid standard
B: Analysis of an extract from *Datura tatula L.* seeds.

Chromatographic conditions

Injection:	10 sec @ 50 mbar
Capillary:	bare fused silica, L= 90 cm, 50 µm id
Buffer:	50 M formic acid, 200 mM trimethyl- -CD
Voltage:	30 kV
Temperature:	20°C
Preconditioning:	4 min flush with run buffer at 1 bar
Sheath liquid:	5 mM ammonium acetate in 50 %, methanol, 4 µL/min
Nebulizing gas:	nitrogen, 10 psi
Drying gas:	nitrogen, 6 L/min, 300 °C
Acquisition:	positive mode, Vcap -4 kV, fragmentor 40 V
SIM:	m/z 209.2, m/z 304.1

Alkaloid	*Datura metel L.*	*Datura tatula L.*	*Datura meteloides DC.*	*Datura innoxia Mill.*	*Datura inermis JACQ*
L-scopolamine	24	1.3	6.0	7.3	1.3
L-hyoscyamine	4.4	21.	5.9	6.0	12
D-hyoscyamine	0.3	1.1	0.4	0.4	0.8

Table 1
Quantitative results of the analysis of an extract from *Datura tatula L.* seeds.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent CE-MS Adapter Kit
- Agilent LC/MSD module with API Electrospray Source
- Agilent CE-ESI-MS Sprayer Kit
- Agilent ChemStation and CE-MS software

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