Introduction

The ELSD overcomes obstacles encountered in traditional HPLC detection. UV/Vis detection is limited by the inability to analyze compounds which contain chromophores with poor extinction coefficients. Alternative detectors such as fluorescence, photodiode array, electrochemical, mass spectroscopy, and refractive index might be considered for special situations. However, these detectors often place restrictions on the class of sample to be analyzed, the choice of mobile phase, and the use of gradient techniques. Sensitivity and cost control must also be considered with these detectors(1).

Evaporative light scattering detection solves many common HPLC detection problems. The ELSD detects any sample less volatile than the mobile phase. Therefore, the ELSD’s response does not depend on the samples optical characteristics. It detects any compound in the sample, regardless of its functional groups. The ELSD gives nearly equivalent response factors for all sample types giving a closer representation of sample composition. The ELSD is sensitive and versatile. It’s able to detect samples in the low nanogram range and is compatible with gradient solvent systems. Difficult samples are detected with great sensitivity and precision.

A reliable, accurate, and economical analytical method is needed for the analysis of rocuronium bromide, pancuronium bromide, pipercuronium bromide, and vecuronium bromide. These muscle relaxants have poor UV chromophores and a gradient solvent system is needed for a HPLC separation. This prohibits the use of traditional HPLC detectors such as ultraviolet and refractive index. Previously reported methods have analyzed vecuronium via mass spectrometry(2) and rocuronium via fluorescence detection with post column derivatization(3). These methods are costly and complicated.

The objective of this study was to develop a rapid HPLC method for the four muscle relaxants using an Evaporative Light Scattering Detector (ELSD). The ELSD response is compared to UV and RI. Multilevel calibration curves were generated and the limit of detection was determined for rocuronium bromide.

Experimental Conditions

The HPLC system consisted of an Alltech (Deerfield, IL) Model 570 Autosampler with a 20 μL loop, a Hitachi (Danbury, CT) L-6200A Intelligent Pump, an Alltech Model 500 ELSD (90°C Tube Temperature, 2.3L/min Gas Flow), a Linear (Thermo Separation Products, San Jose, CA) 205 UV-Vis Detector set at 210nm, a Waters (Milford, MA) Model 410 Differential Refractometer, and Hewlett Packard General Purpose Chemstation. An Alltech Adsorbosphere® UHS C18, 5 μm, 150 x 2.1mm column was used for the separation. Gradient elution with 0.1% TFA in Water and 0.1% TFA in Methanol was used.

HPLC-grade solvents and water were purchased from Burdick & Jackson (Muskegon, MI). Trifluoroacetic acid (TFA) was purchased from Sigma (St. Louis, MO). Vecuronium bromide, rocuronium bromide, and pipercuronium bromide were manufactured by Organon Inc. (West Orange, NJ). Pancuronium bromide was manufactured by Genus Pharmaceuticals, Inc. (Irvine, CA). All standards were diluted in water containing 0.1% TFA

Results and Discussion

Unlike the UV detector, the ELSD’s response does not depend on the analyte’s optical characteristics, nearly equivalent responses are achieved for all muscle relaxants, providing a closer representation of sample composition. The ELSD detects chromophoric and non-chromophoric analytes with nearly equivalent response factors. Although all components have equal concentrations, the UV response for Rocuronium Bromide is not as great as the other muscle relaxants, due to its poorer chromophoric properties.

The ELSD also provides a more stable baseline because it evaporates the mobile phase before detection occurs. Unlike the RI detector, the ELSD does not respond to ambient temperature changes. Even isocratically, the low flowrate used for this analysis does not allow for a stable baseline on the RI detector. The baseline drift seen in the UV chromatogram is caused by changes in TFA molar absorptivity as the methanol concentration in the mobile phase increases. This inhibits accurate quantitation of the muscle relaxants. The baseline drift is not present in the ELSD chromatogram.

The limit of detection (L.O.D.) was determined to be 9 ng of muscle relaxant on column. This was calculated from the signal to noise ratio obtained from a 1.56ng/μL standard using a 20 μL injection volume. The calculated detection limit is the analyte amount that produces a peak height that is three times the noise height. The L.O.D. calculation was based on rocuronium bromide.

Conclusion

The Evaporative Light Scattering Detector coupled with a reverse phase HPLC column provides a rapid and quantitative means for evaluation of muscle relaxants. Using the equipment and procedures described above, muscle relaxants are detectable in low nanogram levels.

For muscle relaxant analysis, the ELSD provides a stable baseline for a simpler and more accurate determination than UV or RI detection.

References


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Detected by ELSD

1. Citric Acid, Sodium Phosphate, Sodium Acetate, Mannitol
2. Rocuronium Bromide
3. Pancuronium Bromide
4. Pipecuronium Bromide
5. Vecuronium Bromide

Detected by UV at 205nm

1. Citric Acid, Sodium Phosphate, Sodium Acetate, Mannitol
2. Rocuronium Bromide
3. Pancuronium Bromide
4. Pipecuronium Bromide
5. Vecuronium Bromide

Detected by RI

1. Citric Acid, Sodium Phosphate, Sodium Acetate, Mannitol

Muscle Relaxants

Column: Adsorbosphere® UHS C18, 5µm, 150 x 2.1mm
Column Temp: 40°C
Sample Conc: 25ng/µL
Mobile Phase: A: 0.1% TFA in Water
              B: 0.1% TFA in Methanol
Gradient: Time: 0 2 12 15
          %B: 35 35 57 57
Flowrate: 0.2mL/min
Detector: ELSD
Drift Tube Temp: 90°C
Gas Flow: 2.3L/min

Calibration Curve

- Vecuronium Bromide
- Rocuronium Bromide
- Pancuronium Bromide
- Pipecuronium Bromide

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