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Application Note SI-01213

Effective HPLC Determination of Fentanyl using ELSD

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Introduction

Fentanyl was first synthesized in Belgium in the late 1950s. With an analgesic potency of about 80 times that of morphine, it was introduced into medical practice in the late 1960s as an intravenous anaesthetic and today fentanyls are extensively used for anaesthesia and analgesia. Illicit use of pharmaceutical fentanyls first appeared in the mid-1970s in the medical community and continues to be a problem in the United States. To date, over 12 different analogs of fentanyl have been produced clandestinely and identified in the US drug traffic. The biological effects of the fentanyls are indistinguishable from those of heroin, with the exception that fentanyls may be hundreds of times more potent. There is, therefore, a requirement to screen urine samples, both from human and animal sources, and to detect and quantify fentanyl present in very low concentrations.

In this experiment, an isocratic HPLC method was used to analyze samples of fentanyl prepared at fixed concentrations over the range 0.5 µg/mL up to 200 mg/mL. The Varian evaporative light scattering (ELS) detector was employed because of its superior performance; the detection method is independent of the optical properties of the compound under consideration.

Instrumentation

Column: Silica C18 5 µm, 50 x 2.1 mm
Detector: Varian ELSD (neb=40 °C, evap=70 °C, gas=1.2 SLM)

Materials and Reagents

Eluent: 60 % 0.25 M Ammonium acetate in water, 40 % Acetonitrile

Sample Preparation

Concentration: 0.5–200 µg Fentanyl/mL

Conditions

Flow Rate: 0.25 mL/min
Injection Volume: 10 µL

Results and Discussion

Figure 1 shows typical chromatograms obtained for two concentrations of fentanyl within the range 0.5–200 µg/mL.

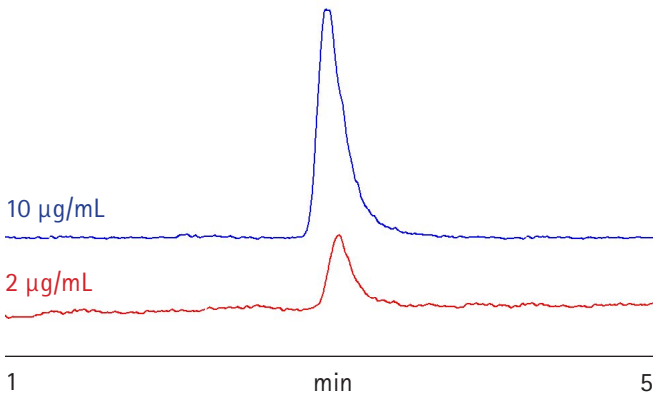

Figure 1. Detection of two concentrations of fentanyl within the range 0.5–200 µg/mL.

The peak area measured for every concentration was used to generate a calibration curve as shown in Figure 2 that indicates good linearity over a wide concentration range. The limit of detection was determined at the 1 µg fentanyl/mL loading as this resulted in a peak signal to noise ratio of 3.4; this LOD represents 10 ng on-column loading.

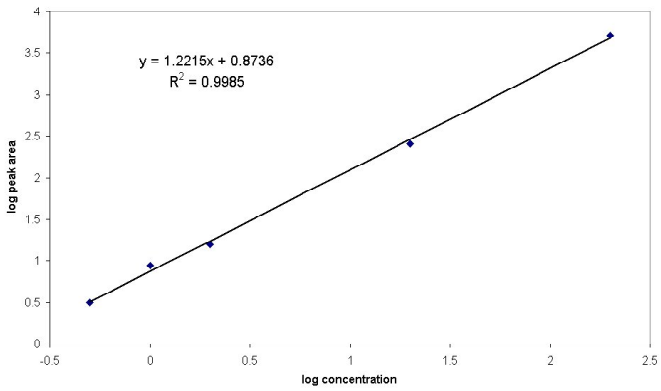

Figure 1. Calibration curve for fentanyl (log-log plot).

Conclusion

Varian ELSD successfully revealed an LOD for fentanyl at 10 ng on column. The Varian ELS detector surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The Varian ELS detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Varian ELS detector provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

These data represent typical results. For further information, contact your local Varian Sales Office.

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Application Note SI-01213 / TB1045