Rapid Quantification of an Enkephalin Analgesic in Cerebrospinal Fluid Using the Agilent 1100 Series LC/MSD

Application Note

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Introduction

D-penicillamine enkephalin (DPDPE) is a synthetic enkephalin used for control of pain. In the past, this pentapeptide has proven difficult to analyze. Because it has a disulfide bridge, immunoassays have not been predictable. Other methods have required radiolabeling, or have proven to be laborious or lacking in sensitivity. This application note demonstrates the rapid, sensitive analysis of DPDPE in cerebrospinal fluid (CSF), using an Agilent 1100 Series LC/MSD quadrupole mass spectrometer.[1]

Experimental

DPDPE was analyzed in combination with an internal standard (IS) of Tyr-D-Ala-Gly-Phe-D-Leu-OH (DADLE). Stock solutions were prepared in mobile phase at 1000 µg/ml and stored at –70°C in high-density polyethylene cryovials. These were diluted each day to 1 µg/ml in sterile saline and used to spike samples prior to extraction.

Standards of DPDPE were prepared at 1, 5, 10, 100, 500, and 1000 ng/ml in naive CSF. Quality control (QC) samples were prepared in CSF from independent stock solutions.

Study samples were obtained from dogs following spinal injection of 0.3 mg/kg DPDPE. CSF samples were drawn at timed intervals up to 24 hours after administration.

CSF standards, blanks, QC samples, and study samples were all processed using the same procedure. First, 5 µl of sample was added to a 0.5-ml high-density polyethylene microcentrifuge tube. Then 5 µl of IS solution was added and the mixture was vortexed for five seconds. This solution was loaded onto a conditioned C_{18} sample preparation pipette tip (Millipore ZipTip). The ZipTip was then rinsed with five 20-µl aliquots of water. The sample was eluted into an autosampler vial with 5 µl methanol.

Duplicate 1-µl injections of each sample were analyzed using the Agilent 1100 Series LC/MSD in selected ion monitoring (SIM) mode. Compounds eluting in the first 45 seconds of each analysis were diverted to waste. The guard column was replaced every 100 injections, but the analytical column was rinsed daily with methanol and was used for the duration of the six-month study.

Results and Discussion

Figure 1 shows typical SIM chromatograms from a CSF study sample. The analytes were free of significant interferences throughout the study. The linearity was excellent in the 1–1000 ng/ml range, with the correlation coefficient being 0.9909 and the intercept being 0.0008.

Instrument Conditions

LC Conditions

Column:	ZORBAX SB-C_{18}, 30 x 2.2 mm, 3.5 µm
Guard cartridge:	MetaChem Intertsil, 5 x 2 mm, 3 µm
Flow rate:	0.4 ml/min.
Injection volume:	1 µl
Mobile phase:	isocratic 75:25 methanol:10 mmol ammonium formate (pH 3)
Autosampler temp.:	4 ± 0.5°C

MS Conditions

Ionization mode:	positive ESI
Drying gas flow:	4 l/min
Nebulizer:	20 psig
Drying gas temp.:	350°C
Vcap:	6000 V
Fragmentor:	80 V
SIM ions:	646.2 DPDPE
	570.7 IS

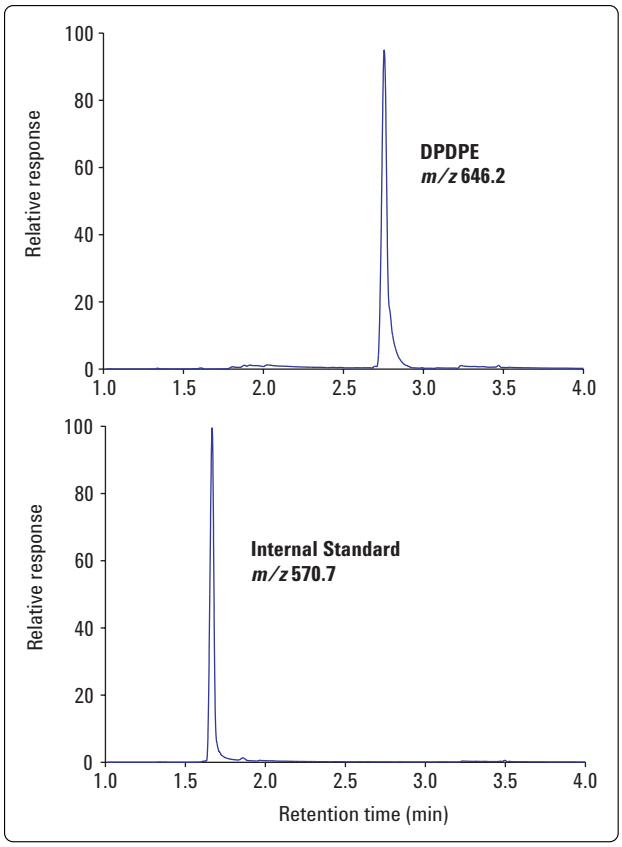

Figure 1. Typical selected ion monitoring chromatograms from study sample

The limit of quantitation (LOQ) was 1.00 ng/ml. At the LOQ, measured concentrations deviated less than ± 7.42% from actual, and the precision was 8.1% relative standard deviation (% RSD).

Tables 1 and 2 show the accuracy and precision for the DPDPE analysis. The accuracy was within 10.2% and the precision was within 9.33%.

Table 1. Accuracy for QC samples of DPDPE (10 replicates on 6 different days)

Concentration (ng/ml)		
Nominal	Observed	Accuracy (% deviation)
5	5.10	10.2
100	103.7	3.72
1000	1016.5	1.65

Table 2. Precision for QC samples of DPDPE (10 replicates on 6 different days)

	Precision (% RSD)	
Concentration (ng/ml)	Within Day	Between Days
5	7.16	9.33
100	4.99	5.39
1000	0.56	1.51

Extraction efficiencies were measured between 97% and 102%. A stability study at three concentration levels showed that the extracted samples were stable for 48 hours, with a relative standard deviation of +/– 7.4%.

Figure 2 shows the concentration versus time profile for DPDPE in the CSF of dogs administered a single spinal injection of 0.3 mg/kg DPDPE. The pharmacokinetic profile observed in dogs was identical to that of similar hydrophilic opioids in other species.

Conclusions

This work demonstrates an improved assay for DPDPE in CSF involving extraction using ZipTip pipette tips followed by selected ion monitoring analysis using the Agilent 1100 Series LC/MSD. The assay developed for DPDPE was fast, simple, sensitive, selective, accurate, and precise. Sample recovery and sample stability were excellent. The sample size requirements were very small (5 µl), making this an ideal assay for small animals.

References

1. Steven Rossi and Tony Yaksh. "Rapid Quantification of the δ-Opioid Receptor Selective Enkephalin DPDPE in Canine Cerebrospinal Fluid by Liquid Chromatography – Mass Spectrometry," *Journal of Chromatography,* **772** (2002), 73–79.

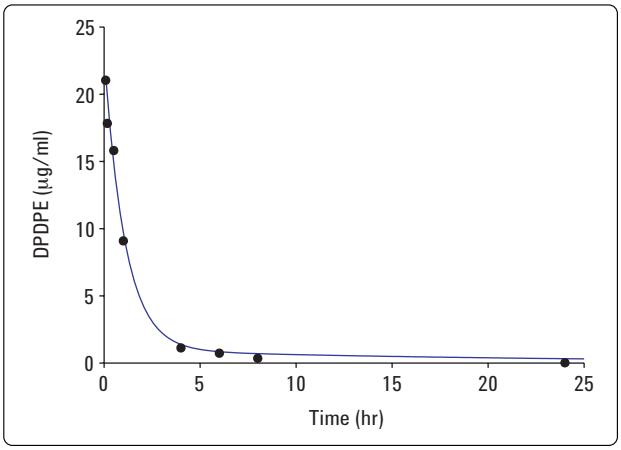

Figure 2. Profile of mean DPDPE concentration versus time in cerebrospinal fluid of three dogs, following spinal injection of 0.3 mg/kg DPDPE

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