Improving the Detection Limits for Highly Basic Neuropeptides Using CESI-MS

Stephen Lock
SCIEX UK

Overview

Who Should Read This: Senior Scientists and Lab Directors with an Interest in Protein Analysis and Proteomics Research.

Focus: High sensitivity neuropeptide detection in biological samples by mass spectrometry.

Goals: To improve the detection limits for highly basic neuropeptides by mass spectrometry.

Problem: Important neuropeptides include Vasoactive Intestinal Peptide (VIP), Pituitary Adenylate Ayclase-Activating Polypeptide (PACAP) and Parathyroid Hormone (PTH). These peptides are present at low physiological levels, so analysis requires low limits of detection. In addition, sample availability is often limited, so analysis from small sample volumes is needed. However, these very basic peptides (isoelectric points > 10) are difficult to analyze by LC-MS methods because they bind to LC auto-sampler components and columns resulting in very poor chromatographic results.

Results: The SCIEX CESI 8000 Plus system enabled the MS detection of the target neuropeptides with over 20 fold more sensitivity than a traditional LC-MS approach. The method was successfully applied to spiked plasma extracts which had been purified by offline solid phase extraction.

Key Challenges:

- Separation and detection of neuropeptides in biological samples
- Carry-over from one sample to the next using LC-MS
- Low limits of detection required

Key Features:

- Neuropeptides were detected in biological samples using a SCIEX CESI 8000 Plus connected to a SCIEX QTRAP® 6500+
- 100 times less carry-over compared with a traditional LC-MS approach using the same Mass Spectrometer.
- 20 x more sensitivity than with a traditional LC-MS approach.

CESI 8000 Plus High Performance Separation – ESI Module

Experimental Design

Sample Preparation for CESI-MS

All chemicals were Reagent Grade and were purchased from Sigma Aldrich, including standards of VIP and PACAP-38. Standard stocks were prepared by dissolving the solid peptides into water to make 0.1 mg/mL concentration standards. These stocks were serially diluted into different solvents.

CESI-MS Analysis

Samples were separated using a Neutral OptiMS cartridge (30 µm ID, 91 cm long) thermostatically controlled at 25° C with a background electrolyte (BGE) of 1% formic acid: 20% Methanol: 79% water. Two injection modes were used; either isotachophoresis (tITP, 5 psi, 99s of a sample containing 100 mM ammonium acetate) or electrokinetic supercharging (EK, 99 s, 10 kV injection after solvent plugs of ammonium acetate and water shown in Table 1). Both methods used the same rinse and separation conditions (Table 1). A SCIEX QTRAP® 6500+ system, fitted with the NanoSpray® III source was used for peptide detection. The source conditions were optimized for low nano-flow rates (source temperature = 50° C, Gas 1 and 2 = 0 psi and curtain gas = 5 psi) with just the ion-spray voltage (1800 V) used to generate spray. The other MRM conditions are shown in Table 2. The MS method was split into 3 periods to cover the CE separation. In the first and last periods (1 minute each) the ionspray voltage was set to zero so that no spray occurred.

Action	Time (min)	Pressure (psi)	Pressure Direction	Voltage (kV)	Solution
Rinse	2.5	100	Forward	0	0.1 Molar HCl
Rinse	3	100	Forward	0	BGE
Rinse	0.75	75	Reverse	0	10% Acetic acid
Solvent plug	60 sec	0.5	Forward	0	250 mM Ammonium Acetate
Solvent plug	10 sec	0.5	Forward	0	Water
Injection	99 sec	0	Forward	10	Sample Vial
Separation	25	5	Forward and Reverse	30	BGE
Separation	2	100	Forward and Reverse	10	BGE
Voltage Ramp down	2	10	Forward	1	BGE

Table 1. CESI method parameters used for separations using electrokinetic injections at 25° C.

Curtain gas	5psi	Peptide	Q1 Mass	Q3 Mass	CE (V)
Gas 1	0 psi	PACAP	567.6	671.8	23
Gas 2	0 psi	PACAP	567.6	647.7	23
ISV	1600 V	VIP	666	663	24
DP	80 V	VIP	666	771.5	27
EP	10 V				
CXP	13 V	Resolution	Q1 Unit	Q3 Unit	
Heater	50°C				

Table 2. MRM conditions used for both LC-MS and CESI-MS methods.

Time [min]	Flow rate (µl/min)	% Water Containing 0.1% Formic Acid	% Acetonitrile Containing 0.1% Formic Acid
1	300	95	5
10	300	50	50
12	300	5	95
13	300	5	95
13.1	300	95	5
16	300	95	5

Table 2. LC-MS gradient method used for the separation of samples at 40° C.

LC-MS Analysis

For the LC-MS analysis an IonDrive™ Turbo V source was used as the separations were run at 300 µL/min. The source conditions were optimised for this flowrate (source temperature = 550° C, curtain gas = 30 psi, gas 1 = 50 psi and gas 2 = 60 psi) with the ionspray voltage at 4500 V. The other MRM conditions were the same as used for the CESI-MS analyses. The LC system was a Nexera system from Shimadzu and samples (10 µL) were injected onto a Phenomenex AERIS PEPTDIE 100 x 2.1mm 1.7 µM XB-C18 HPLC column held at 40° C.

Factors Effecting EK Injections with CESI-MS

When using electrokinetic (EK) injections the pH, salt levels and organic content of the sample have an effect on the peak height. It was found that when the acid concentration was too high, the sensitivity dropped dramatically. So for this application it is recommended that low levels of acetic acid (e.g. 0.05%) be used in the sample solvent (Figure 1). Also the organic content and type of solvent present in the sample impacted sensitivity. In Figure 2 three organic solvents were compared and acetonitrile produced the best response. The level of organic solvent in the sample had an effect on the response and a level of 50 -75% was found to be the best. If 100% organic solvent was used the conductivity of the sample was low and the peak height of an injection dropped.

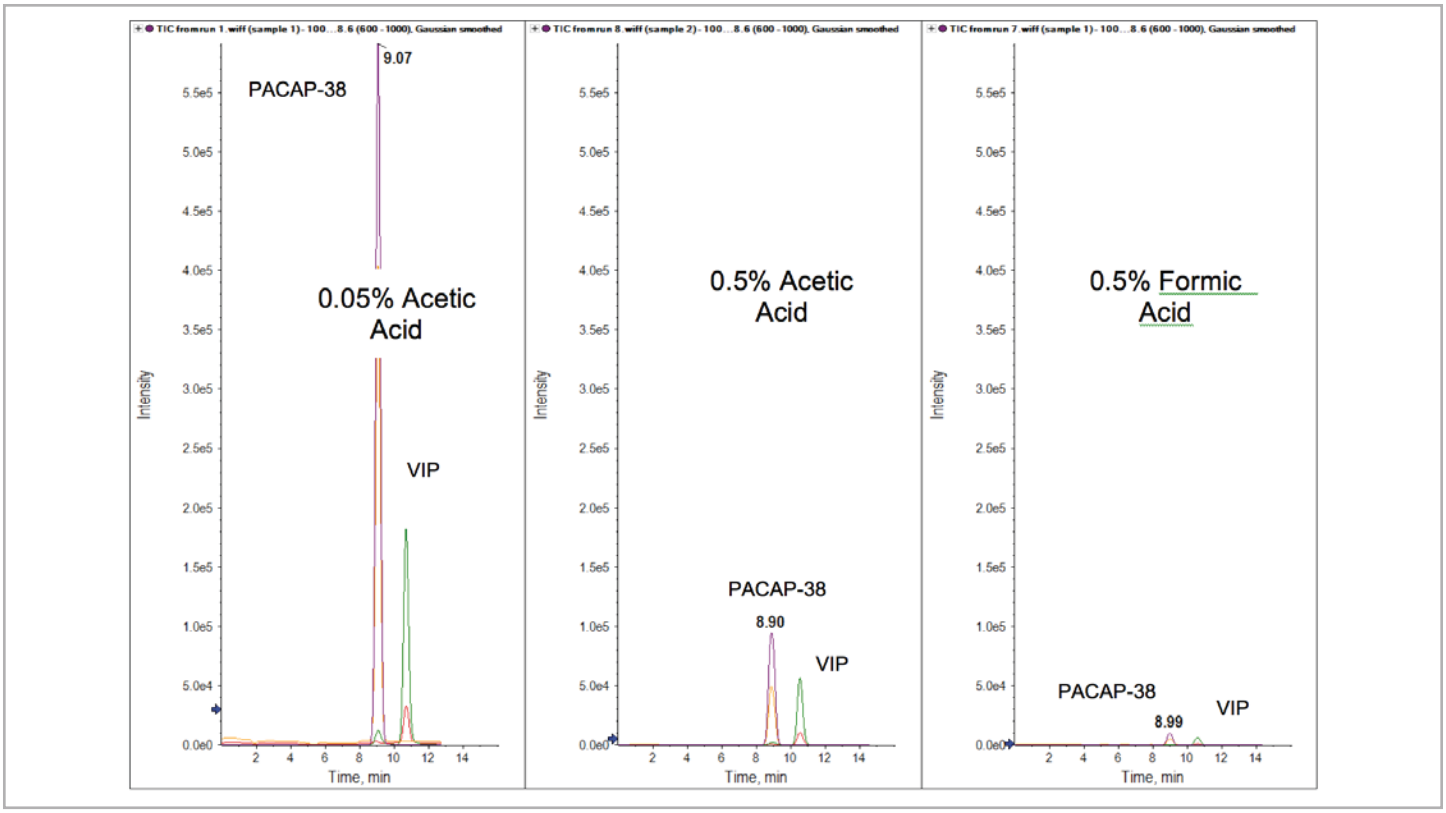

Figure 1. The effect of acid content on CESI-MS sensitivity [PACAP-38 (first peak) and VIP (second peak)].

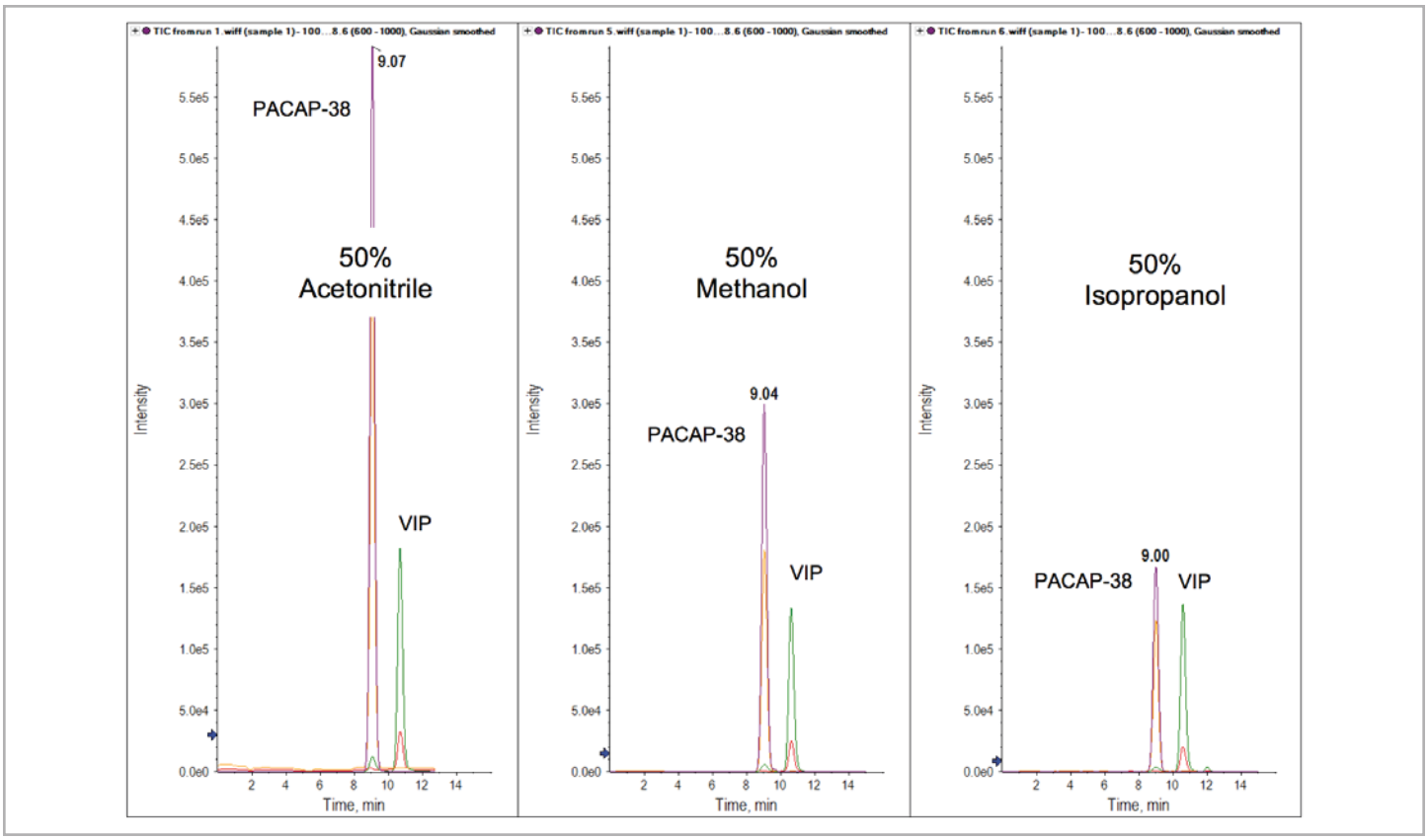

Figure 2. The effect of organic solvent in the sample on the sensitivity [PACAP-38 (first peak) and VIP (second peak)].

Comparison of CESI-MS Separation with LC-MS for Neuropeptide Quantitation

When EK injection was compared with the standard LC-MS approach (Figure 3), the first noticeable difference was that the EK injection was significantly more sensitive than the LC-MS injection. When a blank was injected, after a 10 ng/mL standard, the carryover with CESI-MS was only 0.0069% compared to 0.87% with LC-MS (both systems used standard conditioning methods). The small amount of carryover seen in CESI-MS was later attributed to PACAP-38 on the outside of the capillary. When the EK injection was compared with the tITP injection the tITP injection gave a similar response to the LC-MS analyses (Figure 4) but was slower than the EK approach.

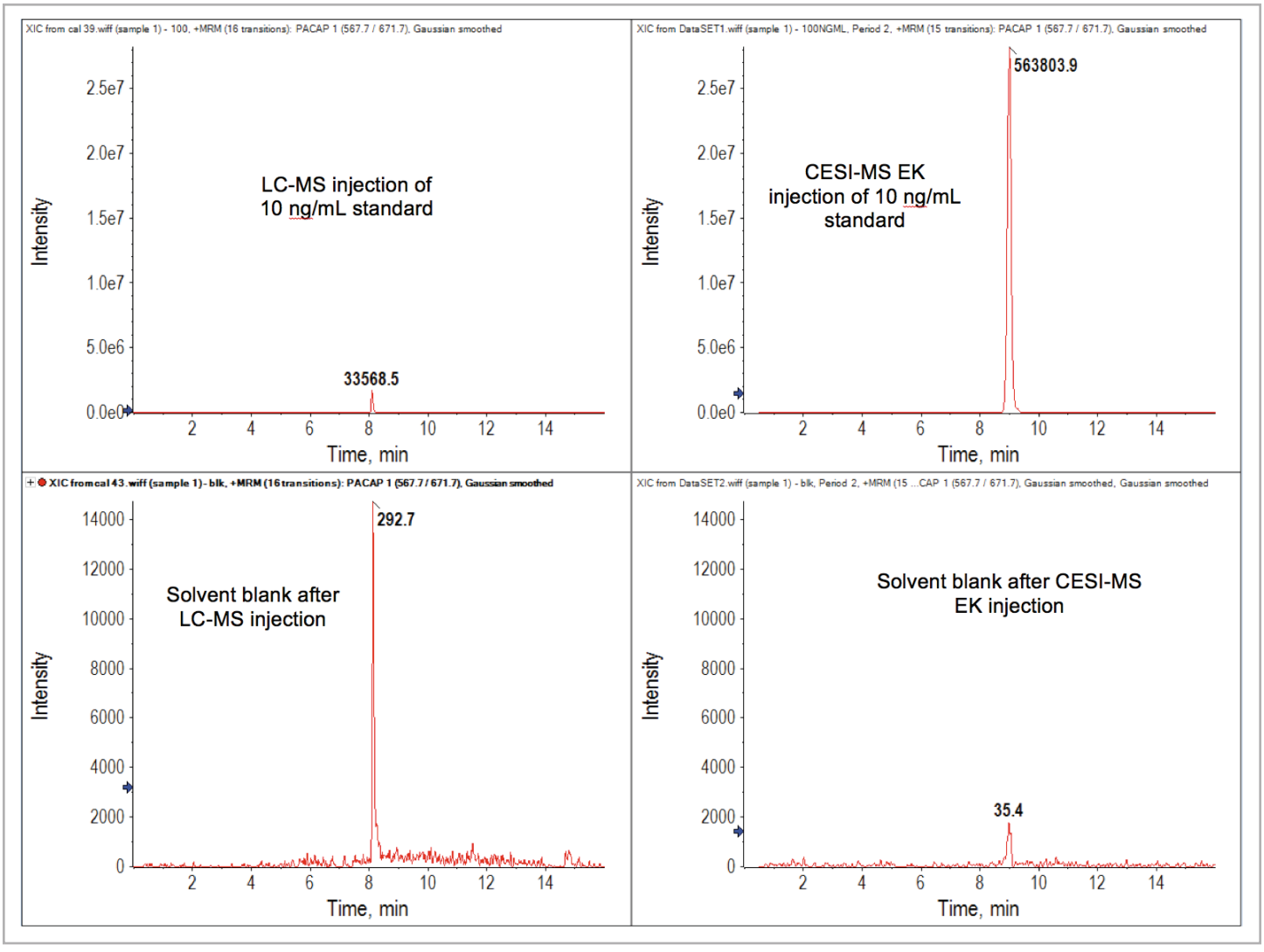

Figure 3. A carryover comparison of CESI-MS vs LC-MS all peaks are labelled with signal to noise for PACAP-38.

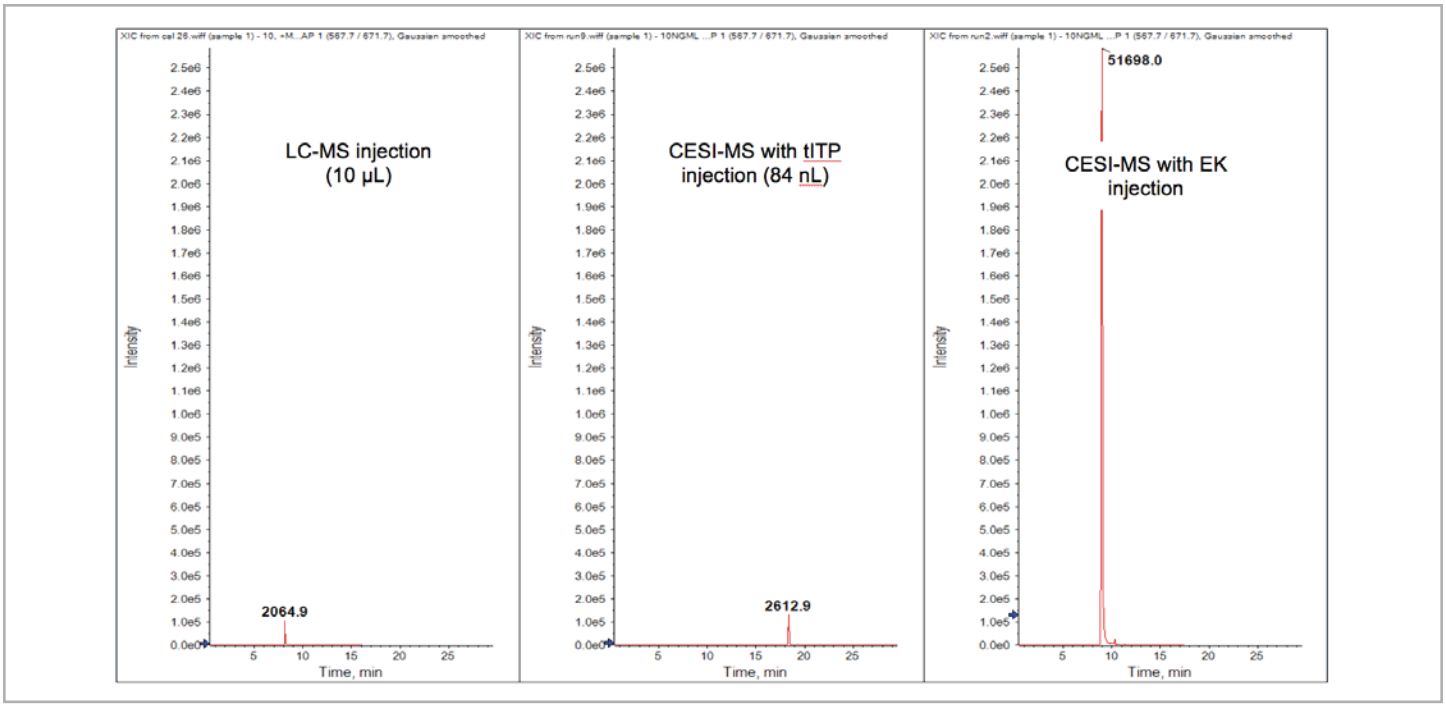

Figure 4. Comparison of a LC-MS with EK and a tITP injection by CESI-MS using a 10 ng/mL (all peaks are labelled with signal to noise for PACAP-38).

Linearity and Robustness of CESI-MS EK Injections

The EK method was further shown to give a linear response over the range 1 – 100 ng/mL (Figure 5) even though no internal standard was used. When it was tested on an SPE extract of a 1 ng/mL spiked plasma sample (produced using a previously developed protocol,[1] the response for PACAP and VIP was high (>1 million cps). In these initial experiments, the CV for the analysis of the same SPE extract was <10% (Figure 6) even without the use of a labelled internal standard. Even though the current sample preparation method is still under development and recoveries are only in the range of 10 - 20% this approach was shown to be reproducible and when compared with the previously published study,[1] which used CESI-MS tITP injections, was 10 times more sensitive.

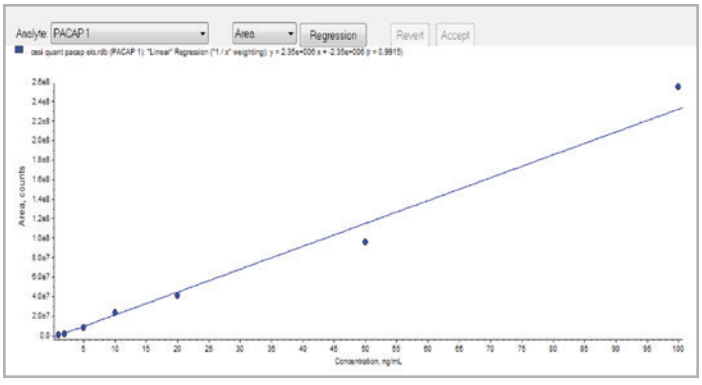

Figure 5. Calibration Line for CESI-MS EK injection approach for PACAP-38 range was 1 – 100 ng/mL (no internal standard was used).

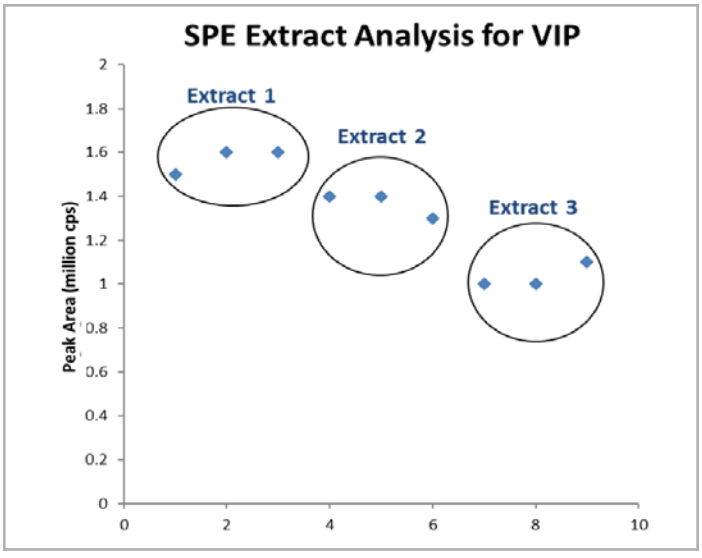

Figure 6. . Three SPE extracts of human serum samples spiked with 1 ng/mL of VIP analyzed by CESI-MS by separate EK injections (no internal standard was used).

Reference

1. Lock, S. 'The application of Capillary Electrospray Ionization to the detection of Neuropeptides'. Conference Proceedings ASMS 2016, San Antonio, June 5-9, 2016.

Who is SCIEX? SCIEX company's global leadership and world-class service and support in the capillary electrophoresis and liquid chromatography-mass spectrometry industry have made it a trusted partner to thousands of the scientists and lab analysts worldwide who are focused on basic research, drug discovery and development, food and environmental testing, forensics and clinical research.

Contact Us: sciex.com/contact-us

Publication number: RUO-MKT-02-7150-A 01/2018

Headquarters
500 Old Connecticut Path, Framingham, MA 01701, USA
Phone 508-383-7800
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices