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# Chemometric Deconvolution of Continuous Electrokinetic Injection Micellar Electrokinetic Chromatography Data for the Quantitation of Trinitrotoluene in Mixtures of Other Nitroaromatic Compounds

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<b>14. ABSTRACT</b>  The chemometric deconvolution of spectral data from continuous electrokinetic injection of mixtures of nitroaromatics for the quantitation of trinitrotoluene (TNT) is presented. Partial least squares regression (PLS) models were constructed for TNT, and the ability of these models to correctly predict TNT concentration in the presence of other nitroaromatic interferents was explored. Spectral similarities between TNT and other nitroaromatic compounds are shown to result in incorrect identification and quantitation of those compounds as TNT when interferent data is not incorporated into the PLS models, highlighting an important limitation of the technique. Using properly calibrated PLS models, TNT concentration is correctly identified and quantified in a solution containing 2,4-DNT, 2,6-DNT, and 4-Amino-2,6-DNT when all three are treated as either interferents, or alternatively as analytes of interest (whether they are also identified and quantified).								
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## 1. Introduction

On-site detection of nitroaromatics and nitramines is an ongoing concern for the Navy and Department of Defense as it relates to the detection of underwater mines, improvised explosive devices, and environmental contamination from training areas. Our efforts have focused on developing electrophoretic methods for the direct analysis of seawater samples [1, 2] for the presence of nitroaromatic and nitroamine explosives and their degradation products.

Most separation techniques require a discrete injection of analyte followed by a separation step in order to establish the identity and concentration of analytes of interest. By the nature of the separation mechanism, electrophoretic processes can replace the separation step with continuous electrophoretic injections. Sample is continuously electrokinetically injected into the capillary, resulting in zones of analyte migrating past the detector. Instead of a discrete peak, whose migration time is indicative of mobility, a plateau rises and maintains its height. Multiple analytes in the sample matrix will result in multiple plateaus, with the electropherogram taking on the appearance of stair steps. This work addresses the issues associated with processing complex continuous electrokinetic injection data sets by implementing partial least square regression analysis to multi-wavelength absorbance data via a diode array for the detection and quantitation of a mixture of trinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 4-amino-2,6-dinitrotoluene.

## **2. Materials and Methods**

### **2.1 Reagents and Materials**

Sodium tetraborate, sodium cholate, and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO). Individual explosives standards, including TNT, 2,4-DNT, 2,6-DNT, and 4-Amino-2,6-DNT, were purchased from AccuStandard (New Haven, CT) at a concentration of 1000 µg/mL in acetonitrile. Fused silica capillary (100 micron i.d., 360 micron o.d.; polyimide coated) was purchased from Polymicro (Phoenix, AZ) with internal diameters of 50, 75, and 100 microns. All capillaries had an outer diameter of 360 microns and were coated with polyimide to impart mechanical stability.

### **2.2 Instrumentation**

All separations were performed on a Beckman Coulter PACE MDQ capillary electrophoresis instrument equipped with a diode array detector detector (Fullerton, CA). Detection occurred from 190-300 nm at 1 nm increments. Capillary temperature was maintained at 25°C and the instrument was utilized at all times per manufacturer recommendations.

### **2.3 Separation Conditions**

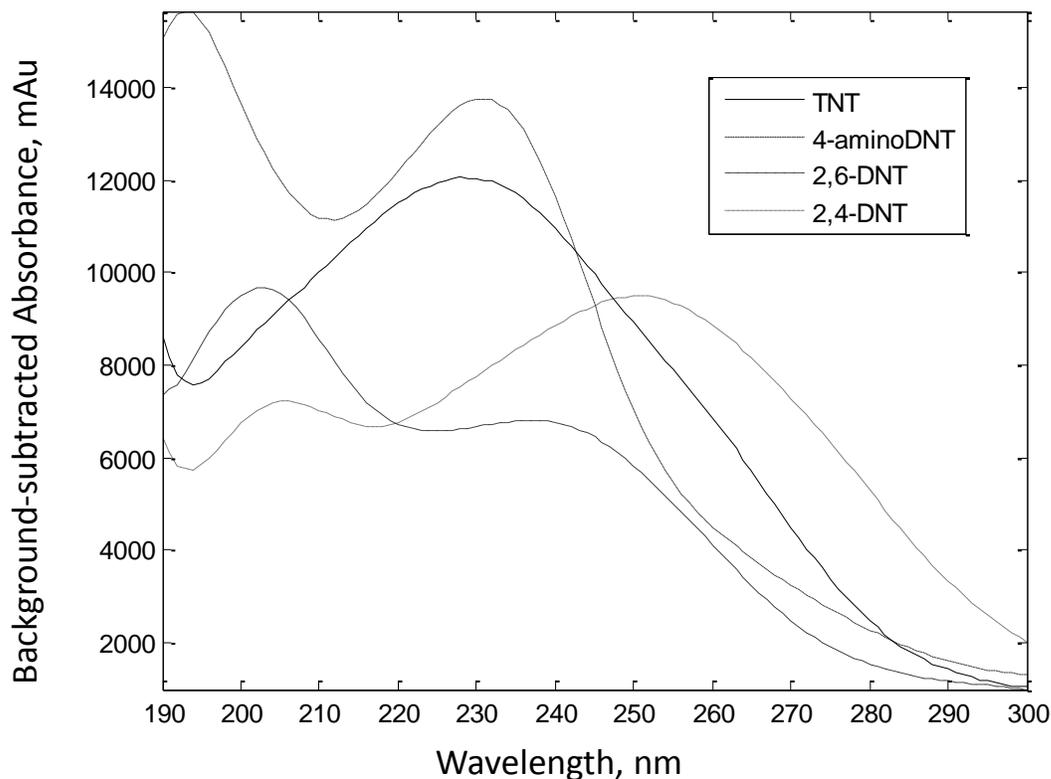
The capillary was rinsed with 1 M NaOH and water, followed by the background electrolyte (BGE). The BGE consisted of 10 mM tetraborate and 80 mM cholate. A voltage of 15 kV was applied with sample at the inlet and BGE at the outlet. The capillary has a total length of 30 cm and an effective length of 10 cm.

### **2.4 Data Set**

Six replicate CIMEKC analysis were performed for each of four analytes (TNT, 2,4-DNT, 2,6-DNT, 4-Am-2,6-DNT) at each of three concentrations (5, 10, and 20 ppm in

acetonitrile) in background electrolyte (BGE) as well as standard BGE blanks. Additionally, several mixtures of the four analytes were analyzed in triplicate.

Each analysis resulted in a matrix of data comprised of UV spectra acquired over time. In the early portion of the analysis, only background electrolyte is moving past the detector. Each analyte begins to appear at the detector at a different time, and past that time, continues to be present. Prior to construction of chemometric models, all data were background-subtracted by averaging 50 spectra extracted from a non-analyte region of the data and then subtracting this average spectrum from all spectra in the data set. Figure 1 shows background-subtracted spectra for 20 ppm TNT, 2,4-DNT, 2,6-DNT, and 4-aminoDNT.

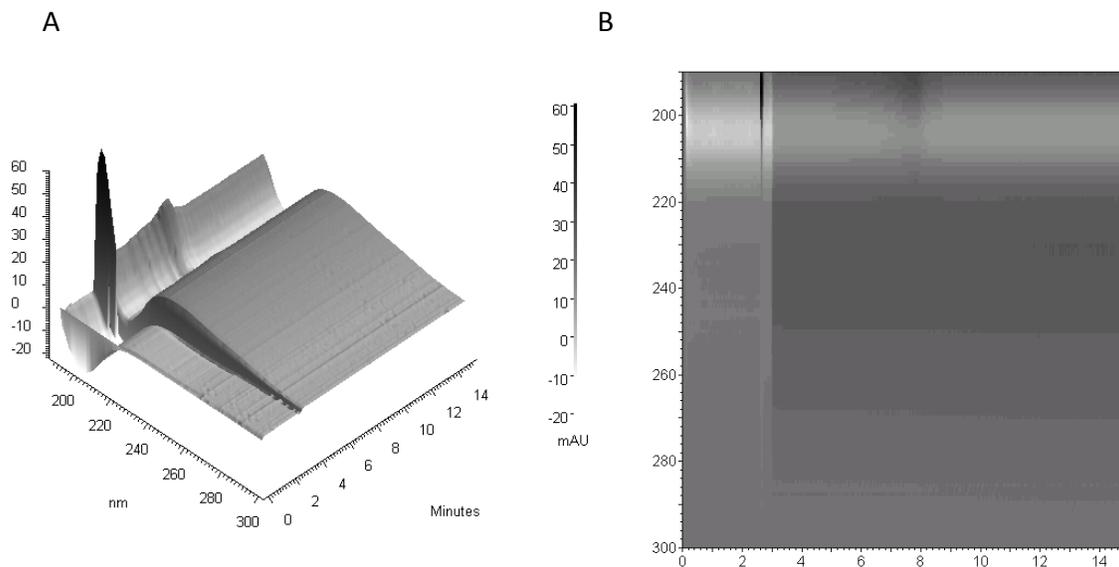


**Figure 1.** Background-subtracted spectra for 20 ppm TNT, 2,4-DNT, 2,6-DNT, and 4-aminoDNT.

Partial least squares (PLS) regression models were constructed from CIMEKC data by extracting 200 spectra from the later regions of three of the six replicate runs for each concentration, as well as the blanks. These models were then evaluated against the remaining three replicate runs, as well as against the analyte mixture runs.

### 3. Results and Discussion

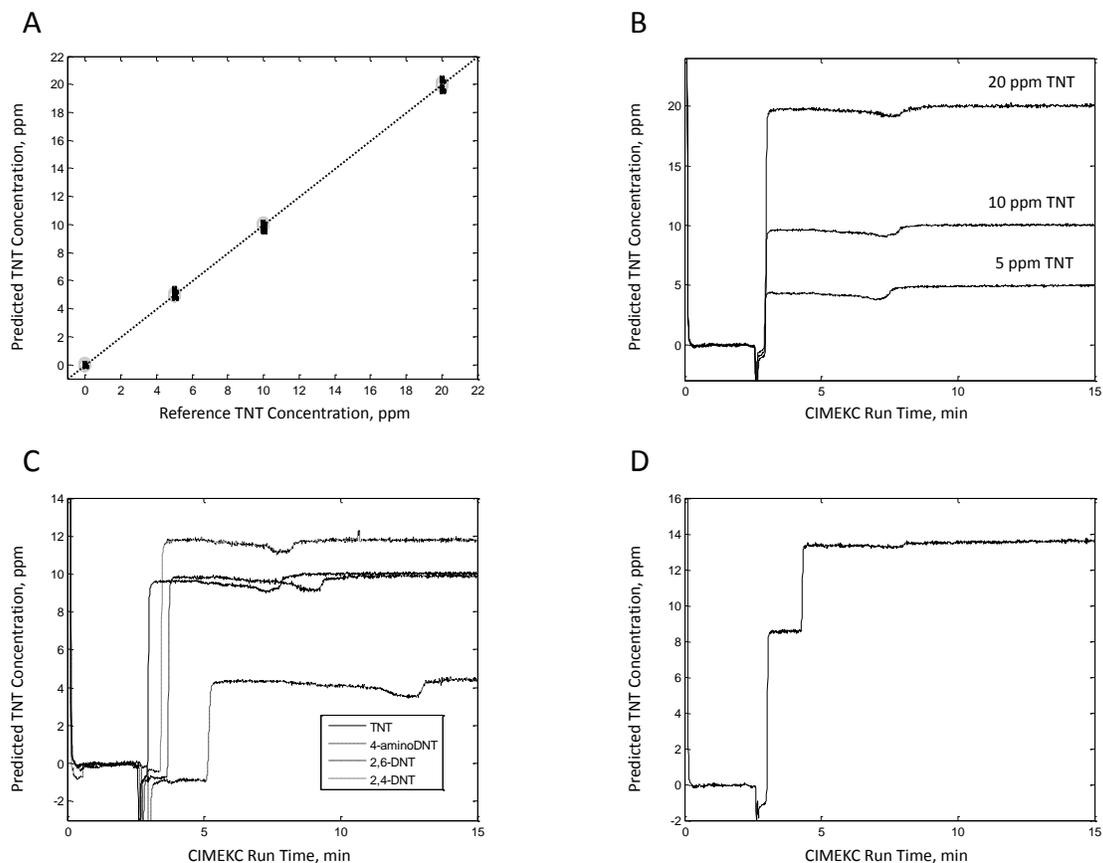
A representative image of the three dimensional data associated with the continuous injection of 20 ppm TNT in BGE is presented in Figures 2A (3D) and 2B (grayscale heatmap). The sharp feature at approximately 2.5 minutes is a system peak associated with solvent from the TNT stock solution and serves as an indicator of electroosmotic flow. The shift in baseline observed at 3 minutes is the TNT passing the detector.



**Figure 2.** A) Representative three dimensional data for the continuous electrokinetic injection of 20 ppm TNT in background electrolyte. B) Grayscale heatmap of the continuous electrokinetic injection of 20 ppm TNT in background electrolyte. Background electrolyte is 10 mM sodium tetraborate, 80 mM sodium cholate. Applied voltage is 15 kV.

Figure 3A depicts the results of a PLS regression model for TNT prediction constructed from spectra extracted from TNT-only CIMEKC runs as shown in Figure 2. This model was constructed from 7 latent variables, as determined from leave-one-out cross validation with the calibration set of spectra. Prediction accuracy was quite good, with a root mean standard error of calibration (RMSEC) of 0.0488 and a root mean error of prediction (RMSEP) of 0.2459. Figure 3B shows the PLS-predicted concentrations of TNT for data sets acquired from analysis of 5, 10 and 20 ppm TNT samples. However, when this model is applied to CIMEKC data acquired from analysis of samples containing the other nitroaromatics, it becomes apparent that this model lacks the selectivity necessary to accurately predict TNT concentration in the presence of other species. This is not surprising, as there is a considerable amount of spectral overlap between the four analytes. Figure 3C depicts an overlay of PLS-predicted concentrations from CIMEKC data acquired from analysis of 10 ppm samples of each analyte. These results give an indication of the relative selectivity of this PLS model for each analyte and thus the bias in predicted value each interfering species is likely to induce if it is present in a mixture. For example, Figure 3D shows the PLS predicted concentration of a mixture of 10 ppm TNT and 10 ppm 4-aminoDNT. The presence of 4-aminoDNT is contributing to the PLS predicted concentration of TNT due to spectral similarities between the spectra of the two compounds.

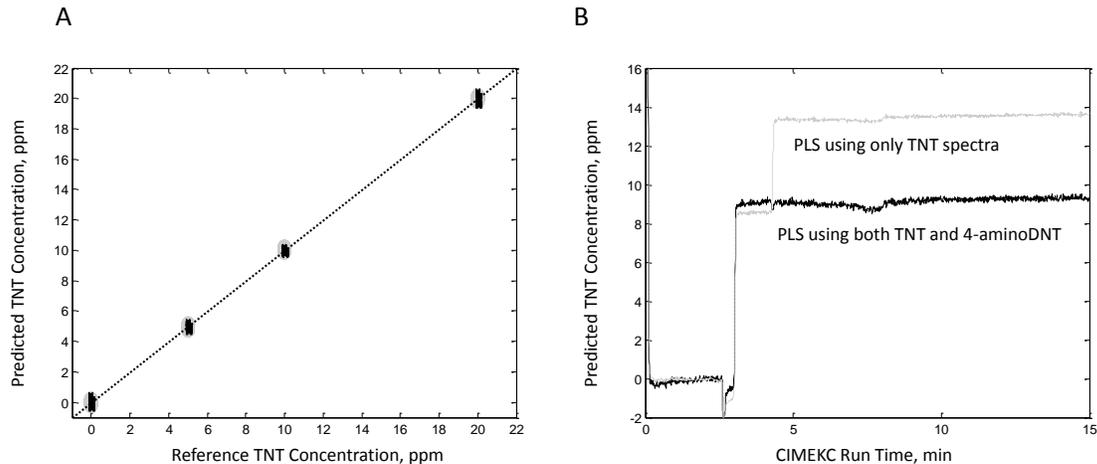
A key benefit of PLS regression (and other multivariate regression techniques) is the so-called first order advantage [3]. This refers to the ability of such techniques to calibrate against the presence of an interfering species at an unknown concentration. This ability is leveraged by incorporating additional spectra into the calibration set used to



**Figure 3.** A) PLS regression model for prediction of TNT. Circles represent predictions from calibration set spectra and crosses represent predictions from test set spectra. B) Overlay of PLS-predicted TNT concentrations for CIMEKC analyses of 5, 10 and 20 ppm TNT solutions in acetonitrile. C) Overlay of PLS-predicted TNT concentrations during CIMEKC analyses of 10 ppm samples of TNT, 2,4-DNT, 2,6-DNT, and 4-aminoDNT in acetonitrile. D) PLS-predicted TNT concentrations during CIMEKC analysis of a mixture containing 10 ppm TNT and 10 ppm 4-aminoDNT in acetonitrile.

construct the PLS model, in essence training the regression model to ignore interferant-related spectra features for the purposes of analyte prediction. Figure 4A shows the results of a PLS regression model constructed from the same data as that shown in Figure 3D, augmented with spectra from CIMEKC analysis of 5, 10 and 20 ppm samples of 4-aminoDNT. In this case, 20 latent variables were used to construct the model, and the resulting RMSEC was increased to 0.0894; roughly twice that of the TNT-only model. The RMSEP, on the other hand, was decreased to 0.1746, one-third of that of the TNT-only PLS model, suggesting that the incorporation of additional calibration set samples

results in greater robustness to run to run variation in the data. Figure 4B shows PLS-predicted concentrations of TNT for spectra acquired during CIMEKC analysis of the sample depicted in Figure 3D. For clarity, the profile shown in Figure 3D is overlaid in gray. As seen in Figure 4B, the incorporation of 4-aminoDNT spectra into the PLS regression model has successfully calibrated the model against this potential interferent. However, this model is not robust against the other potential interferents. That is to say, both the 2,4-DNT and 2,6-DNT samples continue to result in erroneous TNT concentration predictions.

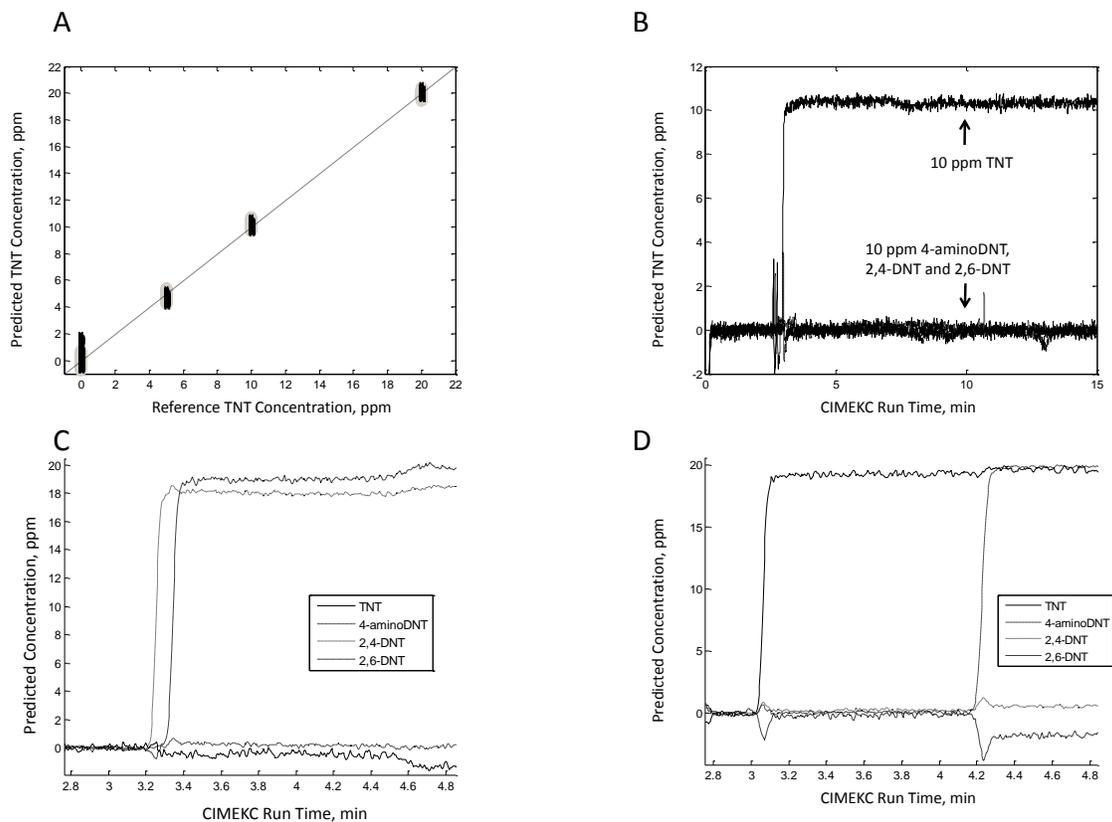


**Figure 4.** A) PLS regression model for prediction of TNT in the presence of 4-aminoDNT. Circles represent predictions from calibration set spectra and crosses represent predictions from test set spectra. B) PLS-predicted TNT concentrations during CIMEKC analysis of a mixture containing 10 ppm TNT and 10 ppm 4-aminoDNT in acetonitrile. The PLS model was constructed using both TNT and 4-aminoDNT spectra.

The benefit conferred by the chemometric first order advantage in this application is connected to the amount of spectral similarity between the analyte and interfering species. If the two spectra are orthogonal, calibration against the interferent is trivial. If they are identical, calibration against the presence of the interferent is rendered impossible. Additionally, as more interfering species are added, it becomes more unlikely that a PLS model will be able to successfully calibrate against all them, resulting

in increased prediction bias due to incorporation of spectral features related to interferants or increased prediction variance due to a reduction in the amount of spectral information related to the analyte.

Figure 5A depicts a PLS model constructed with 25 latent variables using spectra from each concentration of each of the four possible mixture components. As expected,



**Figure 5.** A) PLS regression model for prediction of TNT in the presence of three potential interferants. Circles represent predictions from calibration set spectra and crosses represent predictions from test set spectra. B) Overlay of PLS-predicted TNT concentrations during CIMEKC analyses of 10 ppm samples of TNT, 2,4-DNT, 2,6-DNT, and 4-aminoDNT in acetonitrile using a PLS model constructed from TNT and all interferant spectra. C) Overlay of PLS-predicted concentrations during CIMEKC analyses of a mixture containing 20 ppm 2,4-DNT and 20 ppm 2,6-DNT in a standard background electrolyte matrix using a PLS model constructed from TNT and all interferant spectra. D) Overlay of PLS-predicted concentrations during CIMEKC analyses of a mixture containing 20 ppm TNT and 20 ppm 4-aminoDNT in a standard background electrolyte matrix using a PLS model constructed from TNT and all interferant spectra.

errors of calibration and prediction increase with this model relative to the previous two models, with an RMSEC of 0.2227 and an RMSEP of 0.4185. A prediction bias is seen at the lower end of the calibration range, indicating that the model is not completely

selective against the interferents. Figure 5B shows an overlay of the resulting predicted TNT concentrations for single-component samples at 10 ppm. In no instance was TNT identified in samples containing any of the three interferents. Alternatively, rather than treating all analytes other than TNT as interferents, a PLS model to simultaneously predict the concentration of all four species was constructed from the single component data. Figure 5C shows the predicted concentration of all four components tested against a binary mixture of 2,4-DNT and 2,6-DNT at 20 ppm and Figure 5D shows the predicted concentration of all four components tested against a binary mixture of TNT and 4-Amino-2,6-DNT. These results indicate that all four components can be correctly identified and quantified independent of one another in a single model.

#### **4. Conclusions**

This work has demonstrated the feasibility of a CIMEKC-based approach to multi-analyte detection and quantification with PLS prediction models. This is an important step in transitioning benchtop MEKC detection strategies for trace nitroaromatics in seawater to a miniaturized, mobile platform. PLS regression models were shown to accurately predict TNT concentration in the presence of three other nitro aromatic interfering species, provided the PLS model incorporated calibration data from those interfering species as well as from TNT. It was also shown that such PLS models are capable of providing simultaneous quantification of TNT and the other three nitroaromatics in CIMEKC analyses of mixtures. Importantly, it was observed that when interferent data were omitted from the PLS model, similarities among the UV spectra of TNT and the three nitroaromatic interferents led to significant prediction bias. Thus the utility of CIMEKC coupled with direct PLS regression model prediction as described is

dependent on knowledge of the identity of potential interferents and the extent to which their spectra are similar to that of the target analyte.

## **5. Acknowledgements**

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## GLOSSARY

Am-DNT	– Aminodinitrotoluene
BGE	– Background electrolyte
CE	– Capillary electrophoresis
DNT	– Dinitrotoluene
EOF	– Electroosmotic flow
MEKC	– Micellar electrokinetic chromatography
PLS	– Partial least squares regression
TNT	– Trinitrotoluene

