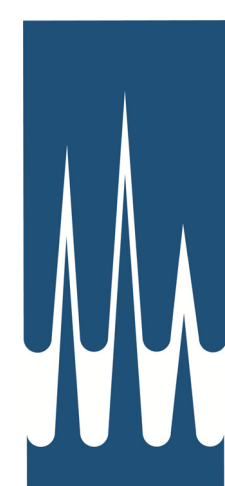# Supercritical Fluid Chromatography/Mass Spectrometry: Negative Ion APCI Mechanisms Associated with the Analysis of Halogenated Environmental Contaminants

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Introduction

Atmospheric pressure ionization (API) methods are subject to a multitude of complicated chemical reactions occurring in the source of a mass spectrometer (MS) during the ionization process. Since it is possible to generate both radical species and protonated molecules that are able to interact with analytes and transfer charge, it is common to observe multiple ionization products which consequently result in a decrease in overall sensitivity for a target analyte. Ideally, conditions need to be determined that maximize the formation of a single ionized species with minimal fragmentation in order to maximize detection limits and decrease cross-talk between MS channels. In one possible approach, oxygen has been used for the ionization of halogenated environmental contaminants under negative API conditions. For example, Zhou et al. reported the measurement of Dechlorane Plus using $[M-X+O]^-$ pseudomolecular ion clusters generated during liquid chromatography/atmospheric pressure photoionization tandem mass spectrometry.[1] Thermodynamic products are observed because the ion source is at atmospheric pressure and equilibrium conditions can be achieved.[2] Generally the formation of $[M-X+O]^-$ clusters (where X is a halogen) by an oxygen enhanced negative chemical ionization process during API-MS does not require an external supply of oxygen since most nitrogen sources contain some level of oxygen due to incomplete fractionation. However, packed column supercritical fluid chromatography (pSFC) introduces new challenges when attempting to do comparable low level analytical work due to the presence of expanding carbon dioxide in the MS source. For the investigation of dopants/reagents for negative ion API processes relevant to environmental contaminants when supercritical chromatography is coupled to mass spectrometry, the non-aromatic halogenated compound Dechlorane Plus (DP) was chosen as a test compound.

Materials and Methods

Individual certified reference standards of anti- and syn-Dechlorane Plus (a-DP and s-DP respectively) were obtained from Wellington Laboratories Inc. (Guelph, ON, Canada). All pSFC experiments were carried out using a Waters Acquity UltraPerformance Convergence Chromatograph (UPC²) (Waters Corp., Milford, MA, USA) system coupled to a Micromass Quattro micro atmospheric pressure ionization (API) Mass Spectrometer (MS) (Waters Corp., Milford, MA, USA) and data were processed using Waters MassLynx software. pSFC separations were performed on a Waters UPC² Trefoil CEL2 column (2.5 µm, 3.0 x 150 mm) using an acetonitrile modified CO_2 gradient elution (data acquired in SIR mode).

Table 1: Summary of Ionization Parameters

| Parameter	Units	Value
Corona Needle	µA	6
Cone Voltage	V	20
Cone Gas Flow	L/Hr	100
Desolvation Gas Flow	L/Hr	500
Desolvation Temperature	°C	350
Source Temperature	°C	100
Make-up Solvent Flow-rate	ml/min	0.4

For infusion of Dechlorane Plus (DP) standards, the MS was configured in negative-ion atmospheric pressure chemical ionization (APCI) mode with the parameters stated in Table 1. Pseudomolecular ion clusters corresponding to $[M-Cl+O]^-$ were generated with these settings for the DP standards. All MS data were acquired in full scan mode by infusion of a 5 µg/ml DP standard (in acetonitrile) that was teed directly into the UPC² eluent/MS make-up solvent at a rate of 10 µl/min. The UPC² eluent was consisted of 90% CO_2 and 10% acetonitrile at a flow-rate of 0.45 ml/min with an ABPR setting of 1500 psi. The optimal MS make-up solvent was found to be acetonitrile with 0.15% triethylamine (TEA).

Results

Optimization of APCI conditions for the formation of the pseudomolecular ion $[M-Cl+O]^-$ of a-DP revealed that m/z 32 (superoxide, O_2^-) was dominating the lower end of the mass spectrum. Source parameters were further optimized to promote the formation of this reactive species and it was noted that its formation was strongly temperature dependent with lower probe temperatures (optimized at 350°C) resulting in significant increases of the superoxide signal ($R^2 = 0.9239$; Graph 1). In the present work, correlations between increased superoxide concentrations and the intensity of the following clusters: $[M-Cl+O]^-$ ($R^2 = 0.9387$; Graph 2), $[M]^-$ ($R^2 = 0.7266$), and $[M+O_2]^-$ ($R^2 = 0.8532$) were demonstrated. The presence of an $[M+O_2]^-$ cluster suggests that the oxidation product formation route proposed by Lépine et al.[3] (see Equation 1) is occurring, while transfer of chlorine to the oxygen atom before dissociation of ClO^- is slow allowing for detection of the intermediate. However, during signal optimization it was found that the presence of this cluster could be minimized by increasing the cone voltage.

Equation 1: $ArCl_n^{-\bullet} + O_2 \rightarrow ArO_2Cl_n^{-\bullet} \rightarrow ArOCl_{n-1}^- + ClO^-$
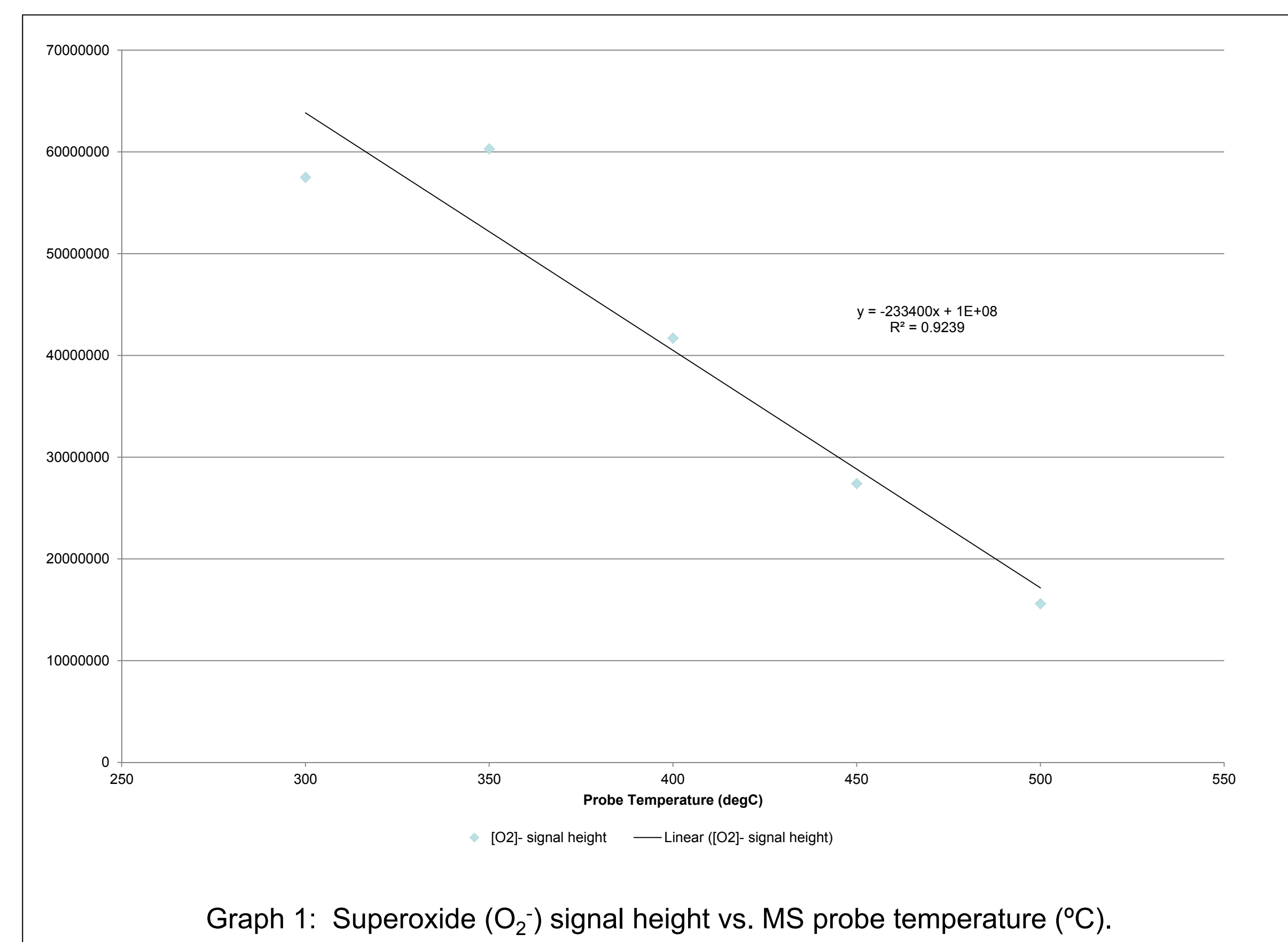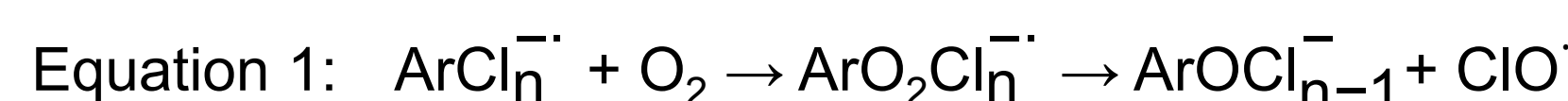
Graph 1: Superoxide (O_2^-) signal height vs. MS probe temperature (°C).
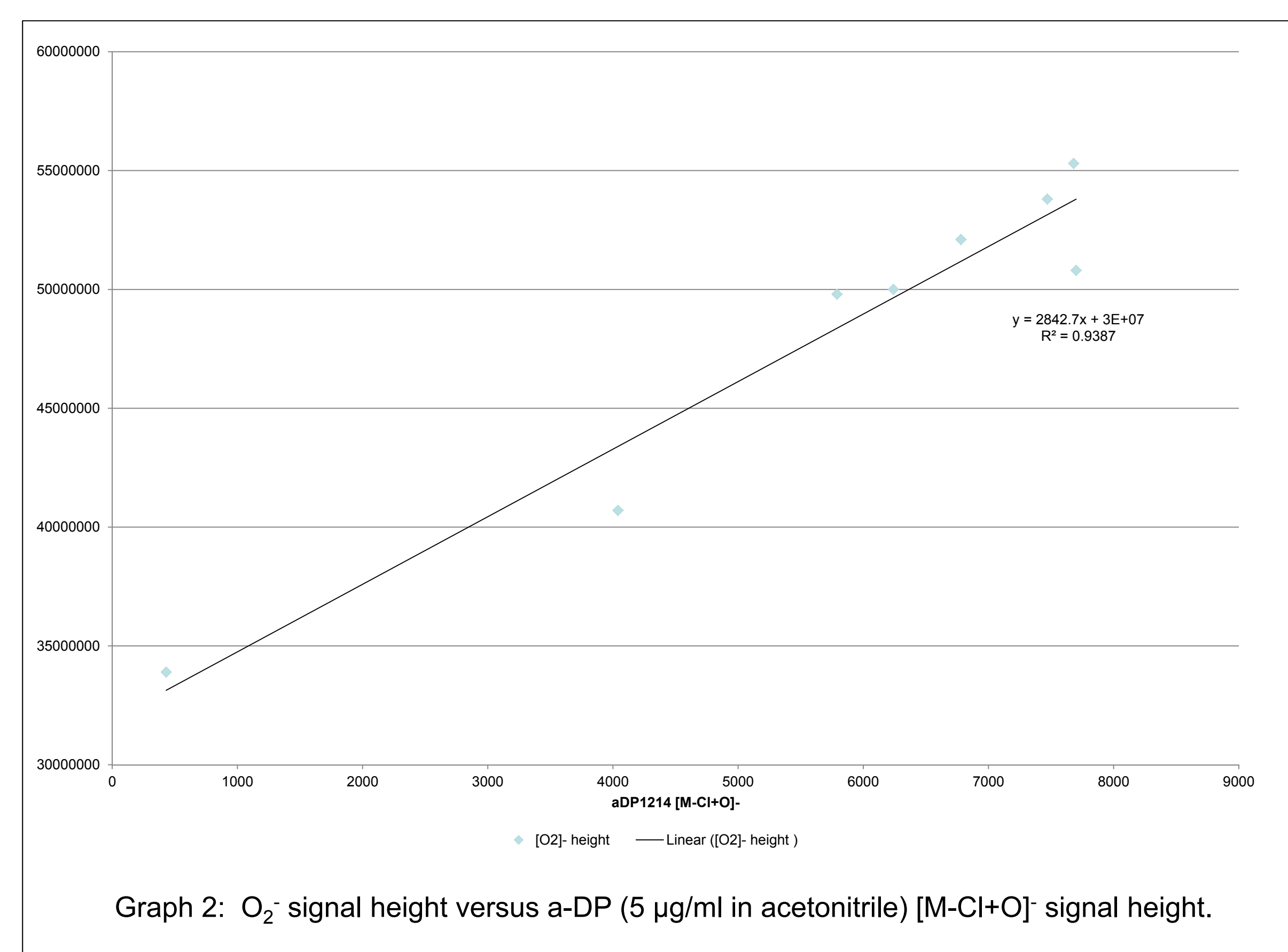
Graph 2: O_2^- signal height versus a-DP (5 µg/ml in acetonitrile) $[M-Cl+O]^-$ signal height.
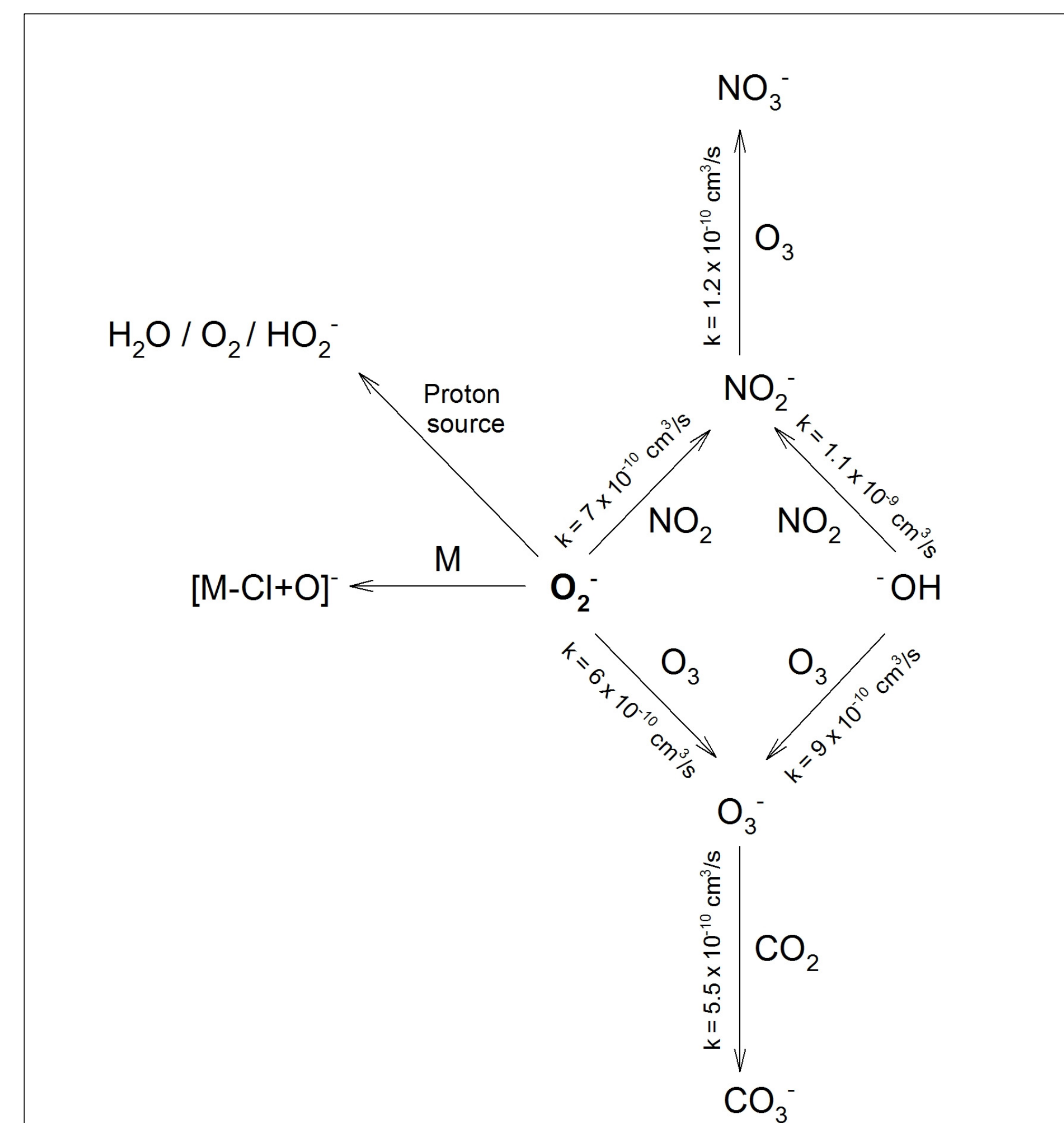
Scheme 1: An illustration of reactive species present in the MS source during APCI as well as possible pathways affecting the ionization of the target analyte M.

In order to gain insight into possible competing ionization pathways, a-DP infusion experiments were conducted with low percentages of ammonium hydroxide. Since superoxide is a powerful nucleophile, it will react with organic species in the absence of protons making it a useful APCI reagent species, but due to its basicity, O_2^- will disproportionate quickly in the presence of proton sources (such as water).[4] It was determined that, at lower NH_4OH concentrations, NH_3 scavenges NO_2 allowing superoxide to be available for reaction with the target analyte. At higher NH_4OH concentrations, the increased presence of water in the MS source quenches the O_2^- species and the dominant ion becomes CO_3^-. This is due to the concurrent presence of hydroxide anions that react with O_3 to produce O_3^- which can then react with CO_2 to produce CO_3^- (Scheme 1). In order to optimize the availability of superoxide in the MS source, and consequently maximize ionization of a-DP, triethylamine was chosen as the APCI reagent due to its ability to scavenge NO_2 without introducing a protic source. Triethylamine may also be a good enough electron donor to aid in the formation of O_2^-. At the optimized level of 0.15% TEA in acetonitrile, a 52% increase in the a-DP signal ($[M-Cl+O]^-$) was observed compared to that obtained when using 0.1% NH_4OH as the APCI reagent.

This information could be useful for other halogenated environmental contaminants since many do form the $[M-X+O]^-$ cluster during negative APCI. Introduction of water into an MS source will be inherently minimized when coupling pSFC to tandem mass spectrometry. The nature of the eluent thus makes this analytical separation technique well suited for this form of ionization (Figure 1).
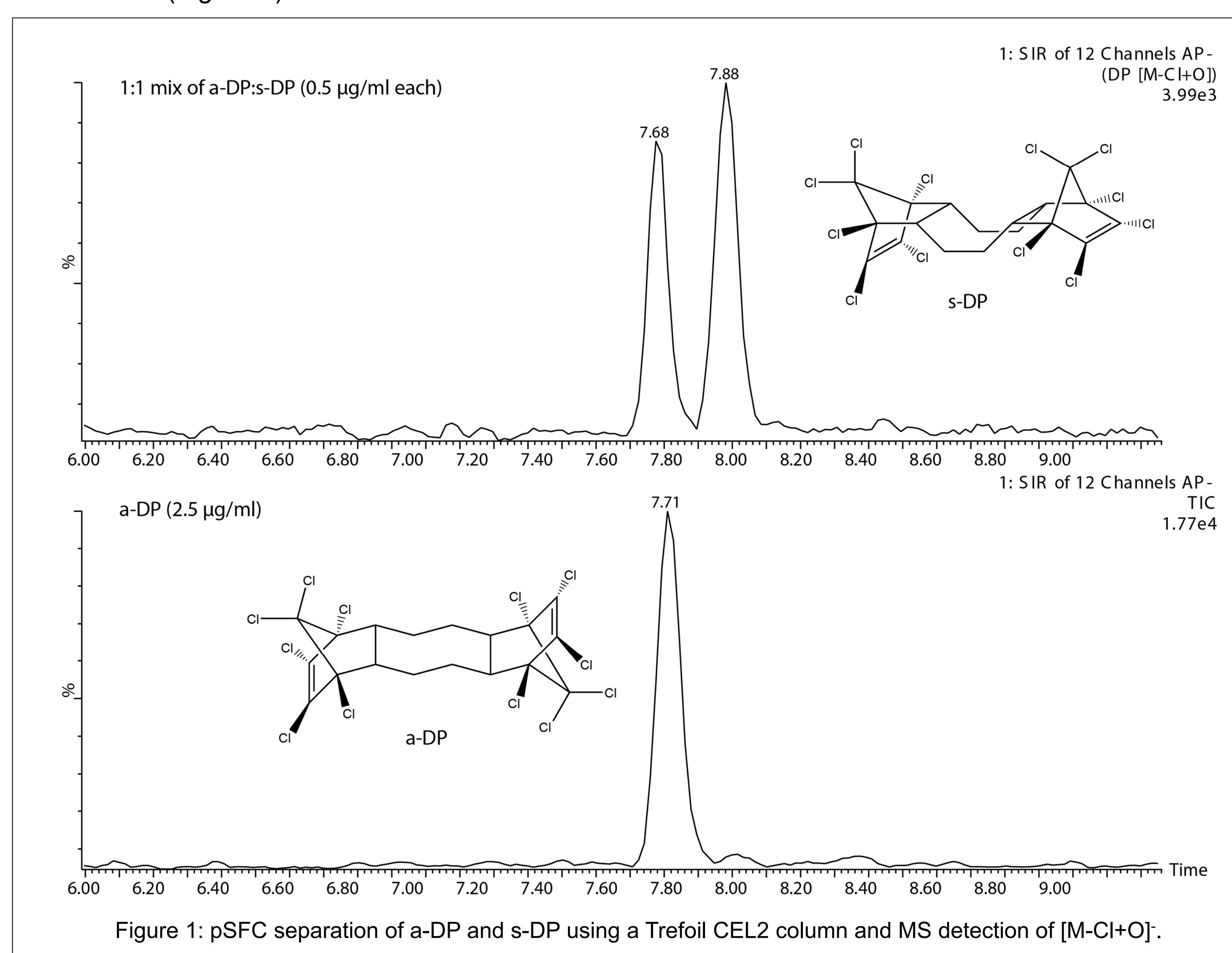

Figure 1: pSFC separation of a-DP and s-DP using a Trefoil CEL2 column and MS detection of $[M-Cl+O]^-$.

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