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Review

Peak compression effects in capillary electrochromatography

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Abstract

Peak compression in CEC is a phenomenon that can generate very narrow peaks with extremely high efficiencies that defy current chromatographic theory. This review article summarises the content of publications in this area up to this date. Two main types of peak compression effects have been observed in the literature. First, an irreproducible and hard to control focusing effect of unclear origin, observed on strong cation exchangers. Second, a reproducible continuous stacking effect caused by sample composition induced system zones demonstrated on several types of stationary phases.

Keywords: Reviews; Electrochromatography; Peak compression

Contents

1. Introduction

When Smith and Evans [1] started to use strong cation exchangers (SCX) as stationary phases in capillary electrochromatography (CEC), primarily to enhance the electroosmotic flow (EOF) at low pH, an unexpected phenomenon occurred that has engaged and puzzled several research groups. During analyses of tricyclic antidepressants, extremely high peak efficiencies of up to 8 million plates/m were obtained (Fig. 1). As this defies current chromatographic theory they concluded that some form of sample stacking possibly contributed to the effect. This article reviews the scientific work that up to this date has been published in this area in order to create a better understanding of this exciting peak compression effect.

2. Stacking and peak compression

In most separation methods, some sort of sample stacking is desirable as it improves separation efficiency and detection limits. However, in most of these methods the stacking process is limited to the initial stages of the analysis. An example of this is the well-known procedure of dissolving and injecting the sample in a solvent with a lower eluting strength than the mobile phase, in order to temporarily concentrate the analytes at the top of the column in liquid chromatography (LC). In capillary electrophoresis (CE), the sample is commonly dissolved in a solvent with lower conductivity than the background electrolyte (BGE) to create a zone with locally higher electric field strength to initially stack the analytes as a narrow band at the boundary between the sample zone and the BGE. Examples of more laborious variants are the isotachophoretic preconcentration and sweeping, which together with other techniques are described in several reviews e.g. [2–4].

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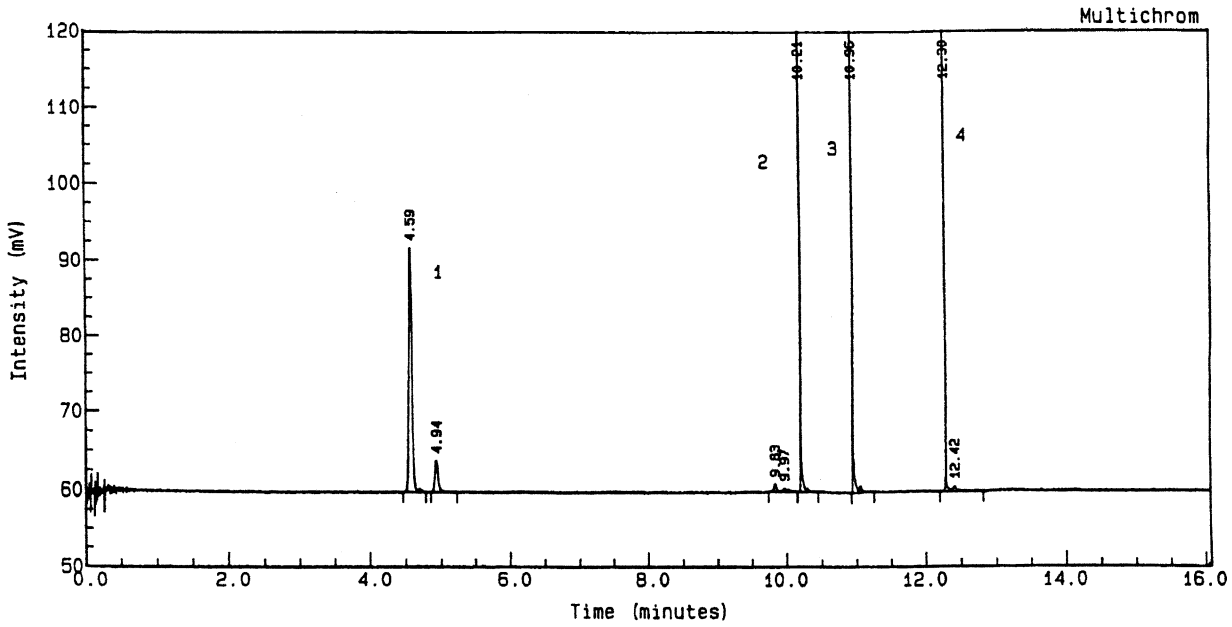

Fig. 1. Electrochromatogram from the first publication on peak compression in capillary electrochromatography. Column: Spherisorb SCX 3 μm, 260 (500) mm × 0.05 mm; mobile phase: 30% 50 mM sodium phosphate buffer, pH 3.5 and 70% acetonitrile; voltage: 30 kV, injection: 2 kV, 30 s; temperature: 30 °C; detection at 210 nm. Solutes: (1) bendroflumethiazide, (2) nortriptyline, (3) clomipramine, (4) methdilazine. From Ref. [1] with permission.

The peak compression effect obtained in the SCX–CEC system reported by Smith and Evans [1], seems to be more of a continuous process. Similar effects have been reported in ion-pair reversed phase LC and supercritical fluid chromatography (SFC). In these systems, peak compression was obtained for analytes that co-eluted with system zones induced by the sample composition. In the ion-pair LC systems high concentrations of organic anions added to the sample were injected to induce ion gradients and disrupt the column equilibrium with the ion-pairing agent (a hydrophobic amine) [5,6]. In SFC, it proved to be a surplus of water in the sample that displaced the organic modifier (an alcohol) from the stationary phase creating a plug containing an excess of alcohol [7–9]. As the displaced alcohol is competing with the analyte for active sites on the stationary phase the analyte will move faster within this plug until it reaches the bulk mobile phase and peak compression is induced.

Ståhlberg [10] as well as Xiang and Horváth [11] have published theoretical models on the migration behaviour of charged analytes in CEC, offering possible explanations for peak compression effects. Ståhlberg showed that the combination of chromatographic and electrophoretic transport mechanisms could give rise to a stabilised zone due to the inhomogeneous field strength and a nonlinear adsorption isotherm. Under these conditions, the peak does not change its shape during migration through the column, suppressing band broadening due to other effects. Xiang and Horváth proposed that the formation of internal gradients in the CEC system could give rise to peak compression effects. The phenomenon was attributed to a combined effect of electrophoretic migration and surface electrodiffusion of the sample components.

3. Peak compression on strong cation exchangers

Further experiments on the SCX stationary phase using longer columns, than those referred to in [1], produced even higher plate numbers in the region of 50 million plates/m [12]. These amazing apparent peak efficiencies raised expectations even more with regard to CEC as a highly efficient separation technique that should combine the best of LC and CE.

As more and more data within the field was produced it was revealed that the phenomenon was irreproducible (Fig. 2) and hard to control [13–16]. Euerby et al. [13] reported excellent peak symmetry and efficiencies of over 16 million plates/m for a basic proprietary compound compared to 56 000 plates/m when using corresponding chromatographic conditions in a pressure driven system. However, the peak compression effect could unpredictably and inexplicably cease to appear, creating highly asymmetric peaks and even peak splitting, and then the peaks would suddenly elute with exceptional efficiencies again, as high as over 40 million plates/m.

Smith and Evans [17] compared the EOF profiles and selectivity of a number of stationary phases used in CEC. They reported that the peak compression effect seen on the original SCX stationary phase with propyl linkers between the sulphonate group and the silica could also be produced on a SCX phase with phenyl linkers. With Symmetry SCX from Waters, which is made from silica with a lower metal content compared to the previous materials, they obtained narrow peaks. However, it was unclear if the peaks were focused.

In 1999 a rather extensive study of the peak focusing effect was presented in a poster by Ferguson et al. [18] (Fig. 3).

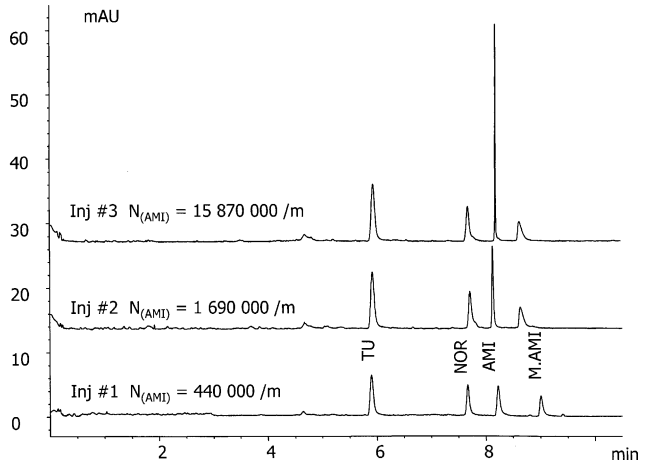

Fig. 2. Three consecutive runs on a SCX column showing the peak compression effect and its lack of reproducibility. Column: in-laboratory derivatised, with propylsulphonate ligands, Nucleosil 120-3 bare silica particles, 3 μm, 250 (335) mm × 0.1 mm; mobile phase: 40% 40 mM sodium phosphate buffer, pH 2.8 and 60% acetonitrile; voltage: 20 kV; injection: 6 kV, 5 s; temperature: 20 °C; detection: UV at 210 nm. Solutes: (TU) thiourea (EOF marker), (NOR) nortriptyline, (AMI) amitriptyline, (M.AMI) *N*-methylamitriptyline. Theoretical plates/m, $N_{(AMI)}$, calculated from peak width at half height. From Ref. [15] with permission.

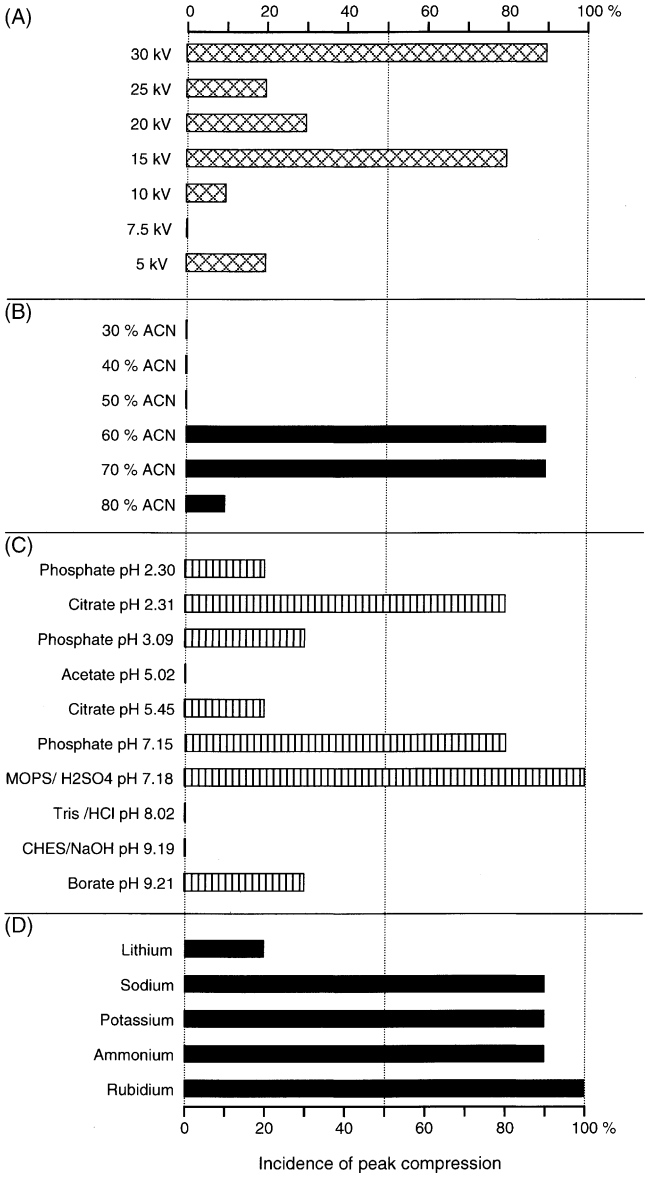

Fig. 3. Summary of results from poster on peak compression on a SCX stationary phase presented at HPLC'99. Figure based on data in Ref. [18] with permission. The effect of (A) voltage over the column, (B) content of acetonitrile in the mobile phase, (C) pH and type of buffer and (D) type of cation in a phosphate buffer on the incidence of peak compression. Columns: Spherisorb SCX 3 μm, 210 (310) mm × 0.075 mm; injection: 10 kV, 10 s; detection at 214 nm. Solute: clomipramine.

Their results indicated that the effect was highly dependent on the voltage, e.g. at 30 and 15 kV the effect occurred in almost every run, occurred rarely at 25, 20, 10 and 5 kV and not at all at 7.5 kV. The composition of the mobile phase also proved to be of importance. When increasing the content of acetonitrile stepwise from 30 to 80% the highly efficient peaks appeared mainly at 60 and 70%. Also the type of buffer and pH influenced the occurrence of focusing as can be seen in Fig. 3C. When changing the type of cation (Li^+, Na^+, K^+, NH_4^+, Rb^+) in a phosphate buffer of pH 2.3 the focusing effect could be obtained in all systems. However, the incidence was low (20%) when using the lithium phosphate buffer. They also mentioned that the buffer concentration and amount of analyte injected has an influence on the focusing process.

In a study by Enlund et al. [19] it was found that the composition of the sample and the injection volume had a major influence on peak compression when the basic analytes eluted with a certain retention compared to the EOF marker. By adjusting the composition of the mobile phase (pH, ionic strength, content of organic modifier) so that the analyte eluted closely behind the EOF marker and then increasing the content of acetonitrile in the sample and the injection volume; a peak compression effect was induced involving apparent efficiences of up to 17 million plates/meter. Contrary to methods reported in the previous papers this effect proved to be reproducible. A mechanism for the effect called "continuous stacking" was proposed (Fig. 4). The higher concentration of acetonitrile in the sample produced a zone with non-equilibrium conditions where the analyte had a higher velocity, due to the lower retention and higher electric field strength, than in the mobile phase. When exiting this zone the elution rate of the analyte is slowed down and the sample zone can catch up to the analyte again. Under these conditions, a continuous stacking can be obtained, trapping the analyte as a very narrow band. Analytes that elute too slowly or too quickly to be caught by the sample zone elute with normal peak efficiencies. It is worth noting that it was the injection volume and not the amount of sample that was essential for the peak compression effect. Hence, a large injection of a diluted sample resulted in a sharp peak while a small injection of a concentrated sample did not. The continuous stacking is probably not the

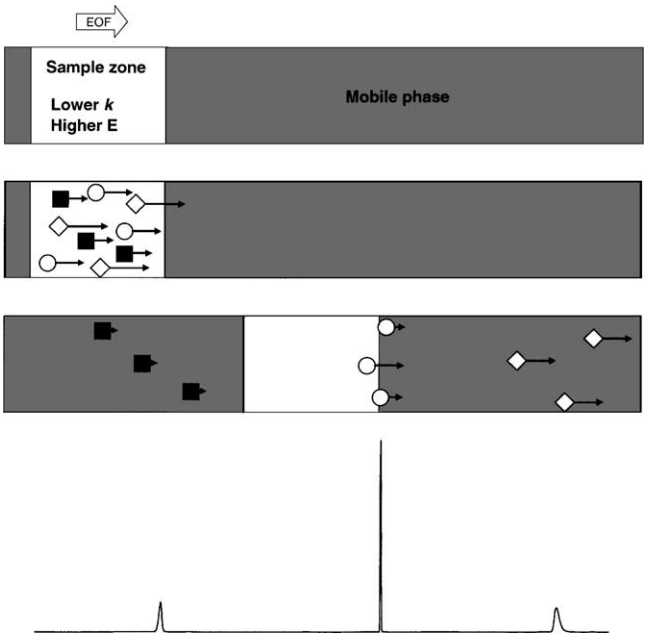

Fig. 4. Schematic representation of the peak compression effect and a resulting constructed electrochromatogram. *E*, electric field strength; *k*, retention factor. A sample zone differing in composition compared to the mobile phase will disturb the equilibriums between the stationary and mobile phases. The elution rate of an analyte will consequently be different when residing inside the sample zone. A higher content of acetonitrile in the sample zone will cause the analyte to migrate through the sample zone at a higher speed than in the mobile phase, due to lower retention factor and higher electric field strength. The analyte can then be strongly retained after passing through the front boundary of the sample zone, producing a continuous stacking that traps the analyte as a very narrow band. An EOF marker would elute just to the right of the compressed peak. From Ref. [19] with permission.

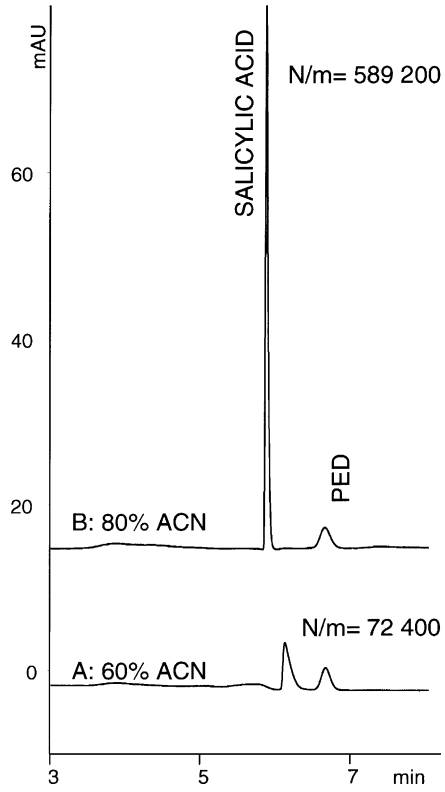

Fig. 5. Electrochromatogram showing peak compression of an anion on a SAX phase. When salicylic acid was injected in a solvent with a high concentration of acetonitrile the peak was focused (B) while the neutral marker remained unaffected. Column: Nucleosil 100-5 SB strong anion exchanger particles, 5 μm, 250 (335) mm × 0.1 mm. Mobile phase: sodium phosphate buffer, pH 6.0 and 60% acetonitrile, ionic strength 30 mM; voltage: −20 kV; injection: −10 kV, 15 s; temperature: 20 °C; current: −29 μA; detection: UV at 210 nm. Solutes: salicylic acid and (PED) 1-phenyl-1,2-ethanediol (EOF marker). Sample A: 31 μM salicylic acid and 72 μM PED dissolved in mobile phase. Sample B: 31 μM salicylic acid and 72 μM PED in sodium phosphate buffer, pH 6.0, 80% acetonitrile, ionic strength 2.1 mM. Theoretical plates/m (*N*/m) calculated from peak width at half height.

explanation for the peak compressions reported by Smith and Evans [1] as their analytes eluted far from the EOF marker.

4. Peak compression on other types of stationary phases

Peak compression in CEC has not only been reported on SCX stationary phases. For example focusing of a positively charged analyte, eluting closely behind the EOF marker has also been observed on underivatised silica by Ferguson et al. [18]. They propose that the sharp peak arises from mismatch of the sample solvent and the mobile phase. Steiner and Lobert [20] reported extraordinary high efficiencies of over 2 million plates/m for a pair of tricyclic antidepressants on bare silica. The peak profiles resembled those seen in SCX systems and the analytes eluted far from the EOF marker which led to the proposal that the peak focusing resulted from a mechanism similar to that seen by Smith and Evans [1] on SCX columns. Furthermore, this effect was irreproducible, e.g. four consecutive injections resulted in plate numbers from 250 000 to 1.5 million. Bare silica particles with different pore size (and surface area) were tested and the results indicated that the magnitude of the peak compression effect appeared to be related to the ion exchange capacity.

In an unpublished investigation within our research group we have been able to induce peak compression during analysis of pharmaceutically related acids using a strong anion exchanger (SAX) as stationary phase for CEC. Similar behaviour was observed on the SAX phase as had previously been seen on the SCX phase [19], i.e. peak compression was induced when the acetonitrile content in the sample plug was higher than in the mobile phase, when large injection volumes were used and when the analyte eluted close to the EOF marker. Fig. 5 shows an example where peak compression was induced for salicylic acid. The peak compression effect obtained using the SAX phase was not as pronounced as it was on the SCX phase and lower plate numbers were obtained. A possible explanation is that the peak shape is affected by a disturbance in the EOF after passing the outlet retaining frit, just before the detection window, since the

empty part of the capillary after the outlet frit has a negative surface charge and the packed bed has a positive charge under the conditions used in this experiment.

Moffatt et al. [21] reported very high efficiencies for neutral/anionic compounds under reversed-phase CEC conditions. They obtained efficiencies of up to 2.5 million plates/m and the peak compression was highly reproducible on a C_{18} column. The effect was seen when the elution of the analyte (determined by the composition of the mobile phase) was synchronous with the elution of sample solvent induced discontinuities in the mobile phase. These discontinuities generate zones of pulsed gradients of weaker or stronger solvents that can result in substantially reduced dispersion. When dissolving the analytes in the mobile phase instead of water the high plate numbers were no longer observed.

Recently, a paper where peak compression is used to improve quantification limits in chiral CEC on a vancomycin-based stationary phase has been published [22]. Peak compression was obtained when the analyte co-eluted with either one of two sample-induced system zones. By tuning the composition of the mobile phase it was possible to selectively sharpen either of the enantiomer peaks (Fig. 6) and if both peaks eluted between the system zones no peak compression was observed. The sample had to be dissolved in a solvent with a sufficiently lower dielectric constant than the mobile phase, e.g. 2-propanol or tetrahydrofuran, to obtain peak compression. The plate numbers for the minor enantiomer increased from 100 000 to 1.4–1.6 million plates/m resulting in a 10-fold improvement of the quantification limit.

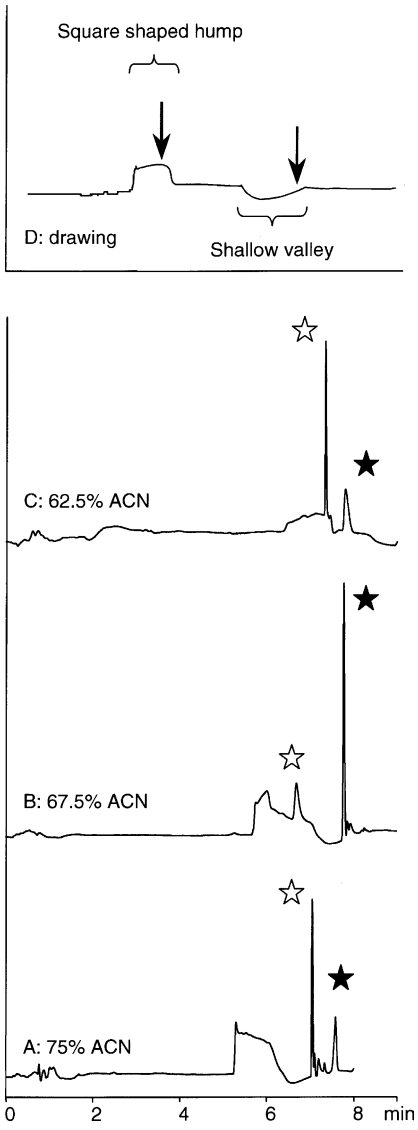

Fig. 6. Peak compression in chiral CEC. Changing the concentration of acetonitrile in the mobile phase influenced which of the enantiomer peaks that was compressed (A–C). Schematic drawing (D) illustrating the two system zones called the “square shaped hump” and the “shallow valley”. Samples A and B: 63 μM mianserin dissolved in 30% 2-propanol. Sample C: 59 μM mianserin dissolved in 24% 2-propanol. Column: Chirobiotic V (Vancomycin) particles, 5 μm, 250 (335) mm × 0.1 mm; mobile phases: triethylammonium acetate buffer, pH 4.8, acetonitrile content as denoted in the electrochromatograms, ionic strength 9.4 mM; voltage: 25 kV; temperature: 15 °C; detection: UV at 214 nm; injection 10 kV, 15 s (A and B) and 15 kV, 25 s (C). From Ref. [22] with permission.

5. Conclusions

The fact that rather few articles have been published within this area nearly 10 years after the phenomenon of extreme peak efficiencies in CEC was first published is indicative of a process that is difficult to understand and investigate. From personal communications, we know that the effect was studied by many groups that never published any articles within this field. It is likely that it is two different kinds of effects that are described in the literature. First, the irreproducible and exceptional focusing effects with unclear origin seen on the SCX phases [1,12–18]. Second, the reproducible and adjustable continuous stacking effect that is due to sample composition induced system zones demonstrated on several types of columns [18,19,21,22]. Even though the origin of the first type of peak compression effect is not fully understood it is likely that both types arise during non-equilibrium conditions, which limits the potential for these effects to generally improve separation efficiencies. However, as described in [22] peak compression can be used to improve detection limits.

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