Recent Developments and Future Challenges in Supercritical Fluid Chromatography

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Supercritical fluid chromatography (SFC) in its various forms has been around for over 30 years during which, it has been applied in numerous fields, including the analysis of fats and oils, pesticides, pharmaceuticals, hydrocarbons and fine chemicals. Even so, despite early promise, it initially failed to make significant inroads into the HPLC market and has only relatively recently started to develop beyond a niche technique.

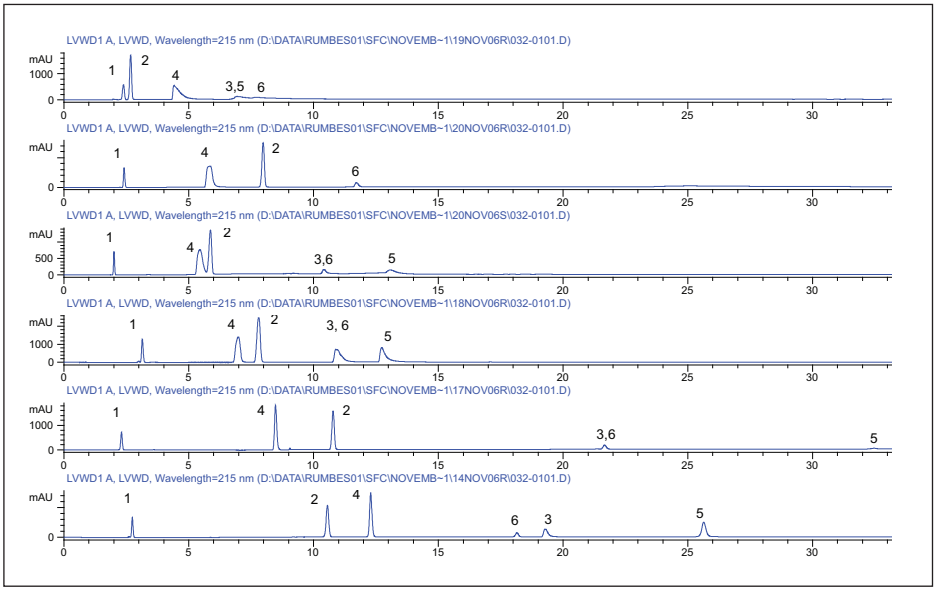
The major attraction of SFC lies in its ability to bridge the gap between more widely used separation techniques, gas and liquid chromatography, by combining the advantages of both and very few of the disadvantages of either. This supercritical fluid (usually carbon dioxide) has a very low viscosity and therefore analytes tend to have a very high diffusivity, which in turn facilitates greater interactions with the stationary phases and greater resolving power. One of the original attractions of this technique was that it would be possible to separate non-volatile compounds (otherwise analysed by HPLC) with a degree of resolution found in gas chromatography. At one stage it was even widely touted as supplanting HPLC for many applications. However, for a variety of reasons, the drive to develop this technique lost its momentum and within a few years only relatively few manufacturers were developing or supporting this technology. One of the factors was that there were technical disagreements as to the relative merits of packed and capillary SFC systems, particularly since all early units were based on either converted HPLC or GC systems. Of these, capillary systems predominated, but unfortunately this hampered further research and development, since they were generally less forgiving to the inexperienced user.

However, in recent years there has been a resurgence of interest in packed column SFC, particularly preparative systems and their application to the isolation and purification of high value products and intermediates for the pharmaceutical industry. Additional advantages include the capability to reduce raw material, processing and waste costs, through the use of an environmentally acceptable (and residue free) solvent. Consequently, more research and development has been focused on this particular technique and both the underlying science and its various subtle aspects are described in detail by Berger [1].

Most modern systems are still based around HPLC technology and hardware and in terms of the separation process, SFC can be regarded as most closely allied to normal phase chromatography and typically uses the same stationary phases. The analyte is typically injected onto a normal-phase type column and then eluted with supercritical CO2, whose polarity is modified by the increased addition of a polar modifier (such as methanol, acetonitrile) or by increased pressure, which itself modifies the properties of the mobile phase. The use of such modifiers is usually essential, since one of the drawbacks of SFC had been the otherwise limited solubility of polar compounds in supercritical CO2. The importance of modifiers and their influence in a diverse range of applications has been extensively reviewed [2].

Modifiers also introduce specific additional interactions such as hydrogen bonding or dipole-dipole in order to modify selectivity. However the higher % modifier, the higher the critical values become and if the critical temperature or pressure is not reached, phase separation will occur. Even so, modifiers are often used in conjunction with separations at room temperature. In such cases, if the temperature is below the critical value, but the pressure is maintained above its own value, then the fluid is regarded as sub-critical. Even though these liquids are more viscous and have lower diffusivity than supercritical ones, in the same review, Berger noted that this did not appear to impact on chromatographic performance [1]. Moreover it appears that there is no discontinuity in moving between the two types of phases and therefore it is possible to perform analyses using both types of liquid phase without any discernible changes in the chromatography. Indeed, many separations are routinely performed under such conditions, particularly where there are concerns over thermal stability. This whole field has been recently reviewed by Lesellier [3].

Despite a lot of early work documenting the impact of various modifiers on selectivity in SFC, there are still a number of issues around how these can be usefully applied. This still limits its applications in, for example, the pharmaceutical industry. The stationary phases used in SFC are



Separation of Benzene (1), Toluene (2), Caffeine (3), Norephedrine (4), Phenanephrine (5) and Uracil (6) on, from top to bottom, Atlantis C18 , Prontosil C18-ace-eps (Mac Mod Analytical), Bonus RP (Agilent), RP Amide (Supelco Inc.), Diethylaminopropyl modified C18 (Princeton Chromatography) and Ethyl Pyridine modified C18 (Princeton Chromatography) phases.
All columns 250 x 4.6 mm x 5 µm packing (except Princeton, 6 µm); oven temperature: 40 0C; flow rate: 1.5 ml min-1; column outlet pressure: 120 bar; modifier (methanol) gradient: 1% for 1 min, increasing to 30% at 1% min-1 and held for 2 min. UV detection at 215nm.

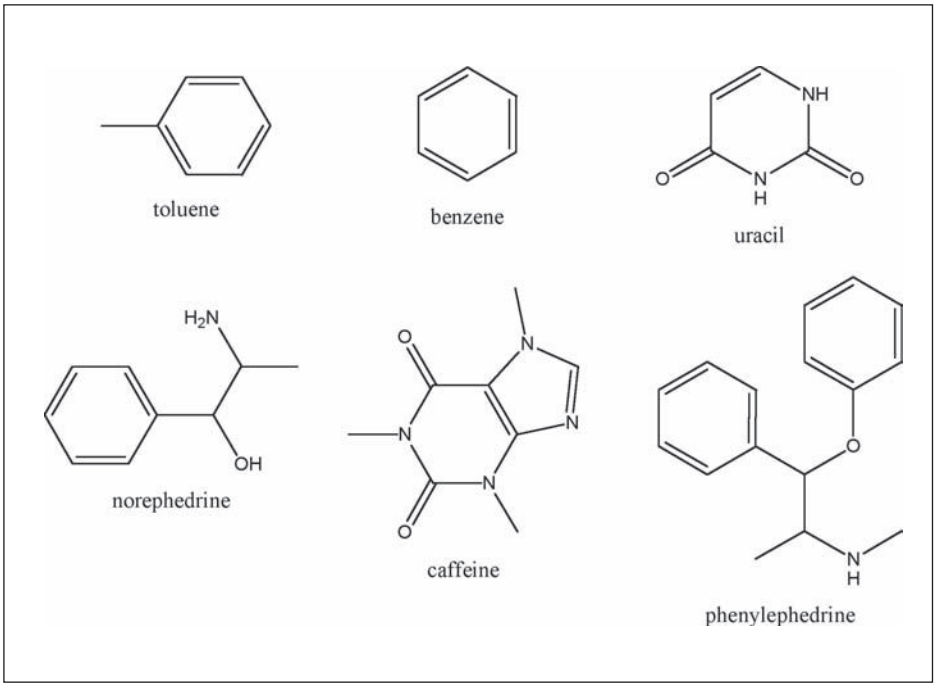


Figure 2. Test compounds for column evaluation.

broadly the same as those used in HPLC and similarly show a wide range of performance and selectivity, as illustrated by the column comparisons shown in Figure 1. In this instance, six aromatic test probes of varying polarity and basicity (Figure 2) were analysed under gradient condition with methanol as modifier. Although all the phases were based on octadecyl silica, these had been modified to varying degrees, resulting in quite marked differences in performance not just in resolution, but in analyte retention as well. As with reversed phase HPLC, the basic compounds all performed better on those phases specifically designed for this chemistry and in some cases, were completely retained by the more conventional C18 phases. More subtle differences are seen with the neutral molecules, where differences in hydrophobicity, silanol activity (end-capping) and even shape selectivity all manifest their influences to varying degrees. Of all the columns studied, the best performance was achieved using an ethyl pyridine modified C18 phase, which has been specifically developed for handling complex mixtures of varying polarity and acidity/basicity.

This specialized phase is not only suitable for the the separation of acidic, basic and neutral molecules, but as demonstrated by Brunelli et al. [4], can separate highly complex mixtures of diverse chemicals. Moreover, by using a mixed modifier (of methanol and acetonitrile) and altering its composition, these authors successfully developed a screening method and futher demonstrated how to tune the selectivity via modifier composition. This type of develoment will hopefully encourage others to investigate this technique further and see it move from strength to strength.
However, despite such progress, development of new methods and applications often still involve a considerable amount of screening and method development. In other fields, SFC has been used to achieve separations normally associated with normal phase chromatography (e.g. chiral separations) but with much greater speeds. One such area has been in the separation and characterisation of complex mixtures such as oligomeric species of alkoxylated chemicals, widely used as surfactants in personal care (shampoos and skin creams) and household cleaning products. They are produced through reacting fat derived alcohols with ethylene (or propylene oxide) to produce highly complex mixtures of oligomers (10 to over 100, repeat units). Such compounds can pose particular problems as they are not only complex, comprising of homologous series in which the incremental difference is based on one small molecular weight oligomer (eg ethylene or propylene oxide) but are also strongly surface active. Although these materials have been analysed by normal and reversed phase HPLC [5], these analyses tend to be time consuming and lack robustness.

However, Berger and Todd [6] demonstrated that is quite possible to analyse the silyl derivatives of these compounds, by packed column SFC, using a methyl end capped silica column heated to 2000C, and elution with carbon dioxide with a pressure gradient at high temperature. This technique is sufficiently robust and reproducible that it can be routinely used to characterize the oligomeric distribution of a number of such products, as with the alcoxylated (n=15)

Degree of Alkoxylation.	Ave. % <7	RSD %	Ave. % >14	RSD %	Average weighted oligomer no.	RSD %
Low	20.13	1.0	24.97	0.9	12.61	0.3
Medium	13.32	2.0	31.52	1.8	13.15	0.2
High	13.14	1.5	37.37	0.9	13.68	0.1

table 1.

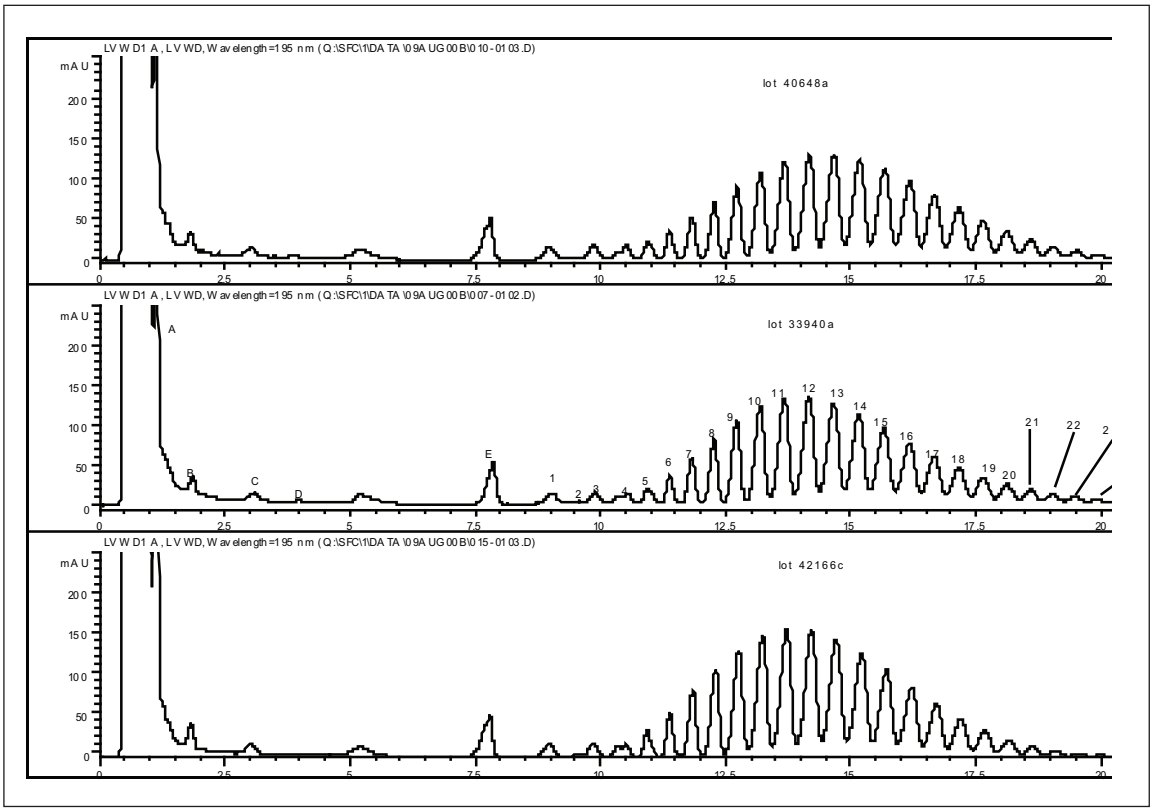


Figure 3. SFC ananlysis of tri-methyl silyl ethers of alkoxylated stearyl alcohol.

Deltabond methyl column (Thermo-Fisher Keystone Scientific, 2.0 mm x 250 mm x 5 mm); oven temperature: 200 0C; flow rate: 0.5 ml min-1; linear pressure gradient: 100 bar held for 1 min, increased to 370 bar at 20 bar min-1, hold at 370 bar for 8 min. UV detection at 200 nm.

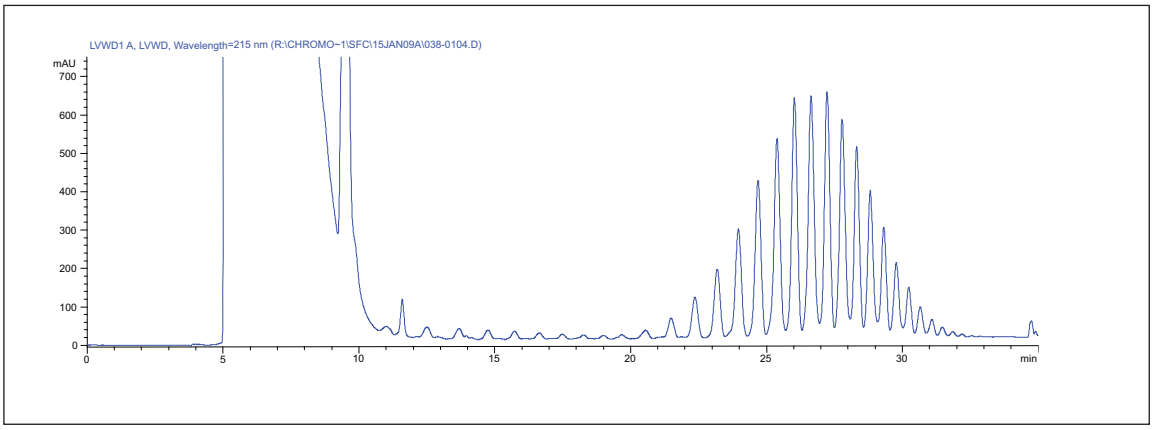


Figure 4. SFC ananlysis of disilazane ethers of alkoxylated stearyl alcohol.

Two Acclaim Polar Advantage C16 columns (Dionex, 4.6 x 250 mm, 5 mm); oven temperature: 40 0C; outlet pressure: 120 bar; flow rate: 2.4ml min-1; linear modifier (acetonitrile): gradient: 1% modifier for 5 min, increased to 20% at 1% min-1 and held at 20% for 5 min. UV detection at 215nm.

By first preparing disilazane derivatives, these authors introduced a phenyl chromophore into these compounds, enabling them to considerably broaden the scope of their investigations to include solvent modifiers (without fear of UV interference) and more readily available stationary phases. In this case, three different stationary phases were compared, both singly and in pairs; as a result of the low back pressures exhibited in this technique, it is more amenable to column stacking. The optimum separation (Figure 4) was eventually achieved using a standard modifier gradient, and two columns in series, both comprising of a C18 phase with embedded sulphonamide functionality combined with the non-polar side chain .

One of the attractions of packed SFC (pSFC) is that it can be used with the many of the same detectors used in HPLC and is readily interfaced with mass spectrometry. In the fine chemicals and polymers industry, many analytes do not have a UV chromophore and therefore this has either to be added to the analytes of interest, or the analyst has to use an alternative detector.

stearyl alcohol shown in Figure 3. Data from such analyses can be exported into spreadsheets and used to characterize the oligomeric distribution to a high degree of reproducibility, as illustrated in Table 1. This in turn, facilitates the detection of subtle differences in oligomer distributions in similar products, enabling it to be used in a variety of applications, from process development through to product comparisons.

However, this type of analysis also illustrates one of the key issues surrounding SFC in that sometimes the resulting method necessitates the use of highly specialized phases from a specific supplier. In this particular application, alternative methods have been developed by Hoffman et al. [7], in which it is possible to achieve similar results using a different phase but under considerably milder separation conditions.

One such detector is the Evaporative Light Scattering Detector (ELSD). As with early MS interfaces, the original problem was that the super critical mobile phase cooled the nebuliser upon exiting it, resulting in freezing and even blocking of the tip. Further developments in nebuliser technology and effective temperature control make it possible to use this detector routinely with SFC [8]. In-house studies have demonstated

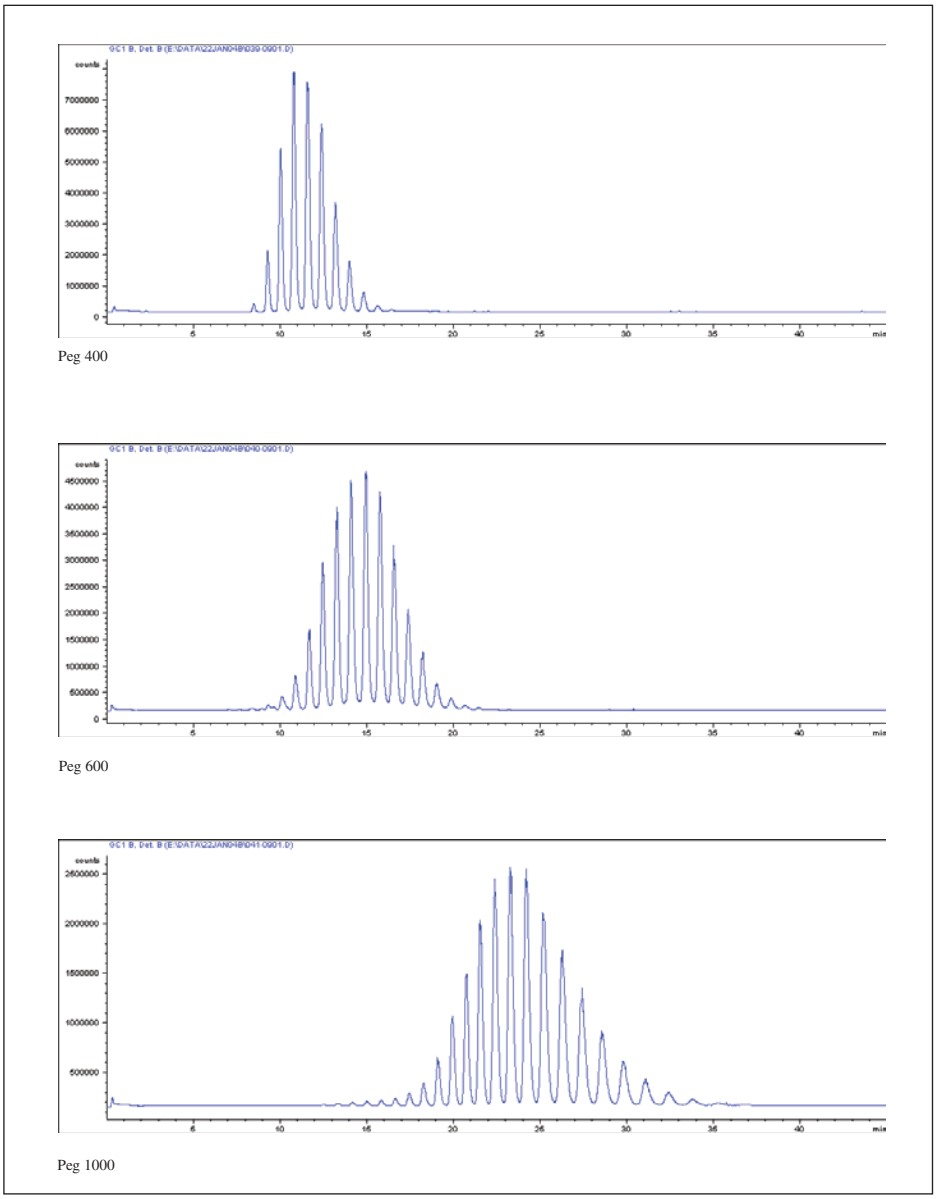


Figure 5. SFC analysis of underivatised PEGs

Diol Column (Princeton Chromatography, 4.6 x 250 mm x 6 mm) column; oven temperature; 40 0C: flow rate: 2 ml min-1; methanol modifier concentration: 5%; linear pressure gradient: 100 bar for 1 min., increased to 250 bar at 10 bar min-1. Detection by ELSD (Polymer Labs 2100).

that underivatised polyethylene glycols can be readily separated with Peg Diol Column and ELSD detection (Figure 5). While developing this separation, it was also noted that a small amount of organic modifier is needed (typically at least 5%) to ensure effective spray formation.

Although the ELSD has found widespread use as a 'semi'-univeral detector, it does have limitations, including limited sensitivity and linear response range and variable response with compound type. Consequently a variety of alternative detectors have been developed, such as the Corona Aerosol Detector (CAD) which has since found increasing use with HPLC and more recently, SFC. [9] Other alternative detectors, such as the acoustic flame detector[10] are also under development although not yet commercially available. However, as these types of detectors and technologies continue to be developed, they will play an increasing role in this technique.

Finally, mention should be given to the hyphenation of SFC with mass spectrometry, although this subject is too large to be adequately covered in this article. Instead, interested parties should refer to the appropriate publications, such as that by Pinkston et al [11], who have extensively compared the relative merits of LC/MS and SFC/MS in the analysis of pharmaceutical compounds. As with LC/MS, the mass spectrometers can range from simple quadrupole units, typically used as detectors (as for mass selective detection and target compound isolation and purification) through to more sophisticated triple quadrupole devices, that are more appropriate for the idenfication of unknown compounds. Developments in interface and ion source design have all facilitated the development of this particular form of hyphenation. This in turn, has significantly contributed to the growing acceptance and future of SFC as a viable and still developing technique.

References

1. *T.A. Berger. Packed Column SFC, Royal Society of Chemistry, 1995*
2. *T.A.Berger, J.Chromatogr.A. 785 (1997) 3-33*
3. *E. Lesellier, J.Sep.Sci. 31 (2008)1238-1251*
4. *C.Brunelli, Y. Zhao, M. Hanna-Brown, P. Sandra, J.Sep.Sci. 31 (2008) 1299-1307*
5. *T.C.G Kibbey, T.B Vavaraski, K.F. Hayes, J.Chromatogr.A. 752 (1996) 155-165*
6. *T.A Berger, B.S. Todd, Chromatographia, 54 (2001) 777-781*
7. *B.J. Hoffman, L.T. Taylor, S. Rumbelow. L. Goff, J.D. Pinkston, J.Chromatogr. A. 1034 (2004) 207-212*
8. *J.T. B Strode, L. T. Taylor, J. Chromatogr. Sci., 34 (1996) 261-270*
9. *K. Takahashi, S. Kinugasa, M. Senda, K. Kimizuka, K. Fukushima, T. Matsumoto, Y. Shibata, J. Christensen., J. Chromatogr. A. 1193 (2008) 151-155*
10. *Z. Xia, K.B. Thurbide, J. Chromatogr. A. 1105 (2006) 180-185.*
11. *J.D. Pinkston, D. Wen, K.L. Morand, D.A. Tirey, D.T. Stanton., Anal Chem. 78 (2006) 7467-7472.*