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Minimizing UV noise in supercritical fluid chromatography. I. Improving back pressure regulator pressure noise

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ABSTRACT

Pressure fluctuations and resulting refractive index changes, induced by the back pressure regulator (BPR) can be a significant source of UV detector noise in supercritical fluid chromatography (SFC). The refractive index (RI) of pure carbon dioxide (CO_2) changes ≈0.2%/bar at the most commonly used conditions in supercritical fluid chromatography (SFC) (40 °C and 100 bar), compared to 0.0045%/bar for water (CO_2 IS 44× worse). Changes in RI cause changes in the focal length of the detector cell which results in changes in UV intensity entering the detector. The change in RI (ΔRI/bar) of CO_2 decreases 8-fold at 200 bar, compared to 100 bar. A new back pressure regulator (BPR) design representing an order of magnitude improvement in the state of the art is shown to produce peak to peak pressure noise (PN_{p-p}) as low as 0.1 bar, at 200 bar, and 20 Hz, compared to older equipment that attempted to maintain PN_{p-p} < 1 bar, at <5 Hz. With this lower PN_{p-p}, changes in baseline UV offsets could be measured as a function of very small changes in pressure. A pressure change of ±1 bar at 100 bar, common with some older BPR's, produced a UV baseline offset >0.5 mAU. A pressure change of ±0.5 bar representing the previous state-of-the-art, resulted in a UV offset of 0.3 mAU. Baseline noise <0.05 is required to validate methods for trace analysis. The new BPR, with a PN_{p-p} of 0.1 bar, demonstrated UV peak to peak noise (N_{p-p}) < 0.02 mAU with a >0.03 min (10 Hz) electronic filter under some conditions. This new low noise level makes it possible to validate SFC methods for the first time.

1. Introduction

Supercritical fluid chromatography (SFC) is a well known technique, widely used in the pharmaceutical industry, mostly for chiral method development [1–3]. Dense carbon dioxide makes up most of the mobile phase, and requires a back pressure regulator (BPR) after the detectors to maintain a single, high density fluid through the system. These BPR's are almost always under electronic control.

In SFC, solute binary diffusion coefficients [4] are 3–5 times higher, and viscosity is 3–5 times lower than in aqueous based mobile phases. Thus, SFC is faster, on the same sized particles and capable of higher efficiency than HPLC. However, SFC has always suffered from a serious lack of sensitivity and has never found a place in trace analysis or regulated environments [5,6].

Criteria for validating a method for trace analysis include the ability to quantitate peaks representing 0.05% of the parent peak with a signal to noise ratio of 10:1. If the main peak is 1AU tall, the noise level must be below 0.05 mAU at the appropriate peak width setting for the peaks. The ASTM test (E1657-98) for diode array detectors in HPLC requires the peak to peak noise (N_{p-p}) to be <0.02 mAU, with a >0.1 min filter. Until recently, this level of noise has not been reported in SFC.

1.1. History of UV noise in SFC

There are no systematic studies of UV noise in the SFC literature. Authors tend to publish only full scale chromatograms on an apparently flat baseline. Chromatograms with an expanded scale, where the baseline noise is visible, are rare. After an extensive search of the literature a relatively small sampling of such chromatograms were found and the peak to peak noise was measured manually. On one system [1–15], noise has always been in the range of 0.3–1 mAU, with PN_{p-p} < ±0.5 bar, with heavy filtering. There is a single literature example [16], using this instrument, in which noise was an order of magnitude lower. A Peltier driven heat exchanger controlled the fluid temperature entering the detector cell. At 35 °C and 45 °C, noise was 0.17 and 0.22 mAU, respectively. However, at 40 °C, noise was approximately 0.02 mAU, with approximately 0.1 mAU wander over 20 min.

Other publications [17–18] with a different instrument show N_{p-p} = 1–4 mAU. The manufacturer describes [19,20] the BPR as a solenoid, driving a pin to open and close an orifice at a rate of 1–20 Hz. Most work in SFC has been performed on

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4.6 mm × 250 mm columns with 5 μm particles. Such columns produce peaks 0.05–0.2 min wide at half height. The electronic signals generated by the detector, thus, consist of frequencies of 1.25–5 Hz, similar to the control loop frequency of the solenoid. SFC is rapidly being converted from the use of columns packed with 5 μm particles to columns packed with 3.5, 3 and sub-2 μm particle. With 3–3.5 μm columns peak signals contain frequencies up to 20 Hz. With sub-2 μm particles, peak signals contain frequencies up to 80 Hz. Any detector noise induced by this BPR cannot be filtered significantly without distorting such peaks.

Another instrument [21] no longer commercially available, showed noise of approximately 0.4 mAU. That instrument used two stage pressure reduction with most of the pressure drop occurring across a mechanical BPR mounted downstream of an electronic BPR.

The literature with respect to baseline noise is complicated by the fact that authors often present results in millivolts, microvolts, or % full scale. Since such ranges are completely arbitrary, they cannot be used directly to determine sensitivity or noise (in absorbance units) or dynamic range without a clear description of a conversion factor. Further, authors almost never state many of the important detector conditions, such as the electronic filter setting, slit size, bandwidth, reference wavelength (if used), conversion factors, cell volume, path length, etc., necessary for others to repeat their work. The fact that so little information is available on detector noise or detection limits, suggests SFC has not competed with HPLC in this regard.

In several recent SFC publications, significantly lower noise levels have been demonstrated [22–23]. The improvement in UV noise has been largely attributed to an improvement in pumping. In this work it is shown that some of this improvement is due to improved BPR noise characteristics.

1.2. Refractive index changes

There are many factors associated with UV detector noise. Some, such as: lamp age, electronic filter setting, and slit width are independent of the mobile phase. However, others, are associated with rapid refractive index changes in the mobile phase, such as: pressure and composition variations caused by the compression strokes of each pump, outlet pressure variations associated with the back pressure regulator (BPR) control, thermal miss-matches in and around the detector cell, drafts, doors missing, etc. It is of interest to attempt to isolate each source of noise to quantitate its effect and potentially improve SFC sensitivity.

In principle, it is widely understood that pressure fluctuations ought to cause rapid density, and RI fluctuations in the flow cell, creating UV noise. Light from the lamps is focused into a conical beam, through the flow cell, onto a slit. This beam produces a large fuzzy "dot" of light covering the slit. The slit limits the amount of light entering the spectrophotometer. If the RI of the mobile phase changes, the focal length of the cell changes. The size and intensity distribution within the "dot" changes, and the amount of light passing through the slit will vary. A change in light intensity causes a change in the detector signal intensity, resulting in a shift in the baseline. Rapid shifts in pressure (pressure noise) should cause rapid shifts in the UV baseline and noise on the UV detector signal at the frequency of the pressure change.

There appears to have been no attempt to correlate measured changes in RI in SFC with changes in pressure, to attempt to understand and potentially optimize UV detector noise. At least three non-SFC papers in the literature [24–26] deal with the RI of pure CO_2 as a function of density under conditions similar to those used in SFC, although in an unfamiliar format for chromatographers.

In this work, the range of RI changes occurring in an SFC detector is estimated from literature values in pure CO_2, translated into a more usable format. A new BPR with lower pressure noise was used to make very small pressure excursions, while measuring the resulting shift in a UV detector signal at a high wavelength, where changes can be attributed to RI changes only. Shifts in the UV baseline were related to pressure changes. An attempt was made to correlate the shift in UV signal intensity with the short term changes in back pressure, and relate the findings to BPR pressure noise requirements for low noise UV operation.

2. Experimental

2.1. Instrumentation

An SFC conversion module (Fusion™ A5, Aurora SFC Systems, Inc., Redwood City, CA) was connected to an HPLC consisting of: a solvent cabinet, Model 1100 degasser, a Model 1200SL binary pump, autosampler, and column oven, and a Model 1100B diode array detector (DAD); all from Agilent Technologies, Waldbron, Germany, converting the HPLC into an SFC.

The HPLC was controlled by a standard Agilent ChemStation. The ChemStation controls total flow rate and %B, all the autosampler functions, column compartment functions and detector settings exactly as in HPLC. The HPLC pump delivers both the CO_2 and the modifier.

The Fusion module is controlled by a small add-on software module that becomes part of the ChemStation graphical users interface. The SFC conversion module consists of a chiller and booster pump that take vapor phase CO_2, condense it, and compresses it to just below the delivery pressure of the Agilent pump. This pre-compresses the fluid. The Fusion module also contains a BPR that controls system outlet pressure. Once connected, the only set point of the Fusion module is the system outlet pressure. The BPR pressure sensor signal was plotted on-line, along with detector signals.

The Agilent binary pump was modified by replacing the active inlet check valves with passive check valves. A second purge valve and an in-line check valve were mounted in series on the outlet of the B side, before the mixing tee. This purge valve allowed the B (modifier) pump to be purged independently. The Fusion module output was the fluid source for the A side of the HPLC pump (1 m of 1/16th in. OD, 0.02 in. ID stainless steel (SS) tube). The compressibility compensation for the A side pump was set to zero (0). This pump does not compress the CO_2 further but only meters the flow of the pre-compressed fluid. The B side was set to 130×10^{-6}/bar for methanol.

The autosampler was modified by replumbing the injection valve. A 3 groove rotor replaced the standard 2 groove rotor and an external 1.25 μL loop was installed (10 cm length of 0.005 SS tubing). A 14 line injector program was written to convert the autosampler to an "external loop" autosampler. The autosampler syringe was used to withdraw air bubbles and sample from vials and then push the sample through the external loop to waste. In this mode the syringe never sees high pressure. A wash pump, with a nominal flow of 2.5 ml/min, located in the Fusion module, was attached to the inlet of the syringe pump in the autosampler, using a length of PEEK 1/16th in. OD tubing. An Agilent BCD card was installed in the rear compartment of the autosampler. The injection program controls contact closures on the BCD card, which turns the wash pump on and off, to wash out the needle and loop before and after each injection.

The DAD was fitted with a 13 μL, 10 mm path length high pressure flow cell. The outlet of the detector cell was connected to the inlet of the BPR in the Fusion module, using another piece of 1 m, 1/16th in., 0.020 in. ID SS tubing.

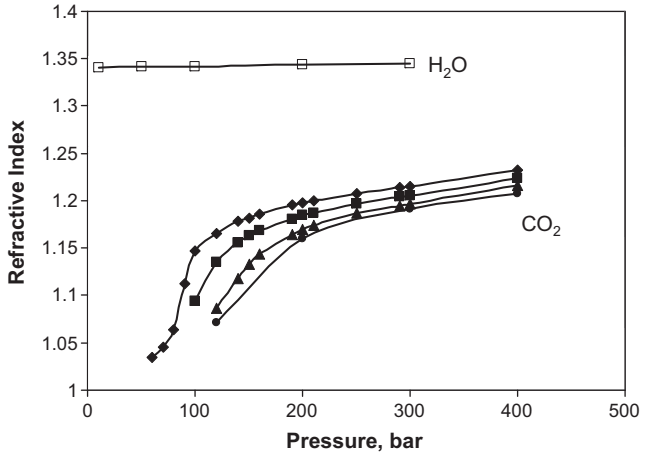

Fig. 1. Refractive index vs density transposed into refractive index vs pressure curves from top to bottom: water at 40 °C, then pure carbon dioxide at 40 °C, 50 °C, 60 °C, and 70 °C (data from Ref. [24]).

2.2. Materials

Carbon dioxide was "beverage grade" purchased from Terry's Supply Co., Sarasota, FL in 50 pound cylinders, without a DIP tube. Methanol was Omnisolve grade from SECO, Aston, PA. Xanthenes (caffeine, theophyline, theobromine >98% pure) were purchased from Sigma Aldrich, St. Louis, MO.

The column was 4.6 mm × 250 mm, 5 μm CN bonded silica, kindly supplied by Agilent Technologies, Little Falls, DE, USA.

3. Results and discussion

3.1. Refractive index

Literature values [24] for the refractive index of pure CO_2 at 40 °C, as a function of density, produced straight line plots. Those authors stated that data collected at 90 °C fell directly on the same line, suggesting that the refractive index was strictly a function of density between 40 °C and 90 °C. With this assumption, plus pressure vs density values from the Encyclopedia des Gaz [27], and from an equation of state [28], the results were translated into a series of pressure vs refractive index curves, under common conditions used in SFC. The shapes of the RI vs pressure curves for pure CO_2 are exactly the same as the shape of the pressure vs density curves. The results are shown at the bottom in Fig. 1, for 40 °C, 50 °C, 60 °C, and 70 °C. Between 100 and 400 bar and 40–70 °C, the RI of CO_2 can vary from 1.07 to 1.25.

The RI of water is 1.333 at 20 °C, and changes much less with pressure than CO_2 as shown as the top line in Fig. 1 (0.0045%/bar, vs 0.025%/bar to 0.2%/bar (CO_2 can be >40× worse)). The RI of methanol, ethanol and acetonitrile are all between 1.33 and 1.36 at 20 °C. Mixing any of these organic solvents with water results in a minor change in refractive index. Most UV detectors were designed for use in HPLC assuming the refractive index is generally between 1.33 and 1.36.

Fig. 1 and the RI of the various modifiers should cause concern for users of SFC. Changing the concentration of any of these modifiers mixed with CO_2, or changing the temperature or pressure, will likely result in much larger changes in refractive index, compared to HPLC. Unfortunately, there does not appear to be any confirming or refuting data in the SFC literature reporting on the refractive index of mixtures of modifiers with CO_2.

Among the most commonly used conditions in SFC, at least in the pharmaceutical industry, have been 100 bar at 40 °C. The RI at 100 bar and 40 °C is poised on a "cliff" as shown in Fig. 1. The RI changes 0.2%/bar at 100 bar, 40 °C, but only 0.025% at 200 bar, 40 °C. "only" is a relative term. Operation below 100 bar will result in even more severe changes in RI.

Although the RI of CO_2–methanol mixtures, at high densities, is not available, we do know that the addition of small concentrations of methanol to carbon dioxide produce pressure vs density curve similar to pure CO_2 but with a slight positive offset [29,30] increasing with methanol concentration. This suggests the RI vs pressure plots of mixtures are likely to be similar to those of pure CO_2, only more exaggerated. All subsequent experiments used constant methanol concentrations at constant temperature, to try to measure only the effect of pressure on UV baseline shifts.

3.2. Pressure noise measurements

The repeatability and peak to peak pressure noise of the BPR in the SFC conversion module was measured at 20 Hz, using 2.0 ml/min, of 10% methanol, at 40 °C and several pressures. The 4.6 mm × 250 mm column packed with 5 μm particles helped dampen flow (and pressure) noise induced by the pumps. The pressure was stepped by as little as 0.1 bar. The data in Fig. 2 show that pressure noise for this specific BPR is less than ±0.05 bar peak to peak at both 100 and 200 bar, while the repeatability is <±0.02 bar (<1 psi).

The composition and flow rate were also changed while holding pressure constant at 150 bar. The results, shown in Fig. 3, indicate no variation in either the absolute outlet pressure value, or the peak to peak pressure noise, even though flow was varied from 1 to 4 ml/min, while the composition was varied from 5% to 40%. A perturbation after each step change in concentration indicates the break-through times. Such breakthrough causes a rapid change in viscosity. Automatic adjustment by the BPR electronics to the new viscosity took a few seconds. These experiments were repeated at 100, 200, and 300 bar. In all cases, pressure noise (PN_{p-p}) < ±0.1 bar. This is the lowest noise reported in SFC by an order of magnitude. Not all BPR's of this design perform this well.

3.3. UV baseline offsets vs pressure

Since Figs. 2 and 3 establish that the BPR is highly repeatable with very low pressure noise, the effect of larger changes in the system outlet pressure on UV detector offsets can be measured accurately. The UV diode array detector wavelength (λ) was set to 280 nm with a 16 nm bandwidth (BW). The reference wavelength (λ_r) was 360 nm with a 40 nm BW. The slit was set to 16 nm. The electronic filter was set to >0.1 min which corresponds to a 2.5 Hz data rate. UV spectra were monitored on-line to insure there was no absorbance at the wavelength used, so that all shifts in the baseline could be attributed to RI changes.

The system outlet pressure was changed approximately every 30–45 s, back and forth between each set of pressures, while the UV signal offset, caused by the pressure perturbation, was measured. At least three steps were made at each pair of pressures. The results from a typical set of measurements are presented in Fig. 4. The lower trace is the system outlet pressure, just downstream of the UV detector. From left to right, there are 3 steps from 120 to 140 bar (20 bar), 3 steps from 120 to 130 bar (10 bar), 3 steps from 120 to 125 bar (5 bar), 3 steps from 120 to 122 bar (2 bar), and 3 steps from 120 to 121 bar (1 bar). The steps are meant to emulate BPR PN_{p-p} of ±10 bar (20 bar total), ±5 bar (10 bar), ±2.5 bar (5 bar), ±1 bar (2 bar), and ±0.5 bar (1 bar).

The upper trace in Fig. 4 shows the UV detector signal responding to the pressure steps. The amplitude of the steps is a direct measure of baseline offset caused by the pressure perturbations and ultimately by the change in the RI of the mobile phase with

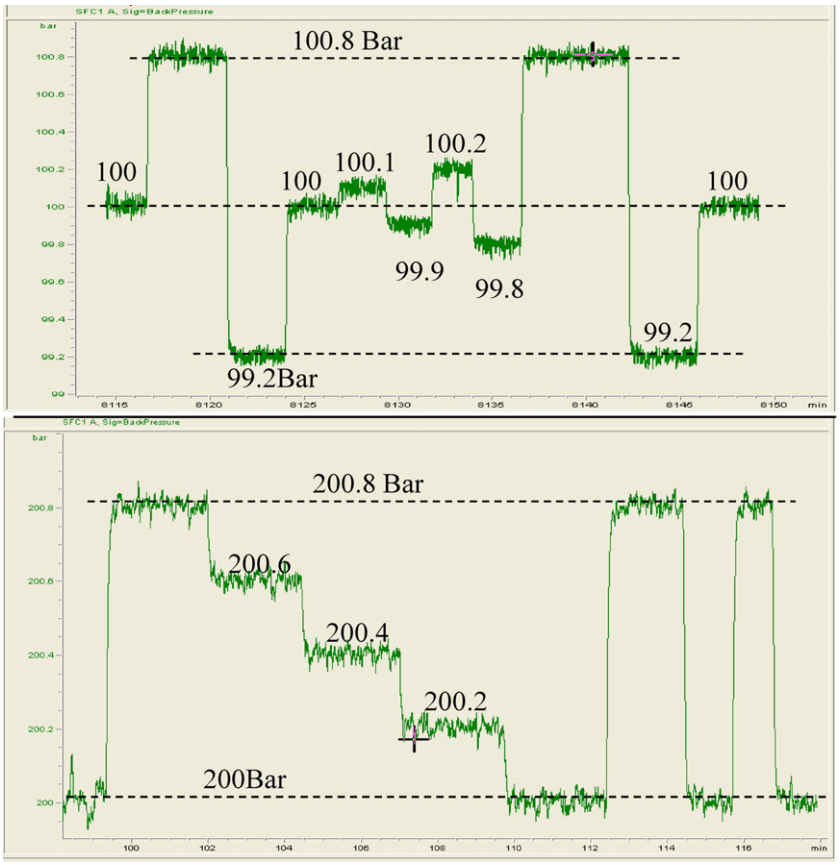

Fig. 2. Small variations in the BPR pressure setting produced low pressure noise on the order of ±0.05 bar, with high reproducibility (±0.01 bar). The data was collected at 20 Hz. Top: near 100 bar; bottom: near 200 bar. Conditions: 2 ml/min 10% methanol, 40 °C.

pressure. These measurements were repeated with the base pressure (the initial pressure) at 90, 100, 120, 140, and 200 bar, with the 20, 10, 5, 2, and 1 bar steps superimposed. Screen captures of the results were printed and the approximate N_{p-p} was manually measured with a caliper. The over-all results were plotted and are presented in Fig. 5A and B. UV detector offsets were the largest in the regions where the RI (and density) changes the most with small changes in pressure, as expected. Operation at higher pressures resulted in decreased detector offsets which should also result in lower UV N_{p-p}.

As recently observed anecdotally [31], large pressure spikes caused significant spikes in the UV baseline. Changing from 90 bar to 110 bar caused a >6 mAU (filter >0.1 min) shift in the UV signal. Such large perturbations (PN_{p-p} = 5–20 bar), similar to those in

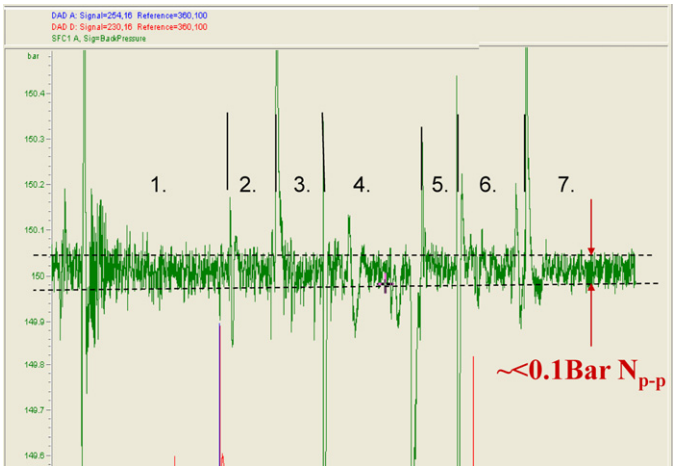

Fig. 3. The 20 Hz pressure signal from the Fusion BPR with flow varied from 1 ml/min to 4 ml/min and composition varied from 5% to 40% at 150 bar. (1) 1 ml/min, 5%, (2) 1 ml/min 10%, (3) 1 ml/min 40%, (4) 2 ml/min 40%, (5) 2 ml/min 5%, (6) 4 ml/min, 5%, and (7) 4 ml/min 40%. All at 40 °C. Similar results were obtained at 100, 200 and 300 bar (results not shown).

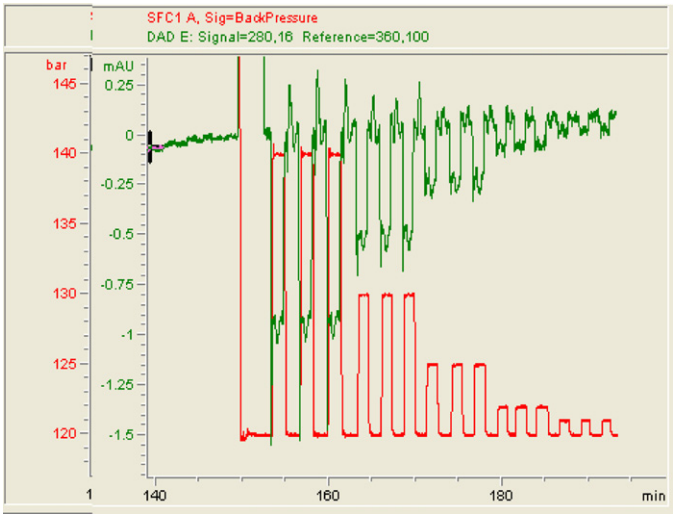

Fig. 4. Pressure steps (bottom trace) and resulting UV signal offsets showing a RI effect at 120 bar. The pressure steps are 20 bar, 10 bar, 5 bar, 2 bar, and 1 bar high. UV offsets are up to 1 mAU tall. 2 ml/min of 10% methanol at 40 °C. UV λ = 280 nm, w/16 nm BW; λ_r = 360 nm, 40 nm BW, 16 nm slit; electronic filter set at >0.1 min (2.5 Hz).

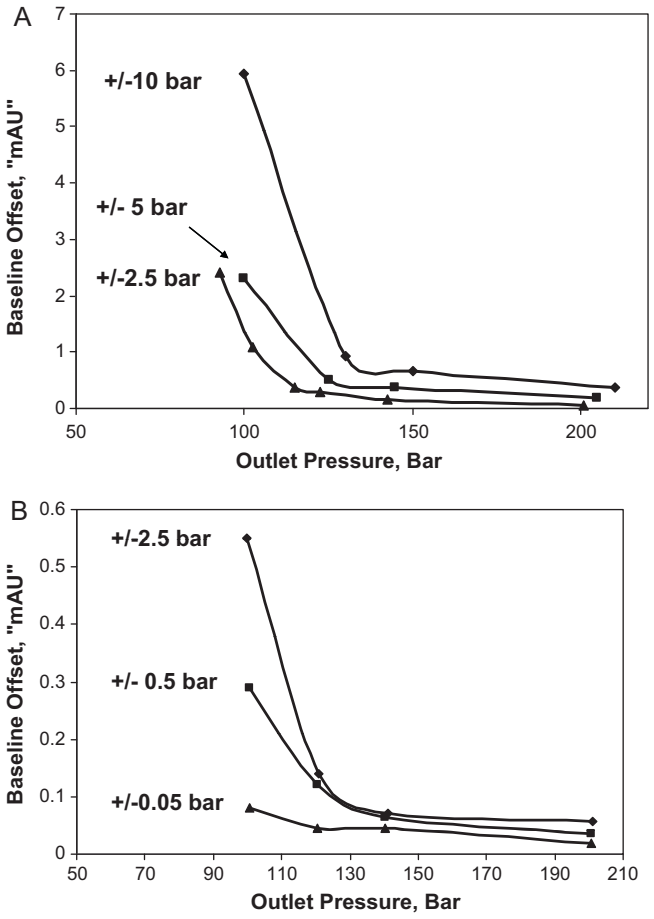

Fig. 5. UV detector signal offsets resulting from pressure steps, between 90 and 200 bar. (A) Results from pressure steps between ±2.5 and ±10 bar. (B) Results from pressure steps between ±0.05 and ±1 bar. The offsets are an indication of the likely UV N_{p-p} resulting from BPR pressure noise of the amplitudes indicated. Conditions: 2 ml/min, 10% methanol, 40 °C; λ = 280 nm; 16 nm BW; λ_r = 360 nm, w/40 nm BW; 16 nm slit; 13 μL, 10 mm path length flow cell. Electronic filter set at >0.1 min (2.5 Hz).

Fig. 5A, will likely only happen highly intermittently (noise spikes). In Fig. 5A the smallest UV shift observed was 0.129 mAU.

Until recently, the quietest electronic BPR's used in SFC exhibited peak to peak pressure noise of ≈±0.5 bar [1–15]. As indicated by the middle curve in Fig. 5B, this BPR should have been capable of producing UV noise of 0.3 mAU or less (with filter >0.1 min). An extensive search of the literature shows 0.3–0.5 mAU noise [1–14] using this BPR, but no better (however [16]). The middle curve in Fig. 5B suggests noise should improve to below 0.1 mAU at higher pressures, which was (almost) never previously observed with older BPR's.

All the curves in Fig. 5B show noise below 0.1 mAU at pressures above 130 bar. A search of the literature reveals a single example [16] of such low noise. Virtually the entire SFC literature exhibits noise >5× worse. It is likely that the novel pumping system employed here is responsible for the low noise observed at these higher pressures and allows observation of the effect of the BPR on UV noise.

3.4. Chromatograms

Chromatograms were run to show the effect of the column outlet pressure on UV baseline noise under realistic analysis conditions, using the low noise BPR. The 4.6 mm × 250 mm, 5 μm CN (Agilent) column was used to separate a test mix of caffeine theophyline and theobromine. The early eluting peaks were found to be <0.05 min wide at half height. Therefore, the electronic filter was set to the next fastest setting (>0.03 min or 10 Hz). The right hand heat exchanger in the column compartment was used to preheat the mobile phase and column to 40 °C. The left hand heat exchanger was optimized at 43 °C to minimize detector noise [32]. Flow remained at 2 ml/min, but the modifier concentration was dropped to 4%. This is lower than the manufacturers recommended minimum modifier concentration for trace analysis (≥5%). All conditions remained the same for the two chromatograms, except for the system outlet pressure.

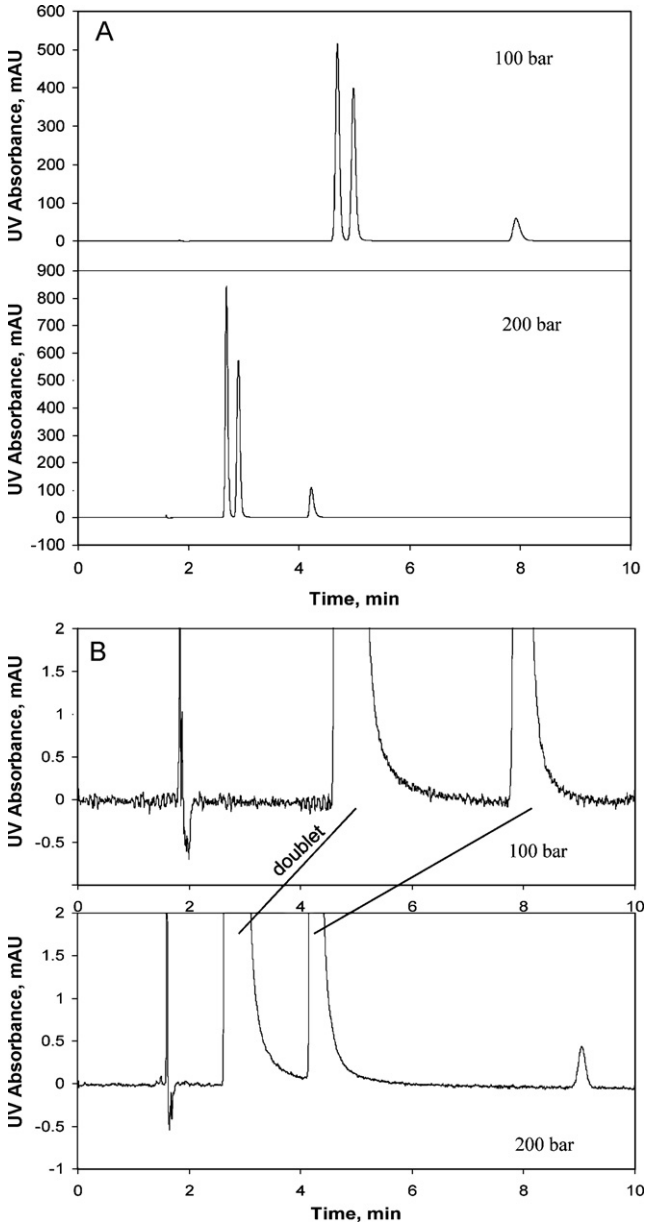

Fig. 6. (A) Chromatograms from injection of a xanthene standard at 2 different pressures, using the low pressure noise BPR. Note the significant decrease in noise when changing from 100 bar to 200 bar. In either case noise is much lower than with previous BPR's. The significant shift to shorter retention due to the pressure increase, is somewhat surprising. However, at low modifier concentration, pressure has an inordinate effect on retention times. Top: 100 bar. Bottom: 200 bar. Conditions 2 ml/min, 4% methanol, 40 °C, wavelength: 275 nm, 16 nm BW, 360 nm ref, 40 nm BW, slit = 16 nm; filter >0.03 min (10 Hz). 5 μL loop, 13 μL load; caffeine, theophyline, theobromine mix; column: Agilent Eclipse 4.6 × 250, 5 μm CN. (B) Same data but on a 300–400 times expanded scale.

Table 1
Retention time and area reproducibility of 10 consecutive injections performed at 100 bar. All other conditions as in Fig. 6.

	RT	Area	RT	Area	RT	Area
	4.682	2637.3	4.973	2194.4	7.921	564.4
	4.685	2631	4.976	2192.6	7.923	565.8
	4.691	2627.3	4.983	2178.8	7.926	559
	4.704	2614.1	4.995	2177.9	7.939	562.5
	4.719	2616.5	5.009	2178.1	7.955	561
	4.71	2610	5.002	2174.1	7.929	559.7
	4.697	2632.7	4.991	2192.1	7.913	564.2
	4.701	2636.8	4.994	2196.5	7.915	565.9
	4.712	2664.2	5.005	2215.2	7.927	570.9
	4.716	2621	5.01	2187	7.923	562.9
Mean	4.7017	2629.09	4.9938	2188.67	7.9271	563.63
Std dev	0.012859	15.60815	0.013172	12.31711	0.012206	3.483947
Rel std dev	0.27%	0.59%	0.26%	0.56%	0.15%	0.62%

Full scale chromatograms at 100 and 200 bar outlet pressure are presented in Fig. 6A. The upper chromatogram was collected at 100 bar. The lower chromatogram at 200 bar. Note the significant shift in retention between the chromatograms, due to the increase in pressure. Such a large shift due to this modest pressure increase is unusual, but the modifier concentration is quite low, and exhibits less dominance over retention vs pressure, under such conditions.

In Fig. 6B the same data is compared but with the scale expanded 300–400 times. The significant decrease in noise from 100 to 200 bar is obvious. At 100 bar, the UV detector N_{p-p} was on the order of 0.08–0.12 mAU (10 Hz). At 200 bar, the N_{p-p} dropped to approximately 0.02 mAU (10 Hz). Thus, increasing pressure from 100 to 200 bar decreased detector noise approximately 4–6 times. The small peak, at just over 9 min, in the 200 bar chromatogram is a "junk peak" not seen in the 100 bar chromatogram.

The UV noise at 100 bar does not meet the requirements for validating a method for trace analysis, since it could not achieve a signal to noise ratio (S/N) >10 for a peak representing 0.05% of a parent peak 1 AU tall. Such a peak would be 0.5 mAU high. A S/N > 10 is required for quantitation, which is N_{p-p} < 0.05 mAU. The chromatogram at 200 bar meets the requirements for validation, in spite of the faster filter setting and the lower-than-the-manufacturers recommended (<5%) modifier concentration for trace analysis.

3.5. Effect of outlet pressure on reproducibility

Ten consecutive injections of the xanthenes mix were made at 100 bar, followed by 9 consecutive injections at 200 bar (ran out of sample). The smallest peaks were over 100 mAU tall at both pressures. The results are presented in Tables 1 and 2 respectively. The retention time and area reproducibility was found to be acceptable in both cases, even with the low modifier concentration (4%). Area reproducibility was slightly improved at 200 bar, compared to 100 bar (0.42%–0.55% compared to 0.56–0.62%). However, retention time reproducibility significantly improved, from 0.15–0.27% to 0.05–0.08%.

Note that an increase in pressure, resulting in an increase in the RI, causes a decrease in the UV signal in Fig. 4. Since the detector is measuring absorbance, a decrease in signal is caused by an increase in light passing through the slit. In Tables 1 and 2 the average peak areas increased slightly (≈6%) when the outlet pressure was raised from 100 to 200 bar.

3.6. Pumping considerations

This work could probably not have been performed without the major improvement in the pumping concept employed [22,23]. The adiabatic compressibility of CO_2 varies from <200 × 10^{-6}/bar to over 900 × 10^{-6}/bar, depending on temperature and pressure. The compression stroke in all other CO_2 metering pumps is up to 30% of the total stroke length, under worst case conditions, and typically accounts for at least 8% of the total stroke length. Large compression and compensation strokes generate large pressure and composition fluctuations at the head of the column. The BPR will undoubtedly interact with any pump induced pressure perturbations reaching the detector cell. By eliminating the compression stroke of the CO_2 pump, most of this noise is eliminated, and the much lower pressure noise of the new BPR can be fully appreciated.

Unfortunately, any pump that still compresses the fluid during the delivery stroke will continue to generate significant pressure and composition fluctuations at the pump, which can be propagated through the column to the detector cell. Extensive hydraulic filtering after the pump but before the injector can help dampen such pressure pulsations but adds gradient delay times into the system.

Table 2
Retention time and area reproducibility of 9 consecutive injections performed at 200 bar. Note improvement in reproducibility compared to Table 1. All other conditions as in Fig. 6.

	RT	Area	RT	Area	RT	Area
	2.687	2771.4	2.903	2330.5	4.222	598.3
	2.686	2763.8	2.902	2322.6	4.222	596.5
	2.684	2789.3	2.9	2345.7	4.219	603.4
	2.683	2765.4	2.899	2327.3	4.216	597.9
	2.686	2772.2	2.901	2327.8	4.219	598.8
	2.684	2774.5	2.9	2332.9	4.216	600.2
	2.683	2799	2.898	2363.6	4.213	604.4
	2.686	2774.7	2.901	2330.7	4.217	599.3
	2.684	2766.1	2.899	2326	4.213	597.2
Mean	2.684778	2775.156	2.900333	2334.122	4.217444	599.5556
Std dev	0.001481	11.71656	0.001581	12.81871	0.003358	2.704215
Rel std dev	0.06%	0.42%	0.05%	0.55%	0.08%	0.45%

All the work reported here employed an older (pre-2000) Model 1100B diode array detector (DAD) from Agilent Technologies. This older detector is substantially "noisier" than newer detectors. Never the less, many "surplus" 1100's (and 1200's) are still deployed in laboratories around the world that could be rehabilitated to provide adequate noise, linearity and detection limits for use in trace analysis for moderately fast SFC chromatography.

4. Conclusions

The pressure noise induced by some older back pressure regulators appears to have contributed a significant fraction of the excessive UV noise observed with previous generations of SFC's. Electronic BPR pressure control electronics and algorithms have generated pressure noise, which in turn created density and refractive index oscillations. The refractive index of CO_2 can vary >40 times more than water as a function of pressure. Earlier BPR's seldom controlled pressure to better than ±0.5 bar, resulting in UV detector noise of 0.5 mAU.

It was demonstrated that it is possible to make a BPR with pressure noise of ±0.05 bar. This dramatic decrease in pressure noise is correlated with an order of magnitude improvement in UV detector noise, making SFC equivalent to HPLC, with the same detector. The results suggest the BPR must produce a pressure noise no larger than 0.1 bar, even at 200 bar, to equal HPLC performance. Higher levels of pressure noise increases UV noise, compared to the same detector used in HPLC.

It appears it is also time to rethink some basics in SFC. Although a large fraction of SFC applications have been performed near 100 bar and 40 °C, with modifier concentrations as low as 4–5%, it is clear that trace analysis would be better performed at a higher pressure and probably higher modifier concentration (subject to appropriate partition coefficients), since UV detector noise (and signal to noise), and reproducibility improves significantly above 100 bar.

It should be an easy matter to observe the pressure noise of any particular SFC and correlate this noise to its UV detector noise. It appears that a pressure noise ≈±0.5 bar generates UV detector noise near 0.3–0.5 mAU, which is more than an order of magnitude too high to perform trace analysis, particularly in a regulated environment. BPR's with a pressure noise >±0.5 bar should be unacceptable for all but the crudest major/minor component applications.

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