Optimum performance laminar chromatography (OPLC) is a high-performance liquid chromatography technology allowing separations of complex samples for both on-line and off-line detection. Innovative modifications, such as the flowing eluent wall (FEW), improves peak shape and purity of isolated fractions. The FEW technology has been integrated into the OPLC purification unit (PU), a new liquid chromatographic accessory, which can easily be integrated into any HPLC system. We have demonstrated the utility of the PU for semi-preparative purification and isolation of quinoxalinones, using HTSorb RP18 flat columns. Samples up to 20 mg were injected and the desired products were isolated with excellent yield and purity (96%).

Introduction
Optimum performance laminar chromatography is the unique liquid chromatography technology which allows high-performance separations of complex samples for both on-line (UV, MS) and off-line detection (visual, densitometry, spray reagents). The OPLC allows the analyst to visualize how the samples constituents are eluting on the stationary phase. In contrast to HPLC, the operator can decompress the system at any time, remove the flat column and observe the migration of the components. The HTSorb can then be reinserted and the chromatographic method adapted to obtain optimum separation. This particularity of the OPLC technique is especially useful for method development.

The original OPLC 50 has seen several innovative modifications to improve its performance. Our latest innovation, the flowing eluent wall or “FEW technology,” has been incorporated in the OPLC PU. The PU was designed as both an analytical and semi-preparative tool to be integrated into any HPLC system. The FEW significantly improves peak shape by confining the sample to a particular zone on the stationary phase. The improved peak shape obtained results in better product purity in semi-preparative mode.

To illustrate the capabilities of the OPLC PU, we shall describe a methodology for purification of reaction mixtures containing quinoxalinones. After an initial method-development step using small-volume injections, scale-up was performed and pure fractions were recovered without modification of the basic chromatographic procedure. A final purity assay was performed to demonstrate the quality of the separations.

Experimental
Material: HTSorb RP18 Flat columns (BIONISIS, France), with a 11 µm particle size and having dimensions 5 cm wide, 20 cm long and 180 µm thick were used for OPLC separations. These flat columns may containing a fluorescent marker (254 nm) for UV observation of elution. Acetonitrile was chromatography grade (SDS, France). Trifluoroacetic acid, synthesis quality, was purchased from SDS. DMSO and the de-ionized water came from VWR (France). Samples used in this study were quinoxalinone derivatives obtained by chemical synthesis and provided by a private research firm.

Instrumentation: The OPLC PU (BIONISIS) is a modification of the OPLC 50 system (BIONISIS) integrating an inlet for the FEW. The hydraulic unit of the separation chamber is equipped with two eluent inlet connections: one for sample injection and the other for the FEW. The FEW is achieved by splitting the solvent into two streams just before the injector (Figure 1). Part of the solvent enters the injector, carries the sample to and through the flat column and on to the detector. The other portion of eluent, dedicated to the FEW, bypasses the injector. It enters the column at the lateral edges, flowing at the same linear flow-rate as the sample stream. The PU was integrated into a Kontron HPLC system equipped with autosampler, UV detectors (0.1 mm–5 mm cells) and a data processing station. The hydraulic unit in the PU applies a homogeneous pressure of 50 bars on the stationary phase in order to obtain well-packed flat column with high plate numbers per centimetre column length.

Figure 1: OPLC purification unit using the few eluent wall.
HPLC method development: The quinoxalinone derivatives were dissolved to 20 mg/mL in DMSO. The stationary phase was rinsed and equilibrated before sample injection. 50 µL (0.2 mg) was injected onto a HTSorb RP18 column at a flow-rate of 0.27 mL/min. Gradient elution was performed over 15 min using 40–99% ACN. Detection was performed between 270–310 nm.

Semi-preparative product purification: The quinoxalinone derivatives were dissolved to 100 mg/mL in DMSO. The chromatographic procedure was identical to above, with the exception up to 16–20 mg were injected onto the column. The major fractions were collected and subsequently re-injected for purity.

Results/Discussion
Experiments performed with the FEW-modified PU have routinely demonstrated linear elution bands on the flat column. The FEW provides finer, more symmetrical peaks and eliminates peak tailing, thus improving the quality of fractioned components during semi-preparative work.

As HTSorb is a very cost-efficient stationary phase for chromatography, we have used the complex sample mixtures as received in all our work. For method development, only one column was necessary. However, for the semi-preparative work a new column was introduced for each sample mixture. Avoiding column regeneration provided an additional savings of time and solvent.

The chromatographic methods described herein were developed in order to have a unique gradient system suitable for both polar and less polar compounds within the same product family. Chromatographic profiles were obtained using 50 µL (approx. 0.2 mg) of sample mixture. Aqueous acetonitrile gradient elution from 40–99% ACN was performed over 15 min. Weakly retained and strongly retained samples all eluted properly under these conditions, as confirmed by off-line observation of the flat columns under a UV lamp.

Chromatograms of four representative products are shown in Figure 2 below. This chromatographic method was maintained for the following semi-preparative isolations. The sample volume and concentration were increased up to 20 mg/injection using a flat column of identical capacity. While the injection volume can represent more than 25% of the column volume under these conditions, as confirmed by off-line observation of the flat columns under a UV lamp.

Figure 2(a)–(d): Purification of different quinoxalinone derivatives using the same OPLC PU chromatographic method.
conditions, resolution and selectivity were maintained. The major fractions of each of the samples were collected. Using the gradient method, it took less than one hour for a complete separation cycle including rinsing, equilibrating and sample purification. One of the benefits of the FEW is that the volume of each collected fraction was very small (approximately 3 mL) and concentrated.

The purity of the major component was further verified by OPLC using the same operating conditions. The purity of product 2C was verified and found to be 98.5% (Figure 3). While the chemical structures of the different products varied considerably, as did their retention times, the main fractions were isolated in good yield with a purity level greater than 96%.

The product purities and yields obtained with the OPLC PU were equivalent, if not better, than what was obtained using an HPLC RP18 column under similar conditions. More interesting however, solvent consumption with the OPLC PU was 40 times less in comparison to the HPLC method.

Conclusion

The OPLC PU was used in developing a generalized semi-preparative isolation procedure for a family of quinoxalinones. Extrapolation from 0.2 mg analytical scale to 20 mg preparative scale was performed with the same chromatographic procedure and column capacity with excellent resolution and selectivity. This was possible in part because of the flowing eluent wall that has been integrated into the purification unit. FEW technology provides greatly improved quality of the separations and allows the isolation of small volume fractions with purities exceeding 96%.

The present semi-preparative work was performed using HTSorb RP18 stationary phase with low column volume (1 mL) and relatively high-volume injections (0.2 mL). Recent laboratory work has also shown that chromatographic methods developed on HTSorb columns are easily extrapolated to high-capacity preparative flat columns. We have obtained efficient separations on 20 mL flat columns with sample injection volumes as high as 5 mL.

These demonstrations show the promise of OPLC technology for purification of crude products with high efficiency. As a final note, it is possible to increase the throughput with the PU by performing parallel separations. Studies in this direction will appear in future communications.

References