



## Low cost postcolumn derivatization for HPLC analysis

*PromoChrom Technologies Pte Ltd*

When the detection sensitivity is not satisfactory or the sample matrix is interfering with the analysis, on-line derivatization may be used to overcome the problem. On-line derivatization may be classified as precolumn and postcolumn approaches. The former can be done using an auto sampler. Although the precolumn approach has simpler configuration, it suffers more interference from sample matrix and thus is more suitable for clean samples. Besides, the application range is limited by the fact that the derivatization reaction can only occur at ambient temperature. Postcolumn derivatization is more tolerant to dirty samples and has wider application range as the reactors can be heated. The disadvantages of post-column derivatization are mainly extra cost for the hardware and more peak broadening.

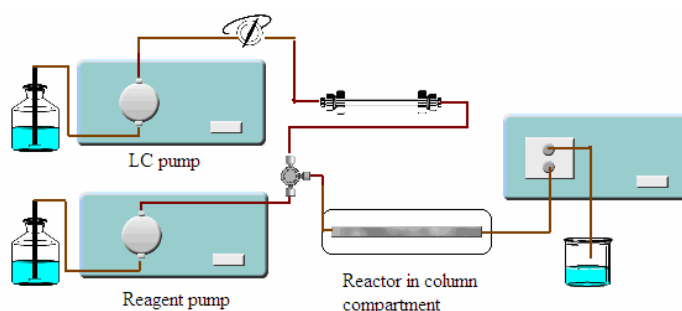
The postcolumn derivatization kit from PromoChrom Technologies reduces the cost considerably while maintaining good performance.

### Working principle

The key component of the kit is the reactor. The Teflon tubing in the reactor is knitted following a special pattern to reduce peak dispersion and to improve mixing. Figure 1 shows how significant the peak broadening can be reduced using the knitted tubing. To improve heat transfer, the tubing is knitted together with metal wire and then fixed into a metal channel using thermal conductive resin. The metal channel is of a shape and dimension that can be well fitted into a column compartment for effective heating.



The derivatization kit reduces cost by making full use of existing HPLC hardware. The following diagram shows the setup of a postcolumn derivatization HPLC system.



The reagent pump can be an HPLC pump. A narrow bore capillary is used to connect the pump to the tee joint. Another function of the tubing is to absorb the pressure pulsation from the pump. The reactor can be housed in a column compartment or a standalone column heater. Although some HPLC column compartment is limited to 80-90 degrees, it is sufficient for many applications. The kit includes another narrow bore tubing for connecting to the detector outlet.

It can be used as a pressure regulator to suppress bubble formation in the detector flow cell when the reaction temperature is very high or the degassing is not very effective. In case two reaction steps are needed for a derivatization, a second kit and a second pump may be added.

### **Advantages and benefit**

The above setting up is very suitable for a non routine post column derivatization using a modular HPLC system. For example, the Agilent 1100 HPLC software can control two to 3 pumps in one system. When there is a need for post column derivatization, an extra pump may be borrowed from another system for quick setting up. As there is no need for purchasing additional LC pump, the cost is only for the derivatization kit, which is below \$700. Compared to a dedicated derivatizer, the cost is below 10%.

### **Applications**

Below are some examples using postcolumn derivatization for HPLC analysis:

<b>Analyte</b>	<b>Derivatization reagent</b>	<b>Temperature and reactor volume</b>	<b>Literature reference</b>
Pantothenic acid in food	Orthophthaldialdehyde, 3-mercaptopropionic acid	99 °C 0.5mm x 40m	J. Chromatogr. A, 1035 (2004) 87-95 C. Pakin et al.
Carbamate pesticides	o-phthaldialdehyde, 2-mercaptoethanol (added to mobile phase)	140 °C 0.5mm x 1.4 m	J. Chromatogr. A, 778 (1997) 103-110 A. Sabala et al.
Formaldehyde in polymer	Nash reagent, dinitrophenylhydrazine	70 °C 0.5 mL	J. Chromatogr. A, 914 (2001) 123-129 J. Michels et al.
Phenothiazines	Peroxyacetic acid	30 °C 0.3mm x8m	J. Chromatogr. A, 890 (2000) 281-287 G. Diehl et al.
Sulfonamides in honey	Fluorescamine, 2-mercaptoethanol	45 °C 0.25 mm x 10 m	J. Chromatogr. A, 1047 (2004) 85-92 K. Mauden et al.
Streptomycin in honey, meat	NaOH	55 °C 10m (ID unknown)	J. Chromatogr. A, 830 (1999) 345-351 P Edder et al.
Vitamin C and derivatives	benzamidine	100 °C 1.5 mL	J. Chromatogr. A, 806 (1998) 340-344 I. Koshiishi et al.

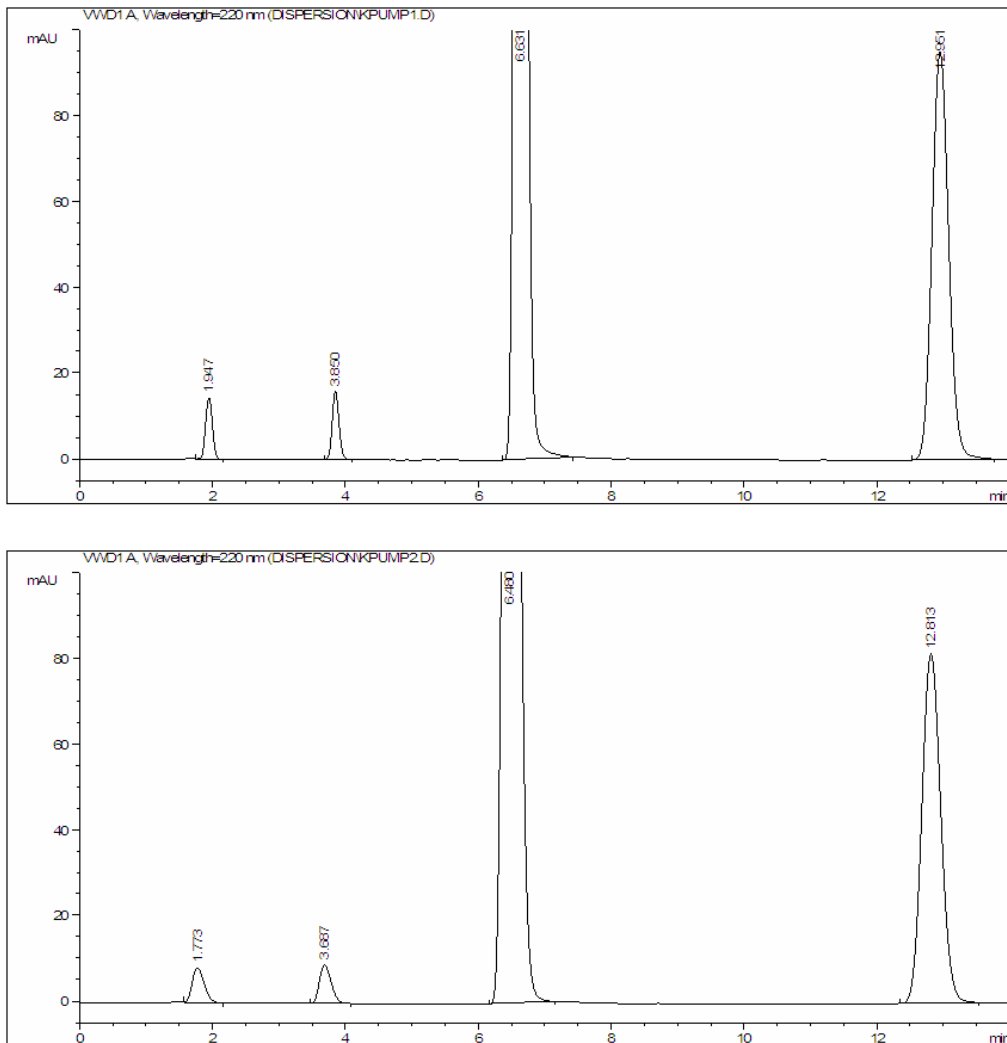


Figure 1. Effect of knitted tubing in reduction of peak broadening.

Top, use a 0.5mm x 4m reactor; bottom, replace the reactor with a 0.5mm x 3m PEEK tubing.

Analytical pump, Agilent 1100 isocratic pump at flow rate 1 mL/min; reagent pump, K-120 LC pump at flow rate 0.5 mL/min; mobile phase and liquid for reagent pump, methanol + water (6:4); detector, Agilent variable wavelength detector at 220 nm; column, XDB C18 4.6 x 150 mm; analytes, uracil 5 ppm, acetophenone 10 ppm; anisole 250 ppm, toluene 700 ppm.



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