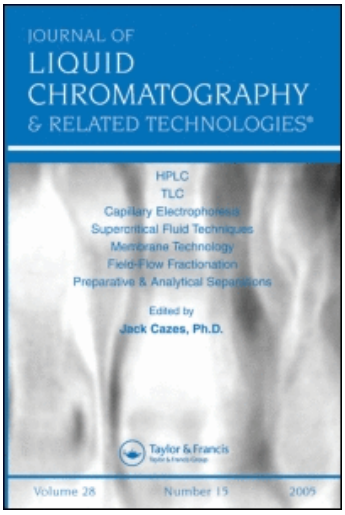

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Measurement of the Dead Volume between Concurrent Detectors in Gel Permeation Chromatography

D. Lecacheux[a]; J. Lesec[a]
[a] Laboratoire de Physico-Chimie des Polymères (C.N.R.S.-L.A. 278) Université Pierre et Marie Curie (Paris VI), Paris Cedex 05, France

To cite this Article Lecacheux, D. and Lesec, J.(1982) 'Measurement of the Dead Volume between Concurrent Detectors in Gel Permeation Chromatography', Journal of Liquid Chromatography & Related Technologies, 5: 12, 2227 — 2239
To link to this Article: DOI: 10.1080/01483918208067631
URL: http://dx.doi.org/10.1080/01483918208067631

JOURNAL OF LIQUID CHROMATOGRAPHY, 5(12), 2227-2239 (1982)

MEASUREMENT OF THE DEAD VOLUME BETWEEN CONCURRENT DETECTORS IN GEL PERMEATION CHROMATOGRAPHY

D. Lecacheux and J. Lesec

Laboratoire de Physico-Chimie des Polymères (C.N.R.S.-L.A. 278)
Université Pierre et Marie Curie (Paris VI)
E.S.P.C.I. - 10, rue Vauquelin - 75231 Paris Cedex 05 - France

ABSTRACT

Multiple detection of gel permeation chromatography effluent is going to become a routine way of polymer characterization in most laboratories. It is then necessary to give attention to the dead volume connecting the cells of concurrent detectors. If neglected, considerable errors may occur in data handling.

We report, in this paper, a method of dead volume measurement. It firstly concerns the coupling of a high pressure gel permeation chromatograph and the "Ouano"-type continuous viscometer, but its general utility is demonstrated. The main conclusion is that the geometric estimate of the dead volume is not suitable, even when an accurate calculation is possible. The method we describe here leads to an experimental dead volume. It is greater than the geometric one (as theoretically proved recently) but it gives the best results.

INTRODUCTION

With the increasing use of Gel Permeation Chromatography (GPC) as a characterization tool for complex polymeric samples, a fast development in dual detection techniques for the effluent is observed. Indeed, it has been known for a long time, except for linear homopolymers, that the size exclusion mechanism does not lead to the separation of macromolecules according to their

molecular weights. As a result, the response of a single concentration detector is often inadequate to give reliable average molecular weights. For the analysis of copolymers with variable composition, the differential refractometer (DRI)-UV detector coupling is particularly used (see the review of Janca (1)). On another hand, the Benoit's universal parameter $[\eta].M$ as a calibration concept (2) requires the fitting of GPC with a molecular size detection, i.e. viscometry (3-4) or light scattering (5-6). But till now, the two detections have never been performed in a single cell. Consequently, accurate data treatment requires the knowledge of the connecting dead volume, ΔV, between detectors so that any pair of experimental points really corresponds to the same species.

From the study of Bruessau (7), the geometric determination of the ΔV value leads to unsatisfactory results, because changes occur in the peak shape during transfer form one cell to the other. That is why this author recommends a method which is suitable to the particular case of two concentration detectors (DRI and UV for instance). According to Cantow (8), the analysis of homopolymers under such conditions gives two similar chromatograms, one of them has to be shifted till their ratio is a straight line parallel to the abscissa. The resulting ΔV value is valid for further calculations. Nevertheless, this method is presented as invalid in a concentration detector-molecular size detector coupling since there is no specific case where the two responses are proportional.

In the laboratory, we have been dealing for a few years (9) with the coupling of a high pressure GPC and an "Ouano"-type continuous viscometer (10). Such a system enables one to rapidly characterize various macromolecular samples (11), even at high temperatures, as recently proved (12). But, the accuracy of the results (especially the viscosity laws) is dependent on the ΔV value. For this reason, we have improved the above described method of ΔV measurement for the concentration detector-molecular size detector coupling.

EXPERIMENTAL

The continuous viscometric detector is an application of the Poiseuille's law. Its principle has been previously described (10-11). Assuming laminar flow through a capillary tube, we can employ the following relationship :

$$P = \frac{8}{\pi} \cdot \frac{l}{r^4} \cdot Q \cdot \eta$$

with - P : pressure drop of the fluid
- Q : flow rate
- l, r : length and radius of the capillary tube
- η : absolute viscosity.

Accordingly, the continuous pressure drop measurement (besides the concentration, C, detection) enables the determination of the intrinsic viscosity $[\eta]$ along with the elution volume (for very low C values) :

$$[\eta] = \frac{1}{C} \ln \frac{P}{P_o}$$

where subscrip, o, refers to pure solvent.

A scheme of the apparatus is given in Figure 1. A stainless steel capillary tube is inserted between the column set and the refractometer (Waters R 401). Two pressure transducers CMAC 5 (Sedeme, 11, rue Simonet - F-75013 PARIS), performing the differential measurement of the pressure drop, are connected by "T" fittings into the system. If it is necessary to select the viscometer length over a wide range, it is important to recognize that the inner diameter of the commercially available 9/1,000-inch capillary fluctuates greatly. By measuring the pressure drops through several capillaries with equal lengths, we observed

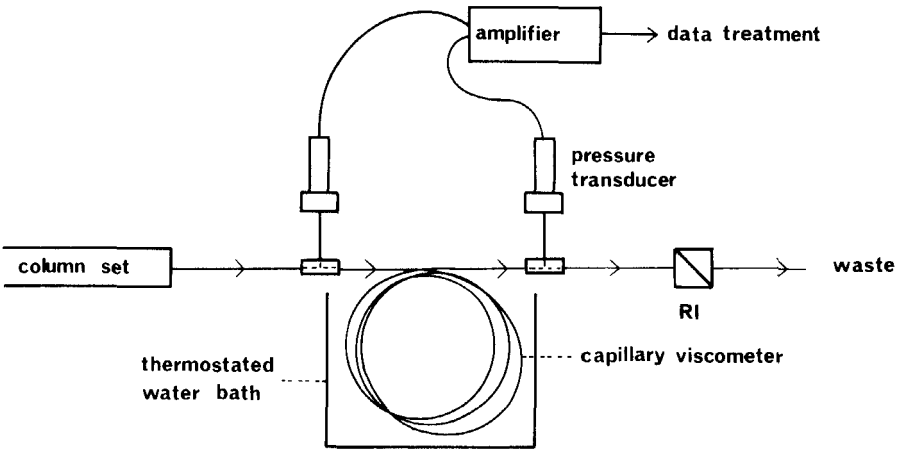

Figure 1 : Scheme of the apparatus.

large discrepancies, the ratio between the extremes being about 3. Poiseuille's law leads to the following range :

$$0.10 < r < 0.14 \text{ mm}$$

Thus, the continuous viscosmeter is required for a precise measurement of the capillary radius. If not, errors in the geometric determination of dead volumes may be as large as 100%.

For our purpose, we have chosen a capillary with r = 0.135 mm and l = 3m. The difference between the coupling of two detectors with cells of negligible volume (I) and the particular case of DRI-continuous viscometer (II) is shown in Figure 2. For the latter, the ΔV value is the sum of two terms : the dead volume of connecting capillaries and half the volume of the viscometer itself. From the Poiseuille's law, we deduced the geometric estimate :

$$\Delta V_G \simeq 0.15 \text{ ml}$$

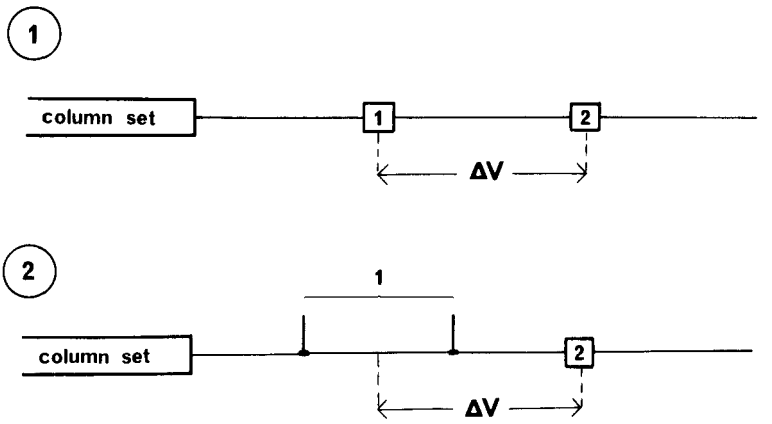

Figure 2 : Difference between the coupling of two detectors with cells of negligible volume (1) and the DRI-continuous viscometer coupling (2).

Routinely, experiments were run under the following conditions :

Solvent : THF ; Flow rate : 1ml/mn ; Temperature : 30°C.
Column set : 4 μ styragel 10^3, 10^4, 10^5 and 10^6 Å

The apparatus was equipped with a system of automatic recording, data treatment and graphic output (12) whose performances are demonstrated in Figure 3, 4 and 5 with a polyurethane sample. After smoothing of the chromatograms (Figure 3), the viscosity law was deduced from universal calibration (Figure 4) as well as the molecular weight distribution shown in Figure 5. In this case, we obtained :

$\bar{M}n$ = 37,000
$\bar{M}w$ = 58,000
$[\eta]$ = 109 ml/g
α = 0.68 (Mark-Houwink exponent)

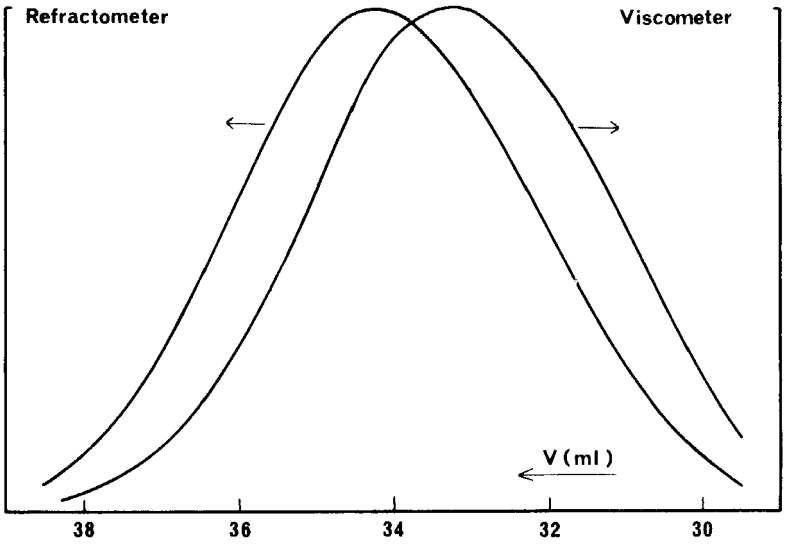

Figure 3 : Polyurethane sample : refractometric and viscometric responses.

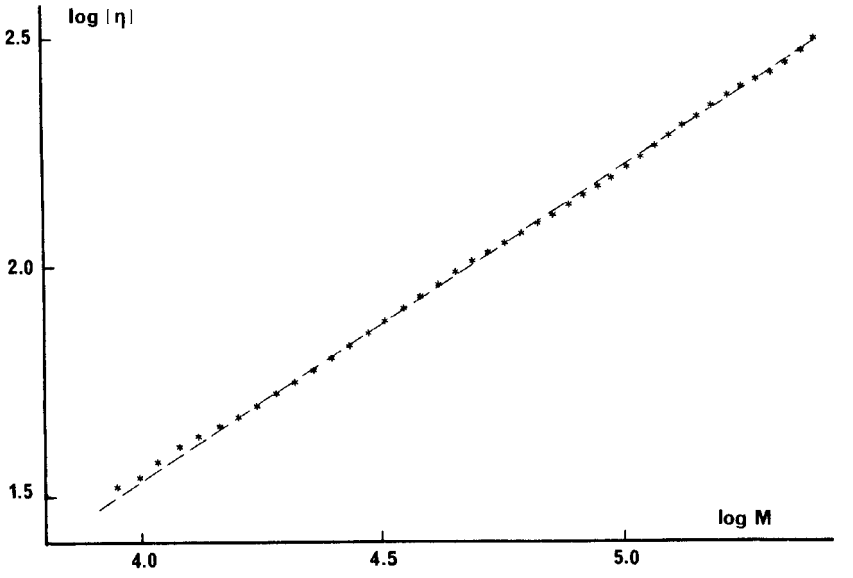

Figure 4 : The experimental Mark-Houwink relationship.

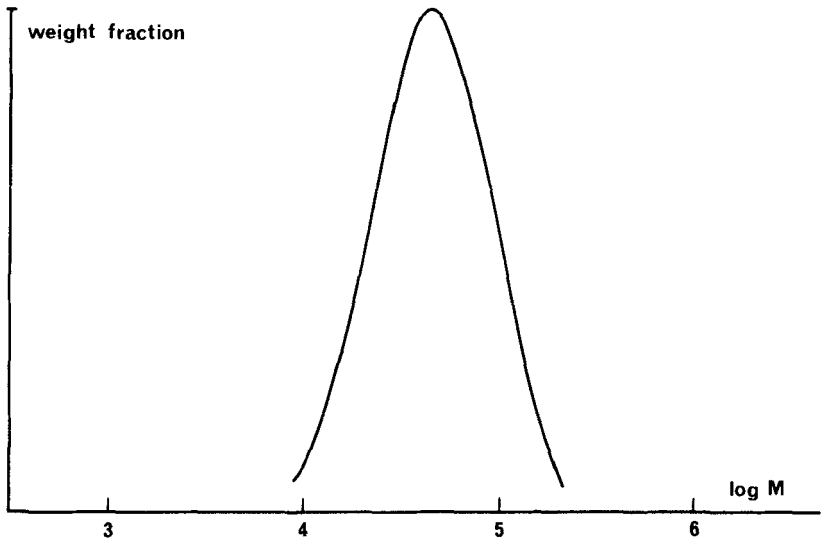

Figure 5 : The molecular weight distribution.

The above mentioned values are linked to a certain ΔV. But before describing the method which provides this ΔV value, we will demonstrate, using a simple model, that a good accuracy is required.

DISCUSSION

From a theoretical viewpoint, we will assume, a column set with a linear calibration curve of molecular weight, M, versus the elution volume, V :

$$\ln M = a - bV$$

Let us consider a macromolecular sample with a molecular weight distribution of the Wesslau type :

$$w(\ln M) = \frac{1}{\beta\sqrt{\pi}} \exp\left(-\frac{1}{\beta^2} \ln^2 \frac{M}{M_p}\right)$$

M_p is the peak molecular weight with peak elution volume V_p and β is a function of polydispersity I :

$$\beta^2 = 2 \ln I$$

The viscosity law for this sample is : $[\eta] = KM^{\alpha}$

Using the following notations :

C(V) concentration chromatogram
S(V) pressure drop chromatogram ($S = P - P_o$)

we obtain, after a few steps :

$$C(V) = Cm \cdot \exp\left(-\frac{b^2}{\beta^2}(V - V_p)^2\right)$$

$$S(V) = Sm \cdot \exp\left(-\frac{b^2}{\beta^2}\left(V - \left(V_p - \frac{\alpha \ln I}{b}\right)\right)^2\right)$$

where Cm and Sm are the respective apexes of the two chromatograms. It appears, when the concentration chromatogram has a gaussian shape, that the pressure chromatogram has also a gaussian equation. The standard deviation is the same but the peak apex of the pressure drop curve is shifted towards lower elution volumes :

$$V'_p = V_p - \frac{\alpha \ln I}{b}$$

(even if the two detections are performed in a single cell). Under routine conditions ($\alpha = 0.7$ and $b \simeq 0.4$), we obtain :

$$V_p - V'_p \simeq 1.75 \ln I$$

and, for instance $V_p - V'_p \simeq 1.2$ ml for $I = 2$.

The above mentioned example for polyurethane is not very far from the Wesslau distribution and we can observe in Figure 3 that

the experimental shift is in satisfactory agreement with the theoretical one.

It is, therefore, easy to predict the consequences of a bad estimate of ΔV : it leads to a wrong value of the expression $\frac{\alpha \ln I}{b}$. If ΔV is underestimated (as when neglected), α will be too high, and vice versa. In addition, the smaller the polydispersity index, the more important the errors become.

Such results can be experimentally confirmed with the assistance of a computer. By varying the ΔV value in the calculations, we obtained, for the polyurethane sample :

- $\Delta V = 0$: $\alpha \simeq 0.8$
- ΔV correct : $\alpha \simeq 0.7$
- $\Delta V \times 2$: $\alpha \simeq 0.6$

with the subsequent errors in the average molecular weights. Consequently, it is very important to use a reliable method of ΔV measurement.

DESCRIPTION OF THE METHOD

The method we describe is based on Cantow's method (7), but modified because of the nature of specific responses of molecular size detectors. Their responses, indeed, are not proportional to the concentration but are proportional either to C.[η] (viscometer) or C.M. (LALLS). The only way to consider them as concentration detectors is to avoid the size exclusion mechanism so as to obtain two similar signals. In our particular case (DRI-viscometer coupling), the first trials were performed by injecting a polymeric sample after removing the columns. But this method is not suitable, since polymer elution is too fast and the chromatograms are very distorted. It is therefore necessary to keep a column set in the system, but one has to select low porosity

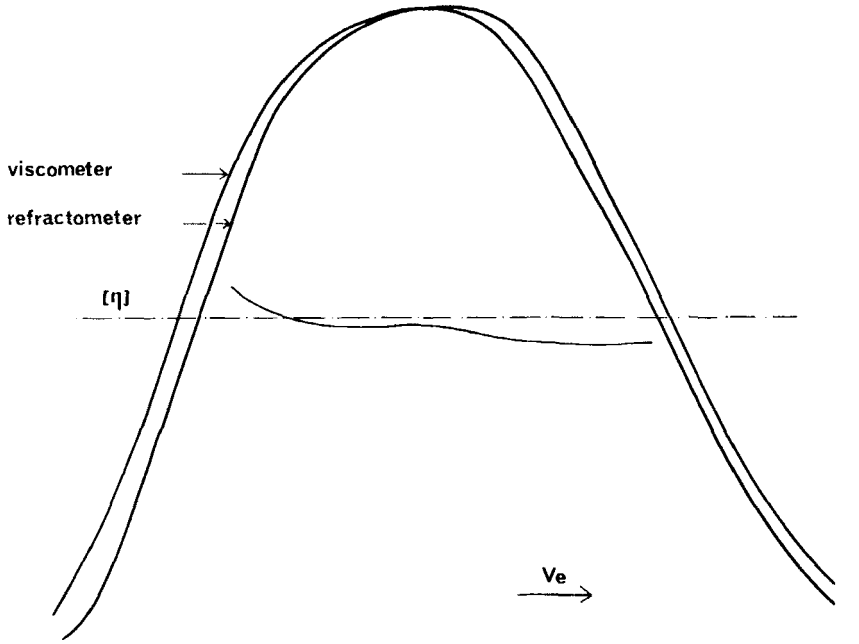

Figure 6 : Underestimated shift volume. After linear regression, [η](V) exhibits a negative slope.

packings so that a high molecular weight polymer is totally excluded and axial dispersion is the only factor responsible for spreading area. Measurements were performed under the following experimental conditions :

- Column set : 2 μ styragel (100 Å and 500 Å)
- Solvent : THF ; Flow rate : 1ml/mn ; Temperature : 30°C
- Sample : polystyrene standard $\bar{M}w \simeq 10^6$ (I < 1.1)
- Injected volume : 100μl ; Concentration 0.5%

In order to precisely measure the dead volume in our system, about twenty injections were made. All parameters were kept constant, except the shift volume (converted in terms of delay

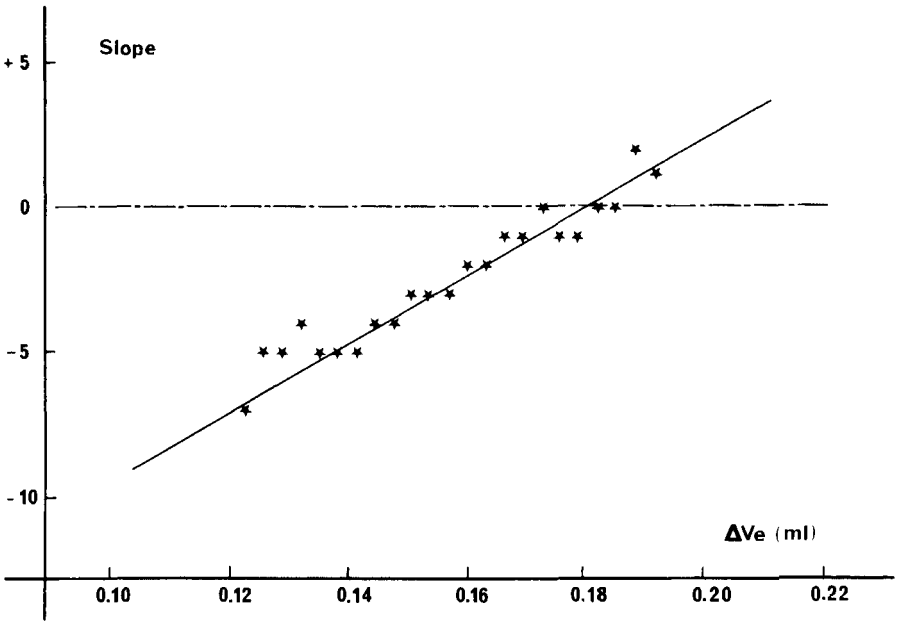

Figure 7 : The experimental shift volume is the abscissa of the intercept.

time in the automatic dual data acquisition system) which was gradually increased in the range of the geometric estimate. In each case, we plotted the apparent intrinsic viscosity $[\eta](V)=\frac{1}{C}\ln\frac{P}{P_o}$ (with the already mentioned notations) versus the elution volume (see Figure 6 as an example). The slope is calculated in arbitrary units using a linear regression method. The experimental ΔV value (ΔV_E) is defined by the zero slope : $[\eta](V)$ is constant and equal to the true intrinsic viscosity $[\eta]$. The slope measurement is relatively imprecise but a linear regression on slopes versus shift volume (shown in Figure 7) enabled us to reduce the uncertainty to a few percent. It leads to :

$$\Delta V_E = 0.18 \text{ ml}$$

This value, greater than the geometric one (ΔV_G = 0.15 ml), confirms the recent result of Bruessau (7). In addition, we compared these two values as delay time for the dual data acquisition in the case of routine experiments. The use of ΔV_E leads to correct viscometric results, when ΔV_G gives Mark-Houwink exponents α systematically overestimated. Both these reasons bring us to recommend the use of this method in preference to the geometric estimate.

CONCLUSION

The results presented in this paper clearly indicate that the shift volume between the detectors in DRI–viscometer coupling has to be carefully taken into account in the treatment of GPC data. Model simulation as well as experimental evidence confirm the need for good accuracy. Therefore, we developed an easy method of dead volume measurement, which can be used for any kind of GPC dual detection, but is especially adapted to a molecular size detector (viscometer, LALLS) coupling. The resulting value appears to lead to satisfactory results in our system. Moreover, it is in good agreement with a recent theoretical model which shows that the geometrical estimate is systematically smaller than the value to be used in the data acquisition, probably due to change in the peak shape during transfer between the two detectors.

REFERENCES

1. Janca, J., J. Liq. Chromatogr., 4 (suppl. 1), 1 (1981).
2. Grubisic, Z., Rempp, P. and Benoît, H., J. Polym. Sci., Part B, 5, 753 (1967).
3. Goedhart, D. and Opschoor, A., J. Polym. Sci., Part A-2, 8, 1227 (1970).
4. Constantin, D., Eur. Polym. J., 13, 907 (1977).
5. Millaud, B. and Strazielle, C., Makromol. Chem., 180, 441 (1979).
6. Jenkins, R. and Porter, R.S., J. Polym. Sci., Polym. Lett. Ed., 18, 743 (1980).

7. Bruessau, R., in Liquid Chromatography of Polymers and Related Materials - II, J. Cazes, ed., Marcel Dekker, Inc., New York, 1980, pp. 73-93.
8. Cantow, H.J., cited in 7.
9. Lesec, J. and Quivoron, C., Analusis, 4, 456 (1976).
10. Ouano, A.C., J. Polym. Sci., Part A-1, 10, 2169 (1972)
11. Letot, L., Lesec, J. and Quivoron, C., J. Liq. Chromatogr., 3, 427 (1980).
12. Lecacheux, D., Lesec, J. and Quivoron, C., to be published.