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When HPLC Was Young

Looking Back to the First International Symposium on Column-Liquid Chromatography, Interlaken 1973

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In 1973, chromatography was no longer a young method; in fact, it was at least 67 years old; the first paper on this technique was published 1906 by *Tswett* [1] based on a discovery made a few years before. *Tswett* used column-liquid chromatography for the separation of plant pigments, and today, we still use column-liquid chromatography which is documented by the name of the series of successful symposia, the first of them taking place in Interlaken, Switzerland, in 1973. So what about HPLC which was young in these years?

The term HPLC was not yet established in the early seventies. Column-liquid chromatography had gained growing interest which was nourished by the successful use of gas chromatography and automated amino-acid analysis with ion-exchange, methods which used instrumentation. The user could buy a 'black box' which was able to perform the separation of a mixture of compounds. Classical liquid chromatography did and does not need instrumentation, but why should it not be possible to perform this type of separation in a 'black box'? It was also clear from theoretical considerations that the separation performance could be improved by using small-diameter stationary phases. However, if the particle diameter is small, it is necessary to use a pump to get the mobile phase pressured through the chromatographic bed. A high separation performance also means that the sensitivity can be high – the separated peaks are narrow and, therefore, higher than with classical column chromatography, which means that the signal-to-noise ratio and the minimum detectable quantity of a compound is improved. Therefore, it makes sense to add another instrument to the set-up, the one which is able to 'see' the separated compounds, and which is called detector. By the end of the sixties, the first modules were commercially available. 'Modern' liquid chromatography was born. How should it be named?

In 1973, most researchers (it was not yet the time of users without research interest) used the term 'high-pressure liquid chromatography' which was sometimes abbreviated as HPLC. This term reflects the fact that technical problems had to be solved: it is not trivial to pump low flow rates in the ml/min range at pressures of 100 bars or higher without pulses and with high reproducibility. Moreover, the need to use a pump is the most obvious difference to classical open-column chromatography. Other people used the term 'high-speed liquid chromatography'. Indeed, it is possible to perform analyses much faster when small-diameter stationary phases are used, because the diffusion-controlled residence time of the sample molecules in a particle of the stationary phase is lowered. Another term was 'high-efficiency liquid chromatography', because small amounts of a sample could be analyzed in short time. The same meaning has 'high-performance liquid chromatography'. It was coined because it was a more striking interpretation of the abbreviation HPLC than the allusion to high pressure. Today, everybody is thinking of 'performance' instead of 'pressure', when HPLC is mentioned. This is perhaps that we do not have to struggle any longer with pressure, we just buy a pump which delivers a pulseless flow at 400 bars or whatever we need. However, in the proceedings of the 1973 symposium the term 'high-performance liquid chromatography' is used only in four of the 45 contributions.

In some sense, the year 1973 was pregnant with HPLC. In 1974, the most successful and leading book of *L.R. Snyder* and *J.J. Kirkland*, 'Modern Liquid Chromatography' was published [2]. For the first time, researchers and the growing number of users of the method had a compendium which explained the theory as well as the practical details and problems of the technique. Without this book, the spreading and rapid introduction of HPLC into every laboratory for quality control or clinical analysis would have been much slower. Many participants of the HPLC '91 symposium in Basel will join me in my grateful statement that 'Modern Liquid Chromatography' was of inestimable value when I started working with HPLC. In 1979, the second edition was published; unfortunately it was the last one.

Because high-pressure liquid chromatography was all in the air, time had come to organize a symposium dedicated to this technique. The 'First International Symposium on Column-Liquid Chromatography' was held from May 2 to 4, 1973, in Interlaken, a famous alpine resort in Switzerland. It was organized by the *Association of Swiss Chemists* (*Schweizerischer Chemiker-Verband*), the members of the scientific committee were *G. Guiochon* (Paris), *J.F.K. Huber* (Amsterdam), and *W. Simon* (Zürich). Today, the scientific committee is much larger, but these three persons are still members of it. 450 participants from 22 countries came to Interlaken to hear and discuss 55 lectures (of 59 scheduled ones). This is more or less the same number of oral contributions as will be presented in Basel this summer, but the amount of scientific information will be much higher and much more difficult to overview. In Interlaken, no posters were presented, whereas today this type of contribution outnumbers the lectures.

In *Chimia*, the journal of the *Association of Swiss Chemists*, a preview with the scientific program was published [3] as well as a report on the meeting (in German) [4]. The proceedings were published in *J. Chromatogr.* [5]. As already mentioned, 45 of the 55 contributions can be found in this special volume. They are grouped into five sections: phase systems, column design, optimization, instrument design, new applications.

Today, a typical HPLC separation is performed in a column of 4.6-mm inner diameter and 10-cm length, the stationary phase has a particle diameter of 5 µm and consists of a chemically bonded phase (often with a very special surface chemistry), and the separation is carried out with gradient elution. A chromatographer of the year 1973 was not familiar with this approach at all. When browsing through the proceedings of the Interlaken symposium, we realize that stationary phases with a particle diameter of less than 10 µm represented the frontier of the research. Typical sizes were 37–44 µm or 56–90 µm, and porous layer beads (PLB's, impermeable glass beads with a superficial chromatographically active layer) were used frequently. Because of the low number of theoretical plates per unit length obtained when using coarse particles, column lengths of 1 m or even more were not uncommon. *Majors* and *MacDonald* were discussing the pro's and con's of PLB and small-particle

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totally porous packings [6], and from the today's point of view, it becomes clear why the PLB's disappeared. The main obstacle for the introduction of small particles was the difficulty to pack them into columns; the balanced-density slurry packing method was not published before 1972 [7]. In 1973, packed columns were not commercially available; therefore, the chromatographers had to pack their own columns, and this was easier with coarse particles (including PLB's) than with fine ones. The situation in these years is characterized in the statement: 'In order to obtain optimal results, we are now in the process of changing the column supports from 30 μm particles to 10 μm or smaller. The results obtained so far are encouraging' [8]. A similar situation was with instrumentation: most groups which presented their results in Interlaken used a home-made chromatograph or bought a pump and a detector and assembled it to an instrument with the help of the machinery shop. The 'Poor Man's Liquid Chromatograph' of *J. Schreiber* which was used in the seventies in many laboratories of Switzerland, including ours, was also presented at Interlaken, but it had been published earlier [9]; with the exception of the pump (a very inconvenient *Orlita* membrane pump whose flow rate was depending on the back pressure of the system) and the recorder everything was home-made.

In 1973, the number of stationary phases was very limited in comparison with what we can choose from today. A lot of research was done in bonding chemistry, including approaches which proved to be less useful, as *e.g.* the ≡Si–N= bonding type [10]. Now, we know that these phases are not stable enough against hydrolysis, and ≡Si–O–Si–C≡ type phases, *e.g.* [11], are preferred. Of course, there were no special phases which today are a matter of course: phases for the separation of carbohydrates, of amino acids, of biopolymers, of enantiomers; phases with restricted surface accessibility for certain types of molecules, especially proteins; phases for affinity chromatography. A method to obtain a phase system with special selectivity is liquid-liquid partition chromatography which was used frequently. Perhaps this approach, where the stationary phase consists of a liquid film coated on a porous support, is forgotten now wrongly, but there is no question that the handling of a liquid-liquid system is more demanding than the use of chemically bonded phases. Spherical particles, prepared in emulsion, were brand-new and not yet commercially available [12][13].

It is also astonishing that gradient elution was used only in five of the 45 papers (there is also one paper where a temperature gradient was used [14]). The striking success of gradient elution was not possible before the widespread introduction of the *C-8* and *C-18* bonded stationary phases (and *vice versa*: these 'reversed' phases are so popular now, because they allow to separate 'everything' with a gradient from H_2O to MeCN or MeOH). In 1973, the reversed phases were only one type of phase among a growing number of other bonded phases, most of them of research interest only.

As today, in most applications a UV detector was used. A rapid-scanning detector with a movable light source was presented in Interlaken [15]; it was able to scan up to 100 spectra per second. It was not before the late seventies that rapid-scanning UV/VIS detectors were commercially available, but what is used today was not introduced before 1982 [16]: the diode array detector allows to obtain information over the whole wavelength range simultaneously and has no movable parts. Also the coupling of liquid chromatography and mass spectroscopy was already done in 1973, but it was not what we are able to do today: the 'coupling' was off-line by collecting fractions, drying them, and introducing them into the mass spectrometer [17]. Spectra (with few fragments) of steroids could be obtained by the field-desorption technique. An interesting application was the fluorescence detection of amino acids after post-column derivatization with *o*-phthaldialdehyde (OPA) [18], only two years after the publication of this reaction as a (non-chromatographic) method for the trace analysis of amino acids [19]. From the today's point of view, the reaction time was long, namely 5 min, but this was no problem, because the separation of 16 amino acids took almost 3 h anyway. In the meantime, the OPA reagent has been improved and the reaction time is now in the range of 10 s.

A really pioneering approach was the use of supercritical fluid chromatography (SFC) with CO_2 as mobile phase [20]. It was not the first application of this technique and not the last one, of course, but the boom of SFC (if it was and is one) was not before the mid-eighties.

From this last example, we can see that it is necessary to present at a symposium also methods and applications which are not in fashion. New developments are not announced in the news, but they are growing in the heads of innovative people in some laboratory, perhaps at a place nobody expects. Fortunately, the series of International Symposia on Column-Liquid Chromatography has always been a forum of open and fruitful discussion. I am convinced that this will also be the case in Basel in June 1991.

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[1] M. Tswett, *Ber. Dtsch. Bot. Ges.* **1906**, *24*, 384.
[2] L.R. Snyder, J.J. Kirkland, 'Modern Liquid Chromatography', John Wiley & Sons, New York, 1974.
[3] *Chimia* **1973**, *27*, 195.
[4] J. Schreiber, K. Schellenberg, P. Schauwecker, *Chimia* **1973**, *27*, 391.
[5] *J. Chromatogr.* **1973**, *83*.
[6] R.E. Majors, F.R. MacDonald, *J. Chromatogr.* **1973**, *83*, 169.
[7] R.E. Majors, *Anal. Chem.* **1972**, *44*, 1722.
[8] M. Vecchi, J. Vesely, G. Oesterhelt, *J. Chromatogr.* **1973**, *83*, 447.
[9] J. Schreiber, *Chimia* **1971**, *25*, 405.
[10] O.E. Brust, I. Sebestian, I. Halász, *J. Chromatogr.* **1973**, *83*, 15.
[11] M. Novotny, S.L. Bektesh, K. Grohmann, *J. Chromatogr.* **1973**, *83*, 25.
[12] K. Unger, J. Schick-Kalb, K.F. Krebs, *J. Chromatogr.* **1973**, *83*, 5.
[13] J.J. Kirkland, *J. Chromatogr.* **1973**, *83*, 149.
[14] C.D. Scott, N.E. Lee, *J. Chromatogr.* **1973**, *83*, 383.
[15] A. Bylina, D. Sybilska, Z.R. Grabowski, J. Koszewski, *J. Chromatogr.* **1973**, *83*, 357.
[16] S.A. George, A. Maute, *Chromatographia* **1982**, *15*, 419.
[17] H.R. Schulten, H.D. Beckey, *J. Chromatogr.* **1973**, *83*, 315.
[18] M. Roth, A. Hampaï, *J. Chromatogr.* **1973**, *83*, 353.
[19] M. Roth, *Anal. Chem.* **1971**, *43*, 880.
[20] D. Bartmann, G.M. Schneider, *J. Chromatogr.* **1973**, *83*, 135.