

Laboratory Chromatography Guide



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“Laboratory Chromatography Guide” – A close look at preparative liquid chromatography

The present “Laboratory Chromatography Guide” is dedicated to preparative liquid chromatography, a common purification technique in most chemical or life science laboratories.

The performance of separations by chromatography is fairly well known in the scientific and industrial communities. Part 1, the “Flash Guide Basics”, gives consideration to this fact, proceeding swiftly through flash chromatography with an emphasis on speed, reliability and reproducibility of the separation.

But there are no rules without exceptions! As usual, problems appear with the most exciting and valuable compounds you want to purify. Therefore, you are personally challenged to understand and solve the purification task as fast as possible. The second part “Preparative Column Chromatography: Theory and Practice” helps you to overcome such drawbacks and leads you back to the shining path of your privileged profession: to understand and explore what modern science offers!

We at Büchi, as a leading supplier of high quality laboratory products and responsive services, wish you a lot of challenging and successful work!

Dr. Ernst Freydl
Büchi Labortechnik AG

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Abbreviations

TLC	Thin-layer chromatography
HPLC	High-performance liquid chromatography
$[C]_{\text{phase 1}}$	Concentration of the compound C in phase 1
GC	Gas chromatography
RPC	Reversed phase chromatography
S_i	Solvent strength
RI	Refractive index
S.I.	Symmetry index
F_m	Delivery rate
V_0	Dead volume
GLP	Good laboratory practice
MPLC	Medium pressure liquid chromatography
LC	Liquid chromatography
UV	Ultraviolet

Introduction

Chromatography has developed very rapidly over the past few years. It was a very long way from the first “capillary pictures” of Runge (1822–1850) through the early work of Tswett, the discoverer of Adsorption Chromatography (1903, separation of plant pigments) to modern HPLC from about 1967. Tswett had in fact adopted the name “Chromatography” for this separation technique (from the Greek chromos = colors, graphein = write).

However, the focal point of this enormous development was clearly in the area of analysis. In preparative chemistry, on the other hand, chromatographic separations are frequently carried out even today by a very simple method, i.e. with the aid of a simple glass column under hydrostatic pressure. The first publications on preparative chromatography under elevated pressure, so-called Flash Chromatography, only appeared towards the end of the seventies. This method too was subsequently further refined. This finally resulted in medium pressure liquid chromatography (called MPLC in the following), which is very efficient but nevertheless readily comprehensible and simple to carry out. At the same time, attempts were made to increase the size of the analytical HPLC systems and thus make them available also for preparative or at least semi-preparative work.

However, closer scrutiny reveals substantial differences between routine analysis and preparative separation. It is therefore essential for a preparative MPLC system to meet the specific requirements for such separations. The following factors must be noted in particular:

- Flexibility in the choice of column. The amount of substance and the required separating power differ for virtually every problem to be solved. Simple and economical adaptation to the particular separation problem must therefore be possible.
- High delivery of the pump. Large columns require large volume flows so that the desired linear flow rate can be achieved.
- Wide pressure range. The trend in preparative chromatography is clearly towards fine-grained adsorbents, which offer substantial resistance to flow.
- The apparatus must be simple to handle. In particular, filling and emptying of the columns as well as operation of the entire remaining system must be capable of being mastered immediately without a prolonged familiarization period. In the preparative laboratory, the liquid chromatography is in general not a specialized unit but rather a universal tool.

This booklet aims to provide both non-specialists and specialists with short and basic as well as with more detailed explanations of the different procedure steps encountered during a liquid chromatography separation.

The first part, “Quick Guide”, is a short, practice-oriented overview of liquid chromatography (LC) for quick reference searches and the second part provides a broader and deeper description of the process, under both practical and theoretical considerations.