Journal of Analytical & Bioanalytical Techniques

Review Article Open Access

Past, Present and Future in Two-Dimensional Liquid Chromatography: A Comprehensive Review

Dr. Uma Shankar MS* and Praveen Basappa

Department of Pharmaceutics, SRM University, Kattankulathur, Chengalpattu District, Tamil Nadu – 603203, India

Abstract

The purpose of this overview is to illustrate the history of 2D liquid chromatography, as well as the concepts that underpin it and the ongoing questions it faces. In the qualification and quantification of closely eluting analytes, two-dimensional liquid chromatography is an analytical tool. Many approaches, such as heart cutting technology, have transformed analyte selection in complicated biological mixtures. Multiple heart-cutting, which uses multiple sampling loops, provides multiple peak samples from the first-dimensional analysis without jeopardizing the temporary overlap of second-dimensional analysis.

Keywords: 2D LC; Multidimensional liquid chromatography; Heart cutting technology; Biological mixtures

Introduction

For the separation of analytes, a traditional separation involves a uni-dimensional column with isocratic or gradient elution mode. The first dimension (1D) column and the second dimension (2D) column in two-dimensional liquid chromatography have distinct selectivity from one other. These methods have shown to be beneficial to individual one-dimensional approaches in terms of resolving power, allowing for the thorough isolation of complex biological mixtures with exceptional resolution and reproducibility [1-3]. Different dimensions are possible, in addition to two-dimensional liquid chromatography, where LC is the first dimension and other techniques.

Various second separation dimensions:

- Supercritical fluid chromatography (LC-SFC)
- Capillary electrophoresis (LC-CE)
- Gas chromatography (LC-GC)

Multidimensional gas and liquid chromatography techniques have enhanced resolution and efficiency while also providing enough data and reproducibility to suit contemporary pharmaceutical industry needs.

The main benefit of the 2D gradient is that it allows the 2D column to focus on the sample contained in the 1D effluent. In basic components of liquid chromatography in gradient elution, it's crucial to remember that the number of components between the solvent mixing point and the column inlet is limited [4, 5]. During the piston stroke in HPLC pumps, commotions in the flow of the different solvents are noted, especially with reciprocating pump designs. It is because the change in the solvent system at the column inlet is delayed is not so unfair by itself because of which a delay can be expected.

History

In 1941, Archer J. P. Martin and Richard L. M. Synge quoted that, "for a liquid-liquid partition chromatography, the capability of a column to isolate compounds was reliant on the liquid-mobile-phase flow rate and the square of the diameter of the particles packed inside the column" [6]. Concerning theoretical plates, it is used to describe a column that can resolve mixtures. The resolution increases when the theoretical plate is lowered. This was the first time the concept of liquid chromatography was introduced, and it took more than 30 years for a theory to become a reality.

Csaba Horvath, a Hungarian-American chemical engineer who invented the first HPLC in 1973, has made significant contributions to understanding and explaining separation science processes [7]. On ion-exchangers and reversed-phase materials, he explained both ionic and hydrophobic interactions. This created a theoretical foundation for learning these procedures.

Later in 1978, F. Erni and R.W. Frei used the notion of two-dimensional column liquid chromatography in their work on the separation of senna glycosides extract by gel permeation chromatography (GPC) [8]. During their investigation, the researchers looked into different gradient or column-switching strategies, which did not always result in the expected features. The possibilities of a two-dimensional gadget like this have been investigated. It starts with a Gel Permeation material-filled column that is then connected to an injection loop device that is joined to a reversed-phase material-filled column. Individual fractions can be separated online from the primary column and then injected into the secondary system. The proportion of fractions in the primary separation is variable, and it is influenced by the injection into the second column.

The parts can be removed and then injected without much loss of resolution using this technique of pre-concentration on the secondary reversed-phase column. With a ten-hour analysis time in the primary dimension and two sample loops, a total of 1.5 mL fractions were collected and transferred to the second dimension. Because of the long collection time in sample loops, separation has been lowered. The technology for two-dimensional chromatography was created in their effort, despite its difficulties.

***Corresponding author:** Uma Shankar MS, Department of Pharmaceutics, SRM University, Kattankulathur, Chengalpattu District, Tamil Nadu – 603203, India, E-mail: umashankar@srmist.edu.in

Received: 03-Aug-2022, Manuscript No. jabt-22-75520; **Editor assigned:** 05-Aug-2022, PreQC No. jabt-22-75520(PQ); **Reviewed:** 19-Aug-2022, QC No. jabt-22-75520; **Revised:** 23-Aug-2022, Manuscript No. jabt-22-75520(R); **Published:** 30-Aug-2022, DOI: 10.4172/2155-9872.1000476

Citation: Uma Shankar MS, Basappa P (2022) Past, Present and Future in Two-Dimensional Liquid Chromatography: A Comprehensive Review. J Anal Bioanal Tech 13: 476.

Other Prominent Studies

Anthony Synge along with Archer Martin: Demonstrated Two-dimensional paper chromatographic separations of 19 amino acids extracted from potato in their Nobel Prize acceptance speech.

Michelle M. Bushey and James W. Jorgenson (1990): Comprehensive two-dimensional (2-D) liquid chromatographic separation system [9]. Using a micro bore cation exchange column operating under gradient conditions as the first dimension. The eluent from this column is filled in one of the two loops connected to an eight-port valve. Then a second pump forces loop material into a second column, a size exclusion column, which is connected to a detector (UV Detector in this experiment).

Theory

It's critical to understand the product rule before diving into the principles of elution in two-dimensional liquid chromatography [10]. Peak Capacity (nc) between the first and last eluting peaks is used to measure the resolution power of each dimension. When all peaks are equally well resolved, the largest number of peaks can be achieved.

Peak capacity may be calculated using equation if the peaks are of equal breadth.

$$nc = 1 + \frac{\text{Retention time of last peak} - \text{Retention time of first peak}}{4 \text{ peak standard deviation} \times \text{Resolution}} \quad (1)$$

In a gradient elution, the peak capacity is much higher than in an isocratic forgiven for a period of time. In iso-cratic chromatography, the phase composition remains constant throughout the runtime, but in gradient elution, the phase strength is gradually increased to provide adequate resolution [11].

As per Linear Solvent Strength Theory (LSST) of gradient elution for reversed-phase chromatography, the relation between retention times, instrumental variables, and solute parameters are shown below.

$$tR = t0 + tD + \frac{t0}{b} \times \ln\left(b \times \left(k0 - \frac{t0}{t0}\right) + 1\right) \quad (2)$$

Where, t_R = Retention time; t_0=Column dead volume; t_D=Gradient delay time

b=Gradient slope; k_0=Solute retention factor

The log of the initial retention factor (K0) is associated with retention duration in the preceding equation, whereas the correlation is linear in isocratic chromatography. This is due to the fact that the solute factor decreases during the gradient program.

When evaluating with isocratic and gradient elution, the correlation of peak width, operational factors, and solute characteristics is taken into account. For both isocratic and gradient elution's, the equations for peak standard deviations are as follows:

$$\sigma_{iso} = \frac{t_0}{\sqrt{N}}(1 + k) \quad (3)$$

$$\sigma_{grad} = \frac{t_0}{\sqrt{N}} G(p)(1 + k_e) \quad (4)$$

Where, σ = Peak standard deviation; t_0 = Column dead time; N = Column Theoretical plate k = Retention factor; G (p) = On-column zone compressor factor; k_e = Retention factor at the time the solute leaves the column

Poppe's method, which is meant to increase the plate count in isocratic elution, is one way to maximise peak capacity in gradient elution. The length of the column and its velocity are both variable. Its primary concept is to fulfill two requirements:

- Operational pressure is at the desired maximum value.
- Column dead volume time and its retention factor decides time scale

This method can also be applied to gradient elution to maximize peak capacity rather than plate count, and for timeline, gradient time should be taken into account rather than dead volume duration of the column [12].

The following are crucial aspects to remember about two-dimensional liquid chromatography:

- On a large scale, 1D separation is usually done using a longer column at low flow rates and intermediate particle size to achieve maximum peak capacity at maximum system pressure.
- As the gradient time is extended, the column dimensions and flow rate are reduced to maintain the maximum permissible pressure.
- To reduce eluent viscosity, column temperatures should be between 40°C and 60°C.
- For faster separation of 2D gradients, a tiny core-shell particles column should be used.

Types of 2D LC

There are two forms of two-dimensional liquid chromatography.

- LCxLC stands for comprehensive two-dimensional liquid chromatography.
- LC-LC chromatography (heart cutting chromatography)

All of the effluents from the 1D column are sampled into the 2D column in comprehensive two-dimensional liquid chromatography (LCxLC). Depending on how they are transferred from the 1D column to the 2D column, it can be operated in "off-line" or "online" mode [13, 14]. Fractions from the 1D column are manually gathered or collected via a fraction collector in offline mode, and then injected into the 2D column. This method is time-consuming and has significant repeatability concerns, resulting in sample contamination during transfer. Effluents from the 1D column are automated for transfer from the 1D column to the 2D column in the online mode of approach. This method is more efficient and repeatable [15-19].

Selected portions of 1D column effluent are introduced to the 2D column using heart cutting liquid chromatography (LC-LC), where a few peaks are precisely focused and a portion is collected and introduced into the 2D column. Several heart-cutting (MLC-LC) uses multiple sampling loops to provide multiple peaks samples from the first-dimensional analysis while avoiding transient interference with the second-dimensional analysis [20-25] Conceptual representation of heart cutting 2D-LC is given in figure 1. (Figure 1)

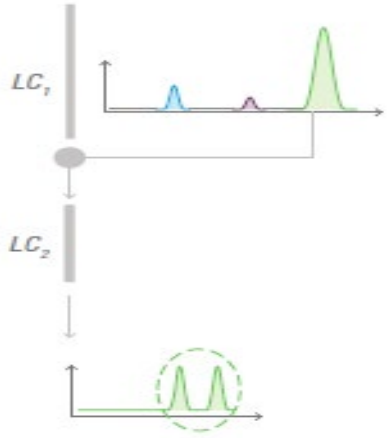

Figure 1: Heart cutting implementation in 2D-LC (Source: Agilent 2D LC primer).

Process of 2D LC

In the first dimension (1D) column, aliquots are injected. This might be an isocratic or gradient separation elution. After that, aliquots of the effluent from the 1D column are fed into a 2D column with a second dimension. This will have an effect on the sample's overall chromatographic resolution. In practice, the 2D column and associated detector function as a chemically selective system that operates on 1D column effluent. Because the selectivity of the 2D column differs from that of the 1D column, peaks that lack resolution with neighbouring peaks on a 1D column will dissociate in the 2D column. The 2D column will multiply the resolving power of the 1D column as long as there is no or minimum remixing of analytes isolated in 1D columns throughout the sampling procedure [26]. A simple representation of the concept of 2D LC is given in figure 2. (Figure 2)

Method Development by LCxLC

Though there is no explicit guideline on how to construct LCxLC methods, several publications provide an introduction [27]. Below mentioned are preliminary steps to be followed.

- **Optimization of 2D separation conditions:** The entire performance of this system benefits greatly from the speed and productivity of 2D separation. Hence, it is recommended to choose a short and narrow column (eg: <5cm & internal diameter of 2.1mm). With a column temperature of higher than 40°C, smaller particle size columns are suggested [28, 29]. The flow rate should not be so low that the gradient flushing time exceeds the gradient time. A Flow representation of the concept of two-dimensional liquid chromatography is given in figure 2. (Figure 3)

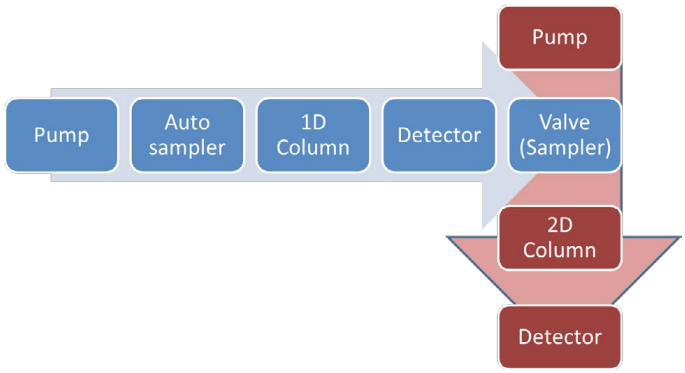

Figure 2: Concept of two-dimensional liquid chromatography.

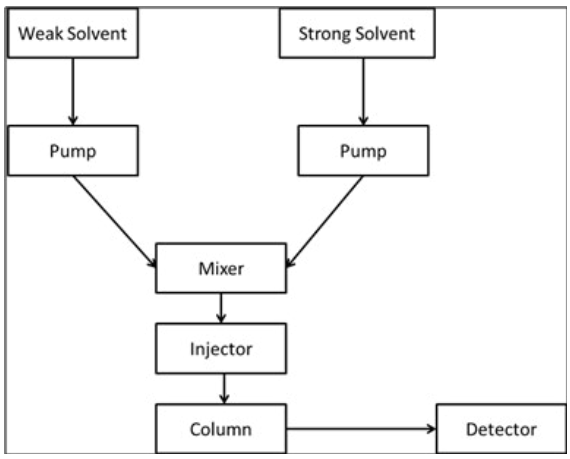

Figure 3: A Flow representation of the concept of 2D LC.

- **Selection of interface loop volume:** In LCXLC, the interface of two separation systems is termed the system's heart. The choices made here have a direct impact on the first and second dimensions, as well as their overall performance. The objective of this specific valve design is to collect 1D effluent and transfer it into a 2D column for isolating materials that are separated in the 1D column [30, 31]. The valve's design is put to the test when performing this action with precision and speed at high pressure. The heart-cut design, for example, includes two valves with two distinct positions that conduct a heart-cut of the peak of interest in the first dimension. (Figure 4)

- **Sampling loops:** Identifying the required storage volume is the product D1 flow rate and sampling time is the first step in determining the loop volume of two samples. When the 1D effluent fills the sample loop, the two-fold increased linear velocity at the tube's Centre causes sample loss when the loop volume is chosen to match the estimated one.

Storage volume = 1D flow rate x sampling time (ts) (1F)

- **Selection of sampling time:** As shown in the equation above, sample time has a significant impact on the interaction of the parameters. Injections of less than 15L into the small column have little effect on peak broadening.

- **Optimization of 1D & 2D instrument conditions:** The 1D instrument's optimization is identical to that of standard HPLC. The 2D instrument's optimization differs slightly from the 1D instrument's [32].

Basic components and their impacts are summarized in table 1. (Table 1)

- **Detection Considerations:** The detectors used in 2D LC separation are identical to those used in 1D LC separation. The speed of detection is the most important factor to consider. This detector is connected to a 2D column, which is running in a quicker mode. As

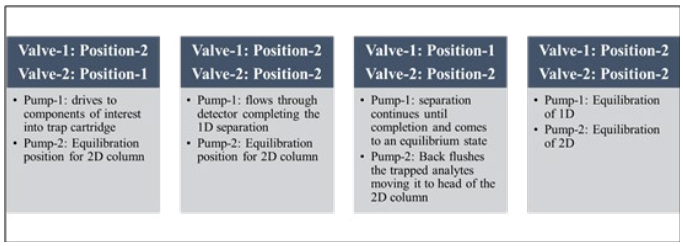

Figure 4: Representation of the loop volume.

Table 1: Basic components of 1D and 2D instrument optimization.

Parameter	Considerations for 1D	Considerations for 2D
Column Dimensions	Larger, similar or smaller diameter	Shorter with larger diameter
Pump gradient delay volume	<200uL	<200uL
Pump flow capability	Low flow capability is important to minimize sampling volume	Increased flow rate limits, this will provide more operational flexibility
Pump Pressure capability	High pressure is not required as columns are operated at their optimal velocities	< 1200bar
System dispersion	Low in terms of volume	Low in terms of volume and time
column Temperature	Higher temperatures favour lower organic content in the eluent	High temperature to be avoided for analysing degradation

a result, the detector must be efficient with very tiny temporal peaks and a greater scan rate. Mass spectrometers (MS) and UV absorbance (PDA/DAD) detectors are the most commonly utilized detectors. ESI (Electrospray ionisation) and MALDI are the most often utilized MD detectors (Matrix-assisted laser desorption ionization) In Mass Spectroscopy, signal suppression caused by the choice of mobile phase additives is a serious problem [33].

Advances & Challenges

In the last few decades, tremendous progress has been made in both hardware and software design. The pumping system's ability to endure a pressure limit of more than 20000 psi has improved as a result of the hardware changes. The delay volumes are the subject of the other enhancements. As discussed in previous chapters, there is a critical requirement to improve delay volumes. Precision under rapid elution circumstances and delay volumes ≤ 100 L are attained.

Conclusion

Finally, we'd like to emphasise that 2D LC's true potential lies not only in the quantification of certain elements or groups of analytes in a sample, but also in the characterization and separation of complex biological mixtures. When performed using multi-dimensional detectors, multi-dimensional chromatography provides more information with respect to characterization than simply providing quantitative data.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgement

The authors acknowledge Department of Pharmaceutics, SRM College of Pharmacy, for extending infrastructural support.

References

1. Stoll DR (2017) Introduction to two-dimensional liquid chromatography—theory and practice. In: Handbook of Advanced Chromatography/Mass Spectrometry Techniques. Elsevier pp 227-286.
2. Franco MS, Padovan RN, Fumes BH, Lanças FM (2016) An overview of multidimensional liquid phase separations in food analysis. Electrophoresis 37: 1768-1783.
3. Ranjbar L, Foley JP, Breadmore MC (2017) Multidimensional liquid-phase separations combining both chromatography and electrophoresis–A review. Anal Chim Acta 950: 7-31.
4. Venkatramani CJ, Zelechonok Y (2003) An automated orthogonal two-dimensional liquid chromatograph. Anal Chem 75: 3484–3494.
5. Stoll DR (2015) Recent advances in 2D-LC for bioanalysis. Bioanalysis 7: 3125-3142.
6. Martin AJP, Synge RLM (1941) A new form of chromatogram employing two liquid phases: A theory of chromatography. 2. Application to the micro-determination of the higher monoamino-acids in proteins. Biochem J 35: 1358-1368.
7. Csaba Horváth. (1979) J Chromatogr Libr 17: 151-158.
8. Erni F, Frei RW (1978) Two-dimensional column liquid chromatographic technique for resolution of complex mixtures. J Chromatogr A 149: 561-569.
9. Bushey MM, Jorgenson JW (1990) Automated instrumentation for comprehensive two-dimensional high-performance liquid chromatography/capillary zone electrophoresis. Anal Chem 62: 978-984.
10. Karger BL, Snyder LR, Horvath C (1973) Introduction to separation science.
11. Schellinger AP, Carr PW (2006) Isocratic and gradient elution chromatography: a comparison in terms of speed, retention reproducibility and quantitation. J Chromatogr A 1109: 253-266.
12. Gilar M, Daly AE, Kele M, Neue UD, Gebler JC (2004) Implications of column peak capacity on the separation of complex peptide mixtures in single-and two-dimensional high-performance liquid chromatography. J Chromatogr A 1061: 183-192.
13. Stoll DR, Li X, Wang X, Carr PW, Porter SEG, et al. (2007) Fast, comprehensive two-dimensional liquid chromatography. J Chromatogr A 1168: 3-43.
14. Česla P, Křenková J (2017) Fraction transfer process in on□line comprehensive two□dimensional liquid□phase separations. J Sep Sci 40: 109-123.
15. Dixon SP, Pitfield ID, Perrett D (2006) Comprehensive multi□dimensional liquid chromatographic separation in biomedical and pharmaceutical analysis: a review. Biomed Chromatogr 20: 508-529.
16. Davis JM, Stoll DR (2018) Likelihood of total resolution in selective comprehensive two-dimensional liquid chromatography with parallel processing: Simulation and theory. J Chromatogr A 1537: 43-57.
17. Chen Y, Wu Y, Liu X, Li B, Hu D, et al (2019) Pulsed elution modulation for on-line comprehensive two-dimensional liquid chromatography coupling reversed phase liquid chromatography and hydrophilic interaction chromatography. J Chromatogr A 1583: 98-107.
18. Donato P, Cacciola F, Mondello L, Dugo P (2011) Comprehensive chromatographic separations in proteomics. J Chromatogr A 1218: 8777-8790.
19. Yan X, Wang LJ, Wu Z, Wu YL, Liu XX, et al. (2016) New on-line separation workflow of microbial metabolites via hyphenation of analytical and preparative comprehensive two-dimensional liquid chromatography. J Chromatogr B 1033: 1-8.
20. Pursch M, Buckenmaier S (2015) Loop-based multiple heart-cutting two-dimensional liquid chromatography for target analysis in complex matrices. Anal Chem 87: 5310-5317.
21. Ouyang Y, Zeng Y, Rong Y, Song Y, Shi L, et al. (2015) Profiling analysis of low molecular weight heparins by multiple heart-cutting two dimensional chromatography with quadruple time-of-flight mass spectrometry. Anal Chem 87: 8957-8963.
22. Zhou Y, Zhang H, Wang X, Qi D, Gu W, et al. (2019) Development of a heart-cutting supercritical fluid chromatography-high-performance liquid chromatography coupled to tandem mass spectrometry for the determination of four tobacco-specific nitrosamines in mainstream smoke. Anal Bioanal Chem 411: 2961-2969.
23. Kula M, Głód D, Krauze-Baranowska M (2016) Application of on-line and off-line heart-cutting LC in determination of secondary metabolites from the flowers of Lonicera caerulea cultivar varieties. J Pharm Biomed Anal 131: 316-326.
24. Yang Y, Zhang Y, Wei C, Li J, Sun W, et al. (2018) Silver ion chromatography for peak resolution enhancement: Application to the preparative separation of two sesquiterpenes using online heart-cutting LC-LC technique. Talanta 187: 252-258.
25. Pursch M, Lewer P, Buckenmaier S (2017) Resolving co-elution problems of components in complex mixtures by multiple heart-cutting 2D-LC. Chromatographia 80: 31-38.
26. Yang SH, Wang J, Zhang K (2017) Validation of a two-dimensional liquid chromatography method for quality control testing of pharmaceutical materials. J Chromatogr A 1492: 89-97.
27. Desmet G, Broeckhoven K (2019) Extra-column band broadening effects in contemporary liquid chromatography: Causes and solutions. TrAC Trends Anal Chem 119: 115619.
28. Hetzel T, Blaesing C, Jaeger M, Teutenberg T, Schmidt TC, et al. (2017) Characterization of peak capacity of microbore liquid chromatography columns using gradient kinetic plots. J Chromatogr A 1485: 62-69.
29. Yang P, Gao W, Zhang T, Pursch M, Luong J, et al. (2019) Two□dimensional liquid chromatography with active solvent modulation for studying monomer incorporation in copolymer dispersants. J Sep Sci 42: 2805-2815.
30. Pursch M, Wegener A, Buckenmaier S (2018) Evaluation of active solvent modulation to enhance two-dimensional liquid chromatography for target analysis in polymeric matrices. J Chromatogr A 1562: 78-86.
31. Haas CP, Biesenroth S, Buckenmaier S, Goor TVD, Tallarek U (2020) Automated generation of photochemical reaction data by transient flow experiments coupled with online HPLC analysis. React Chem Eng 5: 912-920.

32. Xu J, Zheng L, Lin L, Sun B (2018) Stop-flow reversed phase liquid chromatography× size-exclusion chromatography for separation of peptides. Anal Chim Acta 1018: 119-126.

33. Lv W, Shi X, Wang S, Xu G (2019) Multidimensional liquid chromatography-mass spectrometry for metabolomic and lipidomic analyses. TrAC Trends Anal Chem 120: 115302.