

Comprehensive Two-Dimensional Liquid Chromatograph

Nexera-e



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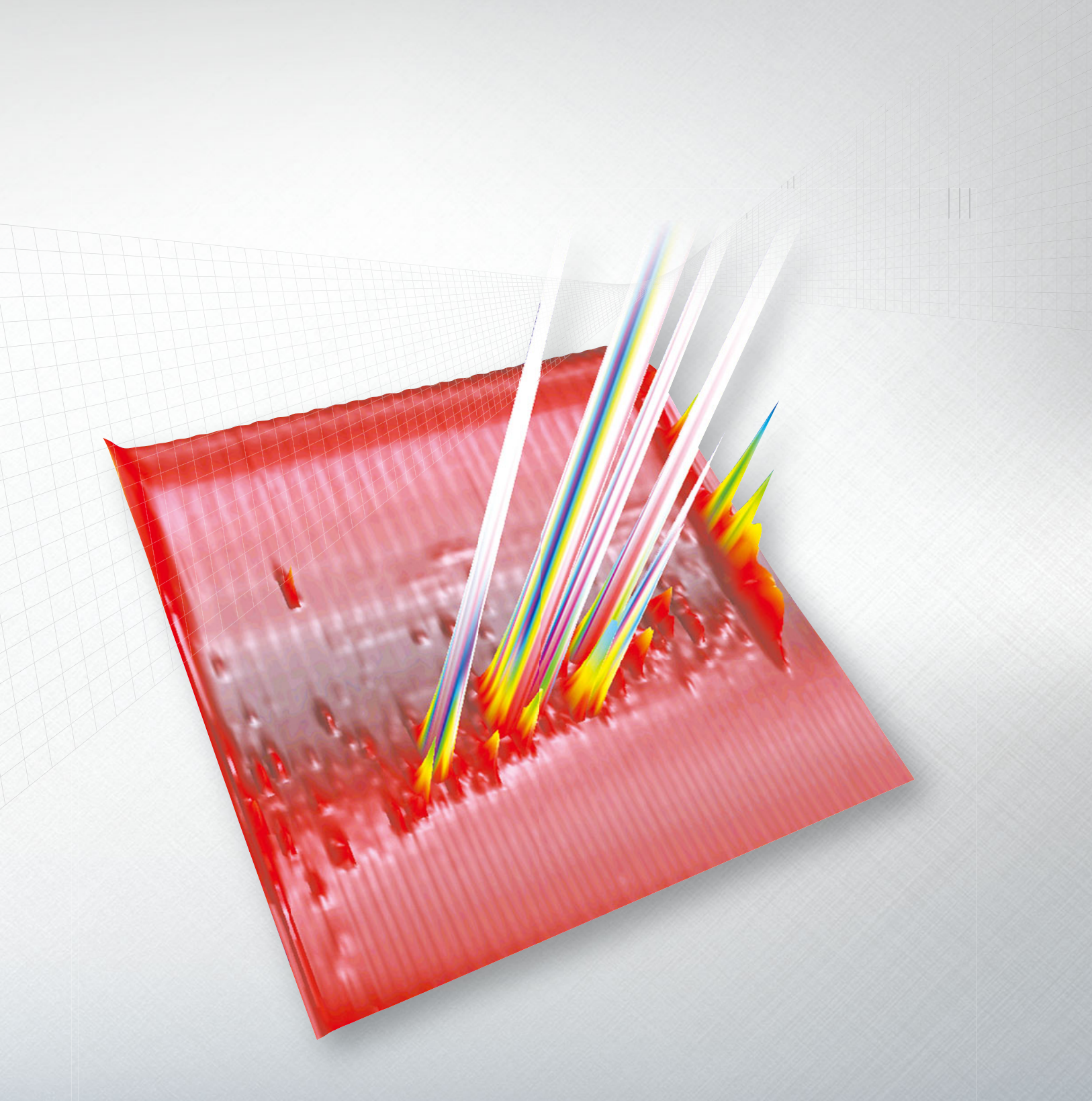
Comprehensive Two-Dimensional Liquid Chromatograph

Novel Separation Method for Complicated Sample Matrices

The comprehensive 2D-LC methodology is a paradigm shift in liquid chromatography separation analysis. By combining two independent separation modes orthogonally in combination with a dual-loop/dual-valve alternate switching design, the highest possible peak capacity is achieved for LC chromatography separation.

The new Nexera-e enables separation of even the most complex mixtures, providing a new level of knowledge and understanding of sample analytes. This is especially beneficial to the analysis of pharmaceutical impurities, proteolytic digests, food extracts, natural products and synthetic polymers.





Comprehensive Two-Dimensional Liquid Chromatograph

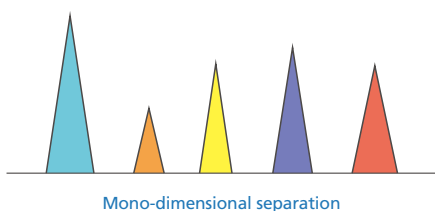
New Paradigm Shift in Liquid Chromatography

Nexera-e

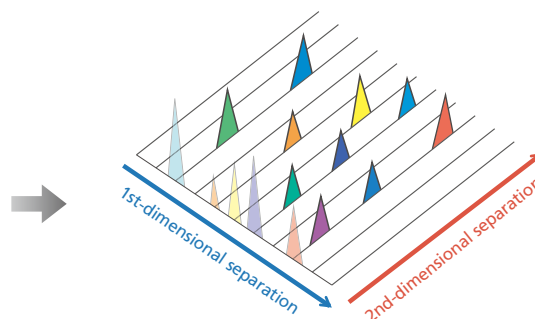
Maximizing the full potential of two-orthogonal separation systems to perform comprehensive two-dimensional separation

Comprehensive 2D-LC is a new analytical methodology combining two independent separation modes orthogonally, greatly increasing separation efficiency. The combination of different modes enables the separation of peaks that are difficult to separate using conventional LC, providing excellent results for the analysis of complex sample matrices.

Unlike other conventional two-dimensional techniques the Nexera-e provides complete separation as a result of the combination of orthogonal 1st and 2nd dimensions. Comprehensive 2D-LC fully utilizes both separation systems to achieve the ultimate separation power.



Insufficient peak separation may be observed for complicated samples.

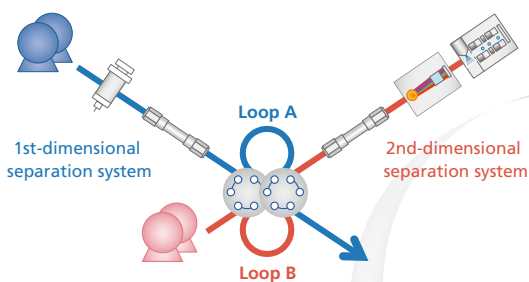


The peaks separated by the 1st-dimensional system will be isolated by the 2nd-dimensional system.

Differences between a conventional 1D-LC and a comprehensive 2D-LC

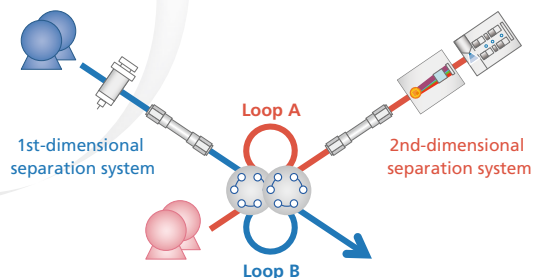
Comprehensive 2D-LC fractionates the 1st-dimensional eluent and successively separates the fraction on-line with the 2nd-dimensional system. Therefore, the 1st-dimensional system intersects with the 2nd-dimensional system at the

dual valves and dual-loops. With valve switching, these loops continue the fractionation of the eluent from the 1st-dimensional system and the injection of the fraction to the 2nd-dimensional system alternately.



The eluent from the 1st-dimensional column is loaded into Loop A. The fraction in Loop B is separated by the 2nd-dimensional column and detected.

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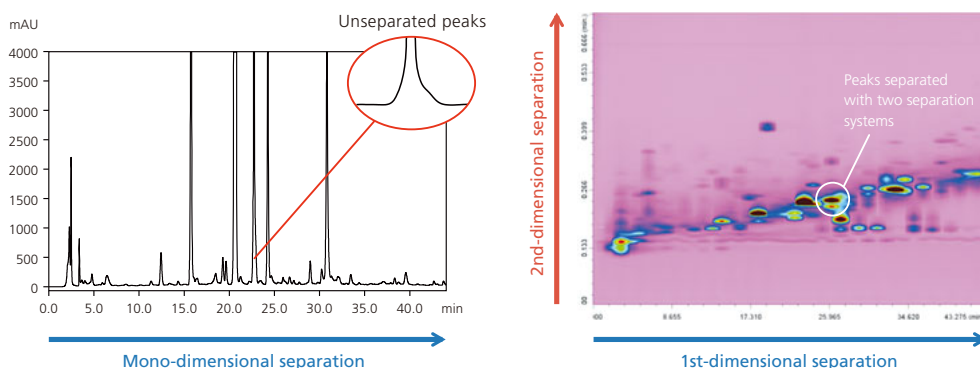


Flow line and mechanism of the comprehensive 2D-LC system

Separation far exceeding conventional 1D-LC

How many peaks are co-eluted under one peak? The more complex the sample, along with a high percentage of similar compounds, means there is a high probability of co-eluting compounds found under single peaks. Even though the

co-eluting compounds may be unable to be separated by mono-dimensional separation, the orthogonal two-dimensional system will provide the best possible result.



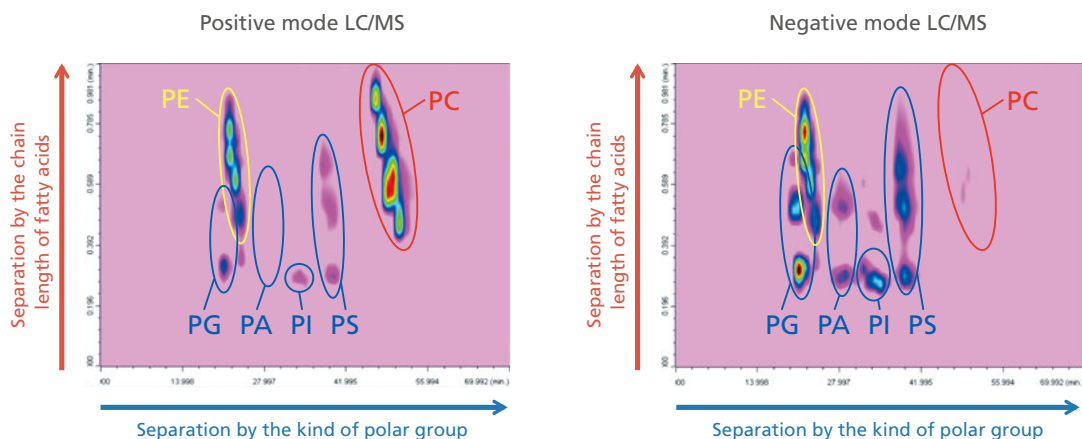
Comparison of the separation of a traditional Chinese medicine sample using Nexera-e and a conventional 1D-LC system

As shown, the figures the unseparated compounds with the mono-dimensional separation system can be separated with the two-dimensional system. Moreover, in the same analysis time, Nexera-e shows more than 200 peaks versus only 74 peaks in the conventional 1D-LC system.

Analysis of samples on a two-dimensional map

On a two-dimensional map, since each 1st-dimensional axis and 2nd-dimensional axis are set according to different separation conditions, the physical properties of compounds and their position on a map correlate.

By grouping a compound with a similar structure, it is possible to match each compound group visually or to analyze the physical properties of an unknown compound.



Simultaneous Determination of 39 Phospholipid Compounds
(PG: phosphatidylglycerol, PE: phosphatidyl ethanolamine, PA: phosphatidic acid, PI: phosphatidylinositol, PS: phosphatidylserine, PC: phosphatidylcholine)

Each phosphatide compound is separated based on its polar group (class) in a normal 1st-dimensional phase separation. And it is separated according to the chain length of fatty acids by a 2nd-dimensional reverse phase analysis. Each compound is grouped for each class and showed in the above figures. Although some components are strongly detected in the positive mode and others in the negative mode, it is possible to acquire both data in one analysis utilizing the Ultra-Fast Polarity Switching [UFswitching] of the LCMS-8050 LC/MS/MS.

Nexera-e Components

Adoption of Nexera X2 components offering ultimate performance

Comprehensive 2D-LC requires excellent performance from each component. For example, the 2nd-dimensional separation system requires ultra-first gradient analysis with a high flow rate and ultra-high pressure. Moreover, in order to analyze differences between samples, excellent retention time reproducibility is required under such analytical conditions. Nexera-e is designed to meet and exceed such exacting

requirements with its high-performance Nexera X2 components. For example, the solvent delivery unit, LC-30AD, provides solvent delivery at a flow rate up to 3 mL/min with a pressure limit of 130 MPa. In addition, the ultra-high-pressure switching valve, FCV-32AH, has a pressure limit of 130 MPa. Such excellent performance expands the range of selection of 2nd-dimensional analytical conditions.



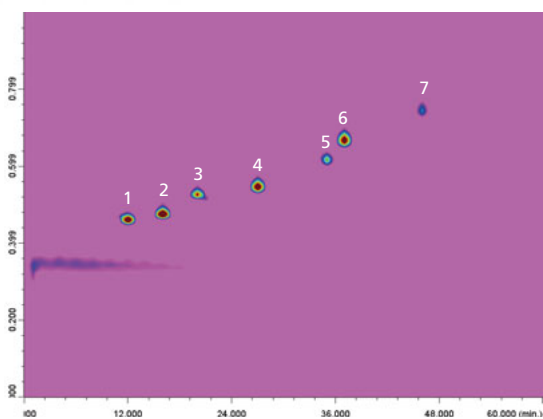
Nexera-e (PDA Model)



The Nexera-e incorporates a Shimadzu designed low-volume switching valve. Comprehensive 2D-LC requires frequent valve switching. Some valve systems may exhibit clogging issues due to abrasion powders. Using the robust technology developed for the Nexera X2 autosampler, this potential problem has been significantly minimized.



Ultra-high speed analysis of comprehensive 2D-LC also requires ultra-high-speed data sampling. The Nexera X2 photo-diode array detector, SPD-M30A, has a high sampling rate up to 200 Hz, while the LCMS-8050 triple quadrupole liquid chromatograph mass spectrometer features ultra-high speed data scanning by employing UF-MS technologies to enable superior data acquisition.



| No. | Compound | Reproducibility of retention time in the second-dimension [%RSD] |
|-----|----------------|--|
| 1 | Acetanilide | 0.43 |
| 2 | Methyl paraben | 0.37 |
| 3 | Acetophenone | 0.66 |
| 4 | Propyl paraben | 0.43 |
| 5 | Butyrophenone | 0.42 |
| 6 | Benzophenone | 0.35 |
| 7 | Hexanophenone | 0.43 |

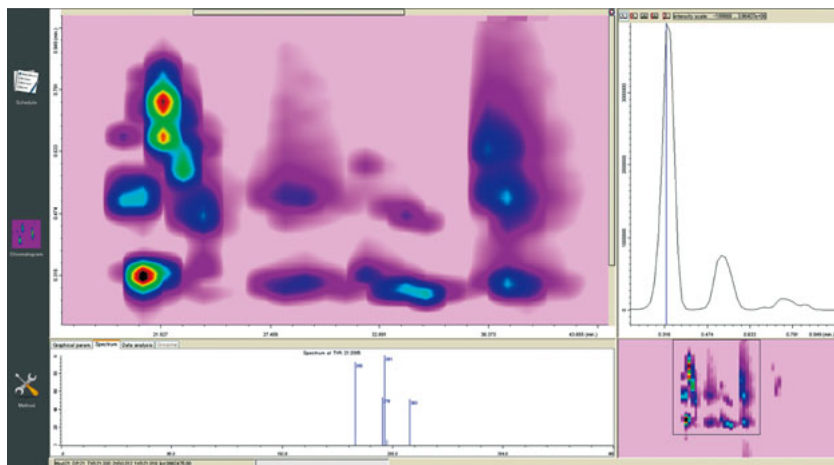
Retention time reproducibility of standard compounds analysis (n=6)

(1: Acetanilide, 2: Methyl paraben, 3: Acetophenone, 4: Propyl paraben, 5: Butyrophenone, 6: Benzophenone, 7: Hexanophenone)

ChromSquare, the data analysis software for 2D-LC

2D Qualitative & Quantitative analysis using Contour Graphics

Acquired data is converted to two-dimensional contour plotting using ChromSquare, the software for comprehensive 2D-LC analysis. A peak on chromatogram is recognized as spot on the contour plot. The qualitative and quantitative data processing are performed for the target spot.

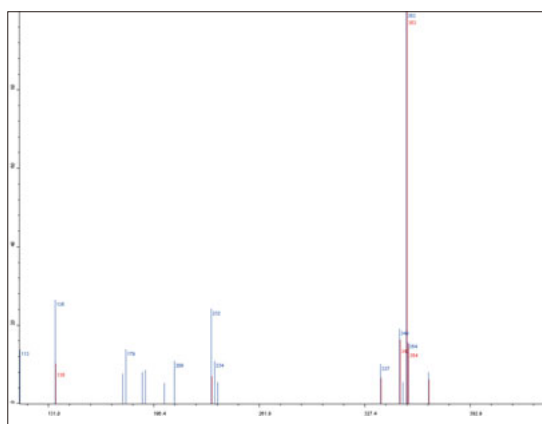


LCxLC/MS data analysis of standard phospholipid sample

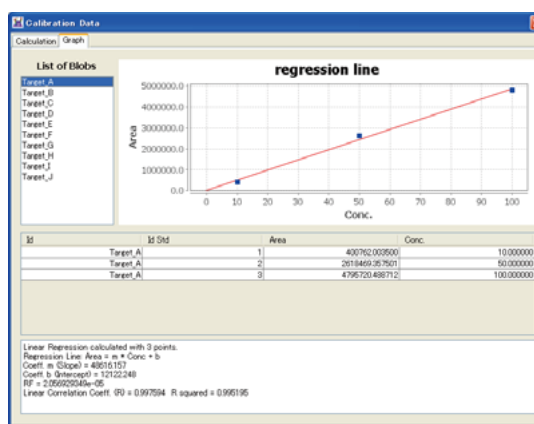
(upper left: Contour plot (magnified view); lower left: MS spectrum; upper right: Second-dimensional chromatogram; lower right: Contour plot (general view))

An MS spectrum is displayed in real time along with a mouse pointer. For example, the compound-specific spot can be easily identified based on its MS spectrum.


In addition, a calibration curve for quantitative analysis can be calculated for each compound based on the contour spot.



MS spectrum of the entire contour spot area (red lines) and MS spectrum of individual data points (blue lines)



Calibration curve

ChromSquare is a product of Chromaleont S.r.l., Italy. 



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