A POWERFUL ANALYTICAL TECHNIQUE IN PHARMACEUTICAL DRUG DISCOVERY: RAPID RESOLUTION LIUID CHROMATOGRAPHY (RRLC)

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Abstract: This review article should focus on the Rapid Resolution Liquid Chromatography (RRLC) has become an increasingly useful approach to achieve higher throughput, improve sensitivity and reduce costs.The potential of high−speed analyses by Rapid Resolution Liquid Chromatography (RRLC) on 1.8 μm porous particles packed into short columns operated at high flow rate was investigated and compared to the performance of 5 μm porous par-ticles packed into conventional colum. In order to display the practicality of RRLC separations, the isocratic analysis of pesticides and the gradient analysis. Often higher temperatures are employed to minimize system back-pressure. With the widespread adoption of RRLC comes the question of HPLC detector compatibility. The analysis time was reduced by a factor of 15, compared to the conventional method

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INTRODUCTION

Chromatography is an effective analytical method for separating, identifying, and determining the chemical components of complex mixtures. The Russian botanist Mikhail Tswett demonstrated it for the first time in 1906 when he separated different plant pigments by passing an extract of the leaves through a glass column packed with calcium carbonate. "chromatography" is derived from the Greek words chroma, which means "colour," and graphein, which means "to write." Utilized for separation of a mixture of components based on their distribution coefficients between two phases: stationary and mobile. The mobile phase is a liquid made up of a solvent or a solvent mixture, while the stationary phase is solid support. The ability of chromatography to separate a mixture of compounds, or "analytes," and determine their identity (chemical structure) and concentration is what gives it its power."Gas", "liquid", and "supercritical fluid chromatography" are the three basic types of chromatography. Liquid chromatography can be further subdivided into ion exchange, size separations, and even gel-based electrophoretic techniques.[1-3].

Liquid chromatography is the process of taking a sample and injecting it into an instrument, then separating the mixture of compounds into individual peaks using a liquid solvent. Individual bands or peaks exit the column, and a relatively universal detector detects them. Mass spectrometry (MS) is a popular detector for both gas and liquid chromatography[4,5])

High-performance liquid chromatography (HPLC) refers to LC techniques that employ small, uniform, rigid supports (<40 μm in theory, but usually 3-10μm in practice). Because of the use of such a support, "system efficiencies" and "plate heights" are improved. There are generally two phases in HPLC these include the Normal and the Reverse phase through which separation takes place. In the normal phase, the stationary phase is polar and the mobile phase is non-polar whereas, in the reverse phase its vice-versa hence hydrophobic separation takes place. RP-HPLC is typically the LC technique of choice for analytical applications as most of the drugs in the pharmaceutical industry are non-polar and utilized due to their fast analysis times, good detection limits, and ease of automation. These qualities make it popular in purification work as well.RP-HPLC Chromatogram has the following consequences:(a) narrow peaks with good detection limits; (b) short separation times; and (c) high operating pressures and fast flow rates[6-8].

Separation resolution and analysis time have steadily improved in High-Performance Liquid Chromatography over the years (HPLC). Column efficiency must be increased for further advancement. This and other systematic investigations resulted in high throughput and high resolution. The primary benefits of high throughput HPLC are increased throughput and lower analysis costs. The use of a shorter column length reduces analysis time. A shorter column, on the other hand, may result in a loss of theoretical plates, resulting in a decrease in chromatographic resolution. To compensate for the potential loss of resolution, "smaller particle size" has resulted in more efficient columns. In 1997, ultra-high pressure liquid chromatography with a capillary column packed with 11.5 m nonporous silica particles was introduced. Since then, HPLC with smaller particles has grown in popularity and a new technique known as RRLCwas introduced which provides these respective specifications for even greater resolution &is gaining attention for various reasons as well.[9]

RRLC refers to Rapid Resolution LiquidChromatography which refers to the technique of using small particles (sub 2 microns) packed into short columns running at a high flow rate. However, such a quick analysis technique is not limited to the pharmaceutical industry. In this study, complete particulars of the RRLC system are provided which applies to a broader field of chemical compound analysis.

The new RRLC technology allows for significant reductions in analysis time while maintaining chromatographic resolution. In addition, using the same approach, gradient elution RRLC was used to analyze nutritional components (catechins) in green tea. Some studies have demonstrated the potential of RRLC on 1.8 m porous particles packed (into short columns operating at a high flow rate) in comparison to the performance of conventional 5 m porous particles packed columns.

RECENT TRENDS IN HPLC CHROMATOGRAPHIC TECHNIQUES

When compared to traditional methods, HPLC is distinguished by Rapid Resolution Liquid Chromatography (RRLC), Ultra Performance Liquid Chromatography (UPLC), Ultra-Fast Liquid Chromatography (UFLC) and (Nano-LC) Liquid chromatography at the nanoscale.

RAPID-RESOLUTION LIQUID CHROMATOGRAPHY CHARACTERISTICS

SERIES 1200 RRLC AGILENT: The 1200 Rapid Resolution LC (RRLC) system was designed to provide LC with ultrafast and highest analysis speed, as well as highly improved sensitivity and pressure while retaining full functionality for standard HPLC applications. It is the world's most rapid, efficient, and adaptable LC system. It has grown in popularity as a means of increasing throughput, improving sensitivity, and lowering costs. The RRLC system enables faster analysis (up to 20x faster than conventional HPLC). This is accomplished by employing sub-2-micron (STM) column particles, which necessitates the use of additional pressure to drive the mobile phase through the column at high flow rates or for long columns.[10-14]

The RRLC flow path is optimized to produce minimal backpressure, and ZORBAX RRHT columns have a particle size distribution that is engineered to produce significantly less backpressure than other STM columns. High temperatures of up to 100°C on certain columns allow for greater selectivity flexibility and lower solvent viscosity, allowing for even faster separation. High flow rates of up to 5ml/min can be used for ultrafast separations. The adjustable delay volume filly can accommodate columns with diameters ranging from 2.1 to 4.6 mm. A low-dispersion tubing kit and low-volume flow cells for narrow bore columns reduce peak dispersion. High data rate detectors keep the resolution of very fast RRLC peaks.

Figure 1. RRLC Instrumentation

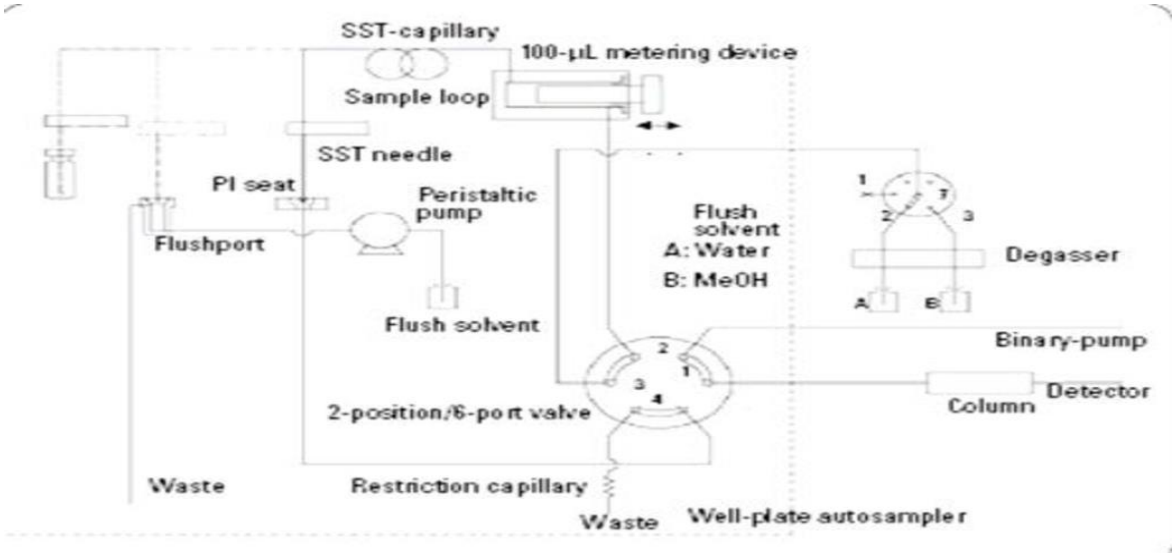


Figure 2. Schematic diagram of RRLC

MAIN PRINCIPLE INVOLVED IN RRLC

HPLC is one of the widely used chromatographic techniques used for the identification, quantification, and purification of the mixture of organic compounds. The mobile phase and stationary phase are utilized through which the analyte passes and further separates. The basic principle involved is Adsorption i.e. a mixture of compounds introduced into the system and they travel according to the relative affinities towards the stationary phase and the compounds which have less affinity towards the stationary phase travels faster and vice versa.

The main Principle is based on Van Demeter's Equation which relates the efficiency of the chromatographic column to the particle size of the column, molecular diffusion and thickness of the stationary phase.[15,16]

$H=A+B/u + Cu$

A-Eddy's diffusion: B- Longitudinal diffusion: C-Concentration: u- Linear Velocity

The resolution is proportional to the square root of N. However, because N is inversely proportional to particle size (Resolution (Rs) equation

$Rs= 4 (\alpha-1/\alpha) (k/k+ 1)$ ----→ Equation 2

The resolution is proportional to the square root of N. However, because N is inversely proportional to particle size (dp).

$N\alpha\ 1/dp$-----→Equation 3

Particle size (1.7m) reduces efficiency (N). N is also inversely proportional to the square of the peak width (W). As the particle size decreases, N consequently, Rs increase, resulting in increased sensitivity. Peak height (H) is also inversely proportional to peak width (W) (W)

$H\ \alpha 1/W$------→Equation 4

Because narrower peaks are taller peaks, sensitivity is increased. Narrower peaks in gradient separations also mean more peak capacity per unit of time, which is desirable in many applications. Peptide mapping, for example.

$N\alpha\ L/dp$-------→Equation. 5

The efficiency (N) of a column is proportional to the column length (L) and inversely proportional to the particle size.

Column efficiency:

$N=16RT^2/W^2$

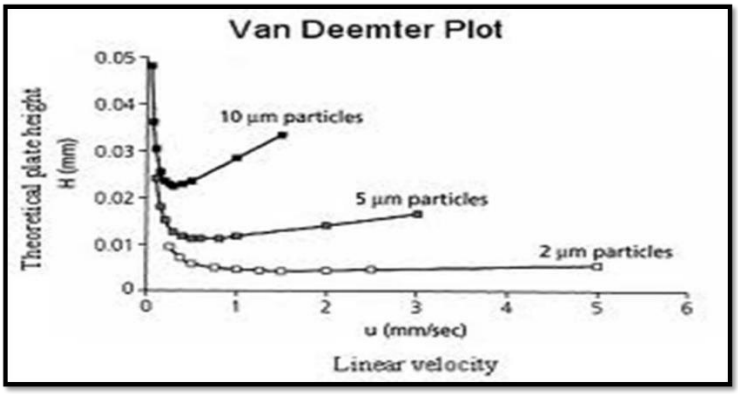


Figure 3. Van deemeter Plot

N-number of theoretical plates: RT-retention time: W-path width

RRLC INSTRUMENTATION

Description: -AGILENT 1200 SERIES OF RAPID RESOLUTION LIQUID CHROMATOGRAPHY
This is the only LC with user-adjustable speed and resolution. The 1200 Rapid Resolution LC (RRLC) System was designed to provide a liquid chromatograph with ultra-fast and high-resolution separation capability while still retaining full functionality for standard HPLC applications. Because STM(sub-two micron)particles are used, additional pressure is required to drive the mobile phase through the column at high flow rates or in long columns. High flow rates of up to 5 ml/min can be used for ultra-fast separations. The adjustable delay volume fully supports 2.1 to 4.6 mm i.d. columns. Additionally, it has Agilent 1200 series "instant pilot" which is found to be cost-effective for a standalone solution for single instrument control, it also has a new chemstation for faster data analysis & review[17-19]

This concept was implemented to provide the greatest amount of flexibility in terms of column dimensions and applications. Whether the rapid resolution separation method is being developed from scratch or adapted from an existing conventional method, having a diverse range of stationary phase chemistries available in a variety of column formats is clearly advantageous.
This System Includes:
→G1379B Micro Vacuum Degasser
→G1312B Binary Pump SL
→G1367C High-Performance HiP Autosampler SL
→(optional) G1330B Autosampler Thermostat
→G1316B Thermostatted Column Compartment SL
→G1315C Diode Array Detector SL (DAD) or G1314E Variable Wavelength Detector SL+ (VWD)

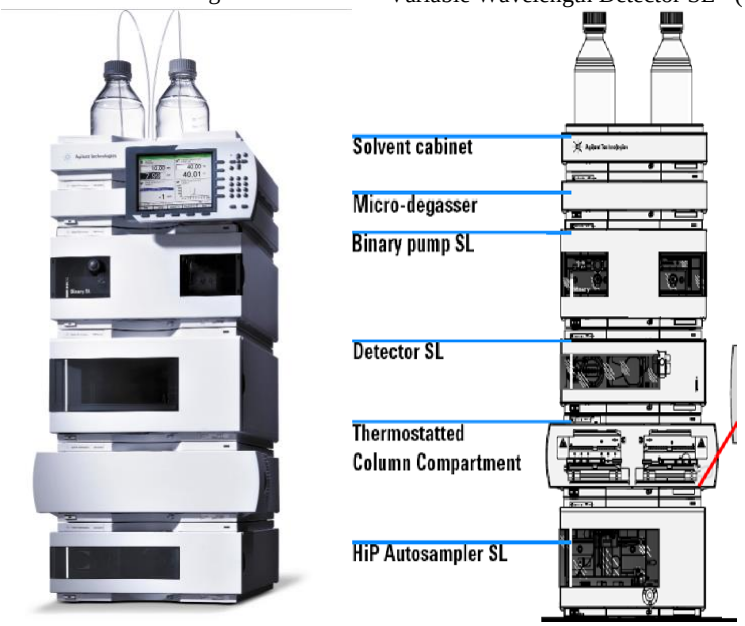


Figure 4. RRLC Instrumentation

MICRO VACCUM DEGASSER: -
The micro vacuum degasser maintains a partial vacuum by using a controlled leak in the degasser's proportional valve and varying the size of the controlled leak within the proportional valve based on the pressure sensor signal.

Figure 5. A micro-Degasser and its supplies which has a flow rate of up to 5ml/min and an internal volume of 1ml

Specifications: -

- **Maximum flow rate:** 0 – 5 ml/min per channel
- **Number of channels:** 4
- **Internal volume per channel:** Typically, 1 ml per channel
- **pH range:** 1 – 14
- **The material in contact with Solvents:** PTFE, FEP, PEEK

- **Weight:** 7 kg (16 lbs)
- **Dimensions:** 13.5 x 17 x 3.1 in. (34.5 x 43.5 x 8 cm)
- **Line voltage:** 100 – 240 VAC, ± 10% Wide-ranging capability
- **Line frequency:** 50 or 60 Hz, ± 5%
- **Power consumption:** 30 VA / 30 W / 102 BTU Maximum
- **Ambient operating temperature:** 0 – 55 °C (32 – 131 °F)
- **Humidity:** < 95%, at 25–40 °C (77–104 °F) Non-condensing
- **Safety standards:** IEC, CSA, UL

PUMPS:

A constant pump pressure (typically 1000-2000 psi) is required to ensure reproducibility and accuracy. Pump system maintenance will help to reduce downtime. Binary and quaternary pumps are commonly used in RRLC.

Binary pumps: -

The pump can mix two solvents for binary gradients and of course, can be used in isocratic mode. we can achieve new levels of productivity by selecting a system from the fast quaternary/binary LC system to the ultra-fast rapid resolution system, processing more samples in less time while maintaining and even improving data quality.

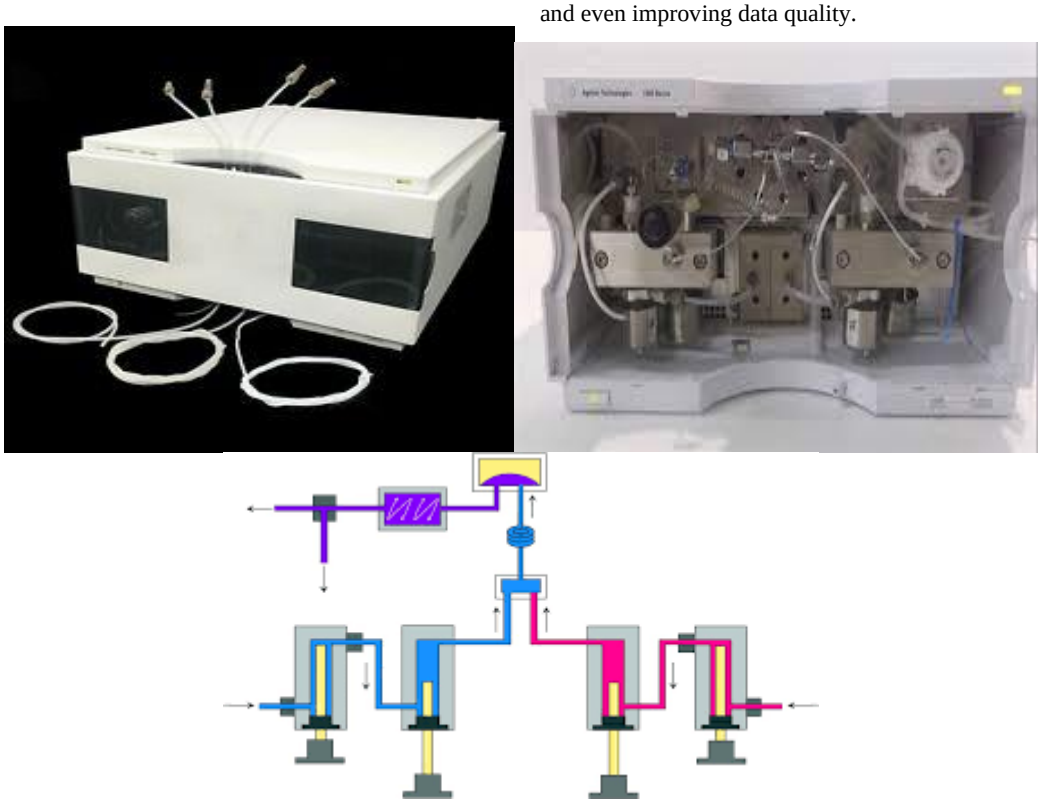


Figure 6. (a) Binary pump and its supplies (b) inner look of a pump in rrlc (c) Color representation of flow in the pump

Specifications: -

- Configurable delay volume: 120 µL, 320 µL and 600-800 µL
- Electronic damping control for lowest baseline,Weight: - 15.5 kg (34 lbs)
- Dimensions (width Ã— depth Ã— height): 180 x 345 x 435 mm (7 x 13.5 x 17 inches)
- Ambient operating temperature: 0--55 Â°C
- Flow range: 0.05 --5.0 mL/m(600 bar)
- Flow accuracy: Â± 1% or 10 Î¼L/min, whatever is greater
- Pressure: Operating range 600 bar (7800 psi) up to 5ml/min
- Perfect choice for fast and precise gradients using LC/MS, as well as UV-only systems
- Fully exploits the speed and separation potential of ZORBAX Rapid Resolution HT Columns.
- Optional: Solvent selection valve for 4 solvents

HIGH-PERFORMANCE AUTOSAMPLER: -

The Agilent 1200 Series G1367B High-Performance Autosampler adds maximum flexibility and fast injection cycles, whenever high sample throughput and speed of analysis are required. The standard metering device provides injection volumes from 0.1â€ “100 Î¼l. A multi-draw kit extends the range up to 1500 Î¼l.

Figure 7. Images showing high-performance autosampler, standard and thermostat autosamplers

WORKING OF AUTOSAMPLER IN RRLS

During the autosampler's injection routine, the sample loop, the inside of the needle, the seat capillary, and the main channel of the injection valve are in the flow path and remain there throughout the run. This means Throughout the analysis; these components are continuously flushed with the mobile phase. Only during sample aspiration is the injection valve removed from the flow path. The pump effluent is directed directly to the column in this position. The outside surfaces of the needle are washed with fresh solvent before injection. This is accomplished through the autosampler's flush port and prevents contamination of the needle seat. A peristaltic pump installed in the autosampler housing refills the flush port of the autosampler with fresh solvent. The flush port holds approximately 680µ l, and the pump delivers 6 ml/min. Setting the wash time to 10 seconds means that the flush port volume is refilled with fresh solvent more than once, which is usually enough to clean the outside of the needle. The autosampler is thus flushed and leaned, resulting in no (or) zero carryover·[20-21]

Specifications: -

High-speed 600-bar injector with new µ-valve design for highest instrument and column robustness at high pressures

• Cycle times < 30 s with overlapped injections

• Highest precision and linearity from 0.1 to 100 µL without loop change

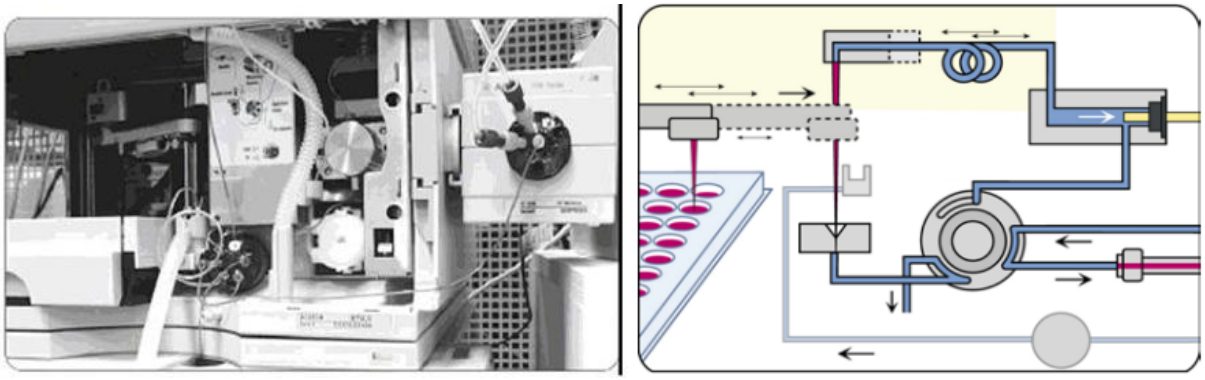


Figure 8. Autosampler SL injection system and Autosampler Flowthrough Design, overlapped injection/delayed volume reduction

COLUMNS: -

The heart of the system is the column where separation occurs. Rapid-resolution liquid chromatography columns have lower particle size-1.8u, compared with 2-10u in conventional columns.lower particle size is to operate at higher pressure(600bar) levels than normal column (400 bar). these columns genuinely provide High resolution (90,000 plates in 4 minutes and produce ultra-fast separation up to 20 times faster.

Thermostatted Column Compartment SL

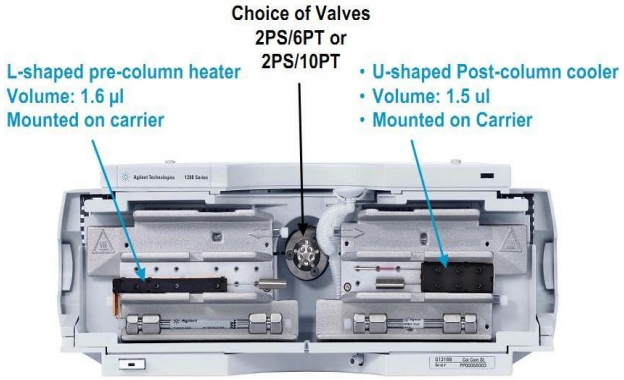


Figure 9. Represents a Thermostatted column in RRLC

For standard applications, the Agilent 1260 Infinity Series G1316A Thermostatted Column Compartment offers stable column cooling and heating up to 80 °C. It fits neatly into a single stack of 1260 Infinity modules, requiring the least amount of bench space. For 600 bar operation, a 2-position/6-port switching valve is optional. The Agilent 1290 Infinity Thermostatted Column Compartment is available for more sophisticated applications and fits seamlessly into any 1260 Infinity system. Columns optimized for 2.1mm, 3 and 4.6 mm I.D are available in RRLC for improving its efficiency.

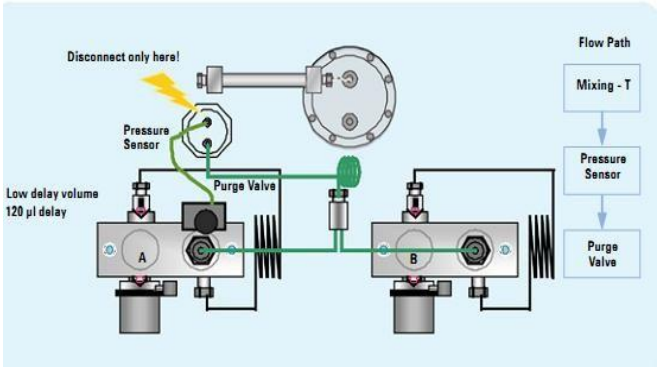


Figure 10. Represents low delay configuration for 2.1mm I'd columns

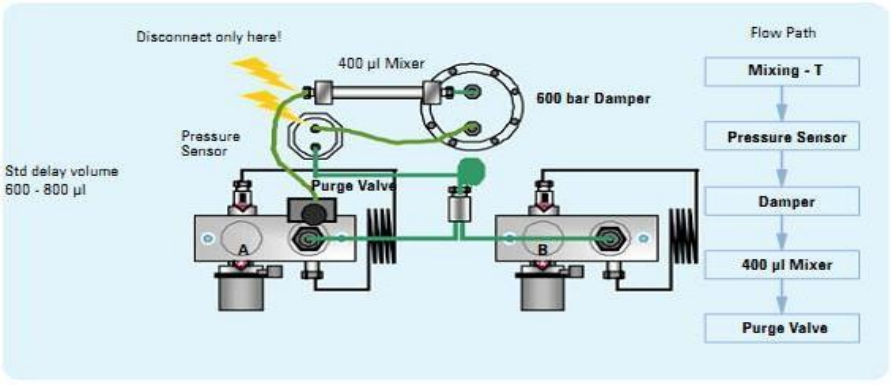


Figure 11. Represents minimum delay volume configuration for 2.1mm i.d column with highest UV sensitivity

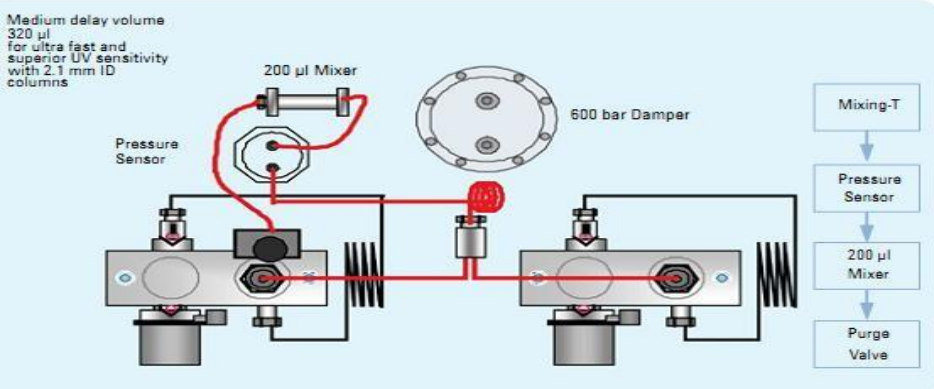


Figure 12. Represents Optimized configuration for 3 and 4.6mm i.d columns i.e., standard delay volume configurations for the respective i.d columns

DETECTORS

The detector detects the presence of a passing compound and sends an electronic signal to a data-acquisition device. Various Detectors available in the Agilent series include: -

1. UV detector flow cells with inner diameters of 2.1, 3.0, and 4.6 mm are available.
2. There are fast UV and MS detectors with data rates of up to 160 Hz (1200 Series VWD SL Plus), 80 Hz (1200 Series DAD SL, MWD SL), and up to 40 Hz for MS applications.
3. The Agilent 1200 series RRLC system is also compatible with the 1200 Series fluorescence detector (FLD) with 37 Hz data acquisition and the 1200 Series refractive index detector (RID). It is possible to progress from 1100 series to 1200 series RRLC in stages.

VWD SL (VARIABLE WAVE LENGTH DETECTOR SL): -

Description: -VWD SL is a low-cost, high-speed detector with a frequency of 55 hertz for maximum resolution and sensitivity in ultra-fast RRLC applications. variable wavelength detector SL for an ultra-fast, programmable single analysis, 1 signal, data rate of 13 Hz Maximum resolution in UFLC with a sampling rate of 55Hz. Three flow cells for narrow and standard bore columns (2.1 - 4.6mm ID), standard: 14ul / 10mm, semi-micro: 5ul / 6mm, and micro: 1ul / 5mm

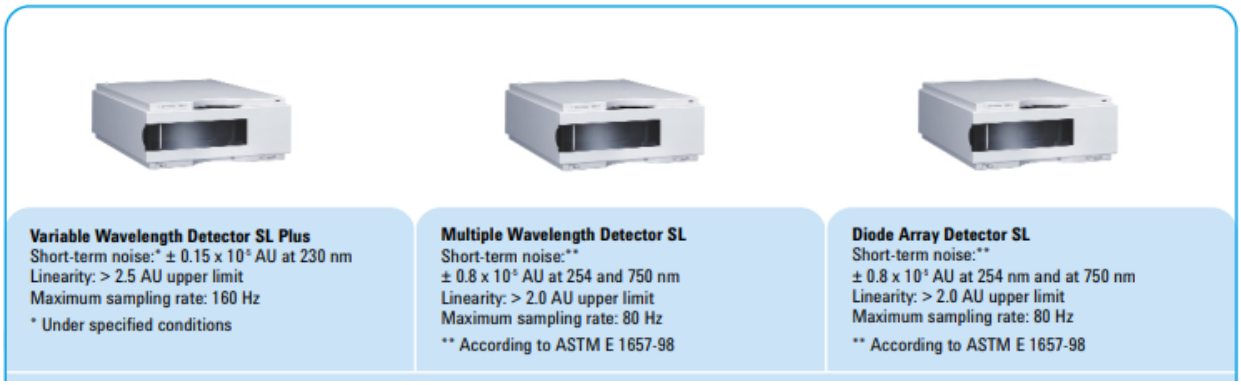


Figure 13. Images showing specifications of Variable wavelength, Multiple wavelength and Diode Array Detectors that are utilized in RRLC

DAD/MWD SL s: (DIODE-ARRAY DETECTOR/MULTIPLE WAVE LENGTH DETECTOR)

The Agilent 1200 Series G1365B MWD multiple wavelength detector can detect wavelengths ranging from low UV to visible. The dual lamp diode-array design provides high sensitivity for trace-level quantification. Simultaneous quantification of compounds of interest and monitoring of impurities at additional wavelengths are possible. It is recommended for use in pharmaceutical quality assurance/quality control laboratories, bioscience applications (proteins, DNA), and as a general-purpose UV detector for routine work. Even in harsh ambient conditions, the temperature management system ensures optimal baseline stability.

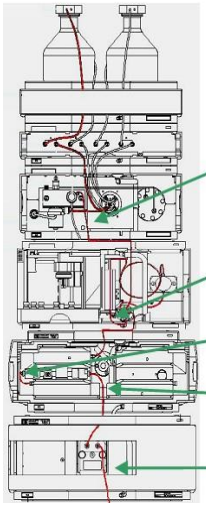
Specifications: -

- Dual lamp design for highest sensitivity from 190 to 950 nm;
- Up to 100% resolution gain in UFLC by 80Hz data rate
- Highest sensitivity in UFLC with < 50uAU noise by – Low noise cell design and electronics, ETC (Electronic Temperature Control), PCC (Post column cooling using TCC SL)
- Diode Array Detector SL for multi-electrical analysis. It has 5 signals, 20 HZ data rate
- Support of 2.1, 3.0 and 4.6mm ID columns by 3 flow cells Standard: 13 ul / 10 mm, Semimicro: 5 ul / 6 mm, Micro: 2 ul / 3 m
- Excellent signal-to-noise – for lowest limits of detection

Figure 13. Image showing specifications of fluorescence, Evaporative light scattering detectors, and LC/MS systems

Table 1: The table represents the Difference between RRL and HPLC Techniques

S.no	RRLC	HPLC
1. Particle size	1.8u	3-10u
2. Analytical column	ZORBAX Eclipse, XBD C18 RRHT	XTERRAC18, ALLITIMAC18
3.Column dimensions (length×i.d)	2.1×4.6mm	150×3.2mm
4. Column temperature	Upto100*c	30*c
5. Injection volume	1.5ul	5ul
6. Flow rate	0.2-20ul/min	0.01-5ml/min



Connection	HPLC	RRLC
Pump to Autosampler	60cm/0.17mm (G1312-67305)	60cm/0.17mm (G1312-67305)
Autosampler to column thermostat inlet	18cm/0.17mm (G1313-87305)	**18cm/0.12mm (G1313-87304)**
Column Thermostat to column	7cm/0.17mm (G1316-87300)	**7cm/0.12mm (G1316-87303)**
Column to Detector (DAD)	38cm/0.17mm (G1315-87311)	**15cm/0.12mm (G1315-87312)**
Detector to Waste (DAD)	PTFE, wide pore (0890-1713)	PTFE, wide pore (0890-1713)
Column to Detector (VWD)	PEEK/0.17 mm (5062-8522)	PEEK/0.17 mm (5062-8522)
Detector to Waste (VWD)	48 cm/0.25mm (5062-8535)	48 cm/0.25mm (5062-8535)

Diagrammatic representation of differences/changes between HPLC & RRLC

APPLICATIONS OF RRLC: -(23-25)

- Rapid Resolution Liquid Chromatography (RRLC) Analysis is utilized for the Quality Control of Rhodiola Rosea Roots and Commercial Standardized Products
- RRLC for analysis and studies on the stability of Shuang-Huang-Lian preparations (a traditional Chinese formula which comprises three medicinal herbs: Flos Lonicerae, Radix Scutellariae and Fructus Forsythia)
- Application of RRLC system to the analysis of some pesticide residue in apple juice
- Preventive doping control screening analysis of prohibited substances in human urine using rapid-resolution liquid chromatography/high-resolution time-of-flight mass spectrometry
- Development of a rapid resolution HPLC method for the separation and determination of 17 phenolic compounds in crude plant extracts using a 1.8 µm, 4.6×50 mm column
- RRLCanalysis of amino acids using pre-column derivatization
- Simultaneous Quantification of a Herbal Combination of Pueraria lobata, Salvia miltiorrhiza and Panax notoginseng by Rapid Resolution Liquid Chromatography
- Comprehensive analysis of the major lipid classes in sebum by rapid-resolution high-performance liquid chromatography and electrospray mass spectrometry
- Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid

chromatography-electrospray ionization tandem mass spectrometry

- Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry
- Simultaneous determination of six bioactive flavonoids in Citri Reticulate Pericarpium by rapid resolution liquid chromatography coupled with triple quadrupole electrospray tandem mass spectrometry
- Development and validation of a rapid-resolution liquid chromatography method for the screening of dietary plant isoprenoids: Carotenoids, tocopherols and chlorophylls
- Development of a new efficient method for isolation of phenolics from sea algae before their rapid resolution liquid chromatographic–tandem mass spectrometric determination
- Rapid Resolution Liquid Chromatography Method for Determination of Malathion in Pesticide Formulation
- Validation and comparison of analytical methods for the determination of uric acid in pulses and cereals by salting out assisted extraction by Rapid resolution liquid chromatography

CONCLUSION

In this study, RRLC outperforms conventional HPLC-based methods in terms of run time and sensitivity.RRLC has recently become a standard method in the pharmaceutical industry which is used to analyze general chemical compounds. Many traditional HPLC methods could easily achieve RRLC separations. The majority of these small particle columns have been used in pharmaceutical analysis. The ultrafast RRLC analysis could be done with satisfactory analytical precision using a 1200 RRLC system equipped with a higher acquisition rate of detector, low dead volume system configuration, and combined with a high-pressure HPLC system with 600 bar pressure capability. In this study, RRLC outperforms conventional HPLC-based methods in terms of run time and sensitivity**.** The ultrafast RRLC analysis could be performed with satisfactory analytical precision using a 1200 RRLC system equipped with a higher acquisition rate of detector, low dead volume system configuration, and combined with a high-pressure HPLC system with 600 bar pressure capability. In an isocratic example, one cycle analysis time of 25 minutes by the traditional HPLC/DAD method could be reduced to 0.8 minutes by the RRLC/MS method, a factor of 31. The same method transfer approach was used in a gradient separation. The RRLC technique is useful not only for pharmaceuticals but also for chemical compounds that are currently analyzed by conventional HPLC on 250mm4.6mm i.d columns**.** Fast RRLC will be used more widely in other HPLC fields in the future.

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