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Evolution of liquid chromatography: Technologies and applications

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Abstract

Liquid chromatographic offers efficient analyte separation employing high pressure pumps. The reversed phase high performance liquid chromatography (RP-HPLC) is widely utilized in the purity testing and quantitative determination of pharmaceuticals and neutraceuticals. The limitations of traditional liquid chromatography such as particle size, resolution and selectivity demanded for the developments and Waters Corporation developed ultra-performance liquid chromatography (UPLC). Ultrafast liquid chromatography (UFLC) is another milestone, which offers faster and efficient separation. Multidimensional UHPLC provides separation of complex molecules. The particle size decrease enhances the resolution of LC separation. Ethylene bridged hybrid (BEH), Charged surface hybrid (CSH) and Peptide separation technology (PST) offer better performance in. The amalgamation of chromatographic and spectroscopic detectors namely fluorescence detector (FD) and mass spectrometry (MS) provides efficient separation. Liquid chromatography (LC) offers the analysis of pharmaceuticals, biological, food materials, and natural products. This review covers technologies and recent pharmaceutical and biomedical applications of liquid chromatography technologies.

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INTRODUCTION

The Russian botanist Mikhail Tswet invented the first version of chromatography for the separation of plant pigments. The colored plant pigments were separated into discrete colored bands on the column (separation glass tube) packed with fine particles of calcium carbonate; hence the name chromatography was given. Later Martin and Synge, demonstrated the use of silica gel as separation media (stationary phase) and principle of partition coefficient in 1940's. A series of developments, chromatographic techniques namely, column, thin-layer (TLC), gas (GC) and liquid (LC) were developed. All these separation techniques offer versatile analysis of natural and synthetic molecules.

The reversed phase high performance liquid chromatography (RP-HPLC) is widely utilized for the identification (purity testing) and quantitative determination of pharmaceuticals and neutraceuticals. Liquid chromatographic methods are widely employed in the pharmaceutical and other industries. High-performance liquid chromatography (HPLC) is most reliable and versatile separation technique for the routine analysis of pharmaceuticals, biological and natural products. The utility of HPLC in the clinical laboratory setup is also significant. HPLC has been extensively utilized in the identification, quantification and purification of large and small molecules, adhering to the ICH guidelines Q2A and Q2B. Modern day developments on the HPLC column and detectors enhanced the

efficiency of separation process. In continuation of the development, Waters Corporation developed ultra-performance liquid chromatography (UPLC), containing 2 μm particles in the year 2004, which offered efficient separation. In this review, newer HPLC technologies and their recent applications are summarized.

HIGH-PERFORMACE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) offers efficient analyte separation employing high pressure pumps hence also known as high pressure liquid chromatography and very high-pressure liquid chromatography (VHPLC).

Principle

In liquid chromatography, the mobile phase is pumped through the packed column, under high pressure. The basic principle involved in the chromatographic separation is adsorption and partition (Basha, 2020) and can be explained through Van Deemter equation (Figure 1, Equation (1)). The equation describes the flow rate (v, velocity) height equivalent to the theoretical plate (H, HETP). The term A refers to eddy mixing, B denotes the diffusion of the particles and kinetic resistance to equilibrium is indicated by C. As the particle size decreases, there is an increase in efficiency with consequent improvement in resolution and sensitivity. The column efficiency (N) is directly proportional to column length (L) and indirectly proportional to the particle diameter (Equation (2)).

$$H = A + B/v + Cv \quad (1)$$

$$N \alpha L/dp \quad (2)$$

Modes of HPLC

HPLC operates through two different modes namely normal phase HPLC and Reverse phase HPLC.

Normal-Phase chromatography

This technique utilizes polar stationary phase and the non-polar mobile phase (Table 1), hence it is most suitable to polar compounds. Most of the pharmaceuticals are non-polar in nature, and can elute faster, which makes this technique not suitable.

Reversed phase chromatography

The nature of stationary phase and mobile phase is exactly opposite to normal phase chromatography, i.e polar mobile phase and non-polar stationary phase, hence this technique is known as reverse phase chromatography. This technique retains more non-polar analyte, and polar analyte being weakly retained moves fast through the column, and elutes earlier. Reverse phase chromatography mode is the most suitable for the analytical and preparative separations of active pharmaceutical ingredients (API), pharmaceutical formulations, chemical substances, biological products and food materials.

TECHNIQUES

The limitations of traditional liquid chromatography system such as size, weight, cost and system complexity demanded for the developments with improvised systems. The realization of particle size (diameter) of separation media influence on the velocity of mobile phase and pressure is basis for the modern day developments in the liquid chromatography (Carr *et al.*, 2011). Presently, ultra-performance LC (UPLC/UHPLC) systems namely rapid-resolution LC (RRLC), rapid separation LC (RSLC) and ultra-fast LC (UFLC) are available.

Ultra-performance liquid chromatography (UPLC)

The efficient separation of molecules requires, lower column length and increased flow rate. Increase in the flow rate in fully porous silica-packed column (stationary phase) develops back pressure and is the major limitation of HPLC. To overcome these ultra-performance liquid chromatography (UPLC) is developed to address the issue, and offered reliable separation (Carr *et al.*, 2011). The revolution in the separation science brought UPLC into the existence. In 2004, Waters Corporation trademarked the Ultra Performance LC (UPLC^{TM}), an more efficient separation technique. The separation on UPLC is on a pressure tolerant reversed-phase (RP) silica / organosiloxane hybrid particles. The narrow size distribution of particles provides more consistent column packing and demonstrated higher peak capacity, speed and sensitivity than conventional HPLC.

In later years, similar separation technique was developed by other manufactures and they coined the term ultra-high performance liquid chromatography (UHPLC) to avoid the trademark violations. Both the technologies (UPLC and UHPLC) works on sub 2-micron particles and are synonymous. UPLC/UHPLC technology facilitates the separation of drugs and their metabolites with high resolution and sensitivity. UPLC/UHPLC technology uses smaller stationary phase particles (< 2 μm) compared with high performance liquid chromatography (HPLC) and the decrease in the particle size in turn increases the separation (Table 2). The sub 2-micron particles of UPLC / UHPLC requires a higher operating pressure (>6000 psi), which is upper limit

Table 1: Stationary phases and mobile phases used in normal phase and reverse phase chromatography

Mode	Stationary phases	Mobile phases
Normal phase		
	1. Cyano propyl bonded with siloxane 2. Amino bonded with siloxane 3. Silica gel with siloxane 4. Diol bonded with siloxane 5. Di methyl amino bonded with siloxane	1. n-Hexane 2. n-Heptane 3. Iso-octane 4. Methylene chloride 5. Ethyl acetate 6. Propylamine 7. Dichloromethane 8. Diethyl ether
Reverse phase		Mixture of water (buffer) and
	1. Octadecyl (C18) 2. Octyl (C8) 3. Butyl (C4) 4. C3 5. Phenyl	1. Acetonitrile 2. Methanol 3. Acetone 4. Tetrahydrofuran 5. 2-propanol.

Table 2: Comparison of HPLC, UPLC / UHPLC, UFLC

Parameter	HPLC	UHPLC	UFLC
Particle size	3 to 10 μ	Less than 2 μ	1.7-2.2 μ
Stationary phase	XTerraC18, Alltima C18	Acquity UPLC BEH C18,C8,rp	Shim-pack XR-ODS column
Column dimension	150 X 3.2 mm	150 X 2.1 mm	75 X 3.0 mm
Column temperature	30 °C	65 °C	40 °C
Flow rate	0.01-5 mL/min	0.6 mL/min	3.7 nL/min
Pressure	>15000 psi	500-6000 psi	~ 200000 psi
Injection volume	5 μL	2 μL	0.1 to 100 μL
Maximum backpressure	300-400 bars	1000 bars	< 35 mpa

of HPLC instruments (Wang *et al.*, 2012). An additional benefit of UPLC is the low consumption of mobile phases (Swartz, 2005).

Ultra-fast liquid chromatography

Ultrafast liquid chromatography (UFLC) is a rapid and cost-effective liquid chromatography. Technique, which offers three times better separation and ten times faster than conventional HPLC. An autosampler of an UFLC analyte injects in less than 10 seconds and an automatic purging system allows faster analysis, with a flow rate ranging from 100 nL/min to 10 mL/min.

The columns are packed with smaller particle size (2.2 μm) to improve peak capacity with high resolution. This technique utilizes low volume of mobile phase to provide high sensitivity and fast analysis.

Multidimensional UHPLC

This instrumental setup enables robust analysis. One-dimensional liquid chromatography (1DLC) suffers with low resolution of molecules, in particular with complex mixtures (e.g, proteome, food). Two-dimensional liquid chromatography (2DLC) permits the elution of primary column fraction for the elution in second column. Primary column fraction can be subjected for the chromatographic separation in second column, 2DLC can be performed through either LC-LC or LC X LC techniques. The major differences between the two techniques are the amount of first column fraction. Separation of co-elute (impurities), particularly the molecules which do not have chromatographic groups is difficult. Multidimensional UHPLC have been developed for the separation of complex molecules and

Table 3: Applications of HPLC, UHPLC and UFLC

Research discipline	Analyte	Instrument
Oncological application	Anastrozole Letrozole Exemestane	HPLC-Luna C18-UV
Chiral separation	D-Asparate D-Serine D-Alanine D-Glutamic acid	HPLC- Pirkle-type- ESI-MS/MS
Analysis of peptides	Mastocarpus stellatus, Saccharina latissima and Codium spp	HPLC- Ascentis Express C18-ESI
Analysis of Vitamins	Vitamin E	RP-HPLC-UV.
Pharmacokinetic studies	Metformin and Teneligliptin	UHPLC-Acquity Cyano-ESI
Formulation stability studies	Diclofenac gel	UPLC- Acquity C18-PDA
Separation of D-cysteine	Cysteine	UHPLC- Pirkle-type-MS
Analysis of Carbohydrates	Fructose, glucose, sucrose, maltose, and lactose	UPLC-ELSD-Amide column

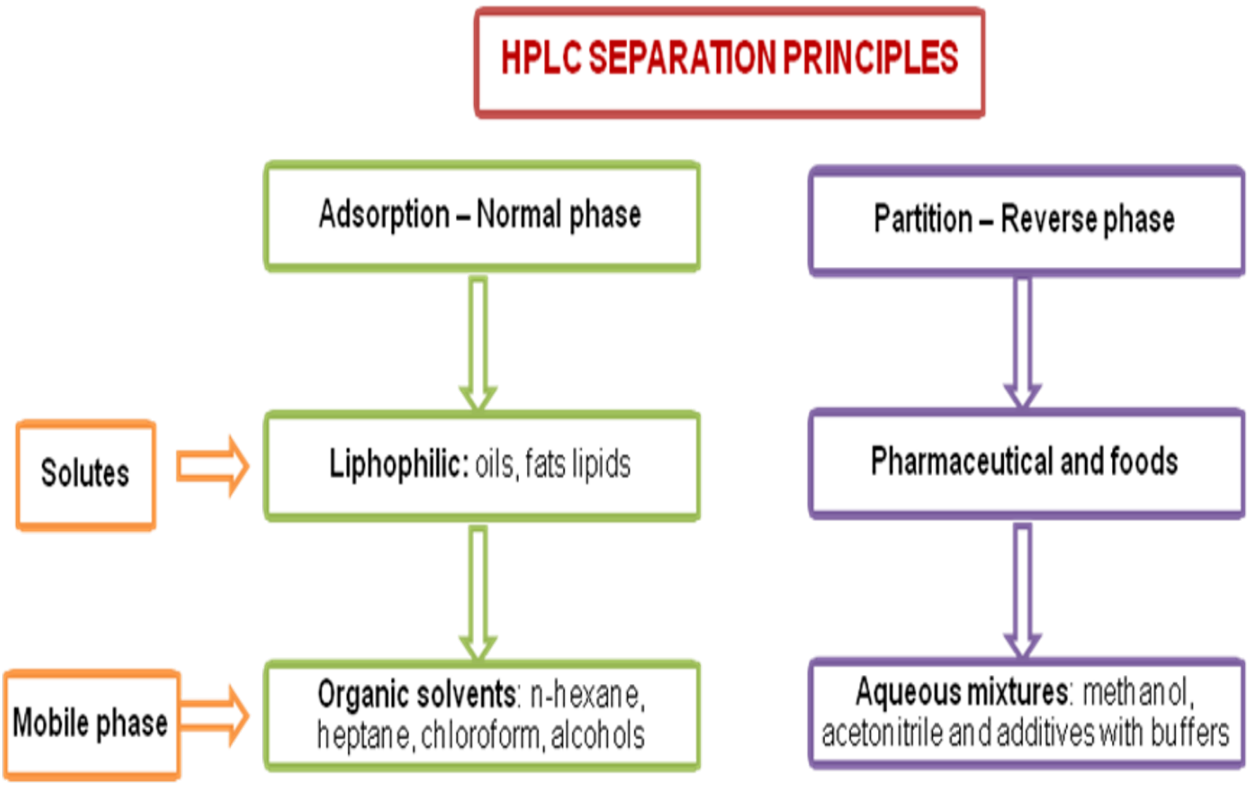

Figure 1: Principles of HPLC separation

offer efficient separation with higher resolution and selectivity.

Particle Technology

The columns of UPLC/UHPLC are made up of ethylene-bridged hybrid derivatized with C18 groups (1.7 μm, 2.1 x 50/100 mm). UPLC efficiency is proportional to the column length and inversely proportional to the column radius (Lynch *et al.*, 2018). The decrease in the particle size by a factor of three increases the efficiency three times greater and the resolution increases by nine times (3^2).

Acquity BEH columns and Xbridge HILIC columns provides high chemical stability in the entire pH range (Jian *et al.*, 2010). Monoliths packed columns overcomes the backpressure issue of HPLC. Monolithic columns offer better performance in chromatography than organic polymers (Chauve *et al.*, 2010; Ghanem and Ikegami, 2011). Charged surface hybrid (CSH) technology uses low-level, charged surface particles to improve peak selectivity and sharpness. The CSH particles offer enhanced peak shape, loading capacity (CSH C18), selectivity (CSH-phenyl-hexyl, CSH-fluoro phenyl). Ethylene bridged hybrid (BEH) has provided incomparable flexibility and precision, enabling chromatographers to push the limits of LC separations. Peptide separation technology (PST) utilizes C18 BEH for the separation of peptides (proteomics analysis).

Hyphenated techniques

The amalgamation of chromatographic and spectroscopic detectors namely UV diode detector (UV-DAD), evaporative light-scattering detector (ELSD), refractive index (RI), fluorescence detector (FD) and mass spectrometry (MS) derives efficient separation and identification. Hirschfield coined the term "hyphenated", which refers to an on-line combination of chromatographic method for separation and spectroscopic detection. High-performance liquid chromatography (HPLC) hyphenated with spectroscopic methods such as mass spectroscopy (LC-MS), photodiode array (HPLC-PDA), Fourier-transform infrared (LC-FTIR) enhanced the separation efficiency.The detectors used in the LC, can be broadly grouped into absorbance, electrochemical and mass spectrometry based detectors. The newer generations detectors offer data-acquisition rates (80 to 200Hz) (Swartz, 2010).

Absorbance detectors (AD)

The fixed wavelength detector (microsized detector) with light emitting diode (LEDs) is the major advancement. The miniaturization in the UV detector cell volumes (0.5 to 2 μL) is the breakthrough in the detector (Schmid *et al.*, 2008). Weaver et al developed laser induced fluorescent detector (LIF) for the analysis of labelled protein and is the latest breakthrough (Desmet and Eeltink, 2013).

Electrochemical detectors (ECD)

ECD offers higher degree of selectivity and sensitivity in the analysis of electrochemically active molecules such as phenols and aromatic amines. Capacitively coupled contactless conductivity detection (CCD) is most suitable for the UPLC system

Mass spectrometer detectors (MS)

The combination of LC with MS identifies and confirms the chemical structure. LC-MS can detect the non-volatile and thermolabile metabolites and drugs (Denoroy *et al.*, 2013). Triple quadrupole, quadrupole-time of flight (Q-TOF) and Orbitrap are used for mass measurement can acquire MS/MS spectra with high reproducibility and allow analyte elemental composition analysis (Patel *et al.*, 2010).

APPLICATIONS OF NANO-LC TECHNIQUES

Liquid chromatography (LC) techniques are most reliable and versatile separation technique for the routine analysis of pharmaceuticals, biological, food materials, and natural products (Table 3).

Pharmaceutical analysis

Ultra-performance liquid chromatography (UPLC) determination is superior compared with HPLC analysis of diclofenac gel. UPLC method together with Acquity column (UPLC) showed higher efficiency compared with C18 columns (HPLC) (Nováková *et al.*, 2006). A cost effective UHPLC method for the quantification and impurity detection of sofosbuvir (anti-viral drug) using DAD and MS detection was reported (Contreras *et al.*, 2017). The presence of Nevirapine in pharmaceutical dosage form is successfully determined using RP-UFLC equipped with C18 column.

Bioanalytical studies

HPLC method for the analysis of aromatase inhibitors anastrozole and letrozole present in the biological samples plasma, and urine was reported using a Luna C18 column (Locatelli *et al.*, 2018). UPLC-MS/MS method for the determination of daclatasvir (DAC) in human plasma was reported using Xevo TQD LC-ESI multiple reaction monitoring and Acquity HSS C18 column (Rezk *et al.*, 2016).Simultaneous determination of gelsemine and koumine in rat plasma by UPLC-MS/MS and Acquity C18 column showed better separation compared with HPLC (Wang *et al.*, 2018). UHPLC-MS method for the bioanalytical determination of milrinone was developed and validated using Acquity C18 column (Chihoho *et al.*, 2012). LBPT a platelet

activating factor receptor present in human plasma was determined using a UPLC-MS/MS method (Wu *et al.*, 2020). LC-MS method have been developed for the simultaneous estimation of Vitamin K1 and Vitamin K2 in human plasma by employing ACE –PFP C18 column and APCI detection (Zhang *et al.*, 2016).

Pharmacokinetic studies

UHPLC-QTOF-MS method for the determination of pharmacokinetic interaction of metformin (MET) and teneligliptin (TEN) in rat plasma using Acquity UPLC HSS Cyano column and positive ion electron spray ionization was developed (Paul *et al.*, 2017). A chiral separation of D-threo-methylphenidate (D-threo-MPH), L-threo-methylphenidate (L-threo-MPH), D-threo-ethylphenidate (D-threo-EPH), L-threo-ethylphenidate (L-threo-EPH) and D,L-threo-ritalinic acid (D,L-threo-RA) in rat plasma was established using UFLC MS/MS method and Astec Chirobiotic V2 column for pharmacokinetic interaction determination (Zhang *et al.*, 2016). UHPLC-MS/MS method for the determination of nifedipine in plasma were developed for high throughput pharmacokinetic screening. UHPLC technique is very suitable for the metabolomics analysis, especially for large-scale untargeted metabolomics. The application of UPLC in analysis of Urinary metabolome of patients suffering from HCC is reported.

Chiral analysis

The amino acid enantiomers (D and L) have different biological functions, bio-distributions and metabolic pathways. Multi-dimensional UHPLC-MS method for the determination of cysteine enantiomers present in the biological samples were determined after efficient reduction of the disulfide bond in cysteine using of 1,4-dithio-D,L-threitol as a reducing agent and the method was applied to cell culture samples Pucciarini *et al.* (2020).

Food analysis

Vitamin E (α-tocopherol) content of human erythrocytes was determined using RP-HPLC-UV. This method was applied for the determination of vitamin E of patients with acute pancreatitis (Solichová *et al.*, 2003). A fast and sophisticated HPLC method was developed for the quantification of samples containing sugars, organic acids and alcohols in food samples. The analysis was performed by employing Hi-Plex H column and Refractive index detector (Zaky *et al.*, 2017). UPLC-ELSD method using Acquity Amide column is developed for the analysis of fructose, glucose, sucrose, maltose, and lactose and identification of the sorbitol and mannitol in chewing gums (Koh *et al.*, 2018). The analysis of folic acid in food products using UFLC method have been developed using C18 column along with PDA detector (Rodríguez *et al.*, 2019).

Phytochemical analysis

HPLC and UFLC methods are utilized in the separation of purine alkaloids and polyphenols present in *Camellia sinensis, Camellia ptilophylla and Camellia assamica* (Li *et al.*, 2017). UFLC-PDA analysis using a Sphere Clone (Phenomenex) reverse phase C18 column for the efficient separation of organic acids present in shoots, leaves, aerial parts, flowering shoots, flowers and fruits was achieved (Pereira *et al.*, 2013).

Peptide Analysis

A peptide analysis for the analysis of short chain peptides from three edible macroalgae namely *Mastocarpus stellatus, Saccharina latissima and Codium spp* was reported by RP-HPLC-QTOF/MS. (Pérez-Míguez *et al.*, 2019). UHPLC–ESI–MS/MS method for the determination of casein peptides in bovine milk have been developed (Liu and Pischetsrieder, 2018).Antioxidant peptides from finger millet (*Eleusine coracana*) protein hydrolysate and amino acid was identified using UPLC-PDA (Agrawal *et al.*, 2019).

CONCLUSION

Ultra-performance liquid chromatography (UPLC)/UHPLC and UFLC technologies offer moe sensitive analysis of pharmaceutical, biological, food, phytoconstituents and diagnostic studies, even in minute quantities. Ultrafast liquid chromatography (UFLC) and multidimensional UHPLC have been developed to offer efficient separation with higher resolution and selectivity.

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Conflict of Interest

Nil

REFERENCES

Agrawal, H., Joshi, R., Gupta, M. 2019. Purification, identification and characterization of two novel antioxidant peptides from finger millet (Eleusine coracana) protein hydrolysate. *Food Research International*, 120:697–707.

Basha, M. 2020. Chromatography. In *Analytical Techniques in Biochemistry* . Humana, New York, NY. Page: 39-59.

Carr, P. W., Stoll, D. R., Wang, X. 2011. Perspectives on Recent Advances in the Speed of High-Performance Liquid Chromatography. *Analytical Chemistry*, 83(6):1890–1900.

Chauve, B., Guillarme, D., Cléon, P., Veuthey, J.-L. 2010. Evaluation of various HILIC materials for the fast separation of polar compounds. *Journal of Separation Science*, 33(6-7):752–764.

Chihoho, A. B., Sage, R. T., Smolenski, A., Vazir, M. L., Rose, N. R., Banner, N. V., Leaver 2012. A clinical assay for the measurement of Milrionone in plasma by UHPLC-MS/MS. *Biomedical Chromatography*, 26(5):566–570.

Contreras, M. D. M., Morales-Soto, A., Segura-Carretero, A., Valverde, J. 2017. Potential of RP-UHPLC-DAD-MS for the qualitative and quantitative analysis of sofosbuvir in film coated tablets and profiling degradants. *Journal of Pharmaceutical Analysis*, 7(4):208–213.

Denoroy, L., Zimmer, L., Renaud, B., Parrot, S. 2013. Ultra high performance liquid chromatography as a tool for the discovery and the analysis of biomarkers of diseases: A review. *Journal of Chromatography B*, 927:37–53.

Desmet, G., Eeltink, S. 2013. Fundamentals for LC Miniaturization. *Analytical Chemistry*, 85(2):543–556.

Ghanem, A., Ikegami, T. 2011. Recent advances in silica-based monoliths: Preparations, characterizations and applications. *Journal of Separation Science*, 34:n/a–n/a.

Jian, W., Edom, R. W., Xu, Y., Weng, N. 2010. Recent advances in application of hydrophilic interaction chromatography for quantitative bioanalysis. *Journal of Separation Science*, 33(6-7):681–697.

Koh, D.-W., woong Park, J., hoon Lim, J., Yea, M.-J., young Bang, D. 2018. A rapid method for simultaneous quantification of 13 sugars and sugar alcohols in food products by UPLC-ELSD. *Food Chemistry*, 240:694–700.

Li, Y. F., Ouyang, S. H., Chang, Y. Q., Wang, T. M., Li, W. X. 2017. A comparative analysis of chemical compositions in Camellia sinensis var. puanensis Kurihara, a novel Chinese tea. *Food Chemistry*, 216:282–288.

Liu, Y., Pischetsrieder, M. 2018. Absolute quantification of two antimicrobial peptides α S2 -casein 182–207 and α S2 -casein 151–181 in bovine milk by UHPLC–ESI–MS/MS in sMRM mode. *Food Chemistry*, 261:15–20.

Locatelli, M., Tinari, N., Grassadonia, A., Tartaglia, A., Macerola, D., Piccolantonio, S., Sperandio, E., D'Ovidio, C., Carradori, S., Ulusoy, H. I., Furton, K. G., Kabir, A. 2018. FPSE-HPLC-DAD method for the quantification of anticancer drugs in human whole blood, plasma, and urine. *Journal of Chromatography B*, 1095:204–213.

Lynch, K. B., Chen, A., Liu, S. 2018. Miniaturized high-performance liquid chromatography instrumentation. *Talanta*, 177:94–103.

Nováková, L., Solichová, D., Solich, P. 2006. Advantages of ultra performance liquid chromatography over high-performance liquid chromatography: Comparison of different analytical approaches during analysis of diclofenac gel. *Journal of Separation Science*, 29(16):2433–2443.

Patel, K., Patel, J., Patel, M., Rajput, G., Patel, H. 2010. Introduction to hyphenated techniques and their applications in pharmacy. *Pharmaceutical Methods*, 1(1):2–13.

Paul, D., Allakonda, L., Satheeshkumar, N. 2017. A validated UHPLC-QTOF-MS method for quantification of metformin and teneligliptin in rat plasma: Application to pharmacokinetic interaction study. *Journal of Pharmaceutical and Biomedical Analysis*, 143:1–8.

Pereira, C., Barros, L., Carvalho, A. M., Ferreira, I. C. F. R. 2013. Use of UFLC-PDA for the Analysis of Organic Acids in Thirty-Five Species of Food and Medicinal Plants. *Food Analytical Methods*, 6(5):1337–1344.

Pérez-Míguez, R., Plaza, M., Castro-Puyana, M., Marina, M. L. 2019. Separation and identification of peptides in hydrolysed protein extracts from edible macroalgae by HPLC-ESI-QTOF/MS. *Algal Research*, 39.

Pucciarini, L., González-Ruiz, V., Zangari, J., Martinou, J.-C., Natalini, B., Sardella, R., Rudaz, S. 2020. Development and validation of a chiral UHPLC-MS method for the analysis of cysteine enantiomers in biological samples. *Journal of Pharmaceutical and Biomedical Analysis*, 177:1–8.

Rezk, M. R., Bendas, E. R., Basalious, E. B., Karim, I. A. 2016. Development and validation of sensitive and rapid UPLC–MS/MS method for quantitative determination of daclatasvir in human plasma: Application to a bioequivalence study. *Journal of Pharmaceutical and Biomedical Analysis*, 128:61–66.

Rodríguez, D. C., Torres, L. N., Parra, J. W. 2019. Analysis of folic acid in white rice by ultra-fast liquid chromatography. *Journal of Physics: Conference Series*, 1388(1):12–37.

Schmid, S., Macka, M., Hauser, P. C. 2008. UV-absorbance detector for HPLC based on a light-emitting diode. *The Analyst*, 133(4):465–469.

Solichová, D., Korecká, L., Svobodová, I., Musil, F., Bláha, V., Žďánský, P., Zadák, Z. 2003. Development and validation of HPLC method for the determination of α-tocopherol in human erythrocytes for clinical applications. *Analytical and Bioanalytical Chemistry*, 376(4):444–447.

Swartz, M. 2010. HPLC Detectors: A Brief Review. *Journal of Liquid Chromatography & Related Technologies*, 33:1130–1150.

Swartz, M. E. 2005. UPLC™: An Introduction and Review. *Journal of Liquid Chromatography & Related Technologies*, 28(7-8):1253–1263.

Wang, L., Wen, Y., Meng, F. 2018. Simultaneous determination of gelsemine and koumine in rat plasma by UPLC-MS/MS and application to pharmacokinetic study after oral administration of Gelsemium elegans Benth extract. *Biomedical Chromatography*, 32(6):e4201.

Wang, Y., Ai, F., Ng, S. C., Tan, T. T. Y. 2012. Sub-2 μm porous silica materials for enhanced separation performance in liquid chromatography. *Journal of Chromatography A*, 1228:99–109.

Wu, M., Liu, A., Zhuang, Q., Jia, R., Zhou, Y., Liu, Y., Wang, H. 2020. Development and validation of a UPLC-MS/MS method for simultaneous determination of LBPT and its metabolites in human plasma. *Bioanalysis*, 12(4):211–220.

Zaky, A. S., Pensupa, N., Áurea Andrade-Eiroa, Tucker, G. A., Du, C. 2017. A new HPLC method for simultaneously measuring chloride, sugars, organic acids and alcohols in food samples. *Journal of Food Composition and Analysis*, 56:25–33.

Zhang, C., Luo, H., Wu, Y., Zhang, J., Zhang, F., Lin, G., Wang, H. 2016. Development and validation of an UFLC-MS/MS method for enantioselectivity determination of d,l-thero-methylphenidate, d,l-thero-ethylphenidate and d,l-thero-ritalinic acid in rat plasma and its application to pharmacokinetic study. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 1011:45–52.