Research Article

UPLC: A Prominent Analytical Technique For Pharmaceuticals

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ABSTRACT

In the modern world pharmaceutical industries are driven to create more efficient tools for estimation and analysis of drugs. Thus the development of a rapid chromatographic method has become essential for the analytical laboratories. Due to recent and rapid advancement in technology, improvement have been done in enhancing particle chemistry performance, improved detector design and in optimizing the system, it resulted in an outstanding performance through the ultra-high performance liquid chromatography (UPLC), which works on the principle of HPLC technique. UPLC brought tremendous improvement in the speed, resolution and sensitivity of chromatographic techniques. Here, column containing bridged ethylsiloxane structure with particle size (less than 2µm) is used, which reduces solvent consumption, decreases the length of column and save time This system is designed in such a way that it can withstand high system back-pressures. UPLC thus has better resolution, assay sensitivity and high sample throughput, which enables greater number of analyses to be performed in short time period and it also imparts cost effective advantage over HPLC analysis.

Keywords: UPLC, HPLC, Analytical techniques, Pharmaceuticals.

INTRODUCTION

UPLC has been considered as an emerging area in the area of analytical separation which retains the principle of HPLC while increasing the attributes of speed, sensitivity and resolution. UPLC works on chromatographic principles by using columns packed with smaller particles (less than 2µm) and higher flow rates to run separation. Therefore, UPLC is a derivative of HPLC whose underlying principle as, efficiency and resolution increases as the column packing particle size decreases. By using smaller particles, the performance of liquid chromatography will be extended to a new limit which is known as Ultra Performance Liquid Chromatography (UPLC). Thus because of above reasons, UPLC stands better than HPLC for its better chromatographic resolution, performs more sensitively, is cost effective as it reduces solvent consumption and possesses high analysis speed.

Principle[1-6]

The basic principle is UPLC governed by an empirical formula known as Van Deemter equation which describes the relationship between flow rate and plate height (HETP). According to this equation, the improved efficiency of UPLC technique is impossible without using smaller particle size than those used in conventional HPLC technique.

The Van Deemter equation:

$H = A + B/v + Cv$,

Where v is linear velocity and A, B and C are constants.Here, A represents the Eddy mixing which is independent of velocity. The value of A is lowest when column particles are uniformly small. B is the natural diffusion tendency of molecules or axial diffusion which is affected by flow rate. This effect is seen at high flow rates, therefore this term is divided by v; C is the kinetic resistance to equilibrium in the separation process.[1-5]

Instrumentation

The design and development of particles having size less than 2 µm is a significant challenge. Disadvantages of high efficiency non-porous 1.5 µm particles, commercially available, is that it suffer from low surface area, which leads to poor loading capacity and retention time. In order to take the advantage of the enhanced speed, better resolution and increased sensitivity, the instrumentation of UPLC should be prepared accordingly by the smaller particles.

To fulfill the purpose, advancement in design of, auto sample, pumps, detector, and data system is required. The basic instrumentation of UPLC is describes as follows:

Sample injector[5]

Sample injection is of much importance in UPLC. Extreme pressures to be achieved in UPLC are not facilitated by conventional injection valves.

To prevent the hazardous effects of extreme pressure fluctuations, the process of sample injection need to be

pulse – free and swept volume also should be minimal for reducing band spreading.

Pumping System[5]

Decrease in particle size, requires greater pressure range. Therefore pumps should be designed in such a way that it is capable of delivering solvent smoothly and reproducibly at such high pressures, which can operate in both isocratic as well as gradient separation modes.

UPLC columns[5,6]

Design of particles < 2.0 µm is a challenging task. To get retention and capacity similar to HPLC, the columns for UPLC must be designed using porous particle that can withstand extreme pressures. For this silica based particles can be used which have good mechanical strength, but they have many disadvantages like tailing of basic analytes, limited pH range, etc. polymeric columns overcome pH limitations but they have low efficiency and limited capacities.

Waters introduced XTerra, the first generation hybrid chemistry. It possessed the properties of both silica and polymeric columns like mechanical strength, high efficiency and wide pH range. To enhance the mechanical strength required by UPLC, the second generation columns were prepared by bridged ethane hybrid (BEH) that can withstand high pressure and is suitable over large pH range. For these BEH columns, efficiency is directly proportional to its length and inversely proportional to the particle size. Hence, the application of these columns resulted in superior separation and improved spectral quality:

- ACQUITY UPLC BEH T M C18 and C8 (straight chain alkyl columns)
- ACQUITY UPLC BEH Shield RP 18 (embedded polar group column)

Agilent introduced regular phases having 1.8 µm particle sizes. The phases used were:

- Zorbax stable Bond C8 and C18 for low pH range.
- Zorbax Extend C18 for high pH range.
- Zorbax XDB-C8 and C18 for general purpose.
- Zorbax-SB CN which provides different reversed phase polarity.

Alltechs offer 1.5 µm particle sizes. The company introduced Platinum HPLC columns which have controlled surface area that offered dual separation modes and extended the polar selectivity range.

- Alltima HP HILIC is a non bonded, silica column for hydrophilic interaction chromatography separations.
- Pro sphere HP ZAP C18 for high speed reversed phase separation.

Bischof introduced three porous phase having 1.8 µm particle size and one non porous silica phase with particle size 1.5 µm.

- Pronto PEARL
- TPP-C8
- ACE EPS (8 % carbon loading)
- C18 EPS (16 % carbon loading)

Detector[5,6]

TUV Detector (Tunable ultraviolet detector)

The conventional absorbance based detectors are concentration sensitive. For UPLC, the flow cell volume would have to be reduced in standard UV/Visible detectors for concentration and signal. Hence for UPLC detection the tunable UV/Visible including new electronics and firmware for supporting Ethernet communications at the high data rates were used. Such detector cells were made up a light guided flow cell equivalent to an optical fiber. The wavelength ranging from 190 to 700 nm is used to operate this detector.

PDA Detector (Photo diode array detector)

The PDA detector is an ultraviolet/visible light (UV/Vis) spectrophotometer that operates between the wavelength ranges of 190 and 500 nm.

ELS Detector

It is an evaporative light scattering detector designed for use in the UPLC system. By using stationary phases of size around 2µm performance similar or even higher has been demonstrated without the adverse effects of high pressure. In addition, the phases of less than 2 µm are generally non-regenerable and thus have limited use.

Advantages[4,5]

1. Decreases run time and increases sensitivity.
2. Provides the selectivity and sensitivity.
3. Maintain the Resolution performance.
4. Expand the scope of Multi-residue Methods.
5. For quick quantification of related and unrelated compound by UPLC's fast resolving power.
6. By using novel separation material of very fine particle size gives faster analysis.
7. Reduction of Operation cost.
8. Less solvent consumption.
9. By Reducing process cycle times, more product can be obtained with existing resources.
10. Give real-time analysis in step with manufacturing processes.
11. Assures end-product quality, including final release testing.

Disadvantages[4,5]

More maintenance requires and reduces the life of the columns of this type due to increased pressure. By using stationary phases of size around 2µm performances similar or even higher has been demonstrated without any adverse effects of high pressure. In addition, the phases of less than 2 µm are generally non-regenerable and thus have limited use.

Applications[4-6]

Analysis of Natural Products and Traditional Herbal Medicine

- For analysis of herbal medicines and natural products, UPLC is widely used.
- For traditional herbal medicines, analytical laboratories need to establish safety parameters for their production.

Study of Metabolomics/Metabolomics

- To develop new medicines, metabonomics studies are carried out in laboratories.
- Metabonomics gives a robust and rapid method to, improves understanding of potential toxicity, for detecting these changes and allows monitoring the efficacy.

Identification of Metabolite

- For drug discovery Biotransformation of new chemical entities (NCE) is necessary.
- Metabolite identification becomes a regulated process when a compound reaches the development stage. It is of the most importance for lab to successfully detect and identify all circulating metabolites of a candidate drug.

Bioanalysis/Bioequivalence Studies

- For toxicity, pharmacokinetic, and bioequivalence studies, quantitation of a drug in biological samples is an important part of development programs.
- UPLC/MS/MS solutions are proven to increase efficiency, productivity and profitability for bioequivalence laboratories.

Dissolution Testing

- For release in drug manufacturing, quality control and dissolution testing is essential in the formulation, development and production process.
- More potent Newer and formulations require increased analytical sensitivity. UPLC gives precise and reliable automated online sample acquisition.

Forced Degradation Studies

- One of the most important factors that impact the safety and quality of pharmaceuticals is chemical stability.
- The common analytical technique for monitoring forced degradation experiments is HPLC with UV and/or MS detection for peak purity, identification of degradation products and mass balance, but these HPLC-based methodologies are very time-consuming and all the degradation products are accurately detected.

Manufacturing/QA/QC

- When manufacturing of a drug product important factors to be considered such as Identity, quality, purity, safety and efficacy. The production of quality pharmaceutical products requires raw materials with proper quality and purity.
- UPLC is used for the highly regulated, quantitative analyses performed in QA/QC laboratories.

Method Development/Validation

- UPLC provide efficiencies in method development: Using UPLC, analysis times becomes as short as one minute, methods can be optimized in one or two hours, separation speed, significantly reducing the time required to develop and validate with UPLC, and efficiency allows for the rapid development of methodologies.

Impurity Profiling

- For the drug development and formulation process; profiling, detecting, and quantifying drug substances and their impurities in raw materials and final product testing is an essential part. Impurity profiling requires high-resolution chromatography, capable of reproducibly and reliably separating and detecting all of the known impurities of the active compound.

Table 1: Comparison between HPLC and UPLC[4-6]

S. No	Characteristics	HPLC	UPLC
1.	Column	150 x 3.2 mm	150 x 2.1 mm
2.	Particle size	3 – 5 µm	Less than 2 µm
3.	Flow rate	3.0 ml/min	0.6 ml/min
4.	Needle wash	Methanol	Methanol
5.	Injection volume	5 µl (std. in 100 % MeOH	5 µl (std. in 100 % MeOH

6.	Column temperature	30 °C	65 °C
7.	Maximum backpressure	35 – 40 MPa	103.5 MPa
8.	Total run time	10 min	1.5 min
9.	Plate count	2000	7500
10	USP resolution	3.2	3.4
11	Delay volume	750 µl	110 µl

Table 2: Drugs recently analyzed by UPLC technique

S. No.	Drug	Column specification	Mobile phase Flow rate	R.T (min)	Ref.
1.	Diclofenac(DCL) & Aceclofenac (ACL)	BEH C-18 (2.1 x 50 mm, 1.7µm)	Acetonitrile : water : formic acid (80:20:0.5 v/v/v), 0.2 mL/min	(ACL) 0.70 (DCL) 0.78	7
2.	Streptomycin, Dihydrostreptomycin, Kanamycin, Gentamicin, Cycloserine, Moxifloxacin, Levofloxacin, 4-aminobenzoic acid Clarithromycin, Roxithromycin, Linezolid PAS	HSS T3 (50.0 x 2.1 mm, 1.8 µm)	A. 10 mM ammonium formate in 0.1% formic acid B. Acetonitrile in 0.1% formic acid(A:B) (80:20 v/v) 200 mL/min.	(STN) 0.56 (DST) 0.56 (KAN) 0.51 (GEN) 0.50 (CSL) 0.63 (MOX) 1.90 (LEV) 1.37 (CLA) 2.34 (ROX) 2.35 (LIN) 1.96 (PAS) 1.42 (ABA) 1.26	8
3.	Aspirin(ASP) &Esomeprozole (ESO)	Zorbax XDB (50 x 4.6mm , 1.8µm)	A. 0.2% ortho Phosphoric acid B. Acetonitrile : Methanol (50:50 v/v)0.7 mL/min	(ESO) 2.40 (ASP) 2.80	9
4.	Atovaquone (AQ)	BEH C18 (2.1 x 50 mm, 1.7 µm)	0.1% Formic acid : Methanol (20:80 v/v) 0.2 mL/min	4.99	10
5.	Atropine sulphate	C18 Hiber HR Purospher Star (100 x 2.1 mm, 2 µm)	Phosphate buffer : Acetonitrile (87:13 v/v) pH 3.5 0.5 mL/min	2.76	11
6.	Terbinafinehydrochloride	BEH C-18 (100 x 2.1 mm, 1.7 µm)	pH 7.5 of 0.1% tri-ethyl amine : Acetonitrile (15:85 v/v). 0.4 mL/min	1.47	12
7.	Dihydrodicyano benzoquinone (DDQ)	Zorbax Eclipse Plus C18 (50 x 2.1 mm, 1.8 µm)	A. 0.02 % Trifluoroacetic acid in Water B. 0.02% Trifluoroacetic acid in Acetonitrile 0.5 mL / min	1.22	13
8.	Eprosartan Hydrochlorothiazide	BEH shield RP C18 (2.1 x 100mm, 1.7µm)	Acetonitrile : 0.05M Phosphate buffer (pH 4.5) (35:65 v/v) 0.4ml/min	(HCT) 0.90 (EPR) 2.07	14
9.	Lansoprazole	Phenomenex luna C18 (25cm x 4.6mm, 5µm)	Methanol : Water (80:20 v/v) 1.0 mL/min	3.90	15
10.	Levodopa, Carbidopa&Entacapone	BEH C18 (2.1 x 100mm, 5µm)	Methanol : Acetonitrile : Phosphate buffer pH 2.8 (30:40:30 v/v/v) 0.3 mL/min	(LD) 0.66 (CRD) 1.43 (ENC) 1.96	16
11.	Losartan potassium & Chlorthalidone	HSS C18, (100 mm x 2.1, 1.8 µm)	A. Phosphate buffer pH 3.0 B. Acetonitrile : Methanol (90:10 v/v) C. 0.4 mL/min	(LOS) 0.72 (PTC) 1.89	17
12.	Emtricitabin, Rilpivirine&Tenofovir	BEH C18 (50 x 2.1mm, 1.7µm)	Acetonitrile : Phosphate buffer pH 3 (55:45 v/v), 0.35 mL/min	(ECT)0.60 (TFV)1.05 (RPV)2.95	18

13.	Naproxen (NAP) & Paracetamol (PAR)	C18 thermo fisher (50 x 4.6 mm, 3µm)	0.4% w/v Acetate buffer : Methanol : Acetonitrile (40:40:20 v/v/v) 0.2 µL/min	(PAR) 1.90 (NAP) 3.00	19
14.	Olanzapine	HSS T-3 C18 (100 x 2.1 mm, 1.8µm)	Phosphate buffer : Methanol (60:40 v/v), 0.8 mL/min	2.43	20
15.	Olmesartanmedoxomil	BEH C18 (100 x 2.1 mm, 1.7 µm)	pH 3.4 Buffer : Acetonitrile (60:40% v/v), 0.3 mL/min	3.41	21
16.	Quetiapinefumarate	Eclipse Plus C18, RRHD (50 x 2.1 mm, 1.8 µm)	A. 0.1% Aqueous triethylamine (pH 7.2) B. Methanol : Water (80:20 v/v)		22
17.	Risedronate	BEH C18 (100 x 2.1 mm, 1.7µm)	Methanol : Water (70:30 v/v) 0.8 mL/min	2.29	23
18.	Sumatriptan	Hypersil Gold C18 (4.6 x 150mm, 8µm)	Methanol : Water (90:10 v/v), 500 µL/min	2.36	24
19.	Amitriptyline, Imipramine, Desipramine, Nortriptyline, Doxepin, Trimipramine& Clomipramine	BEH Shield RP (100 x 2.1 mm, 1.7 µm)	Acetronitrile: Phosphate Buffer pH 8 (35:65 v/v) at initial, 55:45 (v/v) at 3 min and 35:65 (v/v) at 5 min. 0.5 mL/min	(DES) 1.47 (NOR) 1.68 (DOX) 2.03 (IPM) 2..26 (AMI) 2.77 (CLO) 3.35 (TRI) 3.62	25
20.	Atenolol	Zorbax-C18, (50x4.6mm, 1.8µm)	Buffer (1.1g of sodium-1-heptanesulfonate and 0.71g of anhydrous dibasic sodium phosphate in 700ml of water. Add 2ml of dibutyl amine; pH to 3.0 with 0.8 M phosphoric acid) : methanol (70:30v/v) 1.0mL/min	1.15	26
21.	JWH-073 N-(4-hydroxybutyl) JWH-073 N-(3-hydroxybutyl) JWH-018 N-(5-hydroxypentyl) JWH-018 5-hydroxyindole JWH-073 JWH-250 JWH-018 JWH-018 4-hydroxyindole HU-210	HSS T3 (50 mm x 2.1 mm, 1.8 mm)	Methanol–ammonium formate 2 mM (0.1% formic acid) methanol/ 2 mM ammonium formate (formic acid 0.1%) (95:5, v/v) (A) and 2 mMammonium formate (formic acid 0.1%)/ methanol (95:5, v/v) 0.4 mL/min.	4.59 5.13 5.34 6.04 6.26 6.26 6.43 6.44	27
22.	Crizotinib	BEH C18 column (50 x 2.1 mm, 1.7 µm)	Methanol : 0.1%*(v/v)* Ammonium hydroxide (80:20 v/v) 0.4 mL/min.	0.46	28
23.	Lamivudine, Abacavir&Zidovudine	BEH Symmetry C18 (2.1 x 100mm, 1.7 µm)	Phosphate Buffer pH3.0: Methanol (60:40 v/v) 0.25 mL/min.	(LAM) 1.01 (ABC) 1.27 (ZID) 1.61	29
24.	Sitagliptin and Simvastatin	BEH Symmetry C18 (2.1 x 100mm, 1.7 µm)	Phosphate Buffer pH 4.0 with TEA : Acetonitrile (30:70 v/v) 0.4 mL/min.	(SIG) 0.50 (SIM) 1.62	30
25.	Methyl Tosylate, ethyl Tosylate and iso-propyl Tosylate impurities in SorafenibTosylate	RRHD Eclipsed Plus C18 (50 x 2.1mm, 1.8µm)	50mM Sodium Perchlorate in water pH 3.0 : Acetonitrile (60:40 v/v) 0.5ml/min	1.93	31
26.	Metformin, glimepiride and pioglitazone	BEH Symmetry C18 (2.1 x 100mm, 1.7 µm)	Phosphate buffer pH 4.3: Methanol (75:25 v/v) 0.25 mL/min.	(MET) 0.00 (GM) 1.77 (PIO) 2.40	32
27.	Carvedilol	Hypersil BDS-C8, (50x4.6mm, 3µm)	Acetate buffer : Methanol (65: 35 v/v) 1.5mL/min	1.28	33

Table 3: Stability indicating UPLC method

S. No.	Drug	Column specification	Mobile phase Flow rate	R.T (min)	Ref.
1.	Thiocolchicoside (TCC) and Aceclofenac (ACF)	Thermo Scientific hypersil gold C18, (50 x 2.1mm, 1.9µm)	5% Ammonium acetate buffer pH 5: Methanol (40:60 v/v) 250 µL/min	(TCC) 0.69 (ACF) 1.12	34
2.	Amisulpride	C18 (100 x 2.1mm, 1.7 µm)	Buffer : Acetonitrile (50:50 v/v) 0.20 mL/min		35
3.	Aprepitant	BEH C18 (50 x 2.1 mm, 1.7µm)	Phosphate buffer : Acetonitrile (50:50 v/v), 0.5 mL/min.	1.20	36
4.	Candesartan celixetil	Zorbax extended C18 (50 x 4.6, 1.8 µm)	A. 0.1% Triethylamine in water pH 2.2 with TFA B. 0.1% TFA in acetonitrile : Water (95:5 v/v) 0.4 mL/min	2.76	37
5.	Amiloride (AML) Hydrochlorothiazide (HCTZ) Metoprolol (MT) Propranolol (PRO) Amlodipine (AB) Felodipine (FEL)	BEH C18 (50 x 2.1 mm, 1.7 µm)	A. Acetate Buffer pH 4.0 B. 90% acetonitrile and 10% buffer 613 µL/min	(AML) 10.17 (HCTZ) 12.80 (MT) 13.37 (PRO) 16.44 (AB) 17.58 (FEL) 24.35	38
6.	Esomeprazole & Naproxen	BEH C18 (100 x 2.1 mm, 1.7 µm)	Phosphate Buffer pH 2.8 : Acetronitrile (40:60 v/v) 0.5 mL/min.	(ESO) 0.7 (NAP) 1.2	39
7.	Finasteride	BEH phenyl (150 x 2.1 mm, 1.7 µm)	A. Phosphate Buffer B. Acetronitrile : Water (90:10 v/v) 0.22 mL/min	6.35	40
8.	Fluticasone furoate (FF) & Benzalkonium chloride (BKC)	BEH C18 (50 x 2.1mm, 1.7 µm)	Phosphate Buffer : Acetonitrile (45:55 v/v), 0.5 mL/min	(FF) 0.91 (BKC) 1.37	41
9.	Folic acid (FA)	C8 (2.1 x 100 mm, 1.7 µm)	Acetonitrile : 0.005 M 1–Hexane Sulfonic Acid Salt pH 2.5 with Phosphoric acid (10:90 v/v) 0.4 mL/min	1.82	42
10.	Imipramine hydrochloride (IMH)	BEH C18 (100 x 2.1 mm, 1.7 µm)	Acetonitrile : Acetate buffer pH-5 (80:20 v/v/v), 0.3 mL/min	2.13	43
11.	Thiocolchicoside (TCC) &Ketoprofen (KTP)	Thermo Scientific hypersil gold C18 (50 x 2.1mm, 1.9µm)	Phosphate buffer pH 3 : Methanol : Acetonitrile (40:52:08 v/v/v) 250 µl/min	(TCC) 0.59 (KTP) 1.01	44
12.	Lacosamide (LCM)	HSS C18 (100 x 2.1mm, 1.8µm)	Phosphate buffer : Acetonitrile (85:15 v/v), 0.7 mL/min	2.15	45
13.	Lafutidine	BEH-shield RP18 (3.0 x 100 mm, 1.7 µm)	A. Phosphate buffer : Acetonitrile (80:20 v/v) B. Phosphate buffer : Acetonitrile (30:70 v/v) 0.5 mL/min	5.04	46
14.	Levofloxacin hemihydrate	BEH C18 (100 x 2.1mm, 1.7 µm)	Acetonitrile : Phosphate buffer pH 2.50 (23 : 77 v/v), 0.400 mL/min		47
15.	Metoclopramide hydrochloride (MTH)	BEH C18 (50 x 2.1 mm, 1.7 µm)	Phosphate buffer: Acetonitrile (86:14 v/v), 0.5 mL/min	1.01	48
16.	Midazolam (MDZ)	BEH C-18 (100 x 2.1mm, 1.7µm)	Acetonitrile : Phosphate buffer pH3.0 (35:65 v/v), 0.3 mL/min	2.26	49
17.	Nilotinib hydrochloride	Shim-pack XR-ODS II (75 x 3.0 mm, 1.8 µm)	A. Phosphate buffer B. Acetonitrile, 0.6 mL/min	3.99	50

18.	Telmisartan	BEH C18 (100 x 2.1 mm, 1.7 µm)	A. Acetate buffer pH 3.9 : Acetonitrile (90:10 v/v) B. Acetonitrile, 0.3 mL/min	4.17	51
19.	Tolterodine Tartrate	BEH C18 (100 x 2.1 mm, 1.7µm)	A. 0.025% TFA (aqueous) buffer B. 0.025% TFA in Acetonitrile 0.30 mL / min	2.48	52
20.	Tramadol hydrochloride (TMH)	BEH C18 (100 x2.1 mm, 1.7 µm)	Phosphate buffer pH 2.8 : Acetonitrile (60:40 v/v), 0.5 mL/min	2.00	53
21..	Dronedarone	BEH C18 (100 x2.1 mm, 1.7 µm)	Phosphate buffer : Methanol (40:60 v/v), 0.4 mL/min	1.55	54
22.	Fluconazole (FLK)	BEH C18 (100 mm× 2.1 mm, 1.7 µm)	Water : Acetonitrile (80:20 v/v) 0.4 mL/min	1.76	55
23.	Carvedilol	Hypersil BDS-C8, (50x4.6mm, 3µm)	Acetate buffer : Methanol (65: 35 v/v), 1.5mL/min	1.28	56
24.	Iloperidone	HSS C18 (2.1 mm × 100 mm, 1.8 µm)	A. Phosphate buffer B. Acetonitrile : Methanol (80:20 v/v), 0.5mL/min	3.82	57

Table 4: Other applications

S. No.	Component analysed	Column	Mobile phase Flow rate	RT	Ref.
1	Sirolimus in human whole blood	BEH C18 (50 mm x 2.1 mm, 1.7 µm)	10 mM ammonium acetate, pH 5.00 with acetic acid and premixed methanol : acetonitrile (60:40, v/v), 0.400 mL/min.	1.01	58
2	Rutin and Quercetin and their metabolites in Mulberry leaf	BEH C18 (100 mm x 2.1 mm, 1.7 µm)	A. water B. Acetonitrile 0.40 mL/min		59
3	Phytohormones in plant tissue	HSS T3 C18 column (1 x 100 mm, particle size 1.8 µm)	A. water B. 90% aq. acetonitrile, both containing 0.1% HCOOH, 0.3 mmol/L NH4CH3COO (adjusted to pH 4.0 with acetic acid), or 0.3 mmol/L NH4HCOO (adjusted to pH 3.5 with formic acid). 150 µL/min		60
4	Simazine Atrazine Isoproturon Diuron Terbutylazine Alachlor Pentachlorophenol Chlorpyrifos Trifluralin	BEH C18 (100 x 2.1 mm, 1.7 µm)	A. 0.1% formic acid in water B. Acetonitrile 0.5 mL/min	SIM 1.91 AZN 2.37 IPT 2.43 DIU 2.45 TBZ 2.92 ACR 3.44 PCL 3.84 CPY 4.49 TFN 4.54	61
5	Metabolomic analysis in *Arabidopsis thaliana*	AccQ•Tag Ultra column (2.1 mm x 100 mm, 1.7 µm)	A. Acetonitrile (10%), formic acid (6%), ammonium formate in water (84%) B. Acetonitrile 0.7 mL/min.		62
6	Coumarins in flowers of horse chestnut (*Aesculushippocastanum*L., *Hippocastanaceae*)	BEH C18 (2.1 mm x 100.0 mm, 1.7 µm)	A. 1% (v/v) HOAc in H2O] B. Acetonitrile 0.75 mL/min		63

CONCLUSION

From the work described above we can conclude the feasibility UPLC in quantization of pharmaceutical dosage forms. UPLC gives increasing the attributes of speed, sensitivity and resolution then HPLC. This paper summarizes basic principle, instrumentation and most useful qualitative and quantitative applications of UPLC.

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