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Review Article

HPLC: A VERSATILE CHROMATOGRAPHIC APPROACH USED FOR QUALITATIVE AND QUANTITATIVE PURPOSES- A REVIEW

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Abstract

High Performance Liquid Chromatography (HPLC) has been used for the analysis of natural and synthetic compounds. This review gives an overview about some special features involving the mode of operation, mobile phase, detector, column and the flow rate of solvent system employed. This review paper provides useful information regarding the identification, separation and quantification of various natural as well as synthetic compounds.

Keywords: - : HPLC, quantification, phytoconstituents, synthetic

Introduction

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/ Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analysing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure.[1] This method is used for checking the purity of new drug candidates, monitoring changes or in process testing for developing new formulations and quality control or assurance of final drug products. HPLC is a dynamic adsorption process[2] and is a separation technique conducted in the liquid phase in which a sample is separated into its constituent components by distributing between the mobile phase and a stationary phase. HPLC utilises a liquid mobile phase to separate the components of a mixture. The stationary phase can be a liquid or a solid phase. These components are first dissolved in a solvent, and then forced to flow through a chromatographic column under a high pressure.[3]

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HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures. Some axioms of HPLC are: Sample must be soluble; for separation, analytes must be retained and have differential migration in the column; the mobile phase controls HPLC separation; the final analyte solution should be prepared in the mobile phase.[4]

In this review, we discussed the role of HPLC in the qualitative and quantitative determination of natural phytoconstituents and synthetic drug molecules.

HPLC EVALUATION FOR PHYTOCONSTITUENTS [5-45]

For quality control of herbal products, high performance liquid chromatography (HPLC) is a popular method for the analysis of herbal medicines because it is accurate, precise and not limited by the volatility or stability of the sample compounds. HPLC combined with diode array detector (HPLC/DAD), electrochemical detection (HPLC-ED), mass spectrometer (HPLC /MS) have been successfully employed in qualitative and quantitative determination of various types phytoconstituents including alkaloids, flavonoids, tannins, glycosides, triterpenes, sterols *etc*. A brief description of HPLC conditions for some phytoconstituents were discussed in table 1.

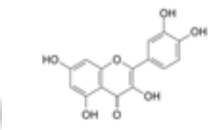
Table 1: HPLC conditions for some phytoconstituents evaluation[5-45]

Sr.no.	Plant and family name	Chemical constituents evaluated	Method	Mobile phase	Column	Detector and wavelength (nm)	Solvent flow rate (mL/min)
1	*Agrimonia eupatoria*, Rosaceae	Procyanidins, kaempferol, 3-*O*-(6-*O*-*p*-coumaroyl)-glucoside and quercetin glycosides	HPLC/DAD/MS	Solvent A- 5% aqueous acetic Solvent B- acetonitrile (2.5%) in aqueous acetic acid in ratio 10:90 Solvent C- acetonitrile	ODS2	Diode array λ_{max} at 280	0.5
2	*Fragaria ananassa*, Rosaceae	Glycosides of quercetin, kaempferol, cyanidin, pelargonidin and ellagic acid, flavanols, derivatives of p-coumaric acidc and ellagitannins	HPLC/DAD/MS	Solvent A- acetic acid/water Ratio: 2:98 v/v Solvent B- acetic acid/acetonitrile/water Ratio: 2:50:48 v/v/v	C-18	Coulometric array λ_{max} at 260, 360 and 500	0.25
3	*Garcinia mangostana*, Guttiferae	α-mangostin	RP-HPLC	Solvent A -0.1 %v/v ortho phosphoric acid Solvent B- acetonitrile	BDS C-18	UV-VIS λ_{max} at 320	1.0
4	*Punica granatum*, Punicaceae	Phytoestrogenic flavonoids anthocyanins and ellagic acid	HPLC	Solvent A -2.5% v/v, solution of acetic acid in water Solvent B -2.5% v/v solution of acetic acid in methanol	RP C-18	UV-VIS λ_{max} at 510	1.0
5	*Capsicum annuum*, Solanaceae	Arbutin	HPLC	Methanol: water Ratio: 90:10	C-18	UV-VIS λ_{max} at 280	0.9
6	*Abutilon indicum*, Malvaceae	Quercetin	RP-HPLC	Solvent A - 0.5% aqeuous solution of Orthophosphoric acid Solvent B -methanol	C-18 ODS	Tunable absorbance λ_{max} 254	1.0
7	*Aesculus hippocastanum*, Hippocastanaceae	Esculin and fraxin	HPLC	Solvent A- acetic acid (1%) Solvent B- methanol Ratio: 84:16 v/v	RP 18	UV λ_{max} at 340	1.0
8	*Alpinia nigra*, Zingiberaceae	Astragalin and kaempferol-3-*O*-glucuronide	HPLC	Solvent A-acetonitrile Solvent B- 0.1 % aqueous acetic acid Ratio: 20: 80	RP 18	UV λ_{max} at 267	1.0
9	*Lonicera edulis*, Caprifoliaceae	4-aminobenzoic acid, rutin, quercitrin, gallic acid	HPLC-ED	Solvent A- methanol 60 % Solvent B- 0.065 M acetic acid 40 % (v/v)	RP C-18	Electrochemical detector	1.0
10	*Michelia alba*, Magnoliaceae	Gallic acid, catechin, rutin ellagic acid and quercetin	HPLC	Solvent A- water-acetic acid (25:1 v/v) Solvent B- methanol	RP C-18	UV λ_{max} at 280	1.0
11	*Vitis* sp. Vitaceae	Anthocyanins	HPLC	Solvent A-1.5% phosphoric acid Solvent B- 1.5% phosphoric acid, 20% acetic acid 25% acetonitrile	C-18	Diode array λ_{max} at 530	1.0
12	*Uncaria tomentosa*, Rubiaceae	Indole alkaloids	HPLC-MS	Solvent A- 30 mili molar ammonium acetate at pH 5 Solvent B- methanol:acetonitrile Ratio: 1:1	RP C-18	Photodiode array	1.0
13	*Terminalia arjuna*, Combretaceae	Arjunic acid, arjunolic acid, arjungenin, arjunetin	HPLC	Acetonitrile:water Ratio: 30:70	ODS2 (RP)	UV-VIS λ_{max} at 220	0.8
14	*Spiraea thunbergii*, Rosaceae	Tulipalin B	HPLC	6% ACN in H_2O	C-18	Photodiode array	0.1
15	*Glycine max*, Fabaceae	Sphingolipids	HPLC	Solvent A- hexane Solvent B - 2-propanol/ethyl acetate/ 88% formic acid Ratio: 50:50:0.5 v/v	Si60	HPLC-ELSD	0.8
16	*Salvia divinorum*, Lamiaceae	Salvinorin A and B	HPLC	Acetonitrile: water Ratio: 1:1 v/v	C-18	UV λ_{max} at 210	0.1
17	*Rubus glaucus, R. adenotrichus*, Rosaceae	Ellagitannins,anthocyanins	HPLC/DAD	Solvent A- 2% aqueous formic acid Solvent B- acetonitrile/water/formic acid Ratio: 80:18:2 v/v/v	ODS-2	Diode array λ_{max} at 200 and 600	0.5
18	*Pseudolarix kaempferi*, Pinaceae	Pseudolaric acids A and B	HPLC	Solvent A- acetonitrile Solvent B-1% aqueous acetic acid, in which acetonitrile was linearly changed from 30% to 60% in 30 min	RP-C18	UV λ_{max} at 260	0.1
19	*Prunus serotina*, Rosaceae,	Flavonoid	RP-HPLC	Solvent A- 0.5% aqueous solution of orthophosphoric acid Solvent B-MeOH	ODS-C-18	UV–VIS λ_{max} at 370	2.0
20	*Prunus cerasus*, Rosaceae	Melatonin	HPLC	0.1 M potassium phosphate buffer with acetonitrile (20%)	OD53	Eight-channel Coul array	0.1
21	*Phyllanthus urinaria, P. virgatus, P.maderaspatensis P. debilis* Euphorbiaceae	Phyllanthin and hypophyllanthin	HPLC	Methanol:water Ratio: 70:30	S10 ODS2	Photodiode array λ_{max} at 220	0.7
22	*Passiflora caerulea, P. incarnate*, Passifloraceae	Flavonoids	HPLC	Methanol water Ratio: 1:1	C-18	UV-VIS λ_{max} at 340	1.0
23	*Sanguinaria canadensis , Dicranostigma lactucoides*, Papaveraceae	Benzo[c] phenanthridine alkaloids	HPLC	Solvent A- 25% acetonitrile Solvent B- 60% acetonitrile (v/v)	C-12	Diode array λ_{max} at 180–550	0.5
24	*Opuntia* sp., Cactaceae	Betaxanthin and betacyanin	HPLC, HPLC-MS	Solvent A- water Solvent B- methanol	RP C-18	Diode array λ_{max} at 482 and 535	1.0
25	*Olea europea*, Oleaceae	Organic acids (oxalic, citric, malic and succinic)	HPLC	0.1% (w/v) phosphoric acid in distilled water	KC-118	UV λ_{max} at 214	0.8
26	*Ocimum spp.* Lamiaceae	Ursolic acid	HPLC	Solvent A- acetonitrile Solvent B- 1.25% H_3PO_4 Ratio: 86:14 (v/v)	RP-ODS	Photodiode array λ_{max} at 206	0.5

27	*Nothapodytes foetida*, Icacinaceae	Camptothecinoids	HPLC	Acetonitrile/H_2O Ratio: 25:75 v/v	C-18	UV λ_{max} at 970	1.0
28	*Mitracarpus scaber*, Rubiaceae	Triterpenic acids	HPLC	Acetonitrile/H_2O Ratio: 85:15 v/v	ODC	UV λ_{max} at 215	0.6
29	*Mammea americana*, Clusiaceae	Coumarins, mammea	HPLC	H_2O:TFA:MeOH Ratio: 15:0.05:84.95 v/v/v	C-18	UV λ_{max} at 290	0.8
30	*Solanum lycopersicum*, Solanaceae	Lycopene	RP-HPLC	Solvent A- acetonitrile Solvent B- 1:1:1 mixture of methanol, hexane and methylene chloride	C-18	Diode array λ_{max} at 471	0.8
31	*Gmelina arborea*, Verbenaceae	Apigenin	RP-HPLC	Acetonitrile and distilled water in volume ratio of 45:55	ODS-3V-C18	UV-970 λ_{max} at 340	1.0
32	*Garcinia parvifolia*, Guttiferae	Mangostin	RP-HPLC	Methanol and water Ratio: 95:5 %v/v	RP C-18	UV λ_{max} at 319	1.0
33	*Cassia alata*, Leguminosae	Kaempferol-3-O-gentiobioside	HPLC	Solvent A- 1.25% aq AcOHy Solvent B- MeCN Ratio: 4:1	SPD	ODS-A 349	0.8
34	*Alpinia nigra*, Zingiberaceae	Kaempferol-3-O-glucuronide	HPLC	Solvent A- acetonitrile Solvent B- 0.1 % aqueous acetic acid Ratio: 20: 80	RP C-18	UV λ_{max} at 267	1.0
35	*Caesalpinia pulcherrima*, Caesalpiniaceae	Gallic acid, catechin, rutin, ellagic acid and quercetin	HPLC	Water-acetic acid Ratio: 25:1 v/v	RP C-18	UV λ_{max} at 280	1.0
36	*Diospyros kaki*, Ebenaceae	Barbinervic acid, Rotungenic acid	HPLC	Solvent A- methanol Solvent B - 0.1% aqueous H_3PO_4 Ratio: 80:20 v/v	SB-C-18	UV 210	1.0
37	*Chrysophyllum roxburghii*, Sapotaceae	Carotenoids	HPLC	Acetonitrile, methanol and ethyl acetate containing 0.05% of triethylamine	C-18	Photodiode array λ_{max} at 300-600	0.5
38	*Eucalyptus polyanthemos*, Myrtaceae	Sideroxylonals	RP-HPLC	Acetonitrile: water containing 0.1% trifluroacetic acid	C-18 RS	Photodiode array λ_{max} at 275	0.75
39	*Malus pumila*, Rosaceae	Anthocyanin	HPLC	Water : methanol	C-18	Photodiode array λ_{max} at 520	1.0
40	*Euphoria longana*, Sapindaceae	Gallic and ellagic acid	HPLC	Solvent A- 0.4% formic acid Solvent B- methanol	RP-18	UV λ_{max} at 270	1.0
41	*Enterophospora infrequens*, Entrophosporaceae	Camptothecin	RP-HPLC	Water and acetonitrile	RP-18	Diode array λ_{max} at 256	0.5

PHYTOCONSTITUENTS EVALUATED *VIA* HPLC[5-45]

Large number of phytoconstituents were quantified *via* HPLC technique for example: flavonoids (quercetin, kaempferol, cyanidin); flavonoid glycosides (rutin); tannins (ellagic acid, ellagitannins); alkaloids (camptothecin, phyllanthin); polyphenols (arbutin); coumarins; triterpenes (ursolic acid, arjunolic acid); carotenoids (lycopene); sphingolipids *etc*. Some of reported quantified phytoconstituents were shown in figure 1.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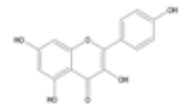
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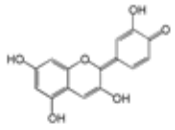
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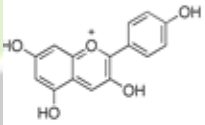
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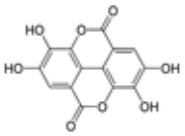
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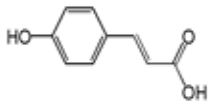
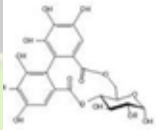
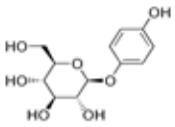
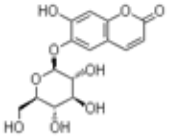

Coumaric acid


Ellagitannins


Arbutin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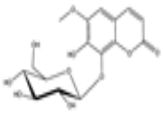
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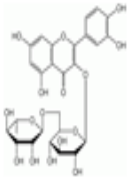
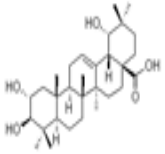
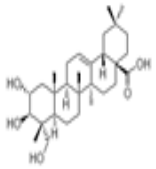
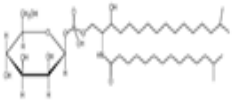
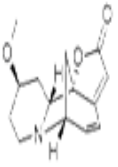

Rutin


Arjunic acid


Arjunolic acid


Sphingolipids


Phyllanthin

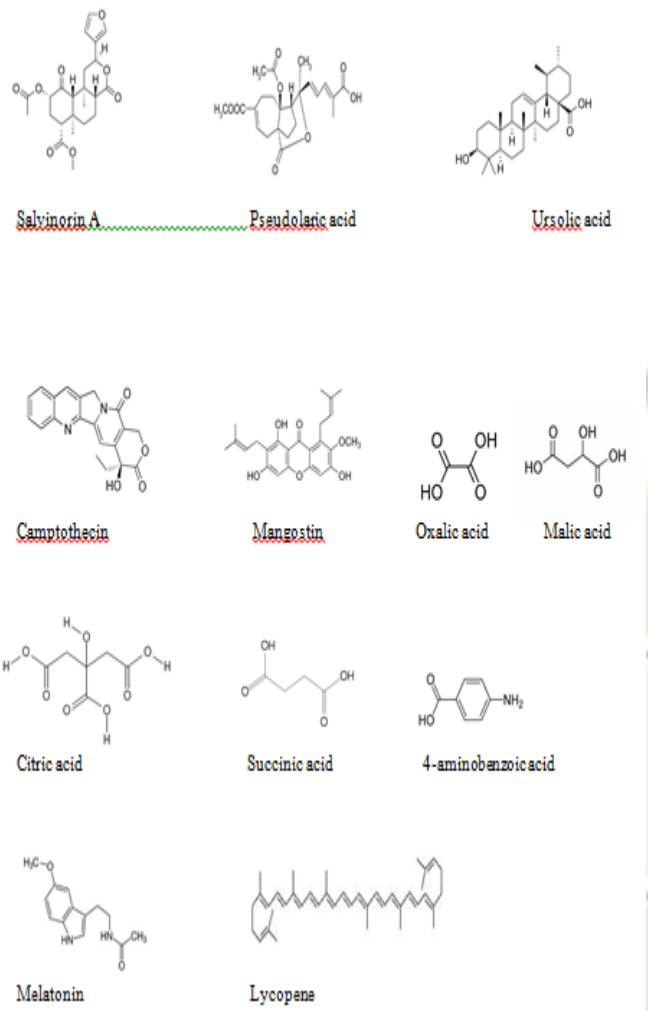


Figure 1: Phytoconstituents quantified *via* HPLC

HPLC EVALUATION FOR SYNTHETIC DRUGS [46-86]

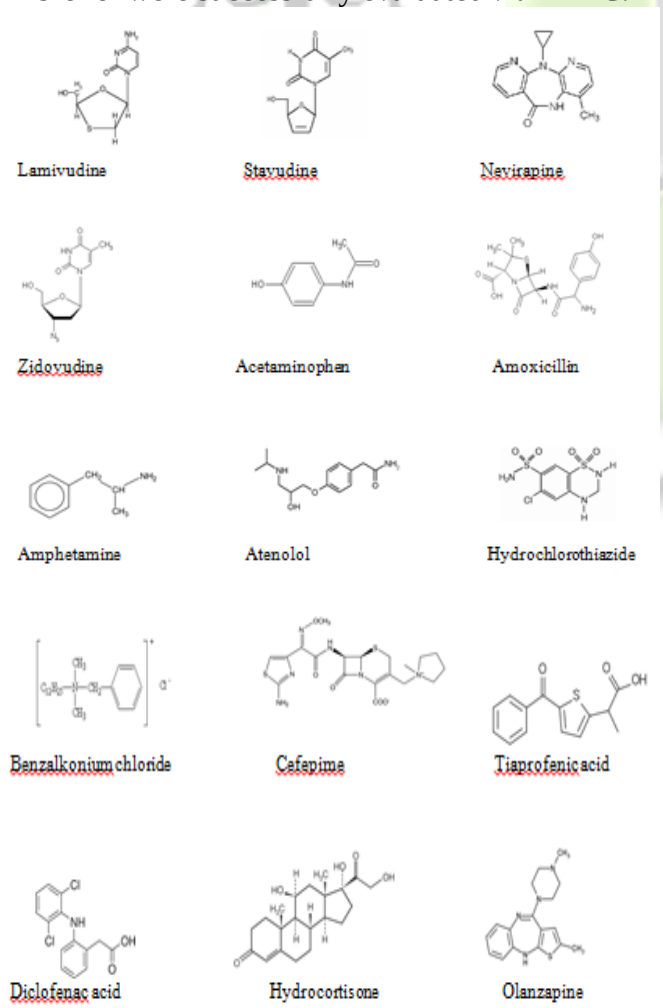
HPLC methods have been used in the determination of drugs in biological fluids and in pharmaceutical dosage forms. HPLC determination method with spectroscopic detection is useful for routine quality control of drugs in pharmaceutical formulations and stability studies. Various types of detectors: photo diode array, ultraviolet (UV), ultraviolet visible (UV-VIS), fluorometric, fluorescence have been employed along with different types of columns (C-8, RP-8, RP C-18, SB-CN, ODS-2) depending upon the synthetic drug to be evaluated. Some of reported synthetic drug molecules evaluated *via* HPLC were shown in table 2 given below.

Sr.no.	Drug	Drug category	Method	Mobile phase	Column	Detector and wavelength (nm)	Flow rate (mL/min)
1	Lamivudine, stavudine, nevirapine, zidovudine	Antiviral	RP-HPLC	Solvent A-50 mM NaH_2PO_4 at pH 3.8 Solvent B- acetonitrile Ratio: 95:5 to 45:55 v/v	C-8	Photo diode array	0.5 to 1.0
2	4-bromo-2,5-dimethoxyphenethylamine	Hallucinogenic	HPLC	Acetonitrile/Potassium phosphate (pH 3.2)	RP-8	Diode array	1.0
3	Acetaminophen	NSAID	RP-HPLC	Mobile phase was prepared by adding 330 ml of methanol to 660 ml of the water pH 3.0 with 10% orthophosphoric acid	C-18	Photodiode array λ_{max} at 193.3	1.78
4	Amoxicillin	Antibiotic	RP-HPLC	Solvent A-95% phosphate buffer (0.01mol/L) pH4.8 Solvent B- 5% acetonitrile mixture	RP C-18	UV229	1.3
5	Amphetamine	CNS stimulant	RP-HPLC	Solvent A-aqueous orthophosphoric acid (pH 2.1) Solvent B-acetonitrile Ratio: 90:10 v/v	C-8, C-18	Diode-array UV λ_{max} at 205	1.5
6	Atenolol	Beta blocker	RP-HPLC	Water: Buffer: Methanol Ratio: 50:35:15	Zorbax SB-CN	UV-VIS λ_{max} at 286	1.2
7	Hydrochlorothiazide	Diuretic	RP-HPLC	Water: Buffer: Methanol Ratio: 50:35:15	Zorbax SB-CN	UV-VIS λ_{max} at 286	1.2
8	Benzalkonium chloride	Preservative	HPLC	Solvent A- potassium dihydrogen orthophosphate buffer (pH 5.5) Solvent B- acetonitrile Ratio: 40:60 v/v	Waters Spherisor CN	UV λ_{max} at 210	1.0
9	Cephalosporins (Cefepime)	Antibiotic	HPLC	Solvent A- 90/10 (5 mM potassium phosphate/ Acetonitrile) Solvent B- 50/50 (5 mM potassium phosphate/ Acetonitrile)	C-18	UV λ_{max} at 254	0.2
10	Ceftriaxone	Antibiotic	RP-HPLC	Methanol:water:acetonitrile Ratio: 80:15:5 v/v/v	C-18	UV-VIS λ_{max} at 270	1.0
11	Tiaprofenic acid, flurbiprofen, diclofenac acid, mefenamic acid	NSAID	RP-HPLC	Methanol:water:acetonitrile Ratio: 80:15:5 v/v/v	C-18	UV-VIS λ_{max} at 270	1.0
12	Hydrocortisone, corticosterone	Corticosteroids	RP-HPLC	Solvent A- water Solvent B- 50% acetonitrile, 17% methanol, 33% isopropanol	C-18	Diode-array	1.0
13	Lercanidipine	Antianginal	RP-HPLC	Solvent A- acetonitrile and an aqueous solution of 1.5% triethylamine, pH adjusted to 3.0 Solvent B- orthophosphoric acid Ratio: 35:65	C-18	UV-VIS λ_{max} at 240	1.0

14	Olanzapine	Atypical antipsychotic	RP-HPLC	KH_2PO_4-methanol-acetonitrile Ratio: 1:1:1	ODS C-18	UV λ_{max} at 271	1.0
15	Rifampicin	Antibiotic	HPLC	Solvent A- 0.05 M potassium dihydrogen phosphate buffer at pH 2.6 Solvent B- acetonitrile Ratio: 55:45 v/v	C-18	Diode array λ_{max} at 254	1.2
16	Phenylbutazone, oxyphenbutazone	NSAID	HPLC	Solvent A- acetonitrile Solvent B- 0.1 M acetic acid	ODS-2	UV-VIS λ_{max} at 240	1.2
17	Paracetamol, acetaminophen, dipyrone	NSAID	HPLC	0.01 M KH_2PO_4-methanol-acetonitrile-isopropyl alcohol Ratio: 420: 20: 30: 30	C-8	UV-VIS λ_{max} at 215	1.0
18	Caffeine	CNS stimulant	HPLC	0.01 M KH_2PO_4-methanol-acetonitrile-isopropyl alcohol Ratio: 420: 20: 30: 30	C-8	UV-VIS λ_{max} at 215	1.0
19	Norfloxacin	Antibiotic	RP-HPLC	Solvent A- 20 mM sodium hydrogen phosphate buffer pH 3.0 Solvent B- acetonitrile Ratio: 88:12 v/v	C-18	UV-VIS λ_{max} at 280	-
20	Nitroxazepine	Antidepressant	RP-HPLC	Phosphate buffer : Acetonitrile Ratio: 70:30	C-18	UV λ_{max} at 265	1.0
21	Milnacipran	Antidepressant	RP-HPLC	Phosphate buffer: acetonitrile Ratio: 72:28 v/v	C-18	UV and spectrofluorometric λ_{max} at 220	1.0
22	Methotrexate	Anticancer	HPLC	Phosphate-citrate buffer (pH 4)- methanol Ratio: 30:70 v/v	RP-8	UV λ_{max} at 342	-
23	Meloxicam	NSAID	HPLC	Solvent A- Phosphate buffer (0.2 N) Solvent B- acetonitrile Ratio: 38:62 v/v	RP C-18	UV λ_{max} at 352	0.5
24	Flutamide	Antiandrogenic	HPLC	Acetonitrile:tetrahydrofuran(THF):water Ratio: 31.5:2.5:66	C-18	UV λ_{max} at 300	1.0
25	Prednisone, betamethasone, flumethasone	Corticosteroids	HPLC	Acetonitrile:water Ratio: 80:20 to 50:50	C-18	UV	1.0
26	Dexamethasone	Corticosteroids	RP-HPLC	Methanol : water Ratio: 50:50	C-18	UV λ_{max} at 254	1.0
27	Chlorzoxazone	Skeletal muscle relaxant	RP-HPLC	Acetonitrile and double distilled water Ratio: 60:40	RP-C-8	UV λ_{max} at 230	1.0
28	Raloxifene	Estrogen receptor modulator	RP-HPLC	Acetonitrile and phosphate buffer Ratio: 30:70 v/v	C-18	UV λ_{max} at 280	1.0
29	Azathioprine	Immunomodulator	RP-HPLC	Acetonitrile:water:methanol Ratio: 25:70:05	RP C-18	UV-VIS λ_{max} at 280	1.0
30	Nebivolol	Beta blocker	RP-HPLC	Methanol-water Ratio: 70:30 v/v	C-18	UV λ_{max} at 282	1.0
31	Gliclazide	Anti-diabetic	HPLC	Acetonitrile and water Ratio: 49:51 v/v pH 2.8)	C18	UV	1.2
32	Cetirizine	Anti-allergic	HPLC	Acetonitrile-water Ratio: 1:1	C-18	UV VIS λ_{max} at 205	2.0
33	Quetiapine	Antipsychotic	HPLC-MS/MS	Methanol/acetonitrile and ammonium acetate buffer 0.01M, pH3.5 Ratio: 31:19:50 v/v/v	C-18	UV	0.4
34	Amlodipine	Antianginal	RP-HPLC	Acetonitrile: potassium dihydrogen phosphate buffer (0.02M, pH 3.0) Ratio: 56:44 v/v	RP C-18	UV λ_{max} at 234	1.0
35	Pantoprazole, Lansoprazole	Proton-pump inhibitor	RP-HPLC	Acetonitrile: phosphate buffer at pH 7.0 Ratio: 60:40 v/v	C-18	UV λ_{max} at 230	1.0
36	Itraconazole	Antifungal	HPLC	Methanol (75% v/v) and water (25% v/v)	C-18	Fluorometric λ_{max} at 250	1.0
37	Alfuzosin	Anti-hypertensive	HPLC	25 % v/v acetonitrile and water 75 % v/v	C-18	Fluorescence λ_{max} at 265- 380	0.5
38	Trimethoprim	Antibiotic	HPLC	Mixture of acetonitrile and 0.5% triethylamine in 1% acetic acid, pH 3 Ratio: 18:82 v/v	C-18	UV λ_{max} at 271	1.5
39	Zidovudine	Anti- hiv	RP-HPLC	Orthophosphoric acid : acetonitrile Ratio: 73:27 v/v	C-18	UV, photo diode array λ_{max} at 246	0.9
40	Nicorandil	Antianginal	HPLC	Methanol and 0.002 mol/L phosphate buffer (pH 7.0) Ratio: 3:7 v/v	ODS C-18	diode array λ_{max} at 240	1.0
41	Ambroxol	Secretolytic	HPLC	Acetonitrile: methanol : 0.5% ammonium acetate (pH 5) Ratio: 44:16:40	C-18	UV λ_{max} at 295	0.8
42	Metformin	Antidiabetic	RP-HPLC	MIxture of Acetonitrile and Ammonium Acetate buffer (pH-3) Ratio: 42: 58	RP- C-18	UV-VIS λ_{max} at 255	0.3
43	Sodium ozagrel	Antiinflammatory	HPLC	Sodium phosphate buffer (0.05 mol/l, pH 3.0) and acetonitrile Ratio: 94: 6 v/v for plasma Ratio: 96: 4 v/v for urine	C-18	Photodiode array λ_{max} at 276	1.5
44	Ketamine	Veterinary anesthetic	HPLC	Acetonitrile:0.03 mol/L phosphate buffer pH (7.2) Ratio: 23:77 v/v	C-18	Photodiode array λ_{max} at 210	1.5

SYNTHETIC MOLECULES EVALUATED *VIA* HPLC [46-86]

Synthetic molecules were successfully evaluated both qualitatively as well as quantitatively *via* HPLC as shown in figure 2. Several pharmaceutically important Antiviral drugs: lamivudine, stavudine, nevirapine, zidovudine; NSAIDS: acetaminophen, meloxicam, paracetamol, phenylbutazone; Antibiotics: amoxicillin, cefepime, rifampicin; CNS stimulants: amphetamine; Beta blockers: atenolol; Preservatives: benzalkonium chloride; Diuretics: hydrochlorothiazide; Antidiabetics: metformin; Anticancer: methotrexate; Corticosteroids: prednisone, betamethasone, dexamethasone; Antianginal: Lercanidipine, Nicorandil; Immunomodulator : azathioprine; Anti-allergic: cetirizine; Antidepressant: Nitroxazepine, milnacipran; Veterinary anesthetic: Ketamine; Anti-inflammatory: sodium ozagrel; Secretolytic: Ambroxol were successfully evaluated *via* HPLC.



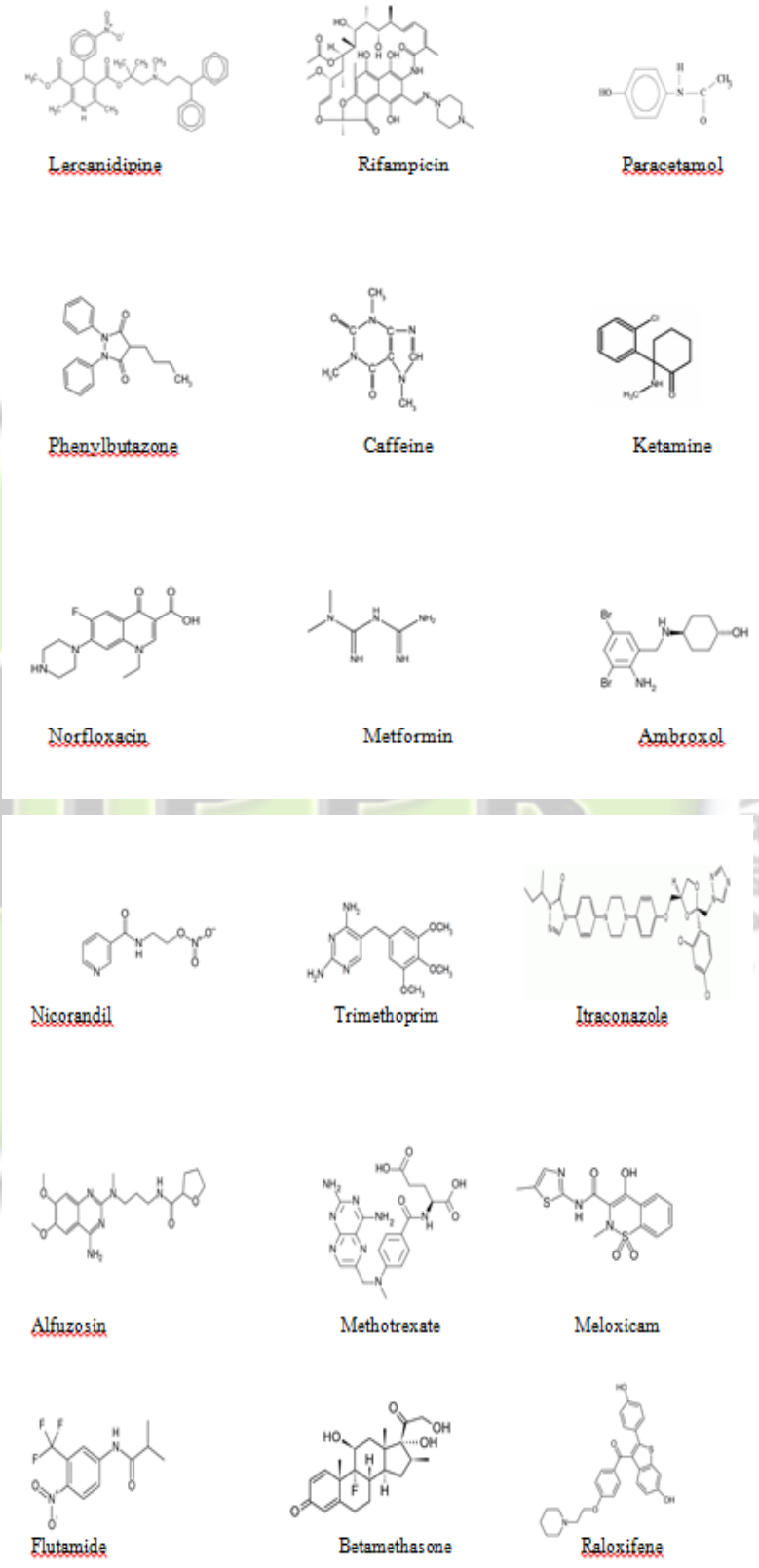


Figure 2: Synthetic drug molecules evaluated *via* HPLC

CONCLUSION

HPLC is one mode of chromatography, one of the most used analytical techniques. HPLC applications can be used effectively for screening analysis as well as quality evaluation of natural as well as synthetic compounds. Owing to the simplicity and efficiency of HPLC specific and rapid determination of various natural and synthetic compounds can be carried out. HPLC can be employed for the routine analysis of natural and synthetic compounds in pharmaceutical formulations and in bulk drug preparations as well as for the quality assurance of related extracts and market samples. Interest in HPLC has increased with improvements in its instrumentation and methods and especially in the last few years, with the combination of hyphenated techniques like HPLC–MS/MS, HPLC/DAD/MS, HPLC-ED.

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