Review Article

Selected HPLC Applications - Quick Separation Guide: A Review

Ravi Sankar P*, Madhuri B, Naga Lakshmi A, Pooja A, Bhargava Sai M, Suresh K, Srinivasa Babu P
Department of pharmaceutical analysis, Vignan Pharmacy College, Vadlamudi, Guntur, Andhra Pradesh, India.
***Corresponding author's E-mail:** banuman35@gmail.com

Received: 15-12-2019; **Revised:** 21-01-2020; **Accepted:** 27-01-2020.

ABSTRACT

HPLC is one of the main useful analytical techniques among all the different chromatographic methods. HPLC is a versatile, safest, and fastest chromatographic technique for quality control of drug components. HPLC is a method for the separation of various elements in plant extracts that resemble a specific and sensitive process. HPLC is required to demonstrate the specificity of stability indicating1 methods and also gives an insight into degradation pathways and degradation products of the drug substance and helps in the education of the structure of the degradation products. HPLC is checking the purity of a compound, presence of impurities, estimation of a mixture of drugs, isolation of identification of drugs, isolation of identification of mixture. High-performance liquid chromatography used to biogenic substances, medical products, food products, environmental samples, and organic industrial products. The essential advantage of the proposed methods is that drugs determined on a single chromatographic system without minor modification in detection wavelengths and mobile phase composition. Even though a combination of these active ingredients would not usually be present in the same tablet formulation, it could provide a useful method for laboratories involved in the routine analysis of these drugs. This review article gives an overview of the essential analysis parameters of a vast number of combinations of pharmaceutical formulations. As a matter of fact, in the current review article, concentrate vital information includes the drug name, the stationary phase, the mobile phase, the mobile phase composition, flow rate and the detector wavelength. These HPLC application separations quick reference guide points out starting points for chromatographic separations also suggest trends in the usage of columns, mobile phase, and detectors, which will both motivate and inform lab's progress and achievement in the analysis.

Keywords: HPLC application quick guide, Pharmaceutical quality control, Stationary phase, Pharmaceutical combination formulations, Validation.

INTRODUCTION

In the modern pharmaceutical industry, HPLC is the vital and integral analytical tool applied in all stages of drug discovery, development, and production. High-performance–liquid chromatography the separation of analytes is fundamental to the differences in the analyte affinity for the stationary phase. The separation of analyte mixtures in modern HPLC is performed in the device called the column. The column is the heart of the chromatography system, and it is the only device where the actual separation of the analyte mixtures takes place. Current HPLC columns, in most cases, are a stainless steel tube packed with very small (1-5 µm) particles of rigid porous materials. HPLC is more related to instrument optimization, column dimensions, and particle geometry-factors that could not have continuous variation during method development[3] except for the small influence from the change of the mobile phase flow rate. The quality of an HPLC column is a subjective factor, which is dependent on the types of analytes and even on the chromatographic conditions used for the evaluation of the overall quality. Long–term column stability (pH and temperature) and batch-to-batch reproducibility are probably the most crucial quality characteristics to be considered in the column selection in the pharmaceutical industry. HPLC for the higher-resolution separation based on molecular weight and hydrophobicity. Approximately 80 % of all divorces are done on 5-10 µm reverse phase C_{18} silica columns. Phase, description, application are tabulated in Table 1. Table 2 shows the essential pharmaceutical applications with HPLC. They are listed according to the combination of drugs. These separations are intended as a guide. Some times conditions will vary from compound to compound and from column to column. Table 3 shows the HPLC applications - Personal separation guide.

Table 1: Application summary

Phase	Description	Application
C_{18}	Optimized for maximum efficiency, superior peak shape, and resolution. Utilizes the same ultra-high purity silica as all ACE phases.	A C_{18} phase for most HPLC applications. Available in a range of particle sizes, from LC/MS and microbore applications through to preparative scale separations.
C_8	Increased bonding density compared to ACE C_{18}. Similarly optimized for maximum efficiency, superior peak shape, and resolution.	Recommended starting point for method development. Also suited to high aqueous conditions and for rapid analysis applications.

C_4	Combines lower hydrophobicity with excellent chromatographic performance. Improved hydrolytic stability and reproducibility compared to conventional C_4 phases.	Use for rapid analysis optimization, when less retention than C_8 or C_{18} is required. Also suitable for the analysis of small proteins.
CN	Suitable use in both normal- and reversed-phase modes much-improved performance, stability, and reproducibility compared to conventional CN phases.	Use to increase the retention of polar compounds — ideal for gradients and rapid screening applications due to fast equilibration capabilities.
PH	Hydrophobicity between C_4 and C_8 phases, with increased polar selectivity. Improved performance, stability and reproducibility compared to conventional phenyl phases.	It offers alternative selectivity for aromatic amine or polar compounds.

Table 2: Selected HPLC quick separation and application guide

Drug	Column	Mobile phase	Flow rate	Detector (nm)
Antibiotics: 15 Quinolones & Fluoroquinolones: Pipemidic acid, Marbofloxacin, Enoxacin, Ofloxacin, Norfloxacin, etc.	C_{18}	Methanol –Acetonitrile – 10 mM Citrate buffer pH- 3.5	1.5 ml\min	UV 280
5- Quinolones antibiotics Nalidixic acid, Norfloxacin, Ofloxacin, Ciprofloxacin& Lomefloxacin	C_{18}	Acetonitrile with tetrabutylammonium acetate, Sodium dodecyl sulfate and citric acid pH-3.4	1ml\min	UV 235
Isoniazid , Rifampicin[4] Pyrazinamide	ODS	Methanol-tetra butyl ammonium hydroxide, pH 3.0 (80:20 v\v)	1 .5ml\min	UV 265
Pyrimethamine, Sulphadoxine[5]	C_{18}	Methanol-Water (50:50,v\v) 2% acetic acid	1.0 ml\min	UV 221
Trimethoprim, Nitrofurantoin	C_{18}	Water- Acetonitrile Triethylamine (65:35:0.1v\v) pH- 6.8 with dilute orthophosphoric acid	1ml\min	UV 270
Amoxicillin trihydrate, Probenecid[6]	C_{18}	0.05Mpotassium dihydrogen phosphate buffer pH- 5.6 adjusted with a mixture of trifluoroacetic acid and Tetrahydrofuran: acetonitrile	0.8ml\min	UV 227
Levofloxacin, Prulifloxacin, Gatifloxacin, Sparfloxacin, Moxifloxacin, Balofloxacin	C_{18}	10mM phosphate buffer pH- 3.1 : Acetonitrile 70:30 v\v	1ml\min	UV 293
Cardiovascular system: Atorvastatin calcium[7] Amlodipine besylate	C_{18}	0.02 M KH_2PO_4- Acetonitrile MeOH (30:10:60 v\v), pH 4.0 with 0.1M O- H_3PO_4	1ml\min	UV 240
Atenolol hydrochloride, Metoprolol succinate Hydrochlorothiazide	C_{18}	10mM Phosphate buffer pH-3.0 adjusted with triethylamine : acetonitrile (50:50 v\v)	1.0 ml\min	UV 235
Telmisartan, Losartan, Valsartan, Olmesartan,[8] Irbesartan, Atenolol	C_{18}	Phosphate buffer with pH-3.3 and acetonitrile 50:50 % v\v	1ml\min	UV 230
Hydrochlorothiazide, Nebivolol hydrochloride[9]	C_{18}	0.1M Sodium phosphate buffer (pH2.5 adjusted with ortho phosphoric acid):Methanol [25:75 v/v	1ml\min	UV 284
Telmisartan, Ramipril[10]	C_{18}	0.01 M potassium dihydrogen phosphate buffer (adjusted to pH 3.4 using orthophosphoric acid): methanol:	1.0 ml\min	UV 210

		acetonitrile (15:15:70 v/v/v)		
Atenolol, Amlodipine[11]	C_{18}	10mM phosphate buffer pH-3.0 : acetonitrile (50:50 v\v)	1.0 ml\min	UV 235
Musculoskeletal Disorders: Paracetamol, Aceclofenac	C_{18}	20mM phosphate buffer, pH – 5.0- Acetonitrile(40:60v\v)	0.8 ml\min	UV 265
Paracetamol, Valdecoxib	C_{18}	20mM octane sulphonic acid pH 3.0 with O- H_3PO_4 – acetonitrile (50:50v\v)	1.0 ml\min	UV 250
Paracetamol, Etoricoxib	C_{18}	Acetonitrile – methanol- water (60:15:25 v\v)	1.0 ml\min	UV 236
Paracetamol, Piroxicam	C_{18}	MeOH- Water pH 4.0 with formic acid (70:30v\v)	1.0 ml\min	UV 227
Paracetamol, Diclofenac[12] sodium, Chlorzoxazone	C_{18}	Phosphate buffer (pH- 6.65) : acetonitrile (60:40 % v\v)	1.0 ml\min	UV 280
Tizanidine, Aceclofenac, Chlorzoxazone,[13] Paracetamol	C_{18}	Phosphate buffer (pH-6.65): Acetonitrile(60:40 % v\v)	1.0 ml\min	UV 280
Alimentary system : Rabeprazole, Itopride	C_{18}	Acetonitrile – phosphate buffer, pH- 7.2 (50:50 v\v)	1ml\min	UV 268
Omeprazole, Domperidone	C_{18}	0.1 M ammonium acetate buffer- methanol (85:15 v\v)	1.5 ml\min	UV- 302
Pantoprazole sodium, Mosapride citrate[14]	C_{18}	Buffer pH -5.0 – Acetonitrile 1.4 ml of orthophosphoric acid in 500 ml of water, pH adjusted to 5.0 with TEA.	1ml\min	UV 276
Gatifloxacin, Ornidazole	C_{18}	Acetonitrile – methanol-water	1.0 ml\min	UV 280
Levofloxacin, Ornidazole	C_{18}	0.05 M KH_2PO_4 buffer pH 6.8 –methanol-acetonitrile	1.5 ml\min	UV -295
Respiratory system : Dextromethorphan hydrobromide, Phenylephrine HCL	ODS -80	Phosphate buffer, pH- 4.7-acetonitrile (40:60v\v), containing 5mM heptane sulphonic acid Sodium, pH-adjusted to2.7 with acetic acid	2.0 ml\min	UV-214
Bromhexine HCL, Amoxicillin	C_{18}	methanol and glacial acetic acid (50:50 v/v)	1.0 ml/min	UV 254
Central nervous system: Fluoxetine, Olanzapine	C_{18}	Buffer – Acetonitrile- glacial acetic acid (55:44:1v\v)	1.0 ml\min	UV 230
Alprazolam, Fluoxetine HCL	C_{18}	Acetonitrile- water pH - 2.75 with 0.1 % H_3PO_4 (75:25 v\v)	1.0 ml\min	UV- 235
Escitalopram oxalate, Clonazepam[15]	C_{18}	buffer and acetonitrile in a ratio of (50:50 v/v)	1.0 ml\min	UV- 240
Analgesic drugs: Antipyrine[16], Hydroxy antipyrine, Acetaminophen	C_{18}	0.0 5M KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 204
Anti viral drugs : Acyclovir[17]	C_{18}	0.1 % phosphoric acid and methanol, acetonitrile	0.8 ml\min	UV 254
Androgen drugs: Testosterone, Testosterone Propionate[18]	ODS	Water\Acetonitrile	1.0 ml\min	UV254,234
Antianginal drugs: Verapamil[19]	C_{18}	0.025 M KH_2PO_4 pH – 3\ Acetonitrile	0.6 ml\min	FLD, 228\312
Antiarrhythmic drugs : Quinidine, Disopyramide Procainamide[20]	RP - 18	0.05 KH_2PO_4 pH – 2.5\ Acetonitrile	1.0 ml\min	UV220 nm, 254 nm, 204 nm
Antiasthmatic drugs: Caffeine, Theophylline	C_8	water-THF (0.1% THF in water, pH 8) acetonitrile (90:10, v/v)	0.8 ml\min	UV 273

Theobromine[21]				
Antibacterial drugs : **Penicillin like**: Ampicillin, Amoxicillin,[22] Penicillin–G, Penicillin – V	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	0.8 ml\min	UV 204 nm
Tetracyclines: Minocycline,[23] Tetracycline Doxycycline	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	0.5 ml\min	UV 350 nm
Miscellaneous : Hydroxy benzotriazole Chloroamphenicol[24] Trimethoprim, Sulfamethoxazole Furazolidone,	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 204 nm, 368 nm, 254 nm
Anticoagulant drugs : Warfarin[25]	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	204 nm, FLD 272\355
Anti-convulsants: Valproic acid,[26] Phenytoin	C_{18}	acetonitrile and 0.05 M phosphate buffer (pH 3.0) 45:55 v/v	1.0 ml\min	UV 360 nm to 210 nm
Anti depressant drugs Bupropion [27], Trazodone Maprotiline, Fluoxetine	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 210 nm
Antiepileptic drugs: [28] Caffeine, Phenytoin Methylphenylsuccinide	ODS	Water\Acetonitrile	1.0 ml\min	UV 204 nm
Antiestrogen drugs: Tamoxifen	RP- 18	0.025 KH_2PO_4 pH – 3\ Acetonitrile	0.7 ml\min	UV 204nm
Anti -histaminic drugs Tetracaine, Promethazine[29] Chlorpheniramine	C_{18}	0.025 KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 204 nm
Antihypertensive drugs: Enalapril, Captopril[30]	C_{18}	0.025M KH_2PO_4 pH – 2\ Acetonitrile	1 ml\min	UV 204 nm
Anti-inflammatory drugs Naproxen[31]	C_{18}	0.025M KH_2PO_4 pH – 3\ Acetonitrile	1ml\min	UV 230 nm
Antiprotozoal drugs: Metronidazole[32]	C_{18}	0.025M KH_2PO_4 pH – 3\ Acetonitrile	1.2 ml\min	UV 320nm
Antitumor drugs: Paclitaxel (Taxol)	ODS	Water\Acetonitrile	1.2 ml\min	UV 204 nm
Antitussive drugs : Dextromethorphan	C_{18}	0.025 M KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV204 nm
Anti-anxiety: Buspirone, Lorazepam, Diazepam	C_{18}	Water:acetonitrile:methanol (45:35:20 v\v)	1.0 ml\min	UV 210nm
Anticancer : Gemcitabine HCL[33], Capecitabine HCL	C_{18}	Acetonitrile:Water:triethyelamine (70:28:2v\v)	1.0 ml\min	UV 260
Capecitabine[34]	C_{18}	Methanol:Acetonitrile:Water (50:30:20:v\v) pH4.6 using Triethylamine	1.0 ml\min	UV 245
Angiotensin converting enzyme (ACE): Enalapril, Captopril	RP-18	Methanol–water 50:50 % v\v pH- 3.2 with orthophosphoric acid	1.0 ml\min	UV 218 nm
Anti-bacterial : Trimethoprim	C_8	Potassiumhydrogenphosphate acetonitrile, methanol and water pH 6.2	1.0 ml\min	UV 260 nm
Anti-fungal : Ketoconazole	C_{18}	Methanol and water (90:10 v/v) adjusted to pH 8.90 with a phosphate buffer.	1.0 ml\min	UV 274 nm
Levamisole and Albendazole[35,36]	C_{18}	Buffer pH 3.5 and acetonitrile (70:30 v\v)	1.0 ml\min	UV 224

β – blockers : Atenolol , Amlodipine	C_{18}	0.1% trifluoroacetic acid Methanol\ Water (50:50, v\v)	1.0 ml\min	UV 276 nm
Bronchodilators: Albuterol[37]	C_8	Potassium dihydrogen ortho-phosphate,1-pentane sulphonic acid sodium salt monohydrate (pH 4.0) and acetonitrile (70:30 v/v)	1.0 ml\min	UV 276 nm and 220 nm
Catecholamines : Norepinephrine, Epinephrine[38] Dihydroxy benzylamine Dopamine	C_{18}	0.025M KH_2PO_4 0.3mM heptane- Sulfonic acid pH – 3 Acetonitrile	0.3 ml\min	UV 204 nm
Calcium channel blockers: Verapamil, Diltiazem	C_{18}	50mM ammonium phosphate: acetonitrile 70:30 v/v pH – 4.5	1.0 ml\min	UV 280 nm
Cardiac glycosides: Digoxin	C_{18}	water–acetonitrile (72:28, v/v)	1.1 ml\min	UV 220nm
Benzodiazepines: Clonazepam	C_{18}	Acetonitrile: Methanol (60:40 v/v).	1.0 ml\min	UV 254nm
Diuretics: Amiloride, Triamterene Furosemide	C_{18}	50 Mm phosphate buffer solution: acetonitrile (50:50 v/v, pH 3.0).	1.0 ml\min	UV 283 nm
Expectorants: Guaiphenesin	C_{18}	0.01M Potassium dihydrogen Phosphate pH-3; Orthophosphoric acid: Acetonitrile (40:60 % v\v)	1.0 ml\min	UV 254 nm
Glucocorticoid drugs: Beclomethasone- dipropionate Prednisolone	ODS	Water\Acetonitrile	1.0 ml\min	UV 254 nm
H_2 – Antagonists Ranitidine, Cimetidine	C_{18}	0.025 M KH_2PO_4 pH – 3\ Acetonitrile	1ml\min	UV 225 nm
Hypnotic drugs : Barbital, Allobarbital, Phenobarbital	ODS	Water\Acetonitrile	1.5 ml\min	UV 204 nm
Keratolytic drugs : Salicyclic acid, Phthalic acid, Benzoic acid	C_{18}	0.05 M NH_4OAC pH – 2.2 \ Acetonitrile	0.2 ml\min	UV 204 nm, 230 nm
Muscle relaxing drugs Papaverine	C_{18}	0.025 M KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 254 nm
Sedative drugs: Diazepam, Oxazepam, Clonazepam, Flunitrazepam	C_{18}	0.025 M KH_2PO_4 pH – 3\ Acetonitrile	1.0 ml\min	UV 210 nm
Sulfa drugs: Sulfanilamide, Sulfadiazine, Sulfathiazole,	ODS	Water\Acetonitrile	1.0ml\min	UV 254 nm
Steroidal: Betamethasone	C_{18}	Methanol-acetate buffer-acetonitrile (33:27:40, v/v)	1.0 ml\min	UV 254nm
Therapeutic drugs : Angiotensin - II, Oxytocin Angiotensin - I, Insulin,	C_{18}	0.1 % TFA\ Acetonitrile	0.5 ml\min	UV 210 nm
Tricyclic antidepressant drugs : Protriptyline, Imipramine Nortriptyline, Doxepin	C_{18}	0.025M KH_2PO_4 pH – 7\ methanol	1.0 ml\min	UV 210 nm
Vitamins : **Fat-soluble:** Vitamins A, D, E	C_{18}	Water\ methanol	1.0 ml\min	UV 210 nm
Water soluble: Aminobenzoic acid, [39] Biotin, Folic acid, Niacinamide	C_{18}	0.05 M KH_2PO_4 pH – 2.5\Acetonitrile	1.0 ml\min	UV 204 nm
Xanthenes : Theophylline	C_{18}	ACN: 50 mM sodium acetate buffer (15:85) pH 6.5	1.0 ml\min	UV 270 nm

Abbreviations: KH_2PO_4, Potassium dihydrogen phosphate; **TFA,** Trifluoroacetic acid; **NH_4OAC,** Ammonium acetate; **TEA,** Triethanolamine; **H_3PO_4**, Phosphoric acid; **MeOH,** Methanol; **ACN**, Acetonitrile.

Table 3: HPLC applications - Personal separation guide

Applications	Column	Detector (nm)	Conditions
Aflatoxins	Si	UV (235), FI, CAD	6 % MeOH\hexane
Aspirin, acetaminophen	C_{18}	UV (254)	10 % AN\H_2O, AcOH, pH 2.5
Anticonvulsants	C_{18}	UV (220)	40 % MeOH \ H_2O
Bromphenacyl acids	C_{18}	UV (254)	15-80 % AN\ H_2O
Catecholamines	C_{18}	UV (270)	6 % MeOH\ H_2O, C_8SO_3, EDTA, PO_4, pH 4.0
Kerbscycle acids	RNH_2	UV (210), RI, CAD	25-250 mM PO_4, pH 2.5
Monosaccharides	CX-Ca	UV (195), RI, CAD	H_2O (80°C)
Nucleic acids	CX-Na	UV (254)	0.4 NH_4HCO_2, pH 4.6
Nucleosides	C_{18}	UV (254)	8 % MeOH\ H_2O, PO_4, pH 5.5
Nucleotides	C_{18}	UV (254)	20 % AN\ H_2O, TBA, PO_4, pH 2.6
OPA amino acids	C_{18}	FI (230\418)	8 % AN\ PO4, pH 1.6 DMSO\MeOH\AN\ H_2O
PNA	C_{18}	UV (254)	80 % AN\ H_2O
Pesticides(carbamate)	C_{18}	UV (192), RI, CAD	50 % MeOH\ H_2O
Pesticides(PO_4)	C_{18}	UV (192), CAD	50 % MeOH\ H_2O
Pesticides(chlorinated)	C_{18}	UV (220) CAD	80 % AN\ H_2O
Peptides(<99 amino acids)	C_8	UV (254)	30 % n BuOH\0.1 % TFA, H_2O
Peptides	C_3	UV (210)	40-70 % AN\ H_2O, PO_4, pH 5.5
Proteins (enzymes)	TSK_{SW}	UV (254,280)	0.1MTris, PO_4,pH 7.0
Proteins (enzymes)	TSK_{DEAE}	UV (280)	50 mM PO_4, pH 7.5+150 mM NaCl
Proteins (structure)	C_3	UV (280)	0.1 % TFA ,75% AN, 0.1 %TFA
PTH amino acids	C_{18}	UV (254)	10 %THF\5 mM AcOH 10 % THF\AN
Polysaccharides	TSK_{PW}	UV (195), RI CAD	H_2O(<20 % AN)
Prostaglandins	C_{18}	UV (192)	35 % AN\ H_2O, PO_4, pH 2.5
Phospholipids	Si	UV (206)	130\5\1.5 AN\MeOH\85 % H_3PO_4
Steroids	C_{18}	UV (230)	60 % MeOH\ H_2O
Tricyclic antidepressants[40]	C_{18}	UV (254)	55 % AN\ H_2O, C_5SO_3, pH 5.5
Theophylline	C_{18}	UV (270)	7 % AN\ H_2O, PO_4, pH 4.0
Triglycerides	C_8	UV (220)	60 % AN\ H_2O
Vitamins (Water soluble)	C_{18}	UV (254)	8 % AN\ H_2O, C_7SO_3,
Vitamins (Fat soluble)[41]	C_{18}	UV (280)	80 % AN\ H_2O

Abbreviations: CAD, charged aerosol detector; **AcOH**,Acetic acid; **H_2O**,Water; **AN**, Acetonitrile; **MeOH**, Methanol; **PO_4**,Phosphate; **THF**,Tetrahydrofuran; **TFA**,Trifluoroacetic acid; **H_3PO_4**,orthophosphoric acid; **DMSO**, Dimethyl sulfoxide; **EDTA,** Ethylenediamine tetraacetic acid; **BuOH**, Butanol; **NH_4HCO_2**,Ammonium formate; **OPA,** o-Phthaladehyde or ortho-Phthaladehyde; **PNA,** Peptide Nucleic acid; **PTH,** phenylthiohydantoin.

CONCLUSION

HPLC is just the leading technique for the trace analysis of organic and inorganic substances. Estimation of trace compounds is very vital in pharmaceutical, biological, toxicology, and environmental studies since even a trace substance can be dangerous or poisonous. The prominent role of HPLC in the pharmaceutical industry[42] is essential, particularly in preformulation research, process development, throughout formulation development and drug discovery, and to verify drug purity. All the work which has been done in pharmaceutical substances, Preparation of pure compounds, trace analysis, food and drug safety analysis[43] where we have to analyze for pesticides and toxic chemicals founds in food and food products all of these things are done routinely and daily rapidly by high-performance liquid chromatography[44] or GC. An essential role of chromatography is the QC of the quality of foods, but of drugs controlling the raw materials and control the finished pharmaceutical products[45] ensuring the safety of the people, we are so dependent in the world today on chemicals, minimum synthetic chemicals made by chemist

which have this mixed blessing and I think primarily of pesticides very good for agriculture, very harmful for humans if they happen to ingest these pesticides. Compare to various analytical bulk drug assay methods[46] HPLC is one of the best methods and also HPLC is the best separation technique for quantitative trace analysis of toxic chemicals, impurities, Manufacturing of high purity products, medicinal uses, and research purpose.

REFERENCES

1. Patel Riddhiben M, Patel Piyushbhai M, Patel Natubhai M, Stability indicating HPLC method development-review, International Research Journal of Pharmacy, 2(5), 2011, 79-87.

2. Malviya R, Bansal V, Pal O.P, and Sharma P.K, High-performance liquid chromatography: A Short Review, Journal of Global Pharma Technology, 2(5), 2010, 22-26.

3. Ravisankar P, Gowthami S, and Devala Rao, A review of analytical method development, Indian journal of research in pharmacy and biotechnology, 2(3), 2014, 1183-1195.

4. Chellini PR, Lages B, Franco PH, et al. Development and validation of an HPLC method for Simultaneous determination of Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol HCL in pharmaceutical formulations, Journal of AOAC international, 98, 2015, 1234-1239.

5. Veeragoni AnilKumar, Vasudeva Murthy Sindgi, Shoba Rani Salta, et al. Stability indicating RP -HPLC method for the Simultaneous Estimation of Pyrimethamine and Sulphadoxine in Bulk and tablet dosage form, Journal of Applied Pharmaceutical Science, 6(03), 2016, 071-076.

DOI: 10.7324|JAPS.2016.60312.

6. Hiren Jani, Mayank Bapna, Jigisha Patel, et al. Development and validation of an analytical method for simultaneous estimation of amoxicillin trihydrate and Probenecid in the combined dosage form, Journal of chemical and pharmaceutical research, 6(6), 2014, 1212-1217.

7. Shah D.A, Bhatt K.K, Shankar M.B, Mehta R.S, Ganhi T.R, Baldania S.L, RP-HPLC determination of Atorvastatin Calcium, Amlodipine besylate combination tablets, Indian Journal Pharmaceutical Sciences, 68(6), 2006, 796-79 DOI: 10.4103|0250-474X.49117.

8. Ravisankar P, Soumya Devi M, et al. Simultaneous Rapid separation of four sartans by isocratic RP-HLC method: Application to the determination of Olmesartan in the pharmaceutical dosage form, Journal of chemical and pharmaceutical sciences, 8(1), 2015, 61-71.

9. Srujani Ch, Varanasi SN Murthy, Novel RP-HPLC method development and Validation for the Simultaneous estimation of Nebivolol hydrochloride and hydrochlorothiazide in bulk and pharmaceutical dosage form, Research Journal of pharmaceutical, biological, and chemical sciences, 5(4), 2014, 1105-12.

10. Kurade V.P, Pai M.G, et al., RP-HPLC estimation of Ramipril and Telmisartan in tablets, Indian Journal Pharmaceutical Sciences, 71(2), 2009, 148-151. DOI: 10.4103|0250-474X.54283

11. Ravisankar P, Krishna Chaitanya M, Devadasu CH, SrinivasaBabu P, DevalaRao G, Validated RP-HPLC method for simultaneous separation and estimation of Atenolol and Amlodipine in combination tablet dosage form, Pharmanest, 4(3), 2013, 418-439.

12.Ravisankar Panchumarthy, Devala Rao Garikapati, Abhinay Akaula, Srinivasa Babu Puttagunta, A validated RP-HPLC method for simultaneous estimation of Paracetamol, Diclofenac sodium, and Chlorzoxazone in combination tablet dosage form, Journal of Chemical and Pharmaceutical Sciences, 6(3), 2013, 181-188.

13. Ravisankar P, Devala Rao G. RP-HPLC method for the separation of Paracetamol, Tizanidine, Aceclofenac, Chlorzoxazole: Application of Tizanidine determination in the tablet dosage form, International Research Journal of Pharmacy, 4(6), 2013, 156-163.

14. Manasa G, Anusha N, Development, and validation of RP-HPLC method for the simultaneous estimation of Pantoprazole and Mosapride in the capsule dosage form, International journal of pharmacy and pharmaceutical sciences, 4, 2012, 293-299.

15. Chusena Narasimharaju Bhimanadhuni, Devala Rao Garikapati, Pasupuleti Usha, Development and validation of an RP-HPLC method for the simultaneous determination of Escitalopram Oxalate and Clonazepam in bulk and its pharmaceutical formulations, International Current Pharmaceutical Journal, 1(8), 2012, 193-198.

16. Patil S, Kumar L, Kohli G, et al. Validated HPLC method for concurrent determination of antipyrine, Carbamazepine, Furosemide, and its application in the assessment of drug permeabilitythroughCaco-2cellmonolayers, A Journal of Scientia pharmaceutical, 80(1), 2012, 89-100. DOI: 10.3797/scipharm.1109-03.

17. Stulzer HK, Tagliari MP, Murakami FS, Laranjeira MC, Development and validation of an RP-HPLC method to quantitative acyclovir in cross-linked chitosan microspheres produced by spray drying. J Chromatogr Sci, 46(6), 2008, 496-500. DOI: 10.1093/chromsci/46.6.496.

18. Gonzalo-Lumbreras R, Garcia-Miguens MA, Izquierdo-Hornillos R, HPLC method development for Testosterone Propionate and cypionate in oil-based injectables, Pharm Biomed Anal, 38(4), 2005, 757-62. DOI: 10.1016/jpba.2005.02.003.

19. Dragan M, Milenovic, Snezana P, et al., Development and validation of an HPLC method for the determination of Verapamil residues in supports, Journal of Analytical Chemistry, 68, 2013, 545-551. DOI: 10.1134/S1061934813060051.

20. James F, Wesley, Fred D, High performance liquid chromatographic analysis of the anti-arrhythmic drugs Procainamide, Disopyramide, Quinidine, Propanolol, and metabolites from serum extracts, Clinical Biochemistry, 14, 1981, 113-118. DOI: https://doi.org/10.1016/S0009-9120 (81)90225-3.

21.Srdjenovic.B, Djordjevic-Milic V, et al. Simultaneous HPLC determination of Caffeine, Theobromine, and Theophylline in food, drinks, and herbal products, Journal chromatography sciences, 46(2), 2008, 144-9. DOI: 10.1093/chromsci/46.2.144.

22. Nidal Batrawi, Shorouq Wahdan, et al, A Validated Stability indicating HPLC method for Simultaneous determination of Amoxicillin and Enrofloxacin Combination in an Injectable Suspension.Sci. Pharm, 6, 2017, 85. DOI: 10.3390/scipharm85010006.

23. Agarwal Alka, Singhvi I.J, Gupta S.K, et al. Method development and Validation for assay of Minocycline in dosage forms by RP-HPLC, Int.J.Chem.Sci, 10(4), 2012, 2191-2198.

24. Jeffrey R, et al, High –Performance liquid chromatographic assay of Chloramphenicol in Serum. Antimicrob Agents Chemother, 14(3), 1978, 439-443. DOI: 10. 1128/aac. 14.3.439.

25. Vijay R. Ramb, Kapil L. Dubala, Pragnesh N. Daveb, Hitendra S. Joshia, Development and Validation of a Stability Indicating HPLC Assay Method for Determination of Warfarin Sodium in Tablet Formulation, Chemical Methodologies, 3, 2019, 328-345.

26. Lucarelli.C, Vill P, Lombaradi E, Prandini P, Brega A, HPLC method for the simultaneous analysis of Valproic acid and other common anticonvulsant drugs in human plasma or serum, Chromatographia, 33, 1992, 37-40.

27. Loboz KK, Gross AS, Ray J, McLachlan AJ. HPLC assay for Bupropion and its major metabolites in human plasma, J Chromatogr B Analyt Technol Biomed Life Sci, 823(2), 2005, 115-21. DOI: 10.1016/j.jchromb.2005.06.009.

28. Khoschsorur G.A, et al., Simple and rapid HPLC method for simultaneous determination of multiple antiepileptic drugs in human serum, Chromatographia, 54, 2001, 345-349.

29. Borkar D.D, Godse V.P, Bafana Y.S, Bhosale A.V, Simultaneous estimation of Paracetamol and Promethazine hydrochloride in Pharmaceutical formulations by an RP-HPLC method, International Journal of chem, Tech Research, 1, 2009, 667-670.

30. NighatShafi, Farhan, Ahmed Siddiqui, et al. Concurrent determination of Diltiazem, Lisinopril, Captopril, Enalapril in Dosage Formulations and Human Serum by Liquid Chromatographic Technique, Journal of liquid chromatography & Related Technologies, 38, 2015, 1466-1473. DOI: https://doi.org/10.1080\10826076.2015.1050503.

31. Mohammad Firoz Khan, Shaila Sharmin Zuthi, et al. A Simple RP-HPLC Method development and validation for the Simultaneous Estimation of Naproxen and Rabeprazole, J APP Pharm Sci, 6(11), 2016, 147-152. DOI: 10.7324/JAPS.2016.601123.

32. Yaswanth Kumar S, Sivaprasad P, et al. RP-HPLC method development and Validation for Simultaneous Quantitative estimation of Metronidazole and Nalidixic acid in tablets, International Journal of Pharmacy and Pharmaceutical Sciences, 7, 2015, 367-371.

33. Rajesh V, Anupama B, Jagathi V, Sai Praveen P, Simultaneous Estimation of Gemcitabine hydrochloride and Capecitabine hydrochloride in Combined tablet dosage form by RP-HPLC Method, Journal of Chemistry, 8(3), 2011, 1212-1217.

34. Ravisankar P, Devala Rao G, Naveen Kumar M, Krishna Chaitanya M, An improved RP-HPLC method for the quantitative determination of Capecitabine in bulk and pharmaceutical tablet dosage form, Pharmacia Lettre, 5, 2013, 249-260.

35. Sowjanya S, Devadasu Ch. Development of RP-HPLC Method for the Simultaneous Quantitation of Levamisole and Albendazole: Application to assay Validation. International journal of analytical chemistry, 5746305, 2018, 9 pages. https:// .org/100.1155/2018/5746305.

36.Ravisankar P, Devala Rao G. Development and validation of RP-HPLC method for determination of Levamisole in bulk and dosage form. Asian J Pharm Clin Res, 2013, 6, 169-173.

37. Gajanan B, Kasawar, Mazahar Farooqui. Development and validation of a stability-indicating RP-HPLC method for the Simultaneous determination of related substances of Albuterol sulfate and ipratropium bromide in nasal solution. Journal of Pharmaceutical and Biomedical Analysis, 2010, 52, 19-29.

38. Arun Mishra, Adesh Upadhyay, Arjun Patra, Sachin Chaudhury, et al. Simultaneous determination of Epinephrine and Norepinephrine by High-Performance liquid chromatography, Sci Pharm, 2009, 77, 367-374.

39. Rada Amid, Jasmina Brboric, et al. RP-HPLC determination of vitamins B_1, B_3, B_6, Folic acid, and B_{12} in Multivitamin tablets. Serb. Chem.Soc, 70(10), 2005, 1229-1235.

40. Regina Helena Costa Queiroz, Vera Lucia Lanchote, et al. Simultaneous HPLC analysis of Tricyclic antidepressants and metabolites in plasma samples, Pharmaceutica Acta Helvetia, 70, 1995, 181-186.

41. Panchumarthy Ravisnkar, Abhishekar Reddy A, Nagalakshmi B, Sai Koushik O, Vijaya Kumar B, Sai Anvith P, The comprehensive review of fat-soluble vitamins, IOSR Journal Of Pharmacy, 5(11), 2015,12-28.

42. Ravi Sankar P, Sai Snehalatha K, Tabassum Firdose Shaik, Srinivasa Babu P, Applications of HPLC in Pharmaceutical Analysis, Int.J.Pharm.Sci.Rev.Res., 59(1), 2019, 117-124.

43. Bokai Ma, XinLei Gou, et al. Application of high-performance liquid chromatography in food and drug safety analysis, Journal of food safety and quality, 7, 2016, 295-4298.

44. Ravi Sankar P, Rajyalakshmi G, Devadasu Ch, Devala Rao G, Instant tips for right and effective approach to solve HPLC troubleshooting, Journal of Chemical and Pharmaceutical Sciences, 7(3), 2014, 191-206.

45. Ravi Sankar P, Sai Geethika, Rachana G, Srinivasa Babu P, Bharmgavi J, Bioanalytical Method Validation, Int.J.Pharm.Sci.Rev.Res., 56(1), 2019, 50-58.

46. Masoom Raza Siddiqui, Zeid A. Alothman, Nafisur Rahman, Analytical techniques in pharmaceutical analysis: A review, Arabian journal of chemistry, 10, 2017, S1409-S1421.

Source of Support: Nil, **Conflict of Interest:** None.