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# A Review on High Performance Liquid Chromatography (HPLC)

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Review Article

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ABSTRACT

Chromatography is defined as a set of techniques which is used for the separation of constituents in a mixture. This technique involves 2 phases stationary and mobile phases. The separation of constituents is based on the difference between partition coefficients of the two phases. The chromatography term is derived from the greek words namely chroma (colour) and graphein (to write). The chromatography is very popular technique and it is mostly used analytically. There are different types of chromatographic techniques namely Paper Chromatography, Gas Chromatography, Liquid Chromatography, Thin Layer Chromatography (TLC), Ion exchange Chromatography and lastly High Performance Liquid Chromatography (HPLC). This review mainly focuses on the HPLC technique its principle, types, instrumentation and applications.

## INTRODUCTION

High Performance Liquid Chromatography which is also known as High Pressure Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid chromatography. The solvent usually flows through column with the help of gravity but in HPLC technique the solvent will be forced under high pressures upto 400 atmospheres so that sample can be separated into different constituents with the help of difference in relative affinities [1-7].

In HPLC, pumps will be used to pass pressurized liquid solvent including the sample mixture which is allowed to enter into a column filled with solid adsorbent material. The interaction of each sample component will be varies and this causes difference in flow rates of each component and finally leads to separation of components of column.

Chromatography can be depicted as a mass exchange process including adsorption. HPLC depends on pumps to pass a pressurized fluid and an example blend through a section loaded with adsorbent, prompting the partition of the specimen segments. The dynamic segment of the section, the adsorbent, is regularly a granular material made of solid particles (e.g. silica, polymers, etc.) 2 µm to 50 µm in size. The segments of the example mixture/blend are isolated from each other because of their distinctive degrees of connection with the retentive particles. The pressurized fluid is commonly a blend of solvents (e.g. water, acetonitrile and/or methanol) and is known as 'mobile phase'. Its organization and temperature plays an important part in the partition procedure by affecting the connections occurring between sample segments and adsorbent [8-15].

HPLC is recognized from traditional ("low weight") liquid chromatography because operational pressures are fundamentally higher (50 bar to 350 bar), while normal liquid chromatography regularly depends on the power of gravity to pass the portable stage through the segment. Because of the small sample amount isolated in scientific HPLC, column section measurements are 2.1 mm to 4.6 mm distance across, and 30 mm to 250 mm length. Additionally, HPLC segments are made with smaller sorbent particles (2 µm to 50 µm in normal molecule size). This gives HPLC high determining or resolving power (the capacity to recognize components) while isolating mixtures, which makes it a prominent chromatographic method [16-25].

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## HISTORY

Preceding HPLC researchers utilized standard liquid chromatographic methods. Liquid chromatographic systems were to an inefficient because of the flow rate of solvents being reliant on gravity. Separations took numerous hours, and some of the time days to finish. Gas chromatography (GC) at the time was more effective than liquid chromatography (LC), in any case, it was trusted that gas stage partition and investigation of extremely polar high atomic weight biopolymers was impossible. GC was ineffectual for some organic chemists due to the thermal instability of the solutes. Accordingly, alternative techniques were hypothesized which would soon bring about the advancement of HPLC.

Taking after on the original work of Martin and Synge in 1941, it was anticipated by Cal Giddings, Josef Huber, and others in the 1960s that LC could be worked in the high-proficiency mode by decreasing the pressing molecule measurement generously beneath the run of the mill LC (and GC) level of 150 µm and utilizing pressure to expand the versatile stage velocity. These expectations experienced broad experimentation and refinement all through the 60s into the 70s. Early developmental exploration started to enhance LC particles, and the innovation of Zipax, an externally permeable molecule, was promising for HPLC technology. The 1970s achieved numerous advancements in equipment and instrumentation. Specialists started utilizing pumps and injectors to make a simple configuration of a HPLC system. Gas amplifier pumps were perfect since they worked at consistent pressure and did not require release free seals or check valves for steady flow and great quantitation.

While instrumentational advancements were important, the historical backdrop of HPLC is principally about the history and development of molecule technology. After the presentation of permeable layer particles, there has been a steady pattern to reduced molecule size to enhance efficiency. However, by decreasing molecule size new issues arrived. The disadvantage from the unnecessary pressure drop is expected to drive versatile liquid through the segment and the trouble of setting up a uniform pressing of to a great degree fine materials. Every time molecule size is diminished altogether, another round of instrument advancement normally should occur to handle the pressure.

## OPERATION

The sample blend to be isolated and dissected is presented, in a discrete little volume (commonly microliters), into the stream of mobile phase permeating through the column. The segments of the sample travel through the segment at various speeds, which are a component of particular physical connections with the adsorbent (likewise called stationary stage). The velocity of every component relies on upon its compound nature, composition of mobile phase. The time at which a particular analyte elutes (rises up out of the column) is called its retention time. The retention time measured under specific conditions is a distinguishing normal for a given analyte [26-36].

Various sorts of columns are available, loaded with adsorbents varying in molecule size, and in the nature of their surface ("surface science"). The utilization of small molecule size packing materials requires the utilization of higher operational pressure ("backpressure") and regularly enhances chromatographic resolution (i.e. the degree of division between sequential analytes rising up out of the column). Sorbent particles might be hydrophobic or polar in nature. Basic mobile phases utilized incorporate any miscible mixture of water with different natural solvents (the most widely recognized are acetonitrile and methanol). Some HPLC systems use without water mobile phases. The aqueous segment of the mobile phase may contain acids, (for example, formic, phosphoric or trifluoroacetic corrosive) or salts to help with the seperation of the sample components. The composition of the mobile phase might be kept constant ("isocratic elution mode") or changed ("inclination elution mode") during the chromatographic examination. Isocratic elution is normally successful in the partition of sample components that are not altogether different in their proclivity for the stationary stage. In gradient elution the organization of the mobile phase is fluctuated ordinarily from low to high eluting quality. The eluting quality of the mobile phase is reflected by analyte maintenance times with high eluting quality delivering quick elution.

The selected structure of the mobile phase (additionally called eluent) relies on upon the force of connections between different example parts ("analytes") and stationary stage (e.g. hydrophobic connections in turned around stage HPLC). Dependent upon their partiality for the stationary and mobile stages analytes partition between the two. During the detachment procedure occurring in the sample. This procedure is like what happens

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amid a liquid–liquid extraction however is continuous, not step-wise. In this case, utilizing a water/acetonitrile angle, more hydrophobic parts will elute (fall off the column) late, once the mobile stage gets more packed in acetonitrile (i.e. in a versatile period of higher eluting quality) [37-45].

## INSTRUMENTATION

The HPLC instrumentation involves pump, injector, column, detector, integrator and display system. In the column the separation occurs. The parts include:

- **Solvent Reservoir**: The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.
- **Pump:** The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector. 42000 KPa is the operating pressure of the pump. This operating pressure depends on column dimensions, particle size, flow rate and composition of mobile phase.
- **Sample Injector:** The injector can be a solitary infusion or a computerized infusion framework. An injector for a HPLC framework should give infusion of the fluid specimen inside the scope of 0.1 mL to 100 mL of volume with high reproducibility and under high pressure (up to 4000 psi).
- **Columns:** Columns are typically made of cleaned stainless steel, are somewhere around 50 mm and 300 mm long and have an inward distance across of somewhere around 2 and 5 mm. They are generally loaded with a stationary phase with a molecule size of 3 µm to 10 µm. Columns with inner diameters of <2 mm are regularly alluded to as microbore segments. Preferably the temperature of the mobile phase and the column should be kept consistent during investigation.
- **Detector:** The HPLC detector, situated toward the end of the column distinguishes the analytes as they elute from the chromatographic column. Regularly utilized detectors are UV-spectroscopy, fluorescence, mass-spectrometric and electrochemical identifiers.
- **Data Collection Devices or Integrator:** Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. The PC coordinates the reaction of the indicator to every part and places it into a chromatograph that is anything but difficult to interpret.

The schematic representation of a HPLC instrument ordinarily incorporates a sampler, pumps, and a locator. The sampler brings the sample into the mobile phase stream which conveys it into the column. The pumps convey the mobile phase through the column. The detector generates a sign relative to the measure of sample component rising up out of the segment, consequently taking into consideration quantitative investigation of the example parts. A computerized microchip and software control the HPLC instrument and give information data. A few models of mechanical pumps in a HPLC instrument can combine numerous solvents in proportions changing in time, producing a sythesis slope in the portable stage. Most HPLC instruments likewise have a column broiler that considers altering the temperature at which the partition is performed [46-53].

## TYPES OF HPLC

Depending on the substrate used i.e. stationary phase used, the HPLC is divided into following types [54-63]:

- **Normal Phase HPLC**- In this method the separation is based on polarity. The stationary phase is polar, mostly silica is used and the non-polar phase used is hexane, chloroform and diethyl ether. The polar samples are retained on column [58].
- **Reverse Phase HPLC**- It is reverse to normal phase HPLC. The mobile phase is polar and the stationary phase is non polar or hydrophobic. The more is the non-polar nature the more it will be retained.
- **Size-exclusion HPLC**- The column will be incorporating with precisely controlled substrate molecules. Based on the difference in molecular sizes the separation of constituents will occur.
- **Ion-exchange HPLC**- The stationary phase is having ionically charged surface opposite to the sample charge. The mobile phase used is aqueous buffer which will control pH and ionic strength [56].

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## APPLICATIONS OF HPLC

The HPLC has several applications in the fields of pharmacy, forensic, environment and clinical. It also helps in the separation and purification of compound [57-83].

- **Pharmaceutical Applications:** The pharmaceutical applications include controlling of drug stability, dissolution studies and quality control.
- **Environmental Applications:** Monitoring of pollutants and detecting components of drinking water.
- **Forensic Applications:** Analysis of textile dyes, quantification of drugs and steroids in biological samples.
- **Food and Flavour Applications:** Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.
- **Clinical Applications:** Detecting endogeneous neuropeptides, analysis of biological samples like blood and urine.

## CONCLUSION

The HPLC is mostly used analytical technique. It is having several advantages. With the use of HPLC one can produce extremely pure compounds. It can be used in both laboratory and clinical science. With the use of HPLC the accuracy, precision and specificity can be increased. The only disadvantage of HPLC is high cost.

## REFERENCES

1. Rogatsky E. Modern high performance liquid chromatography and HPLC 2016 International Symposium. J Chromatogr Sep Tech. 2016;7:e135.
2. Mulubwa M, et al. Development and validation of high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for determination of tenofovir in small volumes of human plasma. J Chromatogr Sep Tech. 2015;6:300.
3. Santini DA, et al. Development of a high performance liquid chromatography method for the determination of tedizolid in human plasma, human serum, saline and mouse plasma. J Chromatogr Sep Tech. 2015;6:270.
4. Lin G, et al. Determination of sodium tanshinone iia sulfonate in rat plasma by high performance liquid chromatography and its application to pharmacokinetics studies. Pharm Anal Acta. 2015;6:383.
5. AL-Jammal MKH, et al. Development and validation of micro emulsion high performance liquid chromatography(MELC) method for the determination of nifedipine in pharmaceutical preparation. Pharm Anal Acta. 2015;6:347.
6. Myron P, et al. Tributylamine facilitated separations of fucosylated chondroitin sulfate (fucs) by high performance liquid chromatography (HPLC) into its component using 1-phenyl- 3-methyl-5-pyrazolone (pmp) derivatization. J Chromatogr Sep Tech. 2015;6:256.
7. Tang M, et al. HPLC analysis of monomer release from conventionally and high temperature high-pressure polymerised urethane dimethacrylate intended for biomedical applications. J Chromatograph Separat Techniq. 2014;5:227.
8. Elshanawane AA, et al. Development and validation of HPLC method for simultaneous estimation of brimonidine tartrate and timolol maleate in bulk and pharmaceutical dosage form. J Chromatograph Separat Techniq. 2014;5:230.
9. Mustafa S, et al. An improved high performance liquid chromatographic method for tryptophan analysis in rat brain administrated by seaweed. J Anal Bioanal Tech. 2014;5:188.
10. Caglar S and Alp AR. A validated high performance liquid chromatography method for the determination of saxagliptin and metformin in bulk, a stability indicating study. J Anal Bioanal Tech. 2014;S12:010
11. Abdallah MA. Validated stability-indicating hplc and thin layer densitometric methods for the determination of pazufloxacin: application to pharmaceutical formulation and degradation kinetics. J Chromatograph Separat Techniq. 2014;5:218.
12. deFigueiredo NB, et al. Determination of 3,4-methylenedioxymethamphetamine (mdma) in confiscated tablets by high-performance liquid chromatography (HPLC) with diode array detector. J Forensic Res. 2010;1:106.
13. Shah I, et al. A novel method for determination of fenofibric acid in human plasma using HPLC-UV: application to a pharmacokinetic study of new formulations. J Anal Bioanal Tech. 2014;S12:009.

e-ISSN: 2320-0812
p-ISSN: 2347-2340

14. Gurupadayya BM and Disha NS. Stability indicating hplc method for the simultaneous determination of ceftriaxone and vancomycin in pharmaceutical formulation. J Chromatograph Separat Techniq. 2013;4:207.
15. Shintani H. HPLC separation of amino acids is appropriate? Pharmaceut Anal Acta. 2013;4:e158.
16. Akan JC, et al. Determination of organochlorine, organophosphorus and pyrethroid pesticide residues in water and sediment samples by high performance liquid chromatography (HPLC) with UV/visible detector. J Anal Bioanal Tech. 2014;5:226
17. Parbhunath OL, et al. Optimization and validation of a reverse-phase high performance liquid chromatography assay with ultra-violet detection for measuring total l-ascorbic acid in food and beverage products. J Anal Bioanal Tech. 2014;5:201
18. Szterk A, et al. Comparison of various detection systems coupled to high performance liquid chromatography for determination of tocopherols in meat. The influence and comparison of the most popular sample preparation method. J Anal Bioanal Tech. 2013;S2:005.
19. Lories IB, et al. High performance liquid chromatography, TLC densitometry, first-derivative and first-derivative ratio spectrophotometry for de-termination of rivaroxaban and its alkaline degradates in bulk powder and its tablets. J Chromatograph Separat Techniq. 2013;4:202.
20. Chierentin L and Nunes Salgado HR. Development and validation of a simple, rapid and stability-indicating high performance liquid chromatography method for quantification of norfloxacin in a pharmaceutical product. J Chromat Separation Techniq. 2013;4:171.
21. Srinivasarao K, et al. Validated method development for estimation of formoterol fumarate and mometasone furoate in metered dose inhalation form by high performance liquid chromatography. J Anal Bioanal Tech. 2012;3:153.
22. Sun H, et al. A Rapid and effective method for simultaneous determination of residual sulfonamides and sarafloxacin in pork and chicken muscle by high performance liquid chromatography with accelerated solvent extraction–solid phase extraction cleanup. J Chromat Separation Techniq. 2012;3:154.
23. Virkar PS, et al. Development and validation of a high performance liquid chromatography method for determination of telmisartan in rabbit plasma and its application to a pharmacokinetic study. J Anal Bioanal Tech. 2012;3:133.
24. Gugulothu DB, et al. A versatile high performance liquid chromatography method for simultaneous determination of three curcuminoids in pharmaceutical dosage forms. Pharmaceut Anal Acta. 2012;3:156.
25. Devika GS, et al. Simultaneous determination of eprosartan mesylate and hydrochlorthiazide in pharmaceutical dosage form by reverse phase high performance liquid chromatography. Pharm Anal Acta. 2011;2:122.
26. Harmita, et al. Optimation and validation of analytical method of cotrimoxazole in tablet and plasma *in vitro* by high performance liquid chromatography. J Bioanal Biomed. 2012;4:26-29.
27. Nardulli P, et al. A combined HPLC and LC-MS approach for evaluating drug stability in elastomeric devices: a challenge for the sustainability in pharmacoeconomics. J Pharmacovigilance. 2014;2:157.
28. Hafez HM, et al. Development of a stability-indicating HPLC method for simultaneous determination of amlodipine besylate and atorvastatin calcium in bulk and pharmaceutical dosage form. Pharm Anal Acta. 2014;5:316.
29. Shintani H. Immobilized enzyme column combined with HPLC and column switching method for the analysis of complicated matrix such as body fluids. PharmaceutReg Affairs. 2014;3:e142.
30. Murthy TGK and Geethanjali J. Development of a validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and rosuvastatin calcium in bulk and in-house formulation. J Chromatogr Sep Tech. 2014;5:252.
31. Suresh Babu VV, et al. Validated HPLC method for determining related substances in compatibility studies and novel extended release formulation for ranolazine. J Chromatograph SeparatTechniq. 2014;5:209.
32. Arayne MS, et al. Monitoring of pregabalin in pharmaceutical formulations and human serum using UV and RP-HPLC techniques: application to dissolution test method. Pharm Anal Acta. 2014;5:287.
33. Praveen C, et al. Method development and validation for simultaneous estimation of ethinyl estradiol and drospirenone and forced degradation behavior by HPLC in combined dosage form. Pharmaceut Anal Acta. 2013;4:231.
34. Abdulla SA, et al. Validated HPLC method for the determination of nisoldipine. Pharm Anal Acta. 2013;S1:004.
35. Sawsan Mohammed AH, et al. Effects of blood collection tubes on determination vitamin-A by HPLC. J Chromat Separation Techniq. 2013;4:184.

e-ISSN: 2320-0812
p-ISSN: 2347-2340

36. Subbaiah PR, et al. Method development and validation for estimation of moxifloxacin HCl in tablet dosage form by RP-HPLC method. Pharm Anal Acta. 2010;1:109.
37. Ahir KB, et al. Simultaneous estimation of metformin hydrochloride and repaglinide in pharmaceutical formulation by HPTLC-Densitometry method. J Chromat Separation Techniq. 2013;4:166.
38. Khodadoust S, et al. A QSRR study of liquid chromatography retention time of pesticides using linear and nonlinear chemometric models. J Chromat Separation Techniq. 2012;3:149.
39. Vali SJ, et al. Separation and quantification of octahydro-1h-indole-2-carboxilic acid and its three isomers by HPLC using refractive index detector. J Chromat Separation Techniq. 2012;3:136.
40. Fayyad MK, et al. Effect of temperature, wavelength, ph, ion pair reagents and organic modifiers' concentration on the elution of cystatin c. stability of mobile phase. J Anal Bioanal Techniques. 2010;1:103.
41. Ndorbor T, et al. Chromatographic and molecular simulation study on the chiral recognition of atracuriumbesylate positional isomers on cellulose tri- 3, 5-dimethylphenycarbamate (CDMPC) column and its recognition mechanism. J Chromat Separation Techniq. 2013;4:176.
42. Hua Z, et al. Extraction and purification of anthocyanins from the fruit residues of *Vacciniumuliginosum* Linn. J Chromat Separation Techniq. 2013;4:167.
43. Rogatsky E. 2D or Not 2D. Column-switching techniques, multidimensional separations and chromatography: approaches and definitions. J Chromat Separation Techniq. 2012;3:159.
44. Al-Sagar KA and Smyth MR. Multi-Dimensional column chromatographic method with uv detection, for the determination of propranolol at therapeutic levels in human plasma. Pharmaceut Anal Acta. 2012;3:197
45. Flores HE and Galston AW. Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. 1982;69:701-706.
46. Reinhardt TA, et al. A Microassay for 1,25-Dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. JCEM. 1983;58.
47. Parker JMR, et al. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and x-ray-derived accessible sites. Biochemistry 1986;25:5425-5432.
48. Shephard GS, et al. Quantitative determination of fumonisins $b_1$and $b_2$ by high-performance liquid chromatography with fluorescence detection. J Liquid Chromatogr. 2006:13.
49. Hamscher G, et al. Determination of persistent tetracycline residues in soil fertilized with liquid manure by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. Anal Chem. 2002;74:1509-1518.
50. Mesbah M, et al. Precise measurement of the g+c content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Evol Microbiol. 1989;39:159-167.
51. Tamaoka J and Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microb let. 1984.
52. Svec F and Frechet MJJ. Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. Anal Chem. 1992;64:820-822.
53. Shintani H. Validation Study in membrane chromatography adsorber and phenyl hydrophobic membrane chromatography adsorber for virus clearance and removal of many other components. Pharm Anal Acta. 2013;S2:005.
54. Badgujar DC, et al. Pathogenicity of mutations discovered in BRCA1 BRCT domains is characterized by destabilizing the hydrophobic interactions. J Cancer SciTher. 2012;4:386-393.
55. Ukuku DO, et al. Effect of thermal and radio frequency electric fields treatments on Escherichia coli bacteria in apple juice. J MicrobBiochem Technol. 2012;4:76-81.
56. Qiao G, et al. Modified a colony forming unit microbial adherence to hydrocarbons assay and evaluated cell surface hydrophobicity and biofilm production of vibrio scophthalmi. J Bacteriol Parasitol. 2012;3:130
57. Pandarinath P, et al. A Python based hydrophilicity plot to assess the exposed and buried regions of a protein. J Proteomics Bioinform. 2011;4:145-146.
58. Lu M, et al. Hydrophobic fractionation enhances novel protein detection by mass spectrometry in triple negative breast cancer. J Proteomics Bioinform. 2010;3:029-038.
59. Morgante PG, et al. Establishment of simple and efficient methods for plant material harvesting and storage to allow dna extraction from a myrtaceae species with medicinal Potential. Int J Genomic Med. 2013;1:109.

e-ISSN: 2320-0812
p-ISSN: 2347-2340

60. Patelia EM and Rakesh Jayesh PT. Estimation of balsalazide by HPTLC-Densitometry method in pharmaceutical formulation. J Chromatograph SeparatTechniq. 2013;4:189.
61. Shah DA, et al. Simultaneous estimation of pregabalin and methylcobalamine in pharmaceutical formulation by HPTLC-densitometry method. J Chromat Separation Techniq. 2013;4:169.
62. Mehta FA, et al. Simultaneous estimation of ambroxol hydrochloride and doxofylline in pharmaceutical formulation by HPTLC-desitometric method. J Chromat Separation Techniq. 2013;4:168.
63. Boadu RF, et al. In vitro activity and evaluation of quality of some selected penicillins on the ghanaian market using developed HPLC methods. Med chem. 2015;5:1-14.
64. Hossain MF, et al. UV-metric, pH-metric and RP-HPLC methods to evaluate the multiple pka values of a polyprotic basic novel antimalarial drug lead, cyclen bisquinoline. Mod Chem appl. 2014;2:145.
65. Sultana N, et al. Development and validation for the simultaneous quantification of prazosin, amlodipine, diltiazem and verapamil in api, dosage formulation and human serum by RP-HPLC: application to in vitro interaction studies. Med chem. 2014;4:770-777.
66. Tamimi L, et al. Pioglitazone HCl levels and its pharmacokinetic application in presence of sucralose in animals serum by HPLC method. Pharm Anal Acta. 2014;5:318.
67. Olbrich J and Corbett J. Development and utilization of reversed phase high performance liquid chromatography methods for a series of therapeutic agents. Mod Chem appl. 2013;1:101.
68. Paranthaman R and Kumaravel S. A Reversed-phase high- performance liquid chromatography (RP-HPLC) determination of pesticide residues in tender coconut water (elaneer/nariyalpani). J Chromatograph Separat Techniq. 2013;4:208.
69. Sheng ZY, et al. The study of analytical identification on main monomer compounds of spoiled grass carp by high performance liquid chromatography of quadrupole time of flight mass spectrometry. J Food Process Technol. 2016;7:600.
70. Amagai T, et al. Determination of nicotine exposure using passive sampler and high performance liquid chromatography. Pharm Anal Acta. 2015;6:399
71. Tyagi A, et al. HPTLC-Densitometric and RP-HPLC method development and validation for determination of salbutamol sulphate, bromhexine hydrochloride and etofylline in tablet dosage forms. Pharm Anal Acta. 2015;6:350.
72. Lu Y, et al. Development and optimization of a rp-hplc method to quantify midazolam in rat plasma after transdermal administration: validation and application in pharmacokinetic study. Pharm Anal Acta. 2015;6:329.
73. Singh A, et al. Active ingredient estimation of clopyralid formulation by reversed phase HPLC. J Chromatogr Sep Tech. 2014;6:257.
74. Sassi A, et al. HPLC method for quantification of halofuginone in human ureter: ex-vivo application. J Chromatogr Sep Tech. 2014;6:255.
75. Sangeetha M, et al. Development and Validation of RP-HPLC method: an overview. J Pharmaceutical Analysis. 2014;3.
76. Ahmad J, et al. Development and validation of RP-HPLC method for analysis of novel self-emulsifying paclitaxel formulation. J Pharmaceutical Analysis. 2013;2.
77. Mehta L and Singh J. RP-HPLC method development and validation for the determination of bupropion hydrochloride in a solid dosage form. J Pharmaceutical Analysis. 2013;2.
78. Ezhilarasi K, et al. A Simple and specific method for estimation of lipoic acid in human plasma by high performance liquid chromatography. J Chromatogr Sep Tech. 2014;5:245.
79. Shintani H. Role of Metastable and spore hydration to sterilize spores by nitrogen gas plasma exposure and DPA analysis by HPLC and UV. PharmaceutReg Affairs. 2014;3:125.
80. Malferrari M and Francia F. Isolation of plastoquinone from spinach by HPLC. J Chromatogr Sep Tech. 2014;5:242.
81. Naveed S. Analytical Determination of Lisinopril Using UV Spectrophotometer and HPLC: an overview. Mod Chemappl. 2014;2:137.
82. Shintani H. Serum or saliva extraction of toxic compounds from methyl methacrylate dental materials and HPLC analysis combined with SPE. Pharmaceut Reg Affairs. 2014;3:123.
83. Rudraraju AV, et al. In vitro metabolic stability study of new cyclen based antimalarial drug leads using RP-HPLC and LC-MS/MS. Mod Chemappl. 2014;2:129.