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A COMPREHENSIVE REVIEW ON LIQUID CHROMATOGRAPHIC TECHNIQUES FOR A FEW US-FDA APPROVED DRUGS

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Abstract: HPLC is one of the most important analytical techniques used for both qualitative and quantitative analysis of the compounds. It works on the principle of adsorption where there are two phases one is stationary phase and the other one is the mobile phase. The Food and Drug Administration (FDA) is in charge of ensuring the security, safety, and efficacy of pharmaceuticals for humans and animals, biological products, medical equipment, food supplies in the country, cosmetics, and radiation-emitting goods in order to safeguard public health. When a medication is approved by the FDA, it indicates that the FDA has concluded that the drug is safe and effective for the intended use. When the medication is taken as prescribed by a licensed professional, its advantages exceed its drawbacks. The present review highlights the HPLC methods developed for recently approved US-FDA drugs.

Index Terms **- H**PLC, analytical technique, mobile phase, stationary phase

1.INTRODUCTION

Liquid chromatography is a separation technique which separates mixture in liquid mobile phase using a solid stationary phase (1). LC and HPLC work the same way except the speed, efficiency, sensitivity, and ease of operation of HPLC is vastly superior. Ordinary LC relies on the gravity force to pass the mobile phase through the column, resulting in a slow flow rate and largely limiting the size of particles being used in column. HPLC, on the other hand, relies on pumps to pass a pressurized liquid through the column, which greatly reduce. HPLC is a high-performance liquid chromatography or high-pressure liquid chromatography. HPLC is a chromatographic technique used to separate a mixture of compounds in analytical chemistry and biochemistry with the purpose of identifying, quantifying or purifying the individual components of the mixture. It involves the injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron in diameter called stationary phase) where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump. These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and packing particles. These separated components are detected at the exit of this tube by detector that measures their amount. The output from the detector is called a liquid chromatogram.

2. TYPES OF HPLC SEPARATIONS (2)

2.1 Normal phase: Separation of polar analytes by partitioning onto a polar, bonded stationary phase.

2.2 Reversed phase: Separation of non-polar analytes by portioning onto a non-polar bonded stationary phase.

2.3 Adsorption: In between normal and reversed. Separation of moderately polar analytes using adsorption onto a pure stationary phase.

2.4 Ion chromatography: Separation of organic and inorganic ions by their partitioning onto ionic stationary phases bonded to a solid support.

2.5 Size Exclusion chromatography: separation of large molecules based in the paths they take through a maze of tunnels in the stationary phase.

3 ADVANTAGES (3)

- Rapid and precise quantitative analysis
- Automated operation
- High-sensitive detection
- Quantitative sample recovery
- Amenable to diverse samples

4 APPLICATIONS

The main purpose of the HPLC technique is to identify, quantify and purify a particular analyte or compound (4). Both quantitative and qualitative analysis can be done. HPLC can be used in water purification, detection of impurities in pharmaceutical industries, HPLC techniques are widely used in forensic, clinical, and food industries in addition to the pharmaceutical industry (5). HPLC is used not only to determine drug substances but also to perform stability studies, plant extracts, proteins, and environmental pollutants. It can also be used to separate isomers (6).

Table 1. Analytical method validation of various drugs approved by FDA

S. No.	Drug (Year of approval)	Active ingredient	Category	Dosage form	Method	Parameters	Reference
1	SYMDEKO (15-06-2017)	Tezacaftor & Ivacaftor	CFTR correctors & Potentiators	Tablet	RP-HPLC	**Mobile phase:** Methanol:0.05 % formic acid (95:5 v/v) **Column:** Inspire C-18 (5 µm, 4.6 mm ×250 mm) **Flow rate:**1 mL/min **Wavelength:** 235 nm **Linearity: TEZ:**-10-50 µg/mL **IVA:**- 15-75 µg/mL **LOD :** 0.011 & 0.09 µg/mL **LOQ:** 0.03& 0.275 µg/mL **RT:** 3.0 & 3.8 min **r^2** :0.999 & 0.9995	(7)
2	SEYSARA (20-12-2017)	Sarecycline	Antibiotic	Tablet	UPLC-MS/MS	**Mobile phase:** 0.1 M Na2Po4 & acetonitrile (50:50 v/v) **Column:** ACQUITY, UPLC, HSS C-18 (2.1×100 mm, 1.8 µm) **Wavelength:** 242 nm **Flow rate:** 1 mL/min	(8)

						Linearity: 50-150 µg/mL **LOD:** 0.157 µg/mL **LOQ:** 0.523 µg/mL **RT:** 3.876 min **r ^{2}** : 0.9998	
3	ERLEADA (14-02-2018)	Apalutamide	Anticancer	Tablet	HPLC	**Mobile phase A:** 0.01m disodium phosphate & acetonitrile(73:27) **Mobile phase B:** Water and acetonitrile (30:70) **Column:** Luna Omega 5µm polar C18, (250×4.6) mm **Flow rate:** 1.0 mL/min **Wavelength**:225 nm **Linearity:** 300-12000 ng/mL **Accuracy:** 96.0 and 106.3 % **r^2 :** 0.999	(9)
4	KRINTAFEL (20-07-2018)	Tefenoquine	Antimalarial	-	HPLC	**Mobile phase:** Methanol & water (80:20 v/v**)** **Column:** Inertsil ODS-3V (150 mm 4.6 mm,5.0 m) **Flow rate:** 1mL/min **Wavelength:** 254 nm **Linearity:** 2-12 µg/mL **RT**: 10.3 min	(10)
5	DIACOMIT (20-08-2018)	Stiripentol	Anticonvulsant	Capsules	HPLC-DAD	**Mobile phase:** Acetonitrile and 50 mM potassium dihydrogen phosphate buffer (60:40 v/v) **Column:** Symmetry C18 (30µm, 75mm × 4.6 mm i.d) **Flow rate:** 1mL/min **Wave length:** 262.5 nm **Linearity:** 1-25 µg/mL **LOD:** 0.024 µg/mL **LOQ:** 0.081 µg/mL **RT**: 1.80 min **r^2:** 0.9996	(11)
6	XOFLUZA	Baloxavir Marboxil	Antiviral	Tablet	RP-HPLC	**Mobile phase:** 0.1% OPA: Methanol (50:50 v/v). **Column:** Kromasil C18 (150 mm×4.6 mm, 5µm) **Flow rate:** 1.0 mL/min **Wavelength:** 220 nm **LOD:** 0.08 µg/mL **LOQ:** 0.25 µg/mL **RT**: 2.199 min **r^2**: 0.999	(12)
7	AEMCOLO (16-11-2018)	Rifampicin	Antibiotic	Tablet	LC MS/MS	**Mobile phase A:** 0.1% formic acid in Milli- Q water **Mobile phase B:** 99.8% Acetonitrile and 0.2% Milli -Q water **Column:** core shell Kintex C18(50×2.1mm,2.6µm) **Flow rate:** 0.8 mL/min **Linearity:** 5-40000 µg/mL	(13)

						RT: 1.1 min **r^2:** 0.9993	
8	VITRAKVI (26-11-2018)	Larotrectinib	Antineoplastic	Capsules	RP-HPLC	**Mobile phase:** KH_2PO_4 & Methanol (1:1) **Column**: Sunsil C 18 (250 mm, 4.6mm, 5µ) **Flow rate:** 1.0 mL/min **Wavelength:** 228 nm **Linearity:** 50-150 µg/mL & 0.217 µg/mL **LOD & LOQ:** 0.065 µg/mL & 0.217 µg/mL **RT**: 3.432 min	(14)
09	XOSPATA (28-11-2018)	Gilteritinib fumarate	Antineoplastic	Tablet	RP-HPLC	**Mobile phase**: 25mm sodium perchlorate: acetonitrile {65:35} **Column:** Homochrom Inertsil C18 (250 mm× 4.6 mm;5µm) **Flow rate**: 1 mL/min **Wavelength**: 310 nm **Linearity**: 5-70 µg/mL **LOD: :**0.53 µg/mL **LOQ**: 1.62 µg/mL **RT**: 6.11 min **r^2**:0.9998	(15)
10	FIRDAPSE (28-11-2018)	Amifampridine	Potassium channel blocker, cholinergic agonist	Tablet	LC-MS	**Mobile phase:** Acetonitrile and iso propanol {90:10}+ 20 mM Ammonium formate in 0.1% Formic acid **Column:** Atlantis HILIC silica C18 (3×50 mm,3 µm particle size). **Flow rate**: 1 mL/min **Linearity:** 0.25 ng/mL to 165 ng/mL **LLOQ**: 0.25 mg/mL	(16)
11	MOTIGRITY (14-12-2018)	Prucalopride	Serotonin receptor agonist(laxative)	Tablet	RP-HPLC	**Mobile phase:** Acetonitrile: 0.02M potassium dihydrogen phosphate (20:80 v/v) **Column:** Grace C18 (150 mm×4.6 mm,5 µm) **Flow rate:** 1 mL/min **Wave length:** 277 nm **Linearity :**2-12 µg/mL **RT:** 5.416 min **r^2** :0.999 **LOD:** 0.367 µg/mL **LOQ**: 1.111 µg/mL	(17)
12	EGATEN (13-02-2019)	Triclabendazole	Anthelmintic	Tablet	LCMS/MS	**Mobile phase:** 0.1% formic acid in acetonitrile & 0.1% formic acid in water **Column:** Gemini NX-C18 **Linearity:** 1- 100 µg/mL **Flow rate:** 0.6 mL/min **LLOQ:** 0.999 µg/mL **RT:** 2.19 min **r^2:** 0.9939	(18)
13	RUKOBIA	Fosetasmvir	Antiviral	Tablet	HPLC	**Mobile phase:** (70:30 v/v)	(19)

	(2-07-2020)					**Column:** C18 (250, 4.6 nm, 5µm) **Flow rate:** 1 mL/min **Wavelength:** 266 nm **Linearity:**3-18 µg/mL **LOD:** 0.67 µg/mL **LOQ**: 0.22 µg/mL. **r^2**: 0.9991 **RT:** 4.8 min	
14	REMDAC (October 2020)	Remdesivir	Antiviral	Parentral	HPLC	**Mobile phase:** O-phosphoric acid & acetonitrile (50:50v/v) **Column:** Intersil ODS-3V **Wavelength:** 246 nm **Flow rate**: 1.2 ml/min **Retention time:** 6.0 min **Linearity:** 25 -2500 ng/mL **LOD & LOQ:** 1.95& 6.49ng/mL	(20)
15	CABENUVA (January 2021)	Rilpivirine & cabotegravir	HIV, integrase inhibitor	Tablet	HPLC	**Mobile phase:** Acetonitrile, 0.1 % formic acid (20:80v/v) **Column:** symmetry C18(4.6×150 mm,3.5) **Flow rate:** 1.0ml/min **Wavelength:** 231 nm **r^2:** 0.999 **Linearity:-** Rilpivirine : 30-450 g/mL Cabotegravir : 20- 300 g/mL **LOD:** 0.375) g/mL (R), 1.238 g/ml (C) **LOQ:** 0.25/mL (R), 0.825 g/mL (C)	(21)
16	TEPMETKO (3-02-2021)	Tepotinib	Antineoplastic	Tablet	RP-HPLC	**Mobile phase**: 0.1% TFA (CF_3CO_2H): MeOH (55:45) **Column:** BDS C18 (4.8mm x 15cm, 5µm) **Wavelength:** 310.0nm **Flow rate:** 1ml/min **Linearity:** 11.25- 67 µg/mL **r^2:** 0.999 **LOD & LOQ:** 0.18 g/ml & 0.55 g/mL	(22)
17	REZUROCK (16-07-2021)	Belumosudil	Kinase inhibitor	Tablet	RP-HPLC	**Mobile phase:** 45% OPA: 55% acetonitrile **Column:** BDS C18 (150×4.6mm,5m) **Flow rate:** 1.0 ml/min **Wavelength:** 225.0 nm **Retention time:** 2.439 min **%RSD:** 0.5 **Linearity:** 12.5- 75µg/ml **r^2:** 0.999 **Accuracy**: 99.97 % **LOD & LOQ:** 0.38 & 1.16 µg/mL	

5. LIMITATIONS

Though there are many advantages of HPLC methods there are certain limitations such as there is no universal detector, less separation efficiency than capillary gas chromatography and more difficult for novices.

6. CONCLUSION

Hence from the above review it is observed that HPLC is one of the most important analytical techniques for quality control of various dosage forms. Though analysis by HPLC is costlier but it is highly sensitive methods most commonly used for the determination of the drugs.

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