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Analytical Methods of Six FDA Approved Molecular Entities Used for the Treatment of Non-Small Cell Lung Cancer: A Concise Review

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ABSTRACT

Analytical method development and validation are continuous inter-dependent tasks associated with research and development, quality control and quality assurance departments as they help to understand the critical process parameters and to minimize their influence on accuracy and precision. Standard and analytical procedures of anticancer drug may not be available in pharmacopeias. The time and effort that are put into developing scientifically sound, robust, and transferrable analytic methods should be aligned with the drug development stage. Therefore it becomes necessary to develop and validate new analytical methods for such drugs. In this review the methods for the analysis of such recently approved new molecular entities in various matrices are presented. This review helps in understanding the further need for the development of analytical methods for the estimation of such drugs.

Keywords: Anti-cancer drug, Non-small cell lung cancer, Analytical methods

INTRODUTION

Approximately one third of all cancer related deaths; lung cancer is the most common and fatal malignancies, accounting for nearly 30% of all cancer-related deaths [1]. Lung cancer accounts for more deaths each year than breast, prostate, and colon cancer combined. The main types of lung cancers are small-cell lung cancer and non-small cell lung cancer (NSCLC) which accounts for approximately 85% of all lung cancers [2]. These cancer cells grow quickly and spread early in the course of the disease. The median survival of patients with untreated metastatic non–small-cell lung cancer is only four to five months, with a survival rate at one year of only 10 percent [3]. One hallmark of cancer is immune evasion, in which the immune system does not mount an effective antitumor response [4]. Programmed cell death 1 (PD-1) is a negative co-stimulatory receptor expressed primarily on the surface of activated T cells [5,6]. The binding of PD-1 to one of its ligands, PD-L1 or PD-L2, can inhibit a cytotoxic T-cell response [7,8]. Tumors can co-opt this pathway to escape T-cell–induced antitumor activity [9-11].

Surgery, radiotherapy, and chemotherapy are the main treatment options for NSCLC. Surgery and radiotherapy are usually considered for localized stage I and stage II of NSCLC; however, these treatment options are not feasible for advanced NSCLC [12,13]. Chemotherapy for advanced non–small-cell lung cancer is often considered ineffective or excessively toxic. However, meta-analyses have demonstrated that, as compared

with supportive care, chemotherapy results in a small improvement in survival in patients with advanced non–small-cell lung cancer [14-17].

Analytical method development and validation for the analysis therapeutic components and associated substances play an important role in the discovery, development and manufacture of pharmaceuticals and natural medicinal compounds where the analytical chemists are responsible for the development and validation of analytical methods. The present active pharmaceutical ingredient stability test guideline Q1A (R2) issued by international conference on harmonization suggests that stress studies should be carried out on active pharmaceutical ingredient to establish its inherent stability characteristics, leading to separation of degradation products and hence supporting the suitability of the proposed analytical procedures [18]. Drug regulatory authority of the country has the responsibility to ensure the quality of drug, as drugs not meeting the quality criteria may cause serious health complications, drug adverse reactions, may produce drug resistance in the individual as well as can increase the risk of morbidity and mortality [19]. Analytical methods are developed for new products when no official methods are available. Alternate Analytical method development and validation though often considered routine, too little attention is paid to them with regards for their potential to contribute to overall developmental time and cost efficiency for better precision and ruggedness [20,21]. These method- related activities are interrelated. Parts of each process may occur concurrently or be refined at various phases of drug development as the iterative, particularly during early drug development phases. Changes encountered during drug development may require modifications to existing analytical methods. These modifications may require additional validation or transfer activities [22]. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [23]. Therefore analytical test procedures for stability samples should be stability indicating and they should be fully validated.

Based on the information and facts that point to the importance of the development of analytical methods, this study will portray a review of the analytical methods already developed for the quantification and identification of specific therapeutic categories of FDA approved drugs used to treat non-small cell lung cancer.

Analytical methods for determining the anti-cancer agents

Afatinib

Afatinib is an orally administered, irreversible tyrosine kinase inhibitor of the ErbB family of receptors. Molecular formula $C_{24}H_{25}ClFN_5O_3$ and molecular weight is 485.9 g/mol. It is potent and highly selective as it irreversibly inhibits signaling from all ErbB family dimers: ErbB1, ErbB2, ErbB3, and ErbB4 [24-26] wide-spectrum pre-clinical efficacy against EGFR mutations [27]. Afatinib was granted the FDA approval for the management of patients with metastatic or locally advanced non-small cell lung cancer (NSCLC). The analytical methods described in the literature for the determination of Afatinib are shown in Table 1. Determination of Afatinib alone was reported in three studies in human plasma using LC-MS/MS and UPLC-DAD, with three other HPLC and RP-HPLC procedures published for its quantitation in tablets.

Table 1: Analytical methods described in literature for determination of afatinib

Method	Conditions	Detection system	Matrices	References
HPLC	Column X-terra RP-18 (4.6 × 250 mm) 5.0 µm. Mobile phase: acetonitrile: potassium dihydrogen phosphate buffer pH adjusted to 3.0 with orthophosphoric acid (60:40, v/v)	254 nm	Tablets	[28-29]
RP-HPLC	Column X-Terra RP-8 (250 mm × 4.6 mm) 5.0 µm. Mobile phase: aqueous potassium dihydrogen orthophosphate buffer adjusted pH 3.0 with (orthophosphoric acid and acetonitrile) and methanol (70:30, v/v)	258 nm	Standard and tablets	[30]
UPLC-DAD	Column Acquity UPLC BEHC18 (2.1 mm × 50 mm) 1.7 µm. Mobile phase: ammonium formate buffer (4 mM, pH 3.2 adjusted with formic acid) and acetonitrile (ACN)	268 nm	Human plasma	[31]
LC-MS/MS	Column Luna-PFP 100Å C18 (50 mm × 2.0 mm) 3.0 µm. Mobile phase: acetonitrile and 0.01 M ammonium formate buffer (pH 4.1) (50 x 50, v/v)	Positive electrospray ionization (ESI) with multiple reaction monitoring (MRM)	Human plasma and urine	[32]
LC-MS/MS	Column Luna-PFP 100Å C18 (50 mm × 2.0 mm) 3.0 µm. Mobile phase: acetonitrile and water (40:60, v/v)	Positive electrospray ionization (ESI) with multiple reaction monitoring (MRM)	Human plasma	[33]

RP-HPLC: reversed phase-high-performance liquid chromatography; UPLC-DAD: ultra-performance liquid chromatography with diode array detector; and LC–MS/MS: liquid chromatography coupled with sequential mass spectrometry.

Ceritinib

Ceritinib [5-Chloro-N4-[2-[(1-methylethyl)sulfonyl]phenyl]-N2-[5- methyl-2-(1-methylethoxy)-4-(4- piperidinyl)phenyl 2,4- pyrimidinediamine] is an oral, small-molecule, ATP-competitive, tyrosine kinase inhibitor of anaplastic lymphoma kinase (ALK) gene [34]. Molecular formula $C_{28}H_{36}ClN_5O_3S$ and molecular weight is 558.1 g/mol. It was approved by the Food and Drug Administration for the treatment of ALK-positive metastatic non-small cell lung cancer (NSCLC) who has progressed on or is intolerant to crizotinib. In enzymatic assays Ceritinib is 20 times as potent as Crizotinib against ALK [35]. The analytical methods described in the literature for the determination of Ceritinib are shown in Table 2. There are no novel analytical methods developed for the estimation of Ceritinib in pharmaceutical dosage forms as it is newly approved by FDA. In this study, there is a predominance of determination by RP-HPLC. However, no HPLC-UV, LC-ESI-MS/MS, LC-MS/MS method has been reported for the determination of crizotinib concentrations.

Table 2: Analytical methods described in the literature for determination of ceritinib

Method	Conditions	Detection system	Matrices	References
RP-HPLC	Column BDS C18 (150 mm × 4.6 mm) 5 µm. Mobile phase: 0.01 N potassium dihydrogen orthophosphate buffer and acetonitrile (55:45 v/v)	320 nm	Standard and capsules	[36]
RP-HPLC	Column Kromosil C18 (4.5 mm × 150 mm) 5 µm). Mobile phase: methanol and water (65:35 v/v)	265 nm	Standard and Capsules	[37]
RP-HPLC	Column Kromosil C18 (4.5 × 150 mm) 5 µm. Mobile phase: methanol and water (65:35 v/v)	265 nm	Standard and capsules	[23]
RP-HPLC	Column KromosilC18 (250 x 4.6 mm) 5 µm. Mobile phase: ortho phosphoric acid and acetonitrile (40:60)	320 nm	Capsules	[38]
LC-MS/MS	Column Ascentis Express C18 (50 mm × 2.1 mm) 2.7 µm. Mobile phase: 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile.	Positive electrospray ionization (ESI) with multiple reaction monitoring (MRM)	Human plasma	[39]

RP-HPLC: reversed phase-high performance liquid chromatography; LC–MS/MS: liquid chromatography coupled with sequential mass spectrometry.

Crizotinib

Crizotinib {CZT, 3-[(1R)-1-(2,6-dichloro-3 fluorophenyl)ethoxy]-5-(1-piperidin-4- ylpyrazol-4 yl)pyridine-2-amine} is a novel small-molecule inhibitor of tyrosine kinases. Molecular formula $C_{21}H_{22}C_{l2}FN_5O$ and molecular weight is 450.3 g/mol. It functions by competitive binding with the ATP-binding pocket of target kinases [40-44]. CZT was granted an accelerated approval by the Food and Drug Administration (FDA) for the treatment of patients with advanced local or metastatic NSCLC that is ALK-positive as detected by FDA-approved test [45]. CRZ is available in the market under the name of Xalkori® capsule and is manufactured by Pfizer, Inc. The analytical methods described in the literature for the determination of Crizotinib are shown in Table 3. In this study, there is a predominance of determination by RP-HPLC and LC-ESI-MS/MS. Both LC-ESI-MS/MS and LC-MS/MS are highly sensitive, these methods could be adapted to quantify intracellular concentrations of these drugs in tumour cells but LC-ESI-MS/MS and LC/MS/MS is not available in most hospital laboratories, reversed phase high performance liquid chromatography (HPLC-UV) may be used instead to measure drug concentration in plasma.

Table 3: Analytical methods described in literature for determination of crizotinib

Method	Conditions	Detection system	Matrices	References
RP-HPLC	Column Syncronis C18 (250 × 4.6 mm) 5.0 µm. Mobile phase: methanol and Buffer (0.3% w/v KH_2PO_4, pH 3) (60:40, v/v)	267 nm	Capsules	[46]
RP-HPLC	Column Prime's C18 (250 nm x 4.6 nm) 5.0 µ. Mobile phase: methanol and Sodium Phosphate Buffer (85:15, v/v)	266 nm	Tablets	[47]
HS-HPLC	Column µ-Bondapack CN(150 mm × 3.9 mm) 5.0 µm. Mobile phase: 1% acetic acid and acetonitrile (70:30, v/v)	264 nm	Human Plasma	[48]
UPLC-MS/MS	Column Acquity UPLC BEH C18 (50 × 2.1 mm) 1.7 µm. Mobile phase and methanol and 0.1% (v/v) ammonium hydroxide (80:20, v/v)	Positive electrospray ionization (ESI) with multiple reaction monitoring (MRM)	Human Plasma	[44]
Micellar enhanced spectrofluorimetric Method	Dilution in methanol until the concentration of 1 µg/ml	404 nm	Human Plasma and standard	[49]
LC-ESI-MS/MS	Column Supelco Discovery C18 (50 × 2.1 mm) 5 µ. Mobile phase: water/formic acid 100:0.3 v/v and methanol/formic acid 100/0.3, v/v)	Multiple reaction monitoring (MRM) with positive electrospray ionization (ESI)	Human Plasma and Mouse Plasma	[50]
LC-ESI–MS/MS	Column Zorbax Eclipse Plus PhenylHexy (2.1 × 100 mm) 1.8 µm. Mobile phase: 5 mM ammonium formatein water containing 0.1% of formic acid and acetonitrile added of 0.1% formic acid	Triple-quadrupole via electrospray ionization (ESI) Jet stream ionization (JSI)	Human serum and CSF	[51]

RP-HPLC: reversed phase-high-performance liquid chromatography; LC–ESI-MS/MS: liquid chromatography- electrospray ionization coupled with sequential mass spectrometry.

Gefitinib

Gefitinib N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-yl propoxy) quinazolin-4-amine, is an anti-neoplastic agent. Molecular formula $C_{22}H_{24}ClFN_4O_3$ and molecular weight is 446.9 g/mol. Gefitinib is currently approved for the treatment of non-small cell lung cancer in patients harbouring epidermal growth factor receptor's (EGFR) activating mutations [52]. It is also used for the treatment of pancreatic cancer, breast cancer and several other types of cancer. Gefitinib is a tyrosine kinase inhibitor that acts on the EGFR which includes Her1 (erb-B1), Her2 (erb-B2) and Her3 (erb-B3). The analytical methods described in the literature for the determination of Gefitinib are shown in Table 4. Literature survey revealed that numerous methods have been developed and reported for estimation of Gefitinib in pharmaceutical formulations. In this study, there is a predominance of determination by RP-HPLC. The advantages of performing quantitative studies by the RP-HPLC technique include wide range of applications, effective for separation of homologs, long stationary phase service life, stabilizes quickly and last but not the least eluents are inexpensive and easy to use.

Table 4: Analytical methods described in literature for determination of gefitinib

Method	Conditions	Detection system	Matrices	References
HPLC-UV	Column: Satisfaction C8+ (250 mm × 3 mm) 5.0 µm. Mobile phase:acetate and acetonitrile (45:55, v/v)	331 nm	Human plasma	[52]
RP-HPLC	Column Agilent C18 (4.6 × 250 mm) 5.0 µ. Mobile phase: triflouroacetic acid (0.1%) and Methanol (35:65, v/v)	246 nm	Tablets	[53]
RP-HPLC	Column YMC-ODS-AQ (150 X 4.6 mm) 5.0 µm. Mobile phase: acetonitrile and 0.5% ammonium dihydrogen phosphate (30:70, v/v)	205 nm	Standard and tablets	[54]
RP-HPLC	Column Hypersil, C18 (250 x 4.6 nm) 5.0 µ. Mobile phase: Methanol: Buffer (3.4 gm of Dihydrogen potassium phosphate was dissolved in 1000 ml of milli-Q water) (85:15, v/v)	247 nm	Tablets	[55]
RP-HPLC	ColumnInertsil C8 (250 × 4.6 mm) 5.0 µ. Mobile phase: 50 mM ammonium acetate adjusted to pH 4.7 ± 0.05 with trifluoroacetic acid (A) and acetonitrile (B) under gradient mode (T_{min}A:B) $T_0$65:35, T_{10}65:35, T_{35}40:60, T_{40}30:70, $T_{40.1}$65:35, T_{45}65:35	300 nm	Standard	[56]
RP-HPLC	Column Inertsil ODS-3 V (250 4.6 mm) 5.0 µm. Mobile phase: 130 mM ammonium acetate and acetonitrile adjusted to pH 5 using acetic acid (63:37, v/v)	260	Standard	[57]
LC-MS/MS	ColumnAlltimaC18 (150 mm × 2.1 mm) 5.0 µm. Mobile phase: acetonitrile and 0.1% formic acid in water (30:70, v/v)	Triple quadrupole mass spectrometer using electrospray ionization (ESI) in positive mode.	Human plasma	[58]
LC– MS/MS	Column Hypersil GOLD C18 (50 × 2.1 mm) 3.0 µm. Mobile phase:10 mmol/L ammonium acetate in acetonitrile and deionised water (99:5, v/v)	positive ionisation mode using Atmospheric pressure chemical ionization(APCI)	Human plasma	[59]
LC-MS/MS	Column Waters X-Terra C18 (50 mm × 2.1 mm) 3.5 µm. Mobile phase: acetonitrile-water (70:30, v/v)	Tandem mass spectrometry with electrospray positive ionization (ESI)	Human plasma, mouse plasma and tissues	[60]
LC-ESI-MS/MS	Column Synergi MAX-RP 80 Å C_{12} column (75 × 2.0 mm^2) 4.0 µ. Mobile phase: acetonitrile and 1.0% formic acid (30:70, v/v)	Triple quadrupole mass spectrometer using turbo ion spray source with positive ionization	Plasma	[61]
RRLC	Column Agilent XDB-C18 column (50 × 4.6 mm) 1.8 µm. Mobile phase: buffer (0.77 g of ammonium acetate dissolved in 1000 mL of water) and acetonitrile (40:60, v/v)	250 nm	Standard	[62]

HPLC-UV: high-performance liquid chromatography with detection by ultraviolet; RP-HPLC: reversed phase-high-performance liquid chromatography; LC–MS/MS: liquid chromatography coupled with mass spectromet: LC–ESI-MS/MS: liquid chromatography- electrospray ionization coupled with sequential mass spectrometry; RRLC: rapid resolution liquid chromatography.

Methotrexate

Methotrexate is an antineoplasm agent with a yellowish appearance, formerly known as amethopterin. It is a folic acid antagonist widely used as an anticancer drug and is official in Indian pharmacopoeia, USP and BP [63-65]. Molecular formula $C_{20}H_{22}N_8O_5$ and molecular weight is 454.4 g/mol. Methotrexate stops thymine production in the cells thus interferes in DNA replication. Moreover, Methotrexate is widely used as an alternative treatment for psoriasis and rheumatoid arthritis, when they are hard to treat by the conventional therapies. Methotrexate is widely utilized in the treatment of a variety of malignancies, including leukemias and solid tumors [66]. However, the use of methotrexate has been limited in clinical treatments, due to its high toxicity.

Furthermore, determination of methotrexate in pharmaceutical formulations has been considered of great importance as quality control process. Different analytical strategies have been proposed to determine methotrexate including high-performance liquid chromatography, spectrophotometric with enzymatic inhibition, and electrochemical detection. The analytical methods described in the literature for the determination of methotrexate are shown in Table 5. In this study, there is a predominance of determination by HPLC and UV absorption spectroscopy. The advantages of performing quantitative studies by the HPLC technique include the specificity, efficiency, speed (due to having a fairly short run-time), and accuracy with facility in monitoring technique.

Table 5: Analytical methods described in literature for determination of methotrexate

Method	Conditions	Detection system	Matrices	References
HPLC	Column C18 (100 x 6 mm) 5µ. Mobile phase: buffer and acetonitrile (92:8, v/v)	303 nm	Standard and Tablets	[67]
HPLC	Column Spherisorb ODS2 (250 x 4.6 mm). Mobile phase: TRIS buffer (0.1M dihydrogen phosphate and 0.01 M TRIS; pH 5.7), methanol and acetonitrile (82:11:7, v/v)	313 nm	Human plasma	[68]
HPLC-UV	Column Agilent Zorbax SB-C18 (50 x 4.6 mm) 5 µm. Mobile phase: water (containing 1% isopropyl alcohol and 0.01% heptafluorobutyric acid) and acetonitrile (containing 1% isopropyl alcohol and 0.01% heptafluorobutyric acid)	280 nm	Standard	[69]
HPLC-UV	Column Eurosphere 100-5 C18 (150 x 4.6 mm). Mobile phase: phosphate buffer (0.01M, pH 3.9) and acetonitrile (85:15, v/v)	307 nm	PBS, plasma, brain homogenate and liver homogenate of rats	[66]
HPLC-UV	ColumnKromasil 100 C18 (250 x 4.6 mm) 5 µm. Mobile phase: 50 mM sodium acetate buffer (pH 3.6) and acetonitrile (89:11, v/v)	307 nm	Human serum	[70]
HPLC-UV	Column Zorbax Eclipse XDB-C8 (150 x 4.6 mm) 3.3 mm. Mobile phase: diluents buffer pH 6.0 and acetonitrile (93:7, v/v)	302 nm	Human serum	[71]
HPLC-DAD	Column Hypersil BDS C18 (250 x 4.6 mm). Mobile phase: methanol and ammonium acetate buffer 0.05 $molL^{-1}$, pH 6.0 (25:75, 30:70, and 35:65, v/v)	303 nm	Biodegradable microparticles	[72]
HPLC-ESI-MS/MS	Column ZORBAX-XDB-ODS C18 (2.1 x 30 mm). Mobile phase: acetonitrile, water and formic acid (74:25:1, v/v)	Triple-quadruple mass spectrometer with an electrospray ionization (ESI) interface.	Human plasma	[73]
RP-HPLC	Column Luna C18 (2) 100 R (250 x 4.6 mm) 5 µm,. Mobile phase: methanol and orthophosphoric acid (1.67% v/v in water) (70:30, v/v).	254 nm	Standard	[74]

RP-HPLC	Column Phenomenx C-18 analytical (4.6 x 250 mm) 5 µm,. Mobile phase: Acetonitrile and Orthophospharic acid (0.1%) (45:55, v/v).	215 nm	Standard and tablets	[75]
UHPLC	Column Titan C18 (100 x 2.1 mm) 1.9 µm. Mobile phase: 100 % (v/v) of phosphate buffer (pH= 7.4).	254 nm	Human urine	[76]
LC-MC	Column ZORBAX XDB-ODS C18 (2.1 x 100 mm) 3.5 µ. Mobile phase: acetonitrile, water and formic acid (74:25:1, v/v).	Triple-quadruple mass spectrometer with an electrospray ionization (ESI) interface.	Human plasma	[77]
LC-MC	Column Agilent Zorbax SB-C18 (150 x 3 mm) 3.5 µm coupled with a C18 guard cartridge (4 × 2.0 mm). Mobile phase: 5 mM ammonium acetate and methanol (70:30, %v/v).	Positive ionization mode with single ionization monitoring (SIM) values of 455 and 441	Mouse plasma, brain and urine	[78]
LC-MS/MS	Column YMC Pack ODS AM (150 mm × 4.6 mm) 5 µm. Mobile phase: methanol and 2.0 mM ammonium acetate buffer (minutes, % mobile phase methanol): (0, 10), (1.5, 70), (4.5, 70), (4.7, 10), (7.5, 10).	Triple quadrupole mass spectrometer equipped with heated electrospray ionization (ESI) and operated in positive ionization mode.	Rat plasma	[79]
Absorption spectrophotometry UV-VIS	Dilution of 5 mg of methotrexate with 10 ml of 0.1N NaOH until the concentration of 100 µg/mL.	259 nm	Tablets	[80]
Absorption spectrophotometry UV-VIS	Dilution of methotrexate stock solution (199.5 mM) was performed with sodium bicarbonate to prepare different concentrations of methotrexate.	372 nm	Macromolecular conjugates drug carrier	[81]
Absorption spectrophotometry UV-VIS	Dilution in 0.1 mol/L hydrochloric acid until the concentration of 10 µg/mL.	307 nm	Polymeric implants	[82]
Absorption spectrophotometry UV-VIS	Aliquot of Methotrexate (4 to 24 μgml^{-1}) in 1 ml of (0.01M) 2, 2-bipyridine solution followed by 1ml of (0.2%) ferric chloride solution and resulting solution was heated and finally 1ml (0.2M) orthophosphoric acid solution was added.	510 nm	Tablets	[83]
Absorption spectrophotometry UV-VIS	Dilution of aliquot (1 mL) in 0.1 mol/L hydrochloric acid to get concentration of 10 µg/mL.	307 nm	PLGA Implants	[84]
Absorption spectrophotometry UV-VIS	Dilution of aliquots of Methotrexate(5 to 30 μgml^{-1}) in (0.01M) MBTH followed by 1ml of (0.7%) ferric chloride solution and resulting solution was heated for 15 min and finally 1ml (0.5N) Hydrochloric acid solution was added.	610 nm	Tablets	[85]

HPLC-UV: high-performance liquid chromatography with detection by ultraviolet; HPLC-DAD: high performance liquid chromatography diode array detector; HPLC–ESI-MS/MS: high performance liquid chromatography- electrospray ionization-coupled with sequential mass spectrometry; RP-HPLC: reversed phase-high-performance liquid chromatography; UHPLC: ultra-high performance liquid chromatography; LC-MS: liquid chromatography coupled with single mass spectrometry; LC-MS/MS: liquid chromatography-coupled with sequential mass spectrometry.

Gemcitabine

Gemcitabine [4-amino-1-[(2R, 4R, 5R)-3, 3-difluoro-4-hydroxy-5-(hydroxymethyl)-oxolan-2-yl] pyrimidin-2-one monohydrochloride] is the most important deoxycytidine analog to be developed since cytarabine; it has geminal fluorine atoms inserted at the 2′, 2′- carbon of the

deoxyribofuranosyl ring which [86-87] belongs to a class of drugs known as antimetabolites, approved by FDA and used to treat a wide variety of solid tumors including pancreatic, breast and non-small lung cancer [88-93], bladder cancer [94]. Its molecular formula is $C_9H_{11}F_2N_3O_4$ and molecular weight is 263.2 g/mol. The analytical methods described in the literature for the determination of Gemcitabine are shown in Table 6. In this study, there is a predominance of determination by RP-HPLC. However, in contrast with methotrexate, less HPLC-ESI-MS/MS method has been reported for the determination of gemcitabine concentrations.

Table 6: Analytical methods described in literature for determination of gemcitabine

Method	Conditions	Detection system	Matrices	References
HPLC	Column Phenomenex Luna C18 column (250 mm× 4.6 mm) 5μ. Mobile phase: acetonitrile and water (10:90; pH adjusted to 7.0 using trietylamine and orthophosphoric acid)	275 nm	Injections	[95]
HPLC	Column TSK gel DEAE-2SW (250 mm x 4.6 mm) 5 µl Mobile phase: 0.06 M Na2HPO4 (pH 6.9) and 20% acetonitrile	254 nm	Cancer cell	[96]
HPLC	Column Waters Spherisorb C18 (4.6 mm × 250 mm) 5 µm. Mobile phase: acetonitrile and (50 mM sodium phosphate and 3.0 mMoctyl sulfonic acid, pH 2.9) aqueous buffer (10:90, v/v)	267 nm	Plasma and tissue culture media	[97]
HPLC-UV	Column Nucleosil 5 NH (250 x 4.0 mm) 5µm. Mobile phase: methanol, cyclohexane and 1,2 dichloroethane (30:50:20, v/v/v)	272 nm	Human plasma	[98]
HPLC-MS/MS	Column Synergi Hydro-RP C18 (150 × 2.1 mm). Mobile phase: 1 mM ammonium acetate (pH 6.8) and acetonitrile (94:6, v/v)	Positive ion electrospray ionization followed by MS/MS	Human plasma	[99]
HPLC- DAD	Column Lichrospher C18 (250 mm × 4.6 mm) 5 µm. Mobile phase: 40 mmol/L acetate ammonium buffer solution (pH 5.5) and acetonitrile (97.5: 2.5, v/v)	268 nm	Human plasma	[100]
RP-HPLC	Column L-7, C18 (25 cm x 4.6 mm) 5µm. Mobile phase: Phosphate buffer : methanol in the ration of (97: 3, v/v)	266 nm	Injections	[101]
RP-HPLC	Column Zorbax RXC8 (250 mm × 4.6 mm) 5µ. Mobile phase: phosphate buffer of pH 3 and methanol (85: 15, v/v)	275 nm	Standard and tablets	[102]
RP-HPLC	CloumnChromosil C18 (250 x 4.6 mm) 5 µm. Mobile phase: Methanol, acetonitrile andwater 80: 15: 5, v/v)	282 nm	Standard	[103]
RP-HPLC	Column Luna C18 (250 mm x 4.6 mm) 5 µ. Mobile phase: methanol and phosphate buffer (40: 60 v/v)	270 nm	Injections	[104]
RP-HPLC	Column Zorbax C8 (250 x 4.60 mm) 5 µ. Mobile phase: methanol and Sodium dihydrogen phosphate pH 3.5 (70: 30, v/v)	230 nm	Nanosponges	[105]
RP-HPLC	Column C18 (250 mm × 4.6 mm) 5 µm. Mobile phase: sodium acetate buffer (pH 4.0) and acetonitrile (75: 25, v/v)	254 nm	Injections	[106]
RP-HPLC	Column Zorbax Rx C8, 4.6 mm x 250 mm) 5µ. Mobile phase: monobasic sodium Phosphate and methanol	275 nm	Injections	[107]
RP-HPLC	Column PhenomenexcolumbusC18 (150 × 4.6 mm) 5 µm. Mobile phase: 50 mM ammonium acetate pH 5.0 in either 2% or 10% methanol	269 nm	Human plasma	[108]

Ion-pair RP-HPLC	Column Thermo Hypersil C18 (250 x 4.6 mm) 5µ. Mobile phase: methanol and phosphate buffer (20 mM, pH 3.1) containing 10 mM sodium 1-heptanesulfonate (17: 83, v/v)	272 nm	Human plasma	[109]
U PLC-MS/MS	Column BEH C18 column (50 mm × 2.1 mm,) 1.7 µm. Mobile phase: methanol and water (containing 2 mM ammonium acetate)	Positive electrospray ionization mode with multiple reactions monitoring (MRM) mode	Rat plasma	[110]
LC-MS	Column YMC ODS-AQ (2.0 mm × 150 mm) 5µm. Mobile phase: methanol and 98% 5 mM ammonium acetate at pH 6.8 (2: 98, v/v)	Negative electrospray ionization conditions having single-ion-monitoring (SIM) mode	Human plasma	[111]
LC–MS/MS	Column Hypercarb column(100 × 2.1 mm) 5 μ m. Mobile phase: 10 mM ammonium acetate, pH 10 and acetonitrile	Triple stage quadrupole mass spectrometer with a heated electrospray ionisation (HESI-II) in positive and negative mode	Tumor tissue	[112]
LC-MS/MS	Column Chrompak-spherisorb-phenyl (3.1 mm x 200 mm) 5 µm. Mobile phase: 50mM formic acid and acetonitrile (9: 1, v/v)	Atmospheric pressure chemical ionization (APCI) source and mass spectrometric positive multi-reaction-monitoring (MRM) mode	Human plasma and tissue	[113]

HPLC-UV: high-performance liquid chromatography with detection by ultraviolet; HPLC-MS/MS: high performance liquid chromatography coupled with sequential mass spectrometry; HPLC-DAD: high performance liquid chromatography diode array detector; RP-HPLC: reversed phase-high-performance liquid chromatography; UPPLC -MS/MS: ultra-performance liquid chromatography coupled with sequential mass spectrometry; LC-MS: liquid chromatography coupled with single mass spectrometry; LC-MS/MS: liquid chromatography-coupled with sequential mass spectrometry.

The contribution of chemistry, pharmacology, microbiology and biochemistry has set a standard in the drug discovery where new drugs are no longer generated only by the imagination of chemists but these new drugs are the outcome of exchange of ideas between biologists and chemists [114]. During the survey of the literature, it was observed that among the chromatographic techniques until now HPLC has been the most widely used system. One of the widely used detectors in HPLC is UV detector which is capable of monitoring several wavelengths concurrently; this is possible only by applying a multiple wavelength scanning program. If present in adequate quantity, UV detector assures all the UV-absorbing components are detected. In liquid chromatography the choice of detection approach is critical to guarantee that all the components are detected [114]. Reversed-phase mode with UV absorbance detection is used by most researchers nowadays, because this provided the best available reliability, analysis time, repeatability and sensitivity. Liquid chromatography combined with mass spectrometry (LC–MS) is considered as one of the most important techniques of the last decade of 20th century [115]. It became the method of choice for analytical support in many stages of quality control and assurance within the pharmaceutical industry [116-117]. Recently HPLC-MS has been used for assay of drugs, which was also observed in the present literature survey. In addition to its application in analyzing the drugs HPLC alone and with hyphenated technique have been applied to analyze the impurities of the pharmaceuticals [118-120] and degradation products [121].

As can be seen in the present survey, most of the methods cited above used toxic chemical solvents. Toxic chemical solvents can cause damage to the equipment used, the environment, and the operators; therefore it is extremely important for the development and validation that the analytical methods to be used are chosen so as to decrease the amount of toxic waste produced [122]. Much effort has been put to decrease the amount of organic solvents involved in API synthesis and drug

product manufacturing. Pharmaceutical companies are now attempting to use more environment-friendly organic solvents which has low cost analysis and consequently reduces energy consumption; requires smaller amounts of solvents or that can identify lower concentrations of the same; can recover toxic solvents; can educate researchers and pharmaceutical companies to use nontoxic solvents and improve environment in order to reduce the risk on the population and analysts [122]. The manufacturers try to exchange more toxic solvents to more friendly ones with similar properties (like replacing benzene with toluene) or look for some new innovations. Substances such as water, supercritical fluids, fluorous phases, surfaces or interiors of clays, zeolites, silica gels, alumina and ionic liquids are taken into consideration as a potential reaction media [123]. Thus, the contribution by universities and research centers to the analysis to the analysis should be taken into consideration so that the overall methods ensure that the pharmaceutical product has a control on impurities, presence of therapeutic agent that developed potency and efficacy of drug, and safety of the same in relation to population.

CONCLUSION

This review is revealed at focusing the role of various analytical instruments in the assay of pharmaceuticals and giving a thorough literature survey of the analytical methods involved in the determination of API concentrations through the development of advance techniques beginning from the HPLC method to the advanced hyphenated techniques. The developed methods supported in understanding the pharmacokinetics, pharmacodynamics, therapeutic drug monitoring, drug interactions with the organism, developing pharmaceutical formulations, and determining the toxicity of these compounds. Nevertheless there is a scope for the development of analytical methods which are time and cost effective and also at the same time environment-friendly.

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