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An insight of development and validation of bioanalytical method in the reference of anticancer drugs by using LC-MS/MS

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Abstract---The bioanalytical analysis of anticancer agents established a more personalized treatment procedure. The importance of validating analytical procedures before they are put into normal usage is widely acknowledged. This novel approach has a lot of promise because it is quick, easy, and only requires a little amount of samples to get the accurate result. The goal of this mini review is to provide a comparative analysis of contemporary research on few anticancer

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agents and their methodology in reference to bioanalytical analysis. We provide practical approaches for determining extraction and clean up, precision and accuracy, selectivity and specificity, chromatographic analysis and its validation. We believe that the liquid chromatographic processes used in the bioanalysis of anticancer medicines, validation standards might have been applied in a variety of ways to counter the failure of an anticancer agent by increasing its therapeutic index approach.

Keywords---anticancer drug, bioanalytical analysis, cancer, chromatography, liquid chromatography.

Introduction

Bioanalytical methods for the analysis and optimization of the anti-cancerous drug are very promising technique because there is a lack of accurate mechanistic behaviour of oncology drug and their treatment. Several bioanalytical methods have been previously applied for the quantification and analysis of anti-tumour drugs (P.Heydari, W., Schinkel, A. H., Beijnen, J. H., & Sparidans, R. Wet al., (2022). It is the need of hour to develop new assay and its validation to fulfil the increasing demand for drugs monitoring. Bioanalytical method is best for fast and high therapeutic quantification of anticancer drug. This method is used in the quantitative analysis of anticancer drugs and their metabolites. It is also used for the demonstration of the preclinical and clinical data with the help of physicochemical and biological technique. It is involved in the quantitative analysis of the drug including pharmacokinetics and bioavailability. In other word, we can define this method as a set of process involved in the drugs collection, storage, analysis, and processing. Bioanalytical method includes gas chromatography, liquid chromatography, high-performance liquid chromatography (HPLC), mass spectroscopy, and chromatography including solid-phase extraction and liquid-phase extraction. The bioanalytical method plays an important role in the brief analysis of different anticancer drugs. The management of cancer has changed dramatically as a result of recent advancements in the treatment. The percentage of cancer patients has increased over time. Previously, studies have been suggested that the treatment of different cancer with drugs therapies showed great success. Therefore, it is must to explore anticancer drugs in the best way possible.

Why Cancer?

Cancer is an abnormal growth of cells that can develop in an uncontrollable manner inside the human body. It can develop anywhere in the body. When cells become damaged it forms a cluster of tumour which can be benign or malignant. The tumour in advanced stage develops into cancerous cells. When cancer spread from one place to another place inside the human body is called metastatic condition. Cancer can be classified as the development on the primary site of origin like carcinoma, sarcoma, leukaemia, breast cancer, prostate cancer, lung cancer, liver cancer, multiple myeloma, lymphoma, melanoma etc. In India, tobacco-related cancer is supposed to increase 3.7 lakhs (27.1%) of the total and

cervix cancer 0.75 lakhs (5.4%). While cancer of gastrointestinal tract was supposed to increase by 2.7 lakhs (19.7%) of the total cancer burden. According to ICMR report, there is a significant increase in head, neck, and lung cancer has been observed in both Indian men and women. While on the other hand, breast cancer is supposed to increase in women. Hence, cancer is the biggest threat to India in the upcoming days. The treatment of cancer depends upon the stage of the diseases. Some cancer patients will have only one treatment while some might face a combination of two different drugs during treatments. The different types of cancer treatment are chemotherapy, surgery radiotherapy, immune therapy, stem cell bone marrow transplant, hormone therapy, etc.

Assessment of anti-cancer drugs

The anticancer drugs for the treatment of cancer have been approved on the basis of cancer-related conditions. Several oncology drugs are trastuzumab (1998), Imatinib (2001), Gefitinib (2003), Erolitinib (2004), and Cetuximab (2004). These oncology drugs have been approved by the FDA drug federation from time to time (. The bioanalytical analysis of various anticancer drugs requires regular monitoring for accuracy. It provides an improved version of the anticancer drugs which has increases its therapeutic index. The following table is showing studies on oncology drugs and their assessment through bioanalytical study (Table 1).

Anticancer drugs for	for appropriate cancer related	d condition and its bioanalytical		
study				

Table 1

Drug Name	Cancer	Bioanalytical study	Reference
Lapatinib, Neratinib or Afatinib	Breast cancer	Label-free LC-MS analysis of HER2+	Di Luca, A., Henry, M.,
		breast cancer cell line	Meleady, P., &
		inhibitor treatment	O Connor, R.et., al (2015)
5-fluorouracil,	Breast Cancer	Development and	van Nuland, M.,
Leucovorin, and		validation of an UPLC-	Venekamp, N.,
Irinotecan		MS/MS method for the	de Vries, N., de
		therapeutic drug	Jong, K. A.,
		monitoring of oral anti-	Rosing, H., &
		hormonal drugs in	Beijnen, J. H.
		oncology	et., al (2019).
Anthracyclines,	Oral cancer	Development and	van Nuland, M.,
Carboplatin, and		validation of an UPLC-	Venekamp, N.,
Paclitaxel		MS/MS method for the	de Vries, N., de
		therapeutic drug	Jong, K. A.,
		monitoring of oral anti-	Rosing, H., &
		hormonal drugs in	Beijnen, J. H.
		oncology	et., al (2019).
Methotrexate (MTX)	Acute	The Relevance of Mass	Ciocan-Cartita,
	lymphoblastic,	Spectrometry Analysis	C. A., Jurj, A.,
	Leukemia,	for Personalized	Buse, M., Gulei,
	Osteogenic	Medicine through Its	D., Braicu, C.,

	sarcoma	Successful Application	Raduly I &
	Cerebral	in Cancer "Omics	Regindan-
	tumors		Neagoe Let ol
	Breast cancer		(2019)
	Ovarian		(201)).
	cancer and		
	Bladder		
	cancer		
Lenvatinib (LENVA)	Hepatocellular	A rapid simple and	Zanchetta M
	carcinoma	sensitive LC-MS/MS	Iacuzzi. V
	and Thyroid	method for lenvatinib	Posocco, B.,
	carcinoma	quantification in	Bortolin, G.,
		human plasma for	Poetto, A. S.,
		therapeutic drug	Orleni, M.,&
		monitoring	Toffoli, G. et.,al
		0	(2021).
Cetuximab	Head & Neck	A simple and rapid LC-	Becher, F.,
	cancer	MS/MS method for	Ciccolini, J.,
		therapeutic drug	Imbs, D. C.,
		monitoring of	Marin, C.,
		cetuximab: a GPCO-	Fournel, C.,
		UNICANCER proof of	Dupuis, C., &
		concept study in head-	Salas, S. et., al
		and-neck cancer	(2017).
		patients.	
Cyclophosphamide	Lung cancer	One-Step Solid	Gao, S., Tao, Z.,
		Extraction for	Zhou, J., Wang,
		Simultaneous	Z., Yun, Y., Li,
		Determination of	$M_{1}, \dots \ll M_{1}a0, \Upsilon$
		Anticancer Drugs and	et., al (2018).
		One Active Metabolite	
		in Human Plasma by	
		HPLC-MS/MS	
Ifosfamide, Irinotecan,	Lung cancer	One-Step Solid	Mehanna, S.,
Etoposide,	0	Extraction for	Bodman-Smith,
Gemcitabine,		Simultaneous	K., Daher, C. F.,
Carboplatin,		Determination of	& Khnayzer, R.
Pemetrexed, Paclitaxel,		Eleven Commonly Used	S. et., al (2020).
Vinblastine, and		Anticancer Drugs and	
Vinorelbine		One Active Metabolite	
		in Human Plasma by	
		HPLC-MS/MS	
Ruthenium(II)	A549 Lung	Rapid quantification of	Mehanna, S.,
	cancer cell	ruthenium(II)	Bodman-Smith,
	line	polypyridyl anti-cancer	K., Daher, C. F.,
		drugs using a selective	& Khnayzer, R.
		ligand dissociation LC- MS/MS method†	S. et., al (2020).
Abemaciclib	Breast cancer	Quantification of	Wickremsinhe

cis>dichloro diammine platinum (cis>-Pt), cis>diammine-1,1- cyclobutane dicarboxylate platinum (CBDCA), and cis>-dichloro-frans- dihydroxy diisopropylamine platinum (CHIP)	Human neoplasma	abemaciclib and metabolites: evolution of bioanalytical methods supporting a novel oncolytic agent Trace Analysis for <i>cis></i> - Platinum Anti-cancer Drugs via LCEC	E. R., & Lee, L. B.et., al (2021). Krull, I. S., Ding, X. D., Braverman, S., Selavka, C., Hochberg, F., & Sternson, L. A. et., al (1983).
Heteroleptic Cu(II) Complexes	Hela and MCF7	Design, Synthesis, Bioanalytical, Photophysical and Chemo- phototherapeutic Studies of Heteroleptic Cu(II) Complexes	Ravi, M., Kumar, M. S., Ushaiah, B., Prasad, C. K., Sudeepa, K., Anitha, N., & Devi, C. S. et.,al (2022)
Sulphoraphane	Breast Cancer	validation of a high throughput bioanalytical UPLC- MS/MS method for simultaneous determination of tamoxifen and sulphoraphane in rat plasma: Application to an oral pharmacokinetic study	Mangla, B., Alam, O., Rub, R. A., Iqbal, M., Singh, A., Patel, K. S., & Kohli, K. et., al (2020).
Matrine, Dictamine, Fraxinellone, and Maackiain	Oral cancer	Pharmacokinetic Characterization and Bioavailability Barrier for the Key Active Components of Botanical Drug Antitumor B (ATB) in Mice for Chemoprevention of Oral Cancer	Bui, D., Yin, T., Duan, S., Wei, B., Yang, P., Wong, S. J., & Hu, M.et., al (2021).
Axitinib or Cabozantinib	Renal cell carcinoma and Oral cancer	Developing a Nationwide Infrastructure for Therapeutic Drug Monitoring of Targeted	Mc Laughlin, A. M., Schmulenson, E., Teplytska, O.,

		Oral Anticancer Drugs: The ON-TARGET Study Protocol	Zimmermann, S., Opitz, P., Groenland, S. L., & Fuxius, S. (2021).
Cyclophosphamide, Docetaxel, Doxorubicin, 5- fluorouracil, Methotrexate, and Tam oxifen.	Breast cancer	Pharmacokinetics of Anti-Cancer Drugs Used in Breast Cancer Chemotherapy	Nagar, S. et., al(2010).
GMC1 (2-(1H- benzimidazol-2- ylsulfanyl)- <i>N</i> -[(Z)-(4- methoxyphenyl) methylideneamino] acetamide)	Prostate cancer	Bioanalytical Assay Development and Validation for the Pharmacokinetic Study of GMC1, a Novel FKBP52 Co-chaperone Inhibitor for Castration Resistant Prostate Cancer.	Ekpenyong, O., Cooper, C., Ma, J., Guy, N. C., Payan, A. N., Ban, F., Xie, H. et., al (2020).
Enzalutamide	Prostate cancer	Development and validation of a bioanalytical method to quantitate enzalutamide and its active metabolite N- desmethylenzalutamide in human plasma: application to clinical management of metastatic castration- resistant prostate cancer patients.	van der Meulen, E., van Oort, I. M., Beumer, J. H., Somford, D. M., Schalken, J. A., Burger, D. M., & van Erp, N. P. et., al (2018).
Glaucocalyxin A (GLA)	Human hepatoma cell line	Effects of glaucocalyxin A on human liver cancer cells as revealed by GC/MS- and LC/MS-based metabolic profiling.	Liu, Y., Lu, S., Zhao, L., Dong, X., Zhu, Z., Jin, Y., & Chai, Y. et., al (2018).
Thiosemicarbazone	Human liver microsomal fractions	LC-MS/MS identification of the principal in vitro and in vivo phase I metabolites of the novel thiosemicarbazone anti-cancer drug, Bp4eT	Stariat, J., Šesták, V., Vávrová, K., Nobilis, M., Kollárová, Z., Klimeš, J.,& Kovaříková, P. et., al (2012).

Method of literature Search

This review is performed from the online search covering the period of past few years. Based on the literature search, the review has been discussed in the following different categories that is sample preparation, extraction, chromatographic analysis, and validation followed by the conclusion and future perspective. The methodologies of all published article have been analysed along with the potential future of bioanalytical analysis in the reference of anticancer drug. The novelty of this review is that it provides a comparative analysis of the four anticancer drugs for various cancer-related conditions. The research publications were found using the search engines PubMed, Google Scholar, Scopus, and Science Direct.

Sample preparation

The sample preparation of any biological sample is widely considered as a laborious and error- prone part of the entire bioanalytical process. The sample preparation for the bioanalytical process includes taking aliquots of samples for analysis which is considered as the initial stage, followed by extraction and sample clean-up, chromatographic analysis, and validation (Nováková, L., & Vlčková, H. et., al (2009). And all the steps are important to achieve a reliable result. All the solvents, reagents, and standards should have been brought for the LC/MS grade.

- Step 1: Preparation of standard solution The standard stock solution of the anticancer drug should be prepared in 100% of acetonitrile solution of LC/MS grade and the solution should be vortexes for 5-6 seconds. The sample should further be stored in glass vial at -20°C (Sharma, A., Thavathiru, E., Benbrook, D. M., & Woo, S. et., al (2017).
- Step 2: Extraction and sample clean up
 - The standard solution is supposed to further dilute in acetonitrile to make an internal standard solution (ISS) of 5ug/ml. The plasma sample preparation includes thawing of plasma sample at room temperature. After thawing, $180\mu l$ of plasma standard has been added to $20\mu l$ of ISS. The sample is vortexes vigorously by 30 second followed by addition of chilled $160\mu l$ of acetonitrile. After vortexing for 10 minutes, the mixture is centrifuge for $21,381 \times g$ for 15 min at 4°C (Sharma, A., Thavathiru, E., Benbrook, D. M., & Woo, S. et., al (2017). The sample clean-up is very important step because inappropriate sample clean up lead to matrix effects, ion enhancements, insufficient validity of the analysis, and ion suppression. The organic phase (supernatant) was collected and transferred into fresh tube and dried under nitrogen gas at a pressure of 15 psi for 10– 15 minutes. The sample residue is ready for LC-MS/MS analysis.
- Step 3: Chromatographic analysis The chromatographic analysis was based on sample, solvent, and column manager. The column manager is composed of thermostatted column compartment, while solvent manager includes binary pump. Mobile phases consisted of LC/MS grade water containing different composition of LC/MS grade acetonitrile in order to evaluate the maximum separation, sensitivity,

and peak resolution. The temperature of the column should be maintained from 20°C to 30°C. The isocratic condition is used to investigate the sensitivity, separation, and peak resolution. The optimal flow rate should ranging in between 0.1– 0.3ml/min (Biswas, N. M., Shard, A., Patel, S., & Sengupta, P. et., al (2018). While samples are queued for analysis, the temperature of the autosampler was held at 4°C to avoid any deterioration. The wavelength range were taken from 250 to 800 nm. Additionally, the wavelength having good peak should be selected.

Validation of bioanalytical method

The validation of bioanalytical method included extraction recovery and matrix effect studies, precision and accuracy, selectivity and specificity, and linearity.

Extraction recovery and matrix effect studies

Extraction recovery is calculated by comparing the area ratio of the extracted sample and un extracted sample. While the matrix effect is determined by the direct or indirect alteration in response of the comparison of post extracted and un extracted samples of the same.

Precision and accuracy

Precision is defined as the ability to interpret agreement across a collection of measurements acquired from multiple samplings of the same homogenous sample under prescribed conditions. While accuracy is defined as the degree to which the determined value is near to the nominal or known real value under certain condition. It is occasionally termed as trueness (Cummings, J., Ward, T. H., Greystoke, A., Ranson, M., & Dive, C. et., al (2008).

Selectivity and specificity

The term "selectivity" refers to a method for determining an analyte in a complex mixture without interference from other matrix components. While specificity is defined as the method which can determine the analyte in the presence of the other component such as degraded product, impurities, and matrix components.

Linearity

Linearity is defined as the method which gives the accurate measurement of the sample that provides sample result is proportional to the concentration of analyte in the sample within a given range, either directly or *via* a mathematical transformation (Lin, Y. T., Médioni, J., Amouyal, G., Déan, C., Sapoval, M., & Pellerin, O. et., al (2017).

Comparative analysis of the effect of anticancer drug validated through bioanalytical analysis

In this section, we will analyse the different anticancer drugs and its bioanalytical study. Focusing on the drug and its cancer-related condition we found that

doxorubicin is supposed to work for more than two cancer related condition such as breast cancer (Dubbelboer, I. R., Pavlovic, N., Heindryckx, F., Sjögren, E., & Lennernäs, H. et., al (2019) and liver cancer (Harahap, Y., Suryadi, H., & Winarti, A. C. et., al 2020). It reduces the progression of cancer cells by blocking topoisomerase enzyme. The development and validation of doxorubicin quantification method in dried blood pot by liquid chromatography-tandem mass spectrometry showed the rapid, sensitive, and specific measurements of doxorubicin concentrations (Han, J., Zhang, J., Zhao, H., Li, Y., & Chen, Z. et., al 2016). Another study suggested that the determination of doxorubicin and its dipeptide prodrug in mice plasma by HPLC with fluorescence detection showed successful development of HPLC-FD method which is very accurate and reliable, might be helpful for preclinical pharmacokinetics study of doxorubicin (Dasari, S., & Tchounwou, P. B. et., al 2014).

Cisplatin is a well-known chemotherapeutic drug known for its action against liver cancer. Besides, it is effective against various types of cancer including ovarian cancer, testicular cancer, head and neck cancer, lung cancer, lymphomas, and sarcomas. It reduces the progression of cancer cell growth by inducing apoptosis in cancer cells (Posocco, B., Buzzo, M., Follegot, A., Giodini, L., Sorio, R., Marangon, E., & Toffoli, G. et., al (2018).). Different analytical procedures were described previously for the quantification and determination of cisplatin and its analogues in biological sample including, plasma, urine, and solid tissues. Among all the methodology and analysis of the cisplatin, highperformance liquid chromatography (HPLC) is the most commonly used method in the practice. Due to the significance of pharmacological analysis, this methodology is considered as a great success in increasing the therapeutic index of the liver cancer via the treatment of cisplatin. Paclitaxel is an anticancer agent which were used to treat the various cancer including lung cancer, cervical cancer, oesophageal cancer, pancreatic cancer, and ovarian cancer. It is administered via intravenous injection. The HPLC-tandem mass spectrometry (HPLC-MS/MS) method was applied on human plasma samples to perform the clinical pharmacokinetic study (Millet, A., Khoudour, N., Guitton, J., Lebert, D., Goldwasser, F., Blanchet, B., & Machon, C. et., al 2021).

It has been used to measure the concentration of paclitaxel in plasma and to determine the pharmacokinetics of these analytes in plasma samples of cancer patients. Pembrolizumab is used in immunotherapy to head and neck cancer, melanoma, stomach cancer, lung cancer, and certain types of breast cancer. It acts against the PD-1 receptor on cell surface by preventing its binding and its activation on cell surface. In the reference of the drug pembrolizumab, we found only few literatures search which demonstrates the analysis of pembrolizumab in human Plasma by LC-MS/HRMS. Thus, the requirement of monitoring of therapeutic drug is increases day by day. Hence, we observe that the approachable way to find out the pharmacokinetics variability is through bioanalytical analysis including high performance liquid chromatography which might be helpful in the dose adjustment during chemotherapy. Figure 1 is explaining how bioanalytical procedure is working (Figure 1) and it is drawn through the help of Chem Draw software.



Figure 1. Layout of bioanalytical procedure

Conclusion and Future Perspective

From the above description, we found that the anticancer drug which went through the bioanalytical analysis supposed to work in the combination, alone, or separately against various kind of cancer. It makes to believe us that bioanalytical analysis of the anticancer agent increases its therapeutic index which surely requires the regular monitoring. New approaches are required to fight against cancer. We believe that this bioanalytical analysis of anticancer drug lesser the side effects and greater the control on cancer patient.

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