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An insight of development and validation of bio-analytical method in the reference of anti-diabetic drugs by using LC-MS/MS

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***Abstract*---**Diabetes mellitus, which has high rates of disability and mortality, is one of the main causes of public health concerns worldwide. It currently poses serious medical and societal issues. Different bio-analytical techniques have been developed to identify the pharmaceutical formulation of the anti-diabetic drug. Due to the continual requirement to achieve improved sensitivity, accuracy, and speed of analysis in complex biological samples, the development of

bio-analytical sample preparation techniques has grown more difficult over time (e.g., blood, serum, plasma, saliva, feces, and urine). This is a mini review, and its goal is to analyze how LC-MS/MS has been used to for the pharmaceutical formulation of the anti-diabetic drugs. The method was validated for linearity, accuracy, precision, specificity, selectivity, and stability.

***Keywords*---**anti-diabetic agent, liquid chromatography, bio-analytical.

Introduction

Globally, the burden of diabetes is increasing at an alarming rate. Studies have shown that India has a highest prevalence of diabetes. There are few factors which is considered as the etiology of the diabetes mellitus and that factors include genetic factors in the terms of Indian genes, obesity, sedentary lifestyle. Its prevalence varies from state to state such as 2.4% and 47.6% in Meghalaya and Delhi respectively. If diabetes left uncontrolled, it can lead to the premature death. The secondary complications of diabetes incudes, heart attack, kidney failure, loss of vision, and stroke [1]. By seeing the scenario of increasing cases of diabetes in India, it is now become important to focus on managements, principles, and medications more precisely. There are so many different forms of anti-diabetic drugs available including oral drugs and injectable drugs. Other recent drugs include di-peptidyl peptidase 4 inhibitors and sodium glucose co transporter 2 inhibitors (SGLT2) which have been approved after several characterization methods [2]. Previously, large number of drugs with different backgrounds such as anti-cancer and anti-neurological has been employed bio-analytical method for the successful estimation and quantification from different naturally occurring compounds [3, 4]. Analytical chemistry and pharmaceutical analysis is the art of determining the composition of the target drug. It involves several crucial steps and can be performed through the HPLC, LC-MS, affinity chromatography etc [5]. Several articles also published the quantification of anti-diabetic drugs with bio-analytical method. Therefore, in this review, papers published between 2017-2022 has been taken to gathered the information from sample preparation till quantification. The following described methodology can be used to for the quantification of anti-diabetic drug (figure 1-3).

Systematic Literature Search

The topic is covered the research publication including review article and original article between the year 2017–2022 by using keywords, “bio-analytical method and anti-diabetic research”. The search engine which we used to accomplished this review is Google Scholar, PubMed, and Research Gate. The articles that appeared to be potentially and completely relevant to our study is considered by the author. We have mentioned our methodology for search in the given flow chart (figure 1).

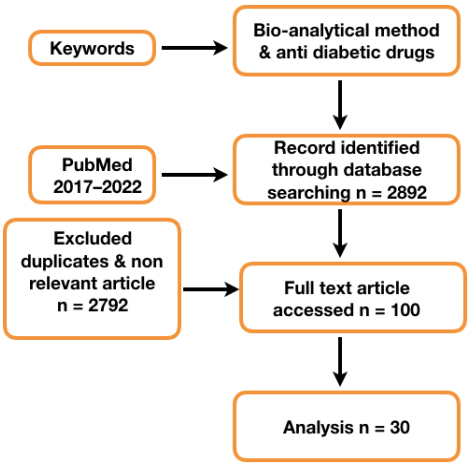

Figure 1: Step-wise systematic literature search

The literature searches as been done through the use of PubMed search engine in which we obtained more than 2000 articles on record. We excluded those articles which does not show the result for LC-MS/MS. After exclusion we obtained 100 full articles. Further, these articles were reduced to 30 review and original article after not finding the relevant information.

Detection Method

The detection method in bio-analytical analysis is broad topic which includes LC-MS (Liquid chromatography-mass spectrometry), CE-MS (Capillary electrophoresis-mass spectrometry), GC-MS (Gas chromatography-mass spectrometry), and HPLC (High performance liquid chromatography). The quantification and determination of the anti-diabetic drugs and their metabolites in bio-analytical analysis includes the following steps mentioned in figure 2.

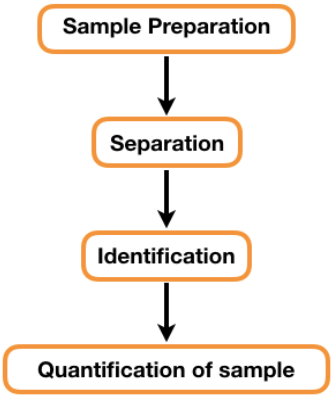

Figure 2: Step wise protocol of the detection method

The sample preparation, separation, quantification and identification has been described below.

Sample preparation

It is the crucial step and is very important to prepare the suitable sample for analysis very carefully since biological samples are complex as compared to others. The liquid liquid extraction (LLE) and solid –phase micro-extraction (SPE) are the two commonly used sample preparation procedures. The micro-extraction technique such as solid-phase micro-extraction (SPME) and liquid phase micro-extraction (LPME) are also considered as a classical sample preparation procedure. Now, the green micro-extraction techniques have been introduced overall the previous technique for sample preparation. Since it reduces the usage of samples size, minimize the consumption of reagents and minimize the risk for operators, therefore the technique is being considered as the most advance technique above all [6]. The anti-diabetic drug Mitiglinde targets the postprandial hyperglycemia, the studies showed the analysis of mitiglinde which involved human plasma as matrix is done through the sample preparation with LLE. While the other novel anti-diabetic drug Linagliptin is prepared through SPE involving human plasma and human urine as a matrix [7]. Table 1 is showing the sample preparation for the analysis of anti-diabetic drug through different bio-analytical procedure.

Table 1: Anti-diabetic drug sample preparation for bio-analytical analysis

S. No	Drug	Sample Preparation	Matrix	Reference
1.	Gliclazide	Solid-phase extraction	Human blood sample	[8]
2.	Metformin,	Liquid phase extraction	Human blood sample	[9]
3.	Linagliptin,			
4.	Sitagliptin,			
5.	Vildagliptin			
6.	Alogliptin			
7.	Liraglutide	Solid phase extraction	Dog blood sample	[10]
8.	Repaglinide	Solvent precipitation reconstitution method	Rats	[11]
9.	Metformin	Protein precipitation	Human blood sample	[12]
10.	Repaglinide	Protein precipitation method	Rabbit plasma samples	[13]

Internal standard

The internal standard is an essential step to handle the handling errors. The physicochemical properties of internal standard should be same as in anti-diabetic drug. The calibration curve of the internal standard will be used. Previous search suggested that the studies used internal standard which is suitable for their studies [14]. Guaiphenesin is used as the internal standard for analyzing anti-diabetic drug in rat plasma [15], while others showing use of deuterated internal standards. Hence, internal standard should be chosen very carefully [16].

Separation and identification through LC-MS/MS

Though there is different kind of separation technique in bio-analytical analysis which has been very well established but in this review we are focusing on LC-MS that is liquid chromatography-mass spectrometry. This analytical method has been reported for the determination of anti-diabetic activity of dapagliflozin [17] and saxagliptin simultaneously with metformin in human plasma. Another study suggested the LC-MS/MS as a highly selective and sensitive method for the bio-analytical study of metformin hydrochloride and canagliflozin in human plasma [18]. The experimental work includes the following given steps (figure 3).

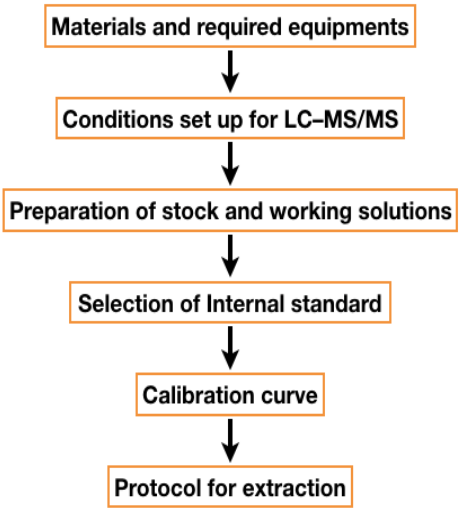

Figure 3: Experimental procedure for LC-MS/MS

The development and validation of an ultrasensitive LC–MS/MS method for the quantification of anti-diabetic drug in human plasma is used frequently because of its successful outcome. This validated method successfully delivers the pharmacokinetics profile of anti-diabetic drug.

Validation and quantification

The method development of LC-MS/MS is validated by the quantification of anti-diabetic drugs in human plasma. The FDA guidelines for the bio-analytical method is followed for the complete validation of the developed method through calculating all the validation parameters are as follows:

Selectivity

The selectivity of the method was investigated by the screening of the blank human plasma and tested anti-diabetic drugs for comparison. The method is able to differentiate and quantify the drugs in the matrix. The LLOQ that is lower limit of quantification quality control, is considered as the lowest concentration of each investigated sample which can be quantitatively estimated with precision and accuracy [18].

Linearity and Sensitivity

For linearity of calibration, the calibration curve should be constructed against the peak area ratio of the anti-diabetic drug vs the standard concentration [19], while sensitivity in LC-MS is directly proportional to the effectiveness of the producing gas-phase ions from sample in solution and its ability to transfer the ions from atmospheric pressure to the pressure zone of the transmission efficiency [20].

Matrix effect

It is defined as the percentage of the absolute peak area of spiked matrix extraction to the peak area of the spiked extracts and has been measured by the matrix factor and internal standard –normalized matrix factor [21]. The following formula should be taken while calculating matrix effect;

$$\text{Matrix factor} = \frac{\text{Mean 1(peak area in the presence of matrix)}}{\text{Mean 2 (peak area in the absence of matrix)}}$$

Recovery

The recovery of the studied anti-diabetic drugs is defined as the absolute peak areas of the five spiked matrix extraction to the peak areas of the five spiked extracts in the same concentration [23].

Accuracy and Precision

It is determined and expressed in terms of the percentage accuracy and co-efficents of variations (RSD%). The precision was determined by the calculating percentage for the concentration obtained from different determinations.

Stability

The stability of the sample is assayed through the concentration of each sample dividing the combined sample concentration. The sample is considered as stable if the assay values are within the acceptable limits of accuracy (i.e., 85–115%) of the nominal value and precision (i.e., CV% 15). It has been categorized under the following classes:

- Short term stability
- Freeze
- Long term stability
- Processed sample stability
- Dry extract stability

It is basically investigated by comparing the recoveries under the different conditions of the sample [17].

Detection of anti-diabetic drugs and their metabolites by LC-MS

The anti-diabetic drug has been recently emerging as a potential research topic based on the published positive action in many diseases. The development in the therapeutic strategies and suggested class of anti-diabetic combination has been introduced in the market. These are distinct classes of anti- diabetic drugs including hypoglycemic agents and hyperglycemic agents undergo bio-analytical validation in order to evaluate their pharmacokinetics properties. The anti-diabetic agents such as sulfonylureas, biguanides, meglitinides, and thiazolidinediones are showing the potential pharmacokinetics property against hypoglycemic condition [23]. A great deal of scientific activities is focused on the treatment of diabetes these days. The drug industries and clinical laboratories are focusing nowadays on development of bio-analytical method. The validated analytical methods are required to quantitate these anti-diabetic drugs individually or simultaneously. As we previously described, more than 100 research has been published on bio-analytical method and anti-diabetic drugs between the year 2017-2022, all are showing their research in different manners. Metformin, a very well established anti-diabetic drugs works great with combination of pioglitazone. Its effect and pharmaceutical analysis has been done through the LC-MS in human sample [24]. SGLT-2 and DPP-4 are effective against diabetes. Empgliflozin is the inhibitor of SGLT-2, while linagliptin and alogliptin are the inhibitors of DPP4. The combination of metformin with these drugs are analyzed through LC-MS and found to be effective against diabetes [25]. Repaglinide is novel anti-diabetic drug which acts as a glucose regulator. The pharmaceutical formulation has been done through the LC-MS in human sample [26]. Furthermore, the combination of metformin with gliclazide or glibenclamide has been investigated as anti-diabetic effective, while the formulation has been characterized with liquid chromatography [27].

Conclusion

The discovery and development of drugs depend mostly on bio-analysis. We have made an effort to emphasize the importance of using good scientific judgment in

order to prevent the rising expenditure of drug development. A thorough analysis of bio-analytical techniques that were approved for quantifying anti-diabetic agent combinations was carried out. The purpose of this article was to examine the validity of bio-analytical procedures and the standard of published records. The review offered a framework for simple examination of alternative methodologies/applicable circumstances to accelerate the development of new assays for more recent anti-diabetic drug combinations. Recent papers noted a very concerning variation in the validation process. These analyses can undoubtedly be taken into account when determining the therapeutic effects of anti-diabetic drugs. It is essential to investigate and contrast the current analytical and bio-analytical methods utilized to determine these drugs, either alone or in combination. LC-MS/MS is the method which is successfully considered as best way for bio-analysis.

References

1. A Simoes, R., A Calixto, L., & S Bonato, P. (2011). Bioanalytical Methods for the Analysis of Hypoglycemic Drugs and their Main Metabolites. *Current Pharmaceutical Analysis*, *7*(3), 141-159.
2. Abdel-Ghany, M. F., Ayad, M. F., et al., (2017). A Mini-Review of Spectrophotometric and Chromatographic Analysis and Bioanalysis of Selected Recently Approved Anti-Diabetic Combinations. *Research Journal of Pharmacy and Technology*, *10*(9), 3161-3172.
3. Abdelwahab, N. S., Morsi, A., Ahmed, Y. M., et al., (2021). Ecological HPLC method for analyzing an antidiabetic drug in real rat plasma samples and studying the effects of concurrently administered fenugreek extract on its pharmacokinetics. *RSC advances*, *11*(8), 4740-4750.
4. Al Bratty, M., Alhazmi, H. A., Javed, S. A., et al., (2017). Development and validation of LC–MS/MS method for simultaneous determination of metformin and four gliptins in human plasma. *Chromatographia*, *80*(6), 891-899.
5. Al Bratty, M., Alhazmi, H. A., Javed, S. A., et al., (2017). Development and validation of LC–MS/MS method for simultaneous determination of metformin and four gliptins in human plasma. *Chromatographia*, *80*(6), 891-899.
6. Elkady, E. F., El-Zaher, A. A., Elwy, H. H., et al., (2015). Validated liquid chromatographic method for simultaneous determination of metformin, pioglitazone, sitagliptin, repaglinide, glibenclamide and gliclazide-application for counterfeit drug analysis. *Journal of Analytical & Bioanalytical Techniques*, (S13), 1.
7. El-Zaher, A. A., Hashem, H. A., Elkady, E. F., et al., (2019). A validated LC-MS/MS bioanalytical method for the simultaneous determination of dapagliflozin or saxagliptin with metformin in human plasma. *Microchemical Journal*, *149*, 104017.
8. Fachi, M. M., Leonart, L. P., Cerqueira, L. B., et al., (2017). A systematic and critical review on bioanalytical method validation using the example of simultaneous quantitation of antidiabetic agents in blood. *Journal of Chromatography B*, *1055*, 61-71.

9. Fachi, M. M., Leonart, L. P., Degaut Pontes, F. L., et al., (2017). Bioanalytical methods for the detection of antidiabetic drugs: a review. *Bioanalysis*, *9*(24), 2015-2025.
10. Filippou, O., Bitas, D., & Samanidou, V. (2017). Green approaches in sample preparation of bioanalytical samples prior to chromatographic analysis. *Journal of Chromatography B*, *1043*, 44-62.
11. Govindaswamy, S., & Dhivya, P. S. (2022). Prevalence and complications of diabetes mellitus In India-A systematic review.
12. Han, D. G., Kwak, J., Seo, S. W., et al., (2019). Pharmacokinetic evaluation of metabolic drug interactions between repaglinide and celecoxib by a bioanalytical HPLC method for their simultaneous determination with fluorescence detection. *Pharmaceutics*, *11*(8), 382.
13. Hess, C., Musshoff, F., & Madea, B. (2011). Simultaneous identification and validated quantification of 11 oral hypoglycaemic drugs in plasma by electrospray ionisation liquid chromatography–mass spectrometry. *Analytical and bioanalytical chemistry*, *400*(1), 33-41.
14. J.S. Page, R.T. Kelly, K. Tang, and R.D. Smith, *J. Am. Soc. Mass Spectrom.* 18, 1582–1590 (2007).
15. Kang, Y. J., Jeong, H. C., Kim, T. E., et al., (2020). Bioanalytical method using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPL-CHRMS) for the detection of metformin in human plasma. *Molecules*, *25*(20), 4625.
16. Kapoor, N., & Thomas, N. (2017). Oral antidiabetic agents: Recently available novel oral antidiabetic agents in India: A clinical review. *Current Medical Issues*, *15*(3), 169.
17. Kulkarni, P., Karanam, A., Gurjar, M., et al., (2016). Effect of various anticoagulants on the bioanalysis of drugs in rat blood: implication for pharmacokinetic studies of anticancer drugs. *Springerplus*, *5*(1), 1-8.
18. Meng, X., Xu, H., Zhang, Z., et al., (2017). Differential mobility spectrometry tandem mass spectrometry with multiple ion monitoring for the bioanalysis of liraglutide. *Analytical and Bioanalytical Chemistry*, *409*(20), 4885-4891
19. Mohamed, D., Elshahed, M. S., Nasr, T., et al., (2019). Novel LC–MS/MS method for analysis of metformin and canagliflozin in human plasma: application to a pharmacokinetic study. *BMC chemistry*, *13*(1), 1-11.
20. Phillippe, H. M., & Wargo, K. A. (2010). Mitiglinide: a novel agent for the treatment of type 2 diabetes mellitus. *Annals of Pharmacotherapy*, *44*(10), 1615-1623.
21. Popa, R., Kamble, S. H., Kanumuri, R. S., et al., (2020). Bioanalytical method development and pharmacokinetics of MCI-92, a sigma-1 receptor ligand. *Journal of Pharmaceutical and Biomedical Analysis*, *191*, 113610.
22. Ramakrishna, B., & Mondal, S. (2021). A review of analytical methods for determination of type-ii antidiabetic drugs in pharmaceuticals and biological matrices. *Asian J Pharm Clin Res*, *14*(1), 69-76.
23. Ramu, B., Chandrul, K. K., & Pandiyan, P. S. (2019). Bio-Analytical Method Development of Repaglinide Drug Delivery Systems. *Journal of Drug Delivery and Therapeutics*, *9*(6), 140-142.
24. Sengupta, P., Bhaumik, U., Ghosh, A., et al., (2009). LC–MS–MS development and validation for simultaneous quantitation of metformin, glimepiride and pioglitazone in human plasma and its application to a bioequivalence study. *Chromatographia*, *69*(11), 1243-1250.

25. Sher, N., Fatima, N., Perveen, S., et al., (2019). Simultaneous determination of anti-diabetic drugs. *Brazilian Journal of Pharmaceutical Sciences*, *55*.
26. Vasconcelos, I., da Silva, P. H. R., Dias, D. R. D., de Freitas Marques, M. B., da Nova Mussel, W., Pedrosa, T. A., ... & Fernandes, C. (2020). Synthesis and characterization of a molecularly imprinted polymer (MIP) for solid-phase extraction of the antidiabetic gliclazide from human plasma. *Materials Science and Engineering: C*, *116*, 111191.
27. Yamuna Pradeepa, J. (2015). *Method Development and Validation for Anti Diabetic Drugs by RP-HPLC* (Doctoral dissertation, Edayathangudy. GS Pillay College of Pharmacy, Nagapattinam).