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RESEARCH ARTICLE

# Review on Development and validation of UPLC methods for quantitative determination of Anti Diabetic drugs in Pharmaceutical dosage forms

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**ABSTRACT**
A review on rapid, simple, and sensitive, stability indicating high performance liquid chromatographic method for the simultaneous determination of Drugs used for Anti-diabetic Drugs for different pharmaceutical samples by using different chromatographic column (hypersil C18, Luna (250x4.6mm, 5 µm), Hypersil c8 (4.6x250nm,5 µm), Inertsil (250x4.6x5µ) column, Waters XTerra RP18, 5 µm, 250 mm X 4.6 mm,C18column, (Kromasil ODS, 5m, 250 × 4.6mm), Zorbax Rx-C18 5µ column (150 x4.6mm)), using different mobile phase (acetonitrile : methanol in the ratio 50:50 v/v., Ammonium acetate: Acetonitrile (30:70v/v), buffer and methanol in 15:85 (v/v)). The method is detected by UV detector in limits ranging from 210-375 nm.
**Keywords:** UPLC, Anti-diabetic, Method Development, Validation, Stability Indicating.

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## 1. Introduction

Analytical method development and its validation is an important aspect in drug discovery process. Development of analytical method producing accurate and precise data is necessary to ensure the quality and safety of the drug. In today's scenario, the most common analytical method employed for estimation of drugs is Reverse Phase Ultra Pressure Liquid Chromatography (RP-UPLC) because of its high sensitivity and speed. Many types of analytical methods are available for estimation of Anti diabetic agents including RPUPLC. This review article briefly discusses analytical methods available for the estimation of currently available Anti diabetic agents specifically focusing on RP

UPLC [1-5]. Pharmaceutical Analysis is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative measurements. In practice, quantifying an analyte in a complex sample becomes an exercise in problem solving.

To be efficient and effective, an analytical chemist must know the tools that are available to tackle a wide variety of problems. Analytical chemistry is divided into two branches qualitative and quantitative. A qualitative method provides information about the identity of atomic or molecular species or functional groups in sample.

A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and biological samples. In pharmaceutical industry, spectrophotometric and chromatographic methods have gained the significance in recent years Spectrophotometric methods. It is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. The electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types [6-12].

Analytical chemistry is the science of making quantitative and qualitative measurements. In practice, quantifying an analyte in a complex sample becomes an exercise in problem solving. To be efficient and effective, an analytical chemist must know the tools that are available to tackle a wide variety of problems. Analytical chemistry is divided into two branches qualitative and quantitative. A qualitative method provides information about the identity of atomic or molecular species or functional groups in sample. A quantitative method provides numerical information as to the relative amount of one or more of the components. Varieties of analytical methods are used for the analysis of drugs in bulk, formulations and biological samples. In pharmaceutical industry, spectrophotometric and chromatographic methods have gained the significance in recent years. Spectrophotometric methods. It is defined as a method of analysis that embraces the measurement of absorption by chemical species of radiant energy at definite and narrow wavelength approximating monochromatic radiation. The electromagnetic spectrum extends from 100-780 nm. Traditionally, analytical chemistry has been split into two main types.

Ultra-performance liquid chromatography (UPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as UPLC. Ultra-performance liquid chromatography (UPLC) is rapidly becoming the method of choice for separations and analysis in many fields. It is an adsorption technique. The basic principle behind this is molecular hydrophobicity [13-20].

## 2. Development of Drug

The drug development process traditionally involves utilizing in vivo screens, which is a time consuming process. It involves a lot of study regarding the potential drug candidate including its pharmacokinetic properties, metabolism and toxicity [22-24]. The drug development process involves some basic steps given below:

- Target identification and screening
- Lead generation and optimization
- Pre-clinical and clinical studies
- Final registration of the drug

**Detection Methodologies for Diabetic Drugs** [25]

- Survey of the literature reveals various methods available for the determination of antiviral drugs like HPLC, capillary electrophoresis, mass spectrometry, liquid chromatography tandem mass spectrometry and spectrophotometric methods. However, most antiviral drugs can be analyzed by following.
- Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS)
- UV Spectrophotometer;
- Ultra-performance liquid chromatography (UPLC)
- Ultra-performance liquid chromatography Tandem Mass Spectrometry (UPLC/MS/MS).
- Most spectrophotometric methods suffer from low sensitivity, high detection limits, tedious experimental conditions and complex procedures for the preparation of samples or standard solutions. In general, UPLC and LC/MS/MS based techniques are expensive, but rapid analysis and a high degree of resolution make them choice of the researchers. Table-2 represents antiviral drugs and their detection techniques.
- Type 2 diabetes mellitus (T2DM) is a worldwide problem affecting approximately 8% of the adult population, with predictions of more than 400 million cases by 2030. The prevalence of T2DM implies an urgent need for new treatments and preventative strategies. The disease results from progressive $\beta$ cell dysfunction in the presence of chronic insulin resistance, leading to a progressive decline in plasma glucose homeostasis. Increased glucagon secretion, gluconeogenesis, renal glucose reabsorption and reduced incretin response are then observed.
- Treatments recommended by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD)

include drugs affecting all of the above processes. In most patients, lifestyle changes and metformin (MET) from biguanides are recommended after diagnosis unless contraindications are present. If the therapeutic goal is not achieved after approximately three months, one of four oral treatment options can be considered in combination with MET: sulfonylureas (SUs), peroxisome proliferator-activated receptor gamma (PPAR*y*) agonists (glitazones), dipeptidyl peptidase 4 (DPP4) inhibitors (gliptins) or sodium/glucose cotransporter 2 (SGLT2) inhibitors (gliflozins). In patients with contraindications for MET, the initial drug one of these four types of drugs will be the initial treatment option. The choice of the treatment is always based on a particular patient and drug properties, with the goal of improving glycemic control and minimizing side effects.

- The present review examines analytical methods used for the determination of giltazones, gliptins and glifozins, the second choice drug options for oral treatment, excluding MET and SUs. Additionally, detection of glinides, relatively new drugs that act as prandial glucose regulators, is discussed. Glinides are not yet included in the recommendations of ADA and EASD, but they are relatively new drugs, preferred for some patients.

## 3. Drugs used for diabetes

**Gliptins**

Gliptins constitute a newer class of agents for treatment of T2DM via the inhibition of DPP4, the enzyme that rapidly inactivates the incretin hormones such as glucagon-like peptide 1 (GLP1) and glucose dependent insulinotropic polypeptide (GIP). GLP1 and GIP serve as important prandial stimulators of insulin secretion and regulators of blood glucose concentrations. Therefore, inhibition of DPP4 prolongs the activity of endogenous GLP1 and GIP, decreasing the elevated blood glucose concentration in diabetic patients. The main drugs from this group are sitagliptin (SIT), vildagliptin (VIL), linagliptin (LIN) and saxagliptin (SAX).

**Gliflozins**

Gliflozins are the newest class of drugs for T2DM. The mechanism of their action implies binding to the SGLT2 resulting in the blockade of the glucose and sodium transport cycle. In patients treated with gliflozins, urinary glucose excretion increases which results in lowering blood glucose concentrations. The main drugs from this group are dapagliflozin (DAP), canagliflozin (CAN), ipragliflozin (IPR) and empagliflozin (EMP).

**Glinides**

Glinides stimulate insulin release through mechanisms similar to that of SUs but their action may be more advantageous for some patients. Repaglinide (REP), mitiglinide (MIT) and nateglinide (NAT) are carboxylic or amino acid derivatives which close the $K_{ATP}$ channels in $\beta$ cells, targeting a low-affinity binding site on the sulfonylurea receptor 1 subunit. Because the effects of these drugs are rapid and short-lived, they are used to curtail postprandial excursions in glucose, thus exposing patients to much less risk of hypoglycemia than SUs.

**Analytical tools for determination of oral antidiabetics**

Pharmaceutical analysis has become one of the most important stages in the therapeutic process. Drug analysis includes analytical investigations of bulk drug materials, the intermediate products, drug formulations, impurities and degradation products. Analytical techniques play a significant role in understanding the chemical stability of the drug, in evaluating the toxicity of some impurities and in assessing the content of drug in formulations. Also, they are fundamental tools in pharmacokinetic studies where the analysis of a drug and its metabolites in biological fluids must be performed. Polypharmacy, which has become an integral part of therapy for many diabetic patients, further supports the importance of drug analyses. To support polypharmacy, methods suitable for two or more components are needed for quality control of such combined formulations as well as for assays in biological samples. This paper presents analytical procedures elaborated for the listed drugs: HPLC/LC-MS, TLC/HPTLC, CE/CE-MS, spectrophotometric (UV/VIS), spectrofluorimetric and electrochemical methods. It is based on a review of the literature from the past ten years (2006-2016).

**Table 1:** LC methods for the analysis of glitazones, gliptins and gliflozins in bulk materials and formulations

| Drug | Sample | Column; mobile phase (*v/v*); flow rate; temperature; $R_T$ | Detection; Ionization, mode |
|---|---|---|---|
| **PIO** | bulk substance; impurities A-E | C18 (150x4.6 mm, 5µm); glacial acetic acid-acetonitrile-ammonium acetate (1:25:25); 0.7 ml/min; *ca*. 7, 9.8 and 21 min | UV269nm |
| **ROS** | formulations | Princeton SPHER C18 (250x4.6 mm, 5µm); phosphate buffer-acetonitrile (73:27); 1.0 ml/min; 6.40 min | UV 243 nm |
| **SAX** | formulations; degradation products | Symmetry C18 (150x4.6 mm, 5 µm); phosphate buffer of pH 4.6-acetonitrile-methanol (40:30:30); 1.0 ml/min; 4.7, 5.2; 2.7, 7.7, 3.7, 7.5 and 8.5 min | UV 208 nmMS, positive ESI |
| | | | |
| **VIL** | bulk substance; degradation products | xBridge C8 (150x4.6 mm, 5µm); acetonitrile-triethylamine 0.3%, pH 7.0 (15:85); 1.0 ml/min; 6.2, 2.4, 4.1, 2.9 and 7.8 min | UV 207nm MS |

| | | | |
|---|---|---|---|
| **DAP** | bulk substance | BDS C18; acetonitrile-phosphoric acid (55:45); 1.0 ml/min; 2.07 min | UV203nm |
| **EMP** | bulk substance | Intersil C18 (150x40 mm, 5μm); 0.01M acetate buffer-methanol (30:70), 2.0 ml/min; 1.22 min | UV260nm |

**Table 2:** UV methods for detection of oral antidiabetics

| **Drug** | **Sample** | **Analytical wavelength (nm); Molar absorptivity; Sandell sensitivity (μg/cm$^2$/001)** | **Linearity range (μg/ml); Precision** | **Solvent** |
|---|---|---|---|---|
| **PIO** | bulk substance | 234; 5.13×10$^2$, 4.39×10$^{-2}$ | 0.2-12 | Methanol |
| **PIO + MET** | bulk substance, formulations | 228.1/228.2 (difference spectroscopy) | 1-5; 0.56-0.59% | 0.1M NaOH |
| **PIO + MET + SU** | tablets | 285 | 0.15-2.4 | methanol |
| **ROS + MET** | bulk substance | 251 | 5-300; 1.11% | 6M urea |
| **SIT** | | 267 | 10-60; 2% | methanol-water |
| **SIT + PIO** | formulations | 267; 0.215068 (SIT) 269; 0.222103 (PIO) | 20-120 (SIT) 2.5-12 (PIO) | methanol |
| **SAX** | formulations | 208; 7.8×10$^3$; 2.516×10$^{-5}$ | 5-40; 0.38% | methanol |
| **DAP** | bulk substance | 203 | 1-5; 0.36% | water |

**Chromatography in reversed phase systems**

In the literature concerning oral antidiabetics, only two reports using reversed-phase chromatography were found. One procedure was described as a stability-indicating method for the determination of PIO in bulk substance and formulations. The main goal of the second study was to obtain experimental lipophilicity data useful for prediction structure-activity relationships for five glitazones, including PIO and ROS. The authors used C18 TLC plates and binary mobile phases containing water and organic modifiers, acetone (50-85%), 1,4-dioxane (40-80%) and methanol (55-95%). After development, the spots of the drugs were visualized in UV light at 254 nm. The obtained results ($R_F$) were then used for calculation of experimental *logP* values from the relationship between the $R_M$ values and the concentration of organic modifier in the mobile phase. The obtained lipophilicity values were compared with some pharmacological properties of the drugs.\

**UV methods for detection of oral antidiabetics**

Direct assays in the UV range or techniques utilizing various derivatives were applied for the drugs mentioned in the present review. The methods were mainly proposed as simple and inexpensive alternatives to HPLC for quantitative measurements of active ingredients in pharmaceuticals. Some of them were proven to be sufficiently selective to detect the drugs in combined dosage forms or in the presence of degradation products.

## 4. Conclusions

In the literature, a broad range of methods have been presented for the analysis of oral antidiabetics in bulk materials and pharmaceuticals. HPLC with UV detection and UV spectrophotometry were mainly used, due to their accuracy, precision and sensitivity. These methods were adequate to analyze the drugs in single component formulations as well as in combinations. Also, TLC/HPTLC with densitometric detection and VIS spectrophotometry were widely used for typical analysis in pharmaceutical formulations. The former method was frequently proposed as an alternative to UPLC while the latter method was used when simplicity and cost effectiveness were required. UPLC-UV and LC-MS are undoubtedly the methods of choice for bioanalytical assays. Bearing in mind the data presented above it could be concluded that higher selectivity of LC-MS was the main difference between the described methods, while sensitivity was similar. Additionally, the LC-MS systems were frequently realized as UPLC techniques. All authors confirmed that UPLC drastically reduced the mobile phase consumption, thus having obvious economic and ecological consequences. At the same time, significant improvements in resolution and sensitivity were achieved.

**List of abbreviations**

ADA: American Diabetes Association
APCI: atmospheric pressure chemical ionization
CAN: canagliflozin
CAA: p-chloranilic acid
CD: cyclodextrin
DAP: dapagliflozin
DPP4: dipeptidyl peptidase 4
EASD: the European Association for the Study of Diabetes
EIS: Electrochemical impedance spectroscopy
EMP: Empagliflozin
ESI: Electrospray ionization
FDA: Food and Drug Administration
GIP: Glucose dependent insulin tropic polypeptide
GLP1: Glucagon-like peptide 1
IMS: Ion mobility spectrometry
IPR: ipragliflozin
LIN: linagliptin
LLE: liquid-liquid extraction
LOD: Limit of detection
LOQ: Limit of quantification
LPME: liquid phase microextraction
MET: metformin
MIP: Molecularly imprinted polymer
MIT: Mitiglinide

MRM: Multiple reaction monitoring
NAT: Nateglinide
OPA: o-phthalaldehyde
Ph. Eur. : European Pharmacopoeia
PIO: pioglitazone
ROS: rosiglitazone
SAX: Saxagliptin
SF: fluorescence
SGLT2: sodium/glucose co-transporter 2
SIM: selective ion monitoring
SIT: Sitagliptin
SPE: solid phase extraction
SRM: selected reaction monitoring
SU: sulfonylurea
T2DM: type 2 diabetes mellitus
UPLC: ultra performance liquid chromatography
USP: US Pharmacopoeia
VIL: vildagliptin

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