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A review of analytical techniques for determination of metformin: present and perspectives

Anil Kumar Tallam[1], Alapati Sahithi[2], Mohana Vamsi Nuli[3]

[1]Department of pharmacy,Shri Venkateshwara University,Rajabpur, NH-24, Venkateshwara Nagar, Gajraula, Uttar Pradesh 244236

[2]Assistant Professor, Department of Pharmaceutical Analysis,Nalla Narasimha Reddy Education Society's Group of Institutions, Narapally, Ghatkesar Mandal, Korremula Rd, Hyderabad, Telangana 500088

[3]Associate Professor, Raghavendra Institute of Pharmaceutical Education and ResearchK.R. Palli Cross, Dist Anantapuramu, Chiyyedu, Andhra Pradesh 515721

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Abstract

Metformin is an oral anti-diabetic drug in preventing complications of type 2 diabetes and it is a good first-line therapy for an over-obese with type 2 diabetes, it is currently available in more than 60 countries worldwide. As a result of the importance of this oral hypoglycaemic agent in the treatment of non-insulin-dependent diabetes mellitus, which leads to end-stage renal disease, this work aims to compile the published analytical methods reported so far in the literature for the determination in biological samples and pharmaceutical formulations. This article narrates different techniques like high-performance liquid chromatography. It can be seen that high-performance liquid chromatography methods have been used extensively. Thus, this paper will help in the selection and development of proper analytical methodologies estimation of Metformin to achieve satisfactory results.

***Corresponding Author**
Anil Kumar Tallam

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Introduction [1]:

The discovery of metformin was beginning with synthesis of galegine like compounds derived from *Gallega officinalis,it is a plant traditionally employed in Europe* as a drug for diabetes treatment formany years. In 1950, Stern *et al*. discovered the clinical usefulness of metformin while working in Paris. They observed that the dose-response of metformin was related to its glucose lowering capacity.

Metformin as a non-insulin oral hypoglycaemic which acts primarily in the liver by reducing glucose output and also by avoiding glucose uptake in the peripheral tissues. These effects are mediated by the activation of an upstream kinase, liver kinase B1 (LKB-1), which in turn regulates the downstream kinase adenosine monophosphate protein kinase (AMPK). AMPK phosphorylates a transcriptional co-activator, transducer of regulated CREB protein 2 (TORC2), resulting in its inactivation which consequently downregulates transcriptional events that promote synthesis of gluconeogenic enzymes and by the inhibition of mitochondrial respiration has also been proposed to contribute to the reduction of gluconeogenesis, as it reduces the energy supply to the cells for this process.

The importance of preventing the type 2 diabetes is to have improved health policies; one of the main reasons is promoting obesity due to the western life style. If they are failed, the pharmacological interventions are used to

reduce diabetes mellitus for the people suffering from impaired glucose tolerance and these will help for effective pharmacological treatment for type 2 diabetes.

Metformin is bigunide class of ant diabetics with anti hyper glycaemicactivity. Metformin is also indicated for the treatment of reduce LDL cholesterol and triglyceride levels and is not associated with weight gain and prevents cardiovascular complications of diabetes. Metformin is not metabolised by kidney and will be excreted as unchanged. It is also recommended for the patient having type II diabetic disease with over obese.Metformin is solid, water soluble and freely soluble as HCl salts.

Due to the importance of this type of drugs in the treatment of type 2 diabetes, here is the use of review of analytical work has reported in the literature for the validation and determination of Metformin along with its combinations which is very useful information for an academic and industrial prospective.

Physical And Chemical Properties [3]

Metformin is white and almost white crystalline powder with the IUPAC name 3-(diamino methylidene)-1,1-dimethylgunidine, having molecular formula $C_4H_{11}N_5$ with the molecular weight 129.197g/mol,melting point of 223-226^{o}C, has water solubility freely soluble as HCl salt, stable under recommended storage conditions,hazardousdecomposition products formed under fire conditions.

Analtyical Methods

Cromatographic Method

A) Reverse- Phase High performance liquid chromatographic:

Umathe*et al*[4]determined a simple reverse phase high-performance liquid chromatographic method which has been developed for determining the concentration of metformin in rat plasma. This method was employed by C_{18} column (300 mm × 2.4 mm i.d.), ammonium acetate (0.15 M) and acetonitrile (90:10; pH-5.5; 1.0 ml/min) as mobile phase and ultraviolet detection at 236 nm and the Acetonitrile was used simultaneously for deproteinize rat plasma and extract metformin. The assay was linear in the concentration range of 0.33 µg-16.6 µg/ml with co-efficient of correlation 0.994 and the retention time was 4.7 min. The method was found to be precise (% CV < 15%), accurate and suitable for pharmacokinetic study of orally administered metformin in rats.

Development And Validation Of RP-HPLC Method for The Analysis Of Metformin was done by saeed *et al* [5], as the reverse phase high performance chromatography method has been developed to quantify Metformin hydrochloride in raw material and pharmaceutical formulations using C18 analyticalreverse phase column. Diazepam was used as an internal standard. Mobile phase consisted of methanol-water(30:70v/v), pumped at a flow rate of0.5ml/minand the retention time was about 4.4min and was detected by UV absorbance at 233nm, this method was linear over the concentration range 0.312-5 mug/ml (R2=0.9995). The limit of detection of Metformin was 0.1mug/ml and the limit of quantitation was0.3mug/ml. The results obtained showed a good results and the method is rapid accurate and economical and selective and it may be used for the quantitative analysis of Metformin in neodipar tablets because of its sensitivity and reproducibility.

Alekya*et al*[6] presenteda work whichis simple and sensitive RP-HPLC Method for the Development and Validation for the simultaneous estimation of Metformin and Vildagliptin in bulk and its pharmaceutical dosage form and their Bio-Analytical studies. Chromatography was carried out on Kromosil C18 (4.6 x 250mm, 5mm) column using Phosphate buffer pH 5.8 and Acetonitrile in the ratio of 80:20 as the mobile phase at a flow rate of 1 ml/min with UV detection at 215 nm. The Retention time of Metformin and Vildagliptin is 2.589 mins and 4.296 mins respectively. The detector response is linear. The Limit of Detection for Metformin and Vildagliptin is 0.06 µg/ml and 0.1 µg/ml and Limit of Quantification for Metformin and Vildagliptin is 0.2 µg/ml and 0.4 µg/ml respectively. The Percentage assay for Metformin and Vildagliptin is 99.6% and 99.2% respectively and Percentage Recovery for average of three different concentrations for Metformin and Vildagliptin is 99.9% and 100.1% respectively. The method was validated by determining its selectivity, robustness, linearity, accuracy and precision. The developed method is simple, fast, sensitive, linear, accurate, rugged and precise and hence can be applied for routine quality control of Metformin and Vildagliptin in bulk and its pharmaceutical dosage form.

Mahesh *et al* [7]proposed method was to develop a simple, fast, sensitive, and validated high-performance liquid chromatography (HPLC) method for the simultaneous estimation of metformin and Vildagliptin in tablets and human plasma. Materials and Methods: The chromatographic separation was accomplished with a fast monolithic column using a mixture of sodium dihydrogen phosphate and sodium dodecyl Sulfate and acetonitrile at pH 4.5 as the mobile phase. In addition, newly developed method was validated as indicated by

International Conference on Harmonization guidelines. Results: The method showed good correlation coeffi cients (r ≥ 0.997) in the range of 0.05-20 μg/mL and 0.1-40 μg/mL for metformin and Vildagliptin, respectively. The accuracy and intraday and interday precision results were within the acceptable range for both analytes. The mean extraction recoveries of metformin and Vildagliptin from human plasma were 97.51% and 97.18%, respectively. Conclusion: The simple, rapid, sensitive, robust and validated analytical method developed was used for simultaneous estimation of metformin and Vildagliptin in formulation and in patient plasma.

b) High performance liquid chromatographic with U.V spectroscopic

Mousumi *et al* [8] proposed a simple, accurate, economical and reproducible HPLC method has been developed for quantitative estimation of metformin hydrochloride from tablet dosage form and formulated microspheres. The developed HPLC method is a reverse phase chromatographic method using Phenomenex C_{18} column and acetonitrile: phosphate buffer (65:35) pH adjusted to 5.75 with o-phosphoric acid as mobile phase and glipizide as internal standard. The linearity was observed in concentration range of 0-25 μg/ml for metformin hydrochloride. Results of analysis were validated statistically and by recovery studies.

A simple, selective, sensitive and precise high-performance liquid chromatographic plasma assay for the hypoglycemic agent metformin is described by Cheng *et at*[9]. Acidified samples of plasma were deproteinated with acetonitrile, washed with dichloromethane and the resulting supernatant injected. Chromatography was performed at 40 degrees C by pumping a mobile phase of acetonitrile (250 ml) in pH 7, 0.03 M ammonium hydrogen phosphate buffer (750 ml) at a flow-rate of 1 ml/min through a silica column. Metformin and the internal standard (atenolol) were detected at 240 nm and were eluted 7.8 and 6.8 min, respectively, after injection. No endogenous substances were found to interfere. Calibration curves were linear (r>0.999) from 10 to 2000 ng/ml. The absolute recovery of both metformin and atenolol was greater than 76%. The detection limit and limit of quantitation were 2.5 and 10 ng/ml, respectively. The intra- and inter-day precision (C.V.) was 12%, or less, and the accuracy was within 6.2% of the nominal concentration. This method is suitable for clinical investigation and monitoring metformin concentration.

Porwal *et al* [10] proposed a simple, sensitive, fast, and economical HPLC method was developed and validated for simultaneous estimation of two fixed dose combinations frequently prescribed in diabetes (Metformin plus Glibenclamide) and hypertension with dyslipidemia (Amlodipine plus Atorvastatin) in Human plasma for the first time. The validated HPLC method was used to quantify the concentration of selected actives in ultra-filtrate. Optimum separation conditions were obtained with Water's Novapack Phenyl (150 mm × 4.6 mm, i.d., 5.0 μm) column with mobile phase consisting of 0.1% Phosphoric acid (pH 3.0) and acetonitrile (ACN) in gradient mode with column oven temperature maintained at 30 °C and elution monitored by a UV detector at 227 nm. Protein precipitation was employed to extract the selected analyte form human plasma. The recoveries were more than 90% for all analytes in cold aqueous 10% trichloroacetic acid (TCA) and acetonitrile. The optimized HPLC-UV was validated in the calibration range of 10–10,000 ng mL^{-1} for Metformin, 25–5000 ng mL^{-1} for amlodipine, 50–10,000 ng mL^{-1} for glibenclamide and 10–5000 ng mL^{-1} for atorvastatin. The mean relative error was least when weighing of $1/\times^2$ was applied for calibration curve. The accuracy of samples for six replicate measurements at LLOQ level was within limit. The precision and accuracy of samples for six replicate measurements at LLOQ level was within limit. The validated method was applied for quantitation of selected analytes in ultra-filtrate from protein binding experiments. A four to five-fold increase in unbound fraction was observed when spiked to human serum albumin. Further the unbound fraction of highly albumin bound drugs was increased nearly to double when incubated with Gly-HSA as compare to HSA.

A simple, precise, and accurate HPLC method for simultaneous estimation of metformin hydrochloride (MET), pioglitazone hydrochloride (PIO), and glimepiride (GLIMP) was developed and validated by Devi *et al* [11], Chromatographic separation of the drugs was performed by using a Phenomenex-ODS-3 (C-18) column (250 × 4.60 mm, 5 μm) with a mobile phase consisting of methanol:acetonitrile:15 mM potassium dihydrogen phosphate (pH 4) in the proportion of 40:35:25 (v/v) at a flow rate of 1 ml/min. Detection was carried out using a UV-SPD-10AVP detector at 240 nm. The retention time for MET, PIO, and GLIMP were 2.85 ± 0.03 min, 4.52 ± 0.03 min, and 7.08 ± 0.02min, respectively. Parameters such as linearity (0.2–50 μg/ ml for MET, 0.2–30 μg/ml for PIO, and GLIMP,

respectively), precision (intra-day % RSD was 1.01–3.24 and inter-day % RSD was 1.54–4.09 for MET; intra-day % RSD was 1.03–2.09 and inter-day % RSD was 2.26–3.10 for PIO; and intra-day% RSD was 1.00–3.15 and inter-day % RSD was 1.58–3.07 for GLIMP), accuracy (99.66 ± 0.14 for MET, 98.46 ± 0.40 for PIO, and 98.62 ± 0.39 for GLIMP), specificity and robustness were calculated in accordance with ICH guidelines. The method was proved to be simple, rapid, precise, accurate, and cost effective.

c) Stability indicating chromatographic method:

A simple and selective HPLC-DADStability study was developed for simultaneous determination of three anti diabetic drugs Metformin(Met), Saxagliptin(Saxa),Sitagliptin(Sita) in their combined formulation described by Sarif Nirough *et al* [12]. The chromatographic separation of these three drugs was achieved using a Inertsil C_{18} (4.6 × 250 mm, 5 μm) analytical column with the gradient elution in isocratic mode of mobile phase using buffer potassium dihydrogen phosphate adjusted pH 4 with orthophosphoric acid: methanol:acetonitrile (70:10:20%v/v) column at ambient temperatureat a flow rate of 1 mL/min, and detection of all the drugs was set at 215 nm using a detector DAD. All the solvents were filtered through 0.45-μm nylon filter, and degassed in an ultrasonic bath previous to use. Measurements were carried out by using injection volume 20 μL and detection at 215 nm. Quantification of these drugs was based on the peak areas, for analysis of forced degradation analysis of samples, the PDA detector was used in scan mode with a scan range of 200–400 nm.

Krishna Karthik Peruru *et al*[13] A simple precise, specific and accurate stability indicating Reversed Phase High Performance Liquid chromatography was developed for the simultaneous estimation of Metformin hydrochloride (Met) and Pioglitazone hydrochloride (Pio) in dosage form the chromatographic separation was performed C8 column (Qualisil BDS 250 mm × 4.6 mm, 5 μm) with a mixture of methanol and water at 45:55 (v/v) containing 0.2% (w/v) n-heptane sulfonic acid (HSA) and 0.2% (v/v) triethyl amine (TEA) as mobile phase and the flow rate was 1 ml/min and eluents were detected at 265 nm. This method shows for PIO with respective correlation coefficient (r2) values of 0.9996 and 0.9997. The described method shows linearity between 100–750 μg/mL for MET and 5–30 μg/mL Drugs were subjected to oxidative, acid base hydrolysis, photolytic, thermal and neutral degradations. This method revealed 14 degradation products and among these products D1, D3, D11, D12 and D14 were identified using impurity standards.

New Validated Stability Indicating RP-HPLC Method for Simultaneous Estimation of Metformin (Met) and Alogliptin (Alo) in Human Plasma based on Liquid chromatography has been developed by Ashutosh *et al*[14]; The drug was spiked in the plasma and extracted with mobile phase by precipitation method. The extracted analyte was injected into X-Terra C18 (4.6 × 150 mm, 3.5 μm, make: ACE) and was maintained at 25°C temperature and effluent was detected at 235 nm. The mobile phase was consisted of sodium dihydrogen ortho phosphate [pH 4.0]: acetonitrile of HPLC Grade (70:30 v/v). The inter-day and intra-day precision was found to be within limits. The Lower limit of quantification (LLOQ) for metformin and Alogliptin were 5.936 and 1.983 μg/mL respectively. The average % recovery for metformin and alogliptin were 100.17 and 99.40-99.55% respectively and reproducibility was found to be satisfactory. The calibration curve for metformin and alogliptin was linear from 300.0 to 700.0 μg/mL (r2=0.997) and 7.5 to 17.5 μg/mL (r2=0.998) respectively. This RP-HPLC method is suitable for determining the concentration of metformin and alogliptin in human plasma and it can have applied for routine analysis for determination of the metformin and alogliptin from dosage form during pharmacokinetic study.

d) High Performance Thin Layer Chromatography Method:

Darshana *et al* [15] performed a simultaneous estimation of two anti-diabetic drugs, Metformin and Glipizide in pharmaceutical dosage form. A normal phase thin layer liquid chromatography plate i.e. Silica gel 60 F_{254} was used as stationary phase and water: methanol:0.5% w/v ammonium Sulfate solution (6:3:1.5 v/v/v) and the λ_{max} was at 236 nm. The linear regression data for the calibration plot showed a good relationship with r = 0.9962 and 0.9930 for metformin and glipizide, respectively. The limits of detection and quantification were 991.30 and 3003.95 ng/band for metformin and 9.57 and 29.01 ng/band for glipizide, respectively. This system gave a good resolution for metformin (R_f value of 0.22 ± 0.01) and glipizide (R_f value of 0.85 ± 0.01). The method was validated for precision and recovery.

Sunil *et al*[16] Estimated Metformin in Bulk drug and in formulation by HPTLC. A normal phase thin layer liquid chromatography plate i.e. Silica gel was used as stationary phase using ammonium sulfate (0.5%): 2-propanol: methanol in the ratio of 8.0:1.6:1.6 (v/v/v) as mobile phase. The linear regression data for the

calibration plot showed a good relationship with r =0.999. Metformin showed Rf value of 0.50±0.03 was scanned at 238 nm and using Camag TLC Scanner 3. The limits of detection and quantification were 95 and 200 ng/spot respectively. The method was validated for precision and recovery. This method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

e) High Performance liquid chromatographic method coupled with M.S:

A sensitive, specific and selective method has been developed for the simultaneous determination of Metformin and Rosiglitazone in human plasma presented by L Zhang *et al*[16]. The method employed states that the high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (ESI-MS/MS) method was performed in human plasma using phenformin as internal standard (IS) has been first developed and validated. Plasma samples were precipitated by acetonitrile and the analytes were separated on a prepacked Phenomenex Luna 5u CN 100A (150 mm x 2.0 mm I.D.) column using a mobile phase comprised of methanol:30 mM ammonium acetate pH 5.0 (80:20, v/v) delivered at 0.2 ml/min. Detection was performed on a Finnigan TSQ triple-quadrupole tandem mass spectrometer in positive ion selected reaction monitoring (SRM) mode using electrospray ionization. The ion transitions monitored were m/z 130.27-->71.11 for metformin, m/z 358.14-->135.07 for rosiglitazone and m/z 206.20-->105.19 for the IS. The standard curves were linear (r(2)>0.99) over the concentration range of 5-3000 ng/ml for metformin and 1.5-500 ng/ml for rosiglitazone with acceptable accuracy and precision, respectively. The within- and between-batch precisions were less than 15% of the relative standard deviation. The limit of detection (LOD) of both metformin and rosiglitazone was 1 ng/ml. The method described is precise and sensitive and has been successfully applied to the study of pharmacokinetics of compound metformin and rosiglitazone capsules in 12 healthy Chinese volunteers.

f) Thin layer chromatography:

A simple precise rapid and accurate thin layer chromatography-densitometry(TLC-Densitometry) had been developed by Regina Andayani *et al*, for the determination of mixed Metformin HCl and glibenclamide in tablet dosage forms. Normal phase thin layer chromatography plate i.e., silica gel $60F_{254}$ was used for stationary phase and mobile phase as methanol: water: glacial acetic acid in the ratio of (6:4:0.25). This system gave a good resolution for Metformin Hydrochloride 0.52 as Rf value and 0.78 for Glibenclamide. Determination was done by densitometer at the absorbance of 237nm and 300nm for Metformin and glibenclamide respectively. The method was validated for precision, accuracy and linearity. Precision of the method were between 0.56-2.02% for Metformin HCl and 0.08%-1.30% for glibenclamide respectively. Accuracy of the method was found to be 88.43-104.54% for Metformin HCl and 97.22-102.88% for glibenclamide respectively and the linearity regression data for the calibration plot showed a good relationship either r=0.999 and 0.996 for Metformin HCl and glibenclamiderespectively. According to the results, this method was in accordance with good validation requirements.

Method Benefits and Limitations:

The application of HPLC to environmental analysis is often hindered by difficulty not experienced in other areas of analysis. Usually the components being determined are at parts per million levels or less and are usually in sample matrices that can yield much interference. In order to develop successful methodology, the prime requirements for an HPLC system are column efficiency and the sensitivity and selectivity of the detection system..

The dominance of HPLC as a premier analytical technique is no accident. The most prominent advantage is its applicability to diverse analytes types, from small organic molecules and ions to large bio molecules and polymers. The successful coupling of HPLC to MS gave it an invincible edge as "the perfect analytical tool" — combining excellent separation capability with the unsurpassed sensitivity and specificity of MS. HPLC–MS is rapidly becoming the standard platform technology for bio analytical testing (drugs in biological fluids), trace analysis for residues in food, forensic and environmental samples, and life science research (3–6). Finally, the excellent precision and robustness of HPLC with UV detection makes it an indispensable tool for quality control (QC). This last point is illustrated by a case study on stability evaluation of a pharmaceutical product

Conventional HPLC has a practical peak capacity (P_c) of ~200 using columns with ~20,000 plates under gradient conditions — not particularly effective for very complex samples The advent of UHPLC has extended P_c to 400–1000 range in a time span of ~60 min (9,12–16). 2D-LC can further increase P_c for comprehensive analysis of very complex samples in proteomics and metabonomics.

HPLC is versatile, quantitative, sensitive, and extremely precise. It can also be time-consuming and arduous, particularly for regulated analysis under good manufacturing practices (GMP). For instance, these are the steps in a typical operation: weighing reference standards; preparing samples and mobile phases; setting up the column and all modules; performing system suitability testing; injecting standards to calibrate the system followed by samples analysis; performing peak integration; reporting; reviewing; and sign-offs. Fortunately, most steps are automated by precision instruments for routine testing and are therefore highly reproducible. Compare it with spectroscopic analysis such as the identification of raw materials using a hand-held Raman spectrometer — point the laser to the sample, press a button and a pass or fail result with GMP documentation is available in seconds. One piece of advice: Don't use HPLC unless you have to quantitative analytes with high accuracy and precision

Conclusion

The present review provides information about the various methodologies which were available in the literature for the determination of Metformin alone and with the combination. The overall analysis of published data revealed that HPLC was the most extensively used analyser for the determination of Metformin as compound and the determination of Metformin in formulation and biological samples were recommended with the HPLC-MS/MS method, since this method combines the HPLC separation ability with MS. Sensitivity and selectivity, allowing all the type of samples containing Metformin. However, HPLC and with U.V detection is applicable because this method provides an accurate results and has a low expense when compared to more expensive devices which has advanced detection technique. This article is carried-out an over view of current state-of-art analytical method for the determination of Metformin.

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