Original Article

Reported Analytical Methods of Metformin: An Extensive Review

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ABSTRACT:
Metformin, a widely prescribed oral antidiabetic drug, plays a crucial role in managing type 2 diabetes by improving glycemic control. Given its extensive use, the accurate and reliable determination of metformin in pharmaceutical formulations, biological fluids, and environmental samples is essential for quality control, therapeutic monitoring, and environmental safety. This review provides a comprehensive overview of the various analytical methods reported for the quantification of metformin. Techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), ultraviolet-visible (UV-Vis) spectroscopy, capillary electrophoresis, and voltammetry are discussed in detail. Emphasis is placed on the sensitivity, specificity, accuracy, and precision of these methods, along with their applicability in different matrices. The review also highlights recent advancements in analytical techniques, including the development of green analytical methods, which offer eco-friendly alternatives to traditional approaches. Challenges associated with metformin analysis, such as matrix effects and interferences, are also explored, providing insights into the ongoing efforts to enhance the robustness of metformin quantification. This article aims to serve as a valuable resource for researchers and professionals involved in the analysis of metformin, facilitating the selection of appropriate methods for various analytical needs.

KEYWORDS: Metformin, UV spectroscopy, HPLC method, Mobile Phase

INTRODUCTION:

Type 2 diabetes is treated with the oral antidiabetic metformin, which is a member of the biguanide class. It works by preventing the liver from producing glucose. It lowers LDL and it may even help some people lose weight [1].Metformin by itself and in combination with other medications including glibenclamide, pioglitazone, and rosiglitazone, metformin is available for purchase. It was first synthesised in 1922 through the heating-reaction of 2-cyanoguanine and dimethylamine hydrochloride [2]. The main side effect of metformin is lactic acidosis; GI effects are among the others. Heart failure, kidney problems, lung diseases, and liver diseases should not be treated with this medication [3].

Method Of Analysis:

The amount of metformin in pharmaceutical goods and neat solutions can be determined using a variety of techniques. We go over a few of these techniques below.

Spectrometric Methods:

Spectroscopy: A quick and easy approach to analyze pharmaceutical formulations of metformin is to use near-infrared reflectance spectroscopy. The method's results were in good agreement with the metformin UV assay method reported in BP 1998. The first set of spectrum data was recorded between 1000 and 2500 nm in wavelength. A technique that uses an atmospheric pressure chemical ionization source as a detector has been proposed for the simultaneous measurement of metformin and glipizide in human plasma. The method has been found sensitive, rapid, simple and suitable for pharmaceutical preparations. A linear and reproducible method has been developed for the simultaneous determination of metformin and glyburide in human plasma. The linearity was seen in the range of 20-2500 ng/mL [4-8].

UV Spectrophotometry: Two brand-new techniques for metformin analysis have been created. It has been discovered that these procedures are straightforward, precise, accurate, and repeatable. Metformin in the range of 2–12 µg/mL and 1–12 µg/mL at 237.6 and 247.4 nm, respectively, was needed for these procedures. Pharmaceutical goods can be applied with these approaches in a satisfactory manner [9].In an alkaline media, the amino group of metformin reacts with ninhydrin to produce violet color chromogen. At 570 nm, the chromogen has been identified spectrophotometrically. The technique is straightforward, sensitive, and has demonstrated a recovery percentage of 97-90% in the absence of excipient influence. Both the bulk and pharmaceutical dosage forms can be successfully used with this strategy [10].For the simultaneous determination of metformin and rosiglitazone in synthetic mixes and coated tablets, a different developed and verified approach has been proposed. Metformin's concentration ranged from 20.0 to 80.0 µg/mL, with an Amax of 236 nm [11].

Chromatographic Methods:

Thin Layer Chromatography: Metformin alone in pure form and with glimepiride in pharmaceutical products was analyzed by a simple and selective salting-out thin layer chromatographic technique. In pharmaceutical formulations, metformin has also been determined simultaneously with sitagliptin. None of the excipients were found to cause interference, and the procedure was discovered to be quick, easy, and accurate. [12] Another strategy that was put forth and confirmed using stability indicating high performance thin layer chromatography (HPTLC) to determine metformin and nateglinide simultaneously in a pharmaceutical dose form. The mobile phase in the investigation consisted of chloroform: acetic acid: ethylenetetraacetic acid (4:6:0.1, v/v/v) and silica gel plates. It was discovered that the metformin method's accuracy was 100.08%[13]. Pharmaceutical formulation comprising of metformin and glyburide was used for the determination of metformin by TLC method.

HPLC Methods: HPLC is the most widely used method for the analysis of metformin in biological fluid and pharmaceutical products.

Drug Profile[14]:

Drug Name: Metformin
IUPAC Name: N,N-dimethylimidodicarbonimidicdiamide
Molecular formula: $C_4H_{11}N_5$
Molecular mass: 129.164 g/mol
Melting point: 224.5°C
Solubility: Freely soluble in water, soluble in alcohol, insoluble in ether and chloroform
Stability: Light sensitive, decomposes when heated emiting fumes of nitric oxide
pka: 12.4
pH: 6.68 (1% aqueous solution)
Appearance: White to off-white crystalline powder
Route of administration: Oral

Absorption: Slow, food delays the absorption of conventional tablets
Bioavailability: 50-60% (with dosages of 0.5-1.5 g)
Plasma-protein binding: Negligible
Volume of distribution: 300-1000 l after a single dose
Half-life : 6.2 h
Distribution: Rapid (peripheral body tissues and fluid)
Metabolism: Not metabolized
Excretion: 35-52% in urine, 20-33% in feces as unchanged drug

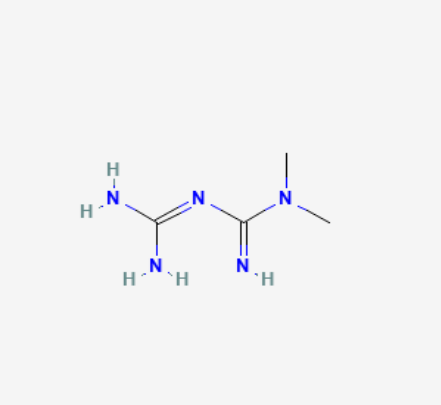

Fig 1: Structure Of Metformin [15]

OFFICIAL I.P. METHODS OF METFORMIN:

SR NO	DRUG	Method	Summary	REFRENCE
1	Metformin hydrochloride	High Performance Liquid Chromatograph	**A stainless steel column;** 30cm x 4mm packed with octadecylsilane bonded to a porous silica **Mobile phase:** A solution containing 0.08%w/v of sodium pentanesulphonate & 0.12%w/v of Nacl **Flow Rate:** 1ml/min Spectophotometer set at 218nm **Injection Volume:** 20µm	16

REPORTED ANALYTICAL METHODS OF METFORMIN:

SR NO	DRUG	METHOD	SUMMARY	REFRENCE
1	Metformin and glimepiride	RP-HPLC	**Column:** Promocil C-18 **Mobile Phase:** Acetonitrile and ammonium acetate buffer 0.05 M pH 3.0 **Flow Rate:** 1mL/min **Conc. Range: -**	17
2	Metformin in human plasma	HPLC	**Column:** Silica column **Mobile Phase:** Acetonitrile (250 mL) in	18

			pH7, 0.03 M diammonium hydrogen phosphate buffer (750 mL) **Flow Rate:** 1mL/min **Conc. Range:-**	
3	Metformin and Rosiglitazone	RP-LC	**Column:** Zorbax XDB C-18 **Mobile Phase:** 10 mM disodium hydrogen phosphate and 5 Mm sodium dodecyl sulphate (34:66, v/v), ph adjusted to 7.1 with orthophosphoric acid **Flow Rate:**1.0mL/min **Conc. Range: -**	19
4	Metformin HCl	RP-HPLC	**Column:**C-18 **Mobile Phase:** Methanol-Water(30:70,v/v) **Flow Rate:** 0.5mL/min **Conc. Range: -**	20
5	Metformin and rosiglitazone in plasma	LC	**Column:** Phenyl column **Mobile Phase:** Acetonitrile-5mM acetate buffer pH 5.5 (75:25, v/v) **Flow Rate:** 1.0mL/min **Conc. Range: -**	21
6	Metformin HCl and pioglitazone HCl	RP-HPLC	**Column: -** **Mobile Phase:** Acetonitrile-water-acetic acid (60:40:0.3), pH adjusted to 5.5 by adding triethylamine **Flow Rate:** 1mL/min **Conc. Range:**0.5-4.0 µg/mL	22
7	Metformin HCl, phenformin HCl, acarbose and voglibose	HPLC	**Mobile Phase:** 30%(0.06% Potassium dihydrogen phosphate & 0.028% disodium hydrogen phosphate) & 70% (acetonitrile) **Flow Rate:** 1 mL/min **Conc. Range:** 0.1-3µg/mL **Column:** Thermo NH2 Analytic column	23
8	Metformin	HPLC-UV	**Column:** Silica column **Mobile Phase:** 0.01 M ammonium acetate pH 5.0 and acetonitrile (40:60, v/v) **Flow Rate:** 1.0mL/min **Conc. Range: -**	24
9	Metformin	HPLC	**Column:**C-18	25

			Mobile Phase: Acetonitrile-KH_2PO_4 **Flow Rate:**0.7mL/min **Conc. Range:**10-5000 µg/mL	
10	Metformin HCl and glyburide	RP-HPLC	**Column:**C-18 **Mobile Phase:** Acetonitrile-Water(60:40,v/v) **Flow Rate:**0.9mL/min **Conc. Range:**0.06-0.24 µg/mL	26
11	Metformin, cimetidine, famotidine and ranitidine in human serum and dosage formulation	HPLC	**Column:** Purospher Star RP 18 **Mobile Phase:** Methanol-water triethylamine (20:80:0.05), pH adjusted to 3 with phosphoric acid 85% **Flow Rate:** 1mL/min **Conc. Range:**5-25 µg/mL	27
12	Metformin, diltiazem, piolitazone and rosiglitazone in pharmaceuticals and human serum	RP-HPLC	**Column:** Hiber, 250-4.6 RP C-18 **Mobile Phase:** Acetonitrile-methanol-water (30:20:50, v/v, pH 2.59 ± 0.02) **Flow Rate:** 1mL/min **Conc. Range:-**	28
13	Metformin, nateglinide and gliclazide in pharmaceutical preparations	LC	**Column:** Nucleosil C-18 **Mobile Phase:** 0.12 M sodium dodecyl sulphate, 10% (v/v) n-propanol, 0.3% triethylamine, adjusted to pH 5.6 **Flow Rate:** 1.0mL/min **Conc. Range: -**	29
14	Metformin, glimepiride, gliquidone and rosuvastatin in pharmaceutical formulations	RP-LC	**Column:** Purospher Star C-18 **Mobile Phase:** Methanol-water (90:10, v/v), pH adjusted to 3 with o-phosphoric acid **Flow Rate:** 1.0mL/min **Conc. Range:**0.25-25 µg/mL	30
15	Metformin and linagliptin	RP-HPLC	**Column:** Waters C-18 **Mobile Phase:** Potassium dihydrogen phosphate buffer (pH 4.6)–methanol (30:70 v/v) **Flow Rate:** 1mL/min **Conc. Range:** 20-800 µg/mL	31
16	Metformin HCl and vidagliptin in tablets	RP-HPLC	**Column:** Grace Cyano Column	32

			Mobile Phase: 25 mM ammonium bicarbonate buffer and acetonitrile (65:35, v/v) **Flow Rate:** 1mL/min **Conc. Range:**25-125 µg/mL	
17	Metformin and linagliptin in pharmaceutical dosage form	RP-HPLC	**Column:**C-18 **Mobile Phase:** Methanol and 0.05 M potassium dihydrogen orthophosphate, 70:30 (v/v), pH adjusted to 4.6 **Flow Rate:** 0.6mL/min **Conc. Range:**400-2400 µg/mL	33

CONCLUSION:

In summary, various analytical methods for metformin quantification and characterization are available, each with its strengths and applications: Chromatographic techniques (HPLC, UHPLC) are the gold standard for their sensitivity and precision, especially when coupled with mass spectrometry. Spectroscopic methods (UV-Visible, FTIR) are cost-effective and suitable for routine quality control but may lack the sensitivity needed for complex samples. Electrochemical methods offer rapid and portable detection, ideal for on-site analysis and point-of-care testing. Capillary electrophoresis (CE), though less common, is gaining traction for its high resolution and speed, particularly in bioanalysis. Emerging techniques like SERS and biosensors promise increased sensitivity and real-time monitoring.

REFRENCES:

1. El Messaoudi S, Rongen GA, de Boer RA, Riksen NP (2011) The cardioprotective effects of metformin. *Curr Opin Lipidol 22*: 2011 445-453.
2. Werner E, Bell J The preparation of methylguanidine and of dimethylguanidine by the interaction of dicyanodiamide and methylammonium and dimethylammonium chlorides respectively. *J Chem Soc Trans 121*: 1921 1790-1795.
3. Sonnett TE, Levien TL, Neumiller JJ, Gates BJ, Setter SM Colesevelam hydrochloride for the treatment of type 2 diabetes mellitus. *Clin Ther 31:* 2009 245-259.
4. O'Neil, Maryadele J The Merck index: an encyclopedia of chemicals,drugs, and biologicals (13th edn.*) Whitehouse Station, NJ: Merck Research Laboratories, USA.* 2001
5. Bristol-Myers Squibb Company Glucophage (metformin hydrochloride) tablets product monograph. *Bristol-Myers Squibb Company, Priceton, NJ, USA* 1995.
6. Habib IH, Kamel MS Near infra-red reflectance spectroscopic determination of metformin in tablets. *Talanta 60*: 2003 185-190.
7. Zhao XH, Song B, Zhong DF, Zhang SQ, Chen XY (Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography-tandem mass spectrometry. *Yao Xue Xue Bao 42*: 2007 1087-1091.
8. Mistri HN, Jangid AG, Shrivastav PS Liquid chromatography tandem mass spectrometry method for simultaneous determination of antidiabetic drugs metformin and glyburide in human plasma. *J Pharm Biomed Anal 45*: 2007 97-106.
9. Abdel-Ghany MF, Abdel-Aziz O, Ayad MF, Tadros MM Validation of different spectrophotometric methods for determination of vildagliptin and metformin in binary mixture. *Spectrochim Acta A Mol Biomol Spectrosc 125*: 2014 175-182.
10. Mubeen G, Noor K Spectrophotometric method for analysis of metformin hydrochloride. *Indian J Pharm Sci 71*: 2009 100-102

11. Mahgoub H, Youssef RM, Korany MA, Khamis EF, Kamal MF Development and validation of spectrophotmetric and HPTLC methods for simultaneous determination of rosiglitazone maleate and metformin hydrochloride in the presences of interfering matrix excipients. *Drug Dev Ind Pharm 40*: 2014 1190-1198.
12. Rezk MR, Riad SM, Mahmoud GY, Abdel Aleem AA Simultaneousdetermination of sitagliptin and metformin in their pharmaceutical formulation. *J AOAC Int 96*: 2013 301-306.
13. Thomas AB, Patil SD, Nanda RK, Kothapalli LP, Bhosle SS, et al. Stability-indicating HPTLC method for simultaneous determination of nateglinide and metformin hydrochloride in pharmaceutical dosage form. *Saudi Pharm J 19*: (2011) 221-231.
14. https://pubchem.ncbi.nlm.nih.gov/compound/Metformin
15. https://pubchem.ncbi.nlm.nih.gov/compound/Metformin
16. "The Indian Pharmacopoeia", Ministry Of Health And Family Welfare, 8th Edition; *Indian Pharmacopoeia Commision*, Ghaziabad; 2022: 2874/75
17. Ahmed R . A simple and convenient method for the simultaneous in vitro study of metformin and glimepiride tablets. *Pak J Pharm Sci 27*: 1939
18. Cheng CL, Chou CH Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B Biomed Sci Appl 762*: (2001) 51-58
19. Kolte BL, Raut BB, Deo AA, Bagool MA, Shinde DB Simultaneous determination of metformin in combination with rosiglitazone by reversedphase liquid chromatography. *J Chromatogr Sci 42*: (2004) 70-73
20. Arayne MS, Sultana N, Zuberi MH *Development and validation of RP-HPLC method for the analysis of metformin*. (2006)
21. Yardimci C, Ozaltin N, Gurlek *A Simultaneous determination of rosiglitazone and metformin in plasma by gradient liquid chromatography with UV detection. Talanta 72*: (2007)1416-1422.
22. Sahoo PK, Sharma R, Chaturvedi SC Simultaneous Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride by RPHPLC Method from Combined Tablet Dosage Form. *Ind J Pharm Sci 70*: (2008) 383-386.
23. Guo D, Nashunchaoketu, Wang J, Liu X, Wu S, et al. Simultaneous determination of four highly polar anti-diabetic drugs in Chinese traditional patent medicines using high performance liquid chromatography. *Se Pu 27*: (2009) 211-215.
24. Huttunen KM, Rautio J, Leppänen J, Vepsäläinen J, Keski-Rahkonen P Determination of metformin and its prodrugs in human and rat blood by hydrophilic interaction liquid chromatography. *J Pharm Biomed Anal 50*: (2009) 469-474
25. Salem H Determination of metformin hydrochloride and glyburide in an antihyperglycemic binary mixture using high-performance liquid chromatographic-UV and spectrometric methods. *J AOAC Int 93*: (2010) 133-140.
26. Arayne MS, Sultana N, Zuberi MH, Siddiqui FA Simultaneous determination of metformin, cimetidine, famotidine, and ranitidine in human serum and dosage formulations using HPLC with UV detection. *J Chromoatogr Sci 48*: (2010) 721-725.
27. Gabr RQ, Padwal RS, Brocks DR Determination of metformin in human plasma and urine by high-performance liquid chromatography using small sample volume and conventional octadecyl silane column. *J Pharm Pharm Sci 13*: (2010) 486-494.
28. Sultana N, Arayne MS, Shafi N, Siddiqui FA, Hussain A Development and validation of new assay method for the simultaneous analysis of diltiazem, metformin, pioglitazone and rosiglitazone by RP-HPLC and its applications in pharmaceuticals and human serum. *J Chromatogr Sci 49*: (2011) 774-779.
29. El-Wasseef DR Simultaneous determination of metformin, nateglinide and gliclazide in pharmaceutical preparations using micellar liquid chromatography. *Int J Biomed Sci 8*: (2012) 144-151.

30. Arayne MS, Sultana N, Tabassum A RP-LC simultaneous quantitation of co-administered drugs for (non-insulin dependent) diabetic mellitus induced dyslipidemia in *active pharmaceutical ingredient, pharmaceutical formulations*. (2013)
31. El-Baqary RI, Elkady EF, Ayoub BM Spectrophotometric methods for the determination of linagliptin in binary mixture with metformin hydrochloride and simultaneous determination of linagliptin and metformin hydrochloride using high performance liquid chromatography. *Int J Biomed Sci 9*: (2013) 41-47.
32. Satheeshkumar N, Pradeepkumar M, Shanthikumar S, Rao VJ Development of validated stability indicating assay method for simultaneous estimation of metformin hydrochloride and vildagliptin by RP-HPLC. *Drug Res(Stuttg) 64*: (2014) 124-129.
33. Vemula P, Dodda D, Balekari U, Panga S, Veeresham C Simultaneous determination of linagliptin and metformin by reverse phase- high performance liquid chromatography method: An application in quantitative analysis of pharmaceutical dosage forms. *J Adv Pharm Technol Res 6*: (2015) 25-28.