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Metformin: Methods of Analysis and Its Role in Lowering the Risk of Cancer

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Abstract

Metformin is the widely used anti-diabetic drug. HPLC is the most widely used method for the analysis of metformin. Others include spectrophotometric and potentiometric methods. The drug is analyzed not only in neat solution but also in pharmaceutical products alone and in combination with other drugs. Studies suggest that metformin can be successfully utilized to reduce the risk of cancer. However, there is a need of a randomized trial to find out if the drug is beneficial among the population at high risk of cancer. This review discusses the different methods utilized for the analysis of metformin and its possible role in resisting the carcinogenesis.

Keywords: Metformin; Analysis; HPLC; Spectrophotometric method; Cancer

Introduction

Metformin is an oral antidiabetic belonging to the class of biguanides used for the treatment of type 2 diabetes (Figure 1). It acts by suppressing the glucose production by the liver. It reduces the LDL cholesterol levels and in some people it promotes weight loss [1]. It is also prescribed for polycystic ovary syndrome (PCOS) [2]. Metformin is sold alone and also in combination with other drugs like rosiglitazone, pioglitazone and glibenclamide. Originally it was synthesized in 1922 by the reaction of dimethylamine hydrochloride and 2-cyanoguanine with heating [3]. Lactic acidosis is the major adverse effect; others include those related to GI. The drug is contraindicated in lung and liver diseases, kidney disorders and heart failure [4]. Table 1 summarizes the pharmacokinetic and physic-chemical properties of metformin HCl.

Methods of Analysis

There are a number of methods employed for the determination of metformin in neat solutions and pharmaceutical products. Some of these methods are discussed below.

Spectrometric methods

Spectroscopy: Pharmaceutical preparations of metformin have been analyzed by a simple and rapid near infra-red reflectance spectroscopic method. The results of the method agreed well with those of the UV assay method of metformin mentioned in BP 1998. The first spectral data was observed within the wavelength range of 1000-2500 nm. For the simultaneous determination of metformin and glipizide in human plasma, a method has been proposed where the atmospheric pressure chemical ionization source was used as a detector. the calibration curve showed linear behavior in the range of 2.0-2000 ng/mL. 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 [5-9].

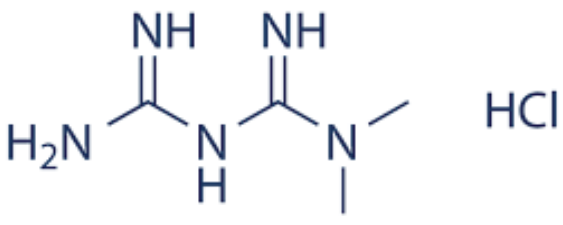

Figure 1: Metformin HCl.

UV Spectrophotometry: Two new methods have been developed for the analysis of metformin. These methods have been found to be simple, specific, accurate, precise and reproducible. These methods required metformin in the range of 2-12 µg/mL and 1-12 µg/mL at 237.6 and 247.4 nm respectively. These methods can be satisfactorily applied to the pharmaceutical products [10]. The amino group of metformin gives violet color chromogen when reacted with ninhydrin in alkaline medium. That chromogen has been determined spectrophotmetrically at 570 nm. The method is simple, sensitive and has shown the percentage recovery of 97-100% without any interference from the excipients. The method can be successfully applied to both the bulk and the pharmaceutical dosage forms [11]. Bhaskar et al. has proposed a simple and rapid method for the simultaneous determination of metformin along with gliclazide and pioglitazone HCl in synthetic samples and combined pharmaceutical products. The spectrophotometric data was coupled to partial least square (PLS). The solutions of metformin were in the range of 5-25 µg/mL and measured between the wavelengths of 200-400 nm in 0.1N HCl [12]. Another developed and validated method has proposed for the simultaneous determination of metformin with rosiglitazone in synthetic mixtures and coated tablets. Metformin was determined at the Amax of 236 nm and the concentration range was 20.0-80.0 µg/mL [13].

A simple, rapid and precise method has been developed for the simultaneous determination of metformin HCl and glibenclamide in

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IUPAC name	N,N-dimethylimidodicarbonimidic diamide
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
pk_a	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

Table 1: Physico-chemical and pharmacokinetic properties of metformin HCl [5,6].

binary mixtures [14]. Another method which can be applied to the pharmaceutical products has been proposed for the determination of metformin and rosiglitazone maleate. The caklibration curves were linear within the concentration range of 1.0-10.0 µg/mL [15]. Metformin has also been determined in a binary mixture containing glyburide along with metformin by two spectrophotometric methods. Metformin was measured at 235 and 227 nm in the two methods respectively. Both the methods followed Beer's law in the range of 20-200 µg/mL [16].

Mass spectrometry: Metformin along with pioglitazone and hydroxypioglitazone in human plasma has been determined by HPLC-elctrospray ionization-tandem mass spectrometry (ESI-MS/MS) method. The chromatographic run time was 4.0 min. The method has found to be simple, selective, robust, economical and accurate [17]. In another method moroxydine (IS-1) was used as an internal standard and the run time was 8.0 min. The recoveries were found in the range of 96.4-112.8% [18]. Metformin has also been determined along with repaglinide in rat plasma by LC-MS/MS-ESI method using phenacetin as an internal standard. The elution of metformin occurred in 1.64 min and the chromatographic run time was 3.5 min [19]. A rapid, sensitive and specific method has been developed for the determination of metformin in plasma. Metformin after precipitation was chromatographed on a C8 column. Intra- and inter day precision were found in the range of 4.4-5.7% and 1.3-2.8% respectively [20]. Metformin in plasma has also been determined and the precision and accuracy were less than 20% and the lower limit of quantification was less than 15% [21]. A method proposed which is efficient with a very short running time (2.0 min) as compared to the other reported methods. The recoveries were between 71-104% and the lower limits of quantification for the method was 7.8 nm/mL [22].

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. Aqueous ammonium sulfate and acetonitrile (7:3, v/v) was used as a mobile phase and silica gel 60 F254 plates were used to perform separation. The Rf value for metformin was found to be 0.73 ± 0.02. The bands were scanned at 237 nm using CAMAG TLC scanner III [23]. Simultaneous determination of metformin has also been performed along with sitagliptin in pharmaceutical formulation. There was no interference found by any of the excipients and the method was found to be simple, accurate and rapid [24]. Another method proposed for the simultaneous determination of metformin with nateglinide in a pharmaceutical dosage form using stability indicating high performance thin layer chromatography (HPTLC) has also been validated. The study included silica gel plates and chloroform: Ethylacetate: Acetic acid (4:6:0.1, v/v/v) as mobile phase. The accuracy of the method for metformin was found to be 100.08% [25]. Pharmaceutical formulation comprising of metformin and glyburide was used for the determination of metformin by TLC method. Silica gel plates were used as stationery phase and water: methanol: Ammonium sulphate (2:1:0.5, w/v) as mobile phase. Determination was made by desitometry at 237 nm and the Rf value was 0.43 ± 0.01. the method was validated for precision and recovery [26]. Normal phase TLC plates and water: methanol: 0.5% w/v ammonium sulfate solution (6:3:1.5, v/v/v) were used as stationery and mobile phase for the determination of metformin in pharmaceutical formulations also containing glipizide. The Rf value of metformin was 0.22 ± 0.01. The limits of detection and quantification were 991.30 and 3003.95 ng/band for metormin respectively [27]. Metformin has been estimated in bulk and formulation by a simple, sensitive and precise HPTLC method. Ammonium sulphate (0.5%): 2-propanolol: methanol (8:1.6:1.6, v/v/v) were used as mobile phase with silica gel F254 plate. The Rf value of 0.5 ± 0.03 was scanned at 238 nm [28].

HPLC methods: HPLC is the most widely used method for the analysis of metformin in biological fluids [29-32] and pharmaceutical products [24]. Table 2 contains the analytical parameters for the assay of metformin HCl by HPLC method.

Analysis of metformin in plasma has been carried out by a number of researchers. A method based on HPLC with electrospray ionization tandem mass (LC-ESI-MS/MS) in positive ionization mode has been developed for the analysis of metformin along with glipizide [33]. A simple, selective and sensitive HPLC method has been proposed for the analysis of metformin. The procedure was carried out using sili ca column. The mean absolute recoveries were 98% and the percent error value of the method was less than 8.3% [34]. Cation-exchange HPLC has been developed for the determination of metformin in urine and plasma. No interference has been found and the method requires only 0.5 mL of the sample. Detection was carried out at 230 nm and the detection limit has been found to be 0.1 mg [35]. A method has been developed for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. The recoveries and limits of quantification were within 76.3-101.9% and 5-22.5 ng/mL respectively [36].

A team developed a simple, economical, accurate and reproducible HPLC method where the linearity was observed within the range of 0-25 mug/mL for metformin HCl in formulations [37]. Metformin and rosiglitazone in pharmaceutical preparation has been determined by an efficient, sensitive and simple method. The limit of detection was in the range of 0.5-1.6 µg/mL and the recovered amount was 100-103.8% [38].

A method has been developed which solved the problems associated with high polarity of metformin. The stability analysis proved that metformin is stable for 3 months. The drug recovery was 98% and the limit of detection and limit of quantitation was 3 and 5 ng/mL respectively [39].

Material	Technique	Column	Mobile phase	Flow rate mL/min	Detection	Conc. Range µg/mL	References
Metformin and linagliptin	RP-HPLC	Waters C-18	Potassium dihydrogen phosphate buffer (pH 4.6)–methanol (30:70 v/v)	1	260	20-800	42
Metformin in human plasma	HPLC	Silica column	Acetonitrile (250 mL) in pH 7, 0.03 M diammonium hydrogen phosphate buffer (750 mL)	1	240	-	43
Metformin in plasma	Ion-pair HPLC	µbondapak C-18	40% acetonitrile, 0.01 M sodium dodecyl sulphate, 0.01 M sodium dihydrogen phosphate, D.I water, adjusted at pH 5.1	1.5	235	-	44
Metformin and linagliptin in pharmaceutical dosage form	RP-HPLC	C-18	Methanol and 0.05 M potassium dihydrogen orthophosphate, 70:30 (v/v), pH adjusted to 4.6	0.6	267	400-2400	45
Metformin in plasma	HPLC-UV	Discovery Reversed Phase C-18	34% acetonitrile and 66% aqueous phase (10 mM KH_2PO_4 and 10 mM sodium lauryl sulphate)	1.3	233	0.125-2.5	46
Metformin and glimepiride	RP-HPLC	Promocil C-18	Acetonitrile and ammonium acetate buffer 0.05 M pH 3.0	1.0	270	-	47
Metformin HCl and vildagliptin in tablets	RP-HPLC	Grace Cyano column	25 mM ammonium bicarbonate buffer and acetonitrile (65:35, v/v)	1.0	207	25-125	48
Metformin HCl in urine and dosage form	RP-HPLC	C-8	33 mM sodium dihydrogen phosphate containing 6.38 mM hexanesulfonic acid sodium salt (Adjusted to pH 3) with phosphoric acid-acetonitrile (93+7, v/v)	1.5	231	0.01-50	49
Metformin, diltiazem, piolitazone and rosiglitazone in pharmaceuticals and human serum	RP-HPLC	Hiber, 250-4.6 RP C-18	Acetonitrile-methanol-water (30:20:50, v/v, pH 2.59 ± 0.02)	1.0	230	-	50
Metformin, cimetidine, famotidine and ranitidine in human serum and dosage formulation	HPLC	Purospher Star RP 18	Methanol-water-triethylamine (20:80:0.05), pH adjusted to 3 with phosphoric acid 85%	1.0	229	5-25	51
Metformin HCl and glyburide	RP-HPLC	C-18	Acetonitrile-water (60:40, v/v)	0.9	254	0.06-0.24	16
Metformin	HPLC	C-18	Acetonitrile–KH_2PO_4 (34:66, v/v)	0.7	-	10-5000	52
Metformin HCl, phenformin HCl, acarbose and voglibose	HPLC	Thermo NH_2 analytical column	30% (0.06% potassium dihydrogen phosphate and 0.028% disodium hydrogen phosphate) and 70% (acetonitrile)	1.0	195	0.1-3 mg/L	53
Metformin in human plasma	HPLC	-	0.01 M potassium dihydrogen orthophosphate (pH 3.5) and acetonitrile (60:40, v/v)	0.01	234	-	54
Metformin HCl and 1-cyanoguanidine in tablet formulation	HPLC-UV	Nova Pak silica column	Ammonium dihydrogen phosphate buffer–methanol (21:79, v/v)	-	232	-	55
Metformin HCl and pioglitazone HCl	RP-HPLC	-	Acetonitrile-water-acetic acid (60:40:0.3), pH adjusted to 5.5 by adding triethylamine	1	230	0.5-4.0	56
Metformin in rat plasma	RP-HPLC	C-18	0.15 M ammonium acetate–acetonitrile (90:10, pH 5.5)	-	236	0.33-16.6	57
Metfromin HCl	RP-HPLC	C-18	Methanol-water (30:70, v/v)	0.5	233	-	58
Metformin and rosiglitazone	RP-LC	Zorbax XDB C-18	10 mM disodium hydrogen phosphate and 5 Mm sodium dodecyl sulphate (34:66, v/v), ph adjusted to 7.1 with orthophosphoric acid	1.0	226	-	59

Metformin, glimepiride, gliquidone and rosuvastatin in pharmaceutical formulations	RP-LC	Purospher Star C-18	Methanol-water (90:10, v/v), pH adjusted to 3 with o-phosphoric acid	1.0	231	0.25-25	60
Metformin, nateglinide and gliclazide in pharmaceutical preparations	LC	Nucleosil C-18	0.12 M sodium dodecyl sulphate, 10% (v/v) n-propanol, 0.3% triethylamine, adjusted to pH 5.6	1.0	254	-	61
Metformin and rosiglitazone in plasma	LC	Phenyl column	Acetonitrile- 5mM acetate buffer pH 5.5 (75:25, v/v)	1.0	245	-	62
Metformin	HPLC-UV	Silica column	0.01 M ammonium acetate pH 5.0 and acetonitrile (40:60, v/v)	1.0	235	-	63

Table 2: Analytical parameters for HPLC methods of metformin assay [42-63].

Potentiometric methods

Method has been developed based on the use of miniaturized potentiometric sensors using β–cyclodextrins for the determination of metformin in biological fluids and pharmaceutical products. Coated wire electrodes have been used and the concentration range from 10^{-6} to 10^{-1} mol/L with the detection limit of 8×10^{-7} mol/L. the method has been compared with the official spectrophotometric methods and has the advantage of simplicity, accuracy and feasibility [40]. Based on the preparation of PVC membrane sensors incorporating metformin-tungstosilicate and metformin-reineckate ion-pairs with o-nitrophenyloctylether and dioctylphthalate as plasticizers respectively, a new, simple and convenient potentiometric method has been developed. These sensors give rapid Nernstian response for 10^{-1}-10^{-5} M metformin in the pH range of 5.0-11.0 [41].

Role in Lowering Risk of Cancer

Metformin is the most commonly and widely used antidiabetic drug for the treatment of type II diabetes and may also reduce the risk of cancer and helps improve the patient's recovery [64-68] like certain vitamins [69,70]. Diabetes has been linked to an increased risk of several types of cancer. There are certain potent risk factors common to both diabetes and cancer like age, sex, obesity, diet, physical activity, alcohol and smoking.

Some mechanisms have been found to be involved in the relationship between the risk of cancer and glucose intolerance. Oxidative stress and glycation end products formed as the result of hyperglycemia at the cellular level can cause cancer development [71]. Increased levels of insulin and Insulin-like Growth Factor I (IGF-I) also promotes cancer cell proliferation [72,73]. Cancer patients having diabetes may face limited choice of treatment because of hyperglycemia and other diabetes complications which may severe their condition [74].

Many researches proposed that may be relevant for metformin in reducing the risk of cancer. Evidences have been found in vitro metformin acts directly on the cancer cells as AMP kinase- dependent growth inhibitor [75,76]. The LKB 1–AMP kinase pathway serves to reduce the consumption of the cellular energy when there is cellular energy depletion. It acts by inhibiting proliferation and motor-dependent protein translation hence complementing the benefits of reduction of circulating insulin level. It has also been found that after certain recent studies that the cancer cells due to this cellular energy deficiency increases the secretion of vascular endothelial growth factor (VEGF) so as to increase the vascular supply resulting in undesired effects [77]. It still remained undetermined if metformin can be utilized as its other beneficial effects in many in vivo models [78,79]. Certain *in vivo* studies in mice have shown that metformin has less anti-neoplastic activity when on control diet as compared to high-energy diet [80]. Such studies may conclude that antidiabetic activity of metformin may contribute to its anti-neoplastic activity and that metformin may have less impact on cancer in less hyperinsulinemic patients.

Pancreatic cancer is considered as one of the deadly form of cancer and diabetes it a known risk factor for this form of cancer. A study was conducted in diabetic patients suffering from pancreatic cancer receiving treatment including insulin injections and oral metformin. It was revealed that the patients taking metformin were found to have a 62% lower risk of developing pancreatic cancer as compared to those not taking metformin. Moreover, it was also found the patients taking insulin injections experienced increased risk of developing pancreatic cancer [81].

Another study comprising of almost 10 years' follow-up has shown that the in diabetic patients not taking metformin there was found 47% high risk of cancer associated death. While the patients taking metformin had 57% reduction in the risk of death due to cancer [82]. Early researches have also detected unexpectedly low cancer incidence and mortality among diabetics on metformin [64,65].

Conclusion

The different analytical methods available for the estimation of the drug have been summed up in the article providing the knowledge of the analysis which can be utilized for the determination of metformin. This review article has provided evidences which supports the role of metformin as an agent which can reduce the risk of cancer however there is still need of further in-depth knowledge to solve the issues like the exact mechanism of action, the characteristics of patient and type of cancer that can influence response to metformin and which therapeutic settings will enhance the benefits of metformin.

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