

## 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 physico-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].

**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 spectrophotometrically 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

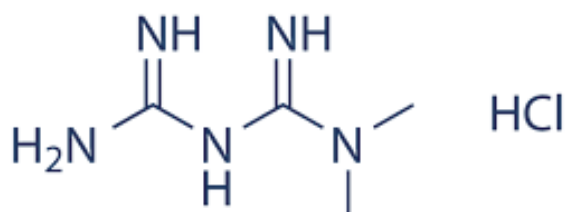


Figure 1: Metformin HCl.

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Received September 28, 2016; Accepted October 17, 2016; Published October 27, 2016

Citation: Gul W (2016) Metformin: Methods of Analysis and Its Role in Lowering the Risk of Cancer. J Bioequiv Availab 8: 254-259. doi: 10.4172/jbb.1000305

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<b>IUPAC name</b>	N,N-dimethylimidodicarbonimidic diamide
<b>Molecular formula</b>	C <sub>4</sub> H <sub>11</sub> N <sub>5</sub>
<b>Molecular mass</b>	129.164 g/mol
<b>Melting point</b>	224.5°C
<b>Solubility</b>	Freely soluble in water, soluble in alcohol, insoluble in ether and chloroform
<b>Stability</b>	Light sensitive, decomposes when heated emitting fumes of nitric oxide
<b>pK<sub>a</sub></b>	12.4
<b>pH</b>	6.68 (1% aqueous solution)
<b>Appearance</b>	White to off-white crystalline powder
<b>Route of administration</b>	Oral
<b>Absorption</b>	Slow, food delays the absorption of conventional tablets
<b>Bioavailability</b>	50-60% (with dosages of 0.5-1.5 g)
<b>Plasma-protein binding</b>	Negligible
<b>Volume of distribution</b>	300-1000 l after a single dose
<b>Half-life</b>	6.2 h.
<b>Distribution</b>	Rapid (peripheral body tissues and fluid)
<b>Metabolism</b>	Not metabolized
<b>Excretion</b>	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 calibration 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-electrospray 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 R<sub>f</sub> 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 stationary 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 R<sub>f</sub> 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 stationary and mobile phase for the determination of metformin in pharmaceutical formulations also containing glipizide. The R<sub>f</sub> value of metformin was 0.22 ± 0.01. The limits of detection and quantification were 991.30 and 3003.95 ng/band for metformin 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 R<sub>f</sub> 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 silica 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 µg/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 <sub>2</sub> PO <sub>4</sub> 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, piolitzone 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 <sub>2</sub> PO <sub>4</sub> (34:66, v/v)	0.7	-	10-5000	52
Metformin HCl, phenformin HCl, acarbose and voglibose	HPLC	Thermo NH <sub>2</sub> 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
Metformin 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  $\beta$ -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  $8 \times 10^{-7}$  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.

### References

1. El Messaoudi S, Rongen GA, de Boer RA, Riksen NP (2011) The cardioprotective effects of metformin. *Curr Opin Lipidol* 22: 445-453.
2. Radosh L (2009) Drug treatments for polycystic ovary syndrome. *Am Fam Physician* 79: 671-676.
3. Werner E, Bell J (1921) The preparation of methylguanidine and of dimethylguanidine by the interaction of dicyanodiamide and methylammonium and dimethylammonium chlorides respectively. *J Chem Soc Trans* 121: 1790-1795.
4. Sonnett TE, Levien TL, Neumiller JJ, Gates BJ, Setter SM (2009) Colesevelam hydrochloride for the treatment of type 2 diabetes mellitus. *Clin Ther* 31: 245-259.
5. O'Neil, Maryadele J (2001) The Merck index: an encyclopedia of chemicals, drugs, and biologicals (13<sup>th</sup> edn.) Whitehouse Station, NJ: Merck Research Laboratories, USA.

6. Bristol-Myers Squibb Company (1995) Glucophage (metformin hydrochloride) tablets product monograph. Bristol-Myers Squibb Company, Princeton, NJ, USA.
7. Habib IH, Kamel MS (2003) Near infra-red reflectance spectroscopic determination of metformin in tablets. *Talanta* 60: 185-190.
8. Zhao XH, Song B, Zhong DF, Zhang SQ, Chen XY (2007) Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography-tandem mass spectrometry. *Yao Xue Xue Bao* 42: 1087-1091.
9. Mistri HN, Jangid AG, Shrivastav PS (2007) Liquid chromatography tandem mass spectrometry method for simultaneous determination of antidiabetic drugs metformin and glyburide in human plasma. *J Pharm Biomed Anal* 45: 97-106.
10. Abdel-Ghany MF, Abdel-Aziz O, Ayad MF, Tadros MM (2014) Validation of different spectrophotometric methods for determination of vildagliptin and metformin in binary mixture. *Spectrochim Acta A Mol Biomol Spectrosc* 125: 175-182.
11. Mubeen G, Noor K (2009) Spectrophotometric method for analysis of metformin hydrochloride. *Indian J Pharm Sci* 71: 100-102.
12. Bhaskar R, Bhaskar R, Sagar MK, Saini V (2003) Multivariate chemometric assisted analysis of metformin hydrochloride, gliclazide and pioglitazone hydrochloride in bulk drug and dosage forms. *Adv Pharm Bull* 3: 79-84.
13. Mahgoub H, Youssef RM, Korany MA, Khamis EF, Kamal MF (2014) Development and validation of spectrophotometric and HPTLC methods for simultaneous determination of rosiglitazone maleate and metformin hydrochloride in the presences of interfering matrix excipients. *Drug Dev Ind Pharm* 40: 1190-1198.
14. Sohrabi MR, Kamali N, Khakpour M (2011) Simultaneous spectrophotometric determination of metformin hydrochloride and glibenclamide in binary mixtures using combined discrete and continuous wavelet transforms. *Anal Sci* 27: 1037-1041.
15. Onal A (2009) Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations. *Eur J Med Chem* 44: 4998-5005.
16. Salem H (2010) Determination of metformin hydrochloride and glyburide in an antihyperglycemic binary mixture using high-performance liquid chromatography-UV and spectrometric methods. *J AOAC Int* 93: 133-140.
17. Jagadeesh B, Bharathi DV, Pankaj C, Narayana VS, Venkateswarulu V (2013) Development and validation of highly selective and robust method for simultaneous estimation of pioglitazone, hydroxypropylglucosamine and metformin in human plasma by LC-MS/MS: application to a pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 930: 136-145.
18. Zhang X, Peng Y, Wan P, Yin L, Wang G, et al. (2014) Simultaneous determination and pharmacokinetic study of metformin and pioglitazone in dog plasma by LC-MS-MS. *J Chromatogr Sci* 52: 52-58.
19. Sharma K, Pawar G, Yadav S, Giri S, Rajagopal S, et al. (2013) LC-MS/MS-ESI method for simultaneous quantitation of metformin and repaglinidie in rat plasma and its application to pharmacokinetic study in rats. *Biomed Chromatogr* 27: 356-364.
20. Chen X, Gu Q, Qiu F, Zhong D (2004) Rapid determination of metformin in human plasma by liquid chromatography-tandem mass spectrometry method. *J Chromatogr B Analyt Technol Biomed Life Sci* 802: 377-381.
21. Heinig K, Bucheli F (2004) Fast liquid chromatographic-tandem mass spectrometric (LC-MS-MS) determination of metformin in plasma samples. *J Pharm Biomed Anal* 34: 1005-1011.
22. Zhong GP, Bi HC, Zhou S, Chen X, Huang M (2005) Simultaneous determination of metformin and gliclazide in human plasma by liquid chromatography-tandem mass spectrometry: application to a bioequivalence study of two formulations in healthy volunteers. *J Mass Spectrom* 40: 1462-1471.
23. Mohamed YA, Mohamed AM, Mohamed FA, Ahmed SA (2015) New Salting Out Stability-Indicating and Kinetic Thin Layer Chromatographic Method for Determination of Glimperide and Metformin HCl Binary Mixture. *J Chromatogr Sci* 53: 1603-1610.
24. Rezk MR, Riad SM, Mahmoud GY, Abdel Aleem AA (2013) Simultaneous determination of sitagliptin and metformin in their pharmaceutical formulation. *J AOAC Int* 96: 301-306.
25. Thomas AB, Patil SD, Nanda RK, Kothapalli LP, Bhosle SS, et al. (2011) Stability-indicating HPTLC method for simultaneous determination of nateglinide and metformin hydrochloride in pharmaceutical dosage form. *Saudi Pharm J* 19: 221-231.
26. Ghassempur A, Ahmadi M, Ebrahimi SN, Aboul-Enein HY (2006) Simultaneous determination of metformin and glyburide in tablets by HPTLC. *Chromatogr* 64: 101-104.
27. Modi DK, Patel BH (2012) Simultaneous determination of metformin hydrochloride and glipizide in tablet formulation by HPTLC. *J Liq Chromatogr. Related Technologies* 35: 28-39.
28. Havele S, Dhaneshwar S (2010) Estimation of metformin in bulk and in formulation by HPTLC. *J Nanomed Nanotechnol* 1: 1-102.
29. Porta V, Schramm SG, Kano EK, Koono EE, Armando YP, et al. (2008) HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies. *J Pharm Biomed Anal* 46: 143-147.
30. Zhang M, Moore GA, Lever M, Gardiner SJ, Kirkpatrick CM, et al. (2002) Rapid and simple high-performance liquid chromatographic assay for the determination of metformin in human plasma and breast milk. *J Chromatogr B Analyt Technol Biomed Life Sci* 766: 175-179.
31. Keal J, Somogyi A (1986) Rapid and sensitive high-performance liquid chromatographic assay for metformin in plasma and urine using ion-pair extraction techniques. *J Chromatogr* 378: 503-508.
32. Huupponen R, Ojala-Karlsson P, Rouru J, Koulu M (1992) Determination of metformin in plasma by high-performance liquid chromatography. *J Chromatogr* 583: 270-273.
33. Ding CG, Zhou Z, Ge QH, Zhi XJ, Ma LL (2007) Simultaneous determination of metformin and glipizide in human plasma by liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr* 21: 132-138.
34. Amini H, Ahmadiani A, Gazerani P (2005) Determination of metformin in human plasma by high-performance liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 824: 319-322.
35. Charles BG, Jacobsen NW, Ravenscroft PJ (1981) Rapid liquid-chromatographic determination of metformin in plasma and urine. *Clin Chem* 27: 434-436.
36. Aburuz S, Millership J, McElnay J (2005) The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 817: 277-286.
37. Kar M, Choudhury PK (2009) HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. *Indian J Pharm Sci* 71: 318-320.
38. Ali AR, Duraidi II, Saket MM, Abu-Nameh ES (2009) Column high-performance liquid chromatographic method for the simultaneous determination of rosiglitazone and metformin in a pharmaceutical preparation. *J AOAC Int* 92: 119-124.
39. AbuRuz S, Millership J, McElnay J (2003) Determination of metformin in plasma using a new ion pair solid phase extraction technique and ion pair liquid chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 798: 203-209.
40. Khaled E, Kamel MS, Hassan HN, Abd El-Alim SH, Aboul-Enein HY (2012) Miniaturized ionophore-based potentiometric sensors for the flow-injection determination of metformin in pharmaceutical formulations and biological fluids. *Analyst* 137: 5680-5687.
41. Hassan SSM, Mahmoud WH, Elmosallamy MAF, Othman AHM (1999) Determination of metformin in pharmaceutical preparations using potentiometry, spectrofluorimetry and UV-visible spectrophotometry. *Acta Chimica Acta* 378: 299-311.
42. El-Baqary RI, Elkady EF, Ayoub BM (2013) 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: 41-47.
43. Cheng CL, Chou CH (2001) Determination of metformin in human plasma by high-performance liquid chromatography with spectrophotometric detection. *J Chromatogr B Biomed Sci Appl* 762: 51-58.
44. Zarghi A, Foroutan SM, Shafaati A, Khoddam A (2003) Rapid determination of metformin in human plasma using ion-pair HPLC. *J Pharm Biomed Anal* 31: 197-200.
45. Vemula P, Dodda D, Balekari U, Panga S, Veeresham C (2015) 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: 25-28.

46. Chhetri HP, Thapa P, Van Schepdael A (2013) Simple HPLC-UV method for the quantitation of metformin in human plasma with one step protein precipitation. *Saudi Pharma J* 22: 483-487.
47. Ahmed R (2014) A simple and convenient method for the simultaneous in vitro study of metformin and glimepiride tablets. *Pak J Pharm Sci* 27: 1939-1943.
48. Satheeshkumar N, Pradeepkumar M, Shanthikumar S, Rao VJ (2014) Development of validated stability indicating assay method for simultaneous estimation of metformin hydrochloride and vildagliptin by RP-HPLC. *Drug Res (Stuttg)* 64: 124-129.
49. El-Gindy A, Nassar MW, El-Abasawy NM, Attia KA, Al-Shabrawi M (2010) Optimization and validation of an RP-HPLC method for direct determination of metformin hydrochloride in human urine and in a dosage form. *J AOAC Int* 93: 1821-1828.
50. Sultana N, Arayne MS, Shafi N, Siddiqui FA, Hussain A (2011) 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: 774-779.
51. Arayne MS, Sultana N, Zuberi MH, Siddiqui FA (2010) Simultaneous determination of metformin, cimetidine, famotidine, and ranitidine in human serum and dosage formulations using HPLC with UV detection. *J Chromatogr Sci* 48: 721-725.
52. Gabr RQ, Padwal RS, Brocks DR (2010) 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: 486-494.
53. Guo D, Nashunchaoketu, Wang J, Liu X, Wu S, et al. (2009) Simultaneous determination of four highly polar anti-diabetic drugs in Chinese traditional patent medicines using high performance liquid chromatography. *Se Pu* 27: 211-215.
54. Yuen KH, Peh KK (1998) Simple high-performance liquid chromatographic method for the determination of metformin in human plasma. *J Chromatogr B Biomed Sci Appl* 710: 243-246.
55. Al-Rimawi F (2009) Development and validation of an analytical method for metformin hydrochloride and its related compound (1-cyanoguanidine) in tablet formulations by HPLC-UV. *Talanta* 79: 1368-1371.
56. Sahoo PK, Sharma R, Chaturvedi SC (2008) Simultaneous Estimation of Metformin Hydrochloride and Pioglitazone Hydrochloride by RPHPLC Method from Combined Tablet Dosage Form. *Ind J Pharm Sci* 70: 383-386.
57. Wanjari MM, There AW, Tajne MR, Chopde CT, Umathe SN (2008) Rapid and Simple RPHPLC Method for the Estimation of Metformin in Rat Plasma. *Indian J Pharm Sci* 70: 198-202.
58. Arayne MS, Sultana N, Zuberi MH (2006) Development and validation of RP-HPLC method for the analysis of metformin. *Pak J Pharm Sci* 19: 231-235.
59. Kolte BL, Raut BB, Deo AA, Bagoool MA, Shinde DB (2004) Simultaneous determination of metformin in combination with rosiglitazone by reversed-phase liquid chromatography. *J Chromatogr Sci* 42: 70-73.
60. Arayne MS, Sultana N, Tabassum A (2013) RP-LC simultaneous quantitation of co-administered drugs for (non-insulin dependent) diabetic mellitus induced dyslipidemia in active pharmaceutical ingredient, pharmaceutical formulations and human serum with UV-detector. *Clin Chim Acta* 425: 54-61.
61. El-Wasseef DR (2012) Simultaneous determination of metformin, nateglinide and gliclazide in pharmaceutical preparations using micellar liquid chromatography. *Int J Biomed Sci* 8: 144-151.
62. Yardimci C, Ozaltin N, Gurlek A (2007) Simultaneous determination of rosiglitazone and metformin in plasma by gradient liquid chromatography with UV detection. *Talanta* 72: 1416-1422.
63. Huttunen KM, Rautio J, Leppänen J, Vepsäläinen J, Keski-Rahkonen P (2009) Determination of metformin and its prodrugs in human and rat blood by hydrophilic interaction liquid chromatography. *J Pharm Biomed Anal* 50: 469-474.
64. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD (2005) Metformin and reduced risk of cancer in diabetic patients. *BMJ* 330: 1304-1305.
65. Bowker SL, Majumdar SR, Veugelers P, Johnson JA (2006) Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. *Diabetes Care* 29: 254-258.
66. Currie CJ, Poole CD, Gale EA (2009) The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 52: 1766-1777.
67. Monami M, Lamanna C, Balzi D, Marchionni N, Mannucci E (2009) Sulphonylureas and cancer: a case-control study. *Acta Diabetol* 46: 279-284.
68. Wright JL, Stanford JL (2009) Metformin use and prostate cancer in Caucasian men: results from a population-based case-control study. *Cancer Causes Control* 20: 1617-1622.
69. Gul W (2014) Menadione: role in cancer prevention and methods of analysis. *WJPS* 2: 1390-1394.
70. Colston KW, Berger U, Coombes RC (1989) Possible role for vitamin D in controlling breast cancer cell proliferation. *Lancet* 1: 188-191.
71. Abe R, Yamagishi S (2008) AGE-RAGE system and carcinogenesis. *Curr Pharm Des* 14: 940-945.
72. Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GY, Kaplan RC, et al. (2009) The role of insulin-like growth factor-I and its binding proteins in glucose homeostasis and type 2 diabetes. *Diabetes Metab Res Rev* 25: 3-12.
73. Pollak M (2008) Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 8: 915-928.
74. Richardson LC, Pollack LA (2005) Therapy insight: Influence of type 2 diabetes on the development, treatment and outcomes of cancer. *Nat Clin Pract Oncol* 2: 48-53.
75. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M (2006) Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. *Cancer Res* 66: 10269-10273.
76. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N (2007) Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 67: 10804-10812.
77. Phoenix KN, Vumbaca F, Claffey KP (2008) Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. *Breast Cancer Res Treat* 113: 101-111.
78. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, et al. (2007) Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 67: 6745-6752.
79. Anisimov VN, Egormin PA, Bershtein LM, Zabezhinskii MA, Piskunova TS, et al. (2005) Metformin decelerates aging and development of mammary tumors in HER-2/neu transgenic mice. *Bull Exp Biol Med* 139: 721-723.
80. Park EJ, Lee JH, Yu GY, He G, Ali SR, et al. (2010) Dietary and genetic obesity promote liver inflammation and tumor-igenesis by enhancing IL-6 and TNF expression. *Cell* 140: 197-208.
81. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL (2009) Antidiabetic therapies affect risk of pancreatic cancer. *Gastroenterology* 137: 482-488.
82. Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, et al. (2010) Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care* 33: 322-326.