Asian Journal of Chemistry Vol. 22, No. 10 (2010), 8273-8279

MINI REVIEW

Determination of Metformin Hydrochloride in Pharmaceutical Formulations

VIPIN K. SHARMA* and A. BHATTACHARYA†
Department of Pharmaceutical Sciences, Gurukul Kangri Vishwavidyalaya, Haridwar-249 404, India
E-mail: sharma_dibru@yahoo.co.in

Metformin hydrochloride is an antidiabetic drug of biguanide category with multiple benefits in type II diabetes mellitus *e.g.*, lowering of HbA_{1c} values, lipid profile regulation and improvement in vascular and hemodynamic indices. Adverse effects are generally tolerable and self-limiting. Alone as well as in combination with other antidiabetic drugs of different categories, it is administered in different formulations. Due to short biological half-life (2-6 h), it is available in sustained release formulations. The present study concentrates on the various analytical methods of metfromin hydrochloride determination in dosage forms as well in blood plasma.

Key Words: Metformin hydrochloride, HPLC, Electrophoresis, Chemical derivatization, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a heterogeneous disorder in which hyperglycemia is the unifying feature. The management of the disease is complex. Life style modification and medical nutrition therapy are the cornevstone of therapy. The medications for the treatment are insulin, biguanides, sulfonyl ureas, meglitinides, phenylalanine derivatives, α-glucosidase inhibitors and thiazolidinediones.

Metformin hydrochloride is a biguanide derivative which is hypoglycemic and commonly available alone and in combination with other antidiabetic drugs. Numerous methods have been developed for the determination of metformin in different matrices. For biological fluids analysis, HPLC and chemiluminescence emission methods have been proposed for the determination of metformin[1-3]. For the detection of metformin in pharmaceutical preparations, a titration in nonaqueous medium with perchloric acid using different indicators and a spectrophotometry have been proposed as standard methods in British and Chinese pharmacopoeia[4,5]. Poly(vinyl)chloride (PVC) matrix membrane sensors based on the use of ion association complexes of metformin with tetrahydroborate[6] and molybdophosphate[7] have been used for direct potentiometric determination of metformin in pharmaceutitcal preparations. Hassan *et al.*[8]

†Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786 004, India.

developed three methods as potentiometry, spectrophotometry and UV-visible spectrophotometry for metformin determination, respectively based on the use of ion association complexes of metformin with reineckate and tungstosilicate as ion exchangers in plasticized PVC matrix. It is on the reaction of metformin with chetysensequinone and 1-*p*-naphthol in alkaline medium to form a fluroresensce product and the formation of Cu-metformin complex by reaction of metformin with copper sulphate in cyclohexylamine. Based on the formation of charge transfer complex with iodine in acetonitrile, direct specrophotometric method has been developed recently[9]. Conductometric titration of metformin with copper sulphate[10], sodium tetraphenylborate and cetylpyridinium bromide[11] have been described. Near infrared reflectance spectroscopic method has also established for the determination of metformin in tablets[12]. Metformin hydrochloride can be determined by atomic absorption spectrophotometry of its copper complex[13], gas chromatography[14-16] and nuclear magnetic resonance spectroscopy[17]. The official assay method for the pure substance and for its tablets involves the reaction of biguanide with nitropentacyanoferrate(III) and hexacyano ferrate(II) in a sodium hydroxide medium and measurement of the absorbance of the coloured product at 525 nm[18]. Recently, the determination of drugs in biological fluids by capillary electrophoresis (CE) in fused silica capillaries have been developed[19-21]. Some of the methods are based on high performance liquid chromatography (HPLC) following some form of protein precipitation or solid-phase extraction of plasma[22-27]. Methods that use solvent extraction and evaporation are time consuming and the methods that use dilution with a protein precipitating agent are fast but sacrifice sensitivity.

Most of the methods used for metformin hydrochloride determination in the pure or in formulation often suffer different problems such as sophisticated operation, time consumption and/or low sensitivity. Some of the analytical methods *e.g.*, spectroscopic determination can be applied for the routine analysis of metformin hydrochloride in pure and pharmaceutical dosage forms. The present study is undertaken to investigate the comparative application of different analytical methods for the assessment of metformin hydrochloride.

Direct spectrophotometric determination: It is considered as a simple, sensitive and accurate method for the determination of metformin hydrochloride in pure form and drug formulations. The method is suitable for the determination of metformin in drug formulations without interference from excipients such as starch, lactose and magnesium stearate indicating that complexation does not occur with these materials under the reactions conditions used. This method is based on the fact that addition of iodine to metfromin hydrochloride in MeCN (methanol and acetonitrile) yields instantaneously a yellow coloured complex.

$$[Mf] + Cl^- + I_2 \rightleftharpoons [Mf]^+ I_2 Cl^-$$

This exhibits an absorption bands at 236 nm in MeCN. The existence of peaks at 360, 286, 260 and 230 nm for the coloured product are attributed to the formation

of the drug complex in MeCN[28]. The iodine molecule acts as electron acceptor. The proposed method has been applied to some pharmaceutical preparations containing metformin hydrochloride. Good agreement with results obtained by the pharmacopoeial method were obtained[29].

Sensitive flow injection chemiluminescence: In recent years chemiluminescence (CL) has obtained considerable attention when coupled with flow-injection analysis (FIA). The chemiluminescence-based FIA methods provide cheap, rapid, simple and reproducible means of detection and therefore, have been accepted for drugs determination[30-34].

It is based on the chemiluminescence reaction between metformin hydrochloride and NBS in alkaline medium using fluorescence as energy transfer agent. Use of cetyltrimethyl ammonium bromide (CTAB) as sensitizer enhances the signal magnitude about 100 times[35]. Number of factors can affect the results obtained as fluorescein concentration, NaOH concentration, NBS concentration, type of the surfactants, flow rates *etc*. For the possible mechanism of the chemiluminescence reaction, it is considered that reducing biguanide group and amino group in the molecular structure of metformin makes the redox between NBS and metformin to be occurred rapidly. The energy generated from the redox reaction may excite fluorescein molecule to its excited state. When it returns to its ground state, fluorescence emission arose. The possible effect of CTAB may be changing the kinetic process of energy transfer, reducing the non-radiative internal transfer process when excited state molecule return to ground state and enhancing the fluorescence quNT yield of fluorescein molecules enhanced greatly. So, energy accepted fluorescein is excited to produce chemiluminescence.

$$NBS + H_2O = NHS + HBrO$$
$$BrO^- + \text{metformin} + OH^- = \text{product}^*$$
$$\text{Product}^* + \text{fluorescein} = \text{fluorescein}^* + \text{product}$$
$$\text{Fluorescein} = \text{fluorescein} + h\nu \ (\lambda_{max} = 534 \text{ nm})$$

where; product* being the excited state of the product, fluorescein* the excited state of fluorescein molecule.

Nuclear magnetic resonance spectroscopy: This method for the determination of metformin hydrochloride in pharmaceutical formulations is simple, accurate and rapid and provides the NMR spectrum which helps to identify and check the purity of the drug. It can be applied for metformin determination in dosage form without any interference from excipients. In this method, deuterium oxide containing maleic acid as internal standard is added. A few specks of the reference standard maleic acid are also added. The excellent solubility of metformin in deuterium oxide in the presence of maleic acid (internal standard)[36,37] and the absence of resonance signals in the region of interest make deuterium oxide an excellent choice of solvent for the NMR procedure. This, together with the convenient downfield resonance yields position of free quantitative analysis. The weight of metformin can be calculated by using the following equation:

$$Ws = (HsMm/HmMm)\ (Im/Is)Ws$$
$$= (2x165.6/6x116.04)\ (Im/Is)Ws$$

where I = integral of signal (mm), H = number of protons contributing to the signal, M = molecular weight and w the weight (mg). The subscribes "s" and "m" stand for internal standard and drug, respectively[38].

Capillary electrophoresis using field-amplified sample stacking technique: The main advantages of capillary electrophoresis over HPLC are the much lower consumption for reagents and time, better mass-sensitive detection limit and high separation efficiency. Because of the small detective volume (nanolitres) in capillary electrophoresis, the concentration sensitivity is typically 1- to 2- orders of magnitude lower than that in HPLC[39]. Using laser-induced fluorescence detection instead of ultraviolet absorbance detection can enhance sensitivity by as much as 3-order of magnitude. Sample stacking techniques applicable during and/or immediately after sample injection can provide comparable sensitivity without the need of special instrumentation[40]. A new sample stacking technique termed as head-column field-amplified sample stacking which is capable of enhancing the sensitivity > 1000-folds has been developed[41].

It is difficult to extract metformin directly from biological fluid by solvent-solvent extraction due to poor partition coefficient in the octanol-water system or in methylene chloride-0.8 M NaOH system or 0.05 in chloroform-*tert*. amyl alchol-0.8 M NaOH system[42]. The pre derivatization with fluorescent agents prior to injection into the column[43] or by ion-pair extraction[44] can be applied for HPLC assay method. All these measures are not suitable for on-column stacking capillary electrophoresis analysis because of the large amount of ions excited in the final sample solution. So, a field amplified sample stacking (FASS) capillary electrophoresis method can be applied for the determination of the concentration of metformin in human plasma after oral administration of metformin tablet or its enteric capsule[45].

Ion pairing liquid chromatography: The ion pair mechanism can be applied to achieve HPLC diode-array detector (DAD) separation and determination of metformin and related impurities (cyanoguanidine, 1-methylbiguanidine, melamine, N,N-2-yl-guanidine) in due time with high resolution. The analytical method is based on an ion exchange mechanism using a stationary phase with benzenesulphonic acid groups chemically bonded to porous silica gel. The mechanism is highly dependent on the pH of mobile phase resulting in a low robustness of the chromatography method. This mechanism has been recently applied only for LC assay of metformin in plasma samples[46]. All analytes of interest can be separated by measuring constant pH, hydrophobicity and thermodynamics.

High performance liquid chromatography: It is suitable for monitoring the entire range of steady-state of metformin concentrations achieved in patients. The use of a short extraction column switching technique can remove a significant portion of the material in the solvent front without significantly changing the overall run time. This maintain the analytical column at optimal performance over hundreds of

analytical runs. Stepensky *et al.*[47] determined the metformin concentration by HPLC method applying a Kontron HPLC system (Kontron, Zurich, Switzerland) and LiChrospher 100 RP-18 column (Merk, Darmstadt, Germany). The detection was at 234 nm and phenformin was applied as internal standard. The mobile phase consisted of 0.01 M Na_2HPO_4 solution (pH = 6.5), methanol and acetonitrile (20:3:6, v/v). The quantification limit was 100 ng/mL.

Non-aqueous titrametry: Metformin hydrochloride concentration can be determined by adding anhydrous formic acid and acetic anhydride to be titrated with perchloric acid. The end point is determined potentiometrically[48,49].

Cation exchange with normal phase LC/MS/MS: Several methods of analysis by HPLC have been published for the determination of metformin in human plasma using various separation modes, such as reverse phase[24,27,49-51] ion pair cation exchange[2,52] and normal phase[3]. However, these methods have exhibited low sensitivity and required relatively large sample volumes, complicated sample preparation procedures including evaporation steps, long analytical intervals, long analytical columns (at least 15 cm) and conventional mobile phase flow rates (at least 1 mL/min).

Liquid chromatography-tandem mass spectroscopy (LC/MS/MS) for analysis of drug in plasma[52,53] involves protein precipitation without an evaporation step for sample preparation procedure and MS/MS detection. It includes simple sample preparation procedure prior to injection into LC/MS/MS and short analytical intervals (3.4 min/sample)[52] of 1-2 min/sample. On another hand, sample preparation by protein precipitation method lacks compound selectivity and robustness due to interference by any other substances. The new method is based on cation exchange solid phase selectivity and exhibited excellent performance in terms of selectivity and sensitivity without resorting extraction and evaporation techniques, robustness, short run time of analysis (7 min/sample) and simplicity of sample preparation[53]. This method can be applied to determine concentration-time profiles of the drug in a clinical pharmacokinetic study of metformin hydrochloride extended-release tablet.

Conclusion

Metformin hydrochloride, a hypoglycemic drug is endowed with multi-beneficial effects in diabetes type II having only one side effect *e.g.*, lactic acidosis which is extremely rare. The drug, metformin hydrochloride is fabricated in various dosage forms of conventional and novel approach in combination with other antidiabetic agents. In the analytical methods applied for metformin determination, spectrophotometric and chemical derivatization can be used for routine analysis. In all the analytical methods applied for metformin hydrochloride analysis, spectrophotometric determination is reliable and appropriate for metformin determination in dosage forms without interference from excipients such as lactose, talc, starch, magnesium stearate *etc*. Also, it is inexpensive and can be performed by spectrophotometer as compared to costly instruments required in LC/MS/MS, capillary electrophoresis, liquid chromatography-tandem mass spectrophtometry, nuclear magnetic resonance *etc*. For high accuracy and precession, HPLC, capillary

electrophoresis and LC/MS/MS can be applied. Furthermore; more of the analytical methods are to be developed that may provide the data accurately without any deviation produced by the other ingredients of the formulations of metformin hydrochloride.

REFERENCES

1. F. Tache, V. David, A. Farca, and A. Medvedovici, *Microchem. J*., **68**, 13 (2001).
2. O. Vesterqvist, F. Nabbie and B. Swanson, *J. Chromatogr. B*., **716**, 299 (1998).
3. K.H. Yuen and K.K. Peh, *J. Chromatogr. B*., **710**, 243 (1998).
4. British Pharmacopoeia, Cambridge University Press: Cambridge (1988).
5. Chinese Pharmacopoeia, Committee of Chinese Pharmacopoeia, Beijing, Vol. II, p. 540 (2000).
6. M.S. Rizk, H.M. Abel-Fattah, Y.M. Issa and E.M. Atia, *Anal. Lett*., **26**, 415 (1993).
7. M.S. Rizk, *Electroanalysis*, **7**, 687 (1995).
8. S.S.M. Hassan, H. Wagiha Mahmoud, M.A.F. Elmosallamy and A.H.M. Othman, *Anal. Chim. Acta*, **378**, 299 (1999).
9. S. Ashour and R. Kabbani, *Anal. Lett*., **36**, 361 (2003).
10. J.M. Calatayud, P.C. Falco and M.C.P. Marti, *Anal. Lett*., **18**, 1381 (1985).
11. M. Abou-Dan, S. Ashour and H. Abou-Dan, *Asian J. Chem*., **13**, 1 (2001).
12. I.H.I. Habib, In. Ph.D. Thesis, Cairo University, Egypt (1997).
13. F.A. Aly and M. El-Rayes, *Egypt J. Pharm. Sci*., **24**, 169 (1983).
14. S.B. Matin, J.H. Karam and P.H. Forsham, *Anal. Chem*., **47**, 545 (1975).
15. J. Brohon and M. Neol, *J. Chromatogr*., **146**, 148 (1978).
16. R.T. Sane, V.J. Banavalikar, V.R. Bhate and V.G. Nayak, *Indian Drugs*, **26**, 647 (1989).
17. S.Z. El-Khateeb, H.N. Assaad, M.G. El-Bardicy and A.S. Ahmad, *Anal. Chim. Acta*, **208**, 321 (1988).
18. British Pharmacopoeia, Her Majesty's Stationary Office, London, p. 293, A-63 (1973).
19. Ch. X. Zhang, Z.P. Sun, D.K. Ling, J.S. Zheng, J. Guo and X.Y. Li, *J. Chromatogr*., **612**, 287 (1993).
20. J.Zh. Song, X.J. Wu, Z.P. Sun, S.J. Tain, M.L. Wang and R.L. Wang, *J. Chromtogr. B*, **692**, 445 (1997).
21. Ch.X. Zhang, Y. Aebi and W. Thormann, *Clin. Chem*., **42**, 1805 (1996).
22. C.J. Bailey and R.C. Turner, *New Engl. J. Med*., **334**, 574 (1996).
23. B.G. Charles, N.W. Jacobsen and P.J. Ravenscroft, *Clin. Chem*., **27**, 434 (1981).
24. L. Benzi, P. Marchetti, P. Cecchetti and R. Navalesi, *J. Chromatogr*., **375**, 184 (1986).
25. C. Lacroix, P. Danger and W. Wojciechowski, *Ann. Biol. Clin*., **49**, 98 (1991).
26. G. Caille, Y. Lacasse, M. Raymond, H. Landriault, M. Perrotta, G. Picirilli, J. Thiffault and J. Spenard, *Biopharm. Drug Dispos*., **14**, 257 (1993).
27. R. Huupponen, P. Ojala-Karlsson, J. Rouru and M. Koulu, *J. Chromatogr*., **583**, 270 (1992).
28. S. Asour and R. Kabbani, *Anal. Lett*., **36**, 361 (2003).
29. British Pharmacopoeia, Her Majesty,s Stationary Office: London, Vol. II, p. 1002 (1993).
30. A.A. Alwarthan and A. Townshend, *Anal. Lett*., **185**, 329 (1986).
31. R.W. Abbott, A. Townshend and R. Gill, *Analyst*, **111**, 635 (1986).
32. B. Li, Z. Zhang and W. Liu, *Talanta*, **54**, 697 (2001).
33. L.P. Palilis and A.C. Calokerinos, *Anal. Chim. Acta*, **413**, 175 (2000).
34. B.J. Hindson and N.W. Barnett, *Anal. Chim. Acta*, **445**, 1 (2001).
35. Z. Wang, Z. Zhang, Z. Fu, W. Luo and X. Zhang, *Ana. Lett*., **36**, 2683 (2003).
36. S.S.M. Hassan, *J Assoc .Off. Anal. Chem*., **61**, 1 (1978).
37. S.Z. El-Khateeb, *Spectrosc.Lett*., **19**, 193 (1986).
38. S.Z. El-Khateeb, H.N. Assad, M.G. El-Bardicy and A.S. Ahmad, *Anal. Chim. Acta*, **208**, 321 (1988).

39. M. Albin, P.D. Grossman and S.E. Moring, *Anal. Chem*., **65**, 485 (1993).
40. A.R.L. Dhien and D.S. Burgi, *Anal. Chem*., **64**, 489A (1992).
41. Ch.X. Zhang and W. Thormann, *Anal. Chem*., **68**, 2523 (1996).
42. E.R. Garrett and J. Tsau, *J. Pharm. Sci*., **61**, 1404 (1972).
43. O. Mutsuko, I. Mastake, K. Masaaki and O. Yosuke, *Anal. Sci*., **9**, 217 (1993).
44. J. Keal and A. Somogyi, *J. Chromatogr*., **378**, 503 (1986).
45. J. Song, H. Chem, S. Tian and Z. Sun, *J. Chromatogr. B*, **708**, 277 (1998).
46. S. AbuRuz, J. Millership and J. McElnay, *J. Chromatogr. B*, **798**, 203 (2003).
47. D. Stepensky, M. Friedman, I. Raz and A. Hoffman, *Drug Metabol. Dispos*., **30**, 861 (2002).
48. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare; Published by the Controller of Publications, Delhi. Vol. I, edn. 4, pp. 469-470 (1996).
49. J.M. Haegy, P. Loirat, B. Marchand, Y. Ravaud, P. Weyne and A. Fournier, *Diabetes Care*, **18**, 779 (1995).
50. M. Zhang, G.A. Moore, M. Lever, J.L. Gardiner, C.M.J. Kirkpatrick and E.J. Begg, *J. Chromatogr. B*, **710**, 243 (1998).
51. X. Chen, Q. Gu, F. Qui and D. Zhong, *J. Chromatogr. B*, **802**, 377 (2004).
52. K. Heinig and F. Bucheli, *J. Pharm. Biomed. Anal.*, **34**, 1006 (2004).
53. N. Koseki, H. Kawashita, M. Niina, Y. Nagae and N. Masuda, *J. Pharm. Biomed. Anal.*, **36**, 1063 (2005).

(*Received*: 12 January 2010; *Accepted*: 7 August 2010) AJC-8972