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Development of normal phase high-performance liquid chromatographic method for determination of metformin and its related impurities in bulk material

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Abstract
A rapid and specific normal phase high-performance liquid chromatographic method has been developed for the determination of metformin hydrochloride and its related impurities in raw material. Elution was performed on a silica column with acetonitrile: 0.05 M dipotassium hydrogen phosphate (20:80% v/v), adjusted to pH 7.0 as mobile phase. The influence of the various experimental parameters on the peaks separation has been studied and the optimum chromatographic conditions were concluded from the results of optimization studies. The linearity and limit of quantitation (LOQ) of the method for the parent drug and related impurities have been determined.

Keywords: Metformin, related impurities, normal phase HPLC, bulk material

1. Introduction
Metformin hydrochloride is a biguanide hypoglycaemic. It is given by mouth in the treatment of type 2 diabetes mellitus [1].The necessity of related impurities control in the substance is imposed by the European Pharmacopoeia [2], the British Pharmacopoeia [3] and the United States Pharmacopoeia [4]. According to the corresponding monographs five impurities (with structure given in Fig. 1) must be controlled in metformin at the following levels: 0.02% impurity A and 0.1% for any other impurity. The analytical methods in the three compendia are based on an ion exchange mechanism using stationary phase with benzenesulphonic acid groups chemically bonded to porous silica gel. A system suitability test imposes separation of metformin against impurity D with a chromatographic resolution of at least 10. Several methods based on ion pair [5-7] and reversed phase [8-9] mechanisms have been reported for the determination of related impurities in metformin. To our knowledge, chromatographic methods for determination of related impurities in metformin based on normal phase mechanism have not been reported-up to the present.
The assay of metformin in different samples has been extensively discussed in literature; for instance, infrared spectrometry or flow injection chemiluminescence based methods [10-11] were recently reported. HPLC has been applied after pre-column fluorescence derivatization for determination of metformin in biological samples has been discussed more often [12-13]. However these methods are not suitable, or present serious limitations, when used for determination of related impurities in metformin.
In this paper, a new method is proposed for separation and determination of related impurities in metformin bulk material. The novelty of the method is given by the use of normal phase silica column and aqueous based mobile phase to achieve separation of all analytes within a reasonable time with high resolution.

2. Materials and Methods
2.1 Reagents and Chemicals
Acetonitrile (HiPersolv™), dipotassium hydrogen orthophosphate (Analar), potassium hydroxide (Analar) and orthophosphoric acid 85% (Analar) used in the chromatography were obtained from BDH Ltd, Poole, England. Metformin HCl and related impurities (A-E) were provided by Mikromol GmbH, Luckenwalde, Germany.
Double distilled water was used in all the analysis.
Metformin HCl bulk materials were obtained from different suppliers were obtained and analysed using the proposed method.

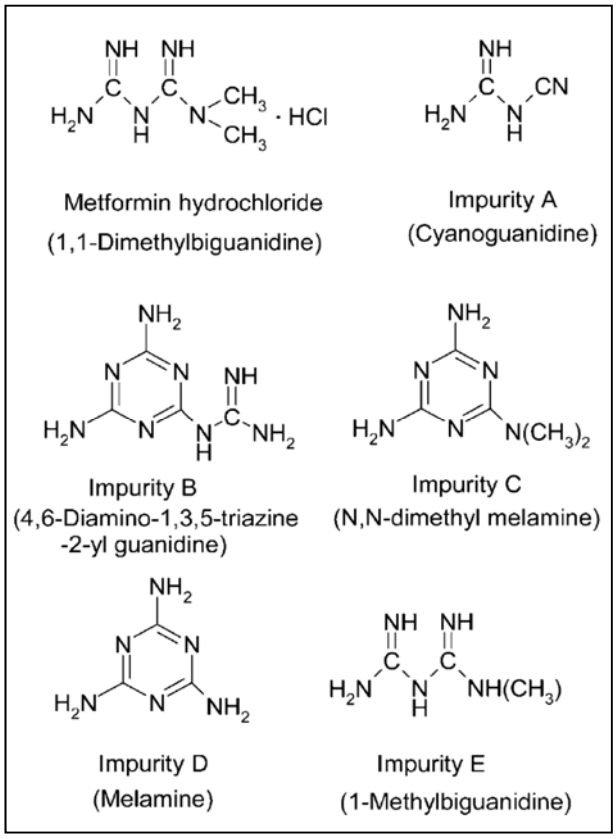

Fig 1: The structures of metformin HCl and related impurities

2.2 HPLC equipment and column

HPLC was performed using a HP-1100 liquid chromatograph equipped with quaternary pumps, auto sampler, thremostated column compartment and variable wavelength absorbance detector. The chromatograms were recorded and the peak areas were calculated using HP Chemstation software (Agilent technologies).

The separation was achieved on Zorbax Sil, 250x4.6 mm i.d., (5µm size particles) column (Agilent technologies) using mobile phases containing various ratios of acetonitrile and 0.05 M potassium dihydrogen orthophosphate; pHs of which were adjusted with potassium hydroxide or orthophosphoric acid. The detection of the analytes was affected at 218 nm.

2.3 Standard solutions

The standard mixture used for method development contained 50 µg/ml of each of the following analytes: metformin, impurities B, C, D and E, and 20 µg/ml impurity A were prepared in 20% aqueous acetonitrile solution by dilution from individual analytes stock solutions containing 500 µg/ml each. Other standard solutions in same solvent were obtained by dilution from the stock solutions and used in the study.

2.4 Samples preparation

Sample solutions containing 5000 µg/ml metformin HCl were prepared in 20% aqueous acetonitrile, 20 µl triplicate injections were made from each sample.

3. Results and discussion

3.1 Separation optimization

In an attempt to optimize the separation of the analytes, the influence of the mobile composition, pH, flow rate and temperature were investigated.

3.1.1 Influence of the mobile phase composition

Acetonitrile was used as the organic modifier for making up the mobile phase; different percentages of acetonitrile (15-35% v/v) were investigated, while keeping the pH of the mobile phase as 7.0, the column temperature at 25 °C and the flow rate at 1 ml/minute.

The variation of the solutes retention with the composition of the mobile phase (Fig. 2); indicated that there was no change in the capacity factor of impurity A and D with the increase of the amount of acetonitrile. Metformin, Impurities B, C and E showed gradual decrease in this parameter up 20% v/v acetonitrile and reached almost a plateau at compositions higher the 20% acetonitrile.

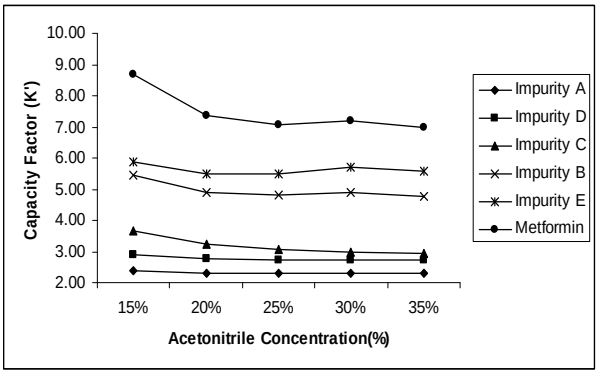

Fig 2: Dependence of capacity factor on acetonitrile content at constant pH, temperature and flow rate

3.1.2 Influence of the mobile phase flow rate

The flow rate of the mobile phase was varied in the interval of (0.5 – 2.0 ml/minute) while keeping the pH of the mobile phase as 7.0, the column temperature at 25 °C and acetonitrile content as 20% v/v. The mobile phase flow rate although it was found to significantly affect the analysis time, however its effect on the analytes retention was proportional and not expected to influence the analytes separation as shown in Fig. 3.

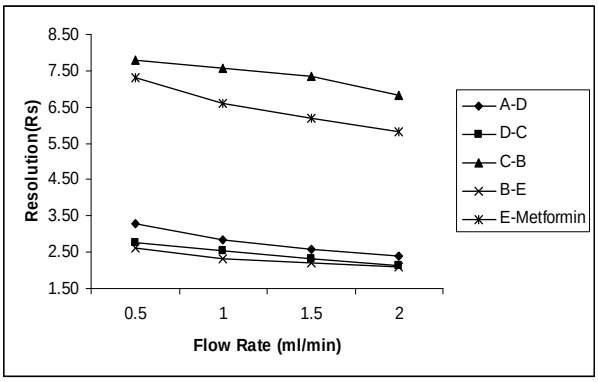

Fig 3: Effect of mobile phase flow rate on the resolution between analytes pairs

3.1.3 Influence of the temperature

The temperature dependences of the six analytes retention were studied within the interval 25- 40 ºC; with a step of 5 ºC. Varying the column temperature does seem to affect the capacity factor of the analytes to any extent as shown in Fig. 4.

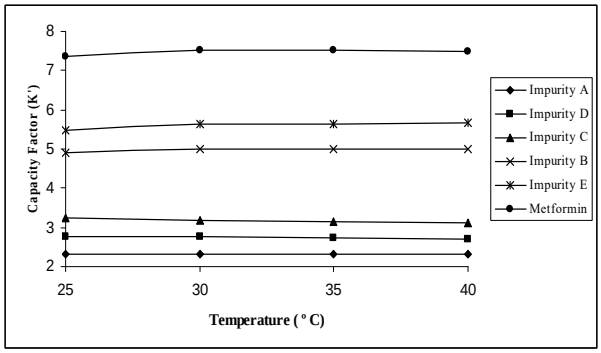

Fig 4: Effect of temperature on the retention factor

3.1.4 Influence of the mobile phase pH

The retention behaviour of the analytes was studied as a function of mobile phase pH over the range of 4.0 – 8.0; while maintaining the acetonitrile percentage as 20% v/v and the column temperature at 25 °C with a flow rate of 1 ml/min. The pH of the mobile seems to play a critical role in the retention behaviour of the analytes and hence the resolution between the analytes pairs, for instance at pH 8.0 there was no baseline separation between impurities A, C and D, at pH 6.0 the resolution between B, C and E impurities was affected and the three analytes eluted as one peak, at pH 5.0 impurity B and E peaks impurity C and metformin behaved similarly and at pH 4.0 impurity B and E co-eluted, while metformin peak appeared as unresolved shoulder at the back of impurity C peak. This pH dependent behaviour could be correlated with the differences in the basicity of the analytes and the extent of their ionization and hence interaction with the stationary phase, since it is proposed that the combination of ion-exchange and interaction with siloxane and silanol groups over the entire range of concentration of the organic solvent are the underlying mechanisms for controlling the retention under normal phase conditions [14].

Base line separation between the adjacent analytes pairs was only achievable at pH 7.0 and a mobile phase containing 20% v/v acetonitrile. Based on these findings it can be concluded that control of pH at 7.0 ± 0.5 is important to obtain the best separation as shown in Fig. 5 and Fig. 6.

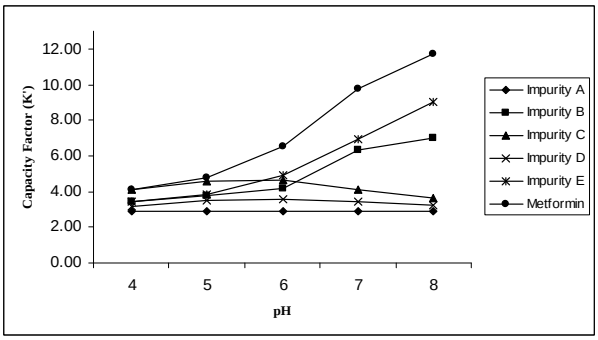

Fig 5: Effect of mobile phase pH on the retention of the analytes

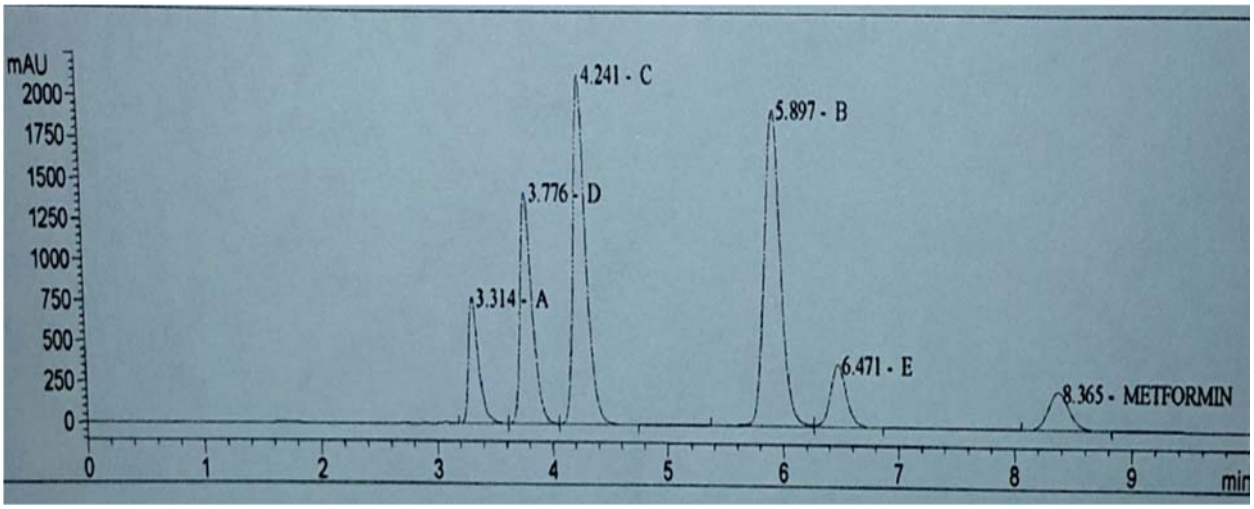

Fig 6: Representative chromatogram of metformin and impurities (A-E) standards mixture under optimized conditions: Acetonitrile: 0.05 M Potassium dihydrogen phosphate (20:80% v/v), pH 7.0

3.2 Method validation

3.2.1 Linearity and Quantitation Limits

Ten standard solutions mixtures in 20% aqueous acetonitrile solution were obtained in the concentration range of 0.5 – 2.5 µg/ml for impurity A, 2 -10 µg/ml for impurities B, C, D and E. Ten standard solutions in 20% aqueous acetonitrile solution were obtained in the concentration range of 10 – 1000 µg/ml for metformin hydrochloride.

Each of the standard solutions was injected three times consecutively and the mean peak areas were used in establishing the calibration functions of the method. The main regression parameters and limits of detection for each of the analytes are given in Table 1.

Table 1: The main regression parameter for the dependence of peak areas on concentration

Analyte	Intercept (a)	Slope (b)	r^2	LOQ (µg/ml)
Metformin	986.94	56.82	0.9996	0.66
Impurity A	5.76	198.34	0.9995	0.25
Impurity B	24.63	236.53	0.9999	0.88
Impurity C	14.55	193.46	0.9999	0.67
Impurity D	12.09	120.51	0.9999	0.59
Impurity E	0.94	36.44	0.9999	0.15

The limit of detection (LOQ) was calculated using the standard deviation of response and the slope of the calibration curve [15].

LOQ values permit determination of the related impurities in metformin below acceptance limits for each of them (1 µg/ml for impurity A, and 5 µg/ml for the others) referring to a solution of 5000 µg/ml metformin dissolved in 20% aqueous acetonitrile.

3.2.2 Bulk material analysis

The validity of the method was confirmed by its application to the analysis of samples obtained from three different suppliers; the results obtained for these samples are shown in Table 2.

Table 2: Results of bulk materials analysis

Supplier	Impurity %				
	A	B	C	D	E
A	ND	0.70	ND	ND	0.67
B	ND	ND	0.56	ND	0.34
C	ND	0.55	ND	ND	0.65

ND: not detected

4. Conclusion

The normal phase mechanism can be applied to the separation and determination of metformin and its five related impurities. The main parameters involved in developing the chromatographic method have been optimized and validated: column temperature, mobile phase composition, pH-value and flow rate. As robustness always represent an important task in developing a chromatographic method, it may be concluded that the major parameters set up finally fulfil the requirements imposed for their validation. The method can be used satisfactorily for quality control of metformin bulk material.

4.1 Conflict of interest

Authors do not have any conflict of interest with the commercial identities mentioned in this article.

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