Journal of Pharmaceutical Research Vol. 10, No. 3, July 2011 : 102-105.

ESTIMATION OF NATEGLINIDE AND METFORMIN HYDROCHLORIDE IN TABLET DOSAGE FORM BY SPECTROPHOTOMETRIC METHODS

Thomas AB*, Patil SD, Kothapalli LP, Nanda RK, Bhosle SS and Deshpande AD
Department of Pharmaceutical Chemistry, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411 018

Received on : 26.04.2011 Revised : 08.07.2011 Accepted : 09.07.2011

ABSTRACT
Three UV spectrophotometric methods have been developed for the simultaneous estimation of Nateglinide (Nat) and Metformin hydrochloride (Met) in combined tablet dosage form. The first method is the Area under curve method, the sampling wavelength ranges selected for estimation of Nat and Met are 214-216 nm and 231-235 nm respectively with linearity in the concentration ranges of 0.5-80 µg /ml and 0.5-40 µg/ml respectively. Second method involves determination using the Multicomponent mode of the UV visible spectrophotometer, the sampling wavelengths selected are 216 nm and 233nm over the concentration range 0.5-80 µg/ml and 0.5-40 µg/ml for Nat and Met respectively. The third method is the second order derivative method, the sampling wavelengths selected for estimation of Nat and Met are 225 nm and 234 nm with linearity in the concentration range 1-70 µg/ml and 1-40 µg/ml respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Nateglinide and Metformin hydrochloride in both bulk and tablet dosage form.

Keywords: *Nateglinide; Metformin hydrochloride; Area under curve method; Multicomponent mode; Second order derivative.*

INTRODUCTION

Nateglinide (Nat) is used in the treatment of diabetes mellitus and is official in U.S.P[1]. Metformin hydrochloride (Met) is prescribed for the treatment of type II diabetes-mellitus, it increases glucose transport across the cell membrane in skeletal muscles and is official in I. P[2] and B.P[3]. Various methods such as, UV[4-6] spectrosctropy, HPLC[7-17] and HPTLC[18] have been reported for individual drugs in formulation as well as combination with other drugs. Not a single UV or HPLC method is reported so far for the simultaneous analysis of Nat and Met in their combined dosage form. Nat and Met are available in combined tablet dosage form for the treatment of diabetes. So a need was felt to develop new methods to analyze the drugs simultaneously. This paper describes three UV spectroscopic methods for the simultaneous determination of Nat and Met in tablet formulation using Area under curve method, Multicomponent mode method and Second order derivative method.

EXPERIMENTAL

Material and Methods

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. The pure drug samples of Nat and Met were obtained from C. C. Lab. Torrent Pharmaceutical Ltd., Indrad, India and USV Limited, Baddi, India as gift samples. The tablet employed in the study was Glinate-MF, (Glenmark Pharmaceutical LTD., Baddi, India)

Preparation of standard stock solution

Standard stock solutions (100µg/ml) of Nat and Met were prepared by dissolving separately 10 mg of each drug each in 100 ml methanol and distilled water respectively.

Preparation of sample stock solution

Twenty tablets were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 10 mg of Met was transferred to a 100 ml volumetric flask and dissolved in 50 ml of methanol. After the immediate dissolution, the volume was made up to the mark with the same solvent. The solution was sonicated for about 30 min and was then filtered through Whatmann filter paper No.41. The solution was suitably diluted with distilled water to obtain sample solutions containing Nat and Met in the concentrations ratio of 3:24.99 µg/ml respectively.

Method A – Area under curve method

Standard stock solutions (100µg/ml) of Nat and Met were prepared by dissolving separately 10 mg of each drug in 100 ml methanol and distilled water respectively. For forming simultaneous equations for Area under curve method (AUC), 214-218 nm and 231-235 nm were selected as the two sampling wavelength

***Correspondence :** dypharmachem@yahoo.co.in

intervals. Fig.1 represents the overlain UV spectra of Nat and Met with their AUC intervals. Nat and Met exhibited linearity in the concentration range of 0.5-80 µg/ml, 0.5-40 µg/ml at their respective selected wavelength interval. Co-efficients of correlation were found to be 0.999 for both Nat and Met. The optical characteristics and regression values for the calibration curves are presented in Table 1. For the simultaneous estimation, mixed standards containing Nat and Met in the ratio of 3:24.99 µg/ml were prepared by appropriate dilution of the standard stock solutions. The AUC of the mixed standard solutions were measured at the selected wavelength intervals. A set of two simultaneous equations were established using the mean absorptivity coefficients of Nat and Met at the selected wavelength intervals.

$A_1 = 140.5\ C_{Nat} + 178.3\ C_{Met}$ …….. (I)
at 214-218 nm (λ_1 - λ_2))

$A_2 = 18.3\ C_{Nat} + 271.0\ C_{Met}$ …….. (II)
at 231-235 nm (λ_3 - λ_4)

Where,
140.5 and 18.3 are mean absorbtivity values of Nat at λ_1 - λ_2 and λ_3 - λ_4 respectively
178.3 and 271 are mean absorbtivity values of Met at λ_1 - λ_2, and λ_3 - λ_4 respectively
A_1, A_2 are the absorbance of mixed standard at λ_1 - λ_2 and λ_3 - λ_4 respectively.

The concentration of Nat and Met in mixed standards and tablet formulation can be obtained by solving equation (I) and (II).

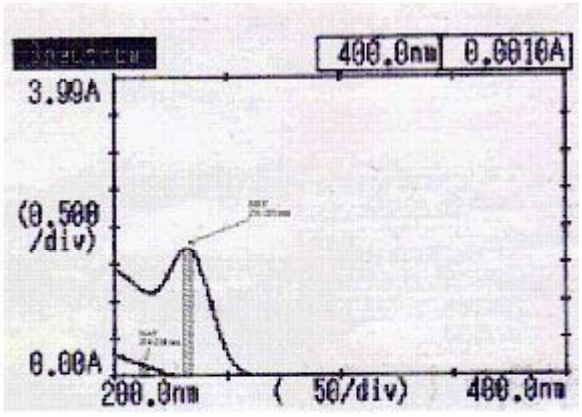

Fig. 1: *Overlay Spectra of Nat and Met in Area under curve method*

Estimation from marketed preparation
Suitable dilutions of tablet sample solution were scanned in the range of 400-200 nm and their AUC's were recorded at the selected wavelength intervals. The concentrations of each drug in sample solutions were calculated using equation (I) and (II).

Table 1: *Optical Characteristics and Validation Data of Nateglinide and Metformin Hydrochloride*

Parameter	Nateglinide			Metformin Hydrochloride		
	Method-A	Method-B	Method-C	Method-A	Method-B	Method-C
Working wavelengths	214-218 nm	216 nm	225 nm	231-235 nm	233 nm	234 nm
Beer-Lambert's Law range (µg/mL^{-1})	0.5-80	0.5-80	1-70	0.5-40	0.5-40	1-40
Precision*						
Interday (%RSD)						
Intraday (%RSD)	0.7596	0.9029	0.8823		0.5716	0.8802
LOD (µg/ml)*	0.8414	0.6969	0.4687	0.6118	0.5323	0.5610
LOQ (µg/ml)*	0.0398	0.1336	1.4685	0.5788	0.2675	0.9037
Regression Values: I. Slope*	0.1201	0.4090	4.46	0.0107 0.0325	0.7998	2.7399
II. Intercept*	0.088	0.020	0.0002		0.087	0.0002
III. Regression Coefficient (r^2)*	0.992 0.998	0.027 0.998	0.0006 0.991	0.257 0.047 0.999	0.006 0.999	0.0005 0.993

** Average of six estimations,*

Method B – Multicomponent mode method
For the analysis of Nat and Met by multicomponent method of analysis, the Multicomponent mode of the UV visible spectrophotometer was used. Standard stock solutions (100µg/ml) of Nat and Met were prepared by dissolving separately 10 mg of each drug in 100 ml methanol and distilled water respectively. For Multicomponent method of analysis, 216 nm and 233 nm were selected as the two sampling wavelengths for Nat and Met respectively. Fig.2 represents the overlain UV spectra of Nat and Met for the multicomponent mode method. The drugs showed linearity in the concentration ranges of 0.5-80 µg/ml, 0.5-40 µg/ml with regression coefficient (r^2) values of 0.998, 0.999 for Nat and Met respectively. Six mixed standards in the ratio of 3:24.99 µg /ml within the Beer's concentration range of Nat and Met were prepared by appropriate dilution of standard stock solutions (100 µg /ml). In the multicomponent mode of the instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions by analysis of the spectral data of sample solution with reference to that of mixed standards.

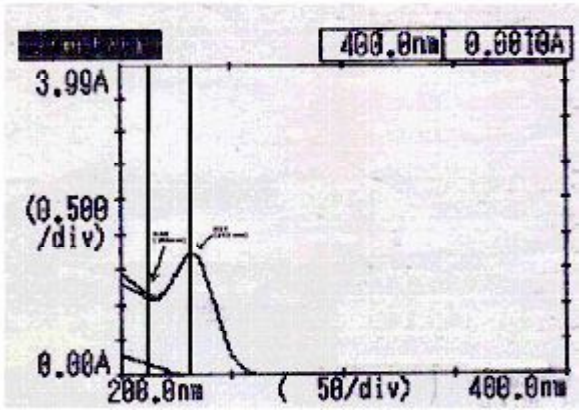

Fig. 2: *Overlain Spectra of Nat and Met in Multicomponent method*

Estimation from marketed preparation

Suitable dilutions of tablet sample solutions were scanned in the range of 400-200 nm in the multicomponent mode and the concentration of each component were obtained by the spectral data of sample solution with reference to that of the pure mixed standards. The analysis procedure was repeated six times with tablet formulation. The developed methods were validated as per ICH guidelines[19-20].

Method C – Second order derivative method[21]

The standard stock solutions were prepared as discussed in Method A. Suitable dilution of both drug solutions (10 µg/ml of Nat and 10 µg/ml of Met) were scanned between 400 and 200 nm using the spectrum mode of the instrument. The absorption spectra thus obtained were derivatised form first to fourth order. The second order derivate spectra were selected for the analysis of both the drugs. For forming simultaneous equation for Second order derivative, 225 nm and 234 nm were selected as the two sampling wavelengths for Nat and Met respectively. Fig.3 represents the overlain UV spectra of both drugs. Nat and Met exhibited linearity in the concentration range of 1-70 µg/ml and 1-40 µg/ml at their respective wavelengths. Co-efficients of correlation were found to be 0.993 and 0.999 for Nat and Met respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For the simultaneous estimation, mixed standards containing Nat and Met in the ratio of 3:24.99 µg/ml were prepared and their absorbances were measured at the selected wavelengths in the second order derivative mode. A set of two simultaneous equations were established using the mean absorptivity coefficients of Nat and Met at the selected wavelength intervals.

$A_1 = 0.4\ C_{Nat} + 0\ C_{Met}$ ..... (III) at 225 nm (λ_5)

$A_2 = 0.008\ C_{Nat} - 0.58\ C_{Met}$ ..... (IV) at 234 nm (λ_6)

Where,

0.4 and 0.008 are absorbtivity values of Nat at λ_5 and λ_6 respectively

0 and -0.58 are absorbtivity values of Met at λ_5 and λ_6 respectively

A_1, A_2 are the absorbance of mixed standard at λ_5 and λ_6 respectively.

The concentration of Nat and Met in mixed standards and tablet formulation can be obtained by solving equation (III) and (IV).

Estimation from marketed preparation

The tablet sample solution was scanned in the spectrum mode in range of 400-200 nm. The absorbances of the sample solutions were recorded at 225 nm and 234 nm in the second order derivative mode. The concentrations of each drug in sample solutions were calculated using equation (III) and (IV). The results of the tablet analysis and its statistical validation data are given in Table 2.

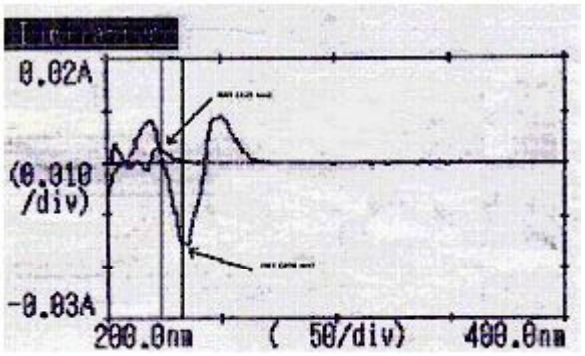

Fig. 3: *Overlay Spectra of Nat and Met in Second order derivative method*

Table 2: *Statistical Validation Data of Tablet Formulation*

Component	Amount present(mg)	Method	% Amount	R.S.D.*
Nat	60	A	100.29	0.7393
	60	B	99.99	0.8427
	60	C	100.56	0.3959
Met	500	A	99.90	0.4109
	500	B	99.78	0.2626
	500	C	99.91	0.8190

** Average of six estimations*

Tablet formulation: Glinate-MF, Glenmark Pharmaceutical LTD, Baddi, India

RESULTS

The optical characteristics and regression value of the calibration curves for the developed methods are presented in Table 1. The mean % content of Nat and Met by three methods was 100.28% and 99.84% respectively with low % RSD (less than 2%). Also the mean % recoveries of Nat and Met were 100.01% and 99.79% respectively. The results of the recovery studies and its statistical validation data are given in Table 3. The results of proposed method were also statistically evaluated using Turkey-Kramer one way ANOVA which showed P>0.05. The calculated F value was 1.032 and 0.0871 for Nat and Met respectively which is less than the standard F value(2.91), indicating that there exist no significant difference between the developed methods for analysis of Nat and Met in the both bulk and tablet formulation.

Table 3: *Statistical Validation of Recovery Studies*

Level of % recovery	Methods	% Recovery*		Relative standard Deviation*	
		Nat	Met	Nat	Met
80	A	99.24	99.40	0.3018	0.3032
	B	99.58	100.01	0.8335	0.3459
	C	100.03	100.13	0.0518	0.8789
100	A	99.77	99.64	0.5106	0.5424
	B	100.19	99.52	0.8067	0.5478
	C	99.77	100.02	0.1967	0.7049
120	A	99.90	99.55	0.7007	0.3666
	B	100.09	99.73	0.7375	0.6186
	C	101.56	100.19	0.3183	0.3804

** Average of three estimation at each level of recovery*

CONCLUSIONS

Nateglinide and Metformin hydrochloride are available in combined tablet dosage form for the treatment of type 2 diabetes. Not even a single UV spectrophotometric method has been reported so far for the estimation of both the drugs in combined tablet dosage form. Here three simple UV spectrophotometric methods; Area under curve method, Multicomponent mode method and Second order derivative method were developed for their simultaneous estimations. The standard deviation, RSD and standard error calculated for three methods are low, indicating high degree of precision of the methods. The RSD is also less than 2% as required by ICH guidelines. The % recovery was between 98-102% indicating high degree of accuracy of the proposed methods. The result of Turkey-Kramer one way ANOVA indicated that there is no significant difference between these methods for the analysis of Nat and Met in bulk and formulation. Hence the developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Nat and Met in both bulk and tablet dosage form.

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pune, MH, India, for providing necessary facilities, and to C. C. Lab. Torrent Pharmaceutical Ltd., Indrad, India and USV Limited, Baddi, India, for the generous gift samples of pure Nateglinide and Metformin hydrochloride.

REFERENCES

1. United State Pharmacopeia, The United States Pharmacopoeial Conventional, first supplement of USP 34 NF 29, 2010.
2. Indian Pharmacopoeia, Ghaziabad: The Indain Pharmacopoeia Commission 2010, p1358.
3. British Pharmacopoeia, London: The Stationery Office 2010; II: 1375-1377.
4. Jain S et al. Asian J Pharm, 2009; 3(3): 218-221
5. Rastogi A, et al. The Pharma Research. 2009; 01: 169-174.
6. Arayne M, et al. Indian J Pharm Sci. 2009, May–Jun; 71(3): 331–335.
7. Bauer S, et al. Journal of Pharmaceutical and Biomedical Analysis. 2003; (31): 551-/555.
8. Panderi I, et al. Analytica Chimica Acta, 2007; (599); 143–150.
9. Sankalia J, et al. Journal of Pharmaceutical and Biomedical Analysis, 2007(44); 196–204.
10. Varanasi K, et al. Journal of Chromatography B, 2008(865); 91–98.
11. O¨ nal A. European Journal of Medicinal Chemistry, 2009(44); 4998–5005.
12. Tirumala R, et al. International Journal of Pharmacy and Pharmaceutical Sciences, 2009; Oct-Dec; 2(2); 162-166.
13. Zhang Z, et al. Journal of Chromatography B, 2007(854); 91–98
14. Ahmadiani A, et al. Journal of Chromatography B, 2005(824); 319–322
15. Yuen K, et al. Journal of Chromatography B, 1998(710); 243–246
16. Shrivastav P, et al. Journal of Pharmaceutical and Biomedical Analysis 2007(45); 97–106
17. Chou C, et al. Journal of Chromatography B, 2001(762); 51–58
18. Ghassempour A, et al. Chromatographia, 2006(64); 101–104.
19. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
20. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996
21. Beckett AH, Stenlake JB, Practical Pharmaceutical Chemistry, IV edition, Part II, 286-288, 296-299