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Simultaneous spectrophotometric estimations of Nateglinide and Metformin hydrochloride in pharmaceutical formulation

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ABSTRACT

Two simple spectrophotometric methods have been developed for simultaneous estimation of Nateglinide (Nat) and Metformin Hydrochloride (Met) from tablet dosage form. First method involves solving simultaneous equations based on measurement of absorbance at two wavelengths 216 nm and 233 nm (λ max of Met). The second method is first order derivative spectroscopic method, wavelengths selected for quantitation were 216.0 nm for Nateglinide and 243 nm for Metformin hydrochloride (zero cross for nateglinide). Beer's law was obeyed in the concentration range of 0.5-80 µg/ml for Nat and 0.5-40 µg/ml for Met in both the simultaneous equation and first order derivative methods respectively. The results of analysis have been validated statistically and by recovery studies. The utility of the developed methods has been demonstrated by analysis of commercial formulation containing these drugs.

Key Words: Nateglinide, Metformin hydrochloride, Simultaneous equation method, First order derivative.

INTRODUCTION

Nateglinide (Nat), chemically is N-(trans-4-isopropyl cyclo hexyl carbonyl)-D-phenylalanine and is official in USP [1]. It is an anti-diabetic agent used as a blood glucose-lowering drug. Metformin hydrochloride (Met), chemically is N, N-dimethylimidodicarbonimidicdiamide hydrochloride. It increases glucose transport across the cell membrane in skeletal muscles and is official in IP [2] and BP [3]. Literature survey revealed spectrophotometric and chromatographic methods reported for estimation of Nat [4-5, 7-10] and Met [6, 11-18] individually or in combination with other drugs. However, there are no analytical methods reported for the simultaneous determination of these drugs in pharmaceutical formulation. The present work

describes two simple, rapid, accurate and precise methods for simultaneous determination of Nat and Met in tablets. The proposed methods were validated as per ICH guidelines [19-20].

MATERIAL AND METHODS

Standard gift samples of Nateglinide and Metformin hydrochloride were provided by C. C. Lab., Torrent Pharmaceutical Ltd., Indrad and USV Limited, Baddi, India respectively. Combined dose tablet formulation containing 60 mg of Nateglinide and 500 mg of Metformin hydrochloride, manufactured by Glenmark Pharmaceutical Limited, India, were purchased from local market. Methanol- AR and distilled water was used as solvent. A double-beam Shimadzu UV- Visible spectrophotometer, model 1700 (Japan), with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used for measurement of absorbance solution.

Preparation of Standard Stock Solution

Accurately weighed quantity of Nat/Met (10 mg) was transferred to 100.0 ml volumetric flask, dissolved in methanol and distilled water respectively and diluted to the mark with distilled water (100 µg/ml).

Method I: Simultaneous Equation Method

For the selection of analytical wavelength, standard solution of Nat (3 µg/ml) and Met (24.99 µg/ml) were prepared separately by appropriate dilutions of standard stock solution with distilled water and scanned in the UV range to determine working wavelength of both the drugs. For Met, the wavelength of maximum absorbance i.e. 233 nm was selected for analysis. For Nat, 216 nm was selected as the working wavelength. A series of standard solutions were prepared in the concentration range of 0.5-80 µg/ml for Nat and 0.5-40 µg/ml for Met. The absorbance of resulting solutions was measured at 216.0 nm and 233.0 nm and calibration curves were plotted. Both the drugs obeyed linearity in the concentration ranges under study. Absorptivity values were determined for both the drugs at the selected wavelengths. Two simultaneous equations (in two variables C_{Nat} and C_{Met}) were formed using mean absorptivity coefficient values as follows:

$$A_1 = 35.1\ C_{Nat} + 4.6\ C_{Met} \quad (1)$$

$$A_2 = 44.5\ C_{Nat} + 68.5\ C_{Met} \quad (2)$$

Where A_1 and A_2 are the absorbances of mixed standard and sample solutions at 216.0 nm and 233.0 nm, respectively. C_{Nat} and C_{Met} are the concentrations of Nat and Met measured in mg/ml, in mixed standard and sample solutions. 35.1 & 4.6 are absorptivity values of Nat at 216 nm and 233 nm respectively. 44.5 & 68.5 are absorptivity values of Met at 216 nm and 233 nm respectively. By applying the Cramer's rule to equation 1 and 2, the concentration C_{Nat} and C_{Met}, can be obtained as follows,

$$C_{Nat} = \frac{A_2\,(44.5) - A_1\,(68.5)}{2199.65} \quad (3)$$

$$C_{Met} = \frac{A_1(4.6) - A_2(35.1)}{2199.65} \quad (4)$$

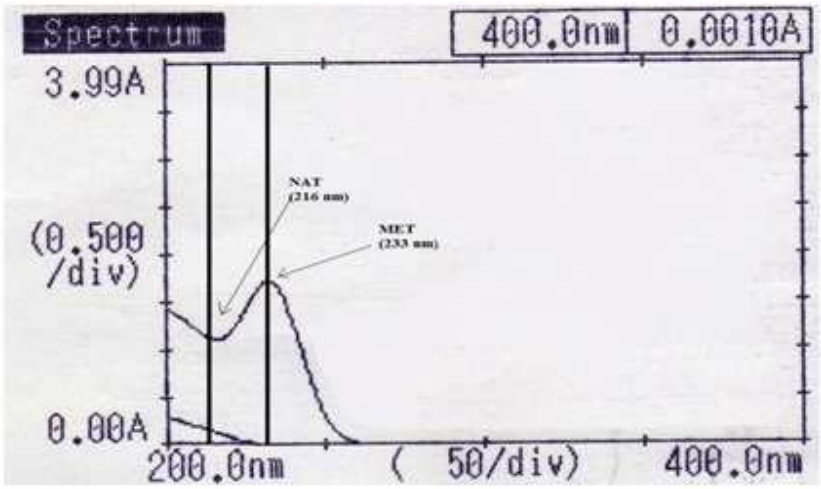

Fig.1: Overlain zero order spectra of Nateglinide and Metformin hydrochloride

Method II: First Order Derivative Method

In this method, solutions of Nat and Met (3µg/ml for Nat and 24.99 Met respectively), were prepared separately by appropriate dilution of standard stock solutions and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized to obtain the first order derivative spectra. From the overlain spectra of both drugs, wavelengths selected for quantitation were 216.0 nm for Nat and 243.0 nm for Met (zero cross for Nat). The calibration curves for Nat (0.5-80 µg/mL) and Met (0.5-40 µg/ml) were plotted at the selected wavelengths. Absorptivity values were then determined for both the drugs and two simultaneous equations (in two variables C_{Nat} and C_{Met}) were formed using mean absorptivity coefficient values and are as follows:

$$A_1 = (-2.2)\, C_{Nat} + (-0.044)\, C_{Met} \quad (5)$$

$$A_2 = 0\, C_{Nat} + (-4.2)\, C_{Met} \quad (6)$$

Where A_1 and A_2 are the absorbances of sample solution at 216.0 nm and 243.0 nm, respectively. C_{Nat} and C_{Met} are the concentrations of Nat and Met measured in g/l, in sample solutions. -2.2 & -0.044 are the absorptivity values of Nat at 216.0 nm and 233.00 nm respectively. Similarly -4.2 is absorptivity values of Met 243.0 nm. By solving the equation 5 and 6, the concentration C_{Nat} and C_{Met} can be obtained.

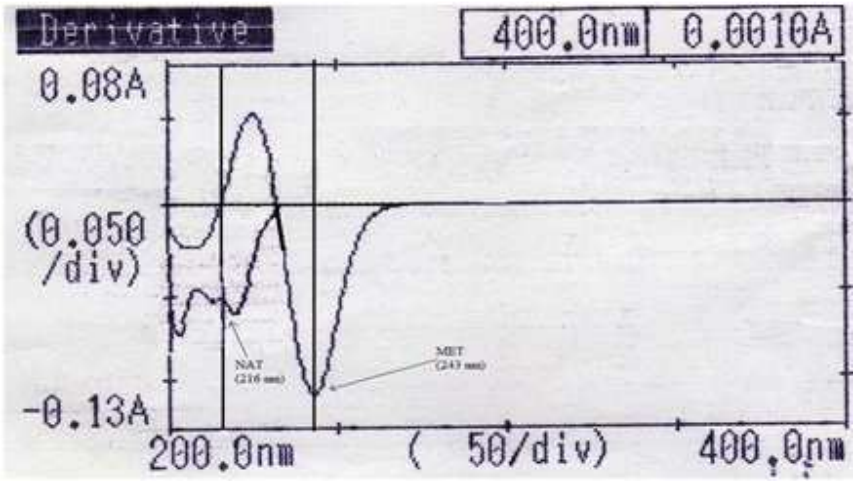

Fig.1: Overlain spectra of Nateglinide and Metformin hydrochloride in first order derivative mode

Analysis of Tablet Formulation by Proposed Method

For the estimation of drugs in commercial formulation, twenty tablets were weighed accurately. The average weight was calculated and then crushed to obtain fine powder. A quantity of tablet powder equivalent to about 10 mg of Met and 1.2 mg of Nat was transferred to 100 ml volumetric flask; 25 ml methanol was added and sonicated for 20 min, volume was then made up to the mark with methanol. The resulting solution was mixed, filtered through Whatmann filter paper and the filtrate obtained was appropriately diluted with distilled water to obtain concentration of 24.99 µg/ml of Met and 3 µg/ml of Nat. The resulting sample solution were analysis by both the developed methods. The results of the tablet analysis are shown in Table No. 1.

Table No – 1: Analysis of Tablet formulation

Method	Drug	Label Claim (mg/tablet)	Amount Found* (mg/tab)	Label Claim* (%)	R.S.D.
I	NAT	60	59.92	99.86	0.9828
	MET	500	500.1	100.02	0.7059
II	NAT	60	59.76	99.60	0.2536
	MET	500	499.2	99.84	0.3237

**Mean of six determinations, Nat – nateglinide, Met – Metformin hydrochloride, R.S.D – Relative standard deviation*

Validation

The proposed methods were validated as per ICH guidelines.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% & 120%). The results of recovery studies, expressed as percent recovery, were satisfactory and are presented in Table No.2

Table No – 2: Recovery Studies data

Level of recovery	Amount of pure added (mg)		Method-I				Method-II			
			Amt of drug recovered (mg)		Percent Recovery		Amt of drug recovered(mg)		Percent Recovery	
	NAT	MET	NAT	MET	NAT	MET	NAT	MET	NAT	MET
80%	2.4	19.99	2.403	19.87	100.13	99.40	2.382	19.93	99.30	99.74
100%	3	24.99	2.993	24.82	99.77	99.34	3.013	24.91	100.44	99.70
120%	3.6	29.98	3.609	29.91	100.27	99.79	3.603	29.88	100.09	99.69
Mean % recovery					100.05	99.51			99.94	99.71
R. S. D.					0.2577	0.2455			0.5843	0.0265

**Mean of three determinations, Nat – nateglinide, Met – Metformin hydrochloride, R.S.D – Relative standard deviation*

Precision

The reproducibility of the proposed methods was determined by analyzing tablets at different time intervals on same day in triplicates (Intra-day assay precision) and on three different days (Inter-day assay precision). Relative standard deviation for the intra-day assay precision was found to be 0.7580 for Nat and 0.5472 for Met in the simultaneous equation method and 0.4607 for Nat and 0.2150 for Met in first order derivative method. The inter-day assay precision coefficient of variance was found to be 0.9010 for Nat & 0.5949 for Met in the simultaneous equation method and 0.9283 for Nat and 0.4724 for Met in the first order derivative method.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and reliable way for quantitative determination of Nat and Met in combined tablet formulation. The linearity was observed in the concentration range of 0.5-80 µg/ml for Nat and 0.5-40 µg/ml for Met for both the simultaneous equation and first order derivative methods respectively. The percent label claim for Nat and Met in tablet was found in the range of 98.33 - 100.88 %. The percent recovery for Nat and Met was found in the range of 98.55 - 101.33 % with standard deviation well below 2 indicating accuracy of the methods. Intra-day and Inter-day precision studies were carried out by analyzing tablet formulation, by the proposed methods, three times on the same day and on three different days, respectively. Standard deviation and coefficient of variance for intra-day and inter-day precision studies was satisfactorily low indicating high degree of precision and reproducibility of proposed methods.

CONCLUSION

Based on the results obtained, it can be concluded that the proposed UV- Spectrophotometric methods (Simultaneous equation method and First order derivative method) for the simultaneous determination of Nat and Met is rapid, economical, accurate, precise and reproducible. The utility of the developed methods have been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be used for quantitative determination of these ingredients in combined dose tablet formulation.

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