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Sensitive and Reproducible Study for UV-Spectrophotometric Method for Analysis of Clopidogrel and Metoprolol in a Combined Tablet Dosage Form

LAKAVATH SURYANARAYANA
Department of Pharmaceutical Chemistry, University College of Pharmaceutical Sciences, Kaktiya University. Warangal-506001,T.S., India

Abstract:
The USP has suggested that the reduction in amount of reagents and materials which are routinely used in HPLC assays that have the potential to cause harm to human health and environment. Therefore, spectrophotometry as a simple, robust, quick and low cost method may be a good alternative if it is combined with calibration methods for determination of a complex mixture in pharmaceutical quality control laboratories. This study is useful because these two drugs are commonly administered simultaneously. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broader availability, suitability and ease of use. With the aim of the present investigation is to develop a simple, sensitive and reproducible UV Spectrophotometric method for analysis of Clopidogrel (CLOP) and Metoprolol (METO) in a combined tablet dosage form and hence an economical method was developed and validated according to the ICH guidelines.

Key Words: Clopidogrel, Metoprolol, UV Spectrophotometry, Validation.

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I. INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of chemicals within a sample as well as single dimensional features such as percent composition and compound identity. The pharmaceutical analysis comprises the procedures necessary to determine the "identity, strength, quality and purity" of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs.

of analytical chemistry

Qualitative analysis: Qualitative inorganic analysis seeks to establish the presence of a given functional group, element or inorganic compound in a sample.

Quantitative analysis: Quantitative analysis seeks to establish the amount of a given element (or) compound in a sample. [1,2]

II. REVIEW OF LITERATURE:

Mital et al., (2012) Development and Validation of First order Derivative Spectrophotometric Method for Simultaneous Estimation of Metoprolol Succinate and Clopidogrel Bisulphate in Tablet Dosage Form [13]. The zero crossing point for metoprolol succinate and clopidogrel bisulphate was 245.7nm and 276.13nm respectively.The LOD and LOQ values were found to be 0.15 and 0.18µg/ml for metoprolol succinate 2 and 4.5µg/ml for clopidogrel bisulphate respectively.

Kalyan et al., (2013) Development and Validation of UV Spectrophotometric Method For Determination Of S (-) Metoprolol Succinate and Clopidogrel Bisulphate In Bulk And Tablet Dosage Form [20]. This method was based on generation of simultaneous equations at 224 nm and 219 nm. Linearity for both drugs was found in the concentration range of 5-30 µg/ml. Assay results of marketed formulation were found to be 101.90% and 101.11% for S (-) MET and CLOP, respectively. Results suggest that the proposed method can be applied in routine quality control studies for assay of S (-) MET and CLOP in bulk and tablet dosage forms.

Kashyap Thumar et al., (2012) RP-HPLC Method for Simultaneous Estimation of Metoprolol Succinate and Clopidogrel Bisulphate from Tablet Dosage.[18]. The determination was carried out on Hibar 250-4.6 RP18

(5um) column using a mobile phase of Methanol: Water: Acetonitrile (70:20:10) pH-3.4 with orthphosphoric acid. The results of analysis have been validated statistically and by recovery studies. The percentage recoveries obtained for Metoprolol succinate and Clopidogrel bisulphate ranges from 99.05-101.28%.

Viral et al.,(2015) New Analytical Methods And Their Validation For The Estimation Of Metoprolol succinate In Bulk And Marketed Formulation [17]. The methods were validated as per ICH guidelines. The LOD and LOQ for estimation of Metoprolol succinate were found as 0.0773, 0.2343 for method A and 0.0667, 0.2021 for method B respectively

Jadhav et al.,(2012) Quantitative Determination of Metoprolol Succinate in bulk and tablet Dosage form through comparative study of UV and derivative Spectroscopy [16]. UV spectrophotometric methods for estimation of Metoprolol Succinate from bulk and tablet formulation in phosphate buffer 6.8. The linearity was observed between 5 – 25 µg/mL. The results of analysis were validated by recovery studies, accuracy, precision, LOD, LOQ and ruggedness. The method was found to be simple, accurate, precise, economical and robust.

Pravin et al., (2012) Development and Validation of Spectrophotometric Method for Clopidogrel bisulfate in pure and in film coated tablet dosage form [15]. The developed estimation method proved to be accurate (accuracy varies between 10.2-5.5%) and precise (Intra day precisions were less than 4.5%). The method is linear over this concentration range as indicated by the *F*-test lack of fit. Analyte recovery was better than 90% at all points on the standard curve, Intraday precision was better than 5% CV, while accuracy was between 98-100% of nominal over this range of the estimation.

III. MATERIALS AND METHODS:

Multi component analysis:

a) Simultaneous Equation Method:

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λ - max of the other (λ1 and λ2), it may be possible to determine both the drugs by the simultaneous equation method. Criteria for obtaining maximum precision, below mentioned ratio should lie outside the range 0.1 – 2.0

(A2/A1) / (ax2/aX1) and (aY2/aY1) / (A2/A1)

The information required is

The absorptivities of X at λ1 and λ2, aX1and aX2

The absorptivities of Y at λ1 and λ2, aY1and aY2

The absorbance of the diluted sample at λ1 and λ2, and A1 and A2

Let Cx and Cy be the concentration of X and Y respectively in the sample. The absorbance of the mixture is the sum of the individual absorbances of X and Y

$$C_y = (A1\ aX1 - A1\ aX2) / (aY1\ aX2 - aY2\ aX1) \quad (1)$$

$$C_X = (A2\ aY1 - A1\ aY2) / (aY1\ aX2 - aY2\ aX1) \quad (2)$$

Equations 1 and 2 are known as simultaneous equations and by solving these simultaneous equations we can determine the concentration of X and Y in sample.

Working steps: prepare the solutions of ciprofloxacin, metronidazole and sample. Scanned under the 2 wavelengths i.e cipro 271nm, and metro320nm. Take the absorbencies of above at 2 wavelengths. Prepare binary mixtures and measure the absorbance's at 2 wavelengths. Substitute the values in simultaneous equation to get the results.

b) Area under curve method:

This method utilizes two wavelength ranges. From the overlain spectra of both the drugs the area under curve is determined at both selected wavelength range, 250-285nm for ciprofloxacin & 306-336nm for metronidazole with in the above selected wavelength ranges, the area under curve was determined for both drugs (cipro & metro – 10µg/ml & 16µg/ml) and sample solutions were analyzed and concentration of cipro & metro in sample solution were calculated using " Cramel's Rule" and " Matrix method".

Working steps: Prepare standard solutions of Clopidogrel and Metoprolol with concentration of 100µg/ml, from this prepare solutions with concentration of 10µg/ml. Prepare sample solution as like in simultaneous method. From the above stock solution 5ml was pipetted out and overlay spectra was taken for CLOP and METO. λmax of clopidogrel was found to be 271nm and metoprolol was found to be 220 nm. Iso absorptive point was found to be 272nm. Substitute the values in Q absorbance ratio equation to get the results.

Reagents and standards: Pharmaceutical grade clopidogrel and metaprolol provided as gift samples by Symed Lab India Pvt Ltd.,Hyderabad, India, were used as working standards. All other reagents and solvents used were analytical grade. Distilled water was used as solvent.

Equipment: UV – Visible spectrophotometer, Make : SHIMADZU, Model : 1800 and Software: UV.

Preparation of solutions:

Preparation of standard solution

Accurately weighed quantity of Clopidogrel (100 mg) and Metaprolol (100 mg) were transferred to two separate 100.0 ml volumetric flask. Dissolve in sufficient water. Then diluted to the mark with the distilled water (Stock solution 100 µg/ml).

A set of standard mixture solutions containing 5-90 µg/ml Clopidogrel and 4-32 µg/ml Metaprolol was made from stock solutions.

Preparation of sample solution

Twenty tablets each containing 70 mg of Clopidogrel and 50 mg of Metaprolol were taken, granules were crushed to fine powder. An accurately weighed powder sample equivalent to 10 mg of powder was weighed and transferred to 100ml volumetric flask, dissolved in sufficient water and filtered through whatman filter paper. The filtrate was made up to volume of 100 ml with distilled water.

IV. RESULTS AND DISCUSSION

The original laboratory (experimental) observations are taken as a raw data and well tabulated systematically for the mathematical, statistical data for analysis purpose.

METHOD VALIDATION: Validation of UV-visible spectrophotometer method was in compliance with recommendations of the ICH guidelines.

Linearity: For this method the calibration curve was prepared and the results obtained were used to calculate

PROCEDURE FOR CALIBRATION CURVE: A series of solutions were prepared using known concentration levels from 5-90µg/ml for clopidogrel and 4-32µg/ml for metoprolol and scanned into UV spectrophotometer by keeping water as blank. The absorbance of the drug was recorded. The calibration curve for the UV spectrophotometric analysis was constructed by plotting the absobance (for simultaneous and Q-absorbance method) and peak area (for AUC) on y-axis and concentration on x-axise the equation of the line by using linear regression by the least square.

RESULTS

Linearity of Metoprolol

S.NO	CONCENTRATION(µg/ml)	SEM	AUC	Q - RATIO
1	4	0.204	0.141	0.011
2	8	0.312	0.317	0.015
3	12	0.424	0.489	0.021
4	16	0.527	0.674	0.024
5	20	0.662	0.873	0.029
6	24	0.820	1.030	0.028
7	28	0.937	1.297	0.034
8	30	1.050	1.453	0.038

Table 6.1 : Linearity Table For Metoprolol

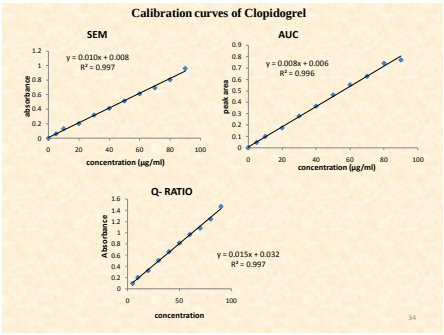

Figure6.5: Calibration curve of Metoprolol

Linearity of Clopidogrel

S.NO	CONCENTRATION (µg/ml)	SEM	AUC	Q- RATIO
1	5	0.061	0.047	0.099
2	10	0.132	0.098	0.020
3	20	0.203	0.173	0.326
4	30	0.320	0.277	0.509
5	40	0.412	0.365	0.661
6	50	0.514	0.462	0.816
7	60	0.613	0.551	0.968
8	70	0.695	0.626	1.081
9	80	0.808	0.740	1.242
10	90	0.961	0.768	1.466

Table 6.2 : Linearity Table For Clopidogrel

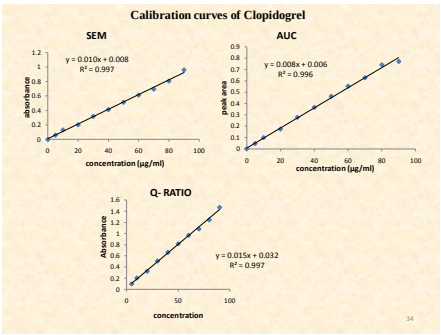

Figure6.5: Calibration curve of Clopidogrel

PRECISION: Precision of the method is studied as intra-day and inter-day precision. Intra-day and Inter-day precision was determined by analyzing the same concentration of the solutions daily for three days. In intermediate precision study, % R.S.D. values were not more than 1.0 % in all the cases

ACCURACY: To assess the accuracy of the proposed method, recovery studies were carried out three different levels i.e. 80%, 100% and 120%. To the pre-analyzed sample solution a known amount standard drug solution was added at three different levels, absorbance was recorded. The % recovery was then calculated and results are given in **Table 6.5 & 6.6.**

LOD AND LOQ: Limit of detection (LOD) and Limit of quantization (LOQ) were determined by using the formula based on the standard deviation of the response and the slope. The LOD and LOQ were calculated by mathematical equation and results are given in **Table:** 6.7 & 6.8.

$$LOD = 3.3\ \sigma/s$$

$$LOQ = 10\ \sigma/s$$

Where, S-slope

σ=standard deviation

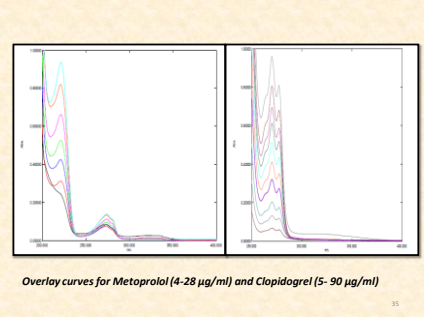

Figure 6.11: Precision spectra for Metoprolol & Clopidogrel

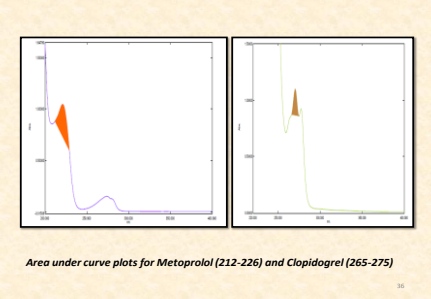

Figure 6.11: AUC for Metoprolol & Clopidogrel

PRECISION FOR CLOPIDOGREL

	SEM			AUC			Q - RATIO		
CONC	5	50	90	5	50	90	5	50	90
DAY 1	0.061	0.520	0.960	0.046	0.461	0.757	0.098	0.722	0.998
DAY 2	0.062	0.521	0.958	0.045	0.460	0.758	0.097	0.720	0.995
DAY 3	0.066	0.521	0.961	0.049	0.462	0.759	0.097	0.716	0.997
AVG	0.063	0.520	0.959	0.046	0.461	0.758	0.097	0.719	0.996
SD			0.0026			0.0042			0.0019
%RSD			0.517			0.995			0.314

Table 6.3: Precision data for Clopidogrel

PRECISION FOR METOPROLOL

	SEM			AUC			Q - RATIO		
CONC	4	16	28	4	16	28	4	16	28
DAY1	0.262	0.526	0.944	0.141	0.672	1.300	0.0117	0.0246	0.0348
DAY2	0.261	0.525	0.940	0.139	0.668	1.299	0.0119	0.0246	0.0346
DAY3	0.257	0.539	0.947	0.140	0.671	1.302	0.0116	0.0247	0.0346
AVG	0.260	0.530	0.940	0.140	0.670	1.30	0.0117	0.0246	0.0345
SD			0.0052			0.0092			0.0005
%RSD			0.916			1.310			0.0211

Table 6.3: Precision data for Metoprolol

ACCURACY OF CLOPIDOGREL

Concentration (µg/ml)	Percent of drug recovered	SEM	AUC	Q- RATIO
40(80%)	98.15	98.89	97.99	98.77
50(100%)	99.08	100.15	99.51	99.6
60(120%)	97.05	115.25	105.3	107.4

ACCURACY OF METOPROLOL

Concentration (µg/ml)	Percent of drug recovered	SEM	AUC	Q- RATIO
16(80%)	98.10	98.10	98.12	98.72
20(100%)	99.7	99.81	99.45	99.45
24(120%)	98.15	98.9	99.12	98.75

Table 6.6 : Accuracy data for Metoprolol & Clopidogrel

LOD AND LOQ

CLOPIDOGREL

METHOD	STANDARD DEVIATION	LOD	LOQ
SEM	0.5171	0.87	2.658
AUC	0.0042	1.73	3.256
Q - RATIO	0.0019	0.14	1.225

METOPROLOL

METHOD	STANDARD DEVIATION	LOD	LOQ
SEM	0.0052	0.579	1.755
AUC	0.0091	0.644	1.953
Q - RATIO	0.0005	1.65	4.18

Table 6.7 : LOD & LOQ data for Metoprolol & Clopidogrel

From statistical data it is clear that the developed method is simple, precise, accurate and economical for simultaneous estimation, area under curve and Q-absorbance of MET and CLOP in combined dosage form. Results suggest that the proposed method can be used for routine quality control studies for assay of MET and CLOP in bulk and combined tablet dosage form.

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