INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.9 | Issue 2 | Apr - Jun - 2020

Journal Home page: www.ijpar.com

Research article *Open Access*

Development and validation of UV spectrophotometric methods for the simultaneous estimation of dexamethasone and neomycin sulphate in bulk drug and pharmaceutical formulations

Manzoor Ahmed, Lavanya Patil M P*, Satishkumar Shetty A, Anil Kumar S M

Department of Pharmaceutical Analysis, National College of Pharmacy, Balraj Urs Road, Shimoga-577201, Karnataka, India.

***Corresponding Author: Lavanya Patil M P**

Email: lavanyapatilmp1996@gmail.com

ABSTRACT

In the present work two simple, precise, accurate and economical spectrophotometric methods have been developed for the simultaneous estimation of Dexamethasone and Neomycin Sulphate in bulk drug and pharmaceutical dosage forms by using distilled ethanol as a solvent. Method A is a Third order derivative method, which involves the measurement of absorbance of one drug at zero crossing point of other; hence wavelengths 230 nm and 262 nm were selected for the estimation of Dexamethasone and Neomycin Sulphate respectively. Method B is a Q-Absorbance ratio method, which is based on the measurement of absorbances at two selected wavelengths 216nm (Iso-absorptive point) and 258nm for the estimation of Dexamethasone and Neomycin Sulphate respectively. Beer's law was obeyed in the concentration range of 1-80µg/mL for Dexamethasone and 5-100 µg/mL for Neomycin sulphate. Working concentrations used in the range of 2-10 µg/mL and 10-50 µg/mL for Dexamethasone and Neomycin Sulphate respectively. The% RSD for intra-day and inter-day precision was within 2% for both the methods.

Keywords: Dexamethasone, Neomycin Sulphate, Third order derivative method and Absorbance Ratio method.

INTRODUCTION

Dexamethasone is a synthetic adrenal corticosteroid with potent anti-inflammatory and immunosuppressant properties. Chemically it is (8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16 octahydrocyclopenta[a] phenanthren-3-one [1]. The anti-inflammatory actions of Dexamethasone are thought to involve phospholipase A_2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes [2, 3].

Neomycin Sulphate is obtained from Streptomyces fradiae, it is a wide-spectrum aminoglycoside active against most gram negative bacilli and some gram positive cocci. Chemically Neomycin Sulphate is(2*R*,3*S*,4*R*,5*R*,6*R*)-5-amino-2-(aminomethyl)-6-[(1*R*,2*R*,3*S*,4*R*,6*S*)-4,6-diamino-2-[(2*S*,3*R*,4*S*,5*R*)-4-[(2*R*,3*R*,4*R*,5*S*,6*S*)-3-amino-6-(aminomethyl)-4,5-dihydroxyoxan-2-

yl]oxy-3-hydroxy-5-(hydroxymethyl)oxolan-2-yl]oxy-3-hydroxycyclohexyl]oxyoxane-3,4-diol; sulfuric acid [4]. Aminoglycosides like neomycin "irreversibly" bind to specific 30S-subunit proteins and 16S rRNAof susceptible bacteria. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to non-functional or toxic peptides and the breakup of polysomes into non-functional monosomes. Eventually bacteria will die because of the lack of functional proteins [6].

The combination of Dexamethasone and Neomycin Sulphate is used to treat bacterial eye infections, conjunctivitis and also it reduces the severity of allergy symptoms such as inflammation, irritation and itchiness [7].

On Literature survey, it was found that no method has been reported for the simultaneous estimation of Dexamethasone and Neomycin Sulphate in combined dosage forms and no method is available in the pharmacopoeias. Few analytical methods have been developed for the determination of Dexamethasone and Neomycin Sulphate individually and in combination with other drugs [8-12]. Hence in the view of the need for a suitable method for routine analysis in combined formulations, attempts were made to develop simple, precise and accurate spectroscopic methods for simultaneous estimation of titled drugs and extend it for their determination in pharmaceutical formulations. The present UV-Spectrophotometric methods were validated according to (ICH) guidelines [13, 14].

MATERIALS AND METHODS

Instrument

For UV-visible Spectroscopy methods, Shimadzu model 1800 double beam UV-visible Spectrophotometer with spectral band width of 1 ± 0.2 nm, wavelength accuracy of ± 0.3 nm and a pair of quartz cuvettes having 1cm path length was used.

Chemicals and reagents

Distilled Ethanol.

Drug sample

Standard Dexamethasone was obtained as gift samples from Micro labs Ltd, Bangalore. Standard Neomycin Sulphate was procured from Brawn Laboratories Ltd, Faridabad, Haryana.

Preparation of standard solutions.

100 mg of Dexamethasone and 100 mg Neomycin Sulphate were weighed and transferred in two different 100 ml volumetric flask. Both the drugs were dissolved in 50 ml of distilled ethanol by ultra-sonication and then volume was made up-to the mark with distilled ethanol to obtain the final concentration of 1000µg/ml (Stock A and A’ solution).

From the above Stock A and A’ solution 10 ml of aliquot was pipetted out into two different 100 ml volumetric flasks and volume was made up-to the mark with the distilled ethanol to obtain a concentration of 100µg/ml (Stock B and B’ solution).

From the above Stock B and B’ solution further dilutions were made to get concentration range from 2-10µg/ml of Dexamethasone and 10-50µg/ml of Neomycin Sulphate.

Preparation of sample solutions

Commercially available formulation DEXCIN eye drops was purchased which contains 0.1% of Dexamethasone and 0.5% of Neomycin Sulphate. From this formulation, 100mg of drug equivalent to Neomycin Sulphate was taken which also contains 20mg of Dexamethasone and transferred to 100 ml volumetric flask, dissolved in 50 ml distilled ethanol and the content was kept in sonicator for 15min. The solution was filtered through Whatmann filter paper No.41, finally the volume was made up-to the mark with distilled ethanol to get the concentration of 1000 µg/mL of NEOM and 200 µg/mL of DEXA and this solution was used as stock “A” solution of the sample.

From the above stock “A” solution, 10 mL of the aliquot was pipetted out and transferred to a 100 mL volumetric flask. The volume was made up-to 100 mL with distilled ethanol to obtain a concentration of 100 µg/mL of NEOM and

20µg/mL of DEXA (stock "B" solution of the sample).

Appropriate aliquots were pipetted out from the sample stock "B" solution in to a series of 10 mL volumetric flasks. The volume was made up to the mark with the distilled ethanol to get a set of solutions having the concentration range of 2, 4, 6, 8 and 10 µg/mL of DEXA and 10, 20, 30, 40 and 50 µg/mL of NEOM.

METHEDOLOGY

Method A: third order derivative method

Third order derivative method involves the measurement of absorbance of one drug at zero crossing point of other: hence wavelengths 230nm and 262nm for the estimation of Dexamethasone and Neomycin Sulphate respectively.

The standard solutions of both the drugs were scanned in the spectrum mode from 400-200nm using UV-Spectrophotometer. These spectrums were converted to Third order derivative spectra by using derivative mode in UV probe software. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components should be as large as possible. From the examination of the Third order derivative spectra of Dexamethasone and Neomycin Sulphate 230 nm and 262 nm were selected as working wavelengths for the Third order derivative spectroscopy.

Method B: Q-absorbance ratio method

The absorbance ratio method is based on the simultaneous equation procedure. It depends on the property of the substance, which obey Beer's law at all wavelengths, the ratio of absorbance at any wavelength is constant value independent of concentration or wavelength. This ratio is also referred to as a **Q-value.** In the quantitative assay of two components in admixture by the absorbance ratio method, absorbances were measured at two wavelengths. One being the λ_{max} of one of the component (λ_2) and other being a wavelength of equal absorptivity of the two components (λ_1), i.e. an iso-absorptivity point (Pernarowski1961).

From the above Stock solutions both the drugs were scanned in the wavelength range of 400-200 nm using UV-Spectrophotometer. With the help of an overlay spectrum absorbance of the solutions was measured at 216.0 nm (Iso-absorptive point) and 258.0 nm (λmax of NEOM).

Validation of the methods

Both the methods were validated according to ICH guidelines by carrying out analysis of six replicate samples. Recovery studies were carried out at three different levels i.e., 80%, 100% and 120% by adding the pure drug to previously analyzed sample. From the amount of the drug found, percentage recovery was calculated.

RESULTS AND DISSCUSSION

The estimation of DEXA and NEOM in bulk and pharmaceutical formulation was found to be accurate and reproducible with a linearity range of 2-10 µg/ml and 10-50 µg/ml respectively for both the methods and the correlation coefficient was found to be 0.9993 and 0.9999 for the method A and 0.9998 and 0.9999 for the method B respectively. The optical characteristics such as linearity range, molar absorptivity, percentage relative standard deviation of recovery studies and precision in each method were calculated and the results were reported in Table 1 for both the methods. Also the regression characteristics like slope (m), intercept (c) and correlation coefficient (r^2) were calculated and are presented in Table 1 for both the methods. The accuracy was found by recovery studies at three different levels i.e. 80%, 100% and 120%. The values of standard deviation were satisfactory and the recovery studies were close to 100%. The % RSD value was less than 2, an indicative of the accuracy of the methods. The results for formulation were reported in Table 2. The absorption spectra of Dexamethasone, Neomycin Sulphate and formulation by Third order derivative method were shown in (Fig. 3, 4 and 5) and calibration curve was shown in (Fig. 6, 7).

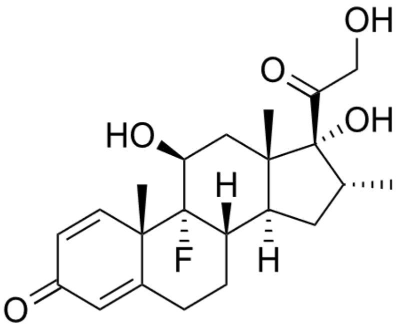

Fig. 1 Structure of Dexamethasone

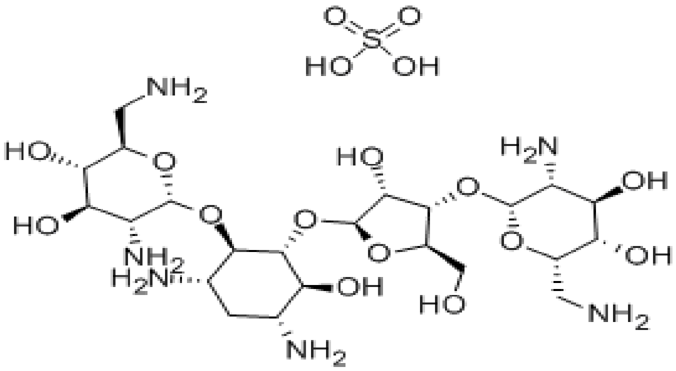

Fig. 2 Structure of Neomycin Sulphate

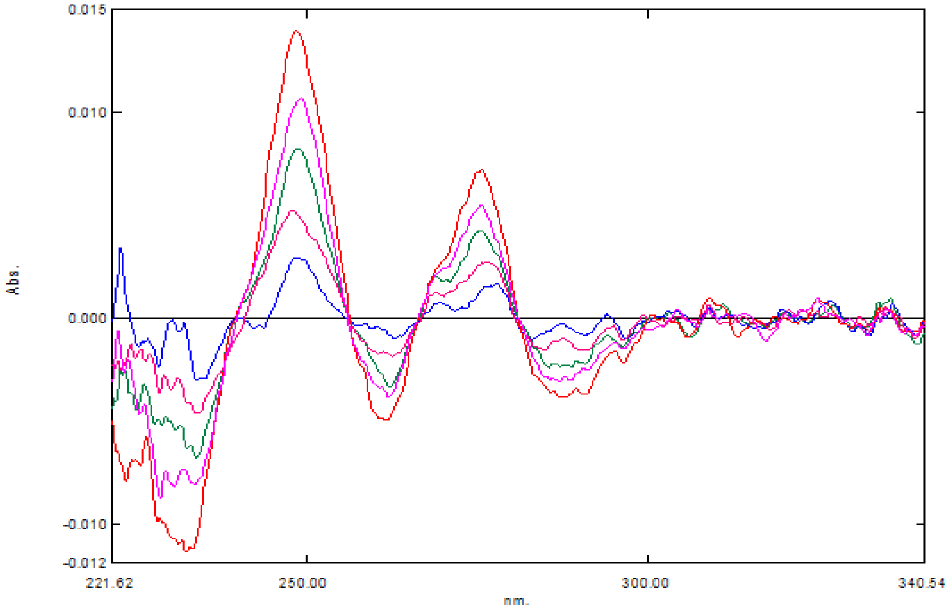

Fig. 3: Third order overlay spectrum of DEXA at 230.0 nm.

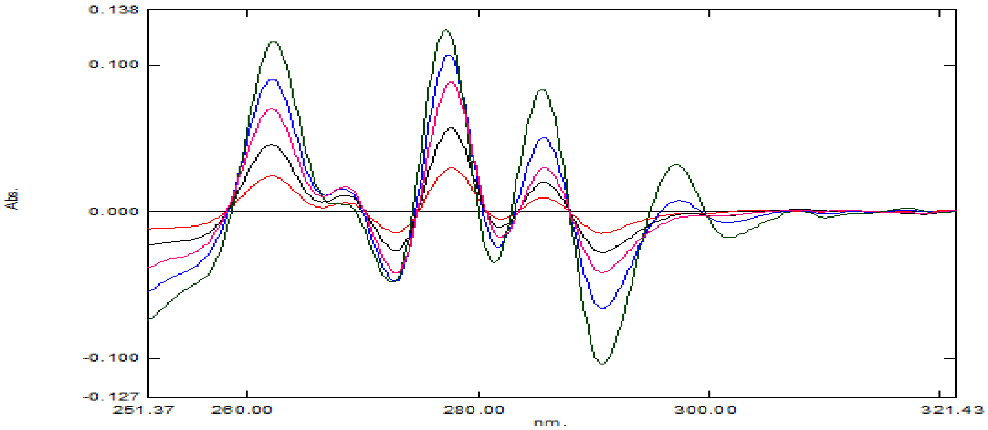

Fig. 4: Third order overlay spectrum of NEOM at 262.0 nm.

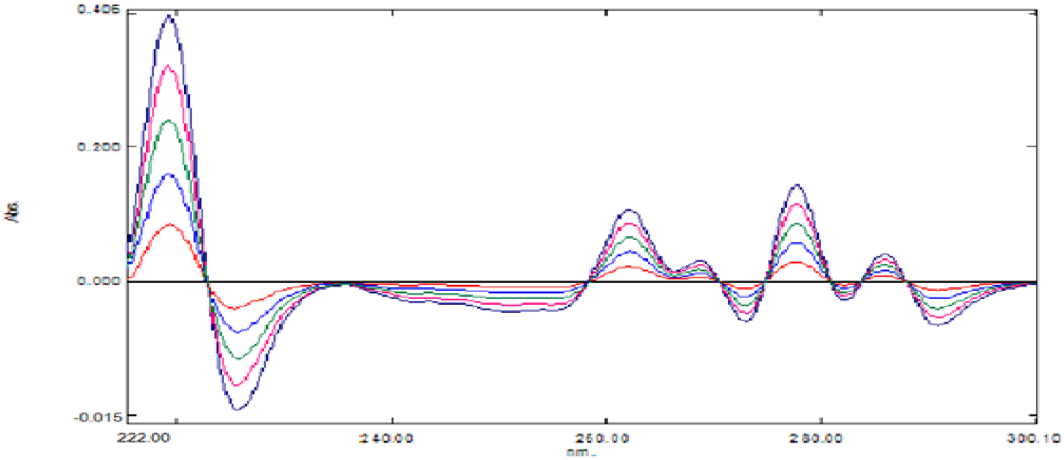

Fig. 5: Third order derivative spectrum of DEXA and NEOM in Formulation at 230.0 nm and 262.0nm.

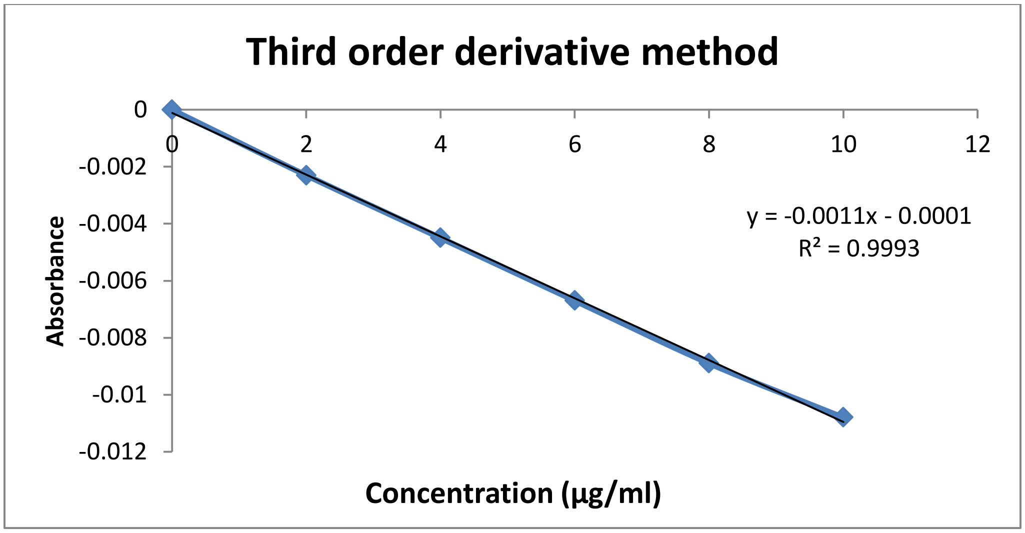

Fig. 6: Calibration curve for DEXA at 230.0 nm by Third Order Derivative Method.

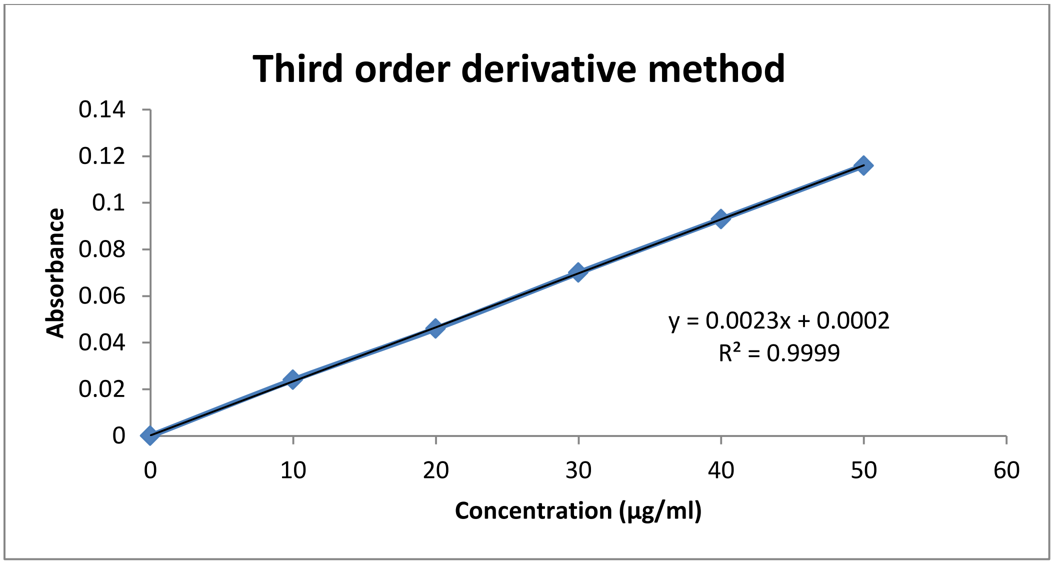

Fig 7: Calibration of Neomycin Sulphate at 262.0 nm by Third Order Derivative method

The spectra of Dexamethasone and Neomycin Sulphate were reported by Q Absorbance ratio method (Fig. 8) and calibration curve was plotted (Fig. 9, 10)

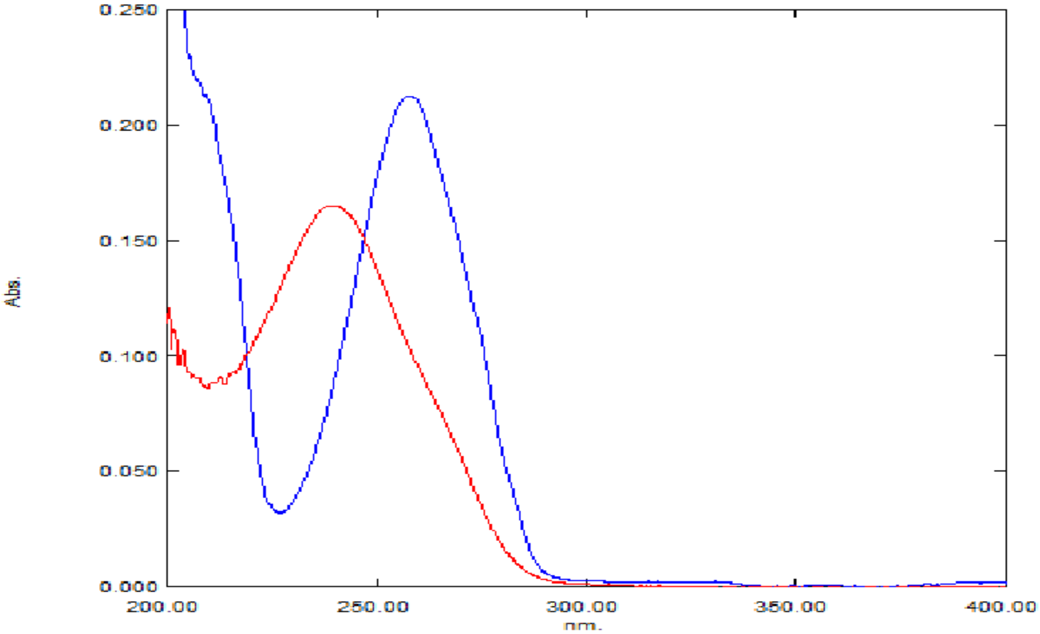

Fig. 8: Iso-absorptive point of DEXA and NEOM.

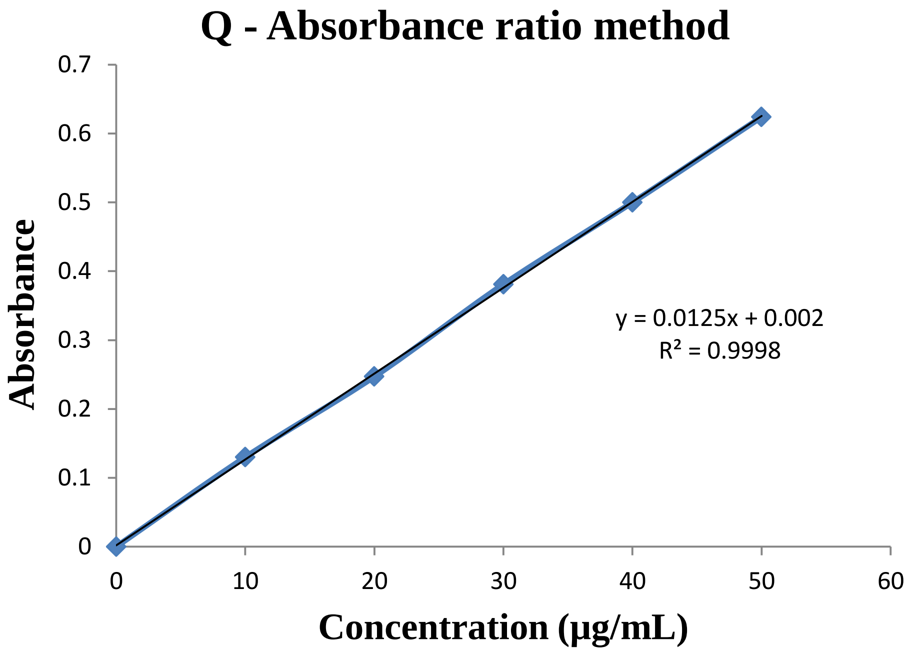

Fig. 9: Calibration curve for DEXA at 216.0 nm by Q - Absorption ratio Method.

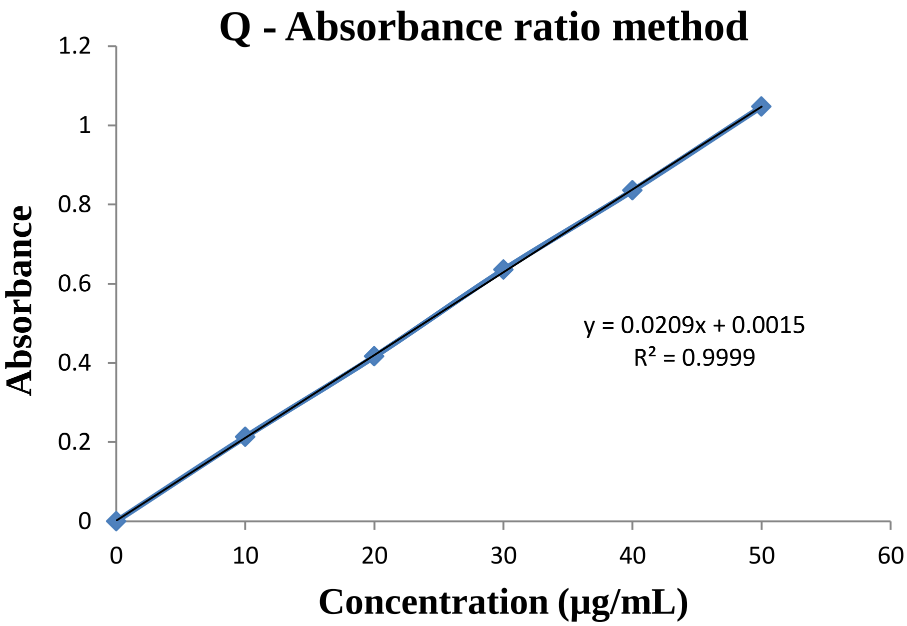

Fig. 10: Calibration curve for NEOM at 258.0 nm by Q - Absorption ratio Method.

Table 1: Optical and validation parameters of UV Spectrophotometric methods.

UV Spectroscopy		**Third Order Derivative Method**		**Q Absorbance Ratio Method**	
Parameters		**DEXA**	**NEOM**	**DEXA**	**NEOM**
Linearity range (µg/ml)		2-10	10-50	2-10	10-50
λmax / wavelength range (nm)		230	262	216	258
Coefficient of correlation		0.9993	0.9999	0.9998	0.9999
Slope*(m)		-0.0011	0.0023	0.0909	0.0209
Intercept*(c)		-0.0001	0.0002	-0.0092	0.0015
Accuracy (%RSD)	**80%**	1.0206	0.14742	0.7938	0.2268
	100%	0.4692	0.09185	0.3572	0.2755
	120%	0.0463	0.67732	0.7886	0.0463
Precision (%RSD)	**Intra-day**	0.1756	0.4265	0.84391	0.70963
	Inter-day	0.6223	0.4850	0.0089	0.0045
Limit of Detection (µg/ml)		0.3354	0.4183	0.4174	0.4087
Limit of Quantification (µg/ml)		1.0163	1.2675	1.2649	1.2384

Table 2: Results of formulation

Method	**Brand name**	**Label claim (mg)**		**%Recovery***		**%RSD***		**Standard Error***	
		DEXA	**NEOM**	**DEXA**	**NEOM**	**DEXA**	**NEOM**	**DEXA**	**NEOM**
A		20	100	99.5	99.06	1.571	0.877	0.00011	0.00048
B	**DEXCIN**	20	100	99.62	99.87	1.2460	0.5205	0.00421	0.00462

CONCULSION

The developed Third order derivative and Q-Absorbance ratio methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Dexamethasone and Neomycin Sulphate. Both the methods were validated as per ICH guidelines.

Acknowledgement

Authors express sincere thanks to the Principal and staff, Department of Pharmaceutical Analysis of National College of Pharmacy, Shimoga for guidance, encouragement and providing laboratory facilities.

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