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ISOABSORPTIVE POINT METHOD FOR THE SIMULTANEOUS ESTIMATION OF NEOSTIGMINE METHYL SULPHATE AND GLYCOPYRROLATE IN INJECTABLE DOSAGE FORM

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ABSTRACT
A simple, precise and accurate method has been described for the quantitative determination of Neostigmine Methyl Sulphate (NEO) and Glycopyrrolate (GLYCO) in bulk and injectable dosage form. The method involves the formation of Q-absorbance equation at 241nm isoabsorptive point and at 260nm, which is λ_{max} of Neostigmine Methyl Sulphate. Linearity of response was obtained in the concentration range of 125-425µg/ml for NEO and 25-85µg/ml for GLYCO. The method was successfully applied to the formulation and no interference was obtained due to excipients. The suitability of the method for quantitative determination was proven by validation, which was performed as per the ICH guidelines.

KEYWORDS: Neostigmine Methyl Sulphate, Glycopyrrolate, Isoabsorptive Point Method

INTRODUCTION

Neostigmine Methyl Sulphate (NEO) is a cholinesterase inhibitor which is used in the treatment of Myasthenia Gravis. It is administered as subcutaneous or intramuscular injection. Chemically, it is dimethylcarbamoyloxy-trimethylanilinium methyl sulphate having the molecular formula $C_{13}H_{22}N_2O_6S$ depicted in Figure 1a [1,2]. Glycopyrrolate (GLYCO) is a synthetic anticholinergic agent and a competitive muscarinic antagonist. It is used in some disorder of gastrointestinal tract and to reduce secretions with certain analgesics. Chemically, it is 1, 1 - dimethylpyrrolidin-1-ium-3-yl 2-cyclopentyl-2-hydroxy phenyl acetate bromide, having the molecular formula $C_{19}H_{28}BrNO_3$ as in Figure 1b [2,3].

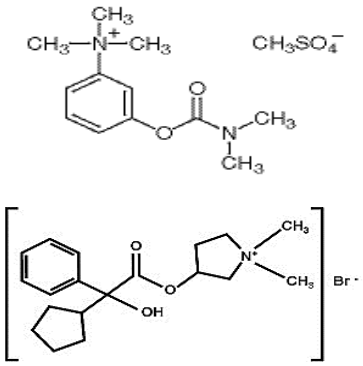
The combination of NEO (2.5 mg) and GLYCO (0.5 mg) is marketed as a 5ml injection (Myopyrrolate) in the ratio of 5:1 respectively. This combination is indicated for the treatment of Myasthenia Gravis. GLYCO is incorporated in the combination with NEO to prevent the serious cardiac side effects observed in the treatment using NEO as a single drug. Literature survey reveals that, analytical techniques such as UV-Spectrophotometery and HPLC are published for determining NEO and GLYCO as individual drugs and for their combinations with other drugs. No methods have been reported for the simultaneous determination of NEO and GLYCO. As far as NEO is concerned, methods are available for its estimation in bulk and formulation by UV-Spectrophotometery [4] and HPLC [5]. Similarly for GLYCO, HPLC [6-9] and bio analytical methods [10,11] have been reported.

MATERIALS & METHODS

Materials

Pure drug samples of NEO and GLYCO were procured as a gift sample from Tablets India Ltd (Chennai) and Vav Life Sciences Pvt Ltd (Mumbai) respectively.

Figure 1a & b. Structure of NEO and GLYCO



These samples were used without further purification. Distilled water was used throughout the study. The injection Myopyrrolate used in the study is manufactured by Neon Laboratories, Mumbai.

Instrumentation

UV-Visible Double Beam Spectrophotometer (Shimadzu 1800) and Precision Balance (Shimadzu ATX224) were used for the study.

Method

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of pure drug containing 1000µg/ml NEO and GLYCO were separately prepared in distilled water. Standard stock solutions were further diluted with distilled water to get working standard solutions in the concentration range of 125-425µg/ml and 25-85µg/ml for Neostigmine Methyl Sulphate (NEO) and Glycopyrrolate (GLYCO) respectively. These were scanned in the wavelength region of 200-400nm and the overlain spectrum was obtained depicted in Figure 2a.

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Figure 2a. Overlain Spectra of NEO and GLYCO at 241nm

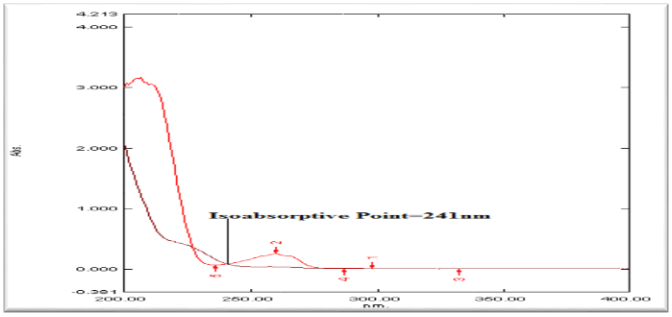
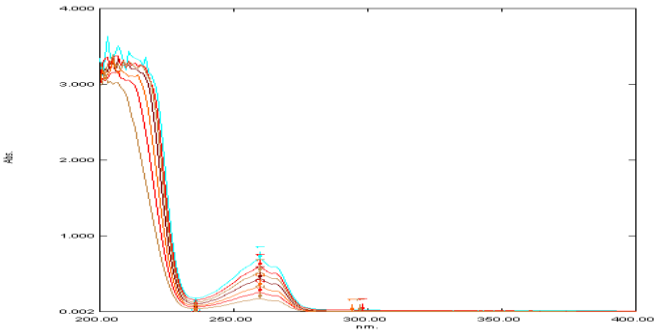
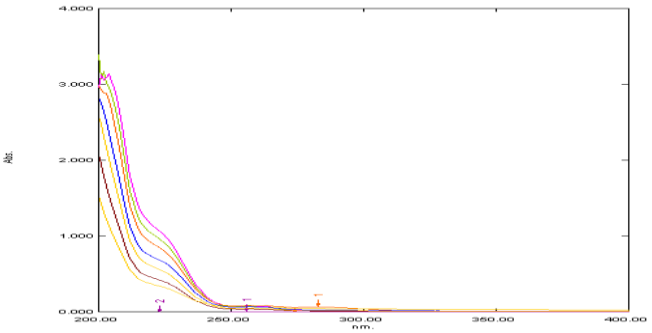


Figure 2b &c. Linearity of NEO and GLYCO





Isoabsorptive Point Method

The criterion to be satisfied for the absorbance ratio method is that the ratio of absorbances at any two wavelengths is a constant value independent of concentration or path length [12]. In this method, the absorbances of both the substances are recorded at two wavelengths of which, one is isoabsorptive point and other is wavelength maxima of any of the drugs. The isoabsorptive point and the wavelength maxima used were 241 nm and 260 nm respectively.

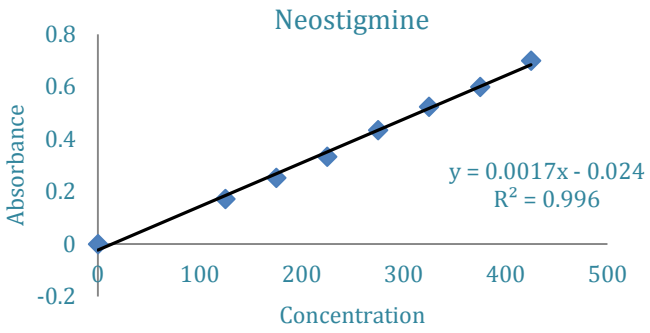
$C_x = (Q_m - Q_y / Q_x - Q_y) * (A / ax_1)$

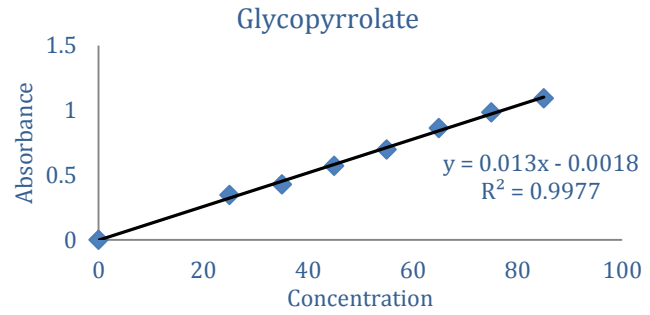
$C_y = (A / ax_1) - C_x$

Assay

3.5 ml volume was accurately withdrawn from the injection containing 2.5mg/5ml NEO and 0.5mg/5ml GLYCO and diluted upto 10 ml with distilled water to get the final concentration 175µg/ml for NEO and 35µg/ml for GLYCO. This solution was further filtered through whatman filter paper and then used. The absorbances of sample solution prepared above were recorded at 241 nm and 260 nm. These values were used in further calculations to determine concentration of both the drugs.

Figure 3a &b. Calibration Curve for NEO and GLYCO





Method Validation

The described method was validated for the assay of both the major components of the bulk drug following the ICH guidelines [13].

Linearity

Linearity was studied by preparing standard solutions of different concentrations. Calibration curves were prepared using standard solutions of 125-425 µg/ml of NEO as seen in Figure 2b and 25-85 µg/ml of GLYCO in Figure 2c.

Specificity

Specificity was performed to check the interference due to other components. A blank sample excluding the active ingredients was analysed.

Figure 3c Specificity

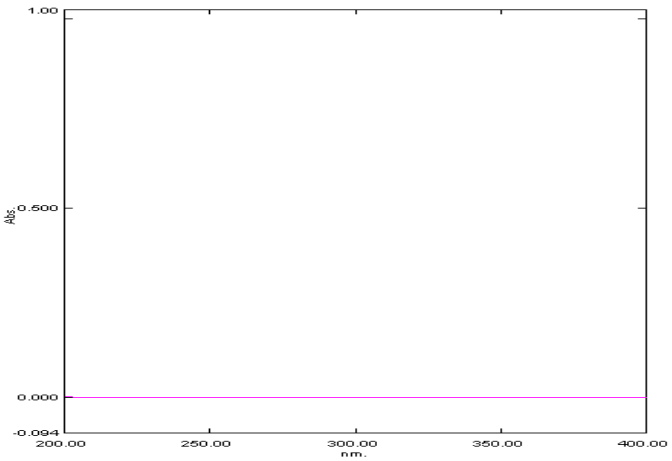


Table 1 Regression Analysis and Validation data

Parameters		NEO	GLYCO
Concentration Range		125-425µg/ml	25-85µg/ml
Molar Absorptivity (L/mol/cm)		4.97	26.6
Sandell's Sensitivity (µg/cm²/0.001absorbance unit)		14.7	1.46
Slope		0.0017	0.013
Intercept		0.0024	0.0018
Correlation Coefficient		0.996	0.9977
Accuracy (recovery n=3)	80%	100.40±0.157	99.00±0.440
	100%	99.49±0.112	98.71±0.130
	120%	100.20±0.0524	98.904±0.353
Repeatability(%RSD, n=6)		0.205	0.0905
Precision (%RSD)	Intraday(n=3)	0.128-0.340	0.057-0.626
	Interday(n=3)	0.128-0.785	0.152-0.474
LOD(µg/ml)		1.10	0.87
LOQ(µg/ml)		3.35	2.66

Precision

Precision of the developed method was performed taking into consideration the repeatability and the intra-day – inter-day variations.

Recovery Studies (Accuracy)

Recovery study of the proposed method was carried out at three different levels i.e. 80%, 100% and 120%, by adding known amount of standard solution at these three different levels. Accuracy was determined by calculating the recovery of NEO and GLYCO by standard addition method. The solutions were prepared in triplicate and absorbance were recorded at the selected wavelengths for NEO and GLYCO.

Limit of detection and quantification

The detection and quantification limits were determined from the standard deviation of response and slope of the least square line parameters.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

where σ = Standard deviation of response; S = slope of the Calibration Curve

Table 2 Precision

Drug	Conc. of Drug (µg/ml)	%RSD(n=3)	
		Intra-day	Inter-day
Neostigmine Methyl Sulphate	225	0.162	0.330
	275	0.128	0.128
	325	0.00	0.244
Glycopyrrolate	45	0.626	0.390
	55	0.00	0.474
	65	0.086	0.229

RESULTS AND DISCUSSION

Linearity

The absorbance were found to be having a direct relationship with concentration. For NEO, the R^2 value was found to be 0.996 as in Figure 3a, while for GLYCO it was found to be 0.9977 in Figure 3b.The regression line analysis was carried out. Y-intercept, slope and regression line equations were calculated and is presented in Table 1.

Specificity

Analysis of blank sample showed no interference due to matrix components depicted in Figure 3c.

Precision

Repeatability

Solutions containing 275µg/ml of NEO and 55µg/ml GLYCO were prepared and analyzed six times. The %RSD was calculated as shown in Table 1.

Intra-day Precision

Solutions containing 225,275,325µg/ml of NEO and 45, 55, 65µg/ml of GLYCO were analyzed three times on the same day. The %RSD was calculated as shown in Table 2.

Inter-day Precision

Solutions containing 225,275,325µg/ml of NEO and 45, 55, 65µg/ml of GLYCO were analyzed on three different days. The %RSD was calculated as shown in Table 2.

Table 3 Analysis of NEO and GLYCO in marketed formulation

Formulation (Injection)	Labelled amount (mg/5ml)		Amount found (mg/5ml)		Label claim ± SD	
	NEO	GLYCO	NEO	GLYCO	NEO	GLYCO
Myopyrolate	2.5	0.5	2.48	0.501	99.29 ± 0.698	100.34 ± 0.369

Assay

The results of assay were used to determine the amount of both the drugs present in marketed formulation. The results obtained proved the efficacy of the developed method to determine both the drugs simultaneously from marketed formulation shown in Table 3.

Table 4 Recovery study of NEO and GLYCO

DRUGS	Recovery level	Amount Present (µg/ml)	Amount spiked (µg/ml)	Total amount of drug (µg/ml)	Amount Recovered (µg/ml)	%Recovery (n=3)	%RSD
NEO	80%	175	140	315	139.46	100.40	0.157
	100%		175	350	174.12	99.49	0.112
	120%		210	385	210.40	100.20	0.054
GLYCO	80%	35	28	63	27.81	99.00	0.440
	100%		35	70	34.41	98.71	0.130
	120%		42	77	41.73	98.90	0.353

Recovery Studies

The percentage recovery for NEO and GLYCO were found to be satisfactory. For NEO it was found to be between 99.49-100.40% while for GLYCO it was found between 98.7-99% shown in Table 4.

Limit of Detection and Limit of Quantitation

The limit of detection and quantification were found to be 1.10µg/ml and 3.35µg/ml for NEO respectively. For GLYCO were found as 0.87µg/ml and 2.66µg/ml.

CONCLUSION

The developed UV method was found to be useful for simultaneous estimation of neostigmine methyl sulphate and glycopyrrolate. The method was also applicable to available marketed formulation. Simplicity and cost efficiency of the method could be understood from the used reagents which is distilled water only. Validation of the method gives evidence of its repeatability.

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