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Pharmaceutical Sciences **Research Article……!!!**

‘‘SIMULTANEOUS DETERMINATION AND VALIDATION OF CODEINE PHOSPHATE AND PROMETHAZINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS”

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KEYWORDS:

Codeine phosphate, Promethazine hydrochloride, Zero order Method, First order derivative, validation.

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ABSTRACT

In the present work two simple and sensitive spectrophotometric methods were developed for the simultaneous estimation of Codeine phosphate and Promethazine hydrochloride in bulk drugs and pharmaceutical dosage forms by using isopropyl alcohol as a solvent. Method A: Zero order method is based on the measurement of absorbance at two selected wavelengths 285 nm and 305 nm for the estimation of Codeine phosphate and Promethazine hydrochloride. Beer’s law obeyed in the concentration range of 40- 200 µg/ml and 15-75 µg/ml with (r^2 = 0.999, %RSD = 0.3931-0.8136 and r^2 = 0.998, %RSD = 0.4270-1.5375) for Codeine phosphate and Promethazine hydrochloride respectively. LOD of both drugs were 0.7379 µg/ml and 0.8605 µg/ml and LOQ were found to be 2.2360 µg/ml and 2.6076 µg/ml for Codeine phosphate and Promethazine hydrochloride respectively. Method B: First order derivative spectroscopic method is based on the measurement of absorbance at two selected wavelengths 293 nm and 322 nm for the estimation of Codeine phosphate and Promethazine hydrochloride respectively. Linearity range was found 40-200µg/ml and 15-75 µg/ml with (r^2 = 0.998, %RSD = 0.3765-0.2605 and r^2 = 0.999, %RSD = 0.6542-1.0510) Codeine phosphate and Promethazine hydrochloride respectively. LOD of both drugs were 0.5135µg/ml and 0.3689µg/ml and LOQ were found to be 1.5562µg/ml and 1.1180µg/ml for Codeine phosphate and Promethazine hydrochloride respectively. In both the methods the % RSD for intra-day and inter-day precision was within 2%.

INTRODUCTION:

Codeine[1-6] **(COD)** is an opioid analgesic. Chemically COD is *(5α, 6α)-7,8-didehydro-4,5-epoxy- 3-methoxy-17-methylmorphinan-6-ol*. It is a narcotic pain-reliever and cough suppressant. COD is used to treat moderate to severe pain. COD is a selective agonist for the mu opioid receptor, but with a much weaker affinity to this receptor than morphine, a more potent opioid drug. COD binds to mu-opioid receptors, which are involved in the transmission of pain throughout the body and central nervous system. The analgesic properties of COD are thought to arise from its conversion to morphine. Binding of COD or morphine to the mu opioid receptor results in hyperpolarization of the neuron, causing an analgesic effect and increased pain tolerance due to reduced neuronal excitability.

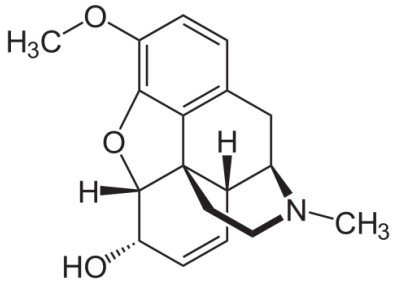

Fig 1: Chemical structure of Codeine

Promethazine[7-11] **(PRO)** is a first-generation antihistamine and anti psychotic drug. Chemically PRO is *N,N-Dimethyl-1-(10H-phenothiazin-10-yl) propan-2-amine*. It is used for the treatment of allergic conditions, nausea and vomiting, and motion sickness. PRO is an antagonist of histamine H_1, post-synaptic mesolimbic dopamine, alpha adrenergic, muscarinic, and NMDA receptors. The antihistamine action is used to treat allergic reactions. Antagonism of muscarinic and NMDA receptors contribute to its use as a sleep aid, as well as for anxiety and tension. Antagonism of histamine H_1, muscarinic, and dopamine receptors in the medullary vomiting center make PRO useful in the treatment of nausea and vomiting.

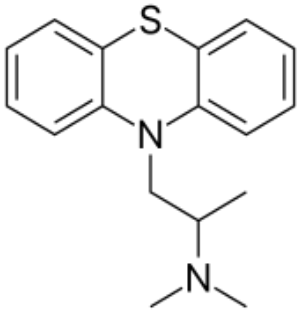

Fig 2: Chemical structure of Promethazine

The combination of COD and PRO is prescribed to treat cold or allergy symptoms such as runny nose, sneezing, and cough.

On literature survey, since codeine is an official drug in IP, several analytical techniques like RP-HPLC, HPTLC and spectrophotometric methods for the estimation of COD individually and in combination with other drugs have been reported. PRO in combination with other drugs has been estimated by HPLC and spectrophotometric methods.

Since, no method has been reported for the simultaneous estimation of COD and PRO in combined dosage form and no method is available in the Pharmacopoeia. In the view of the need for suitable method for routine analysis in combined formulations, a new simple, precise and accurate analytical methods for simultaneous estimation of tilted drugs and extended it for their determination in formulations.

MATERIALS AND METHODS:

Instrument: Spectrophotometric measurements were performed using double-beam UV-Vis spectrophotometer. (1800, Shimadzu) UV-visible Spectrophotometer with spectral band width of 1±0.2 nm, wave length accuracy ±0.3 nm and a pair of quartz cuvettes having l cm path length was used. Volumetric flasks used for preparation of standard solutions and sample solutions were calibrated before use.

Chemicals:

Standard COD was obtained as gift sample from Medrich pharma pvt ltd, Bangalore. Standard PRO was procured from yarrow chem Products, Mumbai.

Methods:

Preparation of standard solutions :

Standard solutions of COD and PRO Prepared by 100 mg powder weighed accurately and dissolved in 100 ml of isopropyl alcohol respectively. Further standard solutions were diluted using isopropyl alcohol to obtain a concentration in the linearity range.

Preparation of sample solution

The syrup consisting of COD and PRO was not available in the local market (AKORN- containing COD-10 mg and PRO-6.2 mg), hence a physical mixture consisting of COD, PRO and all Syrup excipients was prepared by geometrically adding sufficient amount COD, PRO. Oral solution equal to 100 mg of COD was accurately weighed and transferred to 100 ml volumetric flask and dissolved in

isopropyl alcohol. Then further dilutions were done with isopropyl alcohol to get both the concentrations of the drugs within their range respectively.

Procedure for construction of calibration curve:

Required amount of standard solutions of COD and PRO were transferred into the 10 ml volumetric flasks to get the five solutions in the concentration range of 40-200 µg/ml of COD and 15-75 µg/ml PRO separately. All these solutions were scanned in the range of 200 – 400 nm against isopropyl alcohol as blank and spectra were recorded. Zero order spectroscopy, the peak absorbance was measured at 285 nm and 305 nm COD and PRO respectively. Further, for the first derivative spectroscopic method, spectra were converted into first derivative spectra using 8 nm as Δλ with scaling factor 100. First order derivative method, the peak absorbance was measured at 293 nm for COD similarly, the peak absorbance was measured at 322 nm for quantification of PRO. Further, the calibration curves were created for both analytes by plotting a graph between peak absorbance against corresponding concentrations. In addition, regression equations were figured.

Method of estimation

Method A (Zero order method)

From the above standard solution both drugs were prepared and scanned in the wavelength range of 400-200 nm using UV – Spectrophotometer. 285 nm and 305 nm for COD and PRO were selected as the working analytical wavelength.

Method B (First order derivative)

For the estimation of COD and PRO by first order derivative spectroscopy, zero crossing point for both drugs were obtained and the wavelengths were selected in manner such that at the zero crossing of one drug, the other drug should show substantial absorbance. From the first order derivative spectra of standard COD and PRO, zero crossing point of COD was found at 285 nm and zero crossing point of PRO was found at 305 nm and wavelength selected for their estimation was 293 nm for COD and 322 nm for PRO.

VALIDATION PARAMETER:

Validity of the proposed methods were confirmed by performing linearity, limit of detection, limit of quantification, accuracy, precision and stability studies as per the ICH guidelines.

TABLE 1: SUMMARY OF VALIDATION PARAMETERS BY DEVELOPED METHODS.

PARAMETERS	METHOD A		METHOD B	
	COD	PRO	COD	PRO
Wavelenght (nm)	285	305	293	322
Linearity Range (µg/mL)	40-200	15-75	40-200	15-75
Slope (b)	0.002	0.005	-0.016	-0.008
Intercept (a)	0.0027	0.006	0.006	0.048
Correlation Coefficient (r^2)	0.999	0.998	0.999	0.998
LOD (µg/ml)	0.7379	0.8605	0.5135	0.3689
LOQ (µg/ml)	2.2360	2.6076	1.5562	1.1180
Accuracy (mean%)	99.35	99.87	99.98	99.48
Precision (%RSD)				
Intra day	0.5844	0.6287	0.1089	0.8325
Inter day	0.3850	0.6553	0.2461	0.3737

Linearity

The calibration plot provides important information about the linearity of the proposed method. In the present study linearity range was studied in the concentration range of 40-200 µg/ml for COD (Table 2 and Table 4 for Zero order method and First order derivative method respectively). PRO exhibited excellent linearity in the range of 15 to 75 µg/ml (Table 3 and Table 5 for Zero order method and First order derivative method respectively). Summary of validation parameters are listed in Table 1.

TABLE 2: RESULTS OF CALIBRATION CURVES FOR COD AT 285 nm BY ZERO ORDER METHOD.

Concentration (µg/ml)	Absorbance	% CV
40	0.1078	0.4110
80	0.1864	0.8136
120	0.2672	0.6719
160	0.3488	0.3738
200	0.4256	0.3931

Table 3: RESULTS OF CALIBRATION CURVES FOR PRO AT 305 nm BY ZERO ORDER METHOD.

Concentration (µg/ml)	Absorbance	% CV
15	0.0846	1.5375
30	0.1572	0.6968
45	0.2296	0.7287
60	0.3024	0.6857
75	0.4254	0.4270

TABLE 4: RESULTS OF CALIBRATION CURVES FOR COD AT 293 nm BY FIRST ORDER METHOD.

Concentration (µg/ml)	Absorbance	% CV
40	-0.699	0.6942
80	-1.243	0.3765
120	-1.879	0.4687
160	-2.568	0.5245
200	-3.232	1.2605

TABLE 5: RESULTS OF CALIBRATION CURVES FOR PRO AT 322 nm BY FIRST ORDER METHOD.

Concentration (µg/ml)	Absorbance	% CV
15	-0.172	-1.0510
30	-0.314	-0.8630
45	-0.441	-0.6542
60	-0.572	-0.6824
75	-0.693	-0.8075

Limit of detection and quantification limits

The detection and quantification limits were estimated using the linearity curve parameters. The LOD was calculated as 3.3 times the standard deviation of the intercept to the slope of the curve. The LOQ was 10 times the standard deviation of the intercept to the slope of the curve. The low LOD and LOQ values are tabulated in Table 1, indicating the good sensitivity of the proposed methods.

Precision

The precision of the established procedures was also assessed in terms of intra and inter-day by analyzing five concentrations of both analytes in the calibration curve range. For intra-day, solutions were analyzed six times in day and these solutions were investigated for three succeeding days for inter day precision. The result showed low percent RSD, which confirmed that the proposed methods were precise. Statistical validation of data for Intraday and Interday precision methods as shown in Table 6 and Tabel 7.

TABLE 7 : STATISTICAL VALIDATION DATA FOR INTRA-DAY PRECISION.

Components	Method A		Method B	
	COD	PROM	COD	PROM
Mean	100.41	100.47	99.97	99.14
Standard deviation	0.5692	0.6317	0.1306	0.3751
Relative standard deviation	0.5844	0.6287	0.1089	0.8325

n*=6

TABLE 8: STATISTICAL VALIDATION DATA FOR INTER-DAY PRECISION.

Components	Method A		Method B	
	COD	PROM	COD	PROM
Mean	99.97	98.54	99.98	99.95
Standard deviation	0.3841	0.6457	0.2959	0.1691
Relative standard deviation	0.3850	0.6553	0.2461	0.3737

n*=3

Accuracy

Accuracy of the developed procedures were examined by assaying different concentration of both the analytes in the calibration concentration range. The accuracy of the methods was expressed in terms of the percent recovery and percent relative error. The mean percentage recovery was 99.35 % to 99.98 % for COD and 99.98 % to 99.48 % for PRO.

TABLE 8: STATISTICAL VALIDATION DATA FOR ACCURACY DETERMINATION.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A		Method B	
				Total amount recovered (µg)	% Recovey	Total amount recovered (µg)	% Recovery
80%	COD	80	64	143.85	99.98	143.98	99.98
	PROM	30	24	53.98	99.96	83.85	99.72
100%	COD	80	80	159.52	99.98	159.95	99.96
	PROM	30	30	59.65	99.41	59.65	99.41
120%	COD	80	96	175.62	99.78	175.99	99.99
	PROM	30	36	65.23	100.35	65.89	99.83

RESULTS AND DISSCUSION:

Zero order method, the absorbance was measured at 285 nm and 305 nm for the estimation of COD (Fig. 3) and PRO (Fig. 5) respectively.

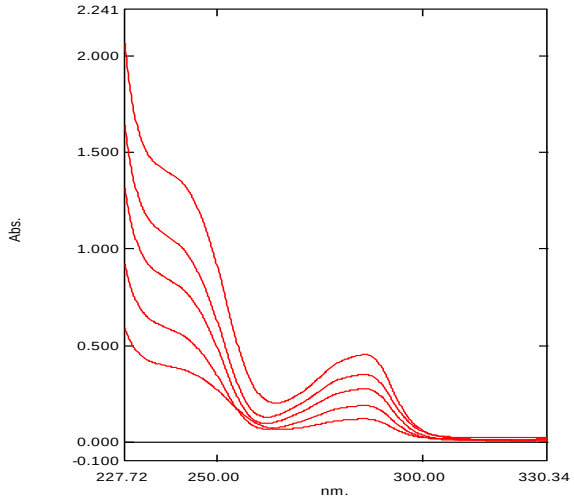

Fig .3 Zero order overlay of COD at 285 nm

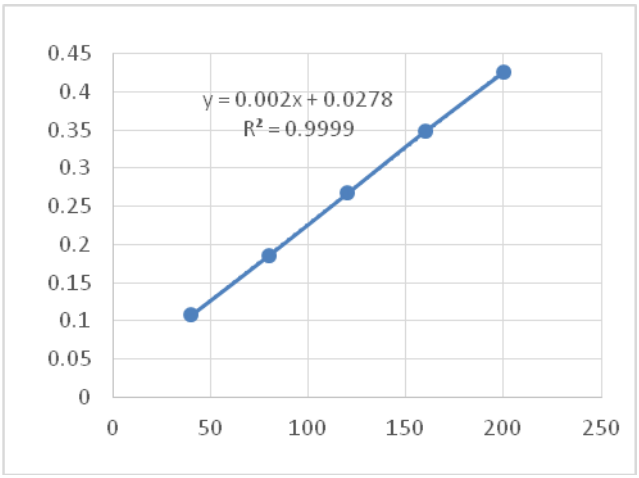

Fig .4 Calibration curve of COD at 258 nm

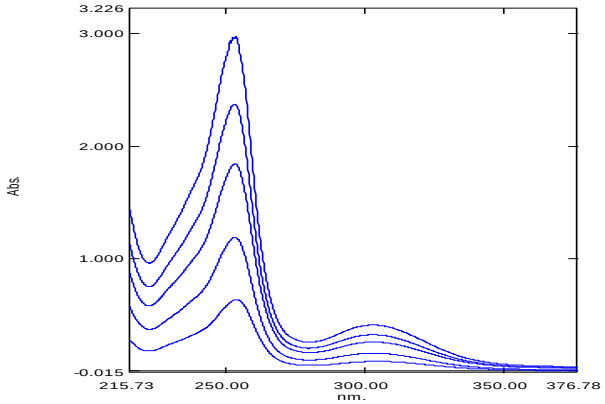

Fig .5 Zero order overlay of PRO at 305 nm

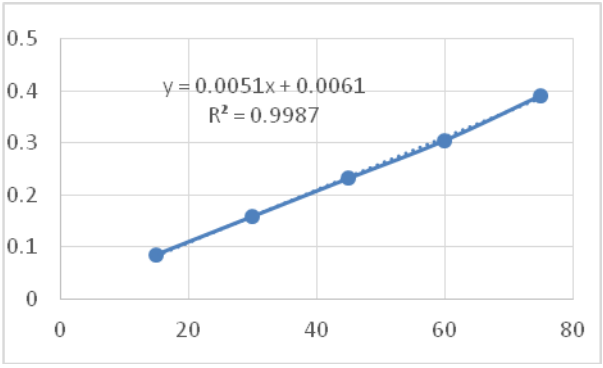

Fig .6 Calibration curve of PRO at 305 nm

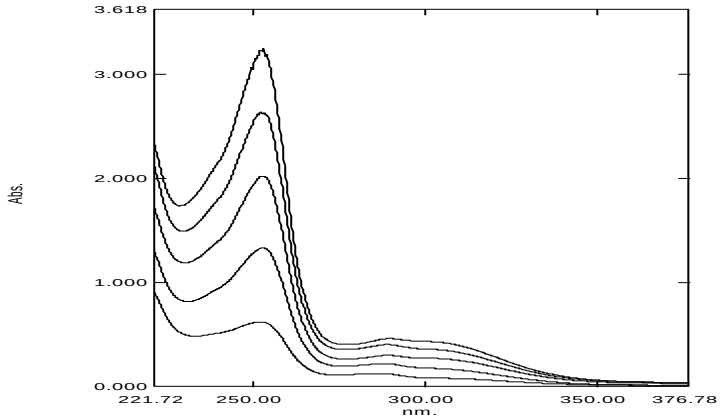

Fig. 7 Zero order UV absorption spectra of COD and PRO in mixture at 285 nm and 305 nm

In first order derivative, the absorbance was measured at 293 nm and 322 nm for the estimation of COD (Fig. 8) and PRO (Fig.10).

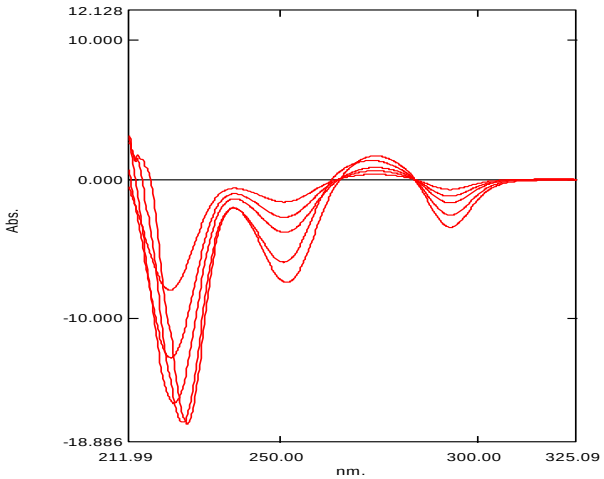

Fig.8 First Order overlay of COD at 293 nm

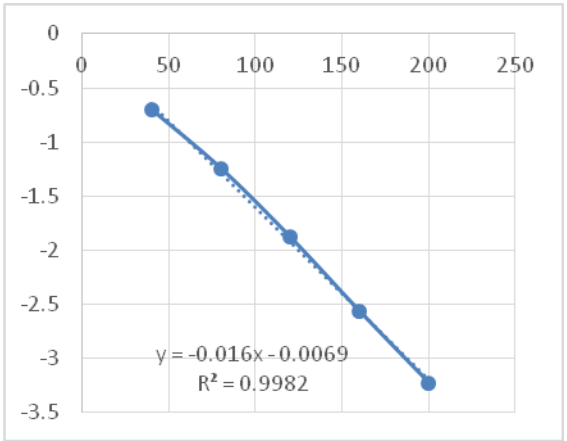

Fig.9 Calibration curve of COD at 293 nm

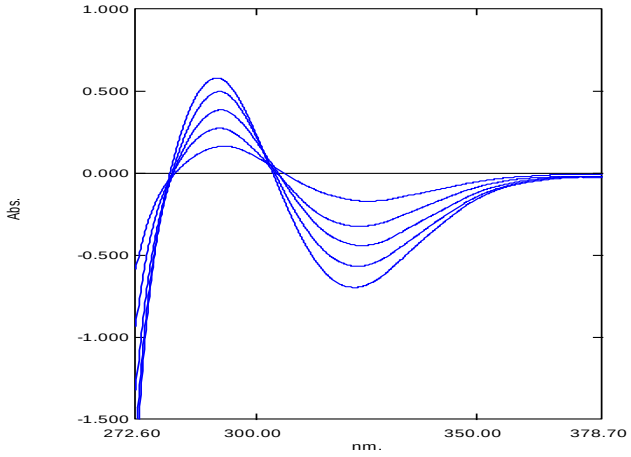

Fig. 10 First Order overlay of PRO at 322 nm

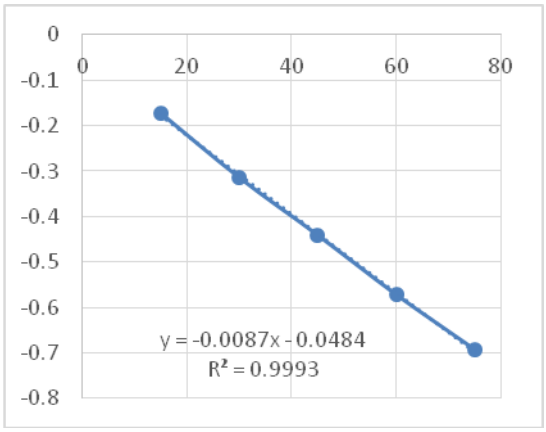

Fig.11 Calibration curve of PRO at 322 nm

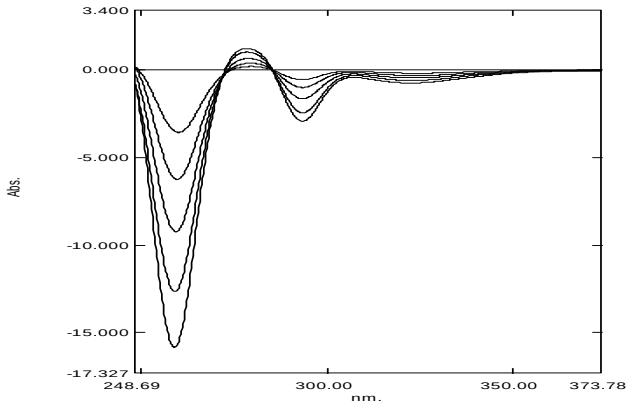

Fig.12 First Order derivative spectrum of mixture at 293 nm and 305 nm and Zero crossing at 298 nm and 322 nm

In our study, a series of COD and PRO solutions were converted into first derivative spectra separately. Different wavelengths 2, 4, 6, 8 nm as Δλ and different scaling factor from 1 to 100 were tried, however, 8 nm as Δλ and scaling factor of 100 showed good resolution of analytes. Hence all spectra were converted using 8 nm as Δλ and scaling factor of 100. First order derivative method, the peak absorbance was measured at 293 nm for COD similarly, the peak absorbance was measured at 322 nm for quantification of PRO.

The % RSD for intraday and inter-day precision was found to be less than 2%. The methods have been validated in assay of active pharmaceutical ingredients. The accuracy of the methods were validated by recovery studies and was found to be significant and within specification limits, with % recovery 99-101%. The assay results were found to be within the acceptable limits.

CONCULSION:

The developed Zero order method and first order derivative methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Codeine phosphate and Promethazine hydrochloride. Both methods were validated as per ICH guidelines.

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ABBREVIATIONS

UV: Ultra violet, %RSD: Percent Relative Standard Deviation; COD: Codeine phosphate, PRO: Promethazine hydrochloride, %CV: Percent Co efficient of Variation.

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