IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

SIMULTANEOUS ESTIMATION OF DROTAVERINE HYDROCHLORIDE AND PARACETAMOL USING SECOND-ORDER DERIVATIVE UV SPECTROSCOPY: METHOD DEVELOPMENT AND VALIDATION

[1]Manjusha Patel, [2]Dr Shiv Shankar Shukla, [3]Dr Ravindra Kumar Pandey

[1,2,3]Columbia Institute of pharmacy Raipur Chhattisgarh, 492001

Abstract

A simple, rapid, accurate, and cost-effective second-order derivative UV spectroscopic method was developed and validated for the simultaneous estimation of Drotaverine Hydrochloride and Paracetamol in combined pharmaceutical dosage forms. The method was based on second-order derivative spectroscopy employing zero-crossing points to resolve overlapping spectra of both drugs without prior separation. Methanol and distilled water (or suitable solvent system) were used as the solvent for preparing standard and sample solutions. The wavelengths selected for estimation were based on the zero-crossing points of each drug, enabling accurate quantification of Drotaverine Hydrochloride and Paracetamol in the presence of one another. The developed method was validated according to analytical validation guidelines for parameters including linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness. Both drugs exhibited good linearity within their respective concentration ranges with satisfactory correlation coefficients. Recovery studies demonstrated acceptable accuracy, while precision studies indicated low percentage relative standard deviation (%RSD), confirming the reproducibility of the method. The proposed method was successfully applied for routine quantitative analysis of Drotaverine Hydrochloride and Paracetamol in tablet dosage forms with satisfactory assay results. Hence, the developed second-order derivative UV spectroscopic method can be effectively employed for quality control analysis in pharmaceutical industries due to its simplicity, sensitivity, and economical nature.

Keywords: Drotaverine HCl; Paracetamol; Derivative UV Spectroscopy; Simultaneous Estimation; Method Validation.

INTRODUCTION

Analytical method development and validation play a crucial role in pharmaceutical quality assurance, ensuring the identity, purity, potency, and safety of drug products. [1]The increasing demand for fixed-dose combination (FDC) formulations in pharmaceutical therapy has intensified the need for robust, accurate, and economical analytical techniques capable of simultaneous drug estimation. Among various analytical approaches, ultraviolet (UV) spectrophotometry has emerged as a widely accepted technique due to its simplicity, rapidity, cost-effectiveness, and applicability in routine quality control laboratories.[2] However, simultaneous estimation of multicomponent formulations presents analytical challenges because of overlapping absorption spectra. To overcome these limitations, derivative spectrophotometric techniques, particularly second-order derivative spectroscopy, have gained considerable attention for enhancing spectral resolution and improving analytical specificity. [3]

Drotaverine Hydrochloride and Paracetamol are commonly formulated together in pharmaceutical dosage forms for the management of pain associated with smooth muscle spasms. The combination exhibits synergistic therapeutic benefits by simultaneously addressing muscular spasm and pain perception.[4] Consequently, the development of a reliable and validated analytical method for their simultaneous quantification is essential to ensure dosage accuracy, therapeutic efficacy, and patient safety. Drotaverine Hydrochloride is an isoquinoline derivative possessing potent antispasmodic activity. Pharmacologically, it acts by inhibiting phosphodiesterase IV enzyme activity, resulting in elevated intracellular cyclic adenosine monophosphate (cAMP) concentrations and subsequent smooth muscle relaxation.[5] Unlike anticholinergic antispasmodic agents, Drotaverine Hydrochloride exerts its action without producing substantial autonomic adverse effects, thereby improving patient tolerability. It is extensively prescribed for gastrointestinal spasms, biliary colic, dysmenorrhea, renal colic, and uterine spasms. Chemically, Drotaverine Hydrochloride is designated as 1-(3,4-diethoxybenzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride and exhibits appreciable ultraviolet absorption characteristics, making it amenable to spectrophotometric determination. Paracetamol, also known as acetaminophen, is a widely used analgesic and antipyretic agent. It exerts its therapeutic effects primarily through inhibition of prostaglandin synthesis within the central nervous system, thereby reducing pain and fever. Owing to its favorable safety profile and broad therapeutic utility, Paracetamol is frequently incorporated into combination therapies intended for symptomatic relief of painful conditions.[6] Chemically, Paracetamol is N-(4-hydroxyphenyl) acetamide and exhibits distinct absorption in the UV region. However, the simultaneous presence of Drotaverine Hydrochloride in combination dosage forms introduces analytical complexity due to overlapping spectra, which limits the applicability of conventional zero-order spectrophotometric methods. Several analytical methodologies have been reported for the determination of Drotaverine Hydrochloride and Paracetamol individually and in combination with other pharmaceutical agents. These include high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), capillary electrophoresis, liquid chromatography–mass spectrometry (LC-MS), and spectrophotometric techniques.[7] Although chromatographic methods provide excellent sensitivity and specificity, they are often associated with high operational costs, extensive solvent consumption, sophisticated instrumentation, and prolonged analysis times. Such limitations may hinder their

routine implementation, especially in resource-limited analytical settings and quality control laboratories. In contrast, UV spectrophotometry represents a practical alternative for routine pharmaceutical analysis owing to its affordability, operational simplicity, and minimal sample preparation requirements. Nevertheless, simultaneous determination of drugs with overlapping spectra remains challenging in conventional UV analysis. Derivative spectroscopy has emerged as a valuable modification of traditional spectrophotometry by transforming normal absorption spectra into derivative spectra, thereby improving spectral discrimination and eliminating background interference. This mathematical transformation enables selective quantification of analytes even in complex mixtures without prior separation.[8] Derivative spectrophotometry involves converting absorbance values into first, second, third, or higher-order derivatives with respect to wavelength. Among these approaches, second-order derivative spectroscopy is particularly advantageous because it enhances spectral resolution and facilitates the identification of zero-crossing points. At these wavelengths, one component exhibits zero absorbance while the other maintains measurable absorbance, thereby enabling selective quantification in multicomponent systems. This technique significantly minimizes spectral overlap and matrix interference, making it highly suitable for simultaneous estimation of pharmaceutical combinations.

The second-order derivative UV spectroscopic method offers several analytical advantages, including improved selectivity, reduced interference from excipients, enhanced sensitivity, and rapid data acquisition. Additionally, it eliminates the need for expensive solvents, sophisticated instrumentation, or complex sample extraction procedures. Such benefits make derivative UV spectroscopy an attractive choice for routine quality assessment of pharmaceutical dosage forms containing Drotaverine Hydrochloride and Paracetamol.[9] Despite the availability of chromatographic methods, there remains a persistent demand for economical and environmentally sustainable analytical approaches aligned with principles of green analytical chemistry.

Method validation is a critical requirement in analytical science to establish the reliability and reproducibility of a developed analytical procedure.[10] International regulatory agencies emphasize the importance of validated analytical methods for ensuring product quality and regulatory compliance. According to the guidelines established by the International Council for Harmonisation, analytical method validation involves systematic evaluation of parameters such as accuracy, precision, specificity, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and range. These parameters collectively demonstrate whether the developed analytical procedure is suitable for its intended purpose.[11] Accuracy refers to the closeness of agreement between experimentally obtained values and true values, while precision evaluates reproducibility under specified conditions. Specificity ensures that the method selectively quantifies analytes without interference from excipients or degradation products. Linearity establishes proportionality between analyte concentration and analytical response across a defined range. Sensitivity-related parameters such as LOD and LOQ determine the minimum detectable and quantifiable concentrations, respectively. Robustness assesses the capability of the method to remain unaffected by minor variations in experimental conditions. Therefore, method validation serves as an essential component in guaranteeing the scientific integrity and regulatory acceptability of analytical findings.[12] In the context of combined pharmaceutical formulations containing Drotaverine Hydrochloride and Paracetamol, the development and validation of a second-order derivative UV

spectroscopic method hold significant analytical importance. A validated method can facilitate rapid routine quality control analysis, reduce operational expenditures, and support efficient pharmaceutical manufacturing processes. Furthermore, the ability to simultaneously estimate both drugs without prior separation contributes to analytical efficiency and sustainability.

Therefore, the present study focuses on the estimation and validation of an analytical method for simultaneous determination of Drotaverine Hydrochloride and Paracetamol using second-order derivative UV spectroscopy. The proposed method aims to provide a precise, accurate, economical, and validated approach suitable for routine pharmaceutical quality control applications. By adhering to internationally accepted validation principles, this analytical strategy seeks to establish a reliable platform for the quantitative assessment of combined dosage formulations containing these therapeutically important drugs.

DRUG PROFILE OF DROTAVERINE HYDROCHLORIDE

Drotaverine hydrochloride is a highly potent spasmolytic drug. It shows excellent properties of smooth muscle relaxant. Its antispasmodic activity is due to inhibition of phosphodiesterase enzyme IV. It causes smooth muscle relaxation by increasing intracellular levels of cyclic adenosine mono-phosphate (cAMP) secondary to inhibition of phosphodiesterase**.**

Chemical Name **:** Drotaverine Hydrochloride

Molecular formula : $C_{24}H_{32}ClNO_4$

Molecular weight **:** 397.507 g/mol

IUPAC Name **:** (1Z)- 1-[(3,4-diethoxxyphenyl) methylidene]-6,7-diethoxy-3,4-dihydro-2H-isoquinoline; Hydrochloride/

Structure

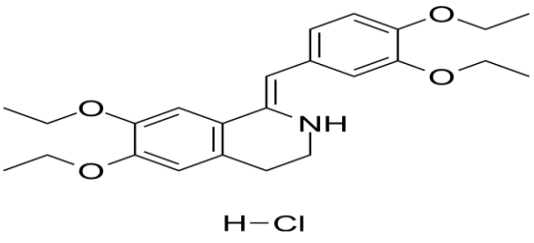

Figure 1.1 Chemical structure of Drotaverine Hydrochloride

Drotaverine hydrochloride is a pale yellow crystalline powder dissolves slightly in chloroform, gently in water, and is 96% soluble in ethanol. Drotaverine hydrochloride calms smooth muscle by increasing intercellular cyclic adenosine monophosphate (cAMP) levels, which suppresses platelet aggregation in a dose-dependent way.

DRUG PROFILE OF PARACETAMOL

Paracetamol (PAR), is considered to be the most frequently used over-the counter medicine worldwide. It is described for the treatment of many symptoms such as headache, cold, fever, muscle aches, and toothache.(Smart chemometrics-assisted spectrophotometric methods for efcient resolution)

Chemical Name: Acetaaminophen **Molecular formula** : C8H9NO2 **Molecular weigh**t: 151.163 g/mol

IUPAC Name : N-(4-hydroxyphenyl) acetamide.

Structure

H N

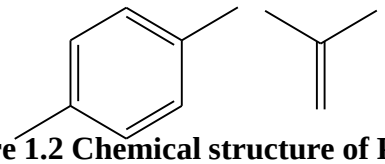

Figure 1.2 Chemical structure of Paracetamol

Material and Methods

Materials: Drotaverine hydrochloride was purchased from Swapnroop Drugs and Pharmaceuticals, Aurangabad, Maharashtra INDIA. The other chemicals and reagents were used of analytical grades.

Table No 4.1 List of Instrument used:

S.No	Instrument used	Company	Model
1.	UV- Visible Spectrophotometer	Shimadzu	UV 1800
2.	UV chamber	Rolex	-
3.	Digital analytical weighing balance	Keroy	120HS

Table No 4.2 List of chemical used:

S.No	Chemical Used	Company	Purity
1.	Methanol	Loba Chemie	99.9%

Materials and Methods

Preparation of Standard Stock Solution

Standard stock solutions of Drotaverine Hydrochloride and Paracetamol were prepared separately by accurately weighing 100 mg of each drug and transferring into individual 100 mL calibrated volumetric

flasks. The drugs were dissolved in a sufficient quantity of methanol and the final volume was adjusted up to the mark using the same solvent to obtain stock solutions having a concentration of 1 mg/mL (1000 µg/mL).

Further dilution of the stock solution was performed using methanol to prepare working standard solutions of concentration 100 µg/mL. Aliquots from the working standard solutions were subsequently diluted to obtain concentrations of 2, 4, 6, 8, 10, and 12 µg/mL for analytical evaluation and calibration studies.

Selection of Maximum Wavelength (λmax)

The absorption maxima (λmax) of Drotaverine Hydrochloride and Paracetamol were determined by preparing individual standard solutions of 100 µg/mL in methanol. The prepared solutions were scanned in the wavelength range of 200–400 nm using a UV–Visible spectrophotometer against methanol as a blank.

The λmax of Drotaverine Hydrochloride and Paracetamol were found to be 230 nm and 248 nm, respectively. These wavelengths were selected for further spectroscopic investigation and method optimization. The obtained absorption spectra were utilized for the development of a second-order derivative UV spectroscopic method to achieve selective and simultaneous estimation of both drugs in combined dosage formulations.

Preparation of Calibration Curve

Calibration curves for Drotaverine Hydrochloride and Paracetamol were prepared by analyzing diluted standard solutions at concentrations of 2, 4, 6, 8, 10, and 12 µg/mL. Each concentration was scanned using a UV–Visible spectrophotometer under optimized analytical conditions.

The absorbance/derivative response of each concentration was recorded at the selected analytical wavelength of 253 nm using the second-order derivative mode. Calibration graphs were constructed by plotting concentration (µg/mL) against corresponding derivative absorbance values. The linearity of the developed method was evaluated by regression analysis to determine the correlation coefficient (R^2), slope, and intercept values.

Method Validation

The developed second-order derivative UV spectroscopic method for simultaneous estimation of Drotaverine Hydrochloride and Paracetamol was validated according to the guidelines established by the International Council for Harmonisation (ICH Q2(R1)) with respect to parameters including linearity, accuracy, precision, specificity, limit of detection (LOD), limit of quantitation (LOQ), and robustness.

Linearity

Linearity of the proposed analytical method was assessed by analyzing standard solutions in the concentration range of 2–12 µg/mL for both Drotaverine Hydrochloride and Paracetamol. The derivative absorbance values were measured and calibration curves were plotted between concentration and analytical response. The regression equation and correlation coefficient were calculated to evaluate the linear relationship between concentration and detector response.

Accuracy

The accuracy of the developed method was determined by recovery studies using the standard addition technique at three concentration levels, namely 80%, 100%, and 120%. Known amounts of standard drug solutions were added to the pre-analyzed sample solution, and the percentage recovery was calculated to assess the closeness of agreement between experimental and theoretical values.

Precision

Precision of the method was evaluated in terms of repeatability (intra-day precision) and intermediate precision (inter-day precision). For repeatability assessment, replicate measurements of selected concentrations were analyzed multiple times within the same day under identical analytical conditions. Inter-day precision was assessed by analyzing samples on different days. The results were expressed as percentage relative standard deviation (%RSD), where values below 2% were considered acceptable.

Specificity

Specificity of the developed method was evaluated to determine its ability to selectively estimate Drotaverine Hydrochloride and Paracetamol in the presence of pharmaceutical excipients and formulation matrices without interference. The second-order derivative technique facilitated selective quantification by minimizing spectral overlap and improving analytical resolution.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The sensitivity of the analytical method was determined by calculating the limit of detection (LOD) and limit of quantitation (LOQ) using the standard deviation of the response and slope of the calibration curve. The following equations were applied:

$$LOD = 3.3 \times (\sigma/S)$$

$$LOQ = 10 \times (\sigma/S)$$

Where:

σ = Standard deviation of the response

S = Slope of the calibration curve

Robustness

Robustness of the developed method was investigated by introducing deliberate minor variations in analytical parameters such as wavelength and solvent composition. The effect of these variations on analytical performance was assessed to determine the reliability and consistency of the method under normal operating conditions.

The validation results demonstrated that the proposed second-order derivative UV spectroscopic method was accurate, precise, selective, and reproducible for the simultaneous estimation of Drotaverine Hydrochloride and Paracetamol in pharmaceutical dosage forms. Therefore, the developed analytical method can be successfully employed for routine quality control analysis.

1 **Linearity and range:** The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. The calibration curve was plotted over a concentration range of 2-12µg/mL for flunarizine dihydrochloride. Accurately measured standard stock solution of flunarizine dihydrochloride (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) was transferred to a series of 10ml volumetric flask separately and diluted up to the mark with methanol and scanned within the range of 200-400nm.

2 **Precision:** It determined by measuring he fixed concentration of the drug solution for 3 times in a day (Intraday) and performed continuously for 7 days (Inter-day).Mean, standard deviation and %RSD need to be calculated

3. **Accuracy:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the proposed method recovery studies was carried out at three different levels 80%, 100% and 120% by addition of known amount of Flunarizine dihydrochloride to a known concentration of the commercial tablet.

4 **LOD & LOQ:** As per ICH guidelines limit of detection and limit of quantification of drotaverine hydrochloride and PCM derived by calculating the signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ).

5 **Robustness**: Robustness of the method was carried out in different parameters with different concentration and wavelength conditions. The absorbance was noted and %RSD was calculated. This study performed on various parameters like change in temperature, and change in wavelength.

Second Order Derivative Method

Derivatizing the absorbance spectrum twice gives this type of spectra. It is a plot of curvature of absorption spectrum against wavelength.

$dA/d\lambda 2=f'(\lambda)$

Second derivative has direct relation with concentration i.e. directly proportional. $d^2A/d\lambda^2$ must be large, large the ratio greater is the sensitivity. The method is useful in obtaining atomic and gas molecular spectra.

Development of 2nd order derivative spectra of Drotaverine hydrochloride: Working standard solution of pure drug was scanned within the range of 200-400nm after baseline correction.The spectral data was processed to obtain second derivative spectrum was shown in figure 5.1

Validation of Drotaverine Hydrochloride

Linearity and Range: The calibration curve was plotted over a concentration range of 2-12µg/mL for drotaverine hydrochloride and paracetamol. Accurately measured standard stock solution of drotaverine hydrochloride and paracetamol. (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) was transferred to a series of 10ml volumetric flask separately and diluted up to the mark with methanol. The absorbance of solution was then measured at 230 & 248 nm for drotaverine hydrochloride and paracetamol respectively. The result of calibration curve was shown in table no. and figure no.

Table No 5.1 Absorbance of Drotaverine Hydrochloride

S.No	Concentration (µg/mL)	Absorbance
1	2	0.137
2	4	0.275
3	6	0.395
4	8	0.508
5	10	0.654
6	12	0.744

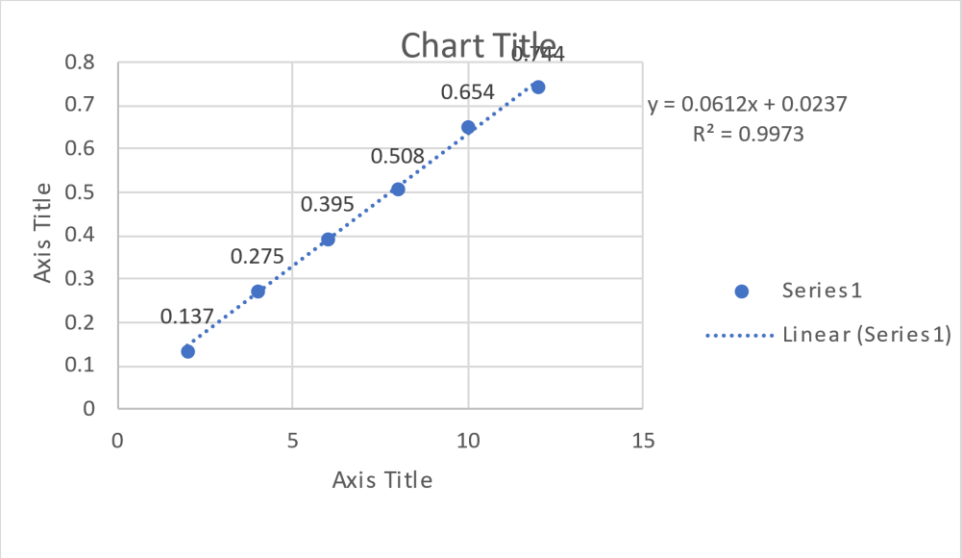

Fig 5.3 Calibration Curve of Drotaverine Hydrochloride

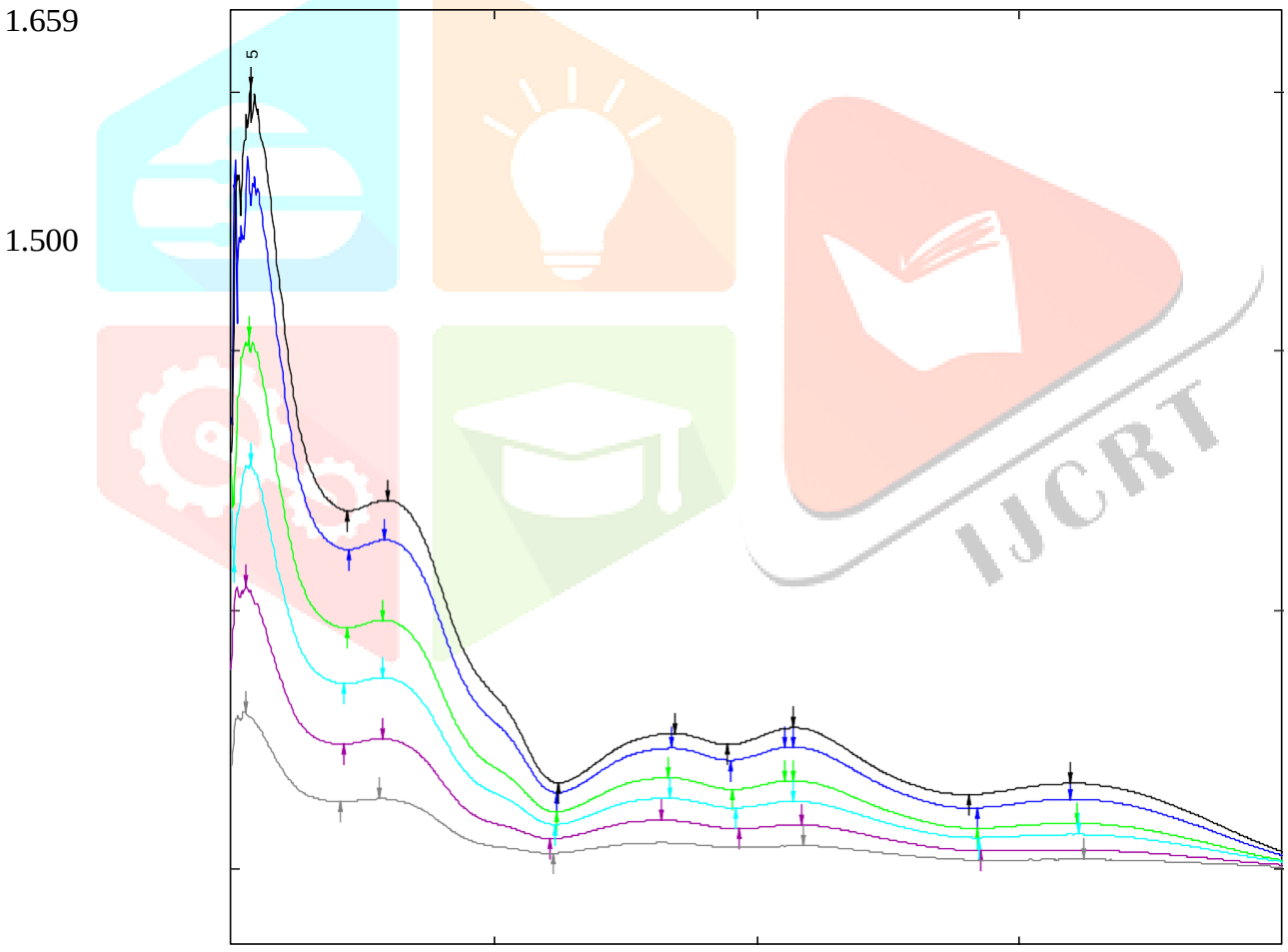

Table No 5.2 Absorbance of Paracetamol

S. No	Concentration (µg/mL)	Absorbance
1	2	0.161
2	4	0.348
3	6	0.543
4	8	0.731
5	10	0.931
6	12	1.105

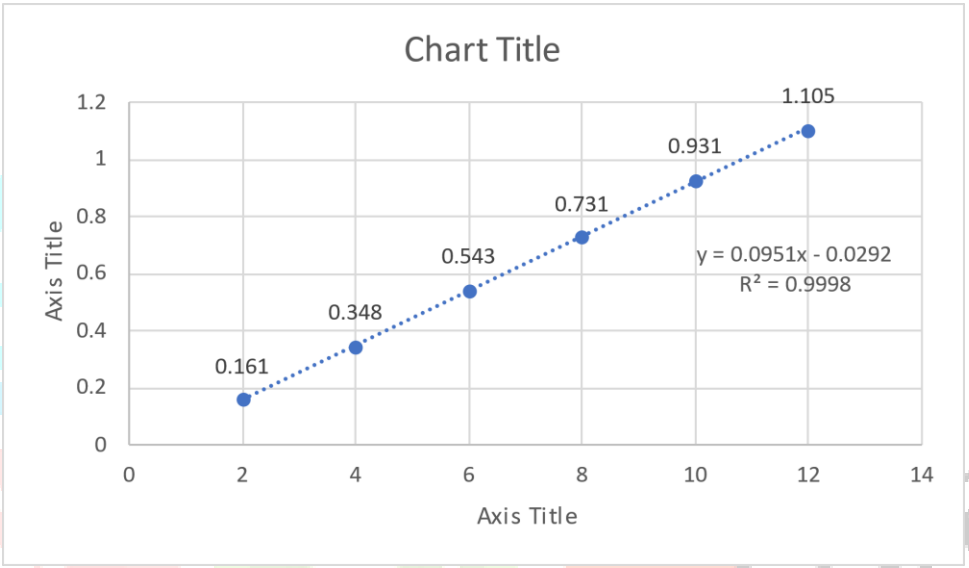

Fig. 5.4 Calibration curve of Paracetamol

Intraday precision: It determined by measuring the fixed concentration both of the drug solution for 3 times in a day. Mean standard deviation and % RSD need to be calculated. In this study the intra-day precision values are within the limits and the % RSD was found to be less than 2 %. The result was shown in table no.5.3

Table No 5.3 Intra-day precision of Drotaverine Hydrochloride

S.No	Time	Conc.	1	2	3	Mean	SD	%RSD
1	10:30am	8	0.653	0.654	0.652	0.653	0.001	0.15314
		10	0.816	0.82	0.811	0.81566	0.00451	0.55284
		12	0.927	0.93	0.935	0.93066	0.00404	0.43426
2	12:30pm	8	0.813	0.817	0.815	0.815	0.002	0.2454
		10	0.685	0.683	0.684	0.684	0.001	0.1462
		12	0.571	0.572	0.581	0.57465	0.00551	0.95842
3	2:30pm	8	0.636	0.632	0.641	0.63632	0.00451	0.70864
		10	0.767	0.768	0.762	0.76566	0.00321	0.41984
		12	0.87	0.872	0.865	0.869	0.00361	0.41491

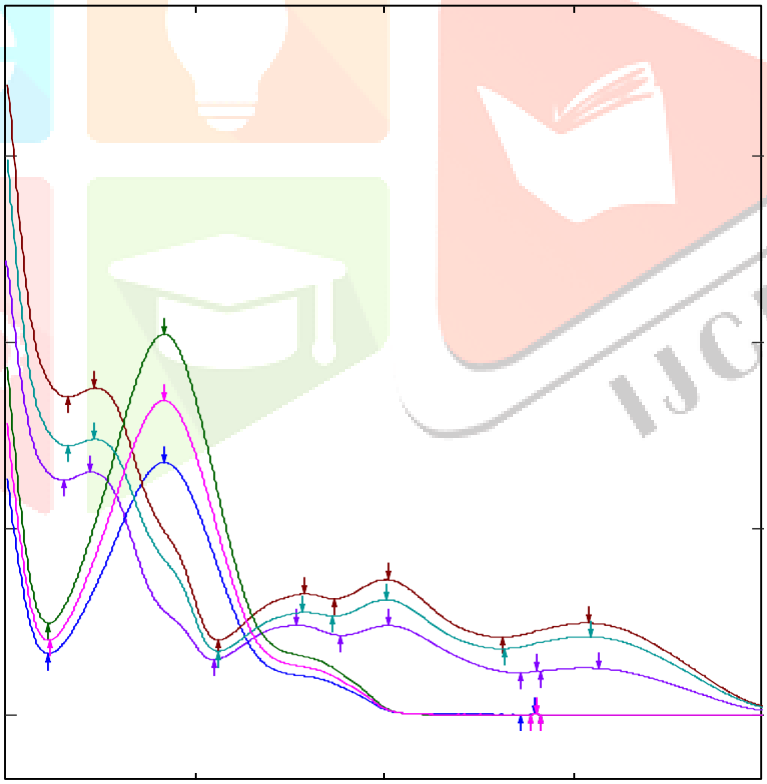

Fig 5.5 Intraday Precision of DRT and PCM Table No 5.4 Intra-day Precision of Paracetamol

S.No	Time	Conc.	1	2	3	Mean	SD	%RSD
1	10:30am	8	0.696	0.689	0.691	0.691994	0.003606	0.521038
		10	0.865	0.862	0.864	0.863666	0.001528	0.176865
		12	1.032	1.011	1.031	1.024621	0.011846	1.156158
2	12:30pm	8	0.615	0.611	0.618	0.61466	0.003512	0.571354
		10	0.764	0.762	0.763	0.763	0.001	0.131062
		12	0.924	0.925	0.921	0.923332	0.002082	0.225452
3	2:30pm	8	0.672	0.673	0.678	0.674328	0.003215	0.476704
		10	0.895	0.893	0.898	0.895331	0.002517	0.281082
		12	1.024	1.021	1.025	1.023332	0.002082	0.20342

Inter-day precision: It determined by measuring the fixed concentration of the drug solution for continuously for 7 days. Mean standard deviation and % RSD need to be calculated. In this study the intra-day precision values are within the limits and the % RSD was found to be less than 2 %. The result was shown in table no

Table No 5.5 Inter-day Precision of Drotaverine Hydrochloride

Conc. (µg/ml)	Absorbance								S.D.	% RSD
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean		
8	0.585	0.587	0.589	0.591	0.594	0.596	0.599	0.59157	0.00503	0.85002
10	0.692	0.693	0.696	0.698	0.702	0.705	0.709	0.69929	0.00632	0.90335
12	0.767	0.769	0.771	0.773	0.775	0.779	0.782	0.77371	0.00538	0.69487

Table No 5.6 Inter-day Precision of Paracetamol

Conc. (µg/ml)	Absorbance								S.D.	% RSD
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean		
8	0.707	0.709	0.711	0.714	0.716	0.723	0.725	0.715	0.00686	0.95883
10	0.883	0.885	0.887	0.891	0.894	0.899	0.905	0.892	0.00794	0.88983
12	0.986	0.989	0.991	0.993	0.995	0.999	1.025	0.99686	0.01309	1.31362

LOD and LOQ: Limit of detection and limit of quantification of drotaverine hydrochloride derived by calculating the signal-to-noise ratio (S/N, 3.3 for LOD and 10 for LOQ). LOD for Drotaverine hydrochloride and Paracetamol was found 1.630768 & 0.479467 respectively and LOQ value was found 4.94172 & 1.45293 respectively.

Recovery Studies:

Table No 5.7Accuracy study of Drotaverine Hydrochloride & Paracetamol

Level	Target conc.	Spiked conc.	Final Conc.	Concentration obtained	%Recovery	Mean	Overall Mean
80	8	6.4	14.4	14.1	7.7	96.25	
	10	8	18	18.02	10.02	100.2	98.24785
	12	9.6	21.6	21.4	11.8	98.33333	
100	8	8	16	16.1	8.1	101.25	
	10	10	20	19.9	9.9	99	99.51999
	12	12	24	23.8	11.8	98.33333	
120	8	9.6	17.6	17.2	7.6	95	
	10	12	22	22.01	10.01	100.1	98.06359
	12	14.4	26.4	26.3	11.9	99.16667	

Conclusion The developed analytical method for Drotaverine Hydrochloride and Paracetamol by using second order derivative spectroscopy is found to simple and precise as percentage recovery was found to be within the acceptable limits. It can be conveniently useful for the routine analysis. Based on the result obtained, it is found that the proposed method is accurate, precise, and reproducible and can be employed for quality control analysis.

References

1. G.R. Chatwal, S.K. Anand, Instrumental methods of chemical analysis, 5th ed, Himalaya Publishing house, Mumbai, 2002, 2.149-2.184.
2. F.R. Stauffer, H. Sakai, Derivative spectroscopy. Applied optics 7, no.1(1968) 61-65.
3. Redasani, V. K. et al. A review on derivative uv-spectrophotometry analysis of drugs in pharmaceutical formulations and biological samples review. J. Chil. Chem. Soc. 63, 4126–4134 (2018). 41
4. Rakesh Tiwle, Saurabh Shrivastava, Suman Shrivastava,.Validation of novel UV Spectrophotometric method for the determination of Ketoconazole in Pharmaceutical Formulation, J Pharm Adv Res, 2020; 3(2): 792-798.
5. Badyal, P. N. et al. Analytical Techniques in Simultaneous Estimation : An Overview. Austin J. Anal. Pharm. Chem. 2, 1–14 (2015)
6. Bhardwaj SK, Dwivedi K, Agarwal DD. A review: HPLC method development and validation. Int J Anal Bioanal Chem 2015;5:76-1.
7. Lavanya G, Sunil M, Eswarudu MM, Eswaraiah MC, Harisudha K, Spandana BN, et al. Analytical method validation: an updated review. Int J Pharm Sci Res 2013;4:1280.

8. Ravichandran V, Shalini S, Sundram KM, Harish R. Validation of analytical methods–strategies and importance. Int J Pharm Pharm Sci 2010;2:18-2.
9. Shweta K, Anita S. A review on analytical method validation. Int J Pharm Res Rev 2016;5:30-6.
10. 4. Daksh S, Goyal A, Pandiya CK. Validation of analytical methods– strategy and significance. Int J Res Dev Pharm Life Sci 2015;4:1489-97.
11. Tijare LK, Rangari NT, Mahajan UN. A review on bioanalytical method development and validation. Asian J Pharm Clin Res 2016;9:6-10.
12. Pasbola K, Chaudhary M. Updated review on analytical method development and validation by HPLC. World J Pharm Pharm Sci 2017;6:1612-30.
13. Geetha G, Raju KN, Kumar BV, Raja MG. Analytical method validation: an updated review. Int J Pharm Biol Sci 2012;1:64-1.