



RESEARCH ARTICLE

**INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH
AND BIO-SCIENCE**

A Path for Horizing Your Innovative Work

**METHOD DEVELOPMENT AND STATISTICAL VALIDATION OF
UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS
ESTIMATION OF TOLPERISONE HYDROCHLORIDE AND
PARACETAMOL IN SYNTHETIC MIXTURE AND COMBINED
DOSAGE FORM**

***MONALI PATEL, Dr. RAGIN SHAH, HIREN KADIKAR,
Dr. PRAGNESH PATANI, MOSAM SHUKLA**

Department of Pharmacy , Pharmaceutical Quality Assurance Laboratory, Arihant School Of
Pharmacy & BRI, Adalaj, Gandhinagar-382421, Gujarat, India.

Corresponding Author Email: monali_patel317@yahoo.in

Accepted Date: 17/01/2012 Published Date: 27/02/2012

Abstract: A simple, novel, sensitive, precise and specific validated spectrophotometric method was developed for simultaneous determination of Tolperisone Hydrochloride (TOL) and Paracetamol (PCM) in synthetic mixture and its dosage form using first order derivative spectroscopic method. Methanol was selected as a common solvent for estimation of Tolperisone Hydrochloride and Paracetamol with λ_{max} at 254 nm and 248nm respectively in methanol. Linearity was obeyed in concentration ranges of 1-70 $\mu\text{g/ml}$ for TOL and 1-80 $\mu\text{g/ml}$ for PCM respectively and calibration curve taken in the concentration range of 3-18 $\mu\text{g/ml}$ for TOL and 2-12 $\mu\text{g/ml}$ for PCM. The Zero Crossing Point (ZCP) of TOL was 254.6 nm and PCM was 248.56 nm. The detection limit and quantification limit were found to be 0.299 and 0.908 $\mu\text{g/ml}$ for TOL and 0.244 and 0.741 $\mu\text{g/ml}$ for PCM respectively. The method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: Tolperisone Hydrochloride, Paracetamol, Derivative Spectrophotometry, Spasmolytic agent.

INTRODUCTION

Tolperisone Hydrochloride (TOL), chemically (R, S) 2-methyl-1-(4 - methyl phenyl)-3- (1-piperidyl) propane -1 one is a piperidine derivative¹ [Figure-1]. It is a centrally acting muscle relaxant which is used in the treatment of different pathological conditions like acute and chronic muscle spasm, electroconvulsive therapy, neurological conditions and orthopedic manipulation - multiocular sclerosis, myelopathy, encephalomyelitis, spondylosis, spondylarthrosis, cervical and lumbar syndrome, Arthrosis of the large joints obliterating arterosclerosis of the extremity vessels, Diabetical angiopathy, thromboangitis obliterans, raynauds syndrome^{2,3}. Tolperisone Hydrochloride is official in japanese pharmacopoeia¹. Paracetamol is N- (4 - hydroxyphenyl) acetamide, a para-aminophenol derivative [Figure 2], has analgesic and antipyretic properties and weak anti-inflammatory activity. Paracetamol is official in Indian Pharmacopoeia⁴, British Pharmacopoeia⁵,

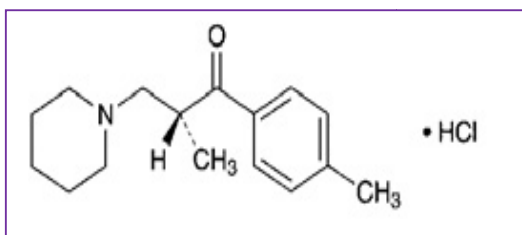


Figure 1 Structure of Tolperisone Hydrochloride

United States Pharmacopoeia⁶ and European Pharmacopoeia⁷. A combination of Tolperisone HCl with Paracetamol has been approved in India in the proportion of 150:500 mg proportion respectively. The literature survey revealed that there are some analytical methods reported for Tolperisone Hydrochloride like Spectrophotometric⁸⁻¹¹, HPTLC¹², RP-HPLC¹³ either individually or in combination with other drug and also reported on biological fluids¹⁴. Many methods have been reported in literature for determination of Paracetamol with other drugs¹⁵⁻¹⁷. However, Literature survey did not reveal any reported methods for the simultaneous analysis of Tolperisone Hydrochloride and Paracetamol in combined dosage form. Hence, aim of present work is to develop simple, feasible, effective and economic validated analytical techniques for quantification of Tolperisone HCl & Paracetamol simultaneously in synthetic mixture as well as combined dosage form.

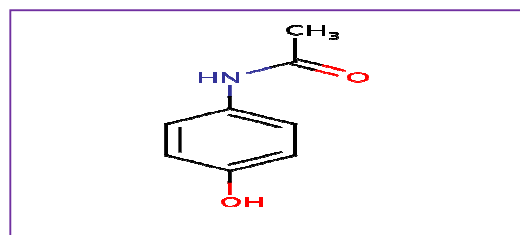


Figure 2 Structure of Paracetamol

MATERIALS AND METHOD**Instrument:**

Shimadzu UV-1800, UV-Visible double beam Spectrophotometer with matching pair of 1 cm quartz cuvettes (Shimadzu Corporation, Kyoto, Japan). The spectral bandwidth is 0.5 nm.

Chemicals:

Tolperisone Hydrochloride reference standard was kindly provided by Zydus Cadila Ahmedabad. The purity of reference standard was 98.9%w/w. Paracetamol was provided by Lincoln Pharmaceuticals Ltd. Marketed Tablet formulation containing Tolperisone Hydrochloride 150mg and Paracetamol 500mg (MYO-MR PLUS) manufactured by Amanath Pharmaceuticals and marketed by Grandix Pharmaceuticals, A Division of Strides Arcolab Ltd, Bangalore, India were used for estimation.

Preparation of Standard Stock Solution:

Standard stock solutions containing Tolperisone HCl and Paracetamol were prepared individually by dissolving 10.0 mg Tolperisone HCl and 10.0 mg Paracetamol in 5ml methanol. The flask was shaken and volume was made up to 10ml with methanol to give a solution containing 1000 µg/ml (Solution SS). 2.5 ml aliquot was withdrawn into 25ml

volumetric flask from solution SS and diluted upto 25ml with methanol. The final stock solution is having concentration of 100 µg/ml for both the drugs. Further dilutions were made from this stock solution to get the required concentration.

Determination of Absorption Maxima:

The standard solution of Tolperisone Hydrochloride (10µg/ml) and Paracetamol (10µg/ml) were scanned in range of 200-400 nm and the λ_{max} were found to be 254 nm and 248nm against methanol as blank for TOL and PCM respectively. The overlain zero order spectra of Tolperisone Hydrochloride and Paracetamol is shown in Figure 3.

Calibration curve for TOL and PCM

Calibration curve of Absorbance Vs Concentration were studied by taking concentrations ranging from 1-70 µg /ml for TOL and 1-80 µg /ml for PCM. Zero order spectra data revealed that Beer's lambert law was obeyed between concentration ranges of 3-18 µg /ml for TOL (Series 1) and 2-10 µg/ml for PCM (Series 2). A set of first order derivative (D_1) Spectra from zero order spectra (D_0) of both the series were prepared and overlay it (Figure 4). Zero Crossing Point (ZCP) was found to be 254.6 nm for TOL and 248.56 nm for PCM. The calibration

curve for Tolperisone Hydrochloride and Paracetamol were shown in Figure 5 and Figure 6. The statistical parameters reported in Table 1.

Spectroscopic measurements:

The Absorbance of the sample and standard solutions of TOL and PCM were recorded from 200-400 nm against methanol as a blank. The first order derivative spectra for each set of solutions were subsequently recorded with $\Delta\lambda = 2$ nm and absorbances were measured at the zero crossing wavelengths of the other drug. The derivative value D_1 amplitudes (in terms of absorbance units) for TOL in the presence of PCM and vice versa measured at 248.56 nm (ZCP of PCM) and 254.6 nm (ZCP of TOL) respectively.

Validation of the Method

The developed method was validated for assay of TOL and PCM in combination in accordance with ICH guidelines¹⁸.

Linearity:

The linearity of measurement was evaluated by analyzing six different concentration of the standard solution of TOL (3-18 $\mu\text{g}/\text{ml}$) and PCM (2-12 $\mu\text{g}/\text{ml}$). A linear relationship was observed between Absorbance Vs Concentration for first order derivative spectra of mentioned concentration range. This range was selected as a linear range for estimation of

TOL and PCM. The linearity data was shown in Table 2.

Precision:

The reproducibility of the proposed method was determined by performing the assay for the same day (intraday assay precision) and on three different days (interday assay precision). Precision studies were performed by preparing nine determinations covering the specified range for the procedure (3 x 3 replicates for each concentration). Low %RSD shows that the method has good precision. The results of repeatability, intraday and inter day precision were expressed in % RSD was tabulated in Table 3(A), 3(B), 4 and 5.

Accuracy

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. The accuracy study was carried out by the analysis of standard additions at three levels that is multi-level recovery studies. To a fixed equivalent quantity of formulation powder as well as synthetic mixture, a known quantity of standard TOL and PCM added at 50%, 100% and 150% level and the contents were re-analysed by the proposed method. The % recovery and %RSD were calculated (Table 6 and 7).

Limit of Detection and Limit of Quantitation:

The LOD and LOQ was separately determined (table 8) based on the standard calibration curve. The residual standard deviation of y- intercept of regression lines may be used to calculate LOD and LOQ. $LOD=3.3*D/S$ and $LOQ=10*D/S$ where , D is the standard deviation of the intercept of regression line and S is the slope of the calibration curve.

Application of the Proposed Method for the Determination of TOL and PCM in Tablets:**Preparation of test solution from synthetic mixture:**

Binary mixture was prepared of combination of both drugs in the ratio of 3:10 (Tolperisone HCl: Paracetamol). Commonly used excipients (starch, lactose, stearates, etc.) in tablet formulation were added and synthetic mixture was prepared. From this synthetic mixture the powder equivalent to 3 mg Tolperisone HCl and 10 mg Paracetamol was transferred into 10 ml volumetric flask. Volume was made upto 10 ml with methanol (Solution A⁰). From this Solution A⁰, 1 ml aliquot was withdrawn into 100 ml flask and diluted upto mark with methanol (Solution B⁰). From this Solution B⁰, 1 ml aliquot was withdrawn into 10 ml

volumetric flask and diluted upto the mark (Solution C⁰ having 3µg/ml TOL and 10 µg/ml PCM) that was used as final test solution. The absorbances of resulting solutions were measured at 254.6 nm and 248.56 nm. The concentration of TOL and PCM present in the sample solution was calculated by using the equation generated from calibration curve of respective drugs.

Preparation of test solution from Tablet Powder:

20 tablets (MYO MR PLUS: TOL 150mg and PCM 500 mg) were accurately weighed individually and the average weight was calculated. The tablet powder equivalent to 150 mg of TOL and 500 mg of PCM was accurately weighed and transferred to 50 ml volumetric flask. Methanol (30 ml) was added and sonicated for 15 min. This solution was filtered through Whatman filter paper and diluted up to the mark with methanol (Solution A). From this Solution A, 1 ml aliquot was withdrawn into 100 ml flask and diluted upto mark with methanol (Solution B). From this Solution B, 1 ml aliquot was withdrawn into 10 ml volumetric flask and diluted upto the mark (Solution C having 3µg/ml TOL and 10 µg/ml PCM) that was used as final test solution. The absorbances of resulting solutions were measured at 254.6 nm and 248.56 nm. The concentration of TOL and PCM present in

the sample solution was calculated by using the equation generated from calibration curve of respective drugs and %purity was calculated (Table 9).

RESULT AND DISCUSSION

Absorption spectra of TOL and PCM showed wavelength of maximum absorbance at 254 and 248 nm respectively (Figure 1). The ratio for TOL: PCM is 3:10 on basis of dose. There is overlapping of both spectra that interfere with the simultaneous determination of this formulation. Therefore, simultaneous estimation of TOL and PCM was not possible using zero order spectrum method. Derivative spectroscopy, based on a mathematical transformation of the zero-order spectra into the derivative spectra, allows a fast, sensitive and precise resolution of a multi component mixture and overcomes the problem of overlapping of a multi-component system. Hence, first order derivative method was performed in which ZCP was found for TOL at 254.6 nm and for PCM at 248.56 nm. Standard calibration curves for TOL and PCM were linear with Correlation coefficients (r) values in the range of 0.9993 and 0.9997 respectively at all the selected wavelengths and the Values were average of five readings. LOD and LOQ were found to be 0.299 $\mu\text{g/ml}$ and 0.908 $\mu\text{g/ml}$ for TOL and

0.244 $\mu\text{g/ml}$ and 0.740 $\mu\text{g/ml}$ for PCM. Precision study showed co-efficient of variation (%CV) values less than 2% for both TOL and PCM respectively in all selected concentrations. The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 50 %, 100 %, 150 % recovery in the range of 99 – 101.6% justifies the accuracy of method. The results obtained from the recoveries of both drugs showed excellent accuracy. The influence of excipients was studied by mixing two drugs with excipients as per the ratio. No interference was observed from the presence of excipient in the amounts, which are commonly present in tablet dosage forms. The results of synthetic mixture and pharmaceutical dosage forms analysis of the combinations are shown in Table 9 which showed good agreement with the labeled claim.

CONCLUSION

The proposed work provides a novel, simple, accurate, economical and convenient method for simultaneous estimation of Tolperisone Hydrochloride and Paracetamol in Synthetic mixture as well as tablet dosage form using first order derivative UV-Spectrophotometry. The proposed method can be applied for routine analysis in laboratory.

ACKNOWLEDGEMENT

The authors are thankful to ZyduS Cadila Ahmedabad and Lincoln Pharmaceuticals Ltd. for providing standard sample of drugs and also to the Arihant School of

Pharmacy & BRI, Adalaj, Gandhinagar for providing facilities to carry out research work.

Table 1**Statistical Parameters from the Calibration Plot**

Statistical Parameters	Observed Value	
	Tolperisone Hydrochloride	Paracetamol
λ_{\max}	254 nm	248 nm
Calibration Curve	3-18 $\mu\text{g/ml}$	2-12 $\mu\text{g/ml}$
Regression equation (Y*)		
Slope(b)	0.0020x	0.0027x
Intercept(a)	0.0011	0.0007
Correlation coefficient(r**)	0.9993	0.9997

* $Y=a+bC$, where 'C' is concentration in mg/ml and Y is absorbance unit.

**Five replicate samples.

Table 2

Linearity data for Tolperisone Hydrochloride and Paracetamol

Sr. No.	TOL ($\mu\text{g/ml}$)	Mean Absorbance at 248.56 nm \pm SD (n=5)	% RSD	PCM ($\mu\text{g/ml}$)	Mean Absorbance at 254.6 nm \pm SD (n=5)	% RSD
1.	3	0.00542 \pm 0.00008	1.54	2	0.00622 \pm 0.00008	1.35
2.	6	0.0107 \pm 0.00016	1.48	4	0.01126 \pm 0.00017	1.49
3.	9	0.016580 \pm 0.00024	1.44	6	0.01716 \pm 0.00021	1.21
4.	12	0.02318 \pm 0.00019	0.83	8	0.02224 \pm 0.00020	0.88
5.	15	0.02872 \pm 0.00030	1.06	10	0.02812 \pm 0.00023	0.81
6.	18	0.03564 \pm 0.00046	1.28	12	0.03316 \pm 0.00022	0.66

Table 3 (A)

Repeatability data for Tolperisone Hydrochloride

Sr. No.	Concentration ($\mu\text{g/ml}$)					
	3	6	9	12	15	18
1.	0.0055	0.0107	0.0167	0.0231	0.0289	0.0359
2.	0.0054	0.0108	0.0168	0.0229	0.0285	0.0355
3.	0.0053	0.0106	0.0167	0.0233	0.029	0.0349
4.	0.0055	0.0109	0.0165	0.0234	0.0289	0.036
5.	0.0054	0.0105	0.0162	0.0232	0.0283	0.0359
Mean	0.00542	0.01070	0.01658	0.02318	0.02872	0.03564
SD	0.00008	0.00016	0.00024	0.00019	0.00030	0.00046
%RSD	1.54	1.48	1.44	0.83	1.06	1.28

Table 3 (B)

Repeatability data for Paracetamol

Sr. No.	Concentration ($\mu\text{g/ml}$)					
	2	4	6	8	10	12
1.	0.0063	0.0114	0.0172	0.0223	0.0281	0.0331
2.	0.0062	0.011	0.017	0.022	0.0285	0.0329
3.	0.0063	0.0112	0.0173	0.0225	0.0279	0.0335
4.	0.0062	0.0114	0.0174	0.0223	0.0281	0.0331
5.	0.0061	0.0113	0.0169	0.0221	0.028	0.0332
Mean	0.00622	0.01126	0.01716	0.02224	0.02812	0.03316
SD	0.00008	0.00017	0.00021	0.00020	0.00023	0.00022
%RSD	1.35	1.49	1.21	0.88	0.81	0.66

Table 4

Intraday precision data for Tolperisone Hydrochloride and Paracetamol

TOL			PCM		
Conc. (µg/ml)	Mean Absorbance at 248.56 nm ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance at 254.6 nm ± SD (n=3)	% RSD
3	0.00493 ± 0.00006	1.17	2	0.00610 ± 0.00010	1.64
9	0.01617 ± 0.00021	1.29	6	0.01680 ± 0.00020	1.19
15	0.02813 ± 0.00026	0.89	10	0.02830 ± 0.00020	0.71

Table 5

Interday precision data for Tolperisone Hydrochloride and Paracetamol

TOL			PCM		
Conc. (µg/ml)	Mean Absorbance at 248.56 nm ± SD (n=3)	% RSD	Conc. (µg/ml)	Mean Absorbance at 254.6 nm ± SD (n=3)	% RSD
3	0.00550 ± 0.00010	1.82	2	0.00633 ± 0.00012	1.82
9	0.01680 ± 0.00026	1.57	6	0.01713 ± 0.00021	1.21
15	0.02857 ± 0.00031	1.07	10	0.02863 ± 0.00025	0.88

Table 6

Recovery data for Tolperisone Hydrochloride

Amt of TOL in Sample (μg)	Amt. of Std. TOL Added (μg)	Total amt. of TOL (μg)	Synthetic Mixture		Tablet Formulation	
			Total amt. of TOL found (μg) Mean \pm S.D.	% Recovery (n=3)	Total amt. of TOL found (μg) Mean \pm S.D.	% Recovery (n=3)
3	0	3	3.02 \pm 0.05508	-	2.95 \pm 0.08737	-
3	1.5	4.5	4.48 \pm 0.04042	99.55	4.46 \pm 0.06028	99.11
3	3	6	6.02 \pm 0.05000	100.33	5.99 \pm 0.02517	99.94
3	4.5	7.5	7.62 \pm 0.05000	101.6	7.60 \pm 0.02517	101.28

Table 7

Recovery data for Paracetamol

Amt of PCM in Sample (μg)	Amt. of Std. PCM Added (μg)	Total amt. of PCM (μg)	Synthetic Mixture		Tablet Formulation	
			Total amt. of PCM found (μg) Mean \pm S.D.	% Recovery (n=3)	Total amt. of PCM found (μg) Mean \pm S.D.	% Recovery (n=3)
10	0	10	9.97 \pm 0.17560	-	10.05 \pm 0.10017	-
10	5	15	14.93 \pm 0.08021	99.53	14.85 \pm 0.16000	99
10	10	20	20.03 \pm 0.09000	100.15	20.27 \pm 0.42532	101.35
10	15	25	25.10 \pm 0.18000	100.4	24.93 \pm 0.34020	99.73

Table 8**LIMIT OF DETECTION (LOD) and LIMIT OF QUANTIFICATION (LOQ) For TOL and PCM**

Parameter	TOL	PCM
LOD	0.299 µg/ml	0.244µg/ml
LOQ	0.908 µg/ml	0.740 µg/ml

Table 9**Data from the analysis of test preparation for TOL and PCM**

Test Preparation	% Assay in Synthetic Mixture	% Assay in Tablet Formulation
TOL (150 mg)	100.66	98.2
PCM (500 mg)	99.66	100.5

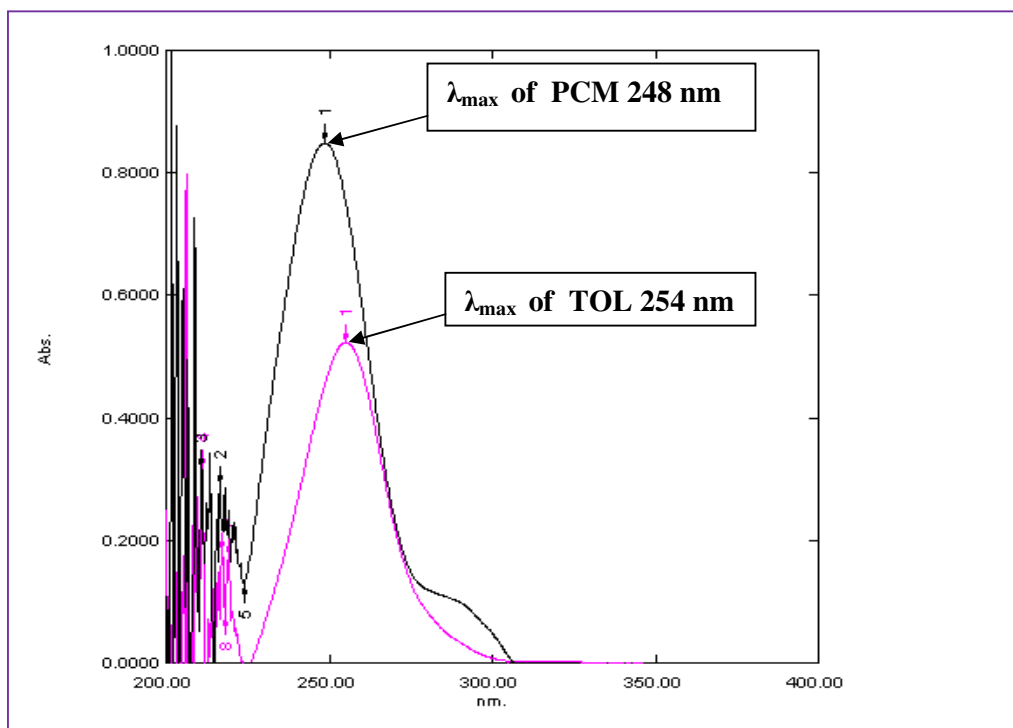


Figure 3: Overlain Spectrum of TOL (10 µg/ml) and PCM (10 µg/ml)

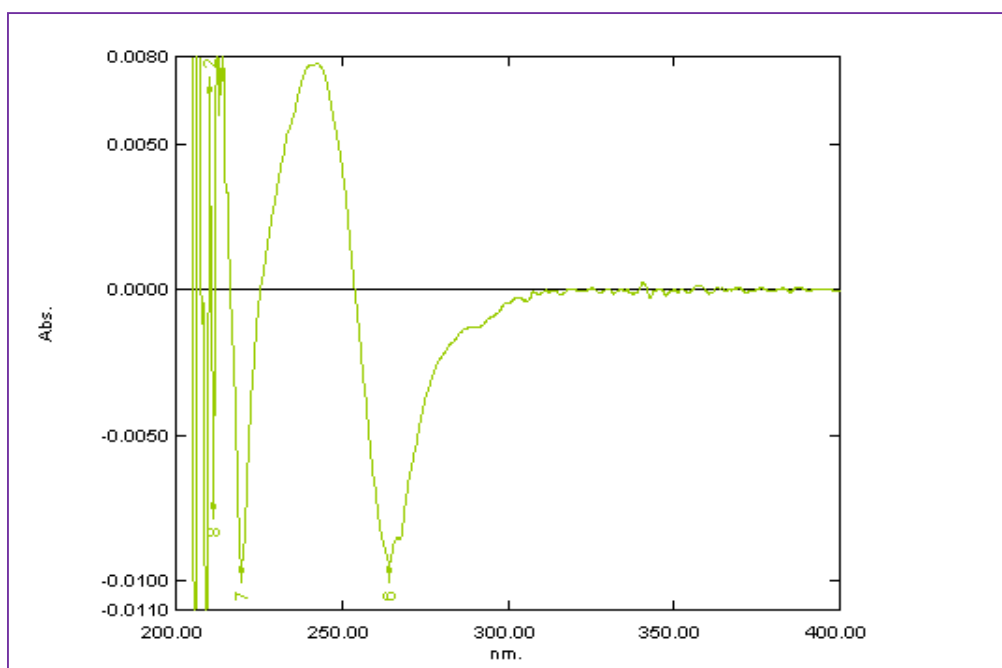


Figure 4 (A): First Order Derivative Spectrum of TOL (3 µg/ml)

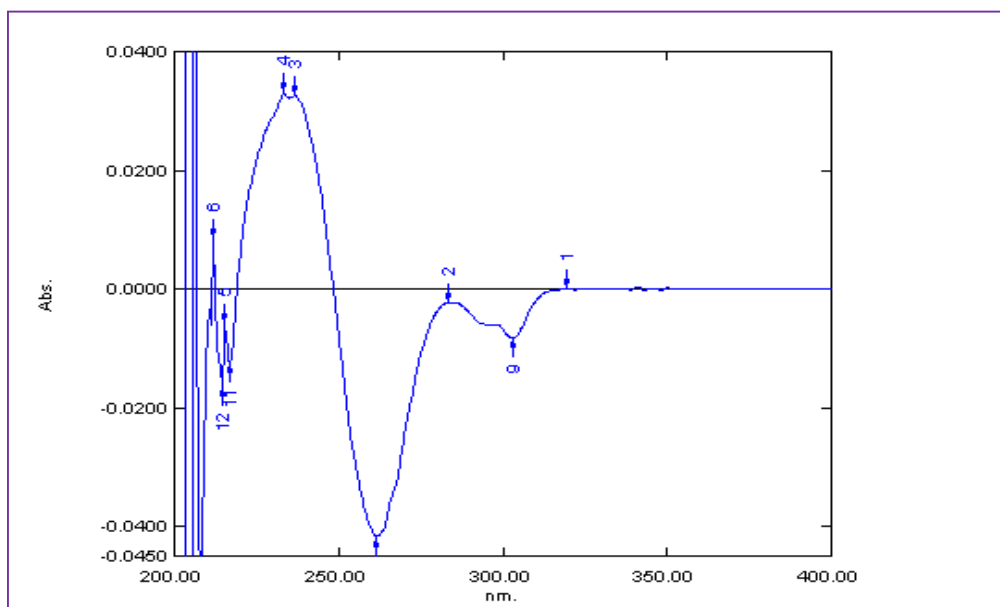


Figure 4 (B): First Order Derivative Spectrum of PCM (10 µg/ml)

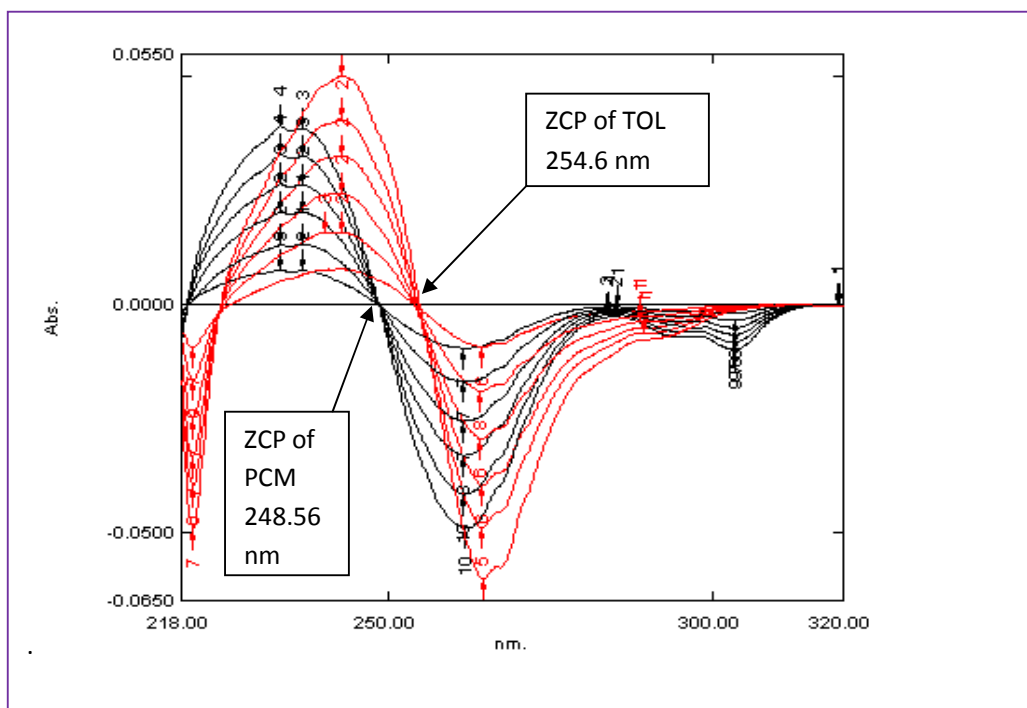


Figure 4: Overlain First Order Derivative Spectra of TOL (3-18 µg/ml) and PCM (2-12 µg/ml)

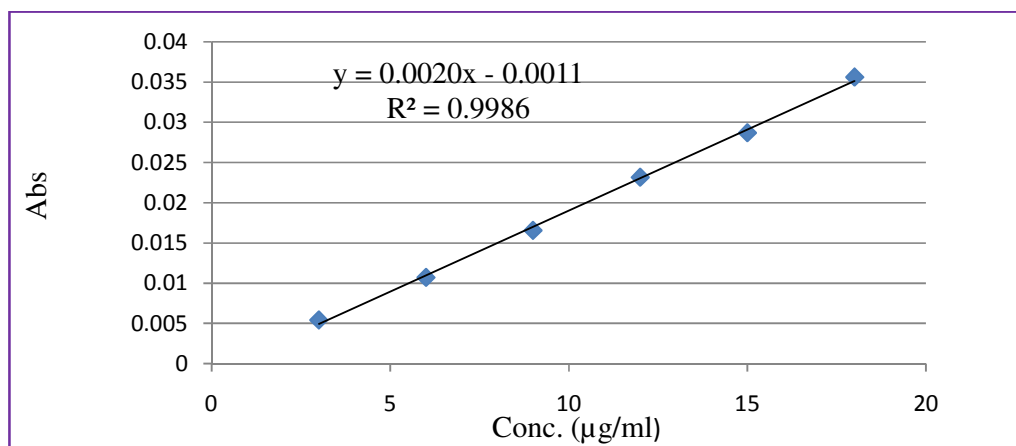


Figure 5: Calibration curve of TOLP standard solution at 248.56 nm

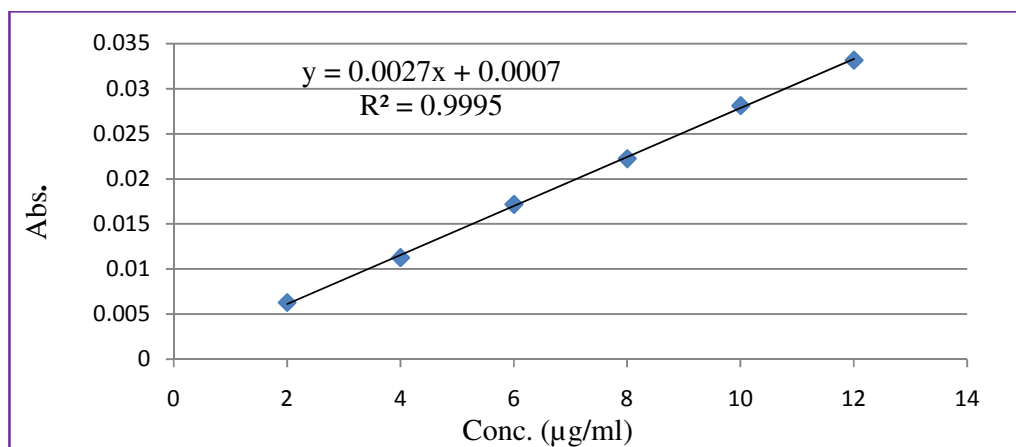


Figure 6: Calibration curve of PCM standard solution at 254.6 nm

REFERENCES

1. Japanese Pharmacopoeia, The ministry of Health, Labour and Welfare, Prefectural office in Japan. 15th edition (2006): 1190.
2. Gayraud M., Reynaud's phenomenon. Joint, bone and spine (2007); 74(1).
3. Muscle relaxants- Wikipedia, (http://en.wikipedia.org/wiki/Muscle_relaxants) 10 January 2011.
4. Indian Pharmacopoeia. Government of India Ministry of Health & Family Welfare; (2007); 3:900-903.
5. British Pharmacopoeia, Government of British (2011); 1: II, III.
6. The United States pharmacopoeia 32, National Formulary-27, Asian Edition, The United States Pharmacopoeia Convention, Rockville (2009); 3: 1266-91.
7. European Pharmacopoeia 5.0: 2184.
8. Jagathi V, Shaiba M, Raghavi K, Sindhura M and Prashanthi R: Assay of tolperisone by extractive spectrophotometry"; RJPBCS - July - September 2010; 1 (3): 654.
9. Praveen PS, Anupama B, Jagathi V and Devala Rao G: Spectrophotometric determination of Tolperisone using 2, 4-dinitrophenylhydrazine reagent. Int. J. Res. Pharm. Sci. 2010; 1 (3): 317-320.
10. Carolin N, Balan P, Chiranjeevi N, Uma M and Rajasekar S: Method development and statistical validation of UV spectrophotometric method. Journal of Pharmacy Research 2011; 4(5): 1356-1357.
11. Badmanaban: Etodolac in combined fixed oral dosage formulation by Spectrophotometry. Research Journal of Pharmacy and Technology (RJPT) 2011; 4 (7).
12. Saisunee L and Boonsom L: Simultaneous determination of Tolperisone and Lidocaine by HPTLC. Journal of Pharmaceutical and Biomedical Analysis 1999; 20: 401-404.
13. Patel MJ, Badmanaban R and Patel CN: RP-HPLC method for simultaneous analysis of Tolperisone Hydrochloride and Etodolac in combined fixed oral dosage formulation. Pharmaceutical Methods- A

Pharmaceutical Journal by In pharma Association 2011; 2(2): 124-129.

14. Bae JW, Park YS, Sohn UD, Myung CS, Ryu BK, Jang CG and Lee SY: HPLC determination of tolperisone in human plasma. Arch Pharm Res 2006; 29(4): 339-42.

15. Deepali G and Pandurang D: Spectrophotometric Estimation of Paracetamol from Tablet Formulations. International Journal of Chemical and Analytical Science 2010; 1(1): 3-5.

16. Nagendra P: Spectrophotometric Estimation of Paracetamol in Bulk and

Pharmaceutical Formulations. E-Journal of Chemistry 2011; 8(1): 149-152.

17. Anuradha SJ, Savita SY and Janhavi RR: Simultaneous Determination of Paracetamol and Lornoxicam by Rp-Hplc in Bulk and Tablet Formulation. International Journal of Comprehensive Pharmacy 2011; 9.

18. ICH Q2 (R1) Validation of Analytical Procedure: Text and Methodology, Geneva, International Conference on Harmonisation (2005).