International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.5, No.2, pp 790-799, April-June 2013

UV Spectrophotometric Estimation Of Pentoxyphylline In Bulk Drug And Its Pharmaceutical Formulation

Lakshmana Rao. Potti[1]*, Narasimharao.Doredla[2], Shaik.Subhani[3], Korrapati. Narsu Kumari[4]

***Corres. Author: potti.lakshman@gmail.com, simham64@gmail.com**
Mobile no: +919492902740

Abstract: A method was developed for the estimation of Pentoxyphylline in bulk drug and its pharmaceutical formulation by using water and 0.1N NaOH as a solvent. Pentoxyphylline show absorbance maxima at 275nm and 271 nm for water and 0.1N NaOH respectively. A Elico model SL-150 Double beam UV-Visible Spectrophotometer was used for quantification. Validation study reveals that the methods are specific, accurate, precise and reproducible. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (S.D < 2.0, %RSD < 2.0) for both accuracy and precision study. High recovery and low percent RSD reveals the reliability of the method for Quantitative study of Pentoxyphylline in pharmaceutical formulation. The method is simple rapid, accurate, precise, reproducible, economic and can be used for the routine quantitative analysis of Pentoxyphylline in bulk drug and its pharmaceutical formulation.
Keywords: Double beam UV-Visible Spectrophotometer, Pentoxyphylline.

Introduction:

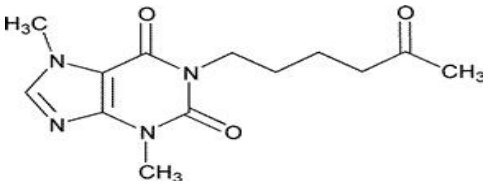
Pentoxyphylline, 1-(5-oxohexyl)-3, 7-dimethylxanthine, is an active haemorheological drug widely used for the treatment of intermittent elucidation and other circulatory disorders [1–4]. Because the drug improves perfusion in the impaired microcirculation of peripheral and cerebral vascular beds, it has also been tried as therapy for cerebrovascular disorders[5–7]. A GC procedure employing trifluoroacetyl derivatization and nitrogen-selective detection has been reported for quantification of Pentoxyphylline [8]. GC methods requiring extensive sample preparation and derivatization tend to be tedious. Previously described HPLC methods for analysis of Pentoxyphylline involve time-consuming extraction procedures [9–11], use an internal standard that is not readily available [12], or use a complex mobile phase; the methods also have a narrow range of linearity [13]. A simple and sensitive HPLC procedure has been described for determination of the concentrations of Pentoxyphylline and one of its major metabolites in rat plasma, but retention times for Pentoxyphylline and the internal standard were too long [14,15]. There is no method for the estimation of Pentoxyphylline by UV spectroscopy. Hence the present work aims for developing simple validated method for Pentoxyphylline.

Experimental

Instrument:

A Elico model SL-150 double beam UV-Visible Spectrophotometer with a pair of 1cm matched quartz cells was used to measure absorbance of resulting solution.

Fig:1- Pentoxyphylline Chemical Structure:



Chemicals

Pentoxyphylline drug was obtained from Micro labs limited, Bangalore. Water used was generated by double distillation. Ferric chloride, Diluted HCl and Sodium Hydroxide were supplied by S.D fine Chemical Ltd., India, Ranbaxy India Ltd.

Method – A

Preparation of stock solution:

Working standard of Pentoxyphylline 100mg was taken and weighed accurately and transferred to an 100ml volumetric flask, dissolve and make up the volume with distilled water to obtain concentration of 1000µg/ml(solution A) from solution A pipette out 10ml of solution into 100ml volumetric flask and make up the volume 100ml with distilled water. The resulting solution has a concentration 0f 100mcg/ml(solution-B).

Determination of λ max:

From the stock solution 25 µg/ml solution was prepared and absorbance was measured at different wavelengths, maximum absorbance was identified as 275nm. The results are shown **in** Table 1.1-1.3 & Fig1.1-1.3.

Preparation of calibration curve:

From stock solution B pipette out 0.5, 1, 1.5, 2, 2.5 and 3ml was transfer in to a 10ml volumetric flask and diluted with water to get a concentration of 5, 10, 15, 20, 25 and 30 µg/ml respectively. The diluted solutions were measured at 275nm.The results are shown in Table 1.4 & Fig1.4.

Analysis of Formulation:

Ten tablets of branded Trental, label claim 400mg of Pentoxyphylline was weighed and average weight was determined and finely powder the tablets. 0.175g of powder sample was taken into 100ml volumetric flask and dissolved in distilled water and make up the volume up to the mark. The solution was filtered through Whatmann filter paper No: 42 and required dilution was made to obtain a final concentration of 100mcg/ml. Appropriate aliquots were taken in such a way that the final concentration in 10ml volumetric flask were within the range of calibration curve. Measure the absorbance at 275nm for testing the drug. The results are shown in Table 1.5.

Recovery studies:

In order to ensure the reliability and stability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were reanalysed by proposed method. To a quantity of formulation equivalent to 10mg Pentoxyphylline was added at 80%, 100% and 120%. This was extracted, diluted and reanalyzed as per the formulation procedure. Absorbance was noted at respective wavelength. Recovery studies were repeated for six times and results are shown in Table 1.6.

Table 1.1: Determination of λ_{max} of Pentoxyphylline (method A)

Concentration	Wavelength(nm)	Absorbance
25µg/ml	240	0.252
	250	0.220
	260	0.438
	270	0.669
	280	0.627
	290	0.276
	300	0.036

Table 1.2: Determination of λ_{max} of Pentoxyphylline (method A)

Concentration	Wavelength(nm)	Absorbance
25µg/ml	260	0.438
	265	0.559
	270	0.669
	275	0.706
	280	0.627

Table 1.3: Determination of λ_{max} of Pentoxyphylline (method A)

Concentration	Wavelength(nm)	Absorbance
25 µg/ml	270	0.669
	271	0.680
	272	0.688
	273	0.701
	274	0.703
	275	0.705
	276	0.697
	277	0.688
	278	0.672
	279	0.652
	280	0.627

Fig:1.1-Determination of λ max of Pentoxyphylline (method A)

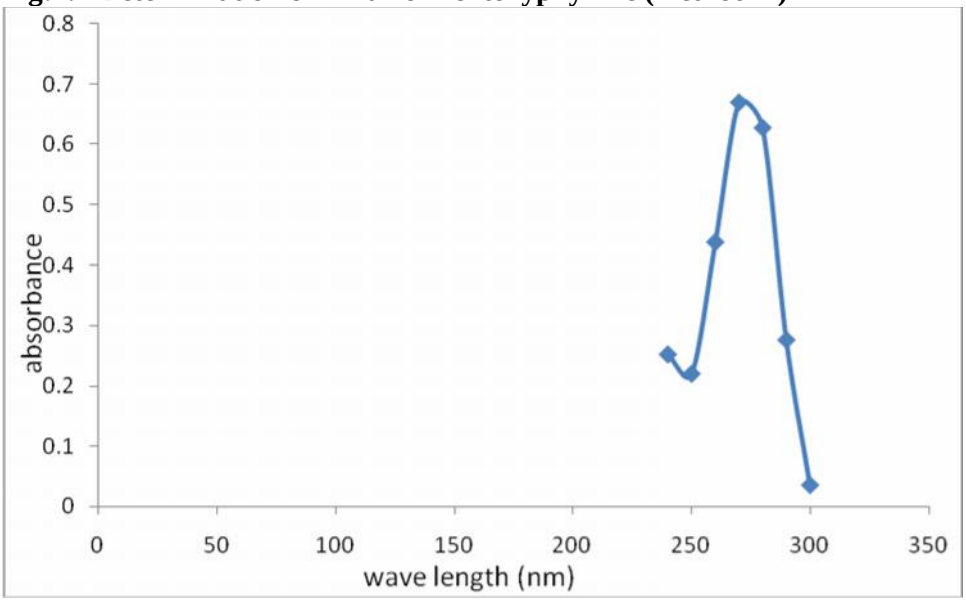


Fig:1.2-Determination of λ max of Pentoxyphylline (method A)

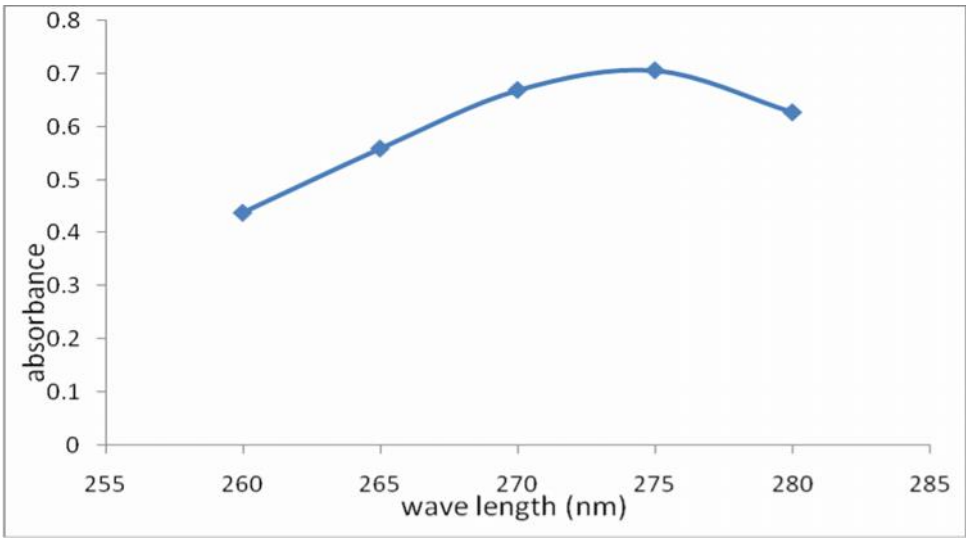


Fig:1.3-Determination of λ max of Pentoxyphylline (method A)

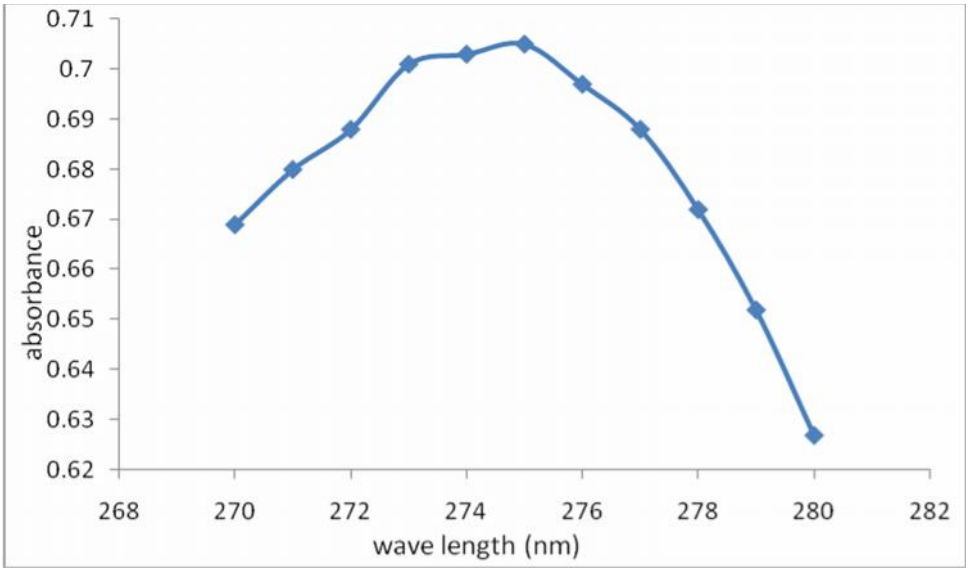


Table 1.4 : Calibration curve of Pentoxyphylline (method A)

S.No	Concentration (µg/ml)	Absorbance
1	5	0.143
2	10	0.270
3	15	0.426
4	20	0.572
5	25	0.704
6	30	0.863

Fig 1.4 : Calibration curve Pentoxyphylline (method A)

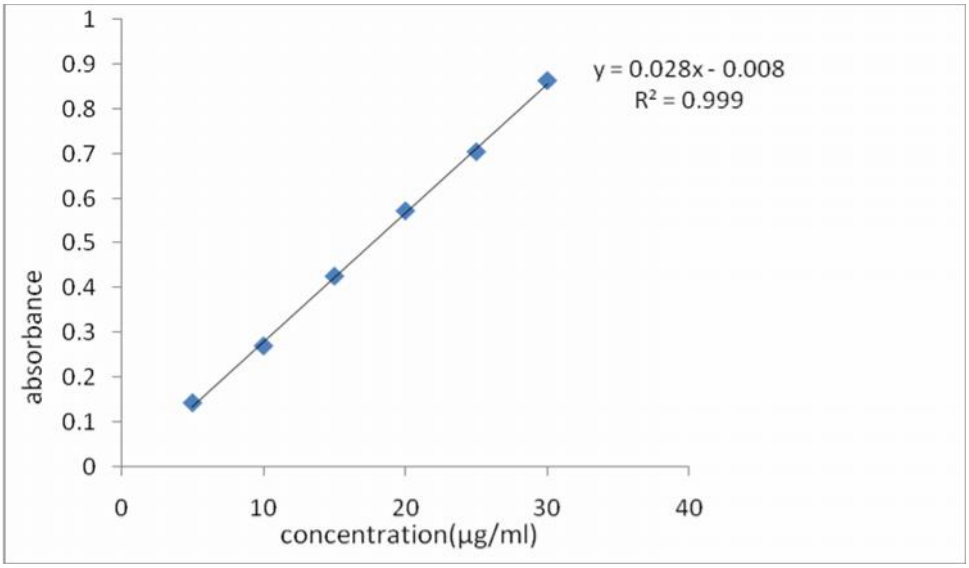


Table 1.5: Analysis of Formulation

S.No	Amount present in(mg/tab)	Concentration in µg/ml	Amount found by proposed method(µg/ml)	*% label claim
1	400mg(Equivalent weight 0.1735g)	10	9.962	99.62
		20	20.174	100.87
		30	29.982	99.94

***Each value is a mean of six observations**

Table 1.6 : Recovery studies

Label	Amount of Drug added(std.solutions)	*%Recovery	%RSD
80%(320mg)	10 µg/ml	104.1%	0.3429
100%(400mg)	20 µg/ml	99.32%	0.1257
120%(420mg)	30 µg/ml	91.59%	0.2478

*** Each value is a mean of six observations**

Method –B

Preparation of stock solution:

Working standard of Pentoxyphylline 100mg was taken and weighed accurately and transferred to an 100ml volumetric flask, dissolve and make up the volume with 0.1N NaOH to obtain concentration of 1000µg/ml(solution A) from solution A pipette out 10ml of solution into 100ml volumetric flask and make up the volume 100ml with 0.1N NaOH. The resulting solution has a concentration 0f 100mcg/ml (solution-B)

Determination of λ max:

From the stock solution 25 µg/ml solution was prepared and absorbance was measured at different wavelengths, maximum absorbance was identified as 271nm. The results are shown in Table 2.1-2.3 & Fig2.1.

Preparation of calibration curve:

From stock solution B pipette out 0.5, 1, 1.5, 2, 2.5 and 3ml was transfer in to a 10ml volumetric flask and diluted with 0.1N NaOH to get a concentration of 5, 10, 15, 20, 25 and 30µg/ml respectively. The diluted solutions were measured at 271nm.The results are shown in Table 2.4 & Fig2.2.

Analysis of Formulation:

Ten tablets of branded Trental, label claim 400mg of Pentoxyphylline was weighed and average weight was determined and finely powder the tablets. 0.175g of powder sample was taken into 100ml volumetric flask and dissolved in 0.1N NaOH and make up the volume up to the mark. The solution was filtered through Whatmann filter paper No: 42 and required dilution was made to obtain a final concentration of 100mcg/ml. Appropriate aliquots were taken in such a way that the final concentration in 10ml volumetric flask were within the range of calibration curve. Measure the absorbance at 271nm for testing the drug. The results are shown in Table 2.5.

Recovery studies:

In order to ensure the reliability and stability of the proposed method, recovery studies were carried out. It was done by mixing known quantity of standard drug with formulation sample and the content were reanalysed by proposed method. To a quantity of formulation equivalent to 10mg Pentoxyphylline was added at 80%, 100% and 120%. This was extracted, diluted and reanalyzed as per the formulation procedure. Absorbance was noted at respective wavelength. Recovery studies were repeated for six times and results are shown in Table 2.6.

Validation of Method (ICH guidelines, 2005)

The method was validated with reference to accuracy, precision, LOD and LOQ

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of drug to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods.

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for six times.

LOD and LOQ

The limits of detection and quantitation, LOD and LOQ, were calculated by use of the equations LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where σ is the standard deviation of the blank and S is the slope of the calibration plot.

The results of validation parameters are shown in table 1.7&2.7.

Table 2.1- Determination of λ_{max} of Pentoxyphylline (method B)

Concentration	Wavelength(nm)	Absorbance
10 µg/ml	**250**	**0.285**
	300	**0.185**
	350	**0.128**

Table 2.2- Determination of λ_{max} of Pentoxyphylline (method B)

Concentration	Wavelength(nm)	Absorbance
10 µg/ml	**260**	**0.359**
	270	**0.401**
	280	**0.346**
	290	**0.292**
	300	**0.284**

Table 2.3- Determination of λ_{max} of Pentoxyphylline (method B)

Concentration	Wavelength(nm)	Absorbance
10 µg/ml	267	0.365
	268	0.372
	269	0.380
	270	0.399
	271	0.404
	272	0.394
	273	0.376
	274	0.332
	275	0.328
	276	0.302
	277	0.292
	278	0.286
	279	0.264
	280	0.256

Fig:2.1-Determination of λ max of Pentoxyphylline(method B)

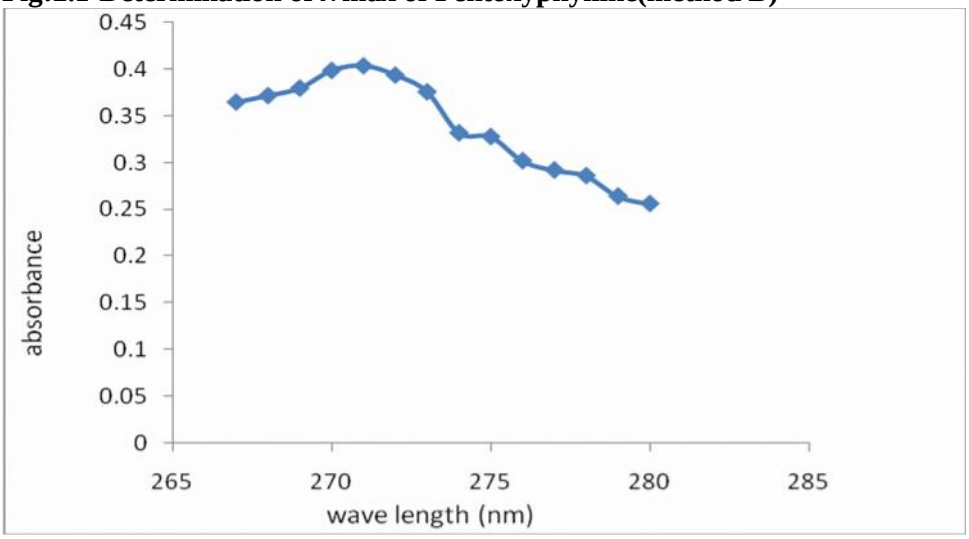


Table 2.4 Calibration Curve of Pentoxyphylline (method B)

S.No	Concentration	Absorbance
1	5	0.142
2	10	0.304
3	15	0.435
4	20	0.584
5	25	0.741

Fig:2.2- calibration curve for Pentoxyphylline (method B)

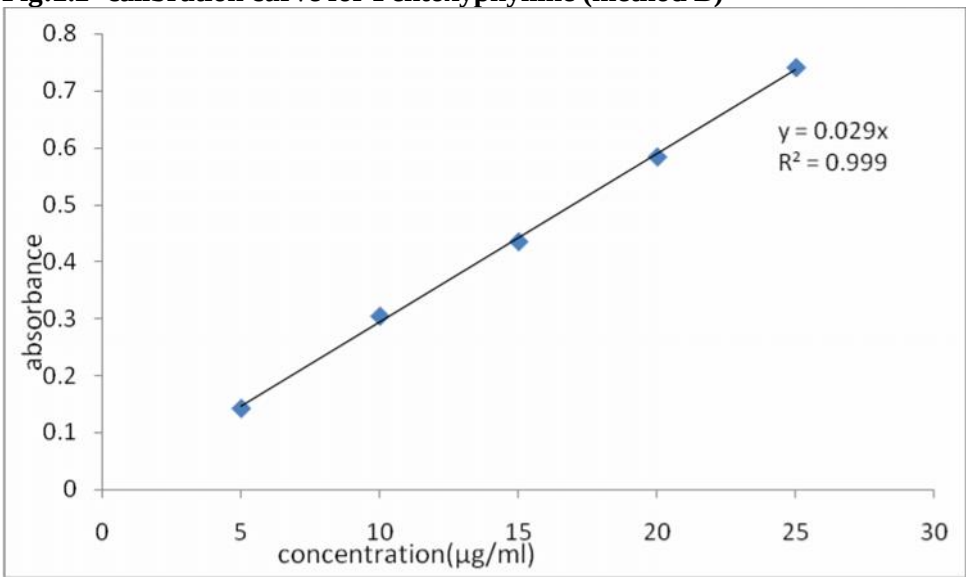


Table 2.5: Analysis of Formulation

S.No	Amount present in(mg/tab)	Concentration in µg/ml	Amount found by proposed method(µg/ml)	*%label claim
1	400mg(Equivalent weight 0.1735g)	10	9.986	99.86
		20	19.26	96.33
		30	29.77	99.23

*** Each value is a mean of six observations**

Table 2.6: Recovery studies

Label	Amount of Drug added(std.solutions)	*%Recovery	%RSD
80%(320mg)	10 µg/ml	107.25%	0.0027
100%(400mg)	20 µg/ml	102.45%	0.0014
120%(420mg)	30 µg/ml	99.05%	0.0045

*** Each value is a mean of six observations**

Table 1.7: Summary of validation of Parameters

Parameters	Method-A
Linearity range (µg/ml)	5-30
Correlation coefficient(r)	0.9997
Y-intercept	-0.004536
Slope	0.02866
Standard deviation(SD)	0.3097
Standard Error(SE)	0.1171
Limit of Detection(LOD)(mcg/ml)	0.30538
Limit of Quantification(LOQ)(mcg/ml)	0.9254
Intraday(%RSD)	0.0075(15mcg/ml)
Interday(%RSD)	0.0075(20mcg/ml)
Repeatability(%RSD)	0.0024(25mcg/ml)
Accuracy(By Recovery Studies)	91-104%

Table 2.7: Summary of validation of Parameters

Parameters	Method-B
Linearity range (µg/ml)	**5-25**
Correlation coefficient(r)	**0.9578**
Y-intercept	**0.1028**
Slope	**0.02573**
Standard deviation(SD)	**0.2512**
Standard Error(SE)	**0.4567**
Limit of Detection(LOD)(mcg/ml)	**0.3380**
Limit of Quantification(LOQ)(mcg/ml)	**1.0242**
Intraday(%RSD)	**0.0060(20mcg/ml)**
Interday(%RSD)	**0.0029(15mcg/ml)**
Repeatability(%RSD)	**0.0062(15mcg/ml)**
Accuracy(By Recovery Studies)	**99-107%**

Results and Discussion:

Estimation of Pentoxyphylline in formulation carried out by UV spectroscopy using different methods in optimised conditions, the percentage of Pentoxyphylline in tablet and the result of analysis shows that the amount of Pentoxyphylline was good agreement with label claim of the formulation. All the validation and quantification parameters were carried out and the comparative results are shown. Linearity range for method A is 5-30 µg/ml and 5-25 µg/ml for method B. Correlation coefficient(r) for method A is 0.9997 and 0.9578 for method B. Limit of detection for method A is 0.30538mcg/ml and 0.3380 for method B. Limit of quantification for method A is 0.9254mcg/ml and 1.0242 for method B. % RSD for intraday precision for method -A was found to be 0.0075 and 0.0060 for method B. % RSD for interday precision for method A was found to be 0.0075 and 0.0029 for method B.% RSD for repeatability was found to be 0.0024 for method A and 0.0062 for method B. Accuracy for method A was found to be 91-104% and 99-107% for method B. The proposed methods for the quantification of Pentoxyphylline in tablet were simple, precise, accuracy, rapid and sensitive. The method are linear in the concentration range reported the developed methods are free form interference due to excipients present in tablets and can be used for routine quantitative estimation of Pentoxyphylline in tablets.

Conclusion:

Literature survey reveals that some methods are reported for determination of Pentoxyphylline. To our knowledge there is no method are report for determination of Pentoxyphylline by spectrophotometry.

The scope and objective of present work is to optimise the spectroscopic conditions to develop U.V methods for the estimation of the drug and same is validated.

Two, simple, accurate and precise methods for estimation of Pentoxyphylline have been described. First method employs estimation by using distilled water as solvent and absorbance at 275nm. Second method involves estimation by using NaoH as solvent and measuring absorbance at 271 nm. Recovery range for method A was 91.5-104% and for method B was 99-107% with the percentage RSD values are NMT 2.It indicates the accuracy of the developed methods and there is no interactions of additives present in the formulations.

So the proposed methods (Method-A&B) were successfully applied for estimation of pentoxyphylline in tablet formulation.

References

1. H.R. Dettelbach and D.M. Aviado, J. Clin. Pharmacol., 25, 8 (1985) .
2. M. Abramowicz, Med. Lett. Drugs Ther., 26, 103 (1984).
3. B. Beermann, R. Ings, J. Mansby, J. Chamberlain, and A. McDonald, Clin. Pharmacol. Ther., 37, 25 (1985) .
4. D.M. Aviado and J.M. Porter, Pharmacotherapy, 4, 297 (1984).
5. J.L. Ambrus, J.M. Anain, S.M. Anain, P.M. Anain, S. Stadler, P. Mitchell, J.A. Brobst, B.L. Cobert, and J.P. Savitsky, Clin. Pharmacol. Ther., 48, 50 (1990) .
6. R. Muller and F. Lehrach, Curr. Med. Res. Opin., 7, 253 (1981.)
7. C.D. Nicholson, Psychopharmacology, 102, 147 (1990).
8. R.V. Smith, E.S. Waller, J.T. Doluisio, M.T. Bauza, S.K. Puri, I. Ho, and H.B. Lassman, J. Pharm. Sci., 75, 178 -82(1986).
9. A. Mancinelli, S. Pace, A. Marzo, and E. Arrigoni Martelli, J. Chromatogr., (1992) 575, 101.
10. G. Musch, T. Hamoir, and D.L. Massart, J. Chromatogr., 495, 215 (1989).
11. J. Szymura-Oleksiak, A. Ślósarczyk, A. Cios, B. Mycek, Z. Paszkiewicz, S. Szklarczyk, and D. Stankiewicz, Ceramics Int.,27, 762 (2001).
12. D.A. Chivers, D.J. Birkett, and J.O. Miners, J. Chromatogr., 225, 261 (1981).
13. J.W. Wong, K.H. Yuen, and K.K. Peh, J. Chromatogr. B, 716, 387 (1998).
14. D.R. Luke and M.L. Rocci, J. Chromatogr., 374, 191 (1986).
15. A. Garnier-Moiroux, J.M. Poirier, and G. Cheymol, J. Chromatogr., 416, 183 (1987).

*****