CHAPTER-7

Pentoxifylline

7.1 A survey of literature for Pentoxifylline indicated the estimation reported by the following methods, Viz., High-performance liquid chromatography electro spray ionization mass spectrometry, High-performance liquid chromatography , Capillary isotachophoresis.Gas chromatography-mass spectrometry, UV Spectro photometric method , Micellar electrokinetic chromatography, Gas chromatography-mass spectrometry. The brief information on above analytical methods are follows.

Wong JW,et al [202] have reported Simple high-performance liquid chromatographic method for determination of pentoxifylline in human plasma. Sastry CSP et al [203] have reported determination of pentoxifylline in pharmaceutical formulations using iodine as oxidizing agent.Dyke TM. et al [204] have reported Detection and determination of theobromine and caffeine in urine after administration of chocolate-coated peanuts to horses. Bhoir IC. et al [205] have reported Separation and estimation of seven vasodilators using packed column supercritical-fluid chromatography. Engelhart DA et al [206] have reported Diltiazem and pentoxifylline determination in postmortem specimens. Proksa,-B [207] have reported Separation of 1-alkyl-3,7-dimethylxanthines by capillary electrophoresis.

Liu ZY et al [208] have reported Studies of the release of long-lasting pentoxifylline sustained release tablets. Meyyanathan SN et al [209] have reported Spectrophotometric determination of pentoxifylline in its dosage forms. Korman M. et al [210] have reported Application of micellar electrokinetic chromatography to the quality control of pharmaceutical formulations: the analysis of xanthine derivatives.Marko V M et al [211] have reported Study of the solid-phase extraction of pentoxifylline and its major metabolite as a basis of their rapid low concentration gas-chromatographic determination in serum.

Mancinelli A et al [212] have reported Determination of pentoxifylline and its metabolites in human plasma by high-performance liquid chromatography with solid-phase extraction. Bauerova K et al [213] have reported Determination of pentoxifylline in serum by high-performance thin-layer chromatography.

Lockemeyer MR et al [214] have reported Analysis of pentoxifylline [oxpentifylline] in rabbit plasma using a Hisep high-performance liquid chromatography

column.Zarapkar SS et al [215] have reported Determination of pentoxi fylline in pharmaceutical preparations using gas chromatography .Sadana GS et al [216] have reported Quantitative high-performance liquid chromatographic determination of pentoxifylline in pharma -ceutical dosage forms. Sane RT et al [217] have reported High-performance liquid chromatographic determination of pentoxifylline in pharmaceuticals.

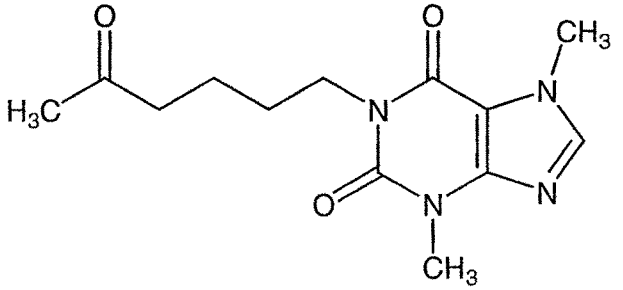
Musch G. et al [218] have reported Determination of pentoxifylline and its 5-hydroxy-metabolite in human plasma by solid-phase extraction and high-performance liquid chromatography with ultra-violet detection. Morton MR et al [219] have reported Lack of theophylline assay interference from pentoxifylline and its metabolites.Lambert WE. et al [220] have reported Simultaneous determination of pentoxifylline and three metabolites in biological fluids by liquid chromatography.Grasela DM et al [221] have reported High-performance liquid-chromatographic analysis of pentoxifylline and 1-(5-hydroxy hexyl)-3,7-dimethylxanthine in whole blood.

Garnier-Moiroux et al [222] have reported High-performance liquid-chromatographic determination of pentoxifylline and its hydroxy-metabolite in human plasma.Luke DR et al [223] have reported determination of pentoxifylline and a major metabolite, 3,7-dimethyl-1-(5-hydroxyhexyl) xanthine, by high-performance liquid chromatography.VonStetten O. et al [224] have reported Direct measurement of pentoxifylline and its hydroxy-metabolite from plasma using HPLC with column-switching techniques.Smith RV et al [225] have reported determination of pentoxifylline and its major metabolites in microbial extracts by thin-layer and high-performance liquid chromatography.
Chivers DA et al [226] have reported Simultaneous determination of pentoxifylline and its hydroxy-metabolite in plasma by high-performance liquid chromatography.

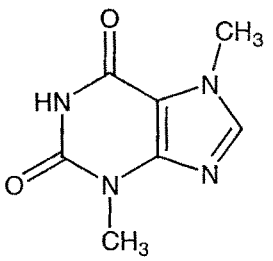
Even though there are several methods reported to estimate pentoxiphylline from plasma samples as well as matrices but no where sertraline separations and estimations from its related compound and process impurities are reported. Hence we have under taken this problem for current work. Developed HPLC method are validated thoroughly to check the suitability and corrective ness as per ICH guidelines

7.2 Method development for the analysis of related substances in Pentoxifylline by HPLC

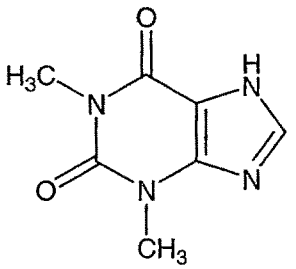
Pentoxifylline and its related compounds & process impurities structures are given below.



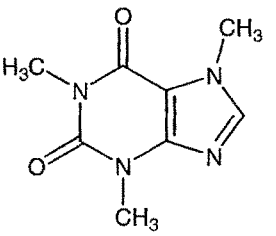
Pentoxifylline STR-10



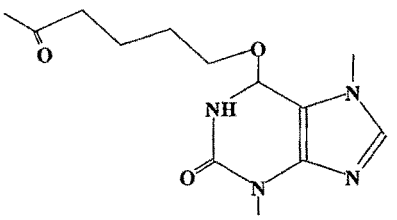
Theobromine STR-11



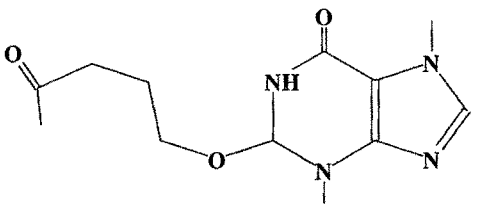
Theofylline STR-12



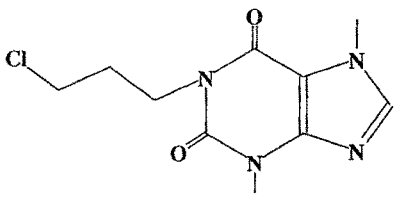
Caffiene STR-13



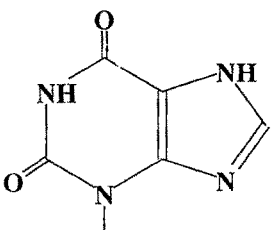
Impurity C[1] STR-14



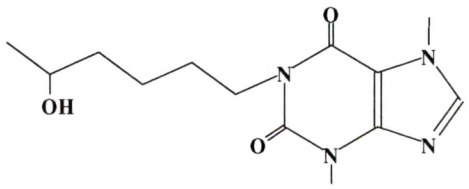
Impurity C STR-15



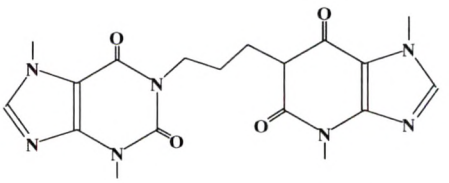
Impurity A STR-16



3-Methylxanthine STR-17

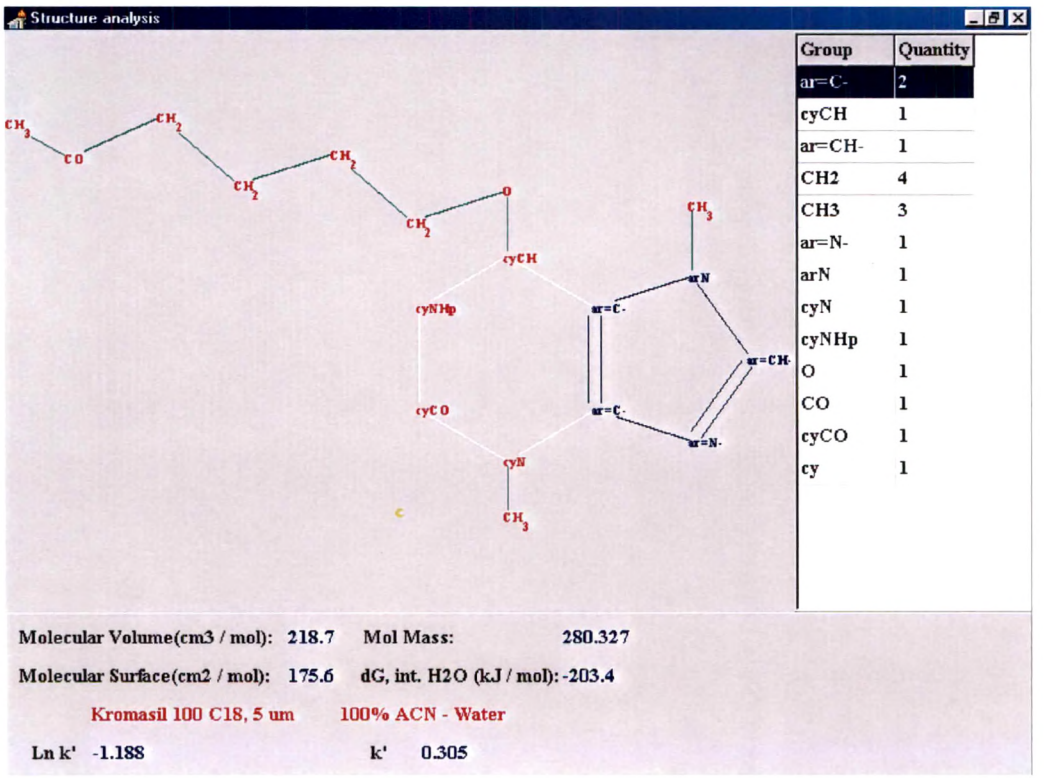


Impurity D STR-18

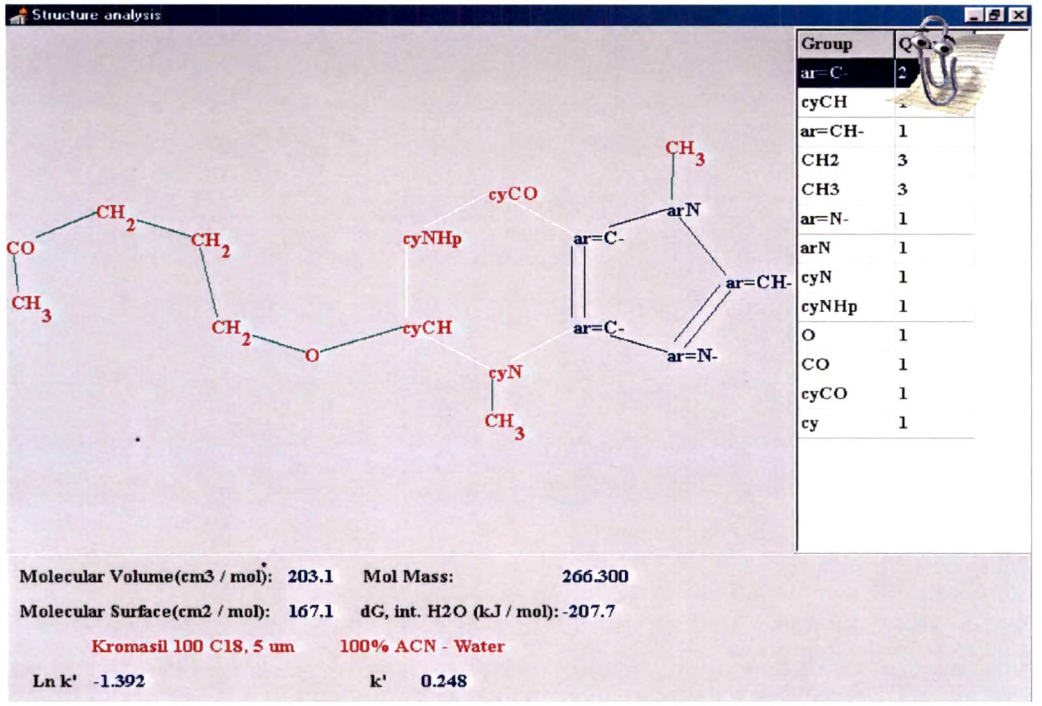


Impurity B STR-19

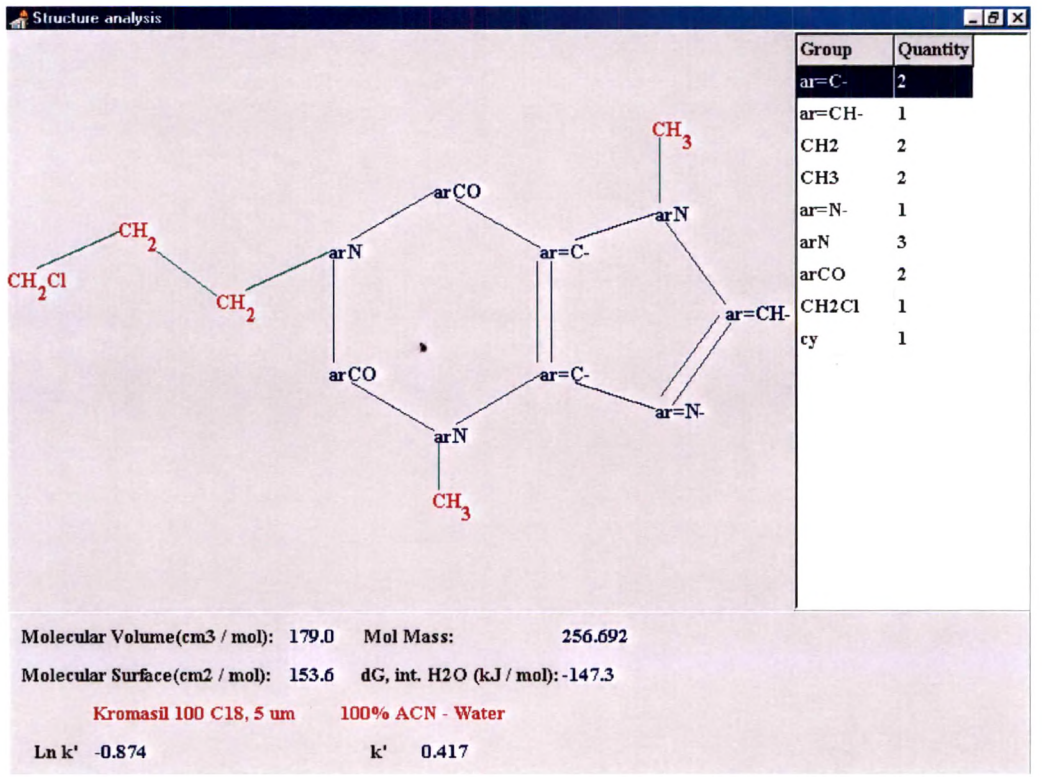
All structures are loaded into chromsword HPLC method development software to deduce structure analysis for method development. Figure-43 to 52 shows Pentoxifylline and its related compounds & process impurities structure analysis charts .



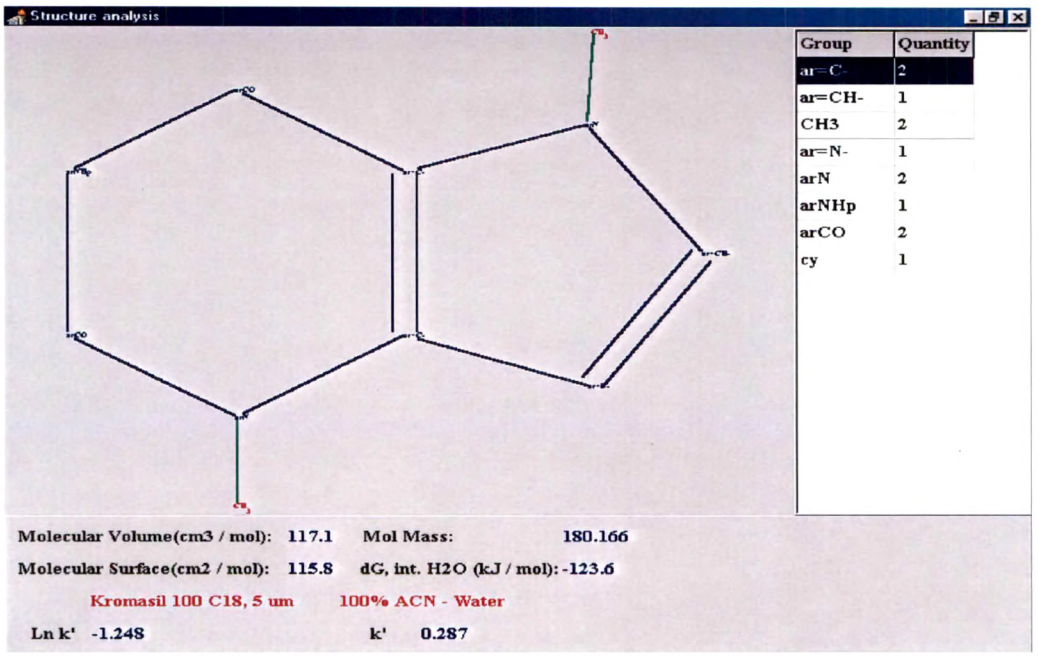
Impurity C[1] structure analysis Figure-43



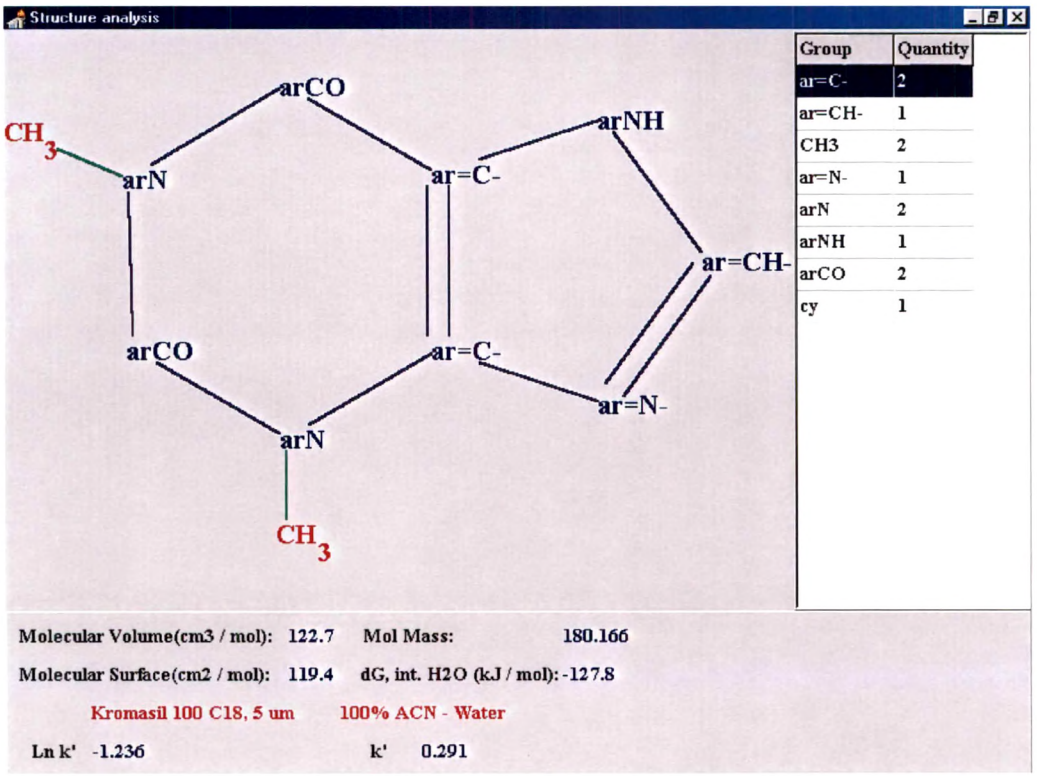
Impurity C structure analysis Figure-44



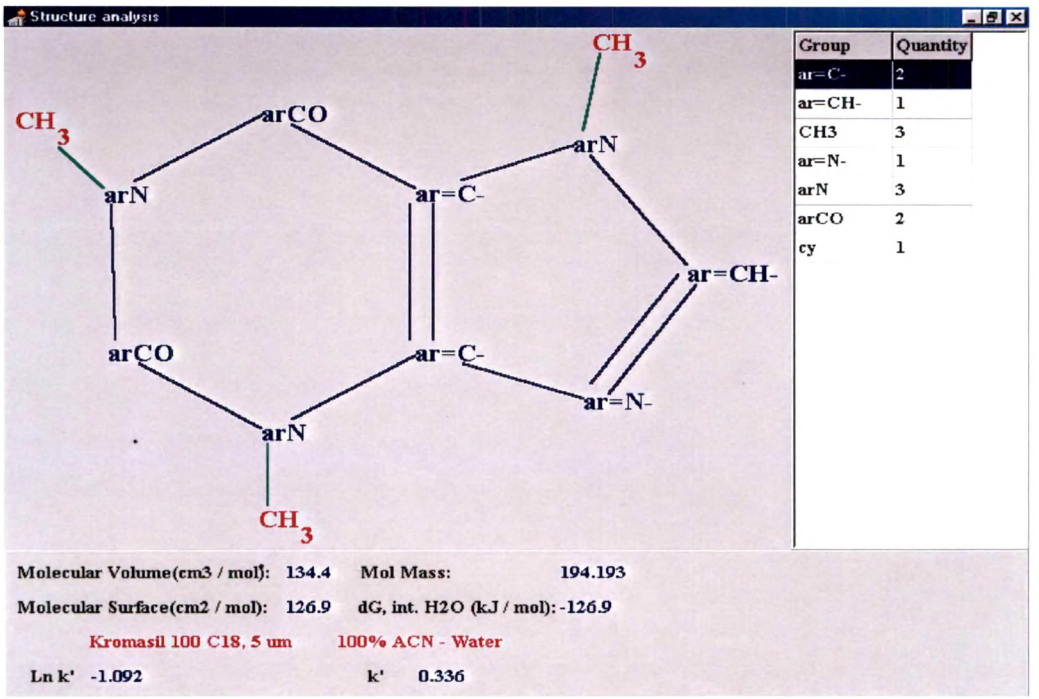
Impurity A structure analysis Figure-45



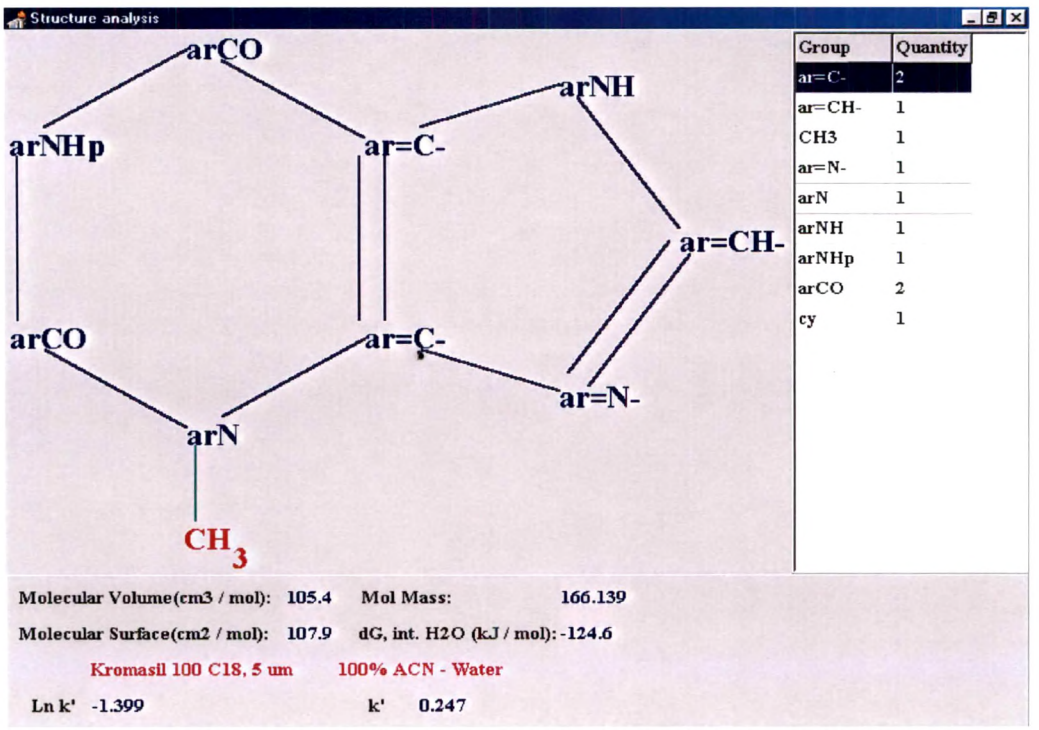
Theobromine structure analysis Figure-46



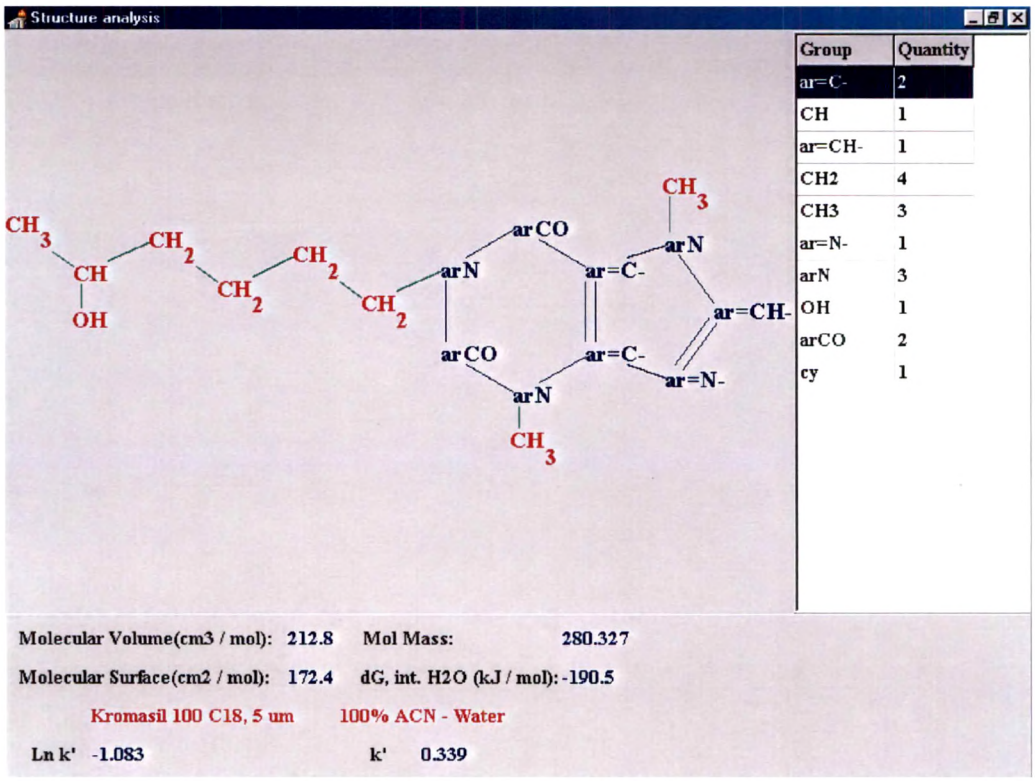
Theofylline structure analysis Figure-47



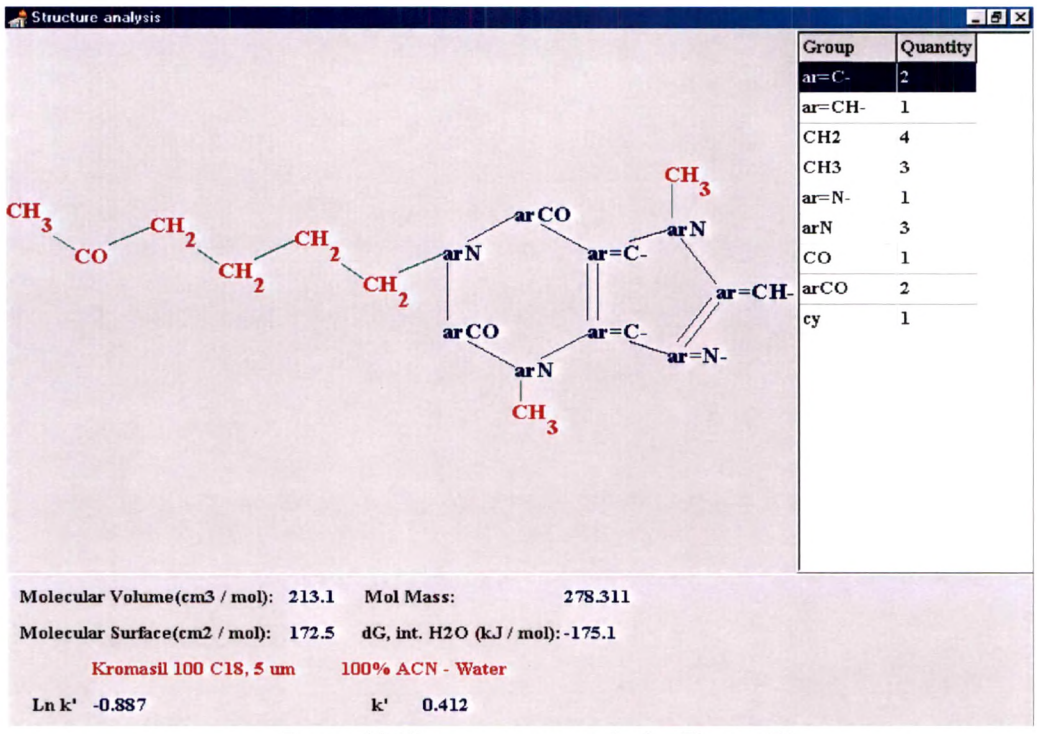
Caffiene structure analysis Figure-48



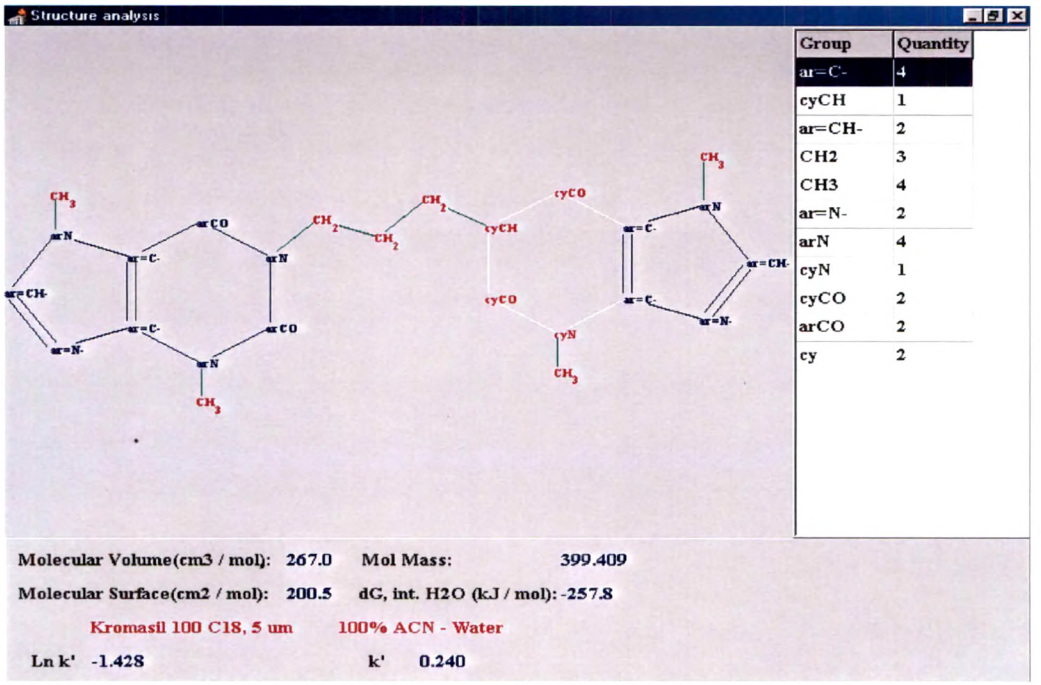
3-Methyl Xanthine structure analysis Figure-49



Impurity D structure analysis Figure-50

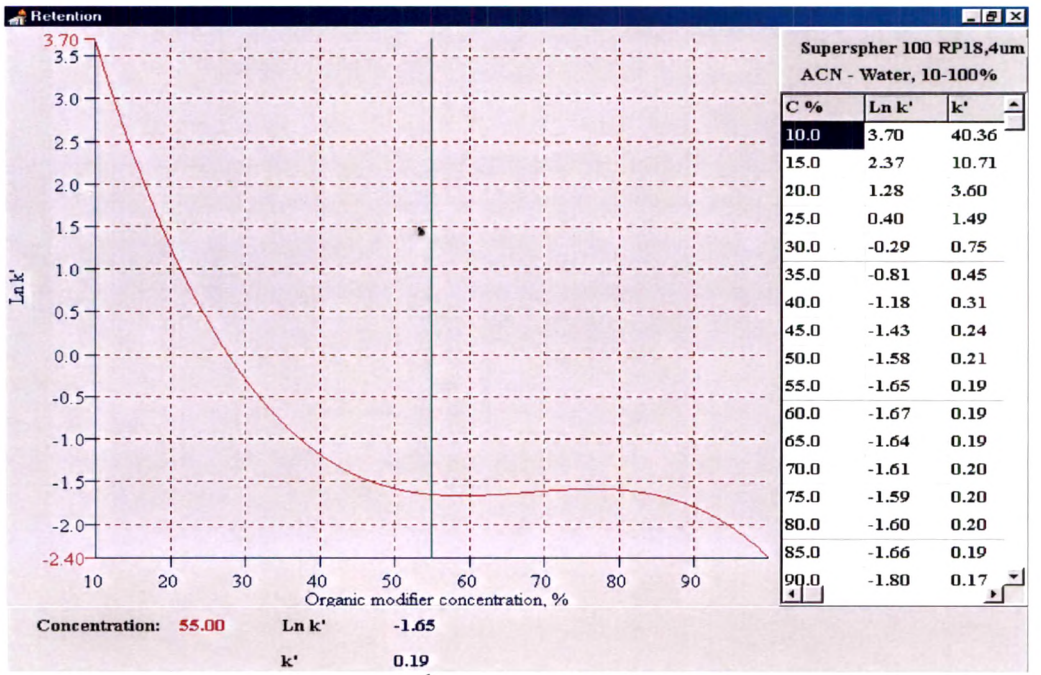


Pentoxifylline structure analysis Figure-51

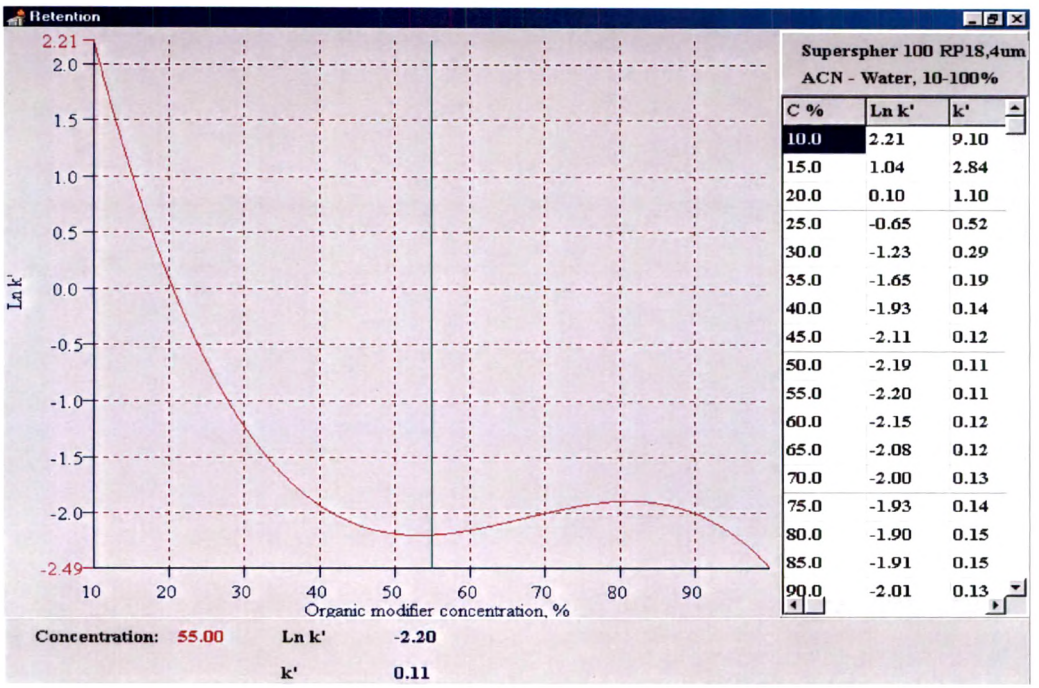


Impurity B structure analysis Figure-52

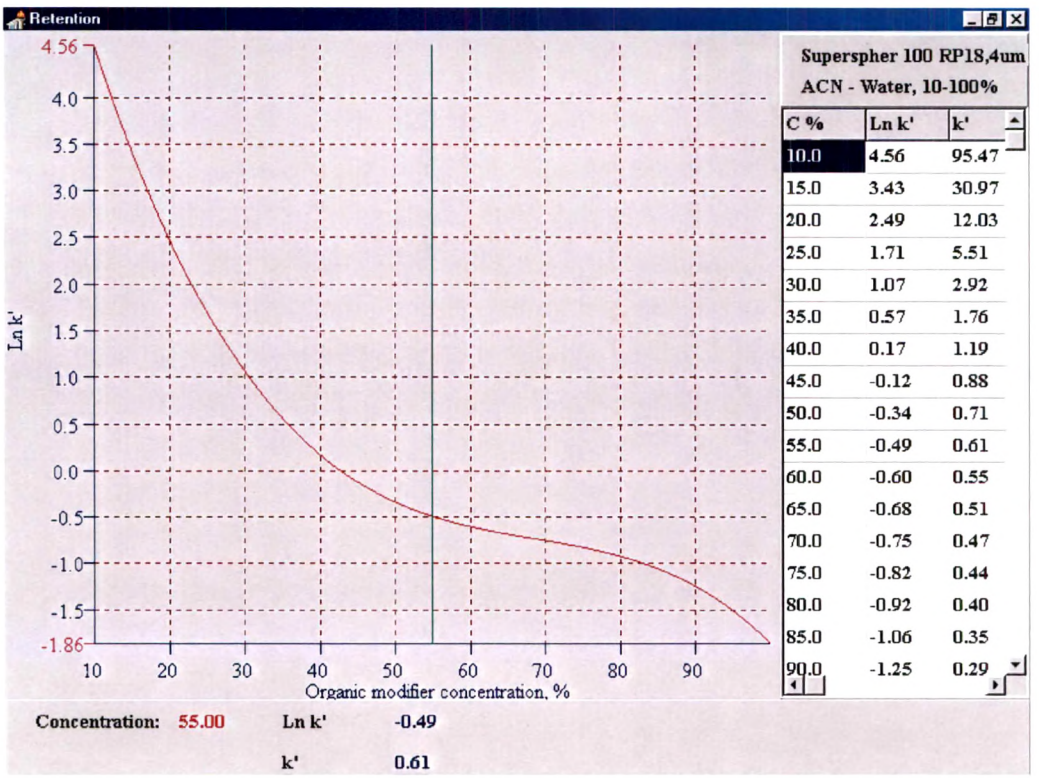
Figure-53 to 62 shows Pentoxifylline and its related compounds & process impurities retention analysis charts .



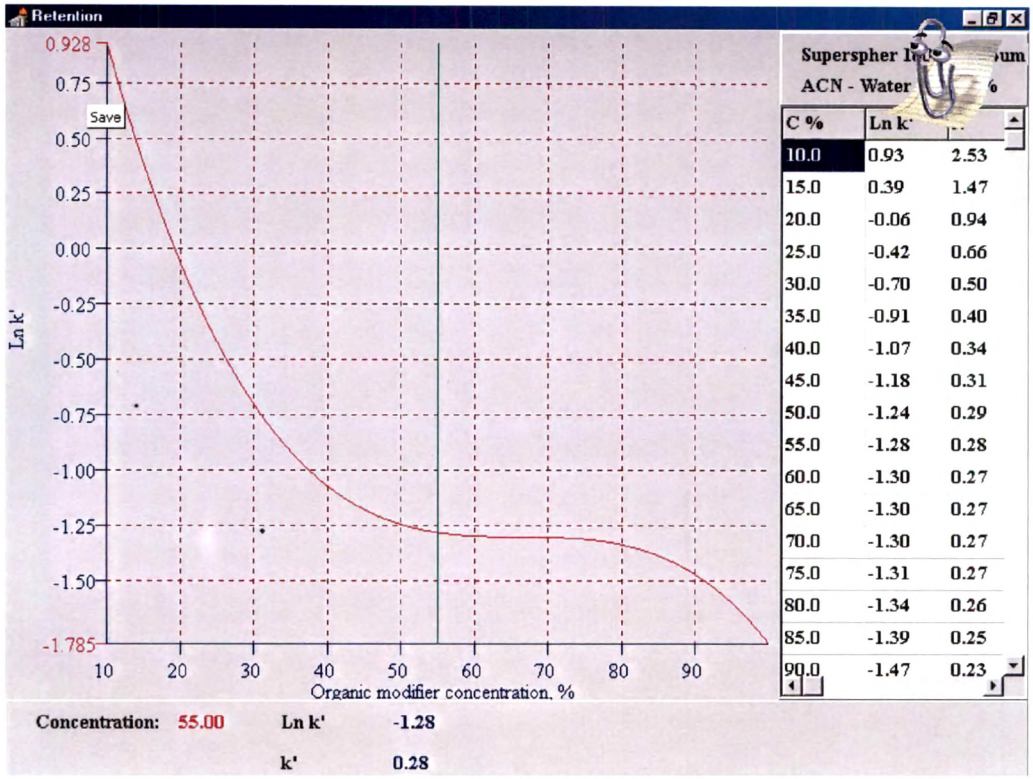
Impurity C^{1} retention analysis Figure-53



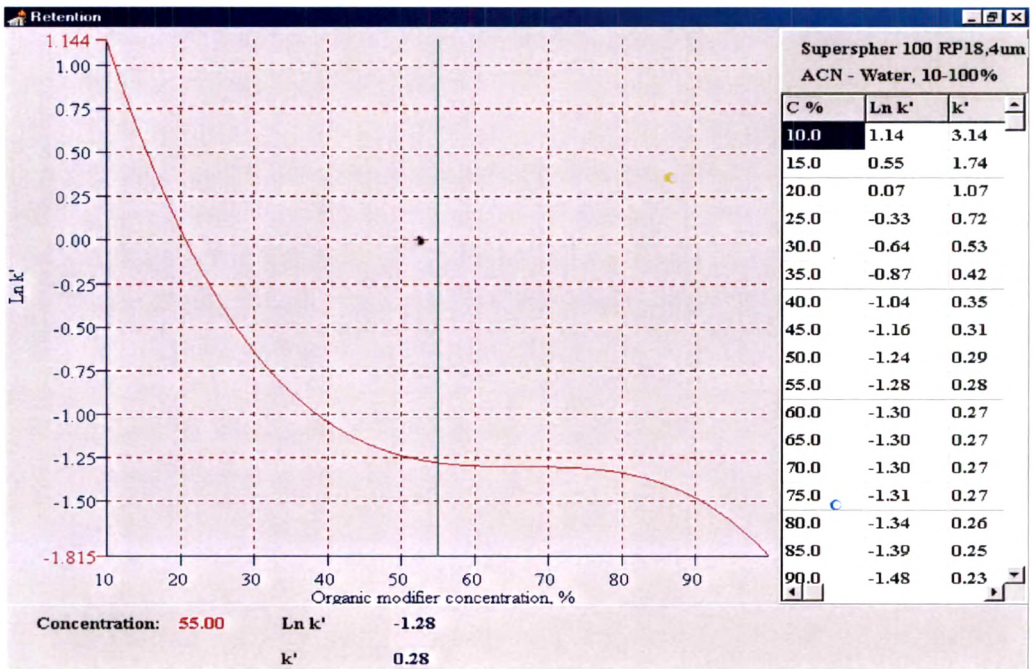
Impurity C retention analysis Figure-54



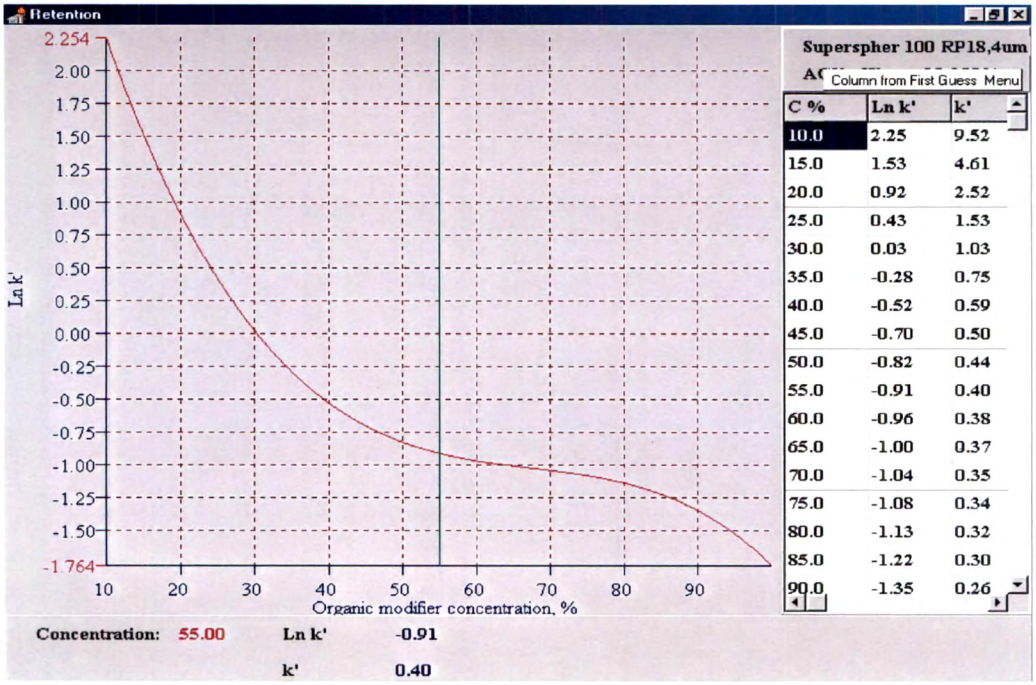
Impurity A retention analysis Figure-55



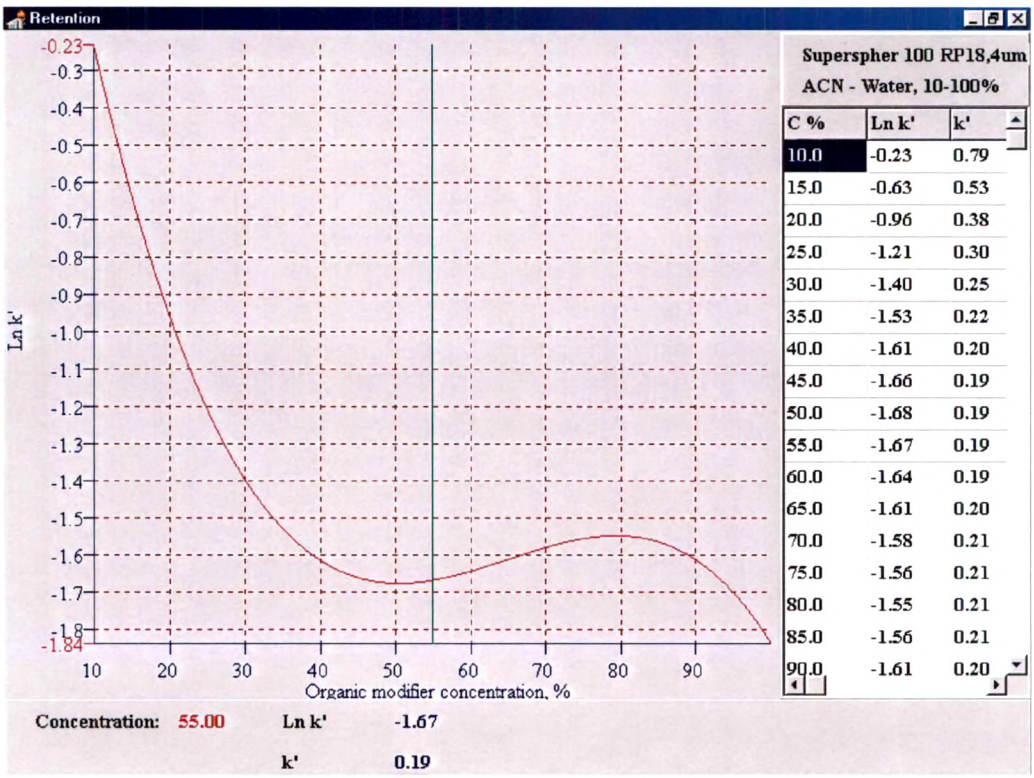
Theobromine retention analysis Figure-56



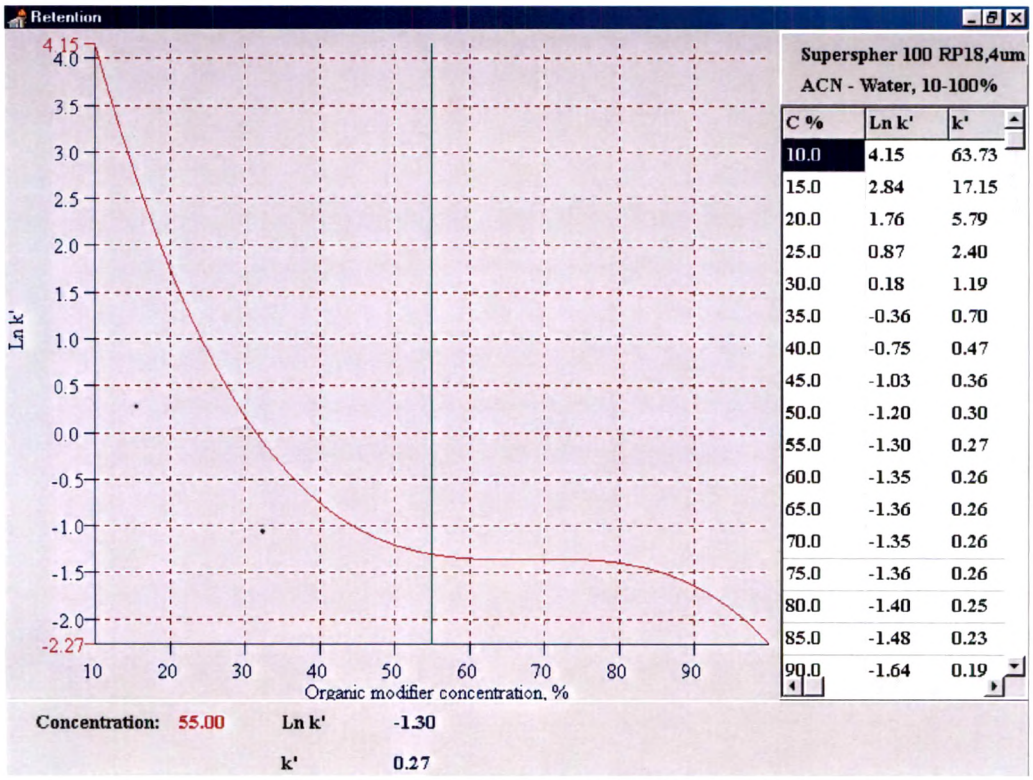
Theofylline retention analysis Figure-57



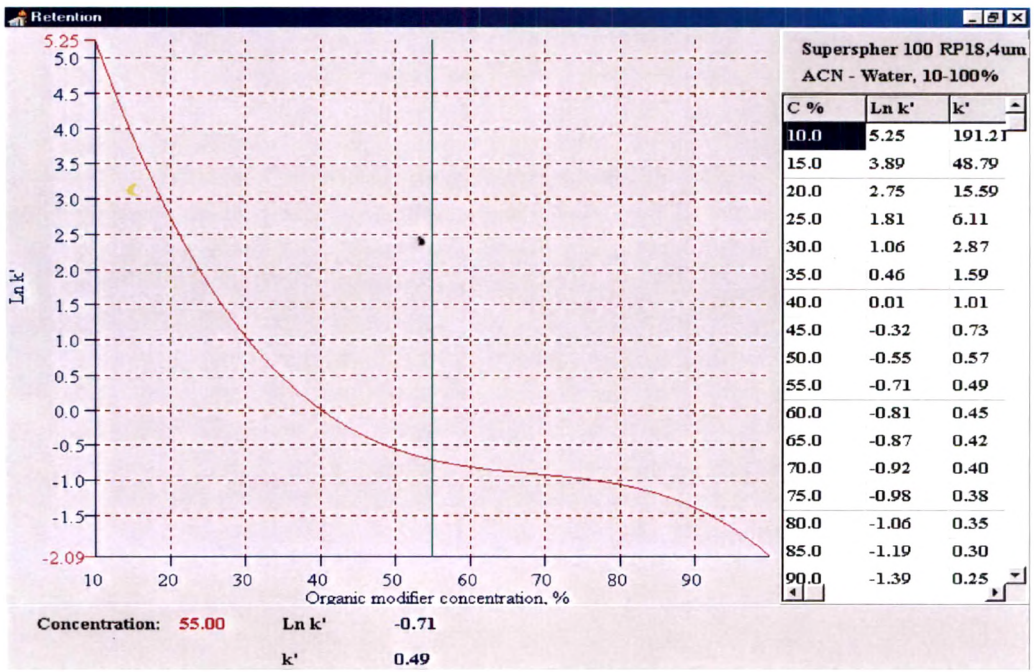
Caffiene retention analysis Figure-58



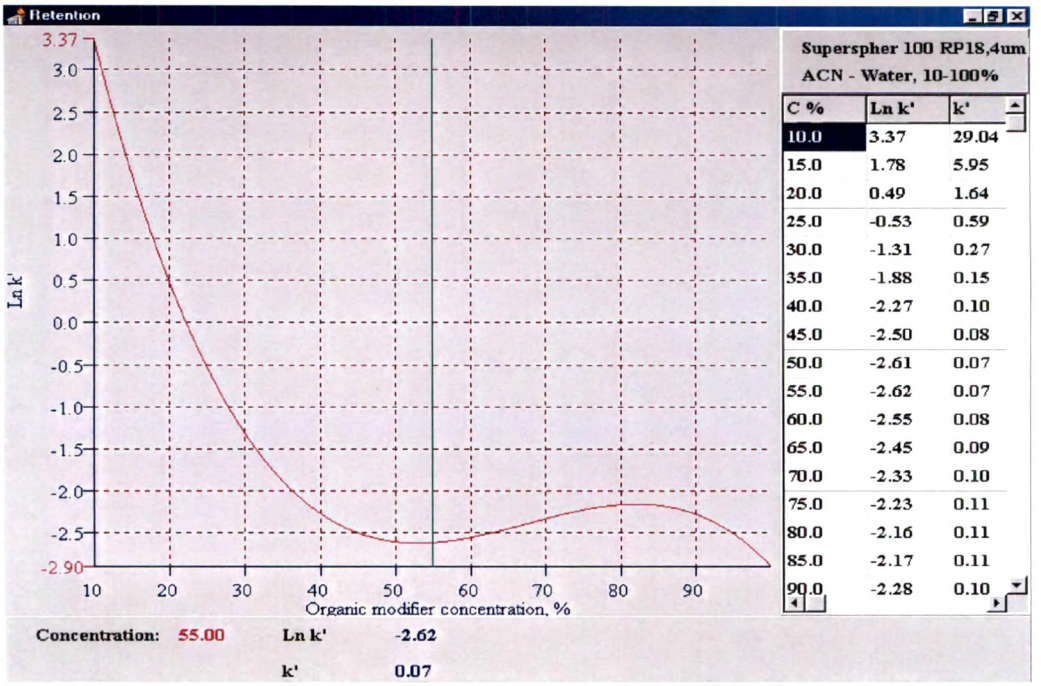
3-Methyl Xanthine retention analysis Figure-59



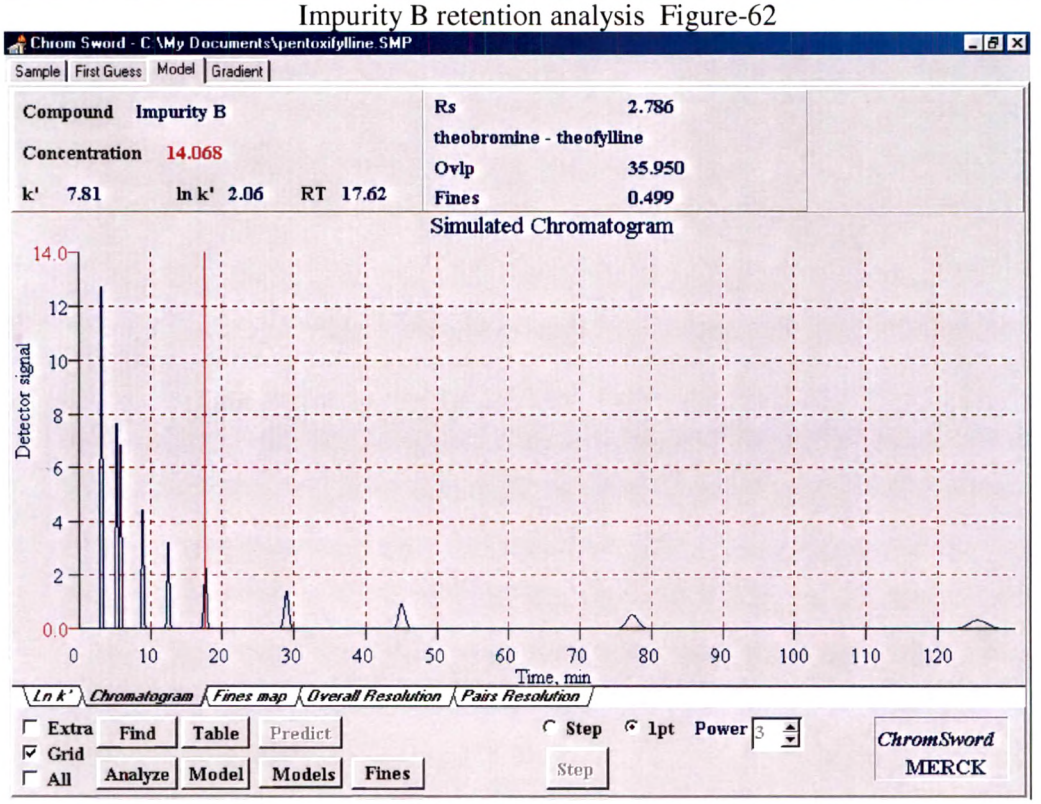
Impurity D retention analysis Figure-60



Pentoxifylline retention analysis Figure-61



Impurity B retention analysis Figure-62



Simulated chromatogram Figure-63

Figure 63 shows the best possible simulated chromatogram. Chromatogram indicated theophylline and theobromine are eluting closely and two impurities are eluting at 78 min and 125.Hence gradient analysis was chosen to resolve all impurities within reasonable run time. Wavelength 280 nm was selected to monitor all impurities. Method is optimized and given below.

Analytical Method :

Reagents and chemicals :

1) Perchloric acid : AR grade
2) Acetonitrile : HPLC grade
3) Methanol : HPLC grade
4) Water : Milli-Q grade

Buffer solution :Transfer 0.8 ml. of perchloric acid (70%w/v) into a 1000ml volumetric flask Dilute upto the mark with water

Mobile phase A : Use Buffer solution as such. Filter and degas prior to use.
Mobile phase B : Mix 150 volumes of acetonitrile ,25 volumes of methanol and 25 volumes of tetrahydrofuran Filter and degas prior to use.

Diluent : Prepare a mixture containing methanol and water in the ratio of 1:1

System suitability:

Solution 1 :Transfer about 10 mg, accurately weighed, each of 3-methylxanthine (3-MEX) and theobromine (THB) into a 10 ml volumetric flask. Dissolve in about 2 ml of 0.2 N aqueous NaOH (sonicate if necessary). Dilute to volume with diluent (1000 µg/ml each of 3-MEX and THB).

Solution 2 :
Transfer about 10 mg, accurately weighed, each of theophylline (THP), caffeine, Imp. A, Imp.B, Imp.C, Imp D and pentoxifylline into a 10 ml volumetric flask. Dissolve in about 5 ml of diluent (sonicate if necessary) and dilute to volume with diluent [1000 µg/ml each of theophylline (THP), caffeine, Imp. A, Imp.B, Imp.C, Imp.D and pentoxifylline].

Pipette out 1.0 ml each of solution 1 & solution 2 into a 10 ml volumetric flask. Dilute to volume with diluent.

Sample preparation :

Transfer about 50 mg accurately weighed pentoxiphylline sample in to a 50 ml volumetric flask. Dissolve in and dilute up to mark with water (1000 µg/ml).

Standard solution preparation :

Prepare a solution containing about 2 µg/ml of each impurity and pentoxiphylline individually (0.2% of sample concentration).

Chromatographic conditions :

Use a suitable high pressure liquid chromatography system equipped with a UV detector set to 280 nm and a column of 250 mm x 4 6mm containing 5µ C18 packing material (suggested column Inertsil C-18, GL Science, Japan).

The system is also equipped to deliver the two phases in a programmed manner as shown in the Table below :

Total flow rate : 1.0 ml / min

Time (in min.)	Mobile Phase A (% v/v)	Mobile Phase B (% v/v)
0	98	2
20	80	20
40	80	20
41	98	2
50	98	2

Procedure: Inject 20 µl system suitability solution into the chromatograph set to above conditions and record the chromatograms upto 50 min. Test is valid only when the resolution between pentoxiphylline and Imp-D is not less than 1.0.

Inject 20 µl of standard solution preparation in triplicate into the chromatograph set to above conditions and record the chromatograms upto 50 min. Calculate the average area of individual component and RSD. Test is valid only when the RSD is not more than 5.0 %.

Inject 20 µl test preparation in duplicate in to the chromatograph set to above conditions and record the chromatograms upto 50 min. Calculate the amount of related

substances & process impurities using the formula given in calculations . Relative retention times of the impurities are :

Sr.No	Name of the component	Relative Retention time
1	3-Methyl Xanthine	0.40
2	Theobromine	0.48
3	Theophylline	0 56
4	Imp-A	0.64
5	Caffeine	0.66
6	Imp – C	0.84
7	Imp – B	0.94
8	Pentoxifylline	1.00
9	Imp – D	1.02

Calculations :

i) Calculate the percentage of Impurity, individually using the formula :

$$\frac{C_{im}}{C_S} \times \frac{r_s}{r_{im}} \times 100$$

Where :

C_{im} = Concentration of impurity individually in standard solution (µg/ml)

C_S = Concentration of sample solution (µg/ml)

r_s = Detector response for impurity individually in sample preparation

r_{im} = Detector response for impurity individually in standard solution

ii) Calculate the percentage of any other impurity, individually using the formula :

$$\frac{C_P}{C_S} \times \frac{r_s}{r_P} \times 100$$

Where .

C_P = Concentration of pentoxifylline in standard solution (µg/ml)

C_S = Concentration of sample solution (µg/ml)

r_s = Detector response for impurity individually in sample preparation

r_P = Detector response for pentoxifylline in standard solution

Limit of impurities :Any impurity, individually not more than 0.2 %.

7.3 Pentoxiphylline Validation Protocol

Purpose :The purpose of this document is to establish the precision, accuracy, linearity of detector response and ruggedness of the analytical method through number of scientific studies and discussions of the data

Scope :This method, upon validation, can be used for the analysis of related substances and process impurities in pentoxiphylline.

Analytical method: As per method given in section 7.2

7.3.1 The experiments designed to study are follows

a System suitability

b. Instrument precision

c. Identification of individual component

d. Solution stability

e. Method precision

f. Linearity and range

g Accuracy

h. Minimum detection limit

i Minimum quantitation limit

j Ruggedness of the method

7.3.2 Stock Solutions :

Diluent : Prepare sufficient quantity by mixing 1 volume of methanol with 1 volume of water.

Solution A : Transfer about 10 mg, accurately weighed, 3-MEX into a 100 ml volumetric flask. Dissolve by adding 2 ml of 0.2N NaOH (sonicate, if necessary) and dilute upto mark with water (about 100 µg/ml).

Solution B : Transfer about 10 mg, accurately weighed, THB into a 100 ml volumetric flask. Dissolve by adding 2 ml of 0.2N NaOH (sonicate, if necessary) and dilute upto mark with water (about 100 µg/ml).

Solution C : Transfer about 10 mg, accurately weighed, THP into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution D : Transfer about 10 mg, accurately weighed, caffeine into a 100 ml volumetric flask. Dissolve in and dilute upto mark diluent (about 100 µg/ml).

Solution E : Transfer about 10 mg, accurately weighed, Imp.A into a 100 ml volumetric flask Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution F : Transfer about 10 mg, accurately weighed, Imp.B into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution G : Transfer about 10 mg, accurately weighed, Imp.C into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution H : Transfer about 10 mg, accurately weighed, Imp.D into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution I : Transfer about 10 mg, accurately weighed, pentoxifylline into a 100 ml volumetric flask. Dissolve in and dilute upto mark with diluent (about 100 µg/ml).

Solution J :Pipette out 5.0 ml each of impurity stock solution A to H into a 50 ml volumetric flask . Dilute to volume with diluent (10 µg/ml of each impurity)

Solution K :Pipette out 5.0 ml each of stock solution A to I into a 50 ml volumetric flask.Dilute to volume with diluent. (10 µg/ml of each impurity and 10 µg/ml of pentoxifylline.

Standard solution /Solution L: Pipette out 2.0 ml of solution K into a 10 ml volumetric flask Dilute to volume with diluent. (2 µg/ml of each impurity, individually and 2 µg/ml of pentoxifylline).

7.3.3 System suitability :

System Suitability solution :
Prepare a system suitability solution as mentioned under the procedure

i) Set up the system as mentioned under the chromatographic conditions.

I. Inject 20 µL of the system suitability solution in duplicate and record the chromatograms upto 50 min

II. Calculate the resolution between pentoxifylline and Imp D peak

Acceptance limit : The mean resolution factor R between pentoxifylline and Imp D peak is not less than 1 0

7.3.4 Identification

Inject 20 µL each of the impurity stock solutions A to I (about 100 µg/ml), individually and record the chromatograms upto 50 min. Note the retention time of each component for identification

7.3.5 Instrument precision

System precision solution :

Transfer about 100 mg, accurately weighed, pentoxifylline WRS into a 100 ml volumetric flask. Pipette out 2 0 ml each of Stock Solution A to H into it. Dissolve in and dilute upto mark with diluent. (100 µg/ml of pentoxifylline and 2 µg/ml of each of impurity individually).

i) Set up the system as mentioned under the chromatographic conditions.
ii) Inject 20 µL of the system precision solution six times and record the chromatograms upto 50 min.
iv) Calculate the relative standard deviation for the detector response for each component individually

Acceptance limit :RSD (%) of detector response for each component is not more than 5 0 %

7.3.6 Solution stability :

i) Inject 20 µl of the system precision solution in duplicate, periodically and record the chromatograms upto 50 min.

ii) Calculate the relative standard deviation of the detector response for each component individually over the entire period.

Acceptance limit : RSD (%) of detector response for each component is not more than 5.0 %

7.3.7 Method precision

i) Prepare a sample solution as directed under the procedure (about 1000 µg/ml).

ii) Set the chromatograhic conditions as mentioned under the method, inject 20 µl of the standard solution in duplicate and record the chromatograms upto 50 min.

iii) Inject 20 µl of **Standard Solution** in duplicate and record the chromatograms upto 50 min. Use this for calculations

iv) Inject 20 µl of the sample solution in duplicate and record the chromatograms upto 50 min

v) Calculate the amount of the impurities present in the sample

vi) Prepare six sets of this sample as directed under the method Spike impurities upto the target levels in each of the sample preparation.

vii) Inject 20 µl of each sample preparation in duplicate into the chromatograph set to the condition mentioned under the method and record the chromatograms upto 50 min .

viii) Calculate the amount of each impurity from the six sets Substract the amount of any impurity already present. Calculate the RSD of each impurity percentage (corrected values) from the six sets

Acceptance limit : RSD (%) of the calculated impurities in the six sets ≤ 5 0 %

7.3.7 Linearity and range :

i) Use solution K (mentioned under section 2.2) for preparing the following linearity solutions.

ii) Linearity solutions.

Table no.6.3.8.1 Dilution and concentration for Linearity study

Level	*% of target*	*Vol. of. Solution K*	*Final Dilution*	*Final concentration of each impurity, individually and pentoxifylline*
		ml	*ml*	*µg/ml*
L1	50	1.0	10.0	1.0
L2	75	1.5	10.0	1.5
L3	100	2.0	10.0	2.0
L4	125	2.5	10.0	2.5
L5	150	3.0	10.0	3.0

iv) Inject 20 µl each of the linearity solution, in triplicate, into the chromatographic system set to the conditions mentioned under the method and record the chromatograms upto 50 min.

v) Calculate the mean and RSD (%) of the detector responses for each linearity level individually of each component.

vi) Plot a graph of the concentration versus mean area count and perform mathematical regression analysis for each component individually.

Acceptance limits :

RSD (%) of area counts for individual components at each level ≤ 5.0 %

Plot of concentration versus detector response for each component is linear. The regression correlation coefficient (r^2) ≥ 0.99

7.3.8 Accuracy : Use the Solution J (10 µg/ml each of impurity as mentioned under section 2.2) for preparing the following solutions.

i) Prepare five sets for five level (70, 85, 100, 115 and 130% of target concentration) recovery study by transferring about 10 mg, accurately weighed, pentoxifylline standard into five 10 ml volumetric flasks separately. Pipette out appropriate volumes of Solution J as shown in the table below and dilute to volume with mobile phase.

(Solution J = 10 µg/ml of each impurity)

Table 7.3.9.1 Dilution and concentration for Accuracy study

Level	*% of target*	*Vol. of. Solution K*	*Final Dilution*	*Final concentration of each impurity, individually*
		ml	*ml*	*µg/ml*
R1	70	1.4	10.0	1.4
R2	85	1.7	10 0	1 7
R3	100	2.0	10.0	2.0
R4	115	2.3	10.0	2.3
R5	130	2.6	10.0	2.6

ii) Inject 20 µl of standard solution, prepared as mentioned in section 2 3, in triplicate and record the chromatograms upto 50 min. Calculate the mean area counts of the components in the standard solution.

iii) Inject 20 µl each of the recovery solution R1, R2, R3, R4 and R5 into the chromatograph in triplicate and record the chromatograms upto 50 mın.

iv) Calculate the mean and RSD (%) of the detector responses for each component from each recovery set.

v) Calculate the amount of each spiked ımpurity ın each set of the recovery sample and calculate the percentage recovery.

Acceptance limit :

Percentage recovery is not less than 95.0 and not more than 105.0 %.

RSD (%) of detector response for each component is ≤ 5.0 %.

7.3.9 Minimum Quantitation level and Minimum Detection level

i) Prepare a stock solution for this study by pipetting out 10 ml of Solution K (10 µg/ml each of impurity + pentoxifylline) into a 50ml volumetric flask. Dilute to volume with dıluent [2 µg/ml each component].(**Solution L**)

ii) Prepare subsequent dıluted solutions as shown ın the table below and inject them in triplicate and record the chromatograms upto 50 min.

iii) Calculate the RSD (%) of the triplicate injections for each level.

Table 7.3.9.1. Dilution and concentration for Limit of detection and limit of quantitation study

Level	*Vol. Of soln L (ml)*	*Final dilution (ml)*	*Final concentration of each impurity, individually (µg/ml)*
D1	5.0	10	1.000
D2	2 5	10	0.500
D3	1 0	10	0.250
D4	0 5	10	0.100
D5	0.2	10	0.050
D6	0 1	10	0.020
D7	0.1	25	0.010
D8	0.1	50	0.004

Acceptance limit :

The MQL of each component is the lowest concentration at which the RSD (%) of the triplicate injections ≤ 5.0 %

The MDL of each component is that concentration at which the detector shows a positive response

7.3.10 Ruggedness of the method :

A sample previously analysed for related substances is reanlaysed by another analyst independently by this method and the results are compared.

7.4 Experimental Data

7.4.1 Reagents and chemicals :

1 Perchloric acid (70% w/v) · AR grad (S.D. Fine-Chem)

2. Acetonitrile : HPLC grade (Ranbaxy)

3 Methanol : HPLC grade (Spectra Chem)

4 Water : Milli-Q grade

7.4.2 Working standards and sample :

Pentoxifylline WRS·# PNT/B/190 (SPARC - Baroda)

Impurity A : # OXP-Imp-A/lot-I (SPARC - Baroda)

Impurity B : # OXP-Imp-B/lot-I (SPARC - Baroda.)

Impurity C . # OXP-Imp-C/lot-I (SPARC - Baroda)

Impurity D	: # OXP-Imp-D/lot-I (SPARC - Baroda)
Theobromine	: # OXP-THB/lot-I (SPARC Baroda.)
Theofylline	. # OXP-THP/lot-I (SPARC Baroda.)
3-Methyl xanthine	: # OXP-3-MEX/lot-I (SPARC Baroda.)
Caffeine	: # OXP- Caffeine /lot-I (SPARC Baroda.)
Pentoxifylline	· # PNT/288(SPIL,Panoli)

7.4.3 Chromatographic system .

Column	4.6 mm x 25 cm, 5 µm, Inertsil C-18 (GL. Science, Japan)
Detector	UV-280 nm
Injection volume	20 µl

The system is also equipped to deliver the two phases in a programmed manner as shown in the table below :

Total flow rate : 1 0ml / min

Time (in min.)	Mobile Phase A (per cent v/v)	Mobile Phase B (per cent v/v)
0	98	2
20	80	20
40	80	20
41	98	2
50	98	2

- Two Shimadzu LC-10AT$_{vp}$ solvent delivery pumps with SIL-10AD$_{vp}$Autoinjector
- Shimadzu SPD-10A$_{vp}$ UV detector with CLASS vp software.

7.4.4 Mobile phase :

Mobile phase A

3 2 ml. of perchloric acid was transferred to a 5000 ml beaker. Add 4000 ml of water. Filtered and degassed This was used throughout the analysis

Mobile phase B

1500 volumes of acetonitrile ,250 volumes of methanol and 250 volumes of tetrahydrofuran were mixed in a beaker. Mixture was filtered and degassed and used for the analysis.

7.4.5 Stock Solutions :

Diluent : Prepared 2.0 lits. of diluent by mixing equal volumes of methanol and water. Filtered and degassed and used for solution preparations.

Solution A : 10.1 mg of 3-MEX was transferred into a 100 ml volumetric flask.. 2 ml of 0.2N NaOH was added to dissolve completely and diluted upto mark with diluent (101 µg/ml).

Solution B : 10.3 mg of THB was transferred into a 100 ml volumetric flask.. 2 ml of 0.2N NaOH was added to dissolve completely and diluted upto mark with diluent (103 µg/ml).

Solution C : 10.0 mg of THP was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (100 µg/ml).

Solution D : 10.0 mg of caffeine was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (100 µg/ml).

Solution E : 10.1mg of Imp-A was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (101µg/ml).

Solution F : 9.9 mg of Imp-B was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (99 µg/ml).

Solution G : 9.9 mg of Imp-C was transferred into a 100 ml volumetric flask. Dissolved in and diluted upto mark with diluent (99 µg/ml).

Solution H : 10.0 mg of Imp-D was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (100 µg/ml).

Solution I : 10.0 mg of pentoxifylline was transferred into a 100 ml volumetric flask.. Dissolved in and diluted upto mark with diluent (100 µg/ml).

Solution J :
5.0 ml each of impurity stock solution A to H were pipetted out into a 50 ml volumetric flask . Diluted to volume with diluent (10 µg/ml of each impurity).

Component	3-MEX	THB	THP	Caffeine	Imp. A	Imp B	Imp. C	Imp. D
Conc. (µg/ml)	**10.1**	**10.3**	**10.0**	**10.0**	**10.1**	**9.9**	**9.9**	**10.0**

Solution K :

5.0 ml each of stock solution A to I were pipetted out into a 50 ml volumetric flask. Diluted to volume with diluent (10 µg/ml of each impurity and 10 µg/ml of pentoxifylline).

Component	3-MEX	THB	THP	Caffeine	Imp. A	Imp. B	Imp.C	Imp.D	PNT
Conc. (µg/ml)	10.1	10.3	10.0	10.0	10.1	9.9	9.9	10.0	10.0

Standard solution /Solution L:
2.0 ml of solution K was pipetted out into a 10 ml volumetric flask. Diluted to volume with diluent. (2 µg/ml of each impurity, individually and 2 µg/ml of pentoxifylline).

7.4.6 System suitability :

System Suitability solution :

Solution 1 :9.8 mg 3-methylxanthine (3-MEX) and 9.9 mg theobromine (THB) were transferred into a 10 ml volumetric flask. Dissolved in about 2 ml of 0.2 N aqueous NaOH and diluted to volume with diluent.

Solution 2 : 10.2 mg theophylline (THP),9.8 mg caffeine, 9 9 mg Imp. A, 10.1 mg Imp.B,10.1 mg Imp.D and 10.2 mg pentoxifylline were transferred into a 10 ml volumetric flask. Dissolved in and diluted to volume with diluent

Solution 3 . 5.0 mg of Imp.C was transferred into a 5 ml volumetric flask. Dissolved in and dilute to volume with diluent Pipette out 1.0 ml each of solution 1 ,solution 2 & solution 3 into a 10 ml volumetric flask. Diluted to volume with diluent.

i) Set up the system as mentioned under the chromatographic conditions.

ii) Injected 20 µl of the system suitability solution in duplicate and recorded the chromatograms upto 50 min.

iii) Calculated the resolution between pentoxifylline and Imp.D peak.

Acceptance limit : The mean resolution factor R between pentoxifylline and Imp.D peak is not less than 1.0

7.4.7 Identification

Injected 20 µl each of the impurity stock solutions A to I (about 100 µg/ml), individually in duplicate and recorded the chromatograms upto 50 min. Noted the retention time of components for identification.

7.4.8 Instrument precision

System precision solution :

Transferred 100.1 mg, of pentoxifylline WRS into a 100 ml volumetric flask. Pipetted out 2.0 ml each of Stock Solution A to H into it. Dissolved in and diluted upto mark with diluent. (1000.1 µg/ml of pentoxifylline and about 2 µg/ml of each of impurity individually).

Table 7.4.8.1 Dilutions & concentrations for system precision

Vol. of stock solution A to H (ml)	Wt. of PNT	Final dilution (ml)	Final Concentrations			
		ml	*3-MEX µg/ml*	*THB µg/ml*	*THP µg/ml*	*Caffeine µg/ml*
2.0	100.1	100	2.020	2.060	2.000	2.000

Contd....

Vol. of stock solution A to H (ml)	Wt. of PNT	Final dilution (ml)	Final Concentrations				
			Imp.A µg/ml	*Imp.B µg/ml*	*Imp.C µg/ml*	*Imp.D µg/ml*	*PNT µg/ml*
2.0	100.1	100	2.020	1.980	1 980	2.000	1001.0

i) Set up the system as mentioned under the chromatographic conditions.

ii) Injected 20 µl of the system precision solution for six times and recorded the chromatograms upto 50 min.

iii) Calculated the relative standard deviation for the detector response for each component.

Acceptance limit :

RSD of detector response for each component is not more than 5 0 %

7.4.9 Solution stability :

i) Injected 20 µL of the system precision solution in duplicate, periodically and recorded the chromatograms upto 50 min.

ii) Calculated the relative standard deviation for the detector response for each component over the period.

Acceptance limit :

RSD (%) of detector response for each component is not more than 5.0 %

7.4.10 Method precision

i) Prepared a sample solution as directed under the procedure (about 1000 µg/ml)

ii) Set the chromatographic conditions as mentioned under the method, injected 20 µL of the standard solution in duplicate and recorded the chromatograms upto 50 min.

iii) Injected 20 µl of standard solution in triplicate and recorded the chromatograms upto 50 min. Used this for calculations.

iv) Injected 20 µl of the sample solution in duplicate and recorded the chromatograms upto 50 min

v) Calculated the amount of the impurities present in the sample

vi) Prepared six sets of this sample as directed under the method. Spiked impurities upto the target levels in each of the sample preparation

Table 7.4.10.1 : Method Precision

Set	Wt. of Sample (mg)	Final Dilution (ml)	Conc. of Sample (µg/mL)	Observed Results in percentage			
				3-MEX	*THB*	*THP*	*Caffeine*
M1	10.1	10.0	1010	2.020	2.060	2.000	2.000
M2	10.2	10.0	1020	2.020	2.060	2.000	2.000
M3	10.4	10.0	1040	2.020	2.060	2.000	2.000
M4	10.0	10 0	1000	2.020	2.060	2.000	2.000
M5	10.1	10.0	1010	2.020	2.060	2.000	2.000
M6	10.2	10.0	1020	2.020	2 060	2.000	2.000

Contd. ..

Set	Wt. of Sample (mg)	Final Dilution (ml)	Sample Conc (µg/mL)	Observed Results in percentage			
				Imp.A	*Imp.B*	*Imp.C*	*Imp.D*
M1	10 1	10.0	1010	2.020	1.980	1.980	2.000
M2	10.2	10.0	1020	2.020	1 980	1.980	2.000
M3	10 4	10 0	1040	2 020	1.980	1 980	2.000
M4	10.0	10.0	1000	2.020	1.980	1.980	2.000
M5	10.1	10.0	1010	2.020	1.980	1.980	2.000
M6	10 2	10.0	1020	2.020	1.980	1.980	2.000

vii) Injected 20 µl of each sample preparation in duplicate into the chromatograph set to the condition mentioned under the method and recorded the chromatograms upto 50 min .

viii) Calculated the amount of each impurity from the six sets. Calculated the RSD (%) of each impurity percentage from the six sets.

Acceptance limit :
RSD of the calculated impurities in the six sets should be ≤ 5.0%.

7.4.11 Linearity and range :

i) Used **Solution K** for preparing the following linearity solutions.

ii) **Linearity solutions.**

Table 7.4.11.1 Dilutions & concentrations for Linearity study

Level	*% of target*	*Vol. of. solution K*	*Final dilution*	*Final Concentrations*			
		ml	*ml*	*3-MEX µg/ml*	*THB µg/ml*	*THP µg/ml*	*Caffeine µg/ml*
L1	50	1.0	10.0	1.010	1.030	1.000	1.000
L2	75	1 5	10.0	1 515	1.545	1.500	1.500
L3	100	2.0	10.0	2.020	2.060	2.000	2.000
L4	125	2.5	10.0	2.525	2.575	2.500	2.500
L5	150	3.0	10.0	3.030	3.090	3.000	3.000

Contd....

Level	*% of target*	*Vol. of. Soln. K*	*Final Dilution*	*Final Concentrations*				
		ml	*ml*	*Imp.A µg/ml*	*Imp.B µg/ml*	*Imp.C µg/ml*	*Imp.D µg/ml*	*PNT µg/ml*
L1	50	1.0	10.0	1 010	0.990	0.990	1.000	1.000
L2	75	1.5	10.0	1 515	1.485	1.485	1.500	1.500
L3	100	2.0	10.0	2 020	1.980	1.980	2.000	2.000
L4	125	2 5	10.0	2 525	2.475	2.475	2.500	2.500
L5	150	3.0	10.0	3.030	2.970	2.970	3.000	3.000

iii) Injected 20 µl each of the linearity solution, in triplicate, into the chromatographic system set to the conditions mentioned under the method and record the chromatograms upto 50 min.

iv) Calculated the mean and RSD (%) of the detector responses for each linearity level, individually, for each component.

v) Plotted a graph of the concentration versus mean area count and performed mathematical regression for each component individually.

Acceptance limits :

RSD (%) of area counts at for each level for individual components ≤ 5.0 % .Plot of concentration versus detector response for each component is linear. The regression correlation coefficient (r^2) ≥ 0.99

7.4.12 Accuracy :

Used **Solution J** for preparing the following solutions.

i) Prepared five sets for five level (70, 85, 100, 115 and 130% of target concentration) recovery study by transferring about 10 mg, accurately weighed, pentoxifylline standard into five 10 ml volumetric flasks separately. Pipetted out appropriate volumes of Solution J as shown in the Table below and diluted to volume with mobile phase.

Solution J

Component	3-MEX	THB	THP	Caffiene	Imp.A	Imp.B	Imp.C	Imp.D
Conc. (µg/ml)	10 1	10.3	10.0	10.0	10.1	9.9	9.9	10.0

Table 7.4.12.1 Dilutions & concentrations for Accuracy study

level	*% of target*	*Vol. of. Solution J*	*Wt of pentoxi-fylline*	*Final Dilution*	*Final Concentrations(µg/ml)*			
		ml	*mg*	*ml*	*3-MEX µg/ml*	*THB µg/ml*	*THP µg/ml*	*Caffeine µg/ml*
R1	70	1.4	10.5	10	1.414	1.442	1.400	1.400
R2	85	1.7	10.0	10	1.717	1.751	1.700	1.700
R3	100	2.0	10.2	10	2.020	2.060	2.000	2.000
R4	115	2.3	9.9	10	2.323	2.369	2.300	2.300
R5	130	2.6	10.3	10	2.626	2.678	2.600	2.600

Contd....

Sr. No.	*Level % of target*	*Vol. of. Soln. J*	*Wt of pentoxi fylline*	*Final Dilution*	*Final Concentrations (µg/ml)*				
		ml		*ml*	*Imp.A µg/ml*	*Imp.B µg/ml*	*Imp.C µg/ml*	*Imp.D µg/ml*	*PNT µg/ml*
R1	70	1.4	10.5	10	1.414	1.386	1.386	1.400	1050
R2	85	1.7	10.0	10	1.717	1.683	1.683	1.700	1000
R3	100	2.0	10.2	10	2.020	1.980	1.980	2.000	1020
R4	115	2.3	9.9	10	2.323	2.277	2.277	2.300	990
R5	130	2.6	10.3	10	2 626	2 574	2.574	2.600	1030

ii) Injected 20 µl of standard solution, prepared as mentioned 3.6, in triplicate and recorded the chromatograms upto 50 min. Calculate the mean area counts of the standard.

iii) Injected 20 µl each of the recovery solution R1, R2, R3, R4 and R5 into the chromatograph in triplicate and recorded the chromatograms upto 50 min.

iv) Calculated the mean and RSD (%) of the detector responses for each set.

v) Calculated the amount of each spiked impurity in each set of the recovery sample and calculated the percentage recovery.

Acceptance limit :

a) Percentage recovery not more than 95.0 and not less than 105.0 %.

b) RSD (%) of detector response for each component is not more than 5 0 %.

7.4.13 Minimum Quantitation level and Minimum Detection level

i) Prepared a stock solution for this study by pipetting out 10 ml of Solution K (10 µg/ml each of impurity + pentoxifylline as mentioned under section 3.6) into a 50ml volumetric flask. Diluted to volume with diluent [about 2 µg/ml each component]-MDL stock solution.

ii) Prepared subsequent diluted solutions as shown in the Table below and injected them in triplicate and recorded the chromatograms upto 50 min.

Table 7.4.13.1 Dilutions & concentrations for Minimum Quantitation level and Minimum Detection level

Level	*Vol. of MDL stock soln K (ml)*	*Final Dilution (ml)*	*Final Concentrations(µg/ml)*			
			3-MEX	*THB*	*THP*	*Caffeine*
D1	5.0	10	1.010	1.030	1.000	1.000
D2	2.5	10	0.505	0 515	0.500	0.500
D3	1 0	10	0 202	0.206	0.200	0 200
D4	0.5	10	0.101	0.103	0.100	0.100
D5	0.2	10	0.040	0.041	0.040	0.040
D6	0.1	10	0.020	0.020	0.020	0.020
D7	0.1	25	0.008	0.008	0 008	0.008
D8	0.1	50	0.004	0.004	0.004	0 004

Contd....

Level	*Vol. of soln L (ml)*	*Final Dil. (ml)*	*Final Concentrations(µg/ml)*				
			Imp.A	*Imp.B*	*Imp.C*	*Imp.D*	*PNT*
D1	5.0	10	1 010	0.990	0.990	1.000	1.000
D2	2.5	10	0.505	0.495	0.495	0.500	0.500
D3	1 0	10	0.202	0.198	0.198	0.200	0.200
D4	0 5	10	0.101	0 099	0.099	0 100	0.100
D5	0 2	10	0.040	0.039	0 039	0.040	0 040
D6	0.1	10	0.020	0 019	0.019	0.020	0.020
D7	0.1	25	0.008	0 007	0 007	0.008	0 008
D8	0.1	50	0 004	0.003	0 003	0.004	0 004

iii) Injected 20 µl each of the recovery solution R1, R2, R3, R4 and R5 into the chromatograph in triplicate and recorded the chromatograms upto 50 min.

iv) Calculated the RSD (%) of the triplicate injections for each level.

Acceptance limit :

The MQL of each component is the lowest concentration at which the RSD (%) of the triplicate injections not more than 5.0 %.

The MDL of each component is that concentration at which the detector shows a positive response.

7.4.14 Ruggedness of the method :

A sample previously analysed for related substances was re-analyzed by another analyst independently by this method and the results are compared.

Acceptance limit :

The difference in the results of the two analysis is not more than 5.0 % of the impurity limit

7.5 Results and Discussions :

7.5.1 Identification :

20 µl of Solution A to Solution I are injected individually and the chromatograms recorded up-to 50 min.

Results & discussion :

Figure-123 to 125, 130 to 135 shows typical chromatograms of individual components

Component		Approx. RT
3-Me-Xanthine	:	12.3 min
Theobromine	:	14.5 min
Theophylline	:	17.2 min
Imp-A	:	19.7 min
Caffeine	:	20.1 min
Imp-C	.	25.3 min
Imp-B	.	28.6 min
Pentoxifylline	:	30 2 min
Imp-D	.	30 8 min.

Figure-136 shows typical chromatogram showing separation of all individual components.

The above results show that all the components are clearly separated and identifiable.

7.5.2 System suitability :

Before starting a set of analysis, the system suitability solution is injected in duplicate. The resolution between the Pentoxifylline and Imp-D is calculated

Results & discussion :

Figure-121 shows a blank chromatogram of pentoxifylline study Figure-122 shows a system suitability chromatogram of pentoxifylline study

The Resolution factor R between Pentoxifylline and Imp-D = 1.41 [Limit : NLT 1.0]As the resolution meets the system suitability requirements the chromatographic system was used for further studies

7.5.3 Instrument precision

System Precision Solution :

i) Set up the system as mentioned under the chromatographic conditions.

ii) Injected 20 µL of the system precision solution six times and recorded the chromatograms upto 50 min

Individual area counts and RSD (%) values are shown in Table below :

Results and discussion :

Figure -122 shows a typical system precision chromatogram.

Table 7.5.3.1 : Instrument precision

Injection	*Detector Response (Area counts)*			
	3-MEX	*THB*	*THP*	*Caffeine*
1	125811	130778	117967	125771
2	126072	129672	117786	125554
3	125831	129895	118458	126021
4	126481	129958	118400	126379
5	127024	130252	118326	126333
6	127186	131002	118194	126415
Mean	126400.80	130259.50	118188.5	126078.8
SD (±)	598.66	527.09	263.57	358.12
RSD (%)	0.47	0.40	0.22	0.28

Contd...

Injection	*Detector Response (Area counts)*				
	Imp.A	*Imp.B*	*Imp.C*	*Imp.D*	*PNT*
1	96133	96664	70573	42644	36702695
2	96084	96574	70512	44447	36640732
3	96343	97134	70323	42611	36730516
4	96634	96950	71048	43071	36782756
5	96654	96845	70555	44134	36763299
6	96730	97632	70938	44045	36860334
Mean	96429.67	96966.5	70658.17	43492	36746722

SD (±)	281.88	382.50	276.36	812.71	74720.58
RSD (%)	0.29	0.39	0.39	1.87	0.20

Acceptance limit :

RSD not more than 5.0 %.

7.5.4 Method Precision :

Impurity found in PNT/288 only Imp.C = 0.027 %

Results and discussion :

Table 7.5.4.1 : Method Precision

Set	Wt. of Sample (mg)	Final Dilution (ml)	Sample Concentration (µg/ml)	Observed Results in percentage			
				3-MEX	*THB*	*THP*	*Caffeine*
M1	10.1	10	1010	0.1973	0.1997	0.1981	0.2066
M2	10.2	10	1020	0.1993	0.2029	0.2001	0.2090
M3	10 4	10	1040	0.2005	0.2032	0.2011	0.2102
M4	10.0	10	1000	0.1902	0.1925	0.1914	0.1999
M5	10.1	10	1010	0.2012	0.2037	0 2020	0.2108
M6	10.2	10	1020	0.2031	0.2063	0.2035	0.2126
Mean				0.1986	0 2013	0.1993	0.2081
RSD (%)				2.2893	2.4009	2.158	2.1717

Contd....

Set	Wt. of Sample (mg)	Final Dilution (ml)	Sample Concentration (µg/ml)	Observed Results in percentage			
				Imp.A	*Imp.B*	*Imp.C**	*Imp.D*
M1	10.1	10	1010	0.1986	0.1978	0.2273	0.2119
M2	10.2	10	1020	0.2006	0.2006	0.2314	0.2148
M3	10.4	10	1040	0 2019	0.2015	0.2336	0.2141
M4	10.0	10	1000	0.1920	0.1916	0 2200	0.2040
M5	10.1	10	1010	0.2023	0.2017	0.2331	0.2141
M6	10.2	10	1020	0.2038	0.2038	0.2361	0.2040
Mean				0.1998	0.1995	0.2302	0.2104
RSD (%)				2.117	2.171	2.854	2.430

* Corrected values (subtracting % impurity found in the sample = 0.027 %)

The above results are well within the acceptance limits and indicates instrument precision. Figure-126 shows typical chromatogram of method precision study. study

[Limit : RSD % impurities calculated from the six sets is NMT 5.0 %]

7.5.5 Linearity and Range :

The linearity of detector (UV) response for impurities was determined by preparing and injecting solutions in the concentration range of 50-150 % of limit conc. are of 3-Me-Xanthine, Theobromine, Theophylline, Caffeine, Imp.A, Imp.C, Imp.B, Pentoxifylline, Imp.D.

Results and discussion :

Figure-127 shows typical linearity chromatogram for various impurities.

Figure-137 to Figure-145 shows typical linearity plots for various impurities

The results of individual impurities is shown in Table 7.5.5.1to 7 5.5.9.

Acceptance limits :

RSD (%) of area counts at for each level for individual components not more than 5.0 % Plot of concentration versus detector response for each component is linear. The regression correlation coefficient (r^2) not less than 0.99

Table 7.5.5.1 : Linearity of 3-MEX

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	62858	62500	62751	62703	0.293
L2	92163	99678	95797	95879	3.919
L3	124255	125243	125699	125065	0.590
L4	158887	165694	162730	162437	2.101
L5	188042	186307	185596	186648	0.674
Slope					62266.9
Intercept					767.20
Correlation coefficient (r^2)					0.9968

Table 7.5.5.2 : Linearity of THB

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	64347	63618	63940	63968	0.571
L2	94682	101764	97543	97996	3.365
L3	127737	127673	128210	127873	0.229
L4	163144	169128	166080	166117	1.801
L5	193407	190246	189579	191077	1.070
Slope				62590.0	
Intercept				470.6	
Correlation coefficient (r^2)				0.9969	

Table 7.5.5.3 : Linearity of THP

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	58790	58357	58528	58558	0.372
L2	86414	93161	89351	89642	3.774
L3	116650	116733	117317	116900	0.311
L4	149207	154405	152133	151915	1.715
L5	176927	174003	173842	174804	1.062
Slope				58953	
Intercept				457.80	
Correlation coefficient (r^2)				0.9970	

Table 7.5.5.4 : Linearity of Caffeine

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	56615	56138	56312	56355	0.428
L2	82996	89737	85653	86128	3.942
L3	111787	111986	112682	112151	0.419
L4	142620	147894	145814	145442	1.827
L5	169054	166255	166030	167113	1.008
Slope				56166	
Intercept				1105.8	
Correlation coefficient (r^2)				0.9969	

Table 7.5.5.5 : Linearity of Imp.A

Level	Detector response (area counts)				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	46455	46151	46259	46288	0.333
L2	68122	74093	70579	70931	4.231
L3	91837	92003	92486	92108	0.366
L4	117350	122095	120007	119817	1.985
L5	139116	137105	136575	137598	0 974
Slope				45842	
Intercept				746.0	
Correlation coefficient (r^2)				0.9967	

Table 7.5.5.6 : Linearity of Imp.B

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	49042	50754	50242	50012	1.757
L2	71454	-	71091	71272	0.360
L3	95647	96043	96885	96191	0.657
L4	122160	120874	122676	121903	0.761
L5	143632	142477	142155	142754	0.544
Slope				47700	
Intercept				1980.4	
Correlation coefficient (r^2)				0.9987	

Table 7.5.5.7 : Linearity of Imp.C

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	32039	32019	31956	32004	0.135
L2	47167	51038	48799	49001	3.966
L3	63566	63919	64238	63907	0.526
L4	81411	84876	83432	83239	2.091
L5	96378	95243	95096	95572	0.734
Slope				32600	
Intercept				195.0	
Correlation coefficient (r^2)				0.9967	

Table 7.5.5.8 : Linearity of Imp.D

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	40032	39738	39921	39897	0.372
L2	59031	63585	60868	61161	3.746
L3	79255	79444	80181	79626	0.615
L4	102076	105752	104388	104405	2.239
L5	120242	118823	118401	119155	0.809
Slope				40352	
Intercept				144.8	
Correlation coefficient (r^2)				0.9957	

Table 7.5.5.9 : Linearity of pentoxifylline

Level	*Detector response (area counts)*				
	Inj. 1	*Inj. 2*	*Inj. 3*	*Mean*	*RSD (%)*
L1	40986	41390	41815	41397	1.001
L2	60325	66331	62343	62999	4.851
L3	79860	80174	80863	80299	0.639
L4	101960	106567	104300	104275	2.209
L5	120706	119120	118646	119490	0.903
Slope					39492 4
Intercept					2707.20
Correlation coefficient (r^2)					0.9963

7.5.6 Accuracy :

Recovery study was performed at 70, 85, 100, 115 and 130 % levels of 0.2 % each of 3-Me-Xanthine, Theobromine, Theophylline, Imp-A, Caffeine, Imp-C, Imp-B, Pentoxifylline, Imp-D).

Figure- 128 shows a typical chromatogram of recovery study.

Table 7.5.6.1 : Recovery of 3-MEX from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.414	13989	98.93
R2	85	1.717	1.6806	97.88
R3	100	2.020	1.9392	96.00
R4	115	2.323	2.2273	95.88
R5	130	2.626	2.5443	96.89
Mean				97.116
RSD (%)				1.332

Table 7.5.6.2 : Recovery of Theophylline from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.400	1.3871	99.08
R2	85	1.700	1.6636	97.86
R3	100	2.000	1.9498	97.49
R4	115	2.300	2.2112	96.14
R5	130	2.600	2.5571	98.35
Mean				97.784
RSD (%)				1.119

6.3 : Recovery of Theobromine from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.442	1.4390	99.79
R2	85	1.751	1.7263	98.59
R3	100	2.060	2.0406	97.60
R4	115	2.369	2.2932	96.80
R5	130	2.678	2.6501	98.96
Mean				98.348
RSD (%)				1.189

I

7.5.6.4 : Recovery of caffeine from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.400	1.4070	100.50
R2	85	1.700	1.6891	99.36
R3	100	2.000	2.0110	100.55
R4	115	2.300	2.2726	98.81
R5	130	2.600	2.5766	99.10
Mean				99.664
RSD (%)				0.813

Table 7.5.6.5 : Recovery of Imp.A from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.414	1.4223	100.59
R2	85	1.717	1.7007	99.05
R3	100	2.020	1.9701	97.53
R4	115	2.323	2.2143	95 32
R5	130	2.626	2.5501	97.11
Mean				97 92
RSD (%)				2.041

Table 7.5.6.6 : Recovery of Imp.B from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.386	1.4367	103.66
R2	85	1.683	1.7037	101.23
R3	100	1.980	2.0241	102 23
R4	115	2.277	2.2560	99.08

R5	130	2 574	2.5763	100.09
Mean				101.258
RSD (%)				1.768

Table 7.5.6.7 : Recovery of Imp.C from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.386	1.3764	99.31
R2	85	1.683	1.7338	103.02
R3	100	1.980	2.0133	101.68
R4	115	2.277	2.2888	100.52
R5	130	2.574	2.6100	101.40
Mean				101.186
RSD (%)				1.364

Table 7.5.6.8 : Recovery of Imp.D from pentoxifylline

Sr. No.	*Level*	*Actual amount added (µg/ml)*	*Amount recovered (µg/ml)*	*% Recovery = Amt found ---------- x 100 Amt. added*
R1	70	1.400	1.4165	101.18
R2	85	1.700	1.6929	99 58
R3	100	2 000	1.9432	97.016
R4	115	2.300	2.3375	101.63
R5	130	2.600	2.6728	102.80
Mean				100.470
RSD (%)				2.171

[Limit : Recovery - 95.0 % - 105.0 %]

7.5.7 Limit of Detection and Quantitation :

5.0 ml of solution L was taken in a 50 ml volumetric flask. This was used as MQl solution. Further dilutions were made and injected in triplicate into the chromatography.

Table 7.5.7.1 to 7.5.7.9 shows the results of the study.

Figure-129 shows typical chromatogram for LOD/LOQ study of various impurities

Results and discussion :

Table 7.5.7.1 : Limit of Detection and Limit of Quantitation Study (3-MEX)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.0100	55395	57048	55534	55992	1.6374
D2	0 5050	27538	27668	27808	27671	0.4879
D3	0.2020	11409	11436	11599	11481	0.8953
D4	0.1010	5858	5700	5671	5743	1.7524
	0.0404	2316	2690	2172	2392	11.1747
	0.0202	1865	1504	1459	1609	13.8289
D7	0.00808	273	777	548	532	47.3747
D8	0.00404	ND	ND	ND	-	-

Table 7.5.7.2 : Limit of Detection and Limit of Quantitation Study (THP)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.03000	52681	53387	52591	52886	0.8242
D2	0.515	26663	26298	26306	26422	0.7889
D3	0.206	10640	10689	10758	10695	0.5542
D4	0.103	5443	5283	5306	5344	1.6187
	0.0412	2290	2417	2296	2334	3.0695
	0.0206	1678	1441	1536	1551	7.6868
D7	0.00824	493	628	621	580	13.0887
D8	0.00412	ND	ND		-	-

Table 7.5.7.3 : Limit of Detection and Limit of Quantitation Study (THB)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.000	56140	57743	56472	56785	1.4900
D2	0.500	28028	28850	28845	28574	1.6558
D3	0.200	12086	11845	12202	12044	1.5120
D4	0.100	6291	6794	6254	6446	4.6794
	0.04	3299	3485	5171	3985	25 8797
	0.02	5668	4047	3860	4525	21.9728 *
D7	0.008	2926	3404	584	1508	65.4844 *
D8	0.004	ND	ND	ND	-	-

Table 7.5.7.4 : Limit of Detection and Limit of Quantitation Study (caffeine)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.000	49369	50626	49495	49830	1.3891
D2	0.500	24723	24846	25087	24885	0.7440
D3	0.200	10408	10541	10634	10527	1.0789
D4	0.100	5318	5492	5516	5442	1.9855
	0.04	2363	2572	2585	2506	4.9702
	0.02	1857	1710	1746	1771	1.3265
D7	0.008	897	1016	1021	978	7.1771
D8	0.004	ND	ND	ND	-	-

Table 7.5.7.5 : Limit of Detection and Limit of Quantitation Study (IMP-A)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.01	40341	41404	40442	40729	1.4406
D2	0.505	20177	20302	20364	20281	0.4696
D3	0.202	8116	8209	8318	8214	1.23084
D4	0.101	4077	3978	4005	4020	1.2730
	0.0404	1680	1818	1702	1733	4.2775
	0.0202	1109	976	1064	1049	6.4447
D7	0.00808	342	403	431	392	11.60925
D8	0.00404	ND	ND	ND	-	-

Table 7.5.7.6 : Limit of Detection and Limit of Quantitation Study (IMP-B)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	0.99	42026	43158	42366	42516	1.36615
D2	0.495	20472	20477	20857	20602	1.07198
D3	0.198	8321	8389	8367	8359	0.41510
D4	0.099	3874	3740	3807	3807	1.7599
	0.0396	1486	1644	1469	1533	6.2951
	0.0198	614	618	806	679	16.15036
D7	0.00792	ND	ND	ND	-	-
D8	0.00396	ND	ND	ND	-	-

Table 7.5.7.7 : Limit of Detection and Limit of Quantitation Study (IMP-C)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	0.99	28148	28868	28296	28437	1.3371
D2	0.495	13798	13885	13983	13888	0.6664
D3	0.198	5543	5642	5632	5605	0.9722
D4	0.099	2595	2562	2442	2533	3.1787
	0.0396	1002	1077	1029	1036	3.6666
	0.0198	698	594	627	639	8.3081
D7	0.00792	ND	ND	ND	-	-
D8	0 00396	ND	ND	ND	-	-

Table 7.5.7.8 : Limit of Detection and Limit of Quantitation Study (IMP-D)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.0	35691	36373	35584	35882	1.927
D2	0.5	18179	18382	18665	18408	1.3259
D3	0.2	7369	7389	7415	7424	1.0584
D4	0.1	3935	3730	3771	3812	2.8456
	0.04	1687	1880	1764	1777	5.4673
	0 02	1515	1198	1449	1387	12.055 *
D7	0.008	ND	ND	ND	-	-
D8	0.004	ND	ND	ND	-	-

Table 7.5.7.9 : Limit of Detection and Limit of Quantitation Study (pentoxifylline)

Level	*Conc. (µg/ml)*	*Inj. 1*	*Inj. 2*	*Inj. 3*	*Mean area*	*RSD (%)*
D1	1.0	39706	41191	39027	39974	2.7685
D2	0.5	30009	32886	32497	31797	4.9089
D3	0.2	10169	10506	10938	10537	3.6580
D4	0.1	9969	8438	7965	8790	11.9162
	0.04	7583	10954	7624	8720	22.814 *
	0.02	12776	7865	10856	10499	23.5726 *
D7	0.008	5134	7858	5892	6294	22.3353 *
D8	0 004	ND	ND	ND	-	-

* values are not considered because of improper integration .

The limit of quantitation and detection for each impurity is as follows :

Table 7.5.7.10 : Limit of Detection and Limit of Quantitation Summary

	Limit of quantitation		*Limit of detection*	
	(µg/ml)	*(%)*	*(µg/ml)*	*(%)*
3-Me-Xanthine	0.1010	0.0101	0.00808	0.00080
Theobromine	0.1000	0.0100	0.00800	0.00080
Theophylline	0.0412	0.0041	0.00824	0.00082
Imp-A	0.0404	0.0040	0 00808	0.00080
Caffeine	0.0200	0.0020	0.00800	0.00080
Imp-C	0.0396	0.0039	0.01980	0.00198
Imp-B	0.0990	0.0099	0.01980	0.00198
Pentoxifylline	0.2000	0.0200	0.00800	0.00080
Imp-D	0.1000	0.0100	0.02000	0.00200

7.5.8 Ruggedness :

Method ruggedness is established by a sample previously analyzed for related substances is re analyzed by another analyst independently by this method and the results are compared

Ruggedness-I
Analyst : ALP

Ruggedness-II
Analyst : NRP

	Pentoxifylline B.No: PNT/288		*Pentoxifylline B.No: PNT/288*	
	By calculation	***By area normalization***	***By calculation***	***By area normalization***
3-Me-Xanthine	ND	ND	ND	ND
Theobromine	ND	ND	ND	ND
Theophylline	ND	ND	ND	ND
Imp-A	ND	ND	ND	ND
Caffeine	ND	ND	ND	ND
Imp-C	0.0253	0.02	0.0272	0.02
Imp-B	ND	ND	ND	ND
Pentoxifylline	ND	ND	ND	ND
Imp-D	ND	ND	ND	ND

7.6 Summary and Conclusions

Acceptance limit		*Actual results*		
System suitability Resolution between Pentoxifylline & Imp-D	NLT 1.0	1.2 to 1.4		
		3-MEX	Theobromine	Theophylline
Precision				
Instrument - RSD (%) of Detector Response for each impurity	≤ 5.0 %	0.4736	0.4048	0.2230
Method - RSD (%) of each Impurity %	≤ 5.0 %	2.2873	2.4009	2.1580
Linearity and Range				
Correlation coefficient (r^2)	≥ 0.99	0.997	0.997	0.997
RSD (%) of detector responses	≤ 5.0 %	3.9196	3.6355	3.7737
Accuracy				
Percentage recovery	95.0 % - 105.0 %	97.116	98.348	97.784
Minimum quantitation level				
RSD (%) at MQL	≤ 5.0 %	1.7524	4.6794	3.0695

Contd..

		Caffeine	Imp-A	Imp-B
Precision				
Instrument - RSD (%) of Detector Response for each impurity	≤ 5.0 %	0.2840	0.2923	0.3944
Method - RSD (%) of each Impurity %	≤ 5.0 %	2.17 7	2.1170	2.1715
Linearity and Range				
Correlation coefficient (r^2)	≥ 0.99	0.997	0.997	0.999
RSD (%) of detector responses	≤ 5.0 %	3.9424	4.2309	1.7570
Accuracy				
Percentage recovery	95.0 % - 105.0 %	99.664	97.92	101.258
Minimum quantitation level				
RSD (%) at MQL	≤ 5 0 %	4.3265	4.2775	1.7599

Contd. ..

		Imp-C	Imp-D	Pentoxifylline
Precision				
Instrument - RSD (%) of Detector Response for each impurity	≤ 5.0 %	0.3911	1.8686	0.2033
Method - RSD (%) of each Impurity %	≤ 5.0 %	2.8544	2.430	-
Linearity and Range				
Correlation coefficient (r^2)	≥ 0 99	0.997	0.996	0.996
RSD (%) of detector responses	≤ 5.0 %	3.9660	3.7460	4.8514
Accuracy				
Percentage recovery	95 0 % - 105.0 %	101.186	100 47	-
Minimum quantitation level				
RSD (%) at MQL	≤ 5.0 %	3 6666	2.8456	3.6580

The results of the study indicates that this method for related substances and process impurities in pentoxifylline is precise, accurate, linear in detector response and rugged

7.7 Recommendations And Limitations

1. This method is recommended for the analysis of pentoxifylline samples for the related substances and process impurities .
2. This method shows precision, linearity and accuracy for all known impurities like 3-Me-Xanthine, Theobromine, Theophylline, Imp-A, Caffeine, Imp-C, Imp-B, Imp-D.