DEVELOPMENT OF NEW ANALYTICAL METHODS FOR SOME RECENT DRUGS

SUMMARY SUBMIITED TO THE MAHARAJA SAYAJIRAO
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DOCTOR OF PHILOSOPHY
(PHARMACY)

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The subject of impurities evaluation of pharmaceutical compounds has been insufficiently addressed in scientific literature up to this time. As a matter of fact Because of the apparent negativity attached to this word in the pharmaceutical world. Hence we have taken this problem and developed related substances and process impurities in some advance drugs. I had not directly jumped in to the work due to the complex nature and insufficiently addressed in scientific literature. We wish to address all types of impurities but we realized problem is too complex Hence we have decided to cover organic related substances and process impurities estimation by High Performance Liquid Chromatography as front end technique.

In **Chapter – 1,** I had discussed in detail about different types of possible impurities with respect to literature and compendial terminology. Fundamentals of chromatography are covered in Chapter **-2.**

In **chapter -3,** I compiled fundamentals of High Performance Liquid Chromatography. Molecular basis of chromatographic fundamentals are covered to promote successful application of chromatographic techniques in pharmaceutical sciences At the end of the compilation entire subject has become so complicated due to detailed discussions of molecular basis.

For simplifying fundamentals **Chapter-4** is covered some important concepts with pictures.

In **Chapter-5**, detailed literature of Sertraline search published was presented to prove the novelty of current work Related substances method development of sertraline was performed with aid of software and some experiments Here structure analysis and retention analysis was derived with the help of Chromsword software. Optimized method was validated for the correctness of intended use . Validation summary and one typical chromatogram showing separation of all components is given below for quick reference.

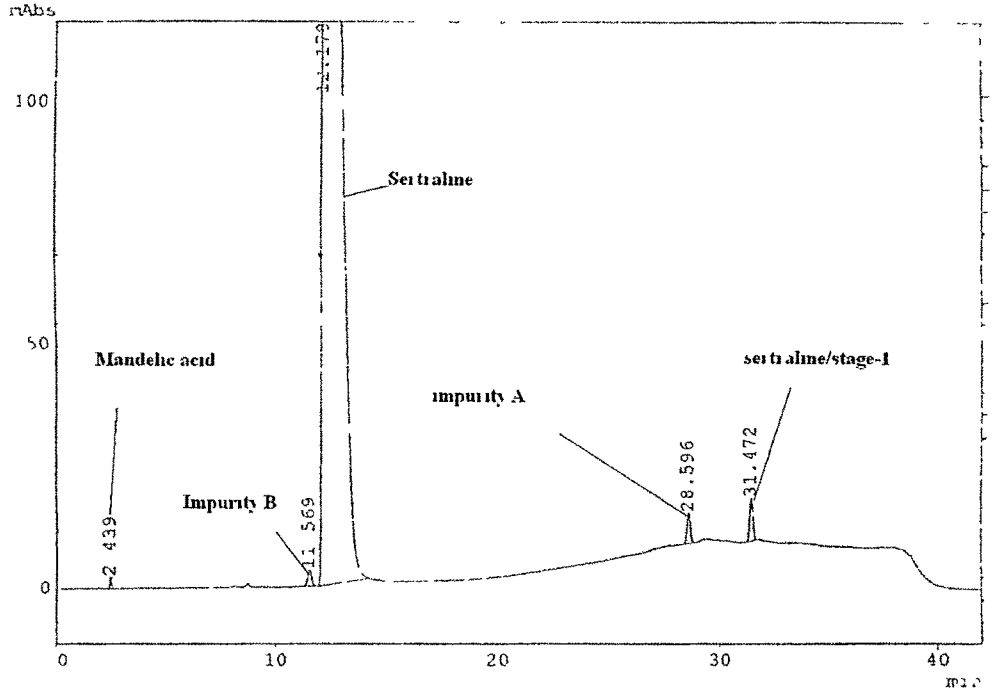
Sertraline related substances method validation Summary

	Acceptance limit	Actual results			
System suitability Resolution between Impurity B & sertraline	NLT 3.0 %	3.59, 3.4, 3.6			
		Mandelic acid	**Impurity B**	**Impurity A**	**Sertraline /I**
Precision					
Instrument - RSD of Detector Response for each impurity	≤ 5.0 %	0.5%	0 5%	1.11%	0 6%
Method - RSD of each Impurity %	≤ 5.0 %	1 47%	1.88%	0 61%	1.37%
Linearity and Range					
Correlation coefficient (r^2)	≥ 0 99	0.9993	0.999	0.9992	0.997
RSD of detector responses	≤ 5.0 %	max. 0.83%	max 2.84%	max. 1.82%	max. 3 12%
Accuracy Percentage recovery	95.0 % - 105.0 %	100.41%	100 25%	100.36%	102 14 %
Minimum quantitation level		0 076%	0.038%	0.039%	0.075%
Minimum detection level		0 008%	0.008%	0.008%	0.008%

The results of the study indicates that this method for related substances in sertraline is precise, accurate, linear in detector response and rugged.Sertraline related substances typical chromatogram is given below

CLASS-LC10 Ver =1 60 SYS=1 Ch=1 REPORT NO=39 DATA=7015V.D20
Sample 7015
ID SYS.PRC
Type Unkn RESOLUTION SOLUTION
Detector SPD-
Operator NRP
Method Name : 7015GR MC1

*** Chromatogram *** Filename 7015V C10



*** Peak Report ***

PKNO	TIME	AREA	CONC [%]
1	2.439	11534	0.0845
2	11.569	43584	0.3194
3	12.178	13440051	98.4915
4	28.596	62457	0.4577
5	31.472	88275	0.6469
		13645900	100.0000

Sertraline assay method was developed on a simple isocratic system to suite many small Manufacturers how ever specificity study performed during validation indicated Developed method is stability indicating and robust method. Summary of Method Validation is given below with typical representative chromatogram.

Summary of Sertraline assay method validation

Sr. No.	Acceptance criteria	Observed value	Limit
1.	System suitability and reproducibility	RSD = 0 204 %	RSD . NMT 2 0 %
2	Accuracy	Recovery = 99.36 % RSD = 1 29 %	Recovery : 98.0 % - 102.0 % RSD NMT 2.0 %
3	Linearity range	Correlation coefficient (r^2) = 0.9995	Correlation coefficient (r^2) NLT 0 999
4.	Precision	RSD : 0 127 %	RSD : NMT 2.0 %
5.	Ruggedness	Variation = - 0 06 %	Variation . ± 0 5 %

All these observations indicate that this method for assay of Sertraline is specific, accurate, precise and is also stability indicating.

In **Chapter-6**, detailed literature search related to Losartan analytical methods published is presented to prove the novelty of current work. Related substances method development of Losartan was performed with aid of software and some experiments. Here structure analysis and retention analysis was derived with the help of Chromsword software However software was failed in predicting correct condition due to closeness between impurities and Losartan. Successful HPLC method was developed to resolve all impurities to base to base separation. Optimized method was validated for the correctness of intended use . Validation summary and one typical chromatogram showing separation of all components is given below for quick reference

Summary of Losartan related substances method validation

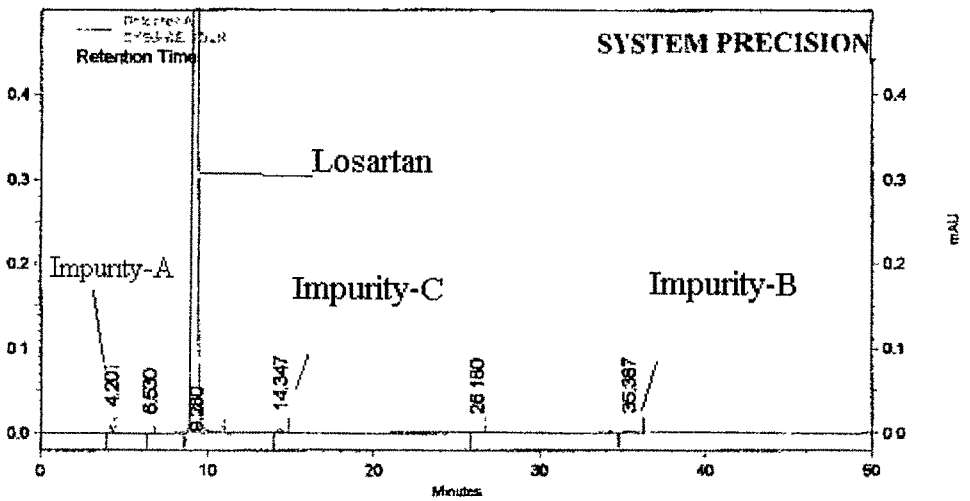
	Accepta -nce criteria	*Actual results*		
System suitability Resolution between Losartan/Imp-C & Losartan	NLT 5	15.22		
		Losartan/ Imp-A	**Losartan/ Imp-B**	**Losartan/ Imp-C**
Precision Instrument - RSD of Detector Response for each impurity	≤ 5.0 %	2.56 %	1.31 %	1.98 %
Method - RSD of each Impurity %	≤ 5.0 %	0 85 %	0 79 %	2 05 %
Linearity and Range Correlation coefficient (r^2)	≥ 0 99	0 994	0.996	0.996
RSD of detector responses	≤ 5 0 %			
Accuracy Percentage recovery	95.0 % - 105 0 %	100 38 %	99 36 %	99.30 %
Minimum quantitation level		0.252µg/ml	0.522µg/ml	0 501µg/ml
RSD at MQL	≤ 5.0 %	0.85 %	3 68 %	2 88 %

The results of the study indicate that this method for related substances in Losartan is precise, accurate, linear in detector response and rugged Typical HPLC chromatogram is Given below.

CLASS-VP V5.02 ***Area % Report***

Method Name: C:\CLASS-VP\METHODS\HPLCMET-11\8009LRSVL.met
Data Name: C:\CLASS-VP\DATA\HPLC-11\138009L.D08.dat
User: SYSTEM
Acquired: 5/13/99 11:53:02 PM
Printed: 5/15/99 11:51:15 AM

Losartan RS validation



Pk #	Retention Time	Area	Area Percent
1	4.207	68555	0.225
2	6.530	6499	0.021
3	9.280	30289558	99.249
4	14.347	70266	0.230
5	26.180	15786	0.052
6	35.387	68110	0.223
Totals		30518774	100.000

Losartan related substances method was taken to for assaying losartan and found suitable but run time and concentration of losartan reduced for optimum results. Optimized method was validated for the correctness of intended use. Validation summary and one typical chromatogram showing separation of all components is given below for quick reference.

Summary of Losartan assay method validation

Sr. No.	Acceptance criteria	Observed value	Limit
1.	System suitability and reproducibility	RSD = 0.17 %	RSD : NMT 2.0 %
2	Accuracy	Recovery = 99 698 % RSD = 0.82 %	Recovery : 98.0 % - 102.0 % RSD NMT 2.0 %
3	Linearity range	Correlation coefficient (r^2) = 0 9996	Correlation coefficient (r^2) NLT 0.999
4	Precision	RSD 0.156 %	RSD · NMT 2 0 %
5.	Ruggedness	Variation = - 0 23 %	Variation ± 0.5 %

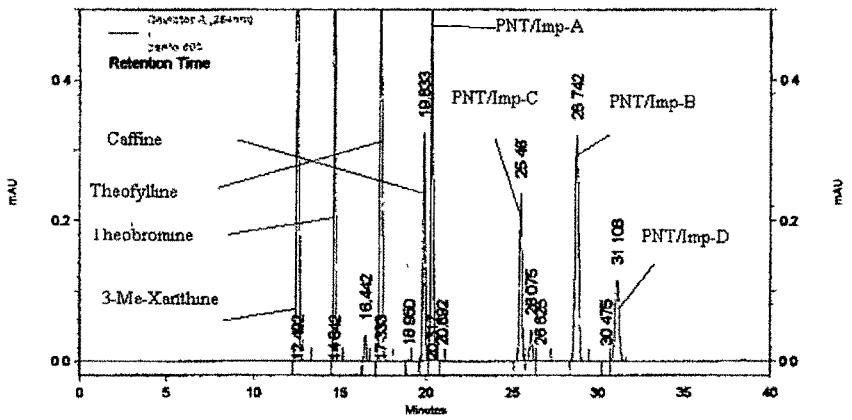
All these observations indicate that this method for assay of losartan potassium is specific, accurate, precise and is also stability indicating.

In **Chapter-7**, detailed literature search related to Pentoxifylline analytical methods published is presented to prove the novelty of current work. Eventhough this drug is little old, related substances and process impurities monitoring HPLC method is not published. Hence I had developed a HPLC method where all impurities of pentoxifylline was addressed. Development was performed with aid of software and some experiments Here structure analysis and retention analysis was derived with the help of Chromsword software. However software was failed in predicting correct condition due to closeness between impurities Successful HPLC method was developed to resolve all impurities to base to base separation. Optimized method was validated for the correctness of intended use. Validation summary and one typical chromatogram showing separation of all components is given below for quick reference

Shimadzu CLASS-VP V5.03 ***Area % Report***

Method Name: C:\CLASS-VP-ver5.03\METHODS\HPLC-11\Pentoxy.met
Data Name: C:\YDC\pento.d03
Acquired: 7/1/99 6:46:44 PM
Seq.Name : C:\CLASS-VP-ver5.03\SEQUENCE\HPLC-11\Pento-stabi.seq
Sample name: I

All component mix for identification



Pk #	Retention Time	Area	Name
1	12.492	5724967	3-Me-Xanthine
2	14.642	5685816	Theobromine
3	16.442	287965	
4	17.333	5458517	Theophylline
5	18.950	8469	
6	19.833	2839590	Caffein
7	20.317	4749684	Imp-A
8	20.892	7250	
9	25.467	2343231	Imp-C
10	26.075	438208	
11	26.625	8628	
12	28.742	4299308	Imp-B
13	30.475	8443	
14	31.108	1887679	Imp-D
Totals		33747755	

Typical HPLC chromatogram of Pentoxifylline is given above.

Summary of Pentoxifylline related substances method validation

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 indicate that this method for related substances and process impurities in pentoxifylline is precise, accurate, linear in detector response and rugged.

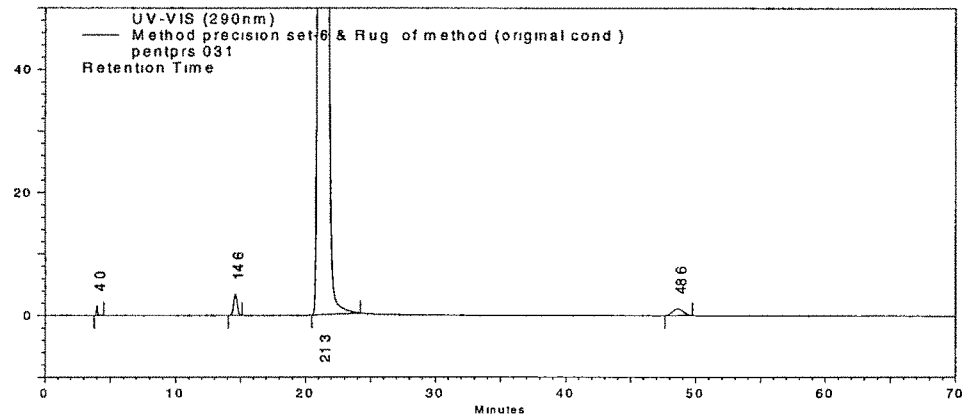
A survey of literature for Pantoprazole indicated the estimation reported by the following methods, Viz., High-performance liquid chromatography-electrospray ionization mass spectrometry, High-performance liquid chromatography, Capillary isotachophoresis.Gas chromatography-mass spectrometry, UV Spectrophotometric method , Micellar electrokinetic chromatography, Gas chromatography-mass spectrometry The brief information on above analytical methods are follows. Detailed summary of literature is discussed in **Chapter-8.** Related substances method development of sertraline was performed with aid of software and some experiments. Here structure analysis and retention analysis was derived with the help of Chromsword software. Optimized method was validated for the correctness of intended use. Validation summary and one typical chromatogram showing separation of all components is given below for quick reference.

Summary of Pantoprazole related substances method validation

Acceptance limit		Actual results		
System suitability				
Resolution between pantoprazole impurity A and pantoprazole sodium	NLT 20.0			
		Pantoprazole impurity A	**Pantoprazole impurity B**	**Pantoprazole sodium**
Precision				
Instrument precision - RSD (%) of Detector Response for pantoprazole impurity B and pantoprazole sodium	≤ 5.0 %	1.88 %	0.42 %	2.89 %
Method precision - RSD (%) of pantoprazole impurity A	≤ 5.0 %	3.90 %	1.10 %	-
Linearity and Range				
Coefficient of correlation (r)	≥ 0 99	1.000	1.000	1.000
RSD (%) of detector responses	≤ 5.0 %	0.82 % max.	0 34 % max.	1.26% max.
Accuracy				
Percentage recovery	95.0 % - 105.0 %	102.01 %	101.96 %	-
Minimum quantitation level (%)	≤ 5 0 %	0.025 %	0.025 %	0.013 %
Minimum detection level (%)		0.013	0.013	0.006%
Ruggedness	*Difference NMT 10.0 % of impurity limit*			
Original condition		0.214	0.203	
Change in analyst		0.212	0.214	
Change in column		0.210	0.213	
Change in column temperature		0.214	0.211	
Change in mobile phase composition		0.204	0.206	
Change in instrument		0.204	0.224	

The results of the study indicates that this method for related substances in pantoprazole sodium is precise, accurate, linear in detector response and rugged.

Representative chromatogram of pantoprazole is given below.



Simple ammonium dihydrogen orthophosphate buffer and acetonitrile mixture is taken to optimize assay method.

Summary of Pantoprazole assay method validation :

Sr. No.	*Acceptance criteria*	*Observed value*	*Limit*
1.	System suitability Tailing factor of pantoprazole peak	1 87	Tailing factor NMT 2 5
3.	Linearity range	Linear regression (r^2) = 0.9999	Linear regression (r^2) NLT 0.999
4.	Instrument Precision	RSD . 1.63 %	RSD : NMT 2.0 %
5.	Method precision	Assay .99 48% RSD 0.125 %	RSD .NMT 2..0 %
6.	Ruggedness	Variation = 0.11 %	Variation : ± 0 5 %

All these observations indicate that this method for assay of pantoprazole is specific, accurate, precise and also stability indicating.

In **Chapter-9**, detailed literature search related to Cephalexin analytical methods published is presented to prove the novelty of current work Even though this drug is old, related substances and process impurities monitoring HPLC method was not published. Hence I had developed a HPLC method where all impurities of Cephalexin were addressed Development was performed with aid of software and some experiments Here structure analysis and retention analysis was derived with the help of Chromsword software. However software was failed in predicting correct condition due to closeness between impurities Successful HPLC method was developed to resolve all impurities to

base to base separation. Optimized method was validated for the correctness of intended use. Validation summary and one typical chromatogram showing separation of all components is given below for quick reference.

Cephalexin related substances method validation summary

Acceptance limit		Actual results		
System suitability Resolution between Delta ADCA and Phenyl acetic acid.	NLT 4.0	4.98 to 6.4		
		α-Phenyl glycine	7-ADCA	Δ^2-7-ADCA
Precision Instrument - RSD (%) of Detector Response for each impurity	≤ 5 0 %	0.65	0.66	0.51
Method - RSD (%) of each Impurity %	≤ 5.0 %	1.35	1.34	1.56
Linearity and Range Correlation coefficient (r)	≥ 0 99	0.9992	0.9995	0.9997
RSD (%) of detector responses	≤ 5.0 %	2.90	2.86	3.25
Accuracy Percentage recovery	95.0 % - 105.0 %	101.43 %	101.00 %	100.64 %
Minimum quantitation level (in %)		0 03 %	0.03 %	0.06 %
RSD (%) at MQL	≤ 5.0 %			

Contd..

		Phenyl acetic acid	7-PADCA	Cephalexin
Precision Instrument - RSD (%) of Detector Response for each impurity	≤ 5.0 %	1.63	0.77	0.47
Method - RSD (%) of each Impurity %	≤ 5.0 %	3.78	1.78	-
Linearity and Range Correlation coefficient (r)	≥ 0.99	0.9985	0.997	0.9997
RSD (%) of detector responses	≤ 5.0 %	4.02	2.15	2.80

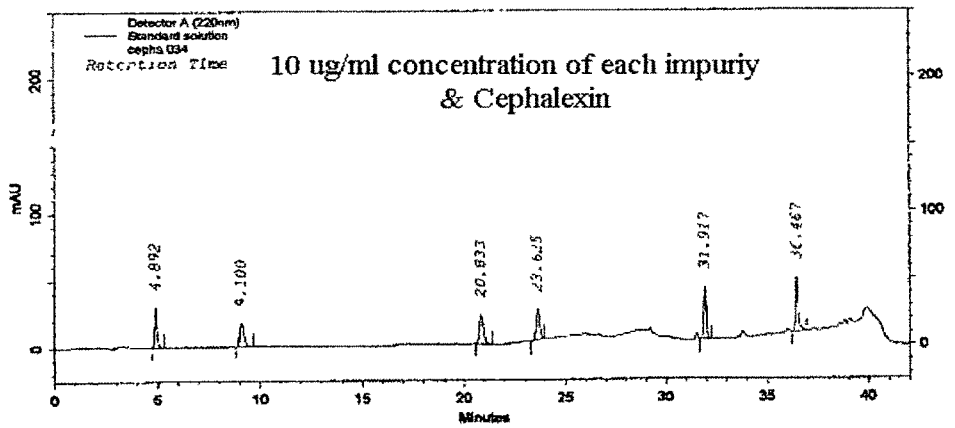
Accuracy Percentage recovery	95 0 % - 105 0 %	98.94 %	100.16 %	-
Minimum quantitation level RSD (%) at MQL	≤ 5.0 %	0 25 %	0 13 %	0 25 %

The results of the study indicate that this method for related substances and process impurities in cephalexin is precise, accurate, linear in detector response and rugged Representative chromatogram of pantoprazole is given below

Shimadzu CLASS-VP V5.03 ***Area % Report***

Method Name: C:\CLASS-VP\METHODS\cephalexin.met
Data Name : C:\CLASS-VP\VLdata\Cepha\cepha.034
Acquired : 10/11/2000 6:34:02 PM
Sample name : Standard solution

Standard solution



Detector A (220nm) Pk #	Retention Time	Area	Area Percent
1	4.892	220788	13.06
2	9 100	237346	14.04
3	20.833	300030	17 74
4	23 625	283407	16 76
5	31.917	312742	18.49
6	36.467	336743	19.91
Totals		1691056	100 00

All references related to current work is given in References section

At last but not the least, representative method validation chromatograms were presented in Appendix section