DEVELOPMENT AND VALIDATION OF GUAIFENESIN BY USING HPLC METHOD

TEJA KUMAR REDDY KONATHAM* V.JYOTHSNA, N.PAVAN
Department of pharmaceutical analysis ,Chilkur Balaji college of pharmacy ,Hyderabad, Telangana.

ABSTRACT
Guaifenesin is an expectorant which is available in tablet dose form and as well as syrup, it is chemically known as (RS)3-2-(-Methoxy phenoxy)-1-2-propanediol.In order to development a simple ,reliable and an accurate method development and validation of Guaifenesin in pharmaceutical dosage form by using reverse phase HPLC and validate the method for its repeatability and reproducibility.

AIM:

Existing literature reveals that Guaifenesin can be analyzed by UV detection, HPTLC, HPLC individually and combination with other drugs in bulk material and pharmaceutical forms.

A comprehensive, validated and simple analytical method development and validation of Guaifenesin is, therefore, crucial. No economic simple and precise[23] HPLC method was there for evaluation of Guaifenesin in bulk and pharmaceutical dosage forms. Therefore, in proposed project a successful attempt has been made to develop, simple, accurate, and economic method for analysis of Guaifenesin tablets.

OBJECTIVE:

The objective of the present work is to develop and validate a HPLC method for dosage of Guaifenesin tablets. To be employed is routine analysis. In the method development of Guaifenesin we have decided to carry out our project work by[24] incorporating the Reverse phase high performance Liquid chromatography (HPLC).Then the development method will be validated according to ICH guidelines for its various parameters

DRUG PROFILE OF GUAIFENESIN:

IUPAC name: (RS) – 3 – (2 – methoxy phenoxy) – 1 -2 – propanediol

Molecular formula: $C_{10}H_{14}O_4$ **Molecular weight:** 198.22 **Category:** Expectorant
Structure:

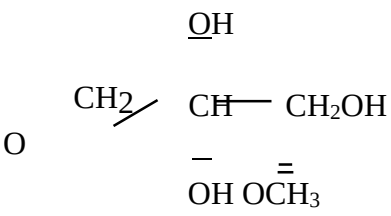


Fig no 5. Structure of guaifenesin

Description: White Solid

Solubility: 5g/100ml (25-^{0}C) in water

Identification:

i. Melting point: 77-81^{0}C

Storage: Store in refrigerator

Mechanism of action:

It acts as expectorant by increasing the volume and reducing the viscosity of secretions in trachea and bronchi. It has been said to aid the flow of respiratory tract secretions, allowing ciliary movements to carry the loosened secretions upward towards the pharynx, thus it may increase the efficiency of cough reflex and facilitate removal of the secretions.

- ✓ Guaifenesin has muscle relaxant and anticonvulsant properties and may be acting as NMDA receptor antagonist
- ✓ Boiling point - 215^{0}C (198mmHg)

Flash point - 215^{0}C/19mmHg

MATERIALS AND METHODS

CHEMICALS USED:

S. No.	Chemicals/standards and reagents	Grade	Laboratories
1	Guaifenesin tablets	AR	Sanzyme
2	Guaifenesin API	NA	Granules
2	Acetonitrile	HPLC	SDFCL
3	Water	HPLC	SDFCL
4	Dipotassium hydrogen phosphate	LR	Merck
5	KOH	LR	Molychem
6	Orthophosphoric acid	LR	Molychem

Table 5. List of chemicals and reagents used

INSTRUMENTS USED:

HPLC

S.no	HPLC	Names
1.	Company	Analytical Technologies
2.	Mode	Isocratic
3.	Software	Chromatographic Workstation
4.	Column	Cosmosil

Table 6. HPLC

UV VISIBLE SPECTROPHOTOMETER

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism
Monochromator	Aberration- correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10g/l) 0.04% or less (340 nm: $NaNo_2$ 50g/l)
Measurement wavelength range	190 700 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	< 1 nm
Recording range	Absorbance: -3.99 3.99 Abs Transmittance: -399 399%
Photometric accuracy	0.004 Abs (at 1.0 Abs), 0.002 Abs (at 0.5 Abs)
Operating Temperature/ Humidity	Temperature range: 4 to 35 C Humidity range: 45 to 85%

Table 7 UV SPECTROPHOTOMETER

LIST OF EQUIPMENT'S:

S.NO	Equipment's	Model	Company
1	Electronic Balance	ECB 300	WENSAR
2	Ultra-Sonicator	SE60US	CITIZEN
3	Heating Mantle	BTI	BIO TECHNICS INDIA
4	pH Meter	DPH 500	GLOBAL
5	Filter Paper (membrane filter cellulose acetate) 0.45mm D	---------	MK-corporation
6	Vacuum pump	Value	VE115N

Table 8. list of Equipments Used

PLAN OF WORK

In order to develop a simple, reliable and an accurate method development and validation of guaifenesin in pharmaceutical dosage form by using Reverse phase HPLC and validate the method for its repeatability and reproducibility.

Plan of the proposed work includes the following steps:

- Selection of drug and literature survey.
- Procurement of raw materials.
- Solubility studies and optimization of conditions.
- Trails for the method development of guaifenesin Setting of the optimized method.
- Establishment of system suitability parameters[20].
- Assay of the drugs(s) in marketed formulations using the proposed method(s).
- Analytical method(s) development using HPLC etc.,
- Validation of the optimized method for guaifenesin

Validation parameters include:

- ❖ System suitability
- ❖ Specificity
- ❖ precision
- ❖ Linearity
- ❖ Accuracy
- ❖ Range
- ❖ Ruggedness
- ❖ Robustness
- ❖ Assay

METHODOLOGY

6.1 SOLUBILITY STUDIES

Solubility studies was performed for Guaifenesin in different solvents based on polarity.

S.No	Solvents	Extent of solubility	Category
1	HCl	10mg in 1ml	Freely soluble
2	NaOH	10mg in 1ml	Insoluble
3	Water	10mg in 1ml	Poorly soluble
4	Toluene	10mg in 1ml	Insoluble
5	Acetonitrile	10mg in 1ml	Soluble
6	Ethanol	10mg in 1ml	Soluble
7	Methanol	10mg in 1ml	Soluble

Table 9. Solubility studies of guaifenesin in polar and non polar solvents:

6.2 Determination of UV spectra by using UV-VISIBLE spectrophotometer

Uv spectra was determined to find out the Wavelength by taking 100mg of Guaifenesin in 100ml of volumetric flask. The solution was scanned between 200- 400nm.The wavelength was found as 260nm

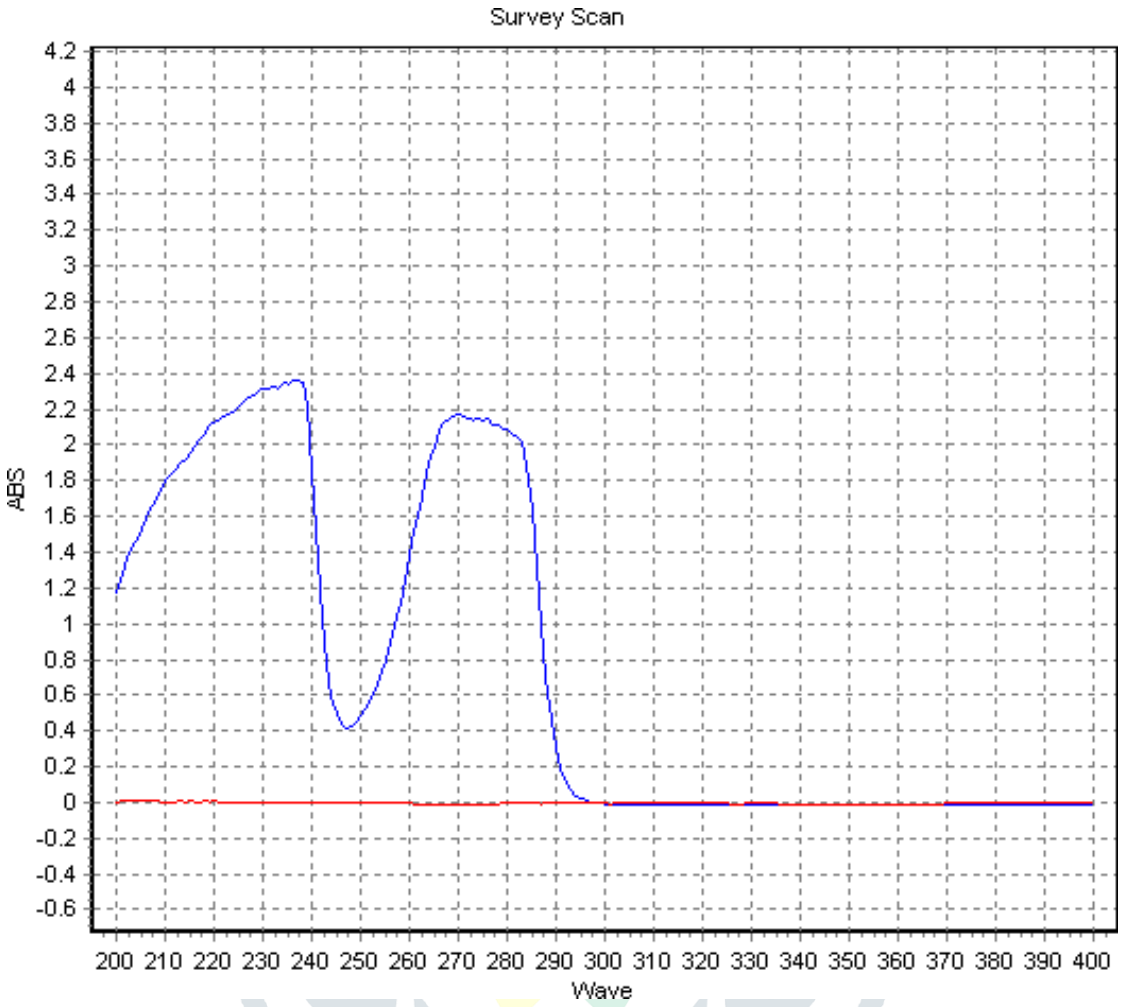


Fig 6: Determination of UV spectra by using UV-VISIBLE spectrophotometer

6.3 OPTIMIZED METHOD

Mobile Phase: Acetonitrile: Phosphate buffer P^H 5(70:30)

Column: Cosmosil packed ($5C_{18}$-MS-II, 4.6 internal diameter 250mm length Make: Analytical) column
Flow Rate: 1.0ml/Min
Temperature: 30˚C **Volume:** 20µl
Detector: 260nm **Procedure:**
Inject 20µL of standard, sample into chromatographic system and measure the areas for the guaifenesin peaks and calculate the % assay by using the formula

PREPARATION OF BUFFER:

Transfer 6.8gms of potassium dihydrogen phosphate in 1000ml volumetric flask and add HPLC water up to mark and adjust the P^H to 5.0 with potassium hydroxide.

PREPARATION OF MOBLE PHASE

Transfer 300ml phosphate buffer in 1000ml volumetric flask and make the volume with Acetonitrle. After mixing sonicated for 20min.

PREPARATION OF THE STANDARD AND SAMPLE SOLUTION OF GUAIFENESIN
PREPARATION OF STANDARD SOLUTION:

Accurately weigh and transfer 100mg of Guaifenesin into 100ml of volumetric flask and add 100ml of mobile phase and sonicate 10min (or) shake 5min and make with water. Transfers the above 10ml solution into 100ml volumetric flask dilute to volume with water.

PREPARATION OF SAMPLE STOCK SOLUTION:

Accurately weigh 20tablets and powedered and transfer equivalent weight to 100mg into a 100ml of volumetric flask and add 10ml of Mobile phase and sonicate 20mins (or) shake 10min and makeup with mobile phase. Transfer above 10ml solution into 100ml of the volumetric flask dilute the volume with Mobile phase and the solution is sonicated before injecting into HPLC system[22].

6.4 ASSAY RESULTS FOR FORMULATION Label claim: Each tablet contains Guaifenesin – 600mg. Average weight of each tablet is 0.646 mg.

Standard preparation:

Accurately weigh 100 mg in 100ml volumetric flask and required amount of mobile phase was added up to mark. The mixture was subjected to sonication for 20 minutes with intermediate shaking for complete extraction of drugs .Filtered by vacuum pump using 0.45µ filter paper.

Sample Preparation:

Accurately weigh equivalent weight to 100 mg in 100ml volumetric flask and required amount of mobile phase was added up to mark. The mixture was subjected to sonication for 20 minutes with intermediate shaking for complete extraction of drugs .Filtered by vacuum pump using 0.45µ filter paper.

Procedure:

Direct comparision method:

By comparing the peak areas of sample and standard solutions , the quatity of the sample can be determined.

$$\text{Peak area} = \text{Peak height Peak width } A_1/A_2 = \alpha C_1/C_2$$

A_1 and A_2 are peak areas of sample and standard. C_1 and C_2 are concentrations of sample and standard α = Response factor .

Result The percentage purity of guaifenesin was found to be 98%.

6.5 VALIDATION PARAMETERS

1. System Suitability:

Tailing factor for the peaks due to Guaifenesin in standard solution should not be more than 2.0.Theoretical plates for Guaifenesin peaks in standard solution should not be less than 2000.

2. Specificity:

Solution of standard, sample, blank and placebo were prepared as per test procedure and injected into the HPLC system.

Acceptance criteria:

Chromatogram of standard and sample should be identical with near Retention time.

Blank interference:

A study to establish the interference of blank was conducted. Diluent was injected into HPLC system as per the test procedure.

Acceptance criteria:

Chromatogram of blank should not show any peak at the retention time of analyte peak. There is no interference due to blank at the retention time of analyte. Hence the method is specific[14].

3. Linearity:

Prepare a series of standard solutions and inject into HPLC system. Plot the graph of standard versus the actual concentration in µg/ml and determine the coefficient of correlation and basis for 100% response.

Acceptance criteria:

Linearity regression coefficient of average peak area response of replicate injections plotted against respective concentration should not be less than 0.999. The % y-intercept as obtained from the linearity data (without extrapolation through origin 0, 0) should be within ±2.0.

Statistical Evaluation:

A graph between the concentration and the average area was plotted. Points for linearity were observed. Using the method of least squares, a line of best fit was taken and the correlation Coefficient, slope and, y-intercept were calculated.

4. Precision:

Preparation of sample:

- Transfer the 100mg of sample into a 100ml of volume at flask and add 100ml of Mobile phase and sonicate 20mins. Transfer the above 3ml solution into 10ml volume metric flask & dilute upto the volume with mobile phase.
- The method precision parameters were evaluated from sample chromatograms obtained, by calculating the % RSD of peek areas from 6 replicate injections[23].

Acceptance criteria: The injection reproducibility requirements are met if the %RSD for peak areas is not more than 2.0 and for retention times are not more than 2.0.

5. Recovery/Accuracy:

Recovery study was performed in the concentration range of 50,100,150% of the target concentration of the test. Minimum 3 concentrations are recommended.

Acceptance criteria:

The average percentage recovery was between 98-102% and Relative standard deviation of these recovery concentrations was less than 2%.

6. Limit of Detection:

The sensitivity of measurement of Guaifenesin by use of proposed method was estimated in terms of the limit of detection (LOD). The LOD was calculated by the use of signal to noise ratio. In order to estimate the LOD value, the blank sample was injected six times and peak area of this blank was calculated as noise level. The LOD was calculated as three times the noise level.

$$LOD = 3.3\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

7. Limit of Quantitation:

The sensitivity of measurement of Guaifenesin by the use of proposed method was estimated in terms of limit of quantitation (LOQ). The LOQ was calculated by the use of signal to noise ratio. In order to estimate the LOQ value, the blank sample was injected six times and the peak area of this blank was calculated at noise level. The LOQ was calculated as ten times the noise value gave the LOQ.

$$LOQ = 10\ \sigma / S$$

Where,

σ = standard deviation of intercepts of calibration curves S = mean of slopes of the calibration curves

The slope S may be estimated from the calibration curve of the analyte.

8. Robustness:

Effect of variation in flow rate:

Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates[22]. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked.

Effect of variation in wavelength:

Prepare the system suitability solution as per the test method and injected into the HPLC with

±2nm variation in wavelength. Evaluate the system suitability values as required by the test method for both wavelengths.

CHAPTER-7 RESULTS AND DISCUSSION

1. SYSTEM SUITABILITY:

Parameter	Guaifenesin	Acceptance criteria
Retention time	3.677	+-10
Theoretical plates	2159	>2000
Tailing factor	0.83	<2.00

Table 10. System suitability data of Guaifenesin

S.no	Sampl name	RT	Area	Theoritical plate count	USP tailing
1.	Injection1	3.518	17115746	737	1.17
2.	Injection 2	3.518	14920550	793	0.97
3.	Injection 3	3.035	18425189	652	0.85
4.	Injection 4	3.677	14449086	2159	0.83

Table 11.Trails of Guaifenesin

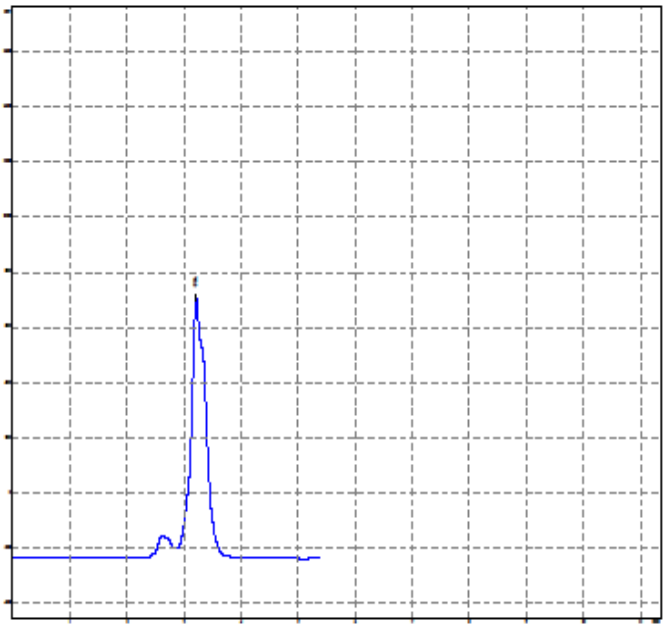
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.36min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:WATER (50:50)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 1639uV, baseline drift: 952192uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	2.613		1399960	1.27	545	-0.000	1.65
2	3.208	GUA	17115746	3.46	737	0.228	1.17
3	4.867		33591	1.08	1711	0.862	1.09
4	5.188		7584	0.00	32530	0.985	4.94

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:46 Page 1/1

Fig 7. Typical Chromatogram of Standard-1

Report:

Fronting in the peak and deformation of the peak

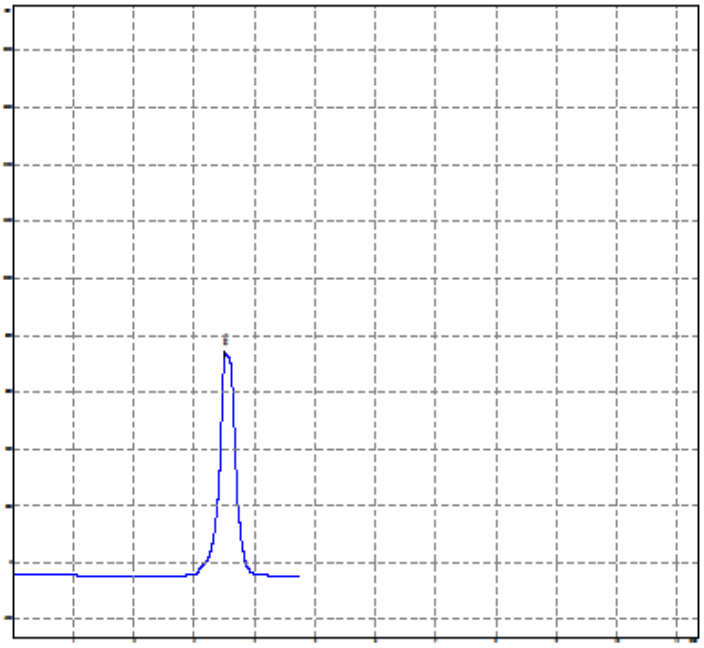
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 4.74min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(50:50)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10PsiV

Baseline noise: 976uV, baseline drift: 782303uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.055		8699	8.32	0	0	0.93
2	2.655		3131	2.43	2834	47.273	1.27
3	3.518	GUA	14920550	0.00	793	62.964	0.97

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:56 Page 1/1

Fig 8. Typical chromatogram of Standard-2

Report:

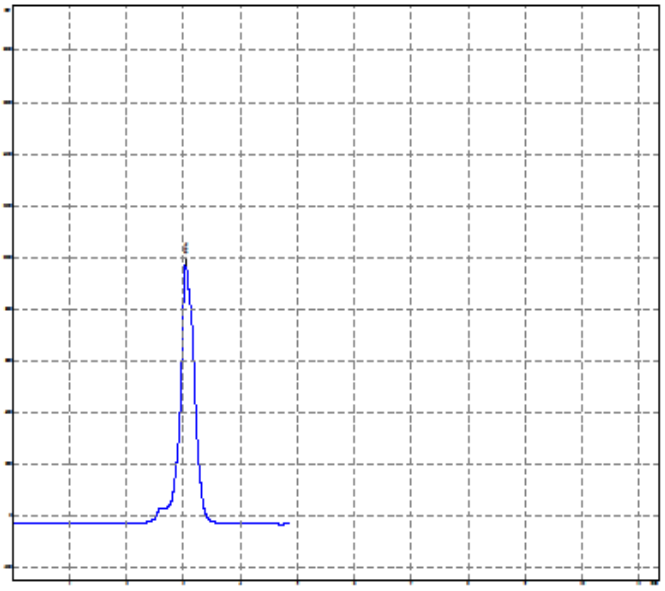
No fronting but deformation of the peak.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 4.84min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(60:40)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 1184uV, baseline drift: 1016227uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.239		59357	2.30	2	0	5.07
2	1.423		9676	4.17	398	4.951	0.54
3	3.035	GUA	18425189	0.00	652	11.690	0.85

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:00 Page 1/1

Fig 9 . Typical Chromatogram of Standard-3

Report:

Fronting of the Peak, so we went to the next trial.

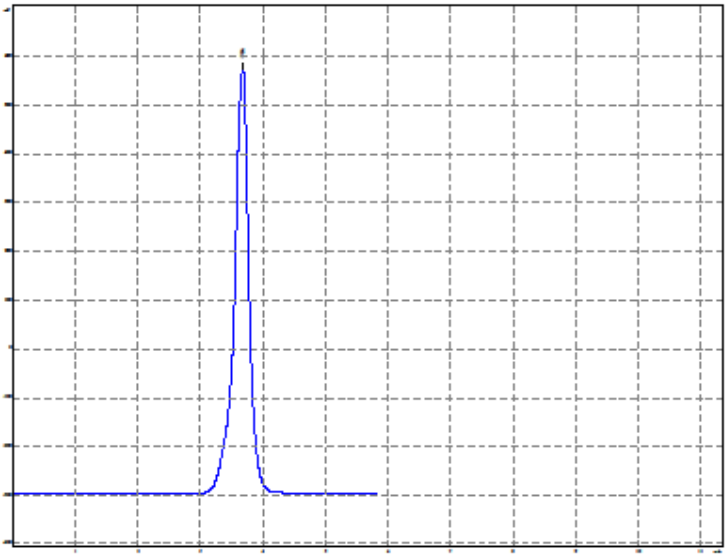
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.82min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 116uV, baseline drift: 875355uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.088		1793	0.10	7	0.006	2.65
2	0.154		15717	1.19	0	0.760	0.96
3	1.106		926	2.33	141	11.640	2.99
4	1.954		392	0.85	522	21.331	0.87
5	2.275		303	3.33	526	25.000	0.81
6	3.677	GUA	14449086	0.00	1159	41.023	0.83

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:02 Page 1/1

Fig 10. Typical Chromatogram of Standard- 4

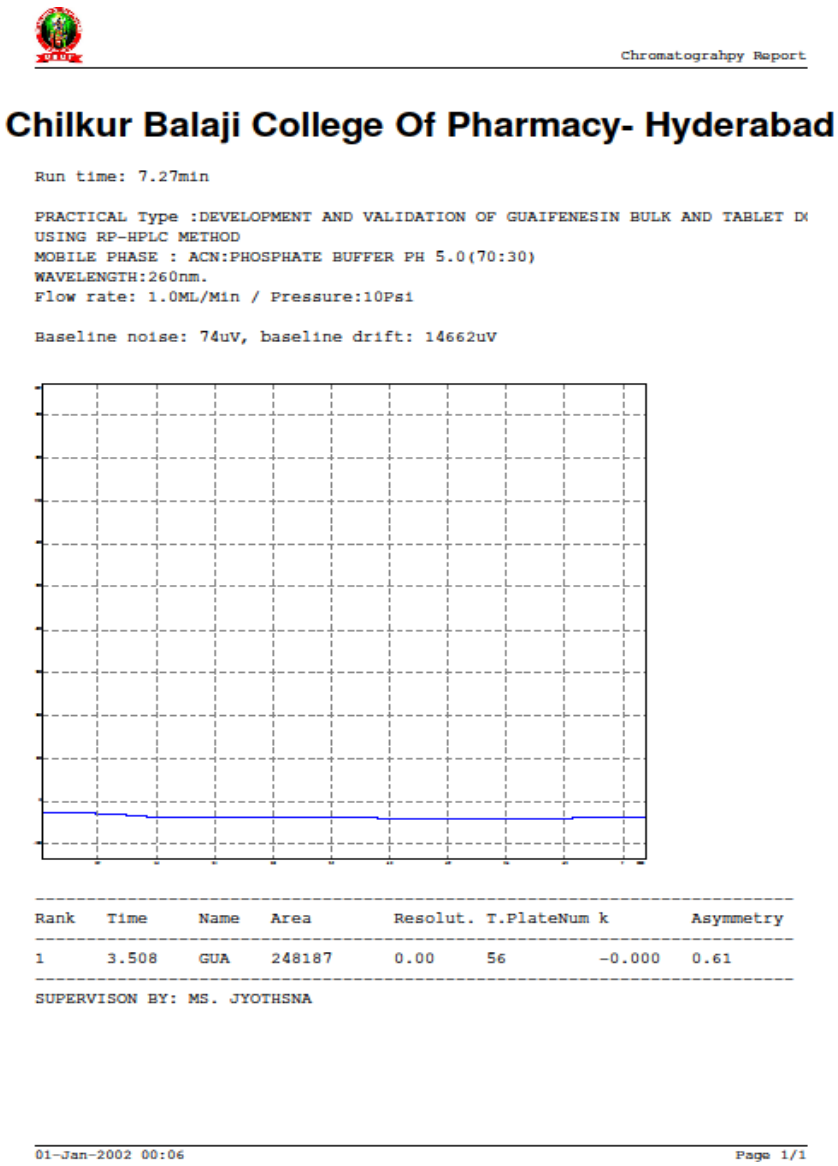
Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time is also less.

RESULT

Results of system suitability study are summarized in the above table. Four consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for the drug which indicate a good system for analysis.

2. SPECIFICITY:



Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 7.27min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 74uV, baseline drift: 14662uV

Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	3.508	GUA	248187	0.00	56	-0.000	0.61

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:06 Page 1/1

Fig 11: Typical chromatogram of the blank

Report:

The baseline is good without any fronting and tailing.

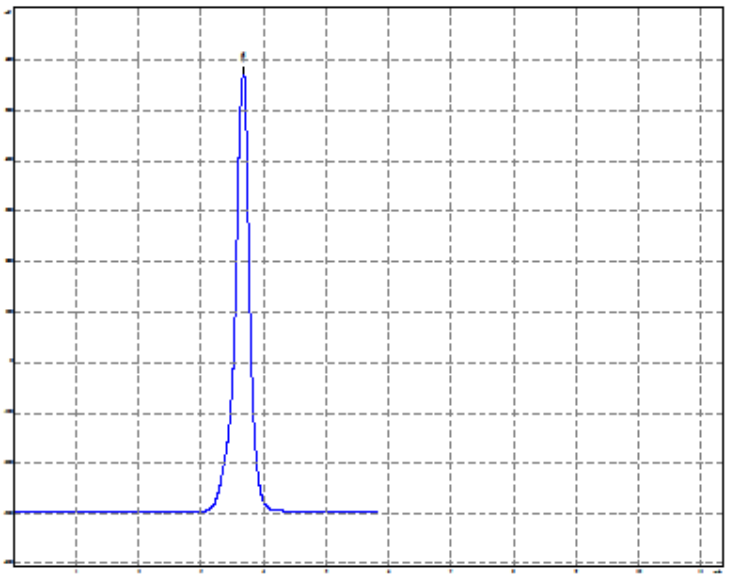
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.82min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 116uV, baseline drift: 875355uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.088		1793	0.10	7	0.006	2.65
2	0.154		15717	1.19	0	0.760	0.96
3	1.106		926	2.33	141	11.640	2.99
4	1.954		392	0.85	522	21.331	0.87
5	2.275		303	3.33	526	25.000	0.81
6	3.677	GUA	14449086	0.00	1159	41.023	0.83

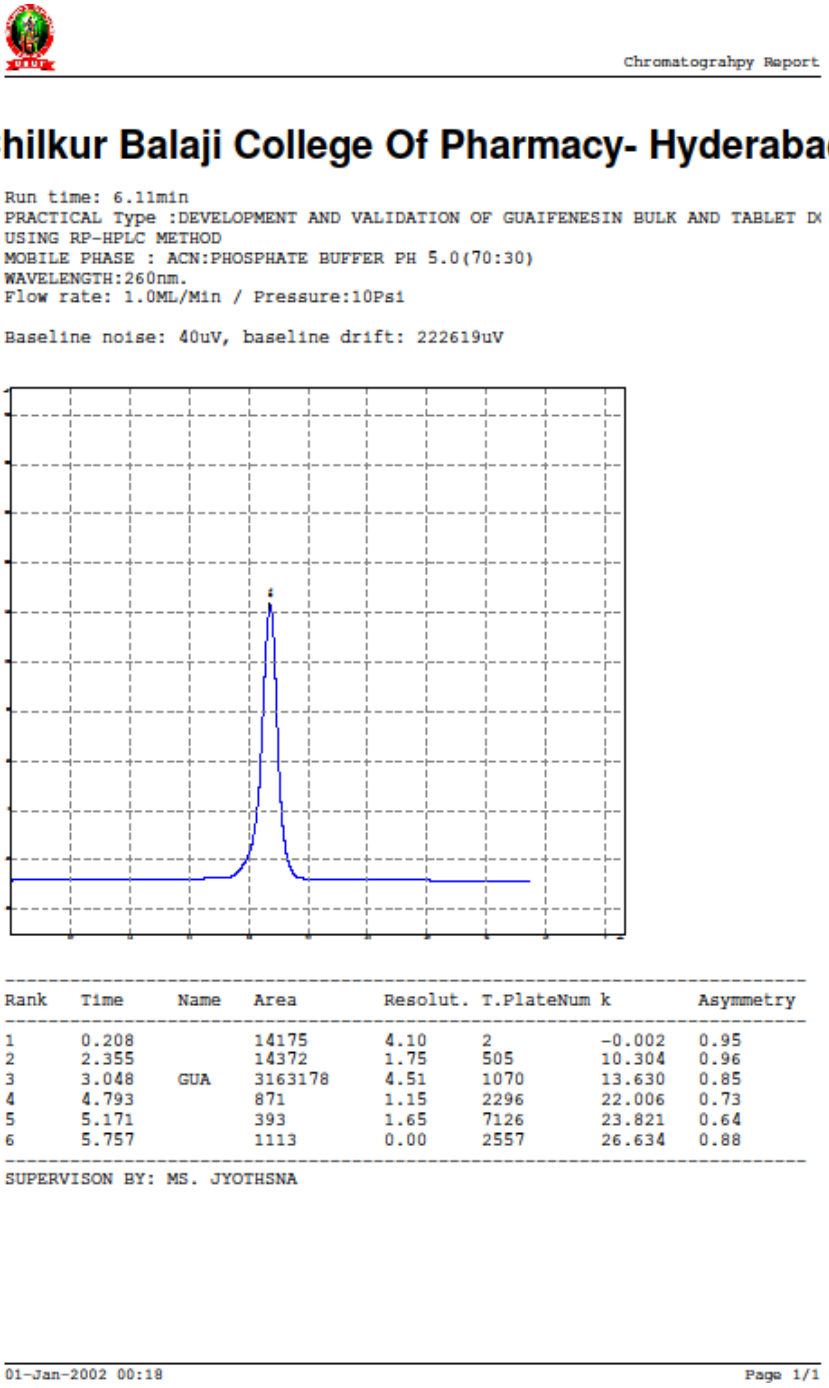
SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:02 Page 1/1

Fig 12: chromatogram representing specificity of standard

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time is also less.



Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.11min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 40uV, baseline drift: 222619uV

Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.208		14175	4.10	2	-0.002	0.95
2	2.355		14372	1.75	505	10.304	0.96
3	3.048	GUA	3163178	4.51	1070	13.630	0.85
4	4.793		871	1.15	2296	22.006	0.73
5	5.171		393	1.65	7126	23.821	0.64
6	5.757		1113	0.00	2557	26.634	0.88

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:18 Page 1/1

Fig 13: chromatogram representing specificity of sample

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

RESULT:

Chromatograms explain that retention time for standard and sample (tablet) product are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective.

3. ACCURACY:

S.NO	Accuracy Level	Injecton	Sample area	RT
1	50%	1	3163178	3.648
		2	3348569	3.645
		3	3215347	3.656
2	100%	1	5648567	3.669
		2	5495826	3.669
		3	5324165	3.668
3	150%	1	6954826	3.667
		2	6856911	3.667
		3	7121432	3.665

Table 12: Accuracy data for Guaifenesin

S.NO	Accuracy level	Sample name	Sample Weight (g)	µg/ml added	µg/ml found	% Recovery	% Mean
1	50%	1	0.3	20	19.5	100	100
		2	0.3	20	20	100	
		3	0.3	20	21	101	
2	100%	1	0.6	40	40	100	100
		2	0.6	40	40	100	
		3	0.6	40	40	100	
3	150%	1	0.9	60	60	100	100
		2	0.9	60	58	99	
		3	0.9	60	60	100	

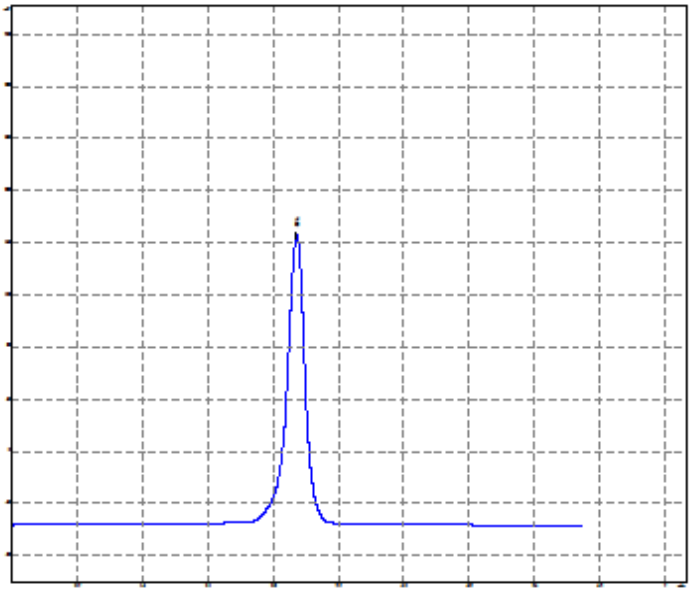
Table 13: Accuracy (%recovery) results of Guaifenesin

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.11min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D(
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 40uV, baseline drift: 222619uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.208		14175	4.10	2	-0.002	0.95
2	2.355		14372	1.75	505	10.304	0.96
3	3.048	GUA	3163178	4.51	1070	13.630	0.85
4	4.793		871	1.15	2296	22.006	0.73
5	5.171		393	1.65	7126	23.821	0.64
6	5.757		1113	0.00	2557	26.634	0.88

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:18 Page 1/1

Fig 14: Typical chromatogram for Accuracy 50 %

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

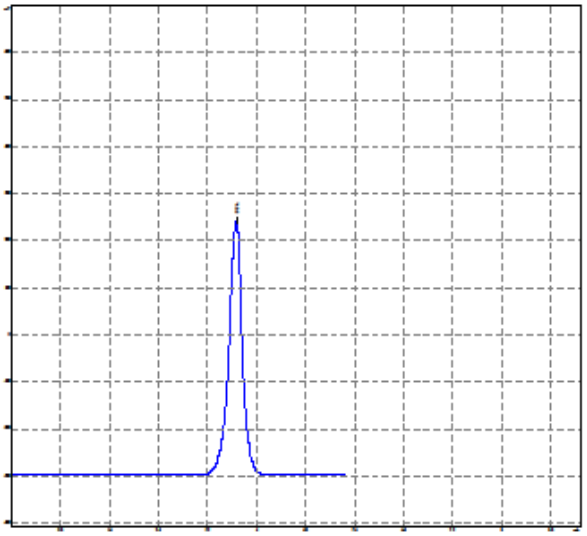
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.44min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 98uV, baseline drift: 541008uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.067		2494	0.55	7	0.005	
2	0.129		2511	3.65	18	0.935	0.72
3	0.633		133	1.57	280	8.495	0.76
4	0.838		108	1.03	1024	11.570	0.68
5	1.025		883	0.16	260	14.375	1.14
6	1.146		5889	3.11	15	16.190	1.38
7	3.669	GUA	5648567	0.00	2879	54.035	0.90

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:20 Page 1/1

Fig 15: Typical chromatogram for Accuracy 100 %

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

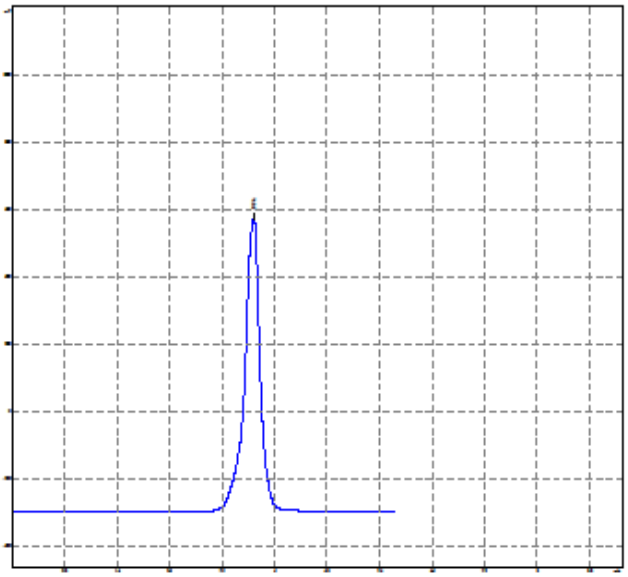
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.82min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 116uV, baseline drift: 875355uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.088		1793	0.10	7	0.006	2.65
2	0.154		15717	1.19	0	0.760	0.96
3	1.106		926	2.33	141	11.640	2.99
4	1.954		392	0.85	522	21.331	0.87
5	2.275		303	3.33	526	25.000	0.81
6	3.677	GUA	6954826	0.00	5003	41.023	0.83

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:21 Page 1/1

Fig 16: Typical chromatogram for Accuracy 150 %

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

RESULT

Results of accuracy study are presented in the above table. The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount.% Recovery was 100.00% for Guaifenesin . All the results indicate that the method is highly accurate**.**

4. PRECISION:

S.no	RT	Area	%Assay
injection1	2.988	4393802	100
injection2	2.973	4070799	100
injection3	3.027	4147528	100
injection4	3.071	4331478	100
injection5	3.048	4372308	101
injection6	2.988	4234578	100
Mean	-	-	100
Std. Dev.	-	-	0.32
% RSD	-	-	0.32

Table 14: Precision data for Guaifenesin

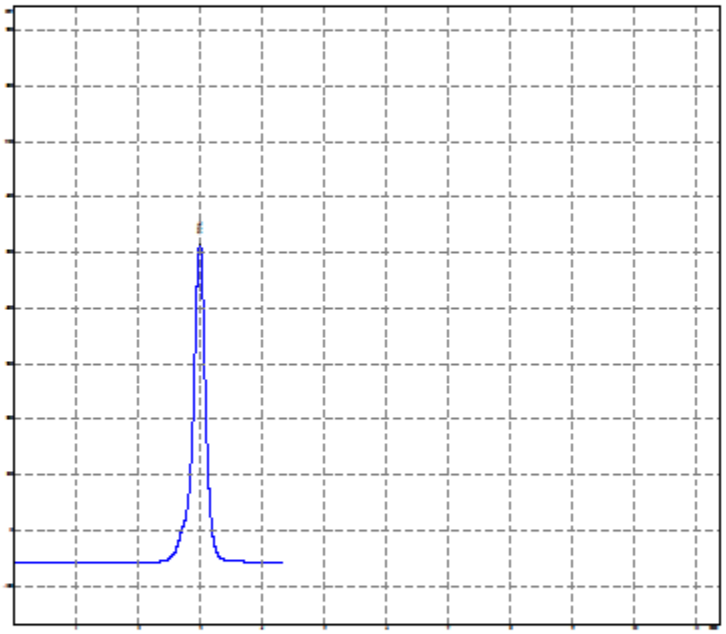
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 4.32min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 672uV, baseline drift: 576363uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.086		37530	4.72	0	0.002	3.62
2	2.988		4393802	0.00	3577	33.812	0.82

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:34 Page 1/1

Fig 17: Chromatogram for precision injection 1

Report:

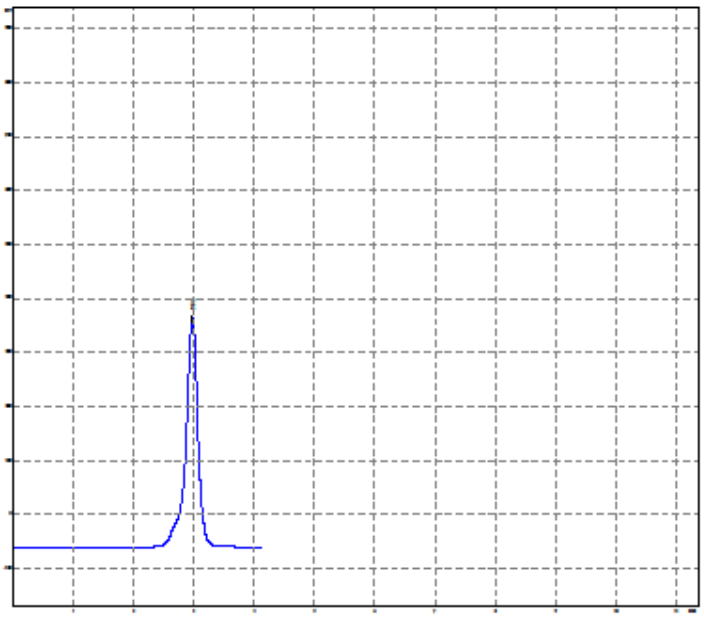
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 4.13min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 312uV, baseline drift: 423057uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.094		240	2.98	10	-0.002	0.73
2	0.658		128	7.57	104	5.988	0.77
3	1.646		106	7.03	1626	16.480	
4	2.973		4070799	0.00	2229	30.572	0.80

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:35 Page 1/1

Fig 18: Chromatogram for precision injection 2

Report:

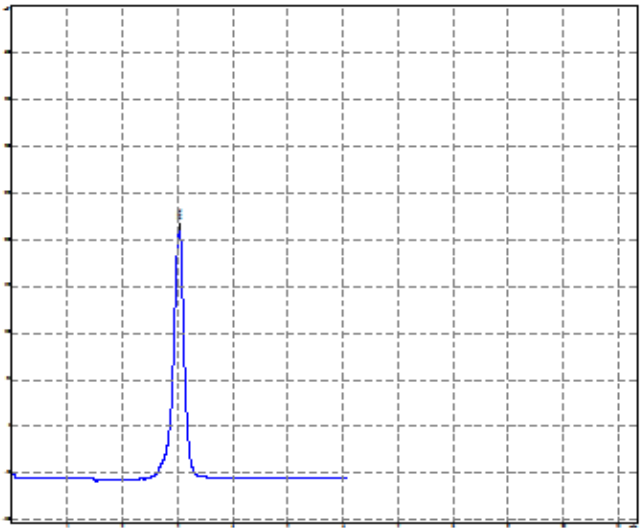
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.07min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 70uV, baseline drift: 272398uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.383		189	1.78	14	-0.001	0.79
2	1.075		134	1.08	150	1.804	0.77
3	1.681		8333	1.35	80	3.385	0.53
4	2.346		3374	2.30	1915	5.120	0.94
5	3.027	GUA	4147528	3.10	907	6.897	0.83
6	4.743		1432	0.83	692	11.373	1.02
7	5.154		128	2.02	6394	12.445	0.52
8	5.855		991	0.00	3031	14.274	0.99

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:37 Page 1/1

Fig 19: Chromatogram for precision injection 3

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

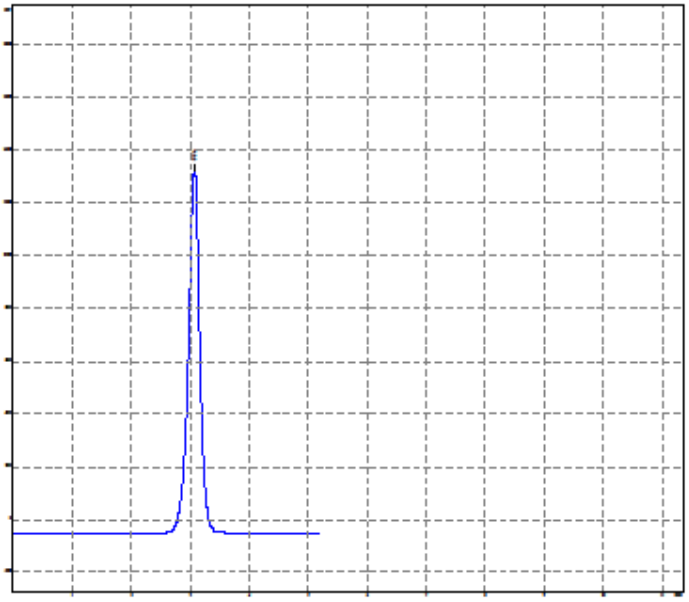
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.19min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 202uV, baseline drift: 1382022uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.238		203	7.95	13	0.002	0.99
2	2.379		8230	2.18	1289	9.017	1.19
3	3.071	GUA	4331478	3.19	22447	11.931	0.92
4	4.480		1346	0.00	1231	17.863	0.92

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:38 Page 1/1

Fig 19: Chromatogram for precision injection 4

Report:

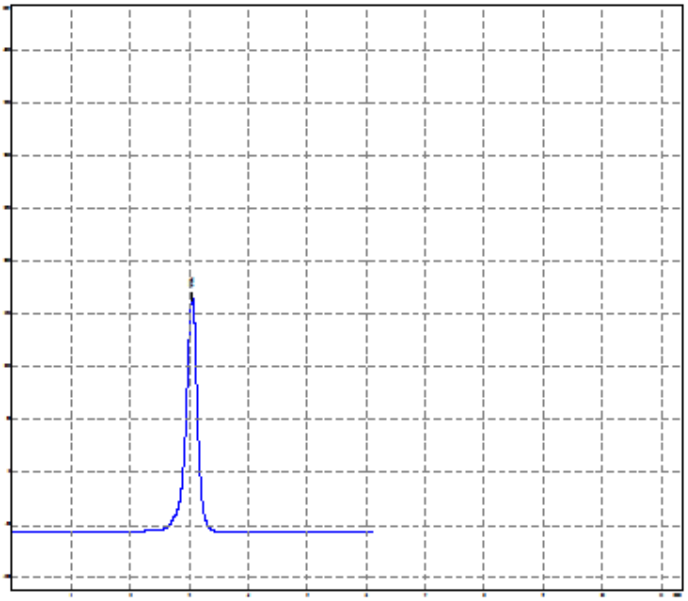
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.11min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 40uV, baseline drift: 222619uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.208		14175	4.10	2	-0.002	0.95
2	2.355		14372	1.75	505	10.304	0.96
3	3.048	GUA	4372308	4.51	560	13.630	0.85
4	4.793		871	1.15	2296	22.006	0.73
5	5.171		393	1.65	7126	23.821	0.64
6	5.757		1113	0.00	2557	26.634	0.88

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:40 Page 1/1

Fig 20: Chromatogram for precision injection 5

Report:

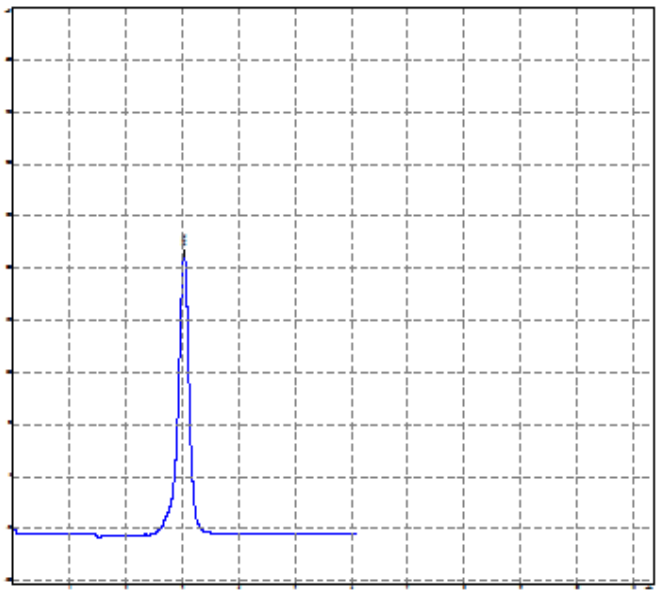
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.07min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 70uV, baseline drift: 272398uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.383		189	1.78	14	-0.001	0.79
2	1.075		134	1.08	150	1.804	0.77
3	1.681		8333	1.35	80	3.385	0.53
4	2.346		3374	2.30	1915	5.120	0.94
5	3.027	GUA	4234578	3.10	870	6.897	0.83
6	4.743		1432	0.83	692	11.373	1.02
7	5.154		128	2.02	6394	12.445	0.52
8	5.855		991	0.00	3031	14.274	0.99

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 00:42 Page 1/1

Fig 22: Chromatogram for precision injection 6

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

RESULT

Results of variability were summarized in the above table. % RSD of peak areas was calculated for various run. Percentage relative standard deviation (%RSD) was found to be less than 2% which proves that method is precise**.**

5.LINEARITY:

s.no	Conc(µg/ml)	RT	Area
1.	10	4.102	942008
2.	15	3.730	14131300
3.	20	3.795	1828907
4.	25	3.872	2270006
5.	30	3.027	2660769
6	35	3.048	3163178
Correlation coefficient (r^2)			0.998

Table 15. : Linearity values of Guaifenesin

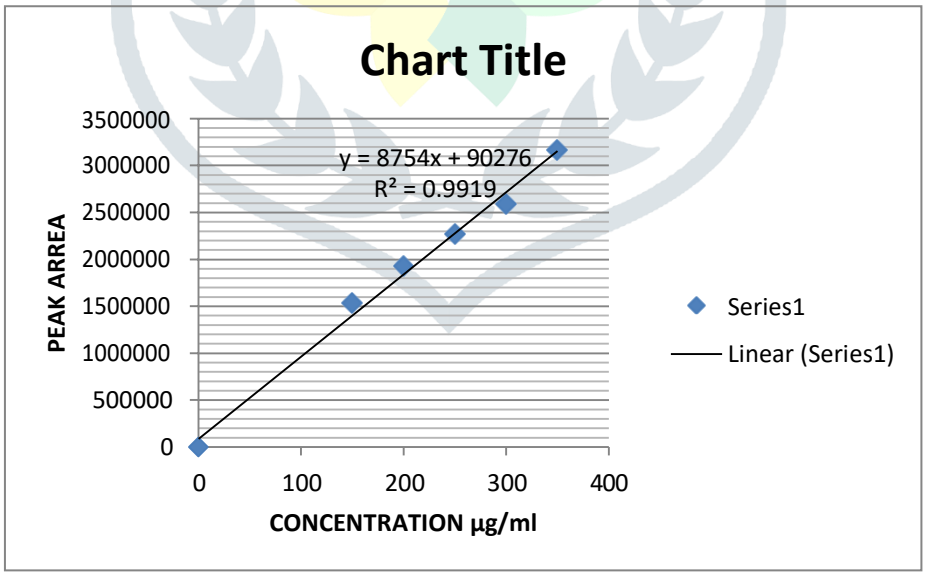


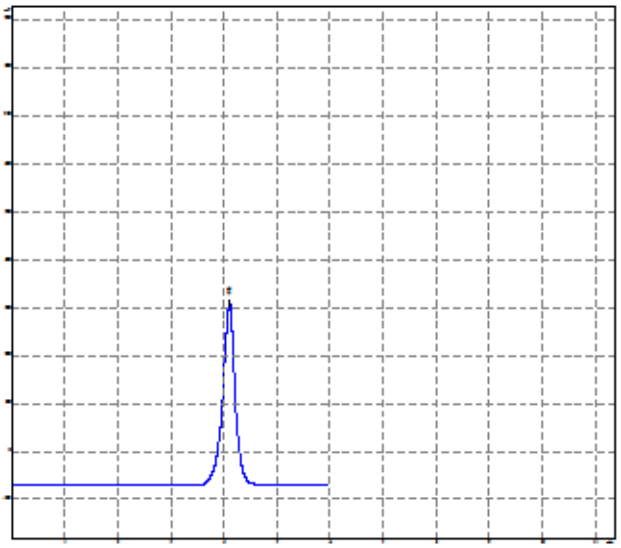
Fig 23.Linearity plot of Guaifenesin

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 5.94min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 92uV, baseline drift: 380176uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.067		4417	0.73	4	0.005	1.27
2	0.479		28869	0.39	4	6.185	0.89
3	0.672		5488	0.39	171	9.080	0.89
4	1.256		21239	0.48	30	17.840	0.94
5	1.587		8688	0.49	201	22.805	0.59
6	1.992		19536	1.35	46	28.880	1.14
7	3.210		13115	1.69	453	47.150	0.86
8	4.102	GUA	942008	0.00	63811	60.530	0.91

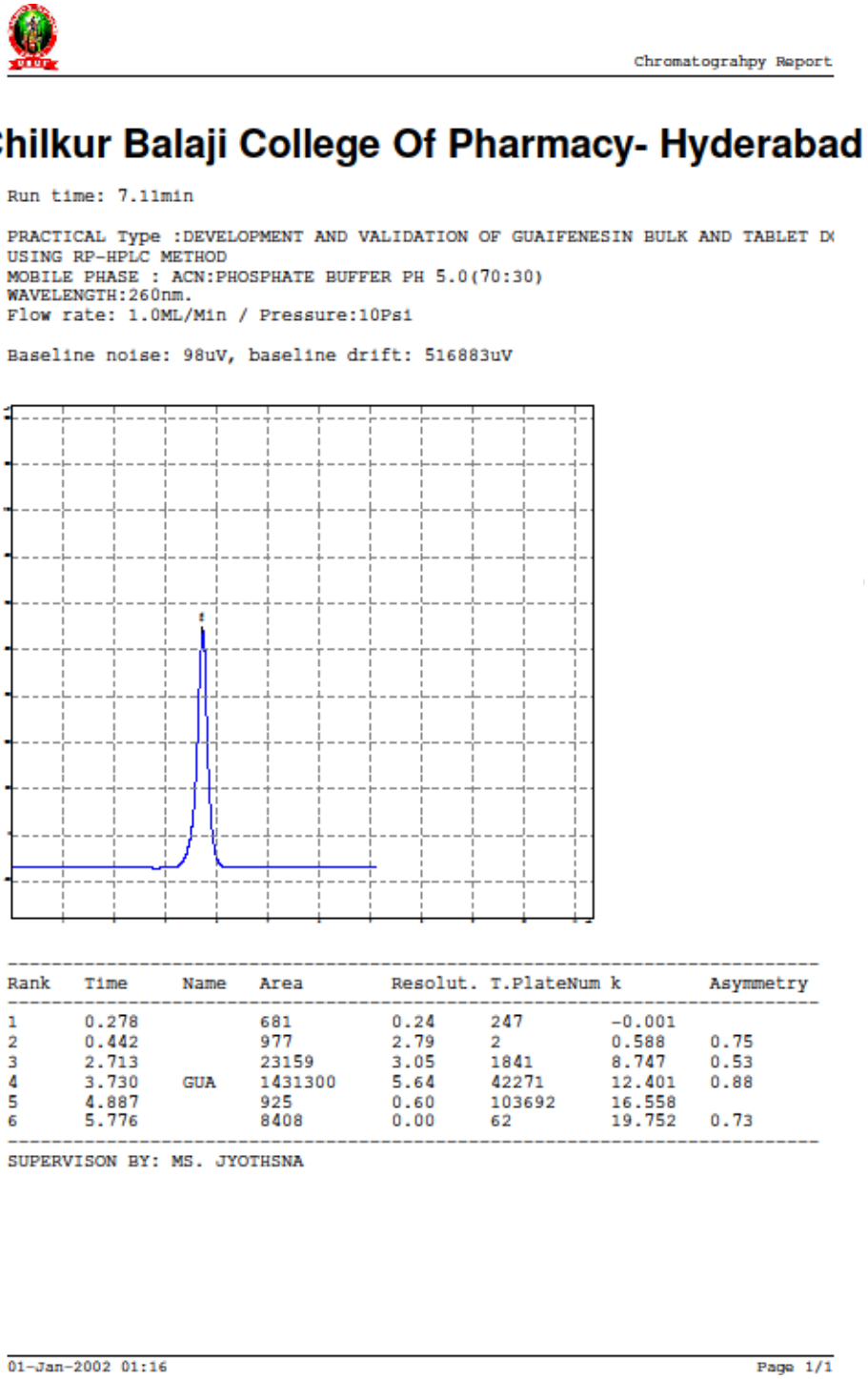
SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:15 Page 1/1

Fig 24: Chromatogram representing linearity 1

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.



Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 7.11min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 98uV, baseline drift: 516883uV

Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.278		681	0.24	247	-0.001	
2	0.442		977	2.79	2	0.588	0.75
3	2.713		23159	3.05	1841	8.747	0.53
4	3.730	GUA	1431300	5.64	42271	12.401	0.88
5	4.887		925	0.60	103692	16.558	
6	5.776		8408	0.00	62	19.752	0.73

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:16 Page 1/1

Fig 25: Chromatogram representing linearity 2

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

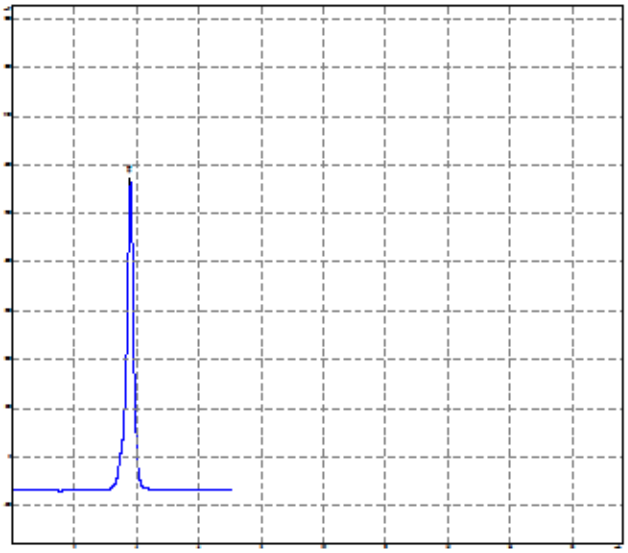
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 7.05min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 152uV, baseline drift: 638037uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.092		2973	1.81	11	0.004	2.91
2	1.417		235225	2.66	18	14.458	0.52
3	3.795	GUA	1828907	2.36	40357	40.400	0.82
4	5.786		28934	0.00	353	62.120	0.60

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:17 Page 1/1

Fig 26: Chromatogram representing linearity 3

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

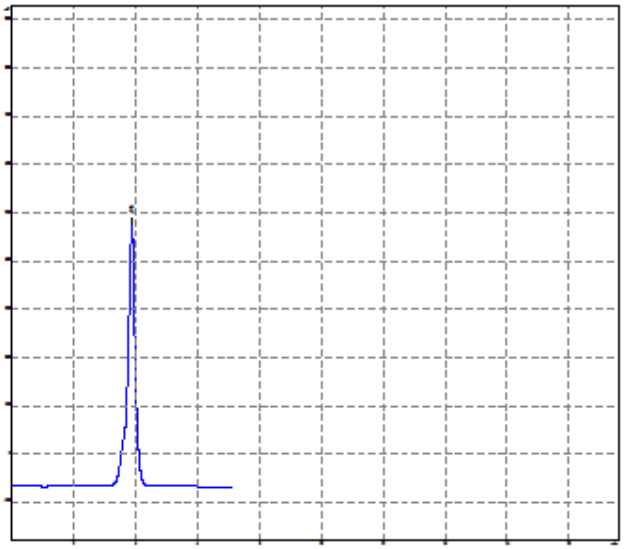
Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 7.14min

PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 116uV, baseline drift: 553365uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.050		698	6.89	4	0	0.61
2	0.954		6734	1.13	601	18.080	0.82
3	1.189		20977	7.46	348	22.780	1.20
4	3.872	GUA	2270006	4.49	20571	76.440	0.82
5	5.873		4371	0.00	3071	116.460	0.75

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:19 Page 1/1

Fig 27: Chromatogram representing linearity 4

Report:

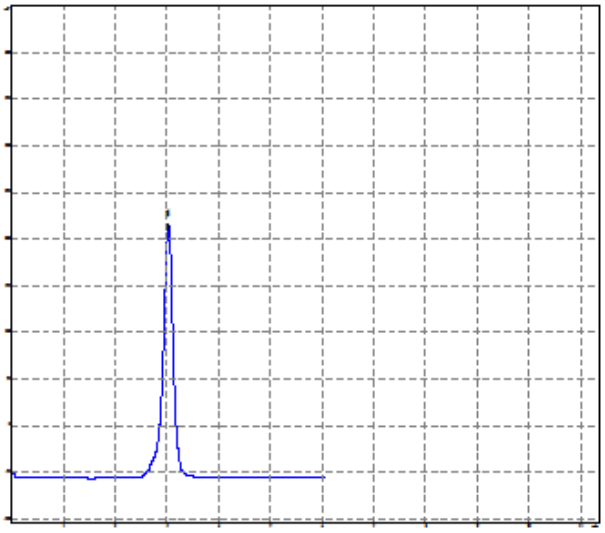
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.07min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 70uV, baseline drift: 272398uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.383		189	1.78	14	-0.001	0.79
2	1.075		134	1.08	150	1.804	0.77
3	1.681		8333	1.35	80	3.385	0.53
4	2.346		3374	2.30	1915	5.120	0.94
5	3.027	GUA	2660769	3.10	2203	6.897	0.83
6	4.743		1432	0.83	692	11.373	1.02
7	5.154		128	2.02	6394	12.445	0.52
8	5.855		991	0.00	3031	14.274	0.99

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:20 Page 1/1

Fig 27:Chromatogram representing linearity 5

Report:

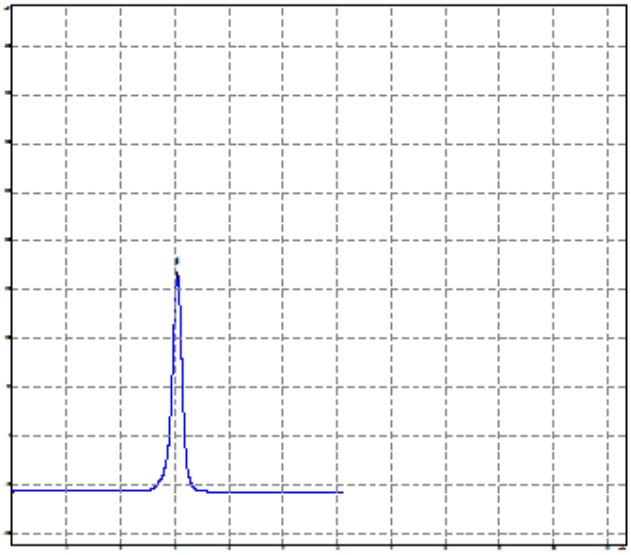
Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

Chromatograhpy Report

Chilkur Balaji College Of Pharmacy- Hyderabad

Run time: 6.11min
PRACTICAL Type :DEVELOPMENT AND VALIDATION OF GUAIFENESIN BULK AND TABLET D
USING RP-HPLC METHOD
MOBILE PHASE : ACN:PHOSPHATE BUFFER PH 5.0(70:30)
WAVELENGTH:260nm.
Flow rate: 1.0ML/Min / Pressure:10Psi

Baseline noise: 40uV, baseline drift: 222619uV



Rank	Time	Name	Area	Resolut.	T.PlateNum	k	Asymmetry
1	0.208		14175	4.10	2	-0.002	0.95
2	2.355		14372	1.75	505	10.304	0.96
3	3.048	GUA	3163178	4.51	1070	13.630	0.85
4	4.793		871	1.15	2296	22.006	0.73
5	5.171		393	1.65	7126	23.821	0.64
6	5.757		1113	0.00	2557	26.634	0.88

SUPERVISON BY: MS. JYOTHSNA

01-Jan-2002 01:22 Page 1/1

Fig 28 Chromatogram representing linearity 6

Report:

Peak is good without fronting and tailing and it has good resolution, symmetry, asymmetry and retention time will also less.

RESULT

A linear relationship between peak areas versus concentrations was observed for Guaifenesin in the range of 10% to 35% of nominal concentration. Correlation coefficient was 0.998 for Guaifenesin which prove that the method is linear in the range of 10% to 35%.

6. ROBUSTNESS:

Prepare the system suitability solution as per the test method and inject into the HPLC system with ±0.2 ml of the method flow. Evaluate the system suitability values as required by the test method for both flow rates. Actual flow rate was 1.0 ml/min and it was changed to 0.8ml/min and 1.2ml/min and inject into HPLC and system suitability was checked[25].

7. LIMIT OF DETCTION:

Minimum concentration of standard component in which the peak of the standard gets merged with noise called the LOD

$$LOD = 3.3* \sigma/S$$

Where;

σ = standard deviation
S = slope

LOD for Guaifenesin =

8. LIMIT OF QUANTIFICATION:

Minimum concentration of standard component in which the peak of the standard gets detected and quantification

$$LOQ = 10*\sigma/S$$

Where;

σ = standard deviation
S = slope

LOQ for Guaifenesin =

CHAPTER-8

SUMMARY

On literature survey it was found that RP –HPLC method was reported for validation of Guaifenesin. For routine analysis purpose it is desirable to establish methods capable of analyzing Guaifenesin in dosage form with less solvent consumption, high resolution, more sensitive, and reduced run time an attempts were being made to develop simple, precise and accurate method. HPLC method for validation of guaifenesin and its assay is developed and validated for various parameters as per ICH guidelines.

The High performance liquid chromatography equipped with UV detector and with Chromatographic Workstation Software. The chromatograms of Guaifenesin were found to be satisfactory with Cosmosil Packed Column $5C_{18}$ – MS - II (4.6 x 250mm) the resolution of the proposed method was found to be satisfactory with peak showing complete base line separation.

The proposed system of mobile phase and stationary phase was suitable for this estimation. System suitability parameters were within the limits as indicated by good number of theoretical plates.

The accuracy of the method was determined by recovery with spiked concentrations at 50%, 100% and 150% levels. The recovery of drug was well within the acceptance limits of 98%-102%.

The method is robust as observed from insignificant variation in the results of analysis on changes in flow rate, temperature, mobile phase composition and pH of the buffer. The linearity was observed over a wide concentration range of 10 - 35µg/ml.

CHAPTER-9

CONCLUSION:

The study is focused to develop and validate HPLC methods for estimation of Guaifenesin in tablet dosage form.

For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robustness, accuracy and precision without any prior separation steps. HPLC method generates large amount of quality data, which serve as highly powerful and convenient analytical tool.

The method shows good reproducibility and good recovery. From the specificity studies, it was found that the developed methods were specific for Guaifenesin.

CHAPTER-10

BIBILOGRAPHY

1. Indian pharmacopoeia 1996 volume1.published by the controller of publications, Delhi.page no: 355
2. Indian pharmacopoeia 2014 volume2 government of India ministry of health and family welfare. Published by the Indian pharmacopoeia commission, Ghaziabad.page no: 1878
3. Text book of analysis, Dr.Ravishankar.
4. Instrumental methods of chemical analysis. Guru deep R.Chatwal, Sharmk.anand.
5. B.K.Sharma.Instrumental methods of chemical analysis
6. A practical approach to pharmaceutical analysis instrumental analysis and manual.Rajesh Kumar Nema.
7. ICH Guideline Q2 Validation.
8. Code Q2A, validation of analytical procedure, ICH Harmonized Tripartite Gudlines, Switzerland ,27 october,1994, 1-5
9. Code Q2B,Validation of analytical procedure Methodology,ICH Harmonized Tripatite Gudlines,Switzerland,6th November,1996,1-5.
10. Institution manual analytical technologic double beam UV Analytical technogical hplc.
12. Available form:htpp/www.Rxlist.com/Amlodipine,Atenolol and Aspirin.
13. Thummala V.Rgavaraju et al.; Dextromorphan impurities in pharmaceutical development and validation of a stability indicating RP-HPLC method for the simultaneous estimation of guaifenisin and

formulation.
14. Senethiraja.m et al.; Reverse phase hplc method for the simultaneous estimation of tebutanile suphate, guaifenesin in cough syrup.
15. CH.Suma et al.; stimulanecous estimation of guaifenesin in pharmaceutical estimation of phenylnephrine in pharmaceutical dosage form by HPLC-PDA method.
16. Vsp.Bhattacharrya et al.; Estimation and validation of stability indicating UV spectrophotometer method for the determination of guaifenisin in presence of its degrading products.
17. C.Ram Babu et al.; A new and sensitive UV spectrophotometric method for determination guaifenesin dosage form.
18.A.Kundu et al.; Stability- indicating hplc method for simultaneous determination of terbutaline suphate bro methoxine hydrochlorile and guaifenesin.
19. Krunal sayathiya et al.; Development and validation of RP –HPLC and HPLC method of analysis for dextromethorphan HBR and guaifenesin in pharmaceutical cough cold preparation and stastical compression of developed method.
20. Senthilraja.m et al.; Reverse phase HPLC method for the simultaneous estimation of tebutaline sulphate ,brohexane HCL and guaifenesin in cough syrup.
21. Nirax.c patel et al.; Simuntaneous estimation of paracetmol ,guaifenesin,chlorophen isomine malate and bromohexine HCI in combined tablet dosage form by reverse phase hplc.
22. Mishra.rk et al.; Stimulation determination of theophline, etoptlline, guaifenesin and ambroxil hydrochloride by validated RP-HLC method in liquid dosage form.
23. Richasah sah and saahil arora journal of advance pharmace education or research reported development and validation of HPLC analytical assay method for amoldipine vestylate tablets.
24. Shalini pachaurietal. (2010) reported development and validation of h HPLC method for analysis of antihypertensive agents in there pharmaceutical dosage form.
25. Permender rathee et al. (2010) reported simultaneous estimation of amilodipine besylate and atenolol as A.P.J and in tablet dosage form by vierodts method using UV spectroscopy.