IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
**Research Article**
September 2018 Vol.:13, Issue:2


# Analytical Method Development and Validation for the Estimation of Guaifenesin and Dextromethorphan by RP-HPLC

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ISSN 2349-7203
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**Submission:** 22 August 2018
**Accepted:** 29 August 2018
**Published:** 30 September 2018



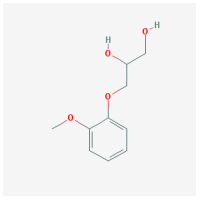
**Keywords:** Guaifenesin, Dextromethorphan, High performance liquid chromatography, Validation

**ABSTRACT**

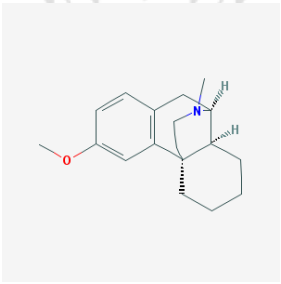
Separation of Guaifenesin and Dextromethorphan[1] was successfully achieved THERMO, $C_{18}$, 250X4.6mm, 5µm,or equivalent in an isocratic mode utilizing 0.1M $KH_2PO_4$ : Methanol (60:40) at a flow rate of 1.0ml/min and eluate was monitored at 280nm, with a retention time of 3.259 and 4.164 minutes respectively using RP-HPLC method for simultaneous estimation of bulk and pharmaceutical formulations. The method was validated and their response was found to be linear in the drug concentration range of 50µg/ml to150 µg/ml for Guaifenesin and 50µg/ml to150 µg/ml for Dextromethorphan. The values of the correlation coefficient were found to 0.999 for Guaifenesin and 1 for Dextromethorphan respectively. The LOD and LOQ for Guaifenesin were found to be 0.597 and 1.991 respectively. The LOD and LOQ for Dextromethorphan were found to be 0.1072 and 0.3572 respectively. The method was extensively validated according to ICH guidelines [2] for Linearity, Accuracy, Precision, Specificity and Robustness.

## INTRODUCTION:

Guaifenesin is an expectorant commonly used in the clearance of mucus from the airways also a centrally acting skeletal muscle relaxant. Chemical name of Guaifenesin; 93-14-1; Guaifenesin; Glycerol guaiacolate; GLYCERYL GUAIACOLATE; Guaifenesin[4]. It act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi.



Dextromethorphan is a drug of morphinan class with sedative, dissociative and Stimulant properties acts centrally (meaning that it acts on the brain) as opposed to locally (on the respiratory tract). It elevates the threshold for coughing, without inhibiting ciliary activity [5]. Dextromethorphan is rapidly absorbed from the gastrointestinal tract and converted into the active metabolite dextrorphan in the liver by the cytochrome P450 enzyme CYP2D6.



## EXPERIMENTAL PROCEDURE:

### Instruments:

WATERS HPLC, Model: Waters 2695, Photodiode array detector (PDA), with an automated sample injector [6], Electronic balance, Ultra-sonicator, Heating mantle, pH meter.

### Reagents:

Potassium Dihydrogenphosphate ($KH_2PO_4$), Dipotassium hydrogen phosphate ($K_2HPO_4$), Water, Methanol, Orthophosphoric acid (OPA), Guaifenesin, Dextromethorphan.

**Standard and sample solutions:**

**Standard:** Accurately weighed quantity of 400mg Guaifenesin and 20mg of Dextromethorphan was taken in a 100ml volumetric flask and 10 ml of methanol was added and made up with methanol to 100ml. Further dilutions were made with water and methanol to get working standard solutions of 100µg/ml.

**Sample:**20 tablets were weighed and crushed, from the powdered tablets, weighed accurately about 980.00mg (400mg Guaifenesin and 20mg of Dextromethorphan) into a 100ml volumetric flask and 10 ml of Methanol was added and made up with methanol to 100ml. Further dilutions were made with water and methanol to get working standard solutions of 100µg/ml.

Separately injected both the standard [7] (5 injections) and sample preparations (1injection) into the chromatographic system and recorded the peak area responses.

**RESULTS AND DISCUSSION:**

**Method Development:**

| **Parameters** | **Optimized Method** |
|---|---|
| Mobile Phase | $K_2HPO_4$: Methanol (60:40) |
| Column | THERMO, $C_{18}$, 250X 4.6mm, 5µm |
| Flow Rate | 1.0ml/Min |
| Temperature | 25˚C |
| Wavelength | 280nm |
| Injection Volume | 10µl |
| Retention Time | Gua:3.259 Dex: 4.164 min |

**Validation Parameters [8]:** System suitability, Accuracy, Linearity, Precision, LOD, LOQ, Robustness, Specificity.

**SYSTEM SUITABILITY:**

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

**PRECISION:**

% 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**.**

**ACCURACY:**

The measured value was obtained by recovery test. Spiked amount of both the drug were compared against the recovery amount. % Recovery was 99% for Guaifenesin and 100.00% for Dextromethorphan. All the results indicate that the method is highly accurate [9]**.**

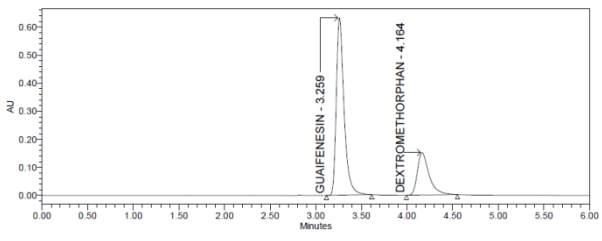
**LINEARITY:**

The linearity of the method was determined at five concentration levels from 50-150(µg/ml). The calibration curve was constructed by plotting peak area versus concentration the slope and intercept values of Guaifenesin Y= 43363x & $R^2$=1 and Dextromethorphan Y=23378x & $R^2$=0.999.

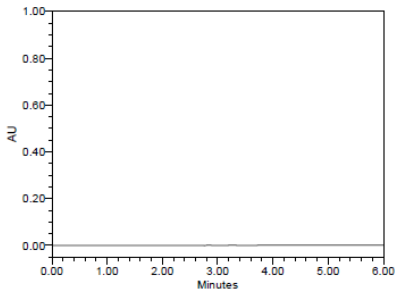
**ROBUSTNESS [10]:**

The results of Robustness of the present method had shown that changes made in the Flow and Temperature did not produce significant changes in analytical results.

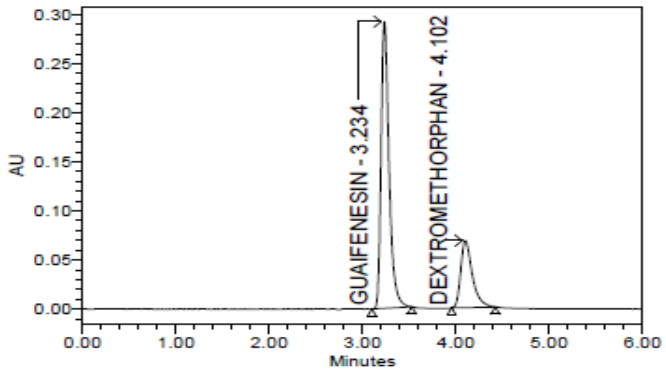
**CHROMATOGRAMS:**




**Standard chromatogram**




**Blank Chromatogram**




**Sample chromatogram**

**Table 1: System suitability data for guaifenesin and dextromethorphan**

| Parameter | Guaifenesin | Dextromethorphan | Acceptance criteria |
|---|---|---|---|
| Retention time | 3.259 | 4.164 | +-10 |
| Theoretical plates | 7596 | 5595 | >2500 |
| Tailing factor | 1.45 | 1.53 | <2.00 |
| % RSD | 0.2 | 0.5 | <2.00 |

**SPECIFICITY:**

**Table 2: Specificity data for Guiafenesin and Dextromethorphan**

| S. No. | Sample name | Guaifenesin area | Rt | Dextromethorphan Area | Rt |
|---|---|---|---|---|---|
| 1 | Standard | 3726649 | 3.359 | 1341704 | 4.164 |
| 2 | Sample | 3739051 | 3.250 | 1322868 | 4.134 |
| 3 | Blank | - | - | - | - |
| 4 | Placebo | - | - | - | - |

**PRECISION:**

**Table 3(A): Precision Data for Guaifenesin**

| S. No. | RT | Area | % Assay |
|---|---|---|---|
| injection1 | 3.250 | 3739051 | 100 |
| injection2 | 3.250 | 3739650 | 100 |
| injection3 | 3.242 | 3732973 | 99 |
| injection4 | 3.242 | 3732125 | 99 |
| injection5 | 3.246 | 3737009 | 100 |
| injection6 | 3.243 | 3735485 | 100 |
| Mean | | | 100 |
| Std. Dev. | | | 0.8 |
| % RSD | | | 0.8 |

**Table 3(B): Precision Data for Dextromethorphan**

| S. No. | RT | Area | % Assay |
|---|---|---|---|
| injection1 | 4.134 | 1322868 | 100 |
| injection 2 | 4.132 | 1326738 | 100 |
| injection 3 | 4.116 | 1321671 | 100 |
| injection 4 | 4.118 | 1322735 | 100 |
| injection 5 | 4.122 | 1321325 | 100 |
| injection 6 | 4.113 | 1320900 | 100 |
| Mean | | | 100 |
| Std. Dev. | | | 0.16 |
| %RSD | | | 0.16 |

**ACCURACY:**

**Table 4(A): Accuracy data for dextromethorphan**

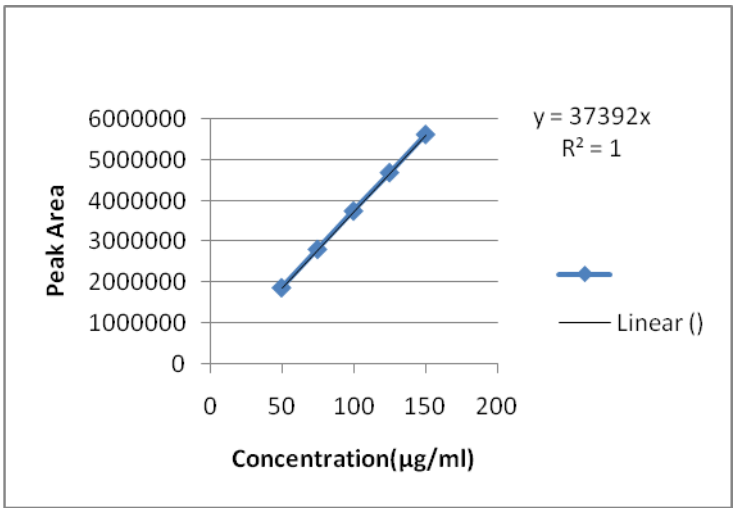
| S. No. | Accuracy Level | Injection | Sample area | RT | % Recovery |
|---|---|---|---|---|---|
| 1 | 50% | 1 | 662941 | 4.102 | 100 |
| | | 2 | 662147 | 4.099 | |
| | | 3 | 662043 | 4.095 | |
| 2 | 100% | 1 | 1325072 | 4.105 | 100 |
| | | 2 | 1326464 | 4.100 | |
| | | 3 | 1326207 | 4.099 | |
| 3 | 150% | 1 | 1980940 | 4.102 | 99 |
| | | 2 | 1980145 | 4.103 | |
| | | 3 | 1982320 | 4.105 | |

**Table 4(B): Accuracy data for Guaifenesin**

| S. No. | Accuracy Level | Injection | Sample area | RT | % Recovery |
|---|---|---|---|---|---|
| 1 | 50% | 1 | 1866611 | 3.234 | 99 |
| | | 2 | 1865356 | 3.233 | |
| | | 3 | 1865316 | 3.233 | |
| 2 | 100% | 1 | 3734210 | 3.244 | 100 |
| | | 2 | 3738762 | 3.240 | |
| | | 3 | 3739706 | 3.241 | |
| 3 | 150% | 1 | 5607485 | 3.244 | 100 |
| | | 2 | 5601456 | 3.248 | |
| | | 3 | 5605099 | 3.248 | |

**LINEARITY:**

**Linearity plot of Guaifenesin**




**Linearity plot of Dextromethorphan**

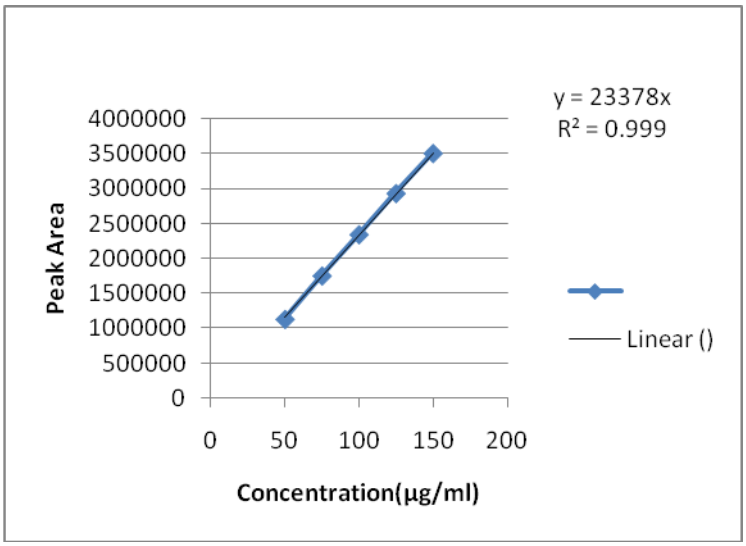



**Table 5(A): Linearity data for Dextromethorphan**

| Sr. No. | Conc(µg/ml) | RT | Area |
|---|---|---|---|
| 1. | 50 | 4.078 | 1125262 |
| 2. | 75 | 4.086 | 1750061 |
| 3. | 100 | 4.095 | 2342288 |
| 4. | 125 | 4.099 | 2933934 |
| 5. | 150 | 4.098 | 3510301 |
| ($r^2$) | | | 1.0 |

**Table 5(B): Linearity data for Guaifenesin**

| Sr. No. | Conc (µg/ml) | RT | Area |
|---|---|---|---|
| 1. | 50 | 3.226 | 1865913 |
| 2. | 75 | 3.234 | 2802714 |
| 3. | 100 | 3.238 | 3737900 |
| 4. | 125 | 3.243 | 4676782 |
| 5. | 150 | 3.246 | 5609376 |
| ($r^2$) | | | 0.999 |

**ROBUSTNESS:**

**Table 6(A): Robustness data for Guaifenesin**

| Parameter | RT | Theoretical plates | Tailing Factor |
|---|---|---|---|
| Decreased flow rate(0.8ml/min) | 4.034 | 8447 | 1.49 |
| Increased flow rate(1.2ml/min) | 2.693 | 7155 | 1.46 |
| Decreased temperature($20^0$c) | 4.037 | 8570 | 1.49 |
| Increased temperature($30^0$c) | 2.701 | 7237 | 1.47 |

**Table 6(B): Robustness data for Dextromethorphan**

| Parameter | RT | Theoretical plates | Tailing factor |
|---|---|---|---|
| Decreased flow rate (0.8ml/min) | 5.054 | 5355 | 1.56 |
| Increased flow rate (1.2ml/min) | 3.404 | 5940 | 1.54 |
| Decreased temperature($20^0$c) | 5.058 | 5451 | 1.56 |
| Increased temperature($30^0$c) | 3.437 | 6019 | 1.55 |

**LOD:** Guaifenesin = 0.597, Dextromethorphan = 0.1072

**LOQ:** Guaifenesin = 1.991, Dextromethorphan = 0.3572

**SUMMARY TABLE:**

| S. NO. | PARAMETER | RESULT (Guaifenesin) | RESULT (Dextromethorphan) | ACCEPTENCE CRITERIA |
|---|---|---|---|---|
| **1** | System suitability<br>Theoretical plates<br>Asymmetry<br>Retention time<br>%RSD | <br>7596<br>1.45<br>3.259<br>0.2 | <br>5595<br>1.53<br>4.164<br>0.5 | <br>Not less than 2500<br>Not more than2<br><br>Not more than 2% |
| **2** | Specificity<br>a) Blank interference<br>b) Placebo interference | <br>Specific | <br>Specific | <br>Specific |
| **3** | Method precision(%RSD) | 0.08 | 0.16 | Not more than 2.0% |
| **4** | Linearity parameter<br>Slope<br>Intercept<br>Correlation coefficient($r^2$) | <br>50-150 mcg/ml<br>0.999 | <br>50-150 mcg/ml<br>1.00 | <br>Not more than 0.999 |
| **5** | Accuracy(Mean % recovery)<br>50%<br>100%<br>150% | <br>99%<br>100%<br>100% | <br>100%<br>100%<br>99% | <br><br><br>97.00– 103.00% |
| **6** | Robustness<br>a) Flow rate variation<br>b) Temperature variation | All the system suitability parameters are within the limits. | All the system suitability parameters are within the limits. | |

**CONCLUSION:**

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 hence the suggested method is more reliable using Rp-HPLC.

**ACKNOWLEDGEMENT:**

I am grateful to SIR CRR College Of Pharmaceutical Sciences, Eluru for providing the necessary facilities.

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