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An Ultra Performance Liquid Chromatographic Method Validation of Guaifenesin and Hydrocodone Bitrartrate

M. David Raju[1]*, Manoranjani M[2], Satyadev T N V S S[3]

[1*,2,3]Department of Chemistry, P B Siddhartha College of Arts and Science, Vijayawada, AP-520010

[1]Email: mdavidraju607@gmail.com

***Corresponding Author:** M. David Raju

* Department of Chemistry, P B Siddhartha College of Arts and Science, Vijayawada, AP-520010

Email: mdavidraju607@gmail.com

KEYWORDS

Guaifenesin, Hydrocodone Bitartrate, Development, Validation, RP-HPLC.

ABSTRACT:

Objective: This investigation demonstrates a stability-indicating and reliable "reverse phase high-performance liquid chromatography" method to simultaneously quantify Hydrocodone Bitartrate and Guaifenesin in the pharmaceutical dosage form.

Methods: Successful separation was accomplished using Agilent C_{18} column (150 mm x 4.6 mm, 3.5µm) with isocratic type of elution using mobile phase containing Acetonitrile + 0.1% Ortho Phosphoric acid buffer (50:50) respectively with 1 ml/min flow rate. The wavelength sensor was attuned at 247 nm to quantify Hydrocodone Bitartrate and Guaifenesin.

Results: Guaifenesin and Hydrocodone Bitartrate peaks were eluted with fine resolution at retention times 2.729 min and 4.127 min respectively. In 20-120 µg/ml concentration range for Guaifenesin and 0.25-1.5 µg/ml Hydrocodone Bitartrate, the calibration graphs were linear, with regression coefficients of 0.9998 and 0.9998 respectively. The suggested High-performance liquid chromatography approach has been shown as sensitive, precise, robust, accurate, and specific and stability indicating through the resolution of Guaifenesin and Hydrocodone Bitartrate from its degradation-based compounds.

Conclusion: The established high-performance liquid chromatography technique was effectively extended to the evaluation of Guaifenesin and Hydrocodone Bitartrate in the pharmaceutical dosage form and the test results appeared satisfactory.

INTRODUCTION

Guaifenesin, also known as glyceryl guaiacolate, is an expectorant medication that aids in the elimination of sputum [1] from the respiratory tract [2]. Chemically it is an ether of guaiacol and glycerine. It is often used in combination with other medications. It is taken by mouth. Guaifenesin is used to try to help with coughing up thick mucus [3, 4] and is sometimes combined with dextromethorphan, an antitussive [5, 6] (cough suppressant), such as in Mucinex DM or Robitussin DM. It is also combined with ephedrine [7, 8] to produce Primatene and Bronkaid tablets for symptomatic relief of asthma [9, 10]. Side-effects of guaifenesin include nausea, vomiting, formation of kidney stones [11, 12], diarrhea [13], and constipation [14]. Nausea and vomiting can be reduced by taking guaifenesin with meals. The risk of forming kidney stones during prolonged use can be reduced by maintaining good hydration and increasing the pH of urine. Rarely, severe allergic reactions may occur, including a rash or swelling of the lips or gums, which may require urgent medical assistance. Mild dry mouth or chapped lips [15, 16] may also occur when taking this medication. Drinking a glass of water is recommended with each dose of guaifenesin.

Hydrocodone, also known as dihydrocodeinone, is an opioid [17, 18] used to treat pain and as a cough suppressant. It is taken by mouth. Typically it is dispensed as the combination acetaminophen/ hydrocodone or ibuprofen/hydrocodone for pain severe enough to require an opioid and in combination with homatropine methylbromide to relieve cough. It is also available by itself in a long-acting form under the brand name Zohydro ER, among others, to treat severe pain of a prolonged duration. Common side effects include dizziness [19, 20], sleepiness [21], nausea, and constipation. Serious side effects may include low blood pressure [22], seizures, QT prolongation [23], respiratory depression, and serotonin syndrome[24, 25]. Rapidly decreasing the dose may result in opioid

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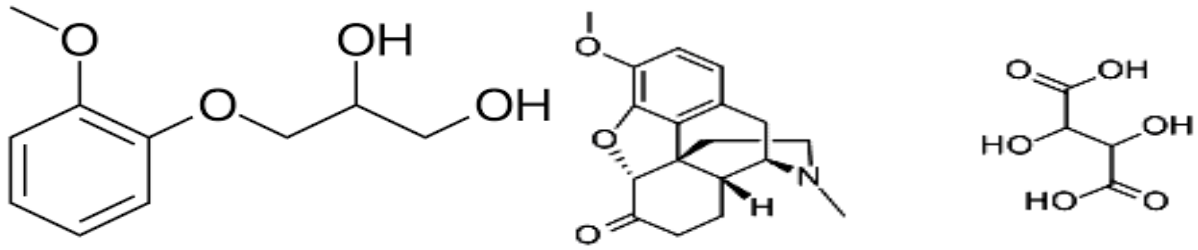
withdrawal. Use during pregnancy or breast feeding is generally not recommended. Hydrocodone is believed to work by activating opioid receptors, mainly in the brain and spinal cord. This paper proposes a novel sensitive stability-indicating RP-HPLC procedure for the assessment of Guaifenesin and Hydrocodone Bitartrate combination. The process proposed enables the rapid assessment of the Guaifenesin and Hydrocodone Bitartrate in bulk drugs and formulation preparations without sample pretreatment with high precision and specificity and with no excipient intervention.



A **B**

Fig. 1: Structure of (A) Guaifenesin and (B) Hydrocodone Bitartrate

MATERIALS AND METHOD

Chemicals: Acetonitrile, HPLC-grade methanol, water were purchased from Merck India Ltd, Mumbai, India. APIs of Guaifenesin, Hydrocodone Bitartrate standards and sample formulation (oral solution with a label claim of 2.5 mg of Hydrocodone Bitartrate and 200 mg of Guaifenesin per 5 ml) were procured from Zydus Cadila Health Care Ltd., Secunderabad.

The Instrumentation: Waters Alliance e-2695 HPLC with quaternary pump, PDA detector with Empower 2.0 software was employed.

Method optimization: To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally a mixture of acetonitrile and OPA with isocractic elution was selected as mobile phase because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as C_8, C_{18} and amino, phenyl columns were tested. From these trials the peak shapes were relatively good with Agilent C_{18} column of 150 x 4.6mm, 3.5 µ with a PDA detector. The mobile phase flow rate has been done at 247nm in order to obtain enough sensitivity. By using above conditions we get retention times of Guaifenesin and Hydrocodone Bitartrate were about 2.727 min and 4.127 min with a tailing factor of 1.22 & 1.04. The number of theoretical plates for Guaifenesin and Hydrocodone Bitartrate were 8841, 6325 which indicate the column's successful output the % RSD for six replicate injections was around 0.54% and 1.09%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Till today there were no UPLC and HPLC methods reported in the literature, but these methods are developed only for routine analysis of the selected drugs in bulk and formulation studies. The developed HPLC method was utilized for the estimation of the combined drugs by *in vitro* method.

Validation procedure [26-31]

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [32, 33].

Preparation of buffer: Take 1 ml of ortho phosphoric acid in 1 Lt of HPLC grade water and filter through 0.45 µ filter paper.

Chromatographic conditions: The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of Acetonitrile and 0.1% OPA (50:50) and agilent C_{18} (150x4.6 mm, 3.5 µ) column with a flow rate of 1.0 ml /min.

Diluent: Mobile phase was used as diluent.

Preparation of Hydrocodone Bitartrate parent stock solution: Parent standard stock solution of Hydrocodone Bitartrate was prepared by appropriately estimating 10 mg of drug in 10 mL volumetric flask. Then the drug was liquified in solvent.

Preparation of the standard solution: Standard Guaifenesin and Hydrocodone Bitartrate solution containing 80µg/ml and 1 µg/ml was prepared by dissolving 1 ml of Hydrocodone Bitartrate parent stock

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solution and 80 mg of Guaifenesin in 100 ml volumetric flask using diluents. Further dilute 5 ml to 50 ml with diluents.

Preparation of the sample solution: Sample solution containing 80 µg/ml of Guaifenesin and 1 µg/ml of Hydrocodone Bitartrate was prepared by dissolving 2 ml of Guaifenesin and Hydrocodone Bitartrate sample (label claim 40mg of Guaifenesin and 2.5 mg of Hydrocodone Bitartrate per ml) in 100 ml of mobile phase solvent blend. Further dilute 5 ml to 50 ml with diluents.

RESULTS AND DISCUSSION

In acquiescence with ICH recommendations, the validity parameters were established [34].

System suitability: In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Guaifenesin	Hydrocodone Bitartrate
USP Plate Count	NLT 2000	8841	6325
USP Tailing	NMT 2.0	1.22	1.04
USP Resolution	NLT 2.0	-	8.51
% RSD	NMT 2.0	0.54	1.09

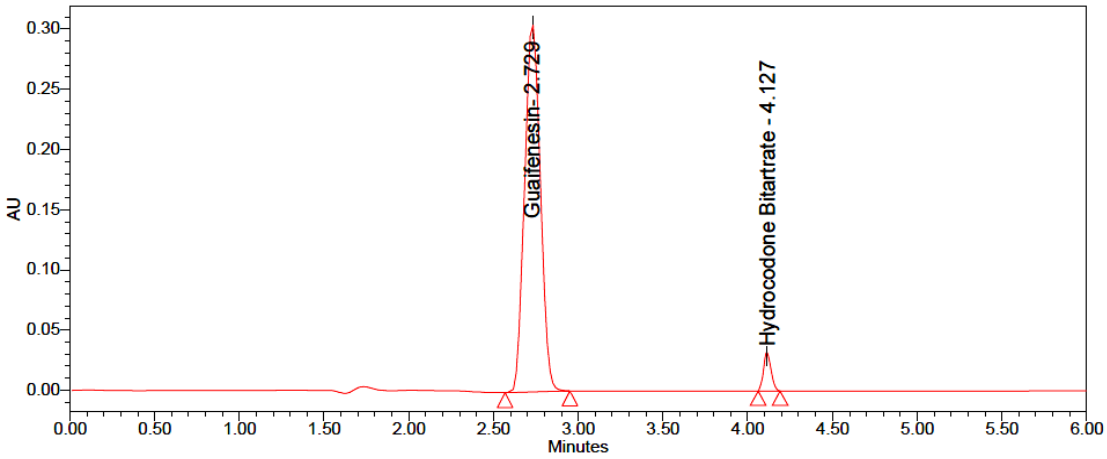

Fig. 2: Chromatogram of standard

Specificity: In this test method placebo, standard and sample solutions were analyzed individually to examine the interference [35]. The below figure shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

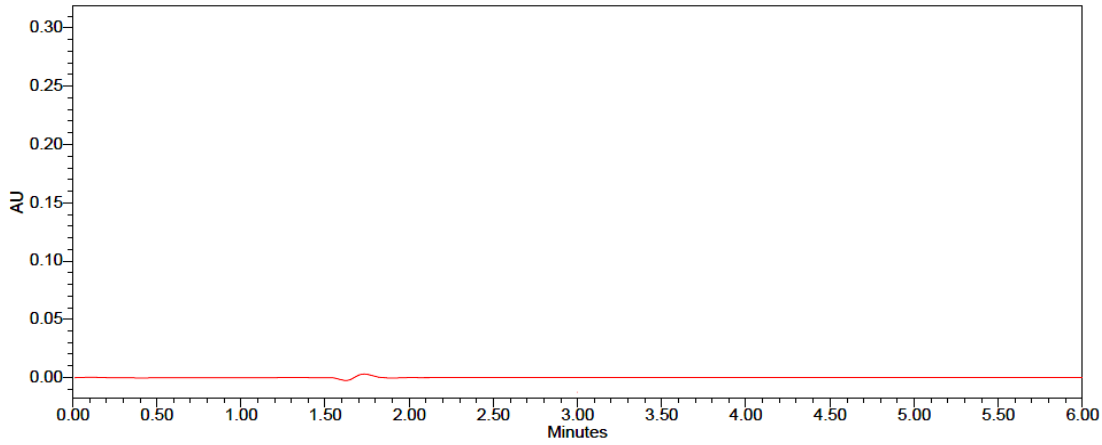

Fig. 3: Chromatogram of blank

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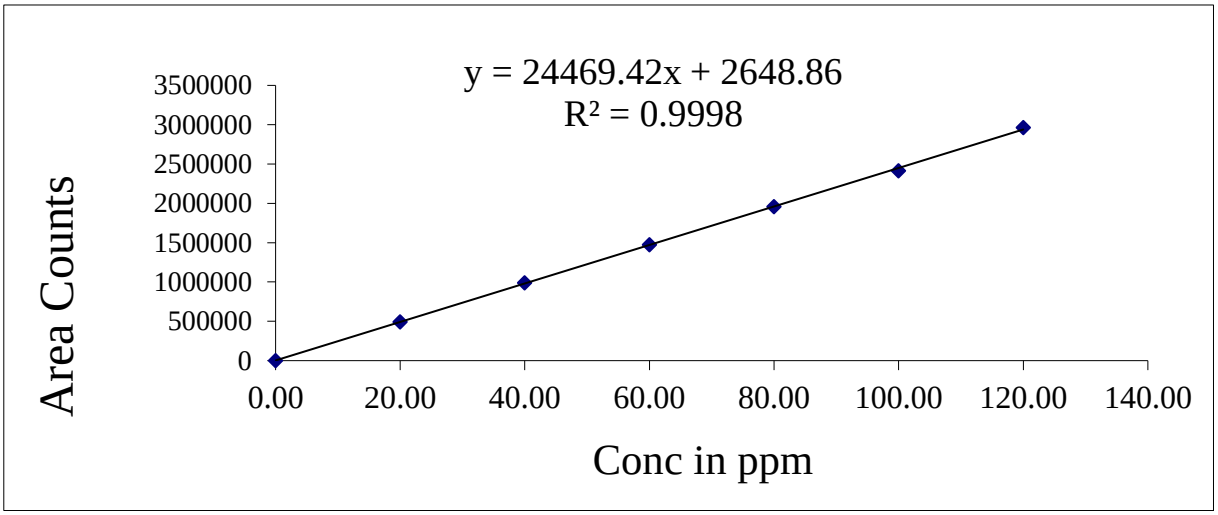
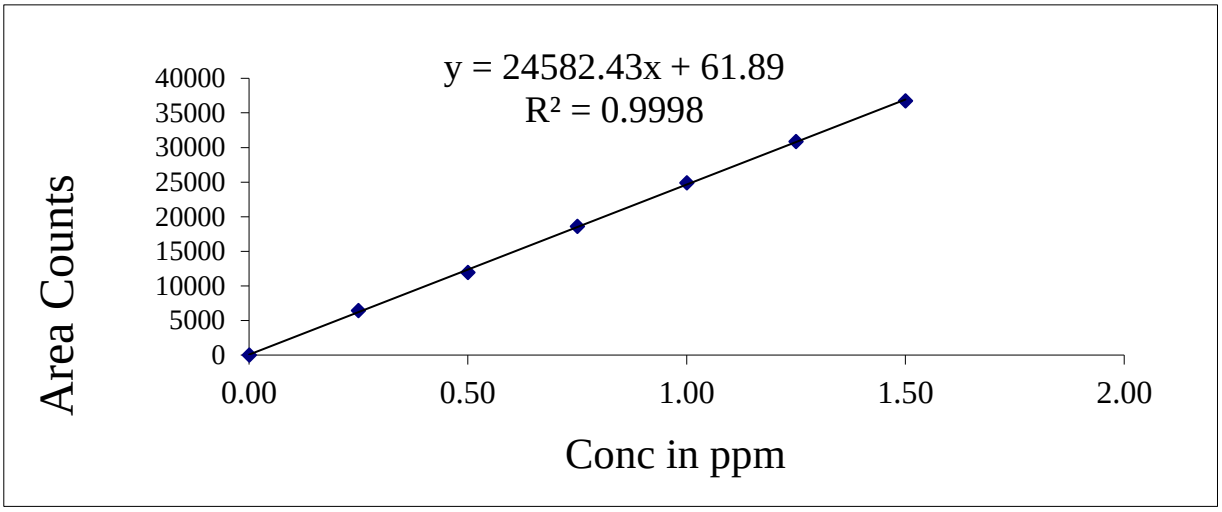
Linearity: During this work, the linearity of area response was checked for both Guaifenesin and Hydrocodone Bitartrate. Chromatographed solutions with concentrations of 20-120 µg/ml of Guaifenesin and 0.25-1.5 µg/ml of Hydrocodone Bitartrate given linear peak response areas. The regression line equation, regression coefficient and Guaifenesin and Hydrocodone Bitartrate calibration curves are shown in fig. 4.

S. No	Guaifenesin		Hydrocodone Bitartrate	
	Conc. µg/ml	Area	Conc. µg/ml	Area
1	20.00	495068	0.25	6427
2	40.00	988745	0.50	11949
3	60.00	1475496	0.75	18598
4	80.00	1956477	1.00	24899
5	100.00	2414459	1.25	30873
6	120.00	2965454	1.50	36745
CC		0.99985		0.99984
Slope		24469.42		24582.43
intercept		2648.86		61.89

Table 2: Linearity of Guaifenesin and Hydrocodone Bitartrate



(A) Guaifenesin



(B) Hydrocodone Bitartrate

Fig. 4: Calibration plots of (A) Guaifenesin (B) Hydrocodone Bitartrate

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Accuracy: The accuracy was determined by assay of Guaifenesin and Hydrocodone Bitartrate in spiked Guaifenesin and Hydrocodone Bitartrate samples according to proposed method. Three diverse quantities (50% quantity degree, 100% quantity degree and 150% quantity degree) [36] of Guaifenesin and Hydrocodone Bitartrate standards were put into samples. The results are given in table 3.

S. No	% Level	Guaifenesin % Recovery	Hydrocodone Bitartrate % Recovery
1	50	100.0	100.0
2	100	99.1	99.2
3	150	99.5	100.1

Table 3: Results of accuracy of Guaifenesin and Hydrocodone Bitartrate

Precision: The precision measurements were assessed using measurements of Guaifenesin (80 µg/ml) and Hydrocodone Bitartrate (1 µg/ml) solution repeated six times within the day. The precision was validated by the RSD measurements of the Guaifenesin and Hydrocodone Bitartrate peak areas, while the accuracy was validated by the Guaifenesin and Hydrocodone Bitartrate percentage content assays. These results are given below table 4.

Intraday precision: Six replicates of a sample solution containing Guaifenesin (80 µg/ml) and Hydrocodone Bitartrate (1 µg/ml) were analysed on the same day [37]. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Guaifenesin				Hydrocodone Bitartrate		
S. No	Conc.(µg/ml)	Area	% Assay	Conc.(µg/ml)	Area	% Assay
1	80	1982574	100.32	1	24524	100.4
2		1983785	100.4		24563	100.5
3		1981652	100.3		24478	100.2
4		1980582	100.2		24875	101.8
5		1981165	100.2		24582	100.6
6		1984968	100.4		24415	99.9
Mean		1982454	100.3		24573	100.6
SD		1668.25	0.085		159.86	0.653

Table 4: Intraday precision results of Guaifenesin and Hydrocodone Bitartrate

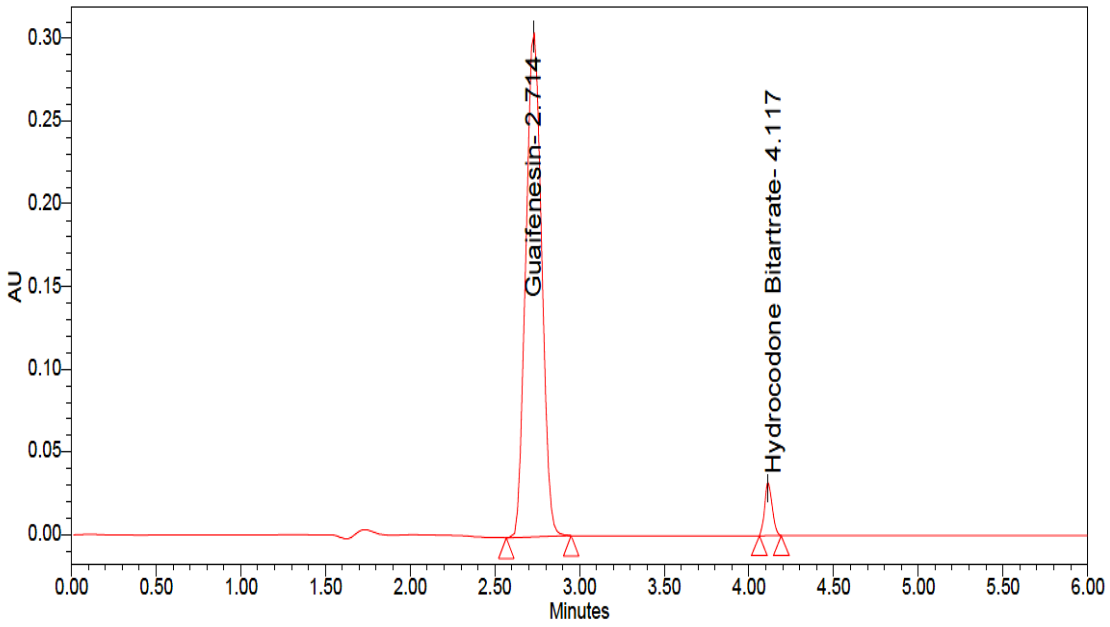

Fig. 5: Chromatogram of method precision

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Intermediate precision: six replicates of the sample solutions were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine mean percent RSD values have been calculated. The results are given in the following table [38].

Inter-day precision: Six replicates of a sample solution containing Guaifenesin (80 µg/ml) and Hydrocodone Bitartrate (1 µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

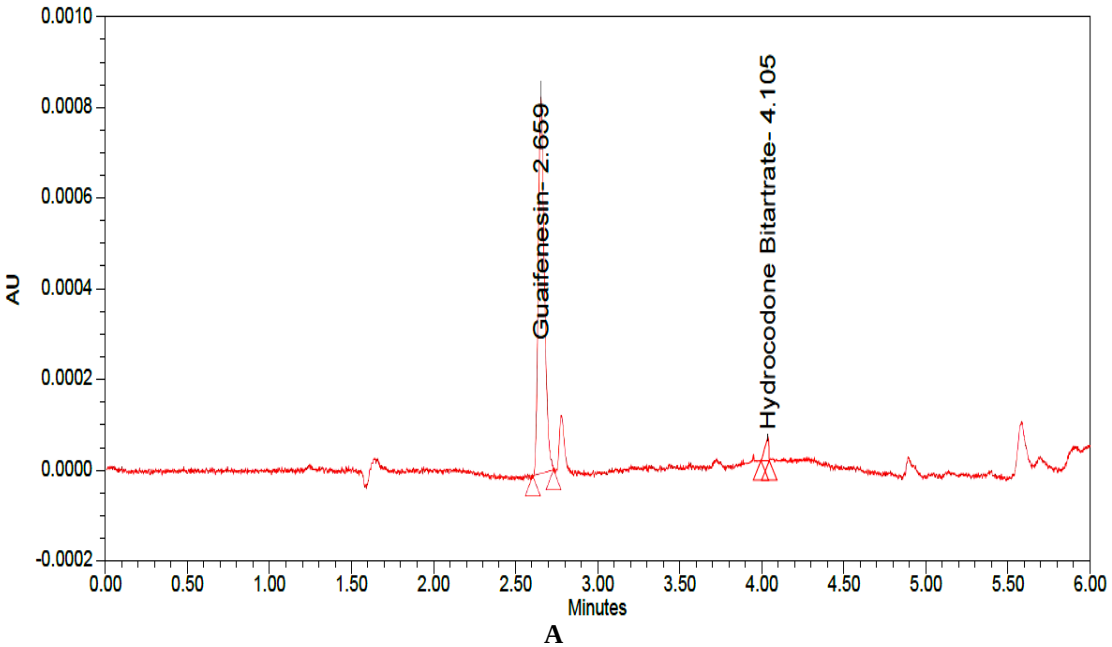
Guaifenesin				Hydrocodone Bitartrate		
S. No.	**Conc.(µg/ml)**	**Area**	**% Assay**	**Conc.(µg/ml)**	**Area**	**% Assay**
1	80	1982214	100.3	1	24524	100.3
2		1980365	100.3		24563	100.4
3		1981475	100.3		24478	100.1
4		1982350	100.4		24175	98.8
5		1982158	100.3		24382	99.7
6		1983451	100.4		24415	99.8
Mean		1982002	100.3		24423	99.9
SD		1024.45	0.051		138.62	0.568

Table 5: Inter-day outcomes of accuracy of Guaifenesin and Hydrocodone Bitartrate

LOD and LOQ: Both LOD and LOQ were measured utilizing a signal-to-noise methodology. LOQ and LOD were defined as the Guaifenesin and Hydrocodone Bitartrate concentration levels that ensuring a peak height of 10 times and 3 times respectively the baseline noise.

Guaifenesin				Hydrocodone Bitartrate			
LOD		**LOQ**		**LOD**		**LOQ**	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.24 µg/ml	3	0.80 µg/ml	10	0.003 µg/ml	3	0.01 µg/ml	10

Table 6: LOD and LOQ for Guaifenesin and Hydrocodone Bitartrate



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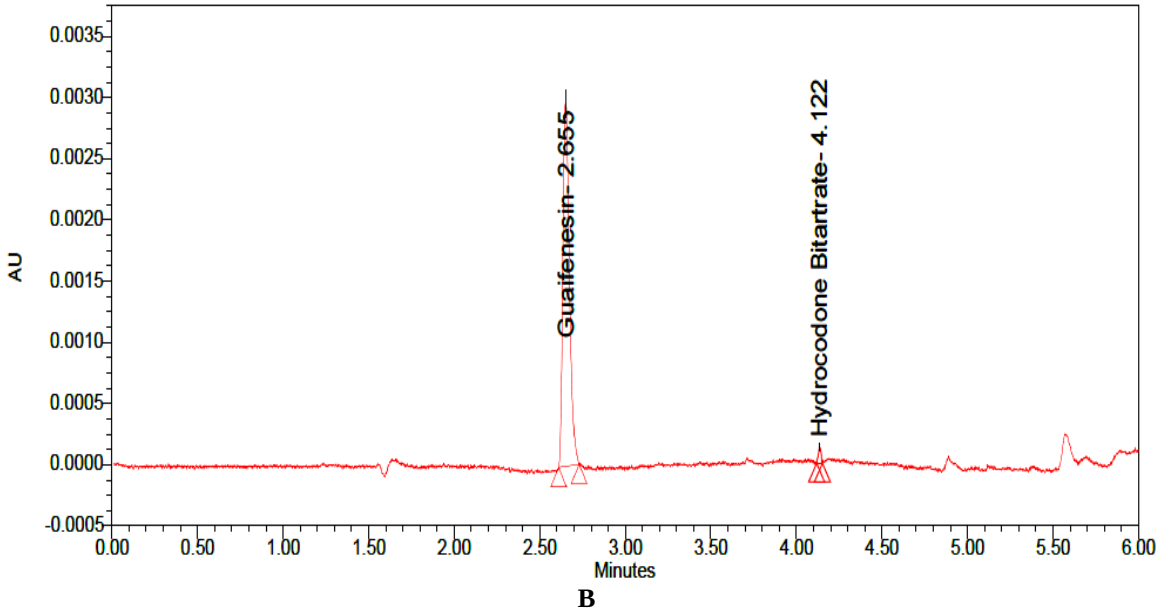

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Robustness: The robustness was measured using peak area measurements of Guaifenesin (80µg/ml) and Hydrocodone Bitartrate solution (1µg/ml) with considerably changed parameters in HPLC assay operating conditions. The changed parameters and peak areas obtained were presented in table **7**.

Title of the Parameter	% RSD	
	Guaifenesin	Hydrocodone Bitartrate
Flow Minus (0.9 ml/min)	0.87	0.66
Flow Plus (1.1 ml/min)	0.45	1.60
Organic Minus (45:55)	0.59	0.67
Organic Plus (55:45)	0.76	0.70

Table 7: Robustness data of (A) Guaifenesin and (B) Hydrocodone Bitartrate

Degradation studies: The Guaifenesin and Hydrocodone Bitartrate sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [39, 40]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [41-44].

Acid degradation: Acid degradation was done by using 1N HCl and 13.2% of Guaifenesin and 12.1% of Hydrocodone Bitartrate degradation was observed.

Alkali degradation: Alkali degradation was done at 1N NaOH and 12.3% of Guaifenesin and 13.0% of Hydrocodone Bitartrate degradation was observed.

Peroxide degradation: Peroxide degradation was performed with 30% hydrogen peroxide and 14.3% Guaifenesin, 14.7% of Hydrocodone Bitartrate degradation was observed.

Reduction degradation: Reduction degradation was performed with 10% sodium bi sulphite solution, 4.5% Guaifenesin and 7.0% Hydrocodone Bitartrate degradation was observed.

Thermal degradation: In thermal degradation the sample was degraded to 4.8% of Guaifenesin and 4.6% of Hydrocodone Bitartrate.

Photolytic degradation: In Photolytic degradation the sample was degraded to 4.3% of Guaifenesin and 3.0% of Hydrocodone Bitartrate.

Hydrolysis degradation: In hydrolysis degradation the sample was degraded to 2.6% of Guaifenesin and 2.6% of Hydrocodone Bitartrate. All degradation results are tabulated in table 8.

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Table 8: Forced degradation results of Guaifenesin and Hydrocodone Bitartrate

Degradation condition	Guaifenesin		Hydrocodone Bitartrate	
	% Assay	% Deg	% Assay	% Deg
Control degradation	99.9	0.1	99.9	0.1
Acid degradation	86.7	13.2	87.9	12.1
Alkali degradation	86.9	12.3	87	13
Oxidation degradation	85.1	14.3	85.3	14.7
Reduction degradation	89.5	4.5	93	7
Hydrolysis degradation	89.9	2.6	97.4	2.6
Thermal degradation	99.1	4.8	95.4	4.6
Photo degradation	99.3	4.3	97	3

CONCLUSION

An Ultra-performance liquid chromatography process for determining the combination of Guaifenesin and Hydrocodone Bitartrate in combined formulation form and pure form has been described in the established method. The present Ultra-performance liquid chromatography process is exemplified by its speed, ease and relatively inexpensive. The successful validity criteria of the proposed approach permit its use in laboratories for quality control.

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CONFLICTS OF INTEREST

None

REFERENCES

1. Altiner A, Wilm S, Däubener W, Bormann C, et al. Sputum color for diagnosis of a bacterial infection in patients with acute cough. Scand J Prim Health Care. 2009; 27: 70–3. doi:10.1080/02813430902759663
2. Patwa A, Shah A. Anatomy and physiology of respiratory system relevant to anaesthesia. Indian J Anaesth. 2015; 59: 533–41. doi:10.4103/0019-5049.165849
3. Atanasova KR, Reznikov LR. Strategies for measuring airway mucus and mucins. Respir Res. 2019; 20: 261. doi:10.1186/s12931-019-1239-z
4. Dash S, Das SK, Samal J, Thatoi HN. Epidermal mucus, a major determinant in fish health: a review. Iran J Vet Res. 2018; 19: 72–81.
5. Knut Schroeder, Tom Fahey. Systematic review of randomised controlled trials of over the counter cough medicines for acute cough in adults. BMJ. 2002; 324: 329–31. doi:10.1136/bmj.324.7333.329
6. Malesker MA, Callahan-Lyon P, Ireland B, Irwin RS. Pharmacologic and Nonpharmacologic Treatment for Acute Cough Associated With the Common Cold: CHEST Expert Panel Report. Chest. 2017; 152: 1021–37. doi:10.1016/j.chest.2017.08.009
7. Magkos F, Kavouras SA. Caffeine and ephedrine: physiological, metabolic and performance-enhancing effects. Sports Med. 2004; 34: 871–89. doi:10.2165/00007256-200434130-00002
8. Laccourreye O, Werner A, Giroud JP, Couloigner V, Bonfils P, Bondon-Guitton E. Benefits, limits and danger of ephedrine and pseudoephedrine as nasal decongestants. Eur Ann Otorhinolaryngol, Head Neck Dis. 2015; 132: 31–4. doi:10.1016/j.anorl.2014.11.001
9. Wagshul FA, Brown DT, Schultek NM, Hahn DL. Outcomes of Antibiotics in Adults with "Difficult to Treat" Asthma or the Overlap Syndrome. J Asthma Allergy. 2021; 14: 703–12. doi:10.2147/JAA.S313480.
10. Thomas M, Bruton A, Moffat M, Cleland J. Asthma and psychological dysfunction. Prim Care Respir J. 2011; 20: 250–6. doi:10.4104/pcrj.2011.00058
11. Bennett S, Hoffman N, Monga M. Ephedrine- and guaifenesin-induced nephrolithiasis. J Altern Complement Med. 2004; 10: 967–9. doi:10.1089/acm.2004.10.967
12. Parmar MS. Kidney stones. BMJ. 2004; 328: 1420–4. doi:10.1136/bmj.328.7453.1420
13. Das S, Jayaratne R, Barrett KE. The Role of Ion Transporters in the Pathophysiology of Infectious Diarrhea. Cell Mol Gastroenterol Hepatol. 2018; 6: 33–45. doi:10.1016/j.jcmgh.2018.02.009
14. Colombo Jennifer M, Wassom Matthew C, Rosen John M. Constipation and Encopresis in Childhood. Pediatr Rev. 2015; 36: 392–401. doi:10.1542/pir.36-9-392
15. Ruiz-Hornillos FJ, Alonso E, Zapatero L, Pérez C, Martínez-Molero I. Clarinetist's cheilitis caused by immediate-type allergy to cane reed. Contact Dermatitis. 2007; 56: 243–5. doi:10.1111/j.1600-0536.2007.00976.x

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 2564 -2573 | ISSN:2251-6727

16. Aydin Erdinc, Gokoglu Ozgur, Ozcurumez Gamze, Aydin Hakan. Factitious cheilitis: a case report. J Med Case Reports. 2008; 2: 29. doi:10.1186/1752-1947-2-29
17. Maltoni M. Opioids, pain, and fear. Ann Oncol. 2008; 19: 5–7. doi:10.1093/annonc/mdm555
18. Frauenknecht J, Kirkham KR, Jacot-Guillarmod A, Albrecht E. Analgesic impact of intra-operative opioids vs. opioid-free anaesthesia: a systematic review and meta-analysis. Anaesthesia. 2019; 74: 651–62. doi:10.1111/anae.14582
19. Chu EC, Chin WL, Bhaumik A. Cervicogenic dizziness. Oxford Medical Case Reports. 2019; 2019: 476–8. doi:10.1093/omcr/omz115
20. Muncie HL, Sirmans SM, James E. Dizziness: Approach to Evaluation and Management. Am Fam Physician. 2017; 95: 154–62.
21. Mullington Janet, Korth Carsten, Hermann Dirk M, Orth Armin, Galanos Chris, et al. Dose-dependent effects of endotoxin on human sleep. Am J Physiol Regul, Integr Comp Physiol. 2000; 278: R947–55. doi:10.1152/ajpregu.2000.278.4.r947
22. Freeman Roy, Abuzinadah Ahmad R, Gibbons Christopher, Jones Pearl, Miglis Mitchell G, Sinn Dong In. Orthostatic Hypotension: JACC State-of-the-Art Review. J Am Coll Cardiol. 2018; 72: 1294–309. doi:10.1016/j.jacc.2018.05.079
23. Tester DJ, Schwartz PJ, Ackerman MJ. Congenital Long QT Syndrome. In Gussak I, Antzelevitch C (eds.). Electrical Diseases of the Heart. London: Springer. 2013; pp. 439–68. doi:10.1007/978-1-4471-4881-4_27
24. Volpi-Abadie J, Kaye AM, Kaye AD. Serotonin syndrome. The Ochsner Journal. 2013; 13: 533–40.
25. Prator B. Serotonin syndrome. J Neurosci Nurs. 2006; 38: 102–5. doi:10.1097/01376517-200604000-00005
26. Manoranjani M. Stability indicating assay method development and validation of Netarsudil and Latanoprost by RP-HPLC and its degradation. JETIR. 2019; 6: 825-32.
27. Prasada Rao P T S R K. Development and validation of Adapalene and Dapsone in bulk and pharmaceutical dosage form by RP-HPLC and its degradation. JETIR. 2019; 6: 754-9.
28. Satya Dev T N V S S. Stability indicating assay method development and validation of Aclidinium Bromide and Formoterol Fumarate by RP-HPLC. JETIR. 2019; 6: 625-9.
29. Manoranjani M. A study of method development, validation and forced degradation for simultaneous quantification of Cisplatin and Fluorouracil in bulk and pharmaceutical dosage form by RP-HPLC. J Pharm Sci & Res. 2021; 13: 155-61.
30. Ramachandran D, Anita Kethipalli, Krishnamurthy Mannam. Bio-analytical Method Development and Validation of Daunorubicin and Cytarabine in Rat Plasma by LC-MS/MS and its Application in Pharmacokinetic Studies. J Pharm Sci Res 2020; 12: 381-6.
31. Prasada Rao P T S R K. A study of method development and validation of Pemetrexed and Cisplatin using RP-HPLC. J Pharm Sci & Res. 2021; 13: 143-8.
32. International conference on the harmonization. ICH harmonized tripartite guideline. Validation of analytical procedures: Text and methodology Q2(R1); 2005.
33. Vijayakumari M, Balasekhar reddy Ch. Stability indicating validated hplc method for the determination of zanubrutinib in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res 2020; 13:159-62.
34. Rajakumari R, Sreenivasa Rao S et.al., Stress degradation studies and development of a validated RP-HPLC method for determination of Tiagabine in presence of its degradation products. Int J Pharm Pharm Sci 2016; 8:230-6.
35. Charu Pandya P, Sadhana Rajput J. Development and validation of stability indicating method RP-HPLC method of Acotiamide. Int J Pharm Pharm Sci 2018; 10:1-8.
36. Birva Athavia A, Zarna Dedania R et.al., Stability indicating HPLC method for determination of Vilazodone hydrochloride. Int J Curr Pharm Res 2017; 9:123-9.
37. Abdul Raziq, Syed Umer Jan et al., Relative comparison of stability and degradation of methylcobalamine tablets of different brands at different storage settings. Int J App Pharm 2021; 13:171-5.
38. Rajakumari R, Sreenivasa Rao S et.al., Stress degradation studies and development of a validated RP-HPLC method for determination of Tiagabine in presence of its degradation products. Int J Pharm Pharm Sci 2016; 8: 230-6.
39. Charu Pandya P, Sadhana Rajput J. Development and validation of stability indicating method RP-HPLC method of Acotiamide. Int J Pharm Pharm Sci 2018; 10: 1-8.
40. Prasada Rao P T S R K. HPLC method development and validation of Lercanidipine HCl and Atenolol, Characterization of its degradants by using LC-MS/MS. Int J App Pharm. 2022; 14: 125-34.
41. Subba Rao Yarlagadda, Subba Rao Mannam, Baby Padmini Jampani. Stability indicating and cost effective analytical method development and validation of Sotorasib by using RP-HPLC. Int J App Pharm 2021; 13: 154-9.

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JCHR (2023) 13(4), 2564 -2573 | ISSN:2251-6727

42. Vejendla, A., Talari, S., Moturu, R. et al. Method development and validation for Cabotegravir and Rilpivirine by using HPLC and its degradants are characterized by LCMS and FTIR. Futur J Pharm Sci **7**, 226 (2021). https://doi.org/10.1186/s43094-021-00355-8.
43. Vejendla, A., Talari, S., Ramu, G. et al. Characterization of novel stress degradation products of Bempedoic acid and Ezetimibe using UPLC–MS/MS: development and validation of stability-indicating UPLC method. Futur J Pharm Sci **7**, 234 (2021). https://doi.org/10.1186/s43094-021-00381-6.
44. Talari, S., Vejendla, A., Deepthi, K. et al. Development and Validation of a New Reverse Phase Ultra Performance Liquid Chromatography Method for the Estimation of Related Substances of the Anticancer Drug Zanubrutinib and Characterization of its Degradants Using Liquid Chromatography Mass Spectrometry. Pharm Chem J (2023). https://doi.org/10.1007/s11094-023-02992-0