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NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DEXTROMETHORPHAN HYDROBROMIDE AND PROMETHAZINE IN ORAL DOSAGE FORMS

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Abstract

Promethazine and dextromethorphan hydrobromide can now be measured simultaneously using a reverse phase high performance liquid chromatography (RP-HPLC) technique that is both sensitive and quick. Using a mobile phase made up of 0.1% O-phosphoric acid and acetonitrile in a 60:40, v/v ratio, chromatographic separation was accomplished on a reverse phase Kinetex C18 column (250 X 4.6 mm, 5 µm). The mobile phase was pumped at a rate of 1.0 milliliters per minute, and a UV detector set to 225 nm was used for detection. The suggested approach was found to be straightforward, quick, accurate, exact, and repeatable. It might be used for routine quality control analysis to determine promethazine and dextromethorphan hydrobromide in pharmaceutical dosage forms at the same time.

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Introduction

Dextromethorphan is an NMDA receptor antagonist used to treat cases of dry cough [1]. Dextromethorphan is a levorphanol derivative and codeine analog commonly used as a cough suppressant and also a drug of abuse. Although similar in structure to other opioids, it has minimal interaction with opioid receptors [2]. Promethazine is a first-generation antihistamine used for the treatment of allergic conditions, nausea and vomiting, and motion sickness. Promethazine, is an N-dimethylaminopropyl derivative of phenothiazine that was developed in France in 1946 [3-4]. Promethazine antagonizes a variety of receptors, allowing it to be used for a number of indications including allergic reactions, pain, sedation, nausea, and vomiting [5-8]. Literature survey reveals that few LC methods are reported for the determination of Dextromethorphan Hydrobromide and Promethazine individually and also combination with other drugs [9-14]. Hence, the purpose of presented work is to develop and validate a simple, rapid, accurate and precise RP-HPLC method for simultaneous estimation of Dextromethorphan Hydrobromide and Promethazine in a combined dosage form.

Experimental

Chromatographic Conditions

Using the Waters HPLC 2695 series, which includes an isocratic binary pump, auto sampler, 2487 dual absorbance detector, and thermostat column compartment linked to Waters Empower software, the chromatographic separation was accomplished. The drugs were analyzed using a Kinetex C18 column (250 x 4.6 mm; 5 µm), with the following parameters: column temperature (30°C), injection volume (20 µL), wave length 225 nm, flow rate 1.0 mL/min, and run time of 35 minutes.

Chemicals and Solvents

Working standards for promethazine and dextromethorphan hydrobromide were provided as gift samples by Aurobindo Labs in Hyderabad, India. Dextromethorphan hydrobromide and promethazine oral solution were purchased from the local pharmacy. The company S.D. Fine Chemicals Ltd. in Mumbai, India, provided us with orthophosphoric acid of AR Grade. The HPLC-grade acetonitrile was purchased from E. Merck (India) Ltd. in Mumbai, India. Water from the Milli Q water purification system with HPLC quality was used for the duration of the study.

Mobile Phase and Diluent Preparation

400 milliliters of acetonitrile were combined with 600 milliliters of 0.1% O-phosphoric acid. The solution was

vacuum-filtered through a 0.45 µm filter after being degassed for five minutes in an ultrasonic water bath. As diluent, the same mobile phase was employed.

Preparation of Standard Stock Solution

A 100 mL volumetric flask was filled with 15 mg of Dextromethorphan Hydrobromide and 6.25 mg of Promethazine working standards, which had been precisely weighed. About 60 mL of diluent was then added, sonicated to completely dissolve it, and the volume was adjusted using the same solvent.

Preparation of Standard Solution

10 mL of the standard stock solution was pipetted into a 100 mL volumetric flask, and the excess was diluted with diluent. Preparation of a sample to a 100 mL volumetric flask, 5 ml of the oral solution was added. The volume was adjusted with diluent after adding around 60 mL of it and sonicating it to dissolve it entirely. Blended thoroughly and passed through a 0.45 µm filter. Additionally, pipette 10 mL of the stock solution mentioned above into a 100 mL volumetric flask, then dilute with diluent until the desired level is reached.

Method Development

To maximize the process, chromatographic parameters such retention duration, peak tailing, theoretical plate count, and resolution were established. To do this, experiments are conducted using various stationary phase types, mobile phase ratios, temperature differences, and flow rates. In order to separate Dextromethorphan Hydrobromide and Promethazine from both themselves and stress degradants, a kinetex C18 (250 × 4.6 mm, 5 µm) column with a temperature of 25±2o^{C} was chosen since it produced well-symmetrical and sharp peaks. Analysis time, peak responsiveness, peak symmetry, column efficiency, and the mobile phase-a blend of OPA (0.1%)—are all taken into consideration. Using a photodiode array detector, acetonitrile = 60:40 (v/v) at a flow rate of 1.0 ml/min was detected at a wavelength of 225 nm. The optimized chromatographic parameters show a good number of theoretical plates, a decent peak shape, and a good resolution. The developed method's typical chromatogram for promethazine and dextromethorphan hydrobromide is shown in Figure 3.

Method Validation

The new analytical technique was verified for criteria such as linearity, accuracy, precision, ruggedness, specificity, and system applicability in accordance with ICH guidelines [15].

Linearity

The capacity of an analytical technique to yield test results that are directly, or through a precise mathematical transformation, proportionate to the drug concentration in the samples within a certain range is known as linearity. Promethazine and dextromethorphan hydrobromide mixed standard solutions at five concentration levels were prepared in order to test linearity. Using produced solutions with concentrations ranging from 25 to 80 µg/mL for Promethazine and 60 to 180 µg/mL for Dextromethorphan Hydrobromide, respectively, it was shown that the detector response for both substances was linear. A linear relationship between the peak area of each sample and the corresponding concentrations of promethazine and dextromethorphan hydrobromide was discovered. Over this concentration range, it was discovered that Beer's law was followed. It is required that the correlation coefficient be at least 0.998. Tables 1 and 2 showed the results of linearity.

Accuracy

Accuracy is the difference between the actual value and the resultant mean value. Recovery experiments were done to evaluate the standard addition method's accuracy. To the predetermined amount of pre-analyzed solution, a known quantity of the standard medication was added. Comparing the area before and after the standard medicine was added allowed for the calculation of the recovery percentage. 50%, 100%, and 150% levels were used for the usual addition procedure. As per the suggested approach, the solutions were examined in triplicate at every stage. Results were shown in Tables 3 and 4, which estimated the percentage recovery and percentage RSD at each stage. For Dextromethorphan Hydrobromide and Promethazine, the suggested approach produced satisfactory recoveries that ranged from 99.2 to 101.1 and 99.9 to 100.3, respectively. This shows that the suggested approach was correct. With accuracy precession a homogenous sample is sampled numerous times under specified conditions, the precision of the analytical process is the degree of agreement between the measurements.

Preparation of Precise Solution

After transferring 10 mL of the standard stock solution to a 100 mL volumetric flask, the volume was diluted using diluent. The remaining six preparations were made in the same manner. For the six preparations used in the precision investigation, the percentage RSD of the results was 0.16 for dextromethorphan hydrobromide and 1.78 for promethazine, both of which were well within the acceptable range of 2.0. The precision study's findings were presented inTables 5 and 6. The sensitivity of The suggested RP-HPLC method's limit of quantification (LOQ) was defined as the lowest concentration accurately assessed, and the limit of detection (LOD) as the lowest concentration providing response. The limits of detection (LOD) and quantification (LOQ) for Dextromethorphan Hydrobromide and Promethazine were determined to be 0.307 µg/mL and 0.674 µg/mL, respectively, and 0.929 µg/mL and 2.045 µg/mL. The method is sensitive for promethazine and dextromethorphan hydrobromide, according to the LOD and LOQ.

System Suitability Test

Dextromethorphan Hydrobromide and Promethazine were completely separated, indicating the method's specificity. In Figure 3, the standard chromatogram of promethazine and dextromethorphan hydrobromide was displayed along with parameters such as tailing factor, resolution, and retention time. Resolution was adequate in this case, and the tailing factor for the peaks of promethazine and dextromethorphan hydrobromide was less than 2%. Promethazine and Dextromethorphan Hydrobromide had respective average retention times of 17.36 and 15.47 minutes for five replicates. The peaks for promethazine and dextromethorphan hydrobromide were distinct and showed a distinct baseline separation. Additionally, analyses were conducted for active promethazine and dextromethorphan hydrobromide, as well as a placebo sample under various circumstances. The results of the investigation showed that the active and placebo samples did not interfere with the peak in the region of promethazine

and dextromethorphan hydrobromide. The approach that was devised was therefore unique to the investigation of this substance. Table 7 provides information on the system suitability parameters.

Assessment of Promethazine and Dextromethorphan Hydrobromide in Oral Tablet

A commercial solution formulation was selected to demonstrate the applicability of the suggested approach forestimating promethazine and dextromethorphan hydrobromide in solution formulations. In a 100 mL volumetric flask, 5 ml of the solution-which contained 15 mg of Dextromethorphan Hydrobromide and 6.25 mg of Promethazine—was transferred, and it was dissolved in 60 mL of diluent. The diluent was used to adjust the volume after the flask's contents were sonicated for fifteen minutes. A membrane filter measuring 0.45 µm was used to filter the mixture. I then pipetted 10 mL of the stock solution mentioned above into a 100 mL volumetric flask and used diluent to dilute it to the appropriate level. There were six injections of the solution into the column. The chromatograms were used to calculate the average peak area of the drug, and the regression equation for the pure drug was used to determine how much of the drug was in the tablet dosage form. Table 8 displays the recommended method's dose formulation assay findings.

Results and Discussion

The RP-HPLC method was developed to establish an accurate and stable assay for the pure medicines Dextromethorphan Hydrobromide and Promethazine in a combination dose form. The kinetex C18 column operated in isocratic mode, utilizing a mobile phase of orthophosphoric acid (0.1%) and acetonitrile (60:40 v/v), yielded peaks with optimal form and resolution. The flow rate was 1 mL/min, and Dextromethorphan Hydrobromide and Promethazine were quantified using a UV detector at 225 nm. Linearity was evaluated by graphing concentration against area throughout the ranges of 60-180 µg/mL for Dextromethorphan Hydrobromide and 25-80 µg/mL for Promethazine, yielding a correlation coefficient of 0.999, indicating a strong linear response beyond 0.995. The percentage recovery was determined to be within the acceptability standards, with a recovery range of 99.2% to 101.1% for Dextromethorphan Hydrobromide and 99.9% to 100.3% for Promethazine. The elevated recovery rate signifies that the proposed approach exhibits considerable accuracy. The relative standard deviation (%RSD) for intra-day and inter-day precision is below 2% for Dextromethorphan Hydrobromide and Promethazine. The detection limits of the proposed method were 0.307 and 0.929 µg/mL, while the quantification limits were 0.674 and 2.045 µg/mL for Dextromethorphan Hydrobromide and Promethazine, respectively, indicating the method's sensitivity. The assay methods were conducted six times, yielding values of 100.91% for Dextromethorphan Hydrobromide and 100.82% for Promethazine. The determined number of theoretical plates was 86,797 for Dextromethorphan Hydrobromide and 10,619 for Promethazine, indicating the column's efficient performance. No interfering peaks were observed in the chromatogram of the formulation during the run period, showing that the excipients utilized in the oral formulations did not affect the simultaneous quantification of Dextromethorphan Hydrobromide and Promethazine by the suggested HPLC approach.

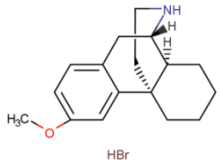

Figure 1: Chemical structure of Dextromethorphan Hydrobromide

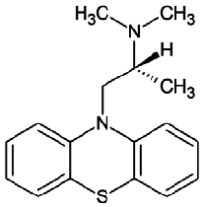

Figure 2: Chemical structure of Promethazine

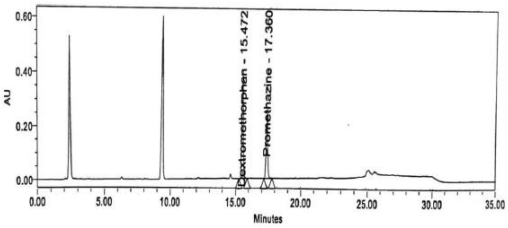

Figure 3: Typical chromatogram of Dextromethorphan Hydrobromide and Promethazine

Table 1: Calibration data of Dextromethorphan Hydrobromide

Concentration (µg/mL)	Peak area (n=5)
60	1758483
90	2623732
120	3481342
150	4325957
180	5235900

Table 2: Calibration data of Promethazine

Concentration (µg/mL)	Peak area (n=5)
25	1216690
40	1781113
50	2417925
65	2989579
80	3547103

Table 3: Recovery study for Dextromethorphan Hydrobromide

Level	Peak area	Amount recovered (µg)	% Recovery
50%	2356708	59.96	99.2
	2360666		
	2372046		
100%	4860773	120.07	101.1
	4871484		
	4873161		
150%	7352312	179.60	99.6
	7367422		
	7349780		

Table 4: Recovery study for Promethazine

Level	Peak area	Amount recovered (µg)	% Recovery
50%	1430770	24.95	99.9
	1432753		
	1432375		
100%	2859728	50.21	100.3
	2863304		
	2866574		
150%	4253632	75.01	100.2
	4242764		
	4267912		

Table 5: Precision study of Dextromethorphan Hydrobromide

Injection number	Peak area
1	2723657
2	2719283
3	2719955
4	2712383
5	2722567
6	2718955
Mean	2719569
SD	4403.7
%RSD	0.16

Table 6: Precision study of Promethazine

Injection number	Peak area
1	1763673
2	1740107
3	1721289
4	1700989
5	1686359
6	1698574
Mean	1722483
SD	30722.9
%RSD	1.78

Table 7: Analytical validation parameters

Parameter	Dextromethorphan Hydrobromide	Promethazine
Linearity (µg/mL)	60-180	25-80
Correlation coefficient	0.9998	0.9992
LOD (µg/ml)	0.307	0.929
LOQ (µg/ml)	0.674	2.045
Theoretical Plates	86797	10619
Tailing Factor	1.28	1.32
Retention Time (min)	15.47	17.36

Table 8: Assay studies

Drug	Label claim (mg)	Amount found (mg)	% Assay
Dextromethorphan Hydrobromide	15	15.25	100.91
Promethazine	6.25	6.26	100.82

Conclusion

Dextromethorphan Hydrobromide and Promethazine can now be measured simultaneously using a straightforward, sensitive, fast, accurate, and precise RP-HPLC approach. The study's outcome complies with ICH requirements and can be effectively used for promethazine and dextromethorphan hydrobromide simultaneous estimation in marketed formulation as well as in combination formulations.

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Not Declared.

Conflict of Interest

No Conflict of interest

Informed Consent

Not Applicable.

Ethical Statement

Not Applicable.

Author Contribution

All authors are contributed equally.

References

1. https://go.drugbank.com/drugs/DB00514
2. https://pubmed.ncbi.nlm.nih.gov/30855804/
3. A new synthetic anti-histamine substance derived from phenothiazine; phenergan, 3,277 R.P. Can Med Assoc J. 1948 Oct; 59(4):322-6.
4. https://pubmed.ncbi.nlm.nih.gov/31335081/
5. https://pubmed.ncbi.nlm.nih.gov/31643746/
6. Smith HS, Cox LR, Smith BR., Dopamine receptor antagonists. Ann Palliat Med. 2012 Jul;1(2):137-42. doi: 10.3978/j.issn.2224-5820.2012.07.09.
7. Adolph O, Koster S, Georgieff M, Georgieff EM, Moulig W, Fohr KJ. Promethazine inhibits NMDA-induced currents - new pharmacological aspects of an old drug. Neuropharmacology. 2012 Aug;63(2):280-91. doi: 10.1016/j.neuropharm.2012.03.006. Epub 2012 Apr 7.
8. Flake ZA, Linn BS, Hornecker JR. Practical selection of antiemetics in the ambulatory setting. Am Fam Physician. 2015 Mar 1;91(5):293-6.
9. Njuguna, Nicholas M. "Development and validation of a liquid chromatograpidc method for the simultaneous determination of diphenhydramine, promethazine, chlorpheniramine and ephedrine in cold-cough syrups." PhD diss., University of Nairobi, 2009.
10. Pippalla, Sreenivas, Arjuna Rao Nekkalapudi, and Venugopal Reddy Komreddy. "A validated stability-indicating reversed-phase-UPLC method for simultaneous estimation of promethazine hydrochloride, methylparaben, propylparaben and sodium benzoate assay of cough suppressant and antihistamine liquid oral

dosage forms." Biomedical Chromatography 38, no. 9 (2024): e5944.
11. Louhaichi, Mohamed R., Sami Jebali, Mohamed H. Loueslati, and Lotfi Monser. "Simultaneous determination of dextromethorphan hydrobromide, pyrilamine maleate and sodium benzoate in a cough cold syrup by LC." Chromatographia 69 (2009): 421-427.
12. Bitar, Yaser. "Separation and assay of three anti-cough drugs pseudoephedrine, dextromethorphan and chlorpheniramine in pharmaceutical forms by using single RP-HPLC Method." Research Journal of Pharmacy and Technology 13, no. 2 (2020): 831-839.
13. Trefi, Saleh. "Simultaneous determination of dextromethorphan and promethazine in pharmaceutical syrups by rapid HPLC method." International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN) 8, no. 2 (2015): 2828-2834.
14. Patil, Amruta Sainath, Sunil V. Amrutkar, and Santaji Nalwade. "Determination of Related Substances in Promethazine Hydrochloride and Dextromethorphan Hydrobromide in Oral Solution by RP-HPLC Method." Journal of Chromatographic Science 62, no. 3 (2024): 273-280.
15. ICH Harmonised Tripartite Guideline, Q2(R1), Validation of Analytical Procedures: Text and Methodology. International Conference on Harmonisation, Geneva, 2005; 1-13.