Available online on 15.06.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Assay of Diphenhydramine HCl in Syrup by High Performance Liquid Chromatography

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Article Info:

Article History:

Received 23 April 2023
Reviewed 14 May 2023
Accepted 27 May 2023
Published 15 June 2023

Cite this article as:

Sulistiyo J, Assay of Diphenhydramine HCl in Syrup by High Performance Liquid Chromatography, Journal of Drug Delivery and Therapeutics. 2023; 13(6):141-144

DOI: http://dx.doi.org/10.22270/jddt.v13i6.5885

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Abstract

Cough is a natural response from the respiratory tract by increasing the clearance of secretions and particles from mucus, irritants, foreign particles, and microbes, so that it can act as a body defense. One way that can be done to relieve cough symptoms is to use symptomatic drugs. Diphenhydramine HCl is an antihistamine that belongs to the first generation H1 receptor antagonist (H1 blocker) group which has sedative and anti-allergic properties. Diphenhydramine HCl is used to treat sneeze, runny nose, watery eyes, itches, skin rashes, and other cold symptoms or other allergies. Diphenhydramine HCl is also used to treat motion sickness and to induce sleep. The purpose of this study was to determine the contents of diphenhydramine HCl syrup sold in the market. This test uses the High Performance Liquid Chromatography (HPLC) method with acetonitrile as a mobile phase; water; triethylamine (50;50;0.5). The results obtained the average content of Diphenhydramine HCl 102.5%. So it can be concluded that the cough medicine sample meets the requirements because the contents are not less than 90% and not more than 110% based on the Indonesian Pharmacopoeia Edition VI 2020.

Keywords: Cough, Diphenhydramine HCl, High Performance Liquid Chromatography (HPLC)

INTRODUCTION

Coughing is a natural response from the respiratory tract by increasing the cleaning of secretions and particles from mucus, irritants, foreign particles, and microbes, so that they can act as the body's defense. There are two types of cough, namely productive cough (phlegm) and non-productive cough (dry). A productive cough is a cough that produces phlegm or mucus (sputum) so it is known as a cough with phlegm. Non-productive cough is a cough without phlegm, this cough is often triggered by food particles, irritants, cigarette smoke, and changes in temperature.[1–4]

One way that can be done to relieve cough symptoms is to use symptomatic drugs. There are many types of drugs used in cough management, depending on the type of cough that has the most effect on cough symptoms. Based on their mechanism of action, cough medicines can be grouped into 3 major groups, namely: expectorants, which have two mechanisms of action. First, it reacts directly by stimulating mucus secretion so that mucus is thinner and easier to remove. Second, by reacting indirectly by irritating the gastrointestinal tract which affects the respiratory system thereby increasing mucus secretion; mucolytic, as the name implies, mucolytic is a cough medicine with phlegm that works by destroying phlegm formations so that the phlegm becomes watery; and antitussive, are drugs that work to reduce the sensitivity of the cough center in the brain to incoming stimuli.[5–7]

Diphenhydramine HCl is an antihistamine which belongs to the first generation of H1 receptor antagonists (H1 blockers) which has sedative and antiallergic properties. Diphenhydramine HCl is used to treat sneezing, runny nose, watery eyes, itching, skin rashes, and other cold or allergy symptoms. Diphenhydramine HCl is also used to treat motion sickness and to induce sleep. Diphenhydramine HCl has physical properties in the form of a white crystalline powder, odorless and has a bitter taste. Diphenhydramine HCl is easily soluble in water, in ethanol, and in chloroform; slightly soluble in acetone; very difficult to dissolve in benzene and in ether.[8,9] Because there are already many companies that produce the drug Diphenhydramine HCl, it is necessary to test the drug preparation with several existing parameters and according to the requirements. A quality drug is a drug that is guaranteed that the drug has the potential or power to be used for its purpose, meets purity requirements, has clear and correct identity and labeling, is packaged in appropriate packaging and is protected from damage and contamination, looks good, and is free from contamination. from defects or damage.[10]

Drugs that are marketed in dosage form in the form of syrup must go through several testing processes. One of them is the determination of the levels of active substance preparations. The author wants to know whether the drug Diphenhydramine HCl in the form of syrup that is circulating in the market is of good quality to be marketed to the public or not.[11,12]

METHODS AND MATERIALS

The method used in this test uses High Performance Liquid Chromatography (HPLC) after dilution. This test was carried out on April 25, 2022. This test was carried out at Laboratory F 2.5. Department of Pharmacy and Food Analysis, Jakarta II Health Polytechnic. The test scheme is carried out in several stages:

Standard Solution

1. Weigh accurately ± 12.5 mg Diphenhydramine HCl BPFI
2. Put into 25.0 ml measuring flask. Add Aquabidest until dissolved, adjusting to the mark. Then homogenized
3. Filtered with a 0.45 µm porosity syringe. accommodated in a test tube. Inject 10 µL into the HPLC tool
4. Perform System Compliance Test and Assay. Measure the peak response at a wavelength of 254 nm

Test Solution

1. Pipette 10 ml of the sample, then put it in a 50.0 ml volumetric flask
2. Added aquabidest up to the boundary mark
3. Filtered with a 0.45 µm porosity syringe. Collected into a test tube. Inject 10 µL into the HPLC tool.
4. Peak response was measured at a wavelength of 254 nm

Mobile Phase

1. Measure Acetonitrile P as much as 250 ml with a measuring cup, put it in a 500 ml Erlenmeyer
2. Aquabidest was measured as much as 250 ml with a measuring cup, put into a 500 ml Erlenmeyer
3. Triethylamine P was measured as much as 2.5 ml with a measuring cup, put into a 500 ml Erlenmeyer
4. Check the initial pH and add glacial acetic acid P to obtain a pH of 6.5 with a pH meter. Aired for 30 minutes with the help of ultrasonic, then cooled

Tools used in the determination of Diphenhydramine HCL levels in syrup preparations by high performance liquid chromatography (HPLC), such as: Erlenmeyer, ultrasonic, measuring cup, beaker glass, syringe, syringe, magnetic stirrer, volume pipette, 0.45 µm membrane filter, pH meters, volumetric flasks, test tubes, test tube racks, filler pipettes, stir bars, and High Performance Liquid Chromatography (HPLC). The materials used were samples, standard Diphenhydramine HCl, aquabidest, acetonitrile p, triethylamine p, glacial acetic acid, ethanol, and methanol for hplc. The sample data as follows:

Sample Data

1. Sample Name: "K" Syrup
2. Production: PT "A"
3. Reg No: DTL7809322737A1
4. Expiration Date: January 07, 2024
5. Composition: Each 1 measuring spoon (5 ml) contains Diphenhydramine HCl 12.5 mg and Ammonium chloride 125 mg

Standard Data

1. Standard Name: Diphenhyidramine Hydrochloridum
2. Exp Date: June 2023
3. Standar Purity: 100 %

In high performance liquid chromatography (HPLC), system suitability tests include: Standard Deviation, Average Standard Deviation (RSD). Then do the calculation of sample content with % Sample Content and finally do the calculation of the Dixon test which is used to select the results of the proficiency test data. If there is data that provides a value outside most of the data set.

as a requirement, based on the Indonesian Pharmacopoeia Edition VI Diphenhydramine Hydrochloride Oral Solution contains Diphenhydramine hydrochloride, $C17H21NO.HCl$, not less than 90.0% and not more than 110.0% of the amount stated on the label.

RESULTS

Table 1: Sample and standard organoleptic data

Description	Sample	Standard
Form	Liquid	Powder
smell	Fragrant	Odorless
Color	Green	White
Flavor	Sweet	Bitter

Table 2: Data on retention time and area

No	Retention Time (Minutes)	Area
1	3,698	1015,42169
2	3,691	904,47369
3	3,684	976,06976
4	3,674	1072,15051
5	3,671	1052,04724
6	3.660	1044,59705
$\bar{x}$	3,6796	1010,79332
%RSD	0,4001	6,12

Table 3: Follow-up Factor Data

No	Follow-up Factor
1	1,5
2	2,25
3	1,75
4	1,75
5	1,25
6	1,5
$\bar{x}$	1,66

Table 4: Standard Weighing Data of Diphenhydramine HCl

Information	Standard Weight (g)
Container Weight	0,0793
Container Weight + Material	0,0920
Container Weight + Residual	0,0795
Material Weight	0,0125

Table 5: Data on retention time and standard and sample area

No	Information	Retention Time	Area
1	Standard	3,674	1072,15051
2	Sample K 1	3,664	1119,79700
3	Sample K 2	3,660	1059,44373
4	Sample K 3	3,661	1046,01184
5	Sample K 4	3,659	1042,40271
6	Sample K 5	3,656	1084,65833

Table 6: Precision Calculation Based on Retention Time

No	Retention Time	$\|xi - \bar{x}\|$	$\|xi - \bar{x}\|^2$
1	3,698	0,0184	0,00033856
2	3,691	0,0114	0,00012996
3	3,684	0,0044	0,00001936
4	3,674	0,0056	0,00003136
5	3,671	0,0086	0,00007396
6	3,660	0,0196	0,00038416
∑	22,078	0,068	0,00097736
$\bar{x}$	3,6796	0,0113	0,0001628293

Table 7: Precision Calculation Based on Area

No	Area	$\|xi - \bar{x}\|$	$\|xi - \bar{x}\|^2$
1	1015,42169	4,62837	21,42180
2	904,47369	106,31936	1130,386372
3	976,06976	34,72356	1205,72561
4	1072,15051	61,35719	3764,70476
5	1052,04724	41,25392	1701,88591
6	1044,59705	33,80373	1142,69216
∑	6064,75994	282,0864	19140,29396
$\bar{x}$	1010,79332	47,0144	3190,04899

Table 8: Dixon Test Sample Content Data

Information	Data	Level %
X1	Sample K 1	99.78%
X2	Sample K 2	100.13%
X3	Sample K 3	101.42%
X4	Sample K 4	103.83%
X5	Sample K 5	107.19%

Table 9: Precision Calculation of Sample Content

No	Level %	$\|xi - \bar{x}\|$	$\|xi - \bar{x}\|^2$
1	107,19	4,72	22,2784
2	101,42	1,05	1,1025
3	100,13	2,34	5,.4756
4	99,78	2,69	7,2361
5	103,83	1,36	1,8496
6	512,35	12,16	37,9422
∑	102,47	2,432	7,58844
$\bar{x}$	107,19	4,72	22,2784

DISCUSSION

The assay was carried out based on the procedures in the Indonesian Pharmacopoeia VI Edition 2020. In preparing the test solution, 10 mL was pipetted into a 50.0 mL volumetric flask aiming to obtain a final concentration of 0.5 mg/ml, and added aquabidest as a sample solvent. Sonication was carried out for ± 5 minutes with the aim of dissolving the sample more completely. After sonication, it was adjusted with aquabidest up to the boundary mark. Filter the test solution with a porous membrane filter of 0.45 µm or smaller, this aims to obtain a solution with smaller particles so that a better separation of components can occur in the column.

In this test, the mobile phase was used with a mixture of acetonitrile p : triethylamine p : aquabidest (50 : 0.5 : 50). The 'degas' technique is carried out to remove dissolved gases. If there is gas in the mobile phase, it can cause bubbles which can cause band widening. The liquid used as the mobile phase must be very pure to avoid the entry of impurities that can interfere with the interpretation of the chromatogram.

The System Suitability Test needs to be carried out every time a high-performance liquid chromatography assay is carried out, this aims to find out that the tool is in good condition and can be trusted so that the tool can be used to carry out a test and obtain accurate analytical data. In the system suitability test, the retention time parameter was 0.38% > 2% and the area precision parameter was 6.1% > 2%. The follow-up factor obtained was 1.7 < 2. So that the area and tailing factor precision parameters met the requirements, while the area area precision parameters did not meet the requirements stated in the procedure for determining diphenhydramine HCl levels.

In this test, resolution calculations were not carried out, because the standard tested was an internal standard because the procedure used Benzophenone while in this test the standard Diphenhydramine HCl was used, which is a compound similar to Benzophenone.

Based on the results of calculating the levels of Diphenhydramine HCl syrup, it was obtained that the average sample K level was 102.5% with an RSD of 3.0%. RSD is calculated to determine the precision of the test data performed. The test data can be said to be precise if the RSD is less than 2%. Based on the test results, the sample concentrations obtained entered the range of requirements for determining the concentration of Diphenhydramine HCl oral solution according to the Indonesian Pharmacopoeia Edition VI. While the RSD samples obtained did not meet the requirements set.

At peak the chromatogram is expected to be as symmetrical as possible and as sharp as possible to ensure that the separation is efficient. RSD results that do not meet the requirements may be due to the occurrence of broadening of the peak chromatogram (band broadening). The widening of the peak chromatogram (band broadening) is influenced by three factors, namely the eddy diffusion factor, longitudinal diffusion, and mass transfer. The eddy diffusion effect is caused by differences in the time of arrival of the solute at the detector which causes the peak chromatogram to widen or become less efficient. To prevent this, the size and shape of the stationary phase particles must be uniform. In longitudinal diffusion, solute molecules tend to diffuse in all directions. The longer the solute is in the column, the greater the tendency to diffuse and this results in a widening of the peak chromatogram. This can be corrected by increasing the velocity of the mobile phase. The mass transfer effect is due to the fact that some of the solute molecules are in the mobile phase and some are in the stationary phase. If the mobile phase flows quickly while some of the solute molecules cannot get out of the stationary phase quickly, then some of the solutes leave the column late. This results in widening of the peak chromatogram as well as making the separation inefficient. The consequent mass transfer effect is improved by decreasing the velocity of the mobile phase. As a result, there is an optimum speed to obtain the most efficient separation possible.[13]

CONCLUSION

Based on the test for determining the concentration of Diphenhydramine HCl syrup by High Performance Liquid Chromatography (HPLC), the average sample K level was 102.5%, so that it can be concluded that the levels of Diphenhydramine HCl in the sample Meet the Requirements (MS) as stated in the requirements of the Indonesian Pharmacopoeia Monograph VI Edition 2020.

CONFLICT OF INTEREST

The authors declare that they have no conflict interests.

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