Quantitative Determination of Dextromethorphan Hydrobromide using High-Performance Liquid Chromatography

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Abstract
Using reverse-phase high-performance liquid chromatography, dextromethorphan hydrobromide was quantified accurately and with high sensitivity, using a C8 type (150mm column), a flow rate of 0.5 ml/min, at room temperature, a wavelength of 295 nm, and an injection volume of 20 µl. The mobile phase consists of (Acetonitrile: Methanol: Hexane: Triethylamine) the ratios were (0.1:10:45:45) with a pH of 10.35. This method was validated by calculating the recoverability, which ranged (104.43%), the correlation coefficient (0.9993), and the linearity was (0.1-90 µg/ml). The method was successfully applied to some pharmaceutical forms containing dextromethorphan hydrobromide.
Keywords: dextromethorphan hydrobromide, antidepressant, RP-HPLC.

1. Introduction

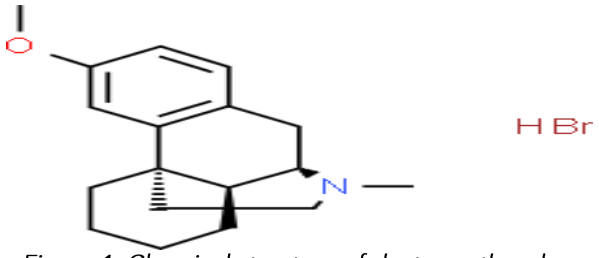

Figure 1: Chemical structure of dextromethorphan hydrobromide.

Dextromethorphan hydrobromide is a safe antibiotic to stop severe coughing fits [1] caused by irritation of the throat and bronchi [2] It has been widely used for more than half a century [3], it is also sometimes used as an antidepressant and pain reliever [4, 5], Figure (1).
Because of the medical importance of this drug, we note its quantitative estimation using several techniques, including high-performance liquid chromatography [6, 7], optical spectroscopy [8, 9], liquid chromatography [10], LC.MS/MS voltammetry
[11] and capillary Electrophoresis [12] and some chemometric methods [13].

Practical part Materials and Reagents

The drug dextromethorphan hydrobromide used as a standard solution is of Indian origin, and acetonitrile was prepared from Supelco (USA), and Methanol, Triethylamine and Hexane were used from Labo chem, and the same materials were used throughout the experiment, while the pharmaceutical preparations contained: Dextromethorphan HBR concentration 15 mg, and the mentioned substances were purchased from the Iraqi local market.

Chromatographic Conditions

Use the HPLC device type Shimadzu, Japan, Kyoto. , which consists of a pump type LC-20AD and a reagent SPD-20A, and a separation column type (150 * 4.6 mm) C8 was used to work in the experiment, and the working conditions were: flow rate of
Ml/min; column temperature 25°C; The wavelength of the reagent is 295 nm; the injection volume was 20 µl, and the mobile phase was (acetonitrile: methanol
:hexane :triethylamine) in the ratio (0.1:10:45:45), the pH was set at 10.35, and filter paper No. 0.45 was used to get rid of impurities until the solutions became clear and measurable.

Solutions

All the solvents used in this work are HPLC grade, and they are supplied from the local market.

Standard Solutions

The standard solution was prepared by dissolving the drug in the mobile phase solution, and then diluted it to the required concentration, and the mobile phase was prepared from (acetonitrile:methanol:hexane:triethylamine) in the proportions (0.1:10:45:45).

Preparation of Standard Solutions

To prepare the drug stock standard solution, dissolve 0.1 g of the drug in a given volume of the mobile phase mixture and then fill in the volume of the same mixture in a 100 ml volumetric vial to obtain a concentration of 1000 µg/ml of the drug, then transfer 2.5 ml from it to a 50 ml volumetric vial and complete Volume of the mobile phase mixture to obtain a concentration of 50 µg/mL of the drug.

2. Results and Discussion

The Best Conditions for the Experiment

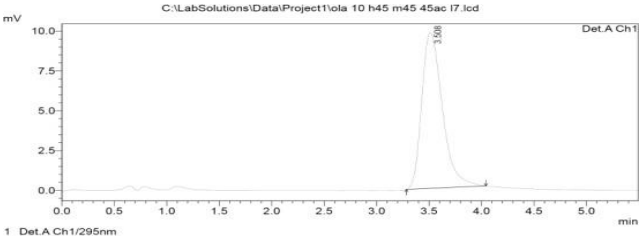

Figure 2: Chromatogram of the optimum wavelength.

The best chromatographic conditions were improved by changing the wavelength of the mobile phase because the

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mobile phase was used throughout the experiment. It was found that the best wavelength gave the highest value in the number of plates is 295 nm. Figure 2 shows the chromatogram for the optimal wavelength, and Table No. 1 shows the number of the plates for each wavelength were used for the study.

Table (1): The number of plates per wavelength.		
Wavelength(nm)	tR	Theoretical plate number
280	3.775	1547.320
285	3.764	1584.014
290	3.741	1559.847
295	3.508	1561.544

Several experiments were also applied to determine the best rate of flow rate, as it was noted that 0.5 ml/min was the best undisputedly, as shown in Figure 3, and Table 2 shows the values of the number of plates for each flow rate.

Table (2): the values of the number of plates for each flow rate.		
Wavelength(nm)	tR	Theoretical plate number
0.5	5.712	1686.408
0.8	3.476	1585.327
1	2.756	1420.553
1.2	2.309	1364.873
1.4	1.684	1126.187

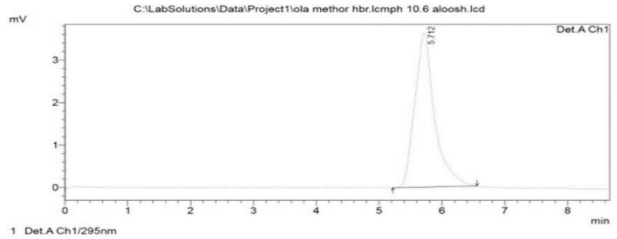

Figure 3: Chromatogram of flow rate.

When studying the pH, we note through the chromatograms that all the used pH did not give an acceptable separation except at pH = 10.35 as in Figure 4.

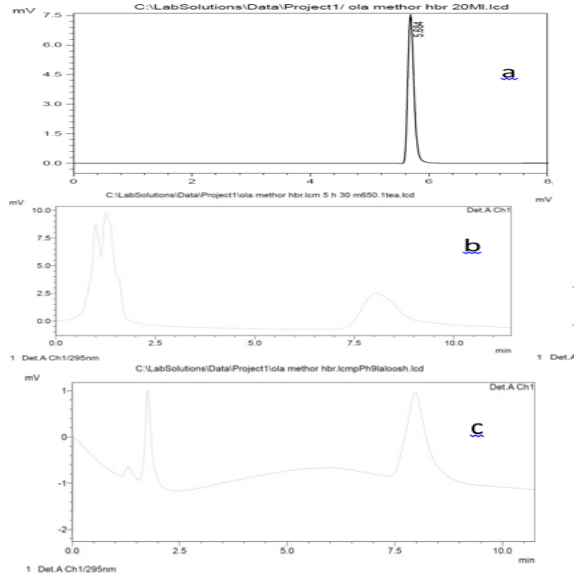

Figure 4: Chromatograms at pH (a) 10.35. (b) 6. (c) 9.

The effect of the ratios of the mobile phase was studied, as it was found that the only mixture that gives the best values for the number of platelets is the mobile phase consisting of (acetonitrile:methanol:hexane:triethylamine) and the ratio was (0.1:10:45:45) and Figure 5 shows a regular chromatogram. For the selected mobile phase. Figure 6 shows irregular chromatograms for a number of studied mobile phase ratios.

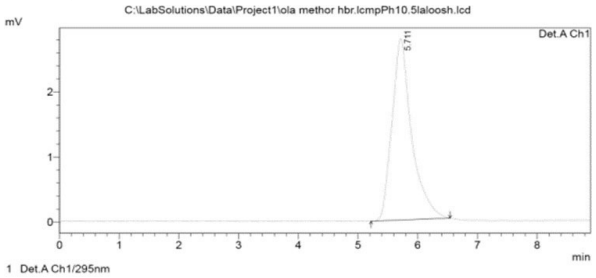

Figure 5: Chromatogram of the best mobile phase.

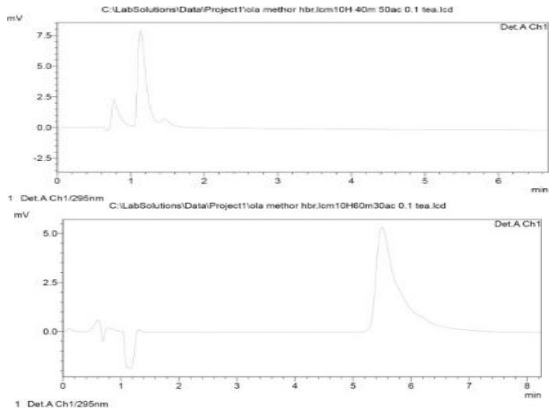

Figure 6: Some irregular chromatograms of the studied mobile phase ratios. Calibration Curve

When constructing the calibration curve for dextromethorphan hydrobromide it was found that the linearity was (0.1-90 µg/ml), Table 3 shows the linearity and correlation coefficient of the drug, and it also shows that the method has good agreement by studying %RSD.

Table (3): Results of the calibration curve of the drug*				
Drugs	LO D (µg.mL-1)	Precision (%RSD)	Linearity (µg.mL-1)	Linear regression equation with coefficient of correlation
Dextromethorphan HBR	0.0015	2.9201	0.1-90	y = 1404.2x - 360.31, 0.9993
*n = 4 %RSD = percent relative standard deviation LOD = limit of detection				

3. Method Accuracy

The accuracy of the method was studied by finding the recoverability. Table 4 the proposed method for quantitatively estimating the drug is of high accuracy, as the recoverability reached (104.43-100), and on this basis the proposed method can be adopted in the quantitative estimation of dextromethorphan hydrobromide.

Table 4. Results of the Recovery Tests for the Drugs*			
Drugs	Conc taken. (µg/mL)	Conc found. (µg/mL)	(%) Recovery

Dextromethorphan HBR	45	46.99	104.43
	90	90.00	100.00
*n = 3			

Method Application

The proposed method was applied to some pharmaceutical preparations available in the local market, and it was found that the method was successful, as the recoverability amounted to (104.43), Table 5.

Table 5. Application of the method to some pharmaceutical forms containing dextromethorphan hydrobromide.

Sample	Conc. taken (µg.mL1) of drug	Weight* found (mg/dosage)	Recovery %
Dextromethorphan HBR / syrup (15 mg\5ml)	45	15.66	104.43
*n = 3			

Conclusion

This method can be used for quantitative determination of dextromethorphan hydrobromide present in pharmaceutical preparations, as the method has been validated and shown to be of high accuracy. This method can be used in the quality control department to check these available pharmaceutical forms.

Reference

1. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang & Dale's pharmacology. Elsevier Health Sciences, 2011.
2. Yuliana T, Gustin S, Alamsyah A, Budiman S, Hardian A, Yun Y, Agma M, editors. HPLC Method for Simultaneous Determination of Dextromethorphan Hydrobromide, Chlorpheniramine Maleate and Potassium Sorbate in Cough Syrup. IOP Conference Series: Materials Science and Engineering; 2021: IOP Publishing.
3. Taylor CP, Traynelis SF, Siffert J, Pope LE, Matsumoto RR. Pharmacology of dextromethorphan: Relevance to dextromethorphan/quinidine (Nuedexta®) clinical use. Pharmacology & therapeutics. 2016;164:170-82. https://doi.org/10.1016/j.pharmthera.2016.04.010
4. Reynolds SM, Mackenzie AJ, Spina D, Page CP. The pharmacology of cough. Trends in pharmacological sciences. 2004;25(11):569-76. https://doi.org/10.1016/j.tips.2004.09.009
5. Morice AH, Shanks G. Pharmacology of cough in palliative care. Current opinion in supportive and palliative care. 2017;11(3):147-51. https://doi.org/10.1097/SPC.0000000000000279
6. Din N, Shafi H, Imran M, Sarwar M, Tahir M, Khursheed S. Validated reversed-phase liquid chromatographic method for simultaneous determination of dextromethorphan and chlorpheniramine in non-biological and biological matrices using PDA detector. Pharm Anal Acta. 2018;9(7):1-6.
7. Sirigiri B, Chengalva P, Parameswari SA, Aruna G. A novel HPLC method for the simultaneous determination of chlorpheniramine maleate and dextromethorphan in bulk and pharmaceutical formulation. International Journal of Pharmaceutical Sciences and Research. 2018;9:1147-51.
8. Rashid QN, Khattab ZT, editors. Spectrophotometric determination of drug compounds in pure forms and in the pharmaceutical preparations. IOP conference series: materials science and engineering; 2018: IOP Publishing.
9. Amin AS, El-Sheikh R, Zahran F, Gouda AAE-f. Spectrophotometric determination of pipazethate HCl, dextromethorphan HBr and drotaverine HCl in their pharmaceutical preparations. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2007;67(3-4):1088-93. https://doi.org/10.1016/j.saa.2006.09.027
10. Amaratunga P, Clothier M, Lorenz Lemberg B, Lemberg D. Determination of dextromethorphan in oral fluid by LC–MS-MS. Journal of Analytical Toxicology. 2016;40(5):360-6. https://doi.org/10.1093/jat/bkw033
11. Rezaei B, Irannejad N, Ensafi AA, Dinari M. Application of modified carbon quantum dots/multiwall carbon nanotubes/pencil graphite electrode for electrochemical determination of dextromethorphan. IEEE Sensors Journal. 2016;16(8):2219-27.
12. Krait S, Heuermann M, Scriba GK. Development of a capillary electrophoresis method for the determination of the chiral purity of dextromethorphan by a dual selector system using quality by design methodology. Journal of separation science. 2018;41(6):1405-13. https://doi.org/10.1002/jssc.201701166
13. Sefid-Sefidehkhan Y, Khoshkam M, Amiri M. Chemometrics-assisted electrochemical determination of dextromethorphan hydrobromide and phenylephrine hydrochloride by carbon paste electrode. Chemical Papers. 2021;75(12):6565-73. https://doi.org/10.1007/s11696-021-01823-4