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RP- HPLC METHOD FOR DETERMINATION OF METHYL 4- HYDROXY BENZOATE AS PRESERVATIVE IN PHARMACEUTICAL FORMULATIONS

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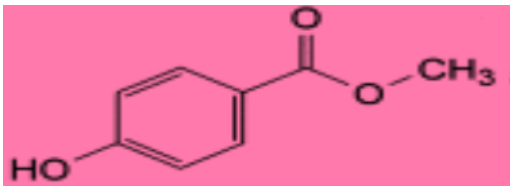
ABSTRACT

A simple, precise and accurate RP-HPLC method for estimation of Methyl 4-hydroxy benzoate in pure form, pharmaceutical formulations and environmental wastewater samples. Chromatographic separation was achieved on L_7Supelco reversed- phase column (25cm × 4. 6mm), 5 microns, using methanol: water (adjusted to pH 4. 8 with 0. 1 N HCL) in the ratio of 45:55 v/v as mobile phase. Flow rate was 1. 0 mL/min and the detection wavelength was 254 nm. The substances satisfactorily resolved with retention time values 5. 34 min for methyl 4-hydroxy benzoate. Linearity for methyl 4-hydroxy benzoate was in the range of 0. 01–0. 12mg/mL (R^2 = 0. 999) for methyl 4-hydroxy benzoate. The proposed method can be used for the estimation of these substances in dosage forms and environmental wastewater samples.

KEYWORDS: Methyl 4-hydroxy benzoate, RP-HPLC, pharmaceutical formulations, environmental wastewater samples.

INTRODUCTION

Methyl 4-hydroxy benzoate(MP) (Figure 1). Other names: Methyl 4-hydroxy benzoate, Methyl parahydroxy benzoate, methylis parahydroxy benzoai.[1] is commonly used as preservative in pharmaceutical, food and cosmetic products because of its anti-fungal and anti-bacterial properties.[1-3]



C8H8O3 **152. 1**

Figure 1: Chemical Structure of Methyl 4-hydroxy benzoate.

Hence a simple and accurate method of analysis was therefore necessary for routine quantitative determination of products in industrial pharmaceutical quality control. Nonspecific assay method (titration) with a long sample preparation time is reported in the United States Pharmacopeia (USP).[4] Many methods have been described for determination of Methyl 4-hydroxy benzoate in pharmaceuticals and cosmetics including gas chromatography[5, 6], spectrophotometry.[7] Separation and quantitation of parabens in pharmaceutical and cosmetic products using capillary electrophoresis (CE) has been reported in the literature.[8] RP-HPLC methods have been reported for the assay of various parabens in cosmetic and pharmaceutical products.[9–15] The purpose of the present study was to develop a speedily and sensitive method for the determination of M. P. using HPLC/UV.

MATERIALS AND METHODS

Apparatus

Chromatographic system consisted of an Shimadzu HPLC model LC-20AT with UV detector model SPD-20A, pump LC-20 AT, degasser model DGU -20A and L_7Supelco column (25cm ×4. 6mm), 5 µm particle size.

Reagents

All chemical used were of analytical or pharmaceutical grade, HPLC grade methanol was used throughout and Methyl 4-hydroxy benzoate standard material was provided from AL-Hokamaa company for pharmaceutical industries (HPI) Mosul-Iraq.

A standard stock solution of Methyl 4-hydroxy benzoate(200 µg/mL) was prepared in methanol. From these standard stock solutions, the mixed working standard solution was prepared to contain 10-120 µg/mL of Methyl 4-hydroxy benzoate.

HPLC method for determining methyl 4-hydroxy benzoate

A series of standard solution containing 10-120 µg/mL of Methyl 4-hydroxy benzoate and the sample solution of

pharmaceutical preparations were applied respectively. 20µl aliquot of each solution was injected on to the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of Methyl 4-hydroxy benzoate. The concentration of the unknown was read from the calibration graph or calculated from the regression equation derived from the concentration and peak area data.

Procedures for pharmaceutical preparations

Syrups and oral drops

The content of 3 containers were mixed well in 500mL dried beaker. A aliquots equivalent to10 mg of Methyl 4-hydroxy benzoate was transferred into 100mL volumetric flask and diluted with ethanol to the volume The determination of Methyl 4-hydroxy benzoate proceeded as. described under HPLC method for determining methyl 4-hydroxy benzoate. Calculate the percentage recovery using a calibration graph previously prepared.

Cream formulations

A quantity of creams containing methyl 4-hydroxy benzoate was transferred into 100 mL separated beaker. A 25 mL of ethanol was then added and the mixture was mixed on a hot plate stirrer at 60°C for 15min. After this period, the solution was cooled and filtered using filter paper (What man 4, England). Then the volume of each filtrate was adjusted to 100 mL with ethanol. Finally, 20 µL of diluted sample was injected into the column. Peak area of methyl 4-hydroxy benzoate was then measured for the determination. methyl 4-hydroxy benzoate concentrations in the samples were then calculated using peak data and standard curves.

Procedure for industrial wastewater samples

To demonstrate the practical applicability of the proposed method, real industrial wastewater samples from AL-Hokamaa company for pharmaceutical industries (HPI) Mosul-Iraq were analyzed by spiked with the concentrations ranging from 0. 04-0. 12 mg/mL of Methyl 4-hydroxy benzoate and determination of these substances as described under HPLC method for determining methyl 4-hydroxy benzoate.

RESULTS AND DISCUSSION

The development of the HPLC method for the determination of preservative in drugs has received considerable attention in recent years because of its importance in routine quality control analysis. The aim of this study was to develop a rapid HPLC method for the determination of Methyl 4-hydroxy benzoate in pure from, its pharmaceutical formulations, and environmental wastewater samples using the most commonly employed L_7 column with UV detection. Different three analytical columns with various stationary phases were tested. Good separation from these three columns was achieved using a supelcoL7(C8) column. The latter was finally used for analysis. A HPLC method was proposed as a suitable method for the for determination of methyl 4-hydroxy benzoate. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of methanol: water: (45:55 v/v), adjust PH to 4. 8 by 0. 1 N HCL, accomplished at 254 nm. The retention time was 5. 34 min at a flow-rate of 1. 0 mL /min and the injection volume was 20 µl. . The mobile phase was chosen after several trials with other solvent combinations. Mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. Figure 2 show a typical chromatogram obtained from the analysis of a standard of Methyl 4-hydroxy benzoate using the proposed method.

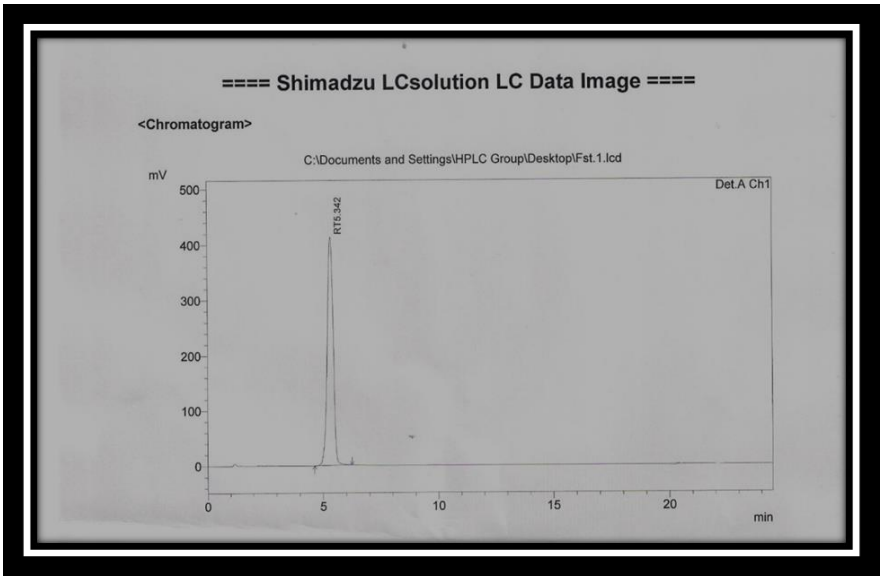


Figure 2: Typical chromatogram of Methyl 4-hydroxy benzoate (0. 08mg/mL).

As shown in this figure, Methyl 4-hydroxy benzoate was eluted forming symmetrical peaks and well separated from the solvent front. Observed retention time (5. 34)min for Methyl 4-hydroxy benzoate allowed a rapid determination of this substance.

Linearity

To determine the linearity of the HPLC method, calibration standard solutions of, Methyl 4-hydroxy benzoate was prepared as in the text. The linear ranges were found to be 0. 01-0. 12 mg /mL for Methyl 4-hydroxy benzoate figure (3). The regression equation and correlation coefficient (r) obtained by least square regression method were y=1E+08x-3178 (y: peak area, x: concentration) and1, for Methyl 4-hydroxy benzoate.

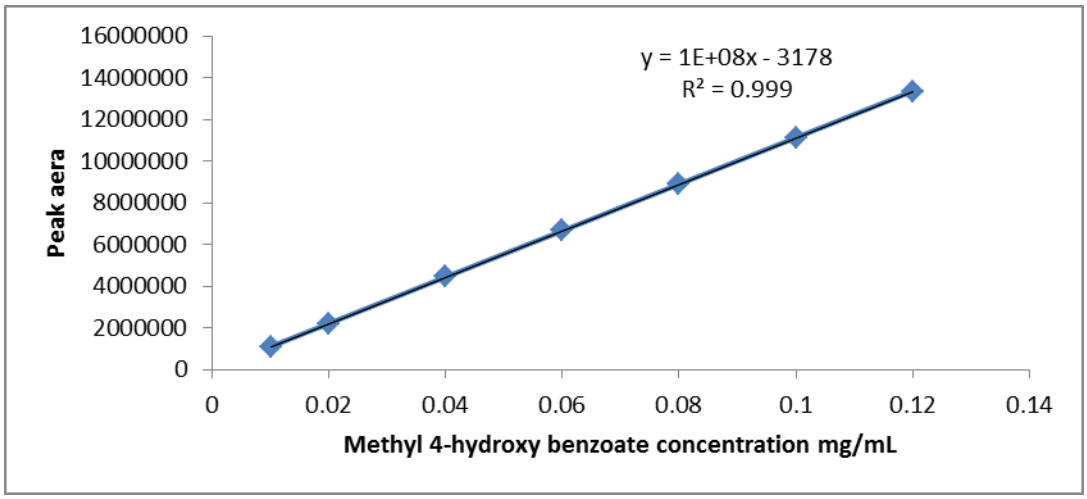


Figure 3: Calibration curve for Methyl 4-hydroxy benzoate.

The limit of detection (LOD) and limit of quantitation(LOQ) were calculated using the standard deviation of the intercepts(σ) and the mean slope (s) of the calibration curves. LOD=3. 3σ/s.[16-19] and it was 0. 2 µg/ml. and LOQ=10 σ/s and it was 0. 66µg/mL for methyl 4-hydroxy benzoate.

Accuracy and precision

Repeatability is given as inter- and intra-day precision and accuracy evaluated by analyzing three different concentrations of methyl 4-hydroxy benzoate.[17] Accuracy of the method was checked for six days at three concentration levels at 0. 01, 0. 06 and 0. 12 mg/ mL in six replicates for Methyl 4-hydroxy benzoate. The results are given in Table 1. The precision of the HPLC method was demonstrated by the relative standard derivation (RSD %) of lower than 2% for intra-day and inter-day. Recovery experiments were performed via standard-addition technique. To fixed and known amount of drug in the pre-analyzed tablet extracts, pure Methyl 4-hydroxy benzoate(standards) was added at three levels and the total amount was found by the proposed method. The experiment at each level was repeated six times. The percent recoveries obtained are given in Table 2. These results indicated a very good reproducibility of this method.

Table 1: Inter- and intra-day precision for Methyl 4-hydroxy benzoate assay by the proposed HPLC method.

Concentration of Methyl4-hydroxy benzoate (mg/mL)	Observed concentration of * methyl 4-hydroxy benzoate	
	Intra – day Mean(n=6)	Inter- day RSD%
0. 01	0. 0106	0. 95
0. 06	0. 0596	1. 20
0. 12	0. 121	0. 86

*Mean of six determinations.

Table 2: % Recovery of Methyl 4-hydroxy benzoate assay by the proposed HPLC method.

Substance	Amount added (mg)	Amount found(mg)*	% Recovery
methyl 4-hydroxy benzoate	0. 04	0. 0401	100. 25
	0. 1	0. 102	102
	0. 12	0. 118	98. 33

*Mean of six determinations.

Analytical application

The proposed method was successfully applied to the assay of Methyl 4-hydroxy benzoate in pharmaceutical formulations. and industrial wastewater samples. No interfering peaks were found in the chromatogram, indicating that the excipients did not interfere with the estimation of the substances by the proposed HPLC method. The results obtained are presented in tables(3) which reveals that there is close agreement between the results obtained by the proposed method and the label claim for the determination of Methyl 4-hydroxy benzoate in pharmaceutical formulations and good agreement between results and known values indicated the successfully applicability of the proposed method for determination of Methyl 4-hydroxy benzoate in environmental samples.

Table 3:Determination of Methyl 4-hydroxy benzoate formulations.

Pharmaceutical formulations	Proposed method found*	Label amount
Oral drop(Finstel- HPI)	0. 806mg/mL Methyl 4-hydroxy benzoate	0. 8mg/mL Methyl 4-hydroxy benzoate
Nystacort cream(HPI)	0. 198mg/gm. Methyl 4-hydroxy benzoate	0. 2mg/gm. methyl 4-hydroxy benzoate
Samilin syrup(HPI)	0. 695mg/mL Methyl 4-hydroxy benzoate	0. 7mg/mL Methyl 4-hydroxy benzoate

***Mean of five determinations.**

Table 4: Determination of Methyl 4-hydroxy benzoate in wastewater samples.

Wastewater samples	Added µg/mL Methyl 4-hydroxy benzoate	Found* µg/mL	Recovery %(n=10)
Industrial wastewater	0. 02 0. 08 0. 12	0. 0202 0. 0796 0. 1215	101 99. 5 101. 25

* mean value of ten determinations

CONCLUSION

This paper describes a reversed-phase HPLC method for determination of Methyl 4-hydroxy benzoate in pure forms, pharmaceutical preparations and wastewater samples. The validation studies show good recoveries, precision and accuracy. In summary, the reported method can be used for the routine quality control analysis of the investigated substances in pharmaceutical preparations and environmental wastewater samples.

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