DOI:10.7598/cst2019.1486
ISSN:2278-3458

Chemical Science Transactions
2019, 8**(2),** 237-243

RESEARCH ARTICLE

HPLC Method for Determination of Paracetamol in Pharmaceutical Formulations and Environmental Water Samples

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Received 21 November 2018 / Accepted 20 December 2018

Abstract: A simple, precise, rapid and accurate reversed phase high performance liquid chromatography method has been developed for the determination of paracetamol in pure from, pharmaceutical formulations and environmental water samples. Chromatography was carried out on supelco L_1 (C_{18}) reversed- phase column (25 cm × 4.6 mm), 5 microns, using a mixture of acetonitril: buffer $pH_{3.0}$ (40: 60 v/v) as a mobile phase at a flow rate of 1.5 $mLmin^{-1}$. Detection was performed at 243nm at ambient temperature. The retention time was found 2.2 minutes. The calibration curve was linear (r= 0.999) over a concentration range from 10 to 100 µg/mL. Limit of detection (LOD) and limit of quantitation (LOQ) were found 3 µg/mL and 9 µg/mL respectively. The method was validated for its linearity, precision and accuracy. The proposed method was successfully applied for the determination of paracetamol in pure form, pharmaceutical formulations and in environmental water samples.

Keywords: HPLC, Paracetamol, Pharmaceutical formulations, Environmental water

Introduction

Paracetamol (acetaminophen or *N*-acetyl-4-aminophenol), is a popular analgesic and antipyretic agent, with the following structural formula[1,2] (Figure 1).

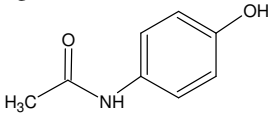

Figure 1. Structure of Paracetamol (Molecular formula: $C_8H_9NO_2$ = 151.2)

Several analytical methods have been devised for the determination of paracetamol. These methods include titrimetric method[3], HPLC methods[4-8], HPLC and UV methods[9-10], HPTLC methods[11-14], LC methods[15-16], UV-spectrophotometric methods[17-25], amperometric method[26] , fluorimetric method[27], chemiluminescence method[28] and voltametric methods[29]. These methods are required expensive or sophisticated instruments and not simple for routine analysis.

High performance liquid chromatography (HPLC) can be used for determination of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as quality control of the finished product. It has the advantages of being accurate, sensitive, rapid, selective and reproducible. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of paracetamol in different type of pharmaceutical formulations and environmental water samples.

Experimental

Chromatographic system consisted of Shimadzu HPLC model LC-20AT with UV detector model SPD-20A and C_{18} supelco column (25 cm ×4.6 mm), 5 µm particle size HPLC condition were given in Table 1.

Table 1. HPLC conditions

Column	Supelco C_{18} (25 cm×4.6 mm), 5 µm
Wavelength	243 nm
Mobile phase	Acetonitrile - pH_3 (40:60)
Retention time	2.2 minutes
Flow rate	1.5 mL/min
Temperature	Ambient
Injection volume	10 µL

Reagents

All chemicals used were of analytical or pharmaceutical grade and HPLC grade acetonitrile were used throughout.

Buffer solution (pH_3)

This solution was prepared by dissolving 5.75 g of monobasic ammonium phosphate in about 80 mL of water, add sufficient acetic acid to adjust the pH to 3 and dilute to 100 mL by distilled water in a volumetric flask[30].

Standard stock solution of paracetamol

1 mg/mL of this solution was prepared in mobile phase. Working standard solutions in a range of 10-100 µg/mL were prepared by dilution from stock solution.

HPLC method for determining paracetamol

A series of standard solution containing 10-100 µg/mL of paracetamol and the sample solution of pharmaceutical preparations were applied respectively. 10 µL aliquot of each solution was injected into the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area *versus* concentration of paracetamol. The concentration of the unknown was calculated from the regression equation derived from the concentration and peak area data, or was read from calibration graph

Procedures for pharmaceutical preparations

The recommended **c**ondition described above and mentioned in the HPLC method has been applied satisfactorily for determination of paracetamol in different type of pharmaceutical formulations.

Tablets

20 tablets were weighed, powdered and transfered an accurately weighed portion of the powder equivalent to 5 mg paracetamol into 100 mL volumetric flasks and diluted with mobile phase to the volume, and the amount of paracetamol was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration.

Syrups and drops

Syrups or drops containing 5 mg of paracetamol was taken into 100 mL volumetric flasks and diluted with mobile phase to the volume and the amount of paracetamol was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration.

Suppositories[31]

Small dish and a glass rod were placed in the dish NLT 5 Suppositories, heated gently on a steam bath until melted, then stirred, cooled while stirring, and weighed. Transfered a weighed portion of the mass, equivalent to 5 mg of paracetamol, to a separator. 30 mL of solvent hexane was added and dissolved. 30 mL of water was added, shaken gently and allowed the phases to separate. Transferred the aqueous layer to a 100 mL volumetric flask, washed the solvent hexane in the separator with three 30 mL portions of water, adding the washings to the volumetric flask, and dilute with Mobile phase to volume. The amount of paracetamol was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration.

Procedure for industrial waste water

To demonstrate the practical applicability of the proposed method, industrial waste water samples from al-hokamaa company for drug industries and medical appliances, Mosul-Iraq, were collected in polyethylene container cleaned with nitric acid and filtered through Whatman No.41 filter paper. Filtered samples were stored at 4 ^{0}C until analyzed which shows negative results, then the samples were spiked with the concentrations ranging from 20-60 μgmL^{-1} of paracetamol and then determined the concentration of paracetamol as described under HPLC method for determining paracetamol. The percentage recovery was calculated using a calibration graph previously prepared.

Results and Discussion

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products. The aim of this study was to develop an accurate, sensitive, rapid, selective and reproducible HPLC method for the determination of paracetamol in pure from, its pharmaceutical formulations and industrial wastewater samples using the most commonly employed L_1 column with UV detection. The detection wavelength of 243 nm was chosen in order to achieve a good sensitivity for quantitative determination of paracetamol in tablets, syrups, drops, suppositories and wastewater samples. The mobile phase consisting of acetonitrile : pH3 (40:60) offered a good separation at ambient temperature under these conditions using a flow rate of 1.5 mL/min and retention time of 2.2 minutes as shown in the chromatogram (Figure 2).

Under the described experimental conditions, the analyte peak were well defined and free from tailing paracetamol was determined by measuring the peak area. A plot of peak

area against concentration gave a linear relationship (r=0.999) over the concentration range 10-100 µg/mL. Using regression analysis, the linear equation Y=3269x -203.8 was obtained where Y is the mean peak area and X is the concentration in mg/mL (Figure 3, Table 2).

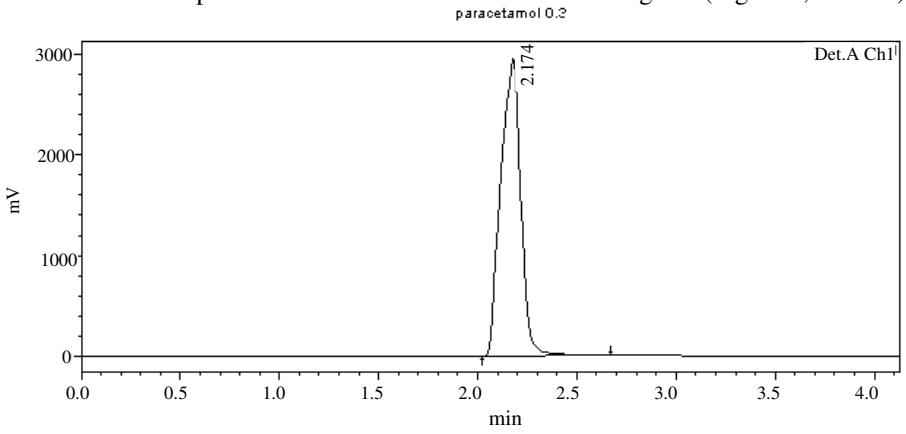

Figure 2 . Typical chromatogram (paracetamole 50 µg/mL)

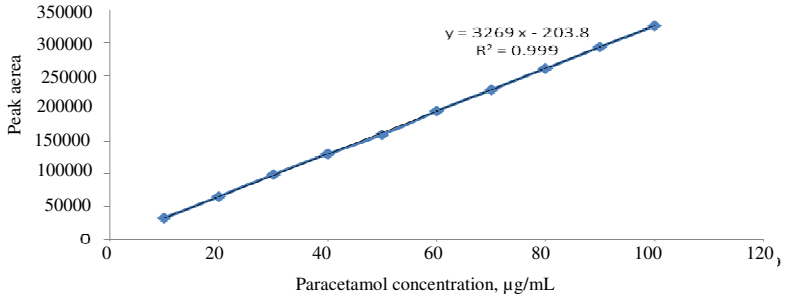

Figure 3. Calibration curve for paracetamol

Table 2. calibration curve data

Linearity range	Intercept	Correlation coefficient	Slope*
10-100 µg/mL	203.8	0.999	3269

**Slope: The ratio between the measured quantity in the analytical technique to the concentration of the substance to be determined quantity to the small change in concentration indicate a good sensitivity (high slope)*[33]

Determination of the limit of detection and limit of quantification (Sensitivity)

The standard deviation at concentration zero was calculated and this value was used for the calculation of the limit of detection and limit of quantification. The limits of detection (LOD) and quantification (LOQ) were calculated using the following formulae: LOD= (3.3σ/s) and LOQ= (10 σ/s) where σ is the standard deviation of the response and s is the slope of the regression line[32]. Limit of detection (LOD) and limit of quantification (LOQ) were found 3 µg/mL and 9 µg/mL respectively. The results indicate that the method was sensitive enough to detect a concentration of 3 ng/mL and able to quantify at a concentration of above 9 µg/mL.

Precision and accuracy

The precision of the method was established by carrying out the analysis of paracetamol (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained are presented in Table 3. To ensure the reliability and accuracy of the method recovery studies were carried out at five different levels. The results of recovery studies were found to be accurate, mean recoveries being 100.51±1.09 (n=6) as shown in Table 3.

Table 3. Method accuracy and precision

Concentration of paracetamol, µg/mL	RSD%	Recovery%
10	0.92	101.6
20	0.85	100.5
40	1.18	101.0
80	1.06	99.95
100	0.80	99.5
Mean(n=6)	0.962	100.51

Analytical application

The proposed method was successfully applied to the assay of paracetamol in pharmaceutical formulations (tablets, syrups, drops and suppositories) and industrial waste water sample. The result of analysis for pharmaceutical formulation Table 4, which reveals that there was close agreement between the results obtained by the proposed method and label claim.

Table 4. Determination of paracetamol in pharmaceutical formulations

Pharmaceutical formulations	Label amount, mg	Found, mg	(Recovery %)*
Colden tablet(NDI)	450 mg/tab	451.8	100.4
Antispasmine tablet(NDI)	350 mg/tab	349.65	99.9
Flu-out tablet(HPI)	350 mg/tab	349.58	99.88
Algesic tablet(HPI)	350 mg/tab	348.0	99.42
Paracetamol tablet(HPI)	500 mg/tab	495.6	99.12
Paradin tablet(NDI)	325 mg/tab	328.4	101.04
Antipyrol syrup(NDI)	120 mg/5 mL	119.0	99.16
Antipyrol drop(HPI)	100 mg/mL	99.3	99.3
Coldin syrup(NDI)	120 mg/5 mL	122.2	101.83
Anti pyrol	120 mg/supp	121.1	100.91
suppositories(HPI)	250 mg/supp	248.5	99.4
	500 mg/supp	496.9	99.38

.**Mean of six determinations*

The results of industrial wastewater samples is presented in Table 5. It shows that the recovery values obtained were closed to 100%.

Table 5. Determination of paracetamol in industrial wastewater samples

Water sample	Paracetamol, µg/mL*		Recovery%
	Taken	Found	
Industrial wastewater	20	20.0	100
	40	40.6	101.5
	60	60.4	100.6

**Mean of ten determinations.*

Comparison of methods

The proposed method was compared with other reported HPLC methods. Table 6 shows the comparison between the present method and another HPLC methods. The present method is more applications than other reported HPLC methods.

Table 6. Comparison of the existing HPLC methods with the proposed method

Parameters	Method 1	Method 2	Method 3	Method 4
Ref	[4]	[5]	[6]	Proposed
Column	C_{18}	C_{18}	C_{18}	C_{18}
Wavelength, nm	243	240	210	243
Linear range µg/mL	10-100 µg/mL	5-120 µg/mL	100-1000	10-100 µg/mL
Mobile phase	Methanol-H_2O(40:60)	H_2O-Acetonitrile –(85:15)	Acetonitile (pH2.5) (15:85)	Acetonitrile – pH 3.0 (40:60)
Retention time. (minutes)	3.03	1.5	5.5	2.2
Flow rate	1.0 mL/min	1.0 mL/min	1.0 mL/min	1.5 mL/min
Application	Tablets	Tablets	Tablets	Tablets ,Syrups, drops, Suppositories and industrial wastewater

Conclusion

In this study, accurate, simple and rapid HPLC method was developed and validated for the determination of paracetamol in pharmaceutical formulations and industrial waste water samples. The method was selective using L_1 analytical column and applicable to pharmaceutical preparations. Thus the developed method was recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in view of its high recovery and precision.

Acknowledgment

The author (Dr. Nief Rahman Ahmad) wishes to express gratitude to the Al-hokamaa state company of drug industries and medical appliance (HDI) Nineveh - Iraq for providing gift samples of paracetamol standard materials and pharmaceutical preparations and for permission and facilities to carry out the research work.

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