WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 15, 124-133. Research Article ISSN 2277– 7105

HPLC METHOD FOR DETERMINATION OF PARACETAMOL IN PHARMACEUTICAL FORMULATIONS AND ENVIRONMENTAL WATER SAMPLES

Nief Rahman Ahmad[1] and Farha Khalaf Omar*[2]

[1]Environmental College, Department of Technology, University of Mosul-Iraq.

[2]Department of Chemistry, Education College for Girls, University of Mosul-Iraq.

Article Received on
13 June 2018,

Revised on 03 July 2018,
Accepted on 23 July 2018

DOI: 10.20959/wjpr201815-12814

***Corresponding Author**
Farha Khalaf Omar
Department of Chemistry,
Education College for Girls,
University of Mosul-Iraq.

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 (25cm × 4.6mm), 5 microns, using a mixture of acetonitril : buffer pH3.0 (40: 60v/v) as a mobile phase at a flow rate of 1.5 $ml.min^{-1}$. 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 3ng/ml and 9 ng/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.

KEYWORD: HPLC, Paracetamol, Pharmaceutical Formulations, Environmental Water Samples.

INTRODUCTION

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

Molecular formula: C8H9NO2ı= 151.2

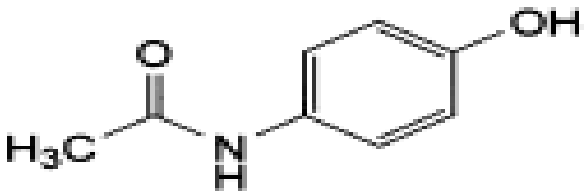

Figure [1]: Chemical structure of Paracetamol.

Several analytical methods have been devised for the determination of paracetamol. These methods include titrimetric method[3], HPLC methods[4,6], HPTLC methods[7,8], LC methods[9], Uv- visible spectrophotometric methods[10,13], amperometric method[14], fluorimetric method[15], chemiluminescence method[16] and voltametric methods.[17] 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.[18,19] 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.

MATERIALS AND METHODS

Apparatus

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

Table (1): HPLC conditions.

Column	**Supelco C_{18} (25cm×4.6mm),5 µm**
Wavelength	243 nm
Mobile phase	Acetonitrile – pH3 (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 (pH3)

This solution was prepared by dissolving 5.75 gm of monobasic ammonium phosphate in about 80 ml of water, add sufficient acetic acid to adjust the pH to 3 and dilute to 100ml by distilled water in a volumetric flask.[20]

A standard stock solution of paracetamol(1 mg/ml) 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. 10µl 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 provided from AL-Hokamaa Company for pharmaceutical industries (HPI) Mosul-Iraq.

Tablets: Weigh and powder 20 tablets. Transfer 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: Take a volume of syrups or drops containing 5 mg of 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.

Suppositories[21]: Tare a small dish and a glass rod, place in the dish NLT 5 Suppositories, heat gently on a steam bath until melted, then stir, cool while stirring, and weigh. Transfer a weighed portion of the mass, equivalent to 5 mg of paracetamol, to a separator. Add 30 ml of

solvent hexane, and dissolve. Add 30 ml of water, shake gently, and allow the phases to separate. Transfer the aqueous layer to a 100-mL volumetric flask, wash 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 C^0 until analyzed which shows negative results, then the samples were spiked with the concentrations ranging from 20-60 µg.ml^{-1} of paracetamol and then determined the concentration of paracetamol as described under HPLC method for determining paracetamol. Calculate the percentage recovery 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 a accurate, sensitive, rapid, selective, and reproducible HPLC method for the determination of paracetamol in pure from, its pharmaceutical formulations and industrial waste water samples using the most commonly employed L_1 column with UV detection. The detection wavelength of 243nm 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].

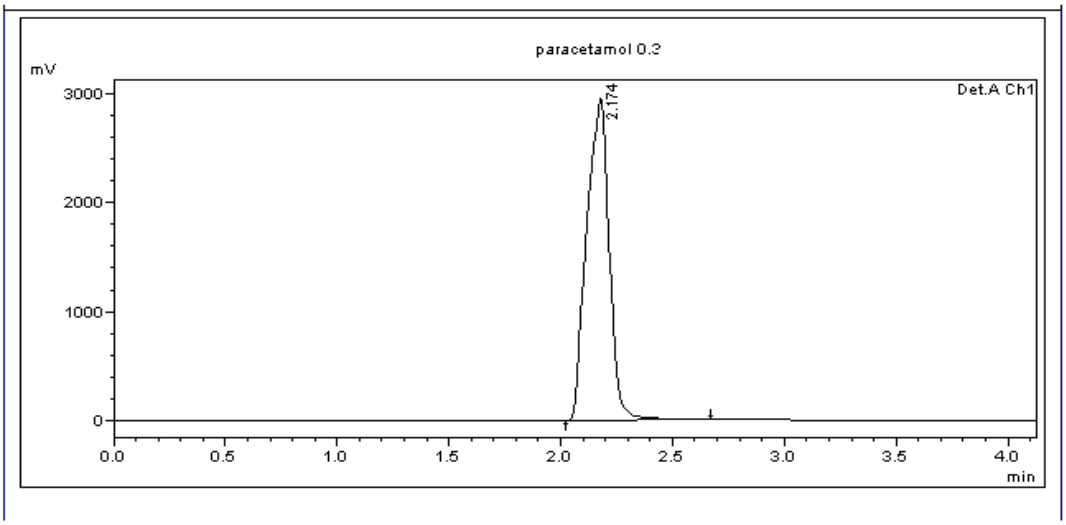

Figure [2]: Typical chromatogram (paracetamole 50 µg/ml).

Under the described experimental conditions, the analytic peak was 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) shows some information about the calibration curve.

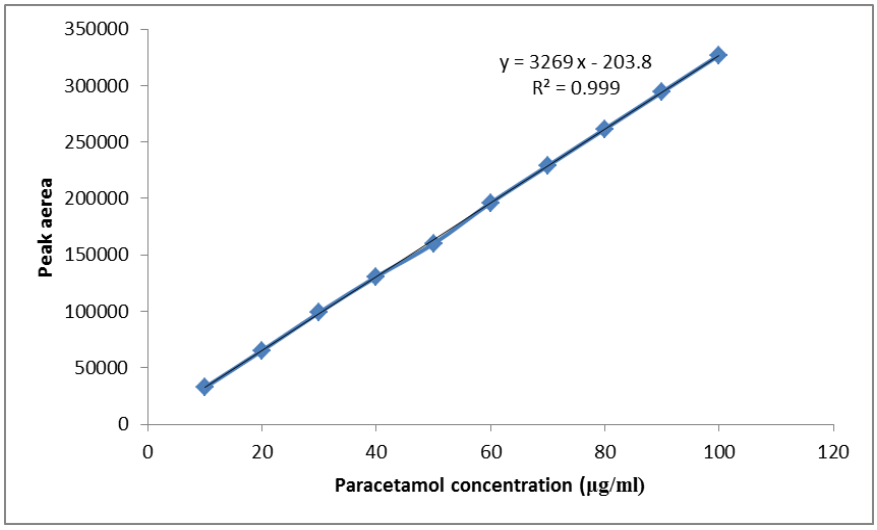

Figure [3]: Calibration curve for paracetamol.

Table (2): Some information about the calibration curve.

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).[22]

Determination 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=(10σ/s) where σ is the standard deviation of the response and s is the slope of the regression line.[23] Limit of detection (LOD) and limit of quantification (LOQ) were found 3ng/ml and 9ng/ml respectively. The results indicate that the method was sensitive enough to detect a concentration of 3ng/ml and able to quantify at a concentration of above 9ng/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 %)★
	450 mg/tab	451.8	100.4
Colden tablet(HPI)	350mg/tab	349.65	99.9
Antispasmine tablet(HPI)	350mg/tab	349.58	99.88
Flu-out tablet(HPI)	350mg/tab	348.0	99.42
Algesic tablet(HPI)	500mg/tab	495.6	99.12
Paracetamol tablet(HPI)	325mg/tab	328.4	101.04
Paradin tablet(HPI)	120mg/5ml	119.0	99.16
Antipyrol syrup(HPI)	100mg/ml	99.3	99.3
Antipyrol drop(HPI)	120mg/5ml	122.2	101.83
Coldin syrup(HPI)			
	120mg/supp	121.1	100.91
Anti pyrol suppositories(HPI)	250mg/supp	248.5	99.4
	500mg/supp	496.9	99.38

★Mean of six determinations

The results of industrial waste water samples (Table. 5) show that the recovery values obtained were closed to 100%.

Table (5): Determination of paracetamol in industrial waste water samples.

Water sample	Paracetamol(µg /ml)★ Taken Found	Recovery%
Industrial waste water	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 other 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)	Acetonitrile (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 waste water

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.

ACKNOWLEDGMENTS

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

REFERENCES

1. British National Formulary (BNF) Royal Pharmaceutical Society, 2016; 70: p.354.
2. Sweetman SC, editors. Martindale, 'The Complete Drug Reference. 36th Edition. London: Pharmaceutical Press, 2009; 108: 1895.
3. British Pharmacopoeia, H.M. Stationery office, London, UK, 2013; 1700.
4. Chandra. R, Sharma D.K,' Quantitative determination of paracetamol and caffeine from formulated tablets by reversed phase-HPLC separation technique, Int. J. Chromatogr. Sci., 2013; 3(2): 31-34.
5. Hamad M. Adress Hasan, Ibrahim H. Habib and Amira A. Khatab, 'RP-HPLC Determination of Paracetamol-containing compounds in quaternary and binary, Eur. Chem. Bull., 2017; 6(7): 330-335.
6. Maslarska, A.; Tencheva, J. Simultaneous determination and validation of paracetamol and codeine phosphate in pharmaceutical preparation by RP-HPLC. Int. J. Pharm and Pharm. Sci., 2013; 5(2): 417-419.
7. Uchadadiya, N, Mehta, F and Sanchaniya, P,' HPTLC-Densitometric Analysis of Eperisone Hydrochloride and Paracetamol in Their Combined Table Dosage Form," Chromatography Research International, 2013; 2013: 1-6.
8. Baheti.K, Shaikh.S, Shah.N and Dehghan.M,' Validated Simultaneous Estimation of Paracetamol and Etoricoxib in Bulk and Tablet by HPTLC Method. Int J Res Pharm Biomed Sci., 2011; 2(2): 672-675.

9. Sultana.N, Saeed Arayne.M, and Nadir Ali.S, 'An Ultra-sensitive LC Method for the Simultaneous Determination of Paracetamol, Carbamazepine, Losartan and Ciprofloxacin in Bulk Drug, Pharmaceutical Formulation and Human Serum by Programming the Detector. American Journal of Analytical Chemistry, 2013; 4: 24-33.
10. Zinati and Monzir S. Abdel-Latif 'Simultaneous Determination of Paracetamol and Tramadol in Pharmaceutical Tablets by Derivative UV-Vis Absorption Spectrophotometry, The Open Analytical Chemistry Journal, 2015; 8: 1-6.
11. Farha Kalaf Omar,' Indirect Spectrophotometric Determination of Paracetamol in Different Pharmaceutical Samples, Iraqi National Journal of Chemistry, 2014; 53: 36-42.
12. Shariati.R, Irandoust.M, Amin.N and Ahmadi.F,' Simultaneous Determination of Paracetamol, Dextromethorphan, Phenylephrine and Chlorpheniramine Using Partial Least Squares, Current Pharmaceutical Analysis, 2013; 9(2): 183-190.
13. Ahmad M. El-Zinati and Monzir S. Abdel-Latif 'Simultaneous Determination of Paracetamol and Tramadol in Pharmaceutical Tablets by Derivative UV-Vis Absorption Spectrophotometry, The Open Analytical Chemistry Journal, 2015; 8: 1-6.
14. Prabakar.S and Narayanan.S,' Amperometric determination of paracetamol by a surface modified cobalt hexacyanoferra graphite wax composite electrode, Talanta., 2007; 72(5): 1818- 1827.
15. Martinez. L, Satinsky.D, Solich.P, Barrales.O, Molina.A,' Fluorimetric optosensing in pharmaceutical analysis Determination of Paracetamol. Journal of Pharmaceutical and Biomedical Analysis, 2007; 45(2): 318-321.
16. Wirat.R, Liawruangrath.S and Townshend.A,' Flow injection chemiluminescence determination of paracetamol, Talanta., 2006; 69(4): 976-83.
17. Anitha Kumary.V, Divya.J, Mary Nancy.T, Sreevalsan.K,' Voltammetric Detection of Paracetamol at Cobalt Ferrite Nanoparticles Modified Glassy Carbon Electrode, Int. J. Electrochem. Sci., 2013; 8: 6610-6619.
18. Nief Rahman Ahmad,' High Performance Liquid Chromatographic Method for the determination of Diclofenac sodium in pharmaceutical preparations and in Environmental Samples, Iraqi National Journal of Chemistry, 2011; 44: 467-473.
19. Nief Rahman Ahmad and Suhaib N. Lottfi,' High performance liquid chromatographic method for the determination of guaifensin in pharmaceutical syrups and in environmental samples, J. Baghdad for Sci., 2013; 10(3): 1014-1023.
20. British pharmacopeia, Her Majesty, Stationary Office, London, 2009; 5107.
21. United States Pharmacopeia and National Formulary, 2018, USP 41, NF 36 .P 38.

22. Kenneth.A.C ,' A Text book of pharmaceutical Analysis "3rd Edn' New York. John Wiley and Sons., 1982; 620.
23. Nief Rahman Ahmed and Labeeb Hasoon Al Sadoon,' Determination of Chlordiazepoxide in Pure Drug Samples, Pharmaceutical Dosage Forms and Environmental Wastewater Samples Using HPLC Method, International Journal of Enhanced Research in Science, Technology & Engineering, 2017; 6(12): 1-5.