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Quantitative Determination of Acetaminophen in the Tablet Dosage Forms by RP-HPLC Method Without Using Buffers

Anuj Kumar Garg[1]*, Sanjay Kumar Bhardwaj[2]

[1]*,[2] Department of Chemistry, S.S.V. College, Hapur-245101 (U.P.) India,

*Corresponding author: Anuj Kumar Garg

*Department of Chemistry, S.S.V. College, Hapur-245101 (U.P.) India,

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ABSTRACT:

The objective of this paper is to develop a method for the determination of acetaminophen in tablets locally available from the pharmacist. We have used a 1220 infinity II LC system of Agilent technologies consisting of a gradient pump with a degasser, variable wavelength detector, Eclipse plus C-18 RP column of size 4.6×250mm, 5 µ. A mixture of methanol–water (30:70 v/v) was used as a mobile phase with a flow rate of 1.0 ml min^{-1}. The separation of acetaminophen was achieved without the use of buffers in the mobile phase. The detector was set at the range of 243 nm. This method was linear over a range of 1-50 µg/ml with correlation coefficients of 0.9998. The average retention time for paracetamol was found to be 4.48 ± 0.03 min. The detection limit and quantitation limit for paracetamol are 0.857 µg/ml and 2.597 µg/ml. The intra-day and inter-day precision expressed as percent relative standard deviation was below 2%. The mean recovery of paracetamol in the dosage form was found to be in the range of 96.0-102.4%. The method can be useful in the validation of tablet dosage forms containing acetaminophen without buffer. The proposed method for drug quantitation is economical, accurate, and rapid

Introduction-

Acetaminophen is also known as Paracetamol and it is p-aminophenol derivatives with the pK_a 9.38. It is used as an analgesic and antipyretic drug. It is used as a pain reliever and fever reducer and is commonly available as a tablet dosage form. It is the most commonly used drug worldwide. On 14 March 2020, France's health minister, Oliver Veran, tweeted that people with COVID-19 symptoms to avoid using ibuprofen and use paracetamol instead, resulting in a disproportionately high purchase of paracetamol medications.[1] In 2019, the sales of all brands under the paracetamol category were nearly Rs 530 crore. They touched Rs 924 crore by 2021 during covid wave in India. Dolo 650 mg became the most branded tablet during covid -19 pandemic. Various side effects were also reported due to an overdose of paracetamol.

The World Health Organization (WHO) "falsified"(Could be a perfect imitation of the original pharmaceutical), substandard (they are products that have been authorized but fail to meet their quality standards)" and unregistered/unlicensed" drug.[2] Counterfeit drugs is now a problem recognized globally[3].

A large number of methods have been used for the determination of acetaminophen. The most common methods are UV- visible spectrophotometric methods [4,5,6], Surface-enhanced Raman spectroscopy [7], NIR [8], HPTLC methods [9], Fluorescence [10] and HPLC methods [11,12,13]. Most of these methods are quite expensive. The most common practice to study acetaminophen is the HPLC method. Most of the methods reported for the study of acetaminophen are based on the use of a buffer with different solvents. Some of these methods are given in Table -1 in which the acetaminophen is determined by RP-HPLC using the buffer.

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S.N.	Mobile phase	Range	Reference
1	Acetonitrile: buffer pH3.0 (40:60v/v) as a mobile phase at a flow rate of 1.5 ml.min^{-1}.	243 nm	N Rahman, & FK Omar [14]
2	0.1 M potassium phosphate monobasic/methanol/glacial acetic acid (95:4:1, v/v/v) was used as the mobile phase.	272 nm	AG Goicoechea et al [15]
3	Mobile phase comprises phosphate buffer (pH 6.8) and acetonitrile in a ratio of 65:35, v/v at a flow rate of 0.7 mL/minute.	243 nm	MS Jahan et. al [16]
4	The mobile phase consists of 40% methanol in 0.4% phosphoric acid.	254 nm	P Kotal et. Al [17]

The methods mentioned above use the buffers in the RP-HPLC method for the determination of acetaminophen. There are always certain limitations and risks associated while using the buffers in RP- HPLC method-

1-The buffers are used when there are ionizable analytes (acidic or basic) present. Small retention is favoured at lower pH for the basic analyte while for the acidic analyte, small retention is favoured at higher pH. The low pH (2-4) suppresses the ionization of weakly acidic analytes, leading to higher retention.

2-The buffers can be used with conc. about 5–20mM.

3-The buffers are only effective within ±1.0 to ±1.5 pH units from their pK_a.

3-The use of a high-pH mobile phase is not feasible with silica-based columns due to the dissolution of the silica support at pH>8.

4- Filtration by using the 0.45-µm membrane is required for all aqueous mobile phases.

5-The salt of the buffer may precipitate in the presence of an organic solvent which can increase the cost of maintenance of HPLC and also the life of the column is decreased.

6-Microorganism can grow in buffers; hence it is mandatory to flush the system after using the buffers.

In order to remove the above difficulties in this method, we have used methanol with water as a mobile phase without using the buffers. Also, methanol is far less expensive than acetonitrile, the most common solvent used in RP-HPLC.Thus, this method is very economical and durable compared to other methods referred to earlier.

Material and Methods –

HPLC grade methanol was procured from Fisher Scientific Pune and HPLC grade water from Fisher Chemicals pharmaceutical formulation. Acetaminophen is used from Merck.

Standard solution of paracetamol

The standard stock solution of paracetamol was prepared by dissolving accurately weighed 10 mg of the CRS acetaminophen in 100 mL of mobile phase (methanol and water 70: 30 v/v) filtering through 0.25 µ nylon membrane. Working standards of paracetamol were prepared 1,2, 5, 10, 20, 30, 40, 50 µg/ml for calibration purposes.

Sample solution preparation

We have purchased a few branded drugs from the local market keeping in view the drugs have the proper shelf life. Twenty tablets of each containing 500 mg of acetaminophen were weighed and finely powdered in a mortar with the help of a pestle. A quantity equivalent to 50 mg of paracetamol was weighed and transferred to a volumetric flask and dissolved in 50 mL of mobile phase i.e. mixture of methanol and water (70: 30 V/V.) This sample solution was stirred magnetically for five minutes and sonicated for 15 minutes. It was diluted to get the solution in the range of calibration after filtering through 0.25 µ nylon membrane. HPLC analysis was done with the Agilent HPLC.

Apparatus and HPLC Conditions

The 1220 infinity II LC system of Agilent technologies consisting of Eclipse plus C-18 RP column of size 4.6×250mm, 5 µm, a gradient pump with a degasser, and a variable wavelength detector with autosampler is used for RP- HPLC analysis. EZ Chrome software is installed on the computer for data acquisition and processing. The mobile phase was methanol and water (70:30, v/v), with a flow rate of 1 mL/min. The detection of the peak was

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carried out at 243 nm. Injection volume (10µL) is used for analysis. The calibration standard and test solutions were analysed without further dilution. The run time of six minutes was taken.

Method validation

Linearity

The standard calibration graph is used to determine the linearity of the method. A total of eight standard solutions were prepared (1,2,5,10,20,30,40,50 µg/ml).10 microlitres of each solution were injected using an autosampler. The results obtained showed that the method is linear for a range of 1–50 µg/ml for acetaminophen. with their coefficient of correlation (r^2) all equal to .9998 (r^2 = 1).The retention time was found to be 4.48 minutes. The linearity of this method was used for finding slope, intercept, and r^2. The results are presented in Table 2. The Slope and Intercept were found to be 619870 and 289856 respectively as given in the figure-1. The chromatograph for one of the standards is also given in the figure-2 at a time of 4.48 minutes.

Table -2 Standard solutions and their corresponding average peak area

S.N.	Conc. in µg/ml	Mean Peak Area
1	1	1181954
2	2	1608534
3	5	3256429
4	10	6298122
5	20	12501242
6	30	18938840
7	40	25183384
8	50	31289829

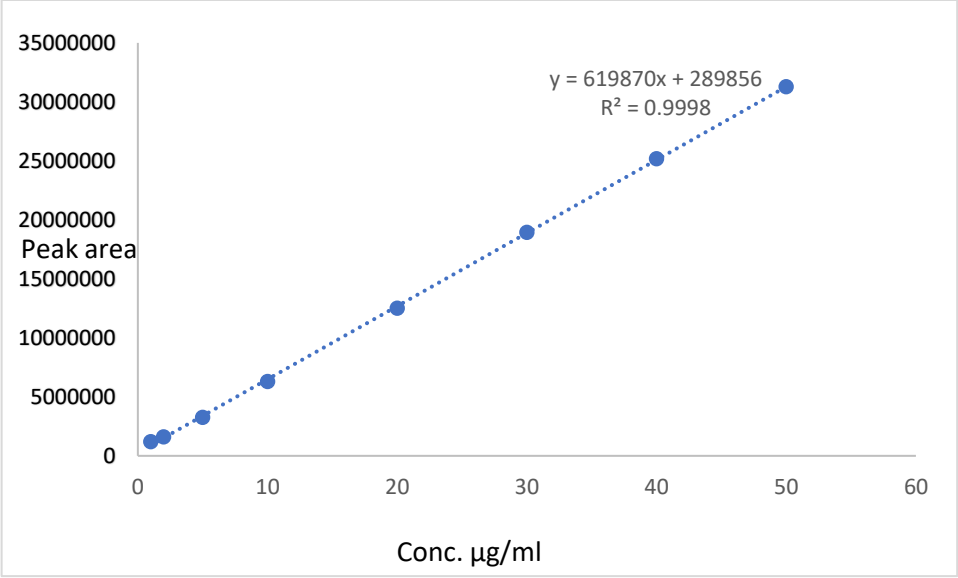

Figure-1 Calibration curve for Acetaminophen

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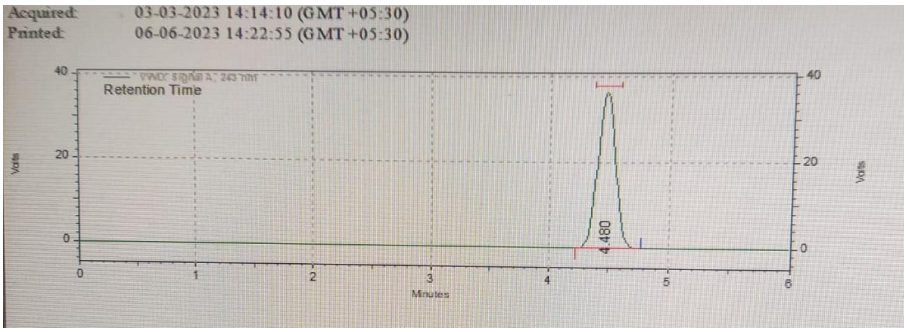

Figure-2 RP-HPLC signal for acetaminophen in the standard solution

Accuracy

The accuracy or trueness of a developed method is defined as 'the closeness of the true value, and the value found experimentally. In the present method, in order to evaluate the accuracy, successive analyses (n = 3) for three different concentrations (2 µg/ml,10 µg/ml, and 30 µg/ml) of standard acetaminophen solution were carried out. Then the accuracy of the method is reported in terms of percentage recovery by the following formula.

% Recovery = (Recovered conc. /Injected conc.) x 100

The results obtained from the determination of accuracy, expressed as percentage recovery, are summarized in Table 3

Table 3. Result of the accuracy of the method

S.N.	Injected true conc. µg/ml	Mean peak area	Mean experimental µg/ml	% recovery
1	2	1545534	2.02	101.28
2	10	6344122	9.76	97.6
3	30	18947840	30.09	100.3

Percentage recovery should be between 97-103 percent as per ICH guidelines [18].

Precision

Precision is defined as 'the closeness of agreement between a series of measurements. Precision is expressed as the standard deviation (*s*) or the relative standard deviation (RSD) of the mean (*x*) of a series of measurements:

RSD = s/*x* × 100

The precision of this method is based on inter and intra-day precisions. Results are presented in Tables 4 and 5. The method was found to be precise since the RSD value is less than 2. The precision of the proposed method is checked by inter-day and intraday from the repeatability of responses after replicate injection (n = 5) of standard solutions (10 µg/ml). The standard solution of 10 µg/ml concentration was analysed for 3 days. The precision analysis was carried out four times within the same day (intra-day variation) and three other days (inter-day variation). The precision was expressed in percentage RSD and comes out to be less than 2 for inter-day and intraday.

Table-4 *Determination of precision* Inter-day

S.N.	Injected conc. (n=5)	Average Peak area	Mean concentration recovered	% recovery	% RSD
1	10	6548839	10.097	100.973	
2	10	6647473	10.256	102.564	
3	10	6365783	9.802	98.019	1.68%
4	10	6487712	9.999	99.986	
5	10	6567483	10.127	101.273	

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Table-05 -*Determination of precision* Intra-day

S.N.	Day	Injected concentration (n=5)	Average Peak area	Mean recovered	%RSD
1	1	10	6288122	9.676651556	0.984%
2	2	10	6390122	9.841202188	
3	3	10	6399150	9.855766532	

LOD and LOQ

The limit of detection (LOD) is the lowest amount of analyte in a sample that can be detected but cannot be quantitated as an exact value. The limit of quantification (LOQ) is defined as 'the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy'. Several methods had been suggested for the determination of LOD and LOQ. The most common approach is

LOD=3.3σ/Slope of the calibration curve

LOQ=10 σ/Slope of the calibration curve

Where σ is defined as the standard deviation of residual responses. The value of LOD and LOQ for acetaminophen comes out to be 0.8570 and 2.597 µg/ml.

Analysis of dosage form

Analysis of the tablet form of the acetaminophen is carried out after preparing the sample in the mobile phase as described in the sample preparation section. A 1.0 mL portion of it is further diluted to 10.0 mL with mobile phase to get the solution in the range of calibration. It is used for injection on HPLC. The results presented in Table 6 indicate the suitability of the method for routine analysis of acetaminophen in the drug products.

Table-06 Analysis of dosage form of tablet containing acetaminophen

S.N.	Sample Code	Paracetamol in drug	Amount found	Assay (%)
1	Sample -1	500 mg	498	99.6
2	Sample-2	500 mg	480	96.0
3	Sample-3	500 mg	509	101.8
4	Sample-4	500 mg	512	102.4

As per the ICH guidelines, the tablet should contain not less than 90% (495mg) and not more than 110% (550mg) of acetaminophen and all of our samples contain the amount of it in the prescribed range.

Conclusion

The current RP-HPLC method developed can be used for the qualitative and quantitative analysis of acetaminophen in the tablet dosage form. The study proved the current method is linear, precise, accurate and specific. The use of a mobile phase without buffers and short run time enhances the utility of the developed method. From the above discussion, it is clear that the current method can be used for acetaminophen in the drug formulation and standards without the use of buffers. The present method proved to be a cost-effective, easy-to-use, and economical method. This method can be used in the pharma industry for assaying the purity of tablets containing acetaminophen.

Conflicts of interest

There are no conflicts of interest.

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