Development of a validated analytical method for Aceclofenac and Paracetamol injection by HPLC method

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ABSTRACT:

A simple, accurate, rapid and precise and validated HPLC method has been developed for the simultaneous estimation of Aceclofenac and Paracetamol injection by using C18 (250mm x 4.6 6 mm id., 5µm) using 0.3M Ammonium acetate: Methanol: ACN in the ratio (27:33:40v/v) as the mobile phase at a flow rate 1ml/min and detected at 260nm. The methods were validated in the terms of linearity, accuracy, specificity, range and precision. The method was found to be precise (%RSD<2) and the method was found to be linear for aceclofenac and paracetamol (>0.99). % recovery for aceclofenac and paracetamol was found to be within the limit.

KEY WORDS:

Aceclofenac, Paracetamol, HPLC, Validation and Method development

INTRODUCTION:

Paracetamol injection,[1-4] are mild analgesic used to treat mild to moderate pain. It can be used to relieve fever. Paracetamol injection as little anti-inflammatory activity. Paracetamol injection is important medication needed in a basic health system. Paracetamol injection can be given through vein or muscle to the patient in the hospital.

Intravenous paracetamol or intravenous acetaminophen they show analgesic and antipyretic agent and it is used worldwide by the patient for fever in adults and children. Intravenous paracetamol is well tolerated in clinical trials, having a tolerability profile than placebo. Adverse reaction emerging from intravenous paracetamol is rare.[5-9]

Aceclofenac belongs to a group of medicines called non-steroidal anti-inflammatory drugs (NSAIDs). Aceclofenac injection is pain relieving medicine. They used for the treatment for osteoarthritis, rheumatoid arthtitis, ankylosing spondylitis and other conditions. It is commonly used in joint pain, muscle pain in cases where oral administration is not possible. It works by blocking the release of certain chemical messengers that cause pain and inflammation (redness and swelling).[10-13]

EXPERIMENTAL:

Chemicals and Reagents Used

Aceclofenac	Gift sample from SPM Drugs pvt ltd
Paracetamol	Gift sample from SPM Drugs pvt ltd
Acetonitrile	HPLC grade
Methanol	HPLC grade
Ammonium acetate	Analytical grade
Orthophosphoric acid (OPA)	Analytical grade
Water	Milli Q water for HPLC

Instruments used

- ✓ Wensar Single Pan Electronic Balance:
- ✓ pH meter (LB-901)
- ✓ Ultra Sonicator (Mark ultrasonic)
- ✓ **Algient** HPLC analytical column (250 x 4.6mm, 5µ)
- ✓ Waters isocratic HPLC system with following configurations:
  - ' 515 solvent delivery system (pump)
  - ' Manual injector with 20 µl loop
  - ' 2487 UV- visible detector
  - ' Young Lin Autochro-3000 data station

Reagents & Standard Solutions preparations

Solubility

The solubility of aceclofenac and paracetamol was confirmed through pharmacopeia.

Selection of wavelength

The wavelength was recorded on UV-visible spectrometer is 260nm.

Preparation of standard stock solution

37.50 mg of Paracetamol was accurately weighed and transferred to the 50ml of volumetric flak and diluted with methanol. Further 2ml was pipetted from the above solution and was made up to 50 ml with mobile phase.

37.47 mg of Aceclofenac was accurately weighed and transferred to the 50ml of volumetric flak and diluted with methanol. Further 2ml was pipetted from the above solution and was made up to 50 ml with mobile phase.

Preparation of sample solution

500.95 mg of sample was accurately weighed and transferred to the 50ml volumetric flask and diluted with methanol. Further 2ml was pipetted from the above solution and was made up to 50ml with mobile phase.

ANALYTICAL METHOD DEVELOPMENT

Effect of nature of stationary phase

Various stationary phase are used for separation and chromatogram were recorded. C18 (250mm x 4.6mm id, 5µ) column.

Effect of ratio of Mobile phase

270ml of 0.3M Ammonium acetate was mixed with 330ml of methanol and 400ml of Acetonitrile and used as mobile phase.

Effect of flow rate

The flow rate of the present condition is 1.0 ml/min is selected; the peak is retained well.

Optimized HPLC Condition

Chromatographic Parameters	Composition
Stationary phase	Agilent HPLC-C_{18} (250 x 4.6mm. id., 5µm)
Mobile phase	0.3M Ammonium acetate: Methanol: ACN
Solvent ratio	(27:33:40v/v)
Detection Wavelength	260nm
Flowrate	1 ml/min
Sample volume	20µl
Column Temperature	Room Temperature

RESULT AND DISCUSSION:

Method development:

With the optimized condition, mobile phase, sample solutions were injected and the chromatogram were recorded. chromatogram was given in the figure 1.

Validation of the developed HPLC method:[14]

Specificity

The specificity of the developed method was checked in the presence of its excipients. The drug was well re-solved and did not show any any interference of placebo solution indicating the specificity of the developed chromatographic method.

Linearity & range:

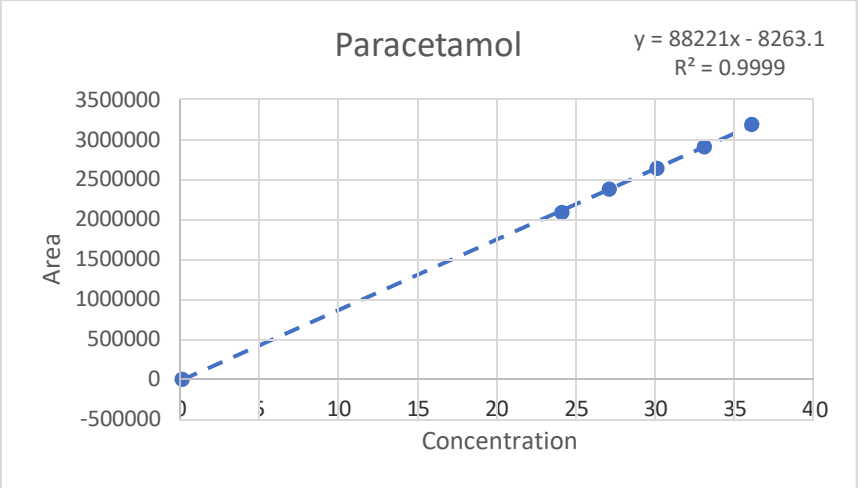
Linearity is the ability of an analytical method to elicit test results that are directly proportional to the concentration of the analyte in samples within a given range and the Range is the interval between the upper and lower levels of the analyte that have been demonstrated to be determined with precision, accuracy and linearity. Linearity and range of the method were

analyzed by preparing calibration curves using different concentrations of standard solutions of Paracetamol and Aceclofenac. The solutions were prepared in concentrations ranging from 24µg/ml to 36µg/ml. The standards were then analyzed by the optimized chromatographic method and the peak areas were noted. The calibration curve was plotted using peak area and concentration of the standard solutions. Standard curve fitting is determined by applying the simplest model that adequately describes the concentration–response relationship using appropriate weighting and statistical tests for goodness of fit.

LINERAITY OF PARACETAMOL: TABLE 1

Concentration (mcg/ml)	Area (n=3)
0	0
24	2084449
27	2375498
30	2634458
36	2904664
39	3184496

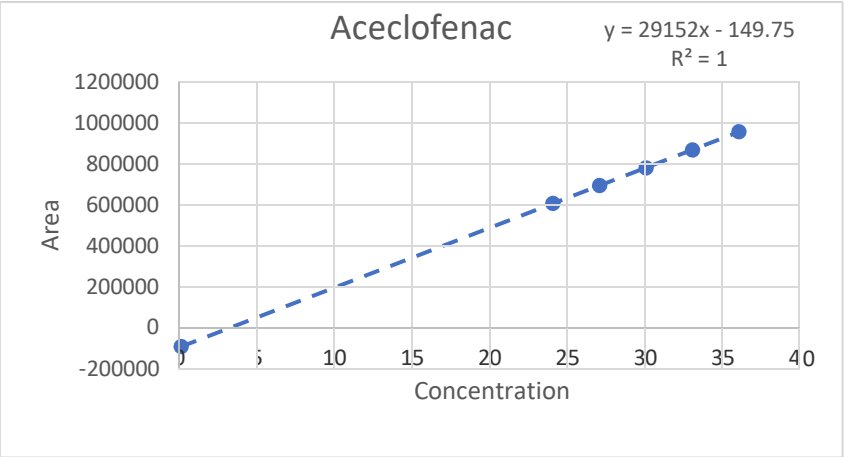
LINEARITY GRAPH OF PARACETAMOL: FIGURE 1



LINEARITY OF ACECLOFENAC: TABLE 2

Concentration (mcg/ml)	Area (n=3)
0	0
24	699595
27	786801
30	873743
33	961436
36	1050304

LINEARITY GRAPH OF ACECLOFENAC: FIGURE 2



Accuracy

Accuracy of the method expresses the closeness of the agreement between the true value, and the obtained value. Standard addition and recovery experiments were conducted to determine the accuracy. Standard addition known amount of drug substances with placebo at 80%, 100% and 120% (LQC, MQC, HQC) of concentration of each level were conducted to determine the accuracy and % recovery was calculated for Paracetamol and Aceclofenac respectively.

ACCURACY OF PARACETAMOL: TABLE 3

Concentration (mcg/ml)	% Recovery (n=3)	Amount found(µg/ml) ± SD (n=3)
27	100.29	26.73
30	100.75	30.22
33	100.29	33.09

ACCURACY OF ACECLOFENAC: TABLE 4

Concentration (mcg/ml)	% Recovery (n=3)	Amount found(µg/ml) ± SD (n=3)
27	100.50	27.13
30	100.82	30.24
33	100.72	33.23

Precision

The precision is a measure of the degree of reproducibility of an analytical method and it describes the closeness of individual measures of an analyte when the procedure is applied repeatedly to multiple samples. Precision is further subdivided into within-run, intra-batch precision or repeatability, which assesses precision during a single analytical run, and between run, inter-batch precision or reproducibility, which measures precision with time, and may involve different analysts, equipment, reagents and laboratoriesThe precision was determined by injecting six separate sample concentration and the % RSD of Paracetamol and Aceclofenac was determined.

PRECISION FOR PARACETAMOL: TABLE 5

Sample no	AREA
Sample -1	2637701
Sample -2	2640078
Sample -3	2639269
Sample -4	2642826
Sample -5	2643058
Sample -6	2643874
Average	2641134.333
SD	2467.836677
%RSD	0.093438514

PRECISION FOR ACECLOFENAC: TABLE 6

Sample no	AREA
Sample -1	875335
Sample -2	876521
Sample -3	876488
Sample -4	877760
Sample -5	878042
Sample -6	878066
Average	877035.3333
SD	1100.548893
%RSD	0.125485126

System suitability:
System suitability of the method was performed by calculating the chromatographic parameters namely, column efficiency, resolution, peak asymmetry factor and capacity factor on the repetitive injection of standard solutions using the following formula.

Capacity factor (k′)

Capacity factor (k′) is a measure of how well the sample molecule is retained by a column during an isocratic separation. The ideal value of (k') ranges from 2-10.

Capacity Factor (k′) = V1 - V0 / V0
Where, V1is the retention volume at the apex of the peak (solute),
V0 is the void volume of the system.

Resolution (Rs)

Resolution (Rs) is the difference between the retention times of two solutes divided by their average peak width. The ideal value of (Rs) is 1.5.

Resolution (Rs) = Rt1 – Rt2 / 0.5 (W1 - W2)
Where, Rt1and Rt2 are the retention times of components 1 and 2,
W1 and W2 are peak widths of components 1 and 2, respectively.

Selectivity (α)

Selectivity (α) is a measure of relative retention of two components in a mixture. The ideal value of α is 2.

Selectivity (α) = V2 – V0 / V1 –V0
Where, V0 is the void volume of the column,
V2&V1 are the retention volumes of the 2nd and 1st peaks, respectively.

Efficiency (N)

Efficiency (N) of a column is measured by the number of theoretical plates per meter. Column with N ranging from 5,000 to 1,00,000 plates/meter are ideal for a good separation.

Column efficiency (N) = 16 (Rt / w)2
Where, Rt is the retention time and w is the peak width.

Peak Asymmetry Factor

Peak Asymmetry Factor can be used as a criterion of column performance. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

Peak asymmetry factor (As) = b/a
Where, a and b are the distances on either side of the peak mid-point.

SYSTEM SUITABILITY PARAMETERS: TABLE 7

System Suitability Parameters	Paracetamol	Aceclofenac
Retention Time	4.44	6.71
Theoretical Plates	8824	13181
Tailing Factor	1.36	1.28
Resolution	0	10.75

CONCLUSION:

After the study of thorough literature survey, there is no method reported for the drug Paracetamol and Aceclofenac injection for simultaneous method.

The chromatographic conditions like detection wavelength, nature and composition of mobile phase, nature of stationary phase, flow rate etc., were optimized for the best possible separation and estimation of the analyte.

The developed HPLC method was validated for their transferability to other laboratories, in terms of specificity, accuracy, precision, linearity and range, detection and quantification limits and system suitability. The validation studies carried out revealed that the developed methods satisfy the ideal characteristics of the analytical methods.

The HPLC method in the present study for the estimation was found to be simple, rapid, accurate, precise, specific and linear. They are thus suitable for the estimation of drug in Parenteral dosage form. The newly developed analytical methods can be used in the following fields:

- Research institutions,
- Academic institutes,

- Quality control department in industries,
- Approved testing laboratories.

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