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Estimation of Tablet Dosage Form Simultaneously with Paracetamol, Caffeine, Propyphenazone Form by RP-HPLC Method

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Anjitha A Pillai*[1], Ch. Alekhya[2], Nagareddy[3]

[1]Assistant Professor, Kerala Academy Of Pharmacy, Kandala, Thiruvanthapuram. Kerala.India 695502

[2]M. Pharm Student, [3]head, Department Of Pharmaceutical Analysis, Talla Padmavati College Of Pharmacy, Orus Kareemabad, India

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Keywords: Specificity, Linearity, Range, Accuracy, Precision, Limit of deduction, Limit of quantitation, Robustness, Ruggedness, RP-HPLC, Validation, Mobile Phase.

ABSTRACT

To validate the method according to ICH guidelines for the estimation of tablet dosage form to minimize repetitious studies and ensure that the validation data are generated under conditions equivalent to the final procedure using sequence of studies like specificity, linearity, range, accuracy, precision, limit of deduction, limit of quantitation, robustness, ruggedness. Review of the literature on Paracetamol, Caffeine, Propyphenazone regarding their physical and chemical properties, various analytical methods are conducted for Paracetamol, Caffeine, Propyphenazone for the development of new analytical RP-HPLC method.

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INTRODUCTION

- Pharmaceutical Analysis plays a vital role in the Quality assurance and Quality control of bulk drugs and their formulations.

- Pharmaceutical analysis is a specialized branch of analytical chemistry that involves separating, identifying and determining the relative amounts of components in a sample of matter.

- It is concerned with the chemical characterization of matter both quantitative and qualitative.

Analytical chemistry may be defined as the science and art of determining the composition of material in terms of elements or compounds contained in it. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative method is the information about the identity of atomic or molecular species or functional groups in sample. A quantitative method provides numerical information as to the relative amount of one or more of these components.

Classification of Instrumental Methods of Analysis

Most of the instrumental techniques fit into one of the three principal areas such as

- Spectroscopy
- Electrochemistry
- Chromatography
- Spectroscopy

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed, scattered, or emitted by atoms, molecules or other chemical species.

Examples: UV Spectrophotometry, Atomic Spectrometry, Infrared Spectrometry, Raman Spectrometry, X-Ray Spectrometry, Nuclear Magnetic Resonance Spectrometry, and Electron Spin Resonance Spectrometry.

Electrochemistry In this, each basic electrical measurement of current like resistance and voltage has been measured alone or in combination for analytical purposes.

Chromatography

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase. Chromatographic methods can be classified most practically according to the stationary and mobile phases.

Modes of Chromatography

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

TYPES OF HPLC TECHNIQUES

Based on modes of chromatography:

1. Normal phase chromatography.
2. Reverse phase chromatography.

Based on the principle of separation:

1. Adsorption chromatography.
2. Ion exchange chromatography.
3. Size exclusion chromatography.
4. Affinity chromatography.
5. Chiral phase chromatography.

Base on elution technique:

1. Isocratic separation.
2. Gradient separation.
3. Based on the scale of operation:

1. Analytical HPLC.

2. Preparative HPLC

Table 1: Classification of Chromatographic methods

Stationary phase	Mobile phase	Method
Solid	Liquid	Adsorption column, thin-layer, ion exchange, High performance liquid chromatography.
Liquid	Liquid Gas	Partition, column, thin-layer, HPLC, paper chromatography, UPLC. Gas – Liquid Chromatography.

The various components of a HPLC system are herewith described.

Solvent container — Pump — damping unit — Injection port --Column

|

Recorder — Detector --- Effluent

SYSTEM COMPONENTS

- Solvent delivery system
- Solvent degassing system
- Gradient elution devices
- Sample introduction systems
- Liquid chromatographic detectors

SYSTEM SUITABILITY PARAMETERS

The parameters that are affected by the changes in chromatographic conditions are

1. Retention time (Rt)
2. Resolution (RS)
3. Capacity factor (k`)

4. Selectivity (α)
5. Number of Theoretical plates (N)
6. HETP
7. Asymmetry factor
8. Tailing factor

METHOD DEVELOPMENT

Basic criteria for new method development of drug analysis;

- The drug or drug combination may not be official in any pharmacopeia.

A proper analytical procedure for the drug may not be available in the literature due to patent regulations.

Getting Started On Method Development

- One approach is to use an isocratic mobile phase of some average organic solvent strength (50%). A better alternative is to use a very strong mobile phase first (80-100%) then reduce %B as necessary. The initial separation with 100% B results in rapid elution of the entire sample, but few groups will separate. Decreasing the solvent strength shows the rapid separation of all components with a much longer run time, with a broadening of latter bands and reduced retention sensitivity.

METHOD OPTIMIZATION

- Selection of stationary phase/column, Selection of the column is the first and the most important step in method development.
- Some of the important parameters considered while selecting chromatographic columns are
- Length and diameter of the column.
- Packing material.
- Shape of the particles.
- Size of the particles.

- The following are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

- Buffer,

- pH of the buffer

- Mobile phase composition.

SELECTION OF DETECTOR

- The detector was chosen depending upon some characteristic property of the analytic like UV absorbance, fluorescence, conductance, oxidation, reduction etc. characteristics that are to be fulfilled by a detector to be used in HPLC determination are,

- High sensitivity, facilitating trace analysis

- Negligible baseline noise. To facilitate lower detection

- Low dead volume

- Nondestructive to sample

METHOD VALIDATION

- Method validation can be defined as (ICH) “establishing documented evidence which provides a high degree of assurance that specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

- Method validation is an integral part of method development it is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity and drug products.

- Simply, method validation is the process of proving that and potency of the drug substances' analytical method is acceptable for its intended purpose.

VALIDATION PARAMETERS

- To minimize repetitious studies and ensure that the validation data are generated under conditions equivalent to the final procedure, the following sequence of studies are recommended.

- Specificity

- Linearity
- Range
- Accuracy
- Precision
- Limit of Detection
- Limit of Quantitation
- Robustness
- Ruggedness

AIM AND SCOPE OF PRESENT WORK

1. To estimate paracetamol, caffeine, Propyphenazone.

2. To validate the method according to ICH guidelines.

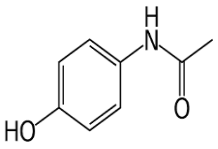
PLAN OF WORK

The experimental work has been planned as follows-

- Review of the literature for paracetamol, caffeine, Propyphenazone regarding its physical and chemical properties, various analytical methods that were conducted for paracetamol, caffeine, Propyphenazone forms the basis for development of new analytical RP-HPLC method for paracetamol, caffeine, Propyphenazone.

PARACETAMOL

Structure -



Chemical formula **:** $C_8H_9NO_2$

Molecular Weight **:** 151.162 g/mol

IUPAC **:** *N*-(4-hydroxyphenyl) ethanamide *N*-(4-

hydroxyphenyl) acetamide

Category **:** Analgesics, Non-Narcotic

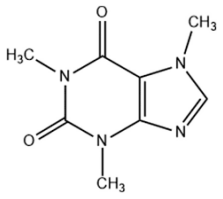
Description **:** Acetaminophen, also known as paracetamol, is commonly used for its analgesic and antipyretic effects.

Its therapeutic effects are similar to salicylates, but it lacks anti-inflammatory, antiplatelet, and gastric ulcerative effects.

- Storage : stored in tightly closed containers protected from light, below 25°C.
- Solubility : Water
- Pharmacokinetics : Bioavailability: 100%
- Protein binding : 25%
- Distribution : distributed throughout the body fluids in a homogeneous way.
- Half-life : 1 to 4 hours.
- Excretion : Renal
- Mode of action : Acetaminophen is thought to act primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1, COX-2, and COX-3 enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, acetaminophen does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory effects.

CAFFEINE

Structure



Chemical formula : $C_8H_{10}N_4O_2$

Molecular Weight : 194.19

IUPAC : 1,3,7-trimethyl-2,3,6,7-tetrahydro-1H-purine-2,6-dione

Category : Central Nervous System Stimulants

Description : A methyl xanthine naturally occurring in some beverages and also used as a pharmacological agent. Caffeine's most notable the pharmacological effect is as a central nervous system stimulant, increasing alertness and producing agitation. It also relaxes smooth muscle, stimulates cardiac muscle, stimulates diuresis, and appears to be useful in the treatment of some types of headache.

Storage : *Store caffeine* at room temperature, between 59 and 86 degrees F (1and 30 degrees C)

Solubility : Water

Pharmacokinetics : Bioavailability: complete absorption, following first- pass metabolism systemic availability 75% (range 52 to 75%).

Protein binding : 25-30%

Distribution : 0.8 to 0.9 L/kg

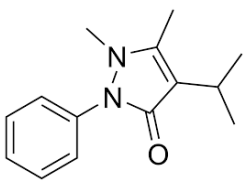
Half-life : 3 to 7 hours in adults, 65 to 130 hours in neonates

Excretion : Renal/Rectal

- Mode of action : Caffeine stimulates medullary, vagal, vasomotor, and respiratory centers, promoting bradycardia, vasoconstriction, and increased respiratory rate.

PROPYPHENAZONE

- Structure



Chemical formula : 1,5-Dimethyl-2-phenyl-4-propan-2-yl-pyrazol-3-one

Molecular Weight : 230.306 g/mol

IUPAC : 1,5-Dimethyl-2-phenyl-4-propan-2-yl-pyrazol-3-one

Category : Anatomical Therapeutic

Description : Propyphenazone is a derivative of phenazone with similar analgesic and antipyretic effects Adverse effects of Propyphenazone, Nitrosamines and as therefore been widely used as a replacement drug for aminophenazone.

Storage : Store between 59°F and 86°F (15°C to 30°C).

Solubility : Water

Pharmacokinetics : Bioavailability: 93 ± 9%

Protein binding : 70%

Distribution : 1.21 ± 0.09 L/kg

Half-life : 1.07 ± 0.16 h and 1.28 ± 0.16 h

Excretion : Renal

Mode of action : Anti-inflammatory agents that are not steroids. In addition to anti-inflammatory actions, they have analgesic, antipyretic, and latelet-inhibitory actions. They are used primarily in the treatment of chronic arthritic conditions and certain soft tissue disorders associated with pain and inflammation.

MATERIALS AND METHODS

Instruments:

- HPLC – WATERS Model NO.2690/5 series Compact System Consisting of

  Inertsil ODS C18 column.

- Electronic balance (SARTORIOUS)

- Digital pH meter(POLOMAN)
- Sonicator (FAST CLEAN)

Chemicals:

- Methanol HPLC Grade
- Purified water HPLC Grade
- O-Phosphoric Acid HPLC Grade
- Acetonitrile HPLC Grade
- Purified KH_2PO_4

Raw Material:

Paracetamol, Caffeine and Propyphenazone Working Standards

METHOD DEVELOPMENT BY HPLC

The objective of this experiment was to optimize the assay method for the estimation of Paracetamol, Caffeine and Propyphenazone simultaneously based on the literature survey made. So here the trails mentioned describe how the optimization was done.

Trail: 1

Mobile Phase: Degassed Methanol and Water in the ratio of 50:50 V/V.

Preparation of Standard Solution:

10mg of Paracetamol, Caffeine and Propyphenazone drugs were weighed separately and dissolved in 10ml of Mobile phase and taken in three 10ml volumetric flasks individually and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from each solution and diluted to 10 ml with mobile phase to get the solution having a combination of 3 drugs.

Chromatographic Conditions:

Flow rate : 1.0 ml/min

Column : Inertsil ODS C18 column

Detector wavelength : 271.2nm

Column temp : Ambient

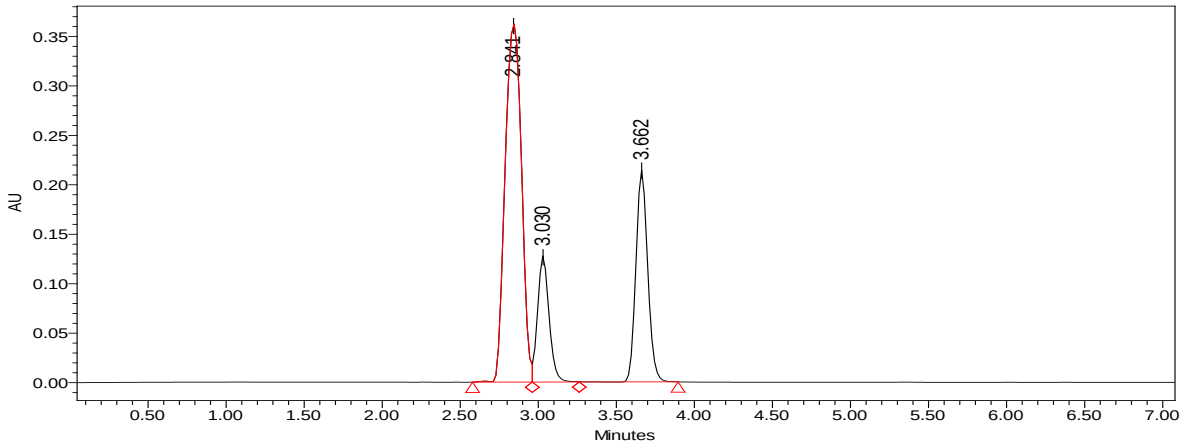
Injection volume : 20µl

Run time : 7min

Retention time for Propyphenazone : 2.8min for Paracetamol, 3.0min for, Caffeine and 3.6min

Observation **:** Propyphenazone got separated, but, Paracetamol and, Caffeine is not separated.

Chromatograph Trail 1



Inference: Propyphenazone got separated but Paracetamol and Caffeine are not separated

S.NO	Name of the peak	Retention time(min)
1	Paracetamol	2.8min
2	Caffeine	3.0min
3	Propyphenazone	3.6min

Trail: 2

Buffer Preparation:6.8g of KH2PO4 in 1000ml of water and sonicate for 20min, then adjust the Ph-5.5 with OPA. Then filter through 0.45µ filter paper.

Mobile Phase: Degassed Methanol, Acetonitrile and Buffer in the ratio of 30:50:20 V/V.

Preparation of Standard Solution:

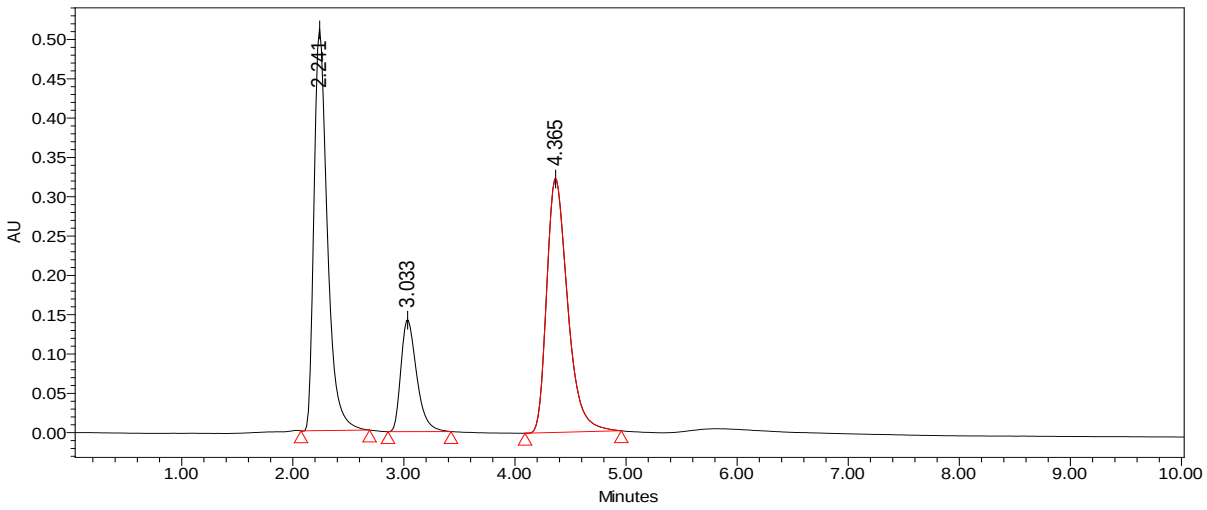
10mg of Paracetamol, Caffeine and Propyphenazone were weighed separately and dissolved in 10ml of Mobile phase and taken in three 10ml volumetric flasks individually and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from each solution and diluted to 10 ml with mobile phase to get the solution having combination of 3 drugs.

Chromatographic Conditions:

Flow rate	: 1.0 ml/min
Column	: Inertsil ODS C18 column
Detector wavelength	: 271.2nm
Column temp	: Ambient
Injection volume	: 20µl
Run time	: 10min
Retention time	: 2.2min for Paracetamol, 3.0min for, Caffeine and 4.3min Propyphenazone

Observation: Three peaks got separated, but the peaks shapes are not good tailing occurred.

Chromatograph Trail 2



S.NO	Name of the peak	Retention time(min)
1	Paracetamol	2.2min
2	Caffeine	3.0min
3	Propyphenazone	4.3min

Inference: Three peaks got separated but tailing occurred

Trail: 3

Buffer Preparation: 6.8g of KH2PO4 in 1000ml of water and sonicate for 20min, then adjust the Ph-5.5 with OPA. Then filter through 0.45µ filter paper.

Mobile Phase : Degassed Methanol, Acetonitrile and Buffer in the ratio of 35:40:25 V/V.

Preparation of Standard Solution:

10mg of Paracetamol, Caffeine and Propyphenazone RS drugs were weighed separately and dissolved in 10ml of Mobile phase and taken in three 10ml volumetric flasks individually and sonicated for 20 minutes to get 1000ppm and 1 ml was taken from each solution and diluted to 10 ml with mobile phase to get the solution having combination of 3 drugs.

Chromatographic Conditions:

Flow rate : 1.0 ml/min

Column : Inertsil ODS C18 column

Detector wavelength : 271.2nm

Column temp : Ambient

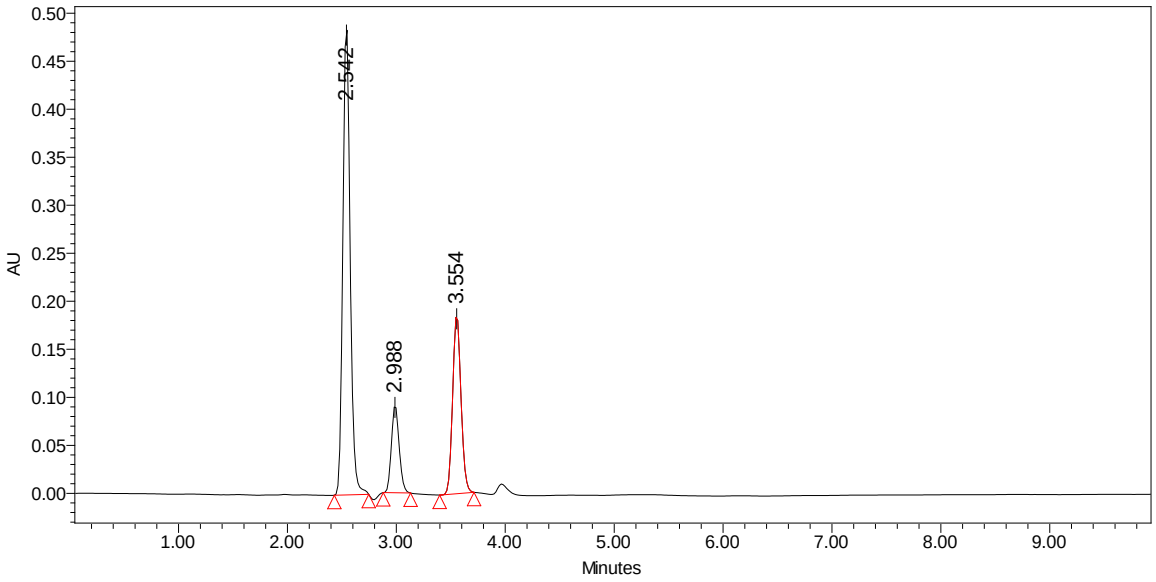
Injection volume : 20µl

Run time : 10min

Retention time : 2.5min for Paracetamol, 2.9min for ,Caffeine and 3.5min for Propyphenazone

Observation: Three peaks got separated completely and the peaks shapes are also good, but resolution is very less.

Chromatograph Trail 3

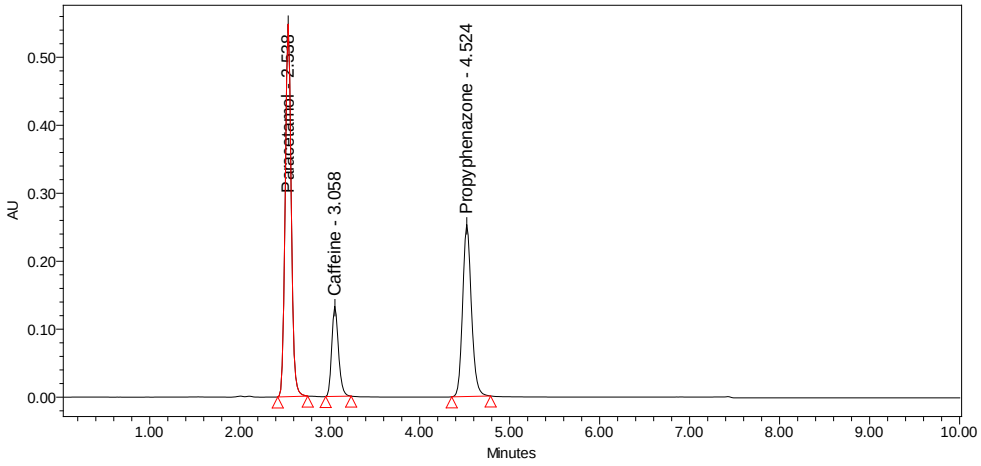


Inference: Three peaks got separated but the resolution is less.

S.NO	Name of the peak	Retention time(min)
1.	Paracetamol	2.5min
2.	Caffeine	2.9min
3.	Propyphenazone	3.5min

OPTIMIZED METHOD

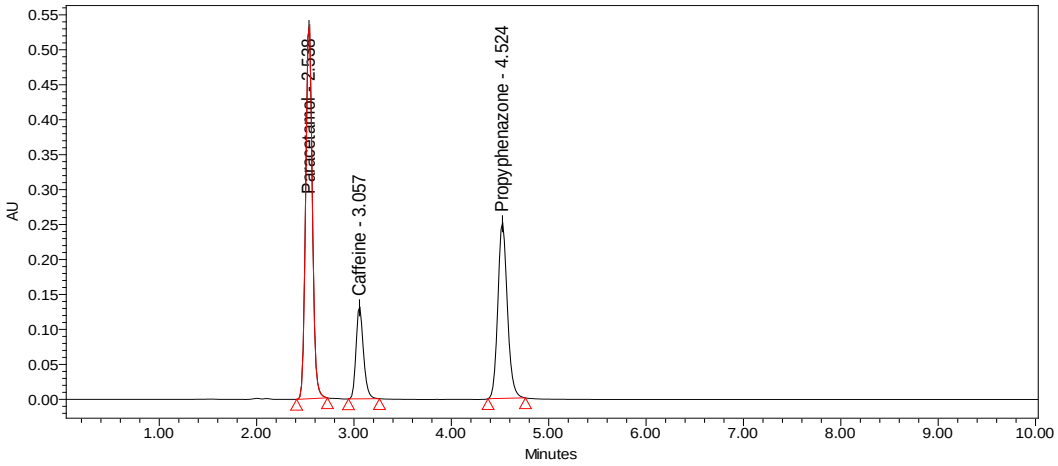
Chromatogram of standard



Inference: Got chromatogram at an RT 2.5minofParacetamol, 3.0minofCaffine and 4.5min of Propyphenazone standard.

S.NO	Name of the peak	Retention time(min)
1	Paracetamol	2.5
2	Caffeine	3.0
3	Propyphenazone	4.5

Chromatogram of sample



Inference: Got the same peak with the same RT as of standard

Sl.NO	Name of the peak	Retention time(min)
1	Paracetamol	2.5
2	Caffeine	3.0
3	Propyphenazone	4.5

VALIDATION DATA

TABLE-1(a): Data of System Suitability of Paracetamol

Injection	RT	Peak Area of Paracetamol	USP Plate count	USP Tailing
1	2.537	2552906	6814.171978	1.010658
2	2.534	2550619	6846.773024	1.063956
3	2.538	2552993	6895.750602	1.070691
4	2.536	2551774	6829.234000	1.062423
5	2.539	2553306	6916.657744	1.068216
Mean	2.536	2552320	6860.517	1.055189
SD	0.001924	1113.557	-------	-------
% RSD	0.07	0.04	-------	-------

TABLE-1(b): Data of System Suitability of Caffeine

Injection	RT	Peak Area of Caffeine	USP Plate count	USP Tailing
1	3.055	704773	7898.033976	1.215745
2	3.052	704479	7569.573768	1.187715
3	3.051	704847	7961.876819	1.216425
4	3.051	706145	8041.623506	1.187447
5	3.051	703985	7938.239165	1.215653
Mean	3.052	704845.8	7881.869	1.204597
SD	0.001732	801.3646	-------	-------
% RSD	0.05	0.11	-------	-------

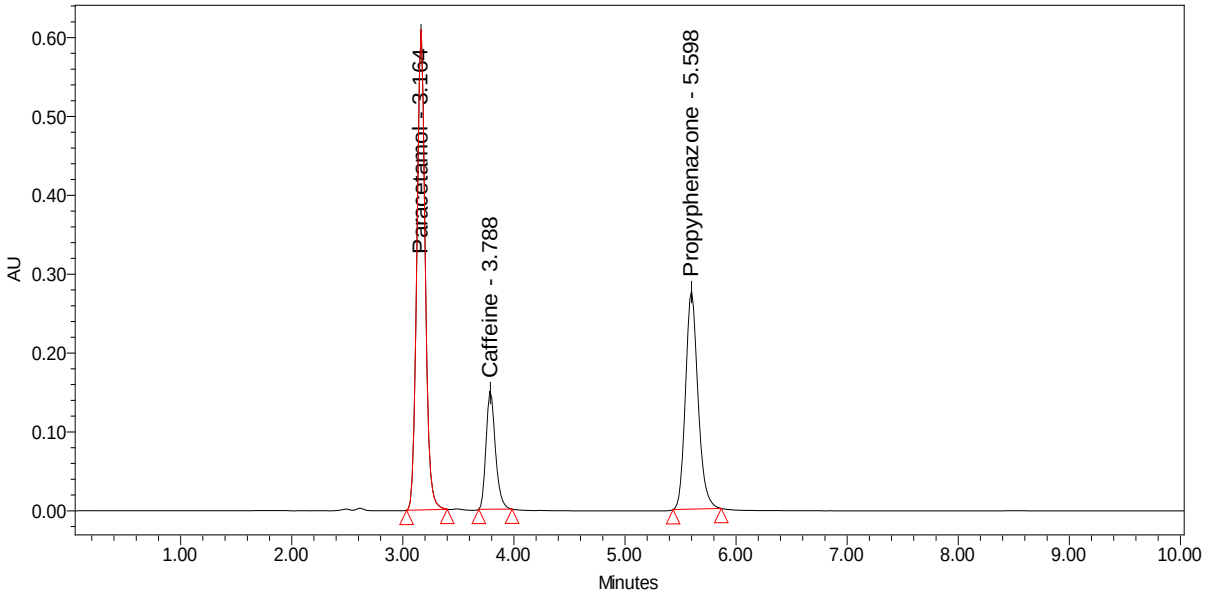
TABLE-1(c): Data of System Suitability

Injection	RT	Peak Area of Propyphenazone	USP Plate count	USP Tailing
1	4.521	1731468	10112.136120	1.155749
2	4.518	1734744	10566.889468	1.149571
3	4.515	1732276	10522.573122	1.156777
4	4.515	1730618	10159.488612	1.148675
5	4.515	1732034	10440.679639	1.159105
Mean	4.5168	1732228	10360.35	1.153975
SD	0.002683	1544.336	-------	-------
% RSD	0.05	0.08	-------	-------

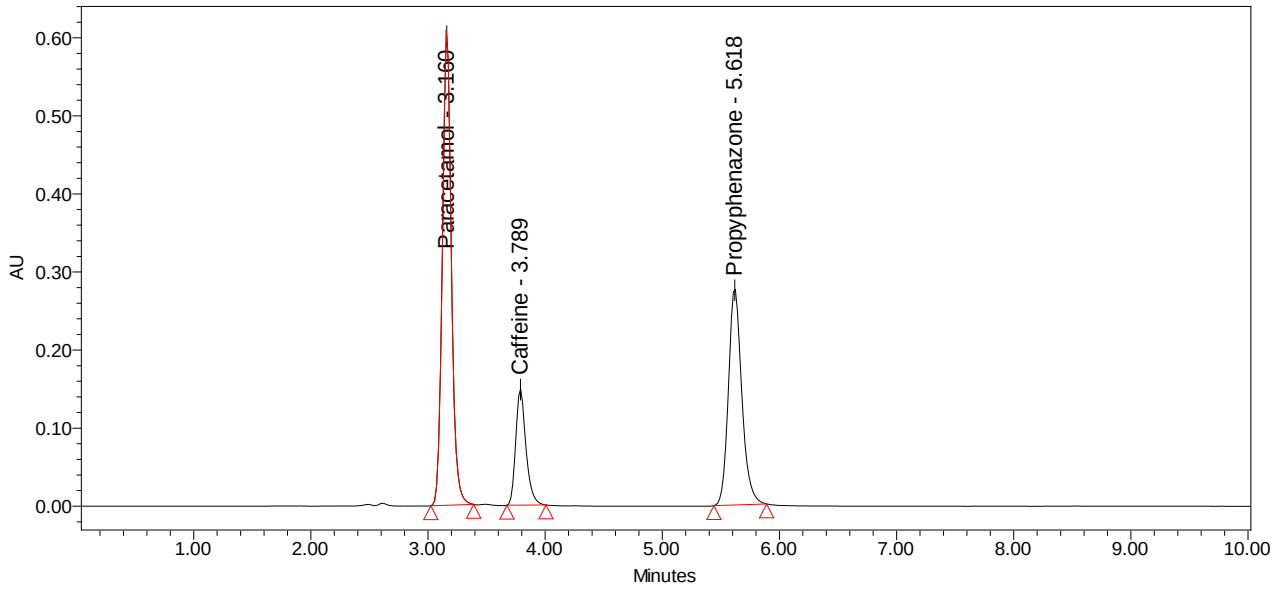
TABLE-1(d): Data of Repeatability (Method precision)

	Injection	Peak Areas of Propyphenazone	%Assay
Concentration 40ppm	1	1732269	99.96
	2	1730734	99.87
	3	1732471	99.97
	4	1733446	100.0
	5	1731468	99.91
	6	1734744	100.1
Statistical Analysis	Mean	1732522	99.96
	SD	1425.87	0.079352
	% RSD	0.08	0.07

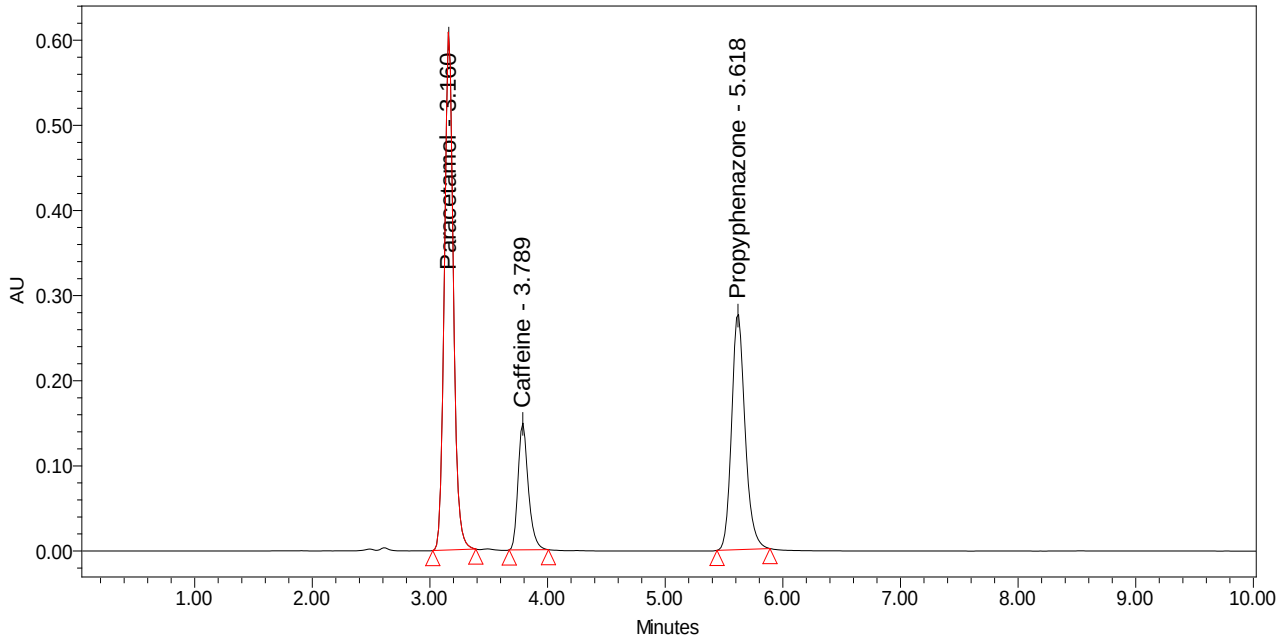
Effect of variation of flow rate (for 0.8ml / min flow)



Chromatogram for robustness standard - 1



Chromatogram for robustness standard - 2



LIMIT OF DETECTION AND LIMIT OF QUANTITATION (LOD and LOQ)

Paracetamol

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= 3.3 \times 305.5$$

$$= 0.015$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= 10 \times 305.5$$

$$= 0.04763$$

CAFFEINE

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= 3.3 \times 98.03$$

$$= 0.018$$

$$LOQ = \frac{10\ \sigma}{S}$$

$$= 10 \times 98.03$$

$$= 0.055$$

PROPYPHENAZONE

$$LOD = \frac{3.3\ \sigma}{S}$$

$$= 3.3 \times 357.7$$

$$= 0.027$$

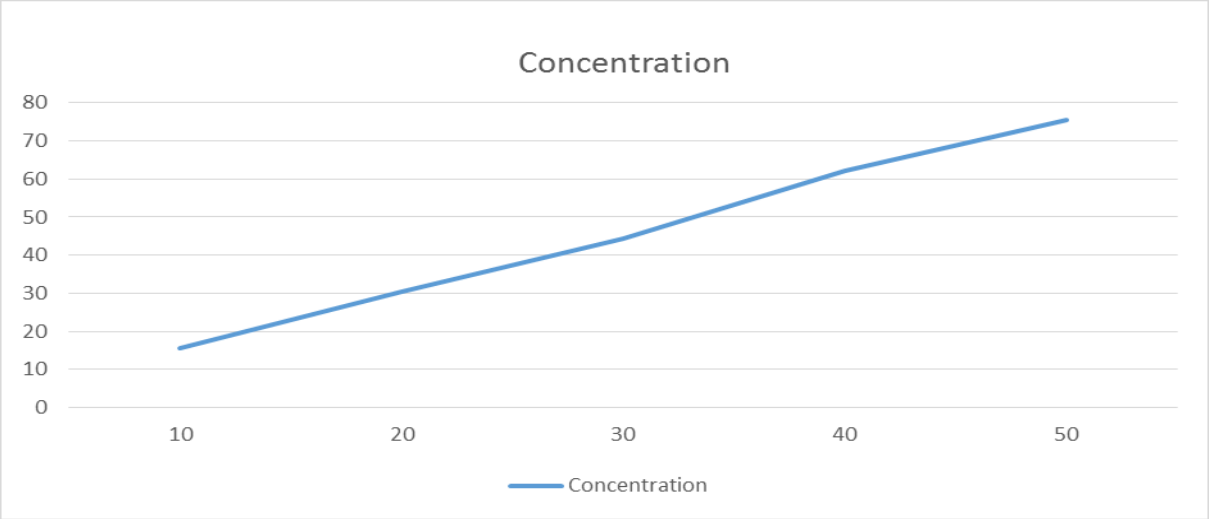
$$LOQ = \frac{10\ \sigma}{S}$$

$$= 10 \times 357.7$$

$$= 0.082$$

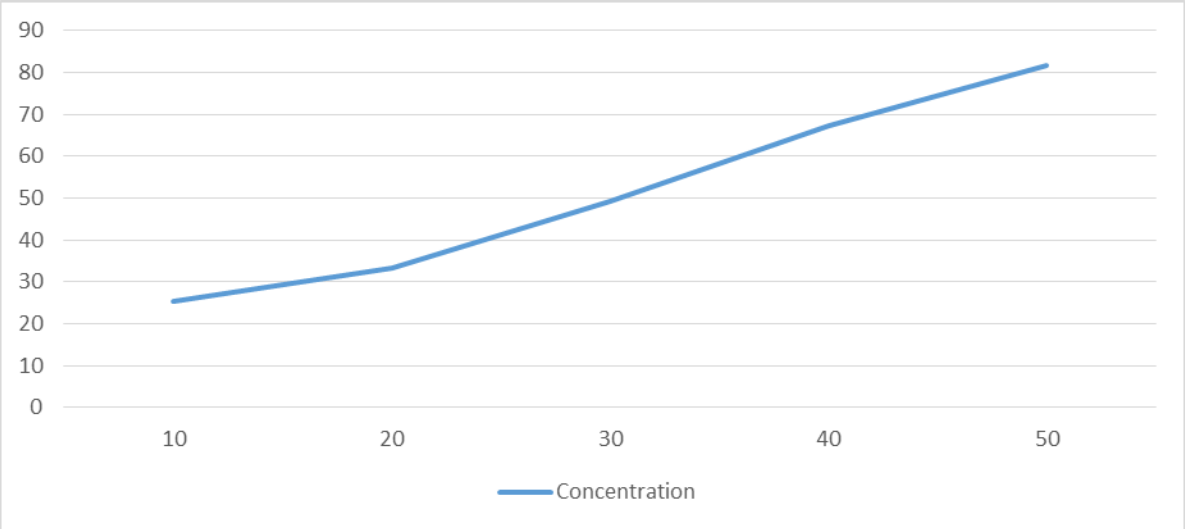
Linearity of Paracetamol

Sr.No	Concentration (µg/ml)	PK.area(p)
1	10	15.5321
2	20	30.256
3	30	44.325
4	40	62.114
5	50	75.479



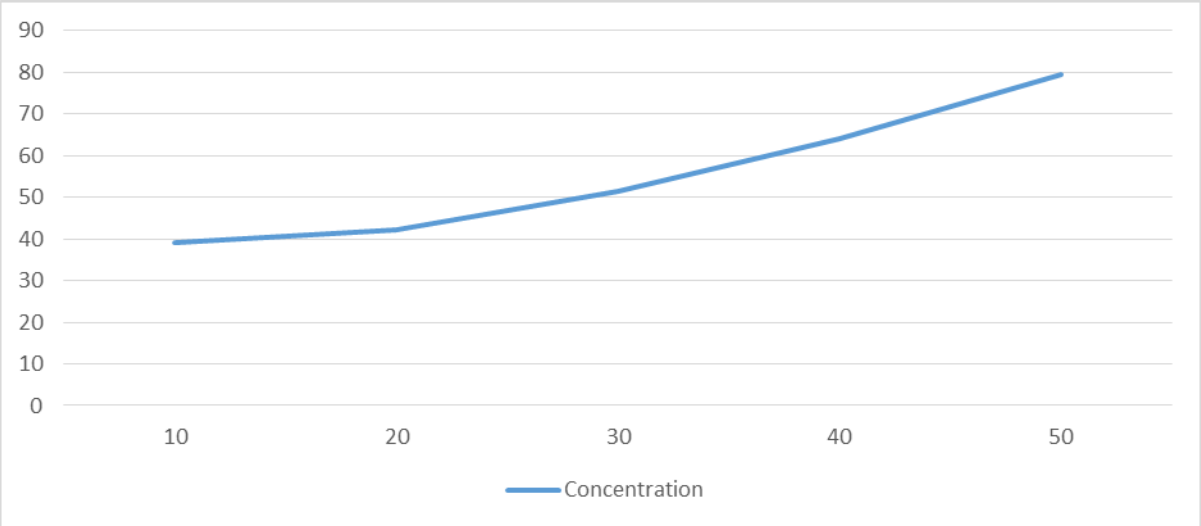
Linearity of Caffeine

S.No	Concentration (µg/ml)	PK.area(p)
1	10	25.521
2	20	33.26
3	30	49.335
4	40	67.426
5	50	81.558



Linearity of Propyphenazone

S.No	Concentration (µg/ml)	PK.area(p)
1	10	39.174
2	20	42.356
3	30	51.389
4	40	64.235
5	50	79.479



RESULTS AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 248.6nm for Paracetamol,271.2nm for Caffeine and 272.4nm for Propyphenazone Common wavelength is 271.2nm. and the peak purity was excellent. Injection volume was selected to be 20µl which gave a good peak area. The column used for study was Inertsil ODS C18, 150*4.6mm, 5µ chosen good peak shape. An ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 0.8ml/min because of good peak area and satisfactory retention time. Different pH and ratios of the mobile phase were studied, mobile phase with ratio of 60:40 Methanol: Phosphate Buffer was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Acetonitrile was selected because of maximum extraction sonication time was fixed to be 10min at which all the drug particles were completely soluble and showed

good recovery. The run time was selected to be 10min because analyze gave peaks around 2.5min for Paracetamol, 3.0min for Caffeine and 4.5min for Propyphenazone and also to reduce the total run time.

The present recovery was found to be 98.0-101.50 and was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. Detection limit was found to be 0.047 for Paracetamol, 0.055 for Caffeine and 0.082 for Propyphenazone. Linearity study was, correlation coefficient and curve fitting was found to be. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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