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A RESEARCH ARTICLE ON ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF HYDROCODONE AND PSEUDOEPHEDRINE USING UPLC

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ABSTRACT

A simple and selective method is described for the determination of hydrocodone and pseudoephedrine in liquid dosage forms. Chromatographic separation was achieved on a Water using mobile phase consisting of a mixture of 60 volumes of Methanol, 40 volumes of water with detection of 243 nm. Linearity was observed in the range 20 - 100 µg/ml for hydrocodone (r^2 =0.999) and 60-140 µg /ml for pseudoephedrine (r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The column used was waters acquity C18125A (100*2.2mm1D) 1.8um using mobile phase as phosphate buffer (PH=6.8) and acetonitrile (ratio=60:40) The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. Lod was found to be 0.32133µg/ml (hydrocodone) and 0.82718µg/ml (pseudoephedrine) Loq was found to be 5.84µg/ml (hydrocodone) and 2.506µg/ml (pseudoephedrine). Assay limit range 99-100.06%. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEYWORDS: hydrocodone, pseudoephedrine, waters acquity, chromatography, assay.

1.1. INTRODUCTION

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY

Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components in to individual components through a porous medium under the influence of solvent.

UPLC refers to Ultra Performance Liquid Chromatography.

UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated.

It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles(less than 2.5µm) & mobile phases at high linear velocities decreases the length of column, reduces solvent consumption & saves time.

According to the van Deemter equation, as the particle size decreases to less than2.5 µm, there is a significant gain in efficiency, while the efficiency does not diminish at increased flow rates linearvelocities Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits, termed Ultra Performance Liquid, Chromatography, or UPLC The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller particles(less than2.5µm) and/or higher flow rates for increased speed, this gives superior resolution and sensitivity.

PRINCIPLE

The UPLC is based on the principal of use of stationary phase consisting of particles less than 2.5 µm (while HPLC columns are typically filled with particles of 3 to 5 µm).

The underlying principles of this evolution are governed by the Van Deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).[8,11]

$H=A+B/v+Cv$

Where;

A, B and C are constants

v is the linear velocity, the carrier gas flow rate.

*The *A* term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform

The *B* term represents axial diffusion or the natural diffusion tendency of molecules.

This effect is diminished at high flow rates and so this term is divided by v.

* The *C* term is due to kinetic resistance to equilibrium in the separation process

Instrumentation

The Ultra Performance Liquid Chromatography have the ability to work more efficiently with higher speed, sensitivity and resolution at a much wider range of linear velocities, flow rates and backpressures to obtain superior results.

The Acquity UPLC system consists of

- Binary solvent manager
- Sample manager including the column heater
- Optional Sample manager
- Pumps
- Detector

Detectors

Types of Detectors

- Optical detectors
- Tunable ultra violet detectors
- Evaporative light scattering detectors
- Fluorescence detector

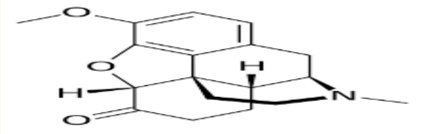
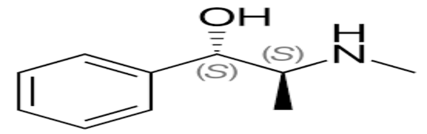
AIM

To develop new UPLC method for the simultaneous estimation of Hydrocodone and Pseudoephedrine pharmaceutical dosage form.

OBJECTIVE

- To develop a rapid and specific stability indicating method for simultaneous estimation of HYDROCODONE AND PSEUDOEPHIDRINE.
- Perform the validation for the developed method.
- Perform assay of the above validated method.

1.9. DRUG PROFILE

	HYDROCODONE	**PSEUDOEPHIDRINE**
CATEGORY	NARCOTIC ANALGESICS, Adrenergic Agonists	nasal decongestants
CHEMICAL STRUCTURE		
CHEMICAL FORMULA	$C_{18}H_{21}NO_3$	$C_{10}H_{15}NO$
MOLECULAR WEIGHT IUPAC NAME HALF LIFE	299.368 g/mol 9-methoxy-3-methyl-1,2,4,4*a*,5,6,7*a*,13-octahydro-4,12-methanobenzofuro[3,2-e]isoquinolin-7-one The half-life of elimination reported for hydrocodone is 7-9 h.	165.2322 g/mol (1S,2S)-2-(methylamino)-1-phenylpropan-1-ol 9-16 hours

METHOD DEVELOPMENT AND VALIDATION

5.3. Solubility Studies

These studies are carried out at 25^{0}C.

***Table 5*: Solubility studies.**

Solvent Name	**HYDROCODONE**	**PSEUDOEPHIDRINE**
Water	Sparingly Soluble	Soluble
Methanol	Soluble	Soluble
Ethanol	Soluble	Sparingly Soluble

Determination of Working Wavelength (λ_{max})

Preparation of standard stock solution of HYDROCODONE

10 mg of HYDROCODONE was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of PSEUDOEPHIDRINE

10 mg of PSEUDOEPHIDRINE was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

RESULTS

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.

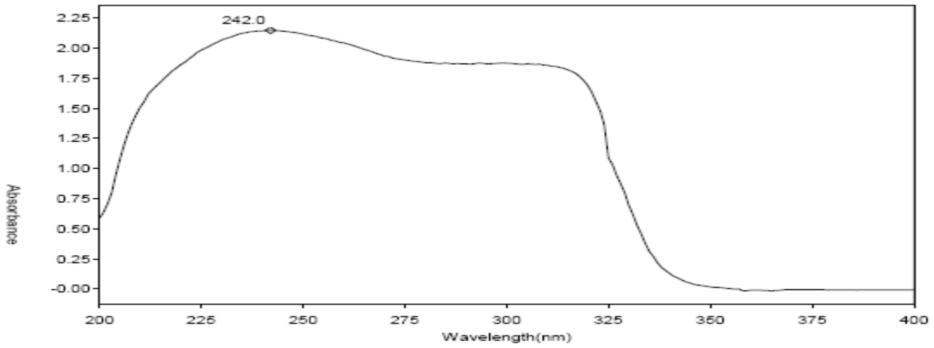


***Fig.* 5. UV-VIS Spectrum of HYDROCODONE (241 nm).**

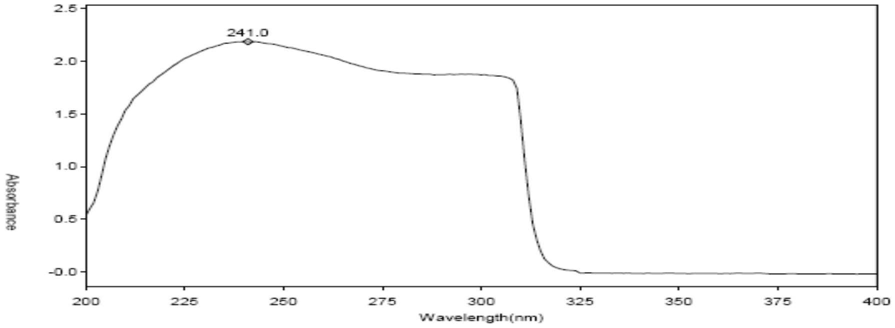


***Fig. 6*: UV-VIS Spectrum of PSEUDOEPHIDRINE (242 nm).**

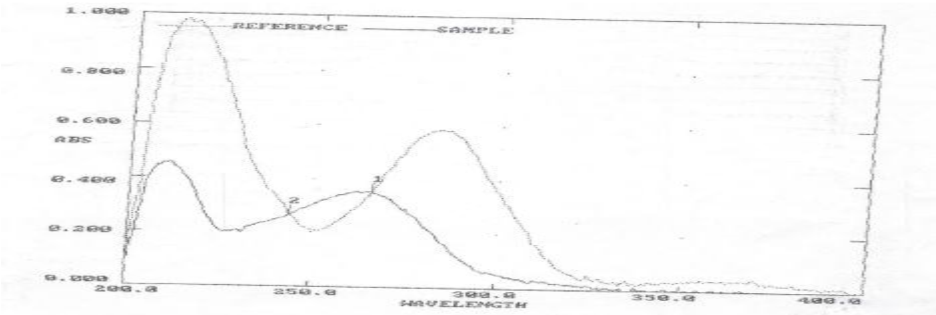


***Fig 7*: UV-VIS overlay Spectrum of HYDROCODONE and PSEUDOEPHIDRINE (243 nm)**

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

***Table 8*: Optimized condition.**

Mobile phase	Methanol: Water: TEA Buffer (60: 40: 0.5%) %v/v/v
Column	PhenomenexC18(250x2.2mm ID) 4.5µm
Flow rate	1.0 mL/min
Column temperature	$30^{o}C$
Sample temperature	$15^{o}C$
Wavelength	243nm
Injection volume	20µL
Run time	8 min

METHOD VALIDATION PARAMETERS

SPECIFICITY

- Blank solution was injected and the chromatogram was recorded for the same as given in Fig. 19
- Placebo solution was prepared and it was injected and the chromatogram was recorded for the same

RESULT

It was observed that diluent or placebo peaks was not interfering with the HDC and PDP peaks.

SYSTEM SUITABILITY

- To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 10µg/mL of HYDROCODONE and 10µg/mL of PSEUDOEPHIDRINE were injected six times and the chromatograms were recorded for the same.

***Table 4*: Results for system suitability of HYDROCODONE.**

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.768	1022197	3851	1.16
2	2.761	1025670	3830	1.12
3	2.767	1041099	3852	1.15
4	2.769	1026496	3868	1.16
5	2.768	1006266	3854	1.14
6	2.769	1033915	3851	1.11
Mean	2.767	1025941	-	-
SD	0.003	11789	-	-
%RSD	0.1	1.1	- -	-

***Table* 5: Results for system suitability of PSEUDOEPHIDRINE.**

Injection	Retention time	Peak area	Theoretical plates	Tailing factor	Resolution
1	3.683	2032157	8972	1.16	12.3
2	3.689	2017044	8964	1.17	12.1
3	3.688	2015194	8952	1.16	12.2
4	3.687	2012644	8948	1.17	12.1
5	3.689	2008604	8937	1.16	12.5
6	3.683	2014157	8955	1.17	12.2
Mean	3.687	2016633	-	-	-
SD	0.003	8121	-	-	-
%RSD	0.1	0.4	-	-	-

RESULT

- the plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

LINEARITY AND RANGE

Preparation of standard stock solution

- Standard stock solutions of HYDROCODONE (10µg/mL) and PSEUDOEPHIDRINE (10µg/mL) were prepared by dissolving 10 mg of HYDROCODONE and 10 mg of PSEUDOEPHIDRINE in 100 mL of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min further dilutions.
- The above prepared dilutions were in injected into the system and the chromatograms were recorded as given in Fig.13,14,15,16 and the results of the chromatograms are given in Table 7 respectively.

***Table 7*: Linearity Preparations.**

Preparations	Conc. obtained (µg/mL)	
	HYDROCODONE	PSEUDOEPHIDRINE
Preparation 1	2	30
Preparation2	4	40
Preparation 3	6	60
Preparation 4	8	70
Preparation 5	10	90

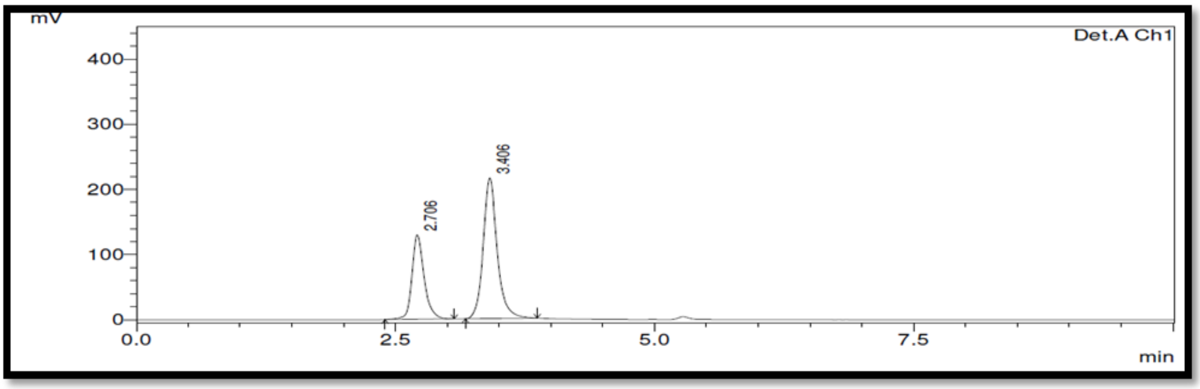


Fig 13**: Chromatogram of linearity for preparation 1.**

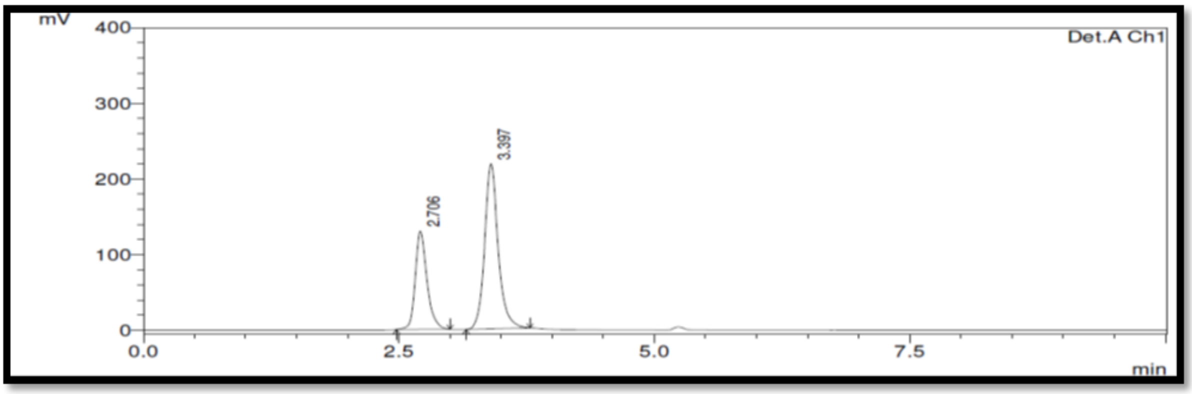


Fig 14: **Chromatogram of linearity for preparation 2.**

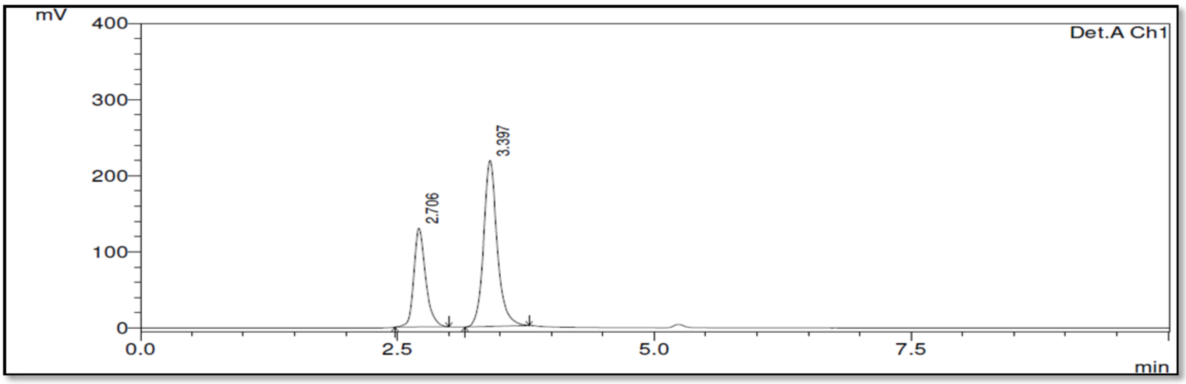


Fig **15: Chromatogram of linearity for preparation 3.**

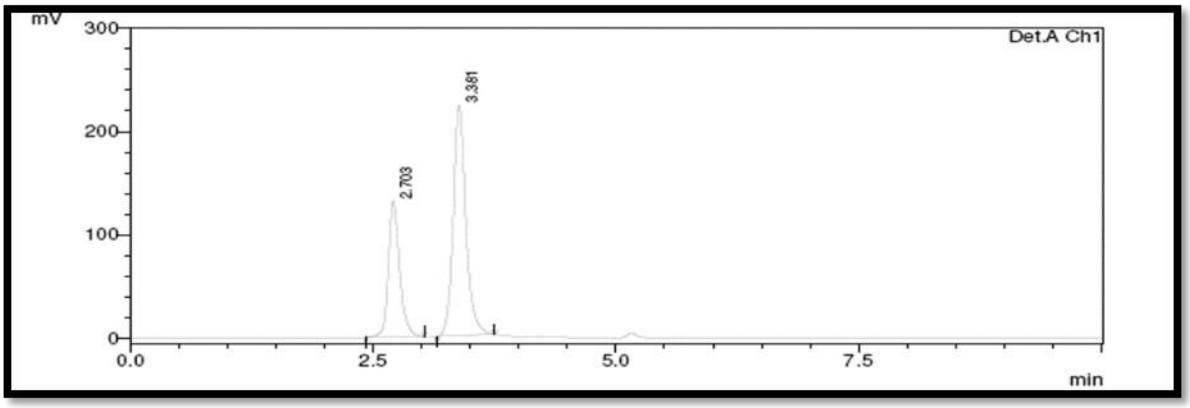


***Fig 16*: Chromatogram of linearity for preparation 4.**

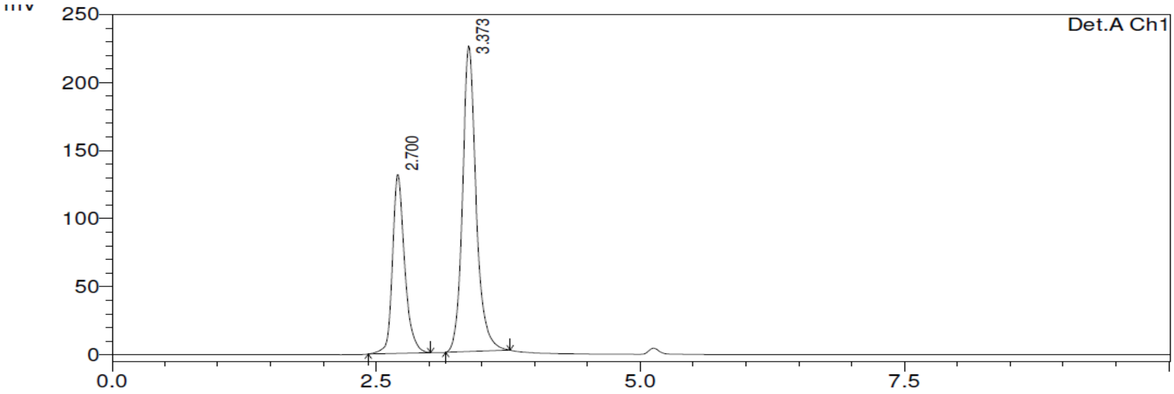


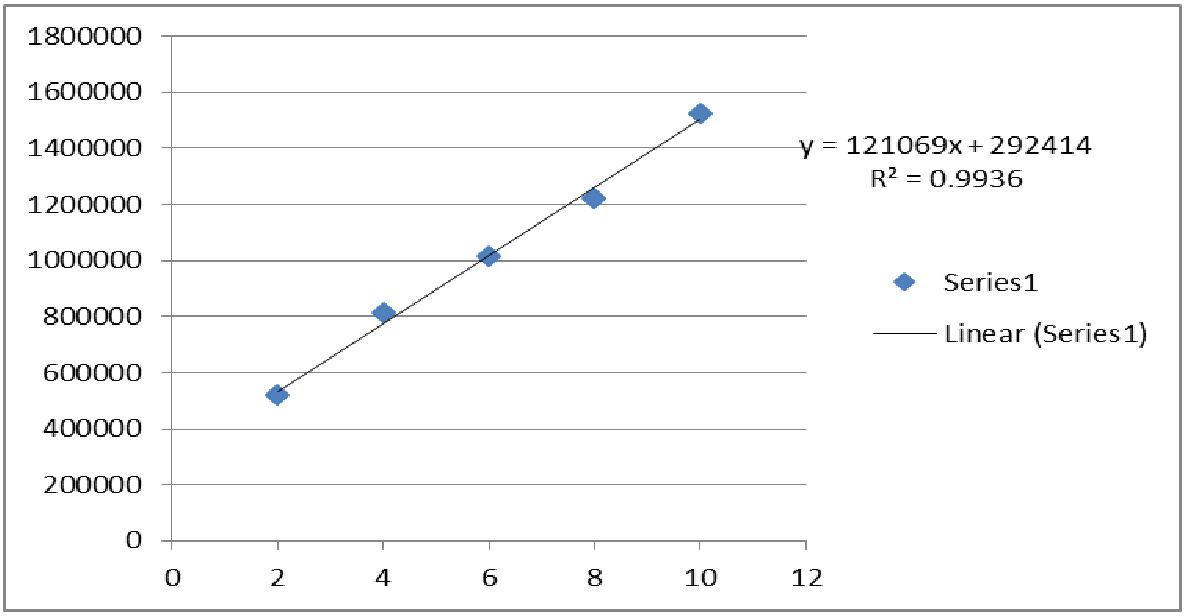
***Fig 17*: Chromatogram of linearity for preparation 5.**

- A graph was plotted for HYDROCODONE and PSEUDOEPHIDRINE against the concentrations of the solutions and the peak areas.
- The correlation coefficient R^2 was determined and was found to be 0.999 for HYDROCODONE and 0.999 for PSEUDOEPHIDRINE.

LINEARITY DATA OF HYDROCODONE TABLE 8.

S.No	Concentration (µg/mL)	Area
1	2	518053
2	4	813525
3	6	1016907
4	8	1220288
5	10	1525360

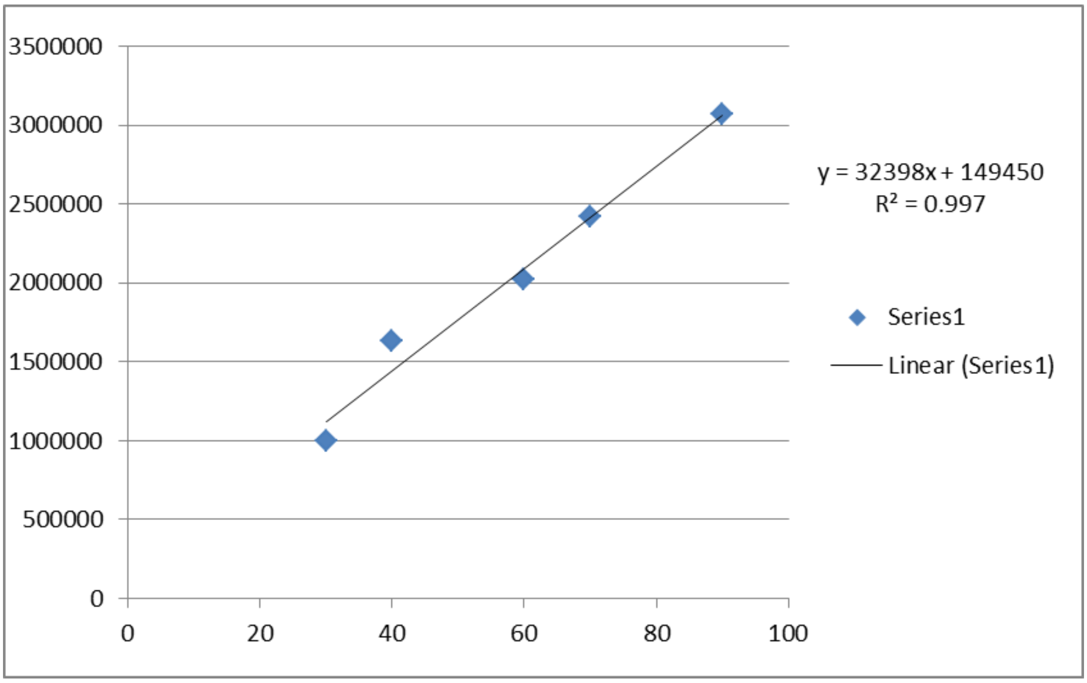
Linearity curve of hydrocodone



LINEARITY DATA OF PSEUDOEPHIDRINE

S.No	Concentration (µg/mL)	Area
1	30	999261
2	40	1634827
3	60	2018534
4	70	2422240
5	90	3067801

Linearity curve of pseudoephedrine



METHOD PRECISION

Table 6: METHOD PRECISION RESULTS FOR HDC AND PDP.

Injection	Hydrocodone		Pseudoephidrine	
	Area	Retention time	Area	Retention time
1	1026668	1.140	2029924	1.137
2	1032616	1.142	2018744	1.141
3	1025772	1.137	2030331	1.139
4	1017071	1.140	1995114	1.142
5	1016907	1.141	2018534	1.137
6	1011321	1.142	2013040	1.143
Average	1021726	1.19	2017615	1.14
SD	7906.218	0.0031	12981.16	0.0028
%RSD	0.77381	0.29	0.643392	0.24

RESULT: The %RSD of 6 determinations of HDC and PDP for System precision found to be within the acceptance criteria of less than 2.0%. Hence the method is precise.

ACCURACY

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table 11,12.

***Table 12*: Results for Recovery of PDP.**

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	AREA
50%	2	1.98	99.3	1022197
100%	6	5.96	99.2	1041099
150%	10	10.1	99.1	1006266

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)*	Percent Recovery *	AREA
50%	30	29.91	99.7	2032157
100%	60	59.99	100.0	2015194
150%	90	91.25	101.4	2008604

Acceptance criteria

The % recovery of HDC and PDP should lie between 98% and 102%.

RESULT

The % mean recovery of HDC and PDP was founded between 99.0 to 100.4

LIMIT OF DETECTION (LOD)

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

HYDROCODONE= (3.3)*(11789)/121069
= 0.32133µg/ml

PSEUDOEPHIDRINE= (3.3)* (8121)/32398
=0.82718µg/ml
Where, σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOD for this method was found to be 0.32133µg/ml for Hydrocodone and 0.82718 µg/ml for Pseudoephidrine.

LIMIT OF QUANTIFICATION(LOQ)

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

HYDROCODONE = (10) * (11789)/12106
= 5.84µg/ml

PSEUDOEPHIDRINE = (10)* (8121)/32398
= 2.506µg/ml
Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

OBSERVATION

The LOQ for this method was found to be 5.84µg/ml for Hydrocodone and 2.506 µg/ml for Pseudoephidrine.

ROBUSTNESS

- The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in the Table.

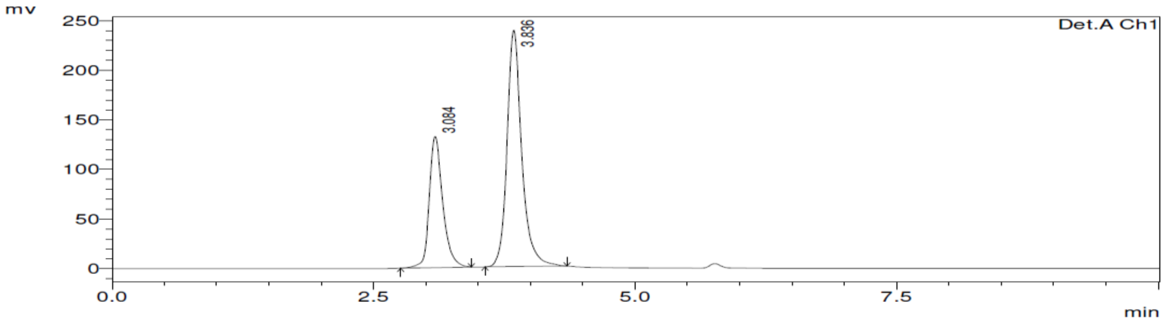


Fig. 24: **Chromatogram of flow rate from 1.0mL/min to 0.8mL/min.**

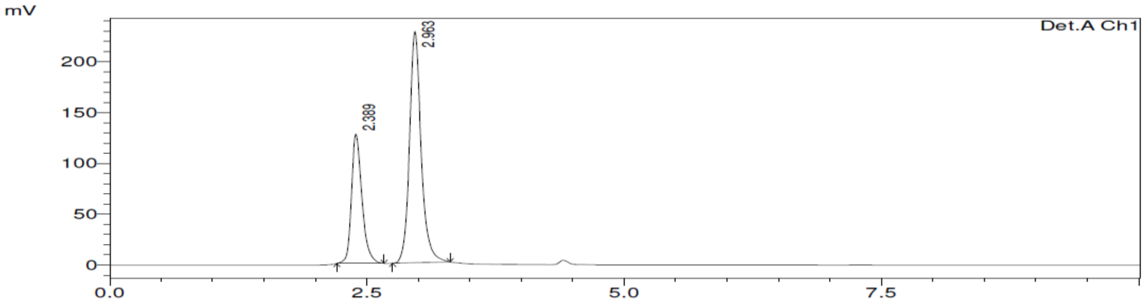


***Fig. 25*: Chromatogram of flow rate from 1.0 mL/min to 1.2 mL/min.**

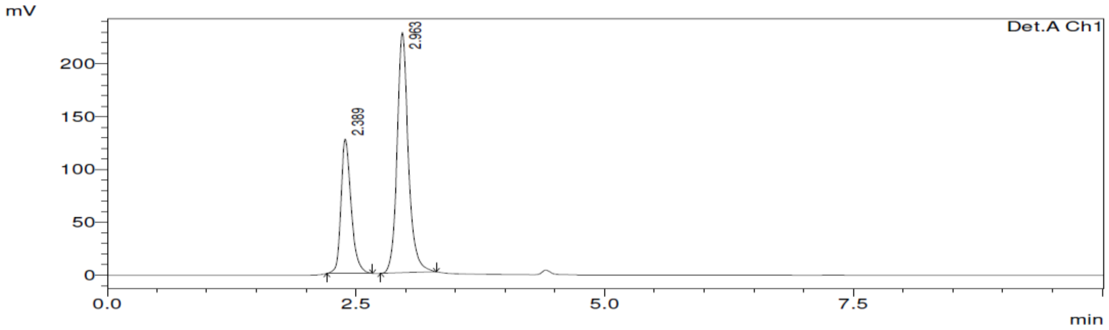


***Fig. 26*: Chromatogram of flow rate from 1.0 mL/min.**

Table 13: **Results for Robustness of HDC and PDP.**

Chromatographic changes		**Theoretical Plates**		**Tailing factor**	
		HDC	**PDP**	**HDC**	**PDP**
Flow rate (mL/min)	0.8	2933	5579	1.23	1.20
	1.0	2621	5294	1.19	1.18
	1.2	2425	4984	1.20	1.16
Temperature(°c)	25	2660	5134	1.20	1.21
	30	2675	5323	1.19	1.25
	35	2775	5521	1.18	1.28

Result

The tailing factor was found to be within the limits on less variation of flow rate and Temperature.

RUGGEDNESS

- The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

RESULTS FOR RUGGEDNESS

Hydrocodone	**%Assay**	**Pseudoephidrine**	**%Assay**
Analyst 01	99.36	**Analyst 01**	100.15
Anaylst 02	99.27	**Anaylst 02**	100.38
% RSD	0.27	**% RSD**	0.57

Observation

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was specified.

ASSAY(PERCENTAGE PURITY)

PREPARATION OF THE SAMPLES

Preparation of Standard solution

About 5 mg of HYDROCODONE and 60mg of PSEUDOEPHIDRINE were weighed into a 100 mL volumetric flask, to this 70mL of mobile phase was added, sonicated and the volume was made up with the mobile phase. Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase.

Preparation of Sample solution

Taken 5mL of Sample Solution which is Equivalent to 5mg HYDROCODONE and 60mg of PSEUDOEPHIDRINE in 100 mL volumetric flask and add 70mL of mobilephase then sonicated it for 30min intermittent shacking after 30min make up volume with mobile phase. Pipetted 5 mL of the clear solution in to 50 mL volumetric flask and make up volume with mobile phase. Filter the solution through 0.45µm filter paper. The resulting solution is used to record the chromatogram (Fig. 7.9).

- $\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$

Table 15: Results For Hydrocodone And Pseudoephidrine.

	HYDROCODONE		PSEUDOEPHIDRINE	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1022197	1026668	2032157	2029924
Injection-2	1025670	1032616	2017044	2018744
Injection-3	1041099	1025772	2015194	2030331
Injection-4	1026496	1017071	2012644	1995114
Injection-5	1006266	1016907	2008604	2018534
Average Area	1024346	1023807	2017129	2018529
Standard deviation	12435.26	6757.01	8977.515	14295.47
%RSD	1.213971	0.659989	0.445064	0.708212
Assay(%purity)	**99.94**		**100.06**	

OBSERVATION

So, the both drugs % assay found to be within the limits. The percentage purity of both HYDROCODONE and PSEUDOEPHIDRINE were found to be within the limits that is 99-100.06%.

CONCLUSION

- Several works involving hplc and uv spectroscopy analysis were reported in few journals, but no work employing stabilities studies using UPLC with this combination were reported
- The detection wavelength for uplc method of the drug is selected as 243nm.
- The method has shown acceptable precision accuracy and adequate sensitivity demands to be use for further studies.
- The established method satisfies the system suitability criteria peak integrity and resolution of the drug peak
- The column used was waters acquity C18125A(100*2.2mm1D)1.8um using mobile phase as phosphate buffer(PH=6.8) and acetonitrile(ratio=60:40)
- System suitability- 10µg/ml %RSD for retention time and peak area was less than 2% The occurrence of good linear relationship was in range of 2 to 10 µg/ml Lod was found to be 1.92(hydrocodone) and 0.78(pseudoephedrine) Loq was found to be 5.84(hydrocodone) and 2.37(pseudoephedrine). Assay limit range 99-100.06%.
- The validation parameters such as precision and accuracy etc… of this method are needed to be well, within the acceptable limits as defined by FDA guidance. No significant intereferences were found.

14. ACKNOWLEDGEMENT

Most importantly I am thankful to the almighty, who is the creator and director of all that initial and final modes to destiny. Finally, I consider this opportunity to express a deep sense of gratitude to my Parents who has always believed in my thoughts and supported me all through. I take the opportunity to express my deep sense of gratitude and respect, Dr.S.H.Rizwan, (Department of pharmaceutical analysis) Deccan School Of Pharmacy for his guidance support and immense encouragement given during the course of seminar work. I am grateful to Prof. Dr.Syed Abdul Azeez, M.Pharm, PGDPMIR, PCCRM, PH.D honorable Principal of Deccan School Of Pharmacy, providing facilities to do this work and for his constant support and encouragement.

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