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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMULTNEOUS DETERMINATION OF DROTAVERINE HYDROCHLORIDE & ACECLOFENAC IN BULK FORMULATION USING RP-HPLC

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Abstract

A novel stability indicating liquid chromatographic assay method was developed and validated as per ICH guide lines for the quantitative estimation of Drotaverine Hcl & Aceclofenac in tablet formulation. An isocratic reverse phase LC-method was developed using Hypersil ODS C18,150× 4.6mm,5µm column and a mobile phase comprising of a mixture of (0.0M Tri-ethyl amine PH 2.16±o.1)TEA Acetonitrile (10:90v/v).The UV-detector set at 224nm with flow rate of 1ml/min. The method is linear between 30µg ml to 70µg ml with R2 value as 0.999,the limit of detection (LOD) is 1.33µg ml and limit of quantification (LOQ) IS 3.99µg ml. The accuracy of the method was found to be in the range of 99.86% to 100.98%.The method precision % RSD were less than 2.Stress degradation studies were done for acid, base ,H2O2 and heat. The proposed method was found to be linear, precise and accurate for the quantitative estimation of Drotaverine Hcl & Aceclofenac in Tablet and can be used for commercial purposes.

Keywords: Drotverine Hcl & Aceclofenac, HPLC, Stress Stability Indicating and Method validation.

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INTRODUCTION

ACECLOFENAC

Non-steroidal anti-inflammatory drug. It is practically insoluble in water, very slightly soluble in ethanol, and in methyl alcohol; highly soluble in acetone and methanol.

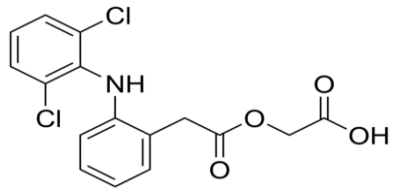

Fig.1: Structure of Aceclofenc

The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is a potent inhibitor of the enzyme cyclooxygenase, which is involved in the production of prostaglandins. It inhibits synthesis of the inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor and prostaglandin E2 (PGE2)production [1,2].

DROTAVERIN

Drotaverine (INN, also known as drotaverin) is an antispasmodic drug, structurally related to papaverine.

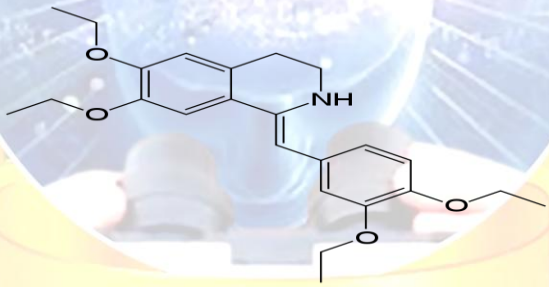

Fig. 2: Structure of Drotaverin .Hydrochloride

Drotaverine is a selective inhibitor of phosphodiesterase 4, and has no anticholinergic effects. Drotaverine has been shown to possess dose-dependant analgesic effects in animal models. One small study has shown drotaverine to be eliminated mainly non-renally [1,2].

HIGH – PERFORMANCE LIQUID CHROMATOGRAPHY

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplest and wide range of sensitivity makes it ideal for the analysis of many drugs in both dosage forms and biological fluids. To gain an appreciation for the growth of HPLC in pharmaceutical analysis one has only to review the last five years of the journal of

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pharmaceutical sciences or journal of chromatography. It is common to gain more than 20 papers on analysis of drugs by HPLC in 2003.The rapid growth of HPLC has been facilitated by the development of reliable, moderately priced instrumentation and efficient columns. Separation efficiencies achievable today are 50 times greater than those available in1970s. When 20thedition of USP was issued in 1980, only 20-25 monographs contained in HPLC analysis. Now, HPLC will be one of the most widely used instrumental methods for purity assays by the times USP published in 1999.

HPLC separation is based on interaction and differential partition of the sample between the mobile liquid phase and the stationary phase. The commonly used chromatographic methods can be roughly divided into the following groups, not necessarily in order of importance.

1. Chiral
2. Ion-exchange
3. Ion-pair/affinity
4. Normal phase
5. Reversed phase
6. Size exclusion

The test method commonly submitted to Center for Drug Evaluation & Research (CDER) is the reverse phase HPLC method. UV detection is the most common detection technique. Reversed phase chromatography, a bonded phase chromatographic technique, uses water as the base solvent. Separation based on solvent strength and selectivity also may be affected by column temperature and pH. In general, the more polar components elute faster than the less polar components. UV detection can be used with all chromatographic techniques. The concern for this type of detector is the loss of sensitivity with lamp aging, and varying sensitivity at the low level depending on design and/or manufacturer. A point to note is that observations on the HPLC chromatograms, by UV detection in combination with reversed-phase HPLC, may not be a true indication of the facts for the following reasons.

Compounds much more polar than the compound of interest may be masked (elute together) in the solvent front/void volume.

Compounds very less polar than the analyte may elute either late during the chromatographic run or retained in the column. Compounds with lower UV extinction co-efficient or different wavelength maxim may not be detectable at the low level relative to the visibility to the analyte since only one wavelength is normally monitored [3].

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MATERIALS AND METHODS:

Instruments Used

Hitachi L-2130 series consisting pump L-2130,Auto sampler,UV-Detector L-2400 Hitachi, Thermostat column compartment connected with Hitachi Elite Lachrome (D-2000Elite) software.

Materials

Active Pharmaceutical ingredient (API)of Aceclofenac & Drotaverin.Hcl was obtained as gift samples from comprime Labs(Hyderbad,India). Aceclofenac & Drotaverin Hcl tablets manufactured by Natco Pharma Ltd were purchased from pharmacy are used in the analysis,its Label claim states that each formulated tablet150mg Aceclofenac & Drotaverin.Hcl.

METHODS

Mobile Phase Selection

On The Basic of Literature Survey, previous Experience and several exploratory efforts. the chromatographic compatibility was achieved by using p^H 2.16 TEA and Acetonitrile (10:90) as an Isocratic elution. This gives the results as a mobile phase.

Wavelength selection

After screening the standard solution over 200 to 400 nm wavelength. On the basis of absorption maxima of Analyte 224 nm was decided as maximum wavelength.

Column selection

Column selection is the most important part in the method development. Applying various column Chemistry. The most suitable selected column was Hypersil ODS $C_{18,}$150×4.6mm.5µ.

Mobile phase preparation

Preparation of Triethyl amine

Pipette out 2 ml of TEA and then dissolve in 200ml of HPLC grade water .Then adjust the P^H to 2.16 with diluted ortho phosphoric acid.

Transfer 100 ml of acetonitrile and 900 ml of TEA to a 1000 ml and sonicated about 10 min for degassing, then finally filtrate the solution with vacum filter using 0.45 µ filter paper [3,4].

Standard Stock solution preparation

10 ml of standard stock solution (Mobile phase) was pipette into 100 mL standard volumetric flask and dilute the volume with diluent up to the mark (the prepared concentration was 10 µg/ml).

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Standard sample solution preparation

0.5 mL of standard stock solution(Mobile phase) was pipette into 10 mL standard volumetric flask and dilute the volume with diluent up to the mark (the prepared concentration was 50 µg/ml).

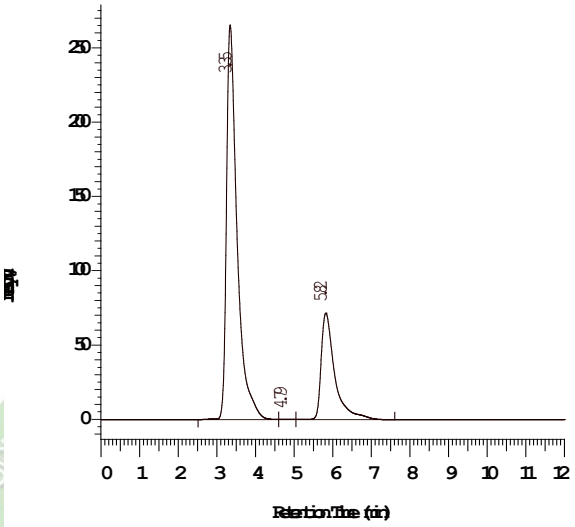

Fig: 3 Overlain Normal spectra of Aceclofenac and Diclofenac Acetonitrile

Obsevation: Change in mobile phase. The peak was observed

Chromatographic conditions:

Column	:	C_{18}, 250 X 4.6 mm, 5µ,
Flow rate	:	1 mL /min
Wavelength	:	224nm
Column temperature	:	20°C
Injection volume	:	10 µL
Run time	:	10 minutes
Diluent	:	Mobile phase
Elution	:	Isocratic
Needle wash	:	ACN:TEA 90:10 (v/v)
Mobile phase -	:	ACN:TEAin the ratio 90:10.
RT	:	3.57 min(A)&6.15min(D).
Buffer preparation	:	0.01M TEA p^H 2.16± 0.1 with dilute phosphoric acid.

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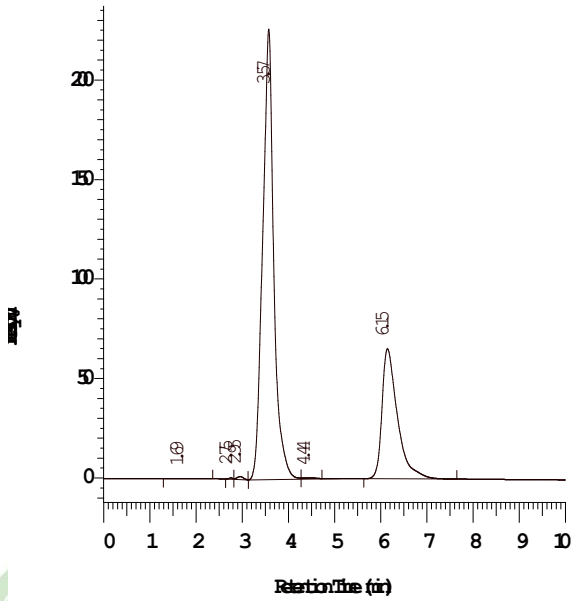

Fig.4: Chromatogram of Drotaverin & Aceclofenac by using ACN:TEA[90:10]

Preparation of sample solutions

Preparation of sample stock solution:Label claim:50mg

Weigh not less than 20 tablets and record the average weigh of tablet.crush the tablets to fine powder in a mortar with pestle. weigh accurately and transfer the tablet powder equivalent to 5omg of Aceclofenac and Diclofenac into a 100ml volumetric flask,add 70ml of diluents, sonicate for 30minutes filter the solution through 0.45µm nylon filter (or) equivalent.

Sample solution preparation

0.5ml of sample stock solution was pipette into 10ml standard volumetric flask and dilute the volume with diluents up to the mark (the prepared concentration was 50µg/ml

Procedure

Inject 20ml of blank solution standard solution, sample solution.

Result & Discussion

Method development

Table: 1 Preparation at 60% to 140% Level:

Linear solutions (%)	Stock solution taken in (mL)	Diluted to volume (mL) with diluents
60%	24(D)+30(A)	100
80%	32(D)+40(A)	100
100%	40(D)+50(A)	100
120%	48(D)+60(A)	100
140%	56(D)+70(A)	100

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Table: 2 Linearity data of Drotaverin

Analyte	Concentration (µL)	Peak area	%RSD	Linear regression Equation
Drotaverine.Hcl	0	0	0	Y=66757X+27685. R^2=0.9986
	24	4023128	200	
	32	4993646	400	
	40	6929130	500	
	48	3119928	600	
	56	3775190	700	

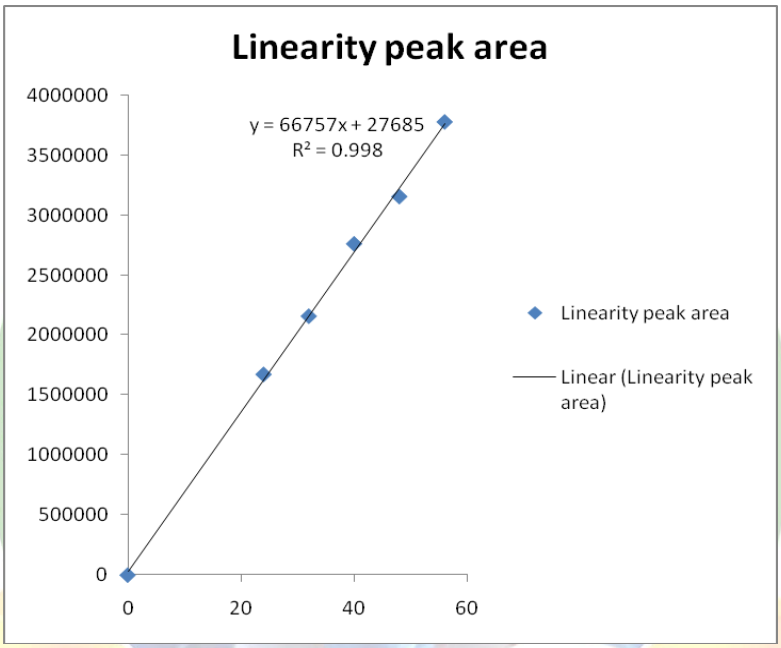

Fig: 5 Calibration curve for Drotaverin

Table 3: Linearity data of Aceclofenac:

Analyte	**Concentration (µL)**	**Peak area**	**% RSD**	**Linear regression Equation**
Aceclofenac	0	0	0	Y=161434X-32034 R^2=0.9992
	30	4023128	200	
	40	4983475	400	
	50	6990474	500	
	60	7697752	600	
	70	9107783	700	

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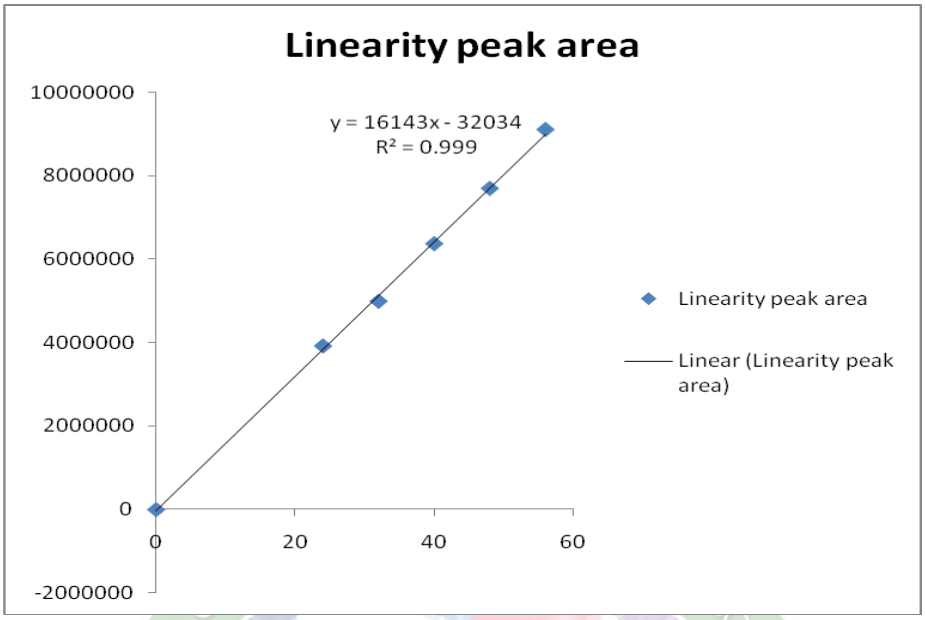

Fig. 6: Calibration curve for Aceclofenac

Accuracy / Recovery:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts(80%, 100%, and 120%) of pure drug of Aceclofenac and Diclofenac Hcl were taken and added to the pre-analyzed formulation of concentration 30µg/ml from the all above data it has been proven that the % recovery is within the limit of 98 to 102% this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2%. Hence this suggests that proposed method is highly accurate. The average percent recoveries obtained as 98% - 102%. Indicating that the method was accurate.

Conclusion

As the recovery results are found and between 98.0% to 102%,the study proves that the method is accurate for the estimation.

Table 4: Data for Aceclofenac analysis

Conc. Of Acceclofenac (API) (µg/ml)	Observed Conc. Of Acceclofenac (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.01	0.86	10.03	0.87
30	30.02	0.30	30.03	0.32
100	99.97	0.13	99.95	0.11

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Table 5: Data for Drotaverine analysis

Conc. Of Drotaverine (API) (µg/ml)	Observed Conc. Of Drotaverine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.005	1.05	10.006	0.24
30	30.003	0.55	30.084	0.41
100	99.84	0.18	99.95	0.18

Method precision: (Repeatability)

Precision was demonstrated by prepared six sample preparation as per the test method representing a single batch.

ACCEPTANCE CRITERIA

PRECISION

Preparation of precision solution

10ml of standard stock solution was diluted in 100ml s.v.f up to volume with diluents Repeat the same procedure for remaining six preparations.

% RSD to be tabulated with obtained readings in tabular form.

Acceptance Criteria

The RSD of areas from six preparations precision Level should not be more than 2%.

METHOD PRECISION (Repeatability)

Precision was demonstrated by prepared six sample preparations as per the test method representing a single batch.

Acceptance Criteria

The RSD of areas from six preparations precision Level should not be more than 2%.

Reproducibility

The intra & inter day precision variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD ≤ 2%) within a day & day today variations for Aceclofenac & Drotaverin.Hcl revealed the proposed method is precision.

LOD (Limit of Detection)

Limit of detection is the lowest concentration of analyte in a sample that can be detected .but not necessarily quantities. under the stated experimental condition.

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LOQ (Limit of Quantification)

Limit of detection is the lowest concentration of analyte in a sample that can be determind with acceptable precision and accuracy under the stated experimental conditions.

Robustness

Influence of small change in chromatographic conditions such as change in flow rate (±0.1ml/min), Temperature (±c^0), wavelength of detection (±2nm)& acetonitrile content in mobile phase (±2%) studied to determine the robustness of the method are also in favour of (Table%RSD≤%) The developed RP-HPLC method for the analysis of Aceclofenac and Drotaverin. Hcl (API).The solution was prepared and Injected with (±0.1) flow rate i.e,,0.9ml/min and 1.1ml/min respectively [5].

Table 6: Summary of Validation Parameters

S.No	Parameters	Limits	Observed Values
1	System suitability	%RSD shouid be NMT 2	0.18
2	Linearity	Correlation coefficient (r^2)NLT 0.99	0.998&0.999
3	LOD	3mg/ml	1.33
4	LOQ	10mg/ml	4.03
5	Accuracy	% Recovery range is 98-102%	98-100%
6	Intraday Interday % Precision	%RSD should be NMT 2	0.87&0.24
7	Ruggedness	%RSD should be NMT 2	0.33&0.65
8	Robustness	%RSD should be NMT 2	0.24&1.56

RESULT & DISCUSSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Drotaverine & Acceclofenac, different chromatographic conditions were applied & the results observed are presented in the thesis. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here develop Sil, C-18, V size (250m*4.6mmØ) column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Acceclofenac was found to be insoluble in water and soluble in acetonitrile & methanol. Drotaverine was found to be insoluble in water and soluble in methanol & acetontrile. Detection wavelength was selected after scanning

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the standard solution of drug over 200 to 800nm. From the U.V spectrum of Drotaverine & Acceclofenac it is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 20 µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay and stability studies which can help in the analysis of Drotaverine & Acceclofenac in different formulations. The future plan for proceeding in this experiment is to identify the degradation products and their toxicity studies .No matter how much the drug is degraded but the toxicity of the degraded product is of prime importance as this can lead to serious toxic reactions. Sometimes a very small amount of degraded product may render the formulation quite toxic that cannot be taken by the patients.

CONCLUSION

The Chromatographic conditions were optimized to develop new method for Aceclofenac &and Diclofenac Hcl in API And tablets dosage forms. The basic Chromatographic conditions were designed to be simple and easy to used and reproduced and were selected after testing the different conditions that affect HPLC analysis. Thus New Method has developed which is precise and validated. The developed method Was found to be specific and validated as for ICH guide lines.

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