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Advance simultaneous determination of paracetamol, thiocolchicoside and aceclofenac in tablets by reverse phase high performance liquid chromatography

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ABSTRACT

Rapid and accurate high performance liquid chromatography method is described for simultaneous determination of paracetamol, thiocolchicoside and aceclofenac from the combination dosage form. The separation of three drugs was achieved on an Inertsil ODS (150 x 4.6 mm i.d.) with 5 µ particle size. The mobile phase consisted of buffer of pH 6.5 and acetonitrile in gradient elution system. The detection was carried out at wavelength 300 nm. The Inertsil ODS column showed the most favorable chromatographic parameters for analysis. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear ranges for paracetamol, thiocolchicoside and aceclofenac were 1250-3750 µg/ ml, 20-60 µg/ ml and 250-750 µg/ ml respectively. The method has been successfully used to assay of combined dosage form i.e. tablets containing 500 mg paracetamol, 8 mg of thiocolchicoside and 100 mg aceclofenac with good recoveries.

Key words: Paracetamol, Thiocolchicoside, Aceclofenac, HPLC.

INTRODUCTION

In this communication a new RP-HPLC method is developed for assay of paracetamol, thiocolchicoside and aceclofenac in combined dosage form.

Paracetamol is chemically N-(4-Hydroxyphenyl) acetamide. It is non steroidal anti-inflammatory, analgesic and anti-pyretic drug.

Thiocolchicoside is a semi synthetic derivative of naturally occurring compound of colchicoside from the seeds of various species of colchicum antumnale (autumn crocus, meadow saffron, Gloriosa upuba), chemically, N-[7(S)-3-(β-D-glucopyronosyloxy)- 1,2-dimethoxy – 10-(Methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo-)[a]heptalen-7-yl]-(S)-acetamide. It is centrally acting muscles relaxant and it also show analgesic activity. It is used in treatment of muscular pain and gout.

Aceclofenac is chemically {[[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy} Acetic acid. It is the non steroidal anti inflammatory, analgesic and anti-inflammatory drug. It is used as anti-inflammatory agent.

Nikhade R.D.[1] and others, Hapse S.A.[2] and others reported UV spectrophotometric methods and Dhaneshwar S.R.[3] and others reported HPLC method for assay of such combined dosage form. In this communication a new

simple, rapid and reliable HPLC method is reported for simultaneous determination of paracetamol, thiocolchicoside and aceclofenac in combination dosage form. This simple method can be used for the routine analysis of this combination formulation. In the proposed work, optimization and validation of the method is presented.

MATERIALS AND METHODS

Materials

Reference standards of paracetamol, thiocolchicoside and aceclofenac were obtained from reputed firm with certificate of analysis. HPLC grade acetonitrile of Qualigens fine chemicals was used for chromatographic separation. Triethylamine and orthophosphoric acid were used of analytical reagent grade from S. D. fine chemicals, HPLC grade water was obtained using Millipore. Standard and sample solutions were prepared in diluent [Buffer pH 6.5: acetonitrile (70:30)].

Instrumentation

The HPLC system used was Waters Alliance HPLC system equipped with auto sampler (2695 separation module) and photo diode array-detector (2996). The chromatogram was recorded and peaks quantified by means of PC based Empower 2 software.

Preparation Standard Stock Solution

Standard Solution was prepared by transferring appropriate amount of paracetamol, thiocolchicoside and aceclofenac in 100 ml volumetric flask and making volume with diluent [Buffer pH 6.5: acetonitrile (70:30)] to get concentration of 2500 µg/ ml paracetamol, 40 µg/ ml thiocolchicoside and 500 µg/ ml aceclofenac.

Sample Solution

A powdered tablet equivalent to 500 mg paracetamol, 8 mg thiocolchicoside and 100 mg aceclofenac was weighed accurately. It was transferred into a 200 ml volumetric flask. It was dissolved in small quantity of diluent [Buffer pH 6.5: acetonitrile (70:30)] and diluted to 200 ml. volume using same diluent. It was further diluted to get 2500 µg/ ml of paracetamol, 40 µg/ ml thiocolchicoside and 500 µg/ ml aceclofenac solution. It was sonicated for 15 minutes and filtered through Whatman filter paper no. 41. First few ml of the filtrate was discarded. The resulting solution was injected into the HPLC system.

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reverse phase inertsil ODS (150 x 4.6 mm id) 5 micron particle size. Mobile phase was consisted of buffer pH 6.5 (Solution A) and acetonitrile (Solution B) with gradient elution system. The buffer was filtered and degassed before use.

Solution A: 2 ml of ortho-phosphoric acid in 1000 ml of water and adjust pH to 6.5 with triethylamine.
Solution B: Acetonitrile.

Gradient system

Time in min	Solution A	Solution B
0.0	77	23
5.0	77	23
7.0	50	50
11	50	50
13	77	23
15	77	23

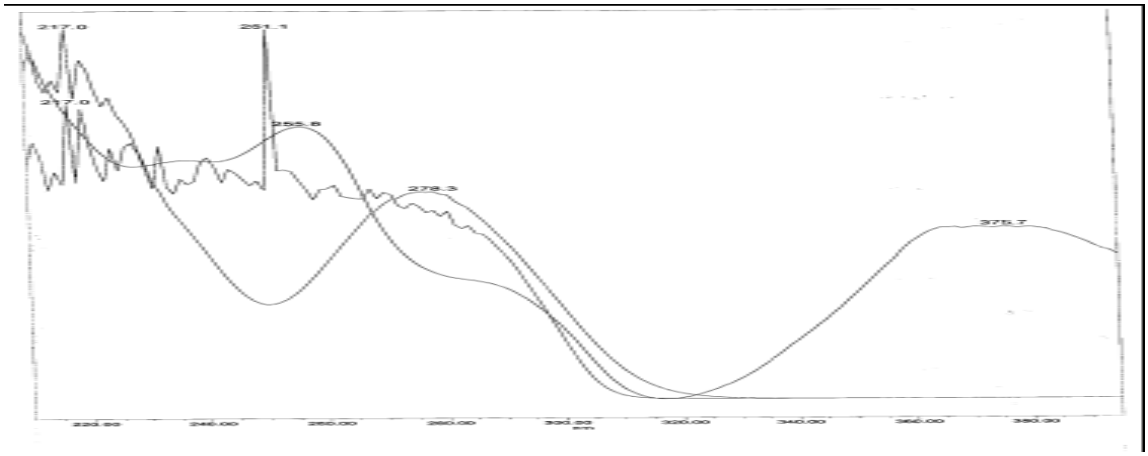
The flow rate of the mobile phase was adjusted to 1.0 ml /min. The detector wavelength was set at 300 nm. The injection volume of the standard and sample solutions was 20 µl.

Method Development

Different columns containing octyl and octadecyl silane stationary phase were tried for the separation and resolution. It was found that Inertsil ODS column offered more advantages over the BDS column. Individual drug solutions were injected into the column and elution pattern of all the three drugs and resolution parameters were studied as a function of pH. In addition to this, UV spectra of individual drugs were recorded at the wavelength range from 200 to 400 nm and the response for optimization was compared. The choice of wavelength 300 nm was

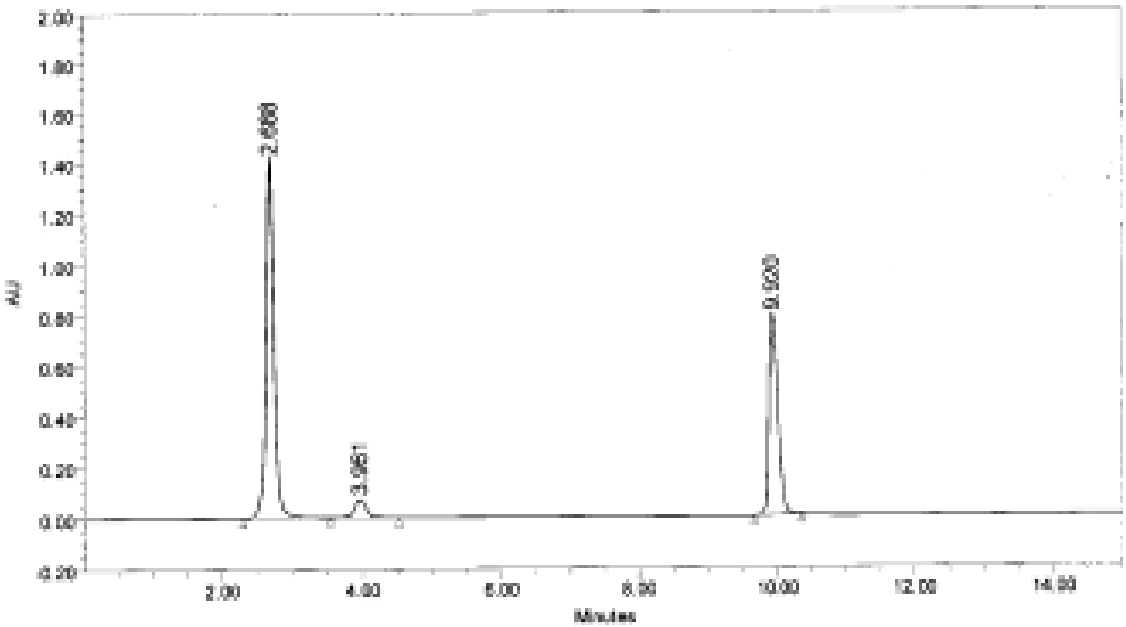
considered satisfactory, permitting the detection of all drugs with adequate sensitivity. (The overlain spectra of three drugs are given in fig 1).

Fig 1 : Overlain spectra of Paracetamol, Thiocolchicoside and Aceclofenac



The pH effect showed that optimized conditions were reached at pH 6.5. It produces well-shaped peaks for all the drugs assayed. At the same time different composition of solution A and solution B checked as gradient elution system to resolve all the peaks from each other. A typical chromatogram of the three drugs is given in fig -2.

Fig -2: A Typical chromatogram of mixture of paracetamol, thiocolchicoside and aceclofenac



The relative chromatographic figures of merit are reported in table -1. The good chromatographic separation indicated that any of these drugs could be used as internal standard for assay of other drugs.

Table – 1: System Performance Parameters for paracetamol, thiocolchicoside and aceclofenac(n = 5)

Drug Substances	Retention time	Symmetry Factor	No. of plates	Resolution factor
Paracetamol	2.70	1.0	3375	-
Thiocolchicoside	3.95	1.0	3049	5.5
Aceclofenac	9.91	1.60	22359	22.4

** Calculated at 5% peak height, + Calculated as N = 16 (t_R/w)^2*

RESULTS AND DISCUSSION

Method Validation

System suitability

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates, tailing factor, and relative standard deviation were determined. The results are shown in table -1, indicating good performance of the system.

Linearity

Under the experimental conditions described above, linear calibration curves for all the three drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the three drugs i.e. (y) v/s concentration (x). The regression analysis data obtained is tabulated in Table -2. The linear ranges were 1250 – 3750 µg/ ml of paracetamol, 20 – 60 µg/ ml of thiocolchicoside and 250 – 750 µg/ ml of aceclofenac.

Table – 2: Linearity – Regression analysis data

Parameters	Pracetamol	Thiocolchicoside	Aceclofenac,
Correlation Coefficient (r)	0.999	0.999	0.999
Intercept (y)*	365612	2793	175804
Slope (m)*	3923	19563	13874

**For equation y = mx + c*

Accuracy

Accuracy of the proposed method was determined by applying the described method to synthetic mixture containing known amount of each drug corresponding to 50 %, 80%, 100 %, 120 % and 150% of the nominal concentration. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under table 3.

Table - 3: Accuracy - %Recovery of each analyte

Drug	Amount of drug taken (mg)	Amount of drug added (mg)	Total amount of drug found (mg)	Percentage Error (%)	% Recovery	RSD (%) N = 6
Paracetamol	50%	259.1	260.8	0.66%	100.7	0.95
	80%	392.6	397.4	1.22%	101.2	0.93
	100%	488.2	486.6	0.08%	99.6	1.56
	120%	563.7	567.6	0.69%	100.7	0.89
	150%	705.9	698.6	1.03%	99.0	1.37
Thiocolchicoside	50%	3.90	3.94	1.02%	101.04	1.02
	80%	6.42	6.51	1.40%	101.4	1.15
	100%	8.05	8.08	0.37%	100.42	1.55
	120%	9.16	9.20	0.43%	100.43	1.43
	150%	12.21	12.29	0.65%	100.69	0.58
Aceclofenac	50%	52.8	53.4	1.14%	101.3	0.4
	80%	82.4	82.9	0.60%	100.5	1.7
	100%	104.8	104.9	0.57%	100.1	1.2
	120%	121.4	122.1	0.57%	100.5	0.7
	150%	154.4	155.8	0.91%	100.9	1.2

Table – 4 : Precision – Method Precision

Experiment No.	Sample weight taken (in mg)	Content in mg/tablet obtained of		
		Paracetamol	Thiocolchicoside	Aceclofenac
1	754.31	504.78	7.98	100.20
2	752.58	505.66	8.04	100.58
3	765.24	510.88	8.14	101.24
4	758.47	509.37	8.13	101.41
5	762.62	510.76	8.14	101.18
6	768.55	513.54	8.16	101.49
	% RSD	0.66	0.88	0.50

Precision

The method Precision was established by carrying out the analysis of powdered tablet containing three drugs. The assay was carried out of all the three drugs using proposed analytical method in six replicates. The value of relative

standard deviation lie well within the limit (0.66 % for paracetamol, 0.88 % for thiocolchicoside, 0.5 % for aceclofenac), indicating the sample repeatability of the method. The results obtained are tabulated in table - 4.

Robustness

The robustness of the method is determined as a measure of the analytical methods capability to be unaffected by small variation in method parameters.

The different variations are as given bellow:
Variation in pH by ± 0.2 units
Variation in wavelength by ± 0.2 nm
Variation in column oven temperature by ± 0.5°C

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Stability of Solution

Stock solution stability was checked for 24 hrs at room temperature. The drug solutions were found to be stable for the specified period. Stock Solution of sample and standard contain 2500 µg/ ml paracetamol, 40 µg/ ml thiocolchicoside and 500 µg/ ml aceclofenac.

Method Application

The validated high performance liquid chromatographic method was applied to simultaneous determination of paracetamol, thiocolchicoside and aceclofenac. Twenty tablets powder containing paracetamol (500 mg), thiocolchicoside (8 mg) and aceclofenac (100 mg) were used. A portion equivalent to 500 mg of paracetamol, 8 mg thiocolchicoside and 100 mg aceclofenac was weighed accurately and was dissolved in 150 ml of diluent. It was sonicated 10 minutes and further diluted to 200 ml to get a solution of concentration of 2500 µg/ ml paracetamol, 40 µg/ ml thiocolchicoside and 500 µg/ ml aceclofenac. A 20 µl of this solution was injected into the chromatograph under the specified conditions. The analyte peaks were identified by comparison with observed retention times with those of respective standards. The peaks areas obtained were used to calculate the amount of drugs present. The assay results, expressed as mg/tablet, are shown in table – 4, which indicates that the amount of each drug in the product meets the requirements.

CONCLUSION

The proposed HPLC method provides a fast, accurate and rugged assay with stability indicating potential for these three drugs in tablet or in solution alone. For the proposed method all the three drugs gave well define three peaks. They were well separated. The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation in comparison to previous methods. The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drugs to the pre analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery obtained indicates non- interference from the excipients used in the formulation.

In previous methods, UV Spectrophotomertic[1-2] methods were used for the estimation of three active ingredients. In the HPLC method[3] HiQ Sil C_{18} column was used. Such column is more expensive than the proposed Inersil ODS Column. Mobile phase suggested in previous method is acetonitrile and water. As the method Suggested in literature is isocratic elution system hence the consumption of solvent was more compare as compared to newly proposed gradient elution system.

The proposed method involves use of acetonitrile, triethylamine, orthophosphoric acid and water. The contribution of acetonitrile in the mobile phase is hardly about 25% of total mobile phase composition. Hence overall cost of analysis is less for proposed method. The proposed method has additional advantages over the existing methods and is more beneficial for analysis of such formulation than the previous methods.

Thus the proposed RP-HPLC method for the simultaneous estimation of paracetamol, thiocolchicoside and aceclofenac in combined dosage forms is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-

HPLC method is strongly recommended for the quality control of the raw material, formulations and dissolution studies.

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