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Simultaneous determination of aceclofenac, paracetamol and tizanidine in tablets by RP-HPLC

R.V.Rele*, R.N.Mali, S.A.Sawant

D.G.Ruparel College, Matunga, Mumbai-400 016, (INDIA)

E-mail : searchnil_2007@yahoo.com

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ABSTRACT

Rapid and accurate high performance liquid chromatography method is described for simultaneous determination of paracetamol, aceclofenac and tizanidine in the combination dosage forms. The separation of three drugs was achieved on stainless steel C₁₈ (250 mm × 4.6 mm) column of 5 μm particle size. Mobile phase consisted of 60:40 of acetonitrile and buffer of pH as 7 respectively. Detection was carried out at 240 nm. Stainless steel C₁₈ column showed most favorable chromatographic parameters for analysis. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear range for aceclofenac, paracetamol and tizanidine was 5-15 μg/ml, 25-75 μg/ml and 0.11-0.32 μg/ml respectively. The method has been successfully used to analyze commercial solid dosage containing 100 mg of aceclofenac, 500 mg of paracetamol and 2 mg of tizanidine with good recoveries.

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KEYWORDS

Tizanidine;
Aceclofenac;
Paracetamol;
Pharmaceutical dosage form;
HPLC.

INTRODUCTION

In this communication the present work proposes a new HPLC method for assay of three drugs i.e. aceclofenac, paracetamol and tizanidine in combined dosage form. Aceclofenac is chemically described as [(2(2',6'-dichlorophenyl amino)phenyl] acetoxy acetic acid. It is orally administered non steroidal anti-inflammatory drug which shows good effect of analgesic properties and good tolerability profile in variety of painful conditions. Paracetamol is chemically described as N-(4-hydroxyphenyl)acetamide. Paracetamol is analgesic, anti-inflammatory and antipyretic drug. Tizanidine is chemically described as 4-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-8-thia-7,9-diazabicyclo[4.3.0]nona-2,4,6,9-tetraen-5-amine which is used as muscle

relaxant. It is centrally acting α-2 adrenergic agonist drug. It is used to treat spasm, cramping and tightness caused by medical problems. Aceclofenac is officially reported in BP^[1] which describes a potentiometric assay method for determination of Aceclofenac in bulk drugs.

Paracetamol is officially reported in BP^[1], IP^[2] and USP^[3]. BP^[1] and IP^[1] suggest titrimetric and UV spectrophotometric methods for assay of paracetamol in bulk and tablet formulation respectively. USP^[3] describes thin layer chromatographic method for determination of paracetamol. Tizanidine Hydrochloride is officially reported in IP^[2] and USP^[3]. HPLC method is reported for determination of tizanidine hydrochloride in bulk and tablet forms in IP^[2] and USP^[3].

In this communication a novel, simple, rapid and

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reliable HPLC method is proposed for simultaneous determination of paracetamol, aceclofenac and tizanidine in combination tablets. Developed method can be used for the routine analysis in quality control laboratories. In the proposed work development, optimization and validation of the method are presented.

EXPERIMENTAL

Materials

Reference standards of paracetamol, aceclofenac and tizanidine were obtained from reputed firms with certificate of analysis. HPLC grade acetonitrile of Qualigens fine chemicals was used for analysis. Triethylamine were used of S.D fine chemicals. HPLC grade water was obtained using Millipore system. Standard sample solutions were prepared in diluent (50:50 with acetonitrile and water).

Instrumentation

The HPLC system, Merck La-chrome Elite HPLC system, equipped with auto sampler (L-2200), isocratic pump (L-2100) and diode array detector was used. The chromatogram was recorded and peaks were quantified by means of PC based Ezchrome Elite software.

Preparation of standard stock solutions.

Standard stock solutions

The stock solution of tizanidine

The About 10 mg of tizanidine hydrochloride was weighed accurately and transferred into 100 ml volumetric flask. About 60 ml of diluent was added and sonicated for 5min to dissolve tizanidine hydrochloride. Volume was adjusted to 100 ml by diluent.

About 40.0 mg of aceclofenac, 200 mg of paracetamol reference standards were accurately weighed and transferred into 100 ml of volumetric flask along with 8ml of Tizanidine standard stock solution and 60 ml of diluent. It was sonicated for 5min to dissolve reference standard completely. Volume was adjusted to 100 ml with diluent.

Sample solution

Twenty tablets of the dosage under study were accurately weighed and average weight of each tablet was determined. Tablets were crushed into fine powder form and from such powder portion equivalent to 100 mg of

aceclofenac, 500 mg of paracetamol and 2 mg of tizanidine was weighed accurately. It was transferred into 100 ml volumetric flask with 60 ml of diluent. Solution was sonicated at 60°C for five minutes. Volume was adjusted to 100 ml with the diluent. Sample was then filtered through whatman filter paper No.41. one ml of this solution was then diluted to 100 ml with the diluent to obtain final concentration as 10µg/ml of aceclofenac, 50µg/ml of paracetamol and 0.2µg/ml of tizanidine.

Chromatographic conditions

Chromatographic separation was performed at room temperature on stainless steel C₁₈ (250mm × 4.6mm) column of 5 micron particle size. Mobile phase consisted acetonitrile and buffer of pH as 7 in the ratio of 60:40. Buffer solution was made up of 0.05 M potassium dihydrogen phthalate. The pH of the solution was adjusted to 7.0 with triethylamine. The mobile phase was filtered and degassed. The flow rate of the mobile phase was adjusted to 1.0 ml/min. The detector wavelength was set at 240 nm. The injection volume of the standard and sample solution was 20µl.

Method development

Different columns containing octyl and octadecyl silane stationary phase were tried for effective separation and resolutions. It was found that stainless steel C₁₈ column offered more advantage over the other columns. Individual drug Solution was injected into column. Elution and resolution parameters of all the three drugs were recorded at the wavelength range of 200 to 400 nm and their response optimization was compared. From the overlain spectra of all the drugs, a suitable wavelength was 240 nm for the detection of all the three

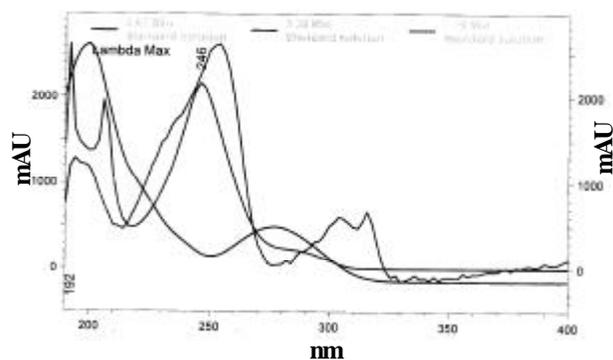


Figure 1 : Overlain spectra of paracetamol, aceclofenac and tizanidine

drugs with adequate sensitivity.(figure 1).The pH study showed that pH 7.0 was most suitable pH for assay of all three drugs . It gave well shaped peaks for the assay of all the drugs. A typical chromatogram of the drugs assayed is depicted in figure 2. The good chromatographic separation indicated that these drugs could be used as internal standards for the assay of other drugs.

RESULTS AND DISCUSSION

The results of analysis showed that the amount of drugs was in good agreement with the label claim of the formulation. Method validation parameters such as system suitability, precisions, accuracy, linearity, robustness, solution Stability, etc., were ascertained.

Method validation

System suitability

System performance parameters of developed HPLC method were determined by injecting standard solution in replicates. Parameters such as number of theoretical plates , tailing factor resolution ,capacity factor and relative standard deviation were determined. The results are shown in TABLE 1. It indicated good performance of system.

Linearity

Under the experimental conditions described above, linear calibration curves for 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 is represented in TABLE 2. The linear

range of concentration was 5-15g/ml for aceclofenac 25-75µg/ml for paracetamol and 0.11-0.32µg/ml for tizanidine.

Accuracy

Accuracy of the method was determined by applying the above method to synthetic mixtures. Synthetic mixtures contained 80%, 100%, and 120% of paracetamol, aceclofenac and tizanidine of the label claim respectively. The accuracy was then calculated

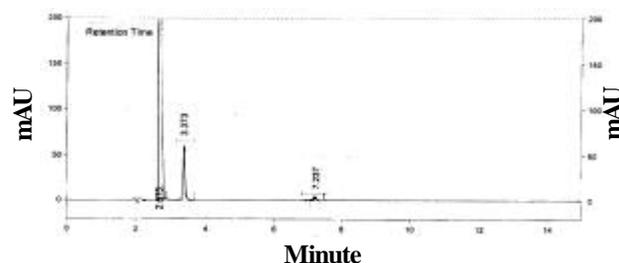


Figure 2 : A Typical chromatogram of mixture of paracetamol, aceclofenac and tizanidine

TABLE 1 : System performance parameters for paracetamol, aceclofenac and tizanidine (n = 6)

| Drug substances | Retention time | Symmetry factor | Capacity factor | No. of plates | Resolution factor |
|-----------------|----------------|-----------------|-----------------|---------------|-------------------|
| Paracetamol | 2.67 | 1.24 | 0.3 | 21707 | - |
| Aceclofenac | 3.38 | 1.22 | 0.7 | 12894 | 7.4 |
| Tizanidine | 7.24 | 1.11 | 2.6 | 23165 | 25.0 |

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

TABLE 2 : Linearity-regression analysis data

| Parameters | Paracetamol | Aceclofenac | Tizanidine |
|-----------------------------|-------------|-------------|------------|
| Correlation coefficient (r) | 0.9905 | 0.9999 | 0.9993 |
| Intercept (y) | 48294 | 7927.3 | 48294 |
| Slope (m) | 23857 | 33174 | 23857 |

$Y = mx + c$

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 | 527.0 | 0 | - | - | - | - |
| | 527.0 | 400.15 | 401.67 | 0.38 | 100.38 | 0.46 |
| | 527.0 | 500.73 | 496.78 | 0.79 | 99.21 | 0.25 |
| | 527.0 | 602.38 | 593.66 | 1.45 | 98.50 | 0.24 |
| Aceclofenac | 105.14 | 0 | - | - | - | - |
| | 105.14 | 81.02 | 81.07 | 0.062 | 100.07 | 0.56 |
| | 105.14 | 101.22 | 101.26 | 0.040 | 100.05 | 0.36 |
| | 105.14 | 120.55 | 121.34 | 0.66 | 100.65 | 0.91 |
| Tizanidine | 2.09 | 0 | - | - | - | - |
| | 2.09 | 1.62 | 1.61 | 0.62 | 99.32 | 0.28 |
| | 2.09 | 2.02 | 2.02 | 0 | 99.76 | 0.20 |
| | 2.09 | 2.42 | 2.43 | 0.62 | 100.15 | 0.42 |

*Average of triplicate analysis

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TABLE 4 : Precision-method precision

| Experiment no. | Sample weight taken (in mg) | Content in mg/tablet obtained of | | |
|----------------|-----------------------------|----------------------------------|-------------|------------|
| | | Paracetamol | Aceclofenac | Tizanidine |
| 1 | 802.9 | 518.5 | 110.8 | 2.164 |
| 2 | 804.3 | 532.0 | 111.1 | 2.170 |
| 3 | 819.8 | 536.5 | 112.9 | 2.174 |
| 4 | 804.3 | 523.5 | 111.3 | 2.164 |
| 5 | 785.6 | 530.0 | 110.5 | 2.140 |
| 6 | 781.2 | 536.5 | 107.2 | 2.094 |
| | % RSD | 1.36 | 1.70 | 1.42 |

as the percentage of analyte recovered by the assay. The results of the analysis are given in TABLE 3.

Precision

The method precision was established by carrying out the analysis of tablets powder blend containing three drugs. The assay was carried out for the drugs using proposed analytical method in six replicates. The values of relative standard deviation were well within limits 1.36%, 1.70% and 1.42% for paracetamol, aceclofenac and tizanidine respectively indicating the sample repeatability of the method. The results obtained are tabulated in TABLE 4.

Robustness

The robustness of the method is determined by small variation in method parameters.

The different variations are as given bellow:

Variation in flow rate by ± 0.2 ml/min.

Variation in mobile phase by ± 0.2 units

Variation in wavelength ± 0.2 nm

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

The stability of the solutions under study was established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the solutions. It confirmed the stability of the drug in the solvents used for the analysis.

Method application

The validated high performance liquid chromatographic method was applied to simultaneous determination of aceclofenac, paracetamol and tizanidine. Twenty tablets were crushed into fine powder form and from such powder portion equivalent to 100 mg of

aceclofenac, 500 mg of paracetamol and 2 mg of tizanidine was weighed accurately. It was dissolved in 100 ml of diluent and further 1ml of this solution was diluted in 100 ml of diluent to obtain $10\mu\text{g/ml}$ of aceclofenac, $50\mu\text{g/ml}$ of paracetamol and $0.2\mu\text{g/ml}$ of tizanidine solution. From this solution $20\mu\text{l}$ was injected into chromatograph under specified conditions. The analyte peaks were identified by comparison with those of respective standards. The assay results expressed as mg/tablet are shown in Table-IV. It indicates the amount of each drug in the product meets the requirement.

CONCLUSION

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. 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 indicated non-interference from the excipients used in the formulations.

Thus the proposed RP-HPLC method is novel method for the simultaneous estimation of paracetamol, aceclofenac and tizanidine in combined dosage forms. It 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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