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DETERMINATION OF PARACETOMOL, DOMPERIDONE AND FLUNARAZINE IN PHARMACEUTICAL DOSAGE FORMS USING RP- HPLC

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ABSTRACT

RP-HPLC method was developed for the simultaneous estimation of Paracetomol, Domperidone and Flunarazine in pharmaceutical bulk drugs and tablet dosage forms. A wavelength 210nm was selected and the mobile phase consists of water and methanol in 50:50% v/v ratios at a flow rate of 1 ml/min were found to be optimum conditions for analysis. the limits of % recovered Shown be in the range of 98-102% the results obtained for Paracetomol, Domperidone Maliate And Flunarizine were found to be within the limits. Hence the method was found to be accurate. The limits of % recovery of drugs were 98-102%. Calibration curve was plotted and correlation co-efficient for the drugs Paracetomol, Domperidone Maliate and Flunarizine found to be 0.999, 0.999 and 1.000. Hence the developed method could be used for simultaneous estimation of Paracetomol, Domperidone Maliate and Flunarizinein pharmaceutical dosage forms.

KEY WORDS: RP-HPLC method, Paracetomol, Domperidone Maliate and Flunarizine

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INTRODUCTION

Chromatography (Chroma means ‘colour’ and graphie means to ‘write’) is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the Analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation[9].Chromatography is defined as a chemical analysis separation process which uses selective adsorption to segregate and identify components of complex mixtures such as solutions, liquids and vapours. Different types of Chromatographic techniques were summarized in to separate the components of a mixture for further use (and are thus a form of purification). Analytical Chromatography is done normally with smaller

amounts of material and is for measuring the relative proportion of analytes in a mixture.

In the modern pharmaceutical industry, High Performance Liquid Chromatography (HPLC) is the major and integral analytical tool applied in all stages of drug discovery, development, and production. Effective and fast method development is of paramount importance throughout this drug development life cycle[11]. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization. Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components.

High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface.Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention (1-8).

Paracetamol also known as acetaminophen or APAP, chemically named N-acetyl-p-aminophenol, is a widely used over-the-counteranalgesic (pain reliever) and antipyretic (fever reducer). Acetaminophen is the name adopted for this pharmacologic agent in the U.S. (USAN) and Japan; paracetamol is approved in a variety of international venues (INN, AAN, BAN, etc.). Common trade names in English-speaking markets are *Tylenol* and *Panadol*. Paracetamol is classified as a mild analgesic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. Though paracetamol is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity. Domperidone (trade names Motilium, Motillium,MotinormCosti, Nomit and Molax) is a medication developed by Janssen Pharmaceutical that is a peripheral, specific blocker of dopamine receptors. It is administered orally, rectally, or intravenously. Domperidone is given in order to relieve nausea and vomiting; to increase the transit of food through the stomach (as a prokinetic agent through increase in gastrointestinal peristalsis); and to increase lactation (breast milk production) by release of prolactin. It is also used in the scientific study of the way dopamine (an important neurotransmitter) acts in the body. Domperidone is available in the form of tablets, orally disintegrating tablets (based on Zydis technology), suspension and suppositories.

Flunarizine is a drug classified as a calcium channel blocker. Flunarizine is a non-selective calcium entry blocker with other actions including histamine H_1 receptor blocking activity. It is effective in the prophylaxis of migraine,occlusive peripheral vascular disease, vertigo of central and peripheral origin, and as an adjuvant in the therapy of epilepsy. It may help to reduce the severity and duration of attacks of paralysis associated with the more serious form of alternating hemiplegia, as well as being effective in rapid onset dystonia-parkinsonism (RDP). Both these conditions share a mutation in the ATP13A gene; flunarizine is not available by prescription in the U.S. or Japan.[3] Flunarizine has been shown to significantly reduce headache frequency and severity in both adults and children. Flunarizine was discovered at Janssen Pharmaceutical in 1968.

Method development and validation of simultaneous estimation of available drugs remain as a challenge for analytical Research scientist .Photo sensitivity stands as a major problem in combination work. To develop an efficient, accurate method for using in the laboratory, all the statistical parameters with acceptance criteria to conclude the major portion of work followed by me. Paracetomol and Domperidone and flunarazine is not official in Indian

pharmacopeia. Literature survey reveals there is no analytical methods were reported for the estimation of Paracetomol and Domperidone and flunarazine. An attempt has been made to develop simple, accurate, sensitive and economic method for effective quantitative determination of Paracetomol and Domperidone and flunarazine in Pharmaceutical dosage forms by using HPLC Validation of the method was done in accordance with ICH guidelines for the assay of active ingredients. The methods were validated for parameters like specificity, system suitability, precision, linearity, accuracy, ruggedness, and the above mentioned methods were suitable to separate the components, characterize and quantify the components.

MATERIALS AND METHODS

HPLC METHOD DEVELOPMENT (9)

Mobile Phase Optimization

Initially the mobile phase tried was Water: methanol(50:50), Water: acetonitrile(50:50) and sodiumdihydrogenorthophosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to methanol and water (75:25)

Wave length selection

UV spectrum of 20mg / ml Paracetamol, Domperidone and Flunarizine in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 210. At this wavelength both the drugs show good absorbance.

Optimization of Column

The method was performed with various columns like C8 column, hypersil column, lichrosorb, and inertsil ODS column. Symmetry C18 (4.5 x 100mm, 5µm, Make: waters) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of standard solution

Accurately weighed 50mg of Paracetamol, 10mg of Domperidone and 5mg of Flunarizine was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of sample solution

Accurately weighed 992.2mg of tablet powder (sample) was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of mobile phase

Accurately measured 750 ml (75%) of methanol and 250 ml (25%) of water were mixed and degassed in an ultrasonic sonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

VALIDATION PARAMETERS (10)

Specificity

Preparation of Mixed Standard solution:

Accurately weighed 50mg of Paracetamol, 10mg of Domperidone and 5mg of Flunarizine was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of Paracetamol solution

Accurately weighed 50mg of Paracetamol was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of Domperidone solution

Accurately weighed 50mg of Domperidone was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of Flunrizine solution

Accurately weighed 50mg of Flunarzine was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Preparation of Test Solution

Accurately weighed 992.2mg of tablet powder (sample) was taken in a 100ml volumetric flask, and made up to the mark with mobile phase.

Intermediate Precision

The above sample solutions were injected for five times in two different days and peak areas were recorded.

Chromatograms were recorded as shown in Fig. 28 to 41 and results are shown in Table 15 to18.

Linearity solution

For linearity determination, five different concentrations were prepared separately i.e. 50%, 75%, 100%, 125% and 150% for the analyte and chromatograms are recorded for the same.

Accuracy solution (Recovery)

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Ruggedness

Intermediate precision of Assay test was performed separately on same batch of sample on two different analysts in different days.

Batch analysis

Assay was demonstrated by injecting 2 batches of test solution as per test method.

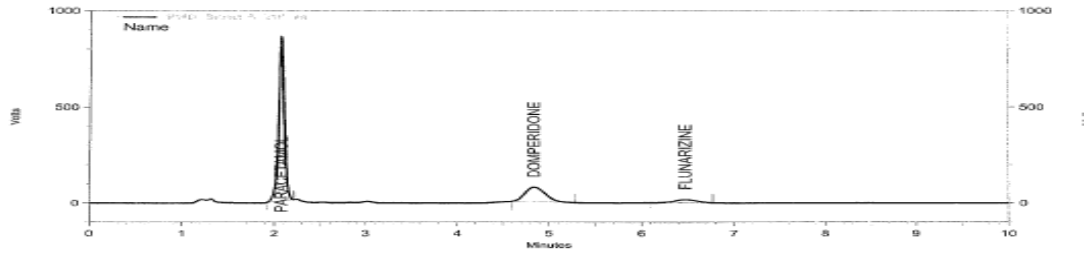
RESULTS AND DISCUSSION

Optimized method

Optimized method was done and it is shown in table-1 and fig-1.

Table-1 Data Showing optimized method

S.no	Stationary phase	Mobile phase	Detection	Flow rate	R_t	Injection volume	Column temp
1	C18(250×4.6 mm, 5µm)	Water: Methanol (50:50)	210 nm	1 ml/min	10 min	20 µl	27 ^{o}C



VWD: Signal A, 210 nm
Results

Pk #	Retention Time	Area	Area Percent	Theoretical plates (USP)	Resolution (USP)	Asymmetry
1	2.077	60428706	71.5929	6308	0.00000	0.95557
2	4.837	19601678	23.2231	2205	10.68629	1.19916
3	6.473	4175632	5.1840	3611	3.88356	0.99054
Totals		84206016	100.0000			

Figure-1 Chromatogram of Optimized method

From the above chromatogram it was observed that all three peaks eluted with good resolution, the theoretical plates and Assymetry are well within the acceptable range. All parameters are satisfied in the case of optimized method.

Validation

Validation of an analytical method is the process to establish by laboratory studies that the performance characteristic of the method meets the requirements for the intended analytical application. Performance characteristics were expressed in terms of analytical parameters.

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The linearity was demonstrated by injecting the analyte over the range of 50% to 150% of standard concentration (Fig-2-5).

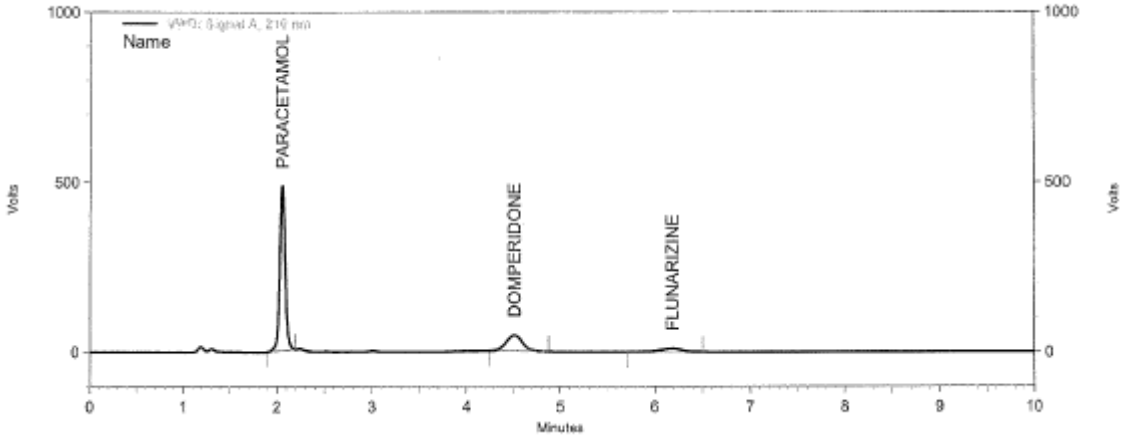


Figure-2 Chromatogram Showing Linearity 50%

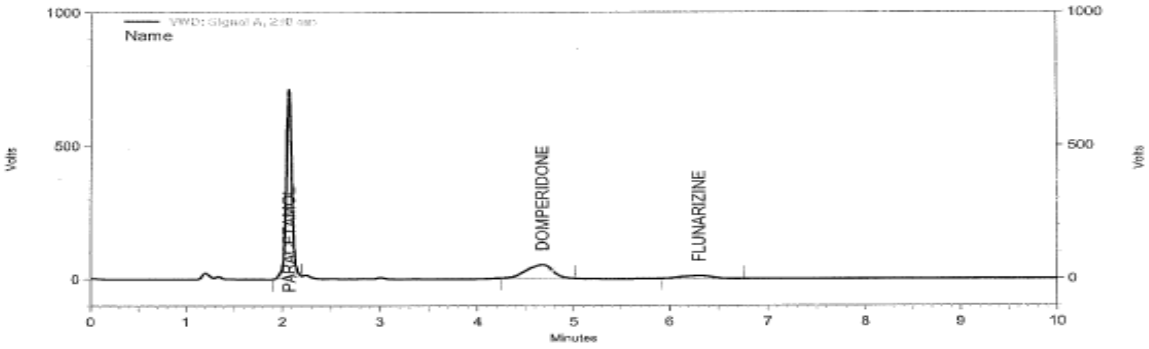


Figure-3 Chromatogram Showing Linearity 75%

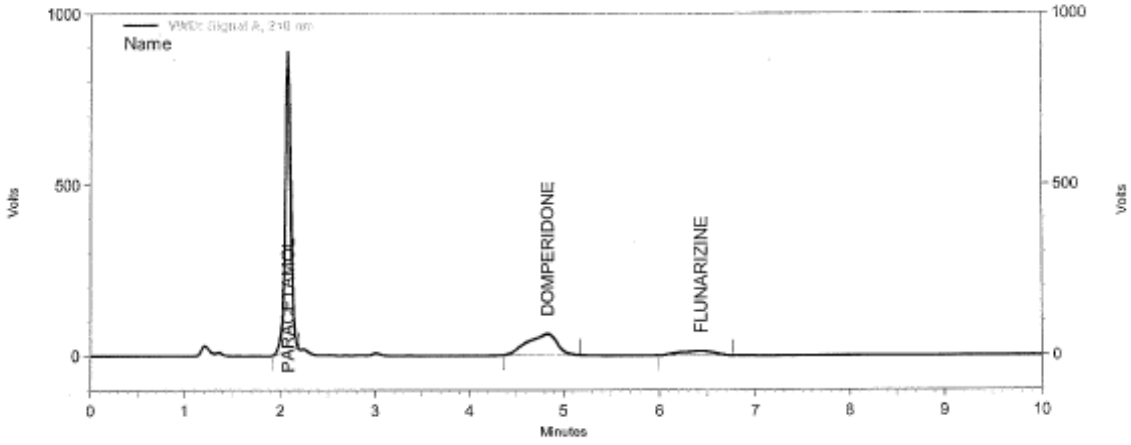


Figure-4 Chromatogram Showing Linearity 100%

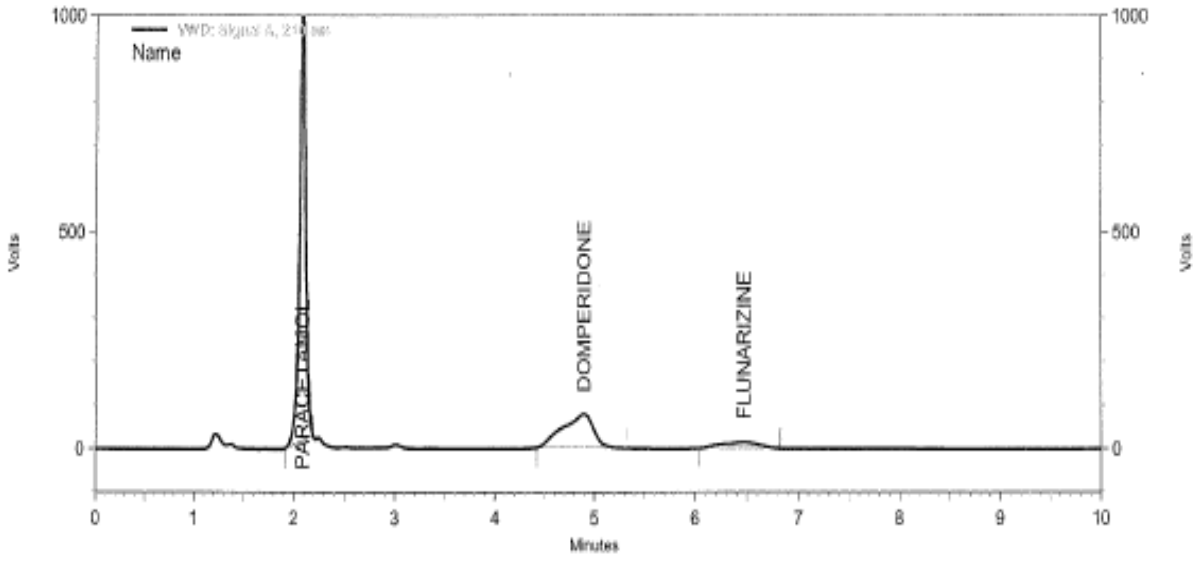


Figure- 5 Chromatogram Showing Linearity 125%

The correlation coefficient is NLT 0.99 The results are shown in fig-6-8

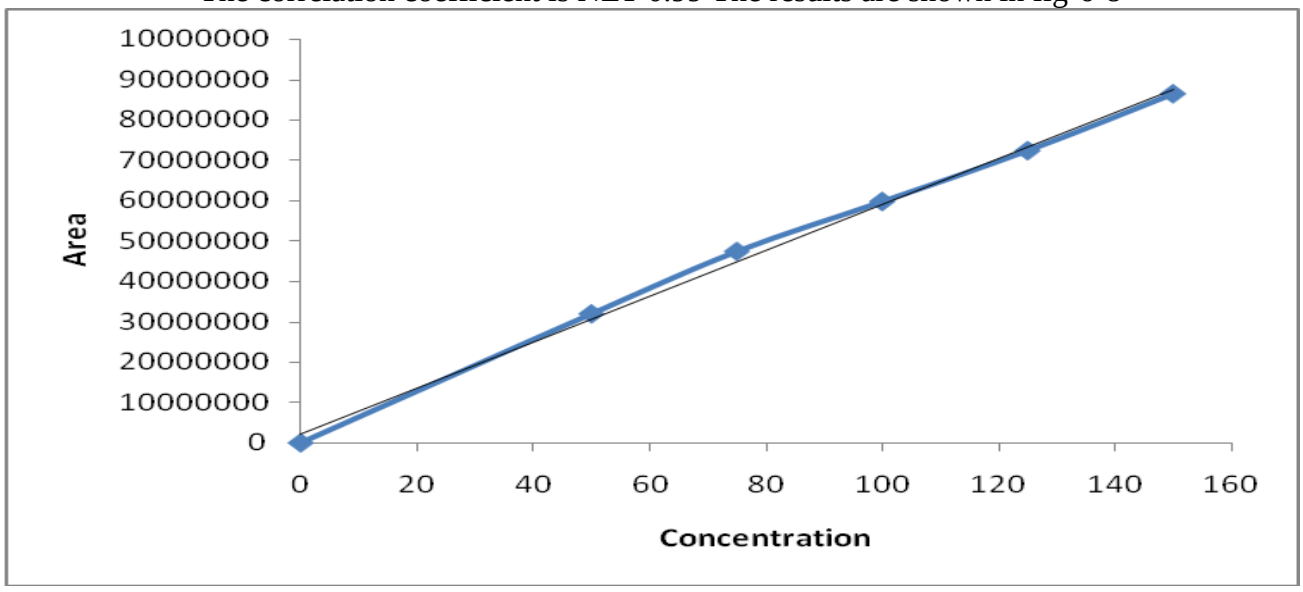


Fig-6 Linearity Graph of Paracetamol

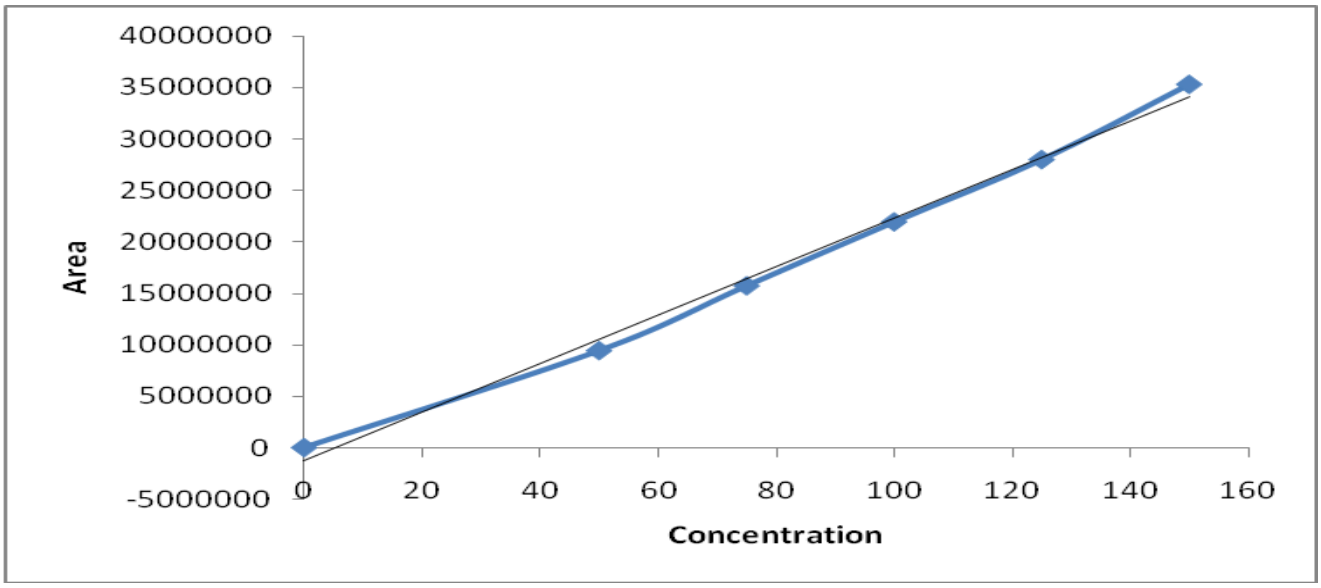


Fig-7 Linearity Graph of Domperidone

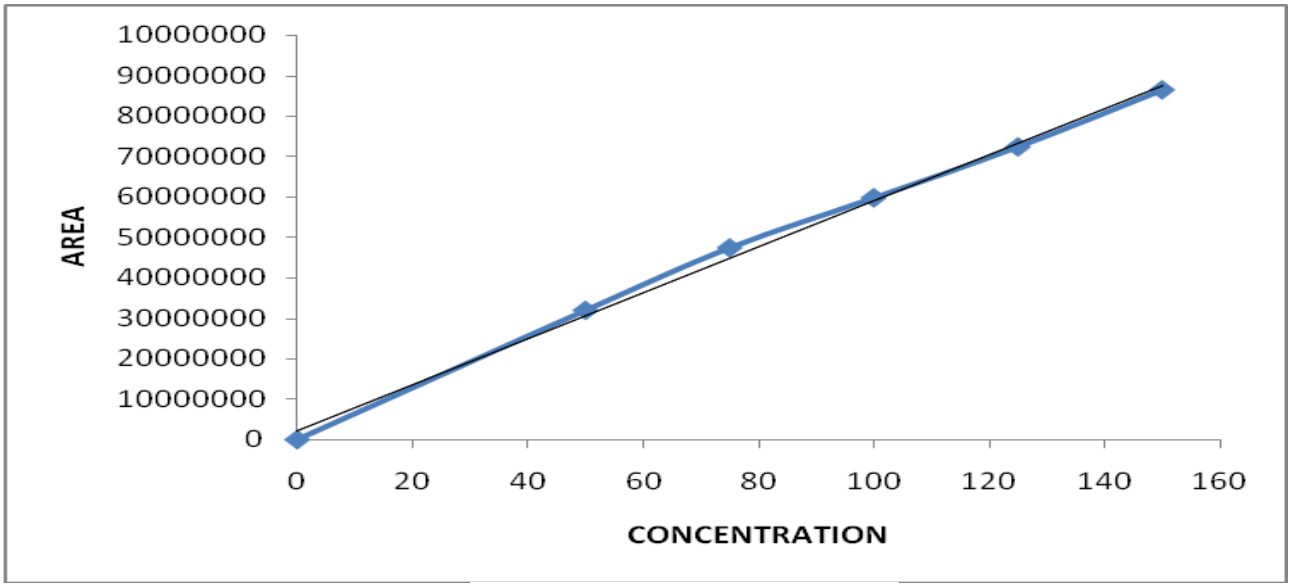


Fig-8 Linearity Graph of Flunarizine

CONCLUSION

Simple, precise, rapid and accurate RP-HPLC method was developed for the simultaneous estimation of Paracetomol, Domperidone and Flunarazine in pharmaceutical bulk drugs and tablet dosage forms. In RP-HPLC method, optimization of chromatographic parameters was done. Parameters optimized were, selection of wavelength, effect of nature of mobile phase, ratio of mobile phase, and effect of flow rate. A wavelength 210nm was selected and the mobile phase consists of water and methanol in 50:50% v/v ratios at a flow rate of 1 ml/min were found to be optimum conditions for analysis. The peaks were well resolved with C_{18}column. System suitability studies were also carried out which includes theoretical plates, resolution and tailing factors etc. The accuracy studies were shown as % recovery for Paracetomol, Domperidone Maliate and Flunarizine at 50%, 100%

and 150% the limits of % recovered Shown be in the range of 98-102% the results obtained for Paracetomol, Domperidone Maliate And Flunarizine were found to be within the limits. Hence the method was found to be accurate. The limits of % recovery of drugs were 98-102%. In the System precision study, %RSD was found to be less than 2%. For ID precision studies 5 replicate injection of Paracetomol, Domperidone Maliate and Flunarizine was performed. %RSD was determined for peak areas of Paracetomol, Domperidone Maliate and Flunarazine the acceptance limit should be not more than 2% and the results were found to be with in the acceptance limits. Using the optimized chromatographic conditions, chromatograms of mixed standard solutions which contained Paracetomol, Domperidone Maliate and Flunarizine were recorded. Retention times were found to be 2.077 min, 4.837 min and 6.437 min for Paracetomol, Domperidone Maliate and Flunarazinerespectively. Calibration curves were obtained by using peak area vs. concentration Calibration curve was plotted and correlation co-efficient for the drugs Paracetomol, Domperidone Maliate and Flunarizine found to be 0.999, 0.999 and 1.000. Precision of the method was studied by making the replicate injections of the standard solutions and standard deviation was determined. The reliability and sensitivity of the method could be seen from recovery studies. There is no interference due to excipients. The proposed method is simple, accurate and rapid. Finally the HPLC method developed could be used for simultaneous estimation of Paracetomol, Domperidone Maliate and Flunarizinein pharmaceutical dosage forms.

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