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Review Article

A REVIEW ON DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHODS FOR ANALYSIS OF ACIDIC DRUGS

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ABSTRACT

High-performance liquid chromatography is one of the fastest, safest and precise technology used for determination and separation of pharmaceutical drugs, impurities and biological samples. High-performance liquid chromatography is versatile and it takes less time for quantification of drugs as compared to old liquid chromatography techniques. This article reviews stability indicating HPLC method developed and validated for acidic drugs and their degradation studies.

Keywords: High-performance liquid chromatography, Acidic drugs, Stability indicating method development and validation

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INTRODUCTION

High-performance liquid chromatography is also known as high-pressure liquid chromatography is an advanced analytical technique that is used for separation, determination, identification, and quantification of active drugs, proteins and impurities [1-2]. HPLC is a type of column chromatography. In column chromatography, the sample is passed through a column with the help of gravity which takes more time for analysis of drugs but in HPLC sample is passed through the column under high pressure up to 400 atmospheres so that fast separation takes place [1-2]. HPLC consist of mobile phase reservoir system, pump, column and detector which records and shows separation result. In HPLC, the pump moves the mobile phase and sample towards the column. Column is the most important part of the HPLC system. In HPLC, column is made up of adsorbent material and most of the columns are made by using silica gel. The elution of the sample depends on the interaction of the sample with the column material and it also depends on the mobile phase. Mobile phase mainly consists of organic liquids such as methanol, acetonitrile and also consists of water. Sometimes buffers are also used as mobile phase. There are two types of elution: (1) HPLC isocratic elution: in which composition of the mobile phase is same during the whole analysis and (2) Gradient elution in which composition is changed during the analysis or separation of sample.

Instrumentation

The instrumentation of HPLC consists of mobile reservoir phase, pump, column, detector and recorder as shown in fig. 1.

Mobile phase reservoir

In HPLC, contents of the mobile phase are stored in a glass container. The mobile phase is a mixture of organic and aqueous liquids in a different proportion which mainly depends on the nature and solubility of drugs [1-7].

Pump

Pump pushes or moves mobile phase and sample to the column under high pressure up to 4000atmospheres. Pump pressure depends on column dimension, particle size, flow rate and composition of mobile phase [1-7].

Column

Column is the most important part of HPLC. Column is made of stainless steel and is 50 mm to 300 mm long. They are generally loaded with the stationary phase or adsorbent and the inner diameter of the column is<2 µm to 5 µm [1-7].

Detector

Detector detects the retention time of the sample. Detector is situated after the column so; it collects the sample and detects its retention time. Different types of detectors used are UV, fluorescence, IR, MASS detectors [1-7].

Recorder

Recorder records the peak, peak area, tailing factor, theoretical plate of the sample [1-7].

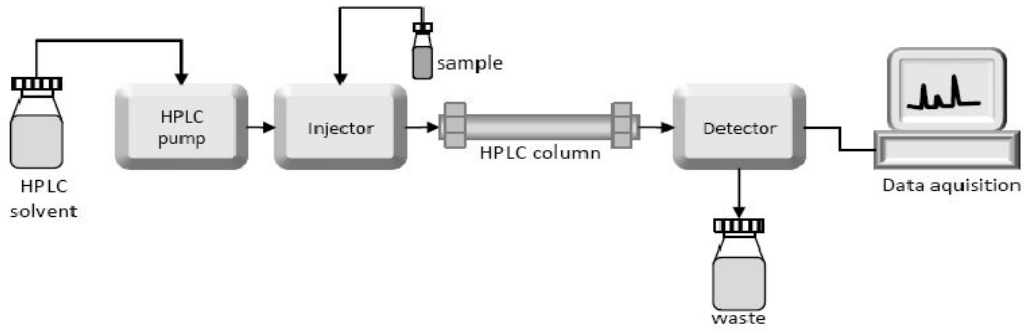


Fig. 1: Instrumentation of HPLC

Introduction of acidic drugs

Most of the drugs used in medicines are either weak acids, weak bases or both weak acids and weak bases. pKa value of drugs gives information about the nature of drug whether it is acidic or basic in nature. pKa value gives information about the strength of acids and bases and also indicates the at which pH drug gets 50% ionized. The acidity of a drug depends on the functional group which is present in their structure. Functional groups such as carboxylic acid, amine, and phenolic groups are responsible for the acidic nature of drug. A number of commonly used drugs are carboxylic acid derivatives. These include NSAID-Aspirin, anticancer agent-Methotrexate, antibacterial agent-Amoxicillin, and the diuretic-Furosemide.

Amoxicillin is an antibacterial agent that belongs to the penicillin class. In amoxicillin, the β lactam ring is present and all penicillins are organic acids having various electron donor groups in their structure. In amoxicillin structure, carboxylate (COO-) and amino NH_2 groups are electron donor and these groups are responsible for the acidic nature of amoxicillin (fig. 2) [3].

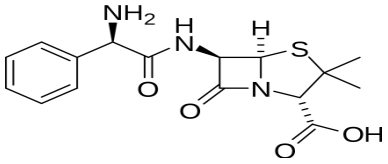


Fig. 2: Chemical structure of amoxicillin

Methotrexate is an anticancer agent which is a folic acid antagonist and it also contains glutamate tail having a carboxylate group which is an electron donor group and hence methotrexate is acidic in nature (fig. 3) [3].

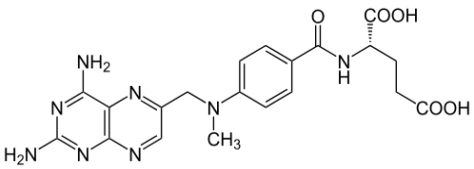


Fig. 3: Chemical structure of Methotrexate

The thiazide diuretics are weakly acidic with a benzothiadiazide 1, 1-dioxide nucleus. The hydrogen atom at the 2-N is the most acidic because of the electron-withdrawing effect of the neighboring sulfone group (fig. 4) [3].

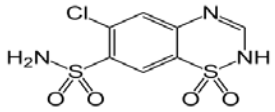


Fig. 4: Chemical structure of Thiazide diuretics

Furosemide has a free carboxylic group in its structure due to which furosemide is strong acid than thiazide diuretics (fig. 5) [3].

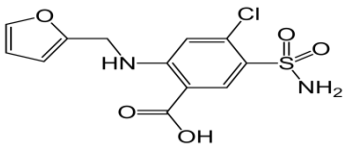


Fig. 5: Chemical structure of furosemide

Barbiturates are cyclic imides used as hypnotic and antiepileptic agents. All barbiturates are derived from barbituric acid which is pharmacologically inactive. Barbiturates contain nitrogen atoms but the lone pair of electrons is not available on nitrogen for the reaction with proton, so barbiturates are weak acid and not a base (fig. 6) [3].

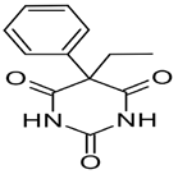


Fig. 6: Chemical structure of Phenobarbitone

Method development and validation for acidic drugs

Acyclovir

For estimation of Acyclovir in bulk and tablet dosage form, new, rapid stability indicating method was developed and validated. For Acyclovir determination, Kromasil ODS C18 (4.6 mm x 250 mm, 5 µm) column was used and MeCN and acetate buffer (pH 4.5) were used as mobile phase in a proportion of 50:50, v/v. The detection was achieved at 253 nm and the run time was 1 ml/min. The retention time was 2.47 min (fig. 7) [4].

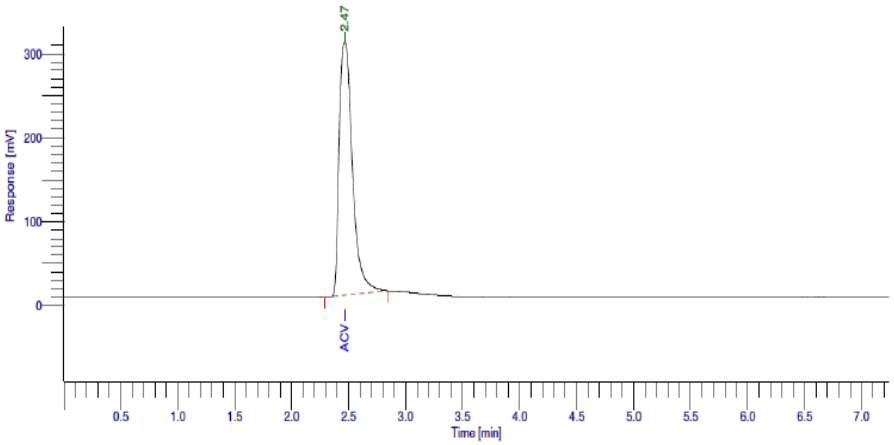
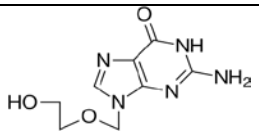
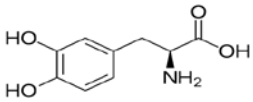
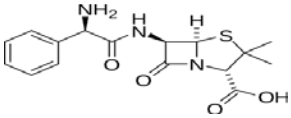
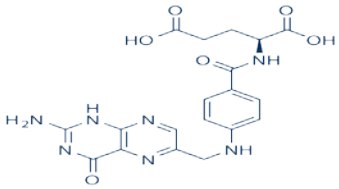
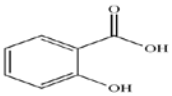
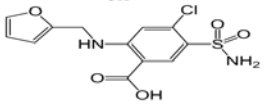
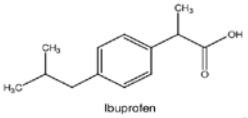
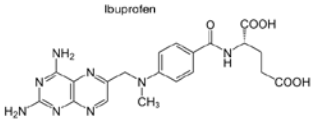
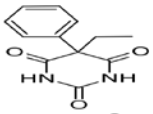
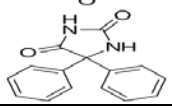


Fig. 7: Chromatogram of acyclovir

Table 1: List of acidic drugs

S. No.	Drugs	Structure	Category	pKa
1.	Acyclovir		Antiviral agent	2.27 and 9.25
2.	Levodopa		Antiparkinson agent	2.3
3.	Amoxicillin		Antibacterial agent	2.62
4.	Folic acid		Hematologic agent	2.7, 4.1and 8.9
5.	Salicylic acid		Keratolytic agent, Antiseptic	3.0
6.	Furosemide		Loop diuretics	3.9
7.	Ibuprofen		NSAIDS	4.2, 5.2
8.	Methotrexate		Anticancer	4.8
9.	Phenobarbital		Sedative	8.1
10.	Phenytoin		Antiepileptic	8.3

Forced degradation study for acyclovir

Acyclovir was subjected to stability indicating a study to check its stability in applied stress conditions. Acid, alkaline, oxidative and thermal hydrolysis stress conditions were applied to acyclovir.

From stability indicating studies, it showed that Acyclovir gets degraded in alkaline and oxidative hydrolysis. 9% drug gets degraded under the alkaline condition and 27% drug gets degraded under oxidative condition (fig. 8-9) [4].

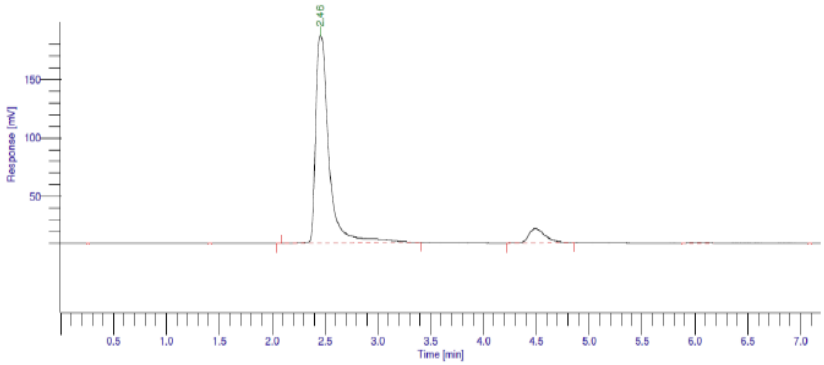


Fig. 8: Chromatogram of alkaline degradation of acyclovir

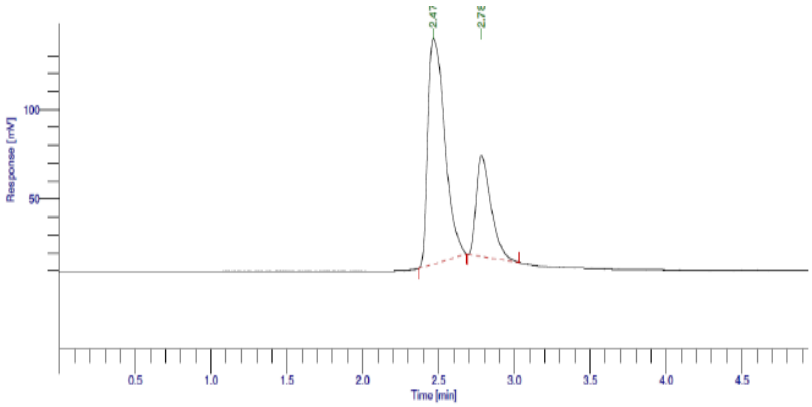


Fig. 9: Chromatogram of oxidative degradation of acyclovir

Levodopa

A new, simple isocratic, RP-HPLC stress indicating method was developed and validated for simultaneous estimation of Levodopa, Carbidopa and Entacapone in a combined dosage form. The separation was carried on Inertsil ODS C18 (4.6 mm x 250 mm, 5 µm) column and mobile phase consisted of water (pH adjusted by using potassium dihydrogen phosphate) and methanol in the ratio of 600:400, v/v. The flow rate was 1 ml/min and column temperature was30 °C. The elution was detected at 257 nm. The retention time for Levodopa, Carbidopa and Entacapone were 3.3 min, 4.1 min, and 9.5 min respectively (fig. 10-11) [5].

Forced degradation study for levodopa

Marketed drug formulation consisting of Levodopa, Carbidopa and Entacapone were subjected to stress conditions of acidic, alkaline, thermal and peroxide degradation. In acidic stress condition, Levodopa gets degraded (fig. 12-14) [5].

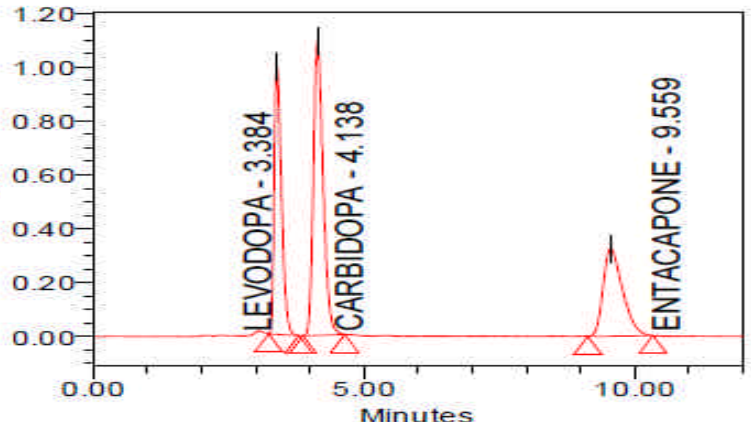


Fig. 10: Standard chromatogram of Levodopa, Carbidopa and Entacapone

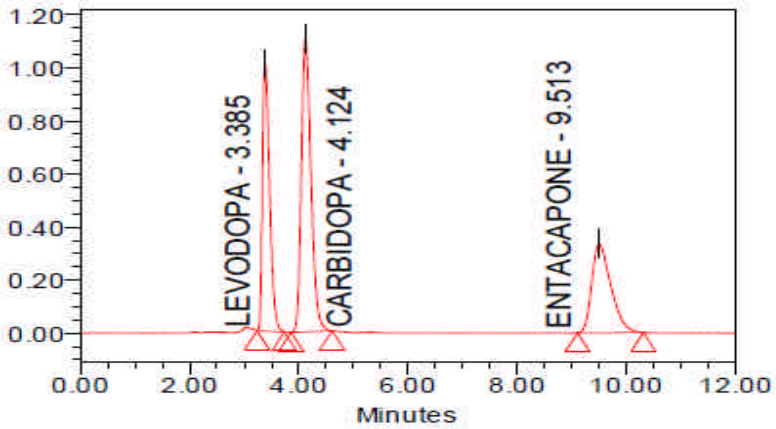


Fig. 11: Formulation chromatogram of levodopa, carbidopa and entacapone

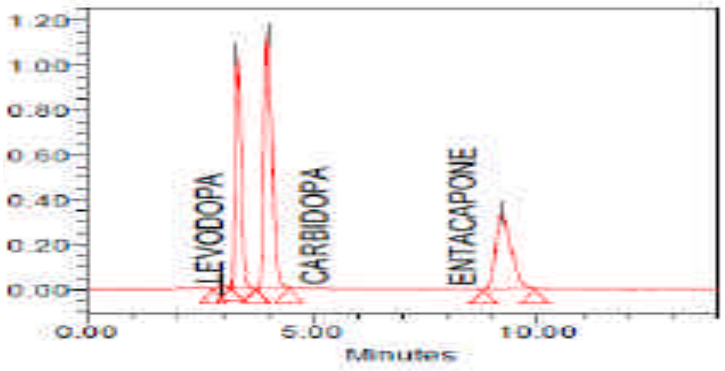


Fig. 12: Chromatogram of acid degradation of levodopa, carbidopa and entacapone

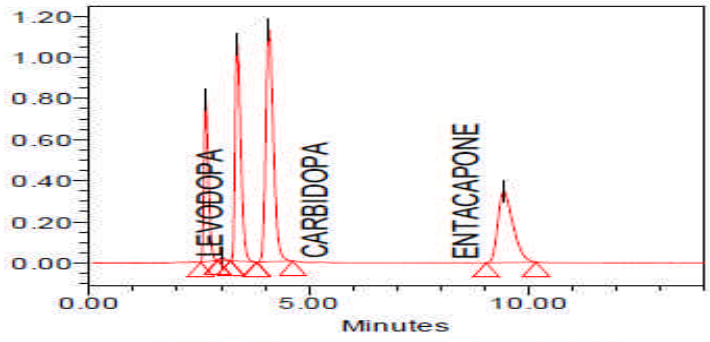


Fig. 13: Chromatogram of heat degradation of levodopa, carbidopa, and entacapone

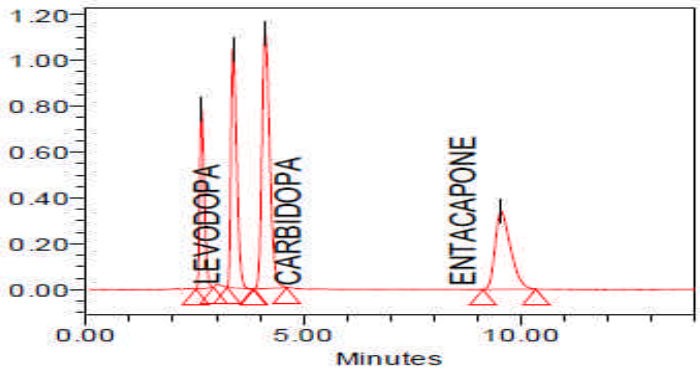


Fig. 14: Chromatogram of alkaline degradation of levodopa, carbidopa, and entacapone

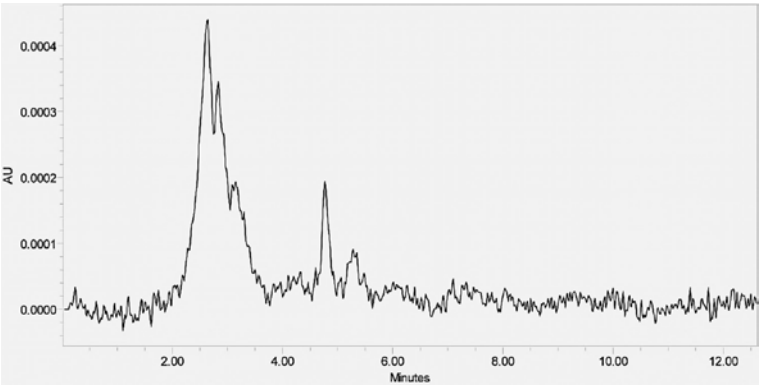


Fig. 15: Chromatogram of working blank solution

Amoxicillin

A new, simple, rapid stability indicating RP-HPLC analytical method was developed for the simultaneous estimation of Flucloxacillin and Amoxicillin in bulk and pharmaceutical dosage form. The chromatographic separation for the two drugs was attained on a Thermosil C18 (4.6 mm x 250 mm, 5 µm) column as the stationary phase at ambient temperature. The mobile phase composed of potassium dihydrogen phosphate buffer (adjusted to pH 3 by using orthophosphoric acid) and Methanol (70:30%, v/v) in isocratic mode at a flow of 1 ml/min, and UV detection at 225 nm. The run time was 8 min (fig. 15-16) [6].

Forced degradation study for Amoxicillin

Amoxicillin and flucloxacillin were subjected to stress conditions of acidic, basic, photolytic and thermal degradation. Amoxicillin degraded in photolytic and thermal conditions (fig. 17)-18) [6].

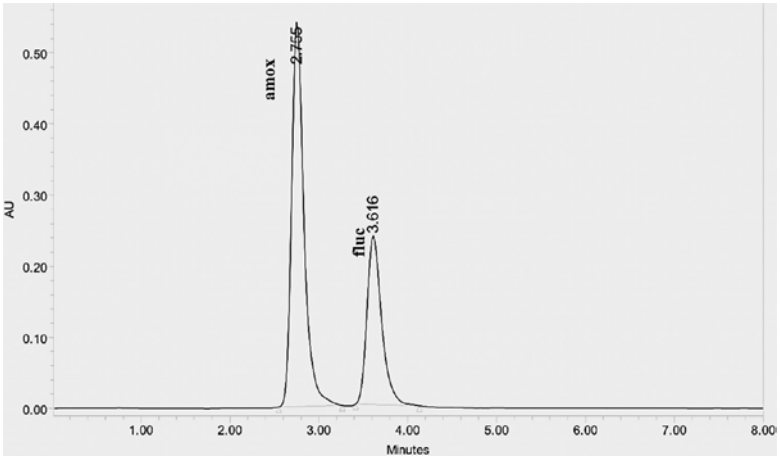


Fig. 16: Chromatogram of the formulation

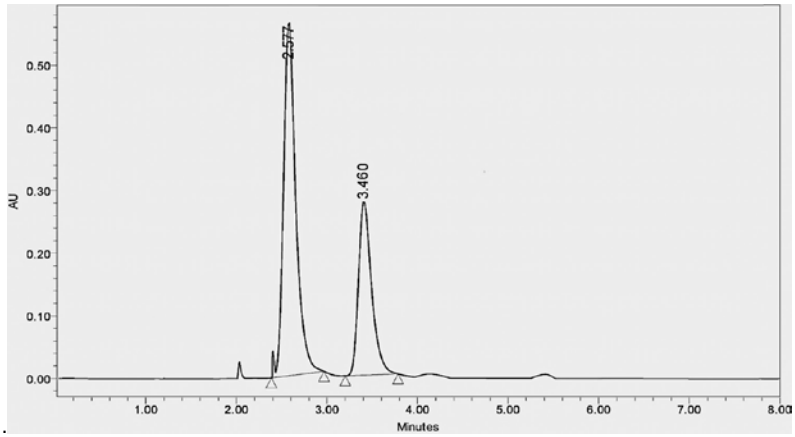


Fig. 17: Chromatogram of photolytic degradation study

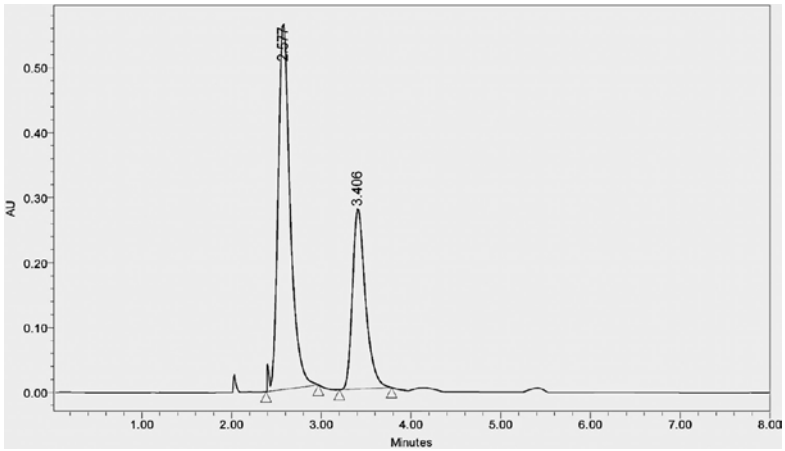


Fig. 18: Chromatogram of thermal degradation study

Folic acid

An isocratic, selective, sensitive reverse-phase high-performance liquid chromatography was developed for the separation and quantification of Methotrexate and Folic acid on the tablet dosage form and further validated. The separation was eluted on Phenomenx C18 column (4.6 mmx250 mm, 5 µm) as stationary phase using mobile phase mixture consisting of acetonitrile and orthophosphoric acid (0.1%) in a ratio of 45:55, v/v. The flow rate was 1 ml/min. The detection was carried out at 215 nm. The retention times were 6.4 min for Methotrexate and 2.8 min for Folic acid (fig. 19) [7].

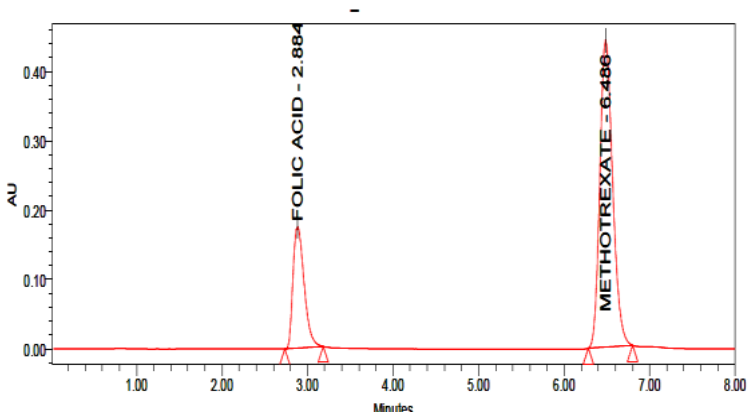


Fig. 19: Typical chromatogram of methotrexate and folic acid

Forced degradation study for folic acid

Marketed formulation of methotrexate and folic acid was subjected to stress conditions such as acidic, alkaline, oxidative, photolytic, reduction, thermal and hydrolysis conditions. Methotrexate and Folic acid are stable under applied stress conditions like thermal, acidic, photolytic stress conditions. No degradants were observed under stability indicating the study of Methotrexate and Folic acid [7].

Salicylic acid

New RP-HPLC stability indicating method was developed and validated for the determination of Salicylic acid in a pharmaceutical dosage form. Shimadzu Prominance L20 AD HPLC equipped with SPD 20A UV-Vis detector was used for the study of the analysis of salicylic acid in a pharmaceutical dosage form. RESTEX allure C18 column (4.6 mm x 12 mm, 3 µm) was used as the stationary phase. For isocratic elution, a mixture of water, methanol, and glacial acetic acid in a ratio of 65:35:1,v/v as mobile phase at a wavelength of 254 nm. The flow rate was 1 ml/min. The retention time was 7.65 min (fig. 20-21) [8].

Forced degradation study for salicylic acid

Salicylic acid was subjected to stress conditions like alkaline, acidic, neutral and photolytic stress conditions. No degradation was found in alkaline, acidic, photolytic and neutral stress conditions [8].

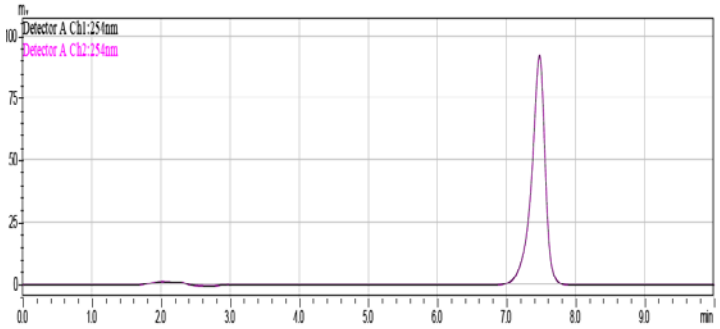


Fig. 20: Chromatogram of salicylic acid (150 ug/ml)

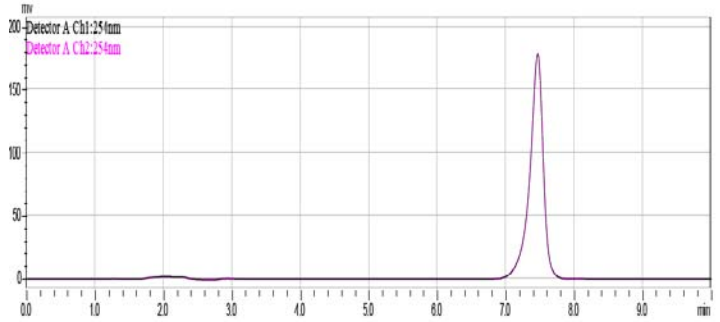


Fig. 21: Chromatogram of salicylic acid (300 ug/ml)

Furosemide

A new, simple stability-indicating method and assay method was developed and validated for the simultaneous estimation of Spironolactone and Furosemide in a pharmaceutical solid dosage form. The separation was carried on SGE SS Wakosill II 5C8RS (4.6 mm x 150 mm, 5 µm) column at ambient temperature. The mobile phase composed of acetonitrile and ammonium acetate, in a ratio of 50:50,v/v. The separation was detected at 245 nm. The flow rate was 1 ml/min. The retention time for Spironolactone and Furosemide was 2.9 min and 7.1 min respectively (fig. 22) [9].

Forced degradation study for furosemide

The marketed formulation of Spironolactone and Furosemide was subjected to acid, alkaline, thermal, oxidation and photolytic degradation. In acidic condition, 8.72% drug degraded (fig. 23) [9].

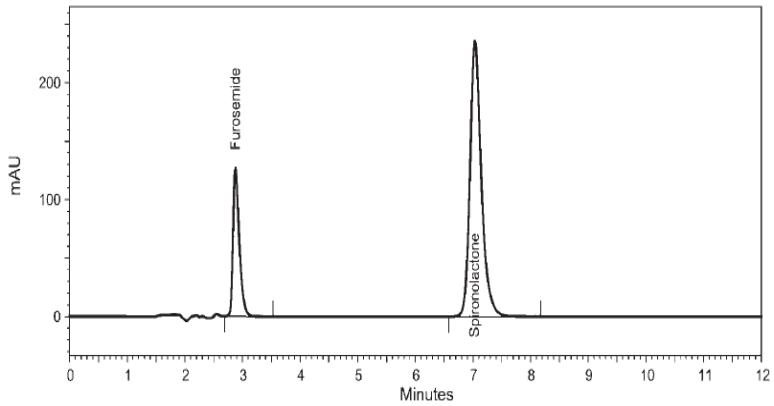


Fig. 22: Chromatogram of spironolactone and furosemide

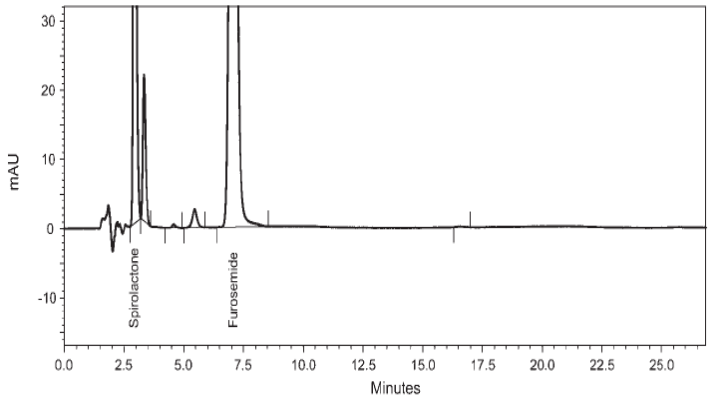


Fig. 23: Chromatogram of alkali degradation of spironolactone and furosemide

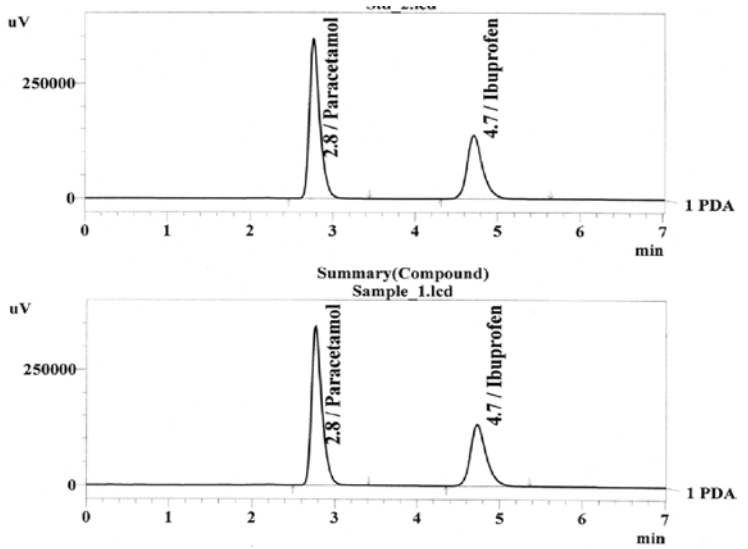


Fig. 24: Chromatograms of blank, standard, and sample

Ibuprofen

A new, accurate, rapid and stability indicating method was developed and validated for the simultaneous estimation of Paracetamol and Ibuprofen in their combined dosage form. The elution was performed on C18 Phenomenex (4.6 mm x 250 mm, 5 µm) column and mobile phase contained phosphate buffer (pH 6.8) and acetonitrile in the ratio of 65:35, v/v. The flow rate was 0.7 ml/min. The elution was detected at 222 nm. The retention time for Paracetamol and Ibuprofen were 2.8 min and 4.7 min respectively (fig. 24) [10].

Forced degradation study for Ibuprofen

The standard mixture of Paracetamol and Ibuprofen was subjected to stability indicating studies such as acidic, basic, reduction and oxidative stress conditions. During stability studies, it was observed that Ibuprofen was stable in acidic condition but it showed degradation in strong basic condition within the limit (fig. 25) [10].

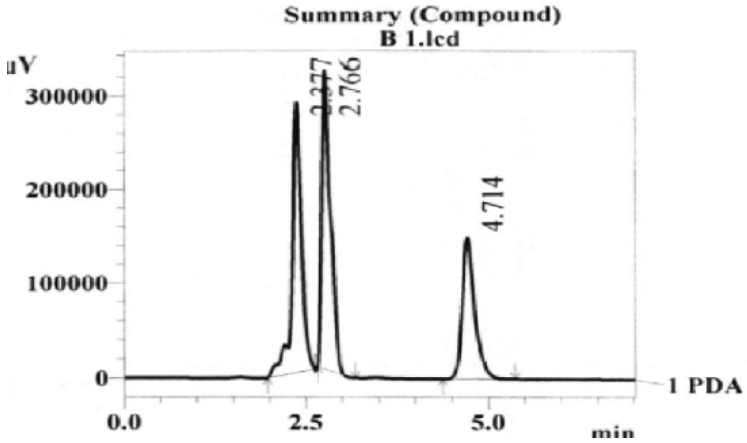


Fig. 25: Chromatogram of base degradation of Ibuprofen

Methotrexate

An isocratic, selective, sensitive reverse-phase high-performance liquid chromatography method has been developed for the separation and quantification of Methotrexate and Folic acid for the tablet dosage form and validated. The separation was eluted on Phenomenx C18 column (4.6 mm x 250 mm, 5 µm) as stationary phase using mobile phase mixture of acetonitrile and orthophosphoric acid (0.1%) in a ratio of 45:55, v/v at a flow rate of 1.0 ml/min. The detection was carried out at 215 nm. The retention times were 6.4 min for Methotrexate and 2.8 min for Folic acid (fig. 26) [11].

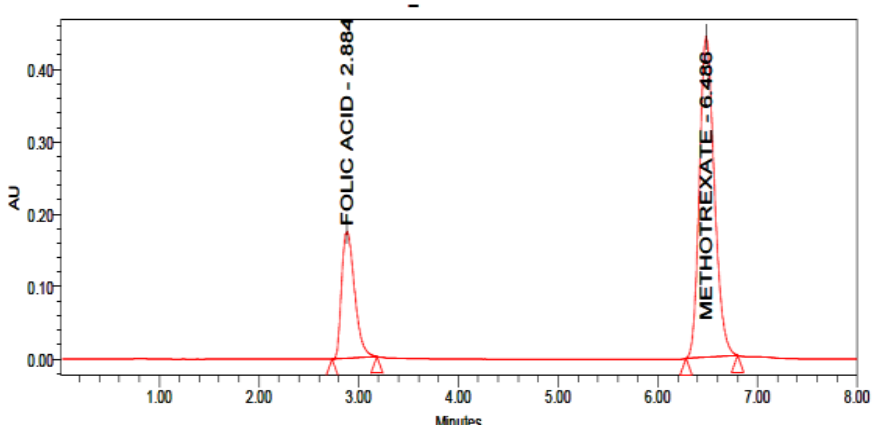


Fig. 26: Typical chromatogram of methotrexate and folic acid

Forced degradation study for methotrexate

Marketed drug formulation of Methotrexate and Folic acid were subjected to stress conditions such as acidic, alkaline, oxidative, photolytic, reduction, thermal and hydrolysis conditions. The methotrexate and folic acid were stable under applied stress conditions like thermal, acidic, photolytic stress conditions. No degradants were observed under stability indicating studied of Methotrexate and Folic acid [11].

Phenobarbitone

A new simple RP-HPLC stability indicating method was developed and validated for quantification of Phenobarbitone in a pharmaceutical dosage form. The elution was carried out on C18 R (4.6 mm x 250 mm, 5 µm) column and acetonitrile and methanol used as mobile phase in a ratio of 65:35,v/v in isocratic mode. The detection was done at 237 nm. The retention time was 4.78 min (fig. 27) [12].

Forced degradation study for phenobarbitone

The working standard of Phenobarbitone was subjected to various stress conditions such as acid, base, oxidation, thermal, dry heat and photolytic stress conditions. From forced degradation studies it was found that Phenobarbitone gets degraded in acid, base, oxidative and thermal stress conditions (fig. 28-31) [12].

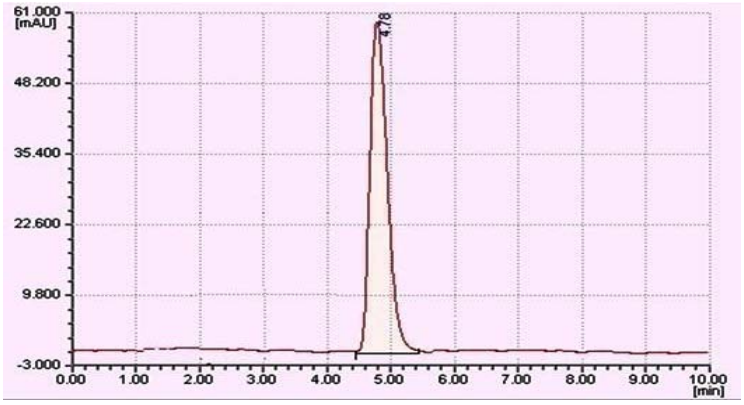


Fig. 27: Chromatogram of phenobarbitone

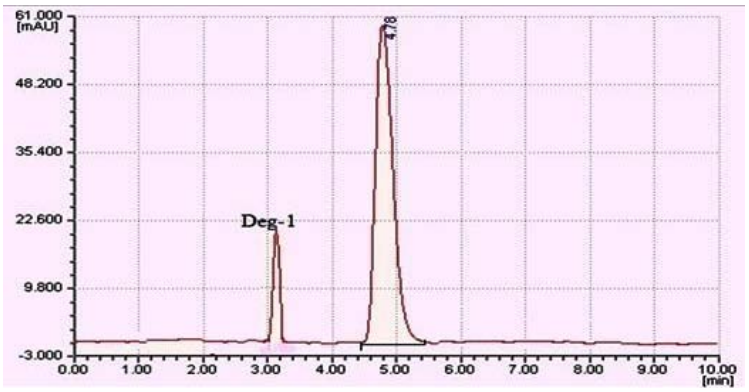


Fig. 28: Chromatogram of phenobarbitone after acid hydrolysis

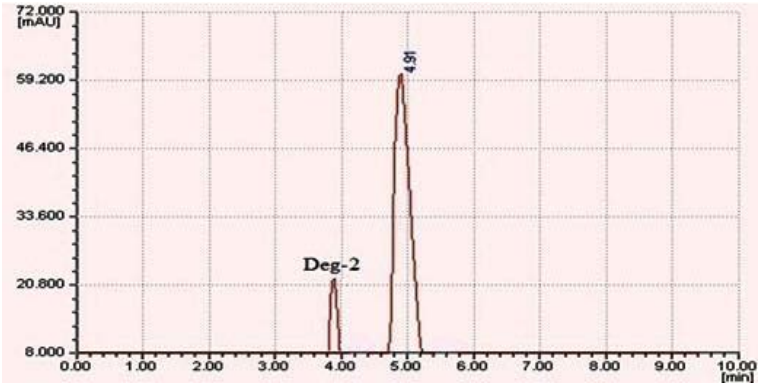


Fig. 29: Chromatogram of phenobarbitone after alkali hydrolysis

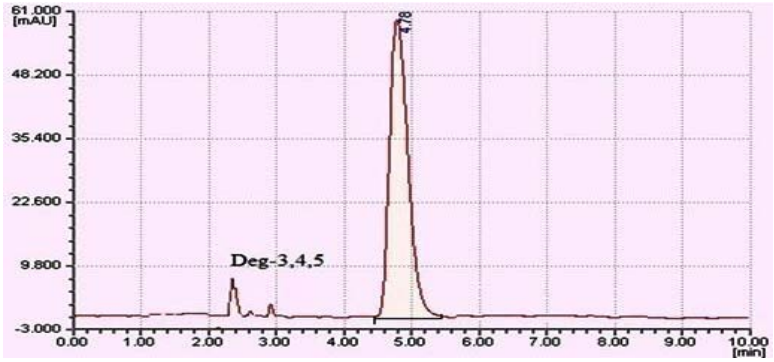


Fig. 30: Chromatogram of phenobarbitone after oxidative hydrolysis

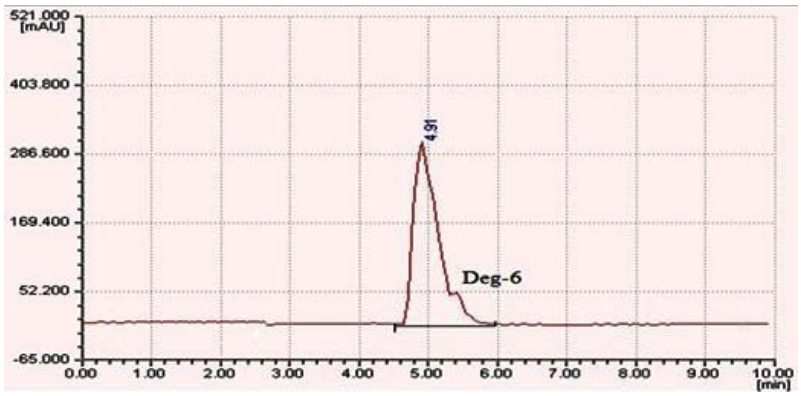


Fig. 31: Chromatogram of phenobarbitone after dry heat degradation

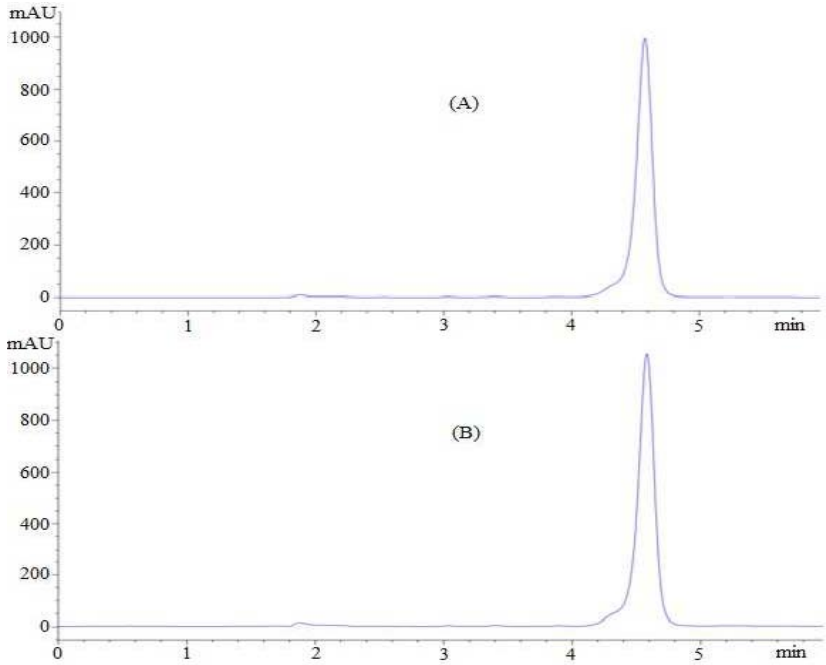


Fig. 32: Typical chromatogram of standard phenytoin (A) and Phenytoin from formulation (B)

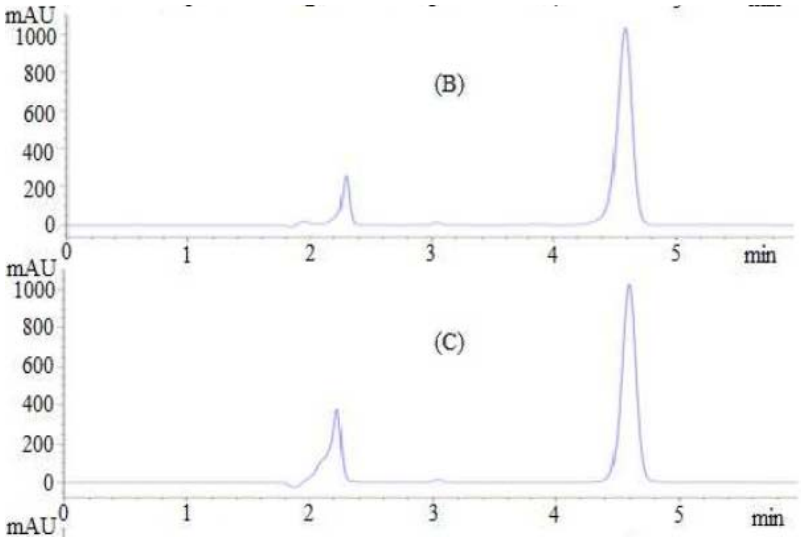


Fig. 33: Chromatogram of oxidative degradation of phenytoin (b), and base degradation (c)

Phenytoin

A simple, new and stability indicating method was developed and validated for the estimation of Phenytoin in a pharmaceutical dosage form. The separation was achieved by using Zorbax C18 (4.6 mm x 250 mm, 5 µm) column at ambient temperature and mobile phase contained acetonitrile and water in a ratio of 50:50%, v/v in isocratic mode. The detection was done at 200 nm and the flow rate was 1 ml/min (fig. 32) [13].

Forced degradation study for phenytoin

Phenytoin subjected to stress conditions such as acid, base, oxidative and photolytic condition and from forced degradation studies it showed that Phenytoin gets degraded in oxidative and basic stress conditions (fig. 33) [13].

CONCLUSION

High-Performance Liquid Chromatography has a wide variety of uses in many fields such as analysis, separation, and identification of pharmaceutical drug, impurity and biological samples. This article gives an overview of analysis and stability indicating method development and validation of acidic drugs.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

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