Determination of Nitrofurantoin in Drug Formulations by High Performance Liquid Chromatography

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Abstract

In the present work the Nitrofurantoin drug is determined in its pharmaceuitical preparation using High Performance Liquid Chromatography (HPLC) technique with L_1 column length 25cm and 4.6mm diameter. The mobile phase of acetonitrile ,and buffer solution pH 3.3 and ratio (30:70) for used.The flow rate was 1.0 m1 min^{-1} , Injection volum was 100 µl of 5ppm of (NFT) . The signal was detected at 254 nm with retention time 4.592 min in the range 5-25 ppm and detectiom limit 0.3425.

This method was succefully applied for the determinarion of Nitrofurantoin its pharmuceuitical preparation with recovery of not less than 106.8%

تحديد نتروفورانتوين في المخدرات عن طريق صياغات عالية الأداء اللوني السائل

عماد طارق حنون حسين حسن خرنوب

مفتاح البحث: النايتروفيورنتين, كروماتوغرافيا, الاسيتونتريل, الاشعة فوق البنفسجية

المستخلص :

تم تطوير طريقة دقيقة وحساسة لتقدير النايتروفيورنتين في المستحضرات الصيدلانية باستخدام تقنية كروماتوغرافيا السائل عالي الاداء. إذ تم حقن 100 مايكروليتر من محلول الدواء ذو التركيز 5 جزء من المليون من العقار بأستخدام عمود نوع L_1 بطول 25سم وقطر 4,6 ملم وباستخدام الاسيتونتريل والمحلول المنظم بنسبة (70:30) كطور متحرك عند سرعة الجريان 1.0 مل . دقيقة $^{-1}$ وباستخدام كاشف الاشعة فوق البنفسجية عندالطول الموجي 254 ناتوميتر وقد تم استقصاء الظروف التجريبية من(نوع العمود , والطور المتحرك , pH, الطول الموجي , الكاشف , معدل سريان الطور المتحرك) تراوحت خطية الطريقة بين (5–25 ppm) وزمن الاحتجاز 4.592 دقيقة, وحد الكشف 0.3425 وكانت معدل الاسترجاعية للطريقة 106.8%.

Introduction

HPLC technique is the best method for separation of consistuents of sample using the ability of the consistuents to migrate at different time through stationary phase under the influence of mobile phase [(1)] .

The principle of separation depend upon the difference in retention volume (V_R) for each analyte in samples .

$V_R = t_R \times F$ _____ (1)

Where V_R = Retention volume , t_R = Retention time and F = FlowRate of mobile Phase .

The chromatogram in HPLC represents the relation between retention time and the response of detector [(2)] .

HPLC classified as Reverse Phase (RP) and normal phase (NP) depending upon physical properties of stationary phase or the polarity of mobile and stationary phases [(3)] .

The efficiency of stationary phase or column is directly proportional to number of High Equivalent theoretical plates (HETP) [(4)] .

$N = 16 \left(\frac{tR}{w} \right)^2$ _____ (2)

Where N= number of HETP , t_R = Retention time , W = width of peak.

Equation (2) indicates that the width of the peak is inversely proportional to the HETP .

The factors that effect on the width of peak are described by Van Deemter equation (3)

$HETP = A + B/U + C$ _____ (3)

Where A , B , C are factors describe the migration of analyte through column .

The separation of analyte depending upon the partition coffient (K) , which is the ratio between concentration of analyte in stationary state (C_S) to concentration of analyte in mobile phase (C_M) .

$K = \frac{Cs}{Cm}$ _____ (4)

Equation (4) indicates the partition coffient is inversely proportional to concentration of analyte in mobile phase . The volume of mobile phase used to pass analyte from column depend upon many analytical parameters such as retention time , flow rate and type of stationary phase[(6)] .

The solvent used in mobile phase should not viscouse and not absorbe by ultra – violet radiation at wave length used for analysis of analyte in sample [(7)] .

Selection of stationary phase column depends upon analyte in sample and the resolution [(8)] .

There are many methods used for quantitative determination of drugs in their formulations such as measured peak height , peak area and internal standard .

The chemical formula of NTF is $C_8 H_6 N_4 O_5$ and used as anti-bacterial klebsiella , staph areus . such as E.coli , it is present in different formulations such tablets , syrup , capsules and injection [(9)] .

Experimental work and Results

Stock standard solution was prepared by dissolving 0.0025 gm of pure NTF in 10 ml of Dimethyl formamide . and the volume was completed in 100 ml volumetric flask . Different concentrations range from 5 – 25 ppm were prepared by dilution .
Buffer phosphate solution was prepared by dissolving 6.8 gm of potassium orthophosphate (K_2HPO_4) in 500 ml of De-ionized water and 30 ml of 0.1M NaOH was added to adjust the pH to 7 , then Diluted to the volume to 1 liter with De-ionized water .

Selection of Analytical parameters

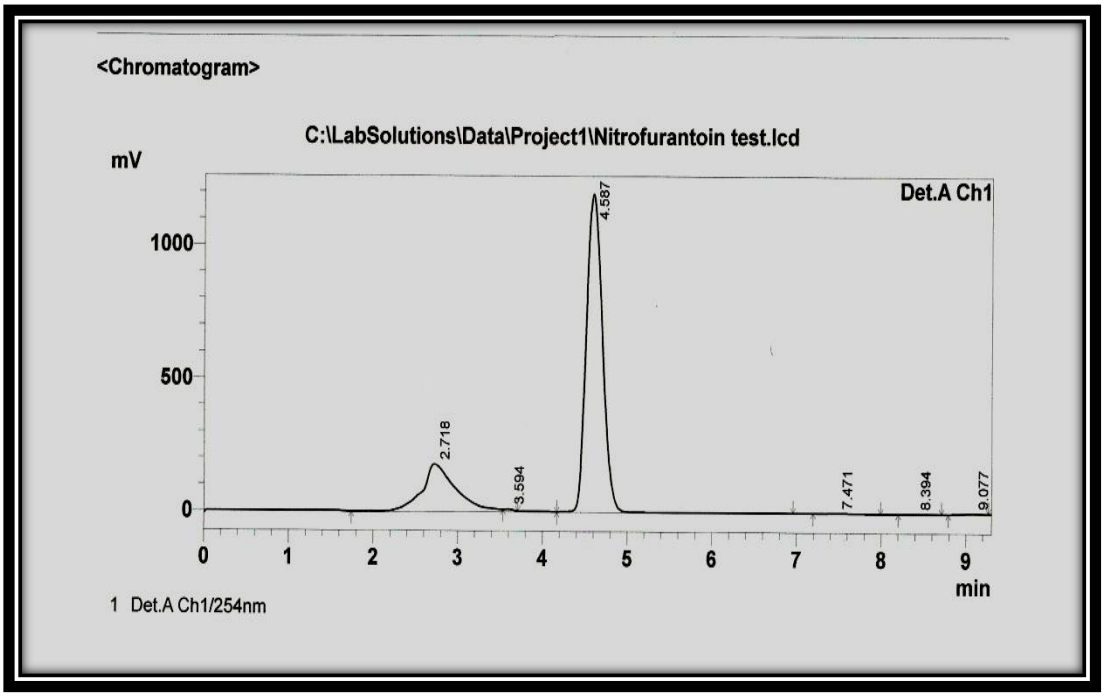
We select L_1 column the best one at length 25 cm and 4.6 mm according to previous workers [10 - 12] .

Table (1) Analytical parameters for separat ion of NTF by HPLC .

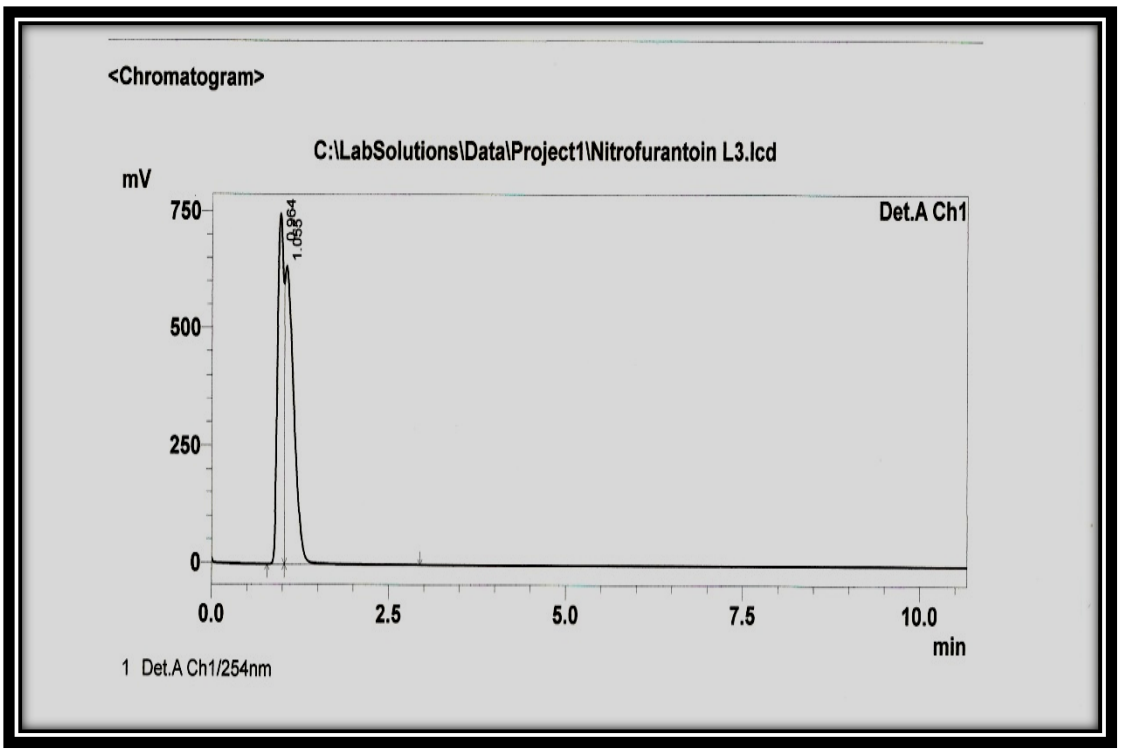
NO	Parameters	Qualitative
1	Mobil phase	L_1 column 25cm and 4.6 diameter
2	Stationary phase	Acetonitrile : Buffer solution pH3.3 (30 : 70)
3	Detector	UV
4	Cencentration	5 ppm
5	Injection Volume	100 µl
6	Flow Rate	1 ml min^{-1}

Many attempts were carried out to use different columns by injection 100 µl of 5 ppm of NTF applying the parameters listed in Table (1).

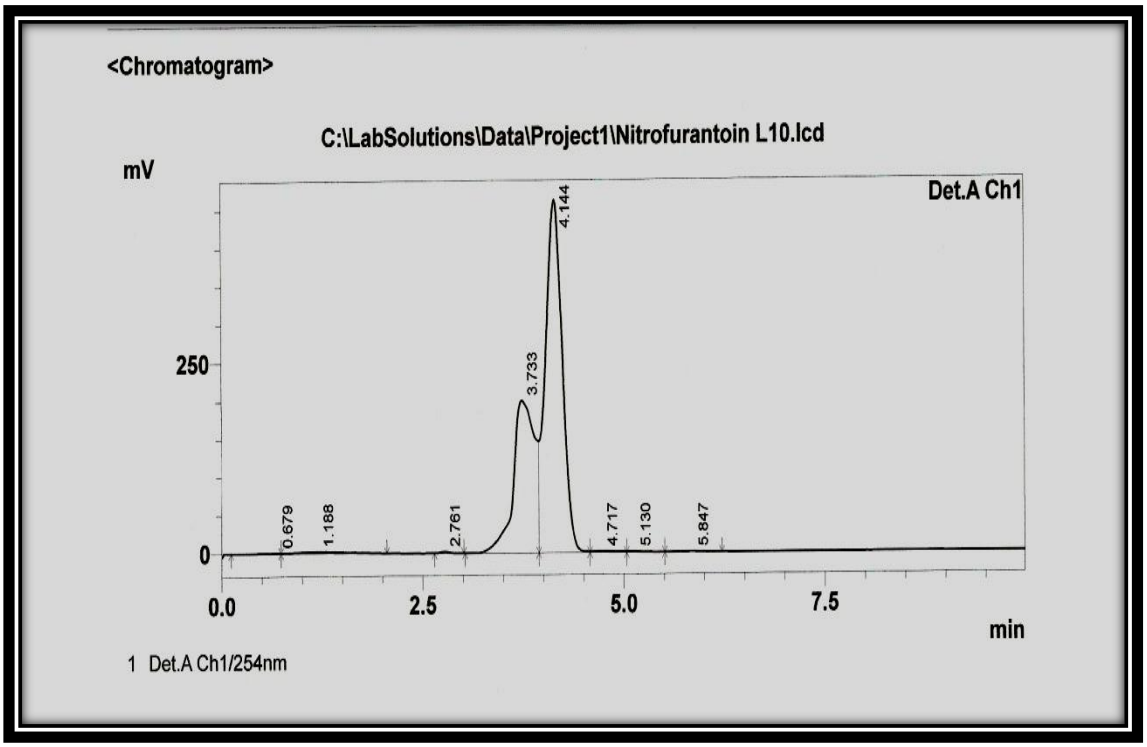
The results obtain in chromatogram (1-3) and Table (2)



Chromatogram (1) separation of NTF at column L_1



Chromatogram (2) separation of NTF of column L_3



Chromatogram (3) separation of NTF of column L_{10}

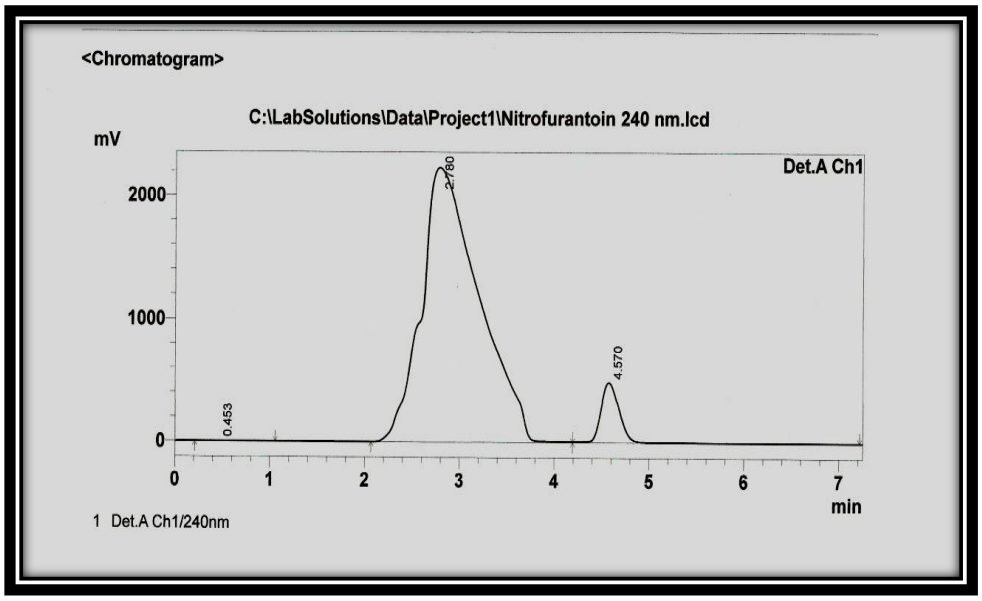
Table (2) Influence of type of column on separation of NTF by HPLC

No	Type of column	Peak height(mv)	Peak area(mv)	Mobile phase	Retention time(min)	HETP	Comments
1	L_1	514428	6610256	Buffer:acetonitril 70:30	4.325	0.2682	Good bond,low HETP and Recovery accepted
2	L_3	635703	5564591	Buffer:acetonitril 70:30	1.005	0.9746	Good bond, high HETP and Recovery low
3	L_{10}	464282	6758560	Buffer:acetonitril 70:30	4.144	1.0000	Two bond,high HETP and Recovery high

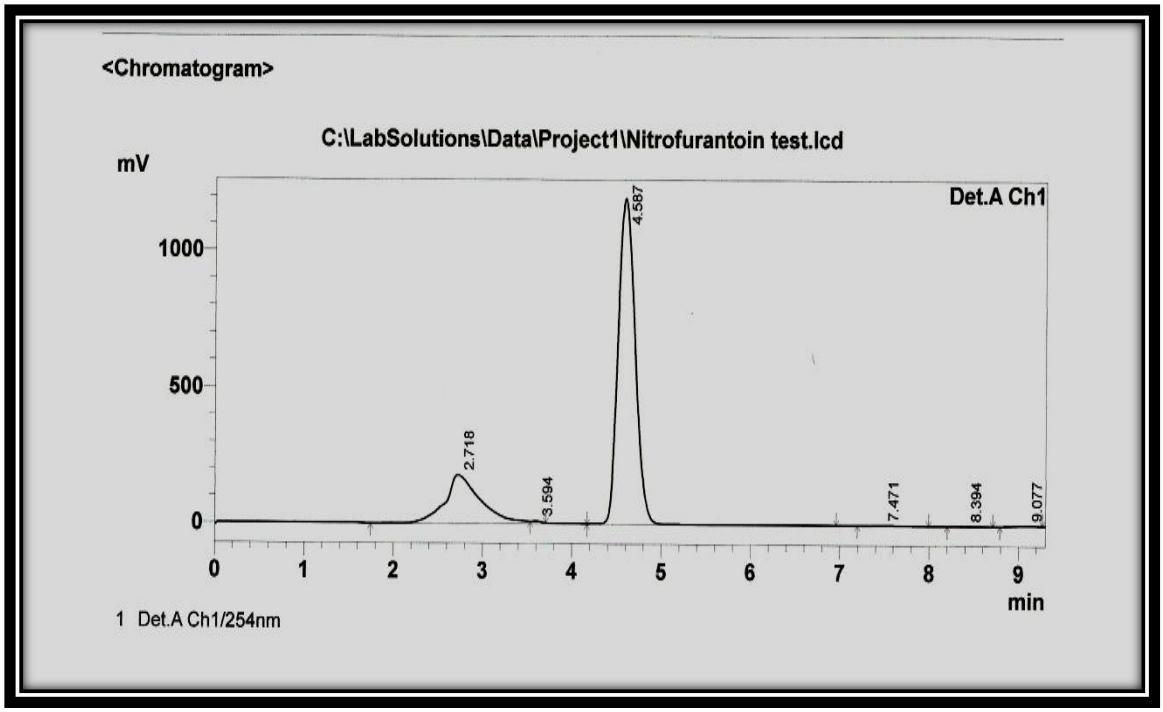
Wave length λ max

absorbance 5 ppm of NTF was run by ultra-violt spectrophotometery , the maximum was found at 254 nm, there fore we used this wave length to carried out many studies to select the maximum absorbance at specific wave length (λ max) by injection 100 µl of 5 ppm of NTF using HPLC conditions technique using list in the table.

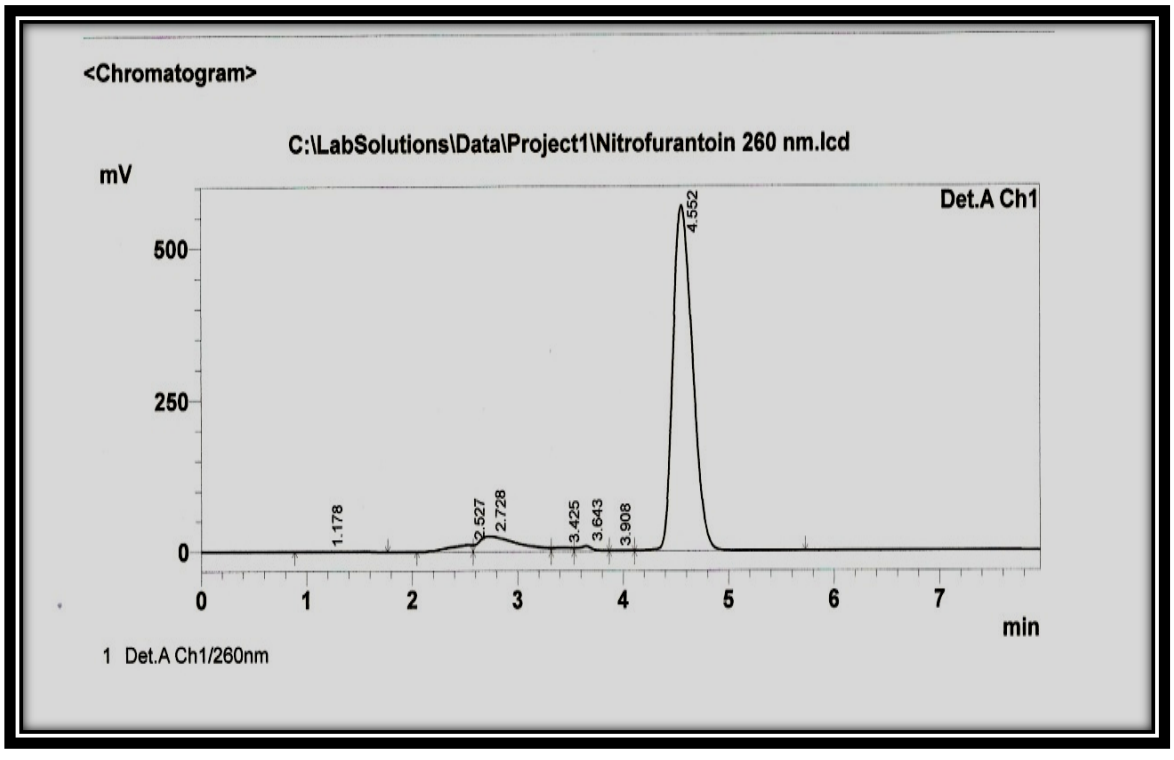
The result obtain are showing in chromatograms (4 -6) and Table (3)



Chromatograms (4) of NTF at 240 nm



Chromatograms (5) of NTF at 254 nm



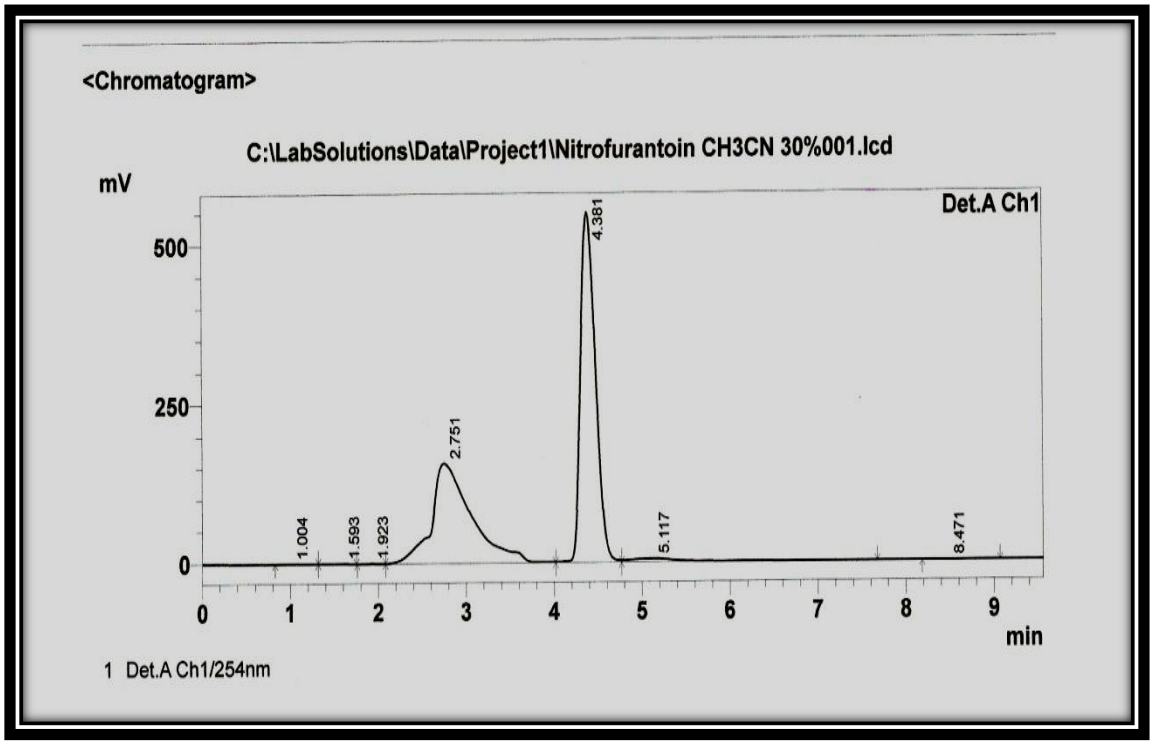
Chromatograms (6) of NTF at 260 nm

Table (3) influence of λ max on separation of NTF by HPLC

No	λ max(nm)	Peak height(mv)	Peak area(mv)	Retention time(min)	HETP	Comments
1	240	489061	6580745	4.570	0.2546	Good bond,high HETP and Recovery low
2	254	1196270	16021615	4.552	0.0838	Good bond,low HETP and Recovery accepted
3	260	569629	7354148	4.587	0.2525	Good bond,high HETP and Recovery low

Mobile phase

The same previous procedurs for selection of mobile phase. The results obtained are listed in table (4) and chromatogram 7



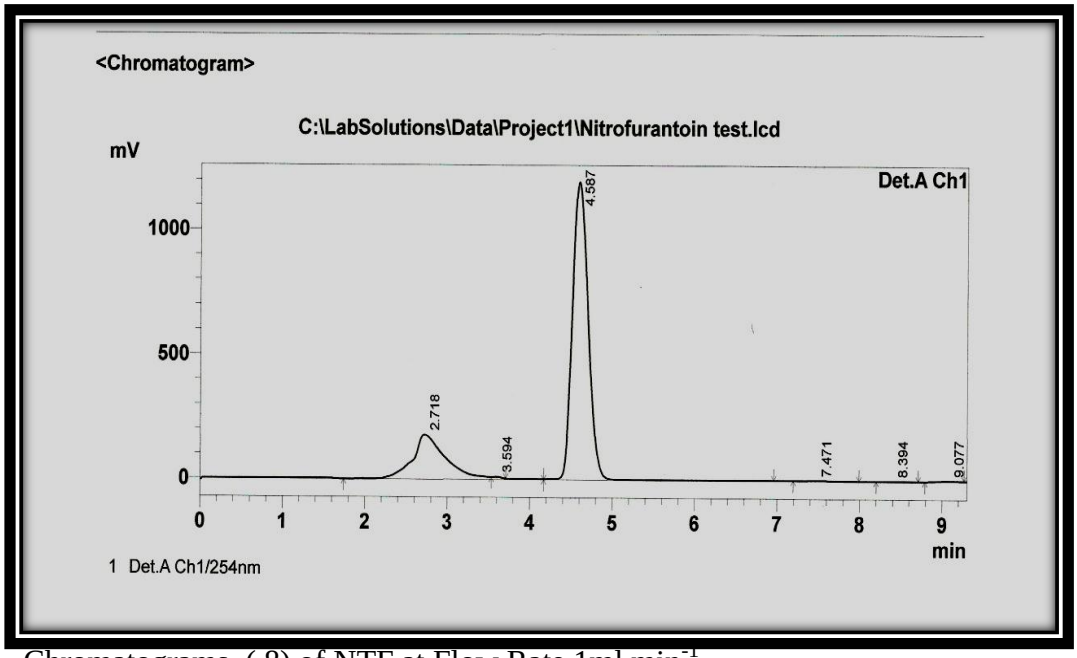
Chromatograms (7) of NTF at mobile phase acetonitril:buffer 30:70

Table(4) Influence of mobile phase on separation of NTF by HPLC

NO	Mobile phase	Peak height(mv)	Peak area(mv)	HETP	Comment
1	Acetonitrile: Buffer 5:95	153082	6326923	0.1596	Good bond,high HETP and Recovery low
2	Acetonitrile: Buffer 10:90	262948	6126309	0.2572	Good bond,high HETP and Recovery low
3	Acetonitrile: Buffer 20:80	427997	6563222	0.2481	Slow bond,high HETP and Recovery high
4	Acetonitrile: Buffer 30 : 70	550343	6478690	0.1303	Good bond,low HETP and Recovery accepted
5	Ether: Nitromethan 50 : 50	43847	2015544	0.4321	Good bond,low HETP and Recovery low

Flow Rate for mobile phase:

The same procedures for selection of mobile was carried out. The results obtain are listed in table (5) and chromatogram (8)



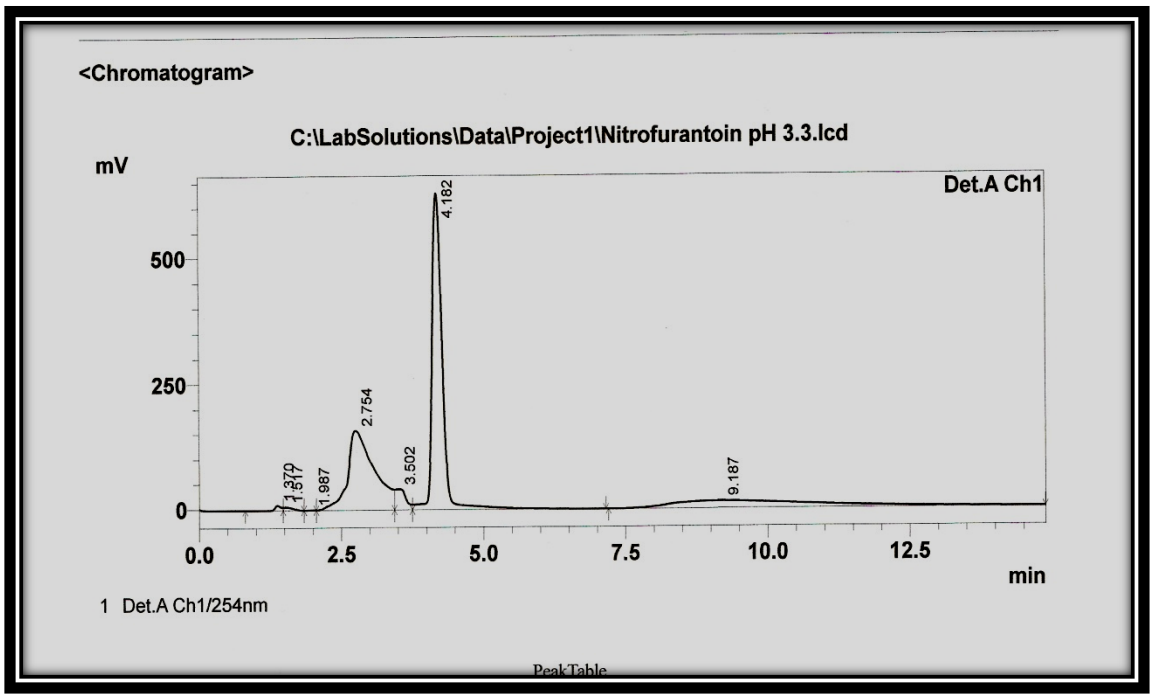
Chromatograms (8) of NTF at Flow Rate 1ml.min^{-1}

Table (5) influence of Flow Rate on separation of NTF by HPLC

NO	Flow Rate(ml/min)	Peak height(mv)	Peak area(mv)	HETP	Retention time(min)	Comment
1	0.5	1403646	31835386	0.3282	8.970	Good bond,high HETP and Recovery high
2	1.0	1191572	15853263	0.1852	4.592	Good bond,low HETP and Recovery accepted
3	1.5	1152296	10829451	0.2699	5.072	Good bond,high HETP and Recovery low

Effect of pH

The same procedure for selection at mobile phase was used for study the pH on separation of NTF by HPLC. The result obtained are listed in Table (6) and chromatogram (9).



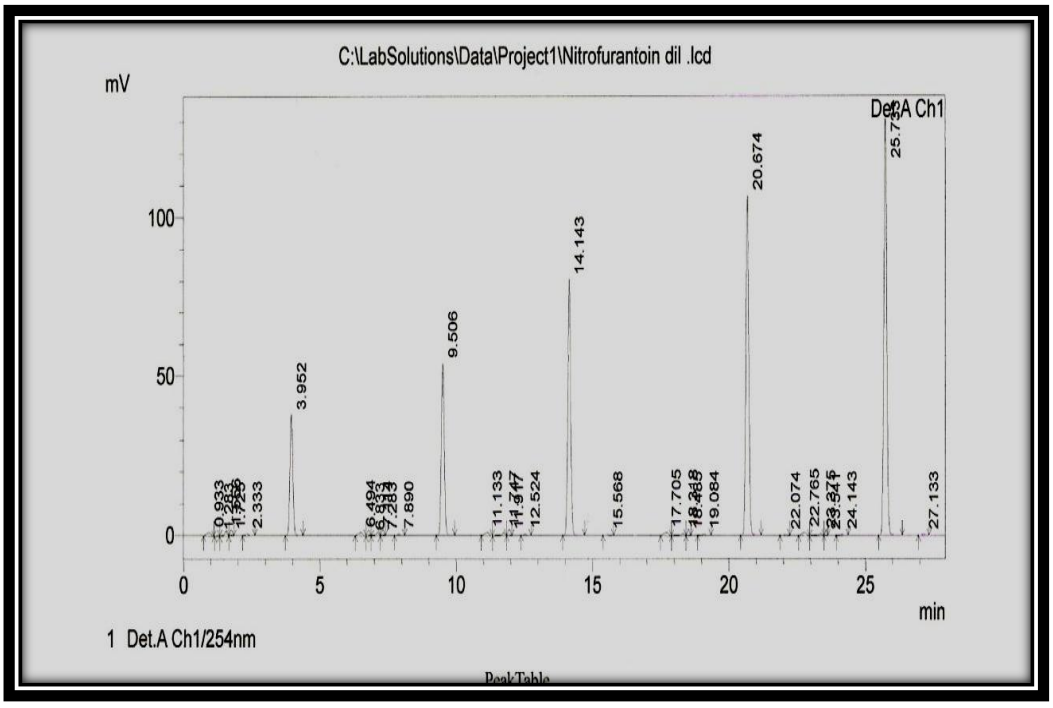
Chromatograms (9) of NTF at pH 5.4

Table (6) influence of pH on separation of NTF by HPLC

NO	pH	Peak height(mv)	Peak area(mv)	HETP	Comment
1	2.2	465515	7137406	0.9887	Refused bond,high HETP and Recovery low
2	3.3	630957	7975315	0.3040	Good bond,high HETP and Recoveryhigh
3	5.4	514428	6610256	0.1123	Good bond,low HETP and Recovery accepted
4	8.5	156918	2263231	0.9064	Refused bond,high HETP and Recovery low
5	9.5	391402	5308065	0.9241	Good bond,high HETP and Recovery low

Calibration Curve

Different concentrations 5-25 ppm were prepared. 100 µl of each concentration of NTF was injected by HPLC technique using L_1 column, acetonitrile : Buffer solution pH 3 (30:70), flow rate 1 ml / min and the chromatogram was registed at at ʎ max 254 nm. The result obtain in Table (7) and Figure (10,11).



Chromatograms (10) of NTF calibration curve

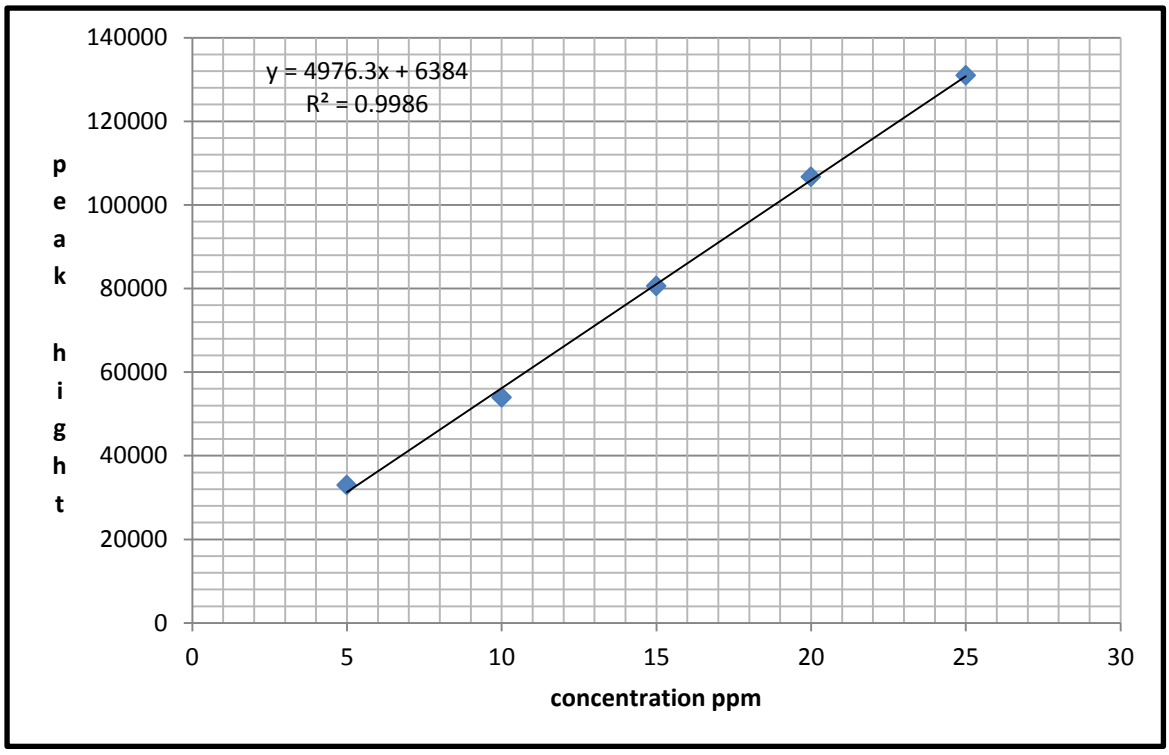


Table (7) influence of calibration curve on separation of NTF by HPLC

NO.1	Conc. (ppm)	Retention time(min)	R.E %	Recovery %	Standard deviation	R.S.D%	HETP
1	5	4.042	6.80	100.8	0.063	0.062	1932.500
2	10	4.045	4.50	99.5	0.063	0.065	1935.000
3	15	4.056	0.67	99.3	0.054	0.054	1925.000
4	20	3.994	0.80	100.8	0.054	0.047	2003.500
5	25	3.987	0.16	100.16	0.014	0.014	1990.500

Detection Limit

It was calculated by using lowest concentration of calibration. The results obtained in Table (8).

Table (8) Detection Limit of NTF in HPLC technique .

NO.	Conc. ppm	Peak height(mv)	Peak area(mv)	HETP
1	5	26465	16202467	0.0542
2	5	27010	16132897	0.0213
3	5	26341	16200896	0.0723

Recovery Percentage

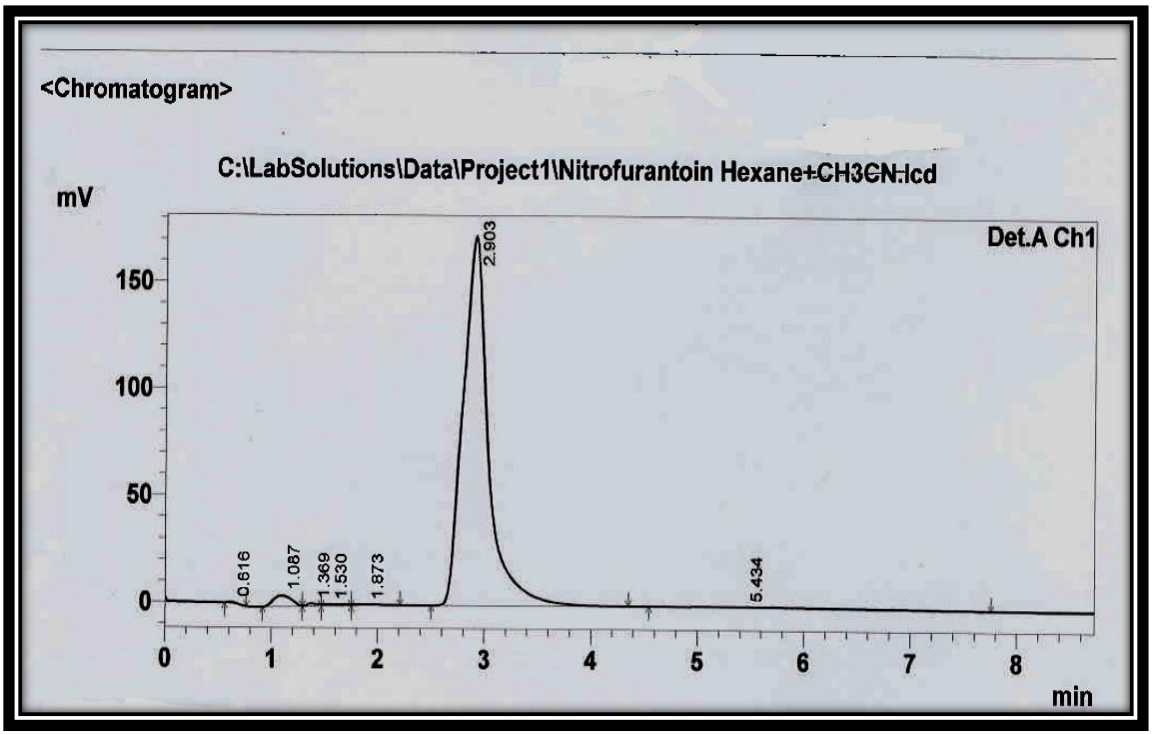
100 µl of 10 ppm of NTF was injected by HPLC using analytical parameter listed in Table (1) , the experimental was repeated three time and the chromatogram for each case was recorded. The results obtained listed in Table (9).

Table (9) Recovery of NTF by HPLC

NO	Conc. . added(ppm)	Conc.(ppm)	Peak area(mv)	Peak height(mv)	HETP	comment
1	10	9.55	18522470	48202	0.1231	Good bond,low HETP and Recovery accepted
2	10	10.10	18603852	49001	0.1500	Good bond,low HETP and Recovery accepted
3	10	9.95	18511103	48390	0.2103	Good bond,low HETP and Recovery accepted

Changing of Rp-HPLC to Np- HPLC the system of HpLc was changed from Rp-HpLc to NP-HPLC by using non polar mobile phase (Benzen and Cyclo hexan) and non polar stationary phase L_{10} . The experiment was applied at the same flow rate, wave length , injection volume and concentration of NTF mentioned in Table (1)

The results obtained are described in Table (10) and chromatogram (12)



Chromatograms (12) of NTF NP-HPLC

Table (10) NP-HPLC for separation of NTF in HPLC

NO	Mobile phase	Peak height(mv)	Peak area(mv)	Retention time(min)	HETP	comment
1	hexon:Benzenل 1 : 1	172549	2866355	2.903	1.0021	Good bond,low HETP and Recovery accepted
2	Ethen : ester 1 : 1	46662	1766406	7.004	1.3271	Good bond,low HETP and Recovery accepted

Analysis of NTF in Drug formulations:.

Tablet

10 tablets containing 10 mg per Tablet from the powder were grounded. 0.57gm was dissolved with 3×25 ml of methanol using ultrasonic shaker for 15 minutes, the

volume was completed to 100 ml with methanol ,and dilute 10 ml from this solution to 100 ml with same solvents . the solution filtered at filter paper witman 41 µm.

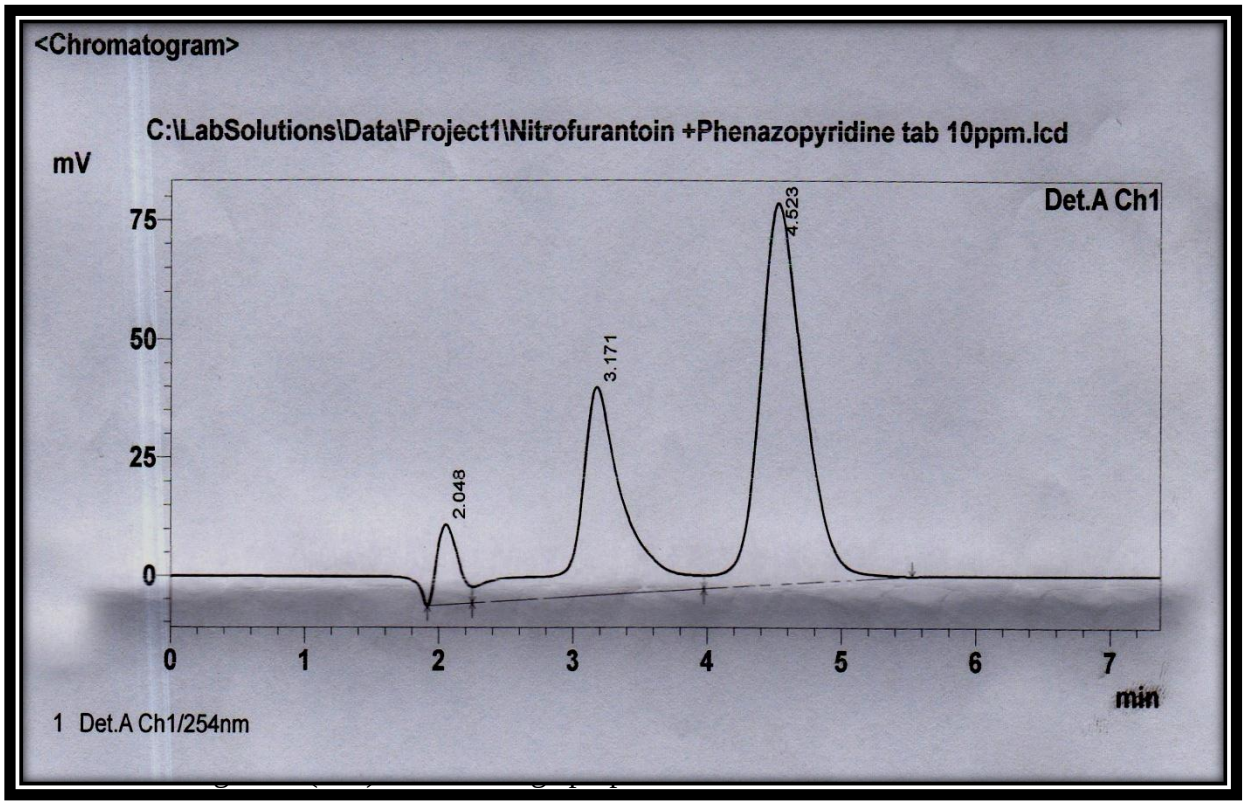


Table (11) druge preparation on separation of NTF by HPLC

substance	Added (ppm)	Found (ppm)	Recovory %
Nitrofurantoin	10	9.51	95.1%
Nitrofurantoin	20	19.9	99.9%

Disccusion:

Fig.13 depicts atypical chromatogram of phenazopyridine HCl and Nitrofurantoin obtained using the described procedure . trials were made to separate the to species using a µ-Bondbak C18 column with different mobile phases , but these trials were not successful . also different ion pairing agent were tried, but no suitabele condition were found for the quantitive analysis of the to species .

The effect of methanol contained and concentration of the phosphate salt on the capacity factor (K) and separation selectivities were studied. The optimum condition incorporated in to the procedure .

Table 7 shows that the HPLC technique in calibration curve used permits the simultaneous separation of the two species in less than 3 minuts . also , included are the correlation coefficient. Slope and intercept obtained by linear regression analysis of the calibration graphs .

Table 11 gives results obtained for the analysis of standard mixture of the two species . the results are accurate and precise.

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