Zagazig J. Pharm. Sci., June 1999
Vol. 7, No. 1, pp. 38 - 43

ISSN 1110-5089

HPLC DETERMINATION OF AMOXICILLIN, AMPICILLIN, PHENOXYMETHYLPENICILLIN, FLUCLOXACILLIN, CLOXACILLIN AND DICLOXACILLIN IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A simple high performance liquid chromatographic procedure was described for the determination of amoxicillin, ampicillin, cloxacillin, dicloxacillin, flucloxacillin and phenoxymethylpenicillin (penicillin V) in single or combined pharmaceutical formulations. The drugs were chromatographed on an Ultrasphere ODS column and (40% methanol containing 9 mM sodium heptane sulphonate, 30 mM potassium dihydrogen phosphate and 0.04% triethylamine) adjusted to pH 5 with 1 M phosphoric acid as mobile phase using penicillin G as internal standard. The flow rate was 1 ml min-1 at 230 nm detection. The results show good accuracy as shown by the % recovery of 99.61, 99.53, 99.9, 100.08, 100.18 and 99.908 and high precision as shown by the relative standard deviation, RSD, 0.744, 0.613, 0.557, 0.731, 0.46 and 0.308.

INTRODUCTION

Amoxicillin and ampicillin are broad spectrum antibiotics effective against many gram positive and gram negative organisms. Cloxacillin,dicloxacillin and flucloxacillin are isoxazolylpenicillin resistant to penicillinase produced by staphylococci.

A co-formulation of amoxicillin and flucloxacillin combines the complementary activities of both to produce a broad spectrum bactericidal activity against a wide range of gram positive and gram negative pathogens. Also a co-formulation of ampicillin with cloxacillin or dicloxacillin extends the antibacterial spectrum to include a wide range of gram positive and gram negative organisms. Phenoxymethylpenicillin is an acid stable pencillin and used mainly in the treatment of streptococcal infections and in rheumatic fever prophylaxis.

Concerning the analysis, iodometric (1-4), potentiometric (5-7) spectro- photometric (8-10), HPTLC(11) and HPLC (12-16) methods were reported for determination of the above penicillins in pharmaceutical preparations. The spectrophotometric and potentiometric assays are often very difficult to carry out and of low sensitivity owing to interference of other ingredients which are generally present in the pharmaceutical preparations. In the present research we introduce a new HPLC method for the determination of amoxicillin, ampicillin, cloxacillin, as well as dicloxacillin, flucloxacillin, and phenoxymethyl-penicillin in different pharmaceutical preparations containing these mixtures.

EXPERIMENTAL

Apparatus:

LDC analytical, 4100 solvent delivery system, Milton Roy, Variable Wave length Detector (VWD).

Reagents

Methanol, HPLC grad, BDH, England. Water, double distilled, Lipco, Egypt. Potassium dihydrogen phosphate, Potassium Hydroxide, Triethylamine and Heptane sulphonate, Fluka, Switzerland. O-Phosphoric acid, Reidel de Haen, Germany.

Stock solutions :

Amoxicillin , ampicillin, cloxacillin and flucloxacillin, 1 mg ml^{-1}, Ribbon, Italy. Penicillin G, 2 mg ml^{-1} and dicloxacillin, 1 mg.ml^{-1}, CID, Egypt. Phenoxymethylpenicillin, 0.615 mg ml^{-1}, The Nile Co., Egypt. All of the standard solutions were dissolved in water except phenoxymethylpenicillin dissolved in methanol.

Authentic mixtures of :

Amoxicillin 1 mg.ml^{-1} and flucloxacillin 1 mg.ml^{-1} . Ampicillin 1 mg.ml-1 and cloxacillin 1 mg.ml^{-1} Ampicillin 1 mg ml^{-1} and dicloxacillin 1 mg ml^{-1}.

Pharmaceutical preparations:

Ampiclox 500 mg capsules, from MUP, Egypt, labelled to contain 250 mg ampicillin as ampicillin trihydrate and 250 mg cloxacillin as cloxacillin sodium, B.N. 96198, average weight of capsule content 620 mg. **Cloxapen** 500 mg capsules, from The Nile Co., Egypt, labelled to contain 250 mg ampicillin and dicloxacillin sodium equivalent to 250 mg dicloxacillin, B.N. A 22, average weight of capsule content 566 mg.

Dipenacid 500 mg capsules, from CID, Egypt, labelled to contain 250 mg ampicillin as ampicillin trihydrate and 250 mg dicloxacillin as dicloxacillin sodium, B.N. 985101, average weight of capsule content 586.2 mg. **Dipenacid** 500 mg vials, from CID, Egypt,

labelled to contain 250 mg ampicillin as ampicillin sodium and 250 mg dicloxacillin as dicloxacillin sodium, B.N. L299303, average weight of vial content 520 mg.

Flumox 500 mg capsules, from Lipson, Egypt, labelled to contain 250 mg amoxicillin as amoxicillin trihydrate and 250 mg flucloxacillin as flucloxacillin sodium monohydrate, B.N. 981A39, average weight of capsule content 602 mg. **Flumox** 1 g vials, from Lipson, Egypt, labelled to contain 500 mg amoxicillin as amoxicillin sodium sterile and 500 mg flucloxacillin as flucloxacillin sodium sterile, B.N. 980778, average weight of vial content 1240 mg.

Ospen 1000 tablets , from the Nile Co., Egypt, labelled to contain 1,000,000 IU phenoxymethyl-penicillin, B.N. B 87, average weight of tablet 760 mg.

Chromatographic conditions :

Mobile phase : 40% methanol containing (9 mM-sodium heptane sulphonate, 30 mM-potassium dihydrogen phosphate and 0.04% triethylamine) adjusted to pH 5 with 1 M-phosphoric acid , degassed and filtered using 0.45 µm membrane filter.

Column : 5 µ Ultrasphere ODS (25) x 4.6 mm)
Detector : 230 nm, 0.2 aufs
Flow rate : 1 ml.min^{-1} , Pressure : 1800 psig
Temperature : ambient , Injected volume : 10µl

Procedures:

A-Preparation of calibrations curves :

From the standard solution of each drug volumes from 0.2-1 ml were pipetted into 10 ml volumetric flasks, then 0.5 ml of the internal standard was added to each flask and completed to volume with water. 10µl of each sample was injected into the column and all measurements were repeated three times at each concentration. The calibration curve was based on the peak area ratio of each compound to that of the internal standard against concentration.

B- Authentic mixtures :

Different aliquots from the authentic mixture stock solutions prepared in ratios claimed to be present in the pharmaceutical preparations within the range recorded in the calibration were transferred to 10 ml volumetric flasks and completed as described under procedure A.

C. Commercial dosage forms :

1- Capsules and Tablets :

The contents of 10 Ampiclox, Cloxapen, Dipenacid, Flumox capsules and Ospen tablets were weighed and the average weight of each was determined, mixed and powdered. An accurately weighed portions of the powders equivalent to 1/10 the average weight of each product were transferred to 100ml volumetric flasks, dissolved in water (except ospen tablets dissolved in methanol) with the aid of an ultrasonic bath, completed to volume and filtered. The first portion of the filtrate discarded.

Different aliquots from the filtrate were transferred to different 10 ml volumetric flasks and completed as under procedure A.

2- Vials :

The contents of 10 Dipenacid or Flumox vials were weighed and the average weight of each was determined and mixed. An accurately weighed portions of the powders equivalent to 1/10 the average weight of each product were transferred to 100 ml volumetric flasks and dissolved in water, completed to volume and filtered. The first portion of the filtrate discarded. Different aliquots from the filtrate were transferred to 10 ml volumetric flasks and completed as under procedure A.

RESULTS AND DISCUSSION

A mixture containing amoxicillin, ampicillin, cloxacillin, penicillin G, penicillin V, flucloxacillin and dicloxacillin was chromatographed on an Ultrasphere ODS column. A number of mobile phases composed of methanol - phosphate buffer - heptane sulphonate were tested in the presence and absence of triethylamine. The mobile phase composed of 40% methanol containing 6mM- sodium heptane sulphonate and 30 mM-potassium dihydrogen phosphate were failed to separate cloxacillin and flucloxacillin (Fig. 1A).

The separation was improved by increasing the morality of heptane sulphonate but the peaks become more tailed (Fig. 1B).The tailing was overcomed by addition of triethylamine. It was found that the mobile phase composed of 40% methanol containing (9mM-sodium heptane sulphonate, 30 mM-potassium dihydrogen phosphate and 0.04% triethylamine) adjusted to pH 5 with 1 M-phosphoric acid flowing at 1ml.min.$^{-1}$ would result in a good baseline and symmetrical peaks with good capacity and resolution factors.

Complete analysis of the seven component mixture was achieved within 25 minutes (Fig. 1C). The retention times were 2.61, 4.55, 5.98, 9.51, 14. 29, 15.64 and 24.91 minutes for amoxicillin, ampicillin, penicillin G, phenoxymethylpenicillin, cloxacillin, flucloxacillin and dicloxacillin, respectively. The internal standard was chosen on the basis that it should be eluted between the peaks and should not interfere with any of the previously mentioned components. Penicillin G was found to be the most suitable internal standard with a retention time of 5.98 minutes.

The peak area ratio of amoxicillin, ampicillin, penicillin V, cloxacillin, flucloxacillin and dicloxacillin

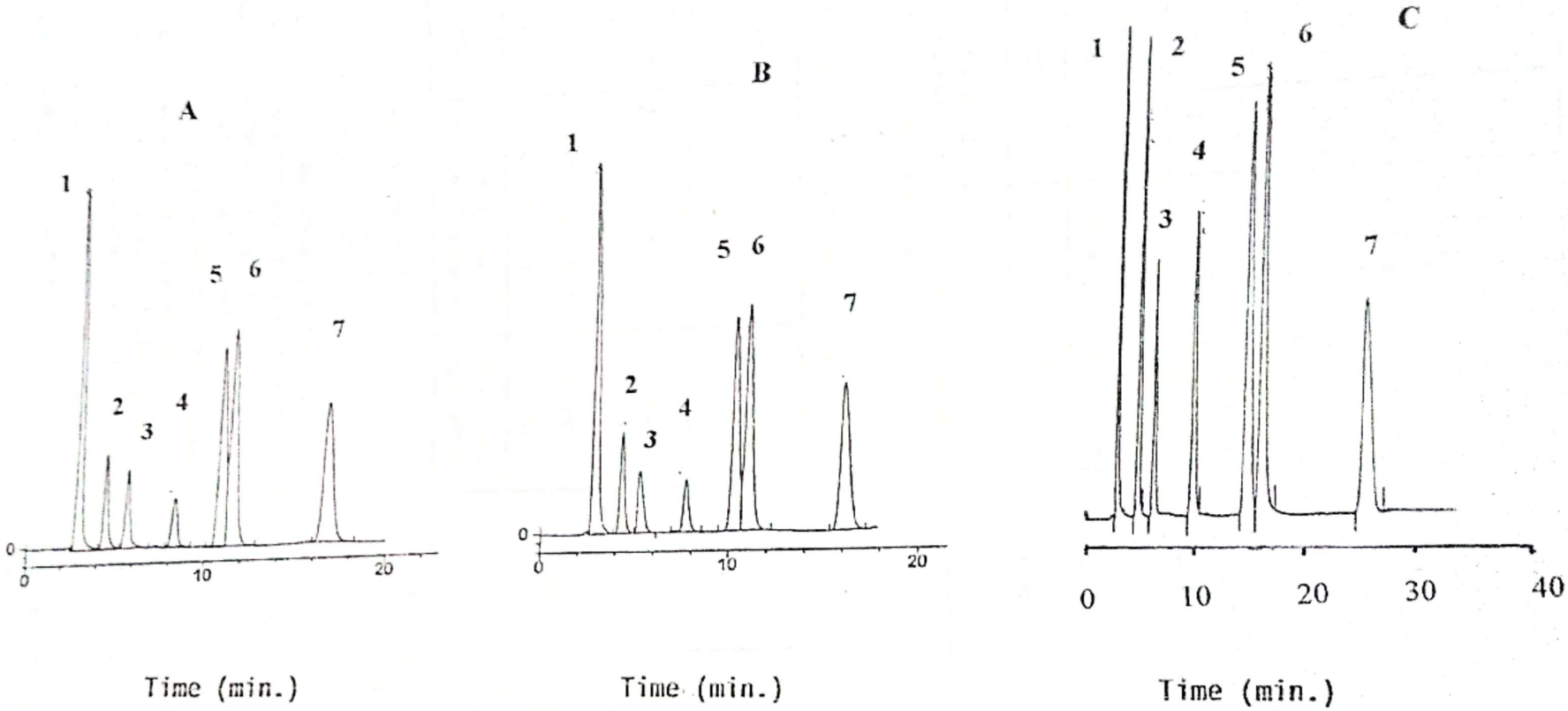

Fig. 1 Effect of molar concentration of heptane sulphonic acid sodium salt in the mobile phase on the separation of 1- amoxicillin 2-ampicillin 3- penicillin V 4-penicillin G (internal standard) 5- cloxacillin 6- flucloxacillin 7- dicloxacillin .

Mobile phase : aqueous methanol 40% containing 30mM KH_2PO_4 and 6 mM heptane sulphonate adjusted to pH 5 with potassium hydroxide ..... (A).

aqueous methanol 40% containing 30mM KH_2PO_4 and 7 mM heptane sulphonate adjusted to pH 5 with potassium hydroxide ..... (B).

aqueous methanol 40% containing 30mM KH_2PO_4 , 9 mM heptane sulphonate and 0.04 % triethylamine djusted to pH 5 with o- phosphoric acid..... (C) .

Column 5 µ Ultrasphere C18, 250x4.6 mm

Detector UV at λ =230

Pharm J Pharm Sci., June 1998
Vol. , No. 1, pp. 38 - 47

to that of penicillin G (the internal standard) exhibit rectilinear relationship within concentration range 2-10 mg% for amoxicillin, ampicillin, cloxacillin, flucloxacillin and dicloxacillin and 1.23 - 6.15 mg % for phenoxymethylpenicillin.

The regression equations and correlation coefficients (r) are given in table 1. The validity of the listed regression equations was tested by the assay of authentic penicillin V or a laboratory made authentic mixtures containing known quantities of amoxicillin and flucloxacillin, ampicillin and cloxacillin , ampicillin and dicloxacillin in ratios equivalent to those found in commercial dosage forms, tables 2, 3, 4 and 11.

Table (1): Regression analysis

Compound	Regression equations	Correlation coefficient (r)
Amoxicillin	Y = 0.418 X + 0.1192	0.999
Ampicillin	Y = 0.219 X - 0.006	0.999
Cloxacillin	Y = 0.481 X + 0.022	0.9999
Dicloxacillin	Y = 0.453 X + 0.052	0.999
Flucloxacillin	Y = 0.392 X + 0.0029	0.999
Phenoxymet-hylpenicillin	Y = 0.285 X + 0.003	0.999

Y=Peak area ratio X= Concentration (mg %)

Table (2) Determination of amoxicillin and flucloxacillin in authentic mixture using the proposed HPLC method.

Amoxicillin			Flucoxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	2.01	100.5	2	1.98	99
5	4.962	99.24	5	5.01	100.2
10	9.91	99.1	10	9.94	99.4
Mean		99.613	Mean		99.533
SD		0.771	SD		0.611
RSD		0.774	RSD		0.613

The results show good accuracy as shown by the % recovery 99.61, 99.53, 99.9, 100.08, 100.18 and 99.908 for amoxicillin, flucloxacillin, ampicillin, coloxacillin, dicloxacillin and phenoxymethylpenicillin, respectively and high precision as shown by the relative standard deviation, RSD 0.744, 0.613, 0.557, 0.731, 0.46 and 0.308 for amoxicillin, flucloxacillin, ampicillin, cloxacillin, dicloxacillin and phenoxymethylpenicillin, respectively.

The proposed method was applied to assay commercial tablets, vials and capsules as in Figs. (2-6) Also results from tables 5, 6, 7,8, 9,10 and 12 show good accuracy and precision.

Table (3): Determination of ampicillin and cloxacillin in authentic mixture using the proposed HPLC method.

Ampicillin			Cloxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	2.01	100.5	2	2.015	100.75
5	4.97	99.4	5	5.01	100.2
10	9.98	99.8	10	9.93	99.3
Mean		99.8	Mean		100.08
SD		0.556	SD		0.73
RSD		0.557	RSD		0.73

Table (4) Determination of ampicillin and dicloxacillin in authentic mixture using the proposed HPLC method.

Ampicillin			Dicloxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	1.98	99	2	2.0044	110.22
5	5.01	100.2	5	5.031	100.62
10	9.97	99.7	10	9.97	99.7
Mean		99.63	Mean		100.18
SD		0.602	SD		0.461
RSD		0.604	RSD		0.46

Zagazig J. Pharm. Sci., June 1998
Vol. 7, No. 1, pp. 38 - 47

Table (5) Determination of amoxicillin and flucloxacillin in Flumox capsules using the proposed HPLC method.

Amoxicillin			Flucoxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	2.02	101	2	1.964	98.2
5	4.953	99.06	5	4.91	98.2
10	9.96	99.6	10	9.78	97.8
Mean		99.88	Mean		98.066
SD		1	SD		0.23
RSD		1	RSD		0.235

Table (6) Determination of amoxicillin and flucloxacillin in Flumox vials using the proposed HPLC method.

Amoxicillin			Flucoxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	2.04	102	2	2.05	102.5
5	5.15	103	5	5.2	104
10	10.04	100.4	10	10.25	102.5
Mean		101.8	Mean		103
SD		1.31	SD		0.866
RSD		1.29	RSD		0.84

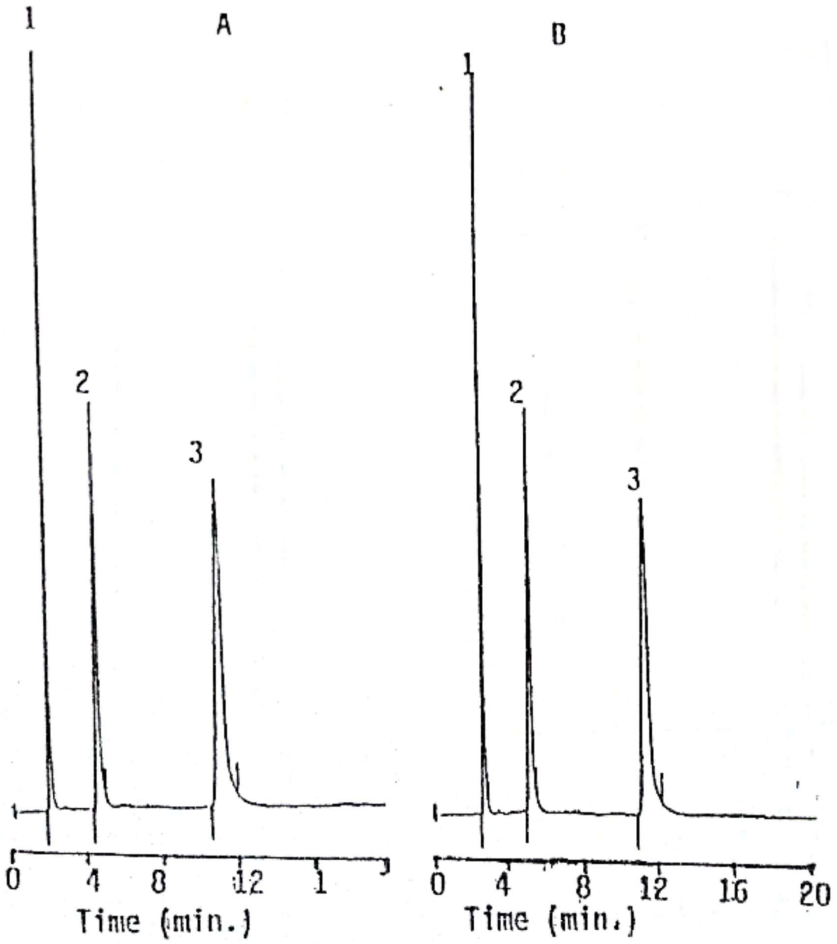

Fig. (2) HPLC Chromatogram of Flumox Vials (A) and Flumox Capsules (B).

1- Amoxicillin 2- Penicillin G 3-Flucloxacillin.

Table (7) Determination of ampicillin and cloxacillin in Ampiclox capsules using the proposed HPLC method.

Ampicillin			Cloxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	1.99	99.5	2	2.03	101.5
5	2.028	100.56	5	5.16	103.2
10	9.97	99.7	10	10.12	101.2
Mean		99.2	Mean		101.96
SD		0.563	SD		1.078
RSD		0.563	RSD		1.057

Table (8) Determination of ampicillin and dicloxacillin in Cloxapen capsules using the proposed HPLC method.

Ampicillin			Dicloxacillin		
Amount added, mg%	Amount found, mg%	Recovery %	Amount added, mg%	Amount found, mg%	Recovery %
2	1.95	97.5	2	1.94	97
5	4.9	98	5	4.81	96.2
10	9.74	97.4	10	9.61	96.1
Mean		97.63	Mean		96.43
SD		0.32	SD		0.49
RSD		0.32	RSD		0.51

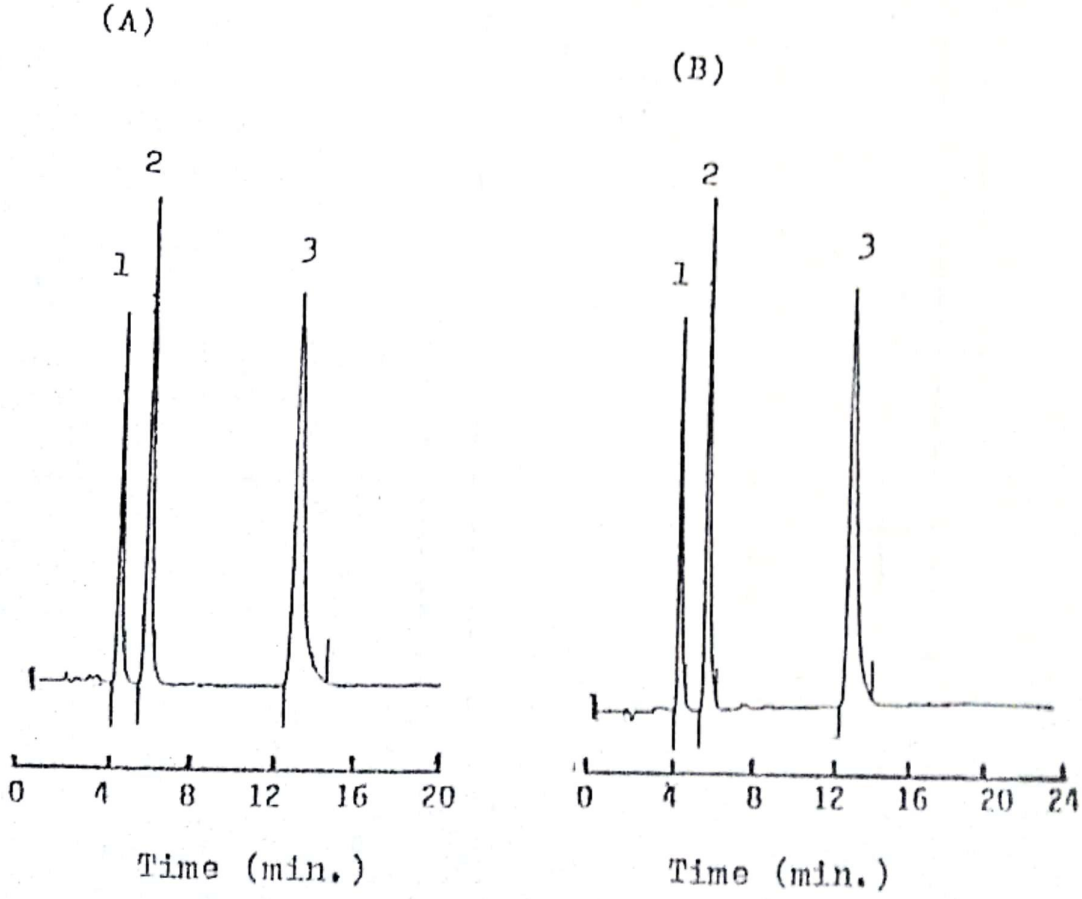

Fig. (3) HPLC Chromatogram of authentic mixture of ampicillin and cloxacillin (A) and Ampiclox capsules (B).

1-Ampicillin 2-Penicillin G 3- Cloxacillin

Zagazig J Pharm Sci, June 1998
Vol 7, No 1, pp 38 - 47

Table (9) Determination of ampicillin and dicloxacillin in Dipenacid capsules using the proposed HPLC method

Ampicillin			Dicloxacillin		
Amount added mg%	Amount found mg%	Recovery %	Amount added mg%	Amount found mg%	Recovery %
2	1.98	99	2	1.96	98
5	4.96	99.2	5	4.92	98.4
10	9.81	98.1	10	9.78	97.8
Mean		98.76	Mean		98.066
SD		0.586	SD		0.305
RSD		0.593	RSD		0.311

Table (10) Determination of ampicillin and dicloxacillin in Dipenacid vials using the proposed HPLC method

Ampicillin			Dicloxacillin		
Amount added mg%	Amount found mg%	Recovery %	Amount added mg%	Amount found mg%	Recovery %
2	1.97	98.5	2	1.95	97.50
5	4.9	98	5	4.86	97.2
10	9.72	97.2	10	9.71	97.10
Mean		97.9	Mean		97.266
SD		0.655	SD		0.208
RSD		0.67	RSD		0.214

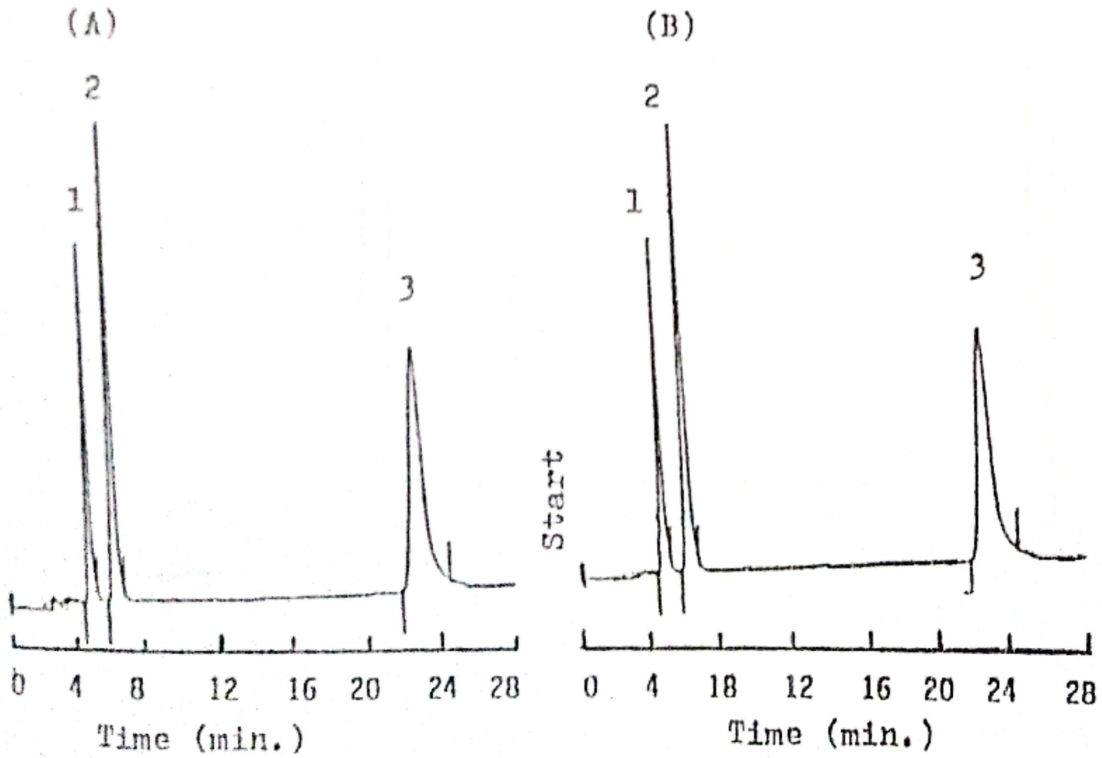

Fig. (4) HPLC Chromatogram of authentic mixture of ampicillin and dicloxacillin (A) and Cloxapen capsules (B).

1- Ampicillin 2- Penicillin G 3- Dicloxacillin

Table (11) Determination of authentic phenoxymethylpenicillin using the proposed HPLC method.

Amount added mg%	Amount found mg%	Recovery %
1.23	1.233	100.244
3.075	3.07	99.84
6.15	6.128	99.64
Mean		99.908
SD		0.3076
RSD		0.308

Table (12) Determination of phenoxmethylpenicillin in Ospen tablets using the proposed HPLC method.

Amount added mg%	Amount found mg%	Recovery %
1.23	1.236	100.487
3.075	3.014	98.016
6.15	6.095	99.105
Mean		99.202
SD		1.238
RSD		1.248

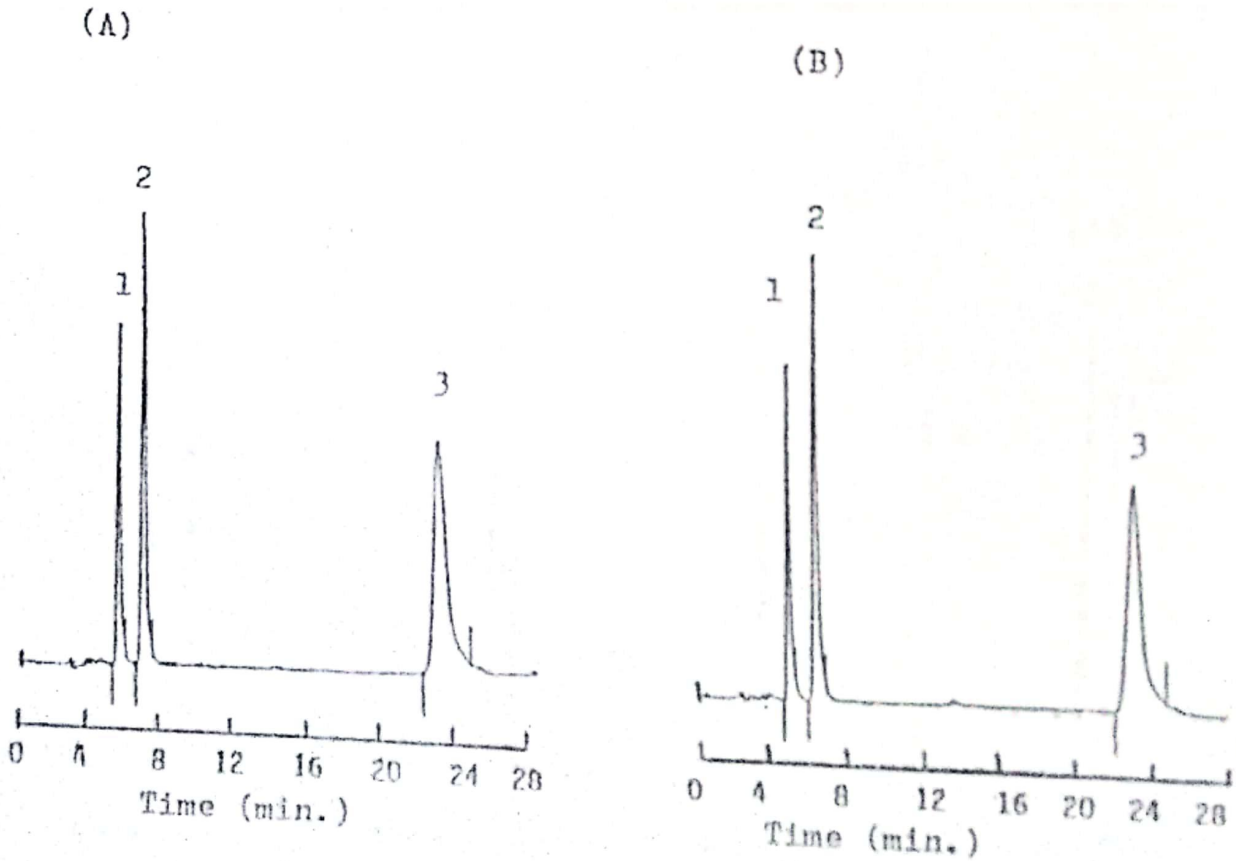

Fig. (5) HPLC Chromatogram of Dipenacid vials (A) and Dipenacid capsules (B).
1 Ampicillin 2 Penicillin G 3 Dicloxacillin

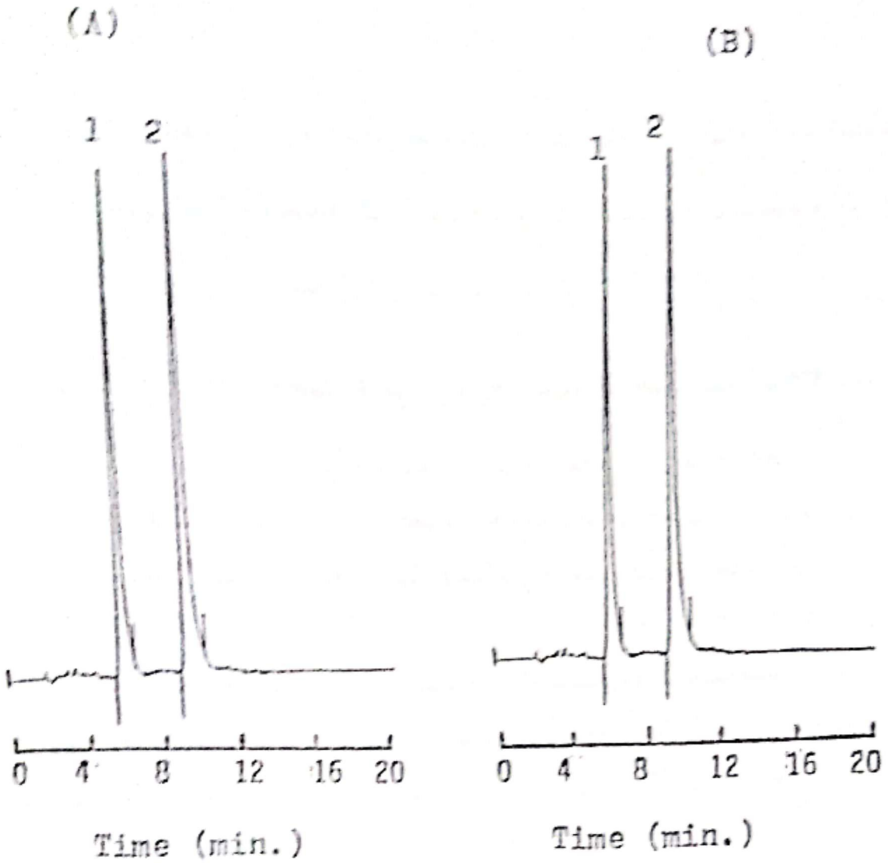

Fig. (6) HPLC Chromatogram of authentic sample of Penicillin V (A) and Ospen tablets (B) .
1- Penicillin V 2- Penicillin G

CONCLUSION

In conclusion , the proposed method is selective, precise, accurate, time saving and can be used in quality control laboratories for analysis of antibiotic preparations containing these compounds in single or combined.

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Received : March 7, 1998
Accepted : April 4, 1998

تقدير مركبات الأموكسيسيلين و الأمبيسيللين والبنيسيللين - ث و الفلوكساسيللين والكلوكساسيللين والداى كلوكساسيللين فى المستحضرات الصيدلية باستخدام كروماتوجرافيا السائل ذو الضغط العالى

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يصف هذا البحث طريقة لتحليل خليط من الأموكسيسيللين والأمبيسيللين والكلوكساسيللين والداى كلوكساسيللين والفلوكساسيللين والبنسيللين - ث باستخدام كروماتوجرافيا السائل ذو الضغط العالى وتم التطبيق على حقن وكبسولات الفلوموكس والداى بيناسيد وحقن الكلوكسابن وكبسولات الأبيكلوكس وأقراص الأوسبن وقد جائت النتائج على درجة عالية من حيث الدقة والحساسية .

وقد أظهرت النتائج نسبة عالية من استرجاع الدواء (٩٩ر٦١ - ٩٩ر٥٣ - ٩٩ر٩ - ١٠٠ر٠٨ - ٩٩ر٩١-١٠٠ر١٨٪) وكذلك أظهرت النتائج أقل معامل إنحراف معيارى نسبى (٠ر٧٤٤ - ٠ر٦١٣ - ٠ر٥٥٧ - ٠ر٧٣١ - ٠ر٤٦٠ - ٠ر٣٠٨) وعلية فالطريقة الجديدة تساعد على سرعة تحليل مثل هذه المركبات بمفردها أو مخلوطه مع الدقة فى النتائج.