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HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF SECNIDAZOLE, OMEPRAZOLE AND AMOXICILLIN MIXTURE IN PURE FORMS AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

An isocratic RP-HPLC method has been developed and validated for rapid simultaneous separation and determination of mixture of secnidazole, omeprazole and amoxicillin in their pure forms and pharmaceutical preparations. The separation was carried out on ACE 5 C₁₈ (25 cm x 4.6 mm) column using a mobile phase of acetonitrile: potassium dihydrogen phosphate buffer (pH 7.5) (60:40, v/v). The flow rate was 1.5 ml/min and the detection was set at 230 nm. The proposed method was successfully applied to the estimation of the cited drugs in their dosage forms.

KEYWORDS

Secnidazole, Amoxicillin, Omeprazole, RP-HPLC and Degradation products.

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INTRODUCTION

Secnidazole is chemically known as (1-(2-Methyl-5-nitroimidazol-1-yl) propan-2-ol). Secnidazole is not official in any pharmacopoeia. A nitroimidazole anti-infective. Effectiveness in the treatment of dientamoebiasis¹.

Secnidazole is structurally related to the commonly used 5-nitroimidazoles metronidazole and tinidazole. These drugs share a common spectrum of activity against anaerobic micro-organisms and they appear particularly effective in the treatment of *amoebiasis*, *giardiasis*, *trichomoniasis* and *bacterial vaginosis*².

Secnidazole also shows activity against *anaerobic bacteria* and *protozoa*. It is used to eradicate *Helicobacter pylori* in peptic ulcer disease with other antimicrobials and proton pump inhibitor^{3,4}.

A literature survey revealed that secnidazole has been estimated in pharmaceuticals by UV, visible spectrophotometry,⁵⁻⁷ and high performance liquid chromatography^{8,9}.

Omeprazole is chemically known as 5-Methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl benzimidazole. It is official in both B.P. 2011¹⁰ where it was determined by titration with standard solution of alkali hydroxide and determining the end-point potentiometrically, and determined by HPLC method in U.S.P. XXXII¹¹. Omeprazole is a proton pump inhibitor, used in treatment of peptic ulcer disease and NSAID-associated ulceration, in gastro-esophageal reflux disease and the Zollinger-Ellison syndrome¹². A survey of the literature revealed that omeprazole has been estimated in pharmaceuticals by UV-spectrophotometric methods,¹³⁻¹⁵ spectrofluorimetry,¹⁶ and HPLC^{17,18}.

Amoxicillin is chemically known as (2S,5R,6R)-6-[[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid trihydrate.

Amoxicillin is bactericidal with a moderate spectrum of antimicrobial activity including many *Gram-positive* and *Gram-negative bacteria* and *some protozoa*. It is used in triple therapy along with secnidazole and proton pump inhibitor in the treatment of peptic ulcer^{4,19}. Amoxicillin trihydrate is official in B.P. 2007,²⁰ where it was determined by chromatographic system. A survey of the literature revealed that amoxicillin has been estimated in pharmaceuticals by UV-visible spectrophotometric,^{21, 22} spectrofluorimetry²³ and HPLC²⁴⁻²⁶.

MATERIALS AND METHODS

Apparatus

Beckman Coulter HPLC instrument. Consort P 400[®] digital PH-meter for PH adjustment.

Materials and Reagents

All solvents and reagents were of HPLC analytical grade. Secnidazole (Amoun Pharmaceutical Industries, El-Obour City, Cairo, Egypt). Omeprazole (Sigma Pharmaceutical Industries, Quesna City, Egypt). Amoxicillin trihydrate (Amoun Pharmaceutical Industries, El-Obour City, Cairo, Egypt). Fladazole[®] tablets labeled to contain 500 mg of secnidazole. (Amoun Pharmaceutical El-Obour City, Cairo, Egypt). Ipiamox[®] tablets labeled to contain 500 mg of amoxicillin. (Amoun Pharmaceutical El-Obour City, Cairo, Egypt). Gastro Loc[®] tablets labeled to contain 20 mg of omeprazole. (Sigma Pharmaceutical Industries, Quesna City, Egypt). Acetonitrile (Merck), potassium dihydrogen phosphate (Sigma-Aldrich) and sodium hydroxide (Fisher Scientific) Buffer preparation: Dissolve 1.36g /L potassium dihydrogen phosphate in 1000ml water, adjust the PH to 7.5 ±0.05 with 10% sodium hydroxide. Mobile phase: was a freshly prepared as binary mixture of acetonitrile: potassium dihydrogen phosphate adjusted to pH 7.5 using sodium hydroxide (60:40, v/v), filtered and degassed using 0.25µm membrane filter.

Standard solution

(25mg, 25mg and 15mg) of secnidazole, omeprazole and amoxicillin were weighted and dissolved in 20 ml of the mobile phase in three 25ml volumetric flasks, the solvent was completed to the mark.

General Procedures

Construction of calibration curves

Working solutions were prepared immediately before use to cover the concentration ranges from (200-600 µg.ml⁻¹) for secnidazole, omeprazole and (120-360 µg.ml⁻¹) for amoxicillin injected into the column and the chromatogram was performed and detected at wavelength 230 nm. A graph was plotted as concentration of each drug against response (peak area) and it was found to be linear for all drugs.

Pharmaceutical preparation

Ten tablets or capsules of each formulation were weighed and powdered. An accurately amounts of the powder equivalent to (25,25 and 15mg) of secnidazole, omeprazole and amoxicillin were

dissolved in 15 ml of the mobile phase, filtered, washed and collected into 25 ml measuring flask and completed to volume with the mobile phase. Working solutions were prepared as in the above procedure and completed as previously mentioned under the general procedure.

Stress Degradation Study

The stress degradation study was carried out according to the ICH requirements (Q IA (R2) and QIB) include acid, alkali and H₂O₂.

Acid degradation

Solution for acid degradation study was prepared in 25 ml volumetric flask, 10 ml appropriate mixed dilutions of the standard stock solutions of omeprazole, secnidazole and amoxicillin, 5 ml of 1 M HCl were added. The solution was set aside at room temperature (25±2 °C) for not less than 1 hour and complete to 25 ml with mobile phase. A 20 µl of each mixture was injected into the column and the chromatogram was obtained no important change in peak area and shape of secnidazole but small degradate product and degradate product of omeprazole and amoxicillin was found.

Alkali degradation

Solution for base degradation study was prepared in 25 ml volumetric flask, 10 ml appropriate mixed dilutions of the standard stock solutions of omeprazole, secnidazole and amoxicillin, 5 ml of 0.1 M NaOH were added. The solution was set aside at room temperature (25±2 °C) for not less than 1 hour and complete to 25 ml with mobile phase. A 20 µl of each mixture was injected into the column and the chromatogram was obtained no important change in peak area and shape of secnidazole, broad peak of omeprazole and appearance of other peak (degradate product) of amoxicillin was found.

Oxidation with H₂O₂

Solution for oxidation degradation study was prepared in 25 ml volumetric flask, 10 ml appropriate mixed dilutions of the standard stock solutions of omeprazole, secnidazole and amoxicillin, 3% H₂O₂ were added. The solution was set aside at room temperature (25±2 °C) for not less than 1 hour and complete to 25 ml with mobile phase. A 20 µl of each mixture was injected into the

column and the chromatogram was obtained no important change in peak area and shape of secnidazole and omeprazole but small degradate product of amoxicillin.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Chromatographic parameters including wavelength detection, mobile phase composition and proportions, pH and flow rate were carefully studied in order to recognize the most suitable chromatographic system. The choice was based on the best resolution in a reasonable time.

Detection wavelength

Spectroscopic analysis of the drugs showed that omeprazole, secnidazole and amoxicillin have maximum absorbance at 290 nm, 310 nm and 230 nm, respectively. Therefore, the chromatographic detection was performed at 230 nm using UV-Vis. detector.

Buffer pH

Choosing suitable mobile phase pH was an important factor. Where omeprazole decomposes in acidic medium. However, an analysis above pH 7.5 was avoided for the following reasons, broad peak of omeprazole and appearance of other peak (degradate product) of amoxicillin. After experimental study, buffer pH 7.5 was optimum one giving good baseline separation.

Several types of buffer (potassium dihydrogen phosphate, acetate and borate buffers) were examined. It was found that potassium dihydrogen phosphate buffer gave the best peak symmetry.

Mobile phase composition and proportions

Mixture of methanol, acetonitrile and potassium dihydrogen phosphate buffer (10: 50: 40 v/v) was tried. However, amoxicillin peak tailing was obtained and retention time was delayed for omeprazole and secnidazole. When acetonitrile was replaced with methanol at the same ratio (60: 40) methanol: buffer, amoxicillin and secnidazole peak tailing were obtained, peak asymmetry retention time was delayed of omeprazole. Therefore, mixture of acetonitrile: potassium dihydrogen phosphate buffer was used for separation. After experimental

trials, it was found that good separation was achieved upon using a mobile phase consisting of acetonitrile: potassium dihydrogen phosphate buffer (pH 7.5) (60: 40, v/v).

So, the optimum chromatographic performances were achieved using, isocratic mobile phase composed of acetonitrile: potassium dihydrogen phosphate buffer (pH 7.5) (60: 40, v/v) in, injection volume 20 µl, detection wavelength 230 nm and flow rate 1.5 ml/min.

Chromatographic Conditions for the proposed HPLC method are listed in Table No.1.

Typical chromatograms for the mixed drugs in their pure forms and pharmaceutical preparations are shown in Figure No.4, 5.

Finally the studies of stress conditions on the chosen drugs are shown in Figure No.6-8.

Method validation

The developed methods were validated according to international conference on harmonization guidelines ICH²⁷.

Linearity and range

The calibration graphs obtained by plotting the values of the peak areas versus the drug concentrations (µg/ml) were found to be rectilinear over the concentration ranges cited in the Table No.2.

The calibration graph was described by the equation:

$$Y = a + bX$$

(Where Y=peak area, a=intercept, b=slope, X=concentration in µg/ml).

Correlation coefficient, intercept and slope for the calibration data are summarized in table (2).

Accuracy

The accuracy of the proposed method was checked by performing recovery experiments through direct method. The results are shown in Table No.3.

Precision

Intraday precision was evaluated by calculating standard deviation (SD) of three independent

concentrations for the mixture of drugs. The SD values revealed the high precision of the method. For inter - day reproducibility, a series was run. The results are summarized in Table No.5.

The limit of detection (LOD) for the proposed HPLC method was calculated using the following equation:

$$LOD = 3.3S/K$$

The limit of quantification, LOQ is defined as;

$$LOQ = 10S/K$$

Where S is the standard deviation of the three replicate determination values under the same conditions as for the drug analysis and K is the slope of calibration graph.

According to these equations, the limits of detection and the limits of quantification were calculated and are listed in Table No.2.

Robustness

The robustness of the method was evaluated by making small changes in the flow rate, pH of mobile phase within a range of ± 0.05 of the optimized pH and mobile phase ratio keeping the other chromatographic conditions constant where the effect of the changes was studied on the percent recovery of drugs. The changes had a negligible influence on the results; data are summarized in Table No.6.

Analysis of pharmaceutical formulations

The validated HPLC method was applied for the simultaneous determination of secnidazole, omeprazole and amoxicillin in individual pharmaceutical mixtures. Results obtained were compared to those obtained by applying reference methods^{9,18,24}, where Student's t-test and F-test were performed for comparison. Results are shown in Table No.4. The calculated *t* and *F* values were less than tabulated values for the cited drugs which in turn indicate that there is no significant difference between proposed method and reference ones.

Table No.1: Chromatographic Conditions for the proposed HPLC method

S.No	Parameters	Conditions
1	Column	ACE 5 C ₁₈ column (25cm x 4.6 mm)
2	Mobile phase	Isocratic mobile phase of acetonitrile: potassium dihydrogen phosphate (60:40, v/v) adjusted to pH 7.5 using sodium hydroxide, filtered and degassed using 0.25µm membrane filter.
3	UV detection	230nm
4	Flow rate	1.5 ml/min
5	Injected volume	20µl
6	Temperature	Ambient
7	Retention time Amoxicillin Secnidazole Omeprazole	1.533 min. 2.267 min. 3.217 min.

Table No.2: Results and characteristic parameters for the simultaneous determination of Omeprazole, Secnidazole and Amoxicillin by the proposed method

Parameters	Omeprazole			Secnidazole			Amoxicillin		
	Conc. taken µg/ml	Conc. found µg/ml	% Recovery	Conc. taken µg/ml	Conc. found µg/ml	% Recovery	Conc. taken µg/ml	Conc. found µg/ml	% Recovery
	200	198.34	99.17	200	197.67	98.84	120	118.94	99.12
	300	300.79	100.26	300	302.30	100.77	180	180.20	100.11
	400	400.48	100.12	400	400.93	100.23	240	241.80	100.75
	500	503.29	100.66	500	500.58	100.12	300	300.06	100.02
	600	597.10	99.52	600	598.53	99.75	360	359.01	99.73
Mean recovery*	-	-	99.95	-	-	99.94	-	-	99.94
N	-	-	5	-	-	5	-	-	5
±SD	-	-	0.5963	-	-	0.7165	-	-	0.5950
±RSD	-	-	0.5966	-	-	0.7169	-	-	0.5953
Regression Equation**	Slope (b)	-	11348.3	-	-	11923.5	-	-	18081.3
	Intercept (a)	-	156180.8	-	-	1185168.2	-	-	221804.4
LOD µgml ⁻¹	-	-	58.522	-	-	58.523	-	-	33.003
LOQ µgml ⁻¹	-	-	195.07	-	-	195.08	-	-	110.01
Correlation coefficient	-	-	9998	-	-	9999	-	-	9999

* Average of three independent procedures, **Y = a + bC where Y is peak area, C is the concentration of the drug in µgml⁻¹

Table No.3: Application for the determination of Gastro Loc[®], Fladazole[®] and Ipiamox[®]

Items	Gastro Loc			Fladazole			Ipiamox		
	Conc. taken µg/ml	Conc. found µg/ml	% Recovery	Conc. taken µg/ml	Conc. found µg/ml	Recovery * %	Conc. taken µg/ml	Conc. found µg/ml	Recovery * %
	200	204.17	102.09	200	201.34	100.67	120	122.06	101.72
	300	305.59	101.86	300	301.90	100.63	180	176.76	98.2
	400	404.27	101.07	400	396.70	99.17	240	241.78	100.74
	500	506.04	101.21	500	508.11	101.62	300	297.97	99.32
	600	598.90	99.81	600	607.37	101.23	360	361.46	100.40
Mean*	-	-	101.21	-	-	100.67	-	-	100.08
N	-	-	5	-	-	5	-	-	5
S.D.	-	-	0.888	-	-	0.929	-	-	1.353
R.S.D.	-	-	0.878	-	-	0.923	-	-	1.352
V	-	-	0.7892	-	-	0.863	-	-	1.831
S.E.	-	-	0.3973	-	-	0.416	-	-	0.6052

*Mean of three different experiments.

Table No.4: Statistical analysis of results obtained by the proposed HPLC method applied on Gastro loc[®], Fladazole[®] and Ipiamox[®] compared with the reference methods

S.No	Statistics	Gastro Loc [®]		Fladazole [®]		Ipiamox [®]	
		Reference method ⁽¹⁸⁾	Proposed method	Reference method ⁽⁹⁾	Proposed method	Reference method ⁽²⁴⁾	Proposed method
1	Mean recovery* ± SD	100.80±0.505	101.21±0.888	101.08±1.28	100.67±0.929	100.03±0.703	100.08±1.35
2	N	5	5	5	5	5	5
3	Variance	0.255	0.7892	1.025	0.863	0.495	1.83
4	<i>t</i> -test**	-	0.8975(2.306) ^a	-	0.5797(2.306) ^a	-	0.073(2.306) ^a
5	F-test**	-	3.092(5.05) ^b	-	1.898(5.05) ^b	-	3.69(5.05) ^b

* Average of three experiments. a and b are Theoretical Student *t*-values and F- ratio at p=0.05.

Table No.5: Results of the intraday and interday precision for the determination of Amoxicillin, Secnidazole and Omeprazole by HPLC method

S.No	Item	Conc.ug/ml	Intraday		Interday	
			Mean ± SD	RSD	Mean± SD	RSD
1	Amoxicillin	120µg/ml	99.47±0.6985	0.7022	100.84±0.7235	0.7174
		240 µg/ml	99.90±0.9820	0.9829	99.55±0.6816	0.6846
2	Secnidazole	200 µg/ml	100.06±1.663	1.662	99.98±1.8033	1.8053
		400µg/ml	99.96±1.052	1.053	100.11±0.4727	0.4722
3	Omeprazole	200µg/ml	99.99±0.6217	0.6218	100.03±0.6738	0.6736
		400 µg/ml	99.93±0.6352	0.6357	100.10±1.6128	1.6110

Table No.6: Results of the robustness for the determination of Amoxicillin, Secnidazole and Omeprazole by HPLC method

S.No	Robustness			
	% of recovery \pm SD			
1	Item	Omeprazole	Secnidazole	Amoxicillin
2	Flow rate 1.6	99.86 \pm 0.961	101.6 \pm 0.982	100.42 \pm 0.575
3	Flow rate 1.4	101.49 \pm 0.82	100.12 \pm 0.55	99.95 \pm 0.712
4	Moile Phase 59:41	101.2 \pm 1.037	100.9 \pm 0.19	98.95 \pm 1.83
5	Moile Phase 61:39	100.51 \pm 1.14	99.69 \pm 1.63	101.06 \pm 0.735

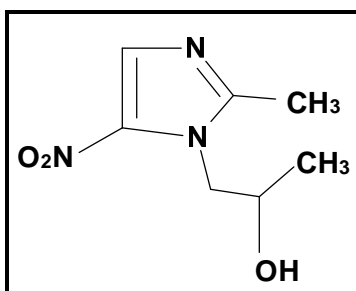


Figure No.1: Structure of Secnidazole

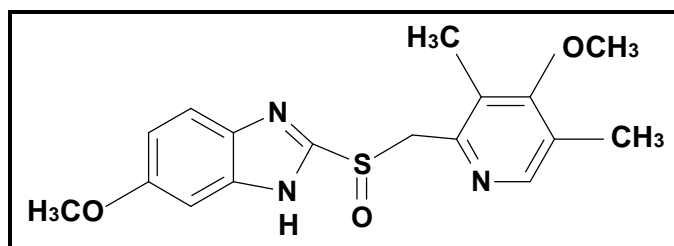


Figure No.2: Structure of Omeprazole

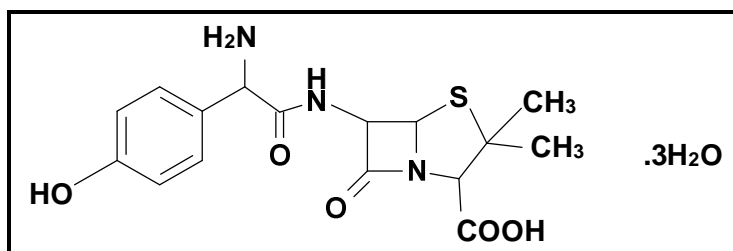


Figure No.3: Structure of Amoxicillin Trihydrate

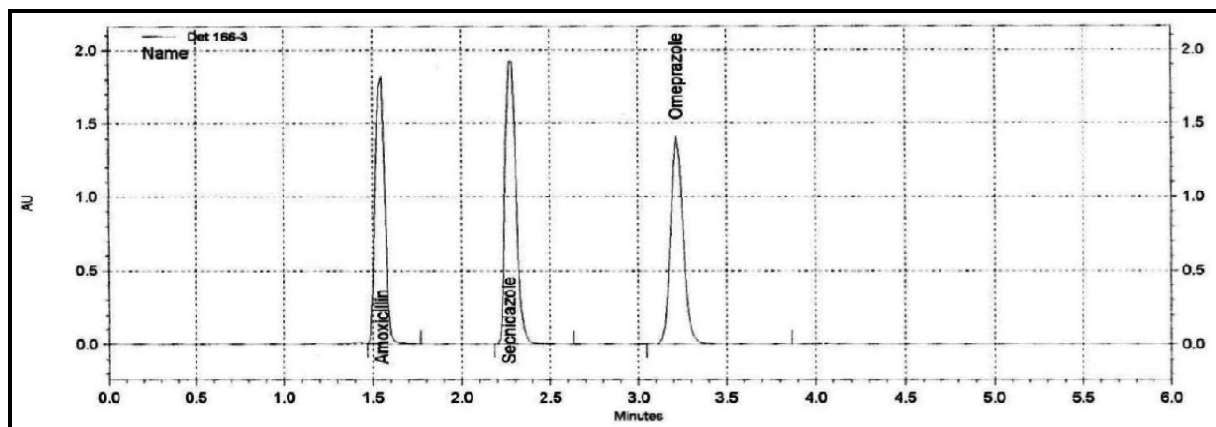


Figure No.4: HPLC chromatogram of authentic mixture of Omeprazole, Secnidazole ($600 \mu\text{gml}^{-1}$) and Amoxicillin ($360 \mu\text{gml}^{-1}$)

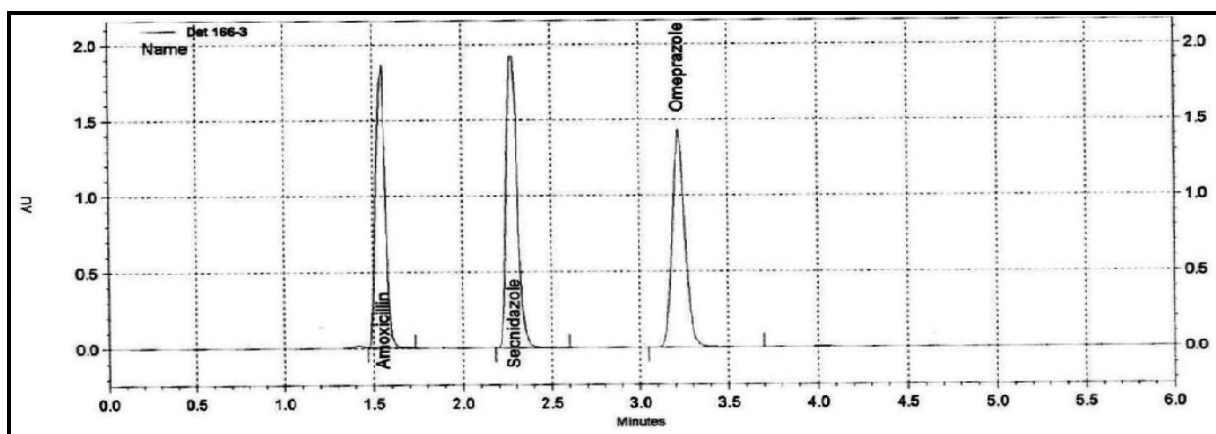


Figure No.5: HPLC chromatogram of mixture of Omeprazole, Secnidazole ($600 \mu\text{gml}^{-1}$) and Amoxicillin ($360 \mu\text{gml}^{-1}$) in pharmaceutical preparation

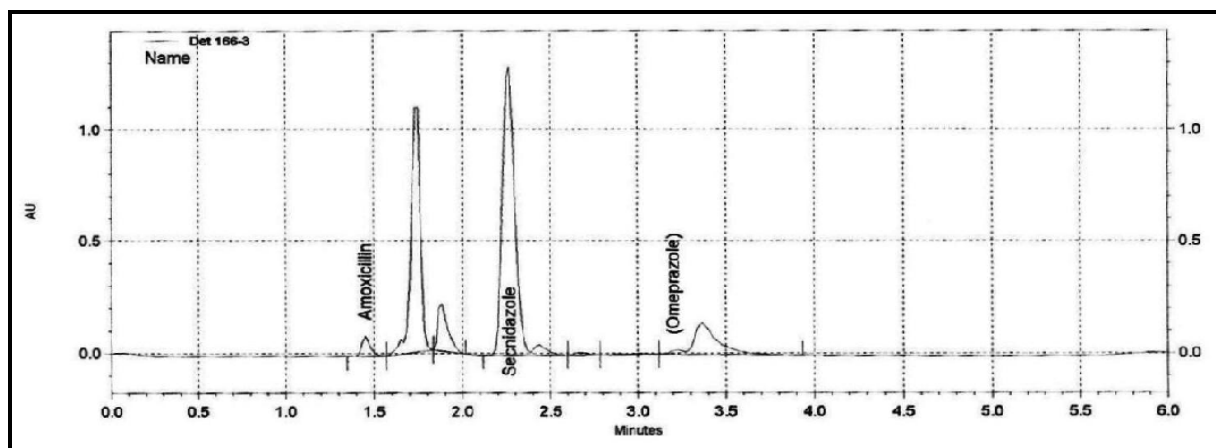


Figure No.6: HPLC chromatogram of authentic mixture of Amoxicillin, Secnidazole and Omeprazole in presence of their acid degradation products

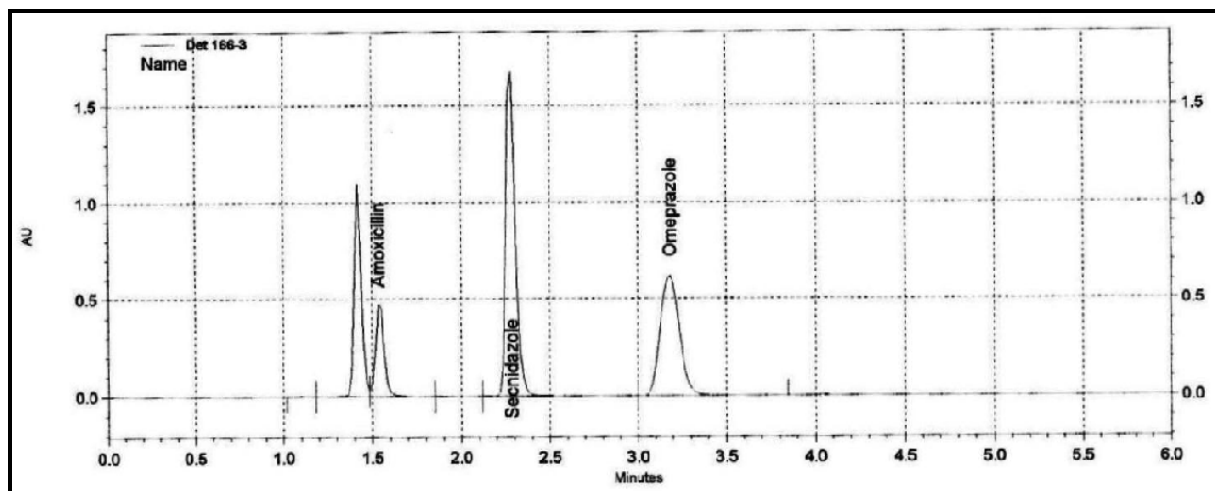


Figure No.7: HPLC chromatogram of authentic mixture Amoxicillin, Secnidazole and Omeprazole in presence of their basic degradation products

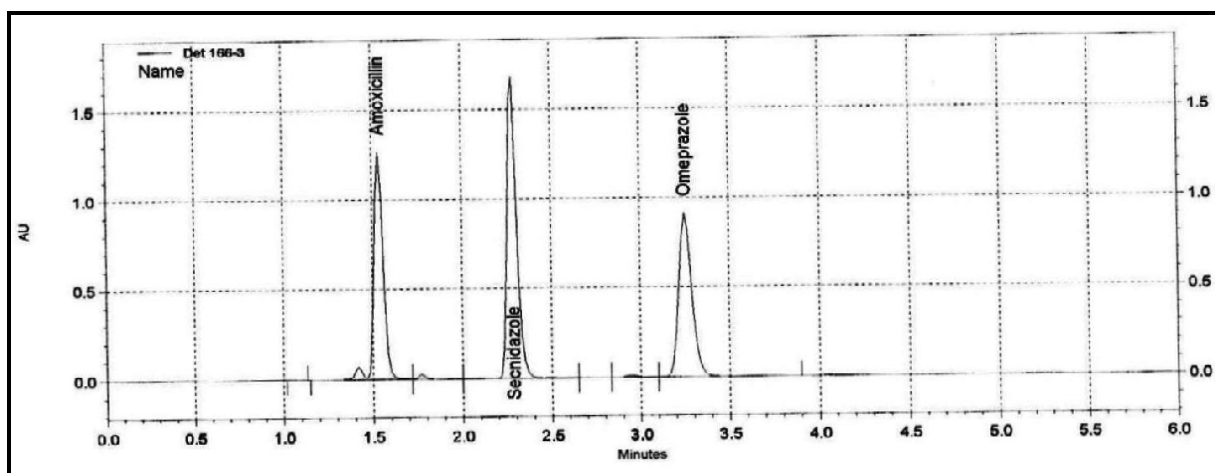


Figure No.8: HPLC chromatogram of authentic mixture Amoxicillin, Secnidazole and Omeprazole in presence of their oxidized degradation products

CONCLUSION

An isocratic RP-HPLC method has been developed for the simultaneous estimation of mixture of secnidazole, omeprazole and amoxicillin. The developed method was validated and it was found to be simple, precise, accurate and sensitive. Excipients present in the tablets and capsules show no interference in the determination. The proposed method can be used in quality control laboratories for routine analysis of secnidazole, omeprazole and amoxicillin in their pure and in their pharmaceutical formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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