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ORIGINAL PAPER

Simultaneous Determination of Amoxicillin, Lansoprazole, and Levofloxacin in Pharmaceuticals by HPLC with UV–Vis Detector

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Abstract
In this study, a specific and rapid high-performance liquid chromatography (HPLC) method has been developed and validated for the simultaneous determination of amoxicillin, lansoprazole, and levofloxacin in pharmaceuticals. Paracetamol was used as internal standard (IS) in the measurements. UV–Vis absorption spectra of the analytes and the IS were taken for the determination of suitable absorption wavelength of UV–Vis detector (diode array detector, DAD) in the HPLC instrument. A reverse-phase C18 column was used in the separation and determination of amoxicillin, lansoprazole, and levofloxacin together with the IS. The pharmaceutical analytes were quantified by the UV–Vis diode array detector in the HPLC using MeOH-0.01 M CH_3COONH_4 (70:30) as the mobile phase. The linear calibration curves of them were measured in the ranges of 15–40 mg/L, 2.5–15.0 mg/L, and 7.5–20.0 mg/L for amoxicillin, lansoprazole, and levofloxacin, respectively. Excellent calibration correlations (R^2: 0.9942, 0.9997, and 0.9974) were obtained. The percentage recoveries of the amoxicillin, lansoprazole, and levofloxacin in commercial pharmaceuticals were obtained as 105.5%, 98.57%, and 102.5%, respectively. The results showed that amoxicillin, lansoprazole, and levofloxacin together with paracetamol IS could be separated and determined simultaneously with low LOD and LOQ values using the proposed HPLC method.

Keywords HPLC · Amoxicillin · Levofloxacin · Lansoprazole · Simultaneous determination

1 Introduction

Amoxicillin (Amox) is a β-lactam antibiotic drug which belongs to the group of penicillin group drugs [1]. It is a moderate-spectrum β-lactam antibiotic used to treat infections caused by penicillin-sensitive Gram-positive bacteria as well as some Gram-negative bacteria [2]. Amoxicillin is named chemically as (2S, 5R, 6R) [[(2R)-2-amino-2 (4 hydoxyphenyl) acetyl] amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptanes-2-carboxyic acid (Fig. 1a) [3, 4]. Lansoprazole (Lanso) is an effective acid pump inhibitor acting at the final enzymatic step of the acid secretory mechanism of parietal cell [5, 6]. It decreases the amount of acid produced in the stomach and used to treat and prevent stomach and intestinal ulcer erosive esophagitis [7]. Lansoprazole is named chemically as (2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulfinyl]-lH-benzimidazole). For treatment of gastric and duodenal ulcers due to infection with campylobacter pylori, this drug is administered in combination with some antibiotics, e.g., with amoxicillin and clarithromycin [5]. The molecular structure of lansoprazole is demonstrated in Fig. 1b. Levofloxacin (Levo) is an oral fluoroquinolone antibacterial agent [8]. Levofloxacin is also named systematically as (−)-(S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-pipe-razinyl)-7oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate [9]. The molecular structure of levofloxacin is given in Fig. 1c. It is a synthetic broad spectrum antibacterial agent active against Gram-positive and Gram-negative bacteria. It acts by inhibiting DNA gyrase [9], and used for the treatment of infections of the respiratory and urinary tract, skin, and soft tissues [10].

There are several analytical methods for the determination of amoxicillin, lansoprazole, and levofloxacin in pharmaceuticals and biological fluids. For the determination of amoxicillin, thin-layer chromatography [11], reverse-phase liquid chromatography [12], liquid chromatography with fluorescence [13], spectrophotometry [14], and high-performance liquid chromatography (HPLC) [15] have been

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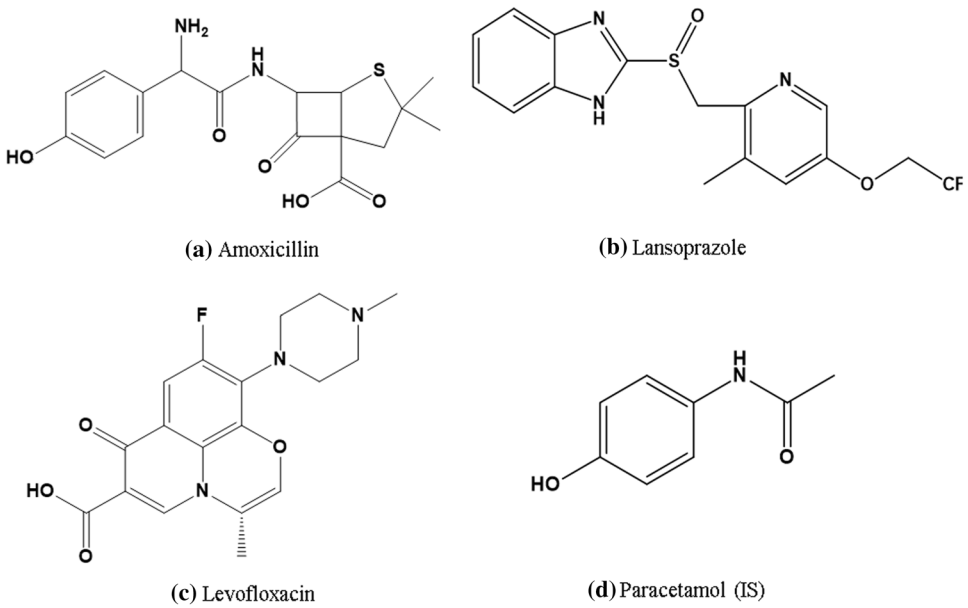


(a) Amoxicillin **(b)** Lansoprazole

(c) Levofloxacin **(d)** Paracetamol (IS)

Fig. 1 Chemical structures of **a** amoxicillin, **b** lansoprazole, **c** levofloxacin, and **d** paracetamol (*IS* internal standard)

used by different researchers. For the determination of lansoprazole, spectrophotometry [5, 17, 18], potentiometry [19], liquid chromatography/tandem mass spectrometry (LC–MS/MS) [20, 21], electrophoresis [22], spectrofluorimetry [23], polarography [24], voltammetry [25], and HPLC [26, 27] have been used. Similarly, levofloxacin can be determined by different methods [28, 29] of spectrofluorometry [30, 31], voltammetry [32], potentiometry [33], spectrophotometry [34–37], and HPLC [38, 39].

Among these methods, HPLC method is the best promised method because of low cost, selective, rapid, and simultaneous determination of the mixtures of pharmaceutical analytes. Mass spectrometric devices have high cost. Electrochemical methods have selectivity problems. Spectrophotometric and spectrofluorimetric devices require complex calculations such as chemometric or derivative processes and they have low selectivity in simultaneous determinations of ternary mixtures. HPLC methods provide both the separation and the detection of the analytes in mixtures during rapid single measurement. A chemical analysis method for rapid and simultaneous determination of lansoprazole, amoxicillin, and levofloxacin is important since the usage of lansoprazole together with these antibiotic drugs. HPLC technique has been widely used for rapid and simultaneous determination of many drug agents in pharmaceuticals, biological fluids, and tissues [40, 41].

In the present work, an HPLC analysis method with UV–Vis diode array detector (DAD) has been developed for simultaneous and rapid determination of amoxicillin, levofloxacin, and lansoprazole drug agents in pharmaceutical tablets. Although the previously described methods were developed for the simultaneous determination of various drug samples, there was no report about simultaneous determination of lansoprazole, amoxicillin, and levofloxacin combination. The novelty of this study is simultaneous and rapid determination of lansoprazole, amoxicillin, and levofloxacin as new pharmaceutical combination. In this HPLC method, paracetamol was used as the internal standard (IS) (Fig. 1d). The improved and validated HPLC method was applied to the commercial drug formulation tablets of amoxicillin, lansoprazole, and levofloxacin.

2 Experimental Procedure

2.1 Materials

Amoxicillin trihydrate, lansoprazole, levofloxacin, and paracetamol (IS) standard materials were obtained from Neutec Pharmaceuticals (Sakarya, Turkey). Largopen drug tablets for amoxicillin analyses were used in the experimental measurements and they were purchased from Bilim Pharmaceuticals Company (Tekirdag, Turkey). Largopen drug tablets contain 1176.47 mg amoxicillin trihydrate as equal to 1000 mg amoxicillin. For lansoprazole, Lansor drug tablets were used and they were purchased from Sanovel Company (İstanbul, Turkey). The formulation of Lansor tablets contains 30 mg lansoprazole, 1000 mg amoxicillin, and 500 mg clarithromycin. Levofloxacin tablets were obtained from the formulation of Tavanic drug produced by Sanofi Aventis Limited Company (Istanbul, Turkey). The Tavanic tablets include 512.6 mg levofloxacin hemihydrate with equal to 500 mg levofloxacin. HPLC-grade methanol (MeOH) was used as mobile phase in the HPLC measurements and it was obtained from Merck Company (Darmstadt, Germany). Ultra-pure deionized

water (18.2 MΩ) was produced by a Milli-Q Gradient A10 water purification system with a Q-Gard®2 and a QuantumTM EX (Millipore Bedford, MA).

2.2 Preparation of Standard and Sample Solutions

After a literature survey to prepare the solutions, amoxicillin [42] and levofloxacin [43] standards and samples were ground and dissolved directly in methanol:water (MeOH:H_2O, 50:50 v/v) mixture. Lansoprazole [44] sample was ground and first extracted with MeOH solvent from its initial material and then the suspension was filtered. The obtained lansoprazole filtrate solution was diluted with MeOH and H_2O to provide 50:50 MeOH:H_2O solvent mixture. MeOH is a suitable solvent for both the dissolutions of all the analytes and the mobile phase in HPLC measurements.

A stock standard solution of amoxicillin trihydrate was prepared at the concentration of 500 mg/L in 250 mL MeOH:H_2O (50:50 v/v) mixture in a flask. As the other stock solutions, lansoprazole, levofloxacin, and paracetamol (IS) solutions were prepared at the concentration of 250 mg/L in 250 mL MeOH:H_2O (50:50 v/v). By taking determined volumes from the stock solutions and by diluting with MeOH:H_2O (50:50 v/v) mixture, the serial concentrations of the calibration solutions were prepared. The standard calibration solutions were prepared as to include the mixtures of amoxicillin, lansoprazole, levofloxacin, and paracetamol (IS). In the calibration standard solutions, the concentrations of amoxicillin were 15, 20, 25, 30, 35, and 40 mg/L, the lansoprazole were 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 mg/L, and the levofloxacin were 7.5, 10.0, 12.5, 15.0, 17.5, and 20.0 mg/L. As the internal standard (IS), 15 mg/L paracetamol was used in each calibration or sample solution. In the pharmaceutical dosage, amounts in the used drugs are 1000 mg amoxicillin, 30 mg lansoprazole, and 500 mg levofloxacin. Therefore, the calibration solution concentrations were prepared by considering these amounts. The second consideration is staying in a linear region. This part requires some pretesting to find the instrument response for each drug. By selecting these concentrations, we tried to bring the drug composition exactly the middle of the calibration concentrations. The amoxicillin sample solutions were prepared by dissolving the amoxicillin of 1000 mg in Largopen drug tablet using MeOH:H_2O (50:50 v/v). Lansoprazole sample solutions were obtained by dissolving Lansor tablets in MeOH. Levofloxacin samples were from Tavanic drug tablets by dissolution in MeOH:H_2O (50:50 v/v). Undissolved solid particles of the drug samples were filtered by 0.2 µm nylon membrane filter. In all the dilutions of the solutions, MeOH:H_2O (50:50 v/v) mixture solvent was used.

2.3 HPLC Measurements

A high-performance liquid chromatograph (HPLC) system (Shimadzu, Japan) equipped with an LC-20AD VP pump, an SIL-20AD VP automated sample injector, and an SPD-20A UV–Vis detector (diode array detector, DAD) was used. A GL Sciences model reverse-phase C18 column (250 mm × 4.6 mm × 5 µm) was used, and the column oven temperature was 25 °C in the HPLC system. The flow rate was set to 1.0 mL/min in isocratic mode. The measurements were performed with 5 µL sample injections. MeOH:10 mM CH_3COONH_4 (70:30 v/v) mixture was used as the mobile phase in the HPLC column [45]. The mobile phase composition, flow rate, and other parameters were determined after pre-tests to separate the chromatographic peaks of amoxicillin, lansoprazole, levofloxacin, and paracetamol (IS).

2.4 UV–Vis Absorption Measurements

An HPLC instrument was used in this study including a DAD system to quantify the drug agents. Therefore, UV–Vis absorption spectra of amoxicillin, levofloxacin, lansoprazole, and paracetamol drug agents were also measured. All the UV–Vis absorption measurements were carried out using a Shimadzu 2600 model UV–Vis spectrophotometer (Japan) and the spectra were recorded between 200 and 400 nm wavelengths. A quartz cell of 1.0 cm was used in the measurements. The solutions of 40 mg/L amoxicillin, 15 mg/L lansoprazole, 20 mg/L levofloxacin, and 25 mg/L paracetamol in MeOH:H_2O (50:50 v/v) solvent were measured on the UV–Vis absorption spectrometer.

3 Results and Discussion

3.1 UV–Vis Absorption Spectroscopy

In this HPLC method, a DAD system was used to quantify simultaneously the amoxicillin, lansoprazole, and levofloxacin analytes together with paracetamol internal standard (IS). First, the UV–Vis absorption spectra of amoxicillin, lansoprazole, levofloxacin and paracetamol were taken separately. The obtained UV–Vis spectra of them are given in Fig. 2. According to UV–Vis absorption measurements, the specific maximums of the absorption bands in the spectrum of amoxicillin were observed at 274, 231, and 205 nm wavelengths. In the spectrum of the lansoprazole, the maximums of the absorption bands were at 286 and 205 nm wavelengths. The levofloxacin was observed with the maximums of the absorption at 330, 292, 258, and 228 nm wavelengths. Lansoprazole and levofloxacin showed high absorption values at the wavelengths below 314 nm, and all of amoxicillin, lansoprazole, and levofloxacin showed high absorption

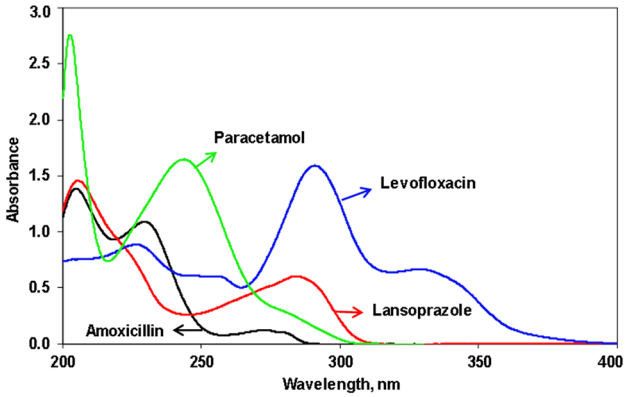


Fig. 2 UV–Vis absorption spectra of amoxicillin, lansoprazole, levofloxacin, and paracetamol

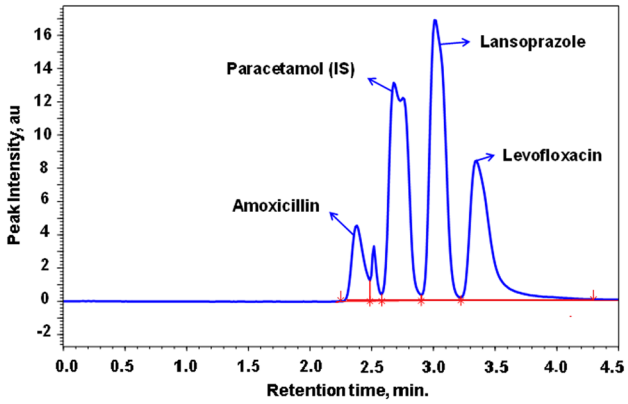


Fig. 3 HPLC chromatogram of amoxicillin, lansoprazole, levofloxacin, and paracetamol standard solution mixture

values at the wavelengths below 292 nm. Simultaneous quantification of amoxicillin, lansoprazole, and levofloxacin is possible at wavelengths below 292 nm. In the HPLC measurements with UV–Vis detector, for the simultaneous quantification of amoxicillin, lansoprazole, and levofloxacin, 265 nm wavelength was selected. At the wavelengths below 230 nm, many organic impurities can give absorption bands. Therefore, if any wavelength of higher absorption wavelengths than 230 nm is selected for the UV–Vis detector of the HPLC, better experimental results can be obtained. The wavelength of 265 nm was selected, in which all the drug agents of amoxicillin, lansoprazole, and levofloxacin as well as paracetamol (IS) have their absorption bands.

3.2 HPLC Chromatography

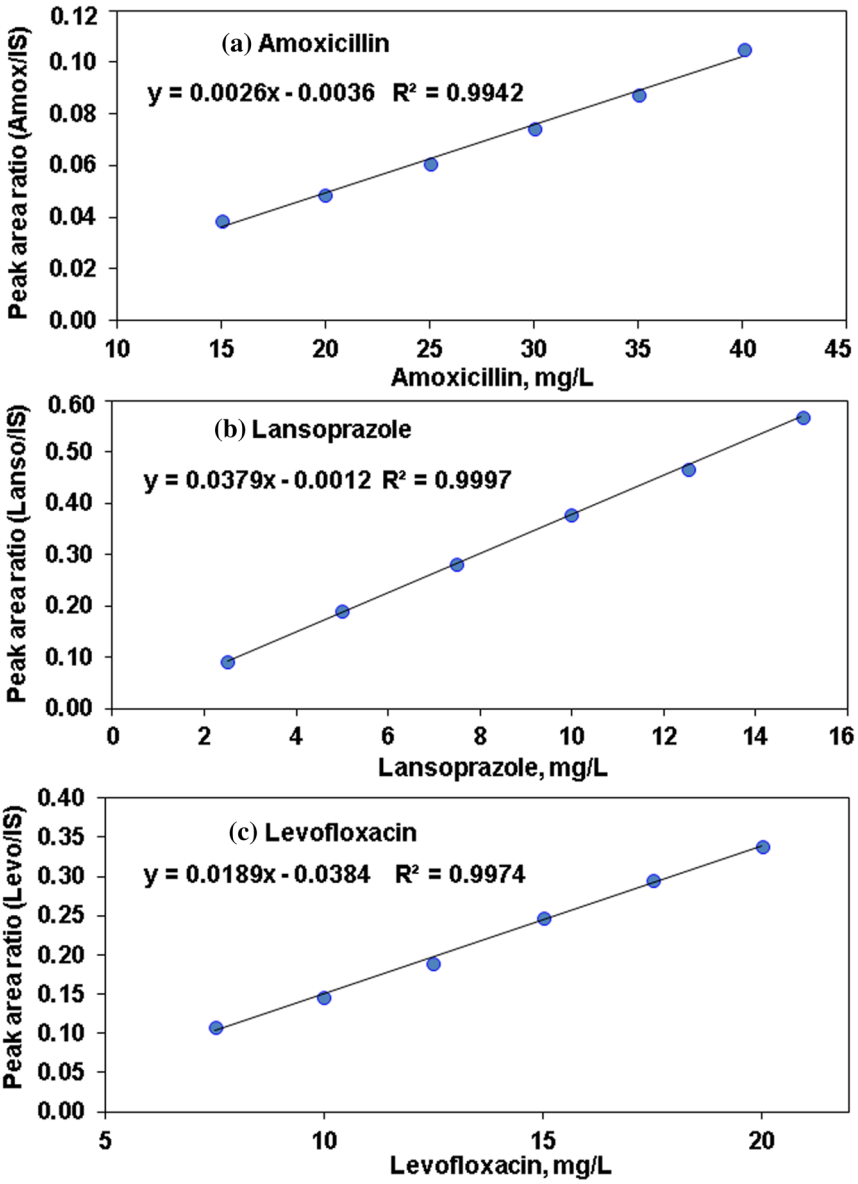
3.2.1 Optimization of HPLC

For the simultaneous determination of amoxicillin, lansoprazole, and levofloxacin, the experimental conditions of the HPLC device were optimized by making pre-tests. The flow rate, mobile phase composition, column type, UV–Vis detector wavelength, and sample injection volume were determined in the pre-test studies. The separation of amoxicillin, lansoprazole, levofloxacin, and paracetamol (IS) was achieved using a reverse-phase C18 column and MeOH:10 mM CH_3COONH_4 (70:30 v/v) mobile phase. The separated chromatogram of amoxicillin, lansoprazole, levofloxacin, and paracetamol (IS) in the optimal conditions of the HPLC measurements is given in Fig. 3. The chromatogram was obtained using the mixture of the standard solutions of the analytes. The peaks could be obtained separately in a short time period from 2.2 to 4.3 min. All the measurements were carried out at the flow rate of 1.0 mL with 5 µL sample injections. The peaks of amoxicillin, paracetamol, lansoprazole, and levofloxacin were represented on the chromatogram in Fig. 3. A small peak was observed next to the amoxicillin peak and this is not related to amoxicillin. This peak comes from lansoprazole. It is hard to find a pure lansoprazole and all comes with a coating as a micro pellets. Therefore, it is hard to remove this peak with the existing chromatographic conditions. It is possible to separate this peak by changing the chromatographic conditions, but this brought other problems like too long elution time of components or overlapped peaks of analytes. The same situation is true for the paracetamol (IS) peak. As seen in the chromatogram, paracetamol (IS) peak has a shoulder. In the same way, this shoulder can be removed by changing the chromatographic conditions, but it requires the same long elution time problem and overlapped analyte peaks. This might bring interference for the calculations. In the calibration calculations, using the peak areas and the ratios of peaks areas to the internal standard peak area, possible interferences were eliminated. After the preparation of all the calibration graphs and quantitative calculations, the obtained results were very promising. All the calculations like calibration calculations and recoveries produced very satisfactory calibration results. Therefore, no further change in the chromatographic conditions has been done. After these optimization experiments, a rapid and simultaneous determination method for amoxicillin, lansoprazole, and levofloxacin mixture was improved.

3.2.2 Calibration Measurements

The calibration curves were obtained by measuring the amoxicillin, lansoprazole, and levofloxacin mixture standard solutions of 15–40 mg/L, 2.5–15 mg/L, and 7.5–20.0 mg/L, respectively. The linarites were established by least-squares linear regression analysis of the calibration curve [46]. The peak areas of amoxicillin, lansoprazole, and levofloxacin were obtained and divided to the peak area of paracetamol (IS). Then, the analyte/paracetamol (IS) peak ratios were

plotted versus their respective concentrations. The linear regression analyses of them were performed on the resultant calibration curves. The obtained calibration curves and their data are given in Fig. 4. The results of correlation coefficients, calibration linear equation, limit of detection (LOD), and limit of quantification (LOD) were calculated from the calibration curves, and they are given in Table 1. The calibration curves for the amoxicillin, lansoprazole, and levofloxacin mixture standard solutions were resulted in good correlation coefficients (R^2) of 0.9942, 0.9997, and



(d)

No	Added (mg/L)			Peak area ratio			Found (mg/L)		
	Amox	Lanso	Levo	Amox/IS	Lanso/IS	Levo/IS	Amox	Lanso	Levo
C1	15	2.5	7.5	0.038177	0.091782	0.108450	15.78	2.45	7.78
C2	20	5.0	10.0	0.049236	0.191737	0.148677	19.95	5.09	9.91
C3	25	7.5	12.5	0.060673	0.282291	0.189675	24.27	7.48	12.08
C4	30	10.0	15.0	0.074417	0.379661	0.245895	29.46	10.04	15.06
C5	35	12.5	17.5	0.087930	0.468042	0.294602	34.57	12.37	17.63
C6	40	15.0	20.0	0.104890	0.570061	0.340141	40.97	15.07	20.05

Fig. 4 Calibration curves of **a** amoxicillin, **b** lansoprazole, **c** levofloxacin, and **d** calibration data

Table 1 Calibration results

Analyte	λ (nm)	Linear calibration equation	R^2	LOD (mg/L)	LOQ (mg/L)
Amoxicillin	265.0	$A = 0.0026C_{Amox} - 0.0036$	0.9942	2.14	7.15
Lansoprazole	265.0	$A = 0.0379C_{Lanso} - 0.0012$	0.9997	0.24	0.81
Levofloxacin	265.0	$A = 0.0189C_{Levo} - 0.0384$	0.9974	0.29	0.95

0.997, and low LOD values of 2.14 mg/L, 0.24 mg/L, and 0.29 mg/L, respectively.

3.2.3 Method Validation

After the optimization of the calibration of amoxicillin, lansoprazole, and levofloxacin mixture using paracetamol IS, the synthetic mixtures at the known concentrations of them were measured for the validation of the proposed calibration method. For this reason, 18 different synthetic mixtures in the concentration range of 15–40 mg/L for the amoxicillin, 2.5–15.0 mg/L for the lansoprazole, and 7.5–20.0 mg/L for levofloxacin were measured using the optimized calibration method. Each of the concentrations was tested three times to provide information on the variation in the peak areas of the samples. The mean recoveries, the standard deviations (SD), and the relative standard deviations were calculated, and they are shown in Table 2. The percent relative standard deviations (RSD %) for amoxicillin, lansoprazole, and levofloxacin were found as 3.91, 2.55, and 2.18%, respectively (Table 2). Additionally, one example chromatogram [V13: Amox: 25 mg/L, Lanso: 15:0 mg/L, Levo: 12.5 mg/L paracetamol (IS): 15 mg/L] of the validation solutions is given in Fig. 5. It was found that the chromatogram was similar to standard solution chromatogram with different intensities of different concentrations. Complete separation time was found as 4.5 min.

3.2.4 Analyses of Pharmaceutical Tablet Samples

The calibrated HPLC method was applied for the determination of amoxicillin, lansoprazole, and levofloxacin mixture in commercial pharmaceutical samples. The experimental results of samples are demonstrated in Table 3. The commercial pharmaceutical tablets were analyzed with good recovery percent values of 105.5%, 98.57%, and 102.5% for amoxicillin, lansoprazole, and levofloxacin in the mixture samples, respectively. Consequently, the experimental

Table 2 Recoveries in the determination of amoxicillin, lansoprazole, and levofloxacin mixture

No	Added (mg/L)			Found (mg/L)			Recovery (%)		
	Amox	Lanso	Levo	Amox	Lanso	Levo	Amox	Lanso	Levo
V1	40	7.5	12.5	40.22	7.68	12.22	100.6	102.4	97.8
V2	35	7.5	12.5	34.46	7.41	12.47	98.5	98.8	99.8
V3	30	7.5	12.5	31.12	7.44	12.93	103.7	99.1	103.4
V4	25	7.5	12.5	25.15	7.37	12.84	100.6	98.3	102.7
V5	20	7.5	12.5	20.23	7.33	12.54	101.2	97.7	100.3
V6	15	7.5	12.5	16.10	7.44	12.99	107.3	99.3	103.9
V7	25	7.5	20.0	25.02	7.53	20.60	100.1	100.5	103.0
V8	25	7.5	17.5	25.86	7.50	17.17	103.4	100.0	98.1
V9	25	7.5	15.0	25.14	7.51	15.46	100.6	100.2	103.0
V10	25	7.5	12.5	26.45	7.35	12.88	105.8	97.9	103.0
V11	25	7.5	10.0	26.34	7.42	10.30	105.4	99.0	103.0
V12	25	7.5	7.5	27.24	7.05	7.28	109.0	94.0	97.1
V13	25	15.0	12.5	28.11	15.17	12.52	112.4	101.1	100.2
V14	25	12.5	12.5	25.82	11.83	12.81	103.3	94.6	102.5
V15	25	10.0	12.5	27.86	9.78	12.82	111.5	97.8	102.6
V16	25	7.5	12.5	26.78	7.42	12.61	107.1	99.0	100.9
V17	25	5.0	12.5	25.44	4.64	12.29	101.8	92.7	98.3
V18	25	2.5	12.5	25.27	2.41	12.78	101.1	96.2	102.2
Mean % recovery							104.1	98.3	101.2
SD							4.06	2.50	2.21
%RSD							3.91	2.55	2.18

V validation, *SD* standard deviation, *RSD* relative standard deviation

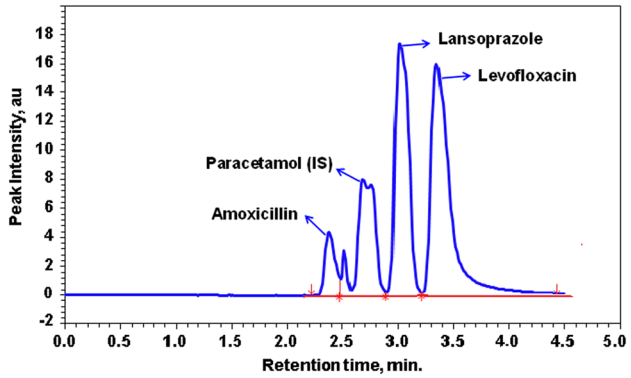


Fig. 5 HPLC chromatogram of amoxicillin (15 mg/L), lansoprazole (15:0 mg/L), levofloxacin (12.5 mg/L), and paracetamol (IS) (15 mg/L) validation (V13) mixture solution

results showed that the proposed HPLC method can be used in the simultaneous and rapid determination of amoxicillin, lansoprazole, and levofloxacin in pharmaceuticals. This method can also be applied to biological samples after simple sample preparation procedures.

3.2.5 Comparison of Experimental Results with Literature

In Table 4, the obtained experimental results were summarized and compared with the results published in the literature. It was found in the literature that the combination mixtures of amoxicillin, lansoprazole, or levofloxacin with other drugs were studied for simultaneous determination using HPLC method. However, this combination mixture of amoxicillin, lansoprazole, and levofloxacin has not been studied with simultaneous HPLC method according to reached literature. The novelty of this work is to improve an HPLC method to determine simultaneously new pharmaceutical combination mixture of amoxicillin, lansoprazole, and levofloxacin. In this improved HPLC method, the separation of amoxicillin, lansoprazole, and levofloxacin analytes was achieved in 4.5 min period and this is better result among the literatures given in Table 4. This HPLC method can be applied to routine analyses.

4 Conclusions

A rapid HPLC method has been developed for the simultaneous determination of amoxicillin, lansoprazole, and levofloxacin in pharmaceuticals together with paracetamol internal standard (IS). These drug agents have been quantified with UV–Vis detector of the HPLC instrument at 265 nm wavelength. With reverse-phase C18 column and MeOH-0.01 M CH_3COONH_4 (70:30 v/v) mobile phase, amoxicillin, lansoprazole, and levofloxacin could be separated, calibrated, and determined in their mixture solutions. The linear calibration curves of them were obtained in the ranges of 15–40 mg/L, 2.5–15.0 mg/L, and 7.5–20.0 mg/L for amoxicillin, lansoprazole, and levofloxacin, with excellent calibration correlations (R^2: 0.9942, 0.9997, and 0.9974) and with low LOD (2.14, 0.24, and 0.29 mg/L), respectively. The percentage recoveries of the amoxicillin, lansoprazole, and levofloxacin in commercial pharmaceuticals were 105.5%, 98.57%, and 102.5%, respectively. The results showed that amoxicillin, lansoprazole, and levofloxacin together with paracetamol IS could be separated and determined rapidly and simultaneously without any separation using proposed HPLC method.

Table 3 Analysis results of amoxicillin, lansoprazole, and levofloxacin in pharmaceutical tablets

No	Added (mg/L)			Found (mg/L)			Found in drug tablets (mg)		
	Amox	Lanso	Levo	Amox	Lanso	Levo	Amox	Lanso	Levo
T1	25	7.5	12.5	25.55	7.32	12.49	1022.1	29.27	499.5
T2	25	7.5	12.5	26.27	7.94	13.20	1050.8	31.77	528.0
T3	25	7.5	12.5	25.67	7.09	12.31	1026.8	28.37	492.2
T4	25	7.5	12.5	26.54	7.31	13.04	1061.5	29.22	521.8
T5	25	7.5	12.5	28.14	7.45	12.94	1125.8	29.81	517.8
T6	25	7.5	12.5	26.10	7.24	12.87	1043.8	28.97	514.9
Mean recovery							1055.1	29.57	512.4
% Recovery							105.5	98.57	102.5
SE							11.89	3.72	4.34
SD							37.61	1.176	13.72
CL (0.05)							23.31	7.29	8.50
% RSD							3.56	3.98	2.68

T tablet, *SD* standard deviation, *RSD* relative standard deviation, *SE* standard error, *CL* confidence level

Table 4 Comparison of simultaneous HPLC measurements with published results in the literature

Mixed analytes	Conditions of HPLC	Results	References
Lansoprazole, clarithromycin, amoxicillin	RP-C18 column; DAD; Mobile phase: 25 mM NH_4Cl buffer, acetonitrile:water (45:55 v/v)	Complete separation time: 20 min.; RSD %: 0.08–2.9	[15]
Amoxicillin, metronidazole, rabeprazole sodium	Hypersil BDS-C8 column; DAD; Mobile phase: Phosphate buffer pH 7 and acetonitrile (70: 30 v/v)	Ranges: 25–250, 25–250, 5–50 µg/mL; RSD %: 1.59–1.83; LOQ: 3.67–1.89 µg/mL; Retention times 2.36, 3.55, 8.72 min	[47]
Garenoxacin, moxifloxacin, levofloxacin	C18 column; DAD; Mobile phase: Acetonitrile, 0.1 M phosphoric acid + sodium hydroxide buffer (pH 3.0), 0.01 M *n*-octylamine (pH 3.0)	Ranges: 4–80, 4–80, 10–200 µg/mL; Complete separation time: 10.7 min.; Quantification limits: 6.0, 8.8, 7.3 µg/mL. SDs: 0.3, 0.3, 0.4	[48]
Amoxicillin, tinidazole, esomeprazole, lansoprazole	LiChrospher® 100 RP-18 column; Mobile phase: 40:60 v/v acetonitrile and phosphate buffer (0.001 M, pH 7.6)	Range: 250–2000, 125–1000, 5–40 and 7.5–60 µg/mL; Retention times 3.11, 5.37, 11.71 and 17.67 min; RSD %: 0.3126, 0.4598, 1.0701 and 0.5072: Lowest determined concs: 8.4, 4.1, 1.8 and 1.6 µg/mL	[49]
Omeprazole, pantoprazole, lansoprazole, rabeprazole	RP Tracer Excel 120 ODS column; Isocratic mobile phase: Phosphate buffer (10 mM)/acetonitrile (53/47, v/v adjusted pH to 7.3 with triethylamine)	Ranges: 20–2500 ng/mL, 20–4000 ng/mL, 20–3000 ng/mL, 20–1500 ng/mL; LOQs: 20 ng/mL; retention times: 5.1–8.1 min	[50]
Amoxicillin, lansoprazole, levofloxacin	RP-C18 column; DAD; Mobile phase: MeOH-10 mM CH_3COONH_4 (70:30 v/v)	Ranges: 15–40, 2.5–15.0, 7.5–20.0 mg/L; LODs: 2.14, 0.24, 0.29 mg/L; Retention times: 2.2–4.5 min	This work

RP reverse phase, *DAD* diode array detector, *RSD* relative standard deviation, *LOD* limit of detection

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