ORIGINAL ARTICLE

Development and Validation of a New RP-UFLC Method for the Assay of Binary Mixture of Perindopril Erbumine and Amlodipine Besylate

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Abstract

Introduction: Perindopril erbumine is a drug used for the treatment of hypertension which acts by inhibition of angiotensin-converting enzyme. Amlodipine besylate is a calcium channel blocker. A new reversed-phase ultra-fast liquid chromatography method has been developed for the simultaneous determination of perindopril erbumine and amlodipine besylate. **Materials and Methods:** An ion pairing agent, tetra butyl ammonium hydrogen sulfate (10 mM) with acetonitrile was used as mobile phase (pH to 3.45) (45:55, v/v) on isocratic mode with flow rate 0.8 mL/min (Detection wavelength 215 nm). **Results and Discussion:** Beer-Lambert's law was obeyed over the concentration range 0.1–20 µg/mL and 0.25–50 µg/mL with linear regression equation y = 28559x – 1188 (R^2 = 0.9999) and y = 20401x + 7.07 (R^2 = 0.9999) for perindopril erbumine and amlodipine besylate, respectively. The limit of quantification values were found to be 0.0912 µg/mL and 0.2123 µg/mL and that of limit of detection values was 0.0291 µg/mL and 0.0691 µg/mL for perindopril erbumine and amlodipine besylate, respectively. The proposed method was validated and found to be simple, precise, accurate, robust, and useful for the routine analysis of pharmaceutical formulations. **Conclusion:** The proposed method was validated and found to be simple, precise, accurate, robust, and useful for the routine analysis of pharmaceutical formulations.

Key words: Amlodipine besylate, perindopril erbumine, reversed-phase ultra-fast liquid chromatography, validation

INTRODUCTION

Perindopril erbumine (PD) [Figure 1a] ($C_{19}H_{32}N_2O_5C_4H_{11}N$) is a tertiary butyl amine salt of perindopril which is the ethyl ester of non-sulfhydryl angiotensin-converting enzyme inhibitor. Perindopril is a pro-drug used for the treatment of hypertension in congestive heart failure and other cardiac diseases.[1,2] Amlodipine besylate (AM) [Figure 1b] is an anti-hypertensive drug approved by FDA in 1987. Amlodipine is a calcium channel blocker.[3]

Duraisamy *et al*. developed a UHPLC-ESI-MS/MS method[4] for the simultaneous estimation of perindopril arginine and amlodipine besylate using Waters ACQUITY UPLC® BEH C18 column and mobile phase mixture consisting of 20 mM ammonium acetate with 0.1% of formic acid and the mobile Phase B consisting of acetonitrile: Methanol (80:20, v/v) with 0.1% of formic acid with flow rate 0.25 ml/min on gradient mode and the linearity was obeyed over the concentration range 0.25–500 ng/ml and 1.0–100 ng/ml for perindopril arginine and amlodipine besylate, respectively. The sample injection volume was 5 µL and the mass spectrometer was operated in the multiple reaction monitoring mode.

Raju and Rao developed a reversed-phase ultra-fast liquid chromatography (RP-HPLC) method[5] for the simultaneous estimation of perindopril and amlodipine using Xterra C18 column and mobile phase mixture, phosphate buffer: acetonitrile in 65:35 (v/v) ratio with flow rate 0.6 ml/min (UV detection 237 nm) in which perindopril was eluted at 5.282 min and amlodipine at 8.506 min, respectively, and the linearity

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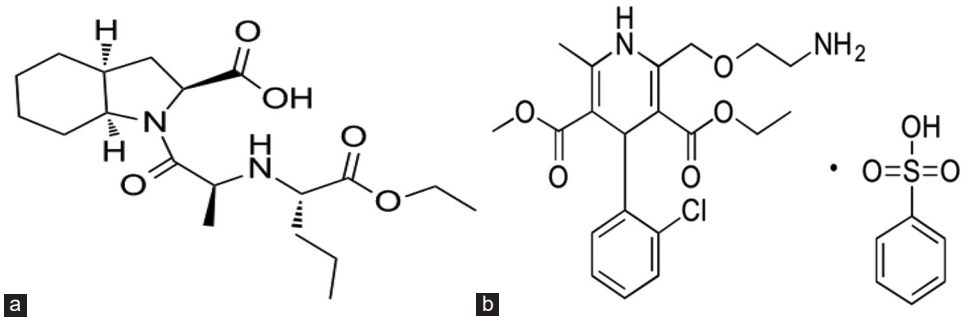

Figure 1: (a) Structure of perindopril, (b) Structure of amlodipine besylate

Table 1: Literature survey

Method	Mobile phase (v/v)	Flow rate (ml/min)	λ (nm)	Linearity (μg/mL)	Reference
UHPLC-ESI-MS/MS (Gradient mode)	Mobile phase A: 20 mM Ammonium acetate: 0.1% Formic acid Mobile phase B: Acetonitrile: Methanol (80:20): 0.1% Formic acid	0.25	-	0.25–500 ng/ml (PD) 1.0–100 ng/ml (AM)	4
HPLC	Phosphate buffer: Acetonitrile (65:35)/0.6/237	0.6	237	10–50 (AM) 200–1000(PD)	5
HPLC	Acetonitrile: Methanol: Phosphate buffer with TEA (pH 5.0) (40:20:40)	1.0	226	60–140 (AM) 48–112 (PD)	6
HPLC	Phosphate buffer (pH 5.0): Acetonitrile (70:30)	1.0	240	10–30 (AM) 5–15 (PD)	7
HPLC	Buffer: Acetonitrile (65:35) and pH adjusted to 2.6 with dilute o-phosphoric acid	1.0	210	10–75 (AM) 8–60 (PD)	8
HPLC	Methanol: Phosphate buffer (27:73)	1.1	270	2–10 (AM) 3–15 (PD)	9
HPTLC	Chloroform: Methanol: Water: Glacial acetic acid: Triethylamine (10:7:5:0.3:0.2)	-	208	1–10 (AM) 1–10 (PD)	10
HPLC	Phosphate buffer (pH 4.6): Acetonitrile (60:40)	1.0	210	1–50 (AM) 1–50 (PD)	10
Spectroscopy	Methanol (First derivative ratio spectroscopy)	-	348 AM) 227 (PD)	10–60 (AM) 20–80 (PD)	
HPLC	0.05M Potassium dihydrogen phosphate buffer (pH 3.0 adjusted with o-H3PO4): Acetonitrile (30:70): 0.002 M Sodium heptane sulfonate	1.0	215	5–200 (AM) 5–120 (PD)	11
TLC-Densitimetry	n-Butanol: Water: glacial acetic acid (40:50:10)	-	365 (AM) 215 (PD)	1–6 (AM) 1–20 (PD)	11
HPLC	0.1% OPA (pH 3.0): Acetonitrile (70: 30)	0.7	230	10–50 (AM) 8–40 (PD)	12
TLC-Densitimetry	Ethyl acetate: Methanol: Toluene: Ammonia solution (33%) (6.5: 2: 1: 0.5)	-	-	1–4 (AM) 2–14 (PD)	13
HPLC	0.01M phosphate buffer solution (pH 2.5 adjusted with 85% phosphoric acid): acetonitrile: tetrahydrofuran (60:40:0.1)	-	218	5–45 (AM) 10–100 (PD)	13
UFLC	10 mM Tetra butyl ammonium hydrogen sulphate (pH 3.45): Acetonitrile (45:55)	0.8	210	0.25–50 (AM) 0.1–20 (PD)	Present work

was obeyed over the concentration range 200–1000 µg/ml and 10–50 µg/ml for perindopril and amlodipine, respectively.

Kalpana *et al*. developed a RP-HPLC method[6] for the simultaneous estimation of perindopril and amlodipine using Inertsil ODS 3V C18 column and mobile phase mixture consisting of acetonitrile: methanol: a mixed buffer of 0.02M potassium dihydrogen phosphate buffer and 0.02M sodium dihydrogen phosphate buffer with 1mL TEA (pH 5.0) (40:20:40) (v/v) ratio with flow rate 1.0 ml/min (UV detection 226 nm) in which perindopril was eluted at 2.9 min and amlodipine at 4.9 min, respectively, and the linearity was obeyed over the concentration range 48–112 µg/ml and 60.0–140.0 µg/ml for perindopril and amlodipine, respectively.

Bhagirath *et al*. developed a RP-HPLC method[7] for the simultaneous estimation of amlodipine and perindopril using C18 column and mobile phase mixture consisting of phosphate buffer (pH 5.0): Acetonitrile (70:30) (v/v) ratio with flow rate 1.0 ml/min (UV detection 240 nm) in which amlodipine was eluted at 7.110 min and perindopril at 3.243 min, respectively, and the linearity was obeyed over the concentration range 5–15 µg/ml and 10–30 µg/ml for perindopril and amlodipine, respectively, within a run time of 10 min.

Jignesh *et al*. developed a RP-HPLC method[8] for the simultaneous estimation of amlodipine and perindopril using Eclipse XDB C-8 column and mobile phase mixture consisting of phosphate buffer: Acetonitrile in 65:35 (v/v) ratio (pH adjusted

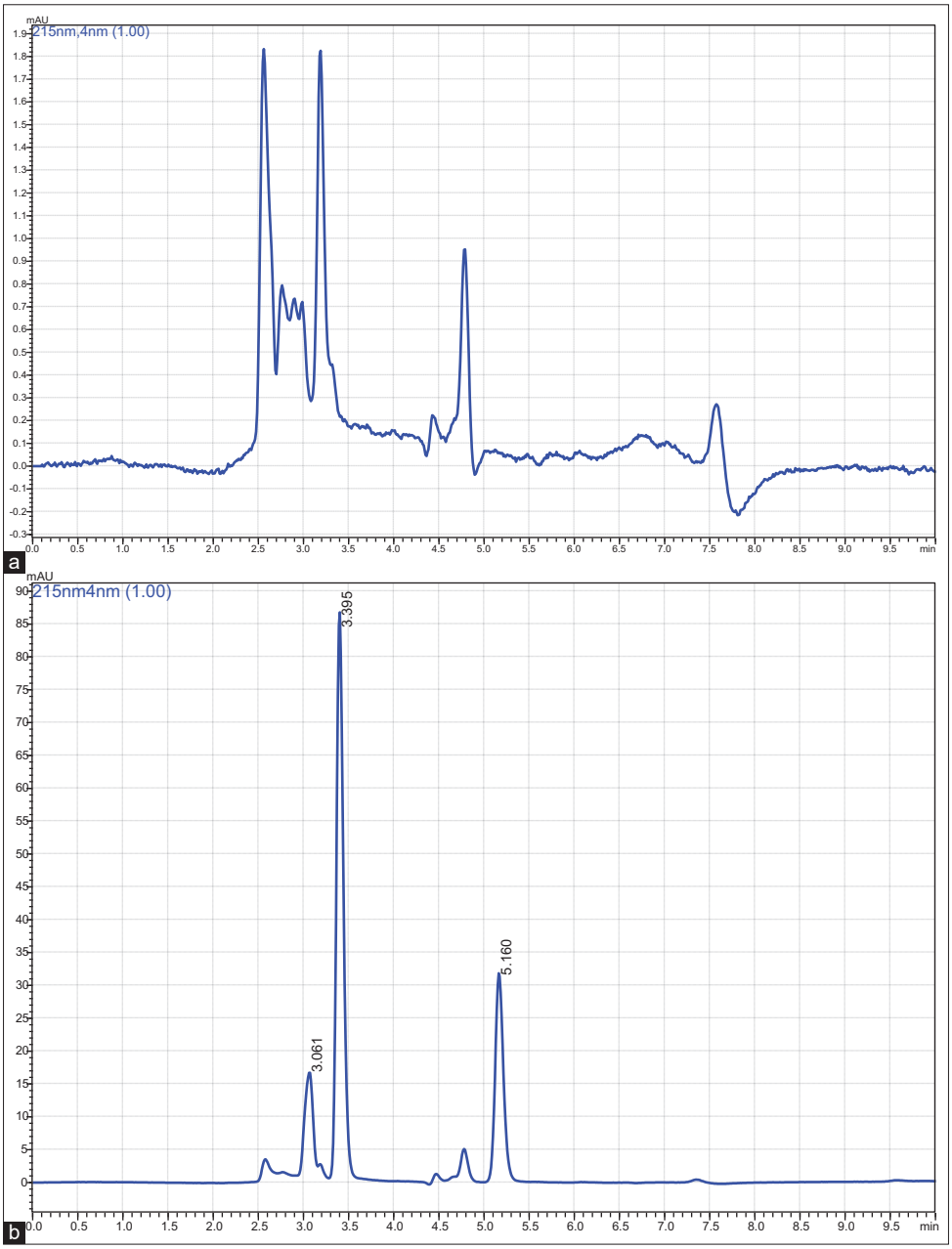

Figure 2: (a) Placebo and (b) representative chromatogram of perindopril erbumine (API) (4 µg/mL) and amlodipine besylate (API) (10 µg/mL)

to 2.6 with dilute orthophosphoric acid with flow rate 1.0 ml/min (UV detection 210 nm) in which amlodipine was eluted at 5.504 min and perindopril at 3.172 min, respectively, and the linearity was obeyed over the concentration range 8–60 µg/ml and 10–75 µg/ml for perindopril and amlodipine, respectively, within a run time of 8 min.

Gunasekar developed a RP-HPLC method[9] for the simultaneous estimation of amlodipine and perindopril using C18 column and mobile phase mixture consisting of methanol: phosphate buffer in 27: 73 (v/v) ratio with flow rate 1.1 ml/min (UV detection 270 nm) in which amlodipine was eluted at 4.2 min and perindopril at 7.3 min, respectively, and the linearity was obeyed over the concentration range 3–15 µg/ml and 2–10 µg/ml for perindopril and amlodipine, respectively.

Ali *et al*. developed a TLC densitometric method[10] and a RP-HPLC method for the simultaneous estimation of amlodipine and perindopril in binary mixtures. The TLC densitometric method involves the separation of these drugs on silica gel 60 F254 TLC plates using Chloroform: Methanol: Water: Glacial acetic acid: Triethylamine (10:7:5:0.3:0.2) where the bands were scanned at 208 nm and the linearity was observed as 1–10 µg for both the drugs. In RP-HPLC method, mobile phase mixture consisting of phosphate buffer: Acetonitrile (60:40) (pH 4.6) was used with a flow rate of 1.0 ml/min (UV detection 210 nm) and the linearity was obeyed over the concentration 1–50 µg/ml for both the drugs.

Samia *et al*. developed[11] a high performance liquid chromatography, TLC-densitometry, and a first-derivative spectrophotometry for the simultaneous determination of amlodipine and perindopril in bulk powder and its tablets. In the first derivative ratio spectra, the amplitudes were measured at 348 nm for amlodipine using 50 µg/mL of perindopril as a divisor and for perindopril, the amplitudes were measured at 227 nm using 30 µg/mL of amlodipine as a divisor. In the ion-pair RP-HPLC method, C18 Zorbax Extend column was used and a mobile phase consisting of 0.05 M potassium dihydrogen phosphate buffer (adjusted to pH 3.0 with *o*-phosphoric acid): acetonitrile (30:70) at a flow rate 1 mL/min using 0.002 M sodium heptane sulfonate as aqueous phase (UV detection

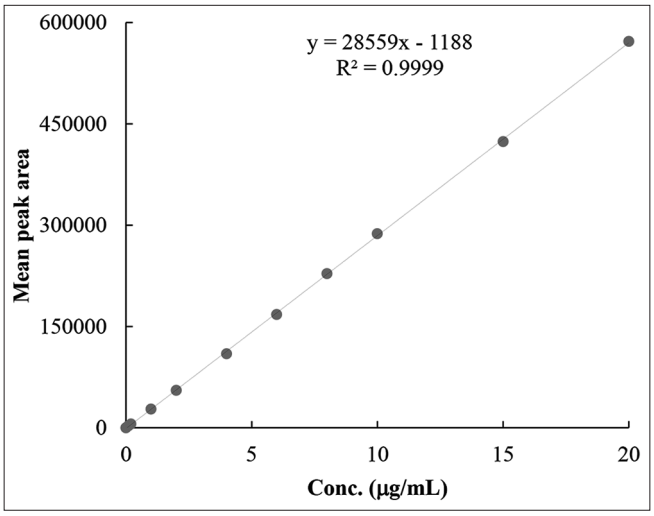

Figure 3: Calibration curve of perindopril erbumine

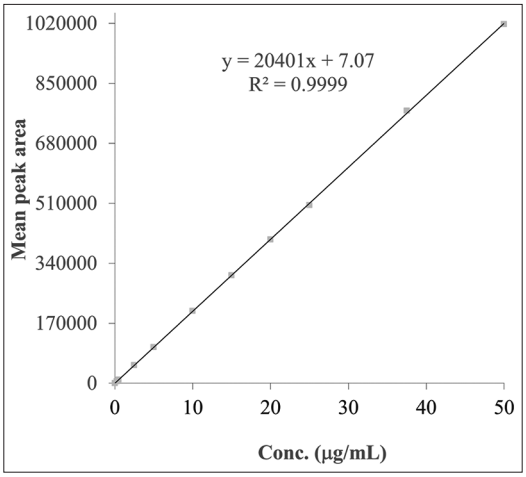

Figure 4: Calibration curve of amlodipine besylate

Table 2: Linearity

Conc. (µg/mL)		*Mean peak area	
PD	AM	PD	AM
0	0	0	0
0.1	0.25	2851	5223
0.2	0.50	5667	10354
1	2.5	27623	51205
2	5	55425	102213
4	10	109658	204688
6	15	167692	306132
8	20	228149	407427
10	25	287432	504731
15	37.5	423742	772136
20	50	572178	1017421

*Mean of three replicates

Table 3: Intraday precision study

Conc. (µg/mL)		*Mean peak area	
PD	AM	PD	AM
4	10	109658	204688
4	10	108897	205198
4	10	108126	205427
4	10	109412	204928
4	10	108954	205218
4	10	109345	205369
Mean		109065.33	205138
SD		512.7197	280.5288
% RSD		0.4701	0.1368

*Mean of three replicates

at 215 nm). In TLC-densitometric method, the separation was carried out with Fluka TLC aluminum sheets silica gel 60 F_{254}, using n-butanol: water: glacial acetic acid (4:5:1) as the mobile phase with TLC scanner 3D densitometer Model 3 S/N 130319 was connected with winCats software.

Bhagyalaxmi *et al*. developed a RP-HPLC method[12] for the simultaneous estimation of amlodipine and perindopril using Phenomenex C18 column and mobile phase mixture consisting of 0.1% of OPA (pH 3.0): acetonitrile: (70:30) with flow rate 0.7 ml/min (UV detection 230 nm) in which amlodipine was eluted at 2.982 min and perindopril at 1.890 min, respectively, and the linearity was obeyed over the concentration range 8–40 mg/ml and 10–50 mg/ml for perindopril and amlodipine, respectively.

Zaazaa *et al*. developed[13] a TLC-densitometry and RP-HPLC method for the simultaneous determination of amlodipine

Table 4: Interday precision study

Drug	Conc. (µg/mL)	Day 1	Day 2	Day 3	*Mean peak area±SD (% RSD)
PD	4	109658	108024	108251	108644.33±885.1679 (0.8147)
AM	10	204688	204712	204585	204661.67±67.4709 (0.0329)
PD	6	167692	167824	167328	167614.67±256.8839 (0.1533)
AM	15	306132	306015	306251	306132.67±118.0014 (0.0386)
PD	8	228149	228245	228156	228183.33±53.5195 (0.0235)
AM	20	407427	407254	407326	407335.67±86.9042 (0.0213)

*Mean of three replicates

Table 5: Accuracy study

Spiked conc. (µg/mL)		Formulation (µg/mL)		Total Conc. (µg/mL)		*Conc. obtained (µg/mL) ±SD (%RSD)		% Recovery	
PD	AM	PD	AM	PD	AM	PD	AM	PD	AM
1	2.5	2	5	3	7.5	2.97±0.0160 (0.54)	7.43±0.0634 (0.82)	99.00	99.07
1	2.5	2	5	3	7.5				
1	2.5	2	5	3	7.5				
2	5	2	5	4	10	3.94±0.0363 (0.92)	9.94±0.0686 (0.69)	98.50	99.40
2	5	2	5	4	10				
2	5	2	5	4	10				
3	7.5	2	5	5	12.5	4.96±0.0322 (0.65)	12.47±0.0611 (0.49)	99.20	99.76
3	7.5	2	5	5	12.5				
3	7.5	2	5	5	12.5				

*Mean of three replicates

Table 6: Robustness study (PD: 4.0 µg/mL and AM: 10 µg/mL)

Parameter	Condition	*Mean peak area		*Mean peak area±SD (% RSD)	
		PD	AM	PD	AM
Flow rate (±0.1mL/min)	0.7	107854	204796	109256±1250.84 (1.1449)	204634.33±194.15 (0.0949)
	0.8	109658	204688		
	0.9	110257	204419		
Detection wavelength (±2 nm)	217	109451	204783	109454±201.53 (0.1836)	204671±121.39 (0.0593)
	215	109658	204688		
	213	109255	204542		
Mobile phase ratio Buffer: Acetonitrile (45:55) (±5%)	50: 50	108145	204743	108782.33±784.15 (0.7208)	204676±73.74 (0.0360)
	45: 55	109658	204688		
	40: 60	108544	204597		
pH (±0.05 unit)	3.40	109352	204326	109477±160.5023 (0.1466)	204635.33±286.6519 (0.1401)
	3.45	109658	204688		
	3.50	109421	204892		

*Mean of three replicates

and perindopril in the presence of their degradation products. In TLC-densitometric method, the separation was carried out with silica gel 60 F254 TLC plates and ethyl acetate: methanol: toluene: ammonia solution (33%) in 6.5:2:1:0.5 ratio as the mobile phase. Linearity was obeyed over the concentration range 2–14 mg and 1–4 mg/ml for perindopril and amlodipine, respectively. In HPLC method, Nucleosil C18 analytical column was used with mobile phase mixture, 0.01M phosphate buffer solution (pH 2.5 adjusted with 85% phosphoric acid): acetonitrile: tetrahydrofuran (60:40:0.1%) (UV detection at 218 nm). Linearity was obeyed over the concentration range 10–100 µg/ml and 5–45 µg/ml for perindopril and amlodipine, respectively.

Table 7: Assay of perindopril erbumine and amlodipine besylate tablets

Brand	Label claim (mg)		Amount found (mg)		% assay	
	PD	AM	PD	AM	PD	AM
Brand I	4	10	3.968	9.912	99.20	99.12
Brand II	4	10	3.914	9.973	97.85	99.73

*Mean of three replicates

In the present study, a new RP-UFLC method has been developed for the simultaneous estimation of perindopril

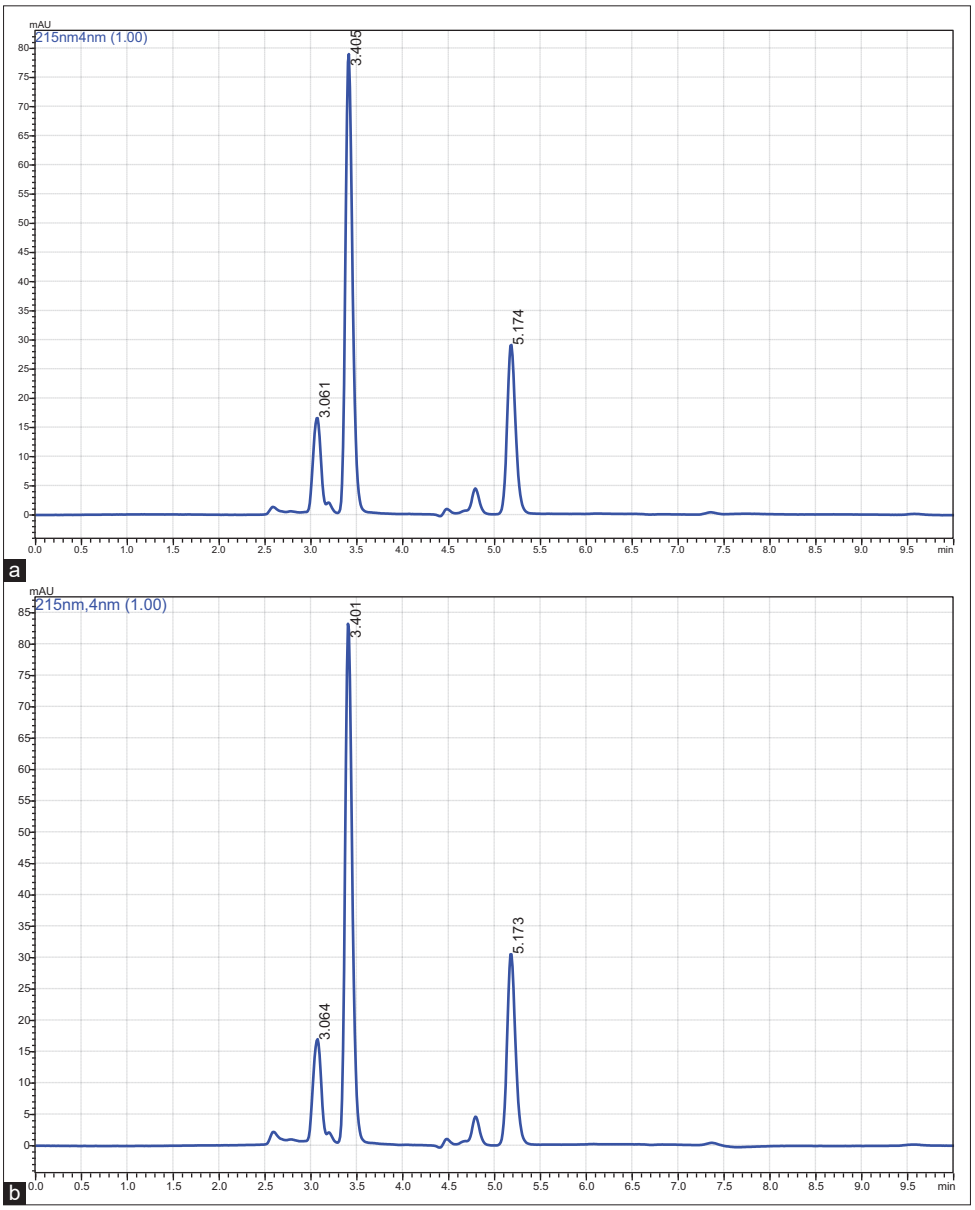

Figure 5: Representative chromatograms of perindopril erbumine and amlodipine besylate tablets. (a) Brand I: Perindopril erbumine (Rt 3.061 min); Theoretical plates: 4892.965; Tailing factor: 1.522. Amlodipine Besylate (Rt 5.174 min and 3.405 min); Theoretical plates: 12413.939; Tailing factor: 1.235; Resolution: 10.292. (b) Brand II: Perindopril erbumine (Rt 3.064 min); Theoretical plates: 4570.894; Tailing factor: 1.452. Amlodipine Besylate (Rt 5.173 min and 3.401 min); Theoretical plates: 12340.095; Tailing factor: 1.228; Resolution: 10.276

erbumine and amlodipine besylate and the method was validated as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation and chromatographic conditions

Shimadzu Model UFLC system SPD-M20A 230V with PDA detector and LC- 20AD pumps and C8 (2) 100A (Luna) Column (250 mm × 4.6 mm i.d. 5 µm particle size) was employed for the present study.

A mixture of tetra butyl ammonium hydrogen sulfate and acetonitrile (45: 55) was used as mobile phase with flow rate 0.8 ml/min (UV detection 215 nm) for the chromatographic separation of perindopril erbumine and amlodipine besylate in tablets.

Preparation of perindopril erbumine and amlodipine besylate solutions

25 mg of perindopril erbumine and amlodipine besylate (API) were individually weighed and transferred into two different 25 mL volumetric flasks, dissolved in HPLC grade acetonitrile (1000 µg/mL) and sonicated for 30 min and dilutions were made with the mobile phase. All the solutions were filtered before use through membrane filter.

Method validation[14]

Linearity, precision, accuracy, and robustness

A series of solutions containing both perindopril erbumine (0.1–20 µg/mL) and amlodipine besylate (0.25–50 µg/mL) were prepared from the stock solution in 10 ml volumetric flasks with mobile phase and sonicated. Each of these solutions were injected into the UFLC system (n = 3) and the peak area was noted from the resulting chromatograms. The mean peak area was calculated and a calibration graph was drawn by plotting the concentration of the drug solutions on the X-axis and the corresponding peak area (n = 3) of the chromatograms on the Y-axis.

The intraday precision studies were conducted on the same day at different equal time intervals and the interday precision studies were conducted on three successive days (Day 1, Day 2, and Day 3) at different concentration levels and the statistical parameters were calculated.

Accuracy studies were performed by spiking the formulation solution with 50, 100, and 150% of API of perindopril erbumine and amlodipine besylate solutions and injected in to the UFLC system. The peak area and thereby the mean peak area were noted and the percentage recovery as well as % RSD were calculated with the help of calibration curve.

In general, in robustness study, small changes such as mobile phase ratio, pH, flow rate, and detection wavelength are incorporated purposefully in the optimized chromatographic conditions and the method was studied and finally the percentage relative standard deviation was calculated.

Assay of perindopril erbumine and amlodipine besylate tablets

The combination of perindopril and amlodipine besylate is available in India with brand names, Coversyl AM (Perindopril: 4 mg and Amlodipine besylate 10 mg) (SERDIA Pharmaceuticals [India] Pvt. Ltd), Coveril AM (Perindopril erbumine: 4 mg and Amlodipine 5 mg) (Johnlee Pharmaceuticals Pvt. Ltd), AMTAS PRP (Perindopril: 4 mg and Amlodipine 5 mg) (Intas Pharmaceuticals Ltd), Prestidia ([Perindopril arginine: 3.5 mg and Amlodipine 2.5 mg] [Adhera Therapeuticals], etc.

Twenty tablets of three different brands available from the local pharmacy store were collected, weighed, and tablet powder equivalent to 4 mg perindopril erbumine and 10 mg Amlodipine besylate was transferred carefully in to three different 10 ml volumetric flasks and mobile phase was added followed by sonication. The solutions were filtered through membrane filter and dilutions were made using the mobile phase and 20 µL of each of these solutions were injected (n = 3) in to the UFLC system and the peak area and mean peak area were calculated and the percentage recovery as well as % RSD was also calculated from the calibration curve.

RESULTS AND DISCUSSION

A new RP-UFLC method has been developed for the simultaneous determination of perindopril erbumine and amlodipine besylate using an ion pairing agent and the method was validated as per ICH guidelines. To change the retention time of ionic analytes, usually ion pairing agents are employed and it is an effective technique of RP-HPLC. Shimadzu Model UFLC system SPD-M20A 230V with PDA detector and LC- 20AD pumps and C8 (2) 100A (Luna) Column (250 mm× 4.6 mm i.d. 5 µm particle size) was employed for the present study.

The previous literature was reviewed thoroughly and some of the highlights are given in Table 1.

Different trial runs were made basing on the literature with various chromatographic conditions. An ion pairing agent, tetra butyl ammonium hydrogen sulfate (10 mM) along with acetonitrile, was used as mobile phase (pH to 3.45) (45:55, v/v) on isocratic mode with flow rate 0.8 mL/min (Detection wavelength 215 nm). The chromatograms obtained for placebo, perindopril erbumine (4 µg/mL), and amlodipine besylate API (10 µg/mL) are shown in Figure 2. Perindopril was eluted at Rt

3.061 min with theoretical plates: 4172.058 and tailing factor: 1.347. Amlodipine in besylate salt form has shown peaks at Rt. 5.160 min and 3.395 with theoretical plates: 12274.238 and tailing factor: 1.232 and resolution 10.278.

Linearity, precision, accuracy, and robustness

Beer-Lambert's law was obeyed over the concentration range 0.1–20 µg/mL and 0.25–50 µg/mL [Table 2] with linear regression equation y = 28559 × −1188 (R^2 = 0.9999) and y = 20401× + 7.07 (R^2 = 0.9999) for perindopril erbumine [Figure 3] and amlodipine besylate, respectively [Figure 4]. The limit of quantification values was found to be 0.0912 µg/mL and 0.2123 µg/mL and that of limit of detection values was 0.0291 µg/mL and 0.0691 µg/mL for perindopril erbumine and amlodipine besylate, respectively. The % RSD is 0.4701 (PD) and 0.1368 (AM) in intraday precision studies [Table 3] whereas for intraday precision study, the values were found to be 0.0235–0.8147 for perindopril erbumine and 0.0213–0.0386 for amlodipine besylate [Table 4] indicating that the method is precise.

The % RSD in the accuracy study was found to be 0.54–0.92 for perindopril erbumine and 0.49–0.82 for amlodipine besylate indicating that the method is accurate with % recovery 98.5–99.2 for perindopril erbumine and 99.07–99.76 for amlodipine besylate [Table 5], respectively. In robustness study, the % RSD was found to be 0.1466–1.1449 and 0.0360–0.1401 [Table 6] for perindopril erbumine and amlodipine besylate, respectively, indicating that the method is robust.

Assay of perindopril erbumine and amlodipine besylate tablets

The two different brands of tablets procured from two different manufacturers were tested for its assay with the optimized chromatographic conditions. The percentage of purity was found to be 99.20–97.85 for perindopril erbumine and that of amlodipine besylate was found to be 99.12–99.73 [Table 7]. The system suitability parameters such as theoretical plates, tailing factor, and the resolution of the chromatograms obtained were within the acceptability criteria and the representative chromatograms were shown in Figure 5a and Figure 5b.

CONCLUSION

The authors have proposed a new RP-UFLC method for the quantification of the binary mixture of perindopril erbumine and amlodipine besylate in tablet formulations. The method was evaluated using an ion pairing agent and the method is proved as simple, precise, accurate, and robust. The method is so suitable for the routine analysis of perindopril erbumine and amlodipine besylate in pharmaceutical industries and no interference of excipients was observed during the study.

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