Supporting Information

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Development of TLC method fors simultaneous estimation of novel combination of amlodipine besylate, rosuvastatin calcium and fimasartan potassium in synthetic mixture

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S1. Experimental

S.1.1. Selection of Solvent

The solubility of Amlodipine besylate, Rosuvastatin calcium, and Fimasartan potassium were checked by the different solvents like methanol, Acetonitrile, chloroform, dichloromethane, water, and toluene where all the drugs were freely soluble in methanol and Acetonitrile. Therefore, methanol has been selected as a common solvent for analysis.

S.1.2. Selection of Analytical Wavelength

The solution of Amlodipine besylate, Rosuvastatin calcium, and Fimasartan potassium were prepared in methanol individually at a concentration of 10 µg/mL and scanned in the wavelength rage of 400-200 nm. Appreciable absorbance was show at 242 nm analytical wavelength, which was selected based on overlay spectrum of Amlodipine besylate, Rosuvastatin calcium, and Fimasartan potassium.

S.1.3. Selection of Stationary Phase

Pre-coated silica gel G 60 – F254 Aluminium sheet (E. Merck, Germany), 10x10 cm, Thickness layer 0.2 mm prewashed with methanol and dried at room temperature.

S.1.4. Calibration Curve

The working solutions of 2 µL to 14 µL were applied to precoated HPTLC plate with help of Hamilton syringe (100 µl) using Linomat 5 sample applicator. The concentration level over the range was found to be 200-1400 ng/band for AML, 400-2800 ng/band for ROS, and 600-8400 ng/band for FIM, respectively. Plates were scanned in optimised chromatographic conditions. The calibration curve was obtained by plotting mean peak area vs concentration with help of winCATS software 1.4.8.

S.1.5. Solution Stability

The stock standard solution of mixture was stored at room temperature for 24 h. and analyzed at interval of 0, 2, 4, 8 and 24h. The working standard solution of 100 µg/mL of Amlodipine besylate, 200 µg/mL of Rosuvastatin calcium, and 600 µg/mL of Fimasartan potassium were prepared and used for the solution stability study. From the above stock solution, 8 µl solutions was applied on precoated TLC plate with the help of Hamilton syringe by applying six times of middle concentration of calibration range.

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S.2. Result and Discussion

S. 2.1. Selection of Analytical Wavelength

The solution was applied in the form of band in concentration range of 200-1400 ng/band for AML, 400-2800 ng/band for ROS, and 1200 – 8400 ng/band of FIM. All the solutions were prepared in methanol. The plate was developed using n-Hexane: n-Butanol: Methanol: Glacial Acetic Acid (5.7:2:2.3:0.1, v/v/v/v) and dry by hair dryer. It was subjected to densitometry measurement in scanning mode in the UV region of 200-800 nm and the overlay spectrum was recorded using CAMAG TLC Scanner 4. The overlay spectra Showed that the drug absorbance appreciably at 242 nm. Therefore, it was selected as detection wavelength. Figure S1 is shows that spectra of detection wavelength.

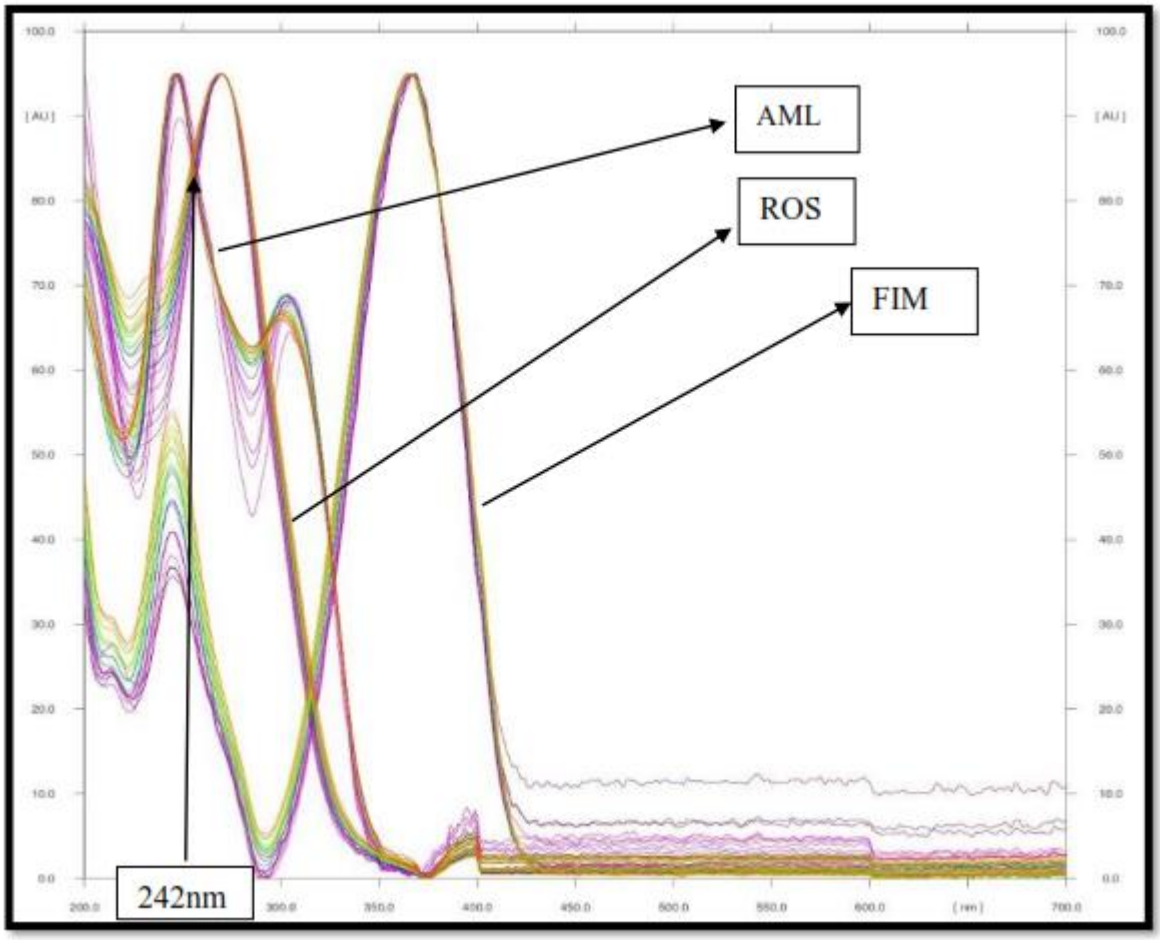

Figure S1: Spectra of calibration curve scanned between 200-800 nm wavelengths for selection of analytical wavelength.

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S.2.2. Linearity and Calibration Curve

Linearity for applied novel HPTLC method was found to be in concentration range AML (200-1400 ng/band) with regression coefficient of 0.9985, for ROS it was (400-2800 ng/band) with regression coefficient of 0.9971, and for FIM it was (1200-8400 ng/band) with regression coefficient of 0.9984. Figure S2–S4 represents the calibration curve.

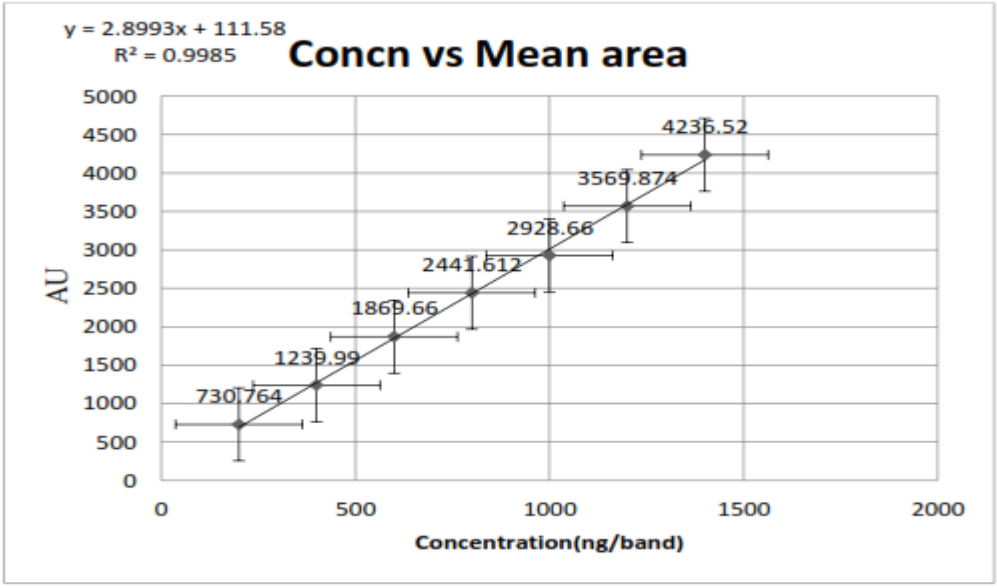

Figure S2: Calibration curve for AML (200-1400 ng/band) at 242 nm.

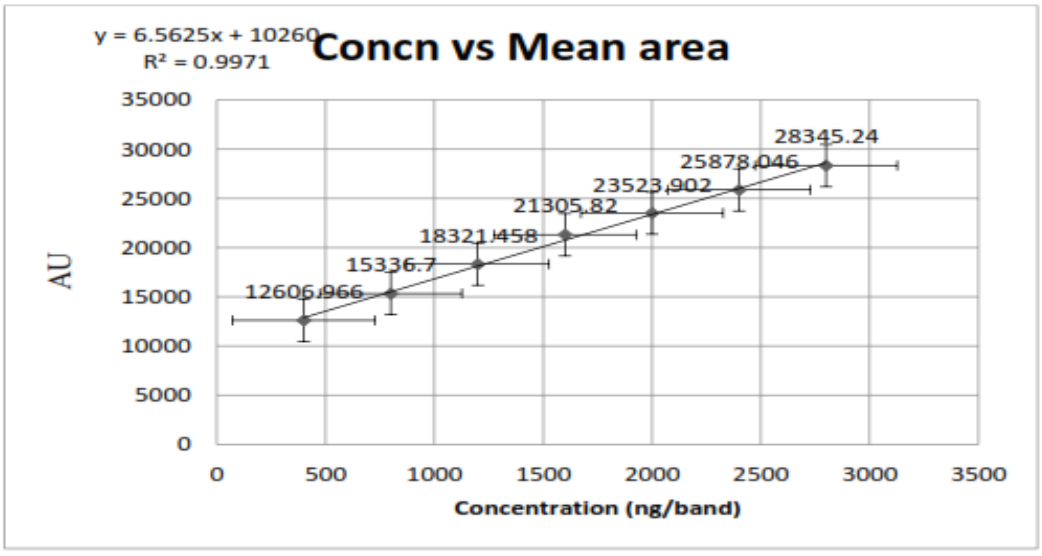

Figure S3: Calibration curve for ROS (400-1400 ng/band) at 242 nm.

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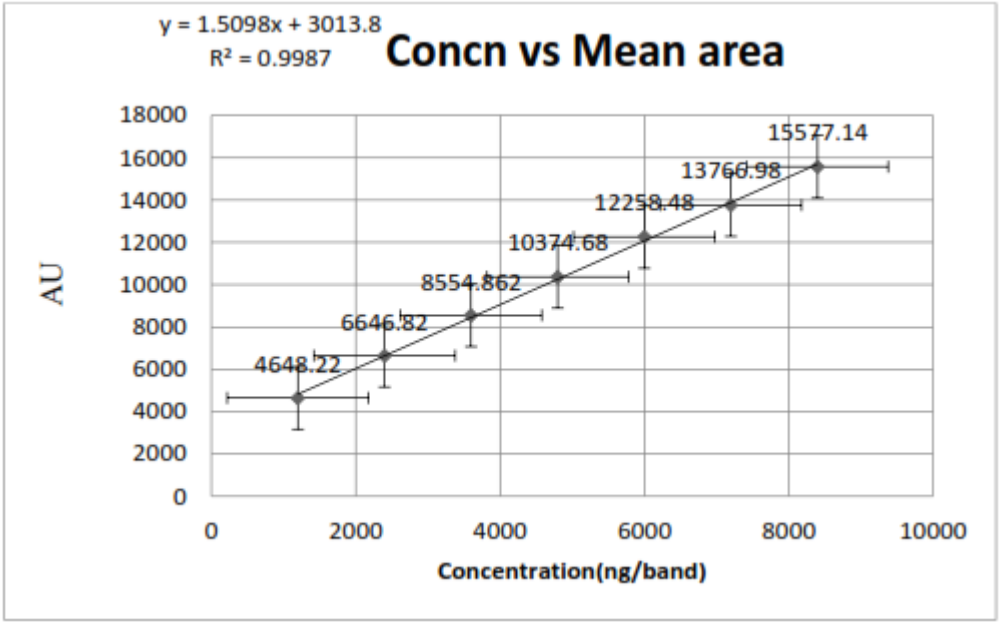

Figure S4: Calibration curve for FIM (1200-8400 ng/band) at 242 nm.

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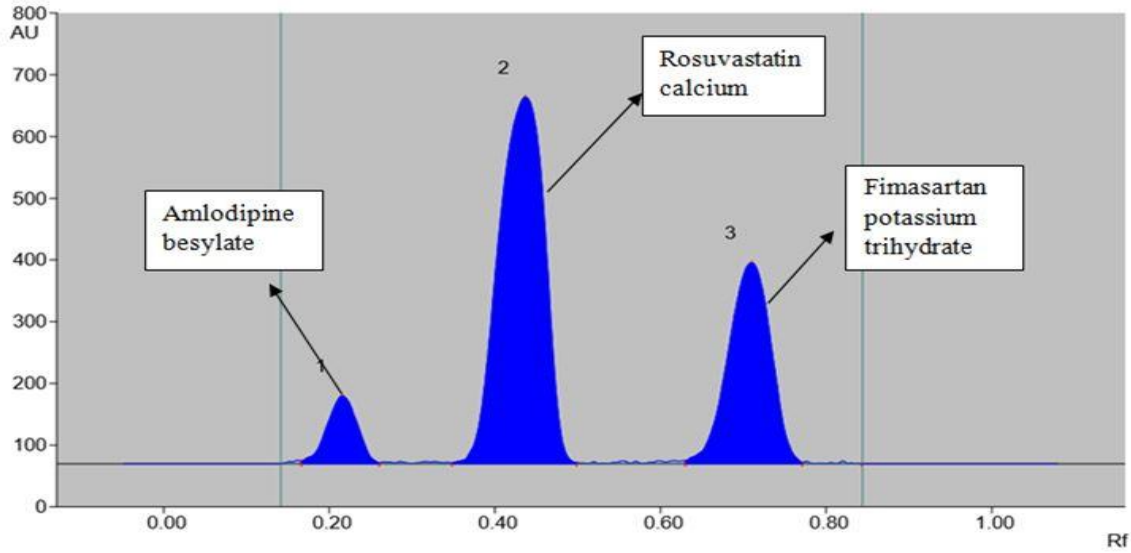

Figure S5: Densitogram of Amlodipine besylate (600 ng/band), Rosuvastatin calcium (1200 ng/band), and Fimasartan potassium (3600 ng/band) using n-Hexane: n-Butanol: Methanol: Glacial Acetic Acid (5.7: 2: 2.3:0.1, v/v/v/v) at 242 nm

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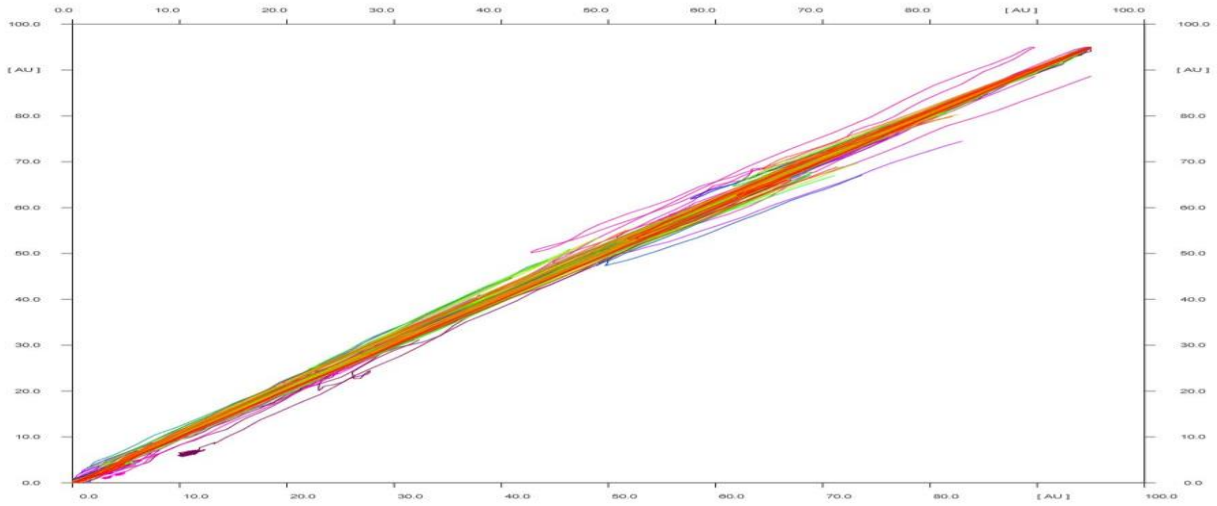

Figure S6: Peak purity spectra of calibration range

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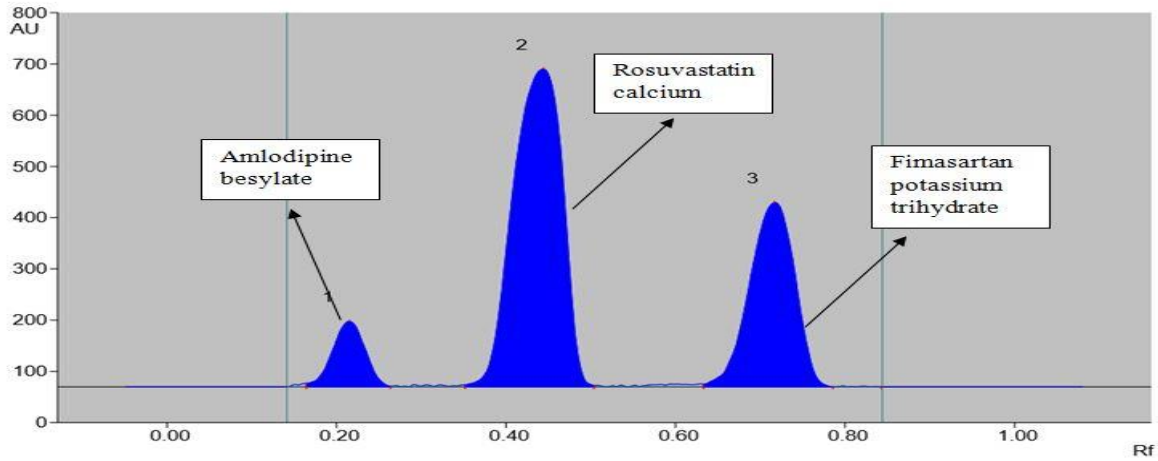

Figure S7: Densitogram of Amlodipine besylate (800 ng/band), Rosuvastatin calcium (1600 ng/band) and Fimasartan potassium (4800 ng/band) synthetic mixture using *n*-Hexane: *n*-Butanol: Methanol: Glacial Acetic Acid (5.7: 2: 2.3:0.1, v/v/v/v) at 242 nm.

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S.2.3. Solution Stability Studies

Solutions of sample and standard sample solution of AML, ROS, and FIM were evaluated at room temperature. The test was carried out over a time period of 2, 4, 8 and 24 h time period and drug was found to be stable with a recovery of >98% which is reported in Table S1.

Table S1: Solution stability studies by HPTLC

Name Drug	Time (h)	Concentration (ng/band)	Mean Area ± SD (n = 3)	% Amount of Drug found	% RSD
	0	800	2458.52± 15.41	101.75	0.62
	2	800	2436.80±54.52	100.25	2.23
AML	4	800	2430.22± 43.46	99.96	1.76
	8	800	2419.33± 47.56	99.50	1.96
	24	800	2386.20±31.72	98.06	1.32
	0	1600	20818.24± 471.68	100.65	2.26
	2	1600	20730.62± 397.37	99.72	1.91
ROS	4	1600	20653.62± 434.24	98.98	2.10
	8	1600	20646.58± 388.03	98.92	1.88
	24	1600	20610.54± 456.03	98.57	2.21
	0	4800	10400± 173.76	101.87	1.67
	2	4800	10293.83 ± 125.52	100.41	1.21
FIM	4	4800	10250.76± 141.25	99.81	1.37
	8	4800	10183.26± 173.81	98.88	1.70
	24	4800	10132.35± 215.63	98.18	2.12

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