

Development and Validation of HPTLC Method for Simultaneous Estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan In Their Combined Tablet Dosage Form

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ABSTRACT

Background: The combination of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan is prescribed for the treatment of hypertension. An Ultra Performance Liquid Chromatography (UPLC) method has been reported for simultaneous estimation of this combination. **Objective:** To develop and validate HPTLC Method for simultaneous estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan in their combined tablet dosage form. **Materials and Method:** The chromatographic separation was performed using aluminum plates pre-coated with silica gel 60F254 as stationary phase and chloroform: butan-1-ol: ammonia (6: 4: 0.1, v/v/v) as mobile phase. Spectro-densitometric scanning was performed at 254 nm. The developed method was validated according to ICH Q2R1 guideline.

Results and Discussion: The linearity was established over a concentration range of 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band with correlation coefficient $r^2 = 0.9952$, 0.9992 and 0.9979 for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively. The R_f values of Amlodipine besylate, Hydrochlorothiazide and Telmisartan were found to be 0.27 ± 0.02 , 0.43 ± 0.02 and 0.14 ± 0.02 respectively. Recovery of drug was achieved in the range of 99.43–101.57%, 100.22–101.54% and 100.12–100.44% for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively by developed method. Limit of detection and limit of quantitation was found to be 8.6, 58.0 and 186.9 ng/band and 26.1,

175.8 and 566.4 ng/band for Amlodipine besylate, Hydrochlorothiazide and Telmisartan, respectively. **Application:** The developed HPTLC method was applied for simultaneous estimation of three drugs in their combined tablet dosage forms and results were found to be in good agreement with the labeled claim. **Conclusion:** The developed HPTLC method was found to be accurate, precise, specific and sensitive. It can be applied for routine analysis (assay) of tablets containing combination of Amlodipine besylate, Hydrochlorothiazide and Telmisartan.

Key words: Amlodipine besylate (AML), Hydrochlorothiazide (HCTZ), Telmisartan (TLM), High Performance Thin Layer Chromatography (HPTLC), Simultaneous estimation.

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INTRODUCTION

Amlodipine besylate (AML): 3-ethyl-5-methyl(4RS)2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (Figure 1) is a widely prescribed anti-hypertensive drug. It is a calcium channel blocker.^{1,2,3,4}

Hydrochlorothiazide (HCTZ): 6-chloro-3,4-dihydro-2H-1,2,4-benzothiazine-7-sulfonamide 1,1-dioxane (Figure 2) is an anti-hypertensive diuretic drug belonging to thiazide class.¹⁻⁴

Telmisartan (TLM): 4'-[4-methyl-6-(1-methyl-1H-benzimidazol-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]-2 biphenylcarboxylic acid (Figure 3) is an angiotensin II receptor antagonist widely used to treat hypertension.¹⁻⁴

From the literature survey, it was found that HPLC, HPTLC, UV-Spectrophotometry and Spectrofluorimetric methods were reported for AML, HCTZ and TLM either individually or in combination with other drugs.⁵⁻¹⁰ Also an UPLC method has been reported for the simultaneous estimation of these three drugs in combination. However this method is complex and makes use of expensive instruments and chemicals which makes it unsuitable for the analysis at the small scale laboratory. Hence it was decided to develop and validate HPTLC method which is both cost effective and simple.

MATERIAL AND METHODS

Instrumentation

A HPTLC system (Camag Muttenz, Switzerland) comprising of semi-automatic sample applicator (Camag Linomat V), Hamilton syringe

(100 µl), Camag TLC scanner IV, Camag WinCATS software, Camag twin trough chamber (10×10 cm), UV cabinet with dual wavelength UV lamps, Electronic analytical balance AUX-220 (Shimadzu) and Ultrasonicator (Toshcon, model 4.5) were used during the study.

Chemicals and reagents

Pure drugs AML, HCTZ and TLM were procured as gift samples from Serdia pharmaceuticals, Mumbai; Colortex Pharmaceuticals Ltd., Surat and Zydus Pharmaceutical Pvt. Ltd., Ahmedabad, India, respectively. Marketed formulations were procured from a local pharmacy containing 5 mg AML, 12.5 mg HCTZ and 40 mg of TLM/tablet as per the label claim. Methanol, Chloroform, Butan-1-ol, Ammonia (AR grade) were obtained from SD Fine Chemicals Limited, Mumbai.

Chromatographic condition

For HPTLC analysis the experiment was performed on aluminium plates pre-coated with silica gel 60F₂₅₄ stationary phase (10×10 cm, prewashed with methanol and activated in an oven at 50°C for 10 min prior to chromatography), using mobile phase comprising of chloroform: butan-1-ol: ammonia (6: 4: 0.1 v/v/v). The solutions were applied on TLC plate in the form of bands of 6 mm width under a stream of nitrogen gas using a Camag Linomat V semi-automatic sample applicator. A constant application rate of 0.1 µl/sec was employed and space between two bands was auto-selected as 10 mm. Ascending development to 80 mm was performed in Camag (10×10 cm) twin trough glass chamber (Muttenz, Switzerland) saturated with the mobile phase for 30 min at room tem-

perature. The developed TLC plate was air dried and scanned between 200 to 400 nm using Camag TLC scanner IV using Win-CATS software. All three components show reasonably good response at 254 nm keeping the slit dimension of 4.00×0.30 mm and scanning speed of 20 mm/sec.

Preparation of solutions

Preparation of standard stock solution

AML (10 mg), HCTZ (25 mg) and TLM (80 mg) were weighed accurately and transferred in 10 ml volumetric flask for AML and HCTZ and 50 ml for TLM. The powder was dissolved and the solutions were diluted up to mark with methanol to get the final concentration of 1000 µg/ml AML, 2500 µg/ml HCTZ and 1600 µg/ml TLM.

Preparation of working standard solution

From the standard stock solutions 1 ml aliquots were taken from AML and HCTZ while 5 ml aliquot was taken from TLM and diluted up to 10 ml with methanol to get the mixture solution containing the concentration of 100 µg/ml AML, 250 µg/ml HCTZ and 800 µg/ml. Further 4 ml of this mixture solution was taken and diluted to 10 ml with methanol to make the final concentration of 40 µg/ml AML, 100 µg/ml HCTZ and 320 µg/ml TLM.

Preparation of calibration curve

The series consisted of different concentration of AML, HCTZ and TLM ranging from 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band, respectively. The working standard solution (5, 10, 15, 20, 25 µl) was spotted and analysed by the proposed method. The calibration curves of peak area versus respective concentration were plotted and correlation coefficient and regression line equation were computed.

Validation of the Proposed Method¹¹

Linearity and range

The linearity response was determined by analyzing five independent levels of calibration curves in the range of 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band for AML, HCTZ and TLM, respectively. The calibration curves of peak area versus respective concentration were plotted and correlation coefficient and regression line equation were computed.

Specificity

The specificity of the method was ascertained by analysing standard drug and sample. The bands for AML, HCTZ and TLM in sample were confirmed by comparing the R_f values and UV spectra of the bands with those obtained from the standard. The peak purity of AML, HCTZ and TLM was assessed by comparing the spectra acquired at three different positions on the band, i.e. peak start (s), peak apex (m) and peak end (e).

Precision

Repeatability of measurement and sample application

For repeatability of measurement working standard solution (15 µl) was spotted on pre coated TLC plate. The plate was developed, dried and the peak area was analysed as described under chromatographic conditions for seven times. For repeatability of sample application working standard solution (15 µl) was spotted on pre coated TLC plate seven times. The plate was developed, dried and analysed as described under chromatographic conditions.

Intermediate precision

Intra-day precision of the proposed method was evaluated by applying 5, 10, 15, 20 and 25 µl of working standard solution on the TLC plate three times on same day and analyzing over the entire concentration

range as described under chromatographic conditions.

Inter-day precision of the proposed method was evaluated by applying 5, 10, 15, 20 and 25 µl of working standard solution on the TLC plates three times on different days and analyzing over the entire concentration range as described under chromatographic conditions.

Accuracy

Accuracy was determined in terms of percent recovery. The proposed method was applied to determine AML, HCTZ and TLM in pharmaceutical dosage form. The recovery experiment was carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards at 80%, 100% and 120%. The details of solution preparation are shown in Table 1. All the solutions were spotted (15 µl) and analyzed as described under chromatographic conditions.

Limit of detection and limit of quantification

Limit of detection was calculated using following equation as per ICH guidelines.

$$LOD=3.3 \times N/S$$

$$LOQ=10 \times N/S$$

Where N is the standard deviation of the Y-intercepts of the five calibration curves and S is mean slope of the five calibration curves.

Analysis of drug formulation

Twenty tablets were accurately weighed, finely powdered and mixed thoroughly. The powder equivalent to 40 mg of TLM (5 mg AML and 12.5 mg HCTZ) was accurately weighed and transferred into 100 ml volumetric flask, 70 ml of methanol was added and the solution was sonicated for 20 min and the volume was made up to the mark with methanol. The solution was filtered through whatman filter paper No. 41. The final solution contained 50 µg/ml, 125 µg/ml and 400 µg/ml of AML, HCTZ and TLM, respectively.

Sample solution (15 µl) was applied on TLC plate and analysed by the developed method. Analysis of marketed formulations was done for three times and percentage of AML, HCTZ and TLM were calculated from the calibration curve.

RESULTS AND DISCUSSION

Selection of wavelength

Zero order spectra of AML, HCTZ and TLM were taken in methanol. The λ_{max} for AML, HCTZ and TLM were found to be 237.5 nm, 270 nm and 297 nm, respectively. Overlain derivative spectra of AML (10 µg/ml), HCTZ (10 µg/ml) and TLM (10 µg/ml) are depicted in Figure 4. The wavelength selected for determination of AML, HCTZ and TLM was 254 nm.

Calibration curve

Calibration curves of AML, HCTZ and TLM were prepared in the range of 200-1000 ng/band, 500-2500 ng/band and 1600-8000 ng/band respectively (n=5). The calibration curves of peak area versus respective concentration were plotted. Calibration curves for AML, HCTZ and TLM were found to be linear in selected range.

Method validation

Linearity and range

The calibration curves were found linear in the given range with a correlation coefficient of 0.9952, 0.9992 and 0.9979 for AML, HCTZ and TLM respectively. A 3D chromatogram showing linearity of AML, HCTZ and

Table 1: Preparation of solution for accuracy study

Pre-analysed Mixture Drug Solution(ml) [AML-100 µg/ml; HCTZ-250 µg/ml; TLM-800 µg/ml]	Standard Mixture Solution Spiked(ml) [AML-100 µg/ml; HCTZ-250 µg/ml; TLM-800 µg/ml]	Volume Made up (ml)	Volume Spotted (µl)
2	-	10	15
2	1.6	10	15
2	2	10	15
2	2.4	10	15

Table 2: Summary of validation parameters

Parameters	AML	HCTZ	TLM
Linearity (ng/spot)	200-1000	500-2500	1600-8000
Correlation Coefficient (r ²)	0.9952	0.9992	0.9979
Precision (RSD)			
Repeatability of measurement (n=7)	0.24	0.97	0.40
Repeatability of sample application (n=7)	0.27	0.95	0.40
Intra-Day (n=3)	0.20-1.22	0.38-1.89	0.40-1.89
Inter-Day (n=3)	0.21-1.87	0.46-0.90	0.44-1.58
Accuracy (% Recovery)	99.43-101.57	100.22-101.54	100.12-100.44
LOD (ng/spot)	8.6	58.0	186.9
LOQ (ng/spot)	26.1	175.8	566.4

Table 3: Data of peak purity

Drug	Standard Drug		Tablet Formulation	
	r (s,m)	r (m,e)	r (s,m)	r (m,e)
AML	0.9999	0.9990	0.9999	0.9991
HCTZ	0.9999	0.9993	0.9999	0.9993
TLM	0.9998	0.9990	0.9998	0.9990

Table 4: Data of recovery study for AML, HCTZ and TLM

Drug	Amount taken (ng)	Amount Spiked (ng)	Total area (Mean ± SD; n=3)	Recovered amount in ng (Mean ± SD; n=3)	Mean % Recovery
AML	300	-	6119.49 ± 101.03	-	-
	300	240	8743.33 ± 132.75	243.38 ± 0.90	101.41
	300	300	9335.41 ± 147.41	298.30 ± 0.74	99.43
	300	360	10061.45 ± 192.50	365.64 ± 0.71	101.57
HCTZ	750	-	879.54 ± 15.27	-	-
	750	600	1568.07 ± 28.04	609.27 ± 0.76	101.54
	750	750	1729.01 ± 31.87	751.67 ± 0.80	100.22
	750	900	1907.38 ± 35.90	909.51 ± 1.11	101.06
TLM	2400	-	2545.04 ± 41.53	-	-
	2400	1920	3523.03 ± 69.79	1926.44 ± 0.44	100.34
	2400	2400	3772.99 ± 74.73	2410.59 ± 1.32	100.44
	2400	2880	4013.84 ± 72.43	2883.41 ± 0.95	100.12

Table 5: Assay data of formulation						
Tablet Formulation	Label Claim (mg)			Assay (%Label Claim) (Mean \pm SD; n=3)		
	AML	HCTZ	TLM	AML	HCTZ	TLM
Brand A	5	12.5	40	100.03 \pm 0.92	99.50 \pm 0.76	100.04 \pm 1.08
Brand B	5	12.5	40	100.32 \pm 0.55	100.50 \pm 0.64	101.28 \pm 1.07
Brand C	5	12.5	40	99.86 \pm 1.18	99.30 \pm 0.97	100.87 \pm 1.18

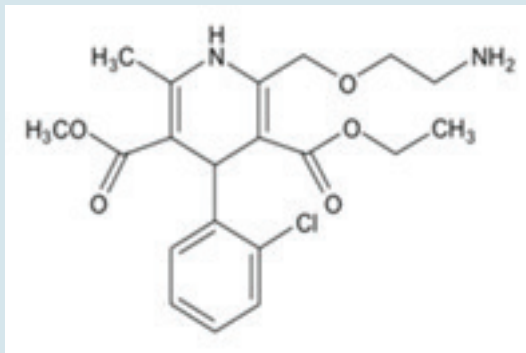


Figure 1: Chemical structure of Amlodipine besylate (AML) (at column width).

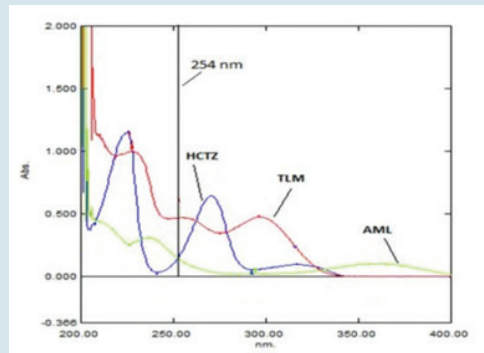


Figure 4: Overlay Spectra for selection of wavelength; Solution of AML, HCTZ and TLM (10 µg/ml, each) was used. (at column width).

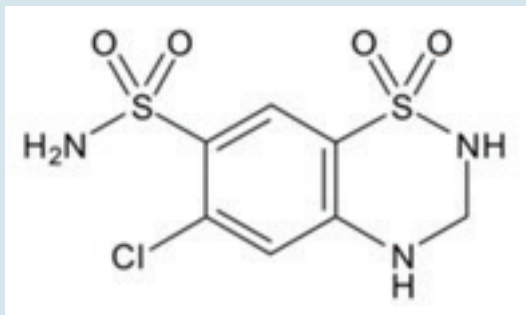


Figure 2: Chemical structure of Hydrochlorothiazide (HCTZ) (at column width).

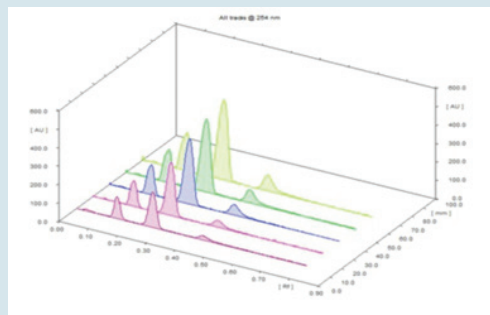


Figure 5: A 3D chromatogram showing linearity of AML, HCTZ and TLM; Rf=0.14: Telmisartan, Rf=0.27: Amlodipine besylate and Rf=0.43: Hydrochlorothiazide (at column width).

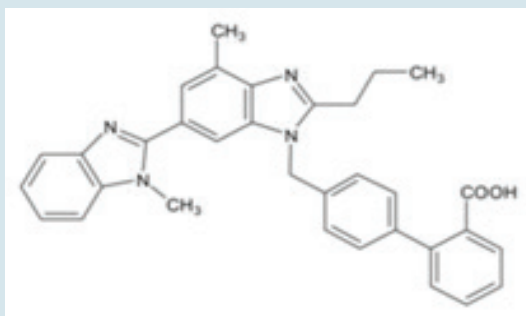


Figure 3: Chemical structure of Telmisartan (TLM) (at column width).

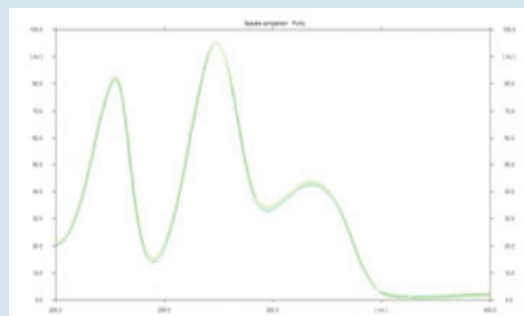


Figure 6: Overlay spectra showing the peak purity of AML standard and sample (at column width).

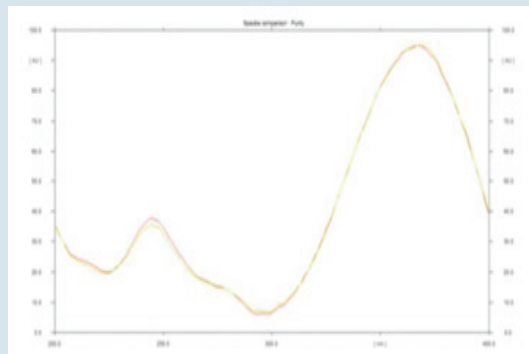


Figure 7: Overlay spectra showing the peak purity of HCTZ standard and sample (at column width).

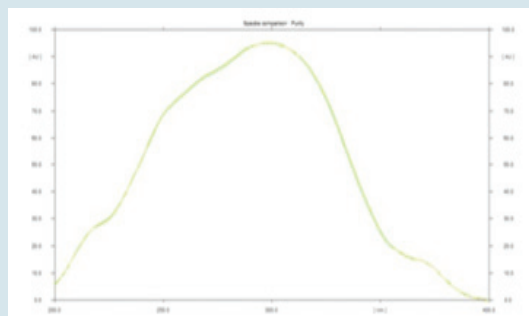


Figure 8: Overlay spectra showing the peak purity of TLM standard and sample (at column width).

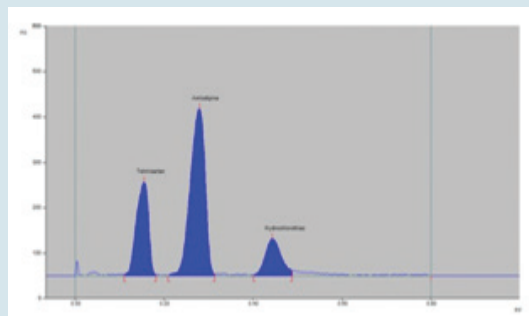


Figure 9: Resolved peaks of AML, HCTZ and TLM in marketed formulation; $R_f = 0.14$: Telmisartan, $R_f = 0.27$: Amlodipine besylate and $R_f = 0.43$: Hydrochlorothiazide, Detection at 254 nm (at column width).

TLM is shown in Figure 5. Regression line equation for AML, HCTZ and TLM were found to be $Y=10.781X+2866.5$, $Y=1.1301X+30.788$ and $Y=0.5094X+1321.7$ respectively.

Specificity

The method was found to be specific as excipient did not interfere with analysis. Also there was good resolution between peaks of AML, HCTZ and TLM. Peak purity of AML, HCTZ and TLM were assessed by comparing spectra acquired at start, middle and end of the band obtained from scanning the spot. The values of r (s,m) and r (m,e) are presented in Table 3. From the overlain peak purity spectrums of AML, HCTZ and

TLM good correlation was obtained which indicate that the peaks are pure and any excipient do not interfere in separation of the drugs (Figure 6, 7 and 8).

Precision

Repeatability of measurement and sample application

The data for repeatability of measurement of peak area is summarized in Table 2. RSD for peak area was found to be 0.24, 0.97 and 0.40 for AML, HCTZ and TLM, respectively. The data for repeatability of sample application is summarized in Table 2. RSD for peak area was found to be 0.27, 0.95 and 0.40 for AML, HCTZ and TLM, respectively.

Intermediate precision

The data for intra-day precision is summarized in Table 2. RSD for peak area was found to be in the range of 0.20–1.22, 0.38–1.89 and 0.40–1.89 for AML, HCTZ and TLM, respectively. The data for inter-day precision is summarized in Table 2. RSD for peak area was found to be in the range of 0.21–1.87, 0.46–0.90 and 0.44–1.58 for AML, HCTZ and TLM, respectively.

Accuracy

The mean of %recovery of AML, HCTZ and TLM was found to be 99.43–101.57%, 100.22–101.54% and 100.12–100.44%, respectively. The data for accuracy is presented in Table 4.

Limit of detection and limit of quantification

The data for LOD and LOQ are presented in Table 2.

Assay of marketed formulations

Tablets were analyzed by the proposed HPTLC method. Results were in good agreement with the label claim. The assay results are shown in Table 5. Chromatogram for AML, HCTZ and TLM in their combined tablet dosage form is shown in Figure 9.

CONCLUSION

An HPTLC method was developed for simultaneous estimation of AML, HCTZ and TLM in bulk. The method was validated as per ICH (Q2 R1) guidelines. The proposed method was found to be specific, accurate, precise and sensitive. The developed method was applied for assay of combination tablets of three drugs and results were found to be in good agreement with the label claim. The proposed method can be applied for routine analysis of combination dosage forms of three drugs.

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CONFLICT OF INTEREST

The author have no conflict of interest.

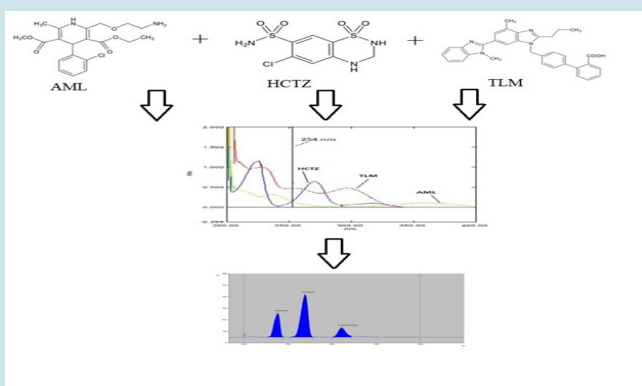
ABBREVIATION USED

AML: Amlodipine besylate **HCTZ:** Hydrochlorothiazide, **TLM:** Telmisartan, **LOD:** Limit of Detection, **LOQ:** Limit of Quantitation, **HPTLC:** High Performance Thin Layer Chromatography, **HPLC:** High Performance Liquid Chromatography, **UPLC:** Ultra Performance Liquid Chromatography, **UV:** Ultra violet, **CV:** Co-efficient of Variance, **SD:** Standard Deviation.

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PICTORIAL ABSTRACT



SUMMARY

- HPTLC method was developed and validated as per ICH (Q2R1) guidelines for simultaneous estimation of Amlodipine Besylate, Hydrochlorothiazide and Telmisartan.
- The analysis was done using aluminium plates pre-coated with silica gel 60F254 as stationary phase and chloroform: butan-1-ol: ammonia (6: 4: 0.1 v/v/v) as mobile phase. The estimation was done at 254 nm.
- The developed method was applied for simultaneous estimation of three drugs in their combined tablet dosage form.
- The assay results were found to be in good agreement with the labelled claim.

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