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Application and validation of eco-friendly TLC- densitometric method for simultaneous determination of co-formulated antihypertensive agents.

Nesrine T. Lamie and Heba M. Mohamed*

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr Al-Aini St. 11562, Cairo, Egypt.

Correspondence author* hmostafa_7@yahoo.com

Abstract:

Owing to the greater consciousness that has arose within the analysis community regarding the colossal negative influence of hazardous chemicals on both health and environment, there is an increasing awareness in developing more environment-friendly practices in different research area. In this context, an eco-friendly TLC-densitometric method was designed, optimized and validated for quantitative analysis of ternary pharmaceutical mixture used for hypertension management, in their pure powdered form, synthetic mixtures and combined pharmaceutical formulation. The proposed method based on the separation of the three co-formulated drugs; Telmisartan (TL), Hydrochlorothiazide (HZ) and Amlodipine besylate (AM) on silica gel F_{254} plates, using green solvents as the developing system. The method was validated with regard to linearity, accuracy, precision, system suitability, and robustness. Comparison of the suggested method to the reported conventional TLC-Densitometric method regarding their validation parameters and greenness profiles was done. The suggested method was found to be more eco- friendly and more solvent/time saving; hence they can be used for quality control analysis of the studied mixture in a safer way.

Keywords:

Telmisartan, Hydrochlorothiazide, Amlodipine, Green, Eco-friendly, TLC-Densitometry

1. Introduction

Many trials and continuous efforts are being made to diminish the injurious impacts of hazardous chemicals on human and environment. As a result, implementation of aspects and principles of Green Analytical Chemistry (GAC) have been increased. [1, 2] Green analytical chemistry conception, signifies the diminishing or eliminating hazardous solvents and chemicals from analytical processes to result in an environment-friendly analysis without conceding the performance criteria of the analytical method.

Several strategies were studied and applied to reduce the harmful impact of the different analytical methodologies. Special consideration went to the use of hazardous solvents [3]; which considered the biggest challenge for greening analytical methods, particularly the chromatographic ones. [4, 5] Either by decreasing contact to a definite hazardous solvent or omission of the different noxious solvents and reagents, researchers can increase the greenness profile of the different analytical procedures. Full exclusion of specific solvents and reagents is not always feasible with no significant waning of the analytical results. As a key in these cases, it is sensible and highly recommended to swap the harmful solvents and reagents by greener alternatives. Instead, if it was not credible to substitute the hazardous reagents by alternatives that are more environment-friendly, their minimization would be a viable opportunity without reducing the effectiveness of the applied method.

Thin-layer chromatography (TLC) is a powerful method equally appropriate for both qualitative and quantitative analytical tasks. Many applications of TLC-Densitometric methods as identification and quantitation of impurities and impurity level targets, constituents, active substances, degradation products, kinetics study, process development and optimization, process monitoring, and cleaning validation have been demonstrated. [6-8] It is superior to other analytical techniques in terms of total cost and time for analysis where it doesn't need tedious

cleanup; with the use of appropriate mobile phases and reagents, all interfering agents will be omitted. It is a very rapid, accurate and precise chromatographic technique for different components assays and can be used for routine quality control of pharmaceutical products. Via elimination and/or minimizing the used of hazardous solvents, TLC methods could be modified to be more eco-friendly.

A co-formulated fixed dose pill composed of Telmisartan (TL); angiotensin II antagonist [9], Hydrochlorothiazide (HZ); a thiazide diuretic, that lowers blood pressure and used in combination therapy with other antihypertensive agents [10] and Amlodipine besylate (AM); a calcium channel blocker, used for stable angina pectoris and adjunct therapy for hypertension [11], Fig. 1, has been recently used for hypertension management as a triple- combination therapy, that proved to have the potential of quicker blood pressure lowering, obtaining target blood pressure, and minimized adverse effects. [12, 13]

Up to our knowledge, very few methods were reported for simultaneous determination of this ternary mixture; by UPLC [14] and RP- HPLC and HPTLC densitometric [15] methods. The reported densitometric method used chloroform: methanol: formic acid (85:15:5 by volume) as the developing system and R_f were 0.73, 0.41 and 0.31 for TL, AM and HZ; respectively.

The goals of our study; firstly, to develop and validate a green TLC densitometric method for the simultaneous determination and quality control analysis of Telmisartan (TL), Hydrochlorothiazide (HZ) and Amlodipine besylate (AM) in their ternary mixture. Secondly, validating the proposed method and statistically comparing the analysis results to that of the cited drugs' official methods. And finally, comparing the proposed method to the reported conventional TLC-densitometric method regarding their validation parameters and greenness profiles, where the suggested method was found to be more eco-friendly and more solvent/time

saving without affecting the method analytical and validation parameters as proved by the study results.

2. Experimental

2.1.Instruments:

The used plates were 20x20 cm, coated with 0.25mm silica gel 60 F254 (Merck, Darmstadt, Germany). The samples was applied to the plates using Camag Linomat 5 auto sampler (Camag, Muttenz, Switzerland) with Camag micro syringe (100 µl). A Camag TLC scanner model 3S/N 1302139 with win CATS software for densitometric evaluation (Camag, Muttenz, Switzerland) was used for scanning.

Material and reagents:

Reference Samples

Standard of TL was generously supplied by National Organization of Drug Control and Research (NODCAR), Cairo-Egypt and HZ and AM standards were generously supplied by Al-Hekma Pharmaceuticals, Cairo, Egypt. All the standards; TL, HZ and AM have their claimed purity of 99.89 %, 100.45% and 99.93 %; respectively according to the official methods. [16]

Pharmaceutical formulations

Telma-AMH 40 tablets-Batch number FT0114029. Each tablet is claimed to contain 40 mg of TL and 12.5 mg of HZ and 5 mg AM. Manufactured by: Glenmark Pharmaceuticals LTD, Mumbai- India.

Chemicals and reagents

All solvents and chemicals used were of analytical grade, Ethyl acetate, methanol and acetone were purchased from Prolabo (VWR, International, West Chester, PA, USA).

2.2.Standard solutions

Stock solutions of TL, HZ and AM (1 mg/mL), where 0.05 g of each drug was accurately transferred into three separate 50-mL volumetric flasks, dissolved in and diluted to volume with methanol.

3. Method

3.1.Chromatographic conditions:

Firstly, the plates were washed and developed with the mobile phase by mixing ethyl acetate: methanol: acetone (7.5: 2.5:0.5 by volume), then activated for 15 minutes by placing in an oven at 100°C prior their use. The three studied drugs solutions were applied as separate compact spots 15mm from the bottom of the plates, 2mm band length, and 150 nL/S dosage speed. The chromatographic tank was saturated with the mobile phase for 20 min. The plates were developed over a distance of 8 cm in an ascending manner then air-dried, and scanned under the following conditions; deuterium lamp as source of radiation, absorbance mode as the scan mode, slit dimension is 3 mm x 0.45 mm, scanning speed is 20 mm s^{-1}, the output as chromatogram and integrated peak area and measurements at 254 nm. Where good separation of the three bands was shown by the difference in the retention factor (Rf) values; AM (Rf = 0.1 ± 0.01), TL (Rf = 0.39 ± 0.01) and HZ (Rf = 0.82 ± 0.01) as shown in Fig. 2 and 3.

3.2.Linearity and construction of calibration graphs

Aliquots (1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 µL) of TL, (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 µL) of HZ and (0.5, 1.5, 2.5, 3.5, 4.5 and 5.5 µL) of AM standard solutions (1 mg/mL) were spotted on TLC plates, using the Camag Linomat autosampler with a micro syringe (100 µL) and analyzed as per the previously mentioned chromatographic conditions. The calibration graphs of TL, HZ and AM were constructed by plotting the mean integrated peak area x 10^{-4} versus the corresponding concentration and then the regression equations were computed.

3.3.Synthetic mixtures preparation and analysis

Into a series of 10-ml volumetric flasks, aliquots of TL, HZ and AM standard solutions (1 mg/ mL) each, were accurately transferred to prepare mixtures containing different ratios of the three drugs. Ten µl from the prepared mixtures were spotted on TLC plates and the procedure was followed as described under linearity and construction of calibration graphs section, the concentrations were calculated from the corresponding regression equations.

3.4.Assay of pharmaceutical formulation

Ten tablets of Telma-AMH 40, were finely powdered. An amount of the powdered tablets equivalent to 100 mg TL was accurately weighted and transferred into 100-ml beaker, then 20 mL methanol was added and the mixture was sonicated for 15 minutes and then filtered into 100-mL measuring flask. The residue was washed (3 x 10 mL methanol) and the volume was completed up to the mark with methanol. Four µL from the filtrate were spotted and analyzed as detailed under "Linearity and construction of calibration graphs" section, concentrations were calculated using the corresponding regression equations for TL, HZ and AM.

4. Results and Discussion

One of Green Chemistry principles is to promote the idea of "environment-friendly" (non-toxic) solvents replacement. [14-16]

Chloroform, is a chlorinated solvent, like carbon tetrachloride in its toxicity and is currently on the list of suspect carcinogens [21, 2], it is highly recommended to minimize its usage and replace it by more environment-friendly substituent. The foremost challenge upon applying the solvent replacement strategy, is to increase the greenness of the method without affecting its validation parameters and effectiveness. In this context, studying the effect of different parameters to obtain maximum resolution was highly essential.

4.1.Optimization of chromatographic conditions

4.1.1. Developing system

In order to obtain the optimum separation, different solvent systems of variable compositions and ratios were tried, which were previously used for similar binary and ternary mixtures, avoiding the mostly used hazardous solvents like chloroform, benzene derivatives and others. In all the trials, the three drugs were applied on TLC plate, developed over a distance of 8 cm in an ascending manner using the different developing systems then air-dried and scanned. The systems tried included; two components system; ethyl acetate: methanol in different ratios (8:2, 2:8, 5:5 v/v) but no good separation was achieved, where two of the three peaks usually overlapped or small differences between R_f values were obtained. Other three component systems were tried; ethyl acetate/ methanol / ammonium hydroxide (5.5:4.5:0.5 by volume). Ethyl acetate: acetone: ammonium hydroxide (8:2:0.2, by volume); the addition of ammonium hydroxide to the system, did not help a lot, where incomplete resolution of the three drugs was obtained. Also, ethyl acetate: methanol: acetone system was tried in different ratios, to obtain optimum separation for the three cited drugs. The obtained TLC chromatograms were promising, and after fine adjustment of the ratios, the best developing system was found to be ethyl acetate: methanol: acetone (7.5:2.5:0.5 by volume), on the basis of minimum tailing and maximum separation of the three drugs with the greatest differences between R_f values, AM (0.1 ± 0.01), TL (0.39 ± 0.01) and HZ (0.82 ± 0.01) (Figures 2 and 3).

4.1.2. Scanning wavelength

Various scanning wavelengths were checked in order to obtain good sensitivity for TL, HZ and AM with minimum noise. Based on the UV-absorption spectra of TL, HZ and AM, The wavelength 296 nm is a λ_{max} for TL, but not for HZ and AM, 270 nm is λ_{max} for HZ, but AM showed very poor absorption. Lower wavelengths were avoided to minimize noise and solvents

UV-cutoff. The wavelength 254 nm was found to be optimum as the three drugs contribute with good absorbance values at this wavelength, where sharp and symmetrical peaks with minimum noise were obtained, as shown in Figures 2 and 3.

4.1.3. Slit dimensions of scanning light beam

The slit dimensions of scanning light beam have to provide a whole coverage of band dimensions on the scanned track with no interference of the adjacent ones. Different slit dimensions were checked and 3 x 0.45 mm was found to be the one of choice that permits maximum sensitivity.

4.2.Method Validation

The proposed TLC-densitometric method was validated by linearity, range, specificity, accuracy, precision, quantitative limit, detection limit, and robustness according to the ICH guidelines, [23]

4.2.1. Linearity

The linearity of the methods was assessed by analysis of different concentrations in the range of 1.0 – 6.0 µg/spot, 0.5 – 3 µg/spot and 0.5 – 5.5 µg/spot for TL, HZ and AM, respectively, the linearity of the calibration graphs was validated by high correlation coefficient values and small intercept values, Table 1.

4.2.2. Range

For accurate and precise results; Beer's law and the concentrations of TL, HZ and AM present in the pharmaceutical preparations were considered while establishing the calibration range. Table 1.

4.2.3. Accuracy

The accuracy was tested by applying the suggested method for determination of different blind samples of TL, HZ and AM within the linearity range. The concentrations were obtained from the corresponding regression equations and the results are shown in Table 1 where good percentage recoveries (100.02 ± 0.939, 99.72 ± 0.791 and 100.11 ± 1.186) were obtained for TL, HZ and AM, respectively.

4.2.4. Precision

Repeatability

Three concentrations of TL (2, 4 and 5 µg/band), HZ (1, 2 and 3 and µg/band) AM (1.5, 2.5, 4.5 µg/band) were analyzed three times intraday using proposed TLC method, results are shown in Table 1, where good RSD % (0.641, 0.834 and 0.774) were obtained for TL, HZ and AM, respectively.

Intermediate precision

The same previously mentioned concentrations were determined on three different days (interday) using proposed TLC method. Results are shown in Table 1, where good RSD % (0.893, 0.992 and 0.805) were obtained for TL, HZ and AM, respectively.

4.2.5. Specificity

Specificity of the methods was checked by the analysis of different synthetic mixtures of TL, HZ and AM containing different concentrations within the linearity range and the dosage form ratio, results are shown in Table 2. Where good percentage recoveries (100.18 ± 0.791, 99.66 ± 1.011 and 101.22 ± 0.717) were obtained for TL, HZ and AM, respectively.

4.2.6. Determination and Quantitation Limits (LOD and LOQ)

They were calculated from the standard deviation (σ) of the response and the slope of the calibration curve (S) according to the following equations: LOD = 3.3 (σ /S) and LOQ = 10 (σ /S). Results presented in Table 1 indicated that the method has acceptable sensitivity for the determination of the studied drugs.

4.2.7. Robustness

In order to evaluate the robustness of the proposed method, the studied ternary mixture was analyzed after altering various parameters in the developed method. The studied parameters were; mobile phase composition, mobile phase volume, saturation time and time taken from chromatographic separation to scanning. It is demonstrated that slight intended variations in the previously mentioned parameters have no significant effect on the analysis of the three drugs in their ternary mixture using the proposed TLC method. The low values of RSD % of peak areas along with the nearly R_f values indicate good robustness of the proposed method, Table 3.

4.3. System suitability

According to ICH [23], system suitability tests are a vital part of many analytical methods, particularly liquid chromatographic methods. They are applied to authenticate that the resolution and reproducibility of the chromatographic system are suitable for the analysis to take place. Parameters like; capacity factor (k) [24], symmetry factor, selectivity factor (α) and resolution (Rs) were calculated as shown in Table 4.

4.4. Analysis of Pharmaceutical preparations

The proposed methods have been successfully applied to assay TL, HZ and AM in Telma-AMH 40® tablets with good percentage recoveries which endorse the suitability of the proposed methods for the routine determination of these components in their combined formulation, Table 5.

4.5. Statistical analysis

The work results obtained for the analysis of TL, HZ and AM in their pure powder forms by the suggested TLC densitometric method were statistically compared with those results obtained by using the official methods.[16] The calculated t and F values are less than the tabulated ones, this indicates that there is no significant difference in regard to accuracy and precision, Table 6.

In addition of being more eco-friendly method, compared to the previously reported HPTLC-Densitometric method [15] which used chloroform: methanol: formic acid (85:15:5 by volume) as a developing system, the suggested method proved to be more linear where the regression parameters showed higher correlation coefficients and smaller intercepts. In addition better recoveries were reported for the analysis of TL, HZ and AM in pure powdered forms and combined tablets. The previously reported method showed higher sensitivity as it was done using HPTLC rather than the regular TLC Densitometry.

5. Conclusion

A significant greenness can be achieved when GAC principles are applied in our analytical procedure. Advances in sample preparation techniques, instrumentation, chemometric treatment of data, environment-friendly organic modifiers etc. can drive noteworthy enhancements in both analytical characteristics and greenness profile of the whole methodology. In the analytical community, we can profit from the green chemistry concepts,

since beside the intrinsic advantages of implementing automated, accelerated, simplified and miniaturized systems, there is also a great conservation of solvents, reagents and energy, less risks to the analyst, and less production of wastes. More progress is predictable over the coming years not only towards more eco-friendly analytical methods but also towards greenness assessment.

The proposed TLC densitometric method represents an advantageous method for determination of TL, HZ and AM in their ternary mixture, for being simple, with no tedious extraction steps, accurate, time and cost saving. In addition, the used mobile phase is eco-friendly where the solvents used are considered greener ones compared to the previously reported one without affecting the effectiveness of the method. The TLC-densitometric method was applicable for the assay of TL, HZ and AM in their powder forms, synthetic laboratory prepared mixtures and pharmaceutical formulations without the need of prior separation and with no interference of additives in the pharmaceutical preparation. The advantages of the TLC method are that several samples can be run simultaneously using a small quantity of mobile phase, thus lowering analysis time and cost per analysis and providing high sensitivity and selectivity. The results obtained indicate that the introduced method can be used as an eco-friendly, simple and accurate method for quality control laboratories.

References

1- P.T. Anastas, Green chemistry and the role of analytical methodology development, *Crit. Rev. Anal. Chem.*1999, **29**, 167–175.

2- L.H. Keith, L.U. Gron and J.L. Young, Green analytical methodologies *Chem. Rev.* 2010, **107,** 2695–2708.

3- C. Capello, U. Fischer and K. Hungerbuhler, What is a green solvent? A comprehensive framework for the environmental assessment of solvents, *Green Chem*. 2007, **9**, 927–934.

4- C.J.Welch, M. Biba, R. Hartman, T. Brkovic, X. Gong, R. Helmy, Greening analytical chromatography, *Trends Anal. Chem*. 2010, **29**, 667–680.

5- F. Tache, S. Udrescu, F. Albu, F. Mica˘le, A. Medvedovici, Greening pharmaceutical applications of liquid chromatography through using propylene carbonate–ethanol mixtures instead of acetonitrile as organic modifier in the mobile phases , *J. Pharm. Biomed. Anal*, 2013, **75**, 230–238.

6- P. Rashmin, P. Mrunali, D. Nitin, D. Nidhi, P. Bharat, HPTLC Method Development and Validation: Strategy to Minimize Methodological Failures, *J. Food Drug Anal*., 2012, **20,** 794-804.

7- E. A. Abdelaleem, N. S. Abdelwahab, Stability-Indicating TLC-Densitometric Method for Simultaneous Determination of Paracetamol and Chlorzoxazone and their Toxic Impurities, *J. chromatogr. Sci*., 2012, **51**, 187-191.

8- P. Phattanawasin, U. Sotanaphun, T. Sukwattanasinit, J. Akkarawaranthorn, Sarunyaporn Kitchaiya, Quantitative determination of sibutramine in adulterated herbal slimming formulations by TLC-image analysis method, *Forensic Sci. Int.*, 2012, **219,** 96-100.

9- S.C. Sweetman, The Martindale: The Complete Drug Reference, 35th ed., Pharmaceutical press, London, UK, 2007

10- M. A. Clark, R. A. Harvey, R. Finkel, J. A. Rey, K. Whalen, Pharmacology, 5th ed., Lippincott Williams and Wilkins, 2011.

11- M.R. Weir, Hypertension, first ed., ACP Press, USA, 2005.

12- S. G. Chrysant, Triple-drug, fixed-dose combinations for the treatment of hypertension: focus on olmesartan/amlodipine/hydrochlorothiazide combination. *Drugs Today (Barc)*, 2011, ***47***, 197-206.

13- A. H. Gradman, Strategies for Combination Therapy in Hypertension, *Curr Opin Nephrol Hypertens*. 2012, **21**, 486-491.

14- S. Nalwade, V. R. Reddy, D. D. Rao and I. K. Rao, Rapid Simultaneous Determination of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide in a Combined Poly Pill Dosage Form by Stability-Indicating Ultra Performance Liquid Chromatography, *Sci Pharm*, 2011, **7**, 69-84.

15- N. Delhiraj and S. Anbazhagan, Validated chromatographical methods for the simultaneous estimation of antihypertensive drugs in multicomponent formulations, *Der Pharma Chemica*, 2012, **4**, 2416-2421.

16- The British Pharmacopoeia, Her Majesty's Stationary office, London, 2012.

17- J. Namiesnik, Green analytical chemistry–some remarks, *J. Sep. Sci*. 24 (2001) 151–153.

18- M. Tobiszewski, A. Mechlinska and J. Namiesnik, Green analytical chemistry-theory and practice, *Chem. Soc. Rev*, 2010, **39**, 2869–2878.

19- R.K. Henderson, C. Jiménez-Gonzalez, D.J.C. Constable, S.R. Alston, G.G.A. Inglis and G. Fisher, Expanding GSK's solvent selection guide: embedding sustainability into solvent selection starting at medicinal chemistry, *Green Chem*, 2011, **13**, 854–862.

20- Heba M. Mohamed, Green, environment-friendly, analytical tools give insights in pharmaceutical and cosmetics analysis, TrAC, 66 (2015) 1–17.

21- H. Greim and U. Reuter, Classification of carcinogenic chemicals in the work area by the German MAK Commission: current examples for the new categories, *Toxicology*, 2001, **166**, 11-23.

22- C. Jiménez-González, A.D. Curzons, D.J.C. Constable and V.L. Cunningham, Cradle-to-gate life cycle inventory and assessment of pharmaceutical compounds, *Int. J Life Cycle Assess*, 2004, **9**, 114–121.

23- International Conference on Harmonization (ICH), Q2B: Validation of Analytical Procedures: Methodology, 62, US FDA Federal Register, 1997.

24- B. Fried and J. Sherma, Thin Layer Chromatography, Marcel Dekker, New York. 1999, **81**, 11-19.

Table 1: Linearity studies and characteristic regression parameters for the proposed TLC-Densitometric method.

Parameter	TL	HZ	AM
Calibration range (µg/band)	1.0 -6.0	0.5 - 3.0	0.5 - 5.5
Linearity			
Slope	0.5937	0.4502	0.2636
Intercept	0.174	0.098	0.0186
Correlation Coefficient	0.9997	0.9994	0.9996
Mean ± % RSD	100.45 ± 0.836	99.96 ± 0.490	99.60 ± 0.682
Accuracy ± % RSD	100.02 ± 0.939	99.72 ± 0.791	100.11 ± 1.186
LOD (µg/band)	0.33	0.16	0.16
LOQ (µg/band)	1.00	0.48	0.48
Precision			
Repeatability *% RSD[a]	0.641	0.834	0.774
Intermediate precision *% RSD[b]	0.893	0.992	0.805

* % RSD[a], * % RSD[b]: the intra-day, inter-day relative standard deviation; respectively (n = 3).

Table 2: Determination of Telmisartan, Hydrochlorothiazide and Amlodipine besylate in their synthetic mixture using the proposed method.

Concentration (µg / band)	TL	HZ	AM
TL:HZ:AM			
4: 1.25: 0.5	100.75	99.82	101.24
2: 2: 2	100.31	98.65	100.72
2: 2: 1	99.65	100.22	101.83
1.5: 1.5: 3	101.07	100.96	102.01
3: 1.5: 3	99.13	98.65	100.32
Mean ± % RSD	100.18 ± 0.791	99.66 ± 1.011	101.22 ± 0.717

Table 3: Robustness of the proposed TLC-Densitometric method for the determination of TL, HZ and AM

Parameter	TL		HZ		AM	
	RSD% of peak area	Rf ± SD	RSD% of peak area	Rf ± SD	RSD% of peak area	Rf ± SD
Mobile phase composition [ethyl acetate: methanol: acetone] (7.5: 2.5: 0.5, 7.7: 2.3:0.5, 7.3: 2.7:0.5)	0.653	0.39 ±0.03	0.892	0.82 ± 0.03	0.621	0.1 ± 0.02
Mobile phase volume [75, 100, 125 mL]	1.522	0.39 ±0.02	0.771	0.82 ± 0.02	0.496	0.1 ± 0.02
Duration of saturation [15, 20, 30 min]	0.381	0.39 ±0.01	1.148	0.82 ± 0.02	0.608	0.1 ± 0.01
Time from chromatography to scan [20, 30, 40 min]	1.462	0.39± 0.01	0.694	0.82 ± 0.01	0.833	0.1 ± 0.01

Table 4: Parameters of system suitability of the proposed TLC-Densitometric method for the determination of TL, HZ and AM

Parameter	AM	TL	HZ	Reference value [19]
Capacity factor (K')	9	1.56	0.22	0 - 10
Symmetry factor	0.93	0.97	1.02	≈ 1
Resolution (Rs)	3.52		5.25	R > 2
Selectivity (α)	5.76		7.09	α > 1

Table 5: Determination of Telmisartan, Hydrochlorothiazide and Amlodipine besylate in pharmaceutical dosage form by the proposed method and application of standard addition technique.

Telma-AMH 40®	Found* %	claimed amount (µg /band)	Standard added (µg/band)	Recovery %
TL	100.36 ± 0.622	1	1.0	100.86
			1.5	101.6
			2.5	101.[illegible]
		Mean		**101.[illegible]**
		RSD %		**0.404**
HZ	99.28 ± 1.05	1	0.5	99.81
			1	98.34
			2	98.17
		Mean		**98.77**
		RSD %		**0.912**
AM	101.47 ± 0.892	2	0.5	98.61
			1	98.07
			1.5	100.87
		Mean		**99.18**
		RSD %		**1.492**

* average of five different determinations.

Table 6: Statistical comparison between the results obtained by the proposed method and the official methods for the determination of Telmisartan, Hydrochlorothiazide and Amlodipine besylate in pure powder form.

Parameter	TL		HZ		AM	
	TLC-Densitometry	official method [a]	TLC-Densitometry	official method [b]	TLC-Densitometry	official method [c]
Mean	100.45	99.89	99.96	100.45	99.60	99.93
S.D	0.84	1.03	0.49	0.52	0.68	0.51
RSD	0.836	1.015	0.490	0.517	0.682	0.51
Variance	0.71	1.05	0.24	0.27	0.46	0.26
n	6	6	6	6	6	6
Student T test	1.034 (2.228)		1.683 (2.228)		0.953 (2.228)	
F	1.478 (5.05)		1.125 (5.05)		1.769 (5.05)	

[a] Non aqueous titration; British Pharmacopoeia 2012.

[b] Zero order spectrophotometric method at λ_{max} 273 nm; British Pharmacopoeia 2012.

[c] HPLC method using C18 column and a mobile phase consisting of acetonitrile: methanol: buffer (15:35:50 by volume) at a flow rate of 1.0 mL /min and detection at 237 nm; British Pharmacopoeia 2012.

[d] Figures in parentheses are the corresponding tabulated values at p = 0.05 .

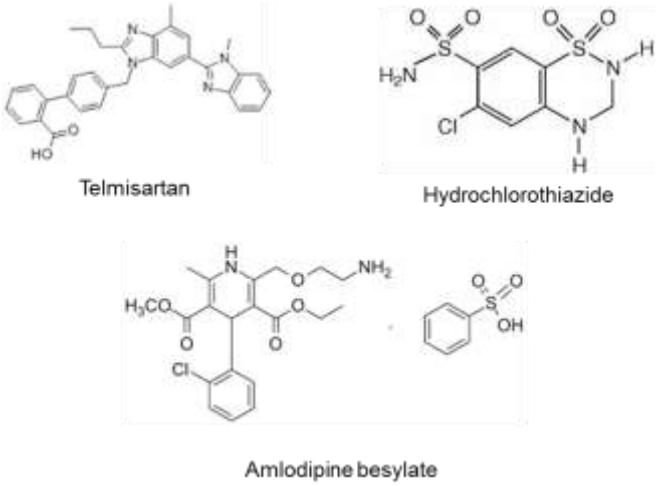

Fig. 1 Chemical Structures of Telmisartan, Hydrochlorothiazide and Amlodipine besylate

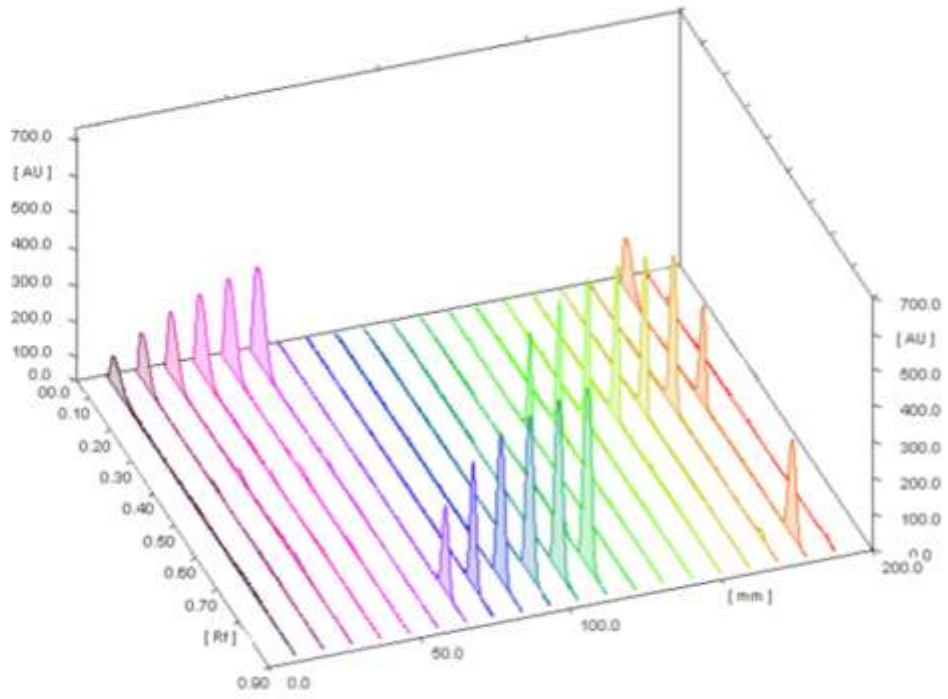

Fig. 2: TLC densitogram of AM (R_f = 0.1 ± 0.01) in the concentration range (0.5 – 5.5 µg/ band), TL (R_f = 0.39 ± 0.01) in the concentration range (1.0 – 6.0 µg /band), HZ (R_f = 0.82 ± 0.01) in the concentration range (0.5 – 3.0 µg/ band) and a mixture of TL, HZ and AM using (ethyl acetate: methanol: acetone, 7.5: 2.5:0.5 by volume) as a developing system at 254nm.

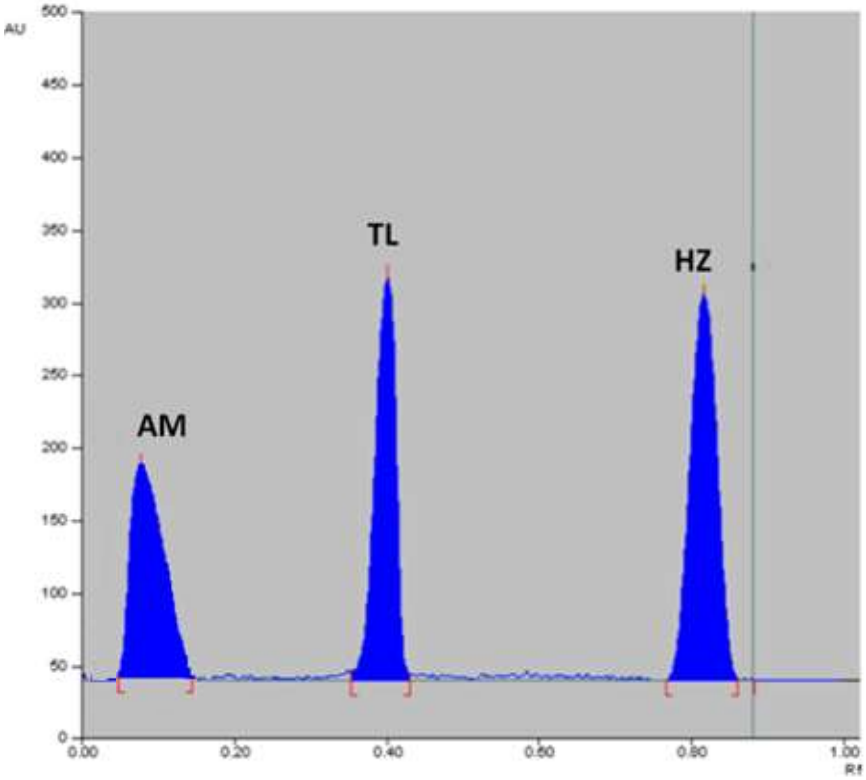

Fig. 3: 2D TLC densitogram of separated peaks of AM (R_f = 0.1 ± 0.01) and TL (R_f = 0.39 ± 0.01) and HZ (R_f = 0.82 ± 0.01) using (ethyl acetate: methanol: acetone, 7.5: 2.5:0.5 by volume) as a developing system at 254nm.