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Der Pharmacia Sinica, 2013, 4(6):16-21

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

HPTLC double development and validation of mefenamic acid and tranexamic acid in combined tablet dosage form

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ABSTRACT

The present study was undertaken to develop and validate simple, rapid, precise, accurate and robust high performance thin layer chromatographic (HPTLC) method for simultaneous estimation of mefenamic acid (MFNC) and tranexamic acid (TXA) in bulk drugs and tablet formulation. Double development technique was employed in the present study in the chromatographic separation of drugs on aluminium plates precoated with silica gel 60 F_{254}. The solvent system used was toluene: methanol (8:2, v/v) for MFNC and methanol: glacial acetic acid (9:1, v/v) for TXA. The densitometric evaluation of separated bands was carried out at 287 nm for MFNC & 525 nm for TXA using 0.2% ninhydrin as derivatizing reagent. Retardation factors of MFNC and TXA were found to be 0.48 ± 0.02 and 0.72 ± 0.02, respectively. Linearity of MFNC and TXA was found in the concentration range of 30 - 400 ng/band and 30 - 500 ng/band, respectively. The % assay (Mean ± S.D.) was found to be 98.91% ± 1.38 & 99.66% ± 1.44 for MFNC and TXA respectively. HPTLC method was validated for linearity, accuracy, precision, specificity, robustness in accordance with International Conference on Harmonisation [ICH] guidelines. The proposed HPTLC method has been successfully applied for the simultaneous analysis of MFNC and TXA in tablet dosage form.

Keywords: Mefenamic acid, Tranexamic acid, Double development, High Performance Thin layer Chromatography, Validation

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INTRODUCTION

Chemically, mefenamic acid (MFNC), is N-[(2, 3-dimethylphenyl) amino] benzoic acid [1]. It is a potent non-steroidal anti - inflammatory drug with analgesic and antipyretic properties [2]. Tranexamic acid (TXA), is trans-4-aminomethyl-cyclohexacarboxylic acid [3]. TXA is a potent anti-fibrinolytic agent that competitively inhibits activation of plasminogen to plasmin [4].

Literature survey revealed that few UV spectrophotometric [5-8], HPLC [9-10] and densitometric [11] methods are reported for TXA. The reported methods for the estimation of MFNC were by UV spectrophotometry [12], HPLC [13-14] and densitometry [15]. Currently HPTLC is a routine analytical procedure. It has been well reported that a number of samples can be run all together with the small quantity of solvent system than in HPLC [16-19].

No research data has been found for simultaneous determination of mefenamic acid and tranexamic acid HPTLC by double development in the tablet formulation and hence the present research study was undertaken.

MATERIALS AND METHODS

Chemicals and reagents

Reference standards of MFNC and TXA were gifted by Emcure Pharmaceuticals Ltd. (Pune, India). PAUSE-MF tablets (250 mg MFNC and 500 mg TXA) were purchased from local market. Analytical grade chemicals and reagents were used in the study and purchased from Merck Chemicals Ltd, Mumbai, India.

Instrumentation and chromatographic condition

HPTLC plates were prewashed with methanol and activated at 105° C in oven for 15 min prior to chromatographic analysis. The sample solution was applied on precoated silica gel aluminium plates $60F_{254}$ (20 × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany) in the form of bands of 6 mm width, located 8 mm from bottom and 15 mm apart with a Camag syringe (100 µl) under the stream of nitrogen gas using a Camag Linomat V (Switzerland) applicator. A constant application rate of 150 nL/sec was used.

The slit dimension was kept at 5 × 0.45 mm and densitometric scanning speed of 10 mm/sec was employed. HPTLC plate was then developed by double development technique at room temperature with 20 mL mobile phase consisting of toluene: methanol (8:2, v/v) for MFNC and methanol: glacial acetic acid (9:1, v/v) for TXA. Linear ascending development was carried out in 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with solvent system. The chamber saturation time for mobile phase was 15 min at room temperature (25 ± 2°C) and relative humidity of 60 ± 5%. The length of chromatographic run was 8 cm. After development the plate was removed from the chamber and air-dried followed by densitometric scanning at 287 nm for MFNC. The plates were then developed with second mobile phase[methanol: glacial acetic acid (9:1, v/v)] as was done for MFNC. After this the plates were scanned after derivatization with 0.2% ninhydrin reagent for TXA at 525 nm using Camag TLC Scanner-III with win CATs software version 1.4.4 in the reflectance - absorbance mode.

Preparation of standard stock solutions

MFNC 10 mg and TXA 10 mg were weighed separately, transferred to separate 10 mL volumetric flasks and dissolved in 10 mL of diluent (CH_3OH :pH 7.2 phosphate buffer 70:30, v/v). From this 0.5 mL of MFNC and 1.0 mL of TXA were transferred to 10 mL volumetric flask and diluted to 10 mL with diluent to obtain final stock solution of concentration 50 µg/mL for MFNC and 100 µg/mL for TXA.

Selection of detection wavelength

After chromatographic development bands were scanned over the range of 200 - 400 nm. It was observed that MFNC showed maximum absorbance at 287 nm (Figure 1). TXA showed poor absorption. Hence after derivatization, the plate was scanned in visible region over the range of 400 - 700 nm and the spectrum was observed. TXA showed maximum absorbance at 525 nm (Figure 2). Hence, detection wavelengths 287 nm and 525 nm were used for analysis of MFNC and TXA, respectively.

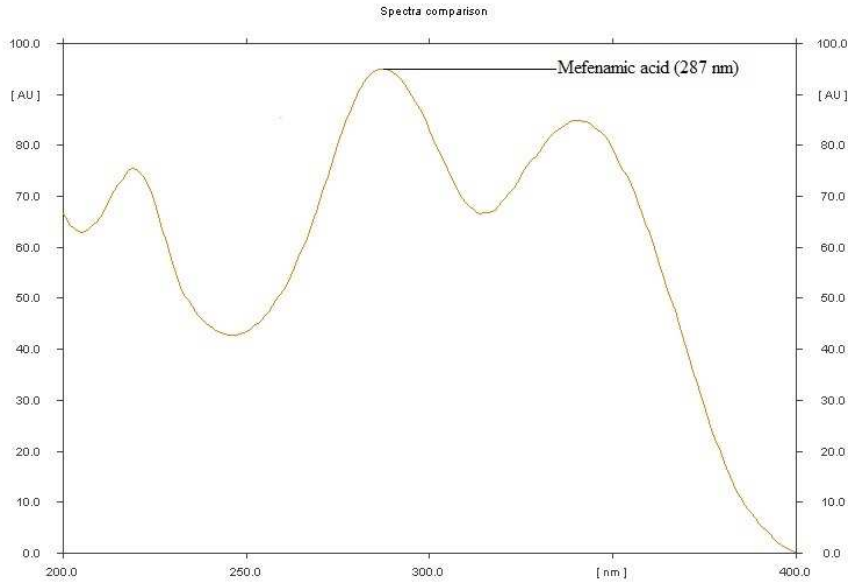

Figure 1: UV Spectrum of standard mefenamic acid

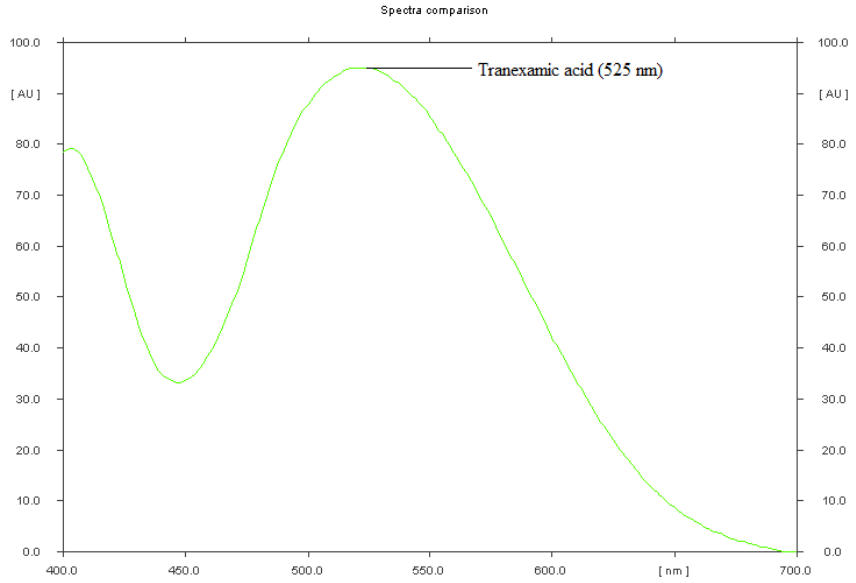

Figure 2: Visible spectrum of standard tranexamic acid after derivatization

Method validation
The proposed HPTLC method was validated as per ICH guidelines Q_2 (R_1) [20].

Linearity and Range
Linearity was determined by applying standard stock solutions to the HPTLC plate in the concentration range of 30-400 and 30-500 ng/band for MFNC and TXA, respectively. The plate was developed using the above mentioned mobile phase and scanned densitometrically. The analysis was repeated six times. Peak area versus concentration was subjected to least square linear regression analysis and the intercept, slope and correlation coefficient for the calibration plot were determined. Regression analysis was carried out using Microsoft excel XP.

Precision
The precision of the method was verified by intraday and inter day precision studies. Intraday studies were performed by analysis of three different concentrations of the drug three times on the same day. The intermediate precision of the method was performed by repeating studies on three different days. The developed method was found to be precise as the relative standard deviation (RSD) values for repeatability and intermediate studies were < 2% as recommended by ICH guidelines.

Sensitivity
Sensitivity involves establishing the limit of detection (LOD) and the limit of quantitation (LOQ). LOD and LOQ were estimated using 3.3 σ/s and 10σ/s respectively, where σ is the standard deviation of the response (y-intercept) and s is the slope of the calibration plot.

Robustness studies
The effects of small, deliberate variation of the analytical conditions on the peak areas of the drugs were examined. Factors varied were mobile phase composition (± 0.1 ml), amount of mobile phase (± 0.5 %), time from application to development (+ 20 min) and from the development to scanning (+ 20 min). One factor at a time was changed to study the effect.

Specificity
Specificity of the method was determined by analyzing standard drug and test samples. The densitograms obtained from standard drug was compared with the densitograms obtained from sample solutions. The peak purity of MFNC and TXA was determined by observing the spectrum at three different regions at peak start (S), peak apex (M) and peak end (E) positions of the band i.e., r (start, middle) and r (middle, end). The developed method was specific as no interference of excipients was observed.

Recovery studies
To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different concentration levels 80, 100 and 120 %. These samples were analyzed as per the procedure and percentage recoveries were calculated.

Analysis of marketed formulation

For analysis of marketed formulation, tablet powder equivalent to 25 mg of MFNC was transferred to a 50 mL volumetric flask and diluted with 30 mL diluent. The solution was ultrasonicated for 20 min and then volume was made up with diluent. Then above solution was filtered through Whatmann no. I filter paper and 1.0 mL of the filtrate was transferred to 10 mL volumetric flask and diluted with 10 mL of diluent to obtain the final concentration of 50 µg/mL for MFNC and 100 µg/mL for TXA.

RESULTS AND DISCUSSION

Linearity

The drug response was linear over the concentration range of 30-400 ng/band and 30-500 ng/and for MFNC and TXA, respectively (Table 1).

Table no. 1: Linear regression data for the calibration curve (n = 6)

Parameters	Mefenamic acid	Tranexamic acid
Linearity range (ng/band)	30-400	30-500
r^2	0.999	0.999
Slope	15.592	14.146
Intercept	344.64	1010.2
Confidence limit of slope	15.116 -16.066	13.775 -14.516
Confidence limit of intercept	230.296 - 458.983	906.546 -1113.768

Precision

The developed method was found to be precise as the RSD values for intra and interday studies were < 2% as recommended by ICH guidelines (Table 2)

Table no. 2: Intra and inter day precision of the HPTLC method (n=6)

Drug	Nominal concentration[a]	Intra/Inter day concentration obtained [a]	Intra/Inter day SD of peak area	% RSD
Mefenamic acid	50	48.6/48.4	13.37/13.45	1.18/1.19
Tranexamic acid	100	99.6/99.5	20.75/20.73	0.85/0.85

[a] *Concentration in ng/band*

Sensitivity

The LOD and LOQ were found to be 8.374 and 25.375 ng/band for MFNC and 8.659 and 26.242 ng/band for TXA, respectively.

Robustness of the method

The standard deviation of peak areas were calculated for each parameter and % RSD was found to be < 2. The low values of the % RSD indicated the robustness of the method (Table 3).

Table no. 3: Robustness testing of mefenamic acid and tranexamic acid
(n=3, 50 ng/band forMFNC AND 100 ng/band FOR TXA)

Parameter	SD of peak area for MFNC	% RSD	SD of peak area for for TXA	% RSD
Mobile phase (toluene) composition (± 0.1 mL)	13.36	1.18	20.75	0.85
Amount of mobile phase (±5%)	13.45	1.19	20.35	0.82
Time of application to development (+ 15 min)	13.36	1.18	20.59	0.83
Time of development to scanning (+ 15 min)	13.45	1.15	20.75	0.89

Specificity

The peak purity of MFNC and TXA studied by observing the spectra at the start, apex and peak end positions of the band i.e., r (S, M) =0.999, 0.998 and r (M, E) =0.998, 0.999, respectively. A good correlation was obtained between standard and sample spectra of mefenamic acid and tranexamic acid, respectively.

Recovery studies

The recovery of the mefenamic acid and tranexamic acid was found to be 99.314 % and 99.232 %, respectively which indicates that the proposed densitometric double development method is reproducible for simultaneous determination of mefenamic acid and tranexamic acid in combined dosage form (Table 4).

Table no. 4: Recovery studies of mefenamic acid and tranexamic acid

Amount taken (ng/band)	Amount added (ng/band)	Total amount (ng/band)	Total amount found (ng/band)	% Recovery	SD	% RSD
	50	40	90	89.7	98.2	1.27	1.28
MFNC	50	50	100	99.4	98.5	0.97	0.98
	50	60	110	109.5	98.3	0.36	0.36
	100	80	180	178.5	99.4	0.79	0.80
TXA	100	100	200	198.6	99.6	0.72	0.72
	100	120	220	218.4	98.5	0.82	0.88

Analysis of marketed formulation

Validity of the proposed HPTLC method was applied for analysis of tablets in six replicate determinations. The percent content of mefenamic acid (Figure 3) and tranexamic acid (Figure 4) in marketed formulation was found to be 98.91% and 99.66 %, respectively.

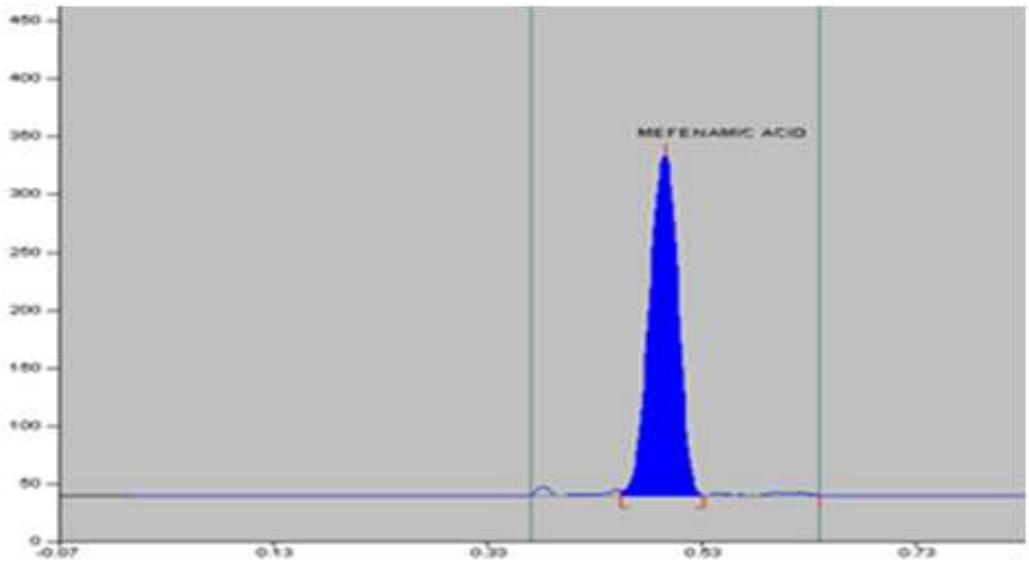

Figure 3: Representative densitogram of mefenamic acid in sample

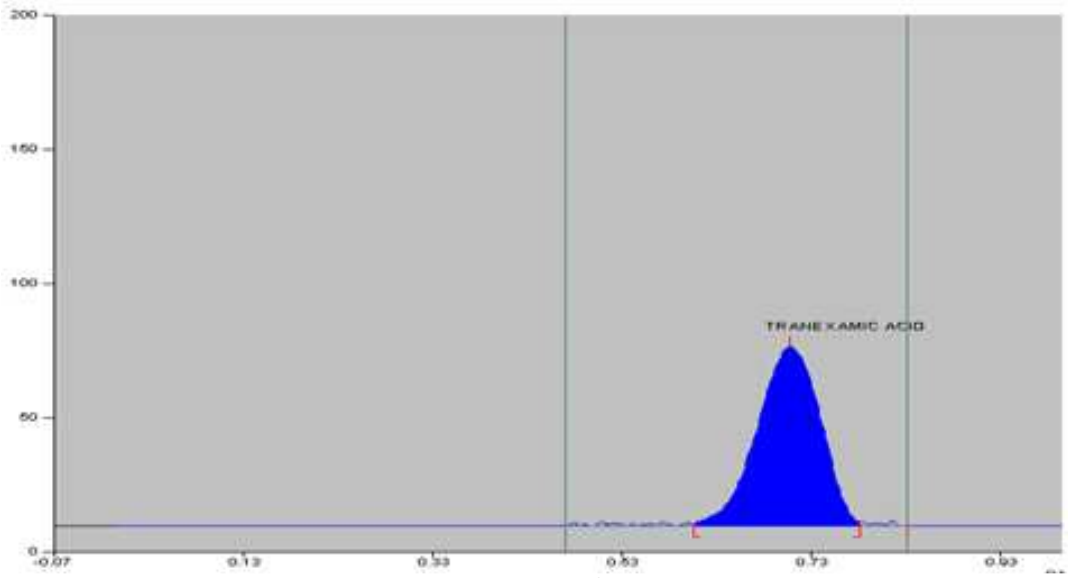

Figure 4: Representative densitogram of tranexamic acid in sample

CONCLUSION

It is shown that the proposed validated HPTLC method was linear, repeatable, accurate, precise, selective, and reproducible proving the reliability of the method [21 - 24]. Hence, the method is suggested for routine quality control of the formulation used in the study [25].

Acknowledgements
The authors are thankful to Emcure Pharmaceuticals Ltd. (Pune, India) for providing gift samples of mefenamic acid and tranexamic acid. Authors are also thankful to Dr. R. N. Purohit, Department of Quality Assurance for providing technical and research support to carry out the study.

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