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Assay Determination of Tranexamic acid in Pharmaceutical Dosage Form (Tablet) Using HPLC and ELS Detector

Kiran R. Patil[a,b], Vipul P. Rane [a,b], Jaiprakash N. Sangshetti[a], Devanand B. Shinde[a*]

[a] Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad- 431004 (MS), India
[b] Wockhardt Research Centre, Aurangabad-431210 (MS), India

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Abstract

A simple, rapid, and precise method is developed for the assay determination of tranexamic acid (Tr) in pharmaceutical dosage form. The method was developed with HPLC, ELS detector and Alltima cyano 100A (250 x 4.6 mm, 5µ) column, the mobile phase composed of aqueous solution of 0.05% v/v trifluoroacetic acid and acetonitrile (60:40 v/v, pH 2.85). The instrumental settings are flow rate of 0.5 mL min^{-1}, column temperature at 30^0C and detector drift tube temperature set at 97^0C, a detector nitrogen gas flow set at 2.6 SLPM, gain 1 and 5 µL sample injection volume. Theoretical plates were 20007. Tailing factor was 1.38. The described method shows excellent linearity over a range of 50 to 150% of assay concentration. The correlation coefficient was 0.9987. The relative standard deviation of six measurements for peak area and retention time less than 2% and 0.5% respectively. The proposed method was found to be suitable and accurate for assay determination of Tr in pharmaceutical preparations.

Keywords:

ELS detector; Tranexamic acid; Pharmaceutical preparations; Validation

1. Introduction

Tr (Fig.1), (trans-4-aminomethyl-cyclohexanecarboxylic acid), is a synthetic amino acid commonly used for controlling abnormal bleeding in a number of diseases (1-3). The analysis of Tr is based on densitometry (4), spectrophotometry (5-8) and HPLC (9-12). The LC methods involve pre and post column derivatization using dansyl chloride, 1-fluoro 2, 4 dinitro-benzene, phenylisothiocyanate, fluorescamine, sodium 2, 6 dinitro-4-trifluoromethyl benzenesulphonate, sodium picrylsulphonate and 2-hydroxynaphthaldehyde. In HPLC, ELS detector has an extensive application base, but it is especially important when UV detection is not feasible. The concept and operation of commercially available evaporative light scattering detectors as sensitive and universal has been discussed thoroughly in the literature (13). The HPLC-ELS detector system also has been extremely useful for the determination of pharmaceutical impurities, raw materials, cleaning verification and small organic compounds. So for to our knowledge, no HPLC – ELS detector method for Tr was published in any journals neither by innovator nor by any other manufacturer. In present research paper attempts were made to determine the chromatographic conditions for the assay determination of Tr in pharmaceutical dosage form (tablet) by using HPLC – ELS detector. After observing the effect of parameters, selected the conditions to provide an optimum and robust method

* **Corresponding Author**
E-mail: proffshinde@yahoo.co.in; dbskiran08@rediffmail.com
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using the optimized chromatographic conditions, the operating conditions used for ELS detector, for example nebulizing gas flow rate, drift tube temperature should be carefully optimized to improve the peak shape.

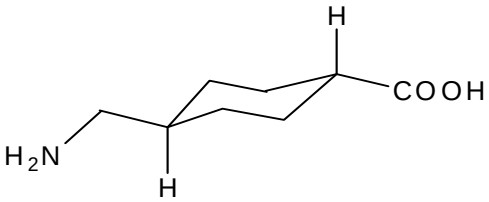


Fig. 1. Structure of Tr.

The isocratic HPLC-ELS detector method developed for the quantitative determination of Tr in pharmaceutical dosage form tablet is precise, accurate and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested and can be conveniently used by quality control department to determine the assay of Tr pharmaceutical preparations. This method does not require derivatization of Tr. This procedure has a cost effective advantage over other techniques for Tr determination, because it uses HPLC system, which is abundant in most pharmaceutical laboratories and relatively inexpensive ELSD.

2. Experimental

2.1. Chemicals

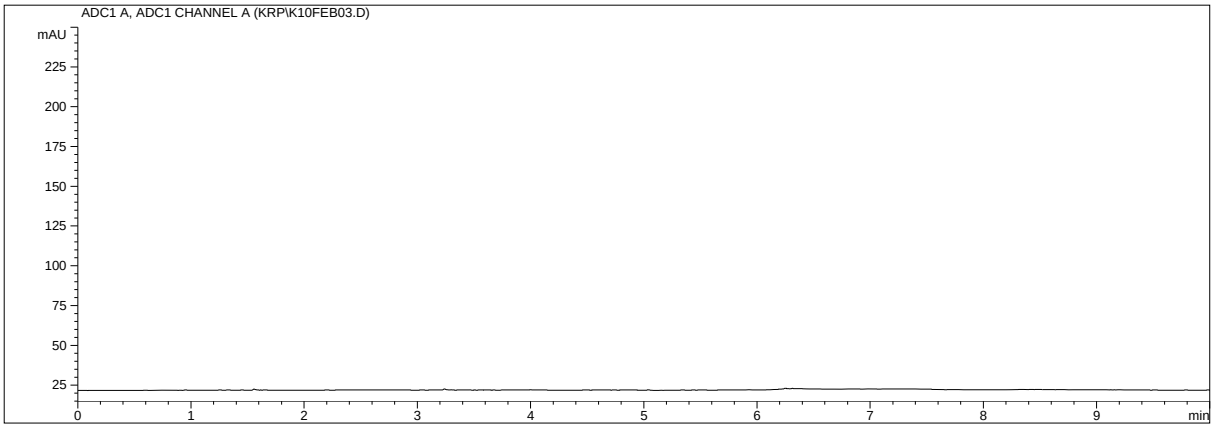
Standard sample of Tr (99.86%) was received from Emcure Pharmaceuticals Ltd. (Pune, India), trifluoroacetic acid and acetonitrile (HPLC grade) were obtained from Merck Fine Chemicals (Mumbai, India). The 0.45 µ nylon filter was obtained from Advanced Micro devices Pvt. Ltd., (Ambala Cantt, India). Double distilled water passed through Pure lab classic (US Filters) and UHP grade nitrogen gas was used during studies. Other chemicals used were have analytical or HPLC grade. Tr tablets contained Tr 500 mg (Pause 500, Emcure pharmaceutical, Pune, India.) was purchased from the market.

2.2. Equipment

HPLC system used was Agilent – 1100 series comprised of degasser, quaternary pump, auto injector, column compartment, variable wavelength detector and integrated with Alltech ELSD 2000ES detector from Alltech associates. The system was controlled through Chemstation software. HPLC column Alltima cyano 100 A, 250 mm x 4.6 mm, 5 µm from Alltech Associates Inc., Lab India model PICO pH meter was used for measure the pH of mobile phase.

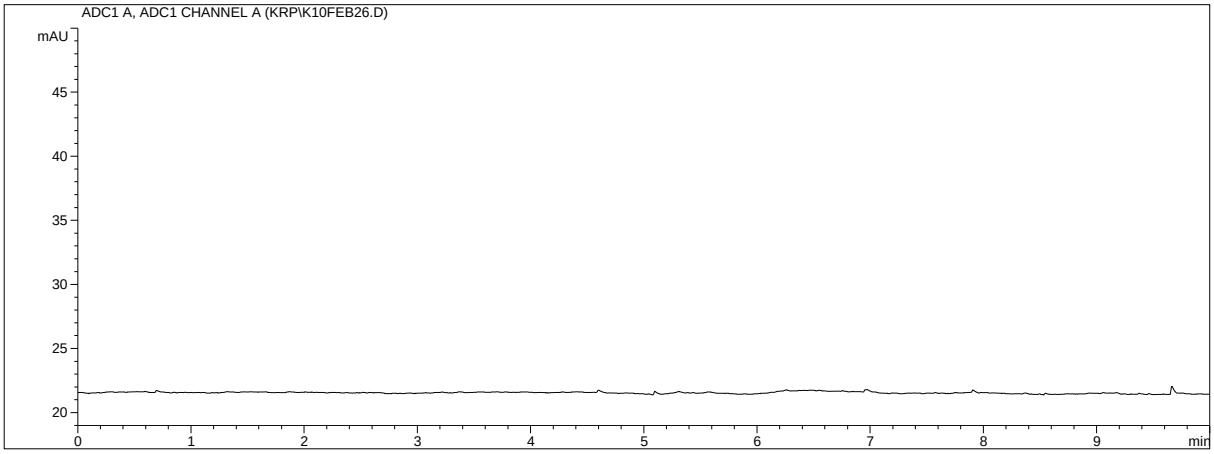
2.3. Chromatographic conditions

The suitable chromatographic conditions of peak was achieved on Alltima cyano 100A, 250 mm x 4.6 mm, 5 µm column using mobile phase composed of aqueous solution of 0.05% v/v trifluoroacetic acid and acetonitrile (60:40 v/v, pH 2.85). The instrumental settings were flow rate of 0.5 mL min^{-1}, column oven temperature at 30 ^{o}C, and detector drift tube temperature set at 97 ^{o}C, a detector nitrogen gas flow set at 2.6 standard liters per minute (SLPM), gain 1 and 5 µL sample injection volume. Water used for standard and sample preparation. A typical chromatograph of water is shown in Fig. 2.



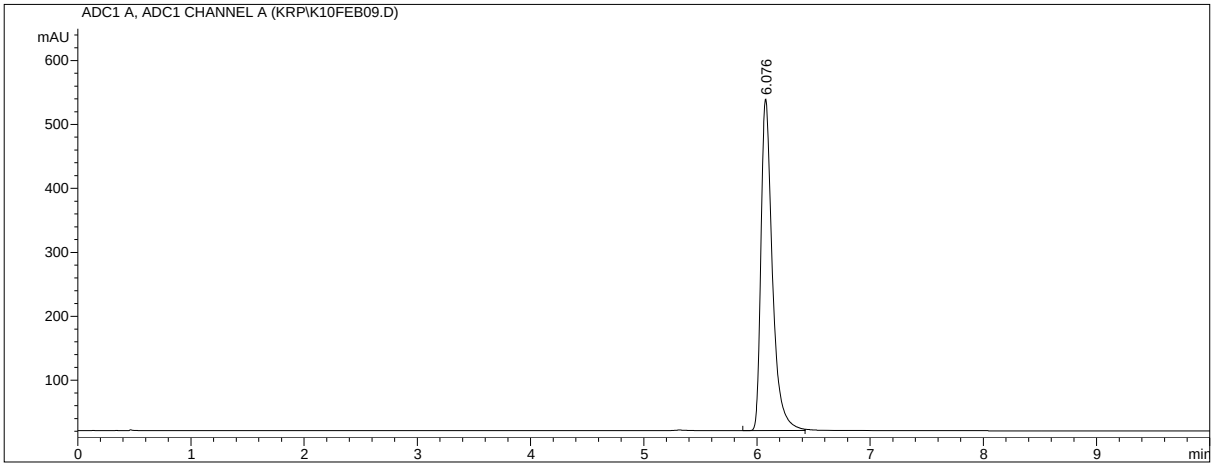
(a)

Fig. 2. (a). Chromatogram of water blank



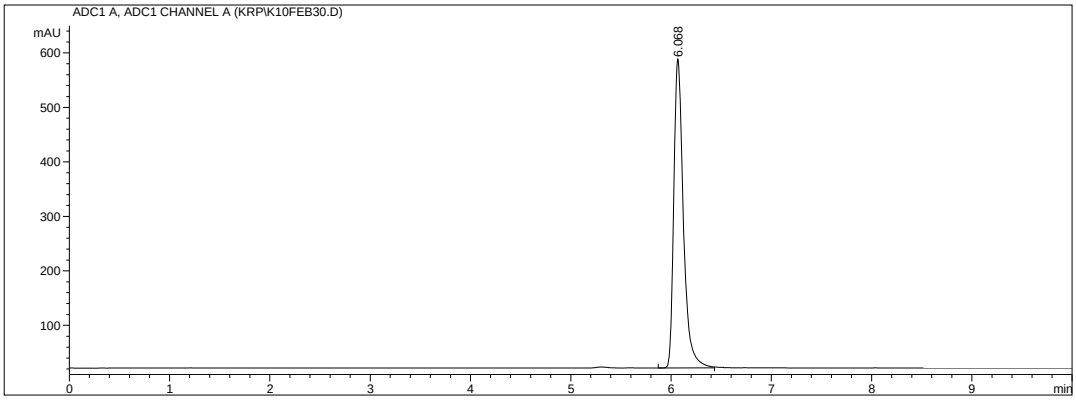
(b)

Fig. 2.(b). Chromatogram of placebo blank



(c)

Fig. 2.(c). Chromatogram of Tr standard solution



d

Fig. 2.(d). Chromatogram Tr tablet solution

2.4. Preparation of standard and sample solution

A stock solution of Tr (1 mg mL^{-1}) was prepared by dissolving 25 mg of drug substance in 25 mL water. Working solution 200 µg mL^{-1} was prepared from stock solution for assay determination. A typical chromatograph of standard Tr shown in (Fig. 2)

Ten tablets were weighed and finely powdered. A quantity of powder equivalent to one tablet containing 500 mg of Tr was transferred in a 100 mL volumetric flask. To this flask, 70 mL water was added, and the solution was sonicated for 25 min. with intermittent shaking. Then volume made up to 100 mL with water and sonicated for 10 min. This solution centrifuged at 10,000 rpm for 10 min. The centrifuged solution filtered through 0.45µ filter. From the filtered solution, 2 mL of solution was transferred into a 50 mL volumetric flask and volume made up with water. A typical chromatograph of Tr tablet solution and placebo shown in (Fig. 2).

2.5. Specificity

Specificity is the ability of the method to asses unequivocally the analyte in presence of components, which may be expected to be present. Typically, these might include impurities, degradants, matrix etc.

2.6. Method validation

2.6.1. Precision

The precision of the assay method was evaluated by carrying out six independent assays of drug product of Tr against reference standard and calculated average assay and % RSD of assay.

The intermediate precision of the method was also evaluated using different analyst and different column of same make in same laboratory.

2.6.2. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined at signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting series of dilute solutions of known concentration (14). Precision study was also carried at LOQ level by injecting six individual preparations of Tr and calculating the % RSD of peak area.

2.6.3. Linearity

Linearity corresponds to the assessment of the relationship between the response (i.e. chromatographic signal) and the concentration of analyte in sample. Test solutions were prepared from stock solution at six concentration levels from 50 to 150% of assay concentration (100, 150, 200, 220, 240 and 300 µg mL^{-1}). The peak area (i.e. chromatographic signal) versus concentration data was performed by least squares linear regression analysis.

2.6.4. Accuracy

The accuracy of the assay method was evaluated in triplicate at three concentration levels, i.e. 160, 200 and 240 µg mL^{-1}.

2.6.5 Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered and assay of drug product recorded in triplicate. The flow rate of the mobile phase was 0.5 mL min^{-1}. To study the effect of flow rate it was changed by 0.1 units from 0.4 to 0.6 mL min^{-1} while the other mobile phase components were held constant as stated in section 2.3. The effect of change in the composition of organic modifier was studied at mobile phase composed by trifluoroacetic acid 0.05%v/v aqueous solution-acetonitrile (58:42 v/v) and (62:38 v/v). The effect of column temperature was studied at 28 ^{o}C and 32 ^{o}C while the other mobile phase components were held constant as stated in section 2.3.

2.6.6. Solution stability and mobile phase stability

The solution stability in the assay method was carried out by leaving both the test solution of drug product and reference standard in tightly packed volumetric flask at room temperature for 48 h. The same sample solution were analysed for every 12 h interval up to the study period. The mobile phase stability was also carried out by assaying the freshly prepared sample solution against freshly prepared reference standard solution for 12 h interval up to 48 h. Mobile phase prepared was kept constant during the study period. The % RSD of assay was calculated for the study period during solution and mobile phase stability experiments.

3. Results and discussion

3.1 Method development and optimization

Objective of chromatographic method development was to achieve peak tailing factor <2, retention time in between 3 to 10 minutes To develop method different stationary phases like C18, CN, different mobile phases containing buffers like ammonium acetate, ammonium formate and trifluoroacetic acid with different pH (3-5) and organic modifier (acetonitrile) were used. For development of chromatographic conditions, Initially ELSD nitrogen gas flow rate at 3.0 SLPM and the drift tube temperature at 100 ^{o}C. The chromatographic condition was achieved using Alltima cyano 100A, 250 mm x 4.6 mm, 5 µm column. Changing the composition of mobile phase optimized the chromatographic method. Alltima cyano column shows better performance as compared to other CN columns.

Next check the effect of drift tube temperature of evaporative light scattering detector and nitrogen gas flow rate. Drift tube temperature was set at 106 ^{o}C and gas flow rate at 3.2 SLPM. But with this condition theoretical plates were down and peak shape was found to be broad.

From above observations, the final HPLC – ELSD operating conditions used as stated in section 2.3.

3.2. Method validation

3.2.1. Precision

The %RSD of assay of Tr during assay method precision study was within 1% and % RSD of area of Tr standard sample within 0.5%. The % RSD of assay results obtained in intermediate precision study was within 0.6% and % RSD of area of Tr standard sample within 0.5% (Table 1).

Table 1. Precision data

	Average assay % (n=6)	Assay % RSD	Standard peak area % RSD (n=6)
"Analyst 1	99.52	0.64	0.18
Analyst 2	99.94	0.52	0.14

n = 6 determinations.

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection was achieved at 2 µg mL^{-1} for 5 µL injection volume. The limit of quantification was achieved at 8 µg mL^{-1} for 5 µL injection volume. The precision for six individual preparations of Tr at LOQ level showed the % RSD of peak area 0.42.

3.2.3. Linearity

Test solutions were prepared from stock solution at six concentration levels from 50 to 150% of assay concentration (100, 150, 200, 220, 240 and 300 µg mL^{-1}). Linearity solutions were injected in triplicate. The calibration graphs were obtained by plotting peak area ratio against the concentration of the drugs. The calibration graph was found to be linear in the aforementioned concentrations with correlation coefficients 0.9987.

3.2.4. Accuracy

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs in the placebo (titanium dioxide). The recovery was performed at three levels, 80%, 100% and 120% of the label claim of the tablet (500 mg of Tr). Placebo equivalent to one tablet was transferred into a 100 mL volumetric flask, and the amount of Tr at 80%, 100% and 120% of the label claim was added to it. The recovery samples were prepared as stated in 2.4. The recovery value ranged from 98.60% to 100.84%. The average recovery of three levels (nine determinations) is 99.80% (0.90) % RSD shown in parenthesis (Table 2).

3.2.5. Robustness

In all deliberate varied chromatographic conditions (flow rate, composition of organic modifier and column temperature) assay value of Tr within 99 to 101%, illustrating the robustness of the method (Table 3)

Table 2. Recovery results

Added (µg) (n =3)	Recovered (µg)	Recovery, %	% RSD
162	160.18	98.88	0.54
200	201.36	100.68	0.28
241	240.6	99.83	0.36

n = 3 determinations

Table 3. Results of robustness study

Sr. no.	Parameter	Variation	Assay % (n=3)
1.	Flow rate (± 20% of the set flow)	a) At 0.4 mL min^{-1}	99.22
		b) At 0.6 mL min^{-1}	99.86
2.	Mobile phase composition	a) At 42 mL	100.57
	(± 2% of organic modifier)	b) At 38 mL	99.95
3.	Temperature (± 2° C of set	a) At 28°C	100.74
	temperature)	b) At 32°C	100.95

n = 3 determinations

3.2.6. Solution stability and mobile phase stability

The solution stability and mobile phase stability experiments were performed as described in section 2.6.6. The % RSD of assay of Tr during solution stability and mobile phase stability experiments were within 1% RSD. No significant change was noticed in these experiments. The data obtained in both the above experiments proves that sample solutions and mobile phase used during assay were stable up to 48 h.

4. Conclusions

The isocratic HPLC-ELS detector method developed for the quantitative determination of Tr in pharmaceutical dosage form tablet is precise, accurate and with short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested and can be conveniently used by quality control department to determine the assay of Tr pharmaceutical preparations. This method does not require derivatization of Tr. This procedure has a cost effective advantage over other techniques for Tr determination, because it uses HPLC system, which is abundant in most pharmaceutical laboratories and relatively inexpensive ELSD.

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