# Gas Chromatographic Analysis of Tranexamic Acid in Pharmaceutical Preparation Using Ethyl Chloroformate as Derivatizing Reagent

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**Summary**: Ethyl chloroformate (ECF) has been used as derivatizing agent for gas chromatographic (GC) determination of tranexamic acid (TA). The elution was from the column HP-5 (30 m x 0.32 mm i.d.) connected with flame ionization detection (FID). Linear calibration curves were obtained with 2-50 ng TA injection on the column. Aminocaproic acid (ACA) when present together with TA could also be separated and determined simultaneously. TA was determined from pharmaceutical preparation. Relative standard deviation (RSD) for analysis was observed within 0.1-0.4 %. The pharmaceutical additives and number of amino acid when present together with TA did not affect the determination of TA.

## Introduction

Tranexamic acid [trans-4-(amino-methyl)-cyclohexanecarboxylic acid] (TA) inhibits breaking of fibrin clots [1, 2]. It is used in a broad spectrum of pre and postoperative interventions and bleeding disorders [2-5]. TA occupies the lysine binding sites of plasminogen thereby inhibiting the formation of a molecular complex required for fibrinolysis [6]. It is also used for the treatment of malignant ovarian tumors [7]. TA reduces menstrual blood losses and is a possible alternative to surgery in menorrhagia and has been used successfully to control bleeding in pregnancy [8]. However, TA is incompletely absorbed from the gastrointestinal tract, possibly due to its amphoteric nature [9]. A simple and selective procedure for the determination of TA from pharmaceutical preparation for quality control could be of analytical interest.

A number of methods for the determination of TA from pharmaceutical preparations and biological fluids have been reported. These methods include high performance liquid chromatography (HPLC) [10-14], HPLC-tandon mass spectrometry (MS) [15], capillary electrophoresis [16], spectrophotometry [12, 17, and 18] and spectrofluorimetry [17-19]. Capillary gas chromatography (GC) has high power of resolution for organic compounds; it does not suffer from the problem of disposing off the eluting solvent. The GC of TA involved 4-fluoro-3-nitrobenzotrifluoride as derivatizing reagent followed by alkylation in an extraction step with tetrabutylammoium as counter ion [20] and 2, 6-dinitro-4-trifluoromethylphenyl derivative of TA esterified with hexafluoroisopropanol and trifluoroacetic anhydride. GC was carried out from SE-30 (3.2 mm x 2.6m) [21]. Ethyl chloroformate (ECF) has been used as gas chromatographic reagent for amines, amino alcohols and acids [22-26]. Chloroformates, in gas chromatography are general purposes derivatizing reagents and their use as esterifying agent have been reviewed [27, 28]. ECF has been used for GC determination of amino-acids [27-29]. Recently TA has been determined by GC combined with mass selective detection [30]. The present work extends the application of ECF for the GC-FID determination of a synthetic amino acid TA from pharmaceutical preparations.

## Results and Discussion

### *Optimization of Reaction Conditions and Separation*

TA and ACA reacted with ECF to form derivatives which were sufficiently volatile and eluted from capillary GC column, each of them indicated single peak and separated well from the derivatizing reagent. GC combined with FID was used to examine the effect of solvent, pH, concentration of derivatizing reagent, reaction time and temperature on derivatization. Each time constant volume (1µl) with split ratio 10:1 was injected and average peak height (n=4) was noted. The condition which gave maximum response was considered optimum. The solvent as

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reaction medium has a significant effect on derivatization and the reaction was examined in aqueous, methanol, acetonitrile, aqueous-methanol, aqueous-acetonitrile and aqueous solution containing pyridine base. A similar GC response (Average peak height) was observed using aqueous-acetonitrile (1:1) or aqueous solution containing pyridine base and aqueous acetonitrile as reaction medium was selected. The effect of pH on the derivatrization was examined between pH 1-12 at unit interval. It was observed that derivatization occurred at pH above 6 and maximum response was observed at pH 9. The carbonate buffer covered the pH range, as reported for amino containing compounds [27]. The addition of pure derivatizing reagent ECF was varied from 0.1 ml to 0.6 ml at an interval of 0.1 ml. The addition of ECF was not observed critical as long excess was available and a similar response was observed with addition of 0.2 ml and above. For quantitative response addition of 0.4 ml was selected. The effect of reaction time between 10 to 50 min. at an interval of 10 min and reaction temperature between 30 to 80°C at an interval of 10°C were examined. The warming temperature was not observed critical, but warning temperature 60-70°C for 20 min. gave reproducible results and was selected. The chloroform was used for extraction of derivatives as has been reported for related compounds [22]. The solution of the derivatives was injected with split ratio of 10:1 on the column HP-5 and separation of TA and ACA was examined. Complete separation was easily obtained (Fig. 1) (Conditions as Experimental).

*Validation and Quantitation by GC-FID*

Repeatability of the separation in terms of reaction time and peak height (n=6) was examined. The relative standard derivations (RSD) were observed 0.2 % and 0.7 %, respectively. The linear calibration curves for TA and ACA as ECF derivatives were obtained within 20-500 µg/ml corresponding to 2-50 ng injected on the column with coefficient of determination ($R^2$) of 0.9881. The limit of detection (LOD) measured as signal to noise ratio (3:1) for TA and ACA was observed 7 µg/ ml and 6 µg/ ml corresponding to 0.7 ng and 0.6 ng respectively. The limit of quantitation measured as signal to noise ratio 10:1 was calculated to 2.1 ng and 1.8 ng for TA and ACA, respectively. The inter and intra day precision with 200 µg/ml TA in terms of peak height (n=5) was obtained with RSD 0.1-0.5 % and 0.3- 0.9%, respectively. ECF also reacts with amino acids to form derivatives, which elute and separate from GC column [28-30]. Their possible interfering effect and separation was examined. The amino acids glycine, L-

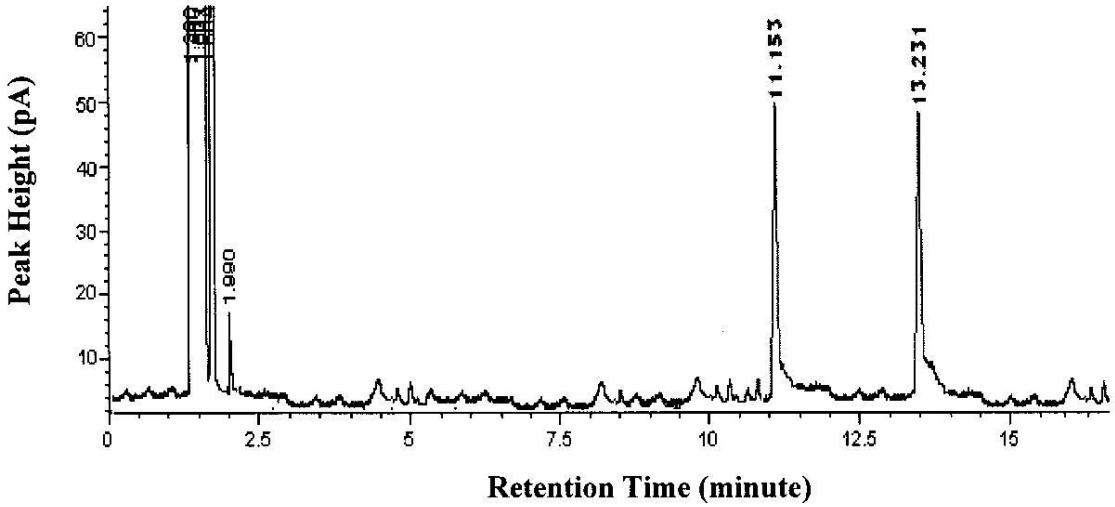


Fig. 1: GC separation of (1) ACA and (2) TA as derivatives of ECF from the column of HP-5 (30m x 0.32 mm i.d.) with film thickness 0.25 µm at initial column temperature 100 °C for 5 min, followed by heating rate 15 °C/ min up to 200 °C with total run time 15 min. Injection port and detector temperatures were maintained at 250 °C and 280 °C. Nitrogen flow rate was 2 ml/min and detection was by FID. Nitrogen was used as make up gas for FID with flow rate 45 ml/min.

valine L-proline, L-threonine, L-asparatic acid, L-glutamine, L-4-hydroxyproline, methionine, arginne, L-phenylalamine, L-histidine, L-tyrosine and L-lysine when added together with TA and ACA, formed the derivatives, extracted in chloroform simultaneously and separated completely from GC column HP-5 and did not affect the determination of TA. The effect of additives present in the pharmaceutical preparation on the determination of TA was examined. The additive methyl parabin, propylparabin, gum acacia, manitol, lactose, fructose, glucose, galactose, sodium chloride, sodium lacuryl sulphate and methyl hydroxy propyl cellulose at least twice the amount of TA was added and analysis was carried out following the analytical procedure. The results obtained were compared with TA standard. The additions of additives did not interface the determination of TA with relative error ± 3.5 %.

*Analysis of Pharmaceutical Preparation*

The determination of TA in pharmaceutical preparations Maxna, Transamine, Btrol and Xed was examined. TA from the pharmaceutical preparation was extracted with water-methanol (1:1 v/v) and analytical procedure was followed. The quantitation was carried from linear regression equation Y=0.067x +0.309. The results of analysis agreed with the labeled values with relative deviation within 3.2-5.3% with RSD 0.1-0.9% (Table 1) A tablet Maxna was also analyzed by standard addition and the results obtained agreed with labeled value with RSD 0.1- 0.32 %.

Table-1: Analysis of Pharmaceutical Preparation.

| S. No | Name of Drug | Amount TA added (µg/ml) | Amount Labeled (µg/ ml) | Amount Found (µg/ ml) (RSD %) |
|---|---|---|---|---|
| 1) | Maxna | | 500 | 477.5 (0.1) |
| 2) | Transamine | | 500 | 483.95 (0.4) |
| 3) | Btrol | | 500 | 477.6 (0.2) |
| 4) | Xed | | 250 | 236.8 (0.2) |
| 1) | Maxna | 25 | 500 | 477.69 (0.6) |
| | | 50 | 500 | 474 (0.1) |
| | | 75 | 500 | 477 (0.3) |

## Experimental

*Chemicals and Reagents*

Ethyl chloroformate (ECF) (Fluka, Switzerland), traexamic acid (TA) (Novartis, Karachi Pak.) 6-aminocaproic acid (ACA) (Alfa Aesar Company A. Johnson Methey USA), methanol and chloroform (E. Merck Germany) were used. The amino-acids glycine, L-valine, L-proline, L-threonine, L-aspartic acid, L-glutamine, L-4-hydroxyproline, L-methionine, L-arginine, L-phenylalanine, L-histidine, L-tyrosine, L-isoleucin, L-cystine and tryptophan (Fluka, Switzerland) were used.

The buffer solutions within pH 1-12 at unit interval at the concentration of (1 M) were prepared from the following: Hydrochloric acid and potassium chloride (pH 1-2), acetic acid and sodium acetate (pH 3-6), ammonium acetate (pH 7), sodium bicarbonate and sodium carbonate (saturated) (pH 8-9), ammonium chloride and ammonia (pH 10-11), sodium chloride and sodium hydroxide (pH 12). The pharmaceutical preparation Maxna (AGP (Private) Ltd. Karachi), Transamine (Hilton Pharma Co., Karachi), Btrol (Bosch Pharmaceutical (Pvt) Ltd Karachi) and Xed (Indus Pharma (Pvt), Karachi) were obtained from the local market (Hyderabad).

*Equipment*

pH measurements were made with Orion 420 A pH meter (Orion Research Inc. Boston, USA) with glass electrode and internal reference electrode. The gas chromatographic studies were carried out on Agilent model 6890 Net work GC system chromatograph (Agilent Technologies Inc. USA) coupled with flame ionization detection (FID), split/splitless injector operated in split mode, hydrogen generator Parker Balson Model H2-90, Analytical gas system (Parker Hannifin Haver Hill, M.A. USA) and pure nitrogen (British Oxygen Company (BOC), Karachi). The gas chromatograph was controlled by the computer with Chemstation software (Agilent Technologies). HP 1300 Laser jet was used throughout the study. Capillary GC column HP-5 (30m x 0.32 mm i.d) with film thickness 0.25 µm (J&W scientific GC column, USA) was used through the study.

*GC Conditions*

Solution (1 µl) was eluted from column HP-5 at column temepature 100 °C for 5 min. followed by heating rate 15 °C/min. upto 200 °C. Injection port and

detector were maintained at 250 °C and 280 °C. Nitrogen flow rate was 2 ml/min. with split ratio 10:1. Nitrogen as make up gas flow rate was 45 ml/min. The hydrogen and air flow rate for FID were 45 ml/min and 450 ml/min, respectively.

*Analytical Procedure*

The solution (0.3-1.2 mL) containing (20 – 500 µg) TA and ACA was added carbonate buffer pH 9 (1 ml), 0.4 ml of pure ECF and 1 ml acetonitrile. The contents were warmed at 70 °C for 20 min. and allowed at room temperature for 10 min. Chloroform (1 ml) was added and contents were mixed well. The layers were allowed to separate and an aliquot of organic layer was transferred to screw capped sample vial. The solution (1 µl) was eluted at operating GC conditions.

*Analysis of Pharmaceutical Preparations for TA*

Ten tablets each Maxna, Transamine, Btrol and Xed were well ground. Maxna (0.5089 g), Transamine (0.5579 g), Btrol (0.534 g) and Xed (0.3356 g) were dissolved in water-methanol (1:1 v/v) separately. The solution was filtered and final volume adjusted to 100 ml. The solution 0.15 ml from Maxna, Transamine and Btrol and 0.3 ml form Xed were taken and processed as analytical procedure. The quantitation was by using external calibration curve.

*Determination of TA from Pharmaceutical Preparation by Standard Addition*

Ten tablets of Maxna was processed as above and three solutions (0.15 mL) each was added TA 25, 50 and 100 µg in sequence. The solutions were further processed as analytical procedure. The quantitation was carried out from increase in response from calibration curve and graphical method.

## Conclusion

Selective and sensitive GC analytical procedure has been developed for the determination of TA after derivatization with ECF using FID. A number of amino-acids and additives investigated did not affect the determination of TA. The method was applied for the determination of TA from pharmaceutical preparations.

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