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RESEARCH ARTICLE

Development and Validation of Reversed Phase-High-Performance Liquid Chromatography, Dissolution Method for Simultaneous Estimation of Aminocaproic Acid in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, accurate, precise, and robust *in vitro* methods developed and validated for measurement of drug release in Aminocaproic Acid tablets. High-performance liquid chromatography (HPLC) method for quantification of drug in dissolution samples of Aminocaproic Acid tablet is developed and validated. 0.1 N Hydrochloric acid is used as dissolution medium and Basket (USP-I) as apparatus at 100 rpm. The sample was withdrawn after 60 min. The developed HPLC method was used for quantitative estimation of drug release in dissolution samples of Aminocaproic Acid tablet. Chromatogram was run through Inertsil ODS 3V, (250 × 4.6 mm), 5 µm. Mobile phase containing buffer solution and methanol in the pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 13.3 g sodium dihydrogen phosphate monohydrate, 500 mg of Heptane-1-sulfonic acid sodium salt, and 1.0 mL of Triethylamine buffer with pH 2.20 adjusted by orthophosphoric acid. Optimized wavelength for Aminocaproic acid was 210 nm. Retention time of Aminocaproic acid was found about 4.0 min; linearity range was 132.605 µg/ml–828.787 µg/ml. The new method was evaluated according to ICH guideline and as far as validation results are concern correlation coefficient value was 0.9999 for the very compound, percentage recovery 100.0%, repeatability results relative standard deviation 0.9 for Aminocaproic acid. The developed HPLC method was found to be a simple and rapid one for regular analysis in professional laboratory.

Keywords: Aminocaproic acid, method development, dissolution, validation, reversed phase high-performance liquid chromatography

DURG PROFILE

Aminocaproic Acid

Description: Antifibrinolytic hemostatic used in severe hemorrhage.

Structure: Figure 1

Appearance: Fine, White, Crystalline Powder.

Molecular weight: 131.175 g/mol.

Molecular formula: $C_6H_{13}NO_2$.

IUPAC name: 6-aminohexanoic acid.

Mechanism of action

Imipramine works by inhibiting the neuronal reuptake of the neurotransmitters norepinephrine and serotonin. It binds the sodium-dependent serotonin transporter and sodium-dependent norepinephrine transporter preventing or reducing the reuptake of norepinephrine and serotonin by nerve cells. Depression has been linked to a lack of stimulation of the post-synaptic neuron by norepinephrine and serotonin. Slowing the reuptake of these neurotransmitters increases their concentration in the synaptic cleft, which is thought to contribute to relieving symptoms of depression. In addition to acutely inhibiting neurotransmitter re-uptake, imipramine causes downregulation of cerebral

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cortical beta-adrenergic receptors and sensitization of post-synaptic serotonergic receptors with chronic use. This leads to enhanced serotonergic transmission.

Pharmacodynamic

Aminocaproic acid works as an antifibrinolytic. It is a derivative of the amino acid lysine. The fibrinolysis-inhibitory effects of aminocaproic acid appear to be exerted principally through inhibition of plasminogen activators and to a lesser degree through antiplasmin activity. Aminocaproic acid may be a possible prophylactic for vascular disease, as it may prevent formation of lipoprotein (a), a risk factor for vascular disease.

Absorption

Rapidly and well-absorbed after oral administration. Bioavailability is approximately 43%. Peak plasma concentrations usually attained 1–2 h following oral administration.[1-4]

Objective and plan of study

The objective of the study was to develop Reversed Phase High-Performance Liquid Chromatography (RP-HPLC) method for dissolution of Aminocaproic acid Tablet and validated method as per International Conference on Harmonization (ICH) Q2 (R1) and to apply validated method for the estimation of Aminocaproic acid in quality control or in research laboratories of pharmaceutical companies.

MATERIALS AND METHODS

Materials

Aminocaproic acid tablets were supplied by Medindia Pharma network. HPLC grade water, methanol, triethylamine, orthophosphoric acid, sodium dihydrogen phosphate monohydrate Heptane-1-sulfonic acid sodium salt, Hydrochloric acid, etc.[5-7]

Instrument

HPLC instrument used A Shimadzu's HPLC (LC-1020C HT) with PDA detector and auto sampler (Shimadzu Corporation, Kyoto, Japan). Software used is Empower-3. Ultraviolet (UV) Spectrophtometer SHIMADZU 1800 double beam UV□Visible spectrophotometer with software LC Solution (Shimadzu Corporation, Kyoto, Japan), dissolution apparatus of Electrolab.

Methods

Dissolution medium preparation

0.1 N hydrochloric acid: Transfer 85.0 mL of hydrochloric acid into a suitable container containing about 5000 mL of water. Dilute up to 10000 mL with water and mix well. Degas it.[8-10]

Dissolution parameters

Apparatus: USP apparatus I (Basket).
Medium: 0.1 N HCl.
Speed: 100 RPM.
Medium Volume: 500 mL.
Time: 60 min.

Diluent

Based on solubility data of drug, dissolution medium selected as a diluent (0.1 N Hydrochloric acid).

Preparation of buffer

Weigh accurately about 13.3 g of Sotassiumdihydrogen phosphate monohydrate and 500 mg of Heptane-l-sulfonic acid sodium salt, transfer into a suitable container containing 1000 mL of water. Sonicate to dissolve it and mix well. Add 1.0 mL of Triethylamine into it and mix well. Adjust the pH to 2.20 ± 0.05 using diluted orthophosphoric acid solution and mix well.

Mobile phase

Prepare a mixture of Buffer solution and methanol in the ratio of 75:25 (% v/v), respectively. Mix well and degas it by sonication.

Chromatographic parameters

HPLC column: Inertsil ODS 3V (250 × 4.6 mm), 5 µm.
Pump Flow: 1.0 ml/min.

Injection volume: 25.0 µL.
Wavelength: UV detector at 210 nm.
Column oven temperature: 50°C.
Sample cooler temperature: 25°C.
Run Time: 10 min.

Preparation of standard stock solutions

Accurately weighed 50.0 mg of Aminocaproic acid standard and transferred to 50 ml volumetric flask. Add 35 mL of diluent and sonication to dissolve it. Dilute to volume with diluent and mix well.

Preparation of sample solutions (for 500 mg tablets)

Place 500 mL of dissolution medium into each of six dissolution vessels, which are placed in water bath, maintained at 37°C + 0.5°C. Individually, weigh each of six tablets and record the weight. Sequentially, place each tablet into a respective basket. Attach the basket to the shaft and shaft lower the basket shaft into vessel. Run the dissolution unit as per the dissolution parameter. Aliquot was withdrawn after 60 min. Filtered through 0.45 µm filter and injected in chromatographic system.

Validation parameters

The method was evaluated as per protocol of ICH guideline. The evaluation parameters took into consideration were system suitability parameters, precision, accuracy, intermediate precision, linearity, robustness studies, etc.[11-14]

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Aminocaproic acid and the solutions were injected 5 times and the parameters such as peak tailing, theoretical plate count, and retention time were determined.

Specificity

Specificity was determine the comparison of diluent, standard solution, and sample solution. We should not find any aminocaproic peak in diluent in this method so the method can be considered as specific.

Accuracy

The accuracy was determined by calculating the extant of recoveries of aminocaproic acid by the method called standard addition. Correct amount of solutions (standard) of aminocaproic acid (each 25%, 100%, and 150%) was added and injected to pre-quantified solution of sample. The quantity of each substance recovered was determined.

Precision

Method precision was evaluated by performing the dissolution using proposed method (dissolution parameters and chromatographic method) on six tablets of aminocaproic acid tablets and calculated % release of aminocaproic acid in each sample. The relative standard deviation (%RSD) for set of six tablets was calculated. The intermediate precision of the method was also evaluated using different day and a different instrument in the same laboratory by carrying out dissolution on six more tablets using proposed method and calculated % release of aminocaproic acid in each sample.

Linearity

The linearity of aminocaproic acid is established by analyzing linearity solutions of different concentrations from 25% to 150% of working concentration method for dissolution. The linearity curve is plotted for peak area versus concentration.

Robustness

Robustness study is performed by analyzing at different chromatographic conditions. These

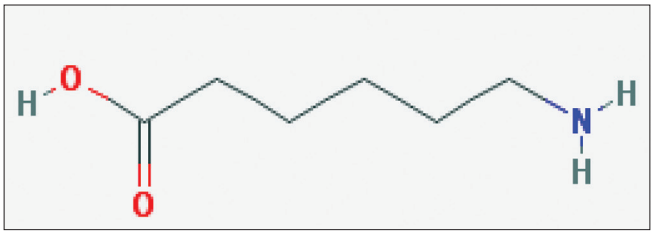

Figure 1: Structure of aminocaproic acid

Table 1: System suitability

Compound	Rt (min)	Area	USP plate count	Tailing factor
Aminocaproic acid	4.25	567599	6606	1.2

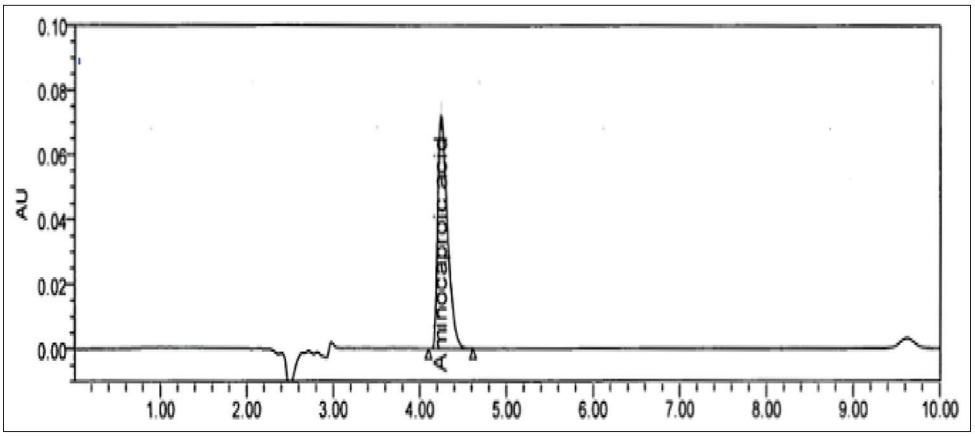

Figure 2: Standard solution chromatogram of aminocaproic acid

Table 2: Results of accuracy

% of Aminocaproic acid standard added (µg/ml)	Amount of standard Aminocaproic acid added (µg/ml)	Amount of Aminocaproic acid standard added (µg/ml)	Amount of Aminocaproic acid standard recovered (µg/ml)	Amount of Aminocaproic acid standard recovered %	Average % recovery	% RSD
25%	250	248.043	250.325	99.1	99.2	0.4
		247.349	250.104	98.9		
		249.552	250.379	99.7		
100%	1000	1010.635	1000.875	101.0	101.1	0.3
		1008.825	1000.511	100.8		
		1014.687	1000.731	101.4		
150%	1500	1505.432	1500.778	100.3	99.8	0.5
		1496.527	1500.089	99.8		
		1489.087	1500.125	99.3		
Overall % recovery					100.0	
Overall % RSD					0.9	

Table 3: Standard solution results of precision and intermediate precision

Sr no.	Peak area of Aminocaproic acid standard (Precision)	Peak area of aminocaproic acid standard (Intermediate Precision)
1	567,599	593,261
2	567,534	593,452
3	567,319	592,964
4	566,914	593,329
5	567,086	593,223
Mean	567,290	593,246
%RSD	0.1	0.0

Table 4: Sample solution results of precision and intermediate precision

Sr no.	Peak area of Aminocaproic acid Sample (Precision)	Peak area of aminocaproic acid Sample (Intermediate Precision)
1	557,171	576,459
2	557,161	582,876
3	560,786	587,685
4	564,211	589,812
5	560,090	580,691
6	567,143	587,849
Mean	561,094	584,229
%RSD	0.7	0.9

parameters included change in flow rate, mobile phase composition, temperature of column, buffer pH, Medium Volume, and RPM. The results obtained with altered conditions are compared against results obtained under normal chromatographic condition.

RESULTS AND DISCUSSION

System Suitability Parameter

The optimized chromatographic developed method resulted in the elution of Aminocaproic acid at 4.25 min. Figure 2 is the representative

chromatogram of standard aminocaproic acid. System suitability results were evaluated taking six replicates of standard at 1000 µg/ml for the compound aminocaproic acid. Table 1 narrates about the results of system suitability parameters.

Accuracy

Recovery of aminocaproic acid 25%, 100%, and 150% was 100.0. Table 2 contains all the results of accuracy studies.

Table 5: Linearity data of aminocaproic acid

Linearity level (%)	Concentration (µg/mL)	Peak area
25	132.605	147,176
50	276.262	302,681
80	442.019	485,212
90	497.272	543,732
100	552.524	610,197
110	607.777	668,376
120	663.029	725,850
150	828.787	904,292
Correlation coefficient: 0.9999	Y-intercept : +2887.9552	
Slope: 1091.0281	Y-intercept bias at 100% level: −0.5%	

Precision

Result of precision as mean area of peak and %RSD for aminocaproic acid standard injection was 567,290 and 0.1. For aminocaproic acid sample injection results were 593,246 and 0.0. Result of intermediate precision as mean area of peak and %RSD for aminocaproic acid standard injection was 559,261 and 0.5. For aminocaproic acid sample injection results were 584,229 and 0.9.

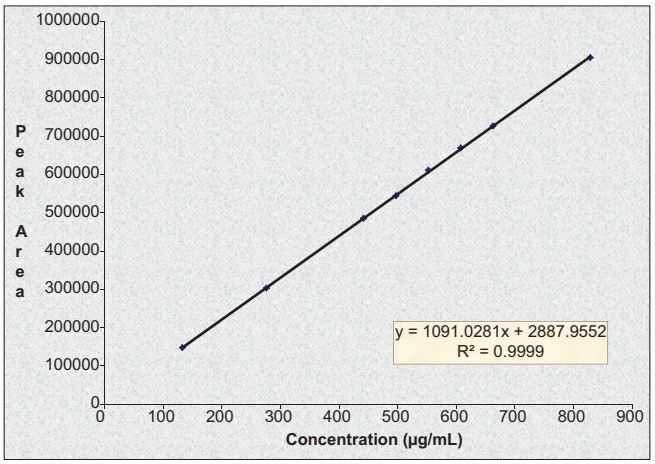

Figure 3: Calibration curve of aminocaproic acid

Table 6: Robustness results comparison with Precision result – Variation in Flow Rate (Sample Solution) (± 0.2 mL/min)

Injection #	% Drug release			
	Flow rate 0.8 mL/min	Actual flow rate 1.0 mL/min	Flow rate 1.2 mL/min	Actual flow rate 1.0 mL/min
1	98	98	98	98
2	98	98	98	98
3	99	99	99	99
4	99	99	99	99
5	99	99	99	99
6	100	100	100	100
Mean	99	99	99	99
% RSD	0.8	0.8	0.8	0.8
Absolute difference	0		0	

Table 7: Robustness results comparison with precision result – variation in column oven temperature (sample solution) (±5°C)

Injection #	% Drug release			
	Column oven temperature: 45°C	Actual column oven temperature: 50°C	Column oven temperature: 55°C	Actual column oven temperature: 50°C
1	98	98	100	98
2	98	98	99	98
3	99	99	100	99
4	99	99	101	99
5	99	99	100	99
6	100	100	101	100
Mean	99	99	100	99
% RSD	0.8	0.8	0.8	0.8
Absolute difference	0		1	

Table 8: Robustness results comparison with precision result – variation of organic solvent (methanol) in mobile phase composition (sample solution)

Injection #	% Drug release			
	−5% Variation of Methanol in Mobile Phase composition (Buffer solution: Methanol [763:237])	**Actual composition of Mobile Phase composition (Buffer solution-B: Methanol [750:250])**	**+5% Variation of Methanol in Mobile Phase composition (Buffer solution: Methanol [738:262])**	**Actual composition of Mobile Phase composition (Buffer solution-B: Methanol [750:250])**
1	101	98	101	98
2	100	98	99	98
3	100	99	100	99
4	101	99	101	99
5	100	99	100	99
6	101	100	101	100
Mean	101	99	100	99
% RSD	0.5	0.8	0.8	0.8
Absolute difference	2		1	

Table 9: Robustness results comparison with precision result – Variation in pH of buffer solution-B For mobile phase (sample solution)

Injection #	% Drug Release			
	pH of Buffer solution-B for Mobile phase 2.00	**Actual pH of Buffer solution-B for Mobile phase 2.20**	**pH of Buffer solution-B for Mobile phase 2.40**	**Actual pH of Buffer solution-B for Mobile phase 2.20**
1	100	98	99	98
2	101	98	101	98
3	100	99	99	99
4	101	99	100	99
5	99	99	99	99
6	100	100	99	100
Mean	100	99	100	99
% RSD	0.8	0.8	0.8	0.8
Absolute difference	1		1	

Table 10: Robustness results comparison with precision result – variation in media volume (sample solution)

Injection #	% Drug release			
	Media volume: 855 mL	**Actual media volume: 900 mL**	**Media volume : 945 mL**	**Actual media volume : 900 mL**
1	99	98	99	98
2	99	98	99	98
3	99	99	99	99
4	99	99	98	99
5	100	99	98	99
6	99	100	100	100
Mean	99	99	99	99
% RSD	0.4	0.8	0.8	0.8
Absolute difference	0		0	

Table 11: Robustness results comparison with precision result – variation in RPM (± 2 RPM) (sample solution)

Injection #	% Drug release			
	RPM: 98	Actual RPM: 100	RPM: 102	Actual RPM: 100
1	100	98	101	98
2	100	98	101	98
3	99	99	101	99
4	100	99	100	99
5	99	99	100	99
6	100	100	100	100
Mean	100	99	101	99
% RSD	0.5	0.8	0.5	0.8
Absolute difference	1		2	

Tables 3 and 4 narrate precision and intermediate precision results.

Linearity [Table 5]

Results of linearity test resulted that mean Y intercept value, slope value, and value of correlation coefficient for aminocaproic acid were + 2887.95, 1091.0281, and 0.9999 at the concentration range of 132.605 μg/ml–828.787 μg/ml (25–150%) [Figure 3].

Robustness

This evaluation had been done by bringing variation in certain chromatographic parameters such as increasing and reducing flow rate, mobile phase composition, temperature of column, buffer pH, Medium Volume, and RPM. All the observed values are given in Tables 6-11.

CONCLUSION

The newly developed analytical method is accurate precise, simple, sensitive, selective, robust, rapid, and cost-effective and can be applied successfully for the estimation of pharmaceutical dosage form without interference in laboratory.

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