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Mini Review

Analytical Methods for the Determination of Thrombolytics

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Abstract

Management of patients with acute myocardial infarction is a great challenge to the physicians nowadays. Multiple clinical trials which included thousands of patients undergoing thrombolytic therapy have demonstrated a substantial improvement in mortality. Thrombolytic therapy exhibits a proven mortality benefit and it requires no specialized equipment and is available in any emergency setting. Thrombolytics act to remove the thrombus that cause myocardial ischemia and restores the coronary circulation and also prevent formation of new clots. The present review describes the analytical methods existing for the determination of Thrombolytics in the literature. This review helps the authors to establish new analytical techniques after a keen verification of the advantages and the disadvantages of the existing methods.

Keywords: HPLC; Spectrophotometry; LC-MS; TLC; HPTLC

Introduction

Thrombolytics are also known as plasminogen activators and fibrinolytic drugs [1]. The plasminogen forms plasmin. Plasmin is a proteolytic enzyme which breaks the crosslink between fibrin molecules. The tissue plasminogen activator is the serine protease which is found on the endothelial cells lining blood vessels and they involved in the breakdown of blood clots. Thrombolytics are used to treat the thromboembolic clot which tends to platelet and fibrin rich [2]. The thrombolytic agents are converted into native plasminogen to plasmin, which hydrolyses the fibrin of thromboembolic that resulting in clot lysis. Thrombolytic therapy is to improve pulmonary artery pressure, arteriovenous, oxygenation, pulmonary perfusion and echo cardio graphic assessment [3,4]. Thrombolytics act to remove the thrombus that cause myocardial ischemia and restores the coronary circulation and also prevent formation of new clots. Drugs used for this treatment include Streptokinase, Alteplase, Reteplase, Tenecteplase, Anistreplase [5].

History

Thrombolytics may be classified as Fibrinolytics (Streptokinase (4-cyclo hexyl pyrrolidine-2-carboxylic acid), Urokinase 3-[4-chloro-1-(diamino methylidene amino) isoquinolin-7-yl] benzoic acid), Alteplase, Reteplase), Anti-fibrinolytics (Aprotinin, Aminocaproic acid (derivative and analogue of the amino acid lysine), Tranexamic acid (trans-4- amino methyl-cyclo hexane carboxylic acid). Streptokinase combines with proactivator plasminogen and forms a complex. This complex act as a catalyst for conversion of plasminogen to active plasmin and this plasmin causes rapid lysis of the clot, plasmin also acts as a catalyst for clotting factor. Urokinase is an enzyme that converts plasminogen to active plasmin which is not clot specific but can cause a generalized lysis when administered through intravenus route. Alteplase is a tissue plasminogen activator. It is an enzyme and allows fibrin enhanced conversion of plasminogen to active plasmin in the absence of fibrin. It enters the systemic circulation and binds to fibrin in thrombus and converts plasminogen to plasmin followed by activated fibrinolysis and limited systemic proteolysis. Reteplase is a recombinant human tissue plasminogen activator, less fibrin specific than tissue plasminogen activator [8]. Tenecteplase is a mutant form of tissue plasminogen activator with a longer duration of action and more specific than tissue plasminogen activator. Aminocaproic acid and tranexamic acid inhibit fibrinolysis by competitive inhibition of conversion of plasminogen to active plasmin. Aprotinin is a serine protease inhibitor [6-9]. In the present review the authors have gathered the analytical methods established so far in the literature for the determination of the above listed anti-gout drugs. The chemical structures of these drugs were shown in figure 1.

Figure 1: Chemical structures of Thrombolytics.

Analytical Methods

Thrombolytics were estimated using different analytical techniques such as HPLC [10-17] and Spectrophotometry [18-20]. Azadi., *et al*. established an ion exchange chromatography technique [21] using 20mM Tris–HCl (pH 8.0) for Streptokinase and Naik., *et al*. developed HPTLC method [22] for the determination of Tranexamic acid and Ethamsylate using a mobile phase mixture consisting of water: acetone: methanol (3:4:3). Fabresse., *et al*. developed LC-MS/MS method [23] for the determination of Tranexamic acid using 2 mM formate buffer (pH 3.8): methanol (5: 95) using Accucore Urea HILIC column (Flow rate 200 µL/min) and Hosiana., *et al*. developed a TLC densitometric method for the determianation of Tranexamic acid in tablets [24] using a mixture of *n*-butanol–glacial acetic acid–water (8: 2: 2). A concise description of these methods was given in table 1 for HPLC methods and table 2 for Spectrophotometry. A list of brands and the type of formulations available for Thrombolytics were given in table 3.

Drug	Mobile phase	λ (nm)	Method	Flow rate(mL/min)	Column	Ref
^{99m}Tc-aprotinin	Triethyl amine: Methanol (40:60) (pH-2.7 ± 0.05)	230	HPLC	1.5	XB- C_{18}	10
Tranexamic acid	Acetonitrile: Water (50:50)	418	HPLC	1.5	HiQ sil C-8	11
Tranexamic acid	Acetonitrile: 0.1M Ammonium acetate (p^H5.0) (25:75)	232	HPLC	1.0	C_{18}	12
Tranexamic acid and Ethamsylate	Methanol: Phosphate bufferpH 3 (35:65)	256	HPLC	1	Thermosil C_{18}	13
Tranexamic acid and Ethamsylate	Water: Acetonitrile: Triethylamine(pH 4) (93:07:01)	220	HPLC	1.0	Intersil C_{18}	14
Tranexamic acid and Mefenamic acid	Phosphate buffer: Acetonitrile(70:30) (pH 6)	215	HPLC	1.0	Hypersil BDS- C18	15
Tranexamic acid and Mefenamic acid	Methanol, 20 mM Acetate buffer (75:25, v/v) pH adjusted to 4.0 using ortho phosphoric acid	370	HPLC	1.0	Phenomenex C_{18}	16
Tranexamic acid and Mefenamic acid	Ammonium acetate: Acetonitrile (30:70)	310	HPLC	2.7 & 3.86	C_{18}	17

Table 1: List of liquid chromatographic methods for the assay of Thrombolytics.

Drug	Method	Reagent	λ (nm)	Ref
Aminocaproic acid	Colorimetry	-	540	18
Tranexamic acid	Spectrophotometry	1 mL of 1% (w/v) salicylaldehyde solution and ethanol	400	19
Tranexamic acid and Pregabalin	Colorimetry	-	418 & 425	20

Table 2: List of spectrophotometric methods for the assay of Thrombolytics.

Drug	Brand names	Dosage forms	Label Claim (mg)	Pharmaceutical company
Aminocaproic acid	Amocap	Vial	5	Celon (Revilon)
	Hamostat	Tablet injection	500; 250	Samarth
	Hemocid	injection	250	GSK
^{99m}Tc-aprotinin	Aprogen	Injection	14	Alkem Laboratories Ltd
	Aprotin	Injection	70	VHB Lifesciences Inc
	Kallistat	Injection	50 ml	Biosena (Biosciences-Pharmakon)
	Aprogen	Injection	70	Alkem Laboratories Ltd
	Transylol	Injection	50 ml	BAYER Pvt.Ltd
	Apronin	Injection	1 vial	Chandra Bhagat Pharma Pvt. Ltd.
	Aprostat	Injection	50 ml	Samarth Pharma Pvt. Ltd.
	Kallistat	Injection	1 vial	Biosena (Biosciences-Pharmakon)
Streptokinase	Cares trepnase	Injection	1 vial	Human pharmacia
	Adplatt	Tablet	75	Grandix
	Antiban	Tablet	75	Blue cross
	Aplatin -75	Tablet	75	Saga lab
	Aptogrel	Tablet	75	Auroindo
	Antiplar	Tablet	75	Emcure
	Asogrel	Tablet	75	As Pharma
	C – Gril	Tablet	75	Triton
	Stapo	Vial	15 lit	Health biotech
	Stapase	Injection	1500000 iu	Candila (oncocare)
	Myokinase	1 vial	1500000 iu	Biocon
	Prokinase	1 vial	0.75 MIU	EMCURE
	Prokinase	1 vial	1.5 MIU	EMCURE
Tranexamic acid	Alexie	Injection	100 /1ml	Altius LS
	B-Clot	Tablets	500	Dynamic Labs
	Capitrax	Tablets	500	Evacare
	Agretax	Tablets	500	Agron Remedies
	CB	Injection	500	USP LS
	Clotawin	Injection	500 /ml	Bestochem
	Cyklokapron	AMP	100	Pfizer
	Examic	Vial	500	Bluebell
	Mouzex	Injection	500 /5ml	Little Greave

Tranexamic acid: Ethamsylate	Myotran ET	Tablet	250:250	Solitaire
	Rauf E	Tablet	500:250	Alna Bio
	Reotran -ES	Tablet	250:250	Rishab (orochem)
	Temsyl	Tablet	250:250	Dewcare
	Trance -ET	Tablet	250:250	Sterkem Pharma
	Trapic E	Tablet	250:250	Sun pharma
Tranexamic acid: Mefenamic acid	Trumps	Tablet	500:250	Kalpataru
	Zutran -M	Tablet	250:500	Zodak
	Tra -MF	Tablet	500:250	Alna(Mepfarma)
	Tranarest MF	Tablet	500:250	Zydus Candila
	Temsyl -T	Tablet	500:250	Dewcare
	Gynae -Pil Forte	Tablet	500:250	PIL
	2-Pin-TX	Tablet	500:250	Novogen Captab

Table 3: List of available marketed formulations of Thrombolytics.

Conclusions

This review article is very much helpful to compare the advantages and disadvantages of any new analytical method for the assay of Thrombolytics.

Bibliography

1. Giri Raja Sekhar., *et al*. "A Review on Thrombolytic Therapy used in Myocardial Infarction (Streptokinase vs Tenecteplase)". *International Journal of Pharmaceutical Sciences Review and Research* 45.2 (2017): 29-32.

2. Andrew Bivard., *et al*. "Review of Stroke Thrombolytics". *Journal of Stroke* 15.2 (2013): 90-98.

3. Douglas Kirk J. "Reperfusion Strategies for Acute Myocardial Infarction: Mechanical vs. Thrombolytic Therapy". *The California Journal of Emergency Medicine* 3.1 (2002).

4. Colleen martin., *et al*. "Systemic Thrombolysis for Pulmonary Embolism: Review". *Pharmacy and Therapeutics* 41.12 (2016): 770-775.

5. Shapiro SS. "Treating thrombosis in the 21st century". *The New England Journal of Medicine* 349.18 (2003): 1762-1764.

6. Gusto V Investigators. "Reperfusion therapy for acute myocardial infarction with fibrinolytic therapy or combination reduced fibrinolytic therapy and platelet glycoprotein IIB/IIIA inhibition: The Gusto V randomized trial". *Lancet* 357 (2001): 1905-1914.

7. Assent III Investigators. "Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: The Assent 3 randomized trial in acute myocardial infarction". *Lancet* 358 (2001): 605-613.

8. Fibrinolytic Therapy Trialists' Collaborative Group "Indications for fibrinolytic therapy in suspected acute myocardial infarction: Collaborative overview of early mortality and major morbidity results from all randomized trials of more than 1000 patients". *Lancet* 343 (1994): 311-322.

9. Gusto Investigators "An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction". *The New England Journal of Medicine* 329 (1993): 673-682.

10. Denholt C and Gillings N. "99mTc-aprotinin – optimisation and validation of radiolabelling kits for routine preparation for diagnostic imaging of amyloidosis". 59 (2016): 171-174.

11. Teli MS., *et al*. "Estimation of Tranexamic acid in its tablet and bulk formulation by RP-HPLC method". *World Journal of Pharmacy and Pharmaceutical Science* 4. 12 (2015): 1404-1415.

12. Ashfaq., *et al*. "Derivatization/chromophore introduction of Tranexamic acid and its HPLC determination in pharmaceutical formulations". *Journal of the Association of Arab Universities for Basic and Applied Sciences* 17 (2015): 51-56.

13. Sravanthi D., *et al*. "Analytical method development and validation for the simultaneous estimation of Tranexamic acid and Ethamsylate by RP-HPLC method in bulk and pharmaceutical dosage form". *Pharma Research Library.*

14. Prashil Nanotkar., *et al*. "Simultaneous estimation of Tranexamic acid and Ethamsylate in combined dosage form by RP-HPLC". *Open Access Scientific Reports* 1 .5 (2012).

15. Priyanka., *et al*. "Stability indicating RP-HPLC method development and validation for simultaneous estimation of Tranexamic acid and Mefenamic acid in tablet dosage form". *European Journal of Biomedical and Pharmaceutical Sciences* 4.6 (2017): 377-386.

16. Natesan S., *et al*. "Improved RP- HPLC method for the simultaneous estimation of Tranexamic acid and Mefenamic acid in tablet dosage form". *Pharmaceutica Analitica Acta* 2.115 (2011).

17. Srivalli Y., *et al*. "Simultaneous method development and validation of Tranexamic acid and Mefenamic acid in its tablet dosage form by RP- HPLC". *International Journal of Innovative Pharmaceutical Sciences and Research* 2.9 (2014): 2189-2198.

18. Shaza W., *et al*. "Development and validation of colorimetric method for the determination of aminocaproic acid in bulk and pharmaceutical formulation". *Journal of Innovations in Pharmaceutical and Biological Sciences* 4.3 (2017): 67-70.

19. Gehad G., *et al*. "Spectrophotometric methods for determination of Tranexamic acid and Etamsylate in pure form and pharmaceutical formulation". *Insight Pharmaceutical Sciences* 5.1 (2015): 1-7.

20. Nawab Sher., *et al*. "Pregabalin and Traxamic acid evaluation by two simple and sensitive spectrophotometric methods". *International Journal of Analytical Chemistry* (2015).

21. Azadi H., *et al*. "Purification of recombinant streptokinases by Ion exchange chromatography". *International Research Journal of Applied and Basic Sciences* 9.5 (2015): 736-742.

22. Naik RY., *et al*. "Development and validation of high-performance thin layer chromatographic method for simultaneous estimation of Etamsylate and Tranexamic acid in tablet dosage form". *International Journal for Pharmaceutical Research Scholars* 4.2 (2015).

23. Fabresse N., *et al*. "LC-MS/MS determination of tranexamic acid in human plasma after phospholipid clean-up". *Journal of Pharmaceutical and Biomedical Analysis* 141 (2017): 149-156.

24. Hosiana., *et al*. "Densitometric determination of Tranexamic acid in tablets: Validation of the method". *Journal of Liquid Chromatography and Related Technologies* 28.20 (2017): 3243-3254.

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