Research Article

Development of Thin Layer Chromatographic (TLC) Method for Identification of Ketorolac Tromethamine Salt

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ABSTRACT

Ketorolac tromethamine was routinely used non-steroidal anti-inflammatory agent. Ketorolac available in tromethamine salt because tromethamine salt of Ketorolac was non-hygroscopic even at high humidity levels and also increase its intrinsic dissolution rate and aqueous solubility. A Thin Layer Chromatography (TLC) method for identification of tromethamine in Ketorolac tromethamine salt was developed. TLC promotes for higher separation efficiencies, shorter analysis time and lower amount of mobile phase required. The drugs were satisfactorily resolved with RF values 0.63 and 0.60 for Ketorolac tromethamine reference standard and test solution respectively. Development of yellow spot with pink to purple colour border conform presence of tromethamine salt in drug substance. It can also be applied for the Preformulation study of drug and fate study of drug in biological fluid.

Keywords: Ketorolac tromethamine, Thin layer chromatography, Tromethamine salt.

1. INTRODUCTION

Non-steroidal anti-Inflammatory drugs (NSAIDs) are used for the management of pain and inflammation associated with musculo-skeletal, joint disorders and operative procedures. The uses of these drugs are prevalent in India due to the high rates of occurrence of rheumatoid disorders. Many NSAIDs have been marketed, the one among is Ketorolac Tromethamine. It is chemically, (±)-5-Benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol (See fig. 1).[1] It is a member of the heterocyclic acetic acid derivative family and is used as an analgesic with an efficacy close to that of the opioid family. It is also a potent antipyretic and anti-inflammatory. It is mainly used for the short term treatment of post-operative pain as it is highly selective for the COX-1 enzyme.[2]

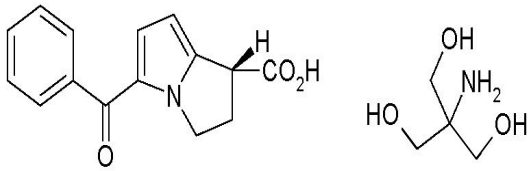

Fig. 1: Structural formulae for Ketorolac tromethamine (MW=376.4)

Why Ketorolac in tromethamine salt

- Tromethamine salt of Ketorolac is non- hygroscopic even at high relative humidity levels.
- Tromethamine salt also increases the intrinsic dissolution rates and aqueous solubility of Ketorolac.[12]

Tromethamine

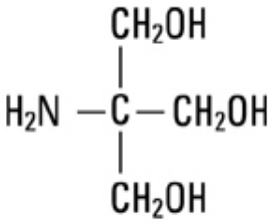

Fig. 2: Structure of tromethamine

Molecular Formula: $C_4H_{11}NO_3$
Molecular Weight: 121.13504
IUPAC Name: 2-amino-2-(hydroxymethyl) propane-1,3-diol (See fig. 2).
Different nomenclature of tromethamine

- 2-Amino-2-Hydroxymethyl- m 1, 3-Propanediol
- Tris(hydroxymethyl)aminomethane

- TRIS AMINO - ANGUS
- Trometamol - INN
- THAM
- Tris
- Tromethamine (United States Pharmacopeia Designation)

Properties

Tromethamine (2-amino-2-hydroxymethyl-1, 3-propanediol) is a mildly alkaline chemical compound. Its chemical structure is that of a trihydric alcohol which also possesses primary amine functionality. Pure Tromethamine is readily available as a white crystalline solid, melting between 168°C and 172°C. TRIS AMINO is readily water-soluble (up to 80 g/100 ml water), and possesses alcohol and glycol solubility as well.

Uses of Tromethamine in pharmaceutical formulation

1) Usually inert substances added to a prescription in order to provide suitable consistency to the dosage form. These include binders, matrix, base or diluents in pills, tablets, creams, salves, etc.
2) Tromethamine buffer demonstrates mild alkalinity, buffering capability and primary aminotriol functionality. These properties can offer the formulator advantageous combinations of active material solubilization into aqueous systems, chemical stabilization of actives against degradation, and pH buffering of solutions. At the same time, the presence of Tromethamine in an aqueous formulation will not compromise favorable toxicological characteristics. These attributes of Tromethamine can be applied to topical, injectable, oral, and ophthalmic systems
3) Tromethamine is the buffer system of choice for a lyophilized dosage form of human recombinant interleukin-1. Not only is its pH-controlling capability for the target pH of 7.5 very good, even at low temperatures, but the polyol nature of Tromethamine permits it to participate in a hydration network surrounding the protein. This is considered a distinct advantage. The polyol nature of Tromethamine buffer also offers storage stabilization against precipitation out of aqueous solution for somatotropin. In this case, the hydrochloride salt of Tromethamine is used to maintain the pH of the solution at about 5.7.[14]
4) Neomycin undecylenate is often used in a propylene glycol or polyol vehicle to treat infections of the ear. Sodium metabisulfite is incorporated as an antioxidant, and the pH of the system is adjusted to 3-5 with benzoic acid. The incorporation of Tromethamine stabilized this formulation against formation of neomycin sulfate precipitate and loss of neomycin activity.[15] Although ethanolamine's also provided similar stabilization; Tromethamine was preferred for its biological tolerance.
5) Mercury-based antimicrobial preservatives such as thiomersal are especially valuable in aqueous ophthalmic preparations. Tromethamine has the ability to prevent the deposition of such preservatives out of the aqueous system onto the walls of plastic containers used for such preparations. At the same time, Tromethamine will stabilize the mercury-based preservatives themselves against chemical decomposition in aqueous solution, greatly lengthening the effective storage life of the preparations. The presence of Tromethamine will also stabilize the active non-steroidal anti-inflammatory drug sodium diclofenac in aqueous ophthalmic formulations and render it more tolerable by the eye.[16]
6) N-Nitrosourea anti-neoplasmic agents are generally quite unstable in aqueous solutions. It is possible to extend the shelf-life of these nitrosourea anti-cancer drugs by including Tromethamine Buffer in their formulation, instead of using carbonate buffers at the same pH[4]. The nitrosoureas have been shown to form a complex with the Tromethamine, and the rate of hydrolytic degradation of the drug, in the form of this complex, is significantly slower than that of the nitrosourea alone. Tromethamine is also used in aqueous formulations of the anti-cancer drug, 5-fluorouracil to buffer the solution at pH 8.2.
7) The arginine salt of fosfomycin has advantageous therapeutic properties, but its water solubility is quite limited. However, when formulated together with Tromethamine, this amino acid-fosfomycin salt becomes hydrosoluble to the extent of about 14%, as well as

becoming more bioavailable in its active form.[18] Examples of some tromethamine salt with their uses and what is improvement when made in tromethamine salt given in table 1.

2. MATERIALS AND METHODS

2.1 MATERIALS

Chemical and reagents:

Free gift sample of Ketorolac Tromethamine were obtained from FDC limited Mumbai. Laboratory grade reagents methanol, dichloromethane, acetic acid and acetone obtained from Modern lab Nasik.

Sample preparations:

- Mobile phase prepare by using dichloromethane, acetone and acetic acid in ratio (95:5:2, v:v:v) respectively.
- Prepare a standard solution of USP Ketorolac tromethamine RS in a mixture of dichloromethane and methanol (2:1) containing 5 mg/ml similarly prepared test solution.
- Freshly prepared alcoholic solution containing 30 mg of ninhydrine /ml for colour development.

2.2 METHOD

Tromethamine test: Prepare a standard solution of USP Ketorolac tromethamine RS in a mixture of dichloromethane and methanol (2:1) containing 5 mg/ml. similarly prepare a test solution of Ketorolac tromethamine containing 5mg/ml. apply 40µl volume of standard solution and test solution to TLC (Thin layer chromatography)plate coated with a 0.25 mm layer of silica gel mixture. Place the plate in chromatographic chamber equilibrated with a mixture of dichloromethane, acetone and acetic acid in ratio (95:5:2) respectively. Seal the chamber and develop the chromatogram until the solvent front has moved about three forth of the length of the plate. Remove plate from chamber and allow the solvent to evaporate. Spray the plate with freshly prepared alcoholic solution containing 30 mg of ninhydrine /ml and heat the plate about 150^0 for 2 to 5 minutes. Yellow spot with pink to purple border developed on plate in the areas where the standard solution and the test solution were applied.[13]

3. RESULT AND DISCUSSION

The position of migrated spots on the chromatogram is indicated by RF Values. These parameters are qualitative and quantitative parameter, characteristics of a substance. R is a function of the partition coefficient. it is constant for a given substance , provided the condition of chromatographic system are kept constant with respect to temperature, type of paper, duration and direction of development , the amount of reservoir humidity etc. the RF defines the movement of a substances relative to solvent front in a given chromatographic system.

RF calculated from the following formula,

$$RF = \frac{\text{Distance travelled by a solute from the origin line}}{\text{Distance travelled by the solvent from the origin line}}$$

Calculations of RF values are given in table 2. RF value of both reference standard and test solution shows nearly same. As per the USP tromethamine test developed TLC plate shows yellow colour spot with violet to pink colour border around it. Fig. 3 shows thin layer chromatographic development assembly. Fig. 4 and fig. 5 are the developed chromatogram of Ketorolac tromethamine RS and test sample having same result as per the USP which conform tromethamine was present in reference standard as well as test sample.

CONCLUSION

A Simple and accurate TLC method has been developed for identification of tromethamine salt. The method was successfully produced yellow spots with violet to pink colour around it as per the tromethamine test in USP. Thus the proposed TLC method promotes high separation efficiency, shorter analysis time and lower amount of the mobile phase. It could also be extended to study the degradation study and analysis of drug from biological fluid.

Table 1: Tromethamine salt with their examples and uses

S. No.	Drugs	Pharmaceutical uses	Special comments on tromethamine salt
1	Dinoprost tromethamine(Prostaglandin F2a)[6]	-Oxytocic agent - Ocular hypertensive	-Tromethamine salt produces a crystalline material and obtained in high purity. -pH solubility study of this salt shows pH≥5 increase in solubility.
2	Carboprost tromethamine[7]	- Oxytocic agent - Hemorrhagic cystitis	-Tromethamine salt produces a crystalline material and obtained in high purity.
3	Prinomide tromethamine[19]	-Anti-inflammatory - Anti-arthritic	- It's crystalline tromethamine salt shows lower acute toxicity than its triethanolamine (trolamine) salt.
4	Lodoxamide trometamol[8]	- Anti-allergy drug -in preventing bronchoconstriction	-High solubility of di-tromethamine salt permits its administration by several routes.
5	Fosfomycin tromethamine[9]	- Antibiotic for urinary tract infections	- It's mono-tromethamine salt shows a six fold increase in rate and extent of absorption over its calcium salt.
6	Ketorolac tromethamine[10, 11]	- Non-steroidal anti-inflammatory	- Non-hygroscopic even at high relative humidity level - Increase dissolution rates and aqueous solubility
7	Tromethamol Glucaldrate	- Stomach antacid	-
8	Desglugastrin tromethamine	- Stimulator of gastric-acid secretion	-

Table 2: Calculation of RF values

S. No	Spots	Distance travelled by the solute from the origin line	Distance travelled by the solvent from the origin line	RF Values
1	Ketorolac tromethamine reference standard spot	3.1	4.9	0.63
2	Ketorolac tromethamine test Sample spot	2.9	4.8	0.60

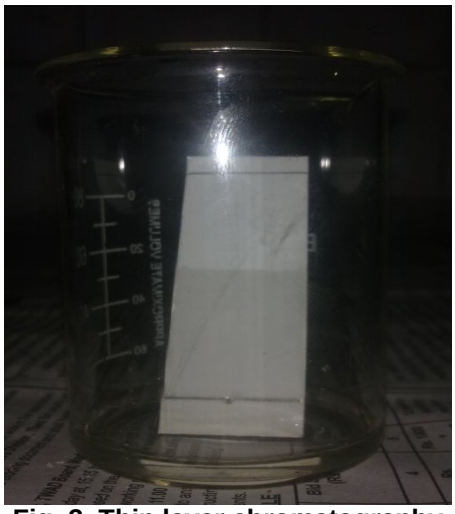

Fig. 3: Thin layer chromatography development chamber

Fig. 4: Developed TLC plate of Ketorolac tromethamine RS sample

Fig. 5: developed TLC plate of Ketorolac tromethamine test sample

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