Research Article

Facile synthesis of Salts of Valganciclovir using Organic acids

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Abstract

Nearly half of all active pharmaceutical ingredients are produced in their salt form. The pharmaceutical salt formation is a significant step in the drug development process. The preparation of novel maleate salt of antiviral valganciclovir offers the substantial improvement in its physicochemical properties. The process of selecting an ideal salt form of given active pharmaceutical ingredient that decides the fate of drug product in terms of efficiency and safety. The pharmaceutical salt synthesized was studied for its in-vitro anti-inflammatory activity by using HRBC membrane stabilization method.

Keywords: Valganciclovir, anti-inflammatory, pharmaceutical salts, polymorphs, XRD

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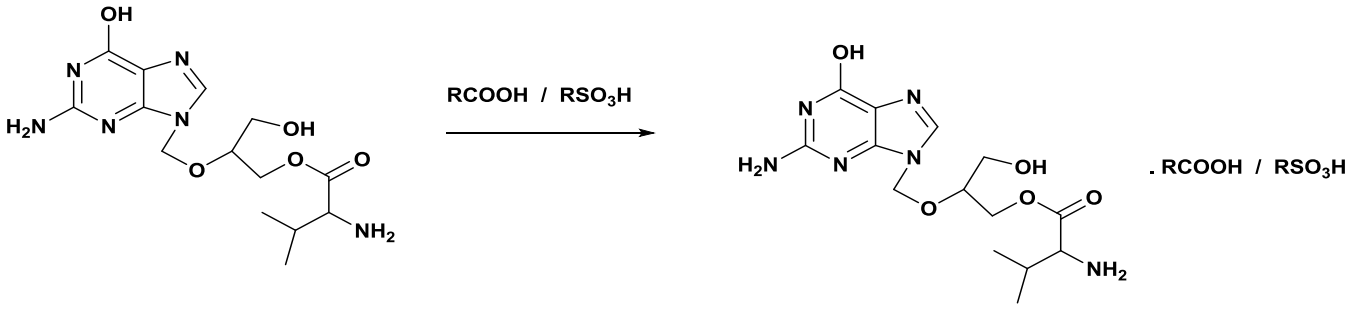
Introduction

Salt formation is a powerful pre-formation technique results in substantial enhancement of drug's physicochemical properties. It offers many advantages to the pharmaceutical products. Enhancement in characteristics of drug molecules like the solubility, dissolution rate, permeability and efficacy of the drug gives better results [1, 2]. Additionally, salts may assist in the improvement of the hydrolytic and thermal stability [3, 4]. It is also important in targeted drug delivery of dosage form [5].The selection of appropriate salt or polymorph as a key step showed distinctive properties of the parent drug. Generally, the salt- forming agents are selected by testing and experience according to the cost of raw materials, the simplicity of crystallization and the amount of yield produced.

Despite the enormous application of salts, a salt formation was associated with the number of adverse effects on the drug's properties and ultimately affects its performance in vivo. Firstly, as counter ions were therapeutically inactive, it leads to increase in total volume to achieve desirable effects, which intern resulted in the increase in the volumes for capsule loading and tablet compaction. It ultimately causes larger dosage units and consequently poses problems at administration [6]. Moreover, introducing counter ions to the drug structure resulted in increase in formation of hydrates and polymorphs which ultimately leads to increase in variability of the drug's pharmaceutical properties. The salts derived from strong acids and bases were generally highly hygroscopic which caused problems related to storage and packing and reduced the shelf life of end product [6].The preparation of antiviral drug valganciclovir and its pharmaceutically accepted salts is disclosed here, which comprises different chemical and physical operations involved in the synthesis of thereof [7-9]. The valganciclovir is used worldwide in the treatment of infections caused by cytomegalovirus and retro virus in AIDS patients [10]. The HRBC membrane stabilization method applied for the analysis in vitro anti-inflammatory activity of given drug molecule [11].



Valganciclovir (free base)

Valganciclovir salt

Where RCOOH , RSO_3H= Maleic acid, Oxalic acid, Benzene sulphonic acid methane sulphonic acid

Experimental

The process of salt formation involves the pairing of the parent drug molecule with a suitable counter ion. The essential prerequisite is the presence of ionisable functional groups in the drug's structure that allow sufficient ionic interaction between the drug and the salt former. The ionic intermolecular forces exist between charged groups in the parent drug and the counter ion. The proper thermodynamic and kinetic control conditions leads to precipitation of salt in the specific polymorphic form.

Procedure

Step I: Preparation of valganciclovir free base:
Charged crude Valganciclovir HCl (5mmol)to round bottom flask containing 6ml water stirred for 5-10 mins to insure complete dissolution ,pH of reaction mass made neutral ~7 by using organic base like triethylamine and extracted with 2X15ml of toluene for removal of water soluble impurities. Toluene layer was collectively dried by using sodium sulphate and evaporated under vacuum at 50-60°C. Yielded solid 88% is valganciclovir free base. Stored it in air tight container till used.

Example 1

Preparation of valganciclovir maleate salt (Crystalline Form):
Added 4ml water to 4.5mmol of prepared valganciclovir free base and stirred to get clear solution, 5.5mmol of maleic acid was added, stirred for 20-minutes at room temp. Reaction mass cooled to 10-15°C followed by slow addition of 30ml of acetone, after completion of addition, stirred for 1-hr at the same temp, centrifuge it under controlled conditions at 10-15°C. The isolated solid, washed with 5ml of acetone to yield a white solid. The product was dried under vacuum at 50-55°C. The obtained yield was 76%.

Preparation of valganciclovir maleate salt (Amorphous Form):
Dissolved 4.5mmol of prepared Valganciclovir free base in 5ml water, 5.5mmol of maleic acid was added, stirred for 20-minutes at room temperature.Reaction mass then transferred to vial and lyophilized. Initially reaction mass was allowed to cool slowly to avoid splashing of reaction mass to avoid loss in yield. After completion of scondary drying, the water content in given product was checked by using Karl Fischer reagent. If it was more than 1% then allowed secondary drying for further few hours.The obtained yield was 68%

Example 2

Preparation of valganciclovir oxalate salt:
Added 5ml methanol to 4.5mmol of prepared valganciclovir free base and stirred at 45-50°C , 0.0025mmol of oxalic acid added and stirring continued at the same temperature for 30-mins then cooled to room temperature. Finally 40ml of n-hexane or acetone added slowly, after completion of addition stirred it for further 1-hr at 10-15°C to insure complete precipitation. Filtered, washed with 5ml of n-hexane. It was dried under vacuum at 50-55°C. The obtained yield was 52%

Example 3:

Preparation of valganciclovir mesylate salt:
Added 5ml Dimethylsulphoxide to 4.5mmol of prepared valganciclovir free base and stirred, 5.5mmol of methanesulphonic acid added slowly. Finally 40ml of n-hexane or acetone added slowly, after completion of addition stirred it for further 1-hr at 10-15°C to insure complete precipitation, but which leads to formation of sticky solid.

Example 4

Preparation of valganciclovir besylate salt:
Added 7ml ethanol to 4.5mmol of prepared valganciclovir free base and 5.5 mmol of benzenesulphonic acid , stirred for 3-hrs at 30-40°C, cooled to 10-15°C and15ml methyl tertbutyl ether added slowly. Stirred it for 1-hr, Filtered. Solid obtained was highly hygroscopic.

Materials and methods

Assay determinations were carried out by titrimetric analysis, sometimes separation of salts done on the centrifuge. The crystallinity study was done by using XRD Wesley Tennyson. The physical and structural properties were studied by measuring melting point, IR (Nicolet). The thermal behavior analyzed by using Differential Scanning Calorimetry (DSC). All the solvents were purchased from Loba Chemicals (India) and were used without further purification.

Results and Discussion

Here we describe the study of preparation of different salts of valganciclovir using various acids in a number of reaction media at different physical parameter. It also deals with study of polymorphs of isolated salts. The method involves the conversion of previously isolated crude valganciclovir HCl into its corresponding free base, followed by extraction of valganciclovir free base in the organic solvent for removal of water soluble impurities. Organic layer collectively evaporated under vacuum to give free base. Then free base dissolved in minimum volume of water or organic solvent followed by addition of suitable mentioned acid to produce corresponding mixture of salt and its counter ion. The resulting solution after precipitation (using antisolvent) or lyophilisation (freez drying) produces corresponding salt. But this method is not applicable to all acids to get expected salt.

For given active pharmaceutical ingredient salts like maleate, oxalate, mesylate and besylate were prepared using above mentioned process.

Maleate salt of valganciclovir

The valganciclovir maleate salt was prepared by two different methods which gives different polymorphic forms of corresponding salts.

First method: It involves precipitation of salt at lower temperature using antisolvent and isolation of salt formed on centrifuge. This showed crystalline nature confirmed by XRD technique.

Lyophilization: Lyophilised valganciclovir maleate confirmed amorphous nature from its XRD.

Oxalate salt: Isolated as free solid having little hygroscopicity.

Mesylate salt: Unable to isolate as it results in sticky solid.

Besylate salt: Resulted solid was highly hygroscopic.

According to **Table 1,** different parameters of different salts studied for its characterization exhibited least hygroscopic. **Figures 1** indicated XRD for amorphous while and **Figures 2** showed crystalline form of Valganciclovir obtained by above process. Quantitative analysis of salts isolated was completed by using conventional titrimetric analysis.

The isolated valganciclovir maleate salt materialized to have advantageous properties like chemical purity, solubility, dissolution rate, morphology, and stability, such as thermal and mechanical stability, stability towards polymorphic conversion.

Table 1 Various parameters studied for different salts

Salt of Valganciclovir	Physical state	Assay	Solubility in water	Hygroscopicity
Maleate (Amorphous)	solid	95%	66mg/ml	Least hygroscopic
Maleate(Crystalline)	solid	98%	62mg/ml	Least hygroscopic
Oxalate	solid	80%	59 mg/ml	Least hygroscopic
Mesylate	Sticky mass	NA	NA	NA
Besylate	Solid(highly hygroscopic)	NA	NA	NA

The in vitro anti-inflammatory activity of valganciclovir maleate was observed by HRBC membrane stabilization method by captivating different concentrations. It is observed that the100 µg/ml concentration exhibited significant anti-inflammatory activity by showing 83.55% protection of HRBC in the hypotonic solution **(Table2).** The standard synthetic drug, Diclofenac was used for anti-inflammatory activity. The lysosomal enzymes produced during inflammation caused a variety of disorders. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilising the lysosomal membranes. The HRBC membranes are equivalent to lysosomal membrane components. The anti-inflammatory activity of drug molecule was measured as a consequence of the inhibition of hypotonicity induced HRBC membrane lysis. The valganciclovir maleate has shown membrane stabilization effect by inhibiting hypotonicity induced breaking of RBC membrane. The stabilization of membrane is nothing but the

stabilisation of lysosomal membrane, which plays a crucial role in controlling inflammatory response by preventing tissue inflammation and damage.

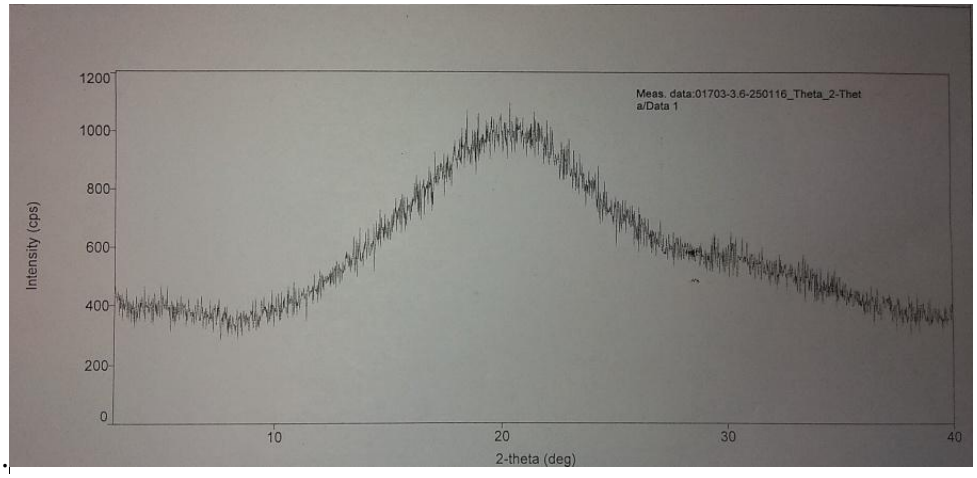


Figure 1 XRD of amorphous valganciclovir maleate

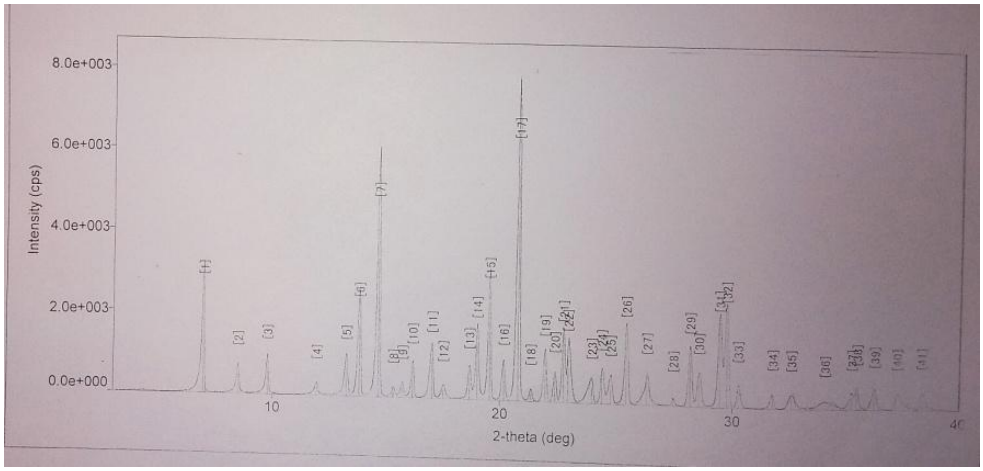


Figure 2 XRD of crystalline valganciclovir maleate

Table 2 Anti-inflammatory results % inhibition for Valganciclovir maleate

Concentration (µg/ml)	**Absorbance**	**% inhibition**
6.25	0.912	32.44
12.5	0.884	34.52
25	0.681	49.55
50	0.291	78.44
100	0.222	83.55
Positive control	1.350	

Conclusion

The prepared novel valganciclovir maleate acts as anti-inflammatory material with improved physiochemical properties such as more solubility, ease of handling, storage stability, and better purity. These physiochemical properties enhance performance characteristics of pharmaceutical product. In vitro anti-inflammatory study of valganciclovir maleate revealed that the activity of valganciclovir maleate was potent enough to show anti-inflammatory behaviour.

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