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Original Article

Comparative Permeation Evaluation of Some New Antiepileptic Drugs

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ABSTRACT

Epilepsy (from the Ancient Greek word (*epilēpsía*) — "seizure") is a common chronic neurological disorder characterized by seizures. The mainstay of treatment of epilepsy is anticonvulsant medications. Some of the new antiepileptic drugs are: Oxcarbazepine, Lamotrizine, Topiramate, Zonisamide. All these new anticonvulsants have shown promising results in the formulation of new dosage forms such as fast dissolving tablets or buccal tablets. For these formulations it becomes important to study their permeation across the buccal membrane and if inherently permeable drugs are chosen their formulations as fast dissolving tablets will be more successful. Since the present study deals with new antiepileptics a suitable spectrophotometric method of analyzing Lamotrigine and Oxcarbazepine was not found during the course of Literature survey, hence a new method was devised and later validated as per the ICH guidelines. However for Topiramate and Zonisamide method reported in the literature survey was followed and these four drug's permeation across bovine cheek mucosa using Franz diffusion cell was studied. Comparative Permeation evaluation of Drugs revealed that Lamotrigine and Oxcarbazepine showed permeation of 30% and 10% respectively which is much larger than that of Topiramate and Zonisamide (6% and 1.28% respectively). This enhanced permeability may be attributed to the slightly lipophilic character of both these drugs as compared to their counterparts and because of their higher permeability they are ideal candidates for the formulation of fast dissolving tablets thus improving their bioavailability.

Keywords: Epilepsy, Franz Diffussion cell, Mucosal membrane, Permeation.

INTRODUCTION

Epilepsy (from the Ancient Greek word (*epilēpsía*) — "seizure") is a common chronic neurological disorder characterized by seizures.[1] These seizures are transient signs and/or symptoms of abnormal, excessive or hypersynchronous neuronal activity in the brain.There has been a marked increase in the finding of the new antiepileptic drugs. Most of these drugs are used as mood stabilizing agents used to treat epilepsy and bipolar disorder (manic-depressive disorder) and in treatment of migraine, obesity and deaddiction[2].

Treatment of epilepsy requires a 100% patient compliance and entails very gradual increments or decrements in the dosages as a very small change in the dose brings about a major change in the drug effectiveness[3]. The mainstay of treatment of epilepsy is anticonvulsant medications. Often, anticonvulsant medication treatment will be life long and can have major effects on quality of life. Some of the new antiepileptic drugs are: Oxcarbazepine, Lamotrizine, Topiramate, Zonisamide .All these new anticonvulsants have shown promising results in the formulation of new dosage forms such as fast dissolving tablets or buccal tablets. For these formulations it becomes important to study their permeation across the buccal membrane and if inherently permeable drugs are chosen their formulations as fast dissolving tablets will be more successful.

Since the present study deals with new antiepileptics a suitable spectrophotometric method of analyzing Lamotrigine and Oxcarbazepine was not found during the course of Literature survey, hence a new method was devised for spectrophotometric analysis of these two drugs and later validated as per the ICH guidelines. However for Topiramate and Zonisamide method reported in the literature survey was followed .Therefore the aim of the present work is to study permeation of these four drugs across bovine cheek mucosa using Franz diffusion cell in order to find the most permeable drug amongst these four for the formulation of fast dissolving dosage forms.

MATERIALS AND METHODS

Materials

Oxcarbazepine and Lamotrigine were a gift sample from Jubiliant Organosis Ltd.(Noida, India). Topiramate and Zonisamide were supplied by Shree Ganesh Dye chem., Ahmedabad, Gujarat and BDR Lifesciences, Gujarat respectively. Acetonitrile A.R. grade was purchased from Shah Chemicals Ltd, .(Secunderabad, India). All other reagents used were of analytical reagent grade.

EXPERIMENTAL

Analytical Method for Lamotrigine

Lamotrigine **(FIGURE 1) is an a**nticonvulsant drug used in the treatment of epilepsy and bipolar disorder. It also acts as a mood stabilizer. It has a bioavailability of 98% and is absorbed 2-3 hours after oral administration[4]. The recommended initial dosing of Lamotrigine begins at less than 1 mg for epilepsy. Generally, the therapeutic range for epilepsy is 300 mg to 500 mg a day. Lamotrigine dosages are generally increased and decreased relatively gradually. A therapeutic response may require weeks or months of subsequent dose escalations, and very small differences in dosage often have noticeably different effects, much more so than with most other psychiatric medications; as little as 10% more or less may make a noticeable difference[5].

As evidenced a suitable spectrophotometric method of analysis of Lamotrigine was not found during the course

of literature survey[4,6] and hence a simple, sensitive, accurate and reproducible spectrophotometric method was devised for analysis of Lamotrigine in bulk and solid dosage forms[7].

Construction of Calibration curve for Lamotrigine.

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of Lamotrigine in 10 ml of AR grade acetonitrile and the volume was made up to100 ml with distilled water. The final concentration of this stock solution being 100µg/mg.

Preparation of standard solution

Stock solution samples were diluted with distilled water to prepare a series of concentration of 10 – 100µg/ml. The solutions were scanned and their absorbencies were measured at 304 nm using acetonitrile in distilled water as blank. All estimations were done in triplicate and the average values were reported.

Analytical Method for Oxcarbazepine

Oxcarbazepine **(FIGURE 2)** is an antiepileptic and mood stabilising drug, used primarily in the treatment of epilepsy and bipolar disorder. Oxcarbazepine is a structural derivative of Carbamazepine, adding extra oxygen to Dibenzepine ring. Oxcarbazepine is 10, 11 –Dihydro -10 – oxo - 5H - dibenz (b, f) azepine-5-carboxamide[8].

This difference helps reduce the impact on the liver of metabolizing the drug, and also prevents the serious forms of anemia occasionally associated with carbamazepine . Oxcarbazepine has recently been found associated with a greater enhancement in mood and reduction in anxiety symptoms than other drugs employed to treat epilepsy. All dosing should be given in a twice-a-day (BID) regimen[9].

Considering the biological importance of Oxcarbazepine and also the limitations associated with the methods reported in the literature survey ,an attempt was made here to develop a simple, rapid, economical and sensitive spectrophotometric method using analytical reagent grade acetonitrile in distilled water as a co solvent for its determination either in pure or in dosage form[7] .

Construction of Calibration curve for Oxcarbazepine

Preparation of standard stock solution

Standard stock solution was prepared by dissolving 10 mg of Oxcarbazepine in 20 ml of AR grade acetonitrile and the volume was made up to100 ml with distilled water. The final concentration of this stock solution being 100µg/ml.

Preparation of standard solution

Different aliquots were taken from stock solution and diluted with distilled water to prepare a series of concentration of 10 – 100µg/ml. The solutions were scanned on spectrophotometer and their absorbencies were measured at about 256nm using acetonitrile in distilled water as blank. The calibration curve was found to be linear in the range of 10 – 80µg/ml. All estimations were done in triplicate and the average values were reported.

This developed method was then validated as per the ICH guidelines on the basis of Linearity, Accuracy. Precision and Robustness.Accuracy was reported as percentage recovery of the drug and precision as percentage relative standard deviation.

Method for Topiramate

Topiramate, **(FIGURE 3)** a sulfamate-substituted monosaccharide (2,3:4,5-bis-O-(-1-methyl)-[beta]-Dfructopyranosesulfamate) is a new second

generation antiepileptic agent[10]. The drug is structurally different from other anticonvulsants and has been proved beneficial in partial and generalized tonic-clonic seizure[11]

Topiramate is an anticonvulsant drug used to treat epilepsy in both children and adults. In children It is also Food and Drug Administration (FDA) approved for, and now most frequently prescribed for, the prevention of migraines. In order to avoid early side-effects (e.g. cognitive dysfunction) the initial dose normally is low and increased in slow steps. The usual initial dose is 25 to 50mg daily in 2 single doses. Recommended increments are 25 to 50mg every 1 or 2 weeks. Common doses for maintenance treatment are 100 to 200mg daily. The highest dose possible is 1,000mg daily in divided doses.

For the estimation of Topiramate the method reported by Raval Kashyap[12] was followed. Estimation of Topiramate was carried out by the reaction of functional group present in it with suitable agent like ammonium molybdate to formed colored products which are determined calorimetrically.

Construction of Calibration curve for Topiramate

Preparation of standard stock solution

100mg of Topiramate was weighed accurately and transferred in to 100 ml volumetric flask, dissolved in water, completed to volume with the same solvent to obtain stock solution of 1000µg/ ml.

Preparation of standard solution

This stock solution was further diluted with water to obtain working solutions in the range of 0-50µg/ ml.

To 4 ml of the solutions containing Topiramate taken in a 10 ml volumetric flask, 1.5 ml of 5% ammonium molybdate and 2ml 2M HCL was added , the solutions were made up to 10ml with distilled water and kept in a water bath for 35 minutes for colour development. The absorbance was measured at different time intervals after dilution against a reagent blank at 750nm.

Method for Zonisamide

Zonisamide **(FIGURE 4)** is a sulfonamide anticonvulsant approved for use as an adjunctive therapy in adults[10]. Zonisamide is chemically 1, 2-benzisoxazole-3- methanesulfonamide. It is a potent sulphonamide agent having antiseizure activity[13]. The recommended starting dose for Zonisamide is 100mg, taken once daily for the first two weeks. Then the dose may be increased up to 400mg once daily.

For the estimation of Zonisamide the method reported by T.A. Dewan and B.A. Patel[14] was followed. The aim of their study was to develop a method for spectroscopic estimation of Zonisamide in bulk and pharmaceutical dosage forms.

Construction of Calibration curve for Zonisamide

Preparation of standard stock solution

Standard stock solution of Zonisamide was prepared by transferring 10mg of Zonisamide in 100 ml volumetric flask and dissolved in methanol, sonicated for 5 min and diluted up to the mark with methanol to get a stock solution containing 100µg/ml of Zonisamide.

Preparation of standard stock solution

Aliquots were diluted with distilled water to prepare a series of concentration of 0-50µg/ml and their absorbance were measured at 240nm. All estimations were done in triplicate and the average values were reported.

Comparative permeation evaluation of new antiepileptic drugs

All these being new drug entities, permeation rate study using Franz diffusion cell were carried out on them. A version of Franz diffusion cell **(FIGURE 5)** was used to study the permeation of drug from bovine mucosal membrane. A fabricated Franz diffusion cell with an internal diameter of 15mm and a diffusion area of 1.76cm^2 was used. The studies were performed using bovine cheek mucosa which is attached between the donor and the receiver compartment[15].

In Vitro Drug Permeation

The institutional animal ethical clearance was obtained from Malla Reddy College of Pharmacy, Secunderabad, Andhra Pradesh (Reg No. CPCSEA/MRCP/2008/ 1217), before conducting the studies.

For the permeation studies across bovine buccal mucosa, the animals were killed in the slaughterhouse, and the bovine buccal mucosa was surgically removed from the oral cavity. It was washed thoroughly and then dipped in 0.2 M ammonia solution. This treatment leads to the separation of buccal membrane from its underlying tissues. The buccal membrane (500–600µm thickness) so obtained was washed with Isotonic Phosphate Buffer (IPB) pH 7.4 and mounted at the junction between the two chambers of Franz diffusion cell with mucosal side upward[16]. The two chambers were then tied securely with the help of silica gum and rubber bands. A measured volume of IPB pH 7.4 was added to the lower chamber of the cell, such that there was no bubble between the membrane and the buffer. The assembly was placed on a magnetic stirrer. The temperature was maintained at 37±0.5°C by circulating water in the outer jacket of the cell with the help of peristaltic pump[17-19]. The buccal mucosa was allowed to stabilize for 24 hours by continuously replacing the buffer in the lower chamber with fresh buffer until no absorbance was obtained at λmax of the drug. This was done to allow the removal of soluble components in the membrane as they may interfere with the analysis of the drug. The upper side of the membrane was kept moistened by IPB pH 6.5. As in the buccal oral cavity, the pH varies from 5.5 to 6.8; therefore, an average of pH6.5 was used. *S*ince after permeation the drug is in the systemic circulation; therefore, to simulate it, a pH of 7.4 was used. Therefore during the permeation study, the upper compartment simulated the buccal cavity pH6.5, and the lower compartment simulated the physiological pH of 7.4 for blood. After stabilization, 100mg of the drug sample for each drug in 1 ml of acetonitrile and 1 ml of phosphate buffer of pH 6.5 was filled into the upper compartment.

The amount of drug permeated was determined by removing samples (2ml aliquots) from the lower chamber at time intervals of one hour. The samples were analyzed by UV-spectrophotometer (Shimadzu, Japan) at 256nm for oxcarbazepine and 304 nm for lamotrigine and 570nm for topiramate and 244.8 nm for zonisamide and percentage drug permeated was determined

RESULTS

The calibration curve for Oxcarbazepine was found to be linear in the range of 10-80mcg/ml with a correlation coefficient of 0.9995 (**TABLE I**) for Lamotrigine linear in the range of 20-100mcg/ml with a correlation coefficient of 0.9992 (**TABLE 2**) for Topiramate linear in the range of 10-50mcg/ml with a correlation coefficient of 0.9993 (**TABLE 3**) and for Zonisamide it was linear in the range of 10-40mcg/ml with a correlation coefficient of 0.9994. (**TABLE 4**)

The results of Accuracy analysis revealed that any small change in the drug

concentration in the solutions could be accurately determined by the proposed analytical methods. In the precision studies, Both in intraday as well as inter day evaluation indicated that the total experimental concentration values were found to be very close to the theoretical drug concentrations. The repeatability of the results indicated precision under the same operating conditions over a short interval of time and Intermediate precision expressed least within-laboratory variations on different days. From robustness, it can be concluded that there was no significant difference between the results from the two spectrophotometers and that the the developed analytical method for Lamotrigine and Oxcarbazepine holds true even with the change of instruments. Lamotrigine (**TABLE 5**), Oxcarbazepine (**TABLE 6**), Topiramate (**TABLE 7**) and Zonisamide (**TABLE 8**) showed permeation of 30% , 10% , 6% and 1.28% respectively .Thus the buccal permeation seen is in the order Lamotrigine > Oxcarbazepine > Topiramate > Zonisamide.(**GRAPH I**)

DISCUSSION

Comparative Permeation evaluation of Drugs revealed that Lamotrigine and Oxcarbazepine showed permeation of 30% and 10% respectively which is much larger than that of Topiramate and Zonisamide. (6% and 1.28% respectively). This enhanced permeability may be attributed to the slightly lipophilic character of both these drugs [19] as compared to their counterparts and because of their higher permeability they are ideal candidates for the formulation of fast dissolving tablets thus improving their bioavailability.

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Table 1. Summary of Calibration curve and validation parameters for Lamotrigine at 304nm

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PARAMETERS	VALUE
Absorption maximum (nm)	304
Beer's Law limit (mcg/ml)	20-100
Correlation coefficient	0.9992
Regression equation	Y=Ax-b
Slope (A)	0.0073
Intercept (b)	0.0081
Accuracy	100%
Precision	Interday 0.0154
	Intraday 0.0115
Robustness	Complies

Table 2. Summary of calibration curve and validation parameters for Oxcarbazepine at 256nm

PARAMETERS	VALUE
Absorption maximum (nm)	256
Beer's Law limit (mcg/ml)	10-80
Correlation coefficient	0.9995
Regression equation	Y=Ax-b
Slope (A)	0.0093
Intercept (b)	0.0064
Accuracy	98.33%
Precision	Interday 0.03
	Intraday 0.02
Robustness	Complies

Table 3. Calibration curve for Topiramate at 750nm

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Sr. No.	Concentration in µg/ml	Absorbance
1	0	0
2	10	0.136
3	20	0.282
4	30	0.418
5	40	0.549
6	50	0.672

Table 4. Calibration curve for Zonisamide at 240nm

Sr. No.	Concentration in µg/ml	Absorbance
1	0	0
2	10	0.193
3	20	0.413
4	30	0.595
5	40	0.808

Table 5. Percentage Drug permeated Lamotrigine

Time in Hrs	Absorbance			Mean absorbance at 304 nm	Conc in mcg/ml	% Drug permeated
	N=1	N=2	N=3			
1	16.75	16.76	16.76	16.76	2295	22.95 %
2	17.03	17.01	17.03	17.03	2332	23.32 %
3	23.98	23.99	23.97	23.98	3285	32.85 %
4	19.75	19.76	19.77	19.76	2706	27.06 %
5	26.57	26.58	26.58	26.58	3641	36.41 %

Table 6. Percentage Drug permeated Oxcarbazepine

Time in	Absorbance	Mean	Conc in	% Drug

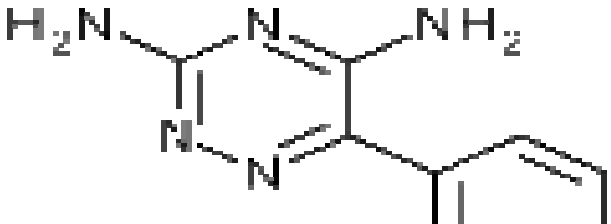
	N=1	N=2	N=3			
1	8.701	8.700	8.701	8.701	935	9.35 %
2	8.805	8.803	8.804	8.804	946	9.46 %
3	8.804	8.804	8.804	8.804	946	9.46 %
4	9.846	9.845	9.844	9.845	1058	10.58 %
5	9.845	9.844	9.846	9.845	1058	10.58 %

Table 7. Percentage Drug permeated Topiramate

Time in Hrs	Absorbance			Mean absorbance at 304 nm	Conc in mcg/ml	% Drug permeated
	N=1 n=1	N=1 n=2	N=1 n=3			
1	8.102	8.106	8.104	8.104	600	6.00 %
2	7.348	7.348	7.347	7.3486	544	5.44 %
3	8.104	8.104	8.104	8.104	600	6.00 %
4	8.55	8.84	8.86	8.550	633	6.33 %
5	7.644	7.645	7.646	7.645	566	5.66 %

Table 8. Percentage Drug Permeated Zonisamide

Time in Hrs	Absorbance			Mean absorbance at 304 nm	Conc in mcg/ml	% Drug permeated
	N=1 n =1	N=2 n =2	N=3 n=3			
1	2.583	2.583	2.582	2.583	128	1.28 %
2	2.663	2.664	2.664	2.664	132	1.32 %
3	2.482	2.482	2.482	2.482	123	1.23 %
4	3.270	3.271	3.270	3.270	162	1.62 %
5	2.583	2.582	2.583	2.583	128	1.28 %



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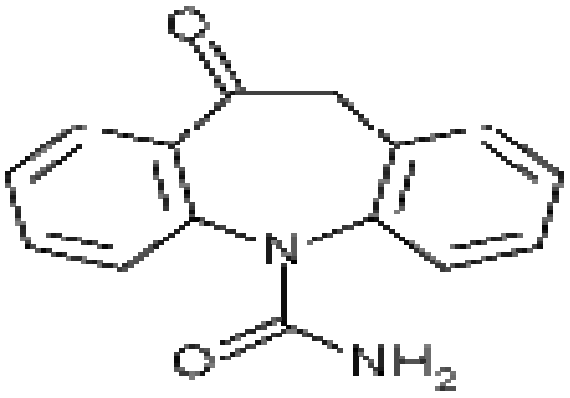
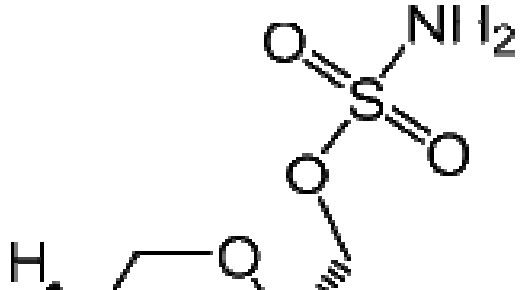


Figure 2. Oxcarbazepine Structural Formula



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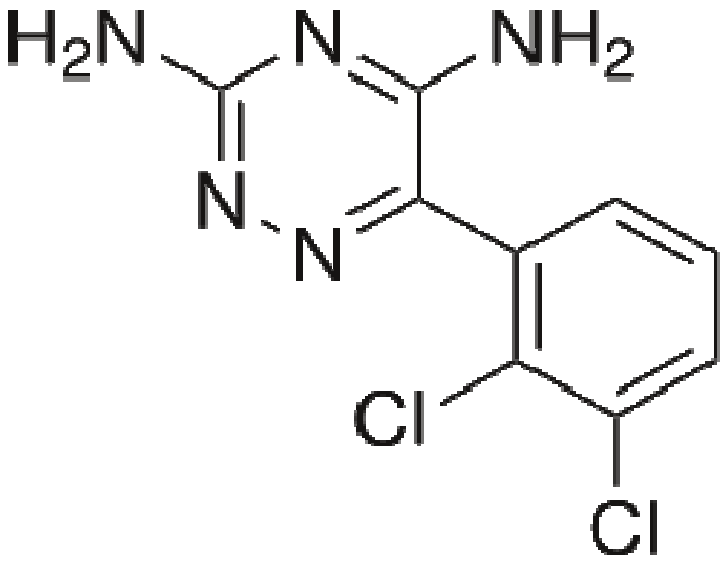
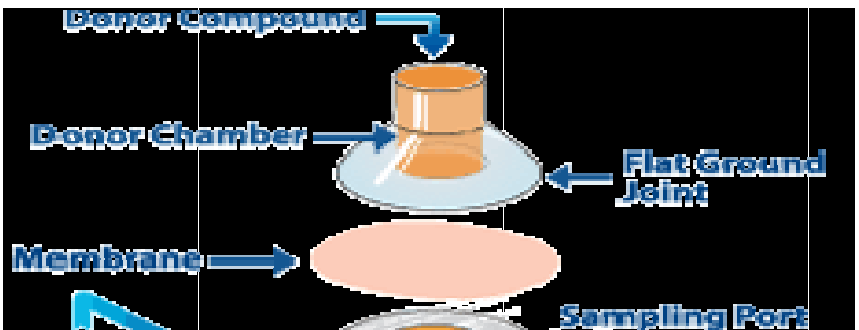


Figure 4. Zonisamide Structural Formula



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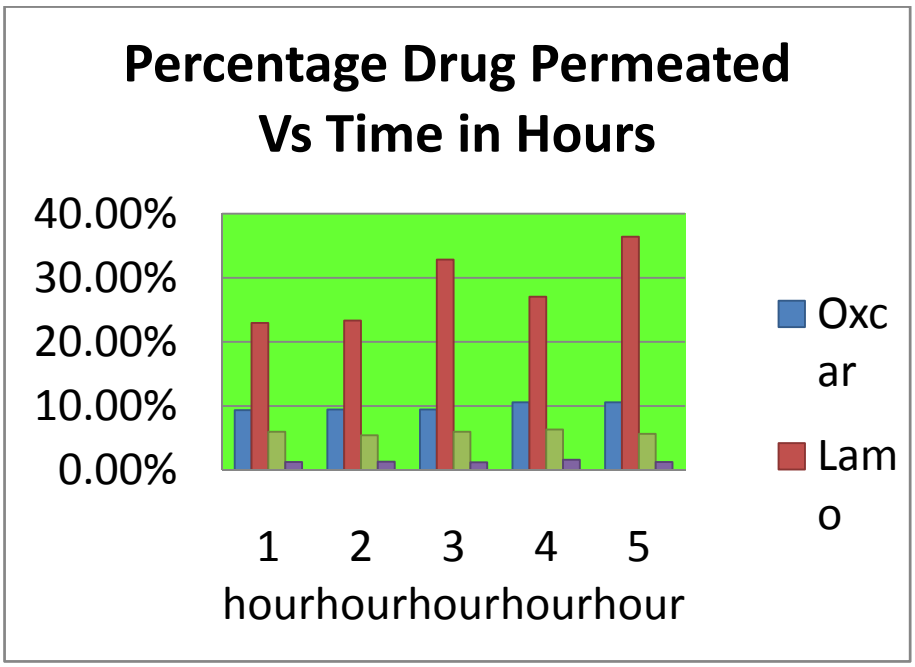

Graph 1. Comparative permeation evaluation of drugs