ความสัมพันธ์ระหว่างความเข้มข้นของยาโทพิราเมตในซีรัมและในน้ำลาย ของผู้ป่วยไทยโรคลมชัก

นางสาวจรีรัตน์ คงฤทธิ์

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RELATIONSHIP BETWEEN TOPIRAMATE CONCENTRATIONS IN SERUM AND SALIVA OF THAI EPILEPTIC PATIENTS

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อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. พ.ต.ท.หญิง ดร.สมทรง ลาวัณย์ประเสริฐ,
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การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความสัมพันธ์ระหว่างความเข้มข้นของยาโทพิราเมตในซีรัมและในน้ำลายของผู้ป่วยโรคลมชักชาวไทยและทดสอบสมการความสัมพันธ์โดยใช้กลุ่มตัวอย่างอีกกลุ่มหนึ่งที่ไม่เกี่ยวข้องกัน ทำการศึกษาในคลินิกผู้ป่วยนอกโรคลมชักแผนกประสาทวิทยาโรงพยาบาลพระมงกุฎเกล้าโดยคัดเลือกผู้ป่วยอายุระหว่าง 15-60 ปีที่ได้รับการรักษาด้วยยาโทพิราเมต ผู้ป่วยกลุ่มแรกได้รับยาโทพิราเมตเพื่อควบคุมอาการชักเพียงชนิดเดียว (จำนวน 10 คน) ใช้ในการสร้างสมการความสัมพันธ์โดยเก็บตัวอย่างเลือดและน้ำลายก่อนรับประทานยาและที่เวลา 1, 2, 4, 6 และ 8 ชั่วโมงหลังรับประทานยา ผู้ป่วยกลุ่มที่ 2 จำนวน 30 คนใช้ในการทดสอบสมการความสัมพันธ์ที่สร้างได้จากผู้ป่วยกลุ่มแรกโดยเก็บเลือดและน้ำลายก่อนรับประทานยาและที่เวลา 2 ชั่วโมงหลังรับประทานยา ทำการวิเคราะห์ความเข้มข้นของระดับยาโทพิราเมตในตัวอย่างซีรัมและน้ำลายด้วยเทคนิคเทอบิไดมีตริกอิมมูโนแอสเส ผลการศึกษาพบว่าระดับยาโทพิราเมต ในซีรัมและในน้ำลายมีความสัมพันธ์กันในเชิงเส้นตรง ($r = 0.919$, $p < 0.001$) โดยสมการความสัมพันธ์ $y = 0.962x + 1.197$ (ใช้ข้อมูลที่วิเคราะห์จากตัวอย่างที่เก็บทั้งก่อนรับประทานยาและที่เวลา1, 2, 4 , 6 และ 8 ชั่วโมงหลังรับประทาน) และเมื่อคำนวณระดับยาโทพิราเมตในซีรัมโดยการแทนค่าความเข้มข้นของยาในน้ำลายของผู้ป่วยจำนวน 30 คน (ไม่ว่าจะเก็บตัวอย่างที่เวลาก่อนหรือ 2 ชั่วโมงหลังรับประทานยาก็ตาม) ลงในสมการความสัมพันธ์พบว่าความเข้มข้นของยาโทพิราเมตที่คำนวณได้ไม่มีความแตกต่างจากความเข้มข้นของยาในซีรัมที่วัดได้จริงจากเครื่องมือ

ผลจากการศึกษานี้สนับสนุนการใช้ตัวอย่างน้ำลายแทนตัวอย่างซีรัมในการติดตามการใช้ยาโทพิราเมตทางคลินิก

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JAREERUT KONGRIT : RELATIONSHIP BETWEEN TOPIRAMATE CONCENTRATIONS IN SERUM AND SALIVA OF THAI EPILEPTIC PATIENTS. ADVISOR : ASSOC. PROF. POL.LT.COL. SOMSONG LAWANPRASERT, Ph.D., CO-ADVISOR : COL. YOTIN CHINVARUN, M.D., Ph.D., 126 pp.

The objective of this study was to determine the correlation between serum and saliva topiramate concentrations in Thai epileptic patients. The correlation equation was verified using saliva and serum topiramate concentrations of unrelated patients. The study was conducted at the Out-patient Epilepsy Clinic, Department of Medicine Neurology Unit, Pramongkutklao Hospital. Patient aged between 15-60 years and receiving topiramate were included into this study. The correlation equation between serum and saliva topiramate concentrations were constructed from 10 patients, receiving topiramate monotherapy. The blood and saliva samples were collected at the time before the morning dose and at 1, 2, 4, 6 and 8 hours after topiramate ingestion. Also, blood and saliva samples of 30 patients were collected at the time before the morning dose and at 2 hours after topiramate ingestion using to verified the correlation equation. Blood and saliva samples were measured by turbidimetric immunoassay technique.

Serum and saliva topiramate concentrations were linearly correlated with a correlation coefficient of 0.919 ($p < 0.001$), and the regression equation of this relationship was $y = 0.962x + 1.197$ (using all the data analyzed from samples collected at the time before and at 1, 2, 4, 6 and 8 hours after topiramate ingestion). Using the correlation equation, saliva topiramate concentrations of 30 patients were used to calculated serum topiramate concentrations. The result showed that there was no significant difference between calculated topiramate concentration and apparent topiramate concentration in serum. The results of this study support the use of saliva as an alternative to serum for monitoring topiramate therapy.

Department :... Pharmacology and Physiology... Student's Signature ……...……

Field of Study : ……Pharmacology…….. Advisor's Signature ……...……...

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CHAPTER I

INTRODUCTION

Epilepsies are among the most common serious neurological disorders worldwide. The incidence of epilepsy ranges from 40 to 70 per 100,000 in most developed counties and from 100 to 190 per 100,000 in developing counties (Sridharan, 2002). Epilepsy encompasses a number of different syndromes, the feature of which is a predisposition to recurrent unprovoked seizures. The clinical manifestations of seizures involve sudden and transitory abnormal symptoms including alternation of consciousness, motor, sensory, psychic or autonomic functions caused by disruption of the normal balance between excitation and inhibition in brain. Control of epilepsy is necessary for preventing acute physical harm and long term morbidity associated with recurrent seizures. Treatment of epilepsy consists of avoidance of potential seizures precipitant, pharmacological treatment and non- pharmacological treatment such as surgery. Overall, antiepileptic drugs are effective in 60-70% of individuals (Elger and Schmidt, 2008). Treatment with antiepileptic drugs does not cure, but only suppresses or prevents recurrence of seizure. Prior to 1993, the choice of anticonvulsant medications was limited to phenobarbital, primidone, phenytoin, carbamazepine and valproate. Besides these " traditional " anticonvulsants, new medications have been approved by the US Food and Drug Administration (FDA). The newer antiepileptic drugs offer the potential advantages of fewer drug interactions, unique mechanisms of action, and a broader spectrum of activity (Laroche and Helmers, 2004).

Treatment of epilepsy is one of the areas where therapeutic drug monitoring (TDM) has made the most significant contributions. The goal of TDM is to optimize patient's clinical outcome by managing their medication regimen with the assistance of measured drug concentrations. Determination of serum concentrations of the antiepileptic drugs, phenobarbital and phenytoin came into routine use soon after the development of sensitive and reliable analytical methods in 1960. Monitoring of antiepileptic drugs such as carbamazepine, valproate and ethosuximide has since then also become widely accepted in clinical practice (Tomson and Johannessen, 2000).

Topiramate is a new broad spectrum antiepileptic drug approved as adjunctive treatment in adults and children 2 years or older with partial seizures, primary generalized seizures and seizures associated with lennox-gastaut syndrome. Topiramate is rapidly absorbed from the

gastrointestinal tract with peak concentrations occurring approximately 2 hours after administration. It is minimally bound to serum proteins and is excreted primarily as unchanged drug while approximately 20 to 30% of a dose is metabolized. Sine topiramate is partly metabolized by the cytochrome P450 (CYP) enzyme system particularly CYP2C19 and glucuronidation reaction (Philip et al., 2002), drugs which induce its associated metabolic pathway are likely to increase the clearance of topiramate. Phenytoin and carbamazepine reduce the half-life of topiramate from 19-23 hours to 12-15 hours in adults, producing a 40 to 50% reduction in topiramate serum concentrations. Valproic acid has a more modest effect, reducing topiramate concentration by 10 to 15% (Buck, 2001). Since topiramate is excreted mostly (approximately 80%) unchanged in urine, dose of topiramate is needed to be adjusted in patients with renal deficiency. It was shown that clearance of topiramate decreased 42% in patients with moderate renal dysfunction (CrCl 30-69 ml/min) and decreased 54% in patients with severe renal dysfunction (CrCl < 30 ml/min) (William, 2000). In children, a significantly higher clearance of topiramate was shown as compared to adults. Topiramate clearance in pediatric patient is approximately 50% higher than that in adults. Steady-state plasma topiramate concentration for the same mg/kg was approximately 33% lower in pediatric patients than in adult patients (Rosenfeld et al., 1999).

Taken together, several factors can affect serum topiramate concentration such as age, concomitant enzyme inducing drugs, metabolic enzyme capacity as well as renal function status of the patients. These factors can cause marked variability in the correlation between topiramate dosage and topiramate serum concentration (Epstein, 1990; Froscher, 2005; Garnett, 2000; Glavser et al., 1999; Graham, 1982). Thus topiramate monitoring would be useful for optimizing the dosing regimen in individual patient besides assessing medication noncompliance, which is an important issue in patients with epilepsy (Langman, 2007).

There are several studies reported that saliva serves as an alternative medium to serum for monitoring of many conventional antiepileptic drugs such as phenobarbital, phenytoin, carbamazepine etc. (Patsalos, 2008). However, few data exist for newer antiepileptic drugs, such as lamotrigine, oxcarbazepine, levetiracetam, gabapentin and topiramate (Baumann, 2007). Regarding topiramate, there is one study examining the relationship between serum and saliva concentration using the specimens collected from 31 epileptic patients (Miles et al., 2003). In that study, topiramate concentrations were determined by fluorescence polarization immunoassay. A strong correlation exists between serum and saliva topiramate concentration, supporting the use of

saliva as an alternative to serum for monitoring topiramate therapy (Miles et al., 2003). Due to the limited data from only one study mostly using specimens of children in a western country treated with topiramate both monotherapy and co-therapy with other antiepileptic drugs, more additional study is encouraged. Thus, the objective of this study is to determine the correlation between serum and saliva topiramate concentrations in Thai adult epileptic patients. The regression equation was constructed from the data using serum and saliva specimens of Thai patients receiving topiramate monotherapy. The equation was then verified using the data analyzed from the specimens of unrelated Thai adult patients receiving topiramate either monotherapy or co-therapy. And, analysis of topiramate concentration was performed using turbidimetric immunoassay.

There are several advantages to perform drug monitoring using saliva as compared to using plasma or serum. Collection of saliva is a noninvasive procedure, does not require the expertise of a professional and risks associated with blood drawing are avoided. Subject's fear and discomfort are diminished and it is much easier to obtain multiple samples of saliva than of blood. Thus, in this regard, the result from this study that provide the information support the use of saliva as an alternative to serum for monitoring topiramate therapy particularly in Thai patients, will be great beneficial.

Hypothesis

Concentration of topiramate in serum and saliva of Thai epileptic patient is linearly correlated.

Objectives

The objectives of this study are to :

1. Determine the correlation between serum and saliva topiramate concentrations in Thai epileptic patients.
2. Verify the correlation equation between serum and saliva topiramate concentrations by using an unrelated group of patients.

Benefit gained from the study

Result from this study provides a relationship between topitamate concentration in serum and saliva samples of Thai epileptic patients. The findings would support the use of saliva as alternative to serum for monitoring topiramate therapy.

CHAPTER II

LITERATURE REVIEW

Topiramate

A. Chemical properties

Topiramate is a sulphamate-substituted monosaccharide with the chemical name of 2, 3 : 4, 5-bis-O-(1-methylethylidene)-β-D-fructopyranose sulphamate (Figure 1). It has a molecular weight of 339.37 and a pKa of 8.7 due to the weakly acidic sulfamate group. The water solubility of topiramate is comparatively high: 9.8 mg/ml at 23 °C (Eadie and Vajda, 1999; Shank et al., 2000).

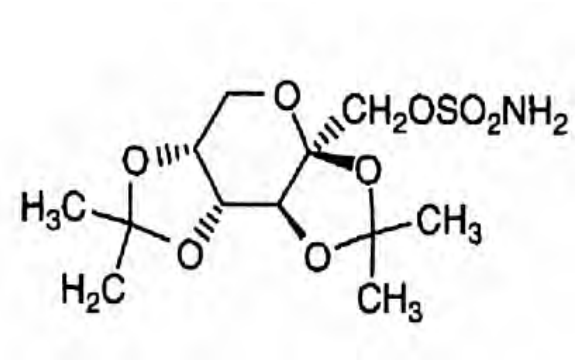

Figure 1 The chemical structure of topiramate (Shank et al., 2000)

B. Pharmacology

1. Mechanism

The mechanism of action of topiramate is unclear. There are three properties that may contribute to topiramate's antiepileptic efficacy. First, action potential elicited repetitively by a sustained depolarlization of the neurons is blocked by topiramate in a time-dependent manner, suggesting of a state-dependent sodium channel blocking action. Second, topiramate increases the frequency at which gamma-aminobutyric acid (GABA) activates GABA (subtype A) receptors, suggesting that topiramate potentiates the activity of this inhibitory neurotransmitter. Finally, topiramate antagonizes the ability of kainite to activate the kainite/alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (non-NMDA) of excitatory amino acid (glutamate) receptor,

but has no apparent effect on the activity of NMDA at the NMDA receptor subtype (Kwan, Sills, and Brodie, 2001; Neil et al., 2006).

Furthermore, topiramate weakly inhibits the type II and IV isoenzymes of carbonic anhydrase. It is assumed that the antiepileptic action of topiramate are independent of this effect (Eadie and Vajda, 1999).

2. Therapeutic use

Topiramate was approved for clinical use in 1996 for adjunctive treatment in adults and children 2 years or older with partial seizures, primary generalized seizures and seizures associated with lennox-gastaut syndrome.

Topiramate is administered orally. In adults and the elderly, the minimal effective dose is at least 100 mg/day but the maintenance dose in most patients is between 200 and 600 mg/day, taken in divided dose. Topiramate dosage should be titrated slowly, usually initiated at 25 mg/day in adults and 1 to 3 mg/kg/day in children. Dosage adjustments are made in 25 mg/week intervals up to 400 to 500 mg/day in adults and 6 to 9 mg/kg/day in children (Neil, 2006).

C. Pharmacokinetic

1. Absorption

Topiramate has rapid and nearly complete absorption, with peak plasma concentration is usually attained within 2-3 hours (Heaney and Shorvon,1999). Topiramate absorption is linear across a wide range of dose. Maximal plasma concentrations (Cmax, 1.73-28.7 μg/ml) were reached within 1.8-4.3 hours (Tmax), as demonstrated in a study in healthy volunteers receving a single dose of 100-1,200 mg of topiramate (Garnett, 2000).

2. Distribution and Protein binding

Topiramate rapidly distributes into all tissue sites and crosses the blood-brain and placental barriers with the drug's volume of distribution being between 0.6 and 0.8 l/kg. Topiramate is only minimally bound to plasma protein (9% to 17%). In a study of 14 adults epilepsy, simultaneous trough samples of venous blood and cerebral spinal fluid (CSF) were collected and analyzed. The median CSF/plasma ratio of total topiramate was 0.85, and the free topiramate concentrations in plasma and CSF were equivalent (Splinter, 2005).

There is a strong correlation between serum and saliva concentration (adjusted $r^2 = 0.97$) (Miles et al., 2003).

3. Metabolism

In patients receiving topiramate monotherapy, about 80% of an administered dose of topiramate is excreted unchanged in the urine. Topiramate is minimally hepatically metabolized (about 20%) to 6 inactive metabolites through glucuronidation, hydroxylation and hydrolysis (Garnett, 2000).

The metabolism of topiramate is induced by enzyme-inducing drugs, for example; phenytoin and carbamazepine induce the metabolism of topiramate and decrease concentrations by 48% and 40%, respectively. Valproaic acid causes a 14% decrease in topiramate concentration (Murphy, 2005). In vitro studies have shown that topiramate inhibits CYP2C19.

4. Elimination

Topiramate has a dual route of elimination, renal clearance predominates in the absence of enzyme induction. In the absence of hepatic enzyme induction, about 80% of an administered dose of topiramate is excreated unchanged in the urine (Johannessen, 1997). Renal clearance is 17-18 ml/min. In the absence of enzyme induction, mean plasma elimination half-life was 19-23 hours in normal volunteers. When topiramate was coadministered with enzyme-inducing antiepileptic drugs, plasma elimination half-life was reduced to 12-15 hours (Garnett, 2000).

Plasma clearance appeared to be ~50% higher in children compared to adult. Consequently, topiramate plasma concentration may be ~33% lower in children than in adults for the same g/kg topiramate dose. These findings are consistent with the pattern of age-related changes in relative clearance (Garnett, 2000; Glaver et al., 1999).

The clearance of topiramate is decreased by 42% in patients with moderate renal impairment (CrCl 30-69 ml/min) and by 54% in patients with severe impairment (CrCl < 30 ml/min). Consequently, half the usual topiramate dose should be used in patients with moderate or severe renal impairment. The topiramate dose in patients with renal impairment should be titrated according to individual patient response (Garnett, 2000; Splinter, 2005).

Hemodialysis increases the clearance of topiramate 4-6 fold. Because this high clearance rate can remove a clinically significant amount of topiramate from the systemic

circulation, a supplemental topiramate dose may be required in patients undergoing hemodialysis (Garnett, 2000).

D. Adverse effect (Neil, 2006)

1. Neurologic

The most commonly observed adverse events associated with the use of topiramate in clinical trials were related to the central nevous system and were observed in both epilepsy and migraine patients.

a) Impaired cognition

Cognitive-related adverse events in adults were mild to moderate in severity and usually occurred in isolation (13%). Rapid titration and higher initial dose were associated with higher incidences of these events. In children, the incidences of cognitive and neuropsychiatric adverse events were generally lower than in adults.

b) Somnolence, fatigue

Somnolence and fatigue were the adverse events most frequently (17%) reported during clinical trials for adjunctive epilepsy.

c) Speech and language disorder, regression

Language regression was reported in 3 pediatric patients between the ages of 5 to 17 years taking topiramate for epilepsy. Adjunctive topiramate was administered at dose ranging from 2.5 to 6 mg/kg/day. Language regression developed 4 to 28 weeks after drug initiation.

2. Endocrine/Metabolic

a) Body temperature above normal

Hyperthermia has been reported following the administration of topiramate. Patients receiving topiramate (especially children) should be monitored for decreased sweating and increased body temperature.

b) Metabolic acidosis

Topiramate has been associated with hyperchloremic, non-anion gap, metabolic acidosis (defined as decreased serum bicarbonate below the normal reference range in the absence of chronic respiratory alkalosis). The inhibitory effect of topiramate on carbonic anhydrase leads to renal bicarbonate loss.

c) Weight loss

Weight loss has occurred in up to 90% of patients treated with topiramate. This effect may be dose-related. A diuretic effect has been suggested to explain weight loss with topiramate.

3. Gastrointestinal

Gastrointestinal tract findings such as anorexia, nausea, vomiting, flatulence, gastroenteritis, taste perversion and constipation have been reported with topiramate use.

4. Psychiatric

a) Disturbance in mood

Depression of mood problems were dose-related for both the add-on epilepsy and migraine population

b) Psychotic disorder

In a retrospective chart review of 80 patients treated with topiramate, five patients were found with developed psychotic symptoms at 2 to 46 days after beginning therapy (Neil, 2006).

5. Renal

Renal stones or nephrolithiasis have been reported in approximately 2% of patients treated with topiramate, an effect attributed to carbonic anhydrase inhibition. It has occurred more commonly in men.

E. Topiramate therapeutic drug monitoring

1. Rationale for therapeutic drug monitoring in epilepsy

The goal of therapeutic drug monitoring is to optimize a patient's clinical outcome by managing their medication regimen with the assistance of measured drug concentrations. It may be useful to distinguish between disease-related and drug-related. Normally, measurement of drug plasma concentrations is more likely to be worthwhile in treatment of disorders where the clinical evidence of therapeutic or toxic effect is difficult to interpret and where therapeutic failures may have serious consequences. This is often the case in the treatment of epilepsy (Tomson and Johannessen, 2000).

A related problem in anticonvulsant therapy is the close relationship between concentrations that are therapeutic and those that produce side effects and toxicity. Although anticonvulsant concentrations cannot ensure that the patient is not experiencing side effects, the monitoring of concentrations can help the patient and clinician decide whether a specific system symptom is likely to be due to the medication. Moreover, it can help the clinician to adjust the medication to minimize the risk of adverse effects (Baumann, 2007).

Determination of serum concentration of the antiepileptic drugs phenobarbital and phenytoin come into routine use soon after the development of sensitive and reliable analytical methods in the 1960s. Monitoring of antiepileptic drugs such as carbamazepine, valproate and ethosuximide has since then also become widely accepted in clinical practice.

Topiramate is one of the new antiepileptic drugs, may be relevant to therapeutic drug monitoring because the proportion metabolized will increase in patients on inducing antiepileptic drug and decrease the serum levels of topiramate markedly (Britzi et al., 2005).

2. Factors associated with individual variation in therapeutic drug concentration of topiramate

a) Age

Topiramate plasma concentrations in children increased linearly and proportionally with dose. Plasma clearance appeared to be ~50% higher in children compared with adult historical data. Consequently, topiramate plasma concentrations may be ~33% lower in children than in adults for the same mg/kg topiramate dose (Garnett, 2000). In general, infants have the highest relative clearance (3-4 times greater than adult values); relative clearance declines with increasing age, until adult values are reached in late childhood or adolescence. These findings are consistent with the pattern of age-related chages in relative clearance. Therefore, children generally need higher mg/kg doses than adults to achieve the same topiramate plasma concentration (Glaver et al., 1999; Mikaeloff et al., 2004).

b) Impaired renal function

Decreased renal function can alter the pharmacokinetics of drugs that are renally eliminated. About 80% of an administered dose of topiramate is excreted unchanged in the urine. Topiramate clearance was reduced 42% in patients with moderate renal impairment and 54% in patients with severe renal impairment (Garnett, 2000). The topiramate dose in patients with renal impairment should be titrated according to individual patient response.

Topiramate plasma concentrations are significantly affected by hemodialysis. In patients with end-stage renal disease, the mean hemodialysis plasma clearance of topiramate was ~ 4-6 times greater than that for an individual with normal renal function (Garnett, 2000). This high clearance rate can remove a clinically significant amount of topiramate from the systemic circulation. The topiramate dosage adjustment should be based on duration of the dialysis period, the clearance rate of the dialysis system, and the patient's effective renal clearance of topiramate.

c) Coadministered drugs (Zanotta et al., 2006)

(a) Carbamazepine

Topiramate plasma concentration was reduced 40% with carbamazepine coadministration compared with plasma concentrations when topiramate was administered alone.

(b) Phenytoin

Clinical pharmacokinetic studies in some patients with epilepsy showed a 25% increase in the concentration of phenytoin when topiramate was added. Because phenytoin metabolism may have been saturated or nearly so in these patients, the modest degree of metabolic inhibition of CYP2C19 with the addition of topiramate may have been sufficient to increase phenytoin plasma concentrations.

When topiramate was given alone, the concentration of topiramate decreased by 48% when phenytoin was added. The enhanced clearance of topiramate is most likely a result of phenytoin-induced enzyme induction (Schadeo et al., 2002).

(c) Valproic acid

Concomitant therapy with valproic acid resulted in a 17% decrease in topiramate levels that is hypothesized to be due to valproic acid induced metabolic clearance of topiramate or reductions in topiramate absorption.

F. Salivary topiramate monitoring

1. The anatomy and physiology of saliva glands

a) Anatomy of saliva glands

Saliva is excreted by the major salivary glands, minor salivary glands and gingival crevices (Langman, 2007). The three pairs of major glands have distinct orifices; the ductus parotid is also called the Ductus of Sten son, while the ductus submandibularis and ductus sublingualis are called ductus Van Wharton and Bartholin, respectively (Figure 2).
(Aps and Martens, 2005).

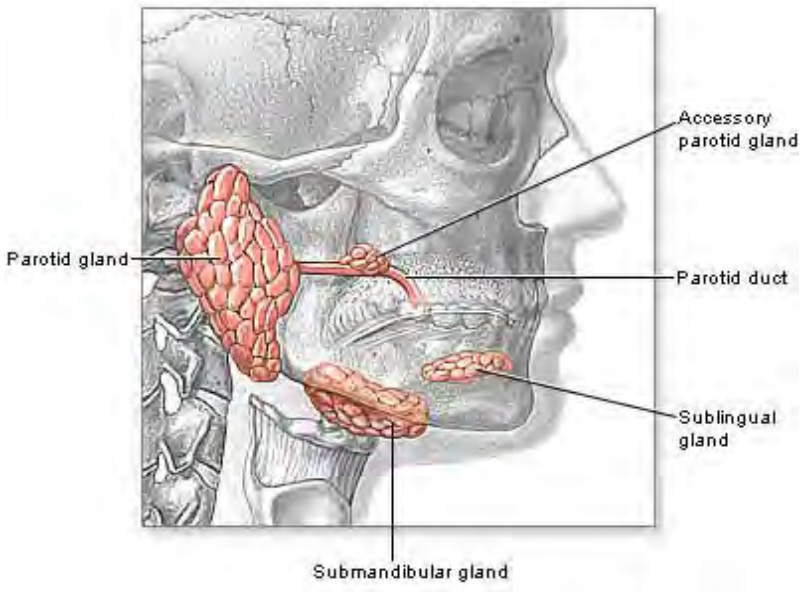

Figure 2 The anatomical positioning of salivary glands (http://www.cssforum.com.pk/)

Salivary glands have a high blood flow. The external carotid arteries enter the submandibular and sublingual glands along with the main ducts and nerves, thereby creating a hilum, although this hilum is not as clearly defined as in larger organs such as the kidney. Within the glands, the vessels follow the subdivision of the secretory duct tree so that each lobule has a distinct and separate blood supply. The direction of the blood flow is countercurrent to the direction of the salivary flow.

b) Composition of saliva (Hold et al., 2006)

Saliva, like other body fluids, is a dilute aqueous fluid containing both electrolytes and protein with an osmolality less than or equal to that of plasma. The total volume of saliva produced each day in adults is 500 to 1,500 ml. Mixed saliva, consists mainly of the secretions of submandibular (65%), parotid (23%) and sublingual (4%) glands, the remaining 8% being provided by the minor numerous glands. These proportions are a function of the type, intensity and duration of stimulation. The important stimulus for secretion is the presentation and ingestion of food. The quantity and quality of the secretion vary with the nature of nutrition. A comparision of the compositions of saliva and plasma is given in Table 6.

Table 1 Parametric compositions of saliva and plasma (Hold et al., 2006)

Parameters	Mixed Saliva	Plasma
Volume	500-1500 ml/day	4.3% of BW*
Rate of flow	0.6 (0.1-1.8) ml/min	
pH	6.7 (5.6-7.9)	7.4
Water (%)	98 (97-99.5)	91.5 (90-93)
Total protein (g/100 ml)	0.3 (0.15-0.64)	7.3 (6-8)
Albumin (g/100 ml)		4.5 (4-5)
Mucin (g/100 ml)	0.27 (0.08-0.6)	
Amino acids (mg/100 ml)	0.1-40	0.98
Electrolytes (mMol/l)		
Potassium	8-40	3.5-5.5
Sodium	5-100	135-155
Calcium	1.5-2	4.5-5.2
Phosphate	5.5-14	1.2-2.2
Chloride	5-70	100-106
Cholesterol (mg/100 ml)	7.5 (3-15)	150-300
Dry Substance (g/l)	6 (3.8)	80

*BW = Body Weight

Saliva contains the usual electrolytes of the body fluids, the principal ions being sodium, potassium, chloride and bicarbonate. They all originate from serum, from which they are actively transported into the acini and striated ducts (Figure 3)

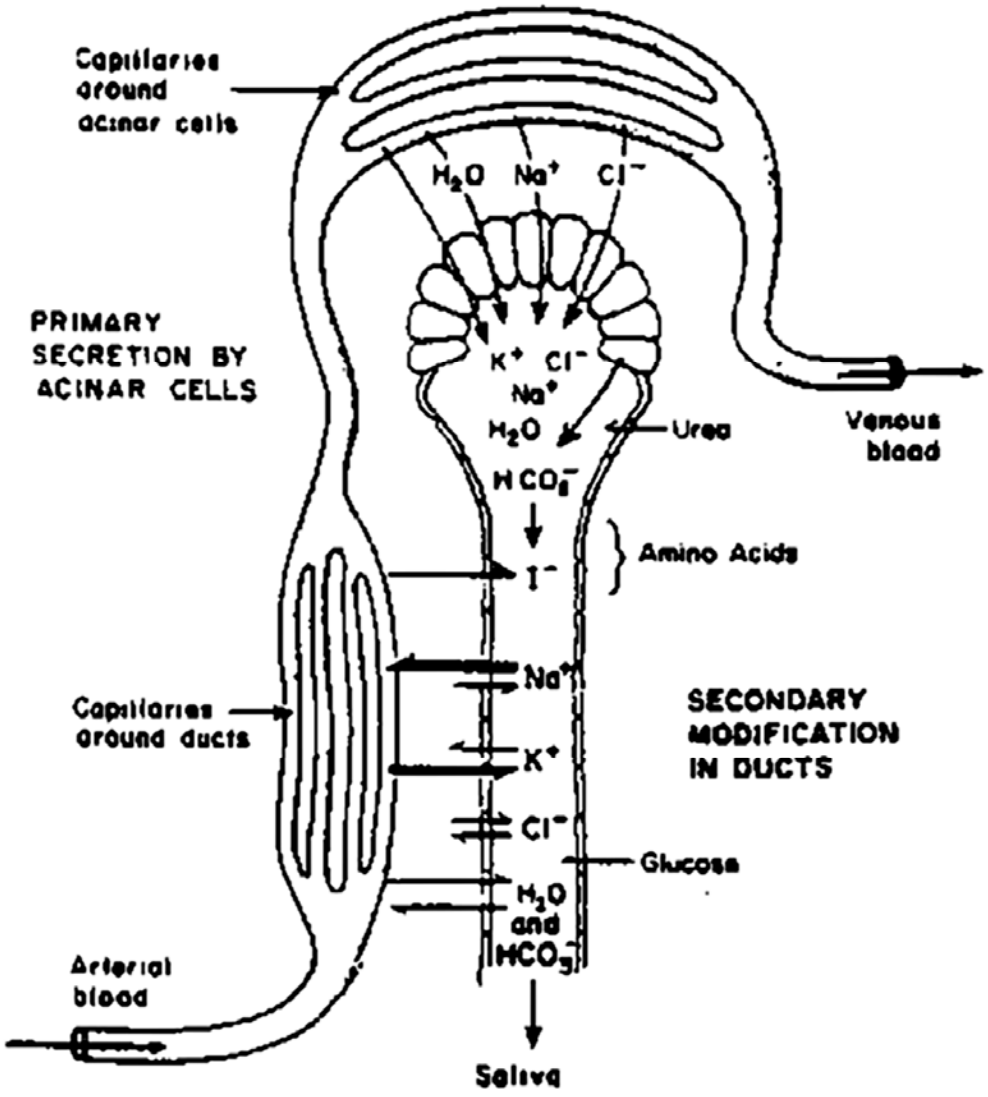

Figure 3 A scheme of electrolyte exchanges during secretion of saliva by parotid gland (Hold et al., 2006)

The organic salivary components, proteins and glycoproteins, are synthesized by the secretory cells. Some highly plasma protein molecule found in saliva, such as immunoglobulins A (MW = 160,000) cannot cross the plasma membrane absolutely, but they may be taken up into cells with membrane vesicle by endocytosis. In addition, plasma protein may also reach the saliva via a more pathological paracellular route (Almeida et al., 2008; Marcotte and Lavoie, 1998; Smith, 2004).

Normal salivary flow rates are in the region of 0.3 ml/min when unstimulated and 1.5-2.0 ml/min when stimulated. Secretion rate depends upon time of the day, the so-called circadian rhythm and the type of stimulation (Aps and Martens, 2005).

The pH value of saliva when flow is stimulated by chewing is variable – ranges from 6.5-7 up to 6.0 – 8.0 have been reported. Furthermore, the pH depends upon the rate of production, salivary pH increasing with increasing flow. The mean pH of saliva is usually accepted to be 6.5 (Graham, 1982).

2. Mechanisms of salivary drug transport (Hold et al., 2006; Aps and Martens, 2005).

Drugs which are not ionizable or are un-ionized within the salivary pH range are candidates for salivary therapeutic drug monitoring. Before any drug circulating in plasma can be discharged into the salivary duct, it must pass through the capillary wall, the basement membrane and the membrane of the glandular epithelial cells. Several mechanism can be involved in the passage of therapeutic substances through the epithelial membrane; a passive diffusion process, an active transport process and ultrafiltration through pores in the membrane.

Most drugs appear to enter saliva by the first mechanism mentioned, a simple passive diffusion process which is characterized by the transfer of drug molecules down a concentration gradient with no expenditure of energy. The rate of diffusion of a drug is a function of the concentration gradient, the surface area over which the transfer occurs, the thickness of the membrane, and diffusion constant that depends on the physico-chemical properties of each drug.

3. Factor affecting saliva drugs concentration (Jusko and Milsap, 1993)

The transfer of drugs from blood (plasma) into saliva depends upon the physicochemical properties of the compounds such as molecular size, lipid solubility, pKa and protein binding. The primary physiologic factors affecting salivary drugs distribution are salivary pH, salivary flow rate, and existing pathophysiology of the oral cavity.

a) Molecular size

Greater lipid solubility was likewise associated with faster transfer rate. Small molecular weight compounds (those with less than 0.4 nm molecular radius) diffuse or undergo ultrafiltration though water-filled "channels" or pores.

b) Lipid solubility

Lipid-soluble molecules pass through all cell membrane lipoidal barriers at rates roughly parallel to their oil/water partition coefficient.

c) pH and pKa

For lipid-soluble acidic or basic drugs, total saliva concentrations are dependent on the degree of ionization of the drug in plasma and saliva. Lipoidal biological membranes are not permeable to charged "ionized" molecules and drug permeability is governed by the pH – partition hypothesis. The degree of ionization of a drug is determined by its pKa and the pH of the solution.

d) Protein binding

The degree of drug protein binding is an important determinant of the availability of drug for diffusion. Drug equilibration between plasma and tissues is usually governed by the "free drug hypothesis". Drug concentrations in saliva usually reflect unbound drug in plasma.

e) Salivary flow rate and salivary clearance

The salivary glands can be considered as a clearance organ to which pharmacokinetic principles incorporating salivary flow rates would apply. As with other clearing organs, especially the kidney, membrane transfer is functionally occurring in both directions and the drug diffuses and equilibrates for most drugs. Salivary clearance may be regarded as the volume of plasma that is depleted of drug per unit of time by the salivary glands and has flow units.

4. Method of salivary drugs determination

a) Collection of saliva sample (Graham, 1982; Hold et al., 2006)

There are 4 methods for collection whole saliva : draining, spitting, suction and swabing (absorbent). Food and drug intake can affect the consistency and appearance of saliva for up to several hours and can contribute to gross contamination. Therefore, saliva samples should be collected at least 2 to 3 hours after taking medication, and individuals should be instructed to rinse their mount with deionised water prior to collection. Saliva should be collected for a period of 5 minutes and put into chilled tubes, kept on ice and frozen until ready for analysis.

Sample preparation and the sensitivity of the analytical method are important factors in the determination of the concentration of the anticonvulsant, or any drugs in saliva samples. The processing of saliva presents many difficulties. For instance, saliva contains particulate matter from oral mucus, mucopolysaccharides and mucoproteins which contribute to its highly mucus, stringy and sticky consistency. Therefore, saliva samples should be kept frozen until they are ready to be thawed and centrifuged prior to assay.

b) Analysis of topiramate in saliva sample (Burton et al., 2006; Bangs, 1996)

Spectrophotometry was initially used for the determination of antiepileptic agents. Chromatography soon followed, first thin-layer chromatography, then gas-liquid chromatography (GLC), and now high-performance liquid chromatography (HPLC).

Various immunoassays have been use for analysis of anticonvulsant drug concentration. These include enzyme immunoassay (EIA), fluorescence polarization immunoassay (FPIA), radioimmunoassay (RIA) and apoenzyme reactivation immunoassay system (ARIS).

Turbidimetric immunoassay is in common used and is read with clinical chemistry analyzers via spectrophotometry. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the topiramate antibody reagent. The topiramate-coated microparticle reagent is rapidly agglutinated in the presence of the anti-topiramate antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically. When a sample containing topiramate is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change.

c) Advantages of monitoring drug concentrations in saliva

(Langman, 2006)

Many drugs, including most anticonvulsants, can be monitored in a variety of body fluids, including blood, serum, plasma, saliva, etc.

Saliva monitoring for various drugs has generally been seen as being more patient-friendly and less expensive than using other body fluids. It lacks of pain, fear of needles, and potential complications of bleeding, infection, and skin discoloration associated with blood or plasma monitoring, which requires a venipuncture. In addition, obtaining blood usually requires the services of a skilled technician, which can add considerable cost. This cost not only includes the cost of technician's salary but also the time and transportation cost of going to the hospital or laboratory where the technician is stationed (Baumann, 2007).

The issue of transportation is especially important where caring for persons with epilepsy. Many adults with epilepsy cannot drive and thus have limited access to hospitals and laboratories. In disadvantaged populations, especially if rural, reliable transporting may not be available.

5. The correlation between serum and saliva topiramate concentration

A study of Mile et al. (2003) examined the relationship between serum and saliva topiramate concentration. Saliva and blood specimens were collected from 31 epileptic patients and topiramate concentration were determined by fluorescence polarization immunoassay. A strong correlation existed between serum and saliva topiramate concentration (adjusted $r^2 = 0.97$, n=30, $p < 0.0001$).

CHAPTER III

MATERIAL AND METHODS

Materials

A. Ethical Approval of Study protocol &

Research Subject Information Sheet (Appendix A)

B. Informed Consent (Appendix B)

C. Case Record From (Appendix C)

D. Education Leaflet (Appendix D)

E. Topiramate Concentration Record Form (Appendix E)

F. Reagents and Chemicals

1. QMS Topiramate Immunoassay Reagent, Thermo Fisher Scientific Inc., U.S.A (Appendix VI) (Lot number 902562, 902092)
2. QMS Topiramate Immunoassay Controls, Thermo Fisher Scientific Inc., U.S.A (Appendix VI) (Lot number 903011)
3. QMS Topiramate Immunoassay Calibrators, Thermo Fisher Scientific Inc., U.S.A (Appendix VI) (Lot number 901322)
4. Topiramate (Lot number 180K0906), Sigma-Aldrich, Co., Ltd., U.S.A

G. Instruments

1. Automated Clinical Chemistry Analyzer (MGC-240, U.S.A)
2. Centrifuge (Hermle Z383 K, U.S.A)
3. Microtube 1.5 ml
4. Disposable syringes 5, 10 ml (Terumo Corporation, Thailand)
5. Needle No.22 (Jelco, Medex Medical Ltd., Thailand)

Methods

A. Study Design

The study was designed to determine the correlation between serum and saliva topiramate concentration and to verify the correlation equation by using the other group of unrelated Thai epileptic patients. Patients who were using topiramate and had good drug compliance were recruited to the study. Saliva and blood samples were collected to determine topiramate concentrations using turbidimetric immunoassay. Then, the difference between the apparent serum topiramate concentrations and the corresponding calculated serum topiramate concentrations was assessed by statistical analysis.

B. Ethical approval

The study protocol was approved by the ethical committee on the protection of rights of human subjects of the Pramongkutklao Hospital (Approval # 1748/2551) (Appendix A).

C. Patients

Thai epileptic patients at the Out-patient Epilepsy Clinic, Department of Medicine Neurology Unit, Pramongkutklao Hospital, Bangkok were recruited. Patient's medical charts were reviewed and the patients were included into the study according to the following criteria.

Inclusion criteria

1. Patients aged between 15 to 60 years old.
2. Patients who suffered from convulsive disorder as diagnosed by physician and treated with topiramate.
3. Patients who continuously received topiramate for at least 1 month and had a good compliance before sample collection.
4. Patients who were not pregnant or lactating women.
5. Topiramate dosage was not changed before or between studying.
6. Patients without history of severe adverse drug reaction.

Exclusion criteria

1. Patients who had been diagnosed of renal or hepatic diseases by physician within 1 month before sample collection.

2. Patients who suffered from pathology of the salivary glands (eg. inflammatory, mumps) as diagnosed by physician.
3. Patients who receiving drug which affected saliva secretion such as anticholinergic drugs, antidepressants drugs before sample collection.
4. Patients who had severe adverse drug reaction and/or other abnormal symptoms during study.

D. Number of patients

Patients sample size was calculated as follow :

$$N = \left(\frac{Z_{\alpha/2} + Z_{\beta}\sqrt{1- r^2}}{r} \right)^2 + 2$$

Where N = number of patients, Z_{α} = Z – value at specific α error, Z_{β} = Z – value at specific β error, r = correlation coefficient from previous study.

The value of "r" used in this study was taken from the correlation coefficient between serum and saliva topiramate concentration, 0.985 reported by Miles et al. (2003).

Assume that $\alpha = 0.05$, $\beta = 0.10$, $Z_{\alpha/2} = 1.96$, $Z_{\beta} = 1.282$

Therefore, $$N = \left(\frac{1.96 + 1.282\sqrt{1- (0.985)^2}}{0.985} \right)^2 + 2$$

$$= \left(\frac{1.96 + 0.220}{0.985} \right)^2 + 2$$

$$= 6.90$$

The lowest number of patients was 7. Therefore, number of patients designed to be included into the study was 10.

E. Procedures

Thai epileptic patients were divided into two groups.

Group I

Ten Thai epileptic patients with topiramate monotherapy were recruited into the study to assess the correlation between serum and saliva topiramate concentrations. Serum and saliva samples of each patient were collected at the time before topiramate ingestion and at 1, 2, 4, 6, and 8 hours after topiramate ingestion.

Group II

Thirty Thai epileptic patients with topiramate either monotherapy or co-therapy with other antiepileptic drugs, were recruited into the study to verify the correlation equation between serum and saliva topiramate concentration obtained from patients in group I. Serum and saliva samples of each patient were collected at the time before topiramate ingestion and at 2 hours after topiramate ingestion.

Each of the recruited patients was followed up for 2 visits and the following activities were performed in individual patient.

1. The first visit (screening)

1.1 Patient's medical charts were reviewed. Patients, age between 15 to 60 years and treated with topiramate, were selected.

1.2 Detail of the study was explained and the research subject information sheet (Appendix A) was provided to the selected patients and their caregivers after they met the physician.

1.3 Patients who were interested in study participation were interviewed. Demographic and pharmacotherapeutic data of individual patient were recorded in the case record form (Appendix C).

1.4 Patients were asked to sign informed consents (Appendix B) if they decided to be recruited into the study.

2. The second visit

Saliva and blood samples of patients were collected as following.

Group I : Blood and saliva samples were collected at the time before topiramate morning ingestion dose and at 1, 2, 4, 6 and 8 hours after topiramate ingestion.

Group II : Blood and saliva samples were collected at the time before topiramate morning ingestion dose and at 2 hours after topiramate ingestion.

Blood samples

Group I

1. Blood samples were collected by venipuncture under aseptic technique by nurse.

2. Three to five millitres of blood were draw at the time before topiramate morning dose ingestion and at 1, 2, 4, 6 and 8 hours after topiramate ingestion.

Group II

1. Blood samples were collected by venipuncture under aseptic technique by nurse.

2. Three to five millilitres of blood were draw at time before topiramate morning dose ingestion and at 2 hours after topiramate ingestion.

At the first collection, each blood sample was divided into 2 portions. One portion was used for clinical blood chemistry analysis, such as blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and complete blood counts. These parameters were analyzer by Professional Laboratory Management Co. Ltd. The other portion of blood sample was used for topiramate analysis.

The blood samples were centrifuged at 3,000 *g* for 10 minutes at room temperature and clear supernatants were stored at -80 ^{o}C until analysis.

Saliva samples

Group I

1. Before saliva sampling, patients were asked to rinse their mouths with plain water to reduce potential contamination with food particles.

2. Patients were asked to spit 0.5-2 ml of saliva into a plastic box at the time before topiramate morning dose ingestion and at 1, 2, 4, 6 and 8 hours after topiramate ingestion.

Group II

1. Before saliva collection, patients were asked to rinse their mouths with plain water to reduce potential contamination with food particles.

2. Patients were asked to spit 0.5-2 ml of saliva into a plastic box at the time before topiramate morning dose ingestion and at 2 hours after topiramate ingestion.

Saliva samples were centrifuged at 3,000 *g* for 10 minutes at room temperature and clear supernatants were stored at -80 ^{o}C until analysis.

F. Validation of the assay procedure for topiramate concentration in serum and saliva samples

1. Analytical method

Topiramate concentration in serum and saliva samples were determined by turbidimetric immunoassay using automated clinical chemistry analyzer.

2. Validation of turbidimetric immunoassay technique for determination of serum topiramate concentration

Validation of the procedure including specificity, sensitivity, precision, and accuracy was reported by the manufacturer, Thermo Fisher Scientific (Appendix F). In addition, verification of the topiramate calibration curve was required each time of the experiment using the topiramate controls. The assay control values should be within the concentration ranges specified in the package insert of topiramate reagent kit. The control topiramate concentration range was shown in Table 2.

Table 2 The control topiramate concentration range

No of topiramate Control	Concentration of topiramate (µg/ml)	Acceptable range of the measured level (µg/ml)
1	2.92	2.33-3.50
2	10.32	8.26-12.39
3	24.99	19.99-29.98

Topiramate controls must be assayed along with the unknown sample on each day of the assay. Recalibration was necessary when controls fell outside the acceptable range or when a new lot number of topiramate reagent was used.

3. Validation of the assay procedure for determination of saliva topiramate concentration

a) Linearity

Stock solution of topiramate was prepared by dissolving 20 mg of topiramate in 10 ml of distilled water to yied the concentration of 2 mg/ml. One millilitre of this stock solution was added to a 10 ml volumetric flask and the volume was adjusted with distilled water to obtain the final stock concentration of 200 µg/ml.

Ten, 20, 40, 80 and 160 microlitres of final stock solution were added to 1.5 ml microtube and the volume was adjusted with saliva obtained from healthy volunteers to achieve the final concentration of 2, 4, 8, 16, and 32 µg/ml. These solutions were ready to be analyzed by automated clinical chemistry analyzer for five time. Linear regression and coefficient of determination (R^2) between apparent final topiramate concentration and measured topiramate concentration were analyzed.

b) Accuracy

Accuracy of the assay procedure was determined by the percentage of recovery which was evaluated by comparing topiramate concentration between the measured concentration and the target concentration of five different concentration of 2, 4, 8, 16 and 32 µg/ml (use data from linearity assay). The percentage of recovery was calculated by the following equation:

$$\% \text{ Recovery} = \frac{\text{Measured topiramae concentration}}{\text{Target topiramate concentration}} \times 100$$

c) Precision

Precision of the assay was evaluated as within day and between day precision. Precision of the assay procedure was assessed from the percentage of coefficient of variation (CV) of each concentration which was calculated as follow :

$$\% \text{ CV} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

Within day precision

Topiramate concentration of 2, 4, 8, 16 and 32 µg/ml from linearity assay was analyzed for five time within 24 hours for within day precision assessment.

Between day precision

Topiramate concentration of 8 µg/ml was analyzed for 4 days (three time in each day) for between day precision assessment.

d) Stability of topiramate in saliva

Ten micrograms/milliliters (µg/ml) of topiramate in saliva was prepared from final stock solution with saliva of healthy volunteers. Saliva topiramate at the concentration of 10 µg/ml was analyzed immediately after preparation, while the remaining were analyzed at 1, 2, 3 and 6 days following keeping at 2-8^{o}C. Stability of topiramate in saliva was expressed as percentage of topiramate concentration remaining at each day in relation to the concentration of the fresh preparation.

G. Serum and saliva topiramate concentration analysis

Serum and saliva samples were analyzed for topiramate concentration using automated clinical chemistry analyzer at Laboratory unit of Bangsai hospital, Ayutthaya. Assay of topiramate in both serum and saliva sample was performed according to the method recommended by Thermo Fisher Scientific. Details of the assay method and its validation were showed in (Appendix F).

The correlation between serum and saliva topiramate concentrations were assessed by linear regression and correlation analysis using data of serum and saliva topiramate concentration of patients in group I. The obtained correlation equation from the linear regression was used for determining the calculated serum topiramate concentrations from the saliva topiramate concentrations of patients in group II. Difference between calculated serum topiramate concentration and apparent serum topiramate concentration was statistically analyzed.

H. Data analysis

1. Demographic and clinical data were described by descriptive statistics. All of the data were presented as mean ± standard deviation (SD).

2. The correlation between serum and saliva topitamate concentrations were assessed by simple linear regression and correlation analysis. The correlation was tested by Pearson's correlation test with $p < 0.05$.

3. Mean difference between apparent serum topiramate concentration and calculated serum topiramate concentration was analyzed by paired t-test. The difference was considered to be statistically significant at $p < 0.05$.

CHAPTER IV

RESULTS

Demographic data

Summary of the demographic characteristic of all patients used in this study were presented in Table 3. Details of the individual patient were shown in Table 18 (Appendix G).

The average age and weight of patients were 38.03 ± 11.02 years old and 56.35 ± 7.21 kg, respectively. Sixteen patients (40%) of all participated patients were male and 24 patients (60%) were female. Two patients (5%) had relatives with family history of seizure.

The average dose of topiramate prescribed for recruited patients were 136.25 ± 82.03 mg/day.

All serum laboratory values were within normal limits. The average serum albumin, AST, ALT, BUN and Scr values were 4.49 ± 0.33 g/dL, 23.45 ± 5.46 U/L, 19.80 ± 8.15 U/L, 13.08 ± 3.38 mg/dl, 0.88 ± 0.22 mg/dl, respectively. The average WBC, RBC, hemoglobin, hematocrit, and platelet values were 5801.82 ± 1518.35 cells/cumm, 4.43 ± 0.50 million cells/cumm, 13.17 ± 1.26 g/dl, 40.52 ± 3.30% and 233424.24 ± 62231.94 cells/cumm, respectively (Table 3).

Table 3 Summary of demographic characteristics of patients

Characteristics of 40 patients	mean ± SD (range or percent)
Age, years old (range)	38.03 ± 11.02 (17-56)
Weight, kg (range)	56.35 ± 7.21 (45-70)
Male/Female, n (%)	16/24 (40/60)
Family history of seizure, n (%)	2 (5)
Dose, mg/day (range)	136.2 5± 82.03 (25-400)
Antiepileptic drugs combination, n (%)	
Carbamazepine	14 (35)
Valproate	13 (32.5)
Phenytoin	4 (10)
Oxcarbazepine	7 (17.5)
Levetiracetam	6 (15)
Serum laboratory values (range)	
Alb, g/dl	4.49 ± 0.33 (3.8-5.3)
AST, U/L	23.45 ± 5.46 (16.0-20.0)
ALT, U/L	19.80 ± 8.15 (8.0-41.0)
BUN, mg/dl	13.08 ± 3.38 (7.0-20.0)
Scr, mg/dl	0.88 ± 0.22 (0.5-1.4)
WBC, cells/cumm	5801.82 ± 1518.35 (2,810-10,580)
RBC, million cells/cumm	4.43 ± 0.50 (3.65-5.74)
Hb, g/dl	13.17 ± 1.26 (10.7-16.2)
Hct, %	40.52 ± 3.30 (34.0-47.0)
Plt, cells/cumm	233424.24 ± 62231.94 (88,000-368,000)

Alb = serum albumin, ALT= Alanine aminotransferase, AST = Aspartate aminotransferase, BUN = blood urea nitrogen, Scr = serum creatinine, WBC = white blood cell, RBC = red blood cell, Hb = hemoglobin, Hct = hematocrit, Plt = platelet

Validation of the assay procedures

Validation of the analytical method for serum topiramate was reported by the company which supplied the reagent kit (Appendix F) .

Validation of the assay procedure for saliva samples was classified into these following topics:

A. Linearity

Linearity was determined using 5 different saliva topiramate concentrations (2, 4, 8, 16 and 32 μg/ml). Each saliva topiramate concentration was analyzed for 5 times. The linear regression equation and coefficients of determination of the linear regression line between target topiramate concentration and the measured topiramate concentrations were $y = 1.01x + 0.186$ and $R^2 = 0.998$, respectively (Figure 4).

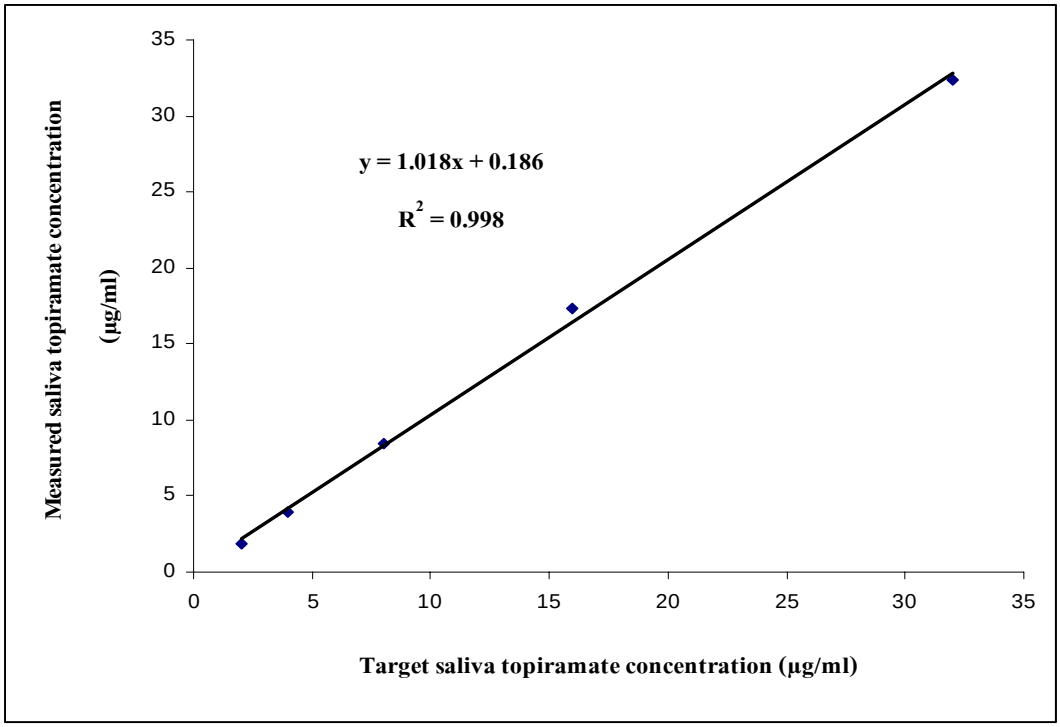

Figure 4 Linear regression line between target saliva topiramate concentration and measured saliva topiramate concentration (Each point represented mean of n = 5)

B. Accuracy

Accuracy of the assay procedure was assessed by the percentage of recovery. Recovery was determined by assaying 5 different saliva topiramate concentrations (2, 4, 8, 16 and 32 µg/ml) (Each concentration was analyzed for 5 times). Use the data from linearity assay, the mean percentage recovery of topiramate in the study was shown in Table 4.

C. Precision

Percentages of coefficient of variation (% CV) of the within day and between day precision assays were shown in Table 5 and Table 6, respectively.

Within day precision was performed using saliva topiramate concentrations of 2, 4, 8, 16 and 32 µg/ml and the % CV was shown to be range from 0.86 to 4.25 (Table 5). Between day precission was performed using saliva topiramate concentration of 8 µg/ml and the % CV was shown to be 0.81(Table 6).

Table 4 Accuracy of the procedure for determining saliva topiramate concentrations

Target saliva topiramate concentrations (μg/ml)	measured saliva topiramate concentrations (μg/ml)	% recovery
2	1.88 ± 0.08	93.8 ± 3.91
4	3.97 ± 0.12	99.3 ± 3.06
8	8.48 ± 0.11	106.02 ± 1.45
16	17.39 ± 0.15	108.69 ± 0.92
32	32.32 ± 0.37	100.95 ± 1.21

The data showed were mean ± SD of n = 5

Table 5 Within day precision of the procedure for determining saliva topiramate concentration

No of the assay	Saliva topiramate concentration (μg/ml)				
	2	4	8	16	32
1	1.80	3.87	8.68	17.50	31.99
2	1.97	3.94	8.45	17.59	32.62
3	1.87	4.08	8.43	17.32	32.17
4	1.80	3.85	8.38	17.29	32.03
5	1.94	4.12	8.47	17.25	32.80
Mean	1.88	3.97	8.48	17.39	32.32
SD	0.08	0.12	0.11	0.15	0.37
Within day precision: % CV	4.25	3.02	1.30	0.86	1.14

Table 6 Between day precision of the procedure for determining saliva topiramate concentration

Precision assay using saliva topiramate conc. of 8 µg/ml No of the assay	Day 1	Day 2	Day 3	Day 4
1	8.68	8.75	8.57	8.52
2	8.45	8.70	8.79	8.76
3	8.43	8.55	8.60	8.53
Mean	8.61			
SD	0.07			
Between day precision: % CV	0.81			

D. Stability of topramate in saliva

Stability of topiramate in saliva samples was determined by keeping saliva samples at 2 - 8 °C for 1, 2, 3 and 6 days. Saliva topiramate concentration of 10 µg/ml was use in the stability test. Percentage of topiramate concentration in saliva was shown to be range between 98.34% to 106.36% as compared to that of the concentration at the first day of preparation (day 0) (Table 7).

Table 7 Stability of topiramate in saliva kept at 2 - 8 °C at various days after preparation

Day	Percent of topiramate concentration
0	100
1	104.30
2	106.36
3	105.09
6	98.34

The relationship between serum and saliva topiramate concentrations

A. The correlation between serum and saliva topiramate concentrations

Serum and saliva topiramate concentrations of patients in group I were shown to be linearly correlated as using the samples collected before topiramate ingestion (0 hour) and at 1, 2, 4, 6 and 8 hours after topiramate ingestion. The correlation equation between serum and saliva topiramate concentrations was y = 0.962x + 1.197 with a correlation coefficient (r) of 0.919, $p < 0.001$ (Figure 5, Table 5A).

The relationships between serum and saliva topiramate concentrations at each time point of sample collection (0, 1, 2, 4, 6 or 8 hours after topiramate ingestion) were also linearly correlated with the correlation equations and correlation coefficient (r) of y = 0.888x + 1.156, r = 0.992; y = 1.011x + 0.967, r = 0.929; y = 1.049x + 1.562, r = 0.873; y = 1.115x + 1.060, r = 0.915; y = 0.835x + 0.962, r = 0.933 and y = 1.017x + 1.082, r = 0.993; respectively (All $p<0.001$) (Figure 6, Table 5A - Figure 11, Table 11A).

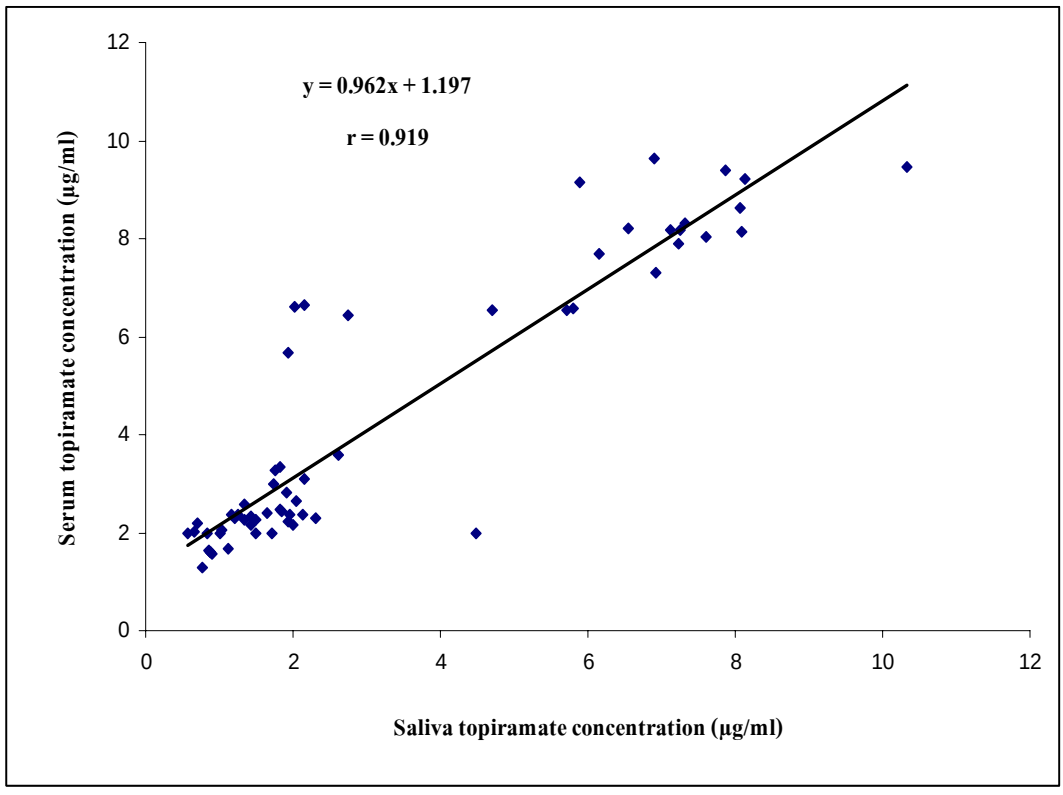

Figure 5 Correlation between serum and saliva topiramate concentrations at the time before topiramate ingestion (0 hr), 1, 2, 4, 6 and 8 hour after topiramate ingestion (n = 60)

Table 5A Statistic test using Pearson's correlation test of the data in Figure 5

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.919[a]	.844	.841	1.12137

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	395.098	1	395.098	314.199	.000[a]
	Residual	72.934	58	1.257		
	Total	468.032	59			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.197	.228		5.252	.000
	saliva	.962	.054	.919	17.726	.000

a. Dependent Variable: serum

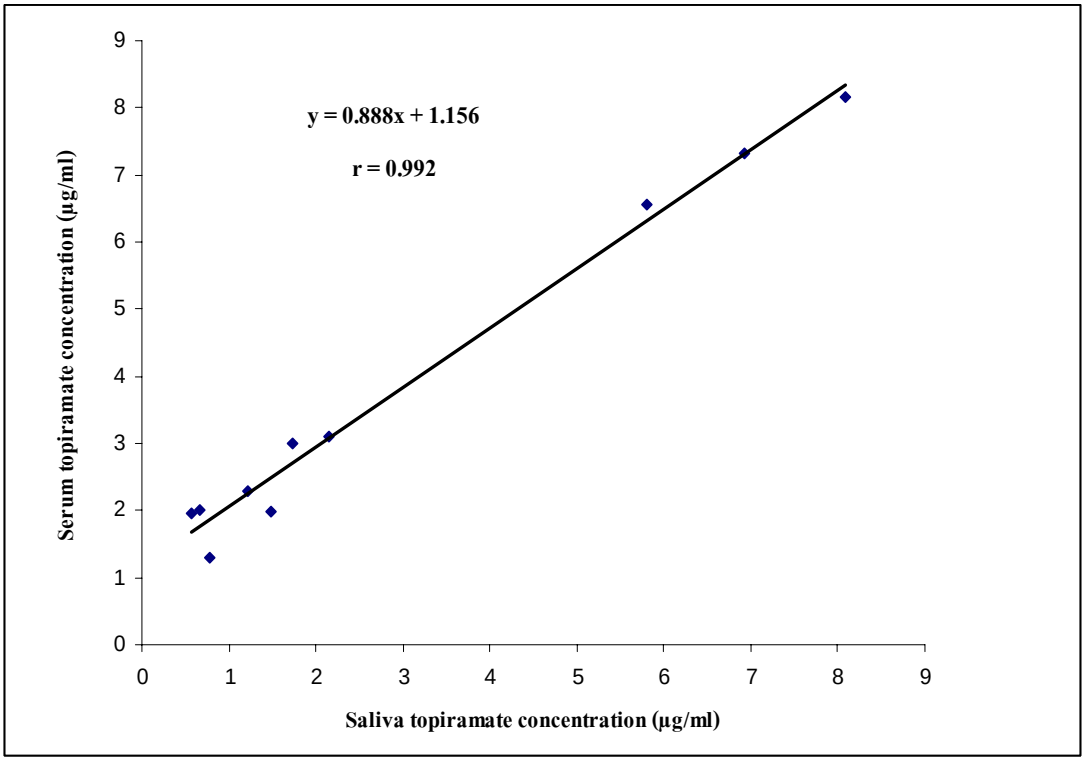

Figure 6 Correlation between serum and saliva topiramate concentrations at the time before topiramate ingestion (0 hr) (n = 10)

Table 6A Statistic test using Pearson's correlation test of the data in Figure 6

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.992[a]	.985	.983	.33517

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	57.506	1	57.506	511.894	.000[a]
	Residual	.899	8	.112		
	Total	58.405	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.156	.157		7.374	.000
	saliva	.888	.039	.992	22.625	.000

a. Dependent Variable: serum

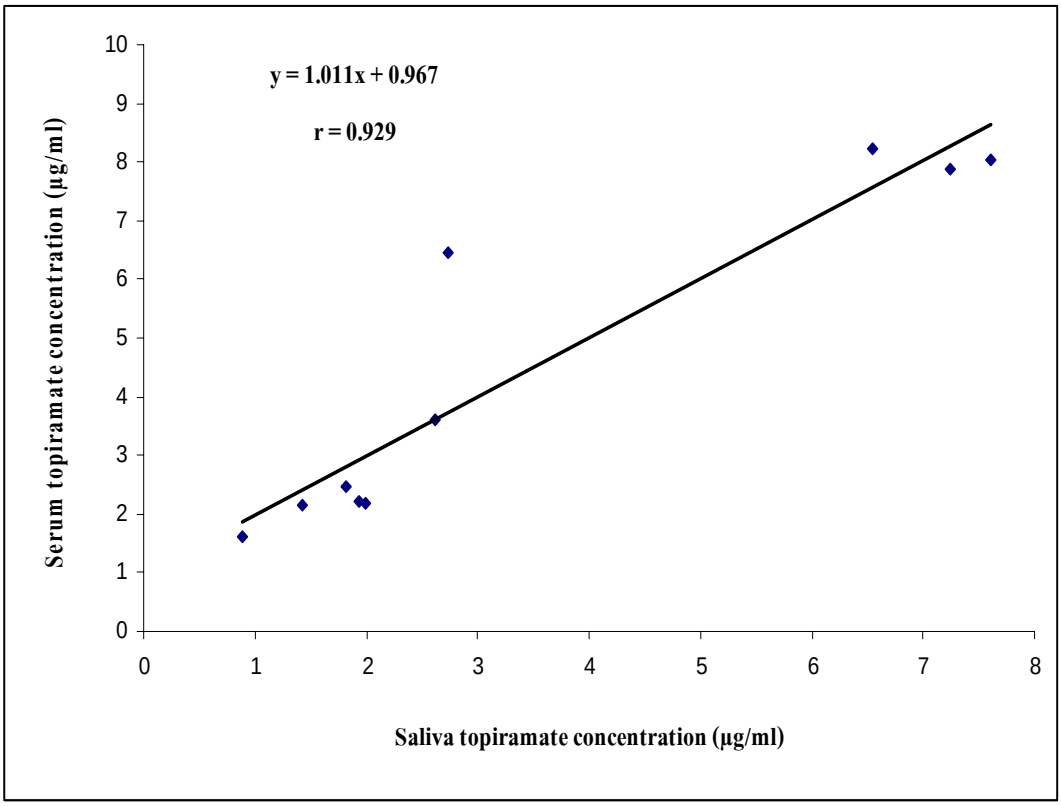

Figure 7 Correlation between serum and saliva topiramate concentrations at 1 hour after topiramate ingestion (1 hr) (n = 10)

Table 7A Statistic test using Pearson's correlation test of the data in Figure 7

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.929[a]	.864	.847	1.09975

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	61.347	1	61.347	50.724	.000[a]
	Residual	9.676	8	1.209		
	Total	71.023	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.967	.604		1.602	.148
	saliva	1.011	.142	.929	7.122	.000

a. Dependent Variable: serum

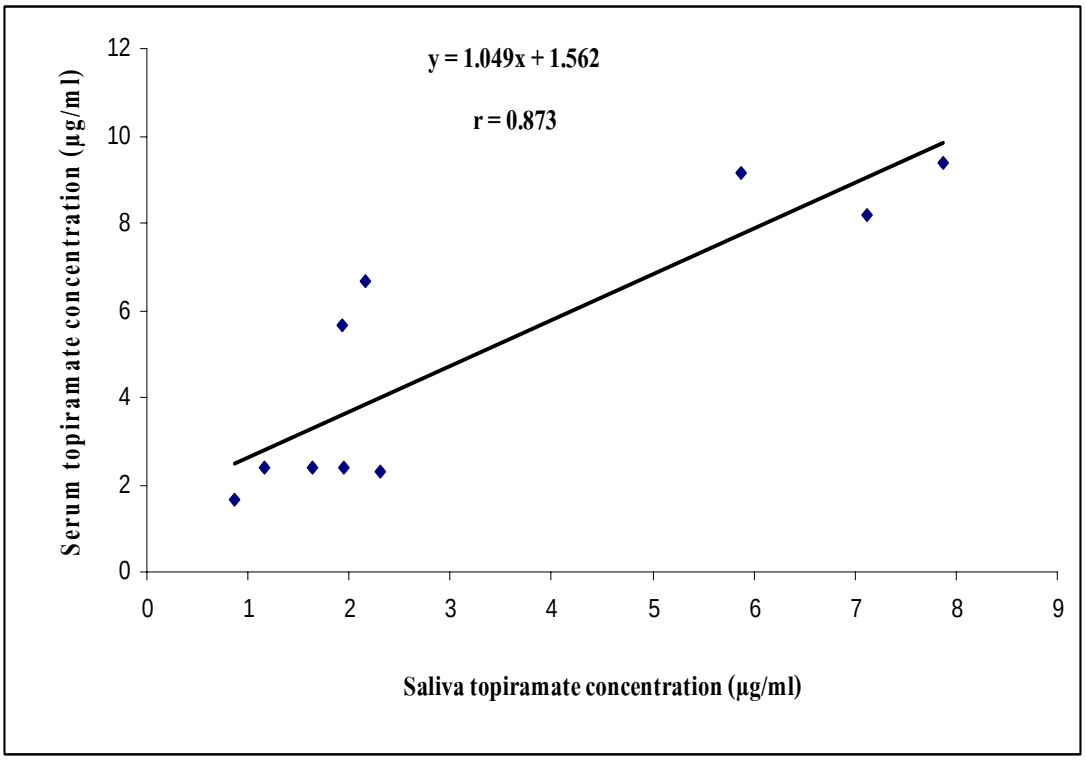

Figure 8 Correlation between serum and saliva topiramate concentration at 2 hour after topiramate ingestion (2 hr) (n = 10)

Table 8A Statistic test using Pearson's correlation test of the data in Figure 8

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.873[a]	.761	.732	1.62728

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	67.633	1	67.633	25.541	.001[a]
	Residual	21.184	8	2.648		
	Total	88.817	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.562	.855		1.827	.105
	saliva	1.049	.208	.873	5.054	.001

a. Dependent Variable: serum

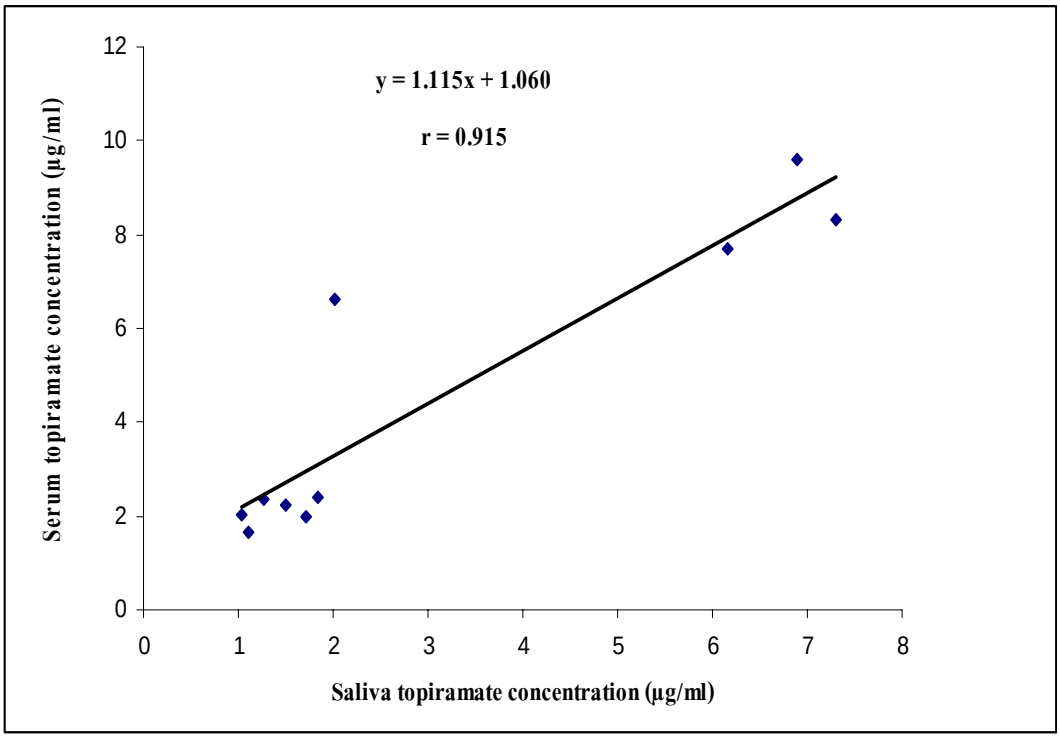

Figure 9 Correlation between serum and saliva topiramate concentrations at 4 hour after topiramate ingestion (4 hr) (n = 10)

Table 9A Statistic test using Pearson's correlation test of the data in Figure 9

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.915[a]	.837	.817	1.35108

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	75.029	1	75.029	41.103	.000[a]
	Residual	14.603	8	1.825		
	Total	89.633	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.060	.686		1.546	.161
	saliva	1.115	.174	.915	6.411	.000

a. Dependent Variable: serum

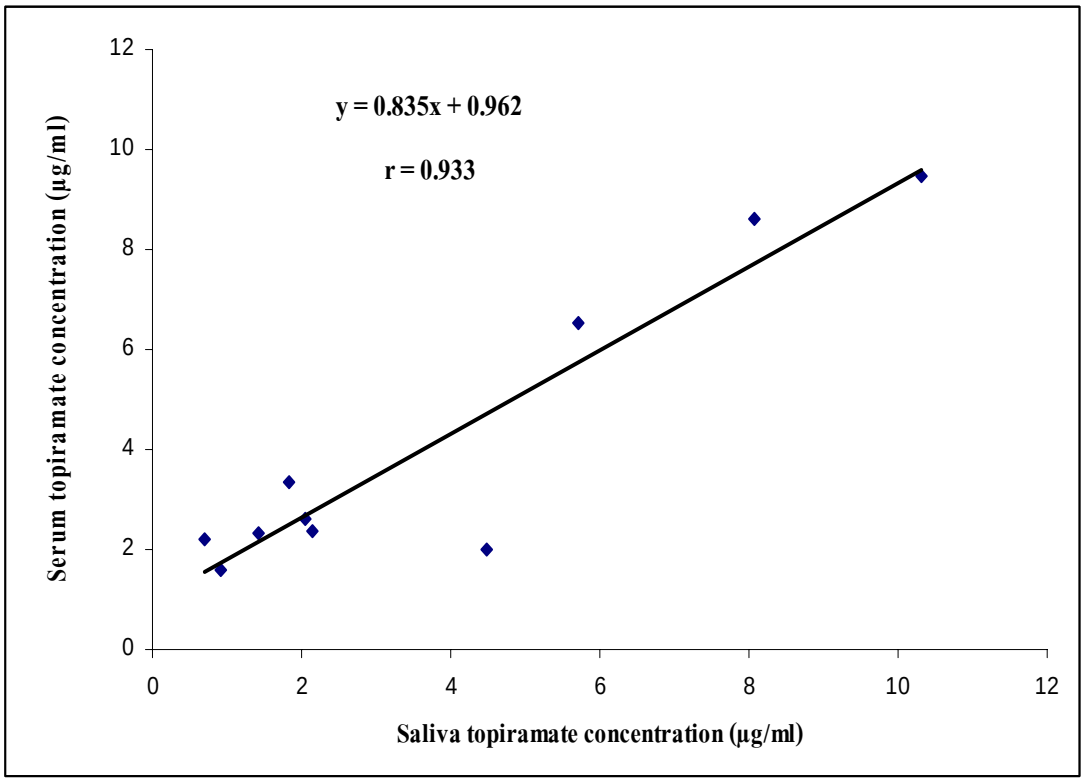

Figure 10 Correlation between serum and saliva topiramate concentrations at 6 hour after topiramate ingestion (6 hr) (n = 10)

Table 10A Statistic test using Pearson's correlation test of the data in Figure 10

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.933[a]	.871	.854	1.12797

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	68.442	1	68.442	53.793	.000[a]
	Residual	10.179	8	1.272		
	Total	78.621	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.962	.557		1.726	.123
	saliva	.835	.114	.933	7.334	.000

a. Dependent Variable: serum

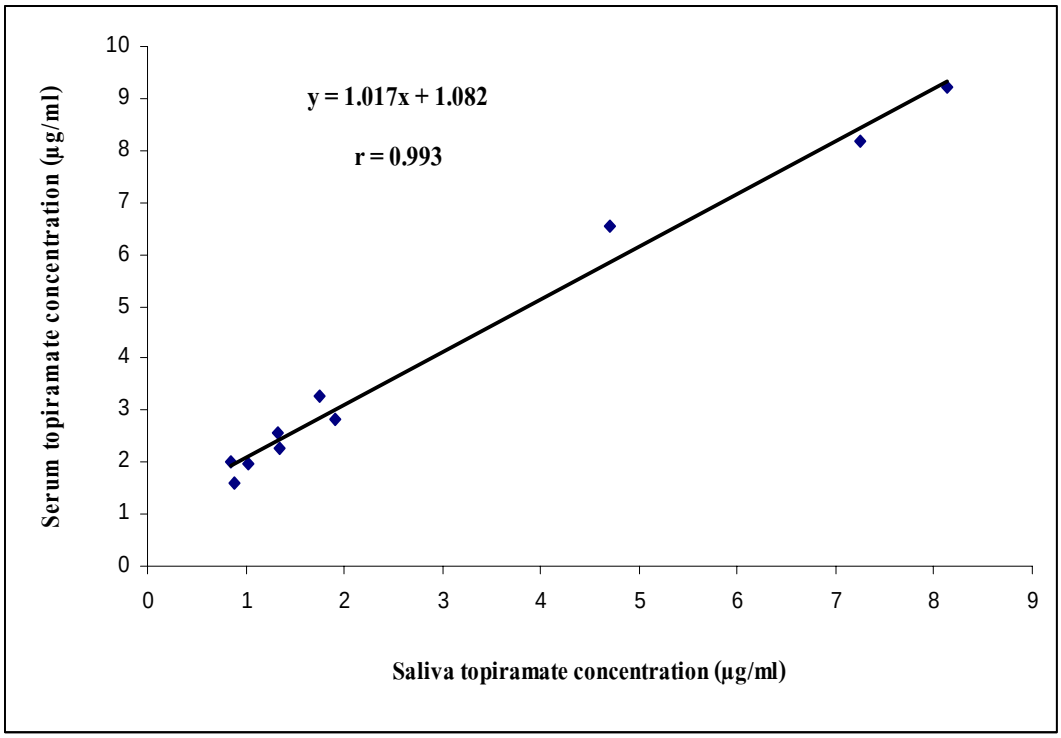

Figure 11 Correlation between serum and saliva topiramate concentrations at 8 hour after topiramate ingestion (8 hr) (n = 10)

Table 11A Statistic test using Pearson's correlation test of the data in Figure 11

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	saliva[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: serum

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.993[a]	.986	.985	.35071

a. Predictors: (Constant), saliva

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	70.917	1	70.917	576.561	.000[a]
	Residual	.984	8	.123		
	Total	71.901	9			

a. Predictors: (Constant), saliva

b. Dependent Variable: serum

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.082	.166		6.517	.000
	saliva	1.017	.042	.993	24.012	.000

a. Dependent Variable: serum

B. Verification of the correlation equations

The correlation equations constructed using serum and saliva samples collected from patients in group I were verified using the samples collected from unrelated group of patients (group II). The group II serum and saliva samples were collected from 30 patients at the time before and 2 hours after topiramate ingestion and analyzed for topiramate concentrations. Calculated serum topiramate concentrations were determined by adding the saliva topiramate concentrations of the patients in group II into the linear regression equation constructed from patients in group I. When the saliva topiramate concentrations of patients in group II collected at either 0 hour or 2 hours after topiramate ingestion, were adding into the linear regression equation construction by the samples of patients in group I collected at either 0 hour, 8 hours or all time points (0, 1, 2, 4, 6, 8 hours) after topiramate ingestion, the calculated serum topiramate concentration was not statistically different from the apparent serum topiramate concentrations (Table 8, 8A, 10, 10A, 11, 11A, 12, 12A, 14, 14A, 15, 15A). In contrast, when the saliva topiramate concentrations of patients in group II collected at either 0 hour or 2 hours after topiramate ingestion, were adding into the linear regression equation constructed by the samples of patients in group I collected at 2 hours after topiramate ingestion, the calculated serum topiramate concentration was statistically significant different from the apparent serum topiramate concentration (Table 9, 9A, 13, 13A).

Table 8 Apparent topiramate concentrations in serum, collected from patients in group II before topiramate ingestion (0 hour) and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 0 hour ($y = 0.888x + 1.156$)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	1.74	1.76	-0.02
2	2.96	2.59	0.37
3	1.33	2.02	-0.69
4	2.66	1.52	1.14
5	2.73	3.29	-0.56
6	3.19	1.84	1.35
7	2.42	2.31	0.11
8	1.65	1.34	0.31
9	2.10	1.97	0.13
10	2.95	3.17	-0.22
11	0.84	1.31	-0.47
12	1.71	2.22	-0.51
13	2.85	3.27	-0.42
14	2.37	3.11	-0.74
15	4.96	4.25	0.71
16	1.64	1.43	0.21
17	2.57	1.70	0.87
18	3.04	3.02	0.02
19	1.76	2.05	-0.29
20	2.84	1.40	1.44
21	1.40	1.22	0.18
22	1.51	1.69	-0.18
23	2.53	2.59	-0.06
24	1.39	1.34	0.05
25	2.16	1.51	0.65
26	2.60	2.22	0.38
27	2.67	2.55	0.12
28	4.43	4.32	0.11
29	3.57	3.02	0.55
30	2.10	3.07	-0.97
			$\Sigma_{di} = 3.57$

Table 8A Statistic test using Pair t-test of the data in Table 8

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	2.4223	30	.90020	.16435
	calculated	2.3033	30	.85831	.15670

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.770	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 serum – calculated	.11900	.59809	.10920	-.10433	.34233	1.090	29	.285

Table 9 Apparent topiramate concentrations in serum, collected from patients in group II before topiramate ingestion (0 hour) and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 2 hour (y = 1.049x + 1.562)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	1.74	2.28	-0.54
2	2.96	3.25	-0.29
3	1.33	2.58	-1.25
4	2.66	2.00	0.66
5	2.73	4.08	-1.35
6	3.19	2.37	0.82
7	2.42	2.93	-0.51
8	1.65	1.78	-0.13
9	2.10	2.53	-0.43
10	2.95	3.94	-0.99
11	0.84	1.74	-0.90
12	1.71	2.82	-1.11
13	2.85	4.06	-1.21
14	2.37	3.87	-1.50
15	4.96	5.21	-0.25
16	1.64	1.89	-0.25
17	2.57	2.20	0.37
18	3.04	3.76	-0.72
19	1.76	2.62	-0.86
20	2.84	1.85	0.99
21	1.40	1.64	-0.24
22	1.51	2.19	-0.68
23	2.53	3.25	-0.72
24	1.39	1.78	-0.39
25	2.16	1.98	0.18
26	2.60	2.82	-0.22
27	2.67	3.21	-0.54
28	4.43	5.30	-0.87
29	3.57	3.76	-0.19
30	2.10	3.83	-1.73
			Σ_{di} = -14.85

Table 9A Statistic test using Pair t-test of the data in Table 9

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	2.4223	30	.90020	.16435
	calculated	2.9173	30	1.01276	.18490

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.769	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	-.49500	.65824	.12018	-.74079	-.24921	-4.119	29	.000

Table 10 Apparent topiramate concentrations in serum, collected from patients in group II before topiramate ingestion (0 hour) and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 8 hour (y = 1.017x + 1.082)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	1.74	1.77	-0.03
2	2.96	2.72	0.24
3	1.33	2.07	-0.74
4	2.66	1.50	1.16
5	2.73	3.52	-0.79
6	3.19	1.87	1.32
7	2.42	2.40	0.02
8	1.65	1.30	0.35
9	2.10	2.02	0.08
10	2.95	3.39	-0.44
11	0.84	1.25	-0.41
12	1.71	2.30	-0.59
13	2.85	3.50	-0.65
14	2.37	3.32	-0.95
15	4.96	4.62	0.34
16	1.64	1.40	0.24
17	2.57	1.70	0.87
18	3.04	3.22	-0.18
19	1.76	2.11	-0.35
20	2.84	1.36	1.48
21	1.40	1.15	0.25
22	1.51	1.69	-0.18
23	2.53	2.72	-0.19
24	1.39	1.30	0.09
25	2.16	1.49	0.67
26	2.60	2.30	0.30
27	2.67	2.68	-0.01
28	4.43	4.70	-0.27
29	3.57	3.22	0.35
30	2.10	3.28	-1.18
			$\Sigma_{di} = 0.8$

Table 10A Statistic test using Pair t-test of the data in Table 10

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	2.4223	30	.90020	.16435
	calculated	2.3957	30	.98200	.17929

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.770	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1 serum – calculated	.02667	.64333	.11746	-.21356	.26689	.227	29	.822

Table 11 Apparent topiramate concentrations in serum, collected from patients in group II before topiramate ingestion (0 hour) and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at all time points (y = 0.962x + 1.197)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	1.74	1.85	-0.11
2	2.96	2.75	0.21
3	1.33	2.13	-0.80
4	2.66	1.60	1.06
5	2.73	3.51	-0.78
6	3.19	1.94	1.25
7	2.42	2.45	-0.03
8	1.65	1.40	0.25
9	2.10	2.08	0.02
10	2.95	3.38	-0.43
11	0.84	1.36	-0.52
12	1.71	2.35	-0.64
13	2.85	3.49	-0.64
14	2.37	3.31	-0.94
15	4.96	4.54	0.42
16	1.64	1.50	0.14
17	2.57	1.78	0.79
18	3.04	3.22	-0.18
19	1.76	2.17	-0.41
20	2.84	1.46	1.38
21	1.40	1.26	0.14
22	1.51	1.77	-0.26
23	2.53	2.75	-0.22
24	1.39	1.40	-0.01
25	2.16	1.58	0.58
26	2.60	2.35	0.25
27	2.67	2.71	-0.04
28	4.43	4.62	-0.19
29	3.57	3.22	0.35
30	2.10	3.27	-1.17
			Σdi = -0.53

Table 11A Statistic test using Pair t-test of the data in Table 11

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	2.4223	30	.90020	.16435
	calculated	2.4400	30	.92903	.16962

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.770	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	-.01767	.62123	.11342	-.24964	.21431	-.156	29	.877

Table 12 Apparent topiramate concentrations in serum, collected from patients in group II at 2 hour after topiramate ingestion and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 0 hour (y = 0.888x + 1.156)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	2.39	2.28	0.11
2	2.67	3.27	-0.60
3	2.29	2.52	-0.23
4	3.16	2.50	0.66
5	3.56	3.46	0.10
6	3.12	3.03	0.09
7	3.33	2.73	0.60
8	2.22	2.35	-0.13
9	3.48	3.86	-0.38
10	3.55	3.46	0.09
11	0.84	2.67	-1.83
12	1.79	1.93	-0.14
13	4.39	2.83	1.56
14	2.92	5.00	-2.08
15	10.89	8.00	2.89
16	1.84	1.61	0.23
17	3.25	2.75	0.50
18	3.85	4.18	-0.33
19	2.69	3.37	-0.68
20	3.39	1.51	1.88
21	1.40	1.51	-0.11
22	1.60	3.28	-1.68
23	2.76	2.88	-0.12
24	1.71	1.59	0.12
25	2.61	1.84	0.77
26	3.39	3.14	0.25
27	10.26	4.61	5.65
28	13.11	12.06	1.05
29	4.32	3.24	1.08
30	2.51	3.46	-0.95
			Σdi = 8.37

Table 12A Statistic test using Pair t-test of the data in Table 12

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	3.6430	30	2.79367	.51005
	calculated	3.3640	30	2.07940	.37965

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.864	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	.27900	1.44526	.26387	-.26067	.81867	1.057	29	.299

Table 13 Apparent topiramate concentrations in serum, collected from patients in group II at 2 hour after topiramate ingestion and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 2 hour (y = 1.049x + 1.562)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	2.39	2.89	-0.50
2	2.67	4.06	-1.39
3	2.29	3.18	-0.89
4	3.16	3.15	0.01
5	3.56	4.29	-0.73
6	3.12	3.78	-0.66
7	3.33	3.42	-0.09
8	2.22	2.97	-0.75
9	3.48	4.75	-1.27
10	3.55	4.29	-0.74
11	0.84	3.35	-2.51
12	1.79	2.47	-0.68
13	4.39	3.53	0.86
14	2.92	6.09	-3.17
15	10.89	9.65	1.24
16	1.84	2.10	-0.26
17	3.25	3.44	-0.19
18	3.85	5.14	-1.29
19	2.69	4.17	-1.48
20	3.39	1.98	1.41
21	1.40	1.98	-0.58
22	1.60	4.07	-2.47
23	2.76	3.60	-0.84
24	1.71	2.08	-0.37
25	2.61	2.37	0.24
26	3.39	3.90	-0.51
27	10.26	5.64	4.62
28	13.11	14.44	-1.33
29	4.32	4.03	-0.29
30	2.51	4.28	-1.77
			$\Sigma di = -16.38$

Table 13A Statistic test using Pair t-test of the data in Table 13

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	3.6430	30	2.79367	.51005
	calculated	4.1697	30	2.45590	.44838

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.864	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	-.52667	1.40584	.25667	-1.0516	-.00172	-2.052	29	.049

Table 14 Apparent topiramate concentrations in serum, collected from patients in group II at 2 hour after topiramate ingestion and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at 8 hour ($y = 1.017x + 1.082$)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	2.39	2.37	-1.98
2	2.67	3.50	-0.83
3	2.29	2.65	-0.36
4	3.16	2.62	0.54
5	3.56	3.73	-0.17
6	3.12	3.23	-0.11
7	3.33	2.88	0.45
8	2.22	2.44	-0.22
9	3.48	4.17	-0.69
10	3.55	3.73	-0.18
11	0.84	2.81	-1.97
12	1.79	1.97	-0.18
13	4.39	2.99	1.40
14	2.92	5.48	-2.56
15	10.89	8.92	1.97
16	1.84	1.60	0.24
17	3.25	2.90	0.35
18	3.85	4.55	-0.70
19	2.69	3.61	-0.92
20	3.39	1.49	1.90
21	1.40	1.49	-0.09
22	1.60	3.51	-1.91
23	2.76	3.05	-0.29
24	1.71	1.58	0.13
25	2.61	1.87	0.74
26	3.39	3.35	0.04
27	10.26	5.04	5.22
28	13.11	13.57	-0.46
29	4.32	3.47	0.85
30	2.51	3.72	-1.21
			$\Sigma_{di} = -1$

Table 14A Statistic test using Pair t-test of the data in Table 14

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	3.6430	30	2.79367	.51005
	calculated	3.6097	30	2.38155	.43481

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.864	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	.03333	1.40522	.25656	-.49139	.55805	.130	29	.898

Table 15 Apparent topiramate concentrations in serum collected from patients in group II at 2 hour after topiramate ingestion and the calculated serum topiramate concentrations which obtained from adding the corresponding saliva topiramate concentration into the linear correlation equation constructed from the samples of patients in group I collected at all time point (y = 0.962x + 1.197)

No of sample	Serum topiramate concentration (μg/ml)		di = apparent – calculated
	Apparent	Calculated	
1	2.39	2.42	-0.03
2	2.67	3.49	-0.82
3	2.29	2.68	-0.39
4	3.16	2.65	0.51
5	3.56	3.70	-0.14
6	3.12	3.23	-0.11
7	3.33	2.90	0.43
8	2.22	2.49	-0.27
9	3.48	4.12	-0.64
10	3.55	3.70	-0.15
11	0.84	2.83	-1.99
12	1.79	2.03	-0.24
13	4.39	3.00	1.39
14	2.92	5.35	-2.43
15	10.89	8.61	2.28
16	1.84	1.69	0.15
17	3.25	2.92	0.33
18	3.85	4.48	-0.63
19	2.69	3.59	-0.90
20	3.39	1.58	1.81
21	1.40	1.58	-0.18
22	1.60	3.50	-1.90
23	2.76	3.06	-0.30
24	1.71	1.67	0.04
25	2.61	1.94	0.67
26	3.39	3.34	0.05
27	10.26	4.94	5.32
28	13.11	13.01	0.10
29	4.32	3.46	0.86
30	2.51	3.69	-1.18
			Σdi = 1.64

Table 15A Statistic test using Pair t-test of the data in Table 15

T-Test

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	serum	3.6430	30	2.79367	.51005
	calculated	3.5883	30	2.25255	.41126

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	serum & calculated	30	.864	.000

Paired Samples Test

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 serum – calculated	.05467	1.41439	.25823	-.47348	.58281	.212	29	.834

CHAPTER V

DISCUSSION

The collection of biological samples for the purpose of determining exposure to various agents is dominated by blood, urine and breath etc. Each of these matrices has advantages and disadvantages with respect to ease of collection, specificity and sensitivity to drug concentrations detected in the matrix, and interpretative value. As an alternative matrix, oral fluid has been investigated as well. Reports concerning the appearance of organic solutions in saliva have been in the scientific literature for over 70 years. One of the earliest systematic studies on the permeability of salivary glands to organic nonelectrolytes was published in 1932. It demonstrated a correlation between the ability of nonelectrolytes to penetrate into saliva and their lipid solubility. It also demonstrated that compounds with low molecular weight can appear in saliva even if they are not lipid soluble. The first look at saliva testing for the presence of drugs was in 1938 with Friedemann's work investigating the excretion of ingested alcohol in saliva. But it has only been in the past 30 years that there has been emphasis on the appearance of other drugs in saliva, such that more than 100 drugs have been evaluated for salivary therapeutic drug monitoring (Langman, 2006). In this study, correlation between topiramate concentrations in serum and saliva was assessed and the correlation equation was tested. Topiramate concentrations in both serum and saliva were measured by turbidimetric immunoassay using automated clinical chemistry analyzer. Accordingly, the discussions were devided into 2 parts. First part was the reliability of turbidimetric immunoassay for serum and saliva topiramate assay while the second part was the relationship between serum and saliva topiramate concentration as well as the confidence of using the correlation equation practically in clinic.

Validation of topiramate assay for serum and saliva samples

Validation of turbidimetric immunoassay for determination of serum topiramate concentration using automated clinical chemistry analyzer and the topiramate reagent kit of Thermo scientific Inc. was reported. Those validation tests included limitation of the procedure, specificity, precision and accuracy of the method (Appendix F). Thus, validation of the method for determination of serum topiramate concentration was not performed in this study. However, calibration curve of topiramate standard of 6 concentrations (0, 2, 4, 8, 16, and 32 μg/ml)

was performed and the calibration equation was recorded in the instrument. Topiramate controls with 3 concentrations were used to verify the standard curve before using the recorded topiramate standard curve for determination of topiramate concentration in samples on each day of the experiment.

Assay control values should be within the acceptable range specified in the topiramate reagent kit package insert (Appendix F). Because, assay of topiramate concentrations in saliva specimens by turbidimetric immunoassay using automated clinical chemistry analyzer has not been recommended by manufacturer, verification of this method for saliva topiramate assay was performed in this study. Linearity of the assay was performed by spiking saliva to yield 5 concentrations of topiramate which were measured by turbidimetric immunoassay using serum topiramate standard curve. The measured saliva topiramate concentration and the target saliva topiramate concentrations were closely correlated with R^2 of 0.998. Accuracy of the assay was assessed by percentage of recovery. Five topiramate concentrations (2, 4, 8, 16, and 32 μg/ml) were measured. Percentage of recovery was range from 93.8 to 108.69%. This range of percentage of recovery was on acceptable range according to the criteria recommended for biological analysis that % recovery should be within 15% of the actual value (CDER and CVM, 2001). Both within day and between day precision of the assay for saliva topiramate analysis were shown by % CV of less than 15% which was acceptable according to the recommendation that % CV determined at each concentration level should not exceed 15% (CDER and CVM, 2001). Stability of saliva topiramate kept at 2-8 °C for various days are presented by percentage of topiramate concentration as compared to the concentration at the first day of preparation demonstrated to be range from 98.34 to 106.36%.

Correlation between serum and saliva topiramate concentrations and verification of the correlation equations

Results from this study demonstrated that serum and saliva topiramate concentrations were linearly correlated with a correlation coefficient of 0.919 (n = 60, $p<0.001$). The regression equation of this relationship was $y = 0.962x + 1.197$. The correlations between serum and saliva concentrations were closely correlated with the correlation coefficient of 0.992, 0.929, 0.873, 0.915, 0.933 and 0.993 (all data were from n = 10, $p<0.001$) at the time point of 0, 1, 2, 4, 6 and 8 hours after topiramate ingestion, respectively. Considering based on the correlation coefficient,

the equations constructed from the data at the time before topiramate ingestion (0 hour) or at 8 hours after topiramate ingestion, the relationships were mostly correlated.

When verification of the correlation equations was performed using saliva and serum samples collected from patients in group II at the time before topiramate ingestion and 2 hours after topiramate ingestion, summary of the result was shown in Table 16. It was shown that the calculated serum topiramate concentration was not statistically different from the apparent serum topiramate concentration at both times of sample collection of group II patients when using the equation constructed from 0 hour, 8 hours or the equation constructed from samples collected at all time points (0, 1, 2, 4, 6, 8 hours). But the different was shown when using the correlation equation constructed from the samples collected at 2 hours after topiramate ingestion. Despite the limited time point collecting of samples in group II patients, the time of saliva collection can be either before or after topiramate ingestion but the correlation equation used should be the equations constructed from the samples collecting at the time before or the whole data of all time points.

Table 16 Summary of the statistic analysis of the difference between apparent serum topiramate concentration and calculated serum topiramate concentration using topiramate concentrations of saliva samples collecting at 0 and 2 hour adding into various conditions of correlation equations

Time that samples in group II was collected	Statistic difference between apparent and calculated serum topiramate concentration (Verification was performed using the correlation equation constructed from samples in group I collected at various time points)			
	0 hour	2 hours	8 hours	0, 1, 2, 4, 6 and 8 hours
0 hour	ns	sig	ns	ns
2 hours	ns	sig	ns	ns

It was recommended that the ideal sampling time for collection of saliva samples to monitoring antiepileptic drugs is just before the ingestion of the next scheduled drug dose. If this is not possible, sampling should be performed at least 3 hours after drug ingestion (Patsalos, 2008). Corresponding to the recommendation above and the reported pharmacokinetic of

topiramate which demonstrated that peak plasma concentration of topiramate is 2 – 3 hours, verification of the correlation equation when constructed by the data collected at 2 hours after topiramate ingestion showed that the calculated serum topiramate concentrations and the apparent serum topiramate concentration was statistically different, implying that this equation was not an appropriate equation. In contrast, when the verification was performed on the correlation equations constructed by the data collected at either 0 or all time points (0, 1, 2, 4, 6 and 8 hours), the calculated and apparent serum topiramate concentrations were not significant different, implying that both equations were appropriate. However, the equation of 0 hour and 8 hours constructed from the smaller data of n = 10, the equation of all time points (0, 1, 2, 4, 6 and 8 hours) constructed from the larger data of n = 60 was probably more appropriate.

Topiramate was officially approved in Germany for combination therapy in July 1999 and for monotherapy in July 2002. The relationship between topiramate serum concentration and efficacy as well as the relationship between serum concentration and toxicity is still under discussion. It was shown that in seizure free patients, topiramate serum concentration was range between 2-25 μg/ml, thus range of an effective serum concentration is very broad. However, an optimal treatment responses is most likely to be found at serum concentration higher than 2 μg/ml, but no further increase in efficacy seems to occur at concentrations above 10.5 μg/ml (Christensen et al. 2003: Froscher, 2005). As a consequence of these conflicting results, the recommended target range of topiramate given in the literature varies between 2-5 μg/ml (Froscher et al.,1999) and 2-25 μg/ml (Glauser and Pippenger, 2000: Froscher, 2005). In this study, range of topiramate in serum of group I patients (n = 10) with topiramate monotherapy was 1.29 – 8.15 μg/ml while the corresponding range of saliva topiramate concentration was 0.58 – 8.08 μg/ml.

In the group II patients (n = 30) with topiramate monotherapy and co-administration with other antiepileptic drugs, serum topiramate concentration was range between 0.84 – 4.96 μg/ml while the range of saliva topiramate concentration was 0.07 – 3.56 μg/ml. Taken together for all patients of group I and group II (n = 40), the range of serum topiramate concentration was 0.84 – 8.15 μg/ml while the saliva topiramate concentration was 0.07 – 8.08 μg/ml (Table 17).

Among the patients (n = 13) who had serum topiramate concentrations below 2 μg/ml (range between 1.29 – 1.99 μg/ml), 4 patients who were given topiramate monotherapy at the dose of 25, 50, 75 or 200 mg/kg/day respectively, while the remaining 9 patients (range of serum

topiramate concentration between 0.84 – 1.76 μg/ml) were given topiramate co-therapy with carbamazepine (n = 4), carbamazepine and valproate (n = 3), phenytoin (n = 1), valproate (n = 1).

Table 17 Range of topiramate concentrations in serum and saliva*

	Serum topiramate concentration (μg/ml)	Saliva topiramate concentration (μg/ml)
group I (n=10)	1.29 – 8.15	0.58 – 8.08
group II (n=30)	0.84 – 4.96	0.07 – 3.56
Total (n=40)	0.84 – 8.15	0.07 – 8.08

*Range of topiramate concentrations in serum and saliva shown in this table is the range concentrations of samples collected before topiramate ingestion (0 hour).

Among 29 patients whose adverse event could be recorded, 6 patients (21%) with serum topiramate concentration range between 2.57 – 8.15 μg/ml, experienced adverse events. The most frequent adverse events were dizziness, speech problems and/or loss of appetite.

Topiramate has been suggested to be less prone to drug interactions than many antiepileptic drugs, significant drug interactions do occur. When topiramate is combined with hepatic enzyme-inducing drugs, e.g. phenytoin, carbamazepine and phenobarbital, the clearance of topiramate may increase two-to three fold (Miles et al., 2003; Mack et al., 2002; Collins et al., 2006). The pharmacokinetic characteristics of topiramate are somewhat atypical to many other antiepileptic drugs. Topiramate clearance has been revealed to be approximately 50% higher in children compared to adults (Miles et al., 2003). Thus, benefit of monitoring topiramate concentration is to enhance efficiency of drug treatment, with more rapid achievement of satisfactory drug effect, fewer and earlier recognized overdosage-type adverse effects and more readily detected noncompliance with therapy (Eadie, 1998). Patients of particulated groups should be considered for topiramate monitoring, e.g. the very young or disabled, those receiving hepatic enzyme-inducing drugs, those with renal dysfunction and patients who have questionable medication compliance.

Using saliva for drug monitoring has several advantages over using serum. The advantages were the ease of collection, non-invasive and less expensive than using other bodies fluids. It lacks of pain, fear of needles, and potential complication of bleeding, infection, and skin discoloration associated with blood or plasma monitoring. Saliva monitoring may be especially beneficial for pediatric patients, for those with fear of needles, and for individuals with poor venous access. Thus, result from this study provides a relationship between topiramate concentration in serum and saliva samples of Thai epileptic patients. The finding would support the use of saliva as alternative to serum for monitoring topiramate therapy.

CHAPTER VI

CONCLUSION

The objective of this study was to determine the correlation between serum and saliva topiramate concentrations in Thai epileptic patients. The study was conducted at the Department of Medicine Neurology Unit, Epilepsy Clinic, Pramongkutklao Hospital. Patients aged between 15-60 years old and receiving topiramate were included into this study. The correlation equation between serum and saliva topiramate concentrations was constructed from 10 patients, receiving topiramate monotherapy. The blood and saliva samples were collected at the time before the morning dose and at 1, 2, 4, 6 and 8 hours after topiramate ingestion. Topiramate concentrations in blood and saliva samples were measured by turbidimetric immunoassay technique. The results showed that serum and saliva topiramate concentrations were linearly correlated with a correlation coefficient of 0.919 (n = 60, $p<0.001$). The regression equation of this relationship was $y = 0.962x + 1.197$. The correlations between serum and saliva concentrations collected at 0, 1, 2, 4, 6 and 8 hours after topiramate ingestion were closely correlated with the correlation coefficient of 0.992, 0.929, 0.873, 0.915, 0.933 and 0.993, respectively (all data were from n = 10, $p<0.001$).

The second part of the study was to verify the correlation equations using serum and saliva samples collected at the time before topiramate ingestion (0 hour) and 2 hours after topiramate ingestion from group II patients receiving topiramate either monotherapy or co-administrated with other antiepileptic medication. The result showed that the calculated serum topiramate concentration was not statistically different from the apparent serum topiramate concentration at both times of sample collection in group II patients when using the equation constructed from samples collected at 0 hour, 8 hours or the equation constructed from samples collected at all time points (0, 1, 2, 4, 6, 8 hours) but the different was shown when using the correlation equation constructed from the samples collected at 2 hours after topiramate ingestion. The results of this study support the use of saliva as an alternative to serum for monitoring topiramate therapy.

These following topics are suggested for further study.

1. Performing the linear regression equation from a larger sample size of sampling collection at 0 or 8 hours, the most appropriate time of sampling collection given the highest R^2.

2. The relationship between saliva topiramate concentration and efficacy as well as relationship between saliva topiramate concentration and adverse events.

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APPENDICES

APPENDIX A

Ethical Approval of Study Protocol &

Research Subject Information Sheet

Q042h/51

คณะอนุกรรมการพิจารณาโครงการวิจัยกรมแพทย์ทหารบก
ชั้น 5 อาคารพระมงกุฎเกล้าเวชวิทยา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า
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ที่ 1348 /2551

วันที่ 15 ธันวาคม 2551

เรื่อง แจ้งผลการพิจารณาโครงการวิจัย

เรียน นางสาวจรีรัตน์ คงฤทธิ์ นศ.ป.โท คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

สิ่งที่ส่งมาด้วย - แบบรายงานสรุปผลการวิจัย

ตามที่ ท่านได้ส่งโครงการวิจัย เรื่อง "ความสัมพันธ์ระหว่างความเข้มข้นของโทพิราเมตในซีรัม และน้ำลายของผู้ป่วยโรคลมชักชาวไทย" [RELATIONSHP BETWEEN TOPIRAMATE CONCENTRATIONS IN SERUM AND SALIVA OF THAI EPILEPTIC PATIENTS] เพื่อพิจารณาระเบียบวิธีวิจัย และจริยธรรมจากคณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก เพื่อประกอบการพิจารณาสนับสนุนการเก็บข้อมูล นั้น คณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก อนุมัติเมื่อวันที่ 15 ธันวาคม 2551 เมื่อท่านได้ทำวิทยานิพนธ์เสร็จสิ้นลง กรุณาส่งวิทยานิพนธ์ของท่านและแบบรายงานสรุปผลการวิจัย มายังคณะอนุกรรมการฯ 1 ชุด

จึงเรียนมาเพื่อทราบ

ขอแสดงความนับถือ

พันเอกหญิง

(เยาวนา ธนะพัฒน์)

ประธานคณะอนุกรรมการพิจารณาโครงการวิจัย กรมแพทย์ทหารบก

เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย (Research Subject Information Sheet)

ชื่อโครงการวิจัย ความสัมพันธ์ระหว่างความเข้มข้นของยาโทรพิราเมตในซีรัมและน้ำลายของผู้ป่วยโรคลมชักชาวไทย

วันที่ชี้แจง.....

ชื่อและสถานที่ทำงานของผู้วิจัย น.ส.จรีรัตน์ คงฤทธิ์
นิสิตปริญญาโท สาขาเภสัชวิทยา จุฬาลงกรณ์มหาวิทยาลัย
ชื่อผู้ร่วมวิจัย รศ.ดร.พตท.หญิงสมทรง ลาวัณย์ประเสริฐ
พ.อ.ดร.นพ.โยธิน ชินวลัญช์

ผู้ให้ทุนวิจัย คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ท่านได้รับการเชิญชวนให้เข้าร่วมในโครงการวิจัยนี้ แต่ก่อนที่ท่านจะตกลงใจเข้าร่วมหรือไม่โปรดอ่านข้อความในเอกสารนี้ทั้งหมด เพื่อให้ทราบว่าเหตุใดท่านจึงได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้ โครงการวิจัยนี้ทำเพื่ออะไร หากท่านเข้าร่วมโครงการวิจัยนี้ท่านจะต้องทำอะไรบ้าง รวมทั้งข้อดีและข้อเสียที่อาจจะเกิดขึ้นในระหว่างการวิจัย

ในเอกสารนี้ อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามผู้วิจัยหรือผู้ช่วยวิจัยที่ทำโครงการนี้เพื่อให้อธิบายจนกว่าท่านจะเข้าใจ ท่านจะได้รับเอกสารนี้ 1 ชุด กลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อน หรือแพทย์ที่ท่านรู้จักให้ช่วยตัดสินใจว่าควรจะเข้าร่วมโครงการวิจัยนี้หรือไม่ การเข้าร่วมในโครงการวิจัยครั้งนี้จะต้องเป็นความสมัครใจของท่าน ไม่มีการบังคับหรือชักจูง ถึงแม้ท่านจะไม่เข้าร่วมในโครงการวิจัย ท่านก็จะได้รับการรักษาพยาบาลตามปกติ การไม่เข้าร่วมหรือถอนตัวจากโครงการวิจัยนี้ จะไม่มีผลกระทบต่อการได้รับบริการ การรักษาพยาบาลหรือผลประโยชน์ที่พึงจะได้รับของท่านแต่อย่างใด

โปรดอย่าลงลายมือชื่อของท่านในเอกสารนี้จนกว่าท่านจะแน่ใจว่ามีความประสงค์จะเข้าร่วมในโครงการวิจัยนี้ คำว่า “ท่าน” ในเอกสารนี้ หมายถึงผู้เข้าร่วมโครงการวิจัยในฐานะเป็นอาสาสมัครในโครงการวิจัยนี้ หากท่านเป็นผู้แทนโดยชอบธรรมตามกฎหมายของผู้ที่จะเข้าร่วมในโครงการวิจัย และลงนามแทนในเอกสารนี้ โปรดเข้าใจว่า “ท่าน” ในเอกสารนี้หมายถึงผู้เข้าร่วมในโครงการวิจัยเท่านั้น

เอกสารชี้แจงข้อมูลแก่ผู้เข้าร่วมโครงการวิจัย (Research Subject Information Sheet)

โรคลมชักเป็นโรคที่มีความผิดปกติทางสมองสามารถพบได้ในทุกกลุ่มอายุ ตั้งแต่เด็กจนถึงผู้ใหญ่ โรคนี้เป็นปัญหาทางด้านสาธารณสุขที่สำคัญปัญหาหนึ่ง โรคลมชักเกิดเนื่องมาจากเซลล์ประสาทภายในสมองมีการส่งผ่านกระแสประสาทซึ่งเป็นสัญญาณไฟฟ้าที่ผิดปกติทำให้เกิดอาการชักขึ้น สาเหตุของโรคลมชักนั้นมีหลายสาเหตุ เช่น สมองได้รับการกระทบกระเทือนจนทำให้เกิดความเสียหายแก่เนื้อเยื่อภายใน ยาหรือสารพิษบางชนิด ความผิดปกติของเลือดที่ไปเลี้ยงสมอง เนื้องอก โรคหรือความเจ็บป่วยที่ทำให้เกิดการทำลายของเซลล์สมอง ปัจจุบันการใช้ยาในกลุ่มยาต้านชักเป็นวิธีการหลักในการรักษาโรคลมชัก โดยยาต้านชักจะเพิ่มการยับยั้งและลดการกระตุ้นของเซลล์ประสาท ดังนั้นเพื่อให้เกิดประสิทธิภาพสูงสุดในการรักษา ไม่มีอาการข้างเคียงจากการใช้ยา ต้องเลือกขนาดยาที่เหมาะสมและมีการตรวจติดตามระดับยาในเลือดประกอบ จึงจำเป็นที่จะต้องติดตามวัดระดับยาร่วมกับการประเมินทางคลินิกของผู้ป่วย เพื่อให้ผลการรักษาเหมาะสมและปลอดภัยต่อผู้ป่วยมากที่สุด โดยทั่วไปในทางคลินิกการตรวจติดตามระดับยาใช้วิธีการเจาะเลือดแล้วตรวจความเข้มข้นของยาในซีรัม มีบางการศึกษาที่ตรวจวัดระดับยาในน้ำลายซึ่งพบว่าความเข้มข้นของยาในน้ำลายนั้นเป็นความเข้มข้นของยาที่อยู่ในรูปอิสระไม่จับกับโปรตีนในซีรัมน่าจะเป็นค่าระดับยาที่มีความสำคัญในการประเมินผลทางเภสัชวิทยาและพิษวิทยาได้ การเก็บน้ำลายเป็นวิธีที่ง่าย ไม่ต้องมีเจ้าหน้าที่เจาะเลือด ผู้ป่วยไม่ต้องเจ็บจากการเจาะเลือด โดยเฉพาะอย่างยิ่งในผู้ป่วยเด็กที่ต้องมีการติดตามวัดระดับยา การเก็บน้ำลายไม่เกิดความเครียด ความกลัว ไม่ก่อให้เกิดความเสี่ยงจากภาวะแทรกซ้อนจากการเจาะเลือด ได้แก่ การติดเชื้อ การเกิดรอยช้ำ รวมทั้งความเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบและเชื้อ HIV เป็นต้น

มีผู้วิจัยบางกลุ่มทำการศึกษาความสัมพันธ์ระหว่างค่าความเข้มข้นของโทพิราเมตในซีรัมและน้ำลายพบว่ามีความสัมพันธ์กัน มีค่าสัมประสิทธิ์สหสัมพันธ์สูงและมีนัยสำคัญทางสถิติ อย่างไรก็ตามเนื่องจากยาโทพิราเมตเป็นยากันชักกลุ่มใหม่ยังไม่มีการตรวจติดตามระดับยาในประเทศไทย ข้อมูลที่ได้จากการศึกษานั้นเป็นการศึกษาในอาสาสมัครชาวต่างประเทศ ยังไม่มีรายงานการศึกษาในอาสาสมัครชาวเอเชียดดยเฉพาะอย่างยิ่งในคนไทย จำเป็นอย่างยิ่งที่จะต้องมีการศึกษายืนยันโดยใช้อาสาสมัครชาวไทย การวิจัยครั้งนี้มีวัตถุประสงค์หลักในการศึกษาความสัมพันธ์ระหว่างความเข้มข้นของยาโทพิราเมตโดยใช้ตัวอย่างซีรัมและตัวอย่างน้ำลายในผู้ป่วยโรคลมชักชาวไทย ทั้งนี้เพื่อให้ได้ข้อมูลในการพิจารณาปรับขนาดและความถี่ของการให้ยาโทพิราเมตในผู้ป่วยชาวไทยเพื่อให้เกิดประสิทธิผลสูงสุดในการรักษา รวมทั้งหาสมการความสัมพันธ์ระหว่างความเข้มข้นของโทพิราเมตในซีรัมและน้ำลาย เพื่อนำข้อมูลที่ได้มาใช้ในการพิจารณาความเป็นไปได้ในการใช้ตัวอย่างน้ำลายในการใช้ค่าความเข้มข้นของโทพิราเมตในน้ำลาย

แทนการตรวจวัดโทพิราเมตในซีรัม ในการติดตามการรักษาผู้ป่วยโรคลมชักชาวไทยที่ได้รับการรักษาด้วยโทพิราเมตต่อไป

ท่านได้รับเชิญให้เข้าร่วมโครงการวิจัยนี้เนื่องด้วยคุณสมบัติที่เหมาะสมดังต่อไปนี้ ท่านมีอายุระหว่าง 15-60 ปี และเป็นผู้ที่ได้รับยาต้ายชัก คือ โทพิราเมต ติดต่อกันเป็นเวลาอย่างน้อย 1 เดือน ไม่มีความผิดปกติในการทำหน้าที่ของตับและไต ไม่เป็นโรคที่มีผลต่อการหลั่งน้ำลาย ไม่ได้รับประทานยาอื่นๆที่มีผลต่อการหลั่งน้ำลาย ก่อนการเก็บตัวอย่างอย่างน้อย 1 สัปดาห์ หากท่านกำลังอยู่ในระหว่างการตั้งครรภ์หรือให้นมบุตร หรือมีอาการแพ้ยา ท่านไม่สามารถเข้าร่วมโครงการวิจัยนี้ได้ โครงการวิจัยนี้จะทำที่หน่วยตรวจผู้ป่วยนอกคลินิกดรคลมชัก โรงพยาบาลพระมงกุฎเกล้า และมีจำนวนผู้เข้าร่วมโครงการวิจัยทั้งสิ้นไม่ต่ำกว่า 60 คน แบ่งผู้เข้าร่วมโครงการเป็นสองกลุ่ม ใช้ระยะเวลาในการเข้าร่วมโครงการ 1 วัน โดยท่านจะได้รับการเจาะเลือดและเก็บน้ำลายเพื่อตรวจวัดระดับยาโทพิราเมต กลุ่มแรกจะได้รับการเจาะเลือดและเก็บน้ำลายในเวลาต่างๆกัน 6 ครั้ง เป็นเวลา 8 ชั่วโมง กลุ่มที่สองเจาะเลือดและเก็บน้ำลาย 2 ครั้ง ก่อนและหลังรับประทานยาโทพิราเมตที่ท่านได้รับอยู่แล้ว ไม่มีการให้ยาอื่นใดในระหว่างการเข้าร่วมโครงการวิจัย ท่านอาจจะได้รับความไม่สุขสบายเล็กน้อยจากการเจาะเลือดเท่านั้น ไม่มีอันตรายอย่างอื่นที่จะเกิดขึ้น ประโยชน์ที่ได้รับจากโครงการวิจัยนี้ท่านจะได้ทราบผลของระดับยาโทพิราเมตที่ท่านได้รับอยู่ ซึ่งเป็นข้อมูลที่แพทย์เจ้าของไข้ของท่านจะนำมาพิจารณาการรักษาที่เหมาะสมต่อท่านในการปรับขนาดยาต่อไป นอกจากนี้ยังเป็นข้อมูลที่สำคัญต่อผู้ป่วยท่านอื่นถึงความเป็นไปได้ในการใช้ตัวอย่างน้ำลายมาตรวจวัดระดับยาแทนการตรวจวัดในเลือด เพื่อลดความไม่สุขสบายจากการเจาะเลือด โดยเฉพาะอย่างยิ่งในผู้ป่วยเด็กและผู้สูงอายุ การเข้าร่วมโครงการวิจัยนี้ท่านไม่ต้องเสียค่าใช้จ่ายใดๆในส่วนของการตรวจวัดระดับยา และท่านจะได้รับค่าตอบแทนเป็นค่าเดินทางมาร่วมโครงการวิจัย ในกลุ่มแรกครั้งละ1,000 บาท กลุ่มที่สองครั้งละ 200 บาท เมื่อมาร่วมในโครงการวิจัยนี้จนเสร็จสิ้นโครงการ หากท่านไม่เข้าร่วมหรือถอนตัวจากโครงการวิจัยนี้จะไม่มีผลกระทบต่อการได้รับบริการ การรักษาพยาบาลหรือผลประโยชน์ที่พึงจะได้รับของท่านแต่อย่างใด หากเกิดอันตรายที่เกี่ยวข้องกับโครงการวิจัยนี้ ท่านสามารถติดต่อได้ทั้งในและนอกเวลาราชการ ที่ น.ส. จรีรัตน์ คงฤทธิ์ หมายเลขโทรศัพท์ 08-5179-0309 และ รศ.ดร.พตท.หญิง สมทรง ลาวัณย์ประเสริฐ หมายเลขโทรศัพท์ 02-2188322-5 ภาควิชาเภสัชวิทยา คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย และ พ.อ.ดร.นพ.โยธิน ชินวลัญช์ แผนกประสาทวิทยา กองอายุรกรรม โรงพยาบาลพระมงกุฎเกล้า ในเวลาราชการ รวมทั้งหากเกิดการเจ็บป่วยหรือบาดเจ็บจากการเข้าร่วมโครงการวิจัยนี้ ทางคณะผู้วิจัยจะเป็นผู้รับผิดชอบค่าใช้จ่ายในการรักษาพยาบาลทั้งหมด รวมทั้งกรณีฉุกเฉินที่อาจจะเกิดขึ้นจากโครงการวิจัย หากท่านมีคำถามที่เกี่ยวข้องกับโครงการวิจัยสามารถสอบถามได้จากผู้วิจัยคือ น.ส. จรีรัตน์ คงฤทธิ์

และผู้ร่วมวิจัยคือ รศ.ดร.พตท.หญิงสมทรง ลาวัณย์ประเสริฐ และ พ.อ.ดร.นพ.โยธิน ชินวลัญช์

หากท่านรู้สึกว่าได้รับการปฏิบัติอย่างไม่เป็นธรรมในระหว่างโครงการวิจัยนี้ ท่านอาจแจ้งเรื่องได้ที่ สำนักงานพิจารณาโครงการวิจัย พบ. เบอร์โทร. 02-3547600 ต่อ 94270

ข้อมูลส่วนตัวของท่านที่ได้จากโครงการวิจัยครั้งนี้จะนำเสนอข้อมูลที่ได้จากโครงการวิจัยเพื่อประโยชน์ทางวิชาการ โดยไม่เปิดเผยชื่อ นามสกุล ที่อยู่ของผู้เข้าร่วมในโครงการวิจัยเป็นรายบุคคล และมีมาตรการในการเก็บรักษาข้อมูลส่วนตัวและข้อมูลที่ได้จากโครงการวิจัย และข้อมูลจะถูกส่งไปให้ผู้ให้ทุนวิจัยหรือคณะกรรมการจริยธรรมกรมแพทย์ทหารบก

ผู้เข้าร่วมในโครงการวิจัยหลังจากได้ลงนามเข้าร่วมโครงการวิจัยแล้วสามารถถอนตัวออกจากโครงการวิจัยได้ตลอดเวลา โดยจะไม่มีผลเสียใดๆเกิดขึ้น และผู้เข้าร่วมในโครงการวิจัยอาจถูกขอให้ออกจากโครงการวิจัยในกรณีดังต่อไปนี้ เช่น เมื่อโรคมีอาการรุนแรงขึ้น หรือมีอาการแพ้ยาเกิดขึ้นในระหว่างการทำวิจัย เป็นต้น หากมีข้อมูลใหม่ที่เกี่ยวข้องกับโครงการวิจัย ท่านจะได้รับแจ้งข้อมูลนั้นโดยผู้วิจัยหรือผู้วิจัยร่วมนั้นทันที

APPENDIX B

Informed Consent

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย (Informed Consent)

รับรองโดยคณะอนุกรรมการพิจารณาโครงการวิจัย พบ.

ชื่อโครงการวิจัย ความสัมพันธ์ระหว่างความเข้มข้นของยาโทพิราเมตในซีรัมและน้ำลายของผู้ป่วยโรค ลมชักชาวไทย

วันที่ลงนาม.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่คาดว่าจะเกิดขึ้นจากการวิจัยอย่างละเอียดและมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามที่ข้าพเจ้าสงสัยด้วยความเต็มใจและไม่ปิดบังซ้อนเร้น จนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการวิจัยนี้ด้วยความสมัครใจ โดยปราศจากการบังคับหรือชักจูง

ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมในโครงการวิจัยเมื่อใดก็ได้ และการบอกเลิกนี้จะไม่มีผลต่อการรักษาพยาบาลที่ข้าพเจ้าจะพึงได้รับในปัจจุบันและในอนาคต

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยเฉพาะในรูปของสรุปผลการวิจัยโดยไม่มีการระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าจะได้รับเอกสารชี้แจงและหนังสือยินยอมที่มีข้อความเดียวกันกับที่นักวิจัยเก็บไว้ เป็นส่วนตัวข้าพเจ้าเอง 1 ชุด

ข้าพเจ้าได้รับทราบข้อความข้างต้นแล้ว และลงนามในใบยินยอมด้วยความเต็มใจ

ลงชื่อ.....ผู้เข้าร่วมโครงการวิจัย

(……..)

ลงชื่อ.....ผู้ดำเนินโครงการวิจัย

(……..)

ลงชื่อ.....พยาน

(……..)

ลงชื่อ.....พยาน

(……..)

ในกรณีที่ผู้เข้าร่วมในโครงการวิจัย อายุน้อยกว่า 18 ปี / ไม่สามารถตัดสินใจได้ด้วยตนเอง และ/หรือ ไม่สามารถลงลายมือชื่อด้วยตนเอง

ข้าพจ้า.....ในฐานะ เป็น.....(ผู้แทนโดยชอบธรรม / ผู้ปกครอง / บิดา-มารดา / สามี-ภรรยา)ของ.....อนุญาตให้..... เข้าร่วมในโครงการวิจัยในครั้งนี้

ลงชื่อ.....ผู้แทนโดยชอบธรรม / ผู้ปกครอง / บิดา-มารดา / สามี-ภรรยา)

(.....)

ลงชื่อ.....พยาน

(.....)

ลงชื่อ.....พยาน

(.....)

APPENDIX C

Case Record Form

Case Record Form (1)

ลำดับที่..... วันที่.....เดือน.....พ.ศ. .....

ชื่อ.....นามสกุล.....

อายุ.....ปี น้ำหนัก.....กิโลกรัม

Inclusion criteria

1. ท่านมีอายุระหว่าง 15-60 ปี ใช่ ไม่ใช่
2. ได้รับยาโทพิราเมต ใช่ ไม่ใช่
3. ไม่อยู่ในระหว่างการตั้งครรภ์หรือให้นมบุตร ใช่ ไม่ใช่
4. ไม่เป็นโรคที่มีผลต่อการหลั่งน้ำลาย ดังนี้
   - 4.1 Cystic fibrosis ใช่ ไม่ใช่
   - 4.2 Multiple sclerosis ใช่ ไม่ใช่
   - 4.3 Graft-versus-host disease ใช่ ไม่ใช่
   - 4.4 Diabetes mellitus ใช่ ไม่ใช่
   - 4.5 Alcoholic liver cirrhosis ใช่ ไม่ใช่
   - 4.6 Acquired human immunodeficiency syndrome ใช่ ไม่ใช่
   - 4.7 Burning mouth syndrome ใช่ ไม่ใช่
   - 4.8 Kidney dysfunction ใช่ ไม่ใช่
5. ไม่รับประทานยาอื่นๆที่มีผลต่อการหลั่งน้ำลาย (ก่อนการเก็บตัวอย่าง อย่างน้อย 1 สัปดาห์) ดังนี้
   - 5.1 Analgesic ใช่ ไม่ใช่ 5.2 Antidepressives ใช่ ไม่ใช่
   - 5.3 Antihypertensives ใช่ ไม่ใช่ 5.4 Cytotoxics ใช่ ไม่ใช่
   - 5.5 Diuretics ใช่ ไม่ใช่ 5.6 Antiarrhythmics ใช่ ไม่ใช่
   - 5.7 Mono-amine-oxidaes inhibitors ใช่ ไม่ใช่ 5.8 Anti nausea agent ใช่ ไม่ใช่
   - 5.9 Antihistamines ใช่ ไม่ใช่ 5.10 Decongestives ใช่ ไม่ใช่
   - 5.11 Anti-spasmodics ใช่ ไม่ใช่

Exclusion criteria

มีอาการข้างเคียงจากยาหรือเกิดอาการผิดปกติต่างๆก่อนการวิจัย ใช่ ไม่ใช่
หรือในระหว่างการวิจัย

6. ผลการตรวจร่างกาย

6.1 General appearance ( ) normal ( ) abnormal……..

6.2 Vital signs BP…….mmHg PR……../min RR……/min BT..... ^{o}C

6.3 Body weight …… Kgs

6.4 HEENT ( ) normal ( ) abnormal……..

6.5 Neck ( ) normal ( ) abnormal……..

6.6 Heart ( ) normal ( ) abnormal……..

6.7 Chest ( ) normal ( ) abnormal……..

6.8 Abdomen ( ) normal ( ) abnormal……..

6.9 Extremities ( ) normal ( ) abnormal……..

7. ผลการตรวจทางห้องปฏิบัติการ

7.1 Hematology

Hemoglobin……..g/dl Hematocrit……%

WBC…….cell/μl Neutrophil……%

Lemphocyte……..%

Monocyte……..%

Eosinophil…….%

Basophil……%

Platelets…….cell/μl

7.2 Boold chemistry

BUN……..mg/dl

Cr……...mg/dl

Liver function test Total bilirubin……..mg/dl

SGOT……...U/L

SGPT……...U/L

Alkaline phosphatase……...U/L

Total protein……g/dl

Albumin……..g/L

สรุปผลการตรวจ

( ) อยู่ในเกณฑ์ปกติ

( ) ผิดปกติ แต่สามารถเข้าร่วมโครงการวิจัยได้

( ) ผิดปกติ ไม่สามารถเข้าร่วมโครงการวิจัยได้

ผู้บันทึก.....

วันที่.....เดือน.....พ.ศ.....

Case Record Form (2)

ข้อมูลทั่วไป

ชื่อ_____ HA. _____ แพทย์_____

เพศ ☐ ชาย ☐ หญิง อายุ_____ปี _____ เดือน น้ำหนัก_____กก.

ที่อยู่_____ โทร. _____

ผู้แทนโดยชอบธรรม_____ อายุ_____ปี เกี่ยวข้องเป็น_____

ผู้มีหน้าที่ดูแลเรื่องยาของผู้ป่วยคือ_____

ประวัติการชักในครอบครัว ☐ ไม่มี ☐ มี_____

อายุเมื่อเริ่มชักครั้งแรก_____ปี_____เดือน รักษามานาน_____ปี_____เดือน

Diagnosis_____ ปัญหาอื่นๆ_____

ชนิดของอาการชัก ☐simple partial ☐ complex partial ☐ partial to 2° Gen.

☐ Gen. Tonic ☐ Gen. Clonic ☐ GTC

☐ Mixed type ระบุ_____

ปัจจัยกระตุ้นให้เกิดชัก 1. มีไข้ 2. ขาดยา 3. อดนอน 4. อื่นๆ

ความถี่ของการเกิดอาการชัก_____ครั้ง/วัน _____ ครั้ง/สัปดาห์ _____ ครั้ง/เดือน

ระยะเวลานานที่สุดที่เคยชัก _____ นาที

ประวัติแพ้ยากันชัก ☐ ไม่มี ☐ มี_____

ผล EEG ☐ ปกติ ☐ ผิดปกติ_____

	Date		
Serum albumin	3.5-5.5 g/dl		
Scr	0.5-2.0 mg/dl		
BUN	10-15 mg/dl		
AST	8-40 unit		
ALT	5-35 unit		
Serum bicarbonate			

Date								
Memory impairment								
Poor judgement								
Confusion								
Fatigue								

Anorexia								
Cardiac arrhythmias								
Stupor								
orther								

Topiramate Dosage Regimen			
Date	Regimen	จำนวน	วันนัด

Non-prescription drug	
Date	รายการ

Compliance assessment						
	Pill count			Self report		
Date	จำนวนที่ต้องใช้	จำนวนยาที่เหลือ	%	Doseที่ต้องกิน	Doseที่ลืมกิน	%

Sample Collection				
	Date / Time			
ครั้งที่				
Last dose				
Blood collection				
Saliva collection				
Analysis				
Serum level				
Saliva level				

APPENDIX D

Education leaflet

ข้อปฏิบัติสำหรับผู้เข้าร่วมโครงการตรวจวัดระดับยาโทพิราเมตในเลือดและน้ำลาย

1. ***รับประทานยาทุกวัน ไม่ควรลืมทานยา*** หากลืมทานยาเมื่อใด ให้ทานทันทีที่นึกได้
2. เมื่อรับประทานยาแล้วบันทึกลงในปฏิทินการรับประทานยาทุกครั้ง
3. เมื่อเกิดอาการชักขึ้นบันทึกลงปฏิทินทุกครั้ง
4. สังเกตว่ามีอาการข้างเคียงจากยาหรือ เช่น

หากมีอาการดังกล่าว ควรหยุดยา และมาพบแพทย์ทันที

<u>ก่อนมาพบแพทย์ จะต้อง</u>

- **นำกล่องยาพร้อมขวดยาทั้งหมดมาด้วย**
- **เฉพาะวันที่แพทย์นัดเพื่อเจาะเลือด <u>ผู้ป่วยอย่าเพิ่งรับประทานยาตอนเช้า</u>**
- **ก่อนมาพบแพทย์ อย่างน้อย 1 สัปดาห์ ควรระมัดระวังอย่าลืมทานยาหรือทานผิดเวลา**
- **ก่อนมาพบแพทย์ อย่างน้อย 1 สัปดาห์ ไม่ควรรับประทานยาอื่นนอกเหนือจากยาที่แพทย์สั่ง หากจำเป็นต้องใช้ ควรจดบันทึกชื่อยาและแจ้งให้แพทย์ทราบ**

APPENDIX E

Topiramate Concentration Record Form

Topiramate concentration record from (group I)

ลำดับที่.....

ชื่อ.....นามสกุล.....

เวลาที่รับประทานยา.....

เวลาที่เก็บตัวอย่าง	เลือด	น้ำลาย	ระดับยาโทพิราเมต (μg/ml)	
			ซีรัม	น้ำลาย
ครั้งที่ 1 (ก่อนรับประทานยา)				
ครั้งที่ 2 (1 ชม. หลังรับประทานยา)				
ครั้งที่ 3 (2 ชม. หลังรับประทานยา)				
ครั้งที่ 4 (4 ชม. หลังรับประทานยา)				
ครั้งที่ 5 (6 ชม. หลังรับประทานยา)				
ครั้งที่ 6 (8 ชม. หลังรับประทานยา)				
ครั้งที่ 7 (12 ชม. หลังรับประทานยา)				

APPENDIX F

Package Insert of Topiramate Reagent Kit

0155227
February, 2008

QMS® TOPIRAMATE
Immunoassay

This Quantitative Microsphere System (QMS) package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service
United States:1-866-737-2396
International:1-317-610-3800
Fax:1-317-610-3888

Key to Symbols Used

IVD	in vitro Diagnostic Medical Device		Caution: Consult Accompanying Documents
LOT	Batch Code/Lot Number		Consult Instructions for Use
R1	Reagent 1	Min Max	Temperature Limitation
R2	Reagent 2		Use by/Expiration Date
REF	Catalog Number	EC REP	Authorized Representative in the European Community
INGRED	Ingredients		Manufacturer
CONC	Concentration		
CONTENTS	Contents of kit		

CE
EC REP

Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10

TOPIRAMATE REAGENTS
REF 0374140

Thermo SCIENTIFIC

NAME

QMS Topiramate **(TPM)**

INTENDED USE

The QMS Topiramate assay is intended for the quantitative determination of topiramate in human serum or plasma on automated clinical chemistry analyzers.

The results obtained are used in the diagnosis and treatment of topiramate overdose and in monitoring levels of topiramate to help ensure appropriate therapy.

SUMMARY AND EXPLANATION OF TEST

Topiramate (2,3:4,5-Di-*O*-isopropylidene-ß-D-fructopyranose) is an anticonvulsant drug approved for use in the treatment of epilepsy and is often prescribed as monotherapy or as one component of a multiple anti-epileptic drug therapy.[1]

PRINCIPLES OF THE PROCEDURE

The QMS Topiramate assay is a homogeneous particle-enhanced turbidimetric immunoassay. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the topiramate antibody reagent. The topiramate-coated microparticle reagent is rapidly agglutinated in the presence of the anti-topiramate antibody reagent and in the absence of any competing drug in the sample. The rate of absorbance change is measured photometrically. When a sample containing topiramate is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic agglutination inhibition curve can be obtained with maximum rate of agglutination at the lowest topiramate concentration and the lowest agglutination rate

at the highest topiramate concentration.

REAGENTS

Reagent Kit

QMS Topiramate, REF 0374140, is supplied as a liquid, ready-to-use, two-reagent kit that contains:

R1	Reagent 1	1 x 22 mL
R2	Reagent 2	1 x 16 mL

Reactive Ingredients

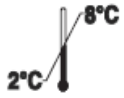
	Ingredient	Concentration
R1	Anti-topiramate Polyclonal Antibody (Sheep)	<5.0%
	Sodium Azide	<0.1%
R2	Topiramate-coated Microparticles	<1.0%
	Sodium Azide	<0.1%

REAGENT HANDLING AND STORAGE

- R1 and R2 Ready for Use.
- Before use, invert several times, avoiding the formation of bubbles.
- Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

- When either the R1 or the R2 reagent cartridge becomes empty, replace both cartridges and verify calibration with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.



The unopened reagents are stable until the expiration date when stored at 2 to 8°C.

Do not freeze reagents or expose them to temperatures above 32°C.

WARNINGS AND PRECAUTIONS

Precautions for Users

- **For in vitro Diagnostic Use.**
- Do not mix materials from different kit lot numbers.

CAUTION: This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, anti-HIV 1/2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

SPECIMEN COLLECTION AND HANDLING

- The following specimen collection tubes may be used for the

QMS Topiramate assay:

	Glass	Plastic
Serum	• No Additive	• Serum Separator Tube (gel) • No Additive
Plasma	• EDTA (K_3)	• EDTA (K_2) • Lithium Heparin • Plasma Separator Tube with Lithium Heparin (gel) • Sodium Heparin

Other specimen collection tubes have not been tested for use with the QMS Topiramate assay. Follow the manufacturer's processing instruction for serum or plasma collection tubes.

- Inadequate centrifugation of the specimen may cause an erroneous result.
- Ensure specimens are free of fibrin, red blood cells, and other particulate matter.
- Remove the plasma or serum from the cells, clot, or gel as soon as possible after collection. Some gel separator tubes may not be suitable for use with therapeutic drug monitoring assays; refer to information provided by the tube manufacturer.[2]
- Specimens removed from the cells, clot, and gel may be stored up to one week at 2 to 8°C. If testing will be delayed more than one

week, specimen should be stored frozen (≤-10°C) prior to being tested. Specimen frozen up to two weeks showed no performance differences from fresh samples. Care should be taken to limit number of freeze-thaw cycles.

PROCEDURE

Materials Provided

- QMS Topiramate Reagents, REF 0374140

Materials Required but not Provided

- QMS Topiramate Calibrators, REF 0374173
- CAL A-F: 1 × 1.0 mL each
- QMS Topiramate Controls, REF 0374181
- Levels 1-3: 1 x 2.0mL each

Assay Procedure

For a detailed description of how to run and calibrate an assay, refer to the instrument specific operations manual.

Specimen Dilution Procedures

Use QMS Topiramate CAL A (0.0 µg/mL) to manually dilute samples outside the reportable range of the assay.

Manual Dilution Protocol

A manual dilution can be performed on patient samples with topiramate concentrations reported as greater than 32.0 µg/mL by making a dilution of the specimen with QMS Topiramate CAL A (0.0 µg/mL) before pipetting the sample into the sample cup. The dilution must be performed so the diluted test results read greater than the assay sensitivity of 1.5 µg/mL. The concentration reported must be multiplied by the manual dilution

factor to obtain the final sample concentration.

$$\text{Final Sample Concentration} = \text{Reported Concentration} \times \text{Manual Dilution Factor}$$

$$\text{Manual Dilution Factor} = \frac{(\text{Volume of Sample} + \text{Volume of CAL A})}{\text{Volume of Sample}}$$

CALIBRATION

The QMS Topiramate assay must be calibrated using a full calibration (6-point) procedure. To perform a full calibration, test the QMS Topiramate Calibrators A, B, C, D, E, and F in duplicate.

Calibration is required with each new lot number. Verify the calibration curve with at least two levels of controls according to the established Quality Control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

Note: QMS Topiramate CAL A is the calibration blank for this assay.

QUALITY CONTROL

As appropriate, refer to your laboratory Standard Operating Procedure(s) and/or Quality Assurance Plan for additional quality control requirements and potential corrective actions.

Recommended control requirements for the QMS Topiramate assay:

- A minimum of two levels of controls spanning the medical decision range should be run every 24 hours.
- If more frequent control monitoring is required, follow the established Quality Control procedures for your laboratory.
- If quality control results do not fall within an acceptable range

defined by your laboratory, patient values may be suspect and corrective action should be taken.

RESULTS

The result unit for the QMS Topiramate assay can be reported as µg/mL or µmol/L. To convert results from µg/mL topiramate to µmol/L topiramate, multiply µg/mL by 2.95.

As with all analyte determinations, the topiramate value should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

Result Error Codes

Some results may contain Result Error Codes. Refer to the instrument specific operations manual for a description of the error codes.

LIMITATIONS OF THE PROCEDURE

Interfering heterophile antibodies occur at a low frequency in the general population. These antibodies can cause autoagglutination of the microparticle reagent leading to undetected erroneously low results.

For diagnostic purposes, the test findings should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

See the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

A therapeutic range for topiramate has not been well established. Some reports in the literature suggest a target range for steady-state concentrations of 2 to 25µg/mL.[3] Inconsistent correlation exists between

levels of circulating topiramate to toxicity, adverse affect or clinical efficacy.[3] Therefore, monitoring topiramate concentration in patients is warranted.

Topiramate drug concentrations should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures. Clinicians should carefully monitor patients during therapy initiation and dosage adjustments. It may be necessary to obtain multiple samples to determine expected variations of optimal (steady-state) concentrations for individual patients.

SPECIFIC PERFORMANCE CHARACTERISTICS

Sensitivity

Limit of Quantitation (LOQ)

The LOQ of the QMS Topiramate assay is defined as the lowest concentration for which acceptable inter-assay precision and recovery is observed (often considered ≤±20% CV with ≤±15% recovery). The LOQ was determined to be 1.5 µg/mL.

Assay Range

The range of the assay is 1.5 µg/mL to 32.0 µg/mL. Report results below this range as <1.5µg/mL.

Accuracy

An accuracy-by-recovery was performed by adding high purity topiramate drug into human serum negative for topiramate. Initially, a serum stock of 32.00 µg/mL topiramate was prepared gravimetrically by adding topiramate to human serum. The stock concentrate was then volumetrically added to human serum negative for topiramate, representing drug concentrations across the assay range. Each sample was assayed in triplicate on an automated clinical chemistry analyzer.

The results were averaged and compared to the target concentration and percent recovery calculated. Results are shown below.

$$\% \text{ Recovery} = \frac{\text{Mean recovered concentration}}{\text{Theoretical concentration}} \times 100$$

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Percent Recovery
32.00	32.48	101.5
24.00	24.50	102.1
16.00	16.74	104.6
8.00	8.35	104.4
6.40	6.61	103.3
3.20	3.47	108.4
2.56	2.67	104.3
1.92	2.11	109.9
1.60	1.65	103.1
1.28	1.33	103.9

Mean percent recovery: 104.6

Linearity

Linearity studies were performed by diluting a high patient pool to concentrations across the assay range. The patient pool was adjusted in order to obtain a 20 to 30% value above the desired reportable range as suggested in NCCLS Protocol EP6-A.[4] The dilutions were made with QMS Topiramate Calibrator A (blank calibrator). Linearity at specific dilutions was considered acceptable if the percent difference was ±10% between the predicted 1st and 2nd order regressed values. Results are shown below.

Estimated Value (µg/mL)	Dilution Factor	Results (µg/mL)	1st Order Predicted Results	2nd Order Predicted Results	Percent Difference (Acceptance Criteria: ±10%)
35.0	0.8750	36.57	36.76	36.78	-0.04%
30.0	0.7500	31.87	31.52	31.53	0.00%
20.0	0.5000	20.86	21.05	21.04	0.06%
15.0	0.3750	15.89	15.82	15.80	0.09%
10.0	0.2500	10.54	10.58	10.57	0.10%
5.0	0.1250	5.28	5.35	5.34	0.06%
3.0	0.0750	3.11	3.25	3.25	-0.02%
2.0	0.0500	2.22	2.20	2.21	-0.13%
1.5	0.0375	1.68	1.68	1.68	-0.25%
1.2	0.0300	1.43	1.37	1.37	-0.36%

Method Comparison

Correlation studies were performed using NCCLS Protocol EP9-A2.[5] Results from the QMS Topiramate assay were compared with results from a commercially available FPIA Immunoassay. The topiramate concentrations ranged from 1.56µg/mL to 30.72µg/mL. Results of the Passing-Bablok[6] regression analysis for the study are shown below.

Slope	0.962
y-intercept	0.228
Correlation Coefficient (R^2)	0.986
Number of Samples	148

Precision

Precision was determined as described in NCCLS Protocol EP5-A2.[7]

A tri-level human serum based commercial control containing topiramate was used in the study. Each level of control was assayed in duplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The within run, between day, total SD, and percent CVs were calculated. Results are shown below.

Sample	N	Mean (µg/mL)	Within Run SD	Within Run CV (%)	Between Day SD	Between Day CV (%)	Total SD	Total CV (%)
1	80	2.94	0.08	2.77	0.06	2.10	0.12	4.22
2	80	10.14	0.18	1.83	0.23	2.34	0.34	3.37
3	80	25.69	0.82	3.23	0.73	2.87	1.14	4.44

Acceptance criteria: <10% total CV

Interfering Substances

Interference studies were conducted using NCCLS Protocol EP7-A2[8] as a guideline. Clinically high concentrations of the following potential interferents were added to serum with known levels of topiramate (approximately 5 and 20 µg/mL). Each sample was assayed using the QMS Topiramate assay, along with a serum control of topiramate. All substances resulted in ≤ ±10% error in detecting topiramate. The results are shown below.

Interfering Substance	Interferent Concentration
Albumin	12 g/dL
Bilirubin	70 mg/dL
Cholesterol	250 mg/mL
Gamma-Globulin	12 g/dL
HAMA Type-1*	normal human level
HAMA Type-2*	normal human level
Hemoglobin	1000 mg/dL
Heparin	185.5 USP/mL

*HAMA = human anti-mouse antibodies

Interfering Substance	Interferent Concentration
Rheumatoid Factor	500 IU/mL
Triglycerides	825 mg/dL
Uric Acid	30 mg/dL

*HAMA = human anti-mouse antibodies

Specificity

Cross-reactivity was tested for the known metabolites of topiramate. Other medications routinely administered with topiramate were also tested to determine whether these compounds affect the quantitation of topiramate concentrations using the QMS Topiramate assay. High levels of these compounds were spiked into serum pools containing low and high therapeutic levels of topiramate. The samples were analyzed and the topiramate concentrations of samples containing interferent were compared to the control serum.

Metabolites

Metabolites of topiramate are found primarily in urine of patients being administered topiramate therapy.[9,10] They are not however seen at clinically significant levels in plasma or serum. The QMS topiramate assay serum and plasma results are unlikely to be affected by metabolism of topiramate drug. The following metabolite was tested for cross-reactivity.

Metabolite	Metabolite Conc (µg/mL)	Low Concentration	High Concentration	Low Concentration	High Concentration
		Percent Cross-Reactivity		Percent Interference	
9-Hydroxy topiramate	4.00	19.75	14.50	18.33	2.55
	8.00	22.63	12.50	44.03	4.49
	32.00	15.56	18.25	137.19	30.62

Drug Interference

Studies using the QMS Topiramate assay were conducted to examine if any of the commonly administered compounds have any effect on the recovery of topiramate concentration. A high concentration of each compound was spiked into normal human serum with known levels of topiramate (approximately 5 and 20µg/mL) and assayed along with a serum control of topiramate. All compound resulted in ≤±10% error in detecting topiramate. The results are shown below.

Compound	Compound Concentration (µg/mL)	Compound	Compound Concentration (µg/mL)
Acetaminophen	31	Lamotrigine	45
Acetozolamide	40	Levetiracetam	124
Alprazolam	2.0	Methysergide	5.2
Amitriptyline	1.0	Metoprolol	5.25
Acetylsalicylic acid	67	Nadolol	121
Atenolol	10.33	Naproxen	509
Caffeine	60	Nimodipine	75
Carbamazepine	30	Nortriptyline	1.0
Chlorthalidone	64	Phenelzine	14.38
Clonazepam	0.18	Phenobarbital	40
Clorazepate	2.0	Primidone	40
Diazepam	5.1	Protriptyline	1.03
Dichlorphenamide	32	Salicylic Acid	598
Ethosuxamide	252	Sulfanilamide	1500
Famotidine	0.97	Tolbutamide	642
Felbamate	243.33	Valproic Acid	100.67
Flurazepam	17.5	Verapamil	1.6
Furosemide	3.7	Viagabatrin	112
Gabapentin	93	Zonisamide	122
Hydrochlorothiazide	6.0		

Drugs that Cross-React

The cross-reactivity of the antibody to ibuprofen, phenytoin and tiagabine at the following concentrations were tested. A high concentration of each compound was spiked into normal human serum with known levels of topiramate (approximately 5 and 20µg/mL) and assayed along with a serum control of topiramate. The results are shown below.

Compound	Conc (µg/mL)	Low Concentration	High Concentration	Low Concentration	High Concentration
		Percent Cross-Reactivity		Percent Interference	
Ibuprofen	500	0.09	0.19	11.38	4.39
Phenytoin	20	5.48	2.25	28.14	4.86
Tiagabine	250	0.49	-0.50	29.60	5.35

Care should be taken when interpreting QMS Topiramate results if any of the above compounds are being administered to the patient.

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TRADEMARKS

Seradyn, Inc.
7998 Georgetown Road
Indianapolis, IN 46268 USA

0155234 February, 2008

QMS® TOPIRAMATE
Immunoassay

This Quantitative Microsphere System (QMS) package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service
United States:1-866-737-2396
International:1-317-610-3800
Fax:1-317-610-3888

Key to Symbols Used

IVD	in vitro Diagnostic Medical Device		Caution: Consult Accompanying Documents
LOT	Batch Code/Lot Number		Consult Instructions for Use
CAL A-F	Calibrators A through F	Max / Min	Temperature Limitation
REF	Catalog Number		Use by/Expiration Date
INGRED	Ingredients	EC REP	Authorized Representative in the European Community
CONC	Concentration		Manufacturer
CONTENTS	Contents of kit		

CE
EC REP

Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10

TOPIRAMATE CALIBRATORS
REF 0374173

Thermo SCIENTIFIC

NAME

QMS Topiramate **(TPM)** Calibrators

INTENDED USE

The QMS Topiramate Calibrator set is intended for use in calibration of the QMS Topiramate assay.

CONTENTS

The QMS Topiramate Calibrator set consists of human serum and <0.1% sodium azide as preservative with the following concentrations of topiramate:

Vial	Concentration (µg/mL)	Quantity	Fill Volume
A	0.0	1	1.0 mL
B	2.0	1	1.0 mL
C	4.0	1	1.0 mL
D	8.0	1	1.0 mL
E	16.0	1	1.0 mL
F	32.0	1	1.0 mL

STANDARDIZATION

There is no internationally recognized standard for Topiramate. The QMS Topiramate Calibrators are prepared by gravimetric dilution of high purity topiramate into human serum free of topiramate.

WARNINGS AND PRECAUTIONS

Precautions for Users

- **For in vitro Diagnostic Use.**
- The calibrators in this set are designed for use as a unit. Do not substitute or mix calibrators with those from other lots.

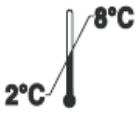
CAUTION: This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, anti-HIV 1/2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

INSTRUCTIONS FOR USE

- Refer to the QMS Topiramate assay package insert, included in the reagent kit, for a complete summary and explanation of the test.
- Calibrators may be used immediately upon removal from 2 to 8°C storage.
- Mix each calibrator by gentle inversion several times before dispensing.
- Carefully squeeze at least four (4) drops of each calibrator into the individual sample cups, avoiding the formation of bubbles.
- After each use, tightly close the caps and return calibrators to 2 to 8°C.

CAUTION: Bubbles may interfere with proper detection of calibrator level in the sample cup, causing insufficient calibrator aspiration that could impact results.

STORAGE AND STABILITY



- Do not expose the calibrators to temperatures above 32°C. Improper storage of calibrators can affect assay performance.
- After first use, the calibrators are stable until the expiration date when stored tightly capped at 2 to 8°C.
- Do not use the calibrators beyond the expiration date.

INDICATIONS OF INSTABILITY OR DETERIORATION

Instability or deterioration should be suspected if there are visible signs of leakage, turbidity, microbial growth, or if the assay does not meet the reagent package insert and/or instrument-specific operations manual criteria.

LIMITATIONS OF THE PROCEDURE

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, storage of product as directed, and good laboratory technique.

TRADEMARKS

Seradyn, Inc.
7998 Georgetown Road
Indianapolis, IN 46268 USA

01552241
February, 2008

QMS® TOPIRAMATE
Immunoassay

This Quantitative Microsphere System (QMS) package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Customer Service

United States:1-866-737-2396

International:1-317-610-3800

Fax:1-317-610-3888

Key to Symbols Used

IVD	in vitro Diagnostic Medical Device		Caution: Consult Accompanying Documents
LOT	Batch Code/Lot Number		Consult Instructions for Use
CONTROL 1-3	Controls 1 through 3	Max / Min	Temperature Limitation
REF	Catalog Number		Use by/Expiration Date
INGRED	Ingredients	EC REP	Authorized Representative in the European Community
CONC	Concentration		Manufacturer
CONTENTS	Contents of kit		

CE

EC REP

Microgenics GmbH
Spitalhofstrasse 94
D-94032 Passau
Tel: +49 (0) 851 886 89 0
Fax: +49 (0) 851 886 89 10

TOPIRAMATE CONTROLS

REF **0374181**

Thermo SCIENTIFIC

NAME

QMS Topiramate **(TPM)** Controls

INTENDED USE

The QMS Topiramate Control set is intended for use in quality control of the QMS Topiramate assay.

CONTENTS

The QMS Topiramate Control set consists of human serum with the following control levels of topiramate:

Vial	Control Level	Quantity	Fill Volume
Level 1	Low	1	2.0 mL
Level 2	Medium	1	2.0 mL
Level 3	High	1	2.0 mL

Each laboratory should establish its own ranges for each new lot of controls. See enclosed control-range card for each specific lot.

WARNINGS AND PRECAUTIONS

Precautions for Users

- **For in vitro Diagnostic Use.**
- The controls in this set are designed for use as a unit. Do not substitute or mix controls with those from other lots.

CAUTION: This product contains human sourced and/or potentially infectious components. Components sourced from human blood have been tested and found to be nonreactive for HBsAg, anti-HIV 1/2, and anti-HCV. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, it is recommended that all human sourced materials be considered potentially infectious and handled with appropriate biosafety practices.

INSTRUCTIONS FOR USE

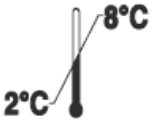
- Refer to the QMS Topiramate assay package insert, included in the reagent kit, for a complete summary and explanation of the test.
- Controls may be used immediately upon removal from 2 to 8°C storage.

  Note: Upon first use, controls must be thoroughly thawed.
- Mix each control by gentle inversion several times before dispensing.
- After each use, tightly close the caps and return controls to 2 to 8°C.

CAUTION: Bubbles may interfere with proper detection of control level in the sample cup, causing insufficient control aspiration that could impact results.

STORAGE AND STABILITY

- Controls must be stored frozen (≤-10°C) until first use.
- After first use, store controls tightly capped at 2 to 8°C. **Do not refreeze.**
- Do not expose the controls to temperatures above 32°C. Improper storage of controls can affect assay performance.



- Controls are stable at 2 to 8°C for 16 weeks after thawing.
- Do not use the controls beyond the expiration date.

INDICATIONS OF INSTABILITY OR DETERIORATION

Instability or deterioration should be suspected if there are visible signs of leakage, turbidity, microbial growth, or if the assay does not meet the reagent package insert and/or instrument-specific operations manual criteria.

LIMITATIONS OF THE PROCEDURE

Accurate and reproducible results are dependent upon properly functioning instruments, reagents, calibrators, storage of product as directed, and good laboratory technique.

TRADEMARKS

Seradyn, Inc.
7998 Georgetown Road
Indianapolis, IN 46268 USA

APPENDIX G

Demographic data of all patients in study

Table 18 Demographic characteristics of each participating patient

No	Sex	Age (year)	Weight (kg)	FH. With seizure	Dose (mg/day)	AEDs combination	Alb (g/dl)	AST (U/L)	ALT (U/L)	BUN (mg/dl)	Scr (mg/dl)	WBC (cells/cumm)	RBC (million cells/cumm)	Hb (g/dl)	Hct (%)	Plt cells/cumm
1	F	40	56	NO	300	-	4.6	20	12	16	0.9	-	-	-	-	-
2	F	48	60	NO	100	-	4.6	33	40	16	0.6	5,410	4.74	13.8	42	230,000
3	F	43	51	NO	200	-	4.3	20	13	16	0.8	7,100	4.73	12.6	38	145,000
4	M	26	48	NO	75	-	4.7	21	24	11	1.1	4,070	5.74	15.2	45	231,000
5	M	24	43	NO	100	-	4.3	20	23	14	0.8	6,100	4.36	13.5	39	156,000
6	F	24	48	NO	100	-	4.0	20	15	16	0.6	5,600	4.28	13.3	36	162,000
7	F	53	65	NO	50	-	4.5	25	16	13	0.9	-	-	-	-	-
8	F	30	60	NO	100	-	4.3	27	30	10	0.8	7,550	4.36	13.0	39	230,000
9	F	42	52	NO	25	-	4.5	17	15	18	0.9	5,380	4.41	13.6	42	185,000
10	M	32	67	NO	200	-	4.7	26	23	11	1.0	5,260	5.33	16.2	47	230,000
11	F	27	52	NO	100	CBZ	4.3	23	20	16	0.8	5,310	4.0	12.0	38	280,000
12	M	25	45	NO	250	VPA,CBZ	4.7	22	14	18	1.2	4,530	4.58	14.6	42	147,000
13	F	49	50	NO	100	OXC,CBZ	4.2	21	24	11	0.6	5,630	3.38	12.4	37	266,000
14	F	53	49	NO	125	LEV	4.5	17	11	13	0.6	4,880	4.02	11.7	36	287,000
15	F	24	47	NO	75	-	4.0	20	22	14	0.8	5,500	3.9	11.6	42	252,000
16	M	52	68	NO	200	VPA,CBZ	4.6	23	18	9	0.9	4,950	4.65	14.6	43	238,000

Table 18 Demographic characteristics of each participating patient (cont.)

No	Sex	Age (year)	Weight (kg)	FH. With seizure	Dose (mg/day)	Drugs combination	Alb (g/dl)	AST (U/L)	ALT (U/L)	BUN (mg/dl)	Scr (mg/dl)	WBC (cells/cumm)	RBC (million cells/cumm)	Hb (g/dl)	Hct (%)	Plt cells/cumm
17	M	45	62	NO	200	LEV	4.8	16	22	14	1.1	-	-	-	-	-
18	M	52	56	NO	50	-	4.2	20	1	16	1.2	6,320	4.0	11.0	35	158,000
19	F	38	52	YES	50	-	4.0	21	15	12	0.9	5,610	3.67	11.6	41	265,000
20	F	42	58	NO	100	VPA,CBZ	4.3	16	12	10	0.8	6,690	4.48	13.0	39	303,000
21	F	26	55	NO	50	CBZ,LEV,VPA	4.4	21	13	20	0.7	-	-	-	-	-
22	F	27	48	YES	50	LEV, OXC	4.5	21	17	14	0.7	2,810	4.08	10.7	34	250,000
23	F	53	62	NO	100	-	4.6	25	13	13	0.7	5,090	3.77	12.3	37	119,000
24	F	39	50	NO	100	VPA	4.2	23	15	14	1.2	5,530	3.65	12.0	38	216,000
25	M	31	65	NO	400	OXC, LEV	5.3	26	18	8	0.9	4,140	4.62	14.3	41	267,000
26	F	46	62	NO	100	VPA,CBZ	4.5	26	15	12	1.0	6,360	4.8	13.2	46	246,000
27	M	48	57	NO	150	VPA,OXC	4.8	29	18	12	1.0	10,580	4.92	14.6	45	263,000
28	M	38	65	NO	200	VPA,CBZ	4.3	23	18	11	0.9	5,630	4.3	12.6	40	272,000
29	F	41	56	NO	100	CBZ,OXC	4.9	20	12	7	0.6	-	-	-	-	-
30	M	48	67	NO	200	VPA,CBZ	4.7	40	36	16	1.4	-	-	-	-	-
31	M	52	70	NO	150	VPA	4.7	27	41	7	0.8	8,190	4.14	13.7	40	368,000
32	F	35	68	NO	100	VPA, OXC	4.9	27	24	15	0.7	6,310	4.67	12.7	40	321,000

Table 18 Demographic characteristics of each participating patient (cont.)

No	Sex	Age (year)	Weight (kg)	FH. With seizure	Dose (mg/ day)	Drugs combination	Alb (g/dl)	AST (U/L)	ALT (U/L)	BUN (mg/dl)	Scr (mg/dl)	WBC (cells/cumm)	RBC (million cells/cumm)	Hb (g/dl)	Hct (%)	Plt cells/cumm
33	M	28	63	NO	150	CBZ,PHT	3.8	24	18	11	0.6	5,430	4.1	11.6	38	261,000
34	F	26	55	NO	100	PHT	4.8	22	31	10	0.5	6,720	4.67	13.8	43	255,000
35	M	19	48	NO	100	PHT	4.7	34	21	8	1.0	6,540	4.59	14.2	43	244,000
36	M	42	57	NO	200	VPA,CBZ	4.3	33	36	20	1.4	4,990	5.47	14.6	46	190,000
37	F	17	46	NO	300	VPA,CBZ	4.8	19	15	18	0.7	4,850	4.35	13.7	43	332,000
38	F	56	48	NO	100	OXC	4.1	18	8	11	0.8	-	-	-	-	-
39	M	36	61	NO	250	CBZ,PHT	5.1	18	15	11	1.0	9,010	5.00	13.6	41	255,000
40	F	44	52	NO	50	VPA,LEV	3.9	34	13	11	1.1	3,390	3.98	13.3	41	88,000
Mean	M =16 F = 24	38.03	56.35	YES= 2 NO= 38	136.25		4.49	23.45	19.80	13.08	0.88	5,801.82	4.43	13.17	40.52	233,424.24
SD	-	11.02	7.21	-	82.03	-	0.33	5.46	8.15	3.38	0.22	1,518.35	0.50	1.26	3.30	62231.94

Patients No 1-10 were patients in group I whereas the remaining were patients in group II.

M = male, F = female, FH = family history, Alb = serum albumin, ALT= Alanine aminotransferase, AST = Aspartate aminotransferase, BUN = blood urea nitrogen, Scr = serum creatinine

WBC = white blood cell, RBC = red blood cell, Hb = hemoglobin, Hct = hematocrit, Plt = platelet

CBZ = Carbamazepine, VPA = Valproate, PHT = Phenytoin, OXC = Oxcarbazepin, LEV = Levetiracetam

BIOGRAPHY

NAME	**Miss Jareerut Kongrit**
DATE OF BIRTH	**19 March 1976**
PLACE OF BIRTH	**Chainat**
INSTITUTION ATTEND	**Ubon Ratchathani University, 2000**
	Bachelor of Pharmacy
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	Chainat, Thailand
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	Amphoe Sapphaya, Chai Nat, Thailand