Original Article

Rapid and Cost-effective Spectrophotometric Method for Rivastigmine Transdermal Patch Quantification

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Abstract:

Introduction: *Several formulations of rivastigmine have been approved for Alzheimer's disease including transdermal patch which increase in compliance and decrease in adverse drug reactions. However, there have been limited reports on direct quantification.* ***Objectives:*** *The aim of this study was to design a simple, rapid, and cost-effective spectrophotometric method for direct quantification of rivastigmine in transdermal formulation. The results were also evaluated for linearity, precision, accuracy, and repeatability.* ***Methods:*** *Rivastigmine was extracted by sonicating in water. The different concentrations of rivastigmine in the patch were investigated for linearity and analyzed for correlation coefficient (r^2). The precision was determined by using intra-day, inter-day, and repeatability techniques. The accuracy was investigated by using spike and unspike techniques.* ***Results:*** *The UV spectrum showed the maximum absorption at 260 nm. The linearity was obtained in this study with r^2 greater than 0.99. The percentages of relative standard deviation from the precision assay were 1.0-3.5%, 0.4-2.5%, and less than 1% for intra-day, inter-day, and repeatability assays, respectively. The percentages of recovery were obtained with the range of 90-110%. The total duration of this method was less than 2 hours.* ***Conclusion:*** *This procedure would be a useful, uncomplicated, rapid and quality-control analytical method for direct quantification of rivastigmine quantity in transdermal patch formulation. This method would be clinically useful for pharmacokinetic study such as drug absorption through skin.*

Keywords: ● *Rivastigmine patch* ● *Quantification* ● *Spectrophometry*

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นิพนธ์ต้นฉบับ

การวัดปริมาณยา Rivastigmine รูปแบบแผ่นแปะด้วยวิธีที่ประหยัดและรวดเร็ว โดยการวัดการดูดกลืนความเข้มแสง

ณัฐพล ใจสุภา และ ศราวุธ จินดารัตน์
ภาควิชาเภสัชวิทยา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

บทคัดย่อ

บทนำ *ยา rivastigmine ที่ได้รับอนุมัติให้ใช้ในผู้ป่วยโรคสมองเสื่อมอัลไซเมอร์มีหลายรูปแบบ ยาในรูปแบบแผ่นแปะถูกพัฒนาขึ้นเพื่อเพิ่มความร่วมมือในการใช้ยา ลดผลข้างเคียงจากยา และลดความถี่จากการบริหารยารูปแบบรับประทาน อย่างไรก็ตามการวิเคราะห์ปริมาณยา rivastigmin ในรูปแบบแผ่นแปะโดยวิธีตรงยังมีค่อนข้างจำกัด* ***วัตถุประสงค์*** *เพื่อออกแบบและศึกษาการวิเคราะห์ปริมาณยา rivastigmine ในรูปแบบแผ่นแปะด้วยวิธีการวัดการดูดกลืนความเข้มของแสง ซึ่งเป็นวิธีที่สะดวก รวดเร็ว และมีค่าใช้จ่ายไม่สูง รวมถึงการทดสอบความน่าเชื่อถือด้วยการวิเคราะห์ค่าความสัมพันธ์เชิงเส้น ความเที่ยงตรง ความแม่นยำ และความสามารถในการทวนซ้ำของเครื่องมือวัด* ***วิธีการ*** *สกัดตัวยา rivastigmine จากแผ่นยาด้วยน้ำบริสุทธิ์ โดยสกัดแผ่นยาในอัตราส่วนที่ต่างๆ กัน แล้ววัดค่าการดูดกลืนคลื่นแสงเพื่อหาความสัมพันธ์เชิงเส้นและวิเคราะห์ค่าสัมประสิทธิ์สหสัมพันธ์ ส่วนการวัดค่าความเที่ยงตรงของวิธีการตรวจนั้นวิเคราะห์จากค่าส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ที่ได้จากการวัดค่าในวันเดียวกัน ต่างวันกัน และการทวนซ้ำ ความเที่ยงวัดโดยการตรวจวัดระดับ rivastigmine ความเข้มข้นต่างๆ และยาหลอก และหาค่าร้อยละการตรวจสอบค่าคืนกลับ* ***ผลการศึกษา*** *ค่าการดูดกลืนคลื่นแสงสูงสุดของยา rivastigmine เท่ากับ 260 นาโนเมตร ความสัมพันธ์เชิงเส้นของอัตราส่วนยา กับค่าการดูดกลืนความเข้มแสงมีค่าสัมประสิทธิ์สหสัมพันธ์มากกว่า 0.99 สำหรับความเที่ยงตรงที่วิเคราะห์จากค่าส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ที่ได้จากการวัดค่าในวันเดียวกัน ต่างวันกัน และความสามารถในการทวนซ้ำของเครื่องมือ มีค่าอยู่ระหว่างร้อยละ 3.5-1.0 ร้อยละ 2.5-0.4 และน้อยกว่าร้อยละ 1.0 ตามลำดับ ส่วนการตรวจสอบค่าคืนกลับได้ผลลัพธ์ระหว่างร้อยละ 90-110 การตรวจวิเคราะห์สารแต่ละครั้งใช้เวลาน้อยกว่า 2 ชั่วโมง* ***สรุป*** *การตรวจหาปริมาณยา rivastigmine จากการศึกษานี้เป็นวิธีที่สะดวก รวดเร็ว และมีค่าใช้จ่ายต่ำ ซึ่งมีประโยชน์ในการศึกษาทางคลินิก และด้านเภสัชจลนศาสตร์ที่เกี่ยวกับกระบวนการดูดซึมยาผ่านผิวหนังได้*

คำสำคัญ: ● *Rivastigmine patch* ● *Quantification* ● *Spectrophometry*

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Introduction

Rivastigmine is one of approved agents used to treat Alzheimer's disease. It is available as capsules, oral solutions, and transdermal patches; however, at present only transdermal formulation of rivastigmine is approved for uses in all stages of the disease[1]. This formulation has been developed due to many beneficial clinical-related aspects including the compliance, pharmacokinetic, and pharmacodynamics issues. For instance, rivastigmine transdermal patch can be given to patients only once every day to reduce the care givers' burden. In addition, this formulation provides smoother plasma level than oral formulation, and hence, decreases undesired side effects at the peak time[2,3]. Rivastigmine provides therapeutic effects by inhibiting the action of acetylcholinesterase and increasing acetylcholine in the synaptic cleft. Approximately 40% of rivastigmine binds to plasma protein. It is predominantly metabolized by non-hepatic processes. Its plasma half-life is approximately 2 h[4]. The patches are available in four sizes; 5, 10, 15 and 20 cm^2 that contain 9, 18, 27 and 36 mg of rivastigmine, and release 4.6, 9.5, 13.3 and 17.4 mg of drug within 24 hours, respectively[5,6]. Rivastigmine is amphiphilic (possessing both hydrophilic and lipophilic properties) and therefore can diffuse through skin and reach the brain sufficiently[7]. Rivastigmine is categorized as class I of the Biopharmaceutics Classification System, in other words, it has high solubility and permeability across the cells[8]. However, certain pharmacokinetic parameters such as AUC_{24h} and C_{max} could be different depending on the application sites. The chest, upper arm and upper back revealed good absorption of rivastigmine whereas the abdomen and thigh showed less absorption[4].

Nevertheless, limited data of direct quantification of rivastigmine in this formulation has been reported. In addition, there has been no assay procedure of rivastigmine transdermal formulation mentioned in the current edition of pharmacopoeias (both USP and BP). One study reported the quantification of rivastigmine in transdermal patch formulation by using high performance liquid chromatography (HPLC) technique[9], however, expensive and high-skilled techniques are required. As a result, this study aimed to develop a simple, rapid, and economical method for direct quantification of rivastigmine in the transdermal patch by using spectrometric technique.

Materials and Methods

Materials

Rivastigmine transdermal patches (Exelon®) of 10 cm^3, each of which contains 18 mg of rivastigmine, and placebo patches were used in this experiment.

Extraction of rivastigmine from transdermal patch

The method employed in this study was modified from previous reports[9,10] by using purified water as an extracting solvent, followed by quantification with spectrophotometer. Firstly, rivastigmine patch was divided into 4 pieces of the same size and put into a tube with 5 ml of polypropylene. Five milliliters of water were then added and sonicated using ultrasonic bath sonicator. To optimize the extraction time, patches were sonicated for 5, 15, 30, and 60 minutes. The obtained extracts were then centrifuged at 10,000g for 5 minutes. The supernatants that were collected from the whole rivastigmine patch (100%), three quarters of the patch (75%), half of the patch (50%), and a quarter of the patch (25%) were analyzed to create the calibration curve.

Scanning for the maximum absorbance (λ_{max}) of rivastigmine

The method was performed according to a previous study[10]. The supernatants obtained from the rivastigmine patch (50% patch proportion) and the placebo patch were scanned to investigate the λ_{max} within the range of 240

to 800 nm by multi-mode plate reader (PerkinElmer®) against water (blank) using CellCarrier™-96 *ultra* plate (PerkinElmer®). The λ_{max} was observed at 260 nm (OD260) and used for further experiments.

Determination of the linearity

The linearity was monitored by plotting the graph between each patch proportion (100%, 75%, 50%, and 25%) against its OD260. The centrifuged solution obtained from each patch proportion was 3-fold diluted by water and its OD260 was recorded. The assays were run in triplicate for each different patch proportion. Linearity was evaluated as correlation coefficient (r^2). The obtained graph was further used as a calibration curve.

Intra-day and inter-day precision assays

Intra-day and inter-day assays were performed to check the precision of the methodology[10]. The samples were measured at 3 different times on the same day (intra-day) and these measurements were repeated on 3 consecutive days (inter-day). The precision was reported as percentages of relative standard deviation (% RSD).

Accuracy test

The accuracy test was investigated with a method modified from Pedroso and Salgado[11], and then evaluated as percentages of recovery (% recovery). The experiment was performed by adding 3 different concentrations of rivastigmine solutions into the extracts (Table 1). The assays were run in triplicate. The recovery percentage of each concentration was calculated by the equation shown below.

$$\% \text{ Recovery} = [(OD260_{spiked} - OD260_{unspiked})/OD260_{add}] \times 100$$

$OD260_{spiked}$ = absobance at 260 nm of extracts or placebo with rivastigmine added;

$OD260_{unspiked}$ = absobance at 260 nm of extracts or placebo without rivastigmine added;

$OD260_{add}$ = absobance at 260 nm of added rivastigmine only

Repeatability

The repeatability was performed by using a method modified from Sharmila et.al[10]. The precision of an instrument was determined by repeating the measurement of samples with the same condition for 20 times.

Results

Rivastigmine λ_{max} verification

The extraction was obtained as a clear solution. The scanning spectrum showed λ_{max} at 260 nm which was not observed from the placebo patch extraction (Figure 1).

Determination of sonication time course

To determine which optimal time point would be appropriated for designated sonication, sonication of rivastigmine patches was performed at different time points ranging from 5 to 60 minutes. The precise linearity was observed in the experimental group that was sonicated for 15, 30, and 60 minutes, with r^2 greater than 0.990 (Figure 2). The result from 30-minute sonication, which showed the highest r^2, was then used for further experiments.

Table 1 Different concentrations of rivastigmine mixture

Condition	Used volume (μL)				Total volume (μL)
	Extracted Sample	Added (spiked) solution	Water	Extracted placebo	
Sample (unspiked)	50	-	100	-	150
Sample (spiked)	50	50	50	-	150
Placebo (unspiked)	-	-	100	50	150
Placebo (spiked)	-	50	50	50	150
Add solution only	-	50	100	-	150

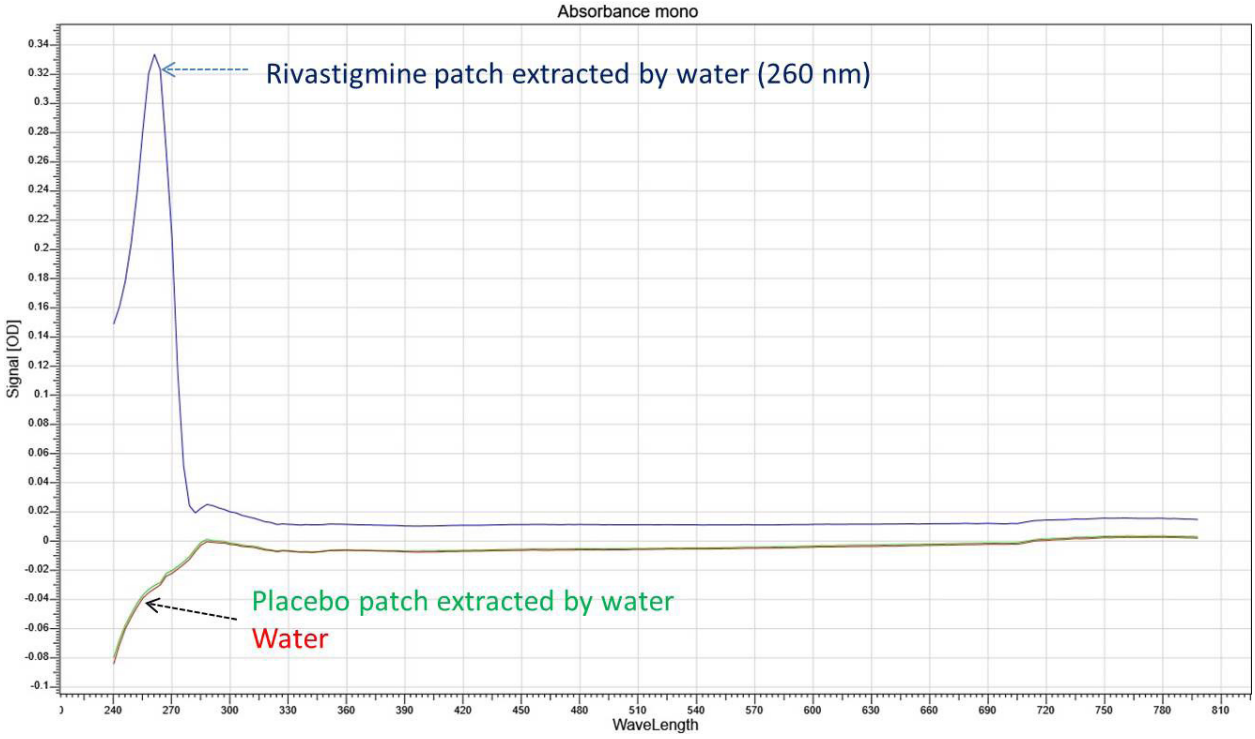

Figure 1 Spectrum of absorption pattern of rivastigmine and placebo patches. The graph is plotted between the wavelength (nm) against the signal (OD).

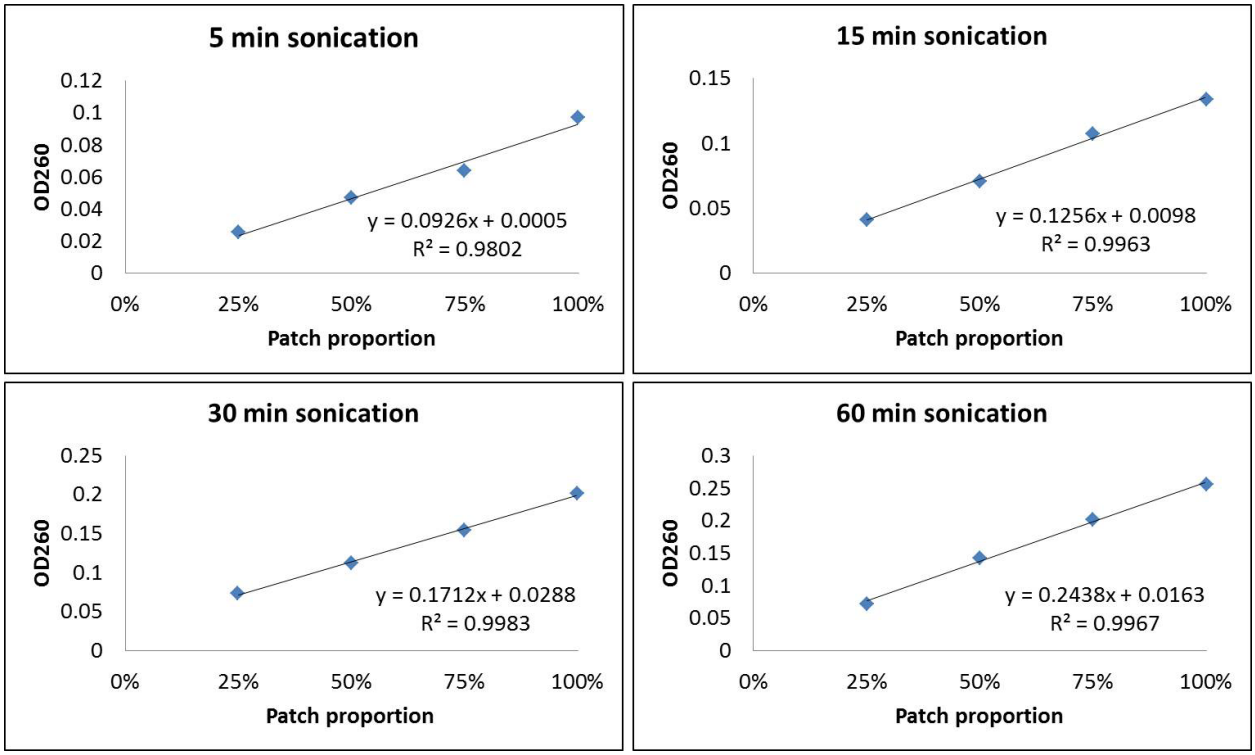

Figure 2 Linear curves obtained from different time points of sonication at 5, 15, 30, and 60 minutes.

Intra-day and inter-day precision assays

The intra-day and inter-day precisions were determined by measuring the samples at 3 different times on the same day and on 3 consecutive days, respectively. The plot of OD260 value against each patch proportion provided the linear correlation with r^2 higher than 0.990 (Table 2). The percentages of RSD obtained from the assays were in the range between 0.2% and 6.5% (Table 2).

Accuracy test

Certain concentrations of rivastigmine were added into the patch and placebo extracts to determine the accuracy. The results were obtained as the percentage of recovery which was acceptable between the ranges of 90-110% (Table 3).

Repeatability

The repeatability test was performed to qualify the instrument, by repeating the measurement 20 times. The results revealed % RSD of less than 1% for every patch proportion (Table 4). The curve constructed from this average showed r^2 of 0.9996.

Table 2 OD260 (average ± SD) and % RSD obtained from intra-day and inter-day assays of water extract of rivastigmine patch. The data presented is the mean of the triplicates.

Patch proportion	Intra-day		Inter-day	
	OD260	% RSD	OD260	% RSD
100%	0.200 ± 0.003	1.45	0.203 ± 0.002	0.75
75%	0.156 ± 0.005	3.15	0.157 ± 0.001	0.86
50%	0.113 ± 0.003	2.53	0.113 ± 0.001	0.43
25%	0.072 ± 0.001	1.09	0.071 ± 0.002	2.30
Regression equation	Y = 0.1705X + 0.0288		Y = 0.1764X + 0.0259	
r^2	0.9997		0.9992	

Table 3 Percentages of recovery determined from accuracy test. Each data was presented as an average ± SD obtained from 3 independent experiments. Placebo was spiked with 50% (spike 1) and 100% (spike 2) of the extract solution.

Patch proportion	Rivastigmine patch			Placebo	
	Spike 80% (%)	Spike 100% (%)	Spike 120% (%)	Spike 1 (%)	Spike 2 (%)
100%	101.06 ± 2.66	101.13 ± 3.02	102.35 ± 1.10	-	-
75%	98.35 ± 1.43	104.93 ± 2.06	100.18 ± 2.39	-	-
50%	101.49 ± 1.71	105.93 ± 2.36	101.90 ± 1.64	-	-
25%	102.05 ± 3.14	108.11 ± 0.94	106.44 ± 1.67	-	-
Placebo	=	=	=	100.13 ± 0.96	102.24 ± 1.51

Table 4 Data obtained from the repeatability test

Patch proportion	OD260 (mean)	SD	% RSD (%)
100%	0.200	0.003	0.381
75%	0.157	0.005	0.630
50%	0.114	0.003	0.469
25%	0.070	0.001	0.382

Discussion

The study of rivastigmine absorption through skin has been of interest to our research team as at present there is only limited available information. The quantitative measurement of rivastigmine residues in the patch after use is one of the most interesting methods because this technique could be used to indirectly estimate drug absorption through skin. Therefore, this research aimed to develop a simple, rapid, and economical spectrophotometric procedure for direct quantification of rivastigmine residues in transdermal patch formulation. Although UV spectrophotometric method is relatively simpler, this analytical technique has recently gained more attentions as found in several publications reporting quantification of the amount of many pharmaceutical products. Rivastigmine is very soluble in water[10], hence, water was used in the extraction process. The scanned spectrum of rivastigmine patches showed λ_{max} at 260 nm. Our data was consistent with another study which observed λ_{max} at 260 nm[10]. The correlation coefficient values (r^2) were higher than 0.99 from the samples that were sonicated for longer than 15 minutes. When the sonicated time was extended, the absorbance was subsequently increased. However, the correlation of each sonication time point was still linear. The results suggested that the sonication time of longer than 15 minutes would give the right % residue result. The calibration curve must be drawn at different times of assay to take into account any errors from the analytical process. In addition, this r^2 followed the International Conference on Harmonization guideline (ICH)[12,13], therefore, this curve could represent a good calibration curve. Although r^2 values were less than 0.999 as reported in many cases, this was sufficient to indicate good linearity since it was obtained by extracting drug existing in the finished product, not by direct weighing the exact amount of standard drug. The calculated % RSD from intra-day and inter-day assays were less than 5% and 3%, respectively, indicating a decent precision[14]. Additionally, % RSD obtained from the reproducibility test was less than 0.63%, and the percentages of recovery from the accuracy test were in the range of -10% to +10%. These results can be accepted according to the guideline mentioned above[13]. These validating parameters suggested a good reliability and confirmed that this analytical procedure could be an alternative used in clinical researches.

Conclusions

Our study indicates a rapid, uncomplicated, cost-effective, and non-invasive procedure which would be clinically useful and could be applied for clinical pharmacokinetic studies in order to quantify rivastigmine residues in transdermal patch formulation. This procedure could be employed for indirect estimation of drug absorption via skin with desirable time courses.

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