The establish of the HPLC method to examine the plasma concentration of lamotrigine and oxcarbazepine

Xue-Ping Zhu*[1], Yao-Dong Zhao[1], Zhi Cheng[1], Ning Min Zhao[2] and Hao Li[3]
[1]The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou City, Henan Province, China
[2]Henan Provincial People's Hospital of Zhengzhou University, Zhengzhou City, Henan Province, China
[3]Zhengzhou University, Zhengzhou City, Henan Province, China

Abstract: To establish the HPLC method to examine plasma concentration of lamotrigine and oxcarbazepine. This study set chlorzoxazone as the internal standard, chromatographic column was Column C18 (200×4.6mm, 5um) of DIKMA company, the mobile phase was methanol, water and trifluoroacetic acid, with rate of 40: 60: 0.0005, at a flow rate of 1 mllmin^{-1}, the detected wavelength was 240 nm. The plasma concentrations of lamotrigine was 0.5-50ug·mL^{-1}, the standard curve was excellent for Y=0.5511C-0.5669, r=0.9940, average recovery was 91.40%; The plasma concentrations of oxcarbazepine was 0.5-50ugmL^{-1}, the standard curve was good for Y=0.4026C-0.5895, r=0.9925 and the average recovery was 89.59%; The three plasma concentrations of lamotrigine were respectively 25μg·mL^{-1}, 10 μg·mL^{-1} and 2μg·mL^{-1} and its five parallel sample for injection RSD were respectively 4.01%, 6.15% and 4.64%; The three plasma concentration of oxcarbazepine were 25μg·mL^{-1}, 10μg·mL^{-1} and 2μg·mL^{-1}, and its five parallel sample for injection RSD were respectively 3.05%, 4.27% and 9.01%. This method was easy to operate, high recovery and high precision, and was applicable to the clinical detection for plasma concentration of lamotrigine and oxcarbazepine.

Keywords: Lamotrigine, oxcarbazepine, plasma concentration, HPLC (High Performance Liquid Chromatography).

INTRODUCTION

Lamotrigine was the new kind of antiepileptic drugs, which could stabilize the presynaptic membrane and inhibited the release of glutamic acid and aspartic acid. Oxcarbazepine was internationally accepted as a good new antiepileptic drug, it was the derivatives of carbamazepine, which could rapidly become the active metabolite monohydroxycarbazepine and epoxide carbamazepine, with less side effect and no adverse drug interactions. These two medicine were with different character, and had narrow therapeutic window and could be affected by many kinds of medicine interactions. Both of the toxic reaction were closed related to the plasma concentration, and could monitor the plasma concentration to adjust the dose of medicine (Yang *et al*., 2006; St. Louis, 2009) in order to increase the effect of curative effect and avoid adverse reaction. This study set chlorzoxazone as the internal standard, and applied HPLC method to examine the plasma concentration of both medicine. It was easy to operate, with high recovery rate, good precision. And it could be used for detect the plasma concentration of lamotrigine and oxcarbazepine.

MATERIAL AND METHOD

Instrument: Diane 680 HPLC, also named Diane 680 high performance liquid chromatograph, (sampler ASI-100, HPLC column TCC-100, detector UVD170U), timing trace oscillator MM-3, varimetric micropipettor, centrifuge was from CENTRIFUGE company, model was 0412-1, Thermostatic water bath 420, ultrasonic cleaner (SONICS company, mode: PS3120 model). Electronic analytical weight scale (shanghai balance instrument factory, model FA1104, weighing range 0-120g, the accuracy 0.1mg), TDZ5-WS multi-tube balancing automatic centrifuge. The vortex shaker (lailida company NH-866), the chromatographic column (DIKMA C18 column, 200x4.6mm, 5um).

Reagents: lamotrigine reference substance (NIFDC, for National Institutes for Food and Drug Control, 100775-200401, applied for content determination), oxcarbazepine reference substance (NIFDC, 100657-200401, applied for content determination), Chlorzoxazone reference substance (NIFDC, 100364-200301, applied for content determination); acetonitrile (tianjin siyou, Q/BSYZ02-2006, level 1 of chromatography pure) and methyl alcohol (Beijing chemical factory, 20110830, analytical pure), purified water.

Preparation of solutions

Reserve liquid: lamotrigine 4.4mg, oxcarbazepine 5.2mg, precision measured, and were respectively dissolved in methanol to 10ml of constant volume and made the concentration respectively 440μg·ml^{-1} and 520μg·m^{-1}.

Internal standard working liquid: use precision weight scale measured the internal standard of chlorzoxazone of 2.4mg, then added into methanol and dissolved in the 100mg of volumetric flask, then made the concentration of 24μg·m^{-1}.

*Corresponding author: e-mail: xueping20125@126.com

Blood sample processing

Blood sampling 100μL had toke, and then added into internal standard working liquid 300μL, vortex help dissolved 3 minutes, 12000r·min^{-1}, then centrifuged for 10 minutes, then took the liquid sample.

Standard blood sample: precision measured the above reservior fluid amount, then diluted with methanol and made it dry. Added suitable amount of blank human plasma, vortex dissolved 3 minutes, added lamotrigine 0.5-50μg·ml^{-1}, oxcarbazepine 0.5-50μg·ml^{-1} of series concentration standard blood samples.

Chromatographic conditions

Chromatographic column C18 column (200×4.6mm, 5μm) were from DIKMA company; Mobile phase: methanol-trifluoroacetic acid-water; Detection wavelength was 240nm; The velocity was 1mL/min; Column temperature was 40^{L}; Sample quantity was 10μL.

Specific inspection

In the above chromatography conditions, this study took the blank plasma and analyzed the sample according to methods of "2.2" Mentioned above. The results showed that sample detection would not be interfered by the plasma endogenous substance. This study had took "2.1" of standard blood, then detected the sample with the methods of "2.2" the sample determination. The record chromatograph chart were shown in fig. 1, fig. 2. The results showed that the retention time of lamotrigine, oxcarbazepine and chlorzoxazone were respectively 7.41 minutes, 15.44 minutes and 18.14 minutes. Each component peak shape was good and the separation was completely.

Linear test

The 50μg·ml^{-1}, 10μg·ml^{-1}, 2μg·m^{-1}, 1μg·ml^{-1}and 0.5μg·m^{-1} of lamotrigine were took according to the above “2.1”. And the standard sample of oxcarbazepine 50μg·ml^{-1}, 10μg·m^{-1}, 2μg·ml^{-1}, 1μg·ml^{-1} and 0.5μg·ml^{-1} were took for the study. Based on the method of “2.2” processing, sample detection, with samples and ratio of internal target peak square Y and concentrations C. Standard curve equation for lamotrigine: Y=0.5511C-0.5669 (r=0.9940); Oxcarbazepine: Y=0.4026C-0.5895 (r=0.9925). It showed that it could had a good linear relations when lamotrigine in plasma was at 0.5-50μg·m^{-1}, oxcarbazepine was at 0.5 - 50μg·ml^{-1}. As shown in fig. 3.

Precision test

Precision measured the liquid under the “2.1”, then added a suitable amount of blank plasma, and configured the 3 blood sample solution of low, medium and high concentration, details was the 3 concentrations of lamotrigine with 25μg·ml^{-1}, 10μg·ml^{-1} and 2μg·ml^{-1}, the oxcarbazepine were 25μg·ml^{-1}, 10μg·ml^{-1} and 2μg·m^{-1}. Each blood sample were processed under the “2.2” method, and continuously determinate 5 batch, calculated the concentration of each sample of lamotrigine RSD were 4.01%, 6.15%, and 4.64%, the precision of the oxcarbazepine were respectively for 3.05%, 4.27% and 9.01%. Details as below table 1.

Stability test

Normal temperature stability

Took the 3 blood samples with low, medium and high concentration, and placed respectively 0 hour, 2 hours and 4 hours at room temperature, then for sample determination, the precision of the calculated concentration was between 2.55% and 8.77%. It showed that it was stable for blood samples been placed 4 hours at room temperature, details was as table 2.

Stability at 4^{L} (48 hours)

Take the above three kinds of concentrations of blood samples, respectively placed at 4^{L} for 0 hour, 24 hours and 48 hours for sample determination, and the calculated RSD were between 3.02%-3.02% of concentration. It showed that blood samples were stable at 4^{L} for 48 hours, as shown in the table 3.

Repeated freezing and thawing test

Took three kinds of concentration of blood sample of above medicine, which kept in 20^{L} below zero at least 24 hours, then were kept at room temperature, and then for sample examination, and repeat the examination for 3 times. Results showed the precision of the concentration of each were between 1.49% and 1.49%, which showed that the plasma samples was in stable condition after 3 times of freeze-thaw test, details seen as table 4.

Recovery ratio test

The 3 kind of concentration of blood sample were detected under the method of “2.2” for 5 times, then compare the area before and after the extraction of the peak area. Then checked the extraction recovery ratio, namely the extraction recovery ratio =A/As x 100%. A was for peak area for drug in plasma sample solution, and as was for the corresponding peak area of standard liquid drugs. The average recovery ratio of lamotrigine was 91.40%, and oxcarbazepine was 89.59%, details as seen in table 5.

DISCUSSION

Chosen wavelength

The maximum absorption wavelength of oxcarbazepine was at 257nm (Ren *et al*., 2010) and the maximum absorption wavelength of lamotrigine was at 225nm (Liu and Tang, 2011). This study had adopted the wavelength of 240nm, the purpose was to take both content into consideration, in order to reduce the influence of baseline drift and impurity peak.

Table 1: The precision of lamotrigine and oxcarbazepine (n=5)

	Lamotrigine (μg·m^{-1})			Oxcarbazepine (μg·ml^{-1})		
	Low	Medium	High	Low	Medium	High
1st Batch	2.80	6.82	22.89	2.19	6.50	24.23
2nd Batch	2.56	6.14	23.29	2.34	5.95	19.22
3rd Batch	2.64	7.00	23.04	2.36	6.53	22.64
4th Batch	2.54	7.06	24.37	2.40	6.29	23.54
5th Batch	2.58	6.34	23.75	2.49	6.02	23.96
RSD	4.01%	6.15%	4.64%	3.05%	4.27%	9.01%

Table 2: The stability of lamotrigine and oxcarbazepine at room (n = 3)

	Lamotrigine (μg·ml^{-1})			Oxcarbazepine (μg·ml^{-1})		
	Low	Medium	High	Low	Medium	High
0 hour	2.30	6.67	20.27	2.69	5.96	20.92
2 hours	2.50	6.49	21.23	2.47	6.18	20.45
4 hours	2.74	7.59	20.38	2.54	6.96	19.51
RSD	8.77%	8.53%	2.55%	4.38%	8.25%	3.54%

Table 3: Stability at 4^{L} of Lamotrigine and oxcarbazepine (n=3)

	Lamotrigine (μg·ml^{-1})			Oxcarbazepine (μg·ml^{-1})		
	Low	Medium	High	Low	Medium	High
0 hour	2.56	6.81	23.04	2.34	6.49	22.64
24 hours	2.49	6.74	20.84	2.42	6.17	20.54
48 hours	2.41	6.13	22.26	2.26	5.99	22.66
RSD	3.02%	5.70%	5.06%	3.42%	4.07%	5.55%

Table 4: Three times of repeated freeze-thaw stability of lamotrigine and oxcarbazepine

	Lamotrigine (μg·m^{-1})			Oxcarbazepine (μg·ml^{-1})		
	Low	Medium	High	Low	Medium	High
The 1st time	2.56	6.81	23.04	2.34	6.49	22 64
The 2nd time	2.50	6.49	21.23	2.47	6.18	20.45
The 3rd time	2.57	6.40	20.48	2.40	6.09	24.11
RSD	1.49%	3.28%	6.10%	2.70%	3.36%	8.22%

Table 5: The average recovery ratio of lamotrigine and oxcarbazepine (n = 5)

	Lamotrigine (%)			Oxcarbazepine (%)		
	Low	Medium	High	Low	Medium	High
1 Batch	104.95	80.56	94.64	83.65	88.70	100.53
2 Batch	95.95	72.53	96.29	89.38	81.20	79.75
3 Batch	98.95	82.68	95.26	90.14	89.11	93.93
4 Batch	95.20	83.39	100.76	91.67	85.84	97.67
5 Batch	96.70	74.89	98.19	90.72	82.15	99.41
RSD	4.01%	6.15%	2.56%	3.55	4.26	9.00%

Selection of internal standard (Including the concentration of internal standard)

Oxcarbazepine was the metabolites of carbamazepine, with similar physical and chemical properties. Clinically, it was not applied along with carbamazepine. Therefore, this study firstly set carbamazepine as internal standard. While, the experiment had proved that the retention time of carbamazepine was approximately 30 minutes under this condition, so carbamazepine could not be replaced as the internal standard. It had reported that chlorzoxazone as the internal standard could had a longer time of retention time than lamotrigine and oxcarbazepine (Li *et al.*, 2009). It had been verified by test, the retention time was around 18 minutes, and resolution was good, so chlorzoxazone was chosen as the internal standard. At the same time, under this condition, the retention time of

oxcarbazepine was about the same time with chlorzpxazone. If both of the concentration was high, it may affect each other, so the configured internal standard solution concentration was 24μg·m^{-1}. According to the sample preparation method, the internal standard concentration was 18μg·m^{-1}.

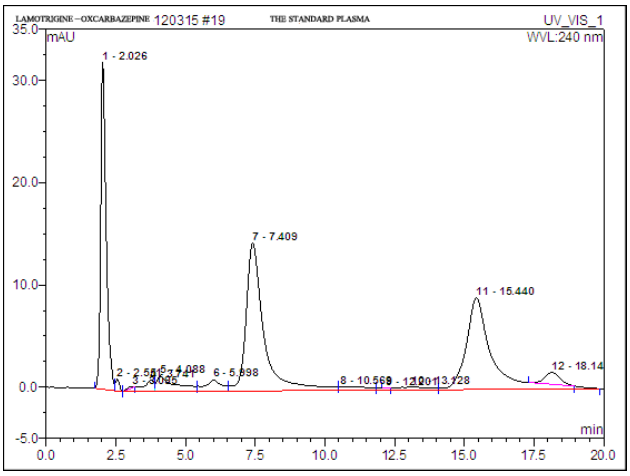

Fig. 1: Sample hromatogram of plasma containing the three samples (A: lamotrigine, B: oxcarbazepine, C: chlorzoxazone)

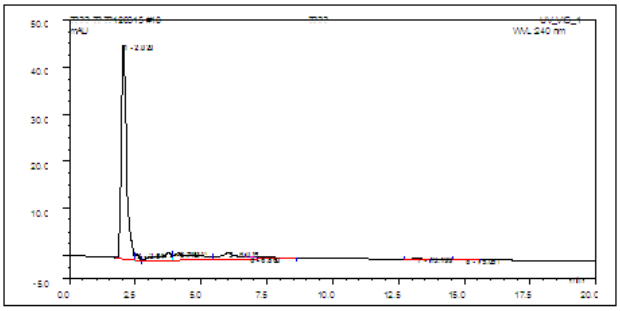

Fig. 2: Chromatograms of blank plasma sample

Choice of mobile phase

Had checked, sample could be separated well at 40% methanol (Du and Li, 2010), when added the blood plasma samples, lamotrigine could interfere with endogenous substances. Therefore, it needed to add some acid in the liquid, in order to change its retention time, and optimize the peak shape. The acetic acid and phosphoric acid both were not able to make lamotrigine perfectly separate with endogenous substances. Therefore, had chose the 0.1% of trifluoroacetic acid to add in the sample. So, the compounds had perfectly separated with endogenous substance and the peak shape was better at the same time. At the same time, the peak shape is better. While, the trifluoroacetic acid could make the volume pressure rise. This study had investigated, the half concentration of trifluoroacetic (0.05%) acid could little influence on the pressure, and could make the compounds be separated well.

Precision inspection

Considering clinical detecting plasma concentration of many people's blood at the same time, so this study had selected 3 concentration in each batch of the 5 batches samples, and investigated the precision. This study did not perform the daytime precision inspection, because it needed to examine the stability of blood sample under the details informed in "2.7.1", which was coincident with this part.

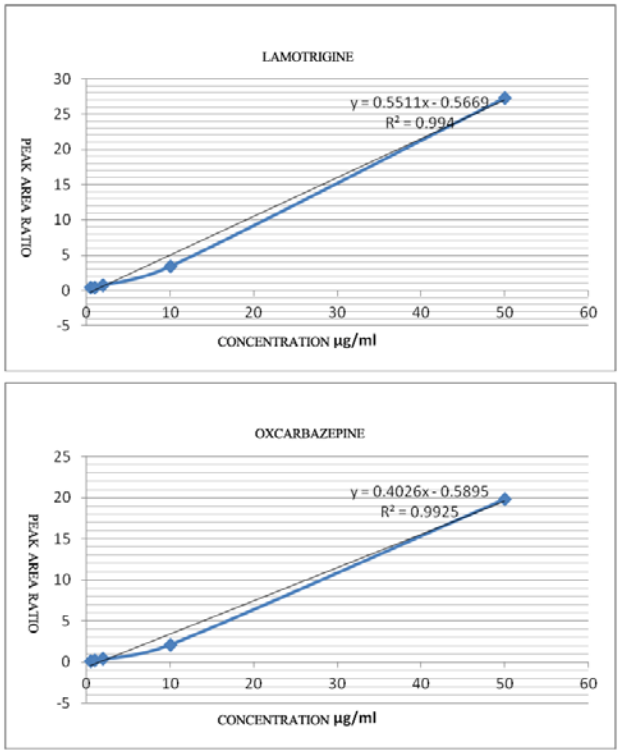

Fig. 3: Standard curve of lamotrigine and oxcarbazepine (5 point method)

Selection of stability test project

In clinical plasma concentration examination work, It needed to keep the blood sample well, in order to do the batch monitoring. Therefore, this study had investigated the stability in room temperature, the stability of 4□, the stability of repeated frozen and thaw, in order to determine the it storage time and storage condition of the sample.

With the popularity of high performance liquid in hospital, it would increase the plasma concentration to be evaluated by high performance liquid chromatography (HPLC) method (He *et al*., 2011). At present, there was no lamotrigine and mass detection method of lamotrigine and oxcarbazepine. It would have the practical significance for the application of HPLC method to examine the plasma concentration.

Lamotrigine and oxcarbazepine had the combined phenomenon in daily clinical field. Therefore, at some time, it was required to detect the plasma concentrations

of both at the same time in clinical testing. Some patients were lack of medical knowledge and could not able to inform the details of medicine he had before under the poisoning symptoms; *In*: some non-standard medical institution, some patients took the Chinese traditional medicine, which contained some unknown chemical composition. It became more important to detect the plasma concentration of many medicines (Jiao *et al*, 2004). Now the β class hospital accreditation clearly announced hospital shall detect the plasma concentration. The plasma concentration examination work would be not only for large hospital, many local hospitals would carry out plasma concentration monitoring too. Considering the two drugs cannot be examined by the large medical instrument like TDX detection. It has strong practical significance to increase the detection project of HPLC method to examine the plasma concentration.

CONCLUSION

This study had applied the HPLC method, which could examine the plasma concentration of lamotrigine and oxcarbazepine at the same time. This method was easy to operate, high recovery rate, good precision, suitable for clinical detection of plasma concentration of lamotrigine and oxcarbazepine respectively, and also suitable to detect the plasma concentration of both at the same time.

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