DOI 10.1007/s11094-019-02077-x
Pharmaceutical Chemistry Journal, Vol. 53, No. 8, November, 2019 (Russian Original Vol. 53, No. 8, September, 2019)

COMPARISON OF QUANTITATIVE ANALYTICAL TECHNIQUES FOR DABIGATRAN IN BLOOD PLASMA OF HUMANS WITH KNEE REPLACEMENTS

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Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 53, No. 8, pp. 59 – 63, August, 2019.

Original article submitted April 21, 2019.

Two different literature methods were used to compare the experimental efficacy and accuracy of dabigatran assays in blood of 30 patients with knee replacements. Blood plasma was collected from patients who underwent anticoagulant therapy and were administered the medicine at a dose of 220 mg. Residual and peak dabigatran concentrations were determined by HPLC-MS and HPLC-MS/MS.

Keywords: dabigatran, HPLC, mass-spectroscopy, pharmacokinetics, anticoagulant.

Peroral anticoagulants are a new class of medicines that appeared recently and are used in pharmacology to prevent thromboembolism [1 – 6].

Three methods are used to estimate the anticoagulant concentration in patient blood for drug monitoring to prevent bleeding and other serious complications. They are:

rapid analysis [7 – 9];

chromatography [10 – 13];

chromogenic analysis [14, 15].

Rapid analysis of human blood for anticoagulant content in clinics, hospitals, and polyclinics often uses coagulometers to monitor the drug concentration in blood of patients on anticoagulants. However, the measurement is made indirectly using activated partial thromboplastin and prothrombin time, ecarin clotting time, etc. [7 – 9].

Chromatographic methods are most common and convenient for drug monitoring and solving pharmacokinetic problems [10 – 13]. The present work used two developed and validated methods for quantitative determination of dabigatran in human blood plasma [14, 16] that corresponded to existing documentation for selectivity, accuracy, linearity, precision, stability, matrix effect, carryover, and recovery [17 – 19]. Data from patients with knee arthroplasty who took the drug for prevention were analyzed. Samples (60) were taken from patients (30) and compared to determine the anticoagulants in blood. The most efficient method for estimating the pharmacokinetic parameters and drug monitoring was determined.

The goal of the present study was to compare the efficiency of methods used for quantitative determination of dabigatran in patient blood plasma and to choose the optimal method for therapeutic drug monitoring and solving pharmacokinetic problems.

EXPERIMENTAL PART

The study included 30 patients with knee replacements. In the post-operative period, they took dabigatran at a dose of 220 mg. Two samples were taken twice from each patient and were divided into two groups, i.e., group 1 before administration and group 2, 3 h after administration of the drug.

Quantitative analysis used two different HPLC methods with mass-spectrometric detection.

Data from the two methods were statistically processed using Kolmogorov—Smirnov and Wilcoxon tests and SPSS Statistics software.

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TABLE 1. Equipment, Reagents, and Reference Standards

Equipment	Method 1 (HPLC-MS/MS)	Method 2 (HPLC-MS)
Chromatograph	HPLC Dionex UltiMate 3000 (Germany)	Agilent 1200 HPLC (USA)
Detector	Bruker micrOTOF-QII mass detector (Germany)	Agilent 6410 mass detector (triple quadrupole) (USA)
Shaker for stirring liquids	Genius 3 (Germany) and Vortex (USA)	Heidolph Reax top vortex shaker (Germany)
Shaker	-	IKA VXR basic Vibrax® with IKA-Werke Type VX 2E for 64 (Germany)
Centrifuge	Eppendorf (Germany)	Eppendorf Minispin® with rotor for 12 tubes (Germany)
N_2 generator	-	Parker Balston NitroFlowLab
Water purification system	Merck Millipore Simplicity® UV (with UV lamp) (Germany)	Millipore Direct-Q 5 UV (France)

TABLE 2. Dabigatran Sample Preparation for Chromatographic Analysis

Sample-preparation conditions	Method 1 (HPLC-MS/MS)	Method 2 (HPLC-MS)
Centrifugation	-	Centrifugation of blood plasma in Eppendorf Minispin®
Preparation of sample with standard	Addition of plasma (200 μL) and internal standard (20 μL)	Transferring supernatant liquid (100 μL) into plastic Eppendorf tubes and treatment with internal standard working solution (250 μL)
Homogenization	Shaking on a laboratory shaker for 15 – 30 sec	Shaking resulting solution on Vortex mixer and leaving for 10 min
Protein oxidation	Addition of MeCN (600 μL), shaking again for 15 – 30 sec, centrifugation at 14,000 rpm for 5 min to precipitate proteins	Centrifugation at 10,000 rpm for 10 min
Preparation of solution after protein precipitation	Placement of an aliquot (700 μL) of resulting mixture into Eppendorf tube and treatment with CH_2Cl_2 (1,000 μL)	-
Additional purification	Shaking emulsion again in laboratory shaker for 2 min and destruction by centrifugation at 14,000 rpm for 5 min	-
Sample volume for chromatography	Sampling (100 μL) upper aqueous layer	Sampled (100 μL) of supernatant layer

TABLE 3. Dabigatran Chromatography Conditions

Chromatographic separation conditions	Method 1 (HPLC-MS/MS)	Method 2 (HPLC-MS)
Mobile phase	0.1% aqueous formic acid—MeCN (80:20)	Solution A (50 mL 0.1 M ammonium acetate and 5 mL formic acid diluted in deionized water to total volume 1 L) and solution B (50 mL 0.1 M ammonium acetate and 5 mL formic acid diluted in MeOH to total volume 1 L) with A:B ratio 70:30
Elution mode	Isocratic	Isocratic
Mobile-phase flow rate, mL/min	0.4	0.6
Stationary phase	Agilent Zorbax SB-CN column (150 × 4.6 mm, 5 μm, 35°C)	Agilent Zorbax SB-CN column (150 × 4.6 mm, 5 μm) at 40°C
Injected sample volume, μL	10	5
Dabigatran retention time, min	4.7	9

TABLE 4. Chromatography Conditions for Dabigatran Detection

Chromatography detection conditions	Method 1 (HPLC-MS/MS)	Method 2 (HPLC-MS)
Electrospray ionization mode	Positive ions	Positive ions
Fragmentation of gas	Argon	N_2
Impact energy, eV	22.2	25
Ion-source potential, V	4500	135
Spray-gas pressure, bar	2.0	2.41
Drying-gas volume flow rate, L/min	7.0	11
Temperature, °C	250	350
Detection in MS mode	Dabigatran m/z = 472.2, internal standard m/z = 476.2	Dabigatran m/z = 472, internal standard m/z = 476
Detection in MS/MS mode	Dabigatran transition m/z 472.2 → 289.1, internal standard transition m/z 476.2 → 293.1	–

TABLE 5. Pharmacokinetic Data for Dabigatran Content in Human Blood Plasma in Two Experiments

Method	Preparation administration	max	min	Mean	SD	Confidence interval
HPLC-MS	before	112.71	9.61	32.54	22.10	7.91
HPLC-MS/MS	before	95.03	6.16	28.49	22.24	7.87
HPLC-MS	after	792.43	35.94	227.63	169.89	60.79
HPLC-MS/MS	after	800.40	24.47	210.54	167.30	59.29

TABLE 6. Normal Distribution (Kolmogorov—Smirnov One-Sample Test)

Parameter	Dabigatran concentration	
	before capsule administration	3 h after capsule administration
N	60	60
Criterion statistics	0.177	0.163
Asymptotic significance p (95%)	0.000	0.000

Table 1 lists the equipment used to determine the drug in blood plasma by the two methods.

Calibrators and quality-control (QC) samples in method 1 were prepared using (Table 1):

matrix solution of dabigatran (1 mg/mL) in DMSO;

internal standard (IS) of deuterated dabigatran [M + 4] (1 mg/mL);

human blood plasma from patients.

Chemical reagents used for sample preparation and preparation of standards and QC samples included:

N_2 (99%);

Ar (99.95%);

MeCN for HPLC (99.9%; LabScan, Poland);

ultrapure H_2O from a Simplicity® UV system (with a UV lamp; Merck Millipore, Germany);

formic acid for HPLC (50%; Fluka, Switzerland);

CH_2Cl_2 (dichloromethane; chemical pure; Khimmed, RF).

Method 2 used the following to prepare calibrators and QC samples (Table 1):

TABLE 7. Statistical Values of Wilcoxon Criteria

Parameter	Dabigatran concentration	
	before capsule administration	after capsule administration
Wilcoxon signed-rank test	– 1.306	– 0.401
Asymptotic significance p (95%)	0.192	0.688

matrix solution of dabigatran (1 mg/mL) in MeOH – DMSO (9:1);

IS of deuterated dabigatran [M + 4] (1 mg/mL);

human blood plasma from patients.

Chemical reagents used for sample preparation and preparation of calibrators and QC samples included:

N_2 (98%);

N_2 (99.99%);

DMSO (99.9%; Panreac);

HCl (37%, Panreac);

MeOH (99.8%; Fisher Scientific);

Ammonium acetate (Merck);

formic acid (85%; Acros Organics);

ultrapure H_2O from a Millipore Direct-Q 5 UV system.

Reference standards

Standard solutions for method 1 were prepared by successive dilution of matrix solutions in H_2O. QC standard solution was prepared by diluting stock solution in H_2O to concentration 0.5 ng/mL.

Standard solutions of analytes and IS were stored in a refrigerator at 4°C for 1 week.

Standard solutions of dabigatran and its deuterated analog for method 2 were prepared using starting matrix solutions of the compounds in MeOH – DMSO (9:1). The concentration of dabigatran and its deuterated standard in the matrix solutions was 10 μg/mL. An IS working solution at concentration 50 ng/mL was prepared by successive dilution of matrix solution in MeOH and 0.1% HCl (9:1).

Sample preparation

Table 2 presents data for extraction of dabigatran from blood plasma by the two methods.

Chromatographic analysis conditions

Table 3 presents the dabigatran chromatographic separation conditions for the two methods.

Detection conditions

Table 4 presents the dabigatran detection conditions for the two methods.

RESULTS AND DISCUSSION

The dabigatran concentration before its administration for method 1 was Max = 112.71 ng/mL; Min = 9.61 ng/mL; average deviation = 22.10 ng/mL; mean = 32.54 ng/mL. After its administration, the values were Max = 792.43 ng/mL; Min = 35.94 ng/mL; average deviation = 169.89 ng/mL; mean = 227.63 ng/mL. Table 5 presents the pharmacokinetic characteristics for the two methods.

A check for normal distributions of dabigatran concentrations before and after administration of capsules used the Kolmogorov—Smirnov one-sample test. Table 6 presents the results.

The asymptotic significance $p < 0.05$ indicated that the concentrations had a normal distribution. Statistically significant differences between concentrations obtained using the different analytical methods were found using the Wilcoxon criterion (Table 7).

The asymptotic significance $p < 0.05$ suggested that differences between medians of dabigatran concentration using the different analytical methods were not statistically significant.

Thus, the results obtained using the two methods were statistically indistinguishable from each other so that either of the developed methods could be used.

REFERENCES

1. V. T. Vavilova, *Med. Sovet*, No. 12, 44 – 47 (2015).
2. I. N. D'yakov, *Remedium*, No. 11, 42 – 43 (2014).
3. "Russian clinical recommendations for diagnosis, treatment, and prevention of complications from venous thromboembolism," *Flebologiya*, **9**(4), 1 – 52 (2015).
4. N. G. Khorev, A. P. Momot, and D. A. Zaloznyi, *Tromb., Gemostaz Reol.*, No. 4(44), 31 – 47 (2010).
5. A. Dabi and A. P. Koutrouvelis, "Reversal strategies for intracranial hemorrhage related to direct oral anticoagulant medications," *Crit. Care Res. Pract.*, URL: https: //doi.org/10.1155/2018/4907164.
6. A. Bromlcy and A. Plitt, *J. Cardiol. Ther.*, **7**(1), 1 – 13(2018).
7. Mosaad Almegren, *Vasc. Health Risk Manage.*, **13**, 287 – 292 (2017).
8. G. Lippi and E. Favaloro, *Clin. Chem. Lab. Med.*, **53**(2), 1 – 13 (2014).
9. V. Taune, M. Skeppholm, A. Agren, et al., *J. Thromb. Haemostasis*, **16**(12), 2462 – 2470 (2018).
10. J. Stangier, K. Rathgen, H. Stahle, et al., *Br. J. Clin. Pharmacol. B*, **64**(3), 292 – 303 (2007).
11. M. Korostelev, K. Bihan, L. Ferreol, et al., *J. Pharm. Biomed. Anal.*, **100**, 230 – 235 (2014).
12. J. Kuhn, T. Gripp, T. Flieder, et al., *PLoS One*, **10**(12), 1 – 19 (2015).
13. E. M. H. Schmitz, K. Boonen, D. J. A. van den Heuvel, et al., *J. Thromb. Haemostasis.*, **12**, 1636 – 1646 (2014).
14. D. A. Sychev, A. N. Levanov, T. N. Shelekhova, et al., *Pharmacogenomics Pers. Med.*, **11**, 127 – 137 (2018).
15. J. Harenberg, S. Kraemer, S. Du, et al., *Semin. Thromb. Hemostasis*, **40**(1), 129 – 134 (2014).
16. X. Delavenne, J. Moracchini, S. Laporte, et al., *J. Pharm. Biomed. Anal.*, **58**, 152 – 156 (2012).
17. *Handbook for Drug Review* [in Russian], Vol. 1, Grif i K, Moscow, 2014.
18. *Guideline on Validation of Bioanalytical Methods (Draft)*, European Medicines Agency, Committee for Medicinal Products for Human Use, London, 2009.
19. *Bioanalytical Method Validation, Guidance for Industry*, U. S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evolution and Research (CDER). U. S. Government Printing Office, Washington, 2001.