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Development of official assay method for loperamide hydrochloride capsules by HPLC

Thi-Anh-Tuyet Le[1], Bao-Tan Nguyen[1], Min-Ho Kim[1], Bit Kim[1], Hyun-Soo Kim[1], Seung-Won Jeong[1], Jong-Seong Kang[2], Dong-Hee Na[3], In-Koo Chun[4], and Kyeong Ho Kim[1,★]

[1]College of Pharmacy, Kangwon National University, Chuncheon 24341, Korea
[2]College of Pharmacy, Chungnam National University, Korea
[3]College of Pharmacy, Chungang University, Korea
[4]The Research Foundation for Pharmaceutical Quality

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Abstract: Currently, the potentiometric titration and the high pressure liquid chromatography (HPLC) method were utilized in Korean Pharmacopoeia XII (KP XII) as well as other pharmacopoeias (USP, EP, BP) for determination of loperamide hydrochloride in raw materials and capsules, respectively. The research objective is to overcome the remaining drawbacks from current methods such as solubility of mobile phase (KP XII), less scientific approach (USP 43) or using paired-ion chromatography reagent which shows some limitations (BP2017 and other formulation monographs). The proposed method was optimized by Design of Experiment (DoE) tool to obtain the satisfied method for determination of loperamide hydrochloride. The optimal condition was performed on the common C18 column (150 mm × 4.6 mm; 5 μm) using isocratic elution with the mobile phase containing 40 mM of potassium phosphate monobasic (pH 3.0) and acetonitrile (56:44), at a flow rate of 0.7 mL/min. The optimized method was validated and met the requirements of the International Conference on Harmonization. The developed method was applied to determine loperamide hydrochloride in capsules and can be used to update the current monograph in KP XII.

Key words: loperamide hydrochloride, capsules, assay, DoE, HPLC

1. Introduction

Loperamide hydrochloride (*Fig.* 1) is chemically named as 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-l]-N,N-dimethyl-2,2-diphenylbutanamide; hydrochloride.[1] It is an μ-opioid receptor agonist and results in antidiarrheal action. The FDA approved loperamide to treat various form of diarrhea as the main indication[2] and chemotherapy-induced diarrhea (especially related to irinotecan) for the off-label uses.[3]

Currently, the method for determination of loperamide hydrochloride in raw material is potentiometric titration in Korean Pharmacopoeia (KP XII),[4] United State Pharmacopoeia (USP 43)[5] and European Pharma-

★ Corresponding author
Phone : +82-(0)33-250-6918 Fax : +82-(0)33-259-5631
E-mail : kyeong@kangwon.ac.kr

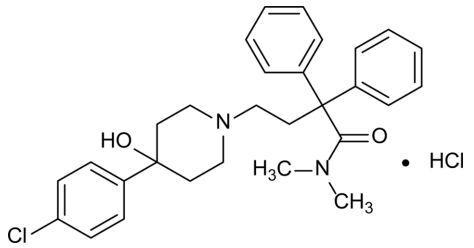

Fig. 1. Chemical structure of loperamide hydrochlorides

copoeia (EP 10.0).[6] For loperamide hydrochloride capsules, HPLC method is mentioned in KP XII, USP 43 and British Pharmacopoeia (BP 2017).[7] However, there are still some limitations in these HPLC methods. In KP XII, the binary eluent containing high concentration of sodium phosphate dibasic at pH 7.0 (approximately 42 mM) and high percentage of methanol (70 %) leads to precipitation in pre-column step. Generally, when using 70 % of methanol, the concentration of potassium phosphate at pH 7.0 should not be more than 35 mM. In addition, the sodium salts is expected to be less soluble in comparison with others.[8] Therefore, there is the solubility problem in the assay test in KP XII. In USP43, the method seems to be unscientific and not robust because of the unrepeatable pH when adding 20 drops of phosphoric acid to mobile phase. Besides, using cyano column with the large particle size (10 μm) and high flow rate (2.0 mL/min) also makes it not become an ideal approach. The method in BP2017 used paired-ion chromatography reagent (sodium octanesulfonate) for eluent and high flow rate (1.5 mL/min) which can cause damage and short column life. Furthermore, the monographs of relatives have some imperfections such as using paired-ion chromatography reagent with high flow rate (loperamide hydrochloride tablets in USP43), using C8 column with high capacity in result (loperamide hydrochloride oral solution in USP 43) and using the complex mobile phase with tetrahydrofuran, acetonitrile, ammonium dihydrogen phosphate and paired-ion chromatography reagent (decanesulfonic acid) (loperamide hydrochloride oral solution in BP 2017). Therefore, developing a simple and robust method for determination of loperamide hydrochloride in capsules to replace the conventional methods is necessary.

Traditionally, the development and optimization for analytical methods have been conducted by one factor at time (OFAT) approach which changes one of factors and constants the remainders. Its major disadvantages are excluding the interaction between factors and increasing the number of experiments which will take time, effort and resources. Design of experiment (DoE) tool was developed to overcome these problems. In this methodology, multiple factors are systematically varied and the results are used to create mathematical models. From these models, it is possible to predict the interaction between factor and find optimal condition.[9,10] Nowadays, application of design of experiment concept in analytical development becomes more popular with several types of concept [11,12] because of its efficiency and variety in design.

In this research, we aimed to seek the optimal condition for alternative HPLC method. The DoE concept was applied to optimize each factors and minimize the number of experiments. The final condition was also validated according to ICH guideline[13] for the reliable and accurate method for quantitating loperamide hydrochloride in commercial capsules.

2. Experimental

2.1. Chemicals and reagents

Loperamide hydrochloride standard was purchased from Sigma-Aldrich (Saint Louis, MO, USA). The capsule formulations were obtained from Youngilpharm and GLPharma. The HPLC-grade acetonitrile, methanol and ethanol were purchased from J.T.Baker (Avantor Inc. – Gyeonggi, Korea), Honeywell Burdick & Jackson (B&J – Ulsan, Korea), Daejung (Siheung, Korea), respectively. Potassium phosphate monobasic was supplied by Kanto Chemical Co. Inc. (Tokyo, Japan).

2.2. Chromatographic conditions

The proposed method was developed by Shimadzu HPLC system from Shimadzu Corporation (Kyoto, Japan) including a DGU-20A5R degasser, LC-20AD

pumps, SIL-20A autosampler, CBM-20A communication bus module, CTO-20AC column oven and SPD-M20A 230V photodiode array detector. For the intermediate precision, Agilent 1100 series HPLC system was utilized. Purified water was newly prepared in laboratory. Other chemicals and reagents for preliminary experiments were analytical grade.

The column Luna C18(2) (150 × 4.6 mm ID, 5 μm) connected with Phenomenex C18 guard column (3 × 4 mm ID) was used throughout the development process. For comparison with the conventional method from other pharmacopoeias, the column INNO CN (250 × 4.0 mm ID, 5 μm) and Aegispak C8 (150 × 4.6 mm ID, 5 μm) were employed.

For optimal condition, the mobile phase contained 40 mM of potassium phosphate monobasic (pH 3.0) and acetonitrile (56:44). The temperature was 35 °C. The flow rate was 0.7 mL/min. Injection volume was 10 μL. Loperamide hydrochloride was detected at 214 nm.

2.3. Sample Preparation

Stock standard solution of loperamide hydrochloride (1 mg/mL) was prepared in 70 % methanol. Final standard solution (10 μg/mL) was diluted by the same diluent.

Sample solution: Transfer the contents of 20 capsules as completely as possible and accurately weigh the amount of powder, equivalent to about 2 mg of loperamide hydrochloride. That required amount powder was transferred to a 20 mL volumetric flask, added with methanol 70 % (about 50 % of the flask capacity) and sonicated for 15 minutes. This solution was diluted to volume with methanol 70 %, mixed and filtered. Transfer 5 mL of this solution to a 50 mL volumetric flask, dilute with methanol 70 % and mix. After dilution, the final solution containing 10 μg/mL of loperamide hydrochloride was used as a sample solution.

2.4. Method development

The preliminary experiments were conducted to initially select a suitable mobile phase composition such as type of organic solvent (acetonitrile, ethanol, and methanol), buffer (type and pH) or mobile phase additives (phosphoric acid, acetic acid, formic acid, and trifluoroacetic acid). According to the design from DoE software, the HPLC condition was optimized easily to obtain the satisfied method.

2.5. Method validation

The final method was validated to meet the requirements of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The validation procedure included:

Specificity: The blank, loperamide hydrochloride standard solution (10 ppm), loperamide hydrochloride in capsules – sample solution (10 ppm) were injected to assess the analyte in presence of other components (mobile phase, excipients, impurities…)

System suitability: Standard solution was injected repetitively to check the stability of the system under the optimal condition.

Linearity and limit of detection (LOD)/limit of quantification (LOQ): The range of standard concentrations from 1 to 30 ppm were selected for linearity test. The LOD and LOQ were based on the signal-to-noise ratio calculated from the chromatograms of the diluted standard solution.

Precision: The repeatability (intra-day and inter-day) and intermediate precision (different HPLC system) were conducted for precision.

Accuracy: The spiked solutions containing standard (80 %, 100 %, 120 %) and sample from formulation were used for measurement.

Robustness: The robustness of this method was assessed by the small variations in percentage of acetonitrile (± 2 %), buffer concentration (± 2 mM), pH of buffer (± 0.2), flow rate (± 0.1 mL/min) and temperature (± 2 °C).

2.6. Method application

The proposed method was applied to determine loperamide hydrochloride in capsules from Youngilpharm and GLPharma. The content of loperamide hydrochloride in capsule was calculated according to the following equation:

Loperamide hydrochloride ($C_{29}H_{33}ClN_2O_2$.HCl) (mg) = $\mathbf{m \times (A_T/A_S)}$

Where:

m is the amount of loperamide hydrochloride weighed for standard solution,

$\mathbf{A_T}$ is the peak area in the sample solution (μAU*s),

$\mathbf{A_S}$ is the peak area in the standard solution (μAU*s).

3. Results and Discussion

3.1. Method development

Initially, the method was developed using the simple acidic mobile phase. The acidic additives (phosphoric acid, acetic acid, formic acid, and trifluoroacetic acid) and the organic solvents (acetonitrile, methanol, and ethanol) were surveyed. The best result of this approach was obtained by the mobile phase containing methanol/0.05 % trifluoroacetic acid (65/35). Besides that approach, another method using the mobile phase containing buffer at acidic pH was also developed. In comparison with the initial condition, the mobile phase containing the buffer gave the better result in peak shape as well as column efficiency and the robustness of procedure. Then, several types of buffer with the suitable pH were also checked to find out the most appropriate buffer (*Table* 1). The comparative chromatograms showed that the combination of potassium phosphate monobasic and acetonitrile were the suitable combination for optimization (*Fig.* 2).

Next step, the screening and optimization concept were designed by DoE. In screening, two-level full factorial design was selected with five factors (buffer pH, buffer concentration, acetonitrile ratio, temperature and flow rate). Four responses were considered including capacity (k'), tailing factor (T_f), number of theoretical plate (NTP) and pressure. The detail screening design and results was shown in the *Table* 2. The analysis indicated that the high temperature positively affected on both four responses. Therefore, the temperature was set at 35 °C and excluded for optimization step. In responses, the highest pressure was 1705 psi which is acceptable so the pressure was also unimportant response and can be excluded.

For optimization, the Box-Behnken design was created with 30 runs divided into 3 blocks. After

Table 1. Buffer types used for preliminary experiments

No.	Type of buffer	pH range	Adjusted pH for experiment
1	Potassium phosphate monobasic	2.0-3.1	2.0 & 3.0
2	Sodium acetate	3.8-5.8	4.0
3	Sodium citrate	4.4-6.4	5.0
4	Potassium phosphate dibasic	6.2-8.2	7.0

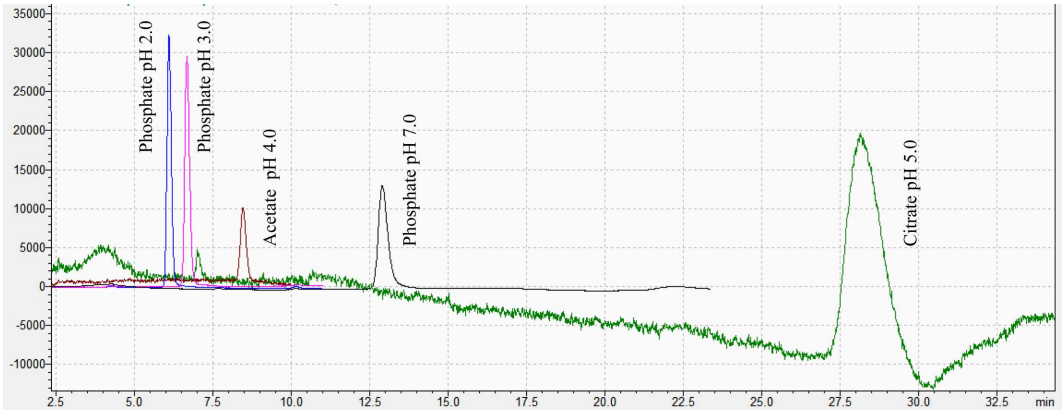

Fig. 2. Comparative chromatogram of buffer type survey

Table 2. Screening design and results

Block	Run	Factors					Responses			
		Buffer pH	Buffer conc.	ACN ratio (%)	Temperature (°C)	Flow rate (mL/min)	Capacity	Tailing factor	Theoreotical Plate	Pressure
1	1	3.1	10	35	35	1.2	14.145	1.185	10188	1248
	2	3.1	10	55	35	0.6	1.150	1.305	7178	496
	3	3.1	50	35	15	0.6	16.169	1.134	11162	925
	4	2.55	30	45	25	0.9	3.430	1.179	8662	1026
	5	2.55	30	45	25	0.9	3.425	1.18	8659	1028
	6	2	10	35	15	0.6	13.562	1.255	10713	939
	7	2	10	55	15	1.2	1.088	1.288	3863	1363
	8	2.55	30	45	25	0.9	3.399	1.181	8693	1023
	9	3.1	50	55	15	1.2	1.207	1.247	4206	1404
	10	2.55	30	45	25	0.9	3.330	1.181	8569	1020
	11	2	50	55	35	0.6	1.107	1.233	7553	520
	12	2	50	35	35	1.2	12.798	1.066	10263	1274
2	13	3.1	10	35	15	1.2	15.562	1.290	8313	1666
	14	2.55	30	45	25	0.9	3.414	1.179	8647	1004
	15	3.1	50	35	35	0.6	12.825	1.100	11585	629
	16	3.1	10	55	15	0.6	0.852	1.329	5472	677
	17	2.55	30	45	25	0.9	3.257	1.178	8573	995
	18	2	50	35	15	1.2	14.48	1.119	8616	1705
	19	2	10	55	35	1.2	0.994	1.259	4554	978
	20	2	50	55	15	0.6	1.032	1.235	6335	695
	21	3.1	50	55	35	1.2	1.145	1.203	5170	1008
	22	2.55	30	45	25	0.9	3.125	1.172	8520	984
	23	2.55	30	45	25	0.9	3.130	1.167	8567	984
	24	2	10	35	35	0.6	10.713	1.162	11524	621

Table 3. ANOVA statistical parameters from optimization results

Response	*p*-value	Adjusted R^2	Predicted R^2	Difference (*)	Adequate Precision
Capacity	<0.0001	0.9991	0.9972	0.002	138.1640
Tailing factor	<0.0001	0.9426	0.8484	0.094	24.2696
Number of Theoretical Plate	<0.0001	0.9774	0.9126	0.065	32.1682

(*) The difference between predicted and adjusted R^2

screening, the range of each factor was adjusted to be smaller and suitable. The domain included buffer pH (2.0-3.0), buffer concentration (30-50 mM), acetonitrile ratio (35-55 %) and flow rate (0.6-1.0 mL/min). From Box-Cox transformation, the capacity and tailing factor responses were transformed to the natural log and inverse square root (k = -1) transformations, respectively. Statistical parameters from ANOVA of both 3 responses demonstrated that the results met the requirements (*Table* 3). Statistical data showed *p*-value < 0.05 which confirmed model significance. The difference between predicted and adjusted R^2 was in reasonable agreement (within 0.2). The adequate precision in ANOVA was sufficiently high (>4) so the model can be used to navigate the design space. From model graphs, the effect of each factor and interactions between the chosen factors on each response can be demonstrated. After analyzing

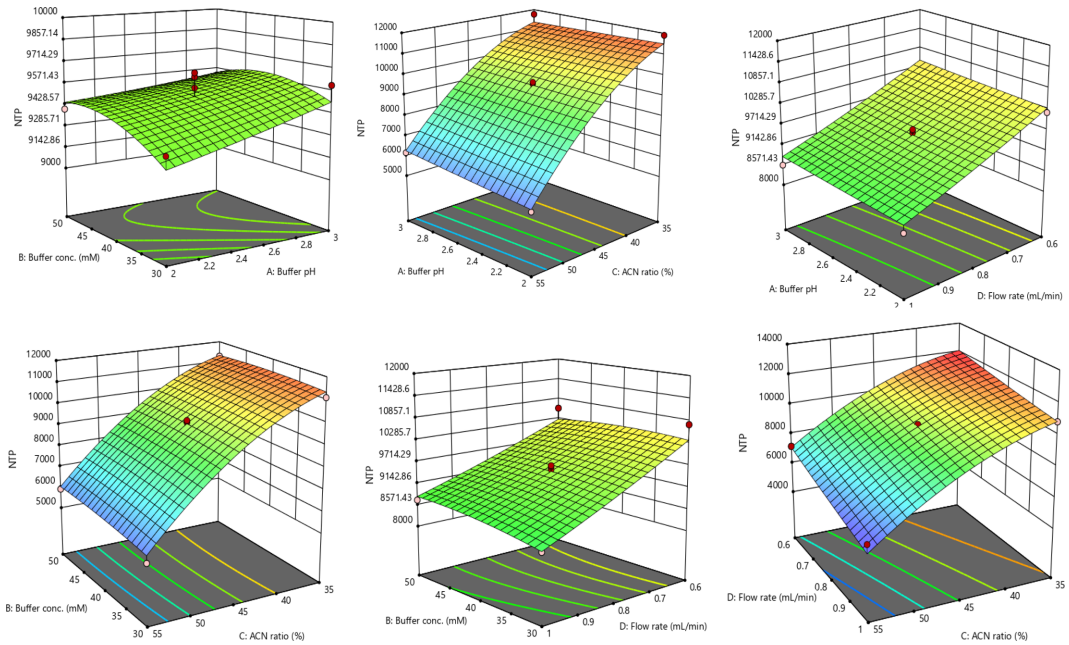

Fig. 3. Interactions between the chosen factors affecting on the number of theoretical plate.

the result, capacity and tailing factor were mainly influenced by percentage of ACN in the mobile phase. To obtain the suitable range of capacity (k'= 2-5), percentage of ACN should not be lower than 40 %. For peak shape, the lower ACN proportion showed the better result in tailing factor. The 3D plots in *Fig.* 3. showed the interactions between the chosen factors affecting on the number of theoretical plate. The higher number of theoretical plate can be achieved when the low ACN percentage combined

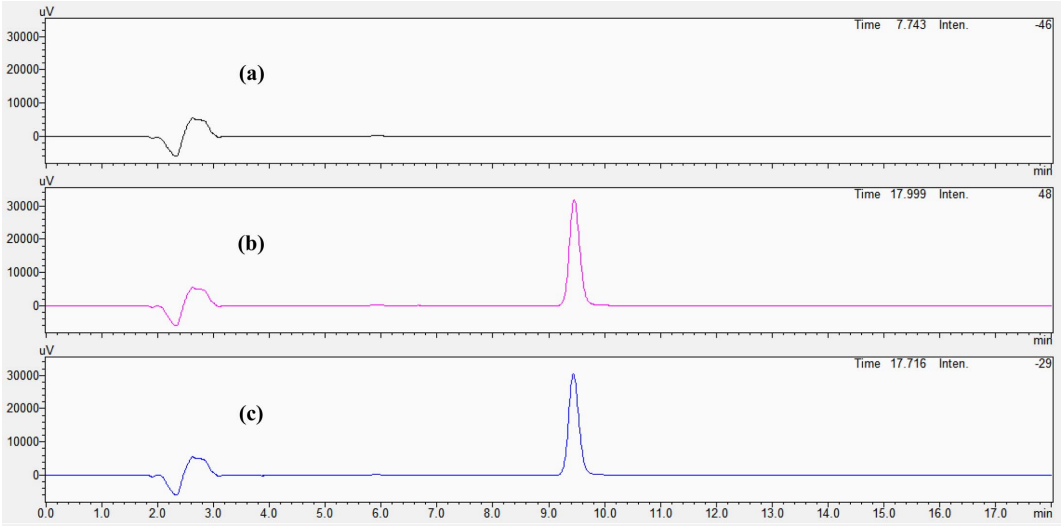

Fig. 4. Typical chromatograms of blank (a), standard solution (b) and sample solution (c). Condition: Column Luna C18(2) (150 × 4.6 mm I.D., 5 µm), mobile phase containing 40 mM potassium phosphate monobasic buffer pH 3.0 and acetonitrile (56:44, v/v), flow rate 0.7 mL/min, injection volume 10µL, detection at 214 nm.

with low flow rate, high buffer pH and middle buffer concentration. The objectives of this optimization are minimizing the capacity and maximizing the tailing factor as well as the number of theoretical plate. After setting these goals in the software, in 100 found solutions, the most suitable solution was chosen (buffer pH 3.0; buffer concentration 40 mM; 44 % ACN in mobile phase and flow rate 0.7 mL/min).

Finally, the recommended condition was applied for a confirmation test ($n = 6$). The results were reached the goals with satisfied parameters of capacity (k'= 3.465), tailing factor (T_f= 1.147) and number of theoretical plate (NTP = 11537).

3.2. Validation

3.2.1. Specificity and system suitability

As illustrated in *Fig.* 4, peak of loperamide was eluted with reasonable capacity and good peak shape. Through the PDA detector, the presence of impurities in the main peak can be easily determined by purity index and similarity curve including similarity (SI) and threshold (t). In *Fig.* 5, the similarity index (SI) is not lower than the threshold index (t) at each sampling point. In addition, the minimum purity index which is the threshold subtracted from the similarity, is positive value for loperamide peak of standard and sample solution. It proved that no contaminated content is detected at position of

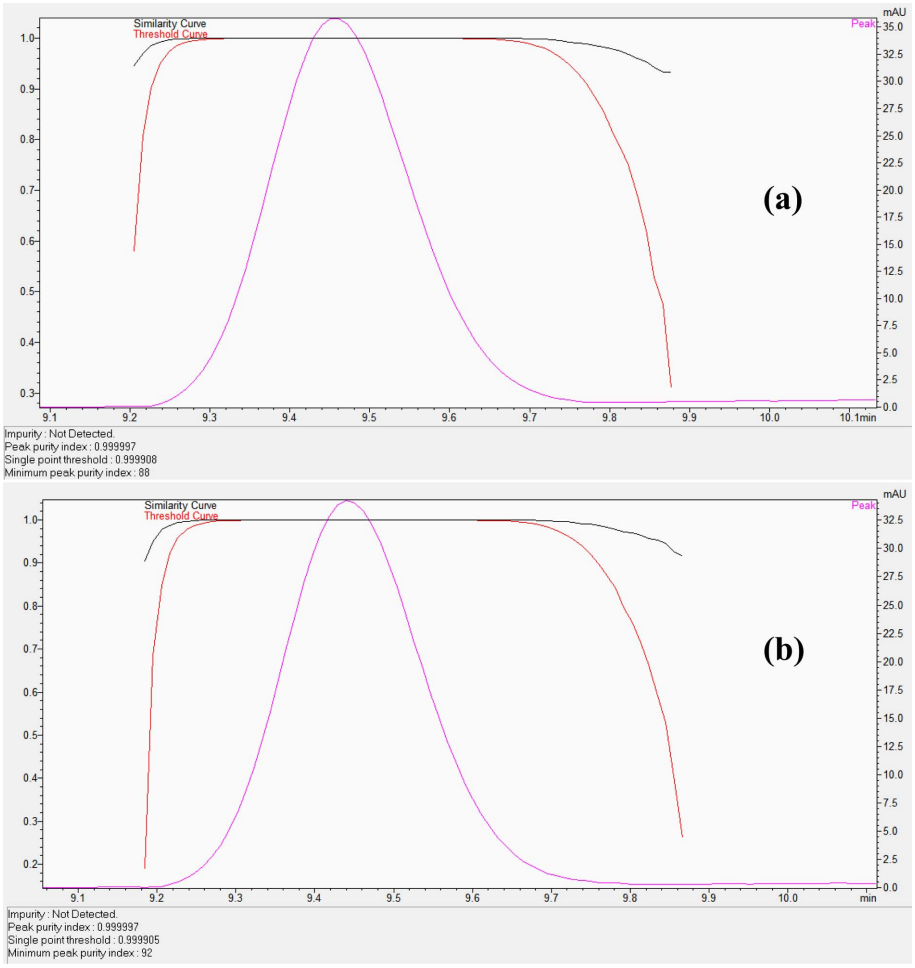

Fig. 5. Similarity curve and purity index of standard solution (a) and sample solution (b).

Table 4. System suitability data (n = 6)

	Retention time	Peak area	Tailing factor	NTP
AVERAGE	9.523	417544	1.133	11604
RSD%	0.29	0.29	0.07	0.18

NTP: Number of Theoretical Plate

loperamide peak in both standard and sample chromatogram.

The system suitability of the proposed method was assessed based on relative standard deviation (RSD) of the retention time, peak area and the value of tailing factor as well as number of theoretical plates (*Table* 4).

3.2.2. Linearity and LOD/LOQ

The sensitivity of the proposed method was determined by calculating the ratio of signal-to-noise. The LOD and LOQ were 0.02 μg/mL and 0.06 μg/mL, respectively [$n = 6$]. The linearity was evaluated with the range from 1 to 30 μg/mL. The proposed method showed good linearity along this range with a correlation coefficient (R^2) > 0.9996 for both 6 sets. Statistical parameters of ANOVA ($p = 0.05$) showed the regression linearity of the method (*Table* 5).

Table 5. Linearity and sensitivity results

Parameter	Loperamide
Regression equation	y = 43626x – 2798.07
Range (μg/mL)	1 – 30
Correlation coefficient (R^2)	0.9998
Number of data points	6
Slope ± SD	43626 ± 954.22
Intercept ± SD	-2798.07 ± 8400.96
LOD (μg/mL)	0.02
LOQ (μg/mL)	0.06

SD: Standard deviation

3.2.3. Precision and accuracy

The repeatability of method was confirmed by the RSD% of both intraday and interday precision (RSD% <1.05 % and < 1.06 %, respectively). For intermediate precision, the method was validated using another system (AGILENT 1100 series) with RSD% of peak area in 6 injections was 0.13 %.

The recovery of loperamide in each spiked samples in accuracy test was in the range of 98.77-101.28 % and the RSD% for each concentration was less than 1.26 %.

The obtained results of precision and accuracy test are shown in detail (*Table* 6).

3.2.4. Robustness

The robustness of the proposed method was proven with small deliberate variations in the ratio of acetonitrile (± 2 %), buffer concentration (± 2 mM), pH of buffer (± 0.2), flow rate (± 0.1 mL/min) and temperature (± 2 °C). The results of method were not influenced (except changes in retention time). In both cases, RSD% of retention time and peak area (n = 6) were not more than 0.47 % and 0.82 %, respectively. Peak shape and column efficiency were also confirmed with tailing factor < 1.15 and number of tailing factor > 11000.

3.3. Application

The proposed method was successfully applied on commercial products. The amount of loperamide hydro

Table 6. Results of repeatability precision and accuracy in validation

Conc. (μg/mL)	Intra-day Precision (n=5)		Inter-day Precision (n=11)		Accuracy (n=3)	
	Recovery	RSD%	Recovery	RSD%	Recovery	RSD%
8	99.64	1.05	99.79	0.94	99.09	0.04
10	100.32	1.02	100.45	1.06	100.08	1.26
12	98.78	0.80	98.96	0.76	99.12	0.22

Table 7. Content of Loperamide hydrochloride in capsules for application (n = 6)

Product name	Claimed value (mg/capsule)	Content	RSD%
Capsule A	2	99.49	1.46
Capsule B	2	99.83	1.83

chloride present in 2 different capsules A and B (n = 6) was 99.49 % (RSD = 1.46 %) and 99.83 % (RSD = 1.83 %), respectively (*Table* 7).

4. Conclusions

The proposed HPLC method was considerably improved to effectively determine loperamide hydrochloride in capsules in comparison with conventional methods of other pharmacopoeias (*Table* 8). The developed method used the most popular column (C18) for convenient application and avoided using paired-ion chromatography reagent which can cause column consumption. Moreover, under DoE concept, HPLC conditions were optimized to obtain the best results in peak shape, column efficiency in reasonable running time with lowest pressure for long column life. The optimal method was successfully validated and obviously proved to be accurate, robust and sensitive. Therefore, this method is appropriate to frequently utilize in quality control and well worth replacing the current method in Korean Pharmacopoeia XII.

Acknowledgements

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Table 8. Comparison with conventional methods from other pharmacopoeias for determination of Loperamide HCl formulation

Pharmaco-poeia	Column	Condition	Capac-ity	Tailing factor	NTP	Pressure (bar)
Proposed method	Luna C18(2) (150 × 4.6 mm ID, 5 µm)	MP: 40 mM KH_2PO_4 pH 3 / ACN (56:44) Temperature 35°C, flow rate 0.7 mL/min, UV 214 nm.	3.809	1.133	11604	57
KP12 (Capsules)	Luna C18(2) (150 × 4.6 mm ID, 5 µm)	MP: 1.8 g/300 mL Na_2HPO_4 pH 7.0 / MeOH (300:700) Flow rate adjusted to retention time 9 min, UV 214 nm.	Miscibility problem			
USP43 (Capsules)	INNO CN (250 × 4.0 mm ID, 5 µm)	MP: ACN/H_2O/H_3PO_4 (500 mL:500 mL:20 drops) Flow rate 2.0 mL/min, UV 220 nm.	1.445	0.925	2276	184
BP2017 (Capsules)	Luna C18(2) (150 × 4.6 mm ID, 5 µm)	MP: Solvent A/ACN (44/55) Solvent A: 0.005M sodium octanesulfonate, per 1000 mL, 1 mL of 13.5M ammonia and 0.5 mL of trimethyl-amine and adjust to pH 3.2 with H_3PO_4. Temperature ambient, flow rate 1.5 mL/min, UV 226 nm.	1.548	1.550	4005	102
USP43 (Tablets)	Luna C18(2) (150 × 4.6 mm ID, 5 µm)	MP: Solvent mixture/Ion pairing solution (55/45) Solvent mixture: MeOH : ACN = 3:1 Ion pairing solution: 2.35 g/L of sodium 1-hexanesul-fonate and 2.88 g/L of $NH_4H_2PO_4$ in water, adjusted with phosphoric acid to a pH of 3.2. Flow rate 2.0 mL/min, UV 219 nm.	28.209	1.407	4930	205
USP43 (Oral solution)	Aegispak C8 (150 × 4.6 mm ID, 5 µm)	MP: 3 g/L KH_2PO_4 pH 3 / ACN (63:37) Flow rate 1.5 mL/min, UV 214 nm.	8.540	1.167	9369	112
BP2017 (Oral solution)	Luna C18(2) (150 × 4.6 mm ID, 5 µm)	MP: THF/ACN/solution A (5/37/58) Solution A containing 0.46 % w/v of ammonium dihydrogen phosphate and 0.61 % w/v of decanesul-fonic acid (pH2.1). Temperature 30 °C, flow rate 1.0 mL/min, UV 219 nm.	27.025	1.061	10002	82

NTP: Number of Theoretical Plate

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Authors' Position

Thi-Anh-Tuyet Le : Graduate student
Bao-Tan Nguyen : Graduate student
Min-Ho Kim : Researcher
Bit Kim : Researcher
Hyun-Soo Kim : Researcher
Seung-Won Jeong : Researcher
Jong-Seong Kang : Professor
Dong-Hee Na : Professor
In-Koo Chun : Chairman of the foundation
Kyeong Ho Kim : Professor