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Original Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR DETERMINATION OF SOLUBILITY OF FUROSEMIDE

Mustafa Sinan KAYNAK[1,2], Selma SAHİN[1*]

[1]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06100, Ankara, TURKEY
[2]İnönü University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 44280, Malatya, TURKEY.

Abstract

The objective of the Biopharmaceutics Classification System is to allow prediction of in vivo pharmacokinetic performance of drug products from in vitro measurements; therefore, it is important to determine the solubility and permeability of drug substances. Furosemide (FSM) is a loop diuretic commonly used in the treatment of edematous states associated chronic renal failure, hypertension, congestive heart failure and cirrhosis of the liver. The aim of this study was to develop and validate an HPLC method for quantification of FSM in the samples obtained from the in vitro solubility studies performed at five different pH values (pH 1.0, 2.9, 3.9, 4.9 and 7.5). Chromatographic separation of FSM was achieved on a reverse phase column (Waters Spherisorb ODS2 C_{18} 250x4.6 mm 5 µm) with a mobile phase consisted of 0.01M KH_2PO_4 (pH 5.5) and methanol (70:30 v/v). Analyses were run at a flow rate 1 mL/min and UV detection was performed at 235 nm. Under these conditions, the retention time of FSM was about 7.0 min. The method was linear in the concentration range of 0.5 to 50 µg/mL, and limit of quantification was 320 ng/mL. Developed and validated HPLC method was proved to be simple, reliable and also suitable as a single method for studying the solubility of FSM as a function of pH. Finally, based on our results, solubility of FSM was dependent on pH. Its solubility was low between pH 1.0 and 4.9, and was high at pH 7.5.

Key words: *Furosemide, HPLC method, BCS, Solubility class, Dose number*

Furosemidin Çözünürlüğünün Tayini için Bir RP-HPLC Metodun Geliştirilmesi ve Validasyonu

Biyofarmasötik Sınıflandırma Sisteminin amacı ilaç ürünlerinin invitro ölçümlerinden invivo farmakokinetik performansının öngörülmesine olanak sağlamaktır; bu nedenle ilaçların çözünürlük ve permeabilitelerinin tayini önemlidir. Furosemid (FSM) karaciğer sirozu, konjestif kalp yetmezliği, hipertansiyon ve kronik böbrek yetmezliğiyle ilişkili ödemin tedavisinde kullanılan bir diüretiktir. Bu çalışmanın amacı beş farklı pH (pH 1.0, 2.9, 3.9, 4.9 and 7.5) değerinde gerçekleştirilmiş in vitro çözünürlük deneylerinden elde edilen örneklerden FSM'in tayini için bir HPLC yöntemi metodu geliştirmek ve valide etmektir. FSM'in kromatogtafik ayırımı bir ters faz kolonuyla (Waters Spherisorb ODS2 C_{18} 250x4.6 mm 5 µm), 0.01M KH_2PO_4 (pH 5.5) ve metanol (70:30 v/v) içeren mobil faz ile gerçekleştirilmiştir. Bu koşullar altında FSM'in alıkonma zamanı yaklaşık 7.0 dk'dır. Yöntem 0.5 to50 µg/mL konsantrasyon aralığında doğrusaldır ve ölçülebilir alt sınır 320 ng/mL'dir. Geliştirilen ve valide edilen HPLC yönteminin FSM'in, pH'nın fonksiyonu olarak, yapılan çözünürlük çalışmaları için basit, güvenilir ve de kullanışlı bir yöntem olduğunu kanıtlanmıştır. Son olarak, sonuçlarımıza göre; FSM'in çözünürlüğü pH'ya bağlıdır. FSM'in çözünürlüğü pH 1.0-4.9 arasında düşük ve pH 7.5'de yüksektir.

Anahtar kelimeler: *Furosemid, HPLC yöntemi, BCS, Çözünürlük sınıfı, Doz sayısı*

***Correspondence**: e-mail: sahin.selma@gmail.com; tel: +90 312 305 12 41; fax: +90 312 310 09 06

INTRODUCTION

The oral absorption of a drug is fundamentally dependent on that drug's aqueous solubility and gastrointestinal permeability. Extensive research into these fundamental parameters by Amidon *et al.* (1) led to the Biopharmaceutics Classification System (BCS) that categorizes drugs into four groups, Class 1(high solubility and high permeability), Class 2 (low solubility and high permeability), Class 3 (high solubility and low permeability), and 4 (low solubility and low permeability). In 2000, the FDA promulgated the BCS as a science based approach to allow waiver of *in vivo* bioavailability and bioequivalence testing for immediate release solid dosage forms for Class 1 compounds, highly soluble and highly permeable drugs, when such drug products also exhibit rapid dissolution (2). The objective of the BCS is to allow prediction of *in vivo* pharmacokinetic performance of drug products from *in vitro* measurements; therefore, it is important to determine the solubility and permeability of drug substances.

Furosemide (FSM; Figure 1) is a loop diuretic used in adults, infants and children for the treatment of edema associated with congestive heart failure, cirrhosis of the liver and renal disease. Oral furosemide may be used in adults for the treatment of hypertension of alone or in combination with other antihypertensive agents (3-5). The therapy should be individualized according to patient response to gain maximal therapeutic response and to determine the minimal dose needed to maintain that response. The usual dose of FSM in edema is 20 to 80 mg given as a single dose. If needed, the same dose can be administered 6 to 8 hrs later or the dose may be increased (4-6). In the case of hypertension, the usual initial dose is 80 mg, usually divided into 40 mg twice as day.

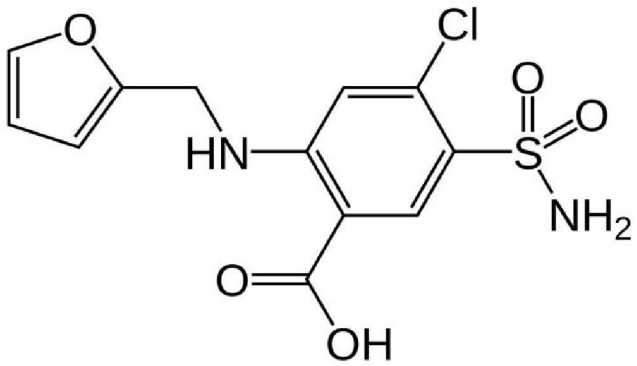

Figure 1. Chemical structure of FSM (3).

Due to its weak acidic properties (pKa=3.9; (7)) FSM is mostly absorbed from stomach and upper small intestine (8). Bioavailability of FSM from tablets varies from 37% to 70% (6-11). Plasma peak concentration (C_{max}) is occurred between 48-90 min (6, 8, 12). The plasma half-life ($t_{1/2}$) of FSM in healthy subjects is about 30-90 mins (13).

Several HPLC methods have been reported for determination of FSM from tablets (14-18), human serum (14, 19-21), dog plasma (22), rabbit plasma (16), human urine (21, 23, 24), intestinal perfusion (25, 26) and milk (27, 28) samples (Table 1). All these methods differ with respect to the mobile phases, columns and detector systems used for the analysis of the compound. Some of the methods have not been formally validated. On the other hand, although the retention time of FSM is about 2 min in the HPLC method reported by Semaan *et al.* (15), the column used in that method is not commercially available.

The initial aim of this study was to develop a simple, rapid and validated HPLC method for quantification of FSM in the samples obtained from the *in-vitro* solubility studies, as the HPLC methods reported in the literature are not specific for our purpose. The second aim was to apply

the developed method for determination of FSM solubility as a function of pH which recommended by FDA Guidance (2).

EXPERIMENTAL

Chemicals and Reagents

FSM was kindly supplied by Sanofi-Aventis (Türkiye). All HPLC grade solvents (methanol, acetonitrile) were purchased from Sigma-Aldrich (Germany). The water used in the HPLC analyses was deionized water obtained from Millipore Simplicity 185 Water Purification Systems (France). All other chemicals were of analytical reagent grade or better and purchased commercially.

Chromatographic System and Conditions

The HPLC system comprised of Water 2690 Separations Module equipped with a Waters 2996 Photodiode Array Detector (Waters, USA). FSM was separated using a Waters Spherisorb ODS2 C_{18} (250x4.6 mm 5 µm; USA) column. The HPLC system was operated at room temperature using a mobile phase was consisted of phosphate buffer (0.01M; pH 5.5) and methanol (70:30, v/v). After mixing, the mobile phase was filtered through a 0.45 µm membrane filter and degassed before use. Analyses were run at a flow rate 1 mL/min and UV detection was performed at 235 nm. Drug concentrations in samples were calculated from peak areas obtained from analyses using Empower™ software.

Standard Stock Solutions

Standard stock solution of FSM was prepared in methanol at concentration of 1 mg/mL. This stock solution was then diluted with the mobile phase to obtain secondary standard stock solution (0.1 mg/mL).

Calibration and Validation

The secondary standard stock of FSM was diluted with the mobile phase to obtain the calibration standards at concentrations of 0.5, 1.0, 2.0, 5.0, 7.5, 10, 20, 30, 40, 50 µg/mL FSM. The peak area was plotted against the corresponding concentration to obtain the calibration graph. Calibration curve and corresponding determination coefficient (r^2) were then calculated by least squares linear regression analysis. The method was validated according to the International Conference on Harmonization (ICH) Guideline and Text on Validation of Analytical Procedures: Text and Methodology Q2R1 (29).

The proposed method was validated as to specificity, linearity, precision and accuracy. Assay specificity was examined in relation to interference from matrix components in drug free buffers used for the solubility of FSM. Linearity of the method was assessed by the calibration equation which was characterized by determination coefficient, slope and intercept. Repeatability was assessed at two levels; method and injection repeatabilities. Method repeatability was determined by single injections from six independent standard solutions containing 10 µg/mL FSM, whereas, injection repeatability was determined from six consecutive injections from the same standard solution containing 10 µg/mL FSM. Repeatability was assessed by calculating the relative standard deviation (RSD %) of the measurements. Inter- and intra-day precision and accuracy of the analytical method were determined at three test concentrations (1, 10, 40 µg/mL) and calculated as percentage recovery. The sensitivity of the analytical method was evaluated by determining the limits of detection (LOD) and quantitation (LOQ). Based on FDA guidance, the signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively.

Table 1. Chromatographic conditions of the reported methods for the separation and determination of FSM.

Samples	Column	Detection	Mobile Phase	Flow Rate (mL/min)	Extraction (+/-)	LOD/LOQ (µg/mL)	Retation Time (min)	Ref.
Tablet Human Plasma	Purosphere C_{18} (250x4.6mm, 5µm)	Ultraviolet 232 nm	MeOH:Water:ACN (pH 3.0) (75:25:5)	0.8	-	0.84/2.55	4.2	(14)
Human Urine	Purosphere C_{18} (250x4mm, 5µm)	Ultraviolet 234 nm	0.05% TFA:ACN:MeOH Gradient	1.0-1.4	+	0.02/0.05	38.5	(23)
Dog Plasma	Spherisorb ODS 25µm	Ultraviolet 235 nm	Water:ACN (70:30) (pH 6.5)	1.0	+	-	-	(22)
Intestinal Perfusion	Symmetry Shield C_{18} (150x4.6mm, 5µm)	Ultraviolet 228 nm	KH_2PO_4:MeOH (0.01M, pH5.5) Gradient	1.5	-	-	17.0	(25)
Intestinal Perfusion	Shimpack ODS (250x4.6mm, 5µm)	Ultraviolet 280 nm	ACN:Water:GAA: TEA(0.1%) (41.5:57.4:0.9:0.1)	1.0	-	2.4/7.2	8.2	(26)
Human Urine	Spherisorb ODS C_{18} (125x4mm, 5µm)	Ultraviolet 270 nm	Ammonium Accetate:ACN (pH 3.0) Gradient	1.5	+	0.125/-	<7.0	(24)
Tablet	Home Made Column C_{18} (50x4.6mm, 3µm)	Ultraviolet 237 nm	KH_2PO_4:MeOH (70:30) (0.01M, pH5.5)	1.0	-	-	2.0	(15)
Human Plasma	Kromasil 100-5 C_{18} (150x4.6mm, 5µm)	Fluorescence Ex:268 nm Em:410 nm	KH_2PO_4:ACN (66:34) (0.02M, pH 3.0)	1.0	+	0.001/0.003	7.9	(19)
Milk	Kromasil C_{18} (150x4.6mm, 5µm)	Amperometric	NaH_2PO_4:ACN (75:25) (0.005M)	1.4	-	-	-	(27)
Tablet Rabbit Plasma	Nucleosil C_{18} (250x4.6mm, 10µm)	Ultraviolet 235 nm	KH_2PO_4:ACN (80:20) (0.02M, pH 4.5)	3.0	+	0.1/0.04	8.1	(16)
Human Plasma	Bondapak C_{18} 150x3.9mm, 5µm)	Fluorescence Exc:230 nm Em:410 nm	KH_2PO_4:ACN (66.7:33.3) (0.02M, pH 3.0)	1.0	+	-	-	(20)
Tablet	Bondapak C_{18}	Amperometric	KH_2PO_4/K_2HPO_4: ACN (30:70) (0.05M, pH 5.5)	1.0	+	0.0015/-	-	(17)
Human Urine Tablets	Bondapak C_{18} (35°C)	Amperometric	KH_2PO_4/K_2HPO_4: ACN (40:60) (0.05M, pH 5.5)	1.0	+	-	-	(18)
Milk	Spherisorb 5 ODS2 (30°C)	Fluorescence Exc:272 nm Em:410 nm	KH_2PO_4:ACN (70:30) (pH 3.0)	-	+	-	-	(28)
Human Plasma	C_{18} Column	Fluorescence Exc:235 nm Em:389 nm	NaH_2PO_4:MeOH (65:35) (0.01M, pH 3.5)	3.0	-	-	-	(21)
Human Urine	C_{18} Column	Fluorescence Exc:235 nm Em:389 nm	Acetic Acid:CHS_3OH (60:40) (pH 3.5)	3.0	-	-	-	(21)

Abbreviations: ACN: Acetonitrile, TFA: Trifluoro Acetic Acid, MeOH: Methanol, GAA: Glaciel Acetic Acid, TEA: Triethylamine, Exc: Excitation, Em: Emission.

Solubility Studies

The method was applied to determine the *in-vitro* solubility of FSM. The saturation solubility of the compound was determined at five different pH values (1.0, 2.9, 3.9, 4.9,7.5) according to FDA guidance (2). Solubility studies were repeated six times at each pH conditions. Five different pH mediums were prepared according to USP 29 (30). Excess amount of FSM was added in a suitable buffer solution and agitated overnight in a horizontal shaker (100 rpm; 37±0.5°C). Sample (1 mL) was withdrawn at the end of the experiment (24 h) and filtered through a La-Pha-Pack HPLC syringe filter (0.45 μm) which has no absorption for FSM. An aliquot (200 μL) of filtered sample was diluted with mobile phase to a final volume of 1 mL, and then 10 μL was injected into HPLC system.

Data Analysis

Saturation solubility of FSM was determined as a function of pH values using the calibration curves. The volume of the buffer required to solve the highest dose strength of FSM was then calculated using these solubility values. For each condition, dose number (Do) was calculated using the dose (D) of FSM, volume (V=250 mL) and solubility (Eq. 1) (31).

$$D_o = \frac{D/V}{S} \qquad \text{Eq.1}$$

All tabulated results were expressed as mean ± standard error (S.E.)

RESULTS AND DISCUSSIONS

Method Development

In order to achieve acceptable peak shape and perform the separation on a suitable run time, various buffer systems were tried systematically. The mixture of a mobile phase was consisted of phosphate buffer (0.01M; pH 5.5) and methanol (70:30 v/v) was capable of a good separation and defined as the optimum conditions. Under optimum conditions, FSM was successfully separated by C_{18} (Waters Spherisorb ODS2 C_{18} 5 μm 250x4.6 mm) column from the matrix components with the retention time of 6.9±0.3 min. Total run time for an assay was approximately 10 min, and the interval between two consecutive injections was 1 min. The representative chromatograms obtained in the standard stock solution of FSM at different pH values (1.0, 2.9, 3.9, 4.9, 7.5) are depicted in Figure 2. FSM was well separated from the matrix components with no interfering peaks in the relevant portion of the trace, and the retention time was not influenced by the pH value of the sample.

Validation of the Method
Linearity

The calibration curve for FSM constructed under optimum conditions and linearity of the method was determined by performing injections at ten different concentration levels in the linear range over 6 different days. The peak area of FSM was plotted against the corresponding nominal concentration to obtain calibration graph. Thus, the method was evaluated linear in the range of 0.5 to50 μg/mL for FSM. The regression equation data are given in Table 2.

Sensitivity

The sensitivity of the analytical method was evaluated by determination of the limits of detection (LOD) and quantitation (LOQ). The signal-to-noise ratios of 3:1 and 10:1 were taken as LOD and LOQ, respectively. The values of LOD and LOQ for FSM were 80 ng/mL and 320 ng/mL, respectively and given in Table 2.

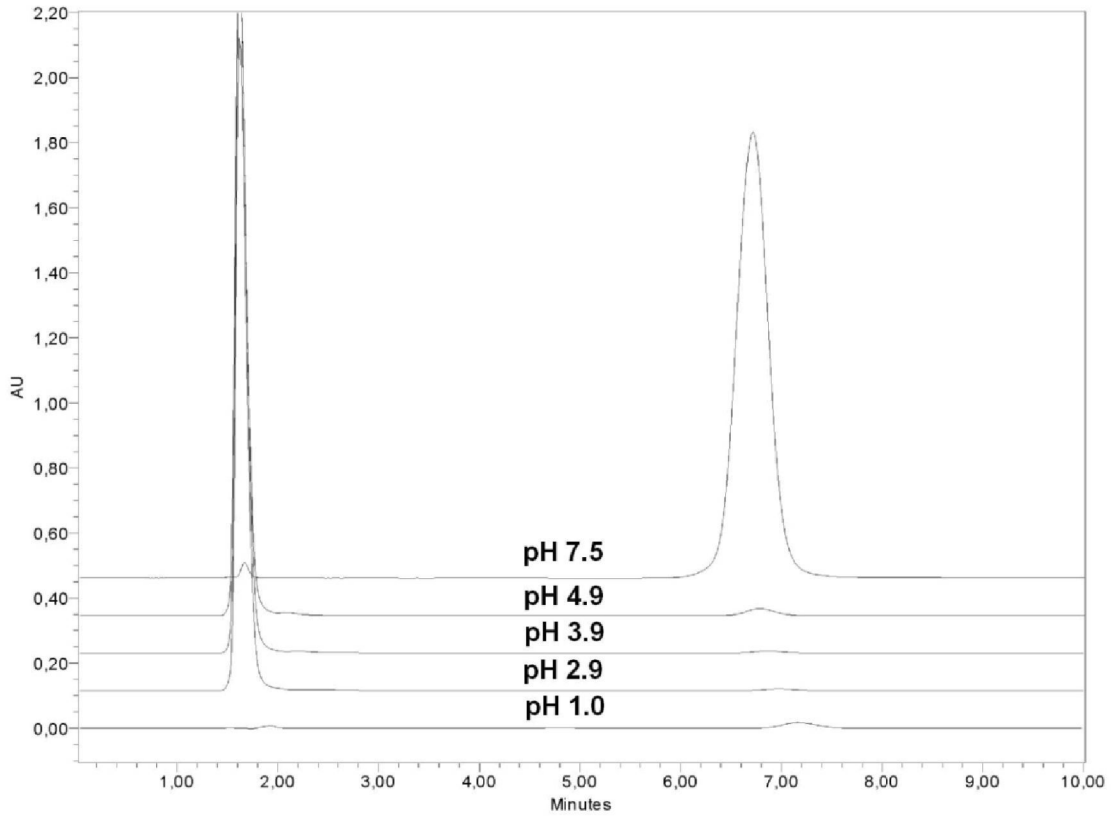

Figure 2. FSM containing sample chromatograms at five different pH values.

Repeatability

In order to measure the method repeatability of the developed method, single injections were made into the HPLC system from six independent standard solutions containing 10 μg/mL FSM. Injection repeatability was determined from six consecutive injections from the same standard solution (10 mg/mL). The relative standard deviations (RSD %) determined for the method and injection repeatabilities were less than 1% and 0.2%, respectively. All these results can be taken as an indication of high repeatability.

Table 2. Linearity and sensitivity data of the developed method (n=6)

	Data
Regression equation*	y = 47526x – 5652
Standard error of intercept	575
Standard error of slope	4133
Determination coefficient (r^2)	0.9989
Linearity range (μg/mL)	0.5 – 50
Number of data points	10
LOD (ng/mL)	80
LOQ (ng/mL)	320

*where y is peak area and x is concentration in μg/mL of FSM

Precision and Accuracy

Three different concentrations of standard FSM solutions (within the linear range) were analyzed six consecutive days (inter-day precision) and six times within the same day (intra-day precision). The RSD values for intra- and inter-day precision were less than 2%, and corresponding Bias values were 15% (Table 3) indicating that the precision and accuracy of the method were satisfactory.

Table 3. Precision and accuracy data of the developed method

	Intra-day			Inter-day		
Added (µg/mL)	**Found* (µg/mL)**	**Precision RSD%**	**Accuracy Bias%**	**Found* (µg/mL)**	**Precision RSD%**	**Accuracy Bias%**
1	1.09 ± 0.01	1.60	-9.69	1.11 ± 0.01	1.79	-11.14
10	9.93 ± 0.08	1.94	0.67	9.92 ± 0.08	1.97	0.78
40	40.3 ± 0.28	1.72	-0.68	40.3 ± 0.32	1.93	-0.68

* mean ± standard error (n=6), Bias % = [(Found-Added)/Added] x 100

Application to Solubility Studies

It is recommended that the pH-solubility profile of the test drug substance should be determined at 37 ±1°C in aqueous media with a pH in the range of 1.0-7.5. According to the FDA Guidance (2), the number of pH conditions for a solubility determination can be based on the ionization characteristics of the test drug substance. When the pKa value of drug is in the range of 3-5, the solubility should be determined at pH=pKa, pH=pKa+1, pH=pKa-1 and at pH = 1.0 and 7.5. FSM has a pKa value of 3.9 (7). Therefore, we determined the solubility of the drug in aqueous media at pH values of 1.0, 2.9, 3.9, 4.9 and 7.5. Our experimental results showed that FSM has pH dependent solubility. Solubility of FSM increased from 0.028 mg/mL to 6.411 mg/mL (Table 4) when the pH medium increased from 1 to 7.5 (Figure 3).

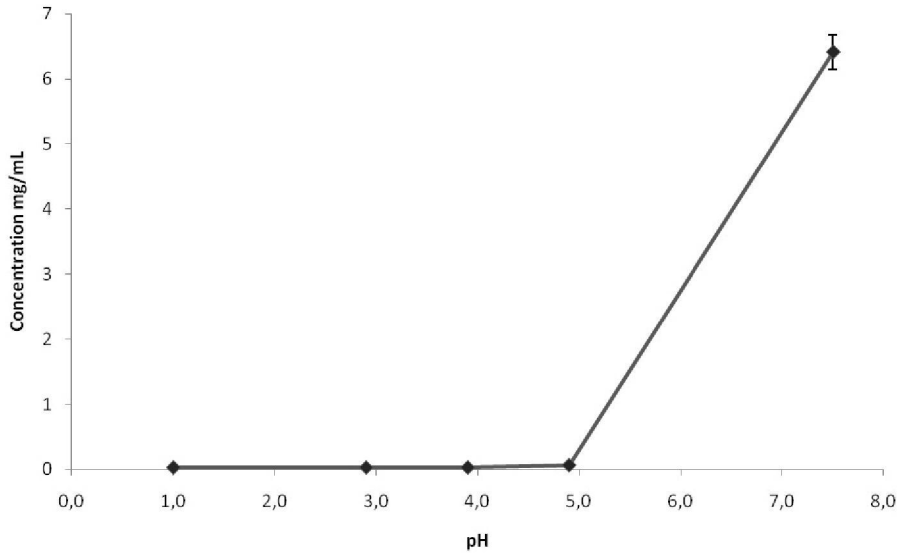

Figure 3. pH solubility profile of FSM (mean ± SE, n=6).

Table 4. Solubility, volume required to solve the highest does strength of FSM and dose numbers

Medium (pH)	Solubility[1] (μg/mL)	Volume required to dissolve FSM[2] (mL)	Dose Number[3] (Do)
1.0	28.4 ± 4.7	2816	11.79
2.9	27.6 ± 1.0	2898	12.76
3.9	28.5 ± 3.0	2807	11.77
4.9	63.3 ± 4.7	1263	5.24
7.5	6410.8 ± 262.1	12	0.05

[1]Determined from solubility experiments; [2]Volume required to dissolve 80 mg of FSM; [3]Dose number was calculated using Equation 1.

CONCLUSIONS

Determination of solubility class of a drug substance according to FDA guidance is very important for the pharmaceutical companies which are taking biowaiver for the immediate release oral solid drug products. Although there are various HPLC methods available for determination of FSM, in this study, a validated HPLC method was for quantification of FSM for particularly solubility studies. Developed and validated method was proved to be simple, reliable and also suitable as a single method for studying the solubility of FSM as a function of pH. Finally, based on our results, solubility of FSM was dependent on pH. Its solubility was low between pH 1.0 and 4.9, and was high at pH 7.5.

REFERENCES

1. Amidon GL, Lennernas H, Shah VP, Crison JR, A Theoretical basis for a biopharmaceutic drug classification: The correlation of in-vitro drug product dissolution and in-vivo bioavailability, Pharm Res 12(3), 413-420, 1995.
2. FDA, Waiver of In-Vivo Bioavailability and Bioequivalance Studies for Immediate-Release Solid Oral Dosage Forms based on a Biopharmaceutics Classification System, C.f.D.E.a.R. (CDER), Editor August 2000, U.S. Department of Health and Human Services Food and Drug Administration: Rockville, MD.
3. Bosch ME, Sanchez AJR, Rojas FS, Ojeda CB, Recent developments in analytical determination of furosemide, J Pharm Biomed Anal 48(3), 519-532, 2008.
4. Castaneda-Hernandez G, Verges J, Pichette V, Heroux L, Caille G, du Souich P, Input rate as a major determinant of furosemide pharmacodynamics: Influence of fluid replacement and hypoalbuminemia, Drug Metab Dispos 28(3), 323-328, 2000.
5. Brater DC, Pharmacology of diuretics, Am J Med Sci 319(1), 38-50, 2000.
6. Granero GE, Longhi MR, Mora MJ, Junginger HE, Midha KK, Shah VP, Stavchansky S, Dressman JB, Barends DM, Biowaiver monographs for immediate release solid oral dosage forms: Furosemide, J Pharm Sci 99(6), 2544-2556, 2010.
7. Ponto LL, Schoenwald RD, Furosemide (frusemide). A pharmacokinetic / pharmacodynamic review (Part I), Clin Pharmacokinet 18(5), 381-408, 1990.
8. Klausner EA, Lavy E, Stepensky D, Cserepes E, Barta M, Friedman M, Hoffman A, Furosemide pharmacokinetics and pharmacodynamics following gastroretentive dosage form administration to healthy volunteers, J Clin Pharmacol 43(7), 711-720, 2003.
9. Ponto LL, Schoenwald RD, Furosemide (frusemide). A pharmacokinetic / pharmacodynamic review (Part II), Clin Pharmacokinet 18(6), 460-471, 1990.

10. Hammarlund MM, Paalzow LK, Odlind B, Pharmacokinetics of furosemide in man after intravenous and oral administration. Application of moment analysis, Eur J Clin Pharmacol 26(2), 197-207, 1984.
11. Kelly MR, Cutler RE, Forrey AW, Kimpel BM, Pharmacokinetics of orally administered furosemide, Clin Pharmacol Ther 15(2), 178-186, 1974.
12. Abou-Auda HS, Al-Yamani MJ, Morad AM, Bawazir SA, Khan SZ, Al-Khamis KI, High-performance liquid chromatographic determination of furosemide in plasma and urine and its use in bioavailability studies, J Chromatogr B 710(1-2), 121-128, 1998.
13. Brater DC, Seiwell R, Anderson S, Burdette A, Dehmer GJ, Chennavasin P, Absorption and disposition of furosemide in congestive heart-failure, Kidney Int 22(2), 171-176, 1982.
14. Sultana N, Shamim S, Gul MAS, Arayne MS, Simultaneous determination of gemifloxacin and diuretics in bulk, pharmaceutical dosage forms and human serum by RP-HPLC, Quim Nova 33(7), 1590-1593, 2010.
15. Semaan FS, Santos-Neto AJ, Lancas FM, Cavalheiro ETG, Rapid HPLC-DAD determination of furosemide in tablets using a short home-made column, Anal Lett 38(10), 1651-1658, 2005.
16. el-Saharty YS, Simultaneous high-performance liquid chromatographic assay of furosemide and propranolol HCL and its application in a pharmacokinetic study, J Pharm Biomed Anal 33(4), 699-709, 2003.
17. Barroso MB, Alonso RM, Jimenez RM, Simultaneous determination of the diuretics triamterene and furosemide in pharmaceutical formulations and urine by HPLC-EC, J Liq Chromatogr Relat Technol 19(2), 231-246, 1996.
18. Barroso MB, Jimenez RM, Alonso RM, Ortiz E, Determination of piretanide and furosemide in pharmaceuticals and human urine by high-performance liquid chromatography with amperometric detection, J Chromatogr B Biomed Appl 675(2), 303-312, 1996.
19. Gomez C, von Plessing C, Godoy CG, Reinbach R, Godoy R, Method validation for the determination of furosemide in plasma by liquid-liquid extraction and high-performance liquid chromatography with fluorescence detection, J Chil Chem Soc 50(2), 479-482, 2005.
20. Najib N, Idkaidek N, Beshtawi M, Bader M, Admour I, Alam SM, Zaman Q, Dham R, Bioequivalence evaluation of two brands of furosemide 40 mg tablets (Salurin and Lasix) in healthy human volunteers, Biopharm Drug Dispos 24(6), 245-249, 2003.
21. Lovett LJ, Nygard G, Dura P, Khalil SKW, An Improved Hplc Method for the Determination of Furosemide in Plasma and Urine, J Liq Chromatogr 8(9), 1611-1628, 1985.
22. Lima EQ, Albuquerque MM, Rodrigues OG, Alencar JRB, Medeiros FPM, Neto PJR, Technological development of 40mg furosemide tablets: equivalence and bioavaibility study in dogs, Pesq Vet Bras 27(11), 462-466, 2007.
23. Baranowska I, Markowski P, Baranowski J, Development and validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples, Anal Sci 25(11), 1307-1313, 2009.
24. Moreira V, Moreau RLM, Liquid chromatographic screening test for some diuretics of doping interest in human urine, J Liq Chromatogr Relat Technol 28(17), 2753-2768, 2005.
25. Chawla S, Ghosh S, Sihorkar V, Nellore R, Kumar TRS, Srinivas NR, High-performance liquid chromatography method development and validation for simultaneous determination of five model compounds, antipyrine, metoprolol, ketoprofen, furosemide and phenol red, as a tool for the standardization of rat in situ intestinal permeability studies using timed wavelength detection, Biomed Chromatogr 20(4), 349-357, 2006.
26. Valizadeh H, Zakeri-Milani P, Islambulchilar Z, Tajerzadeh H, A simple and rapid high-performance liquid chromatography method for determining furosemide, hydrochlorothiazide, and phenol red: Applicability to intestinal permeability studies, J AOAC Int 89(1), 88-93, 2006.
27. Guzman A, Agui L, Pedrero M, Yanez-Sedeno P, Pingarron JM, Flow injection and HPLC determination of furosemide using pulsed amperometric detection at microelectrodes, J Pharm Biomed Anal 33(5), 923-933, 2003.
28. Shaikh B, Development and Validation of a Liquid-Chromatographic Method for the Determination of Furosemide, a Diuretic, in Bovine-Milk, J Agric Food Chem 43(8), 2117-2121, 1995.

29. ICH, Q2(R1) Validation of Analytical Procedures: Text and Methodology, C.f.m.P.f.H. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Editor 2005.
30. Kim RB, Fromm MF, Wandel C, Leake B, Wood AJ, Roden DM, Wilkinson GR, The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J Clin Invest 101(2), 289-294, 1998.
31. Kasim NA, Whitehouse M, Ramachandran C, Bermejo M, Lennernas H, Hussain AS, Junginger HE, Stavchansky SA, Midha KK, Shah VP, Amidon GL, Molecular properties of WHO essential drugs and provisional biopharmaceutical classification, Mol Pharmaceutics 1(1), 85-96, 2004.

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