doi.org/10.36721/PJPS.2023.36.4.REG.1113-1119.1

Development and validation of a stability-indicating HPLC method to determine the impurity profile of fosaprepitant dimeglumine in the injection formulation

Hanbing Liu[1], Lili Huang[1], Xin Hu[1], Huaqing Lin[1*], Yuanjian Wang[2] and Yongjie Yang[2]
[1]Guangdong Provincial Key Laboratory of Advanced Drug Delivery Systems, Guangdong Pharmaceutical University, Guangzhou, China
[2]Bostal Drug Delivery Co., Ltd., Guangzhou, China

Abstract: Fosaprepitant dimeglumine, an injectable phosphorylated prodrug of aprepitant, has been approved for preventing chemotherapy-induced nausea and vomiting. A novel stability-indicating HPLC method was designed and validated to determine process- and degradation-related impurities of fosaprepitant dimeglumine in an injection formulation. Chromatographic separation was done on a NanoChrom C18 (250 mm×4.6 mm, 5µm) column at a column oven temperature of 35°C. Mobile phase A had 0.5 M ammonium dihydrogen phosphate solution (pH fixed to 2.2 with orthophosphoric acid) and acetonitrile at 80:20 ratio and mobile phase B had methanol and acetonitrile at 70:30 ratio. The formulations underwent forced degradation conditions, like acidic, basic, thermal oxidation and photolytic conditions. The designed HPLC approach was validated per International Conference of Harmonization (ICH) guidelines, including limit of detection (LOD), specificity, limit of quantitation (LOQ), accuracy, linearity, precision and robustness. The results showed that this method is specific, sensitive, precise, accurate and robust.

Keywords: Fosaprepitant dimeglumine, stability-indicating, impurity profile, HPLC.

INTRODUCTION

Chemotherapy-induced nausea and vomiting (CINV) as a frequent side effect of anticancer treatment has a negative effect on patient health-related quality of life and weakens the chemotherapy effectiveness (Aapro *et al*., 2015; Chen *et al*., 2022; Lindley *et al*., 1992; Ballatori and Roila, 2003). Fosaprepitant dimeglumine (FA) is a prodrug of aprepitant and a widely used NK-1 receptor antagonist approved as part of an antiemetic regimen to prevent nausea and vomiting due to moderately and highly emetogenic chemotherapy (Garnock-Jones, 2016; Rapoport *et al*., 2018). Several recent randomized, double-blind studies have indicated that a triple-antiemetic regimen including a single dose of intravenous FA is not inferior to a triple-antiemetic regimen including 3-day oral aprepitant (Zhang *et al*., 2020; Yang *et al*., 2017; Weinstein *et al*., 2016). Intravenous FA provides a useful addition to antiemetic therapy regimens.

FA is chemically introduced as deoxy-1-(methylamino)-D-glucitol[3-[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl) phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methy l]-2,5-dihydro-5-oxo-1H-1,2,4-triazol-1-yl]phosphonate (2:1) (salt). The chemical structures of FA and its impurities are shown in table 1.

Currently, a large body of literature on FA focuses on clinical research, including its safety and effectiveness (Saito *et al*., 2013; Ruhlmann *et al*., 2016; Willier *et al*., 2019; Cabanillas *et al*., 2019; Dranitsaris *et al*., 2022), but literature about the determination of impurities in FA drug products is limited. Azuma *et al* (2013) established an LC–MS/MS bioanalytical approach to determine fosaprepitant and aprepitant in plasma of humans and applied it to a single 150mg intravenous infusion of fosaprepitant in healthy Japanese subjects. Skrdla *et al* (2006) studied the hydrolysis efficiency of fosaprepitant on an HPLC column at different temperatures and investigated the hydrolysis kinetics of fosaprepitant in acetonitrile-0.1% v/v aqueous H_3PO_4 (50:50, v/v) at different temperatures. Shaikh *et al* (2020) designed a stability-indicating approach for the determination of organic impurity in fosaprepitant API-based quality by design, which included the validation and quantitation of only impurity A (aprepitant) and impurity D (dibenzyl fosaprepitant). In addition, there are no official methods for determining impurities in FA drug products in major pharmacopoeias (The United States Pharmacopeial Commission, 2020; Chinese Pharmacopoeia Commission, 2020; British Pharmacopoeia Commission Secretariat, 2020; European Directorate For Quality of Medicines & Health Care 2019; Pharmaceuticals and Medical Devices Agency of Japan, 2021). Hence, this study is focused on the design and validation of a novel stability-indicating reverse-phase HPLC method to determine process- and degradation-associated impurities of FA in dosage form. This method would be of great value for ensuring safe and sustainable quality products.

MATERIALS AND METHODS

Chemical agents and reagents

Fosaprepitant dimeglumine (97.85%), impurity A (99.80%), impurity B (88.47%), impurity C (98.41%),

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

impurity D (98.94%), impurity E (98.26%) and impurity F (95.58%) reference standards were obtained from Viwit Pharmaceutical Co., Ltd. (ZaoZhuang, China). Fosaprepitant dimeglumine for injection (150mg) was manufactured by a private company (GuangZhou, China). Ammonium dihydrogen phosphate was purchased from Macklin (Shanghai, China), orthophosphoric acid was purchased from Aladdin (Shanghai, China) and methanol and acetonitrile were purchased from CINC (Shanghai, China). HPLC analysis was done using water from a Milli-Q ultrapure water system (Darmstadt, Germany).

HPLC instrumentation and chromatographic conditions

Chromatographic separation was done on a Shimadzu LC2030C (Shimadzu, Kyoto, Japan) attached to a quaternary pump, a temperature-controlled autosampler and column thermostat and a photodiode array (PDA) detector. Shimadzu Labsolution software version 6.108 SP3 was used for chromatographic data processing. The NanoChrom C18 (250mm×4.6 mm, 5µm) column was applied for analysis at a sample cooler temperature of 5°C and a column oven temperature of 35°C. Mobile phase A contained 0.5M ammonium dihydrogen phosphate solution (pH fixed to 2.2 with orthophosphoric acid) and acetonitrile at 80:20 ratio. Mobile phase B contained methanol and acetonitrile at 70:30 ratio. The following gradient elution program was used: 0 min, 27% B (3 min); 22 min, 35% B; 26 min, 50% B (14 min); 45 min, 80% B (10 min); 56 min, 27% B; and 60 min, 27% B. The test solution injection volume was 10µl and the detection wavelength was set at 210nm.

Sample solution preparation

Water and acetonitrile (30:70, v/v) were applied as diluents. The standard stock solution was obtained by dissolving FA in the diluent to achieve the 0.06mg/ml concentration. The stock solution was then diluted to obtain a final standard solution concentration of 6µg/ml. Preparation of the individual impurity standard solutions was done by dissolving impurities in the diluent to achieve the concentrations as follows: impurity A, 240µg/ml; impurity B, 24µg/ml; impurity C, 24µg/ml; impurity D, 9.6µg/ml; impurity E, 24µg/ml; and impurity F, 24µg/ml. The individual impurity standard solutions were considered a working standard solution and applied for the validation assessments. The sample stock solution was prepared by dissolving powdered FA for injection to obtain a 4.8mg/ml concentration and the sample nominal concentration was finally diluted to achieve 1.2mg/ml.

Forced degradation conditions

Forced degradation studies on FA formulations can identify possible degradants, which can help to validate whether the HPLC method is stable. All stress degradation assessments were done using a primary sample concentration of 4.8mg/mL. Acid hydrolysis was done in 1 N HCl at 60°C for 10 min. Base hydrolysis was performed in 1 N NaOH at 60°C for 10 min. Oxidation assessments were conducted in 3% v/v H_2O_2 at 60°C for 10 min. Photolytic degradation studies were performed in a photo stability chamber/600 Wh/m^2 in UV light and 1.2 million lux hours in visible light. Thermal degradation studies were performed in an oven at 80°C for 12h. The specimen was further diluted to obtain a final concentration of 1.2mg/ml.

STATISTICAL ANALYSIS

All the statistical tests were performed using Excel® 2021 software (version 2304 Build 16.0) and RSD (%) was determined.

RESULTS

Development and optimization of the HPLC method

Adequate separation of FA and its impurities was done using various types of columns (Phenyl, C18), aqueous phase solutions (Phosphoric acid-water, KH_2PO_4 and $NH_4H_2PO_4$ buffer with different pH values) and organic modifier (methanol and acetonitrile) were investigated. Although the phenyl column performed well for the separation of aromatic compounds (Kalariya *et al*., 2015), C18 column exhibited a better resolution performance than that of the phenyl column in this experiment. Phosphoric acid-water solution, KH_2PO_4 and $NH_4H_2PO_4$ buffer solution all can obtain good peak shapes, but the performance of the phosphoric acid-water solution was poor regarding the resolution for FA and its impurities compared to buffer solution. The type of buffer and pH values (2.0, 2.2, 2.4) of buffer exhibit no effect on the separation of impurity A and impurity F, because impurity A and impurity F coeluted as a single peak, but methanol and acetonitrile show different performances for the separation of FA and its impurities. Compared to acetonitrile, methanol appears to perform better for coeluting impurity A and impurity F; however, the FA peak and impurity C peak produce a poor resolution simultaneously. Subsequently, different ratios of acetonitrile and methanol (90:10 and 70:30) were examined. FA and its impurities were better separated when the mixed solutions of methanol and acetonitrile were used as the organic phase compared to methanol or acetonitrile alone. The separation of FA and its impurities would increase when the proportion of methanol increased, but the resolution between FA and impurity C did not perform better. Finally, we added acetonitrile to aqueous phase to form buffer-acetonitrile system to improve the resolution between FA and impurity C. By further adjusting the gradient elution program and the proportion of acetonitrile in the aqueous phase, a clear separation characterized by desirable peak shapes and plate numbers was achieved under the chromatographic condition introduced Section 2.2.

Table 1: Chemical structures of fosaprepitant dimeglumine (FA) and its impurities

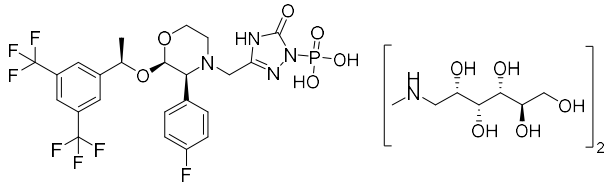
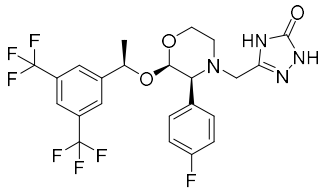
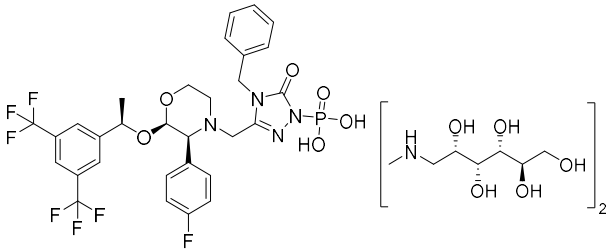
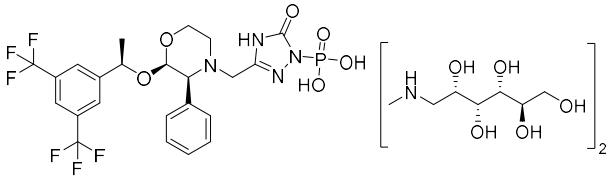
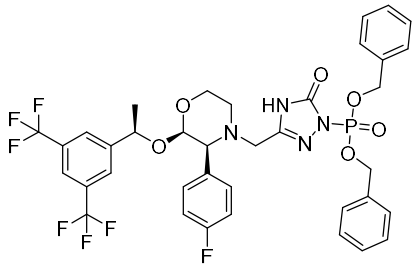
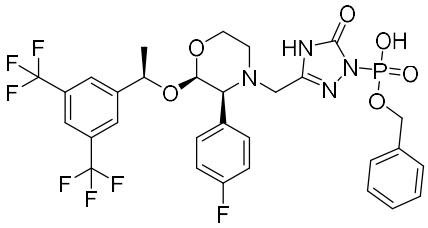
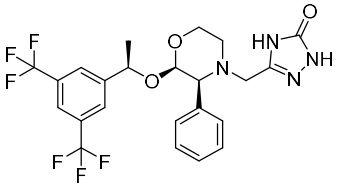
Compound	Structure	Source
FA		API
Impurity A		Degradation
Impurity B		Process
Impurity C		Process
Impurity D		Process
Impurity E		Process
Impurity F		Process

Table 2: Results of the forced degradation of FA for injection

Stress conditions	Degradants formed (%)		Mass balance (%)	Peak Purity Index
	Impurity A	SUM		
Acidic degradation	10.22	10.31	100.62	0.9167
Basic degradation	0.83	0.83	98.86	0.9191
Oxidation degradation	0.31	5.39	98.81	0.9170
Thermal degradation	11.93	12.34	99.76	0.9173
Photolytic degradation	0.43	0.43	101.4	0.9387

Table 3: LOD, LOQ and linearity of FA and its impurities.

Compound	LOD (µg/ml)	LOQ (µg/ml)	Concentration range	Linear equation	r^2	RRF
FA	0.085	0.284	0.284-4.734	Y=10371x-705.85	0.9998	1.00
Impurity A	0.056	0.186	0.186-49.692	Y=19148x+1240.9	0.9999	1.13
Impurity B	0.097	0.265	0.265-4.411	Y=11907x-597.32	0.9997	1.06
Impurity C	0.091	0.302	0.302-5.040	Y=8165x-541.88	0.9994	0.80
Impurity D	0.042	0.141	0.141-1.884	Y=17093x+1621.7	0.9964	1.01
Impurity E	0.042	0.140	0.140-4.677	Y=16246x-150.49	0.9999	0.96
Impurity F	0.044	0.147	0.147-4.913	Y=19148x+1240.9	0.9998	1.25

Table 4: Results of the precision of FA for injection

		Precision	
Compound	System precision	Repeatability	Intermediate precision
FA	0.3	1.3	1.3
Impurity A	—	1.9	1.4
Impurity B	—	2.7	2.5
Impurity C	—	2.5	1.8
Impurity D	—	2.5	2.1
Impurity E	—	2.0	2.5
Impurity F	—	1.6	2.0

Table 5: Results of accuracy for FA for injection

	Recovery (%)					
Recovery level	Impurity A	Impurity B	Impurity C	Impurity D	Impurity E	Impurity F
50%	97.19	100.60	108.52	101.31	107.20	98.90
100%	96.90	96.21	104.20	105.49	101.69	99.75
150%	95.99	106.96	102.29	98.39	101.05	96.29
RSD%	0.61	4.76	2.72	4.57	2.87	1.60

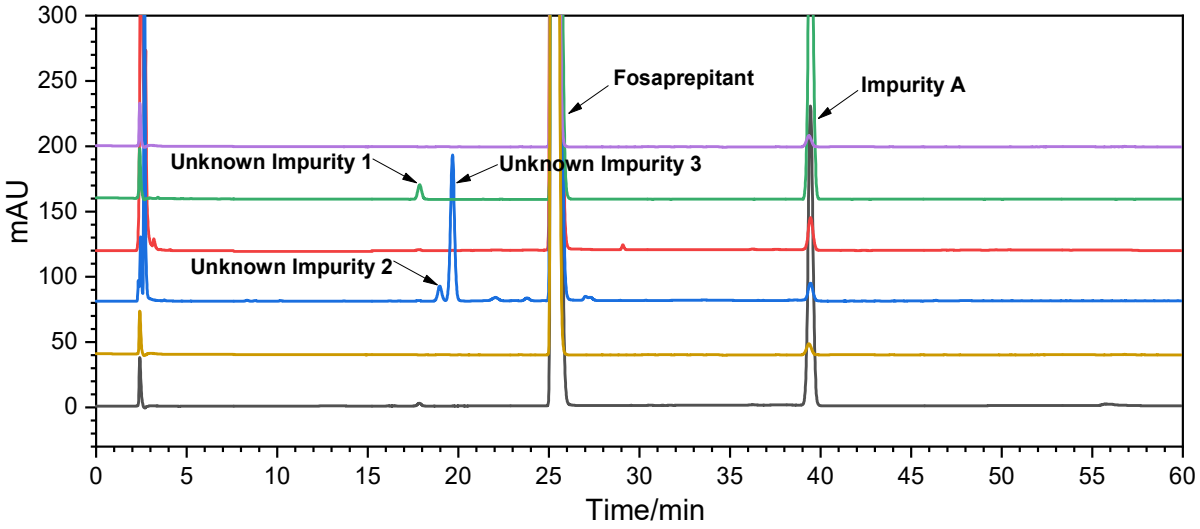


Fig. 1: Chromatograms of the forced degradation samples. Acidic degradation-black line; Basic degradation-red line; Oxidation degradation-blue line; Thermal degradation-green line; Photolytic degradation-purple line.

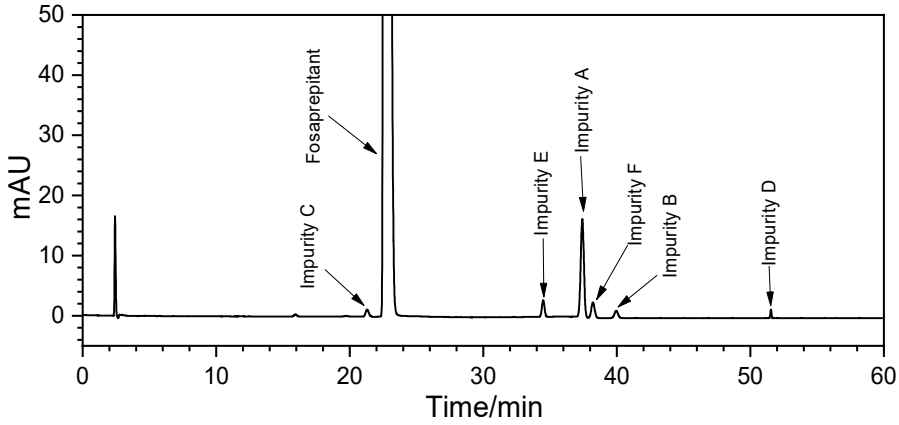


Fig. 2: HPLC chromatograms of FA and its impurities.

Forced degradation assessments

FA significant degradation was found under acidic, oxidation and thermal degradation and its mild degradation was found under photolytic and basic degradation (fig. 1). The peak purities were checked using a PDA detector for all degradation specimens, which can ensure the FA peak spectral homogeneity.

Assay studies were conducted for these degradation specimens by comparing the samples with the FA standard solution and checking the sample mass balance. The results of forced degradation of FA for injection are presented in table 2.

Method validation

The designed method was validated per ICH guideline Q2(R1) (*ICH, 2005*), including specificity, limit of detection (LOD), limit of quantitation (LOQ), precision, linearity, accuracy and robustness.

Specificity

Specificity is the method ability to assess the analyte in the existence of potential impurities and degradation products. The specificity studies for process-associated impurities were evaluated through diluting the working standard solution and spiking it into the FA formulation solution at a specific concentration (fig. 2). The resolution between the chromatographic peaks of each component is greater than 1.5, the number of theoretical plates of FA is greater than 3000 and the symmetry factor of the chromatographic peaks of each impurity is between 1.0-1.2. In the chromatograms recorded in blank solution and placebo solution, no interference peaks occurred at the FA retention time and its impurities.

LOD and LOQ

The LOD and LOQ for FA and its impurities were calculated at signal-to-noise (S/N) ratios of respectively 3:1 and 10:1, through the injection of a series of diluted solutions with known levels. Table 3 presents the LOD and LOQ values for FA and its impurities.

Linearity and range

The working standard solution was quantitatively diluted to perform the linearity assessments at seven different concentrations from LOQ to 200% of the each impurity specification level (LOQ, 12.5%, 25%, 50%, 100%, 150% and 200%). Linearity evaluation was done through drawing a calibration curve and indicating the plot of the impurity area versus analyte concentration. The correlation coefficient (r^2) and regression equation values of the calibration curves were calculated. The relative response factor (RRF) of impurities A, B, C, D, E and F was calculated by the ratio of the slope of each impurity to the slope of FA (table 3).

Precision

The precision was evaluated through the injection of FA standard solution six times and the peak area standard deviation (RSD) was determined. The method precision was approved by intermediate precision and repeatability. Repeatability was evaluated through the injection of six individual preparations of FA into an injection solution spiked with 0.08-2% of its six impurities (% of impurities considering 1.2mg/mL FA). The percent relative standard deviation (RSD) of the area for all impurities was determined. The intermediate precision was assessed by various analysts and instruments and the analysis was performed on different days. The RSD(%) for system precision is 0.3, the RSD(%) for method repeatability is below 5.0% and the RSD(%) for Intermediate precision of the method is below 5.0% (table 4).

Accuracy

The method accuracy for FA and its impurities was assessed in triplicate with three concentrations of 50%, 100% and 150%. The average percentage recovery and percent RSD for each impurity were determined at each level (table 5). The impurities of FA showed percentage recovery ranging from 95.99%-108.52%, with RSD (%) ranging from 0.61 to 4.57.

Robustness

The robustness was done by altering the experimental conditions and the system suitability parameters were determined. The evaluated variables were the column temperature (±2°C), pH of the mobile phase buffer (±0.2) and flow rate (±0.2mL/min). The resolution, retention time and tailing for the peaks of FA and its impurities were unaffected under any modified chromatographic conditions.

Solution stability

The prepared sample solution and standard solution of FA was investigated at room temperature (20-30) and refrigerated conditions (2-8) for 24h, 36h and 48h. The sample solution was stable for room temperature for 24h and refrigerated conditions for 48h. The standard solution was found to be stable for room temperature for 48h and refrigerated conditions for 48h.

DISCUSSION

This method was developed to obtain good separation between FA and its impurities. In addition to detecting process impurities in the formulation, a stability-indicating HPLC method can detect impurities generated by degradation. During method development and optimization research, appropriate chromatographic parameters were utilized to efficiently separate FA and its known impurities. Forced degradation studies have shown that this method can detect potential degradation-related impurities. In the process of research, it was found that impurity A was the main degradation impurity, and impurity D was unstable and rapidly degraded in solution. When calculating the relative response factor, because some impurity reference substances were not meglumine salts, fosaprepitant dimeglumine was detected as the peak area of fosaprepitant under chromatographic conditions; therefore, fosaprepitant dimeglumine should be converted to fosaprepitant and calculated.

CONCLUSION

A stable HPLC method was developed and validated to determine FA process- and degradation-related impurities in injection formulations. The forced degradation study revealed that the method is stable. Method validation findings proved that the method was precise, specific, robust and accurate. Hence, the proposed method is applicable for routine impurity analysis in FA formulations and also for stability studies.

ACKNOWLEDGMENT

This research was supported by Department of Science and Technology of Guangdong Province and Guangdong Academy of Traditional Chinese Medicine Joint Scientific Research Project (No.2016A020226038).

REFERENCES

Aapro M, Carides A, Rapoport BL, Schmoll HJ, Zhang L and Warr D (2015). Aprepitant and fosaprepitant: A 10-year review of efficacy and safety. *Oncologist*, **20**(4): 450-458.

Azuma J and Fukase H (2013). Pharmacokinetics of a single 150-mg intravenous infusion of fosaprepitant: Effects of concentration and infusion time in healthy Japanese men. *Clin. Pharmacol. Drug Dev.*, **2**(4): 394-399.

Ballatori E and Roila F (2003). Impact of nausea and vomiting on quality of life in cancer patients during chemotherapy. *Health Qual. Life Outcomes*, **1**: 46.

British Pharmacopoeia Commission Secretariat (2020). British Pharmacopoeia. The Stationery Office, London, UK.

Cabanillas Stanchi KM, Ebinger M, Hartmann U, Queudeville M, Feucht J, Ost M, Koch MS, Malaval C, Mezger M, Schober S,Weber S, Michaelis S, Lange V, Lang P, Handgretinger R and Döring M (2019). Efficacy, safety and feasibility of antiemetic prophylaxis with fosaprepitant, granisetron and dexamethasone in pediatric patients with hemato-oncological malignancies. *Drug Des. Devel. Ther.*, **13**: 3439-3451.

Chen W, Zhao Y, Dai Y and Nie K (2022). Gastro-intestinal inflammation plays a critical role in chemotherapy-induced nausea and vomiting. *Eur. J. Pharmacol.*, **936**: 175379.

Chinese pharmacopoeia commission. (2020). Chinese Pharmacopoeia. China Medical Science Press, Beijing, China.

Dranitsaris G, Moezi M, Dobson K, Phelan R and Blau S (2022). A real-world study to evaluate the safety and efficacy of three injectable neurokinin-1 receptor antagonist formulations for the prevention of chemotherapy-induced nausea and vomiting in cancer patients. *Support Care Cancer*, **30**(8): 6649-6658.

European Directorate for the Quality of Medicines and Health Care. (2019). European Pharmacopoeia. European Directorate for the Quality of Medicines. Strasbroung.

Garnock-Jones KP (2016). Fosaprepitant dimeglumine: A review in the prevention of nausea and vomiting associated with chemotherapy. *Drugs*, **76**(14): 1365-1372.

ICH (2005). Q2(R1) Validation of analytical procedures: Text and methodology. ICH, European Medicines Agency, 7 Westferry Circus, Canary Wharf, London, E14 4HB, UK.

Kalariya PD, Talluri MV, Patel PN and Srinivas R (2015). Identification of hydrolytic and isomeric N-oxide degradants of vilazodone by on line LC-ESI-MS/MS and APCI-MS. *J. Pharm. Biomed. Anal.*, **102**: 353-365.

Lindley CM, Hirsch JD, O'Neill CV, Transau MC, Gilbert CS and Osterhaus JT (1992). Quality of life

consequences of chemotherapy-induced emesis. *Qual. Life Res*, **1**(5): 331-340.

Pharmaceuticals and medical devices agency of Japan. (2021). The Japanese Pharmacopoeia. The Ministry of Health, Labor and Welfare. Tokyo, Japan.

Rapoport BL, Jordan K and Weinstein C (2018). Neurokinin 1 receptor antagonists in the prevention of chemotherapy-induced nausea and vomiting: Focus on fosaprepitant. *Future Oncol.*, **14**(1): 77-92.

Ruhlmann CH, Christensen TB, Dohn LH, Paludan M, Rønnengart E, Halekoh U, Hilpert F, Feyer P, Kristensen G, Hansen O, Keefe D and Herrstedt J (2016). Efficacy and safety of fosaprepitant for the prevention of nausea and emesis during 5 weeks of chemoradiotherapy for cervical cancer (the GAND-emesis study): A multinational, randomised, placebo-controlled, double-blind, phase 3 trial. *Lancet Oncol.*, **17**(4): 509-518.

Saito H, Yoshizawa H, Yoshimori K, Katakami N, Katsumata N, Kawahara M and Eguchi K (2013). Efficacy and safety of single-dose fosaprepitant in the prevention of chemotherapy-induced nausea and vomiting in patients receiving high-dose cisplatin: a multicentre, randomised, double-blind, placebo-controlled phase 3 trial. *Ann. Oncol.*, **24**(4): 1067-1073.

Shaikh JK, Farooqui M and Asema Sy UK (2020). Quality by design approach for development and validation of stability indicating RP-HPLC method for fosaprepitant dimeglumine. *Asian J. Chem*., **32**(9): 2158-2164.

Skrdla PJ, Abrahim A and Wu Y (2006). An HPLC chromatographic reactor approach for investigating the hydrolytic stability of a pharmaceutical compound. *J. Pharm. Biomed. Anal.*, **41**(3): 883-890.

The United States Pharmacopeial Convention. (2020). United States Pharmacopeia. United Book Press, Baltimore, USA.

Weinstein C, Jordan K, Green SA, Camacho E, Khanani S, Beckford-Brathwaite E, Vallejos W, Liang LW, Noga SJ and Rapoport BL (2016). Single-dose fosaprepitant for the prevention of chemotherapy-induced nausea and vomiting associated with moderately emetogenic chemotherapy: results of a randomized, double-blind phase III trial. *Ann. Oncol*., **27**(1): 172-178.

Willier S, Cabanillas Stanchi KM, von Have M, Binder V, Blaeschke F, Feucht J, Feuchtinger T and Döring M (2019). Efficacy, safety and feasibility of fosaprepitant for the prevention of chemotherapy-induced nausea and vomiting in pediatric patients receiving moderately and highly emetogenic chemotherapy-results of a non-interventional observation study. *BMC Cancer*, **19**(1): 1118.

Yang LQ, Sun XC, Qin SK, Cheng Y, Shi JH, Chen ZD, Wang QM, Zhang HL, Hu B, Liu B, Zhang QY, Wu Q, Wang D, Shu YQ, Dong J, Han BH, Wang KM, Dang CX, Li JL, Wang HB, Li BL, Lu JG, Zhang ZH and Chen YX (2017). Efficacy and safety of fosaprepitant in the prevention of nausea and vomiting following highly emetogenic chemotherapy in Chinese people: A randomized, double-blind, phase III study. *Eur J Cancer Care (Engl)*, **26**(6): e12668.

Zhang Z, Yang Y, Lu P, Li X, Chang J, Zheng R, Zhou L, Chen S, Chen X, Ren B, Gu W, Jiang X, Peng J, Huang M, Feng G, Shen P, Zhang Q, Zhang B, Huang Y, He J, Chen Y, Cao J, Wang H, Li W, Wan H, Nan K, Liao Z, Zhang C, Lin Z, Zhong D, Xu Q, Liu H, Sun T, Deng Y and Zhang L (2020). Fosaprepitant versus aprepitant in the prevention of chemotherapy-induced nausea and vomiting in patients receiving cisplatin-based chemotherapy: A multicenter, randomized, double-blind, double-simulated, positive-controlled phase III trial. *Ann. Transl. Med.*, **8**(5): 234.