THE EFFECT OF MEMBRANE FILTRATION ON THE ACCURACY OF QUANTITATIVE DETERMINATION OF APREPITANT IN AN AQUEOUS MEDIUM

Ubushaev SV ✉, Sepp VV, Bakulin KS, Sen OYu

Pirogov Russian National Research Medical University, Moscow, Russia

Validation of analytical methods for poorly soluble BCS Class II/IV drugs like Aprepitant in aqueous media is complicated due to instability of samples at the stage of preparation. The goal of the research is to detect the mechanism of loss of Aprepitant analytical signal during filtration and determine the conditions for an exact diagnosis. In this paper, the effect of membrane materials (PES, PVDF, Nylon, PP) on analyte concentration measurements via UV spectrophotometry was examined. Filtration of aqueous solutions is established to cause significant analyte losses ranging from 15.1% (PP) to 83.9% (PES), depending on the initial concentration. It is proved that the main loss factor is not passive adsorption, but mechanically induced heterogeneous nucleation on the membrane surface, which is confirmed by an increase in optical scattering within the 300–500 nm range. Fraction filtration revealed abnormal desorption effects on PES filters. It has been shown that the use of an acidic medium (0.1 M HCl) prevents nucleation due to protonation of the molecule: filtration losses are reduced to statistically insignificant values (<1.6%). To ensure the accuracy of aprepitant analysis, it is recommended to exclude filtration of neutral aqueous solutions, replacing it with centrifugation, or to use acidic dissolution media.

Keywords: aprepitant, membrane filtration, induced nucleation, method validation, adsorption, sample preparation, spectrophotometry, dissolution test

Author contribution: Ubushaev SV — development of research design, conducting experiments to assess the effects of filtration and composition of the medium, collection and interpretation of spectral data, writing the main text of the manuscript; Sepp VV — scientific guidance of the work, formulation of a working hypothesis, critical analysis of the obtained patterns, editing and approval of the final version of the article; Bakulin KS — participation in the validation of the analytical method, processing of the obtained results, work with equipment and materials, technical verification of data; Sen OYu — concept development, provision of resources, manuscript writing.

✉ **Correspondence should be addressed:** Sandji V Ubushaev
Ostrovityanova str., 1, Moscow, 117997, Russia; sandjik.ubushaev@gmail.com

Received: 21.01.2026 **Accepted:** 11.02.2025 **Published online:** 25.02.2026

DOI: 10.24075/dc.2026.002

ВЛИЯНИЕ МЕМБРАННОЙ ФИЛЬТРАЦИИ НА ТОЧНОСТЬ КОЛИЧЕСТВЕННОГО АНАЛИЗА АПРЕПИТАНТА В ВОДНОЙ СРЕДЕ

С. В. Убушаев ✉, В. В. Сепп, К. С. Бакулин, О. Ю. Сен

Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва, Россия

Валидация аналитических методик для малорастворимых веществ (БКС II/IV), таких как апрепитант, в водных средах осложнена нестабильностью проб на этапе подготовки. Цель исследования — выявить механизм потери аналитического сигнала апрепитанта при фильтрации и определить условия для точного анализа. В работе изучено влияние материалов мембран (PES, PVDF, Nylon, PP) на концентрацию вещества методом УФ-спектрофотометрии. Установлено, что фильтрация водных растворов приводит к критическим потерям аналита, варьирующим от 15,1% (PP) до 83,9% (PES) в зависимости от исходной концентрации. Доказано, что основным фактором потерь является не пассивная адсорбция, а механически индуцированная гетерогенная нуклеация на поверхности мембраны, подтвержденная ростом оптического рассеяния в области 300–500 нм. Фракционная фильтрация выявила аномальные эффекты десорбции на фильтрах PES. Показано, что использование кислой среды (0,1 М HCl) предотвращает нуклеацию за счет протонирования молекулы: потери при фильтрации снижаются до статистически незначимых величин (<1,6%). Для обеспечения точности анализа апрепитанта рекомендуется исключить фильтрацию нейтральных водных растворов, заменив ее центрифугированием, либо использовать кислые среды растворения.

Ключевые слова: апрепитант, мембранная фильтрация, индуцированная нуклеация, валидация методики, адсорбция, пробоподготовка, спектрофотометрия, тест растворения

Вклад авторов: С. В. Убушаев — разработка дизайна исследования, проведение экспериментов по оценке влияния фильтрации и состава среды, сбор и интерпретация спектральных данных, написание основного текста рукописи; В. В. Сепп — научное руководство работой, формулировка рабочей гипотезы, критический анализ полученных закономерностей, редактирование и утверждение окончательной версии статьи; К. С. Бакулин — участие в валидации аналитической методики, обработка полученных результатов, работа с оборудованием и материалами, техническая проверка данных; О. Ю. Сен — разработка концепции, предоставление ресурсов, написание рукописи.

✉ **Для корреспонденции:** Санджи Викторович Убушаев
ул. Островитянова, д. 1, г. Москва, 117997, Россия; sandjik.ubushaev@gmail.com

Статья поступила: 21.01.2026 **Статья принята к печати:** 11.02.2026 **Опубликована онлайн:** 25.02.2026

DOI: 10.24075/dc.2026.002

Selection of an acceptable surfactant-free dissolution medium is common when developing analytical methods and dissolution tests for poorly soluble surfactant-free pharmaceutical substances. Pure and registered substances commonly utilize recommended non-aqueous solvents and assay methods. Aqueous media are however preferred in early drug development and pilot trials due to biopharmaceutical considerations or technological limitations.

Aprepitant is used as a model compound due to its low water solubility. According to literature, it exhibits a solubility of about 3–7 micrograms/ml between pH 2 and 10 and a sharp increase in solubility at pH 1 (about 130 micrograms/ml at pH 1.0). Based on the provided *in vitro* data, the substance demonstrates solubility values of 15.6 micrograms/ml (pH 1.2), 0.79 micrograms/ml (pH 4.5) and < 0.3 micrograms/ml (pH 6.8–7.4). Dissolution remains limited even in the presence of surfactants (PBS pH 6.6 + 0.1% SDS) [1–3]. These features make aprepitant an ideal model for studying hydrophobic substance behavior and the effects observed during their preparation for assay.

The goal of the study is to present practical criteria and recommendations when choosing an aqueous medium and sample preparation methods (including filtration) that minimize the systematic loss of the analytical signal.

MATERIALS AND METHODS

Materials. Aprepitant (99.6% purity, Fuxin Long Rui Pharmaceutical Co., Ltd., China). Purified water was produced by a laboratory water distiller. 95% ethanol. 10 ml disposable syringes. Syringe membrane filters of various materials and pores: PES 13 mm 0.45 microns, PVDF 25 mm 0.45 microns, Nylon (NY) 13 mm 0.22 microns, Polypropylene (PP) 13 mm 0.45 microns. All filters are hydrophilic.

Solution preparation. Aprepitant solutions were prepared as alcohol concentrates (e.g., aprepitant in 95% ethanol) that are diluted with purified water to target concentrations. The total organic phase content in solutions was less than 1%. Freshly prepared solutions were analyzed with and without syringe filtration. Sample stability was monitored by measuring sample parts with a spectrophotometer over time. The first 2 ml after filtration was discarded if it was not a fraction collection.

Instrumental analytical methods. The absorption spectra were recorded using the double-beam UV-visible spectrophotometer UV-2600 (Shimadzu Corp., Japan) with 1 cm quartz cuvettes. The measurements were carried out in the range of 185–500 nm in 0.2 nm increments (the corresponding dissolution medium was placed in the reference cell). Data were acquired and initially processed using UV-Probe software, version 2.42 (Shimadzu). Linearity and other key parameters were tested near operating concentrations.

Samples were weighed on Pioneer PA214 analytical scales (Ohaus Corporation, USA) with a 0.1 mg graduation (discreteness) and a standard deviation (reproducibility) of 0.1 mg.

Calibration and calculation of losses. The calibration dependence was determined using the straight-line method $y = a \cdot x + b$, where y is the absorbance (Abs.) at 210 nm, and x is the concentration (µg/ml). The loss of substance during filtration was calculated as follows:

$$\text{Loss,\%} = \frac{(C_{\text{prior to filtration}} - C_{\text{following filtration}})}{C_{\text{following filtration}}} \times 100\%.$$

Experimental observations. A series of measurements were carried out using different initial concentrations with sequential membrane filtration (fraction sampling through the same membrane), and acidic (0.1 M HCl) treatment to compare the solubility and stability of solutions.

RESEARCH RESULTS

Calibration curve

Data-based calibration dependence (table 1): $y = 0{,}02983 \cdot x + 0{,}01318$, $R^2 = 0{,}99541$ (an alternative approximation of $y = 0{,}0273 \cdot x + 0{,}0097$, $R^2 = 0{,}9999$ was obtained for a separate series of measurement, and the data were used to calculate concentrations of some control solutions).

Effect of filtration and membrane material

The research has shown that filtration of aqueous aprepitant solutions through membranes made of various materials (PES, PP, Nylon, PVDF) results in significant and hardly predictable losses of an analytical signal. The loss value varied in the wide 15–84% range depending on the initial concentration and polymer type. Summary of experimental series is provided in (table 2).

None of the investigational materials provided complete inertia with respect to aprepitant in an aqueous medium. The most critical losses were observed on PES membranes at high concentrations (up to 84%) and Nylon membranes (up to 59%). The high variability of the results (RSD of control solutions up to 7.5%) indicates that the process is not limited to simple adsorption.

Dynamics in sequential filtration (fraction filtration)

During sequential filtration of a sample in 10 mL fractions, fundamental differences in material behavior were found (Figure).

PES membranes have a traditional saturation profile of the adsorption centers: their losses decreased to 38% for the first fraction and to 10% for the third fraction. An abnormal concentration growth (+20% to the initial level) was seen for the fourth fraction. It proves there is desorption of the accumulated substance or failure of the adsorption layer when the stream hydrodynamics is changed.

In contrast to this, PVDF filtration demonstrated a chaotic variability of losses (23–39%) without any pronounced saturation trend accompanied with a lower solution stability in time.

Table 1. Calibration aprepitant solutions (Abs. at 210 nm)

C, µg/ml	Abs. (mean)	SD (n = 3)	RSD, %
0,885	0,0387	0,0015	3,95
1,815	0,0660	0,0010	1,52
4,268	0,1437	0,0015	1,06
7,304	0,2383	0,0035	1,47
9,45	0,2795	0,0167	5,96
11,332	0,3587	0,0031	0,85

Table 2. Loss of aprepitant during filtration with various filters of aqueous solutions at different concentrations

Membrane material	Initial concentration, µg/ml	Substance loss, %
PP (polypropylene)	4.27	15.1
	7.3	26.6
	1.93	51
Nylon	5.61	23.3
	7.3	59.3
PES (polyethersulfone)	0.89	32.8
	9.45	83.9
PVDF	1.87	34.8

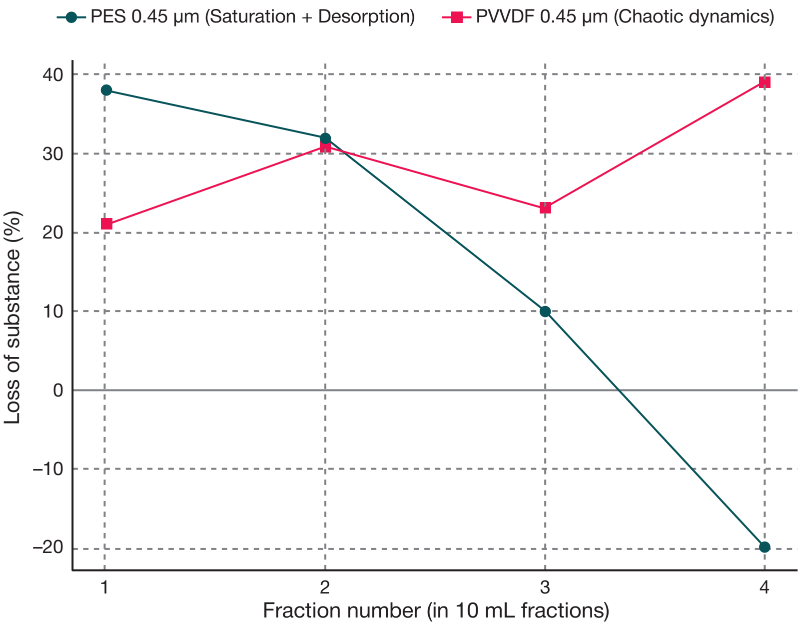

Fig. Dynamics of losses in fraction filtration

The role of mechanically induced nucleation

Experiments confirm that substance losses result from both adsorption and transitions of dissolved substances into a solid phase triggered by mechanical stress.

1. The mechanical action effect: passing the solution through a syringe without a filter led to a background signal in the 500 nm range. It proves that nucleation (crystal nuclei) from the oversaturated solution is provoked by pressure changes and turbulence.
2. Spectrum shift: in supersaturated solutions (36.8 micrograms/ml), a simultaneous drop in the absorption peak (210 nm) and an increase in light scattering in the long-wavelength region (300–500 nm) were recorded, which confirms that suspended particles were formed.
3. The filter as a crystallization center: when trying to filter supersaturated solutions, the membrane initiated heterogeneous nucleation, completely removing matter from the filtrate. That was not the case with transparent solutions.

Experiments in an acidic medium (0.1 M HCl) have shown that the protonated molecule of aprepitant formed a stable true solution, without pronounced effects of crystallization and adsorption (filtration losses for solutions of 17.9 micrograms/ml and 91.12 micrograms/ml were 1.6% and 0.7%, respectively).

DISCUSSION OF RESULTS

Our data correlate with studies indicating that membrane filtration is a critical stage of sample preparation, which can significantly distort the results of quantitative determination. In particular, when studying the equilibrium solubility of pharmaceutical substances, the concept of the "distortion effect of filter" (DEF) was introduced, showing that adsorption on the membrane surface can lead to unpredictable errors [4]. Our results confirm this thesis for aprepitant.

The analysis of the obtained data has, apparently, revealed two processes that reduce the analytical signal.

1. Polymer matrix adsorption. Like in similar papers [5], we observed the dependence of losses on material hydrophobicity. High PES and Nylon losses are in line with data [6] that take the materials as problematic for hydrophobic analytes and recommend using PTFE as a more inert alternative. The membrane saturation effect

recorded in fraction filtration is also described for other classes of compounds.

2. Induced heterogenous nucleation. This appears to be the dominant factor for oversaturated aprepitant solutions. In contrast to the pure adsorption described for true solutions, we observed an optical density increase in the non-analytical region (300–500 nm) and a sharp drop in concentration after a mechanical action. We assume that the microporous structure of the filter and the shear stress during passage through the pores serve as triggers of the phase transition. This explains why losses can exceed 80%. It occurs because the filter does not just resorb the molecules, but also initiates avalanche crystallization of the entire sample.

The use of an acidic medium (0.1 M HCl) eliminated the problem, converting aprepitant into an ionized and more soluble form. This is consistent with the principles described in [7], where the pH of the medium and ionic strength is referred to as key factors in sorption control. It can also be noted that in many studies and in the regulatory documentation for aprepitant, an acidified mobile phase in HPLC is used.

CONCLUSIONS

The purpose of the study has been achieved as critical factors of sample preparation affecting the validity of aprepitant analysis were identified.

The main mechanism of aprepitant loss in neutral aqueous media is not only adsorption, but also mechanically and superficially induced membrane nucleation.

As far as the hypothesis for future research goes, we suggest that filtration stress testing (comparing centrifugation and filtration under different conditions) can be a component in validating analytical techniques for low-solubility drugs.

Practical use: In practice, priority should be given to centrifugation. If filtration is unavoidable, it is necessary to validate not only the filter material, but also the pre-saturation volume, considering the filter as a consumable with a certain “distortion effect” (DEF). The most effective method of sample stabilization is to transfer the substance to a true solution by protonation (using an acidic medium), which eliminates the influence of the filter material.

References

1. Liu J, Zou M, Piao H, Liu Y, Tang B, Gao Y, Ma N, Cheng G. Characterization and Pharmacokinetic Study of Aprepitant Solid Dispersions with Soluplus®. Molecules. 2015 Jun 19; 20(6): 11345–56. DOI: 10.3390/molecules200611345. PMID: 26102068; PMCID: PMC6272549.
2. Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K, et al. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm. 2004 Nov 5; 285(1–2): 135–46. DOI: 10.1016/j.ijpharm.2004.08.001. PMID: 15488686.
3. Nazlı H, Mesut B, Özsoy Y. In Vitro Evaluation of a Solid Supersaturated Self Nanoemulsifying Drug Delivery System (Super-SNEDDS) of Aprepitant for Enhanced Solubility. Pharmaceuticals (Basel). 2021 Oct 27; 14(11): 1089. DOI: 10.3390/ph14111089. PMID: 34832871; PMCID: PMC8621193.
4. Völgyi G, Csicsák D, Takács-Novák K. Right filter-selection for phase separation in equilibrium solubility measurement. Eur J Pharm Sci. 2018 Oct 15; 123: 98–105. DOI: 10.1016/j.ejps.2018.06.031. Epub 2018 Jun 30. PMID: 29969669.
5. Michlig N, Lehotay SJ, Lightfield AR. Comparison of filter membranes in the analysis of 183 veterinary and other drugs by liquid chromatography-tandem mass spectrometry. J Sep Sci. 2024 Feb; 47(3): e2300696. DOI: 10.1002/jssc.202300696. PMID: 38356232.
6. Tomas MK, Mijatović A, Nevistić MB, Šarkanj B, Babić J, Kovač T. How different microfilters affect the recovery of eleven EU-regulated mycotoxins. Arh Hig Rada Toksikol. 2023 Apr 4; 74(1): 8–15. DOI: 10.2478/aiht-2023-74-3693. PMID: 37014685; PMCID: PMC10231880.
7. Zheng YH, Carter E, Zou S, Williams CF, Chow AT, Chen H. Using syringe filtration after lab-scale adsorption processes potentially overestimates PFAS adsorption removal efficiency from non-conventional irrigation water. J Environ Qual. 2025 Jan-Feb; 54(1): 20–30. DOI: 10.1002/jeq2.20640. Epub 2024 Oct 16. PMID: 39414564; PMCID: PMC11718135.

Литература

1. Liu J, Zou M, Piao H, Liu Y, Tang B, Gao Y, Ma N, Cheng G. Characterization and Pharmacokinetic Study of Aprepitant Solid Dispersions with Soluplus®. Molecules. 2015 Jun 19; 20(6): 11345–56. DOI: 10.3390/molecules200611345. PMID: 26102068; PMCID: PMC6272549.
2. Wu Y, Loper A, Landis E, Hettrick L, Novak L, Lynn K, et al. The role of biopharmaceutics in the development of a clinical nanoparticle formulation of MK-0869: a Beagle dog model predicts improved bioavailability and diminished food effect on absorption in human. Int J Pharm. 2004 Nov 5; 285(1–2): 135–46. DOI: 10.1016/j.ijpharm.2004.08.001. PMID: 15488686.
3. Nazlı H, Mesut B, Özsoy Y. In Vitro Evaluation of a Solid Supersaturated Self Nanoemulsifying Drug Delivery System (Super-SNEDDS) of Aprepitant for Enhanced Solubility. Pharmaceuticals (Basel). 2021 Oct 27; 14(11): 1089. DOI: 10.3390/ph14111089. PMID: 34832871; PMCID: PMC8621193.
4. Völgyi G, Csicsák D, Takács-Novák K. Right filter-selection for phase separation in equilibrium solubility measurement. Eur J Pharm Sci. 2018 Oct 15; 123: 98–105. DOI: 10.1016/j.ejps.2018.06.031. Epub 2018 Jun 30. PMID: 29969669.
5. Michlig N, Lehotay SJ, Lightfield AR. Comparison of filter membranes in the analysis of 183 veterinary and other drugs by liquid chromatography-tandem mass spectrometry. J Sep Sci. 2024 Feb; 47(3): e2300696. DOI: 10.1002/jssc.202300696. PMID: 38356232.
6. Tomas MK, Mijatović A, Nevistić MB, Šarkanj B, Babić J, Kovač T. How different microfilters affect the recovery of eleven EU-regulated mycotoxins. Arh Hig Rada Toksikol. 2023 Apr 4; 74(1): 8–15. DOI: 10.2478/aiht-2023-74-3693. PMID: 37014685; PMCID: PMC10231880.
7. Zheng YH, Carter E, Zou S, Williams CF, Chow AT, Chen H. Using syringe filtration after lab-scale adsorption processes potentially overestimates PFAS adsorption removal efficiency from non-conventional irrigation water. J Environ Qual. 2025 Jan-Feb; 54(1): 20–30. DOI: 10.1002/jeq2.20640. Epub 2024 Oct 16. PMID: 39414564; PMCID: PMC11718135.