JPPT | Compounding and Stability Study

Multidisciplinary Approach to Deciphering Etoposide Infusion Reactions and Potential Role of Polyethersulfone Filter Membranes

Nicholas Miles, PharmD; Andi Masters, PhD; Zeruesenay Desta, PhD; Jennifer L. Goldman, MD; Sarah L. Suppes, PharmD; and Emma M. Tillman, PharmD, PhD

PURPOSE Etoposide, a topoisomerase II inhibitor used clinically to treat cancer, has been associated with severe anaphylactic infusion related adverse drug reactions (ADRs). In a previous study we identified a hydrophilic polyethersulfone filter as a possible cause of increased rates of pediatric etoposide infusion reactions. In this multidisciplinary follow-up analytical study, we aimed to assess the chemical structure of etoposide after passing through the same hydrophilic polyethersulfone filter.

METHODS An etoposide 0.4 mg/mL infusion was prepared under aseptic conditions and then passed through a standard IV infusion set with an in-line filter in place. Samples were taken in triplicate using a needle-less access system to include sampling sites directly from the IV bag port and from the IV tubing both before and after the in-line filter. Samples were diluted into mobile phase, then an aliquot was injected into a high-performance liquid chromatography mass spectrometry HPLC-MS (Thermo TSQ Quantum Ultra) system coupled to a Diode Array Detector (DAD) (Thermo Dionex Ultimate 3000). Etoposide was monitored using a selected reaction monitoring scan (SRM) of 606.2/228.8 and wavelengths of 210, 220, 254, and 280 nm for 30 minutes.

RESULTS No detectable differences were observed upon comparing the three samples. Based on these results, a chemical change in etoposide resulting from an in-line filter is unlikely to be the primary cause of increased rates of infusion reactions.

CONCLUSION Pharmacists working in healthcare systems, observe many ADRs, but rarely have the resources necessary to investigate the potential etiology or causality. This report highlights importance of multi-disciplinary collaboration to investigate serious ADRs.

ABBREVIATIONS ADR, Adverse drug reactions; CMH, Children's Mercy Hospital; DAD, diode array detection; HPLC, high-performance liquid chromatography; HPLC-DAD-MS/MS, high-performance liquid chromatography diode array detection mass spectrometry; MS, mass spectrometry; PVC, polyvinyl chloride; RH, Riley Hospital for Children; SRM, selected reaction monitoring

KEYWORDS drug-related side effects and adverse reactions; Etoposide; polyethersulfone filter

J Pediatr Pharmacol Ther 2023;28(7):643–648

DOI: 10.5863/1551-6776-28.7.643

Introduction

Adverse drug reactions (ADRs) are an unintended reaction to a drug given at a normal dose.[1] Cancer chemotherapy is often associated with ADRs due to narrow therapeutic windows.[2] Etoposide is a common chemotherapeutic agent used in a wide variety of both adult as well as pediatric cancers such as leukemia, lymphoma, neuroblastoma, and osteosarcomas.[3] Common adverse effects of etoposide include hematologic toxicities such as leukopenia and thrombocytopenia, as well as gastrointestinal toxicities such as nausea, vomiting, and diarrhea. While these adverse events are expected and occur in many patients, etoposide is known to cause anaphylactic type reactions during infusion administration and has been reported to occur in approximately 0.7% to 2% of patients according to the package insert.[2–5] Symptoms of these infusion-related reactions can range from mild to life-threating and include dyspnea, flushing, erythema, chest pain, hypotension, chills, fever, and more.[2–5]

There have also been studies questioning whether the use of an in-line filter during etoposide administration may be a possible component behind the increased rates of infusion reactions in the pediatric population.[6–8] In a recent study conducted as a collaboration between Riley Hospital for Children (RH) in Indianapolis, IN, and

Children's Mercy Hospital (CMH) in Kansas City, MO, we identified an increased rate of etoposide infusion reactions in pediatric cancer patients that correlated with a 2017 implementation of in-line filters at CMH.[9] Filtration of compounded pharmaceutical solutions serves multiple roles including removing unwanted particles, air, and precipitates, as well as sterilization of the final solution. In-line filtration is a common practice in pediatric drug delivery.[10] Interactions have been reported between the pharmaceutical solution being filtered and the type of filter.[11] These types of interactions include sorption of the drug to the filter causing a decrease in available drug to the patient, leaching of a chemical substance from the walls of the filter into the IV solution, swelling of the membrane causing increased resistance to flow, and chemical interactions between the solution and components of the filter.[11] The aim of this study was to assess for any measurable change in the chemical structure of etoposide and potential degradation products that may be associated with an in-line filter equivalent to those used at CMH from 2017–2020.

Methods

To investigate the possible drug-filter interaction observed in pediatric patients,[9] clinicians aware of the increased infusion related reactions occurring more frequently with in-line filters sought collaboration with the Clinical Pharmacology department at Indiana University School of Medicine to utilize expertise and equipment that could further investigate the interaction in a small analytical study.

Preparation of Clinical Etoposide Product. Etoposide 100 mg/5 mL (Accord, Durham, NC) was added to a 250-mL bag of 0.9% sodium chloride (Baxter, Deerfield, IL) to achieve a final concentration of 0.4 mg/mL, which is the recommended maximum concentration to minimize risk of precipitation per the package insert.[3] The various solvents and additional components contained within the etoposide formulation are listed in the Table, along with their relative concentrations and the maximum recommended FDA doses for each component.[3,12] The infusion was compounded under standard aseptic technique by the Hematology-Oncology Outpatient Infusion Pharmacy at RH. After preparation, the infusion bag was spiked with non-polyvinyl chloride (PVC) tubing (Becton, Dickinson and Company, Franklin Lakes, NJ) and the in-line Supor IV-5 filter (low protein binding polyethersulfone filter) (ICU Medical, San Clemente, CA), was placed. The infusion set, that is, filter and tubing, were then primed with the etoposide solution to fully saturate the in-line filter and expose it to the solution, as well as ensure solution was available at all sampling sites prior to sample collection. No solution was wasted from the infusion set prior to sample collection. Next, 5 mL samples were collected in triplicate from the following three locations: 1) the injection port of the compounded IV bag, 2) a needle-less access port on the tubing located before the in-line filter, and 3) needle-less access port after the in-line filter. Analysis was performed immediately following sample collection.

Chemical and Reagents. Etoposide (95.0%–105.0% purity) was purchased from Sigma Aldrich (St. Louis, MO). Methanol, acetonitrile, water, and ammonium acetate were purchased from Fisher Scientific (Fairlawn, NJ). All solvents were LC/MS grade.

Stock Solution. A stock solution of etoposide (1 mg/mL) was prepared in methanol. Prior to analysis the etoposide stock solution was diluted to 100 ng/µL in mobile phase A (acetonitrile: 5 mM ammonium acetate, 20:80; v/v). Then a 10-µL aliquot was injected into the HPLC-UV-MS/MS system.

HPLC-UV-MS/MS Conditions. From each sample, 20 µL was transferred to a polypropylene tube and 180 µL of mobile phase was added. The samples were vortex mixed for 30 seconds then transferred to a high-performance liquid chromatography (HPLC) vial with a polypropylene insert. An aliquot (10 µL) of each

Table. Etoposide Formulation Inactive Ingredients*

Ingredients	Etoposide vial (20 mg/mL, 5 mL vial)[3]	Concentration for administration (Etoposide 0.4 mg/mL)	Potency per unit dose (100 mg)	Accepted FDA Thresholds[12]
Ethanol	0.305 mL/1 mL	0.0061 mL/1 mL	0.61% (v/v)	62% (w/v)
Benzyl alcohol	30 mg/1 mL	0.6 mg/1 mL	150 mg	180 mg
Anhydrous citric acid	2 mg/1 mL	0.04 mg/1 mL	250 mg	510 mg†
Polysorbate 80	80 mg/1 mL	1.6 mg/1 mL	0.16% (w/v)	58% (w/v)
Polyethylene glycol 300	650 mg/1 mL	13 mg/1 mL	1.3% (w/v)	44.22% (w/v)

FDA, US Food and Drug Administration

* Additional inactive ingredients of etoposide are listed including the concentration of each component in the undiluted vial, concentration in the final diluted preparation, and the accepted FDA threshold for each inactive ingredient.

† Maximum daily exposure per FDA guidance.

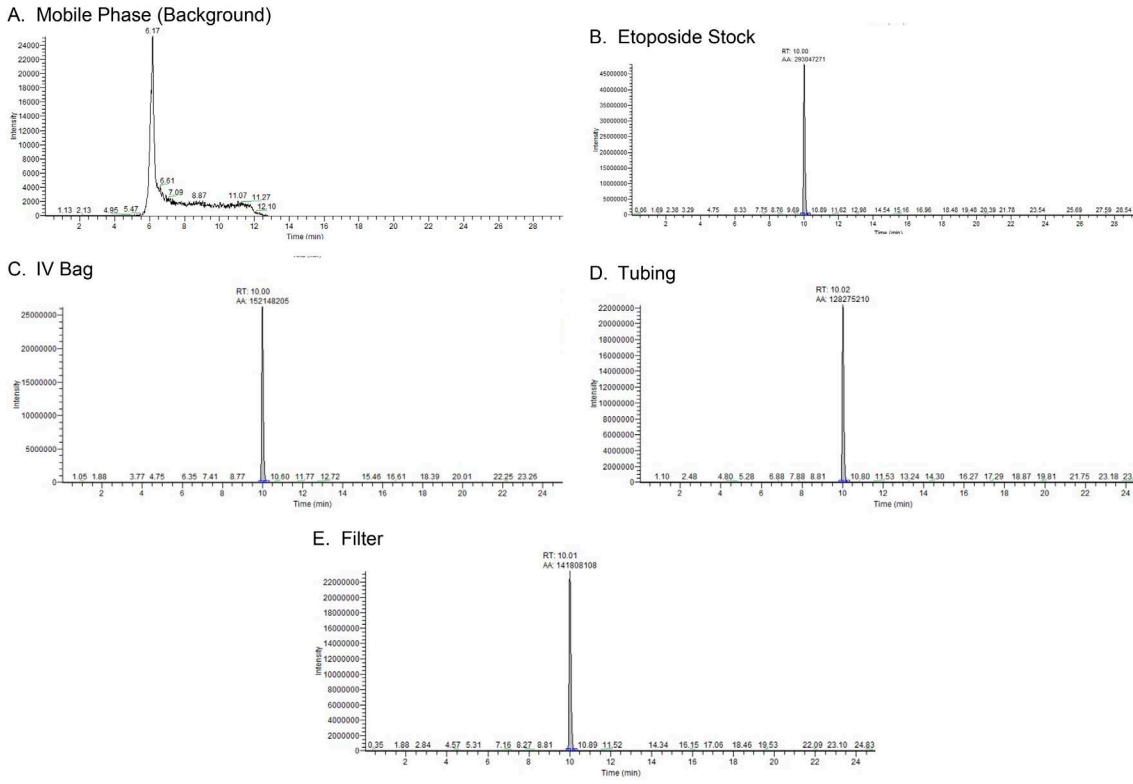
sample was injected to the high-performance liquid chromatography diode array detection mass spectrometry (HPLC-DAD-MS/MS) system as described below. All samples were run in triplicate. The chromatograms of the DAD and mass spectrometry (MS) were analyzed for any possible degradation, metabolite formation, or excipients as described below.

HPLC-UV-MS/MS Conditions. An Accela HPLC pump coupled with a Leap PAL HTC autosampler performed the chromatographic analysis of etoposide using reverse phase chromatography at ambient temperature on an Inertsil ODS-3 150 × 4.6 mm 5-micron column. The mobile phase was delivered via gradient at a constant flow rate of 800 µL/min. Mobile phase A was acetonitrile: 5 mM ammonium acetate in water (20:80; v/v) and mobile phase B was acetonitrile: 5 mM ammonium acetate in water (80:20; v/v). The gradient began after 5 minutes at 100% of mobile phase A and then increased to 100% mobile phase B by 10 minutes in a linear fashion and held at 100% mobile phase B until 27 minutes. At 27.1 minutes the mobile phase was stepped to 100% mobile phase A and held until 30 minutes. A Thermo Dionex Ultimate 3000 PDA detector at wavelengths 254 and 280 nM monitored the column effluent. The effluent from the PDA detector then went directly into a Thermo TSQ Quantum Ultra triple-quadrupole mass spectrometer equipped with an electrospray probe. Analysis was performed using the selected reaction monitoring (SRM) scan mode of the etoposide ammonium adduct m/z, 606.2/228.8. XCalibur (version 4.0) controlled the HPLC-UV-MS/MS system. All analysis was done in triplicate to assure accuracy.

Results

Except etoposide, no additional peaks were detected in the chromatograms of any of the samples collected during the duration of the 30-minute run at SRM 606.2/228.8 or wavelengths 210, 220, 254, or 280 nm compared to etoposide standard (Figure 1). The etoposide standard produced an absorbance peak at a range of 9.99 to 10.03 minutes into the run. A peak of similar intensity can be seen at 9.91 minutes of the IV bag port sample, 9.94 for the tubing pre-filter sample, and 9.93 for the tubing post-filter sample. Similar peaks were seen at all measured wavelengths for each sample. While additional peaks were not observed in the SRM scan of etoposide standard or at

Figure 1. HPLC-MS/MS SRM chromatographs

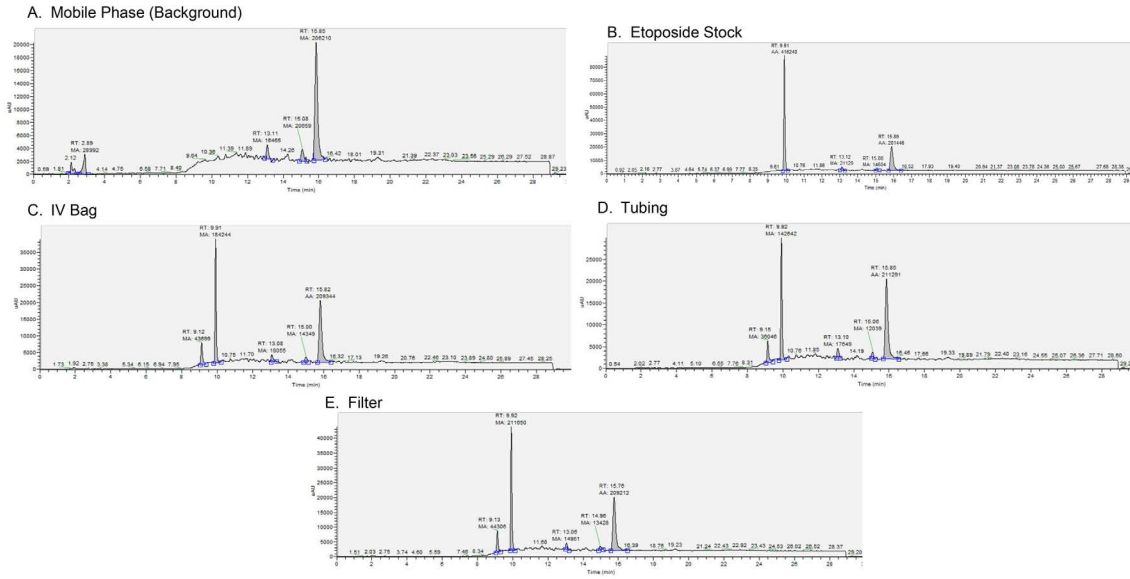


HPLC-MS/MS, high-performance liquid chromatography-mass spectrometry/mass spectrometry; IV, intravenous; SRM, selected reaction monitoring

various wavelengths; they were consistent across the different sample locations with no samples producing a peak unique to a specific wavelength or time between the different sample sites.

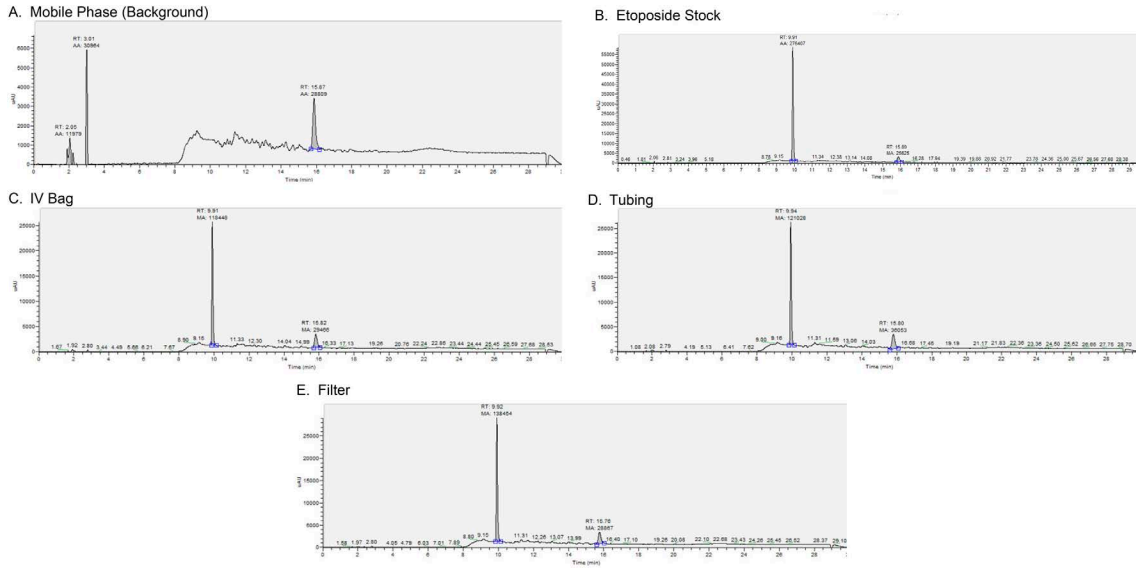
Mass spectrometry data showed an average peak area of 151563474 (SD 25679732; CV 16.9%) for the IV bag sample, 109870416 (SD 18738338; CV 17.1%) for the pre-filter tubing sample, and 154166029 (SD 21523693; CV 14.0%) for the post-filter sample. Full results from HPLC-DAD-MS/MS analysis are shown in Figure 1. To clearly appreciate the similarity of the HPLC-MS/MS Chromatographs, Figure 2 shows HPLC-UV Λ = 254 nm, and Figure 3 shows HPLC-UV Λ = 280 nm for all experimental conditions.

Figure 2. HPLC-UV Λ = 254 nm



HPLC-UV, high-performance liquid chromatography-ultraviolet; IV, intravenous

Figure 3. HPLC-UV Λ = 280 nm



HPLC-UV, high-performance liquid chromatography-ultraviolet; IV, intravenous

Discussion

We report the importance of interdisciplinary collaboration to investigate potential causes of ADRs that are observed in a clinical setting. Specifically, our results highlight two key findings: 1) HPLC-DAD-MS/MS did not detect a measurable difference between pre- and post-filter samples of etoposide infused through a hydrophilic polyethersulfone in-line filter; and 2) the absence of a chemical change of etoposide does not rule out interactions between other components of the medication solution and the filter.

To our knowledge, this is the first study to evaluate chemical composition of etoposide pre- and post-filter. There were no chemical changes in etoposide as shown by no significant difference in peak absorbance intensity in any of the three sampled conditions and compared to the etoposide standard. We did not fully rule out all the potential existence of an interaction between etoposide IV solution and the studied polyethersulfone membrane filter, but no chemical change appeared on the chromatograms.

Beyond a chemical change to etoposide mediated by a polyethersulfone membrane, other possibilities exist that could contribute to the observed increases of infusion reactions when filtering etoposide. The IV solution used to prepare etoposide for infusion contains benzyl alcohol which may potentially interact with the polyethersulfone membrane used in the IV-5 filter.[11–14] Another potential cause of infusion reactions is the method utilized for filter sterilization. A possible link has been proposed between the use of ethyl oxide gas in the sterilization of biomedical equipment that cannot tolerate heat sterilization and hypersensitivity reactions in pediatric patients.[15] The Supor IV-5 filter used to conduct this analysis and match those used at the institution in which we identified the possible link between filtration and increased rates of infusion reactions in pediatric patients previously, is sterilized using ethyl oxide gas.[14–16]

Based on these results, we deduce that it is unlikely that a chemical change of etoposide mediated by an in-line filter is the cause of the increased incidence of pediatric infusion reactions observed in recent observational clinical studies.[6,9] While other possible explanations exist such as ethyl oxide gas used for IV filter sterilization, an increased risk of infusion reactions has not yet been widely reported despite the common use of these filters in medical practice.

We believe this study not only contributes to the limited literature discussing the potential for drug-administration interactions, but it also illustrates the importance of a multidisciplinary team needed to investigate a clinical hypothesis. In this case, pharmacists and a physician identified this increase in etoposide infusion reactions that coincided with a protocol change to use inline filters for etoposide infusion.[9] They hypothesized that the filer could have a potential role in this uptick in reactions but did not have the necessary skill-sets to fully investigate. The clinical team sought out experts in HPLC-DAD-MS/MS, which is a very specialized skill and requires sophisticated equipment to successfully complete the analysis. Pharmacists working in healthcare systems, observe many ADRs, but rarely have the time, skill sets, and resources necessary to investigate the potential etiology or causality of the ADR. Drug-drug interactions or drug-nutrient interactions are often considered by pharmacist when evaluating ADRs, but interactions with tubing, filters, and components of drug sterilization may not be on the radar of pharmacists, therefore we believe it is important to share our experience of multi-disciplinary collaboration to investigate serious ADRs.

Article Information

Affiliations. Division of Clinical Pharmacology (NM, ZD, ET), Indiana University School of Medicine, Indianapolis, IN; Indiana University Comprehensive Cancer Center (AM), Indiana University School of Medicine, Indianapolis, IN; Division of Clinical Pharmacology (SLS), Toxicology, and Therapeutic Innovation, Children's Mercy Hospital, Kansas City, MO; Department of Pediatrics (JLG), Children's Mercy Hospital and the University of Missouri-Kansas City, Kansas City, MO.

Correspondence. Emma M. Tillman, PharmD, PhD; emtillma@iu.edu

Disclosure. The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. The authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Submitted. November 15, 2022

Accepted. March 7, 2023

References

1. World Health Organization. Medicines: safety of medicines – adverse drug reactions. Fact sheet N 293. Updated October 2008. Accessed May 11, 2020. http://whqlibdoc.who.int/hq/2002/WHO_EDM_QSM_2002.2.pdf
2. Turgay Yagmur I, Guzelkucuk Z, Yarali N, et al. Evaluation of hypersensitivity reactions to cancer chemotherapeutic agents in pediatric patients. *Ann Allergy Asthma Immunol*. 2020;124(4):350–356.
3. Etoposide [package insert]. Accord Healthcare; 2020.
4. Ogle KM, Kennedy BJ. Hypersensitivity reactions to etoposide. A case report and review of the literature. *Am J Clin Oncol*. 1988;11(6):663–665.
5. Sambasivan K, Mahmoud S, Kokache A, et al. Hypersensitivity reactions to etoposide phosphate. *J Oncol Pharm Pract*. 2014;20(2):158–160.
6. Ronsley R, Jacques L, Potts JE, et al. Association between in-line filtration and Type I hypersensitivity reactions in pediatric oncology patients receiving

intravenous etoposide. *Pediatr Hematol Oncol*. 2021; 38(3):208–215.
7. Pringle NR, Gilbar PJ, Grewal GD. Immediate severe hypersensitivity reaction to etoposide phosphate: case report and review of the literature. *J Oncol Pharm Pract*. 2022;28(4):1019–1023.
8. Stockton WM, Nguyen T, Zhang L, Dowling TC. Etoposide and etoposide phosphate hypersensitivity in children: Incidence, risk factors, and prevention strategies. *J Oncol Pharm Pract*. 2020;26(2):397–405.
9. Tillman EM, Suppes SL, Miles N, et al. Risks and mitigation strategies to prevent etoposide infusion-related reactions in children. *Pharmacotherapy*. 2021;41(8):700–706.
10. Perez M, Decaudin B, Abou Chahla W, et al. Effectiveness of in-line filters to completely remove particulate contamination during a pediatric multidrug infusion protocol. *Sci Rep*. 2018;8(1):7714.
11. McKinnon BT, Avis KE. Membrane filtration of pharmaceutical solutions. *Am J Hosp Pharm*. 1993;50(9):1921–1936.
12. Inactive Ingredients in Approved Drug Products. FDA Center for Drug Evaluation and Research, Office of Pharmaceutical Quality. Accessed October 25, 2022. https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm
13. DiFiore M. An investigational study on the impact of an incompatible filtration of benzyl alcohol using a polyethersulfone membrane. "Single-Use Technologies: Bridging Polymer Science to Biotechnology Applications", Ekta Mahajan, Genentech, Inc., USA Gary Lye, University College London, UK Eds, ECI Symposium Series. 2015.
14. IV-5 Filter with Supor Membrane [package insert]. PALL Medical; 2019.
15. Belen B, Polat M. Type I allergic hypersensitivity reactions due to ethylene oxide sterilised leucocyte filters in patients with thalassaemia: report of four cases. *BMJ Case Rep.* 2015;2015.
16. Masin G, Polenakovic M, Ivanovski N, et al. Hypersensitivity reactions to ethylene oxide: clinical experience. *Nephrol Dial Transplant*. 1991(6 suppl 3):50–52.