

## A Subject Review on Some Analytical Methods for Determination of Fosfomycin Drugs

**Ali Khalil Mahmood**

Department of Chemistry, College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad, Iraq.  
[ali.khalil.mahmood@gmail.com](mailto:ali.khalil.mahmood@gmail.com)

**Khalid Waleed S. Al-Janabi**

Department of Chemistry, College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad, Iraq.  
[Khalid.Janabi@gmail.com](mailto:Khalid.Janabi@gmail.com)

**Takleef Dheyab Sallal**

Ministry of Education, Baghdad, Iraq.  
[sallaltakleef535@gmail.com](mailto:sallaltakleef535@gmail.com)

**Article history: Received 13 March 2022, Accepted 3 April 2022, Published in July 2022.**

**Doi: 10.30526/35.3.2826**

### Abstract

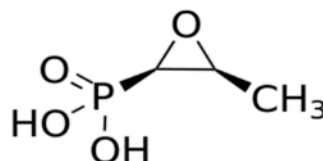
Medicines comprising fosfomycin are prescribed for urinary tract infections. These drugs are available for oral use as tromethamine and calcium, while fosfomycin-sodium and disodium are given for intravenous (IV) and intramuscular (IM). Many quantitative analytical methods have been reported to estimate Fosfomycin in blood, urine, plasma, serum, and pharmaceutical dosage formulations. Some techniques were spectrophotometric, mass spectrometry, gas chromatography, high-performance liquid chromatography, and electrochemical methods. Here we perform a rapid narrative review that discusses and comparison between them of various analytical methods for the determination of Fosfomycin-containing drugs.

**Keywords:** Review, Fosfomycin, Drugs, Medicines.

### 1. Introduction

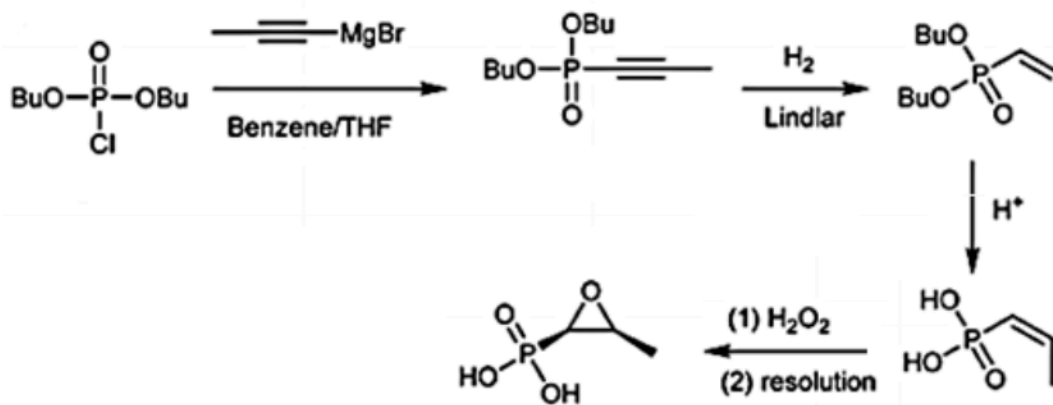
Fosfomycin (Fig.1) is a broad-spectrum antibiotic that inhibits the phosphoenol pyruvate transferase enzyme involved in the synthesis of peptidoglycan (found in the cell wall of Gram-positive and negative bacteria)[1,2]. *S. aureus*, staphylococci, penicillin-resistant *S. pneumonia*, *Enterococcus* species, *Escherichia coli* (*E. coli*), *Klebsiella pneumonia*, *Citrobacter*, and *N. meningitides* are often referred to as meningococcus, in addition to

Enterobacteriaceae and carbapenemase strains[3,4]. This drug's intravenous (IV) is widely prescribed in combination with other antimicrobial agents; it has an excellent safety profile with long-term administration[5]. This naturally occurring antibiotic agent was discovered in 1969[6].



**Figure 1.** Chemical structure of Fosfomycin

The first chemical synthesis (**Figure 2**) was described by (Christensen, B. G.) et al. in 1969, based on epoxidation of the cis-Propenylphosphonic acid since diverse synthesis (chemical synthesis) of this drug has been described. Currently, there are three stages[6].



**Figure 2.** The first chemical synthesis of Fosfomycin

## 2. Results and Discussion

This literature review demonstrated various analytical methods for the quantitative determination of fosfomycin[7–32]. Many of these techniques were reported, as shown in (Table 1) below. Now Fosfomycin has been used to treat particularly urinary infections that occur from bacteria. Furthermore, fosfomycin differs from other antibiotics that are non-cross-resistance for Fosfomycin indicated in clinical application[33].

There was research to estimate fosfomycin in pharmaceutical preparations or biological fluids. HPLC methods were used utilizing different mobile phases of several solvents with different degrees of dilution having diverse columns. The first method used to determine the above drug in urine in 1970 was Thin-layer paper and gas-liquid chromatography with linearity ranging from (1-5) $\mu\text{g}$  [7]. Also, it was found that the minimum linearity range for determining fosfomycin was (0.01-0.4)  $\mu\text{g}\cdot\text{mL}^{-1}$  in chicken muscle, liver, and kidney using HPLC-MS/MS [23]. The highest linearity was over (50-5000)  $\mu\text{g}\cdot\text{mL}^{-1}$  for determining fosfomycin in urine using capillary gas chromatography[14] with varying detection limits. It was also noted that the spectrophotometric methods were not widely used for assaying the above drug. Mostly, the simple chemical structure of the drug led to not being able to react with different reagents to estimate it in the visible region of the spectrum. It was observed that there were three methods

for the spectrophotometric determination of fosfomycin; the first method was at the visible region,  $\lambda_{\max} = 605$  nm, with a specific absorptivity of  $4.59 \times 10^4$  L. mol<sup>-1</sup>.cm<sup>-1</sup> and linearity =  $(0 - 28 \times 10^{-6})$ M [15]. However, it has been estimated within the ultraviolet region at  $\lambda_{\max} = 254$  nm, with specific absorptivity equal to  $(4.59 \times 10^4$  L.mol<sup>-1</sup>.cm<sup>-1</sup> and its linearity =  $(30-70)$   $\mu\text{g.mL}^{-1}$ . In addition Flow Injection Spectrophotometric method used for determination of it in urine at visible region,  $\lambda_{\max} = 960$  nm, Linearity =  $(3 \times 10^{-6} - 6 \times 10^{-4})$  mol.L<sup>-1</sup>, T = 90°C, r = 0.9969, LOD=  $1 \times 10^{-6}$ , RSD =1.2%, flow- rate = 0.2 mL.min<sup>-1</sup>. The drug is currently widely used to treat urinary tract infections with only one dose in the form of oral granules.

**Table 1.**Some analytical methods for the determination of Fosfomycin

Methods	sample	Results	Years	Ref. No.
<b>Thin-layer, paper and gas-liquid chromatography</b>	Urine	Linearity= $(1-5)\mu\text{g}$ , $R_F = (0.16-0.86)$ , preparative PC crude (30-50)%, TL plates loaded up to 150 $\mu\text{g}$ of drug in (2-5) $\mu\text{L}$	1970	7
<b>Specific ion monitoring method</b>	Blood and urine	Linearity= $(0.5-10)$ and $(0.25-50)$ $\mu\text{g.mL}^{-1}$ for microbiological and Sim methods respectively, Total Re. 25-28.1	1981	8
<b>Gas chromatography</b>	Biological and bacterial culture medium	apillary column OV 17-01, LOD = $1\mu\text{.mL}^{-1}$ , assay of 100 microliters.	1987	9
<b>anion-exchange chromatography</b>	Plasma	$\lambda_{\max} = 272$ nm, leanirity $(5-100)\mu\text{g.mL}^{-1}$ , mobile phase, is 0.4 mM of phthalate-buffer (pH =8.5), r= 0.999, RSD = $(0.6-5.3)$	1993	10
<b>capillary zone electrophoresis</b>	Serum	Linearity $(10-100)$ $\mu\text{g. mL}^{-1}$ , Re.= $(0.5-18)\%$ , RSD < 2%, buffer mobile phase (200 mM sodium borate + 10 mM phenylphosphonic acid)	1993	11
<b>High performance liquid Chromatography</b>	Plasma	Linearity = $(10-80)\mu\text{g.mL}^{-1}$ , IC-Pak, column (4.6x50) mm, pH =8.5, mobile phase acetonitrile: borategluconate v/v (12:88), r=0.999.	1993	12
<b>Capillary electrophoresis</b>	Serum and cerebrospinal fluid	Linearity = $(2.5-200)$ $\mu\text{g/mL}^{-1}$ , LOD = $(1.0- 2.5)$ $\mu\text{g. mL}^{-1}$ in serum and aqueous fluids respectively, r= 0.999, SD = 94.5%.	1994	13
<b>Capillary gas chromatography</b>	Urine	Linearity= $(50-5000)\mu\text{g.mL}^{-1}$ , LOD equal 10 $\mu\text{g. mL}^{-1}$ , CV = 0.006, r = 0.999	1996	14
<b>spectrophotometric method</b>	pharmaceutical manufacture	$\lambda_{\max} = 605$ nm, E= $4.59 \times 10^4$ L. mol <sup>-1</sup> .cm <sup>-1</sup> , Linearity = $(0-28 \times 10^{-6})$ M.	1999	15
<b>Gas chromatography</b>	Plasma	Commercial Complexes of Drug(CCd) : a levorotatory, Ca (-) salt, a racemic, Ca (+/-) salt, and (THAM) salt.	1999	16
<b>Gas chromatography</b>	Chicken plasma	Linearity= $(1-150)\mu\text{g.mL}^{-1}$ , column: HP-5 capillary, detector: flame ionisation (FID)LOD and LOQ = $(1$ and $2.1)$ $\mu\text{g mL}^{-1}$ respectively, Re. 109%. $R^2 = 0.997$ , CV= $(2.8$ and $5.1)$ .	2001	17
<b>Flow Injection Spectrophotometric</b>	Urine	$\lambda_{\max} = 960$ nm, Linearity = $(3 \times 10^{-6} - 6 \times 10^{-4})$ mol.L <sup>-1</sup> , T = 90°C, r = 0.9969, LOD= $1 \times 10^{-6}$ , RSD=1.2%, flow- rate = 0.2 mL.min <sup>-1</sup> .	2002	18
<b>Capillary electrophoresis analysis</b>	Biological fluids	$\lambda_{\max} = 254$ nm, Linearity = $(3.5$ or $6.8-1000)$ mg./mL, LOD= $(0.62-2)$ , LOQ= $(2.0-6.8)$ $\mu\text{g.mL}^{-1}$ , RSD= $(3.2-5.6)$ , R> 0.9994.	2004	19

<b>capillary zone electrophoresis</b>	Pus	$\lambda$ max = 254 nm, Linearity = (20 -7800) mg/mL/, LOD = 4.5 LOQ = 15, RSD = ( 2.4-8.2)%,, Re.= (75.4-90.0)%, R= 0.9956.	2005	20
<b>liquid chromatographic/tandem mass spectrometric</b>	Plasma	Column, ultimate XB-CN, Linearity = (0.1-12) mg .mL <sup>-1</sup> , LOD < 0.02 $\mu$ g.mL <sup>-1</sup> , RSD = (2.4-8.2)%, Er. = (1- 4.2)%, R<10.6%.	2007	21
<b>High performance liquid chromatography/tandem mass spectrometry</b>	Chicken serum	Linearity(0.1-50)mg.mL <sup>-1</sup> , column (150x4.6) mm, mobile phase, (20:80)% acetonitrile : water, Re. (95 to 108)%, Er.= (-7-7.8)%, CV. < 10%, Sd =(0.001-0.006).	2011	22
<b>High performance liquid Chromatography</b>	Chicken Muscle, Liver and Kidney	Con. Rang (0.1-0.28) $\mu$ g.g <sup>-1</sup> , CV.=(0.23-15.1)%, Re. = (81106%, 92-102% 99-107% for muscle, liver and kidney respectively, Er. = (0.3-3.76)%, LOQ=0.1 $\mu$ g.mL <sup>-1</sup> .	2011	23
<b>liquid chromatography– tandem mass spectrometry</b>	Plasma	Linearity =(50 -1200) ng.mL <sup>-1</sup> , column ACE, 150 mm $\times$ 4.0 mm, mobile phase acetonitrile: water (30:70)%, RSD =(2.2-6.7)%, r <sup>2</sup> > 0.999, LOQ = 50ng.mL <sup>-1</sup> ,	2014	24
<b>High performance liquid Chromatography</b>	Urine, Plasma	Linearity =(1 -2000), and (0.1-10) $\mu$ g/mL, Re. = (68, 72)%, precision (4.7%, 3.1)%, accuracy (1.7% and 1.2%) for plasma and urine respectively.	2014	25
<b>High performance liquid Chromatography</b>	Plasma	Linearity = (5.000–2000) $\mu$ g.mL <sup>-1</sup> , column, (2.1 x 50 mm), 5.0 $\mu$ m, accuracy = ( 0.1- 3.9) %, precision =(2-8.2)%, Re. = 83.6%, limits of agreement (-2.6-30.6%). RSD =(2.2-6.7)%, R <sup>2</sup> = 0.9998, LOQ = 0.75 $\mu$ g.mL <sup>-1</sup> .	2015	26
<b>liquid chromatography– tandem mass spectrometry</b>	Plasma	Linearity = (15-150) and (100-750) $\mu$ g/ml, column HILIC (150 $\times$ 2.1) mm x 5 m, with the precision =(4.0-6.4)% and (2.0–11.0)%, accuracy = (-1.1- 11.5) % and (0.6–7.8)%, R = 0.9976 and 0.9969.	2015	27
<b>High performance liquid Chromatography</b>	Urine, Plasma	Linearity =(0.75–375) $\mu$ g.mL <sup>-1</sup> , column HILIC, column 1.7 $\mu$ m, (2.1 $\times$ 100)mm, ,accuracy = ( 2.1- 3.2) %, precision =( 1.5% -1.7)%, Re. = (99-103)%, RSD =(2.2-6.7)%, R <sup>2</sup> = 0.9998, LOQ = 0.75 $\mu$ g.mL <sup>-1</sup> .	2017	28
<b>High performance liquid Chromatography</b>	Urine, aqueous fluids, plasma	Column, PS C18, Linearity: (12.5-800 $\mu$ g/mL), (62.5-4000 $\mu$ g/mL), (1-160 $\mu$ g/mL) for plasma, urine and aqueous fluid respectively, LOQ= plasma ( $\leq$ 6.5%, $\leq$ 8%), urine, ( $\leq$ 5.80, $\leq$ 6.30) %, and aqueous fluid ( $\leq$ 10.6, $\leq$ 12) %.	2017	29
<b>High performance liquid Chromatography</b>	Lysogeny broth (antimicrobial resistance)	Linearity = (1-1000) $\mu$ g.mL <sup>-1</sup> , column Kinetex (2.1 $\times$ 50) mm, 2.6 $\mu$ m, (pH 4.76), precision <15%, accuracy ( $\pm$ 85 and 115% ).	2018	30
<b>Liquid Chromatography Electrospray Ionization Mass Spectrometry</b>	Plasma and Dialysate	Linearity = (25.0 – 700) $\mu$ g. mL <sup>-1</sup> , precision, (1.1-1.2)%, accuracy (5.9% to 0.9%) respectively, Recovery $\geq$ 87%, Matrix effects (2.2- 4.3)%, R <sup>2</sup> =0.999.	2021	31
<b>spectrophotometric method</b>	pharmaceutical manufacture	$\lambda$ max = 254 nm, E= 4.59x104 L. mol <sup>-1</sup> .cm <sup>-1</sup> , Linearity = (30-70) $\mu$ g.mL <sup>-1</sup> , recovery was (98.75-101.00) %, RSD = 0.41%.	2021	32

### 3. Conclusions

Fosfomycin has a versioning mechanism of great action, lower toxicity, wide antibacterial activity, and excellent pharmacokinetic properties, in addition to its bioavailability [33]. An important literature review of different quantitative analytical methods for estimating fosfomycin in its pharmaceutical preparations was obtained. Many instrumental methods for determining fosfomycin have been developed, such as spectrophotometry, liquid chromatography with mass spectrometry, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography, and gas chromatography. However, chromatographic methods are the most used for quantitative analysis of these drugs in pharmaceuticals because these methods provide precise and accurate results.

### References

1. Raz, R. Fosfomycin: An Old—New Antibiotic. *Clinical Microbiology and Infection* **2012**, *18*, 4–7.
2. Patel, S.S.; Balfour, J.A.; Bryson, H.M. Fosfomycin Tromethamine. *Drugs* **1997**, *53*, 637–656.
3. Samonis, G.; Vardakas, K.Z.; Tansarli, G.S.; Dimopoulou, D.; Papadimitriou, G.; Kofteridis, D.P. Fosfomycin. *Clin Microbiol Rev* **2016**, *29*, 321–347.
4. Michalopoulos, A.S.; Livaditis, I.G.; Gougoutas, V. The Revival of Fosfomycin. *International journal of infectious diseases* **2011**, *15*, e732–e739.
5. Zhanel, G.G.; Zhanel, M.A.; Karlowsky, J.A. Intravenous Fosfomycin: An Assessment of Its Potential for Use in the Treatment of Systemic Infections in Canada. *Canadian Journal of Infectious Diseases and Medical Microbiology* **2018**, *2018*.
6. Christensen, B.G.; Leanza, W.J.; Beattie, T.R.; Patchett, A.A.; Arison, B.H.; Ormond, R.E.; Kuehl Jr, F.A.; Albers-Schonberg, G.; Jardetzky, O. Phosphonomycin: Structure and Synthesis. *Science* **1969**, *166*, 123–125.
7. Shafer, H.; Vandenheuvel, W.J.A.; Ormond, R.; Kuehl, F.A.; Wolf, F.J. Characterization of Phosphonomycin by Microchromatographic and Related Techniques. *Journal of Chromatography A* **1970**, *52*, 111–117.
8. Longo, A.; di Toro, M.; Pagani, E.; Carezzi, A. Simple Selected Ion Monitoring Method for Determination of Fosfomycin in Blood and Urine. *Journal of Chromatography B: Biomedical Sciences and Applications* **1981**, *224*, 257–264.
9. Dessalles, M.C.; Levieux, J.; Souleau, M.; Mahuzier, G. Determination of Fosfomycin in Biological Fluids by Gas Chromatography. *Pathologie-biologie* **1987**, *35*, 200–204.
10. Pianetti, G.A.; Moreirade Campos, L.M.; Chaminade, P.; Baillet, A.; Baylocq-Ferrier, D.; Mahuzier, G. Application of ion chromatography with indirect spectrophotometric detection to the sensitive determination of alkylphosphonic acids and fosfomycin. *Analytica Chimica Acta* **1993**, *284*, 2, 30, 291-299.
11. Baillet, A.; Pianetti, G.A.; Taverna, M.; Mahuzier, G.; Baylocq-Ferrier, D. Fosfomycin Determination in Serum by Capillary Zone Electrophoresis with Indirect Ultraviolet Detection. *Journal of Chromatography B: Biomedical Sciences and Applications* **1993**, *616*, 311–316.
12. Pianetti, G.A.; Baillet, A.; Traore, F.; Mahuzier, G. Chromatographic Analysis of Some Alkylphosphonic Acids Using a Conductimetric Detection. Application to Fosfomycin Determination. *Chromatographia* **1993**, *36*, 263–267.

13. Levêque, D.; Gallion, C.; Tarral, E.; Monteil, H.; Jehl, F. Determination of Fosfomycin in Biological Fluids by Capillary Electrophoresis. *Journal of Chromatography B: Biomedical Sciences and Applications* **1994**, *655*, 320–324.
14. Dios-Vieitez, M.C.; Goni, M.M.; Renedo, M.J.; Fos, D. Determination of Fosfomycin in Human Urine by Capillary Gas Chromatography: Application to Clinical Pharmacokinetic Studies. *Chromatographia* **1996**, *43*, 293–295.
15. Hu, Y.-L.; Feng, Y.-Q.; Zhang, Q.-H.; Da, S.-L. Determination of Fosfomycin by Indirect Spectrophotometric Method. *Talanta* **1999**, *49*, 47–52.
16. Webster, G.K.; Bell, R.G. Gas Chromatographic Analysis of Fosfomycin in Plasma for Pharmacokinetic Analysis. *Journal of AOAC International* **1999**, *82*, 620–624.
17. Hernandez, E.; Loste, A.; Bregante, M.A.; Garcia, M.A.; Solans, C. Determination of Fosfomycin in Chicken Plasma Samples by Gas Chromatography: Application to Pharmacokinetic Studies. *Chromatographia* **2001**, *54*, 365–368.
18. Tzanavaras, P.D.; Themelis, D.G. Flow Injection Spectrophotometric Determination of the Antibiotic Fosfomycin in Pharmaceutical Products and Urine Samples after On-Line Thermal-Induced Digestion. *Analytical biochemistry* **2002**, *304*, 244–248.
19. Petsch, M.; Mayer-Helm, B.X.; Sauermann, R.; Joukhadar, C.; Kenndler, E. Capillary Electrophoresis Analysis of Fosfomycin in Biological Fluids for Clinical Pharmacokinetic Studies. *Electrophoresis* **2004**, *25*, 2292–2298.
20. Petsch, M.; Mayer-Helm, B.X.; Sauermann, R.; Joukhadar, C.; Kenndler, E. Determination of Fosfomycin in Pus by Capillary Zone Electrophoresis. *Journal of Chromatography A* **2005**, *1081*, 55–59.
21. Li, L.; Chen, X.; Dai, X.; Chen, H.; Zhong, D. Rapid and Selective Liquid Chromatographic/Tandem Mass Spectrometric Method for the Determination of Fosfomycin in Human Plasma. *Journal of Chromatography B* **2007**, *856*, 171–177.
22. Dieguez, S.; Soraci, A.; Tapia, O.; Carciocchi, R.; Perez, D.; Harkes, R.; Romano, O. Determination of Antibiotic Fosfomycin in Chicken Serum by Liquid Chromatography-Tandem Mass Spectrometry. *Journal of liquid chromatography & related technologies* **2011**, *34*, 116–128.
23. Pérez, D.S.; Soraci, A.L.; Dieguez, S.N.; Tapia, M.O. Determination and Withdrawal Time of Fosfomycin in Chicken Muscle, Liver and Kidney. *Int. J. Poult. Sci* **2011**, *10*, 644–655.
24. Papakondyli, T.A.; Gremilogianni, A.M.; Megoulas, N.C.; Koupparis, M.A. A Novel Derivatization Method for the Determination of Fosfomycin in Human Plasma by Liquid Chromatography Coupled with Atmospheric Pressure Chemical Ionization Mass Spectrometric Detection via Phase Transfer Catalyzed Derivatization. *Journal of Chromatography A* **2014**, *1332*, 1–7.
25. Parker, S.L.; Lipman, J.; Roberts, J.A.; Wallis, S.C. A Simple LC–MS/MS Method Using HILIC Chromatography for the Determination of Fosfomycin in Plasma and Urine: Application to a Pilot Pharmacokinetic Study in Humans. *Journal of Pharmaceutical and Biomedical Analysis* **2015**, *105*, 39–45.
26. Parker, S.L.; Lipman, J.; Dimopoulos, G.; Roberts, J.A.; Wallis, S.C. A Validated Method for the Quantification of Fosfomycin on Dried Plasma Spots by HPLC–MS/MS: Application to a Pilot Pharmacokinetic Study in Humans. *Journal of Pharmaceutical and Biomedical Analysis* **2015**, *115*, 509–514.
27. Martens-Lobenhoffer, J.; Bode-Böger, S.M. A Validated Method for the Quantification of Fosfomycin in Human Plasma by Liquid Chromatography–Tandem Mass Spectrometry. *Journal of Chromatography B* **2015**, *990*, 164–168.
28. Wijma, R.A.; Bahmany, S.; Wilms, E.B.; van Gelder, T.; Mouton, J.W.; Koch, B.C.P. A Fast and Sensitive LC–MS/MS Method for the Quantification of Fosfomycin in Human Urine and

- Plasma Using One Sample Preparation Method and HILIC Chromatography. *Journal of Chromatography B* **2017**, 1061, 263–269.
29. El-Najjar, N.; Jantsch, J.; Gessner, A. A Rapid Liquid Chromatography-Tandem Mass Spectrometry for the Quantification of Fosfomycin in Plasma, Urine, and Aqueous Fluids. *Journal of Chromatography B* **2017**, 1061, 57–64.
  30. Gandhi, A.; Matta, M.; Garimella, N.; Zere, T.; Weaver, J. Development and Validation of a LC-MS/MS Method for Quantitation of Fosfomycin–Application to in Vitro Antimicrobial Resistance Study Using Hollow-fiber Infection Model. *Biomedical Chromatography* **2018**, 32, e4214.
  31. Shopova, T.; Hüppe, T.; Wolf, B.; Sessler, D.I.; Volk, T.; Groesdonk, H. v; Kreuer, S.; Maurer, F. Quantitative Determination of Fosfomycin in 10 ML of Plasma and Dialysate by Hydrophilic Interaction Liquid Chromatography Electrospray Ionization Mass Spectrometry. *Journal of Chromatographic Science* **2021**, 59, 165–174.
  32. Chaudhari, G.D.; Patil, S.D.; Chaudhari, H. v; Itnare, D.G.; Patil, P.J.; Sonar, K. v; Patil, P.R. Development and Validation of UV Spectroscopic Method for Estimation of Fosfomycin In Fosfomycin for Injection. *Am. J. PharmTech Res* **2021**, 11, 13–20.
  33. Cao, Y.; Peng, Q.; Li, S.; Deng, Z.; Gao, J. The Intriguing Biology and Chemistry of Fosfomycin: The Only Marketed Phosphonate Antibiotic. *Rsc Advances* **2019**, 9, 42204–42218.