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UHPLC-MS-Based Analysis of Fluvoxamine in Rabbit Aqueous Humour and Serum: Method Development and Validation

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Abstract

Background/Objectives: Fluvoxamine (FLU) is a selective serotonin reuptake inhibitor and one of the most potent agonists of the sigma-1 receptor. Emerging evidence shows that FLU exerts protective effects in multiple organs, making it a promising candidate for topical ocular therapy. Developing an FLU eyedrop for glaucoma can address a significant treatment gap with potentially fewer side effects compared with conventional therapies. To optimise formulation development, precise quantification of FLU in ocular compartments such as aqueous humour, as well as systemic circulation, is essential to characterise drug absorption, ocular bioavailability, and safety. **Methods**: We developed and validated a UHPLC-MS method for FLU detection in aqueous humour and serum using simple sample preparation steps. **Results**: The 11-min-long reverse phase chromatography followed by SRM-based mass spectrometry detection provides a highly selective and sensitive FLU detection method. Our method was proved to be linear in the 0.0625–1.5 µg/mL range and was validated according to the EMA guidelines. **Conclusions**: The simplicity of sample preparation, the tolerable matrix effects, and the favourable detection parameters provide a robust tool for preclinical pharmacokinetic and pharmacodynamic studies of FLU's ocular protective effects.

Keywords: fluvoxamine; UHPLC-MS method; EMA guidelines; aqueous humour; sigma-1 receptor; serum

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1. Introduction

Fluvoxamine (FLU) is one of the most potent agonists of the sigma-1 receptor (S1R), a ligand-regulated endoplasmic reticulum chaperone protein with a crucial role in cellular homeostasis [1,2]. FLU is also a selective serotonin reuptake inhibitor, and its protective effect via S1R agonism is best known and extensively studied in the central nervous system, where it modulates neuroplasticity, reduces ER stress, enhances mitochondrial function, suppresses neuroinflammation, and provides neuroprotection in neurodegenerative and psychiatric disorders [3,4]. However, emerging evidence shows that FLU's agonism exerts protective effects in multiple organs, including the heart, lung, and kidney, by modulating cellular stress responses, inflammation, cell survival pathways, and fibrosis [5–7].

Glaucoma is a chronic progressive optic neuropathy characterised by elevated intraocular pressure (IOP), leading to retinal ganglion cell and optic nerve neurodegeneration and resulting in irreversible vision loss [8]. Current treatments focus on lowering IOP to prevent or delay optic nerve damage. In the eye, S1R activation protects retinal cells from stress-induced degeneration, maintaining retinal structure and function and reducing chronic inflammation and oxidative damage [9]. Recently, our studies demonstrated that FLU exhibits anti-fibrotic properties in trabecular meshwork (TM) cells, reducing the fibrotic markers [10,11] associated with impaired aqueous humour outflow, which is a primary cause of elevated intraocular pressure (IOP) in glaucoma. Such protective properties make FLU a promising candidate for topical ocular therapy aiming to prevent or slow glaucoma and related optic neuropathies.

Developing an FLU eyedrop formulation aims to utilise its S1R agonist properties to reduce TM fibrosis and lower IOP, thus addressing a significant treatment gap with potentially fewer side effects compared with conventional therapies.

The topical route is ideal for glaucoma as it allows targeted drug delivery to the anterior segment of the eye, minimising systemic exposure and improving patient compliance.

To optimise a new eyedrop formulation development, precise and robust quantification of FLU in ocular compartments such as aqueous humour—as well as in serum to assess systemic exposure—is essential for characterising drug absorption, ocular bioavailability, and safety. Existing methods (Table S1) with UV or fluorescence detection or LC-MS assays target pharmaceutical formulations (e.g., tablets) or plasma at higher concentrations, mostly lacking the sensitivity and specificity needed for the analysis of complex biological fluids like aqueous humour in low volume or serum in preclinical ophthalmic models [12–16]. Other methods have the necessary sensitivity, but the sample preparation requires multiple steps or multiple chromatographic separations, hindering its application for higher sample numbers needed in the clinical validation studies [17–20].

Thus, developing a simple, sensitive, selective, and relatively cheap UHPLC-MS method is critical to accurately measure FLU levels during eyedrop formulation and pharmacokinetic studies, facilitating optimisation of dosing and therapeutic efficacy in glaucoma treatment.

This paper presents the development and validation of a UHPLC-MS methodology optimised for FLU detection in rabbit aqueous humour and serum. No prior UHPLC-MS method has been reported for FLU quantification in ocular compartments, highlighting the analytical novelty of this study. The method addresses chromatographic separation, sample preparation, matrix effects, and detection parameters to provide a robust tool for preclinical pharmacokinetic and pharmacodynamic studies of FLU's ocular protective effects.

2. Results and Discussion

The method was developed on an Acquity H-class ultra-high performance liquid chromatography (UHPLC) system coupled with a 5500QTRAP tandem mass spectrom-

eter. An 11-min gradient was applied, and the MS analysis was carried out using SRM acquisition [21]. The observed retention time was 8.77 min (Figure 1).

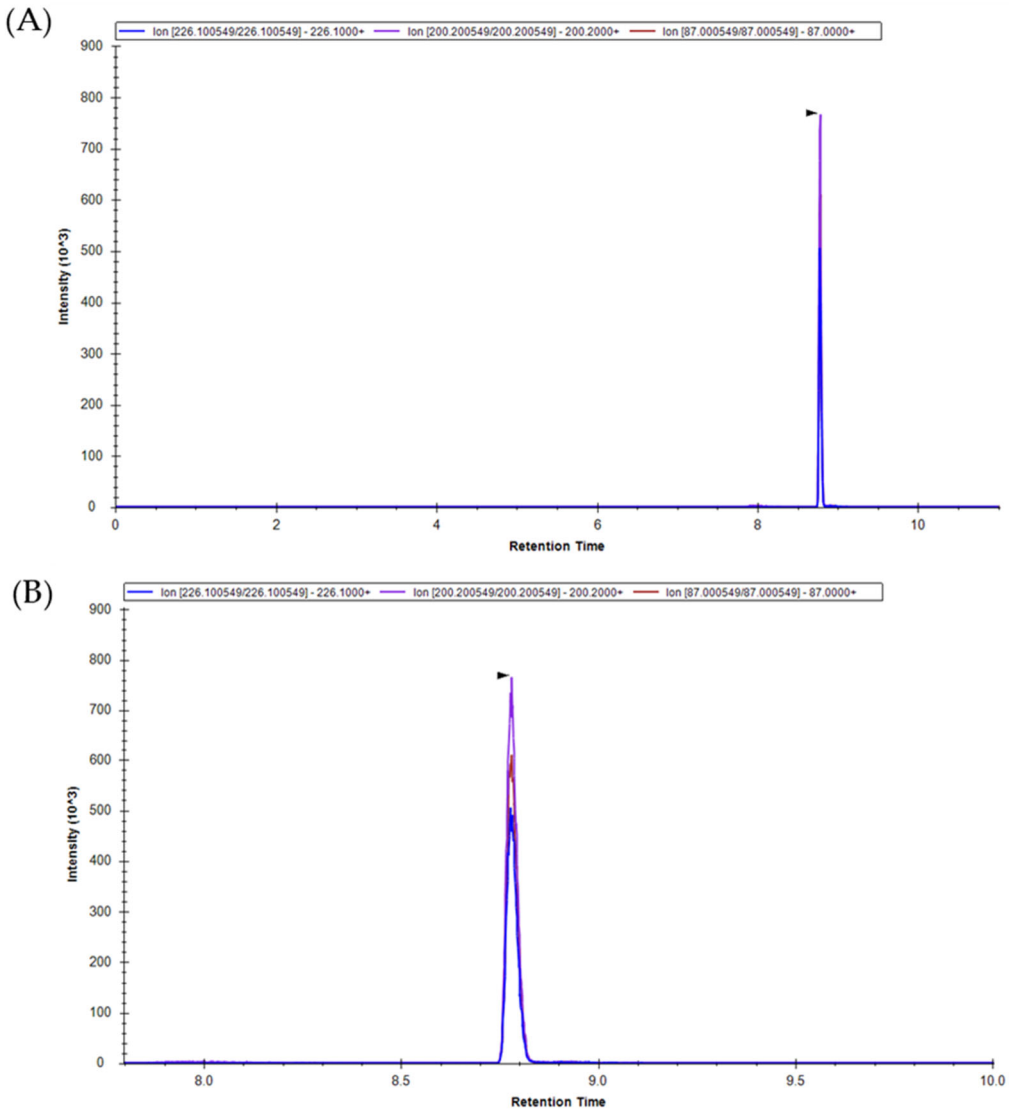

Figure 1. Representative SRM spectra of FLU in AH matrix. (**A**) Full spectrum. (**B**) Zoomed spectrum highlighting the peak corresponding to FLU. The x axis shows the retention time in minutes, while the y axis shows the intensity. The individual SRM transitions are indicated.

The developed LC-MS method was further validated according to the guidelines of the European Medicines Agency (EMA) [22]. The selectivity, carry-over, linear range, lower limit of quantification (LLOQ), upper limit of quantification (ULOQ), accuracy, precision, recovery, matrix effect, and stability were tested.

2.1. Sensitivity, Calibration Curve, and Range

A mixture of aqueous humour (AH) samples collected from untreated eyes was used as a matrix. Dilution series in the range of 0.0625–5 µg/mL of FLU were prepared in water and the matrix. To determine the linear range, namely the LLOQ and ULOQ, the calibration range was investigated in three independent runs and two replicates over three days. The calibration equation was $y = 244{,}670 \times x^2 + 178{,}400 \times x - 52{,}852$ ($R^2 = 0.9986$), and the LLOQ was 0.0625 µg/mL, while the ULOQ was 2 µg/mL. The calibration range was defined by the LLOQ, which is the lowest calibration point, and the ULOQ, which is the highest calibration point. According to our results, the linear range was between 0.0625

and 1.5 µg/mL (Figure 2). Our results are in accordance with the EMA recommendations; the accuracy of each calibration standard was less than 20% of the nominal concentration for the LLOQ value and less than 15% for all other concentrations (Table A1) [22].

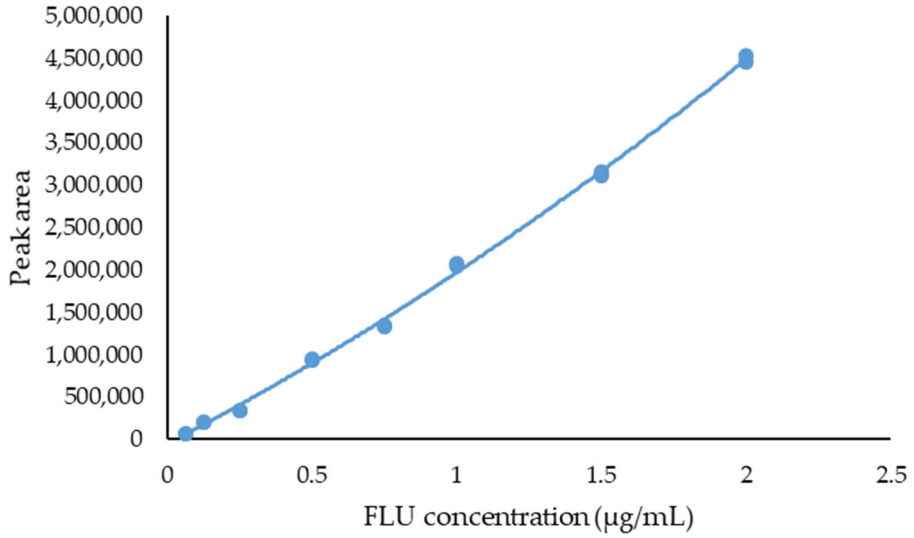

Figure 2. Calibration curve. The y axis shows the peak area (AUC) for the FLU concentrations, shown on the x axis (details shown in Table A1).

2.2. Selectivity and Carry-Over

In order to determine the selectivity and carry-over, a blank sample (matrix without FLU) was used. In the case of selectivity of the method, the analyte response was 2.36% of the LLOQ (Table A2), fulfilling the EMA criteria.

During the analysis of the carry-over, the analyte response for FLU in the blank sample after injection of the standard with an FLU concentration corresponding to the ULOQ was 19.54% (Table A2). All of our registered data were below 20% for the analyte response, being in accordance with the EMA guidelines [22].

2.3. Accuracy, Precision, and Recovery

During method validation, the accuracy, precision, and recovery of intraday and interday runs were examined (Table 1).

Table 1. Accuracy, precision, and recovery of FLU.

Analyte	Nominal Concentration (µg/mL)	Within Run (n = 5)				Between Runs (n = 15)			
		Mean (µg/mL)	SD	CV%	Recovery (%)	Mean (µg/mL)	SD	CV (%)	Recovery (%)
FLU	0.0625	0.064	0.002	2.511	101.888	0.068	0.004	6.118	109.120
	0.25	0.222	0.007	3.224	88.680	0.227	0.013	5.912	90.957
	0.75	0.677	0.017	2.533	90.288	0.715	0.057	7.931	95.276
	1.5	1.390	0.017	1.228	92.655	1.507	0.111	7.391	100.447

As expected, the intraday variation was less than the interday variation, and in all cases, the accuracy, recovery, and precision (CV%) values at all concentrations were less than 15% of the nominal concentration, fulfilling the EMA validation criteria [22].

2.4. Matrix Effect

The matrix effect was examined through the analysis of three replicates of low-concentration (0.25 µg/mL) quality controls (QCs) and high-concentration (1.5 µg/mL)

QCs from five different batches. The results of the matrix effect analyses are shown in Table A2.

The matrix effect was positive in the case of the AH matrix, indicating suppression compared with the aqueous matrix.

The precision (CV%) was below 1.5%, the matrix effect was less than 8%, and the recovery at each examined concentration was found to be less than 11% of the nominal concentration. The obtained results are in accordance with the EMA criteria, requiring less than 15% for the above-mentioned values [22].

2.5. Stability

During the method validation, for the freeze-thaw stability, the stability of the analytes in the working solutions at 4 °C was examined. The stability of the analyte in the matrix was evaluated through the analysis of two replicates of low-concentration (0.25 µg/mL) and high-concentration (1.5 µg/mL) QCs, and the results are shown in Table 2.

Table 2. Summary of the results of the stability tests of FLU.

Analyte		**Fluvoxamine**	
QC (µg/mL)		0.25	1.5
Stability after three freeze-thaw cycles	Mean (µg/mL)	0.271	1.636
	SD	0.003	0.009
	CV (%)	0.965	0.527
	Recovery (%)	108.420	109.073
Stability in working solution	Mean (µg/mL)	0.316	1.601
	SD	0.003	0.135
	CV (%)	1.052	0.844
	Recovery (%)	126.340	106.730

Our results indicate that three freeze-thaw cycles did not alter the concentration of the target molecule in the samples stored at −70 °C, nor did the incubation in working solution at 4 °C. Testing stability is critically important when storing samples, but the EMA does not specify a precise limit in this case. Based on the results, the samples tolerate freezing and thawing well, but according to our recommendations, the working solution must be prepared fresh on the day of the measurement.

2.6. Examination of FLU Content of Aqueous Humour and Serum Samples

To demonstrate that our method is suitable for analysing FLU concentrations in complex biological samples, we processed and analysed AH and serum samples from animals treated with FLU. During method development, we tried to use the simplest possible sample preparation method. In the case of AH, the sample was diluted, reducing the amount of interfering compounds. As serum is a more complex sample compared with AH, dilution was not applicable. Protein precipitation is one of the most popular techniques used to prepare biological fluid samples for LC-MS/MS analyses [23,24], and we applied acetone precipitation followed by drying and redissolving the supernatant. Filtering using membranes with a low molecular weight cutoff is often applied in the examination of small molecules [25] by reducing the complexity of the biological samples, which could not be applied as there was a considerable unspecific binding of FLU to the membrane and the cartridge (Figure S1). Using the simplified sample preparation and the developed UHPLC-MS method, we determined the concentration of FLU in both sample types. Our data show that FLU was quantifiable in the AH samples of the animals treated with FLU

eye drops. With one exception, it was not detectable in the control samples. The results are shown in Table A3. Although FLU was detectable in the serum samples from these animals, it was only present in extremely low concentrations. The results of the serum samples are shown in Table A4. The FLU concentrations observed in the different sample types can be seen in Figure 3.

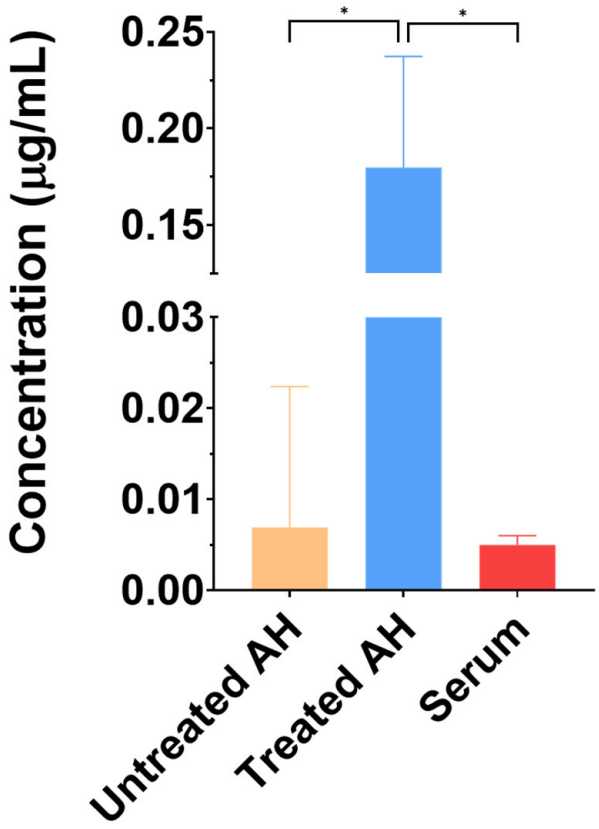

Figure 3. Concentration of FLU in AH and serum samples. The mean flu concentration in µg/mL along with the standard deviation (SD) are shown on the y axis, measured in the samples (n = 5) indicated on the x axis. * Statistically significant difference ($p < 0.05$).

These results indicate that the treatment increased the concentration of FLU in the AH samples originating from the treated eyes compared with the untreated eyes. Low FLU levels could be detected in the serum, suggesting limited systemic exposure; however, the available data do not allow for definitive conclusions regarding systemic absorption.

The results of our exploratory study indicate that the UHPLC-MS method optimised by our group is suitable for the reliable analysis of AH and serum samples containing FLU. At the same time, we have to note some limitations. Our method was tested only on AH and serum; using multiple sample types could make the method more generally applicable. The sensitivity of the method and the ULOQ could be further improved; however, because the achieved performance met the analytical requirements for FLU quantification in AH and serum samples, further optimisation was not pursued. The robustness of the analytical method was not tested, representing another limitation. Moreover, the low volume of available AH samples poses a challenge, as it requires working with extremely small sample amounts.

Along with the above limitations, our method can provide a relatively simple tool for preclinical pharmacokinetic and pharmacodynamics studies of FLU's ocular protective effects.

3. Materials and Methods

3.1. Chemicals and Reagents

AccQ-Tag Ultra eluent A and B were purchased from Waters (Milford, MA, USA). LC–MS-grade water was acquired from VWR Ltd. (Radnor, PA, USA). Fluvoxamine-maleate was purchased from Merck (St. Louis, MO, USA).

3.2. Instruments and Software

To conduct the analyses, a Waters ACQUITY H Class ultra-performance liquid chromatography system controlled by Empower 3 software (build number: 3471, Waters, Milford, MA, USA), coupled with a 5500 QTRAP (Sciex, Framingham, MA, USA) mass spectrometer controlled by Analyst software (version 1.6.3, Sciex, Framingham, MA, USA), was used. MS data were processed using Skyline software (version 23.1.0.455) [26].

3.3. Collection of Aqueous Humour and Serum Samples

Five male New Zealand white rabbits (purchased from S & K LAP Kft, Kartal, Hungary), approximately 10 weeks old and weighing 2.0–2.2 kg, were used in this study conducted at the Test Facility of Aurigon Labs Ltd. (Dunakeszi, Hungary). The rabbits were housed individually in cages within a temperature- and humidity-controlled room. They had ad libitum access to tap water suitable for human consumption via drinking bottles changed daily. Food (S & K LAP rabbit mixed diet produced by Cargill Takarmány Zrt., Budapest, Hungária) was provided daily, with an average intake of about 30–60 g/kg/day. Animals received topical treatment with FLU eye drops twice daily in one eye for two weeks, while the contralateral eyes served as controls and were treated with the vehicle only. Following euthanasia by intravenous overdose of sodium pentobarbital at 150 mg/kg, 50–100 µL of aqueous humour samples were withdrawn from each eye and transferred into sterile microcentrifuge tubes. The collection was conducted under sterile conditions, and the samples were immediately frozen on dry ice for further analysis. Blood samples were collected from the marginal ear vein of all animals prior to euthanasia. Approximately 0.5 mL of blood was drawn into plastic vials containing clot activator (MiniCollect® TUBE 0.5/1 mL CAT Serum Clot Activator). Immediately after collection, the samples were gently inverted several times for mixing. Serum was separated via centrifugation within 20–40 min of collection at 2000× *g* for 10 min at room temperature. Aliquots of approximately 200 µL serum were placed in pre-labelled plastic reaction tubes and immediately frozen on dry ice.

3.4. Preparation of QC Samples and Standard Solutions

The standard solution of FLU was prepared in water at a 2.5 mg/mL final concentration. The calibration standards were prepared from the standard solutions through serial dilutions with water in the range of 0.0625–5 µg/mL of FLU, with calibration points of 0.0625, 0.125, 0.25, 0.5, 0.75, 1, 1.5, 2, and 5 µg/mL. Parallel to that, a pool of AH samples collected from the untreated eyes was formed and used as a matrix, and FLU was spiked into that to have the same concentration as that in the water. For quality control (QC), the AH pool was spiked with FLU standard at 4 concentrations: 0.0625 µg/mL, 0.25 µg/mL, 0.75 µg/mL, and 1.5 µg/mL. QC samples were used for analysis of the recovery, matrix effect, intra- and interday precision, and stability.

3.5. Chromatographic Separation and Mass Spectrometric Analysis

Liquid chromatographic separation was performed on an Acquity H-Class UHPLC system (Waters, Milford, MA, USA) controlled by Empower 3 software (Waters, Milford, MA, USA). The separation of FLU from other components was carried out on an AccQ-tag

Ultra C18 column (1.7 µm; 2.1 × 100 mm, Waters, Milford, MA, USA) guarded by an Acquity in-line filter (0.2 µm; 2.1 mm, Waters, Milford, MA, USA). The 11-min chromatographic separation was performed according to the Waters Corporation application note. Briefly, solvent A was 100% AccQ-tag Ultra eluent A, solvent B was 10% AccQ-tag Ultra eluent B in LC–MS grade water, solvent C was LC water, and solvent D was 100% AccQ-tag Ultra eluent B. The flow rate was 0.60 mL/min, and the column temperature was 43 °C.

The PDA detector was set to a 260 nm wavelength with a 10 points/s sampling rate. SRM-based targeted mass spectrometry analyses were carried out on a 5500QTRAP (Sciex, Framingham, MA, USA) mass spectrometer controlled by Analyst software (version 1.6.3., Sciex, Framingham, MA, USA). The eluates from the LC column were ionised using electrospray ionisation with a 5500 V spray voltage, and the positive ion mode SRM spectra were recorded. The detailed parameters of the SRM experiment are presented in Table 3. The other acquisition parameters were as follows. The ion source gas 1 was set to 30 psi, the ion source gas 2 was 50 psi, the curtain gas was 30 psi, and the source temperature was 500 °C. The applied declustering potential was 120 eV, and 23 eV of collision energy was used for fragmentation.

Table 3. Transitions used for selected reaction monitoring (SRM) analysis. Q1 m/z = parent ion; Q3 m/z = fragment ion.

ID	Q1 *m/z*	Q3 *m/z*
FLU	319.1	226.1
FLU	319.1	200.2
FLU	319.1	87

The registered chromatograms were analysed with Empower 3 software, and the SRM spectra were analysed with Skyline software (version 23.1.0.455) [26]. The area under the curve (AUC) values for individual transitions were summed and used for calculating the concentration of FLU. The acquired SRM data were uploaded to the Panorama website [27] (https://panoramaweb.org/University%20of%20Debrecen/Fluvoxamine/project-begin.view, accessed on 28 November 2025), and they are publicly available.

3.6. Method Validation

The developed LC–MS method was validated according to the European Medicines Agency (EMA) ICH guideline M10 with bioanalytical method validation and study sample analysis [22]. The linearity, selectivity, accuracy, precision, intra- and inter-day variability, carry-over, recovery, matrix effect, lower limit of quantification (LLOQ), and upper limit of quantification (ULOQ) were determined following the EMA recommendations. Calibration curves in the 0.0625–1.5 µg/mL range in water and an AH matrix were recorded according to the EMA guidelines; three independent runs and two technical replicates over three days were recorded.

At the LLOQ, the accuracy of each analyte should be within ±20% of the nominal concentration; at all the other concentrations, the accuracy should be within ±15%; and at least 75% of the calibration points should fulfil the above criteria [22]. In order to determine the selectivity, blank samples were analysed without the addition of the analyte. The criteria were that there should be no observed interfering peaks at the studied retention times. Selectivity assessment should confirm that no significant signal from interfering substances is present at the analyte's retention time in blank samples. Any response due to interfering components should not exceed 20% of the analyte response at the LLOQ level for each matrix.

The accuracy and precision were determined by analysing QC samples in 0.0625, 0.25, 0.75, and 1.5 μg/mL concentrations. Intra- and inter-day variability was determined by the analysis of five replicates of the QC samples in the same day (intraday) and for 2 days. The carry-over of the system was tested via the analysis of blank samples after the ULOQ of the calibration curves. The matrix effect was examined through the analysis of three replicates of low-concentration (0.25 μg/mL) and high-concentration (1.5 μg/mL) QCs from 5 different matrix batches compared with the same concentration. The matrix effect is defined as an alteration of the analyte response due to interfering and unidentified components in the sample matrix. We used the following formula to calculate the matrix effect:

$$(\text{ME}): \text{ME} = [(\text{Cw} - \text{Cm})/\text{Cw}] \times 100 \quad (1)$$

where Cw is the concentration of the analytes in MilliQ water and Cm is the concentration of the same concentration of the analytes spiked into the matrix. The accuracy and coefficient of variation should not be more than 15%. The freeze-thaw stability of the samples was tested through the analysis of the QC samples after three freeze-thaw cycles at −70 °C according to the EMA guidelines with the following criteria. Low and high QC samples (0.25 μg/mL and 1.5 μg/mL, respectively) should be thawed and analysed according to the same procedures as the test samples. QCs should be kept frozen for at least 12 h between thawing cycles. Freeze-thaw stability of the QCs should be performed using freshly prepared calibration standards and QCs. The number of validated freeze-thaw cycles shall be at least three. The stability of the working solutions of the FLU was determined at 4 °C by using the lowest and highest concentrations of the solutions.

3.7. Examination of FLU-Containing Samples

The AH and serum samples were kept at −80 °C until the analysis. For the AH samples, no specific sample preparation except dilution was carried out. To each 8 μL of AH, 32 μL of MQ water was added, mixed with the pipette, and used for UHPLC-MS analysis. In each case, 5 μL was injected into the UHPLC-MS system.

For the serum samples, we performed an acetone precipitation and dried the supernatant. Then, the redissolved pellet was cleared via centrifugation and used for analysis. Briefly, 600 μL of acetone (−20 °C) was added to 100 μL of serum, incubated at −20 °C for 4 h, vortexed in every half hour, and then centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was collected and dried in a vacuum concentrator (ThermoScientific, San Jose, CA, USA). The dried samples were redissolved in 100 μL of MQ water, centrifuged again using the above parameters, and finally the supernatants were transferred into glass vials before 10 μL was injected into the UHPLC-MS system. For both the AH and serum samples, two technical replicates were applied.

The statistical analysis was carried out using GraphPad Prism 8.0.1 for Windows (GraphPad Software, Boston, MA, USA, www.graphpad.com, accessed on 15 November 2025), a Mann–Whitney U test was applied, and the results were considered statistically significant at $p < 0.05$.

4. Conclusions

We developed a fast LC-MS method for the quantitative examination of FLU and validated it, taking into account the EMA guidelines. The developed simple method was optimised for the measurement of FLU in ocular compartments and serum. This validated UHPLC-MS method fills a critical analytical gap, providing essential guidance for preclinical pharmacokinetic and pharmacodynamic studies of FLU in ocular tissues. Immediate applications include dose-ranging studies and comparative formulation testing, directly facilitating preparation of eyedrop formulations for phase I clinical trials.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ph19020260/s1 Figure S1: Purification of 10 µg/mL FLU sample with 3 kDa spin column. Table S1: Comparison of the reported analytical methods for the analysis of FLU.

Author Contributions: Conceptualisation, É.C. and J.H.; methodology, A.G., J.H., A.T.-N., S.D., B.S. and G.K.; software, A.G.; validation, A.G., A.T.-N., S.D., B.S. and M.V.; formal analysis, A.G., S.D., B.S. and G.K.; investigation, J.H., A.T.-N., S.D., B.S. and M.T.; resources, A.F. and É.C.; data curation, J.H., A.T.-N., B.S., M.T., G.K. and A.G.; writing—original draft preparation, A.G. and J.H.; writing—review and editing, J.H., A.F. and É.C.; visualisation, A.G. and M.T.; supervision, J.H., A.F. and É.C.; project administration, J.H., A.F. and É.C.; funding acquisition, A.F. and É.C. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Handling and care of animals were conducted according to the Guide for the Care and Use of Laboratory Animals, NRC, 2011. This study was conducted in compliance with the principles of Hungarian Act 1998: XXVIII regulating animal protection (latest modified by Act 2011 CLVIII) and Government Decree 40/2013 on animal experiments. The institution Aurigon is registered at the authority as an experimental animal usage facility and has an authorisation for animal use. The necessary permission of the Institutional Animal Care and Use Committee (IACUC) at Aurigon was obtained prior to commencing the study. The animal study protocol was approved by the Institutional Review Board of NÉBIH (PE/EA/43-2/2021, 7 January 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The acquired SRM data are publicly available at the Panorama website (https://panoramaweb.org/University%20of%20Debrecen/Fluvoxamine/project-begin.view (accessed on 28 November 2025)).

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Conflicts of Interest: Author Bálint Szokol was employed by the company Vichem Chemie Research Ltd., and author Anna Takácsi-Nagy was employed by the company PannonPharma Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AH	Aqueous humour
EMA	European Medicines Agency
LC-MS	Liquid chromatography–mass spectrometry
LLOQ	Lower limit of quantification
SRM	Selected reaction monitoring
UHPLC-MS	Ultra-high performance liquid chromatography tandem mass spectrometry
ULOQ	Upper limit of quantification

Appendix A

Table A1. Calibration parameters.

Compound		Fluvoxamine
Abbreviation		Flu
t_R (min)	MQ	8.77
	Matrix	8.77
LLOQ (µg/mL)	MQ	0.0625
	Matrix	0.0625
ULOQ (µg/mL)	MQ	2
	Matrix	2
Linear range (µg/mL)	MQ	0.125–1
	Matrix	0.0625–1.5
R^2	MQ	0.9984
	Matrix	0.9986
Calibration equation	MQ	$Y = 24{,}980X^2 + 716{,}960X - 21{,}348$
	Matrix	$Y = 244{,}670X^2 + 1{,}781{,}400X - 52{,}852$

Table A2. The matrix effect and recovery for the determination of fluvoxamine.

Analyte	QC (µg/mL)	Average Conc. (µg/mL)	SD	CV% (RSD%)	Recovery (%)	Matrix Effect (%)
Fluvoxamine	0.25	0.223	0.003	1.303	89.067	6.873
	1.5	1.390	0.012	0.858	92.678	7.384

Table A3. Concentration of fluvoxamine in the AH samples.

Description	Animal ID	FLU Concentration (µg/mL)
SigmaDrops_Day_15_AH	101	0.1856
	102	0.1028
	103	0.2412
	104	0.1429
	105	0.2264
Control_Day_15_AH	101	0.0346
	102	N.D.
	103	N.D.
	104	N.D.
	105	N.D.

Table A4. Concentration of fluvoxamine in the serum samples.

Description	Animal ID	FLU Concentration (μg/mL)
Male rabbit serum	101	0.0053
	102	0.0040
	103	0.0044
	104	0.0066
	105	0.0047

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